Brain penetrant liver X receptor (LXR) modulators based on a 2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole core

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Abstract

Liver X receptor (LXR) agonists have been reported to lower brain amyloid beta ($A\beta$) and thus to have potential for the treatment of Alzheimer's disease. Structure and property based design led to the discovery of a series of orally bioavailable, brain penetrant LXR agonists. Oral administration of compound **18** to rats resulted in significant upregulation of the expression of the LXR target gene ABCA1 in brain tissue, but no significant effect on $A\beta$ levels was detected.

The two isoforms of the liver X receptor, LXR α and LXR β , are ligand activated transcription factors belonging to the nuclear receptor class.¹ The LXRs regulate genes involved in lipid and cholesterol metabolism. Oxysterols have been shown to be endogenous ligands of the LXRs.²

The majority of the published work describing efforts to discover LXR modulators has been directed towards treatment of atherosclerosis;³ however, the LXRs have also been proposed as targets for the treatment of cancer,⁴ atopic dermatitis⁵ and Alzheimer's disease (AD).^{6,7}

The most prominent hypothesis explaining the relevance of LXRs to AD is as follows.⁸ The genes coding for ATP-binding cassette protein A1 (ABCA1) and apolipoprotein E (apoE) are LXR target genes. Upregulation of ABCA1 leads to increased export of cholesterol from the cell, contributing to the lipidation status of apoE. Lipidated apoE associates with soluble amyloid beta (A β) in the CNS and allows it to be cleared in microglia.⁹ The involvement of other mechanisms has also been postulated,¹⁰⁻¹² including a role for the anti-inflammatory effects of LXR activation.¹³ Several published studies have demonstrated reductions in brain A β upon treatment with **1** (T0901317, Figure 1) or **2** (GW3965) in rodents;¹⁴⁻¹⁹ however, no reduction in A β levels was observed in other experiments.^{10, 20-21} The relative importance of LXR α vs LXR β is also uncertain. While the latter is ubiquitously expressed, including in the brain, LXR α is expressed in microglia.⁷ A knockout study showed that elimination of either LXR isoform increases amyloid plaque levels, while another set of experiments indicated that LXR α plays a critical role in A β clearance.¹⁶ Recently a group at Merck reported that **3**, administered subcutaneously to rat at 100 mg/kg/day for 4 d, led to increased levels of A β in CSF.²² In a 3-week study in Tg2576 mice, dosed subcutaneously at 50 mg/kg/day for 3 weeks, a *ca.* 60% increase in brain ABCA1 protein was observed along with a trend towards decreased soluble A β , which however did not reach statistical significance.

As one aspect of a multifaceted program to discover clinically useful LXR modulators, we sought brain penetrant compounds as potential AD therapeutics. Unacceptable increases in liver triglycerides have been observed in animal studies with LXR modulators and it has been

hypothesized that LXR β selective compounds may reduce this liability.²³⁻²⁴ In this letter, we describe the discovery and characterization of orally bioavailable LXR β selective agonists which enter the CNS and regulate ABCA1 expression in the brain.

Figure 1. Literature LXR agonists

We recently described application of Contour[®], our proprietary structure based drug design software,²⁵ to the discovery of piperazine **4** (VTP-766).²⁶ Compound **4** is a potent LXR β agonist with moderate selectivity for LXR β over LXR α (27x in binding and 9x in cellular assays), which activates the expression of the LXR target genes ABCA1 in THP1 cells and SREBP1c in HepG2 cells. It is >100x selective over four other nuclear receptors (PXR, MR, RXR and ROR γ t) and

has IC₅₀ values > 25 μ M against recombinant CYP2D6 and CYP3A4. On the other hand, **4** is a submicromolar inhibitor of recombinant CYP2C9 and liver microsome stability varies between species. Low concentrations of **4** in mouse brain after oral administration suggested that this compound would not be suitable to explore the effects of LXR β selective agonist on brain A β levels. We were also concerned about possible toxicity resulting from the aniline functionality in **4**.²⁷⁻²⁸

As a replacement for the central piperazine core, we conceived of the two regioisomeric 2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazoles **A** and **B** shown in Figure 2. Initially we prepared compounds **5** and **6**, direct analogs of **4**, in which the piperazine ring has been replaced by **A** and **B** respectively. Regioisomer **5** possessed generally similar potency to **4**, while **6a**, the more potent enantiomer of **6**, was 5-10x less potent than **4**, depending on which assay is examined (Table 1). As a result, we focused on analogs of **5** incorporating central scaffold **A**.

Figure 2. Piperazine replacements and initial analogs synthesized

Table 1.

Cpd No	Binding ^{a,b}		Cell ^{a,c}	
	LXR α Ki (nM)	LXR β Ki (nM)	LXR α EC ₅₀ /Eff (nM/%)	LXR β EC ₅₀ /Eff (nM/%)
1	24	17		
4	81	3	244/60	21/56
5	71	6	214/79	36/56
6a ^d	383	26	1376/74	273/56
6b ^d	1365	135	2241/74	507/58

^a Results are the average of at least two replicates. ^b Compound binding to the LXR ligand binding domain (LBD) was measured by displacement of radiolabeled **1**. ^c Modulation of LXR transcriptional activity was measured in HEK293 cells transiently transfected with a plasmid containing the Gal4-DNA binding domain (DBD) fused to the LBD of either LXR α or LXR β and a plasmid which has Gal4 response elements upstream of the firefly luciferase gene. Compound efficacy was determined relative to the maximum effect achieved with **1**. ^d Enantiomers of **6** were separated by chromatography on a chiral column.

Compounds with MW <500, tPSA 40-90 Å² and one or no hydrogen bond donors are usually favored for blood brain barrier penetration.²⁹ We explored successive removal of the right and left hand hydroxymethyl groups in **5** to give **7** and **8** (Table 2). Compound **8** (MW = 451.5, tPSA = 89 Å², HBD = 0) conforms to the favored property ranges for CNS penetration, demonstrated improved potency and cellular activity towards LXR α and retained a similar level of binding and cellular potency on LXR β . It was also more efficacious on both isoforms. The compound is metabolically stable in liver microsomes and does not significantly inhibit CYP3A4 and CYP2D6, although it is a CYP2C9 inhibitor (IC₅₀ = 2110 nM, Table 3).

Based on a model of **7** in the LXR β binding site generated by Contour[®],²⁵ we anticipated that the key interactions of the sulfone oxygen with the Leu330 backbone NH observed in the X-ray structure of **4** bound to LXR β (PDB code: 5I4V), and in X-ray structures of other methyl sulfone containing LXR agonists (PDB codes: 3KFC, 3L0E, 4RAK), would be maintained. Since the N

to N distance in **A** is 4.29 Å vs 2.89 Å for piperazine, the position of the pyrimidinyl group of **5** within the binding site would be offset by 1.4 Å compared to the same group in **4**. On this basis, we expected that the SAR on the left hand side of the molecules would differ from that observed in analogs of **4**. Thus the majority of our synthetic effort was directed modification of the left hand heteroaryl ring.

Table 2. LXR bioassay data of 2-pyrimidinyl analogs^a

Cpd No	Het	Z	Binding ^{a,b}		Cell ^{a,c}	
			LXR α Ki (nM)	LXR β Ki (nM)	LXR α EC ₅₀ /Eff (nM/%)	LXR β EC ₅₀ /Eff (nM, %)
5		CH ₂ OH	71	6	214/79	36/56
7		H	63	6	249/67	46/43
8		H	20	7	111/104	28/91

^aSee footnotes in Table 1.

Table 3. CYP inhibition and liver microsome stability of selected compounds

Cpd.	CYP3A4	CYP2D6	CYP2C9	HLM	MLM	RLM
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No.	IC ₅₀ (nM)	IC ₅₀ (nM)	IC ₅₀ (nM)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)
4	29,450	>30,000	608	43	-	69
5	7,700	7,750	493	>60	-	>60
7	3,050	-	373	>60	-	187
8	>30000	25700	2110	>60	>60	>60
9	12,800	20,950	1,867	>60	>60	>60
10	>30000	>30000	5210	>60	50	>60
11	21100	15750	5043	>60	>60	>60
12	-	24200	5752	>60	>60	>60
13	12920	5370	6616	>60	-	
14	15400	12710	858	>60	-	>60
15	2500	27000	1445	>60	-	>60
16	>30000	25650	3815		>22	
17	>30000	>30000	5625	>60	>60	>60
18	>30000	7800	8297	>60	66	>60

Transposition of the pyrimidine ring nitrogens of **8** afforded regiosomers **9** and **10** (Table 4).

Compound **10** was equipotent with **8** against LXR β and was a less potent CYP2C9 inhibitor.

Introduction of a methyl group afforded **11** which possessed approximately 2x improved cellular potency and similar CYP2C9 potency to **10**, although CYP3A4 and CYP2D6 inhibition worsened. In an effort to render the compounds less hydrophobic, replacement of the CF₃ group with a methoxy group was explored. Compounds **12** and **13** had comparable cellular potency against LXR β to **11**, although 2-methoxy-4-pyrimidinyl compound **12** is a partial agonist while 6-methoxy-4-pyrimidinyl compound **13** is a full agonist. Compound **12** also displayed 17x selectivity for LXR β over LXR α in the cellular assay, retained metabolic stability and was only a moderate CYP2C9 inhibitor. Compound **13** demonstrated increased inhibition of CYP2D6 compared to other analogs. Fluorinated methoxypyrimidines **14** and **15** were also potent. Once again, the 2-methoxy-4-pyrimidinyl compound **14** was more isoform selective and less efficacious than the 6-methoxy-4-pyrimidinyl analog **15**. Both of these compounds suffered from increased CYP2C9 inhibition and **15** was a more potent inhibitor of CYP3A4 than other analogs. Replacement of the methoxypyrimidine ring in **12** and **13** with a 2-methoxy-4-pyridinyl

group gave **16**, with potency and efficacy similar to **13**, but a loss of metabolic stability in mouse liver microsomes. Deletion of the methyl group from **16** gave **17**, which regained metabolic stability, although it suffered from a 2x loss in potency. Modeling indicated that the isopropyl group on the bicyclic core could be replaced with t-butyl to give **18**. This change led to a 2x increase in binding potency, although only a nominal increase in cellular potency.

Table 4. LXR bioassay data of 4-pyrimidinyl and 4-pyridinyl analogs^a

Cpd No	Het	R	Binding		Cell	
			LXR α Ki (nM)	LXR β Ki (nM)	LXR α EC ₅₀ /Eff (nM/%)	LXR β EC ₅₀ /Eff (nM/%)
9		i-Pr	204	13	567/57	109/50
10		i-Pr	186	7	199/88	35/81
11		i-Pr	32	3	72/95	18/78
12		i-Pr	218	17	271/5	16/29
13		i-Pr	22	4	57/91	18/81
14		i-Pr	136	13	491/45	15/29

15		i-Pr	7	1	37/96	17/87
16		i-Pr	51	6	94/88	22/83
17		i-Pr	86	13	189/72	45/67
18		t-Bu	48	5	166/83	38/77

^aSee footnotes in Table 1.

Table 5. 4 h Mouse Studies

Cpd No	Dose (mpk)	Plasma (nM)	Brain (nM)	LXR β EC ₅₀ /Eff (nM/%)	ABCA1 induction in brain
1	30	3,768	8,048		4.5
9	3	2,893	3,153	109/50	2.2
11	1	143	212	18/78	3.1
12	1	2		16/29	
14	1	50		15/29	
18	3	1,075	1,018	38/77	3.1

Compounds **9**, **11**, **12**, **14** and **18** were dosed orally to wild type C57BL mice at 1 or 3 mg/kg.

Plasma drug levels were measured after 4 h (Table 5). Compounds **9**, **11** and **18** achieved drug levels $\geq 5\times$ LXR β EC₅₀ in plasma at 1 or 3 mg/kg. Brain drug levels for these three compounds were $\geq 10\times$ LXR β EC₅₀ at the same doses and all three compounds elicited $\geq 2\times$ induction of ABCA1 mRNA levels over vehicle control. Compounds **9**, **11** and **18** were advanced into a 4 h study in male Sprague-Dawley rats in which they were dosed orally at 5 mg/kg and plasma and brain drug levels and brain ABCA1 levels were determined 4 h after dosing (Table 6).

Compound **18** exhibited the highest level of ABCA1 mRNA induction in rat brain in this study and was advanced into a 5 d rat study at 10, 3 and 1 mg/kg/day. At the lowest dose, **18** achieved a similar level of ABCA1 induction as **1** at 30 mg/kg (Table 7). Despite robust LXR activation in brain, as evidenced by >4x increases in ABCA1 mRNA levels, neither **1** nor **18** elicited significant changes in levels of A β 1-40 or A β 1-42 in rat cerebrum or CSF (Figure 3).

While many of the studies showing an affect of LXR agonists on A β levels have been performed with transgenic mice, engineered to overexpress A β , the study of Suon *et al.* employed wild type rats.¹⁹ In the Suon study, subcutaneous administration of **1** at 30 mg/kg/day for 3 or 6 days resulted in significant increases in the levels of A β 1-40 and A β 1-42 in CSF. A small, but significant, decrease in soluble A β 1-40 in brain was observed after 6 d, but not 3 d, in the same study. The reasons for the discrepancy between these results and ours are unclear. The Suon study also reported marked increases in ApoE in brain and CSF upon treatment with **1** at 30 mg/kg/day after 3 and 6 days.¹⁹ ApoE levels were not measured in our study.

Table 6. Four hour rat study at 5 mg/kg

Cpd No	Plasma (nM)	Brain (nM)	ABCA1 induction in brain
9	1,745	2,064	1.6
11	423	825	2.5
18	1,215	2,282	2.8

Table 7. Five day rat study

Cpd No	Dose (mg/kg/day)	Plasma (nM)	Brain (nM)	ABCA1 induction in brain
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1	30	2,796	5,364	5.0
18	10	1,566	5,041	7.8
18	3	463	1,561	9.3
18	1	187	641	4.7

Figure 3. Aβ1-40 and Aβ1-42 levels in rat cerebrum and CSF after treatment with with **1** and **18** for 5 d.

Figure 4. X-ray structure of compound **7** bound to LXR β (PDB code: 5KYA). The heterodimer of the ligand binding domains (LBDs) of human LXR β and human RXR β was co-crystallized with compound **7** and the structure solved to a resolution of 2.6 Å. Key residues in the vicinity of the ligand binding site are shown (carbon of ligand and protein in green and white respectively, nitrogen in blue, sulfur in yellow, and oxygen in red). One of the sulfone oxygens of **7** hydrogen bonded to the backbone NH of Leu330, while the hydroxymethyl group forms a hydrogen bond with His435.

Figure 5. X-ray structure of compound **14** bound to LXR β (PDB code: 5KYJ). The heterodimer of the ligand binding domains (LBDs) of human LXR β and human RXR β was co-crystallized with compound **14** and the structure solved to a resolution of 2.7 Å. Key residues in the vicinity of the ligand binding site are shown (carbon of ligand and protein in orange and white respectively, nitrogen in blue, sulfur in yellow, and oxygen in red). One of the sulfone oxygens of **14** hydrogen bonded to the backbone NH of Leu330.

Compounds **7** and **14** were cocrystallized with the heterodimer of the LXR β -RXR β LBDs (PDB codes: 5KYA and 5KYJ). In both X-ray structures, one of the methyl sulfone oxygens forms a

hydrogen bond with the backbone NH of Leu330 and the i-Pr group occupies a hydrophobic pocket formed by Phe271, Ile309, Leu313, Phe340, Leu345, Phe349 and Ile353 (Figures 4 and 5). Despite the increased length of **7** compared to **4**, the hydroxymethyl group in **7** forms a hydrogen bond with the imidazole ring of His435, similar to that seen in the complex of **4** with LXR β (PDB code: 5I4V). The methoxy oxygen of compound **14** does not lie within hydrogen bonding distance of the imidazole ring of His435. The lack of this interaction may be responsible for the somewhat reduced efficacy of **14**, compared to **4** and **7**.

The preparations of **5** and **7** are outlined in Scheme 1. Acylation of Meldrum's acid with (R)-N-Boc valine (**19a**), followed by heating the adduct to reflux in EtOAc afforded tetramic acid **20a**. A two step reduction of **20a** with NaBH₄, followed by BH₃.Me₂S,³⁰ provided a hydroxypyrrolidine which was oxidized with Dess-Martin periodinane to give 3-oxopyrrolidine **21a**. The fused pyrazole in **22a** was introduced by treatment of **21a** with DMF dimethyl acetal followed by hydrazine. Copper catalyzed arylation of **22a** with the appropriate meta-bromophenyl methyl sulfone, followed by removal of the Boc group, provided **23a** and **23b** as the exclusive regioisomeric products. These two pyrrolidines reacted smoothly with ethyl 2-chloro-4-(trifluoromethyl)pyrimidine-5-carboxylate at room temperature and the products were reduced with DiBAL at -70 °C to give **5** and **7** respectively. The same sequence of steps, starting with (R)-N-Boc tert-leucine (**19b**), provided t-butyl substituted bicycle **23c**.

Scheme 1. Synthesis of **5** and **7**. Yields are quoted for the case where R = i-Pr. a Meldrum's acid, DCC, DMAP, CH₂Cl₂, 0 °C to rt, 16 h (100%); b EtOAc, reflux, 2 h (100%); c NaBH₄, HOAc, CH₂Cl₂, -15 °C to -5 °C, 6 h; d BH₃.Me₂S, THF, reflux, 1 h (34% over two steps); e Dess-Martin periodinane, CH₂Cl₂, 0 °C to rt, 4 h (75%); f (MeO)₂CHNMe₂, reflux, 3 h; g N₂H₄.H₂O, HOAc, EtOH, reflux, 4 h (90% over two steps); h methyl 4-bromo-2-(methylsulfonyl)benzoate (for **23a**) or 1-bromo-3-(methylsulfonyl)benzene (for **23b**), (1R,2R)-N¹,N²-dimethylcyclohexane-1,2-diamine, K₂CO₃, CuI, dioxane, reflux; i HCl, dioxane, CH₂Cl₂ (97% over two steps); j ethyl 2-chloro-4-(trifluoromethyl)pyrimidine-5-carboxylate, i-Pr₂NEt, EtOH, rt, 5 h; k 1 M DiBAL in hexanes, PhMe, -70 °C, 4 h.,

The preparation of **6** is depicted in Scheme 2. Michael addition of Cbz-Gly-OMe (**24**) to α,β -unsaturated ester **25**, followed by Dieckmann condensation, were effected in one pot with KOt-Bu. Acid-catalyzed decarboxylation of the Dieckmann product afforded oxopyrrolidine **26**. The fused pyrazole was introduced by the same method shown in Scheme 1 Steps f and g to give **27**,

which underwent copper catalyzed arylation to provide regioisomer **28** as the major product. Hydrogenation unmasked the pyrrolidine nitrogen which underwent S_NAr reaction with ethyl 2-chloro-4-(trifluoromethyl)pyrimidine-5-carboxylate to afford diester **29** which was reduced with DiBAL to afford **6**. The enantiomers of **6** were separated by preparative SFC on a OJ-S column.

Scheme 2. Synthesis of **6**. a KOt-Bu, THF, rt to reflux, 3.5 h; b aq HCl, reflux, 20 h; c (MeO)₂CHNMe₂, reflux, 1 h; d N₂H₄·H₂O, HOAc, EtOH, reflux, 1 h; e 1-bromo-3-(methanesulfonyl)benzene, (1R,2R)-N¹,N²-dimethylcyclohexane-1,2-diamine, K₂CO₃, CuI, dioxane, reflux; f H₂ (1 atm), Pd/C, 2:1 EtOH/EtOAc, 4 h; g ethyl 2-chloro-4-(trifluoromethyl)pyrimidine-5-carboxylate, i-Pr₂NEt, THF, rt, 18 h; h 1 M DiBAL in hexanes, PhMe, -70 °C, 5.5 h.

Target compounds **8-15**, in which Het = pyrimidine, were prepared by S_NAr reaction of **23b** with the appropriate chloropyrimidines (Scheme 3). Analogs **16-18**, in which Het = pyridine, were prepared by Buchwald-Harwig amination of the appropriate chloro- or bromopyridines (Schemes 3 and 4).³¹

Scheme 3. Synthesis of **8-15**, **17** and **18**. a For **8-15**: HetCl, i-Pr₂NEt, i-PrOH; b For **17**: 4-bromo-2-methoxypyridine, Pd(Pt-Bu₃)₂, NaOt-Bu, toluene, 120 °C, 90 min; c For **18**: 4-bromo-2-methoxypyridine, RuPhos, RuPhos precatalyst, Cs₂CO₃, THF.

Scheme 4. Synthesis of **16**. a 2,4-dichloro-6-methylpyridine, Pd₂(dba)₃, XPhos; b Cs₂CO₃, Pd₂(dba)₃, BippyPhos, MeOH.

We discovered a series of structurally novel, low molecular weight LXR agonists. Three compounds were found to be orally bioavailable and to distribute to the brain in a 4 h study in mice. Significant upregulation of the LXR target gene ABCA1 in brain was observed with these three compounds. Compound **18** was advanced into a 5-day rat study. As in the 4 h mouse study, the compound was detected in brain and there was an increase in ABCA1 expression levels; however, no effect on A β levels was observed in either rat cerebrum or CSF. The lack of LXR-mediated A β clearance with this series of compounds led us to discontinue efforts to optimize them for treatment of Alzheimer's disease.

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References and Notes

1. Ottow, E.; Weinmann, H., Nuclear receptors as drug targets: A historical perspective of modern drug discovery. In *Nuclear Receptors as Drug Targets*, Ottow, E.; Weinmann, H., Eds. Wiley-VCH Verlag GmbH & Co KGaA: Weinheim, Germany, 2008; pp 1-17.
2. Janowski, B. A.; Grogan, M. J.; Jones, S. A.; Wisely, G. B.; Kliewer, S. A.; Corey, E. J.; Mangelsdorf, D. J., Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 266.
3. Tice, C. M.; Noto, P. B.; Fan, K. Y.; Zhuang, L.; Lala, D. S.; Singh, S. B., The Medicinal Chemistry of Liver X Receptor (LXR) Modulators. *J. Med. Chem.* **2014**, *57*, 7182.
4. Pencheva, N.; Buss, C. G.; Posada, J.; Merghoub, T.; Tavazoie, S. F., Broad-spectrum therapeutic suppression of metastatic melanoma through nuclear hormone receptor activation. *Cell* **2014**, *156*, 986.
5. Fowler, A. J.; Sheu, M. Y.; Schmuth, M.; Kao, J.; Fluhr, J. W.; Rhein, L.; Collins, J. L.; Willson, T. M.; Mangelsdorf, D. J.; Elias, P. M.; Feingold, K. R., Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. *J. Investig. Dermatol.* **2003**, *120*, 246.
6. Whitney, K. D.; Watson, M. A.; Collins, J. L.; Benson, W. G.; Stone, T. M.; Numerick, M. J.; Tippin, T. K.; Wilson, J. G.; Winegar, D. A.; Kliewer, S. A., Regulation of cholesterol homeostasis by the liver X receptors in the central nervous system. *Mol. Endocrinol.* **2002**, *16*, 1378.
7. Zelcer, N.; Khanlou, N.; Clare, R.; Jiang, Q.; Reed-Geaghan, E. G.; Landreth, G. E.; Vinters, H. V.; Tontonoz, P., Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10601.
8. Hong, C.; Tontonoz, P., Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nature Reviews Drug Discovery* **2014**, *13*, 433.

9. Jiang, Q.; Lee, C. Y. D.; Mandrekar, S.; Wilkinson, B.; Cramer, P.; Zelcer, N.; Mann, K.; Lamb, B.; Willson, T. M.; Collins, J. L.; Richardson, J. C.; Smith, J. D.; Comery, T. A.; Riddell, D.; Holtzman, D. M.; Tontonoz, P.; Landreth, G. E., ApoE promotes the proteolytic degradation of A β . *Neuron* **2008**, *58*, 681.
10. Sandoval-Hernandez, A. G.; Arboleda, G.; Buitrago, L.; Moreno, H.; Cardona-Gomez, G. P., Role of Liver X Receptor in AD Pathophysiology. *PLoS One* **2015**, *10*, e0145467.
11. Sandoval-Hernandez, A. G.; Hernandez, H. G.; Restrepo, A.; Munoz, J. I.; Bayon, G. F.; Fernandez, A. F.; Fraga, M. F.; Cardona-Gomez, G. P.; Arboleda, H.; Arboleda, G. H., Liver X Receptor Agonist Modifies the DNA Methylation Profile of Synapse and Neurogenesis-Related Genes in the Triple Transgenic Mouse Model of Alzheimer's Disease. *J. Mol. Neurosci.* **2016**, *58*, 243.
12. Sandoval-Hernández, A. G.; Restrepo, A.; Cardona-Gómez, G. P.; Arboleda, G., LXR activation protects hippocampal microvasculature in very old triple transgenic mouse model of Alzheimer's disease. *Neurosci. Lett.* **2016**, *621*, 15.
13. Zhang-Gandhi, C. X.; Drew, P. D., Liver X receptor and retinoid X receptor agonists inhibit inflammatory responses of microglia and astrocytes. *J. Neuroimmunol.* **2007**, *183*, 50.
14. Fitz, N. F.; Cronican, A.; Pham, T.; Fogg, A.; Fauq, A. H.; Chapman, R.; Lefterov, I.; Koldamova, R., Liver X receptor agonist treatment ameliorates amyloid pathology and memory deficits caused by high-fat diet in APP23 mice. *J. Neurosci.* **2010**, *30*, 6862.
15. Koldamova, R. P.; Lefterov, I. M.; Staufenbiel, M.; Wolfe, D.; Huang, S.; Glorioso, J. C.; Walter, M.; Roth, M. G.; Lazo, J. S., The Liver X Receptor Ligand T0901317 Decreases Amyloid β Production in Vitro and in a Mouse Model of Alzheimer's Disease. *J. Biol. Chem.* **2005**, *280*, 4079.
16. Terwel, D.; Steffensen, K. R.; Verghese, P. B.; Kummer, M. P.; Gustafsson, J.-A.; Holtzman, D. M.; Heneka, M. T., Critical role of astroglial apolipoprotein E and liver X receptor- α expression for microglial A β phagocytosis. *J. Neurosci.* **2011**, *31*, 7049.
17. Donkin, J. J.; Stukas, S.; Hirsch-Reinshagen, V.; Namjoshi, D.; Wilkinson, A.; May, S.; Chan, J.; Fan, J.; Collins, J.; Wellington, C. L., ATP-binding cassette transporter A1 mediates the beneficial effects of the liver X receptor agonist GW3965 on object recognition memory and amyloid burden in amyloid precursor protein/presenilin 1 mice. *J. Biol. Chem.* **2010**, *285*, 34144.
18. Riddell, D. R.; Zhou, H.; Comery, T. A.; Kouranova, E.; Lo, C. F.; Warwick, H. K.; Ring, R. H.; Kirksey, Y.; Aschmies, S.; Xu, J.; Kubek, K.; Hirst, W. D.; Gonzales, C.; Chen, Y.; Murphy, E.; Leonard, S.; Vasylyev, D.; Oganessian, A.; Martone, R. L.; Pangalos, M. N.; Reinhart, P. H.; Jacobsen, J. S., The LXR agonist TO901317 selectively lowers hippocampal A β 42 and improves memory in the Tg2576 mouse model of Alzheimer's disease. *Mol. Cell. Neurosci.* **2007**, *34*, 621.
19. Suon, S.; Zhao, J.; Villarreal, S.; Anumula, N.; Liu, M.; Carangia, L.; Renger, J.; Zerbinatti, C., Systemic treatment with liver X receptor agonists raises apolipoprotein E, cholesterol, and amyloid- β peptides in the cerebral spinal fluid of rats. *Mol. Neurodegener.* **2010**, *5*, 44.
20. Vanmierlo, T.; Rutten, K.; Dederen, J.; Bloks, V. W.; van Vark-van der Zee, L. C.; Kuipers, F.; Kiliaan, A.; Blokland, A.; Sijbrands, E. J. G.; Steinbusch, H.; Prickaerts, J.; Luetjohann, D.; Mulder, M., Liver X receptor activation restores memory in aged AD mice without reducing amyloid. *Neurobiology of Aging* **2011**, *32*, 1262.

21. Wang, S.; Wen, P.; Wood, S., Effect of LXR/RXR agonism on brain and CSF A β 40 levels in rats [version 2; referees: 1 approved, 2 approved with reservations]. *FL1000Research* **2016**, 5, 138.
22. Stachel, S. J.; Zerbinatti, C.; Rudd, M. T.; Cosden, M.; Suon, S.; Nanda, K. K.; Wessner, K.; Dimuzio, J. M.; Maxwell, J.; Wu, Z.; Uslander, J. M.; Michener, M. S.; Szczerba, P.; Brnardic, E.; Rada, V.; Kim, Y.; Meissner, R. S.; Wuelfing, W. P.; Yuan, Y.; Ballard, J.; Holahan, M.; Klein, D. J.; Lu, J.; Fradera, X.; Parthasarathy, G.; Uebele, V. N.; Chen, Z.; Li, Y.; Li, J.; Cooke, A. J.; Bennett, J.; Bilodeau, M.; Renger, J., Identification and In Vivo Evaluation of Liver X Receptor β (LXR β) Selective Agonists for the Potential Treatment of Alzheimer's Disease. *J. Med. Chem.* **2016**, 59, 3489.
23. Bradley, M. N.; Hong, C.; Chen, M.; Joseph, S. B.; Wilpitz, D. C.; Wang, X.; Lusis, A. J.; Collins, A.; Hseuh, W. A.; Collins, J. L.; Tangirala, R. K.; Tontonoz, P., Ligand activation of LXR β reverses atherosclerosis and cellular cholesterol overload in mice lacking LXR α and apoE. *J. Clin. Invest.* **2007**, 117, 2337.
24. Lund, E. G.; Peterson, L. B.; Adams, A. D.; Lam, M.-H. N.; Burton, C. A.; Chin, J.; Guo, Q.; Huang, S.; Latham, M.; Lopez, J. C.; Menke, J. G.; Milot, D. P.; Mitnaul, L. J.; Rex-Rabe, S. E.; Rosa, R. L.; Tian, J. Y.; Wright, S. D.; Sparrow, C. P., Different roles of liver X receptor alpha and beta in lipid metabolism: Effects of an alpha -selective and a dual agonist in mice deficient in each subtype. *Biochem. Pharmacol.* **2006**, 71, 453.
25. Liu, Z.; Lindblom, P.; Claremon, D. A.; Singh, S. B., Structure-based design technology Contour and its application to drug discovery. *RSC Biomol. Sci.* **2012**, 24, 265.
26. Zheng, Y.-J.; Zhuang, L.; Fan, K. Y.; Tice, C. M.; Zhao, W.; Dong, C.; Lotesta, S. D.; Leftheris, K.; Lindblom, P. R.; Liu, Z.; Shimada, J.; Noto, P. B.; Meng, S.; Hardy, A.; Howard, L.; Krosky, P.; Guo, J.; Lipinski, K.; Kandpal, G.; Bukhtiyarov, Y.; Zhao, Y.; Lala, D.; Van Orden, R.; Zhou, J.; Chen, G.; Wu, Z.; McKeever, B. M.; McGeehan, G. M.; Gregg, R. E.; Claremon, D. A.; Singh, S. B., Discovery of a novel, orally efficacious Liver X Receptor (LXR) β agonist. *J. Med. Chem.* **2016**, 59, 3264.
27. Hecht, S. S.; El-Bayoumy, K.; Tulley, L.; LaVoie, E., Structure-mutagenicity relationships of N-oxidized derivatives of aniline, o-toluidine, 2'-methyl-4-aminobiphenyl, and 3,2'-dimethyl-4-aminobiphenyl. *J. Med. Chem.* **1979**, 22, 981.
28. Duan, J.-D.; Jeffrey, A. M.; Williams, G. M., Assessment of the medicines lidocaine, prilocaine, and their metabolites, 2,6-dimethylaniline and 2-methylaniline, for DNA adduct formation in rat tissues. *Drug Metab. Dispos.* **2008**, 36, 1470.
29. Wager, T. T.; Hou, X.; Verhoest, P. R.; Villalobos, A., Moving beyond Rules: The Development of a Central Nervous System Multiparameter Optimization (CNS MPO) Approach To Enable Alignment of Druglike Properties. *ACS Chem. Neurosci.* **2010**, 1, 435.
30. Courcambeck, J.; Bihel, F.; De Michelis, C.; Quelever, G.; Kraus, J. L., Design of potential new HIV protease inhibitors: enantioconvergent synthesis of new pyrrolidin-3-ol, and pyrrolidin-3-one peptide conjugates. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1421.
31. Surry, D. S.; Buchwald, S. L., Dialkylbiaryl phosphines in Pd-catalyzed amination: a user's guide. *Chemical Science* **2011**, 2, 27.

4

LXR α EC₅₀ (efficacy): 214 nM (79%)

LXR β EC₅₀ (efficacy): 36 nM (56%)

Poor CNS penetration

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LXR α EC₅₀ (efficacy): 166 nM (83%)

LXR β EC₅₀ (efficacy): 38 nM (77%)

Good CNS penetration

Brain ABCA1 expression ↑