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LXR ANTAGONISTS WITH A 5-SUBSTITUTED PHENANTHRIDIN-6-ONE SKELETON: SYNTHESIS AND LXR TRANSREPRESSION ACTIVITIES OF CONFORMATIONALLY RESTRICTED CARBA-T0901317 ANALOGS

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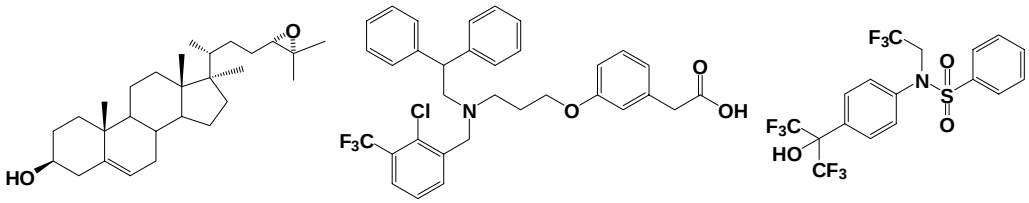
Abstract – Conformationally restricted heterocyclic analogs of carba-T0901317, a liver X receptor (LXR) antagonist, were prepared via the palladium catalized cyclization reaction as a key step. *In vitro* transactivation assay revealed that the structural modification altered the nature of the activity from LXR-agonistic to LXR-antagonistic.

INTRODUCTION

Liver X receptors (LXR) are members of the nuclear receptor superfamily and are involved in the regulation of cholesterol, lipid, and glucose metabolism.[1,2] LXRs are ligand-activated transcription factors which bind to DNA as heterodimers with retinoid X receptor (RXR). In macrophages, liver, and intestine, activation of LXRs induces the expression of genes involved in lipid metabolism and reverse cholesterol transport.[3] The potential to prevent or even reverse atherosclerosis by modulating the expression of these genes makes LXR an attractive therapeutic target for the treatment of atherosclerosis. Several LXR agonists (Figure 1) have been reported to date,[4-6] but currently available synthetic LXR agonists also activate triglyceride synthesis in the liver by the activation of sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (FAS), and this limits the utility of these synthetic LXR agonists.[7] Therefore, novel, specific LXR ligands are needed.

We have been engaged in the design and synthesis of nuclear receptor ligands, and recently we have focused on LXR ligands.[8,9] Now, we would like to report LXR ligands of a new structural type, generated by modification of carba-T0901317.[10]

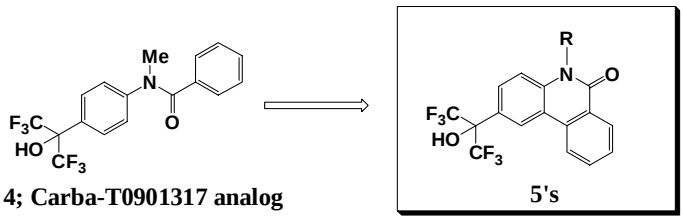


1; EPC **2; GW-3965** **3; T0901317**

Figure 1. Structures of known LXR agonists, EPC (**1**), GW3965 (**2**) and T0901317 (**3**)[1-5]

RESULTS AND DISCUSSION

We designed 5-substituted-phenanthridin-6-one derivatives (**5**'s) as conformationally restricted heterocyclic analogues of carba-T0901317 (**4**), expecting to increase the LXR-agonistic activity. Carba-T0901317 is an amide-surrogate of the representative LXR agonist, T0901317, and exhibits LXR-agonistic activity similar to that of T0901317.[10]

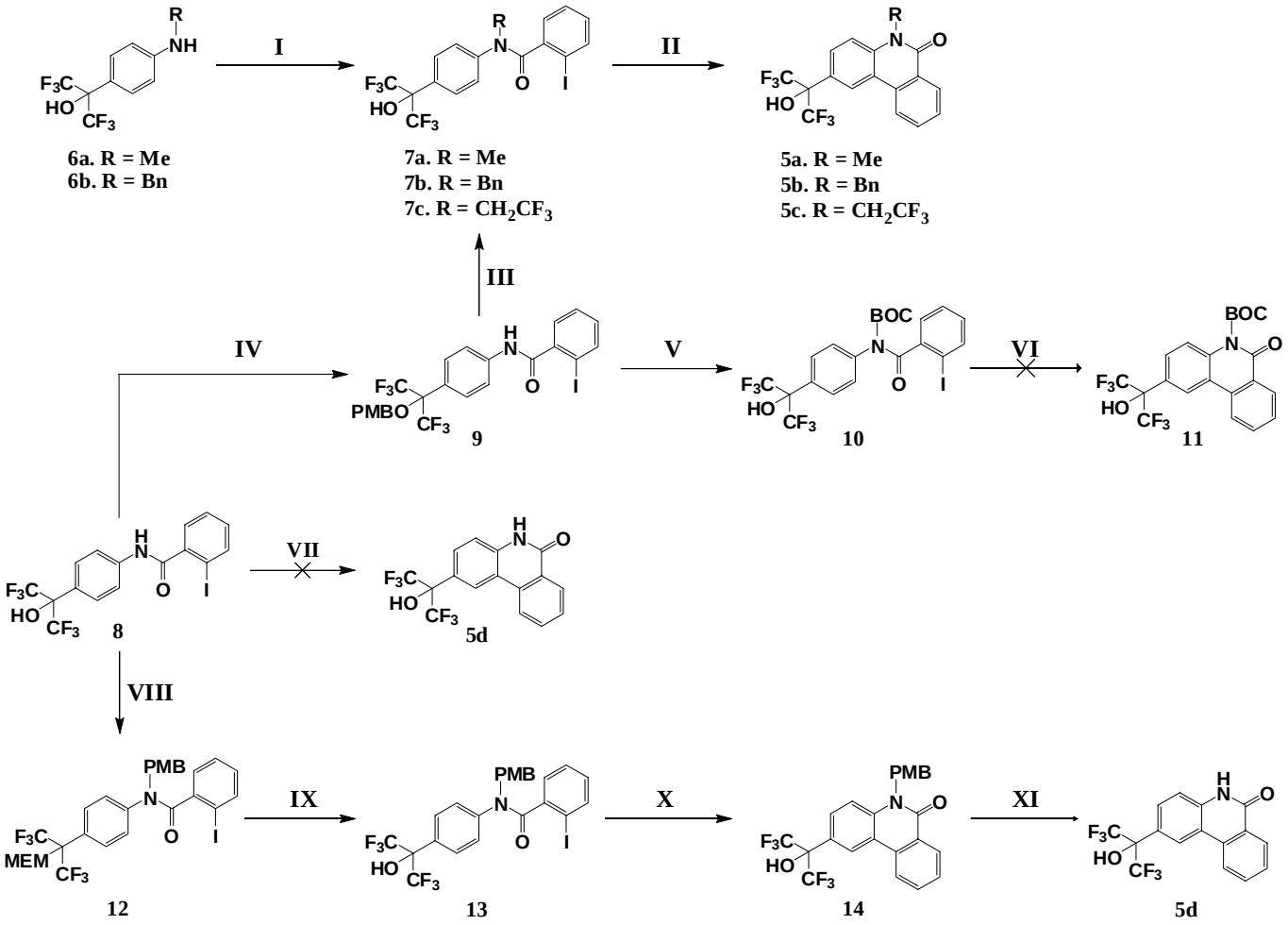


4; Carba-T0901317 analog **5's**

Figure 2. Structural development of 5-substituted phenanthridin-7-one derivatives (**5'’s**)

We planned to synthesize these compounds by the reaction of 2-(4-(substituted amino)phenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol with 2-iodobenzoyl chloride, followed by intramolecular cyclization.[11] As expected, the intermediate amides **7a**, **7b**, which have a methyl, benzyl, or 2,2,2-trifluoroethyl group as a 5-substituent, respectively, were efficiently synthesized by the reaction of the corresponding substituted 2-(4-(substituted amino)phenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol with 2-iodobenzoyl chloride. The hydroxyl group of **8**, which was prepared by the reaction of 2-(4-aminophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol with 2-iodobenzoyl chloride was preprotected with a *para*-methoxybenzyl (PMB) group (PMBBr-NaH-DMF) to affford **9** quantitatively. The amide nitrogen of **9** was 2,2,2-trifluoroethylated by using $TfOCH_2CF_3$-NaH-DMF, then deprotected the PMB group with ceric ammonium nitrate (CAN)-MeCN-H_2O to afford **7c** in about 20% yield (two steps).

The intramolecular cyclization of **7a**-**7c** proceeded smoothly with the use of $Pd(OAc)_2$-$PCy_3.HBF_4$-Cs_2CO_3[12] as the coupling reagent. Intramolecular cyclization of **8** to afford the



Scheme. 1. Synthetic routes to 5-substituted phenanthridin-6-one derivatives (**5'**s)
Reagents and comments. I) 2-iodobenzoylchloride, TEA, DCM, 82-97%; II) $Pd(OAc)_2$, $PCy_3.HBF_4$, Cs_2CO_3, DMA, 80-90%; III) 1) $TfOCH_2CF_3$, NaH/DMF, 23%, 2) CAN, MeCN/H_2O, 93%; IV) PMBBr, NaH, DMF, quant.; V) 1) $(Boc)_2O$, DMAP, MeCN, 72%, 2) CAN, MeCN/H_2O, 48%; VI) $Pd(OAc)_2$, $PCy_3.HBF_4$, Cs_2CO_3, DMA; VII) $Pd(OAc)_2$, $PCy_3.HBF_4$, Cs_2CO_3, DMA; VIII) 1) MEMCl, NaH, DMF, 51%; 2) PMBBr, NaH, DMF, 81%, IX) $TiCl_4$, DCM, 67%; X) $Pd(OAc)_2$, $PCy_3.HBF_4$, Cs_2CO_3, DMA, 99%; XI) CAN, MeCN/H_2O, 34%.

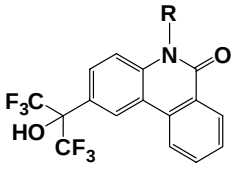
5-unsubstituted phenanthridin-6-one (**5d**) did not proceed with $Pd(OAc)_2$-$PCy_3.HBF_4$-Cs_2CO_3 or under the other coupling conditions examined. Thus, we devised a different route, i.e., protection of the amide-nitrogen of **8** with a Boc (*tert*-butoxycarbonyl) group, intramolecular cyclization, and subsequent deprotection. The hydroxyl group of **8** was preprotected with a PMB group (PMBBr-NaH-DMF) to affford **9** quantitatively. The amide nitrogen of **9** was protected with a Boc group by using $(Boc)_2O$-DMAP-MeCN, then deprotected the PMB group with ceric ammonium nitrate (CAN)-MeCN-H_2O to afford **10** in about 35% yield (two steps). However, the intramolecular cyclization of **10** again failed with $Pd(OAc)_2$-$PCy_3.HBF_4$-Cs_2CO_3 and other coupling conditions examined. Therefore, we next tried PMB as a protecting group. The hydroxyl group of **8** was preprotected with a 2-methoxyethyl (MEM) group (MEMCl-NaH-DMF), then the amide nitrogen was protected with a PMB group by using PMBBr-NaH-DMF to afford **12** in about 40% yield (two steps). The MEM was

deprotected with $TiCl_4$-DCM to afford **13** in 67% yield. In this case, intramolecular cyclization of **13** proceeded smoothly with $Pd(OAc)_2$-$PCy_3.HBF_4$-Cs_2CO_3 to afford the cyclized 5-PMB-substituted phenanthridin-6-one (**14**) in 99% yield. The PMB group was removed with ceric ammonium nitrate (CAN)-MeCN-H_2O to afford the desired **5d** in 34% yield, with significant recovery (50%) of the starting **14** (these reaction conditions were not optimized).

The intramolecular cyclization proceeded when the nitrogen substitutent was alkyl, benzyl, or *para*-methoxybenzyl, but not when it was hydrogen or the Boc group. It is clear that cis-amide conformation is critical for the cyclization to form the 5-substituted (or unsubstituted) phenanthridin-6-one skeleton. Therefore, we speculated that **7a-c** and **13** can adopt cis-conformation, while **8** and **10** can not. To test the validity of this idea, spectroscopic analysis and computer-aided conformational analyses of **7a-c**, **8**, **10**, and **13** are in progress.

The values of in vitro transactivation activity of the compounds in the present series are summarized in Table 1, along with those of T0901317 and carba-T0901317 as the positive control. Both T0901317 and carba-T0901317 exhibited potent pan LXR-agonistic activities, i.e., the EC_{50} values of T0901317 and carba-T0901317 for LXRα and LXRβ are 0.34, 0.97 and 0.09, 0.25 μM, respectively. But none of the

Table 1. In vitro transactivation assay of the present series of compounds as LXR ligands.



	LXR α[a)]		LXR β[a)]	
R	**EC_{50} (μM)**	**IC_{50} (μM)**	**EC_{50} (μM)**	**IC_{50} (μM)**
H	**ia[b)]**	**5**	**ia[b)]**	**5.1**
CH_3	**ia[b)]**	**4.1**	**ia[b)]**	**> 100[c)]**
CH_2CF_3	**ia[b)]**	**8**	**ia[b)]**	**> 100[d)]**
$CH_2C_6H_5$	**ia[b)]**	**> 30[e)]**	**ia[b)]**	**> 10[f)]**
T0901317	**0.34**	**ia[b)]**	**0.09**	**ia[b)]**
carba-T0901317	**0.97**	**ia[b)]**	**0.25**	**ia[b)]**

a) Compounds were screened for agonist activity on LXR-GAL4 chimeric receptors in transiently transfected HEK-293 cells. The EC_{50} value is the molar concentration of the test compound that affords 50% of the maximal reporter activity. The IC_{50} value is the molar concentration of the test compound that affords a 50% decrease in the maximal reporter activity of 100 nM T0901317. b) ia means inactive at 100 μM. c) 65% inhibition at 100 μM. d) 61% inhibition at 100 μM. e) 65% inhibition at 30 μM. f) 54% inhibition at 10 μM.

conformationally restricted analogues of 5-(un)substituted phenanthridin-6-one (**5a**-**c**) exhibited LXR-agonistic activitiy, but all exhibited characteristic LXR-antagonistic activity. In the case of LXRα, **5a-c** exhibited similar IC_{50} values of micromolar order, while the bulkier benzyl derivative, **5e**, showed somewhat decreased LXRα–antagonistic activity. For LXRβ, only **5a** exhibited a micromolar order of IC_{50} value, and the other 5-substituted phenanthridin-6-one derivatives (**5b**-**d**) did not show apparent LXRβ–antagonistic activity.

Therefore, compounds **5b**, and **5c** exhibited LXRα-selective antagonistic activities in our assay system.

In summary, we have designed and synthesized a series of 5-(un)substituted phenanthridin-6-one derivatives of carba-T0901317, and found that conformational restriction changed the activity of the compounds from LXR-agonistic to LXR-antagonistic.

ACKNOWLEDGEMENTS

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