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Design of a novel class of biphenyl CETP inhibitors

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ABSTRACT

A new class of CETP inhibitors was designed and prepared. These compounds are potent both in vitro and in vivo. The most active compound (**12d**) has shown an ability to raise HDL significantly in transgenic mouse PD model.

Atherosclerosis and its clinical consequences, coronary heart disease (CHD), stroke and peripheral vascular disease, are the leading causes of death and represent a heavy burden to the health care systems around the world. For example, based on Centers for Disease Control and Prevention (CDC), in the United States alone, approximately 13 million patients have been diagnosed with CHD, and greater than one half million deaths are attributed to CHD each year.[1] Furthermore, this toll is expected to grow over the next 10–25 years as an epidemic in obesity and diabetes continues to grow.[1]

It has long been recognized that in mammals, variations in circulating lipoprotein profiles correlate with the risk of atherosclerosis and CHD. The clinical success of HMG-CoA reductase inhibitors,[2–5] especially the statins, in reducing coronary events is based on the reduction of circulating Low Density Lipoprotein cholesterol (LDL-C), levels of which correlate directly with increased risk for atherosclerosis. More recently, epidemiologic studies have demonstrated an inverse relationship between High Density Lipoprotein cholesterol (HDL-C) levels and atherosclerosis, leading to the conclusion that low serum HDL-C levels are associated with an increased risk for CHD. In fact, a substantial number of people who develop atherosclerosis have plasma cholesterol levels in the 'desirable' range while having abnormal low HDL (<35 mg/dl). Therefore, low HDL levels are not only recognized as a risk factor for the disease, but are considered the single best predictor of an individual's likelihood of developing CHD.[6–9]

Metabolic control of lipoprotein levels is a complex and dynamic process involving many factors. One important metabolic control in man is the cholesteryl ester transfer protein (CETP), a plasma glycoprotein that catalyzes the movement of cholesteryl esters from HDL to the apoB containing lipoproteins, especially VLDL. Under physiological conditions, the net reaction is a hetero-exchange in which CETP carries triglyceride to HDL from the apoB lipoproteins and transports cholesterol ester from HDL to the apoB lipoprotein. It has been established clinically that pharmacological inhibition of CETP will lead to increased HDL-C concentrations. However, there remains uncertainty as to whether a CETP inhibitor can provide clinical benefit to high risk CHD patients. More studies are needed to determine if increasing HDL-C via CETP inhibition will reduce atherosclerosis in patients.[10–12] A number of CETP inhibitor scaffolds have been reported from this laboratory and other research organizations in the world, most notably the tetrahydroquinoline core of Pfizer's torcetrapib and the acylaminobenzenethiol core of the Roche/Japan Tobacco inhibitor dalcetrapib.[13–24] Tocetrapib was the first CETP inhibitor that underwent large PhIII trials involving more than 15,000 patients.[25–27] All ongoing clinical trials with torcetrapib were discontinued abruptly in December 2006 when the investigators from one of the trials (ILLUMINATE) reported an increase in all-cause mortality associated with torcetrapib though positive lipid targets were met.[25–27] Discussion surrounding the withdrawal of torcetrapib from development is still ongoing,

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centered around the question of whether the increased rate of deaths associated with torcetrapib resulted from inhibition of CETP, off-target effects, or some combination of these two factors.[17,28–30]

This letter will discuss the design of a new class of biphenyl CETP inhibitors which ultimately led to the discovery of anacetrapib. We initiated an effort to design a proprietary CETP inhibitor scaffold utilizing Pfizer's torcetrapib as a model. In examining torcetrapib, we reasoned that breaking the nitrogen containing ring of the tetrahydroquinoline core would afford compounds that were synthetically accessible and without the added complexity of stereogenic centers. As shown in Figure 1, the initial acyclic variants were promising, as they were low micromolar inhibitors of CETP (as in compound **1**, IC_{50} = 3.89 μM), but efforts to increase the potency via alternate substitutions on either the carbamate or the pendant benzylic group were largely unsuccessful. We investigated the incorporation of a conformational constraint in an attempt to increase intrinsic potency of the series. The heterocyclic

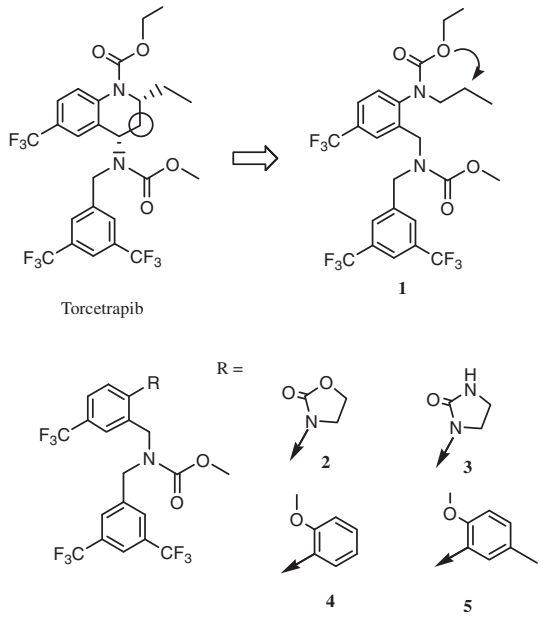
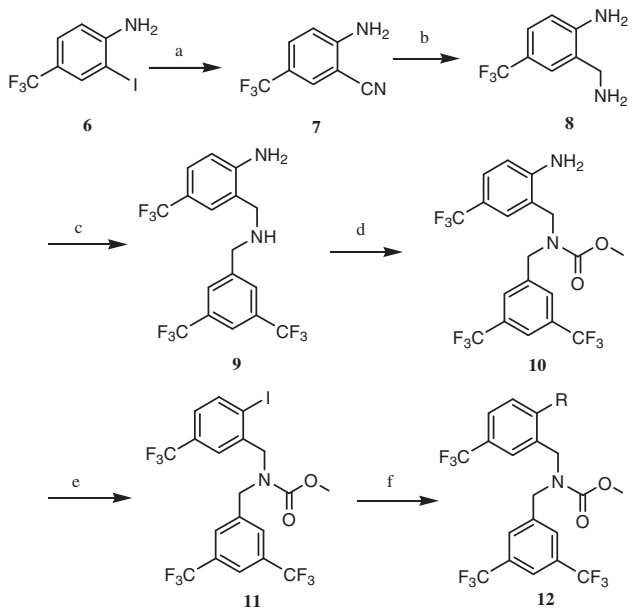
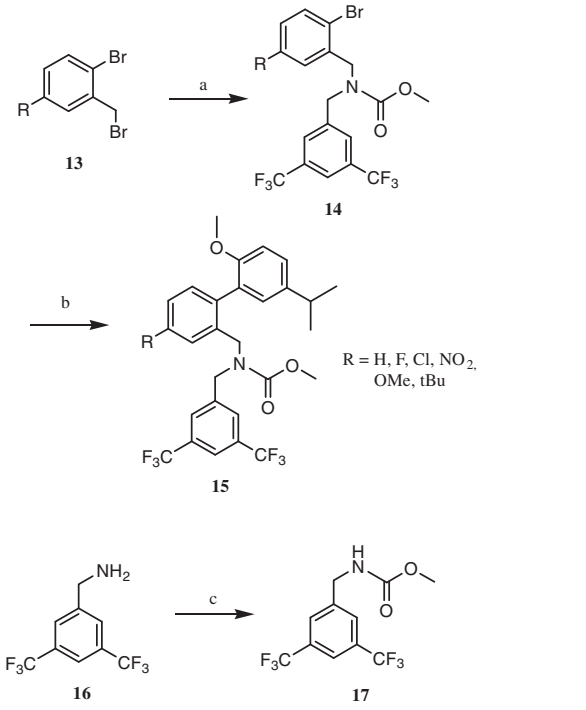


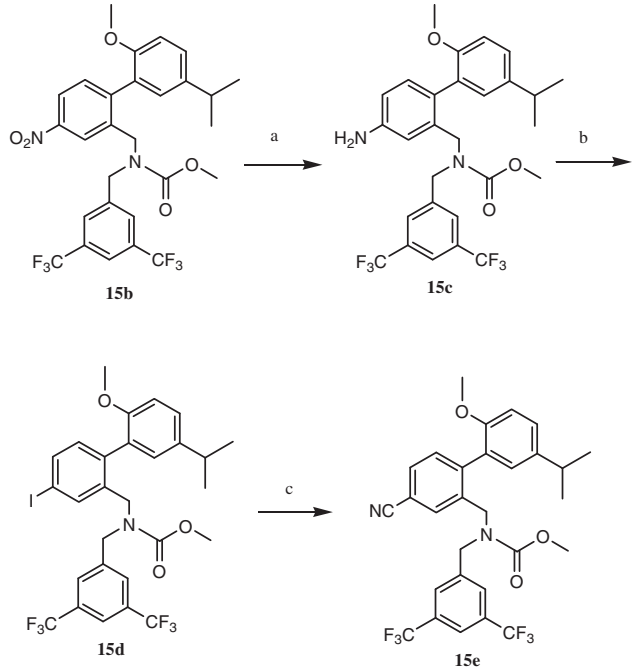
Figure 1. Design of a novel biphenyl class of CETP inhibitors.



Scheme 1. Synthesis of the biphenyl analogs. Reagents and conditions: (a) CuCN, DMF, 100 °C, 76%; (b) H_2, Raney Ni, NH_4OH, 40 psi, 60%; (c) 3,5-di-trifluoromethylbenzyl bromide, *N*-methylmorpholine, dimethoxyethane, rt, 55%; (d) MeOC(O)Cl, *N*-methylmorpholine, DME, 0 °C, 80%; (e) iso-amyl nitrite, I_2, $CHCl_3$, reflux, 85%; (f) R-$B(OH)_2$, $Pd(OAC)_2$, acetone/H_2O (4:1), reflux. 20–80%.

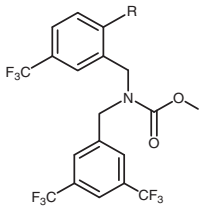


Scheme 2. Synthesis of the substituted biphenyl analogs. Reagents and conditions: (a) NaH, **17**, THF, 0 °C, 70–90%; (b) 2-methoxy-5-isopropyl phenyl boronic acid, $Pd(OAC)_2$, acetone/H_2O (4:1), 80 °C, 40–70%; (c) methyl chloroformate, CH_2Cl_2, 25 °C, 95%.



Scheme 3. Synthesis of the substituted biphenyl analogs. Reagents and conditions: (a) H_2, Pd/C, MeOH, 25 °C, 90%; (b) *n*-amyl nitrite, I_2, $CHCl_3$, reflux, 70%; (c) CuCN, DMF, 100 °C, 85%.

Table 1
SAR of the substituted top phenyl analogs



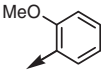
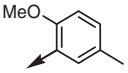
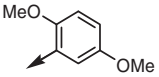
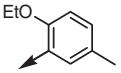
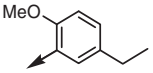
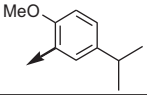
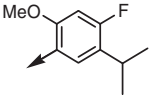
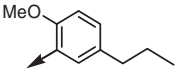
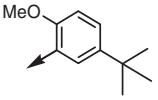
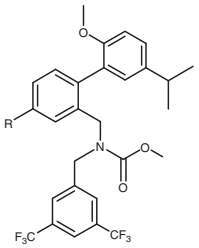
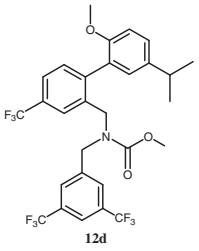
Compound R	R	IC_{50} (nM)
4		1601
5		469
12a		444
12b		4494
12c		210
12d		108
12e		117
12f		753
12g		2590

Table 2
SAR of the middle substituted phenyl analogs



Compound	R	IC_{50} (nM)
12d	CF_3	108
15a	H	3832
15b	NO_2	134
15c	NH_2	4664
15d	I	365
15e	CN	224
15f	OMe	694
15g	F	1725
15h	Cl	1430
15i	*t*BU	9514

Table 3
The effects of dose related HDL increase for compound **12d** on the transgenic mouse


12d

PO dose (mg/kg)	HDL start (mg/dL)	HDL end (mg/dL)	Increase of HDL (%)
1	32.5	44.5	37
3	26.0	37.1	43
10	27.2	41.8	54
30	22.5	41.8	86

Male cynoCETP mice, $n = 5$ for each dose.

compounds oxazolidinone **2** and imidazolidinone **3**, however, were found to be inactive. Replacement of the heterocycles with a substituted phenyl ring showed low micromolar activity and compound **4** was our first active compound in the biphenyl series. Incorporation of a 2,5-disubstitution pattern to better approximate the way torcetrapib displays its pendant groups led to compound **5** which was 10-fold more potent than **1** in vitro. Further, compound **5** also elicited a significant increase in HDL-C (40%@100 mpk) in a pharmacodynamic assay[31] utilizing transgenic mice expressing cynomolgus monkey CETP, and was the first such compound to do so. This letter will discuss the synthesis and SAR of this series of biphenyl CETP inhibitors.

The general approach to the preparation of these compounds is exemplified by the synthesis of analog **12** as shown in Scheme 1. Thus, commercially available 2-iodo-4-trifluoromethylaniline **6** was mixed with copper cyanide in DMF at 100 °C for 24 h to afford aminocyanide **7**. Hydrogenation of **7** with Raney nickel at 40 psi gave 2-amino-5-trifluoro benzylamine **8**. Alkylation of **8** with 3,5-di-trifluoromethyl-benzyl bromide and *N*-methylmorpholine at room temperature in dimethoxyethane produced compound **9**. Carbamate **10** was obtained in good yield by treatment of **9** with methyl chloroformate. Treatment of compound **10** with iso-amyl nitrite and iodine in chloroform under reflux conditions gave the corresponding iodide **11**. Suzuki coupling of iodide **11** with commercially available boronic acids and $Pd(OAc)_2$ gave the final compounds **12** (Scheme 1).

In a similar fashion, the other biphenyl analogs were prepared as shown in Scheme 2. Alkylation of commercially available benzyl bromides **13** with NaH and compound **17** provided **14** in good yield. Suzuki coupling of **14** with commercially available 2-methoxy-5-isopropyl phenyl boronic acid and $Pd(OAc)_2$ gave the final compound **15**. Compound **16** was obtained in excellent yield by treatment of 3,5-bis-trifluoromethyl benzylamine **17** with methyl chloroformate (Scheme 2).

Several compounds were prepared from **15b** by functional group manipulations. Hydrogenation of **15b** with Pd/C in methanol at 40 psi gave the aniline **15c** in good yield. Treatment of **15c** with *n*-amyl nitrite and iodine in chloroform under reflux conditions afforded iodide **15d**. **15e** was obtained by treatment of **15d** with copper cyanide in DMF at 100 °C (Scheme 3).

All final compounds were evaluated in a fluorescence-based assay measuring the compound's ability to inhibit CETP-mediated neutral lipid transfer.[32] The results are reported as IC_{50}s. As can be seen in Table 1, the 2-methoxy-5-alkyl substituted pattern on the phenyl ring is preferred and 5-methyl-2-methoxy phenyl analog (**5**) is used as the starting point to discuss the SAR. 2-Ethoxy

analog **12b** is less active than the 2-methoxy analog **5** due to the steric effect. Both 5-methyl and 5-methoxy groups are equally potent (**5** and **12a**, respectively). 5-Ethyl analog **12c** is twice as active as methyl analog **5**. The isopropyl analog **12d** is the most active CETP inhibitor in the series with IC_{50} 108 nM. Adding a F atom at the 4-position of phenyl ring did not have much effect on potency (**12d** vs **12e**). The analogs with 5-*n*-propyl and 5-*t*-butyl groups (**12f** and **12g**, respectively) are less active than the isopropyl analog **12d** because of the steric hindrance.

In a separate SAR study (Table 2), we attempted to determine the effect of the substitutions on the central phenyl ring, using compound **12d** as a comparator. The unsubstituted analog compound **15a** was approximately 30–40-fold less potent than **12d**. A clear SAR was observed when halogens were introduced and the order was I > Cl > F (**15d**, **15h**, and **15g**, respectively). Substitutions with NO_2 (**15b**) and CN (**15e**) were less potent than the trifluoromethyl group (**12d**) but better than any of the other groups in this study.

In light of these in vitro SAR studies, we selected compound **12d** to benchmark in our transgenic mouse pharmacodynamic assay.[31] Compound **12d** was evaluated for its ability to raise HDL utilizing a BID dosing regimen. Thus, the animals were given a first dose of **12d** at the beginning of study, an equivalent dose 7 h later and blood was collected 24 h post the first dose. The difference in HDL-C levels between $t = 24$ and $t = 0$ h was then determined. Compound **12d** showed a good dose-dependent increase in HDL-C levels. At 30 mpk, it raised HDL-C up to 86%. It was also active as low as 1 mpk, raising HDL by 37% (Table 3).

In summary, we have discovered a new class of CETP inhibitors that are potent in both in vitro and in vivo assays. The most active compound of these inhibitors has shown an ability to raise HDL significantly in a transgenic mouse PD model.

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31. *Transgenic mouse pharmacodynamic assay*: B6.cynoCETP male mice 12–16 weeks of age were used. Pre-dose blood samples were collected by retro-orbital bleed. Compounds were formulated in DMSO/cremophor/saline at a 4:4:92 ratio and screened at 10 or 20 mg/kg PO BID. Twenty-four hours after the first dose, the mice were euthanized and blood was collected by cardiac puncture. HDL-C was measured on the pre- and post-bleed samples using a HDL-E biochemical assay kit from Wako Chemical USA (cat# 431-52501), following manufacturer's instructions.
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