Bioorganic & Medicinal Chemistry Letters 20 (2010) 1432–1435

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and discovery of 2,3-dihydro-3,8-diphenylbenzo[1,4]oxazines as a novel class of potent cholesteryl ester transfer protein inhibitors

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ARTICLE INFO

Article history:
Received 2 December 2009
Revised 20 December 2009
Accepted 22 December 2009
Available online 4 January 2010

Keywords:
Cholesteryl ester transfer protein
CETP
CETP inhibitors
HDL-C
Atherosclerosis

ABSTRACT

2,3-Dihydro-3,8-diphenylbenzo[1,4]oxazines were identified as a new class of potent cholesteryl ester transfer protein inhibitors. The most potent compound **6a** (IC_{50} = 26 nM) possessed a favorable pharmacokinetic profile with good oral bioavailability in rat (*F* = 53%) and long human liver microsome stability ($t_{1/2}$ = 62 min). It increased HDL-C in human CETP transgenic mice and high-fat fed hamsters. The structure and activity relationship of this series will be described in this Letter.

Low levels of high density lipoprotein-cholesterol (HDL-C) and high levels of small-dense low density lipoprotein-cholesterol (LDL-C) are independent risk factors for the development of atherosclerosis and eventually coronary heart disease (CHD), which remain the leading cause of death in the developed countries.[1–3] HDL-C plays important role in removal of excess cholesterol from peripheral cells to the liver for metabolic degradation in a reverse cholesterol transport (RCT) process.[4–6] Therefore, the increase in HDL-C level offers a new and promising therapeutical principle for treatment of CHD.[7]

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that mediates the transfer of cholesteryl ester (CE) from HDL to very low density lipoprotein (VLDL) and LDL in exchange for triglyceride.[8,9] The general net effect of this process is a reduction in atheroprotective HDL-C level and with increase in proatherogenic VLDL-C and LDL-C levels. However, the potential antiatherogenic role of CETP in participation of reverse cholesterol transport has also been suggested.[4,10–12] In spite of controversies regarding the roles of CETP in the progression of atherosclerosis,[13] the growing body of evidence showing the profound beneficial effects of low levels of LDL or high levels of HDL suggests that specific and effective inhibition of CETP to raise HDL-C level[13] may outweigh the risk and provide a potential therapeutic benefit for patients with CHD.

Considerable efforts have been made toward the development of potent and selective CETP inhibitors. The most advanced inhibitors include irreversible inhibitor JTT-705 (Fig. 1, Roche and Japan Tobacco, phase III),[14,15] which has moderate potency, Torcetrapib (Pfizer, phase III, discontinued),[16–18] which was withdrawn from phase III studies in December 2006 due to hypertension observed, and Anacetrapib (Merck, phase III).[19] 3,4-Dihydro-2,5-diphenyl-α-trifluoromethyl-1-quinolineethanol derivatives were discovered in our laboratories as potent CETP inhibitors.[20,21] In order to improve aqueous solubility, 2,3-dihydro-3,8-diphenylbenzo[1,4]oxazines **6** were designed and synthesized as a new series of potent CETP inhibitors.[22,23]

The synthesis of 2,3-dihydro-3,8-diphenylbenzo[1,4]oxazines **6** is shown in Scheme 1. Reduction of 2-bromo-6-nitrophenol with $Na_2S_2O_4$ provided 2-amino-6-bromophenol **1**. α-Bromoketone **2** was prepared in two steps by methylation of 3-(1,1,2,2-tetrafluoroethoxy)benzoic acid with MeLi at low temperature to give methyl ketone, and bromination of this intermediate with bromine in acetic acid to yield **2**. Alkylation of 2-amino-6-bromophenol **1** with α-bromoketone **2** by using base Cs_2CO_3 in CH_3CN produced phenoxy ketone **3** in 88% yield. Intramolecular reductive cyclization of **3** to racemic 8-bromo benzoxazine **4** was accomplished in quantitive yield by using $NaBH(OAc)_3$ in the presence of 1 equiv of TFA. Suzuki coupling of aryl bromide **4** with 3-trifluoromethoxybenzene boronic acid gave compound **5** in high yield. In the presence of catalytic amount of Lewis acid ytterbium trifluoromethanesulfonate, N-alkylation of **5** with commercially available ~90% enriched (*S*)-1,1,1-trifluoro-2,3-epoxypropane occurred to produce a mixture of four diastereomers **6a** (3*S*, α*S*), **6b** (3*R*, α*R*), **6c** (3*R*, α*S*), and **6d** (3*S*, α*R*).[24] The mixture was separated by silica gel column

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doi:10.1016/j.bmcl.2009.12.096

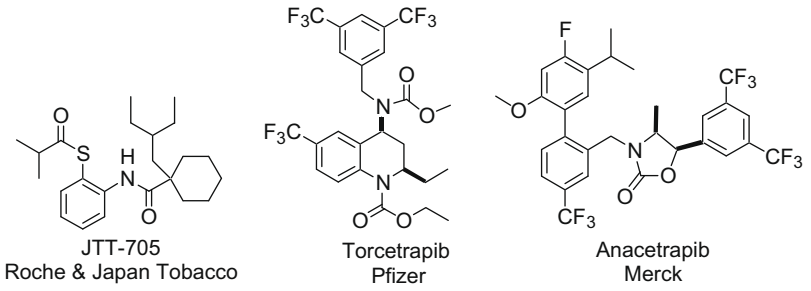
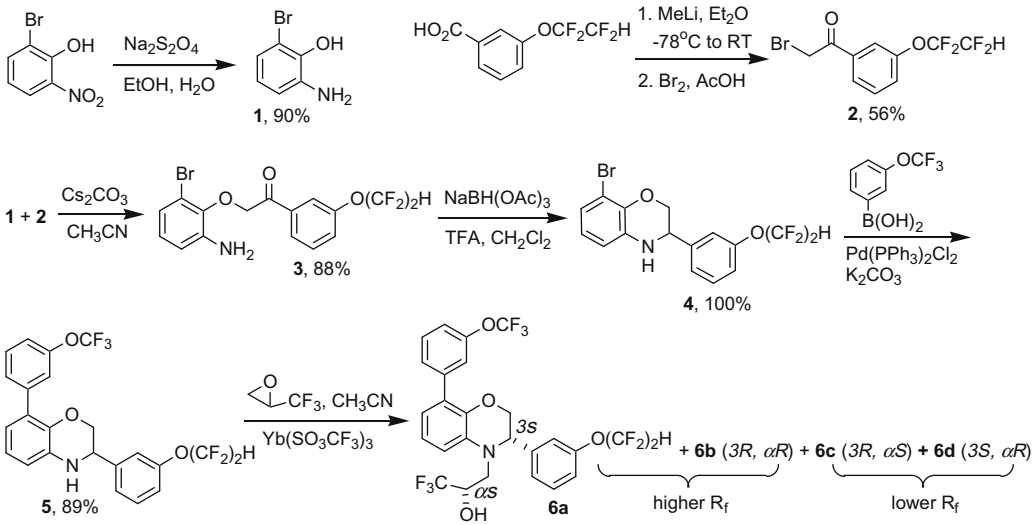


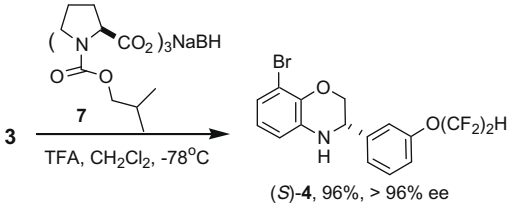
Figure 1. Structures of CETP inhibitors.



Scheme 1.

chromatography into two diastereomeric pairs. The higher R_f pair consisted of **6a** (*3S*, *αS*) as a major component and **6b** (*3R*, *αR*) as a minor one, while the lower R_f pair made up of **6c** (*3R*, *αS*) as a major component and **6d** (*3S*, *αR*) as a minor one**.**

The optically pure compounds were prepared either by chiral HPLC resolution of the advanced intermediate, racemic compound **5**, or by enantioselective synthesis. Eq. 1 shows the example of asymmetric reductive cyclization of **3** to (*S*)-**4** in 96% yield and >96% ee by employing chiral sodium triacyloxyborohydride **7** at −78 °C.[25] Following the reaction sequence in Scheme 1, the enantiomer (*S*)-**4** was carried through Suzuki coupling and N-alkylation with ~90% enriched (*S*)-1,1,1-trifluoro-2,3-epoxypropane to obtain two separable **6a** (*3S*, *αS*) and its diastereomer **6d** (*3S*, *αR*) in 75% and 18% yield, respectively.



(1)

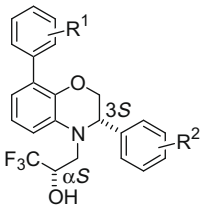
By modification of the top and right-side phenyl substituents, analogs were prepared and evaluated for their ability to inhibit human CETP, some of which are illustrated in Table 1. Except optically pure compounds indicated in the parenthesis, all of the other compounds listed in Table 1 are high R_f diastereomeric pairs of (*3S*, *αS*) as major components and (*3R*, *αR*) as minor ones. It was demonstrated in all of this class of compounds prepared, the potency of lower R_f diastereomeric pairs of (*3R*, *αS*)- and (*3S*, *αR*)-isomers was consistently much poorer than that of its counterpart, higher R_f diastereomeric pairs of (*3S*, *αS*) and (*3R*, *αR*), therefore, the data of lower R_f diastereomers are not listed in Table 1.

As exemplified in diastereomers **6a–d**, the (*3S*, *αS*) stereochemical configuration (**6a**) possessed the highest potency among the four diastereomers. R^1 replacement of 3-OCF_3 in **6e** with 3-F in **7** decreased the potency dramatically. However, when the number of *F*-atom increased to 3,5-difluoro **8** or 3,4,5-trifluoro **9**, the potency increased.

The SAR of substituent R^2 on the right-side phenyl ring was also investigated. Moving the OCF_2CF_2H group from *meta*- in **6e** to *ortho*- in **13** or *para*-position in **14** dramatically reduced the potency. The OCF_3 group also exhibited preference for *meta*- (**15b**) to *para*-orientation (**16**). The 3-OCF_2CF_2H group on the right-side aryl ring was 2–5-fold more potent than the short 3-OCF_3 by comparison of **6a** with **15a**, **8** with **11**, and **9** with **12**. None of other compounds (**17**–**23**) carrying different R_2 was as potent as **6e** (R_2 = 3-OCF_2CF_2H).

Scheme 2 is the chemical approach developed to quickly explore the heteroaryl and aliphatic replacements of the right-side phenyl group. Cyclization of 2-amino-6-bromophenol **1** with chloroacetyl chloride provided lactam **25** in quantitive yield. Suzuki reaction of **25** with 3-trifluoromethoxybenzene boronic acid and protection of the resulting intermediate as *N*-Boc afforded compound **26**. Aminoether **27** was obtained by DIBAL-H reduction of lactam **26** to hemiaminal followed by reaction with EtOH in the presence of *p*-toluene sulfonic acid. Treatment of aminoether **27** with Lewis acid such as $BF_3{\cdot}OEt_2$ generated in situ an *N*-acyliminium

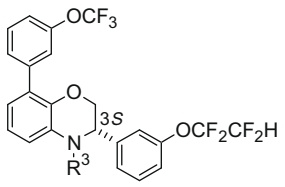
Table 1
In vitro inhibition of human CETP by derivatives with substitution variations on 3,8-diphenyl groups

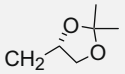
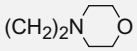


Compd[a]	R^1	R^2	% Inhibition at 1 μM	IC_{50} (nM)
6a (3*S*, α*S*)	3-OCF_3	3-$O(CF_2)_2H$	91	26
6b (3*R*, α*R*)	3-OCF_3	3-$O(CF_2)_2H$	26	ND
6c (3*R*, α*S*)	3-OCF_3	3-$O(CF_2)_2H$	14	ND
6d (3*S*, α*R*)	3-OCF_3	3-$O(CF_2)_2H$	23	ND
6e	3-OCF_3	3-$O(CF_2)_2H$	96	45
7	3-F	3-$O(CF_2)_2H$	79	322
8 (3*S*, α*S*)	3,5-F_2	3-$O(CF_2)_2H$	100	50
9 (3*S*, α*S*)	3,4,5-F_3	3-$O(CF_2)_2H$	101	41
10 (3*S*, α*S*)	3,5-$(CF_3)_2$	3-$O(CF_2)_2H$	59	580
11 (3*S*, α*S*)	3,5-F_2	3-OCF_3	100	193
12 (3*S*, α*S*)	3,4,5-F_3	3-OCF_3	94	94
13	3-OCF_3	2-$O(CF_2)_2H$	6	ND
14	3-OCF_3	4-$O(CF_2)_2H$	33	ND
15a (3*S*, α*S*)	3-OCF_3	3-OCF_3	69	133
15b	3-OCF_3	3-OCF_3	65	107
16	3-OCF_3	4-OCF_3	38	ND
17	3-OCF_3	3-H	43	ND
18	3-OCF_3	3-Cl	62	214
19	3-OCF_3	3-CF_3	37	ND
20	3-OCF_3	3-OCH_3	29	ND
21	3-OCF_3	3-OEt	56	490
22	3-OCF_3	3-SCF_3	68	360
23	3-OCF_3	3,4-OCF_2O	48	271

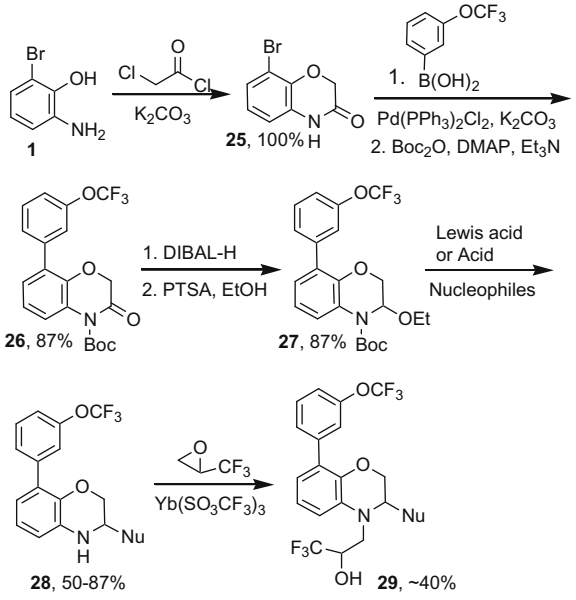
ND, not determined.
[a] Except optically pure compounds indicated in the parenthesis, all of the other compounds were high R_f diastereomeric pairs of (3*S*, α*S*) as major components and (3*R*, α*R*) as minor ones.

Table 2
In vitro inhibition of human CETP by derivatives with terminal chain variations on *N*-atom



Compd[a]	R^3	% Inhibition at 1 μM	IC_{50} (nM)
6a (3*S*, α*S*)	$CH_2CH(OH)CF_3$	91	26
30a (3*S*, α*S*)	$CH_2CH(OH)CH_2F$	77	14
30b (3*S*, α*R*)	$CH_2CH(OH)CH_2F$	55	147
31 (3*S*)	$CH_2C(O)CF_3$	5	ND
32 (3*S*, α*S*)	$CH_2CH(OH)CH_2OH$	27	ND
33		25	ND
34a (3*S*, α*R*)	$CH_2CH(OH)CH_3$	50	88
34b (3*S*, α*S*)	$CH_2CH(OH)CH_3$	28	ND
35 (3*S*)	$CH_2C(O)CH_3$	5	ND
36	$CH_2CH(OH)CH(CH_3)_2$	86	129
37	$CH_2C(OH)(CH_3)_2$	29	560
38		0	ND
39	$(CH_2)_2OH$	29	ND
40	$(CH_2)_3OH$	10	ND
41	$(CH_2)_2OCH_3$	25	ND
42	$(CH_2)_2NH_2$	2	ND
43	$(CH_2)_2N(CH_3)_2$	9	ND
44	Pr	39	190
45	Et	24	ND
46	CO_2CH_3	16	ND

ND, not determined.
[a] Except optically pure compounds indicated in the parenthesis, all of the other compounds were high R_f diastereomeric pairs of (3*S*, α*S*) as major components and (3*R*, α*R*) as minor ones.

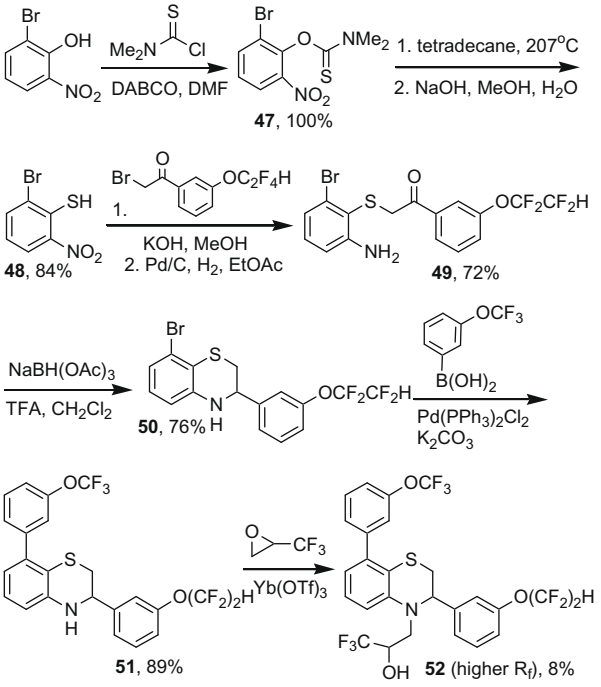


Scheme 2.

ion,[26] which was trapped by nucleophiles such as 2-ethylthiophen or allyltrimethylsilane leading to dihydrobenzo[1,4]oxazines **28**. Reaction of **28** with ~90% enriched (*S*)-1,1,1-trifluoro-2,3-epoxypropane in the presence of catalytic quantity of $Yb(OTf)_3$ produced target **29**. As in the case of preparation of **6**, the two higher and lower R_f bands were separated by SiO_2 flash chromatography into two diastereomeric pairs.

By utilizing aminoether **27** as a late stage common intermediate, many analogs **29** were prepared bearing *Nu* as furan, thiophen, 2-methoxythiophen, 3-methoxythiophen, 2-ethylthiophen, 3-methylthiophen, 3-ethylthiophen, allyl, 1-hydroxypropyl, 1-methoxypropyl, and 3,3-dimethylbutan-2-one. Among them, the 2-ethylthiophen derivative was the most potent (IC_{50} = 502 nM). Further exploration was limited due to commercial availability of such class of nucleophiles carrying OCF_2CF_2H or OCF_3 substituents, which contributed good inhibitory potency to the right-side phenyl analogs such as **6e** and **15b**.

Focus was then placed on the studies of 1,1,1-trifluoro-2-propanol moiety on the *N*-atom. The results are summarized in Table 2. The stereocenter of the chain was very important for the inhibitory potency as demonstrated in compounds, **6a** and **6d** (Table 1), **30a** and **30b** (10-fold difference), as well as **34a** and **34b**. Surprisely, changing trifluoromethyl in **6a** to monofluoromethyl in **30a** resulted in a twofold more potent inhibitor **30a**. However, human liver microsome stability of **30a** ($t_{1/2}$ = 21 min) was much shorter than that of **6a** ($t_{1/2}$ = 62 min). Replacement of trifluoromethyl in **6a** with hydrophilic hydroxymethyl group in **32** was detrimental to the inhibition of CETP. Modification of it with less lipophilic methyl in **34a** or elimination of trifluoromethyl in **39** resulted in decreased inhibitory potency as well. When the hydroxyl group in **6a** and **34a** was oxidized to ketone functionality in **31** and **35**, the CETP inhibitory potency abolished.



Scheme 3.

It seems that CETP prefers inhibitors with lipophilic groups in the molecules, therefore, it would be interesting to replace the *O*-atom in the morpholine ring of **6** with *S*-atom. The synthesis of *S*-analog **52** is outlined in Scheme 3. 2-Bromo-6-nitrobenzenethiol **48** was prepared in three steps in 84% yield by first reaction of 2-bromo-6-nitrophenol with *N,N*-dimethylcarbamoyl chloride to thiocarbamate **47**, then *O*- to *S*-rearrangement occurred over 200 °C followed by hydrolysis with NaOH. The rest of reaction sequence to target **52** was similar to that of **6**. The inhibitory potency of **52** (IC_{50} = 166 nM) was about fourfold lower than its *O*-analog **6e** (IC_{50} = 45 nM).

Compound **6a** was the most potent inhibitor prepared in this series with IC_{50} = 26 nM in a CETP SPA assay and IC_{50} = 1.0 μM in a human plasma ^{3}H-CE HDL assay. In the studies of human liver microsome cytochrome P450 inhibition assay 1A2, 2C9, 2C19, 2D6, 3A4-T, and 3A4-M, compound **6a** showed no significant inhibitory effect on the P450 isozymes tested (IC_{50} >100 μM). It also displayed long human liver microsome stability ($t_{1/2}$ = 62 min).

The pharmacokinetic (PK) profile of **6a** was studied in rats orally dosed at 10 mg/kg using sesame oil as the vehicle, and iv dosed at 2 mg/kg using 10% EtOH:10% solutol:80% D5W as the vehicle. The oral bioavailability in rats was 53% with significant plasma exposure (AUC = 19.8 μg h/mL) and slow oral absorption (T_{max} = 11.5 h). It also exhibited low systemic clearance (CL = 4.6 mL/min kg) and low volume of distribution (V_{ss} = 0.73 L/kg).

In vivo efficacy studies, treatment of female and male human CETP transgenic mice on a normal chow diet with **6a** orally for 5 days resulted in an increase in HDL-C. At 3, 10, and 30 mg/kg doses of **6a**, the female mice plasma HDL-C was increased 4%, 8%, and 24%, respectively, and the male mice plasma HDL-C was increased 11%, 10%, and 28%, respectively. High-fat fed hamsters were also treated with **6a** orally for 5 days. At 10 and 30 mg/kg doses of **6a**, the hamsters plasma HDL-C was increased 9% and 16%.

As we expected, **6a** displayed higher aqueous solubility (11 μg/mL at pH 2.0, and 2.7 μg/mL at pH 7.4) than its *C*-analog, (*2R*, *αS*)-3,4-dihydro-2-[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-5-[3-(trifluoromethoxy)-phenyl]-α-(trifluoromethyl)-1(*2H*)-quinolineethanol (<1 μg/mL at pH 2.0, and <1 μg/mL at pH 7.4).[20,21]

In conclusion, we have discovered 2,3-dihydro-3,8-diphenylbenzo[1,4]oxazines as a novel class of potent CETP inhibitors. The most potent compound **6a** exhibited favorable pharmacokinetic profile with good oral bioavailability in rat (*F* = 53%). It was a weak inhibitor of P450 isozymes and had long human liver microsome stability ($t_{1/2}$ = 62 min). It increased HDL-C in human CETP transgenic mice and high-fat fed hamsters. Its aqueous solubility was also improved.

Acknowledgment

We thank Jef Proost for chiral HPLC resolution of compound **5**.

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