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Discovery and SAR of cinnolines/quinolines as liver X receptor (LXR) agonists with binding selectivity for LXRβ

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ABSTRACT

A series of cinnolines/quinolines was prepared and it was found that 4-phenyl-cinnoline/quinolines with either a 2′,3′ or 2′,5′-disubstituted benzyloxy moiety or the 1-Me-7-indole methoxy moiety on the meta position of the 4-phenyl ring showed good binding selectivity for LXRβ over LXRα. The LXRβ binding selective modulators displayed good activity for inducing ABCA1 gene expression in J774 macrophage cell line and poor efficacy in the LXRα Gal4 functional assay. **26**, **37** and **41** were examined for their ability to induce SREBP-1c gene expression in Huh-7 liver cell line and they were weak partial agonists.

1. Introduction

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.[1,2] They are ligand-activated transcription factors and bind to DNA as obligate heterodimers with retinoid X receptors (RXR). In macrophages, liver, and intestine, activation of LXRs induce the expression of several genes involved in lipid metabolism and reverse cholesterol transport including ATP binding cassette transport A1 (ABCA1), ATP binding cassette transport G1 (ABCG1) and apolipoprotein E (ApoE). The potential to prevent or even reverse atherosclerotic process by increasing the expression of these genes makes LXR an attractive drug target for the treatment of atherosclerosis which is one of the leading health concerns in the United States.[3] Several LXR pan agonists (Fig. 1), such as GW3965[4], TO901317,[5] and **WAY-254011**,[6] have been shown to increase expression of several genes involved in lipid metabolism and reverse cholesterol transport including ABCA1, ABCG1 and ApoE. These compounds reduced or even reversed atherosclerotic processes in mouse models of atherosclerosis. Currently available synthetic LXR pan agonists, however, also activated triglyceride (TG) synthesis in the liver by the up regulation of sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) which limits the utility of these LXR synthetic agonists. Several strategies[1,2] have been proposed for the improvement of the therapeutic window of LXR agonists including LXRβ subtype selective agonists, partial agonists, and gene or tissue specific agonists. The first hypothesis is based on the observation that LXRα is the predominant isoform expressed in the liver and that activation of LXRα may be responsible for the TG liability in vivo. Therefore, LXRβ selective LXR modulators may have less impact on TG synthesis, but may be effective in macrophage reverse cholesterol transport. Thus our new efforts were focused on the identification of LXRβ selective modulators. A recent study has shown that ligand activation of LXRβ reversed atherosclerosis and cellular cholesterol overload in mice lacking LXRα and ApoE.[7] This observation provided strong in vivo support for LXRβ as a drug target for the treatment of atherosclerosis. Unfortunately, there are only minor structural differences in the ligand binding domains (LBD) of LXRα and LXRβ that can be exploited to obtain highly β-selective ligands. The two LXR isoforms α/β share a high sequence identity (78%) and residue differences are located far away from the ligand binding pocket.[8] This high similarity in the binding pocket of the two LXR isoforms constitutes a serious obstacle to the development of highly β-selective ligands. Nevertheless, a modest level of LXRβ selectivity in a small molecule has been achieved. N-Acylthiadiazoline **5** with preferential affinity for LXRβ in scintillation proximity assays (SPA) measuring total binding (LXRβ IC_{50} 0.3 μM, LXRα IC_{50} 9.8 μM) has been reported.[9] Func-

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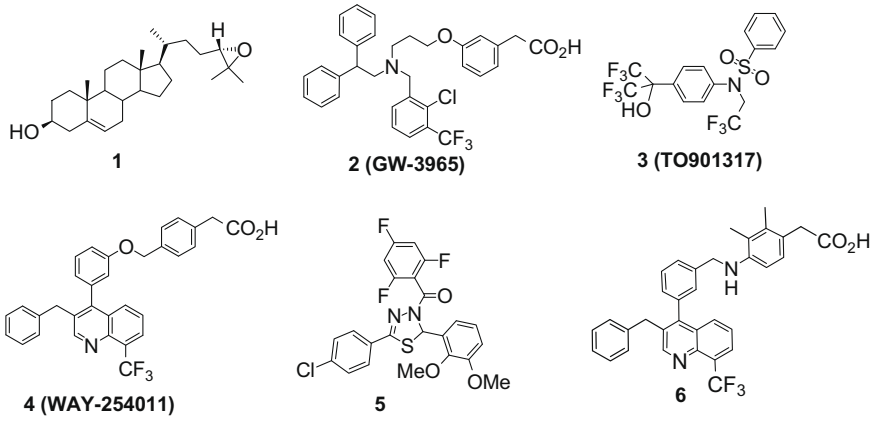


Figure 1. Known LXR agonists.

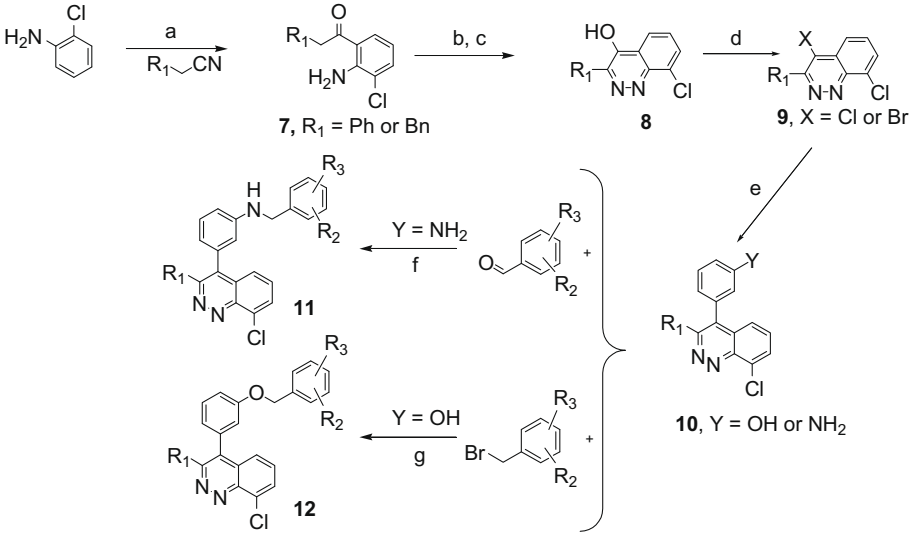
tional selectivity was also confirmed using macrophages derived from LXR-null mice: compound **5** showed significantly higher expression of ABCA1 in LXRα-null macrophages than in LXRβ-null macrophages. We previously identified quinoline-based agonists with a 2′,3′,- or 2′,5′-dimethylphenyl acetic acid moiety (such as **6**) or a naphthalene acetic acid moiety that showed good binding affinity for LXRβ and moderate to good binding selectivity for LXRβ over LXRα.[10] The premise for the design of these compounds was to gain selectivity via ligand interaction with LXRβ Ile_{277}/LXRα Val_{263}. However, compound **6** also exhibited cross-activity with peroxisome proliferator-activated nuclear receptors (PPAR). In an effort to eliminate PPAR activity and maintain or improve LXRβ selectivity we prepared a series of substituted cinnolines/quinolines in which the acetic acid of **6** was removed and a variety of substitutions on the adjunct phenyl ring were examined.

2. Results and discussion

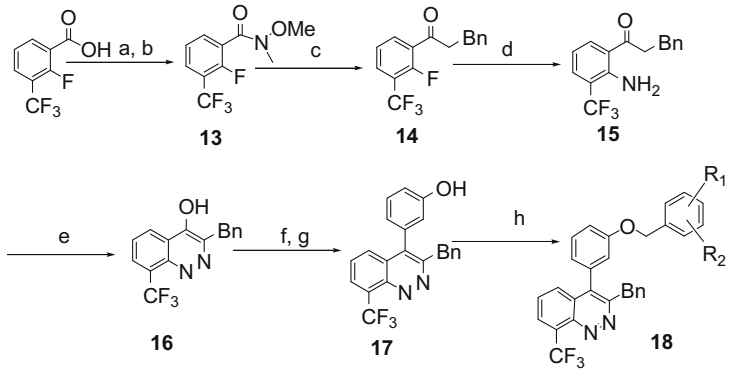
2.1. Synthetic chemistry

In an attempt to enlarge the chemical diversity of LXRβ selective ligands, we prepared a series of cinnoline derivatives as shown in Scheme 1 (8-chloro-cinnolines) and Scheme 2 (8-CF_3-cinnolines). Following literature precedent for analogous transformations 2-amino-3-chloro-benzophenones **7** were synthesized via the Sugasawa reaction[11] between an aniline and a nitrile in the presence of stoichiometric amounts of $AlCl_3$ and BCl_3. Conversion of **7** into the corresponding cinnoline phenol derivatives **8** was readily achieved in two steps by diazotization ($NaNO_2$/acid) of the 2-aminobenzophenones **7** followed by cyclization upon heating at 80 °C. Refluxing the phenol **8** with phosphorus oxychloride or phosphorus oxybromide afforded good yields of the corresponding cinnoline halo derivatives **9**. Reaction of **9** with phenylboronic acid under standard Suzuki conditions provided **10**. Reductive amination of **10** (Y = NH_2) with benzyl aldehydes gave the desired 8-chloro-benzyl-amine cinnolines **11**. 8-Chloro-benzyl-ether cinnolines **12** were prepared from **10** (Y = OH) under standard Mitsonobu conditions or alkylations with benzyl bromides under basic conditions.

For 8-CF_3-cinnolines because CF_3 group does not survive the Sugasawa reaction conditions (BCl_3–$AlCl_3$), an alternative synthesis was developed as shown in Scheme 2. The Weinreb amide **13**[12] was prepared using standard conditions from commercially available 2-fluoro-3-trifluoromethylbenzoic acid. Treatment of **13** with phenylethylmagnesium bromides gave fluorobenzophenones **14**. Conversion of **14** into aniline **15** was accomplished with ammonium hydroxide at 140 °C in a steel pressure reactor. The aniline



Scheme 1. Reagents and conditions: (a) BCl_3/$AlCl_3$, 30–60%; (b) $NaNO_2$/H^+; (c) 80 °C, 20–80% over all yield for step (b) and (c); (d) $POBr_3$/DMF or $POCl_3$, 90–100%; (e) arylboronic acids, K_3PO_4, $Pd(PPh_3)_4$, dioxane, 30–85%; (f) benzyl amines, $NaBH(OAc)_3$, DMF, 30–90%; (g) benzyl bromides/K_2CO_3 or benzyl alcohol, DIAD, PPh_3; 30–90%.



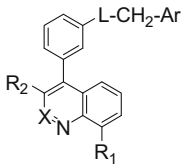
Scheme 2. Reagents and conditions: (a) $SOCl_2$, 84% (b) NMeOMe; (c) $PhCH_2CH_2MgCl$, 81% for step (b) and (c); (d) NH_3/DMF, 90%; (e) $NaNO_2/H^+$, 20–80%; (f) $POBr_3$, DMF, 34% for step (e) and (f); (g) K_3PO_4, $Pd(PPh_3)_4$, dioxane, 74%; (h) benzyl bromides, K_2CO_3, or benzyl alcohol, DIAD, PPh_3, 30–90%.

15 was then converted to 8-CF_3 cinnolines **18** in the same manner as shown in Scheme 1. Quinoline analogs (**39** to **42**) were prepared as previously described.[6,13]

2.2. LXR receptor binding assays

Results from the quinoline series[10] showed that 2′,3′-dimethylphenyl acetic acid and 2′,5′-dimethylphenyl acetic acid substituted quinolines enhanced LXRβ binding selectivity. The substituents on the phenyl acetic acid moieties were proposed to interact with the LXRβIle277/LXRαVal263 residue, which is the only amino acid sequence difference within the LBD. Initially, several cinnoline acetic acids (a combination of the phenyl acetic acid moieties and the cinnoline scaffold) were prepared[14] and they showed similar LXRβ binding potency and selectivity as the quinoline-based compounds **4** and **6**. However, these acetic acid substituted cinnolines also had unwanted PPAR activity, activating all three subtypes of the PPAR. It is speculated that the phenyl acetic acid moiety is responsible for the PPAR activity, and we therefore focused our new efforts on the synthesis of analogs without the acid moiety. The LXR binding affinity of the newly synthesized compounds was evaluated in radioligand binding assays as reported.[6] As a reference, TO-901317 (compound **3**) was tested in our binding assays and was found to be a potent LXR pan agonist (Table 1). As would be expected from the earlier quinoline series,[10] non-substituted (**19**) or mono substituted benzyl analogs (**20** and **21**) showed potent

Table 1
Relative binding affinities of compounds **19**–**42** for LXR receptor α and β



Compound	X	R^1	R^2	Ar	L	hLXRβ IC_{50} (nM)*	hLXRα IC_{50} (nM)*	Ratio α/β
3						9	14	1.4
4[6]						2	10	5.0
6[10]						4	58	15
19	N	Cl	Bn	Ph	O	46	180	3.9
20	N	Cl	Bn	2-Cl–Ph	O	14	92	6.7
21	N	Cl	Bn	3-CF_3–Ph	O	10	62	6.2
22	N	Cl	Bn	2-Cl–3-CF_3–Ph	O	24	837	36
23	N	Cl	Bn	2-Cl–5-CF_3–Ph	O	12	147	12
24	N	Cl	Bn	2-CF_3–5-Cl–Ph	O	85	1826	21
25	N	Cl	Bn	2-CF_3–5-F–Ph	O	44	1191	27
26	N	Cl	Bn	2-Cl–3-CF_3–Ph	NH	40	1124	28
27	N	Cl	Bn	2-CF_3–5-F–Ph	NH	62	>1000	>16
28	N	Cl	Ph	2-CF_3–5-Cl–Ph	O	65	332	5.1
29	N	Cl	Ph	2-Cl–3-CF_3–Ph	NH	19	179	9.6
30	N	CF_3	Bn	2-CF_3–5-Cl–Ph	O	57	>1000	>17
31	N	CF_3	Bn	2-CF_3–5-F–Ph	O	38	1194	31
32	N	CF_3	Bn	2-F–3-CF_3–Ph	O	63	1336	21
33	N	CF_3	Bn	2-Cl–3-CF_3–Ph	O	43	2927	68
34	N	CF_3	Bn	1-Me–2-indole	O	42	>1000	>23
35	N	CF_3	Bn	1-Me–7-indole	O	14	>1000	>71
36	N	Cl	Bn	1-Me–7-indole	NH	24	>1000	>41
37	N	Cl	Bn	1-Me–7-indole	NH	24	603	25
38	N	Cl	Ph	1-Me–7-indole	NH	10	62	6.2
39	CH	CF_3	Bn	1-Me–7-indole	NH	34	1415	41
40	CH	CF_3	Bn	1-Me–7-indole	O	20	668	33
41	CH	CF_3	Bn	1-Me–2-indole	NH	39	1123	29
42	CH	CF_3	Bn	1-H–7-indole	NH	38	134	3.5

*Results are given as the mean of at least two independent experiments. The standard deviations for these assays were typically ±30% of mean or less.

(IC_{50} < 50 nM) binding affinity for LXRβ, but their subtype binding selectivity against LXRα was low (<10-fold). Better binding selectivity was observed with disubstituted analogs, such as 2-Cl–3-CF_3 substituted compound **22** (LXRα/β ratio of 36). The binding selectivity dropped to 12-fold when moving the CF_3 substituent from position 3 to position 5 (compound **23**). The selectivity improved to 21-fold when switching the orientation of the two substituents from 2-Cl–5-CF_3 (compound **23**) to 2-CF_3–5-Cl (compound **24**), however, the LXRβ binding affinity decreased from 12 nM for compound **23** to 85 nM for compound **24**. Compared to **24** the 2-CF_3–5-F analog **25** showed similar binding affinity (LXRβ IC_{50} 44 nM) and selectivity (27-fold) toward LXRα.

Modifications on the linker region, C_3- and C_8-position were undertaken. As it was seen in the quinoline series[6,10,13] that the analogs with a $NHCH_2$ linker (compound **26** vs **22**; **27** vs **25**) showed a similar potency or selectivity profile. However, the introduction of phenyl substituent on the 3-position of the cinnoline ring resulted in a big loss in term of binding selectivity (compound **28** vs **24**; **29** vs **26**) although the LXRβ binding affinity was similar between the pairs. In our SAR studies on quinoline-based compounds, we have shown that the 8-Cl moiety can be altered to a CF_3 moiety without compromising the LXR binding affinity.[13] Interestingly, all the 8-CF_3 compounds with 2,3 or 2,5-disubstituted phenyls (compounds **30**–**33**) and N-methyl-indoles (**34** and **35**) seem to have good binding selectivity for LXRβ (α/β > 17). 1-Me-7-indole **35** was the most selective compound in this 8-CF_3 cinnoline series which had good (71-fold) binding selectivity over LXRα. The compound also showed potent binding affinity (LXRβ IC_{50} 14 nM). While the 8-Cl-3-benzyl indole **36** was also potent (24 nM) and selective (42-fold) the 8-Cl-3-phenyl indole analog **37** was potent (LXRβ IC_{50} 10 nM) but not selective (6.2-fold) against LXRα which is consistent with results obtained by other 3-phenyl cinnoline analogues (**28** and **29**). Several closely related quinoline-based 1-methyl-indoles (**39**–**41**) were prepared and they had basically the same LXRβ binding affinity and selectivity as the corresponding cinnolines (**34**–**37**). Compared to the 1-methyl indoles the 1-H indole **42** showed similar binding affinity (LXRβ IC_{50} of 38 nM) but reduced selectivity (α/β 3.5-fold).

2.3. Molecular modeling

In order to understand the modest LXRβ selectivity (α/β 71-fold) of the cinnoline compound **35**, we docked this ligand into a previously solved in-house X-ray structure of **WAY-254011**.[6] The best scoring pose of **35** from the docking studies is shown in Figure 2. A similar binding mode was also observed for compound **39** of the quinoline series.

Ligand recognition within the binding site was achieved by a hydrogen bond interaction between the N1 atom of the cinnoline ring and the Nε atom of His_{435} residue, while the N-3 benzyl group was completely surrounded by hydrophobic Phe residues (271, 340, and 349)[6] in the C2 pocket. The benzyloxy linker was able to extend the indole group further out towards the β sheet region and orient the N-methyl group of the 7-indole towards the conservative amino acid difference $Ile_{277}(β)/Val_{263}(α)$ at a distance of 5.3 Å to the Cδ atom of Ile_{277} residue. At this distance, the non-bonded energy contribution of this methyl group from any differential interaction with $Ile_{277}(β)/Val_{263}(α)$ residues are probably small, but important for ligands selectivity. The lack of selectivity observed for the 1-H-7-indole **42** (α/β 3.5-fold) analog of the quinoline series when compared to the 1-Me-7-indole analog **39** (α/β 41-fold) further supports our hypothesis that extending groups, that is, methyl in this region of the pocket, the ligand was able to make some differential interactions with $Ile_{277}(β)/Val_{263}(α)$ residue and enhance selectivity. It is also possible that additional contributions to ligand selectivity could come from differential interaction of residues outside the immediate binding site (>5 Å) which are different in the two isoforms. Such effects could originate from shift of one or more conserved residues within the binding pocket which in turn could propagate to residues differences outside the binding site. We believe such shifts in second shell residues are responsible for the selectivity differences seen in the case of the 3-phenyl substituted analogs versus 3-benzyl substituted analogs, that is, compound **28** versus **24**; and **29** versus **26**. For example in order to accommodate the larger 3-benzyl group within the C2 pocket, the Phe residues within the C2 pocket would have to adopt alternate rotamer states which could affect residues differences seen in the outer shell of two isoforms.

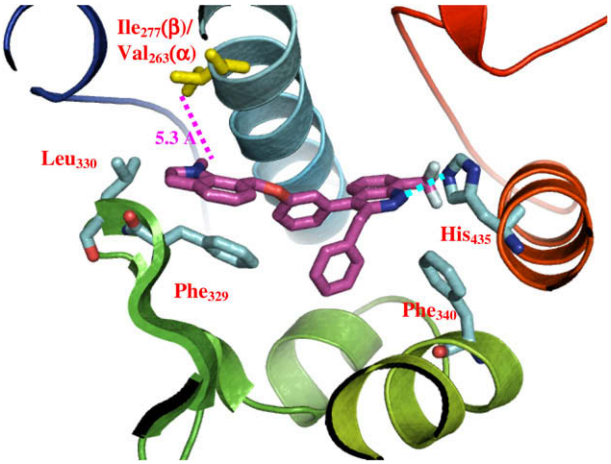


Figure 2. Compound **35** docked into the LXRβ/**WAY-254011** pocket (ligand is shown in magenta). Only key residues of the binding site are shown for simplicity. Residue difference, that is, $Ile_{277}(β)/Val_{263}(α)$ closest to the ligand is highlighted in yellow and distance from Cδ of Ile_{277} to 7-methyl group of indole is shown in magenta. Hydrogen bonds to key residues are shown as dotted cyan line.

2.4. Functional assays

A few binding selective LXR agonists were tested in Gal4 functional transactivation assays (Table 2, for assay conditions see Ref. 6). In these assays, all binding selective compounds (**22**, **26**, **31**, **33**, **35, 37, 39**, and **41**) proved to be LXRβ partial agonists, reaching a maximum activation of 75% when compared to the literature standard TO901317. The binding selective compounds showed large efficacy differences in the Gal4 transactivation assays between LXRβ and LXRα, although their potency differences were only 2–7-fold. Compared to the reference compound **3** and pan agonist **4** these binding selective compounds showed reduced potency with very weak partial agonism on LXRα (1–23% efficacy), suggesting potentially less lipogenic impact on the triglyceride synthesis. Furthermore, when profiled in the J774 macrophages, all of the binding selective compounds in Table 2 showed good potency (0.7 μM or less) and high efficacy (71% or greater) for stimulating an endogenous LXR target gene ABCA1. As expected, the compounds in this new series (without the acetic acid moiety) had no cross activity against PPARα, γ, and δ receptors (data not shown) as measured in transiently transfected cell lines. As stated previously, LXRβ selective compounds should have less impact on the triglyceride synthesis when compared to LXR pan agonists. In this regard, cinnoline **26** and **37**, and quinoline **41** were tested in SREBP-1c gene expression in Huh-7 cells and they showed much reduced potency (EC_{50} > 1.0 μM for the three compounds) and lower efficacy (34% for **26**, 37% for **37**, and 34% for **41**) relative to pan agonists **3** (EC_{50} 0.067 μM, 100%) and **4** (EC_{50} 0.168 μM, 103%).

Table 2
Gene expression activity and Gal4 transactivation activity for binding selective compounds

Compound	LXRβ IC_{50} (nM)	α/β ratio	Transactivation LXRβ EC_{50} (μM, %eff)[a,b]	Transactivation LXRα EC_{50} (μM, %eff)[a,b]	Macrophage ABCA1 EC_{50} (μM, %eff)[a,b]
3	9	1.4	0.178 (100%)	0.135 (100%)	0.034 (100%)
4[6]	2	5.0	0.09 (63%)	0.24 (90%)	0.041 (115%)
22	24	36	6.54 (54%)	15.3 (6%)	0.18 (81%)
26	40	28	3.63 (20%)	11.1 (1%)	0.35 (95%)
31	38	31	3.65 (61%)	8.41 (9.5%)	0.067 (71%)
33	43	68	8.60 (52%)	18.1 (5.5%)	0.12 (91%)
35	14	71	1.16 (67%)	8.32 (23%)	0.068 (109%)
37	24	25	2.78 (35%)	6.00 (11%)	0.70 (129%)
39	34	41	4.17 (75%)	12.0 (15%)	0.076 (104%)
41	39	29	10.18 (53%)	13.5 (13%)	0.109 (82%)

[a] Results are given as the mean of two to three independent experiments. The standard deviations for these assays were typically ±50% of mean or less. The percentage of efficacy is relative to reference compound **3**.
[b] LXR transactivation assay used Huh7 cells transfected with human LXR LBD fused to Gal4 DBD; ABCA1 gene regulation by LXR ligands was measured J774 (murine) cells.

2.5. ADME profile

While the principle goal of this study was to probe the LXRβ selectivity, drug-like properties of analogs were also evaluated. Using human and mouse liver microsomes the in vitro metabolic stability was investigated and the majority of the compounds tested showed poor metabolic stability ($t_{1/2}$ < 10 min, <40% remain at 30 min) in both species. Upon oral administration (10 mg/kg) in mice **35** had no detectable plasma exposure which is consisted with the poor metabolic stability ($t_{1/2}$ < 5 min). Poor absorption may also be a factor since the compound had poor aqueous solubility (2 μg/mL) and high *c* Log *P* (7.82) value.

3. Conclusion

In summary, a series of novel cinnolines/quinolines was found to be potent LXRβ binders. This work has led to >60-fold LXRβ binding selective modulators even though there is only one amino acid difference in the ligand binding domain (LXRβIle277/LXRα Val263). It was found that cinnolines/quinolines with a 2′,3′ or 2′,5′-disubstituted phenyl or1-Me-7-indole moiety showed good (>25-fold) binding selectivity for LXRβ over LXRα. Those LXRβ binding selective modulators showed good potency (0.7 μM or less) and efficacy (71% or greater) for inducing ABCA1 gene expression in macrophages and no PPAR agonist activity. Cinnolines **26**/**37** and quinoline **41** also showed poor efficacy in the LXRα Gal4 functional assay and they were weak partial agonists for inducing SREBP-1c gene expression in Huh-7 liver cells.

4. Experimental

4.1. General

Solvents and chemicals were purchased from VWR and Aldrich Chemical Co. and were used without further purification. Anhydrous and deuterated solvents as well as fine chemicals were purchased from Aldrich Chemical Co and used without further treatment. High-resolution mass spectra were taken on a Waters LC-TOFMS instrument and were measured to within 5 ppm of the calculated values. ^{1}H NMR spectra were taken on a Bruker DPX300 (300 MHz) instrument and delta values (δ) were measured in ppm using tetramethylsilane as an internal standard (δ = 0 ppm). High-performance liquid chromatography (HPLC) was performed with an Agilent 1100F series instrument with auto sampler and a diode array detector (Xterra RP18, 3.5u, 150 × 4.6 mm column, 1.2 mL/min, 85/15–5/95 solvent A–solvent B for 10 min, hold 4 min, solvent A: ammonium formate buffer (pH 3.5), solvent B: ACN/MeOH 1:1). Appropriate safety practices were observed during all laboratory functions.

4.1.1. 1-(2-Amino-3-chlorophenyl)-2-phenylethanone (7a)

2-Chlorophenylaniline (2.92 g, 23 mmol) in 25 mL of 1,2-dichloroethane was added dropwise to a solution of 25.3 mL (25.3 mmol) of BCl_3 in xylene at 0–5 °C. 5.38 g (46 mmol) of benzyl cyanide and 3.37 g (25.3 mmol) of $AlCl_3$ were added to the suspension, and the reaction mixture was stirred at 80 °C for 20 h and cooled to 0 °C. 2 N HCl was added to the mixture and the mixture was then refluxed for 30 min at 80 °C and extracted with dichloromethane. The organic phase was washed with 1 M NaOH, dried, and evaporated to yield 1.5 g of **7a** as a gray solid (27%). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H, *J* = 8.2 Hz), 7.41 (d, 1H, *J* = 7.7 Hz), 7.40–7.20 (m, 5H), 6.85 (br s, 2H), 6.62 (t, 1H, *J* = 7.8 Hz), 4.27(s, 2H); MS *m/z* 246.

4.1.2. 8-Chloro-3-phenylcinnolin-4-ol (8a)

A solution of sodium nitrite (0.60 g, 8.4 mmol) in water (1.5 mL) was added dropwise to a solution of **7a** (1.5 g, 6.1 mmol) in acetic acid (20 mL) and sulfuric acid (3.0 mL). After being stirred for 20 min at 80 °C the solution was poured into iced water and the pH was adjusted to ~6 with 2 N sodium hydroxide. The aqueous layer was extracted with ethyl acetate. The combined organics were dried over $MgSO_4$ and concentrated. The material was purified via column chromatography using 5–50% ethyl acetate in hexane as the eluent to yield 0.20 g of **8a** as a pale yellowish gum (13%). ^{1}H NMR ($CDCl_3$) δ 10.32 (s, 1H), 8.30 (d, 1H, *J* = 7.0 Hz), 8.14–8.11 (m, 2H), 7.75 (d, 1H, *J* = 7.7 Hz), 7.52–7.40 (m, 3H), 7.34 (t, 1H, J = 7.7 Hz); MS (ES) *m/z* 257.0.

4.1.3. 3-Benzyl-8-chlorocinnolin-4-ol (8b)

Prepared from 2-chlorophenylaniline and 3-phenylpropanenitrile according to the procedures for **7a** and **8a** as a white solid in 56% yield for the two steps. ^{1}H NMR (DMSO-d_6) δ 13.2 (s, 1H), 8.02 (d, 1H, *J* = 8.1 Hz), 7.92 (d, 1H, *J* = 7.5 Hz), 7.38 (t, 1H, *J* = 7.7 Hz), 7.35–7.25 (m, 4H), 7.25–7.15 (m, 1H), 4.07 (s, 2H); MS (ES) m/z 271.0.

4.1.4. 4-Bromo-8-chloro-3-phenylcinnoline (9a)

A solution of **8a** (0.19 g, 0.74 mol) and $POBr_3$ (1.0 g, 3.5 mmol) in DMF (10 mL) was heated to 50 °C for 30 min. The reaction was poured into ice-water, adjusted to pH to ~10 by diluted ammonium hydroxide and extracted with ethyl acetate. The combined organics were concentrated to yield **9a** (0.15 g, 64%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.22 (d, 1H, *J* = 8.6 Hz), 8.00 (d, 1H, *J* = 7.4 Hz), 7.90–7.82 (m, 2H), 7.80 (t, 1H, J = 7.5 Hz), 7.62–7.50 (m, 3H); MS (ES) *m/z* 318.8; HPLC purity 100% at 10.2 min; HRMS calcd for $C_{14}H_9BrClN_2$: 318.9632; found (ESI, $[M+H]^+$): 318.9630.

4.1.5. 3-Benzyl-4-bromo-8-chlorocinnoline (9b)

Prepared from **8b** according to the procedure for **9a** as a a pale yellow solid in 82% yield. ^{1}H NMR (acetone-d_6): δ 8.20 (d, 1H, *J* = 8.5 Hz), 8.12 (d, 1H, *J* = 7.4 Hz), 7.96 (t, 1H, *J* = 7.4 Hz), 7.41 (d,

2H, *J* = 7.5 Hz), 7.30 (t, 2H, *J* = 7.2 Hz), 7.25–7.20 (m, 1H), 4.85 (s, 2H); MS (ES) *m/z* 333.0; HRMS calcd for $C_{15}H_{11}BrClN_2$: 332.9789; found (ESI, $[M+H]^+$): 332.9786.

4.1.6. 3-(8-Chloro-3-phenylcinnolin-4-yl)phenol (10a)

Compound **9a** (0.16 g, 0.5 mmol) was taken into DME/EtOH (10 mL/2 mL). Then 3-hydroxyphenylboronic acid (0.16 g, 1.0 mmol) was added followed by 2 M Na_2CO_3 (1.5 mL, 3.0 mmol) and finally $Pd(PPh_3)_4$ (0.06 g, 0.05 mmol). The reaction was refluxed for 2 h and concentrated. The resulting material was purified via column chromatography using 5–50% ethyl acetate in hexane to elute out 0.14 g (84%) of **10a** as a pale yellow solid. ^1H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.15 (d, 1H, *J* = 7.5 Hz), 7.82 (t, 1H, *J* = 8.6 Hz), 7.64 (d, 1H, *J* = 8.6 Hz), 7.53–7.48 (m, 2H), 7.40–7.30 (m, 3H), 7.26 (t, 1H, J = 7.9 Hz), 6.85–6.83 (m, 1H), 6.76–6.68 (m, 2H); MS (ES) m/z 330.9; HRMS calcd for $C_{20}H_{14}ClN_2O$: 333.0789; found (ESI, $[M+H]^+$): 333.0805.

4.1.7. [3-(8-Chloro-3-phenylcinnolin-4-yl)phenyl]amine (10b)

Prepared from **9a** and 3-aminophenylboronic acid according to the procedure for **10a** as a a pale yellow solid in 55% yield. ^1H NMR (DMSO-d_6) δ 8.14 (d, 1H, *J* = 7.4 Hz), 7.81 (t, 1H, *J* = 8.6 Hz), 7.68 (d, 1H, *J* = 8.3 Hz), 7.58–7.54 (m, 2H), 7.41–7.35 (m, 3H), 7.11 (t, 1H, J = 7.7 Hz), 6.65–6.60 (m, 1H), 6.50–6.40 (m, 2H), 5.24 (br s, 2H); MS (ES) m/z 332.1; HPLC purity 96% at 9.3 min; HRMS calcd for $C_{20}H_{15}ClN_3$: 332.0949; found (ESI, $[M+H]^+$): 332.0937.

4.1.8. 3-(3-Benzyl-8-chlorocinnolin-4-yl)phenol (10c)

Prepared from **9b** and 3-hydroxyphenylboronic acid according to the procedure for **10a** as a pale yellow solid in 47% yield. ^1H NMR ($CDCl_3$) δ 7.84(d, 1H, *J* = 7.3 Hz), 7.50 (t, 1H, *J* = 8.5 Hz), 7.45–7.35 (m, 2H), 7.30–7.20 (m, 2H), 7.20–7.00 (m, 4H), 6.71 (d, 1H, J = 7.6 Hz), 6.61 (s, 1H), 5.40 (br s, 1H), 4.48 (d, 1H, *J* = 13.8 Hz), 4.42 (d, 1H, *J* = 13.8 Hz); MS (ES) *m/z* 347; HPLC purity 98.4% at 10.2 min; HRMS calcd for $C_{21}H_{16}ClN_2O$: 347.0946; found (ESI, $[M+H]^+$): 347.0932.

4.1.9. [3-(3-Benzyl-8-chlorocinnolin-4-yl)phenyl]amine (10d)

Prepared from **9b** and 3-aminophenylboronic acid according to the procedure for **10a** as a a pale yellow solid in 72% yield. ^1H NMR (acetone-d_6): δ 7.99(d, 1H, *J* = 7.3 Hz), 7.71 (t, 1H, *J* = 7.3 Hz), 7.56 (d, 1H, *J* = 8.5 Hz), 7.26 (t, 1H, *J* = 7.7 Hz), 7.20–7.10 (m, 5H), 6.89–6.86(m, 1H), 6.61 (s, 1H), 6.50 (d, 1H, J = 7.4 Hz), 4.46 (s, 2H); MS (ESI) *m/z* 346; HPLC purity 97.8% at 9.8 min.

4.1.10. 2-Fluoro-*N*-methoxy-*N*-methyl-3-(trifluoromethyl)-benzamide (13)

A couple of drops of DMF was added to a suspension of 2-fluoro-3-(trifluoromethyl)benzoic acid (5.0 g, 24.0 mmol), $SOCl_2$ (10 mL) and dichloromethane (50 mL). The reaction was refluxed for 4 h and concentrated. The residue was dissolved in 50 mL of chloroform and cooled in an ice bath. N,*O*-Dimethylhydroxylamine hydrochloride (4.0 g, 41.2 mmol) and pyridine (7 mL) were added. The reaction was then warmed to room temperature, stirred over night and concentrated. The residue was dilute with ether, washed with diluted HCl and sodium carbonate. The organic layer was dried over $MgSO_4$ and concentrated to give an oil which was used for the next reaction without further purification (5.1 g, 84%). ^1H NMR (DMSO-d_6) δ *7.84–7.92 (m, 2H), 7.51 (t, 1H, J* = 7.8 Hz), 3.66 (s, 3H), 3.16 (s, 3H); MS (ESI) *m/z* 252.1; HPLC purity 100% at 7.8 min; HRMS calcd for $C_{10}H_{10}F_4NO_2$: 252.0642; found (ESI, $[M+H]^+$): 252.0651.

4.1.11. 1-[2-Fluoro-3-(trifluoromethyl)phenyl]-3-phenylpropan-1-one (14)

To a cooled (0 °C) solution of **13** (5.0 g, 20 mmol) in THF (50 mL) was added phenethyl magnesium chloride (50 mL of 1.0 M solution in THF) and the reaction was warmed to room temperature. After 2 h the reaction was poured into 2 N HCl and extracted with ether. The organic layer was dried ($MgSO_4$) and concentrated. The product was purified by column chromatography (eluent 5% EtOAc/hexane) to give **14** as a clear oil (4.8 g, 81%). ^1H NMR (DMSO-d_6) δ 8.14 (t, 1H, *J* = 8.2 Hz), 8.02 (t, 1H, *J* = 7.8 Hz), 7.53 (t, 1H, *J* = 7.8 Hz), 7.30–7.25 (m, 4H), 7.20–7.17 (m, 1H), 3.37 (t, 2H, J = 7.5 Hz), 2.94 (t, 2H, *J* = 7.5 Hz); MS (ES) *m/z* 297.0; HPLC purity 100% at 10.7 min.

4.1.12. 1-[2-Amino-3-(trifluoromethyl)phenyl]-3-phenylpropan-1-one (15)

A solution of **14** (4.8 g, 16.2 mmol) and ammonium hydroxide (150 mL of 30% solution) in DME (50 mL) was heated to 140 °C in a steel pressure reactor. After 3 h the reaction was cooled to 0 °C, the steel pressure reactor was opened and the reaction was partitioned between water and EtOAc. The organic layer was dried ($MgSO_4$) and concentrated to give **15** as a yellow oil (4.3 g, 90%). ^1H NMR (DMSO-d_6) δ 8.18 (d, 1H, *J* = 7.4 Hz), 7.66 (t, 1H, *J* = 6.8 Hz), 7.29 (br s, 2H), 7.28–7.25 (m, 4H), 7.20–7.15 (m, 1H), 6.71 (t, 1H, J = 7.8 Hz), 3.37 (t, 2H, *J* = 7.3 Hz), 2.92 (t, *J* = 7.3 Hz, 2H); MS (ES) *m/z* 293.9; HPLC purity 100% at 11.0 min; HRMS calcd for $C_{16}H_{14}F_3NO$: 294.1100; found (ESI, $[M+H]^+$), 294.1110.

4.1.13. 3-Benzyl-4-bromo-8-(trifluoromethyl)cinnoline (16)

To a solution of **15** (4.2 g, 13.8 mmol) in AcOH (70 mL) and H_2SO_4 (10 mL) was added a solution of $NaNO_2$(1.8 g in 10 mL H_2O). The reaction was then heated to 70 °C. After 1.5 h the reaction was cooled, poured into water and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated to give a dark solid which was dissolved in DMF (30 mL). $POBr_3$ (2.5 g, 8.7 mmol) in DMF (30 mL) was added and the mixture was heated to 75 °C. After 1 h the reaction was cooled and poured into water. The aqueous layer was extracted with EtOAc and the organics were dried ($MgSO_4$) and concentrated to give a solid. The solid was triturated with MeOH and filtered to give **16** (1.7 g, 34%) as a light yellow solid. ^1H NMR (DMSO-d_6) δ 8.47 (d, 1H, *J* = 8.7 Hz), 8.44 (d, 1H, *J* = 7.1 Hz), 8.13 (t, 1H, *J* = 7.8 Hz), 7.40–7.30 (m, 4H), 4.79 (s, 2H); MS (ES) *m/z* 366.7.

4.1.14. 3-[3-Benzyl-8-(trifluoromethyl)cinnolin-4-yl]phenol (17)

A solution of **16** (1.7 g, 4.6 mmol), 3-hydroxyphenylboronic acid (0.84 g, 6.0 mmol), $Pd(PPh_3)_4$ (300 mg), and K_3PO_4 (3.0 g) in dioxane (50 mL) was heated to reflux. After 6 h the reaction was cooled and poured into water and extracted with EtOAc. The organic layer was concentrated and the product was purified by column chromatography (eluent 10% EtOAc/hexane) to give **17** as a white solid (1.3 g, 74%). ^1H NMR (DMSO-d_6) δ 9.82 (s, 1H), 8.34 (d, 1H, *J* = 7.2 Hz), 7.90 (t, 1H, *J* = 8.0 Hz), 7.78 (d, 1H, *J* = 8.6 Hz), 7.39 (t, 1H, *J* = 7.1 Hz), 7.25–7.10 (m, 3H), 7.10–6.95 (m, 3H), 6.78–6.76 (m, 2H), 4.40 (m, 2H); MS (ES) m/z 381.1; HPLC purity 94% at 10.6 min; HRMS calcd for $C_{22}H_{15}F_3N_2O+Na^+$: 403.1029; found (ESI, $[M+Na]^+$ calcd): 403.1029.

4.1.15. 3-Benzyl-4-[3-(benzyloxy)phenyl]-8-chlorocinnoline (19)

A mixture of **10c** (0.05 g, 0.14 mmol), benzyl bromide (0.06 g, 0.35 mmol), and cesium carbonate (0.20 g, 0.61 mmol) in DMF (6 mL) was stirred at room temperature for 1 h. The reaction was quenched with water and extracted with ethyl acetate. The organic residue was purified by semi-preparative HPLC (Column: Phenomenex C18 Luna 21.6 mm x 60 mm, 5 μM; solvent A: Water (0.1% TFA buffer); solvent B: acetonitrile (0.1% TFA buffer); solvent gradient: time 0: 0% B; 10 min: 100% B; hold 100% B 5 min. Flow rate: 22.5 mL/min) to provide **19** (41 mg, 67%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, J = 7.4 Hz), 7.73 (t, 1H, J = 8.6 Hz), 7.48 (t, 1H, J = 8.0 Hz), 7.45–7.30 (m, 6H), 7.22–7.10 (m, 4H), 6.98–6.96 (m, 3H), 6.89 (d, 1H, J = 6.5 Hz), 5.10 (d, 1H, J = 12.1 Hz), 5.03 (d, 1H, J = 12.1 Hz), 4.38 (d, 1H, J = 16.2 Hz), 4.34 (d, 1H, J = 16.2 Hz); MS (ES) m/z 436.8; HPLC purity 98.6% at 11.7 min; HRMS calcd for $C_{28}H_{22}ClN_2O$: 437.1415; found (ESI, $[M+H]^+$): 437.1411.

4.1.16. 3-Benzyl-8-chloro-4-{3-[(2-chlorobenzyl)oxy]phenyl}-cinnoline (20)

Prepared from **10c** and 2-chlorobenzyl bromide according to the procedure for **19** as a gum in 60% yield. ^{1}H NMR (DMSO-d_6) δ 8.09 (d, 1H, J = 7.2 Hz), 7.74 (t, 1H, J = 7.9 Hz), 7.72–6.90 (m, 14H), 5.15 (d, 1H, J = 11.7 Hz), 5.12 (d, 1H, J = 11.7 Hz), 4.41 (d, 1H, J = 14.7 Hz), 4.36 (d, 1H, J = 14.7 Hz); MS (ES) m/z 470.8; HPLC purity 99.8% at 12.1 min; HRMS calcd for $C_{28}H_{21}Cl_2N_2O$: 471.1025; found (ESI, $[M+H]^+$): 471.1016.

4.1.17. 3-Benzyl-8-chloro-4-(3-{[3-(trifluoromethyl)benzyl]-oxy}phenyl)cinnoline (21)

Prepared from **10c** and 3-trifluoromethylbenzyl bromide according to the procedure for **19** as a a gum in 54% yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, J = 7.6 Hz), 7.82–7.70 (m, 4H), 7.64 (t, 1H, J = 7.6 Hz), 7.50 (t, 1H, J = 7.8 Hz), 7.39 (d, 1H, J = 8.5 Hz), 7.25–7.10 (m, 4H), 7.05–6.85 (m, 4H), 5.21 (d, 1H, J = 12.6 Hz), 5.18 (d, 1H, J = 12.6 Hz), 4.35 (s, 2H); MS (ES) m/z 504.8; HPLC purity 98.2% at 11.9 min; HRMS calcd for $C_{29}H_{21}ClF_3N_2O$: 505.1289; found (ESI, $[M+H]^+$): 505.1309.

4.1.18. 3-Benzyl-8-chloro-4-(3-{[2-chloro-3-(trifluoromethyl)-benzyl]oxy}phenyl)cinnoline (22)

Prepared from **10c** and 2-chloro-3-trifluoromethylbenzyl bromide according to the procedure for **19** as a gum in 37% yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, J = 7.3 Hz), 7.92 (d, 1H, J = 7.7 Hz), 7.88 (d, 1H, J = 7.8 Hz), 7.74 (t, 1H, J = 8.6 Hz), 7.62 (t, 1H, J = 7.9 Hz), 7.53 (t, 1H, J = 7.8 Hz), 7.41 (d, 1H, J = 8.6 Hz), 7.30–7.10 (m, 4H), 7.10–6.90 (m, 4H), 5.27 (d, 1H, J = 12.9 Hz), 5.20 (d, 1H, J = 12.9 Hz), 4.42 (d, 1H, J = 14.7 Hz), 4.36 (d, 1H, J = 14.7 Hz); MS (ES) m/z 538.7; HPLC purity 99.6% at 12.2 min; HRMS calcd for $C_{29}H_{20}Cl_2F_3N_2O$: 539.0899; found (ESI, $[M+H]^+$): 539.0898.

4.1.19. 3-Benzyl-8-chloro-4-(3-{[2-chloro-5-(trifluoromethyl)-benzyl]oxy}phenyl)cinnoline (23)

Prepared from **10c** and 2-chloro-5-trifluoromethylbenzyl bromide according to the procedure for **19** as a gum in 66% yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, J = 8.4 Hz), 7.96 (s, 1H), 7.79–7.77 (m, 2H), 7.73 (t, 1H, J = 8.6 Hz), 7.52 (t, 1H, J = 7.9 Hz), 7.40 (d, 1H, J = 8.4 Hz), 7.27 (dd, 1H, J = 7.8, 2.0 Hz), 7.20–7.10 (m, 3H), 7.10–6.90 (m, 4H), 5.25 (d, 1H, J = 12.7 Hz), 5.19 (d, 1H, J = 12.7 Hz), 4.40 (d, 1H, J = 14.9 Hz), 4.35 (d, 1H, J = 14.9 Hz); MS (ES) m/z 539.0; HPLC purity 98.4% at 12.2 min; HRMS calcd for $C_{29}H_{20}Cl_2F_3N_2O$: 539.0899; found (ESI, $[M+H]^+$): 539.0886.

4.1.20. 3-Benzyl-8-chloro-4-(3-{[5-chloro-2-(trifluoromethyl)-benzyl]oxy}phenyl)cinnoline (24)

Prepared from **10c** and 5-chloro-2-trifluoromethylbenzyl bromide according to the procedure for **19** as a pale yellow solid in 43% yield. ^{1}H NMR (DMSO-d_6) δ 8.09 (d, 1H, J = 7.4 Hz), 7.90–7.80 (m, 2H), 7.74 (t, 1H, J = 8.5 Hz), 7.68 (dd, 1H, J = 8.5, 1.5 Hz), 7.52 (t, 1H, J = 8.2 Hz), 7.41 (d, 1H, J = 8.5 Hz), 7.40–7.10 (m, 4H), 7.05–6.90 (m, 4H), 5.26 (d, 1H, J = 12.9 Hz), 5.20 (d, 1H, J = 12.9 Hz), 4.41 (d, 1H, J = 14.5 Hz), 4.36 (d, 1H, J = 14.5 Hz); MS (ES) m/z 539.0; HPLC purity 97.6% at 12.3 min; HRMS calcd for $C_{29}H_{20}Cl_2F_3N_2O$: 539.0899; found (ESI, $[M+H]^+$): 539.0866.

4.1.21. 3-Benzyl-8-chloro-4-(3-{[5-fluoro-2-(trifluoromethyl)benzyl]oxy}phenyl)cinnoline (25)

Prepared from **10c** and 5-fluoro-2-trifluoromethylbenzyl bromide according to the procedure for **19** as a gum in 38% yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, J = 6.9 Hz), 7.90–7.80 (m, 2H), 7.74 (t, 1H, J = 7.4 Hz), 7.63 (d, 1H, J = 9.2 Hz), 7.52 (t, 1H, J = 7.3 Hz), 7.50–6.85 (m, 9H), 5.26 (d, 1H, J = 13.0 Hz), 5.22 (d, 1H, J = 13.0 Hz), 4.41 (d, 1H, J = 14.4 Hz), 4.36 (d, 1H, J = 14.4 Hz); MS (ES) m/z 523.0; HPLC purity 99.4% at 12.0 min; HRMS calcd for $C_{29}H_{20}ClF_4N_2O$: 523.1195; found ($[M+H]^+$): 523.1171.

4.1.22. [3-(3-Benzyl-8-chlorocinnolin-4-yl)phenyl][2-chloro-3-(trifluoromethyl)benzyl]amine (26)

Compound **10d** (0.05 g, 0.14 mmol) and 2-chloro-3-trifluoromethylbenzaldehyde (0.1 g, 0.48 mmol) were mixed in DMF (2 mL) and then treated with $NaBH(OAc)_3$ (0.1 g, 0.47 mmol) and acetic acid (1 mL). After stirring at 50 °C for 1 h the mixture was quenched with water and then extracted with ethyl acetate. The organic residue was purified by silica gel chromatography using 5–50% EtOAc/hexanes as eluent to provide **26** (45 mg, 60%) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 7.82 (dd, 1H, J = 6.6, 1.7 Hz), 7.64 (d, 1H, J = 7.6 Hz), 7.59 (d, 1H, J = 7.8 Hz), 7.50–7.05 (m, 10H), 6.72 (dd, 1H, J = 8.2, 2.2 Hz), 6.55 (d, 1H, J = 6.6 Hz), 6.27 (br s, 1H), 4.50–4.30 (m, 4H); MS (ESI) m/z 538; HPLC purity 100% at 12.0 min; HRMS calcd for $C_{29}H_{21}Cl_2F_2N_3$: 538.1059; found ($[M+H]^+$): 538.1059.

4.1.23. [3-(3-Benzyl-8-chlorocinnolin-4-yl)phenyl][5-fluoro-2-(trifluoromethyl)benzyl]amine (27)

Prepared from **10d** and 5-fluoro-2-trifluoromethylbenzaldehyde according to the procedure for **26** as a gum in 31% yield. ^{1}H NMR (DMSO-d_6) δ 7.83 (dd, 1H, J = 6.9, 1.2 Hz), 7.66 (dd, 1H, J = 8.8, 5.3 Hz), 7.55–7.00 (m, 11H), 6.70–6.67 (m, 1H), 6.55 (d, 1H, J = 7.5 Hz), 6.23–6.21 (m, 1H), 4.55–4.35 (m, 4H); MS (ES) m/z 522.1; HPLC purity 97.3% at 11.9 min; HRMS calcd for $C_{29}H_{21}ClF_4N_3$: 522.1355; found (ESI, $[M+H]^+$): 522.1368.

4.1.24. 8-Chloro-4-(3-{[5-chloro-2-(trifluoromethyl)benzyl]oxy}-phenyl)-3-phenylcinnoline (28)

Prepared from **10a** and 5-chloro-2-trifluoromethylbenzyl bromide according to the procedure for **19** as a yellow solid in 27% yield. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, 1H, J = 6.1 Hz), 7.70–7.10 (m, 11 H), 7.05 (d, 1H, J = 8.3 Hz), 6.92 (d, 1H, J = 7.4 Hz), 6.81 (br s, 1H), 5.17–5.12 (m, 2 H); MS (ES) m/z 525.1; HPLC purity 100% at 12.0 min; HRMS calcd for $C_{28}H_{18}Cl_2F_3N_2O$: 525.0743; found (ESI, $[M+H]^+$): 525.0754.

4.1.25. [3-(8-Chloro-3-phenylcinnolin-4-yl)phenyl][2-chloro-3-(trifluoromethyl)benzyl]amine (29)

Prepared from **10b** and 2-chloro-3-trifluoromethylbenzaldehyde according to the procedure for **26** as a pale yellow solid in 39% yield. ^{1}H NMR (DMSO-d_6) δ 8.12 (d, 1H, J = 6.6 Hz), 7.70–7.30 (m, 9 H), 7.15 (t, 1H, J = 7.8 Hz), 6.68 (d, 1H, J = 9.7 Hz), 6.58 (t, 1H, J = 6.2 Hz), 6.50–6.40 (m, 2H), 4.37 (d, 2H, J = 6.1 Hz); MS (ESI) m/z 524; MS (ESI) m/z 522; HPLC purity 100% at 11.8 min; HRMS calcd for $C_{28}H_{19}Cl_2F_3N_3$: 524.0903; found (ESI-FT/MS, $[M+H]^+$): 524.0911.

4.1.26. 3-Benzyl-4-(3-{[5-chloro-2-(trifluoromethyl)benzyl]oxy}-phenyl)-8-(trifluoromethyl)cinnoline (30)

Prepared from **17** and 5-chloro-2-trifluoromethylbenzyl bromide according to the procedure for **19** as a gum in 64% yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, J = 7.2 Hz), 7.95–7.80 (m, 3H), 7.74 (d, 1H, J = 8.2 Hz), 7.68 (d, 1H, J = 8.4 Hz), 7.54 (t, 1H, J = 7.9 Hz), 7.25–6.95 (m, 8H), 5.26 (d, 1H, J = 12.9 Hz), 5.20 (d, 1H, J = 12.9 Hz), 4.42 (d, 1H, J = 14.7 Hz), 4.37 (d, 1H, J = 14.7 Hz); MS

(ESI) *m/z* 573; HPLC purity 100% at 12.2 min; HRMS calcd for $C_{30}H_{20}ClF_6N_2O$: 573.1163; found (ESI, $[M+H]^+$): 573.1156.

4.1.27. 3-Benzyl-4-(3-{[5-fluoro-2-(trifluoromethyl)benzyl]oxy}-phenyl)-8-(trifluoromethyl)cinnoline (31)

Prepared from **17** and 5-fluoro-2-trifluoromethylbenzyl bromide according to the procedure for **19** as a yellow oil in 19% yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 7.2 Hz), 7.92–7.85 (m, 2 H), 7.74 (d, 1H, *J* = 8.4 Hz), 7.65 (dd, 1H, *J* = 9.8, 2.8 Hz), 7.55 (t, 1H, *J* = 8.3 Hz), 7.45 (td, 1H, *J* = 8.3, 1.2 Hz), 7.30–6.95 (m, 8H), 5.26 (d, 1H, J = 12.9 Hz), 5.20 (d, 1H, *J* = 12.9 Hz), 4.42 (d, 1H, *J* = 14.7 Hz), 4.37 (d, 1H, *J* = 14.7 Hz); MS (ES) *m/z* 557.2; HPLC purity 100% at 12.0 min; HRMS calcd for $C_{30}H_{20}F_7N_2O$: 557.1458; found (ESI, $[M+H]^+$): 557.1440.

4.1.28. 3-Benzyl-4-(3-{[2-fluoro-3-(trifluoromethyl)benzyl]oxy}-phenyl)-8-(trifluoromethyl)cinnoline (32)

Prepared from **17** and 2-fluoro-3-trifluoromethylbenzyl bromide according to the procedure for **19** as a yellow foam in 11% yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 7.2 Hz), 7.92–7.87 (m, 2H), 7.82 (t, 1H, *J* = 6.9 Hz), 7.75 (d, 1H, *J* = 8.2 Hz), 7.53 (t, 1H, *J* = 8.3 Hz), 7.47 (t, 1H, *J* = 8.0 Hz), 7.28–6.93 (m, 8H), 5.25 (d, 1H, J = 12.3 Hz), 5.18 (d, 1H, *J* = 12.3 Hz), 4.40 (d, 1H, *J* = 15.1 Hz), 4.37 (d, 1H, *J* = 15.1 Hz); MS (ES) *m/z* 557.2; HPLC purity 96.7% at 11.9 min; HRMS calcd for $C_{30}H_{20}F_7N_2O$: 557.1458; found (ESI, $[M+H]^+$): 557.1450.

4.1.29. 3-Benzyl-4-(3-{[2-chloro-3-(trifluoromethyl)benzyl]oxy}-phenyl)-8-(trifluoromethyl)cinnoline (33)

Prepared from **17** and 2-chloro-3-trifluoromethylbenzyl bromide according to the procedure for **19** as a yellow solid in 37% yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 7.2 Hz), 7.93–7.87 (m, 3H), 7.74 (d, 1H, *J* = 8.0 Hz), 7.62 (t, 1H, *J* = 7.8 Hz), 7.54 (t, 1H, *J* = 8.3 Hz), 7.29–6.99 (m, 8H), 5.27 (d, 1H, J = 12.8 Hz), 5.20 (d, 1H, *J* = 12.8 Hz), 4.42 (d, 1H, *J* = 14.6 Hz), 4.37 (d, 1H, *J* = 14.6 Hz); MS (ES) *m/z* 573.1; HPLC purity 97.7% at 12.1 min; HRMS calcd for $C_{30}H_{20}ClF_6N_2O$: 573.1163; found (ESI, $[M+H]^+$): 573.1159.

4.1.30. 3-Benzyl-4-{3-[(1-methyl-1*H*-indol-2-yl)methoxy]-phenyl}-8-(trifluoromethyl)cinnoline (34)

DIAD (0.1 g, 0.51 mmol) was added slowly to a mixture of **17** (0.11 g, 0.3 mmol), PPh_3 (0.1 g, 0.38 mmol) and (1-methyl-1H-indol-7-yl)-methanol (0.08 g, 0.49 mmol) in 10 mL of diethyl ether. After being stirred for 1 h, the reaction was concentrated and purified by silica gel chromatography (ether/hexanes) to give **34** as yellow foam (17 mg, 11%). ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 7.2 Hz), 7.87 (t, 1H, *J* = 8.5 Hz), 7.74 (d, 1H, *J* = 7.8 Hz), 7.54–7.50 (m, 2H), 7.46 (d, 1H, J = 8.2 Hz), 7.28–7.20 (m, 1H), 7.20–6.90 (m, 9H), 6.59 (s, 1H), 5.32 (d, 1H, J = 12.4 Hz), 5.23 (d, 1H, *J* = 12.4 Hz), 4.41 (d, 1H, *J* = 15.0 Hz), 4.38 (d, 1H, *J* = 15.0 Hz), 3.32 (s, 3H); MS (ES) *m/z* 524.1; HPLC purity 98.3% at 11.8 min; HRMS calcd for $C_{32}H_{24}F_3N_3O + Na^+$: 546.1764; found (ESI, $[M+Na]^+$): 546.1775.

4.1.31. 3-Benzyl-4-{3-[(1-methyl-1*H*-indol-7-yl)methoxy]-phenyl}-8-(trifluoromethyl)cinnoline (35)

Prepared from **17** and (1-methyl-1H-indol-7-yl)methanol according to the procedure for **34** as a yellow foam in 45% yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (d, 1H, *J* = 7.0 Hz), 7.91 (t, 1H, *J* = 8.5 Hz), 7.77 (d, 1H, *J* = 7.8 Hz), 7.57 (d, 1H, *J* = 7.9 Hz), 7.54 (d, 1H, *J* = 8.3 Hz), 7.30–7.28 (m, 2H), 7.25–7.10 (m, 5H), 7.10–6.95 (m, 4H), 6.45 (d, 1H, J = 3.2 Hz), 5.46 (d, 1H, *J* = 11.2 Hz), 5.37 (d, 1H, *J* = 11.2 Hz), 4.45 (d, 1H, *J* = 15.4 Hz), 4.40 (d, 1H, *J* = 15.4 Hz), 3.96 (s, 3H); MS (ES) *m/z* 524.1; HPLC purity 96.0% at 11.8 min; HRMS calcd for $C_{32}H_{25}F_3N_3O$: 524.1940; found (ESI, $[M+H]^+$), 524.1943.

4.1.32. *N*-[3-(3-Benzyl-8-chlorocinnolin-4-yl)phenyl]-*N*-[(1-methyl-1*H*-indol-7-yl)methyl]amine (36)

Prepared from **10d** and 1-methylindoline-7-carbaldehyde according to the procedure for **26** as a pale yellow solid in 50% yield. ^{1}H NMR (DMSO-d_6) δ 8.07 (d, 1H, *J* = 7.4 Hz), 7.74 (t, 1H, *J* = 8.6 Hz), 7.54 (d, 1H, *J* = 8.3 Hz), 7.47 (d, 1H, *J* = 8.0 Hz), 7.29 (t, 1H, *J* = 7.9 Hz), 7.25–6.90 (m, 9H), 6.62 (br s, 1H), 6.51 (d, 1H, *J* = 6.6 Hz), 6.40 (d, 1H, *J* = 3.1 Hz), 6.34 (t, 1H, *J* = 5.0 Hz), 4.60–4.56 (m, 2H), 4.42 (d, 1H, J = 14.0 Hz), 4.39 (d, 1H, *J* = 14.0 Hz), 3.98 (s, 3H); MS (ESI) *m/z* 489; HPLC purity 99.0% at 11.8 min; HRMS calcd for $C_{31}H_{26}ClN_4$: 489.1841; found ($[M+H]^+$): 489.1860.

4.1.33. 3-(3-Benzyl-8-chlorocinnolin-4-yl)phenyl][(1-methyl-1*H*-indol-2-yl)methyl]amine (37)

Prepared from **10d** and 1-methylindoline-2-carbaldehyde according to the procedure for **26** as a pale yellow solid in 35% yield; ^{1}H NMR (DMSO-d_6) δ 8.06 (d, 1H, *J* = 7.4 Hz), 7.65 (t, 1H, *J* = 8.3 Hz), 7.46 (t, 1H, *J* = 6.4 Hz), 7.40 (d, 1H, *J* = 7.9 Hz), 7.30–6.90 (m, 9H), 6.60–6.45 (m, 4 H), 4.45–4.35 (m, 4H), 3.71 (s, 3H); MS (ES) m/z 489.1; HPLC purity 98.4% at 11.7 min; HRMS calcd for $C_{31}H_{26}ClN_4$: 489.1840; found (ESI, $[M+H]^+$), 489.1839.

4.1.34. [3-(8-Chloro-3-phenylcinnolin-4-yl)phenyl][(1-methyl-1*H*-indol-7-yl)methyl]amine (38)

Prepared from **10b** and 1-methylindoline-7-carbaldehyde according to the procedure for **26** as a pale yellow solid in 58% yield. ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H, *J* = 7.2 Hz), 7.76 (t, 1H, *J* = 8.5 Hz), 7.67 (d, 1H, *J* = 8.6 Hz), 7.50–7.10 (m, 8H), 6.96 (d, 1H, *J* = 6.5 Hz), 6.90 (t, 1H, *J* = 7.4 Hz), 6.76 (dd, 1H, *J* = 8.2, 1.5 Hz), 6.58 (s, 1H), 6.46 (d, 1H, *J* = 7.4 Hz), 6.35 (d, 1H, *J* = 3.1 Hz), 6.20 (t, 1H, *J* = 4.9 Hz), 4.48 (d, 1H, *J* = 4.9 Hz), 3.85 (s, 3H); MS (ES) *m/z* 474.9; HPLC purity 100% at 11.5 min; HRMS calcd for $C_{30}H_{24}ClN_4$: 475.1681; found ($[M+H]^+$): 475.1689.

4.1.35. {3-[3-Benzyl-8-(trifluoromethyl)quinolin-4-yl]phenyl}-[(1-methyl-1*H*-indol-7-yl)methyl]amine (39)

Prepared from [3-[3-benzyl-8-(trifluoromethyl)quinolin-4-yl]-phenyl]amine[13] and 1-methylindoline-7-carbaldehyde according to the procedure for **26** as a foam in 36% yield. ^{1}H NMR (methanol-d_4) δ 8.87 (s, 1H), 8.06 (d, 1H, *J* = 7.4 Hz), 7.84 (d, 1H, *J* = 8.5 Hz), 7.56 (t, 1H, *J* = 7.9 Hz), 7.46 (d, 1H, *J* = 7.9 Hz), 7.32 (t, 1H, *J* = 7.9 Hz), 7.25–6.85 (m, 9H), 6.55–6.53 (m, 1H), 6.39 (d, 1H, J = 3.1 Hz), 4.57 (d, 1H, *J* = 6.1 Hz), 4.54 (d, 1H, *J* = 6.1 Hz), 4.06 (d, 2H, *J* = 4.0 Hz), 3.95 (s, 3H); HPLC purity 98.6% 12.1 min; HRMS calcd for $C_{33}H_{27}F_3N_3$: 522.2152; found (ESI, $[M+H]^+$), 522.2142.

4.1.36. 3-Benzyl-4-{3-[(1-methyl-1*H*-indol-7-yl)methoxy]-phenyl}-8-(trifluoromethyl)quinoline (40)

A mixture of 3-(3-benzyl-8-trifluoromethyl-quinolin-4-yl)-phenol (0.20 g, 0.5 mmol) and triphenylphosphine (polymer-bound, ~3.2 mmol/g, 1 g) in DCM (5 mL) was stirred at room temperature for 30 min and then treated with 1-methyl-1H-indol-7-yl)methanol (0.08 g, 0.5 mmol) and diisopropyl azodicarboxylate (0.2 g, 1 mmol) in 5 mL of DCM. After stirring for 2 h the solid was removed and the liquid was washed with water and then extracted with ethyl acetate. The organic residue was purified by silica gel chromatography using 0–100% EtOAc/hexanes as eluent to provide **40** (160 mg, 61%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.03 (s, 1H), 8.16 (d, 1H, *J* = 6.7 Hz), 7.70–7.65 (m, 2H), 7.58–7.49 (m, 2H), 7.31–7.10 (m, 6H), 7.05–6.90 (m, 5H), 6.44 (d, 1H, J = 3.0 Hz), 5.44 (d, 1H, *J* = 11.2 Hz), 5.34 (d, 1H, *J* = 11.2 Hz), 4.04 (d, 1H, *J* = 5.3 Hz), 4.00 (d, 1H, *J* = 5.3 Hz), 3.94 (s, 3H); MS (ESI) *m/z* 523; HPLC purity 100% at 12.2 min; HRMS calcd for $C_{33}H_{26}F_3N_2O$: 523.1992; found (ESI, $[M+H]^+$), 523.1997.

4.1.37. {3-[3-Benzyl-8-(trifluoromethyl)quinolin-4-yl]phenyl}-[(1-methyl-1*H*-indol-2-yl)methyl]amine (41)

Prepared from [3-[3-benzyl-8-(trifluoromethyl)quinolin-4-yl]-phenyl]amine[13] and 1-methylindoline-7-carbaldehyde according to the procedure for **26** as a white foam in 29% yield. ^{1}H NMR (DMSO-d_6): δ 8.96 (s, 1H), 8.12 (d, 1H, J = 7.1 Hz), 7.75 (d, 1H, J = 8.9 Hz), 7.57 (t, 1H, J = 8.2 Hz), 7.46 (d, 1H, J = 7.8 Hz), 7.40 (t, 1H, J = 8.2 Hz), 7.27 (t, 1H, J = 7.6 Hz), 7.25–6.85 (m, 7H), 6.60 (br s, 1H), 6.48 (d, 1H, J = 7.5 Hz), 6.40–6.35 (m, 2H), 4.40 (d, 2H, J = 7.5 Hz), 4.00 (d, 1H, J = 5.0 Hz), 3.96 (d, 1H, J = 5.0 Hz), 3.72 (s, 3H); MS (ESI) m/z 522; HPLC purity 100% at 12.1 min; HRMS calcd for $C_{33}H_{27}F_3N_3$: 522.2152; found (ESI, $[M+H]^+$), 522.2151.

4.1.38. {3-[3-Benzyl-8-(trifluoromethyl)quinolin-4-yl]phenyl}-(1*H*-indol-7-ylmethyl)amine (42)

Prepared from [3-[3-benzyl-8-(trifluoromethyl)quinolin-4-yl]-phenyl]amine[13] and 1H-indole-7-carbaldehyde according to the procedure for **26** as a foam in 82% yield. ^{1}H NMR (DMSO-d_6): δ 11.1 (s, 1H), 8.92 (s, 1H), 8.12 (d, 1H, J = 7.1 Hz), 7.70 (d, 1H, J = 8.7 Hz), 7.60 (t, 1H, J = 7.6 Hz), 7.43 (d, 1H, J = 7.9 Hz), 7.33 (t, 1H, J = 2.6 Hz), 7.30–6.90 (m, 17H), 6.80 (d, 1H, J = 8.2 Hz), 6.55–6.40 (m, 3H), 4.50 (d, 2H, J = 6.8 Hz), 3.92 (s, 2H);MS (ES) m/z 508.2; HPLC purity 94.6% at 11.9 min; HRMS calcd for $C_{32}H_{25}F_3N_3$: 508.1994; found (ESI, $[M+H]^+$), 508.1992.

4.2. Docking calculations

Docking studies were carried out using Schrodingers Glide program.[15] Protein preparation was done on the in-house X-ray structure of hLXRβ/**WAY-254011** using the Protein Preparation Wizard, a comprehensive protein preparation tool which adds hydrogens, adjusts protonation states for ionizable residues, modifies tautomeric forms for histidine residues, and repositions hydrogens (e.g., side chain hydroxyl hydrogens). This was followed by restrained minimization to allow hydrogens to be freely minimized while allowing for sufficient heavy atom movement (0.3 A) to alleviate potential steric clashes. The final refined structure was then visually examined for correct formal charges, bond order and protonation states. The receptor grid generation was done with the scaling factors for van der Waals radii of non-polar atoms (those with an absolute partial charge less than 0.25) set to 0.9. Ligand was built using the Maestro interface and prepared for docking using the LigPrep utility. Flexible docking of the ligand was performed using the standard precision[16,17] (SP) followed by post-minimization of 5 best poses eliminate poses with close intraligand distances and high strain energies.

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