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Communications to the Editor

Discovery of Chiral *N,N*-Disubstituted Trifluoro-3-amino-2-propanols as Potent Inhibitors of Cholesteryl Ester Transfer Protein

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Introduction. Epidemiological studies have demonstrated an inverse relationship between serum high-density cholesterol (HDL-C) levels and the incidence of coronary heart disease (CHD).[1] Low levels of HDL-C represent a significant independent risk factor in CHD whether these patients have elevated plasma low-density cholesterol (LDL-C).[2] Several clinical studies have shown reduced CHD events with treatments that raised HDL-C.[3,4] Multiple approaches have been identified with the potential to elevate HDL-C levels.[5] Here we describe our efforts to identify a novel simple class of potent cholesteryl ester transfer protein (CETP) inhibitors.

CETP is a plasma glycoprotein that mediates the transfer of cholesteryl ester (CE) from HDL to very low-density lipoprotein (VLDL) and LDL with a balanced exchange of triglyceride (TG).[6−8] CETP lowers HDL-C by moving CE from HDL that is known to protect against CHD into proatherogenic VLDL and LDL. In contrast, CETP deficiency in humans results in hyperalphalipoproteinemia, i.e., elevated HDL-C.[9] Analysis for CETP polymorphism in the Framingham Offspring Study identified a B2B2 allele in men associated with significantly reduced CETP activity, elevated HDL-C, and a reduced risk for CHD.[10] Administration of TP-2, a CETP-neutralizing monoclonal antibody, to hamsters produced a dramatic reduction in CETP activity with a concomitant elevation of HDL-C.[11] Recently, a CETP inhibitor, which binds to CETP via a disufide bond, has been shown to raise HDL-C, lower LDL-C, and inhibit the progression of atherosclerosis in rabbits.[12] On the basis of these observations, a CETP inhibitor is expected to block the balanced exchange of CE and TG between HDL and VLDL or LDL such that the LDL-C/HDL-C ratio and risk of CHD are significantly reduced. Preferably, a CETP inhibitor would be specific for CETP and not disrupt the integrity of other lipoproteins.

Small-molecule CETP inhibitors from a variety of structural classes including sterols, polycyclic natural products, and heterocycles are known.[7] Each class of known inhibitors appears to require a central core ring for activity. Representative members of these classes typically exhibit modest micromolar IC_{50} values for inhibiting CETP-mediated transfer of [^{3}H]CE from HDL to LDL under buffered assay conditions in vitro.[7] To date, no CETP inhibitor class has been described that exhibits submicromolar activity when this CETP-mediated transfer process is assayed in the presence of human plasma.[7] However, recently a series of tetrahydronaphthalene derivatives have been reported with low-nanomolar potency in vitro[13] and a series of tetrahydoquinolines derivatives have been reported (activity not given).[14]

In this Communication we describe the identification and initial modification of *N*,*N*-disubstituted trifluoro-3-amino-2-propanols as an unusually simple class of CETP inhibitor. Subsequent optimization produced the chiral (*R*)-(+)-propanol derivative **1a** having low- nanomolar potency in vitro under buffered conditions. The activity of **1a** was also maintained at submicromolar levels in the presence of human serum. This novel class thus represents the first alicyclic series exhibiting significant inhibitory activity for the CETP system.

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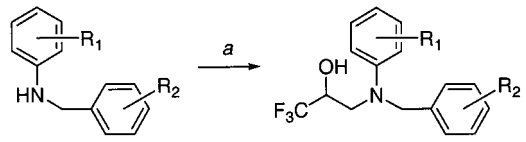
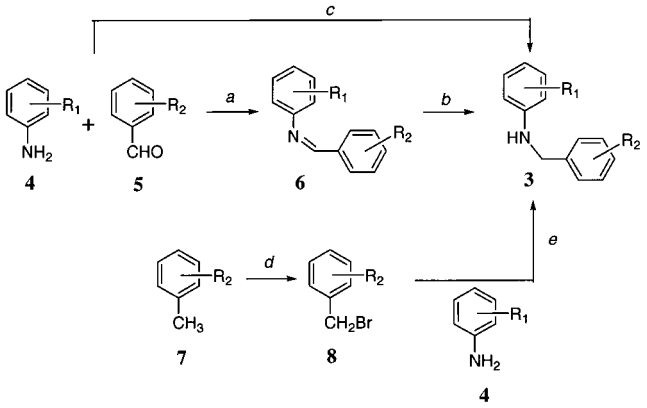
Scheme 1[a]



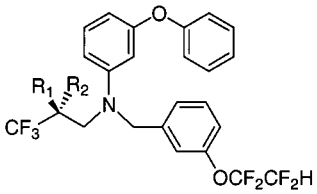
[a] Reagents: (a) 1,1,1-trifluoroepoxypropane, $Yb(CF_3SO_3)_3$, CH_3CN, 50 °C, 2 h.

Scheme 2[a]



[a] Reagents: (a) cyclohexane, heat, $-H_2O$; (b) $NaBH_4$, CH_3OH; (c) $NaBH(OAc)_3$, AcOH, DCE, rt; (d) NBS, AIBN, CCl_4, heat; (e) cyclohexane, heat.

These results have been presented at the 220th ACS National Meeting, Washington, DC, in which **1a** was identified as SC-795.[15]



(*R*)-**1a** $R_1 = H, R_2 = OH$

(*S*)-**1b** $R_1 = OH, R_2 = H$

Chemistry. *N*,*N*-Disubstituted trifluoro-3-amino-2-propanols **2a**−**k** were readily prepared from the ring-opening reaction of commercially available 1,1,1-trifluoro-2,3-oxirane of unspecified enantiomeric composition with the appropriate *N*-benzylaniline **3** (Scheme 1). This reaction proceeded smoothly in the presence of ytterbium(III) triflate in warm acetonitrile. The ytterbium triflate helps the reaction proceed at lower temperature and minimizes the need for large excess quantities of this volatile epoxide due to thermal loss. This epoxide ring-opening reaction proceeded with complete regioselectivity, since none of the isomeric 2-amino-3-propanol product was detected. The required *N*-benzylanilines **3** were conveniently prepared by standard reductive amination or alkylation sequences (Scheme 2). The aniline **4** was treated with an appropriate benzaldehyde **5** and dehydrated to generate imine **6** which was then reduced with sodium borohydride to give the *N*-benzylaniline **3**. Reduction of imine **6** to **3** with [^{3}H]sodium borohydride provided a convenient entry to radiolabeled analogues.[17] Alternatively, direct reductive amination of a mixture

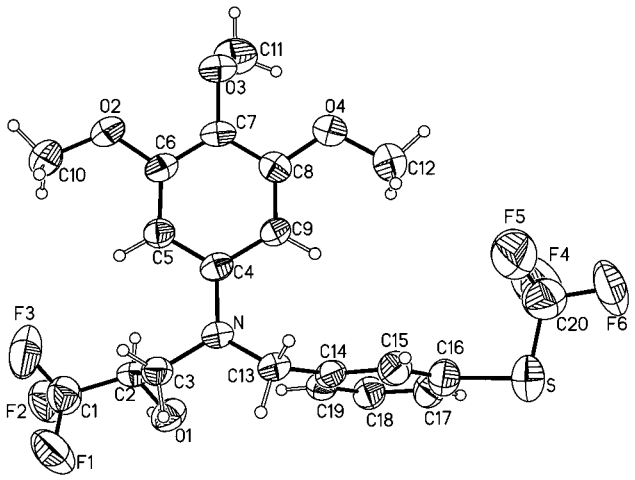
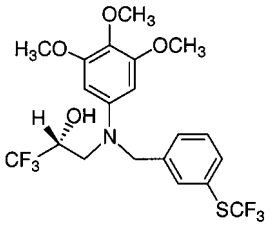


Figure 1. ORTEP drawing of **9** from X-ray analysis.

of **4** and **5** with sodium triacetoxyborohydride also gave **3**. In cases where the benzaldehyde **5** was difficult to access, radical bromination of a suitable toluene **7** gave the bromomethylbenzene **8** which could be used to prepare **3** by reaction with an excess of aniline **4**.

Surprisingly, the potent 3-amino-2-propanol **2k** was shown by analytical chiral chromatography to consist of a 7:1 mixture of enantiomers. Independent chiral GC analysis confirmed that the commercial 1,1,1-trifluoro-2,3-oxirane reagent also contained a 7:1 mixture of the individual (*S*)-(−)- and (*R*)-(+)-enantiomers. The individual enantiomers of **2k** were conveniently isolated by standard preparative chiral chromatography. The activity[16] was shown to reside in the minor (+)-isomer, **1a**, which was prepared independently from the reaction of *N*-(3-phenoxyphenyl)[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]amine with the known chiral epoxide, (*R*)-(+)-1,1,1-trifluoro-2,3-epoxypropane.[17] To confirm the (*R*)-configuration at the chiral carbon in (+)-**1a**, a crystalline analogue **9** was prepared from the reaction of *N*-(3,4,5-trimethoxyphenyl)[[3-(trifluoromethylthio)-phenyl]methyl]amine with (*R*)-(+)-1,1,1-trifluoro-2,3-epoxypropane, and its structure was determined by X-ray crystallography (Figure 1).



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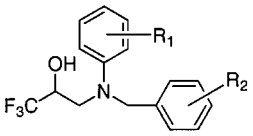
Results and Discussion. An initial chemical lead **2a**, identified through screening a combinatorial library, had promising activity (IC_{50} 40 μM) as a CETP inhibitor in a simple buffer assay.[16,18,19] In a similar assay but in the presence of human serum which provided the source of the LDL, VLDL, and human CETP, the inhibitory activity (IC_{50} > 200 μM) was markedly reduced for **2a**. The IC_{50} value in human serum is indicative of the inhibitory activity in the target tissue, human blood, when other plasma lipoproteins are present. We suspect that this value is lower than that in buffer due to nonspecific binding of the inhibitors to nontarget blood

Table 1. Structure and CETP Inhibitory Properties of *N*,*N*-Disubstituted Trifluoro-3-amino-2-propanols



inhibitor	R_1	R_2	IC_{50} (μM) in buffer[a]	IC_{50} (μM) in human serum[a,b]
2a	3-F	3-CF_3	40	>200
2b	H	3-CF_3	15	>200
2c	3-F	H	>100	ND
2d	3-F	3-CH_3	>100	ND
2e	3-F	2-CF_3	>100	ND
2f	3-F	3-OCF_3	12	>200
2g	3-F	4-OCF_3	15	ND
2h	3-phenoxy	3-OCF_3	1	40
2i	4-phenoxy	3-OCF_3	25	ND
2j	3-phenoxy	4-OCF_3	4	120
2k	3-phenoxy	3-OCF_2CF_2H	0.2	6

[a] ref 18. [b] ND, not determined.

Table 2. Structure and CETP Inhibitory Properties of Chiral *N*,*N*-Disubstituted Trifluoro-3-amino-2-propanols

inhibitor	IC_{50} (μM) in buffer[a]	IC_{50} (μM) in human serum[a,b]
1a	0.02	0.6
1b	0.8	20
9	5	ND

[a] Ref 18. [b] ND, not determined.

proteins. The simplicity of the chemistry needed to prepare **2a** suggested that a wide variety of structural modifications could be readily incorporated to explore the structure−activity relationships (SAR) with this interesting class (Table 1).

As summarized below, several key structural changes provided insights into the SAR and suggested a direction for further modifications. Removing the aniline 3-F group from **2a** to give **2b** (IC_{50} 15 μM) retained activity, while removing the benzylic 3-CF_3 group as in **2c** or replacing the benzylic 3-CF_3 substituent in **2a** with a 3-CH_3 moiety as in **2d** produced a significant reduction in potency. These results indicated that the benzylic 3-CF_3 substituent was an important contributor to the activity while the aniline ring was open to broader modification. Similarly, moving the benzylic 3-CF_3 group to an *ortho* position as in **2e** also dramatically reduced activity (IC_{50} > 100 μM). *Ortho* substituents would likely affect the respective orientation of the two phenyl rings, and this in turn may be critical for potency. Alternatively, changing the benzylic 3-CF_3 group in **2a** to a 3-OCF_3 group as in **2f** (IC_{50} 12 μM) improved the potency by over 3-fold. Activity similar to **2f** was also observed with the 4-OCF_3 substituent as in **2g**.

Modifying the 3-F substituent in **2f** with a 3-phenoxy group as in **2h** (IC_{50} 1 μM) identified the first low-micromolar inhibitor, and more importantly, **2h** now exhibited some inhibitory activity in the presence of human serum (IC_{50} 40 μM). Moving the 3-phenoxy aniline substituent in **2h** to the 4-position as in **2i** (IC_{50} 25 μM) significantly reduced activity. Similarly, moving the benzylic 3-OCF_3 group in **2h** to the 4-position as in **2j** (IC_{50} 4 μM) also reduced activity. Subsequent extension of the benzylic 3-OCF_3 group in **2h** using a 3-(1,1,2,2-tetrafluoroethoxy) moiety identified **2k** (IC_{50} 0.2 μM) with nanomolar potency, and **2k** also had significant potency in the presence of human serum (IC_{50} 6 μM).

When **2k** was analyzed by chiral chromatography, an unexpected 7:1 ratio of enantiomers was observed. The individual enantiomers were obtained by preparative chiral chromatography and analyzed for CETP inhibitory activity. The minor (+)-enantiomer **1a** had an IC_{50} 0.02 μM, while the major (−)-enantiomer **1b** exhibited much less activity (IC_{50} 0.8 μM). The minor (+)-enantiomer **1a** also displayed submicromolar activity (IC_{50} 0.6 μM) in the presence of human serum. Moreover, **1a** could be prepared independently from the known chiral epoxide, (*R*)-(+)-1,1,1-trifluoro-2,3-epoxypropane,[17] suggesting that the (+)-**1a** enantiomer contained the (*R*)-configuration. Since no unequivocal structural data has been presented on such compounds, we prepared a related crystalline analogue **9** from the same sample of chiral (*R*)-(+)-1,1,1-trifluoro-2,3-epoxypropane,[17] determined its structure by X-ray analysis (Figure 1), and confirmed that the alcohol configuration in **9** was indeed (*R*). From these data we conclude that the structure of (+)-**1a** also has the (*R*)-configuration.

More detailed biochemical binding studies comparing the relative affinities and binding properties of **2k**, **1a**, and **1b** have been completed and are reported separately.[16] The results of these efforts demonstrate that these inhibitors reversibly block both CETP-mediated TG and CE transfer. They associate with HDL and LDL but do not disrupt the structure of these lipoproteins. Their CETP inhibitory activity is highly specific, since they do not inhibit phospholipid transfer protein or lecithin cholesterol acyl transferase. Competition experiments showed that the potent reversible inhibitor **1a** prevented CE binding to CETP and that **1a** bound approximately 5000-fold more efficiently to CETP than CE.

In conclusion, we have discovered chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanols as a new simple class of potent CETP inhibitors. The most potent inhibitor **1a** also exhibited submicromolar activity in the presence of human serum. We are currently optimizing this series to identify even more potent analogues.

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Supporting Information Available: Experimental details and analytical data for the preparation of compounds **1a**, **1b**, **2a**−**k**, and **9** as well as details of the X-ray structure for **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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