Discovery and optimization of indoline derivatives as new LXR agonists

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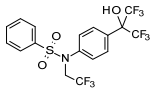
1- INTRODUCTION

- Liver X receptors are members of the nuclear receptor superfamily of ligand activated transcription factors.
- They act by forming heterodimers with retinoid X receptor (RXR).
- Agonism of LXRs upregulates several genes involved in lipid metabolism :
  - *Cholesterol transport genes that modulate Reverse Cholesterol Transport (RCT)*
    - ABCA1, ABCG1, ABCG5, ABCG8 –desired effects
    - Important function of both LXRα and LXRβ (macrophage, intestine)
  - *Triglyceride synthesis genes*
    - SREBP1c and FAS –undesired effects
    - LXRα is the predominant isoform in the liver
- Oxysterols (e.g. 24,25-epoxycholesterol) are LXR endogenous ligands.

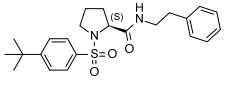
- Objective of the program : Identification of orally active partial LXRs agonists (devoid of side effects) and subtype selective ligands (β-selective preferred) to elucidate the functional roles of LXRs.

2- HTS

- Compounds were assayed for agonist activity on LXR-GAL4 chimeric receptors in transfected Cos-7 cells. Results are expressed as the induction rate normalized to the activity of the reference T0901317.
- Proline derivative hit **1** was identified through a transactivation cell based HTS assay. the objective was to improve potency and Emax using a structure-based design strategy.



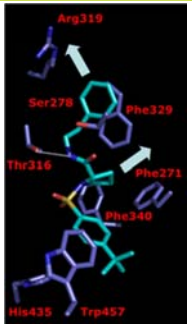
T0901317



1

hLXRα EC_{50} = 1.4 µM (Emax =45%)
hLXRβ EC_{50} = 2.8 µM (Emax =15%)

3- DOCKING OF COMPOUND 1

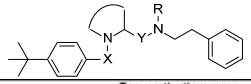


Docking in hLXRβ (PDB : 1P8D). Only key residues of the binding pocket displayed in blue. Docking solution for **1** depicted in cyan.

- Compound **1** was docked into the pocked of human LXRβ (PDB ID : 1P8D). Ligand was submitted to the *Ligprep* module of *Maestro* and docked using *Glide*.
- Compound **1** is nicely positioned to create an H-bond between amide NH and Thr316.
- Improvement in binding could be achieved through :
  - Additional Van der Waals interactions with aromatic residues of the pocket (Phe271, Phe329).
  - Introduction of polar residues to get an H-bond with Arg319.

(Depicted as light blue arrows in the figure)

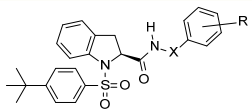
4- POTENCY IMPROVEMENT



Cpd		X	R	Transactivation assay hLXRα EC_{50} (nM)	hLXRα Emax (%)	hLXRβ EC_{50} (nM)	hLXRβ Emax (%)	Microsomal stability Human %remaining 30 min	Murine %remaining 30 min
1		SO_2	H	1367	45	2755	15	0	5
2		SO_2	H	NC	0	NC	0	NT	NT
3		SO_2	Me	NC	0	NC	0	NT	NT
4		CO	H	NC	0	NC	0	NT	NT
5		SO_2	H	387	28	863	8	1	6
6		SO_2	H	375	38	1007	25	1	1
7		SO_2	H	228	48	583	62	2	1
8		SO_2	H	44	48	213	50	5	1
9		CH_2	H	640	10	NC	1	NT	NT
10		SO_2	H	NC	1	NC	1	NT	NT

- *(S)*-configuration is required for activity (**1** vs **2**).
- Linker X : SO_2 preferred over CO (**1** vs **4**).
- Aromatic addition increases activity (**5,8** vs **1** and **7** vs **6**).
- Amide : C=O (**10** vs **8**) and NH (**3** vs **1**) mandatory for activity.
- Microsomal stability needs to be improved.

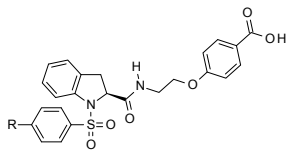
5- MICROSOMAL STABILITY IMPROVEMENT



Cpd	R'	X	hLXRα EC_{50} (nM)	hLXRα Efficacy (%)	hLXRβ EC_{50} (nM)	hLXRβ Efficacy (%)	Microsomal stability Human % remaining 30 min	Murine % remaining 30 min
8	H	$(CH_2)_2$	44	48	213	50	5	1
11	H	CH_2	72	45	446	32	12	1
12	H	$(CH_2)_3$	184	51	399	32	6	0
13	H	COCH$_2$	90	64	281	70	17	3
14	H	O(CH$_2$)$_2$	98	49	240	23	8	3
15	3-COOH	CH_2	268	34	1109	3	0	77
16	4-COOH	CH_2	333	48	ND	17	56	52
17	4-CH$_2$COOH	CH_2	324	33	ND	27	81	74
18	3-CH$_2$COOH	CH_2	1084	48	ND	16	21	83
19	3-COOH	O(CH$_2$)$_2$	1065	40	ND	25	2	41
20	4-COOH	O(CH$_2$)$_2$	178	71	997	35	63	30

- Change in the X-linker length has little effect on the activity (Cpds **8-14**).
- Microsomal stability is increased with the addition of a carboxylic acid in para position of the phenyl ring.

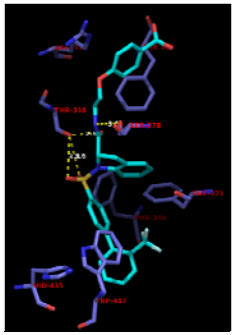
6- INTERACTIONS WITH T457 AND H435



Cpd	R	Transactivation assay hLXRα EC_{50} (nM)	hLXRα Efficacy (%)	hLXRβ EC_{50} (nM)	hLXRβ Efficacy (%)	Microsomal stability Human % remaining 30 min	Murine % remaining 30 min
20	tBu	178	71	997	35	63	30
21	2-CF_3-Ph	5	94	113	113	76	29
22	4-F-Ph	429	66	1186	14	82	76
23	2,4-diF-Ph	198	92	1087	38	87	58
24	2-Me-4-F-Ph	42	91	545	67	74	45
25	2-OMe-4-F-Ph	142	95	1017	58	69	54
26	2-Cl-4-F-Ph	18	102	276	87	76	48

- Biphenyl moieties bring high activity with :
  - Ortho substitution inducing high potency
  - Para substitution improving microsomal stability

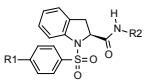
7- CO-CRYSTAL OF CPD 21 in hLXRβ LBD



- Position of **21** in X-ray similar to the one proposed in docking of **1**.
- H-bond between SO_2 and alcohol of Thr316.
- H-bond between NH and Ser278.
- Van de Waals contacts (His435, Trp457, Phe271, Phe340).

Co-crystal of Cpd **21** and hLXRβ LBD.

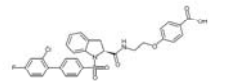
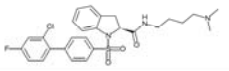
8- MODULATION OF α/β SELECTIVITY



Cpd	R1	R2	Transactivation assay hLXRα EC_{50} (nM)	hLXRα Efficacy (%)	hLXRβ EC_{50} (nM)	hLXRβ Efficacy (%)	Microsomal stability Human % remaining 30 min	Murine % remaining 30 min
27	2-CF_3Ph		62	82	409	77	93	52
28	2-CF_3Ph		23	88	90	105	5	7
29	2-CF_3Ph		38	58	70	95	47	24
30	2-CF_3Ph		134	65	133	75	58	30
31	2-CF_3Ph		101	80	41	93	55	42
32	2-CF_3Ph		143	94	153	93	74	79
33	2-Cl-4-F-Ph		269	68	228	92	92	79

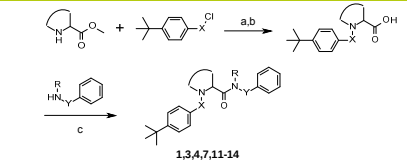
- Acidic moiety gave LXRα activity (e.g. **27**)
- Amides or amines led to dual LXR α/β agonists (**28-32**)

9- PHARMACOKINETIC PROFILE

Cpd	Structure	Cmax (ng/mL)	AUCinf (h.ng/mL)	Tmax (h)
26		2666	5089	1
32		900	5821	3

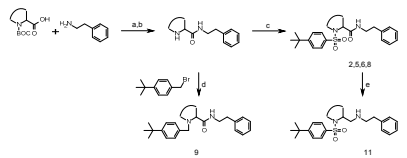
Plasma profile : 30 mpk po in C57Bl6 mice

10- SYNTHESIS



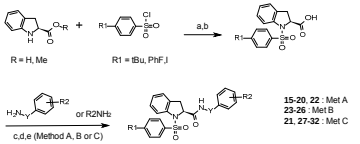
1,3,4,7,11-14

(a) (i) NEt_3, THF, rt ; or (ii) NEt_3, CH_2Cl_2, rt or NMM, CH_3CN, reflux ; (b) (i) NaOH, Dioxane, H_2O, rt ; or (ii) LiOH, THF, H_2O, rt or LiOH, Dioxane, H_2O, rt; (c) (i) resin PS-carbodiimide, HOAt, CH_2Cl_2, rt ; or (ii) EDCI, DIPEA, CH_2Cl_2, rt ; or (iii) EDCI, DIPEA, CH_2Cl_2, rt



2,5,6,8 — 9 — 11

(a) resin PS-carbodiimide, HOAt, CH_2Cl_2, rt ; (b) TFA, CH_2Cl_2, rt ; (c) (i) IRA400, CH_2Cl_2, rt (ii) sulfonyl chloride, PS-DIEA, CH_2Cl_2, rt ; (d) Na_2CO_3, acetonitrile, rt (e) BH_3-SMe_2, THF, reflux



R = H, Me — R1 = tBu, PhF,I

15-20, 22 : Met A
23-26 : Met B
21, 27-32 : Met C

If R=Me, (a) (i) NEt_3, THF, rt; or (ii) NMM, CH_3CN, rt; (b) (i) NaOH, Dioxane, H_2O rt; or (ii) LiOH, THF, H_2O, rt ;
If R=H, (a) LiOH, CH_3CN, H_2O, rt
Method A : (c) (i) oxalyl chloride, toluene, DMF, rt (ii) Et_3N, CH_2Cl_2, rt or (i) EDCI, HOAt, CH_2Cl_2, rt ; (d) (ii) LiOH, THF, H_2O, rt; or (ii) HCl dioxane (4N), AcOEt
Method B : (c) (i) EDCI, HOAt, CH_2Cl_2, rt; (d) (i) ArB(OH)$_2$, PdCl$_2$dppf (cat), DME/H2O, Na_2CO_3; (e) (i) HCl dioxane (4N), AcOEt
Method C : with R1=I (c) ArB(OH)$_2$, PdCl$_2$dppf (cat), DME/H_2O, Na_2CO_3; (d) (i) EDCI, HOAt, CH_2Cl_2, rt; or (ii) oxalyl chloride, toluene, DMF, rt; (iii) Et_3N, CH_2Cl_2, rt

11-SUMMARY

The discovery and structure activity optimization of new indoline agonists of LXR has been developed. Compounds with different LXRα/β profiles and good PK parameters have been identified. Derivatives bearing acidic moieties are more LXRα selective. LXRβ activity was restored by switching to a tertiary amino group leading to dual LXRα/β agonists (e.g. **31**).
This series has been further developed delivering two clinical candidates.

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