Stereospecific Inhibition of CETP by Chiral *N*,*N*-Disubstituted Trifluoro-3-amino-2-propanols

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ABSTRACT: Chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanols represent a recently discovered class of compounds that inhibit the neutral lipid transfer activity of cholesteryl ester transfer protein (CETP). These compounds all contain a single chiral center that is essential for inhibitory activity. (*R*,*S*)SC-744, which is composed of a mixture of the two enantiomers, inhibits CETP-mediated transfer of [^{3}H]cholesteryl ester ([^{3}H]CE) from HDL donor particles to LDL acceptor particles with an $IC_{50} = 200$ nM when assayed using a reconstituted system in buffer and with an $IC_{50} = 6$ μM when assayed in plasma. Upon isolation of the enantiomers, it was found that the (*R*,+) enantiomer, SC-795, was about 10-fold more potent than the mixture, and that the (*S*,−) enantiomer, SC-794, did not have significant inhibitory activity ($IC_{50} >$ 0.8 μM). All of the activity of the (*S*,−)SC-794 enantiomer could be accounted for by contamination of this sample with a residual 2% of the highly potent (*R*,+) enantiomer, SC-795. The IC_{50} of (*R*,+)SC-795, 20 nM, approached the concentration of CETP (8 nM) in the buffer assay. These chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanols were found to associate with both LDL and HDL, but did not disrupt overall lipoprotein structure. They did not affect the on or off rates of CETP binding to HDL disk particles. Inhibition was highly specific since the activities of phospholipid transfer protein and lecithin cholesterol acyl transferase were not affected. Competition experiments showed that the more potent enantiomer (*R*)SC-795 prevented cholesteryl ester binding to CETP, and direct binding experiments demonstrated that this inhibitor bound to CETP with high affinity and specificity. It is estimated, based on the relative concentrations of inhibitor and lipid in the transfer assay, that (*R*)SC-795 binds approximately 5000-fold more efficiently to CETP than the natural ligand, cholesteryl ester. We conclude that these chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanol compounds do not affect lipoprotein structure or CETP-lipoprotein recognition, but inhibit lipid transfer by binding to CETP reversibly and stereospecifically at a site that competes with neutral lipid binding.

Cholesteryl ester transfer protein (CETP),[1] a carrier of cholesteryl ester (CE) and triglyceride (TG), mediates the exchange of neutral lipids between lipoprotein particles in the plasma (*1*−*3*). Since the lipid transfer process is driven by mass action resulting from differences in the relative concentrations of CE and TG in the various lipoprotein fractions, the net effect of CETP action is to lower HDLc and to elevate LDLc. It has therefore been hypothesized that inhibitors of CETP might provide anti-atherogenic therapeutic benefit by elevating HDLc and lowering LDLc. Support for this idea was recently obtained in a rabbit atherosclerosis model using a CETP inhibitor (*35*). Several CETP inhibitors have been described, including monoclonal antibodies (*4*), apolipoproteins (*5*, *6*), amphipathic peptides (*5*, *7*−*9*), amphipathic small molecules such as cholesteryl sulfate and lipopolysaccharide that modify lipoprotein structure (*5*, *10*), as well as various small molecules including sterols, polycyclic natural products and heterocyles (*11*−*19*). Some compounds have been shown to irreversibly inactivate CETP following cysteine modification, suggesting that a cysteine residue may reside in or near the neutral lipid binding site (*12*, *13*, *20*−*22*, *35*). A well-characterized amino steroid, U-95594, was shown to inhibit CETP by a competitive mechanism, but the relatively high K_i (12.9 mol %) probably limits its usefulness (*12*). The precise inhibitory mechanisms of the other small molecule inhibitors described to date remain to be characterized.

A new class of compounds, chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanols, was recently discovered in a random screen for CETP inhibitors (*23*). The key structure−activity features of these inhibitors include the chiral trifluoromethyl and alcohol groups β to the disubstituted amine (Figure 1). These compounds were found to inhibit CETP-mediated neutral lipid transfer using an in vitro assay with reconstituted purified components and to inhibit CETP in plasma. The present study was undertaken to further under-

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[1] Abbreviations: CETP, cholesteryl ester transfer protein; LDL, low-density lipoprotein; LDLc, low-density lipoprotein cholesterol; HDL, high-density lipoprotein; HDLc, high-density lipoprotein cholesterol; LCAT, lecithin cholesterol acyl transferase; PLTP, phospholipid transfer protein; CE, cholesteryl ester; TG, triglyceride; TSE, Tris/saline/EDTA buffer; BSA, bovine serum albumin.

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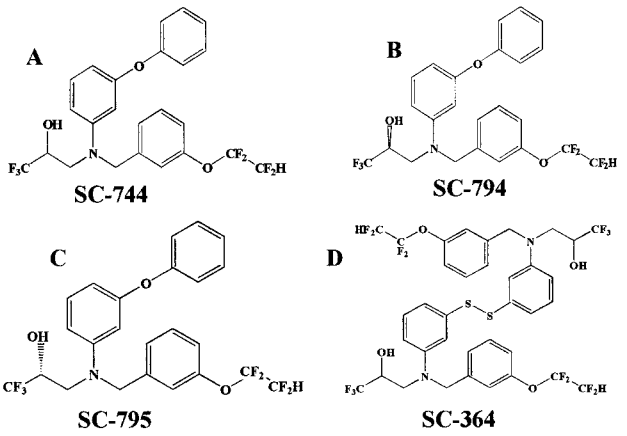

FIGURE 1: *N*,*N*-Disubstituted 1,1,1-trifluoro-3-amino-2-propanol CETP Inhibitors. (A) SC-744 mixed stereoisomers; (B) SC-794, the purified *S*,- enantiomer; (C) SC-795, the purified *R*,+ enantiomer; (D) SC-364, a bis-disulfide compound in the same class.

stand the mechanism of inhibition by these compounds. We show that these chiral *N*,*N*-disubstituted trifluoro-3-amino-2-propanols bind stereospecifically to CETP with high affinity and inhibit neutral lipid transfer by a competitive mechanism.

MATERIALS AND METHODS

Assay of CETP Inhibition Using Purified Components (Reconstituted Buffer Assay). The ability of compounds to inhibit CETP activity was assessed using an in vitro assay that measures the rate of transfer of radiolabeled cholesteryl ester ([^{3}H]CE) from HDL donor particles to LDL acceptor particles (*24*). Human recombinant CETP was obtained from the serum-free conditioned medium of CHO cells transfected with a cDNA for CETP and purified as described (*24*). To measure CETP activity, [^{3}H]CE-labeled HDL, LDL, CETP and assay buffer (50 mM tris(hydroxymethyl)aminomethane, pH 7.4; 150 mM sodium chloride; 2 mM ethylenediaminetetraacetic acid; 1% bovine serum albumin) were incubated in a final volume of 200 μL, for 2 h at 37 °C in 96 well plates. Inhibitors were included in the assay by diluting from a 10 mM DMSO stock into 16% (v/v) aqueous DMSO. The inhibitors were then diluted 1:1 with CETP in assay buffer, and then 25 μL of that solution mixed with 175 μL of lipoprotein pool for assay. Following incubation, LDL was differentially precipitated by the addition of 50 μL of 1% (w/v) dextran sulfate/0.5 M magnesium chloride, mixed by vortex, and incubated at room temperature for 10 min. A portion of the solution (200 μL) was transferred to a filter plate (Millipore). After filtration, the radioactivity present in the precipitated LDL was measured by liquid scintillation counting. Correction for nonspecific transfer or precipitation was made by including samples that did not contain CETP. The rate of [^{3}H]CE transfer using this assay was linear with respect to time and CETP concentration, up to 25−30% of [^{3}H]CE transferred.

The potency of test compounds was determined by performing the above-described assay in the presence of varying concentrations of the test compounds and determining the concentration required for 50% inhibition of transfer of [^{3}H]-CE from HDL to LDL. This value was defined as the IC_{50}.

Assay of CETP Inhibition in Human Plasma. Blood was obtained from healthy volunteers recruited from the personnel of Monsanto Company, St. Louis, MO. Blood was collected in tubes containing EDTA (EDTA plasma pool). The EDTA human plasma pool, previously stored at −20 °C, was thawed at room temperature and centrifuged for 5 min to remove any particulate matter. [^{3}H]CE-HDL (*25*) was added to the plasma to a final concentration of 25 μg/mL cholesterol. Inhibitors dissolved as 20−50 mM stock solutions in DMSO were serially diluted in DMSO, and 4 μL of each of the serial dilutions of inhibitor or DMSO alone were then added to the 396 μL of plasma. After mixing, triplicate aliquots (100 μL) were then transferred to wells of 96-well round-bottomed polystyrene microtiter plates (Corning, Corning, NY). Plates were sealed with plastic film and incubated at 37 °C for 4 h. "Test" samples contained plasma with dilutions of inhibitor compounds. "Control" samples contained plasma with DMSO diluted to the same concentration as the test samples, but without inhibitor. "Blank" samples were prepared as "control" samples, but were left in the micro tubes at 4 °C instead of 37 °C for the 4 h incubation and were then added to the microtiter wells at the end of the incubation period. VLDL and LDL were precipitated by the addition of 10 μL of precipitating reagent (1% (w/v) dextran sulfate (Dextralip50)/0.5 M magnesium chloride, pH 7.4) to all wells. The wells were mixed on a plate mixer and then incubated at ambient temperature for 10 min. The plates were then centrifuged at 1000*g* for 30 min at 10 °C. The supernatants (50 μL) from each well were then transferred to Picoplate 96 plate wells (Packard, Meriden, CT) containing Microscint-40 (Packard, Meriden, CT). The plates were heat-sealed (TopSeal-P, Packard, Meriden, CT) according to the manufacturer's directions and mixed for 30 min. Radioactivity was measured on a microplate scintillation counter (TopCount, Packard, Meriden, CT). The maximum percentage transfer in the control wells (% transfer) was determined using the following equation:

$$\%\ \text{transfer} = \frac{[\text{dpm}_{\text{blank}} - \text{dpm}_{\text{control}}] \times 100}{\text{dpm}_{\text{blank}}}$$

The percentage of transfer relative to the control (% control) was determined in the wells containing inhibitor compounds as follows:

$$\%\ \text{control} = \frac{[\text{dpm}_{\text{blank}} - \text{dpm}_{\text{test}}] \times 100}{\text{dpm}_{\text{blank}} - \text{dpm}_{\text{control}}}$$

IC_{50} values were then calculated from plots of % control versus concentration of inhibitor compound.

Assay of Time-Dependent CETP Inactivation. The ability of compounds to inactivate CETP in a time-dependent manner was assessed as described previously (*20*). Samples of CETP and inhibitor were incubated for 15 h at 37 °C. To measure CETP activity, the samples (incubated and nonincubated control) were then added with [^{3}H]CE-labeled HDL, LDL, and assay buffer as described above in the reconstituted buffer assay.

Determination of Lipoprotein Integrity by Agarose Electrophoresis. A Paragon Lipoprotein (Lipo) Electrophoresis Kit (Beckman, Fullerton, CA) was used for the elctrophoretic separation of lipoproteins, according to the manufacturer's instructions. Inhibitors were incubated with LDL or HDL at concentrations 1000-fold above the IC_{50} value (as determined by in vitro buffer assay) for 2 h at 37 °C. Cholesteryl sulfate

(100 μM), a compound previously shown to significantly alter the electrophoretic migration pattern of lipoproteins (*5*), was used as a positive control. After electrophoresis, the lipoproteins in the gel were immobilized in a fixative solution, the gel dried to a film, and the film stained to visualize lipoproteins.

Phospholipid Transfer Protein and Lecithin Cholesterol Acyl Transfer Assays. The phospholipid transfer assay was adapted from published methods (*26*, *27*). Liposomes were prepared (*28*) using L-α-phosphatidyl choline, type V-E from frozen egg yolk (Sigma Chemical Co., St. Louis, MO), (dipalmitoyl-1-^{14}C) phosphatidyl choline (Dupont NEN). Acceptor lipoproteins (90.5% HDL cholesterol, Sigma Chemical Co., St. Louis, MO) were diluted in 10 mM Tris buffer such that the protein concentration was 5 mg/mL and the final concentration in the reaction mixture was 250 μg. Phospholipid transfer protein was isolated from a 300 mL pool of human plasma according to the method of Lagrost et al. (*29*). The PLTP enzyme peak fractions were pooled, aliquoted, and stored at −20 °C. For assay, PLTP was diluted 25-fold in 10 mM Tris buffer so that the final reaction mixture contained 8.4 μg/mL protein. Each of following were added to reaction tubes: 0.2 mL of 10 mM Tris buffer, 0.05 mL of 5 mg/mL bovine lipoprotein, 0.10 mL of [^{14}C]DPPC labeled vesicles, and 0.05 mL of 25-fold diluted PLTP or 10 mM Tris buffer as control. Reactions were incubated at 37 °C in a shaking water bath for 30 min. The reaction was stopped by placing the tubes in an ice bath and adding 0.3 mL of 500 mM NaCl, 215 mM $MnCl_2$, and 445 units heparin (Sigma Chemical Co., St. Louis, MO). After 30 min the vesicles were precipitated by centrifugation at 14 000 rpm in an Eppendorf microfuge. From the supernatant, 0.5 mL was added to scintillation vials and counted for radioactivity transferred to the acceptor lipoprotein. Cholesteryl sulfate was used as a positive control for inhibition of PLTP.

Measurement of LCAT activity was modified from the procedure of Chen and Albers (*30*) using LCAT isolated from human plasma and partially purified on a phenyl Sepharose Cl-4B column as described (*31*). A synthetic analogue of phosphatydyl choline (PLA2-1) prepared by Dr. Patrick Lennon (Monsanto Corporate Research) was used as a positive control for LCAT inhibition.

Surface Plasmon Resonance Measurements of CETP Binding to HDL Particles. The study was performed using a BIAcore 2000 system (BIAcore, Inc., Piscataway NJ). Biotinylated recombinant HDL disk particles were prepared as described (*32*). HDL (0.22 mg/mL) was immobilized onto the SA sensorchip at a density of about 600 resonance units via biotin-streptavidin interaction. The immobilized HDL was then exposed to 1-min injections of 38 nM CETP solution (in 10 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 7.6 buffer) at a flow rate of 30 μL/min. The CETP/HDL complex was allowed to dissociate from the surface at the end of the association phase for 400 s. The entire association and dissociation regions of CETP/HDL interaction were fitted with a global fitting kinetics program supplied with the BIAcore system, from which the association rate constant, k_a ($s^{-1}M^{-1}$), the dissociation rate constant, k_d (s^{-1}), and the equilibrium dissociation constant K_d (M, obtained from dividing k_d by k_a) were obtained.

Inhibition of Cholesteryl Ester Binding to CETP. The ability of compounds to inhibit cholesteryl ester binding to CETP was assessed using an assay in which radiolabeled cholesteryl ester ([^{3}H]CE) was transferred from HDL to CETP (*24*). Samples containing [^{3}H]CE-HDL alone, [^{3}H]-CE-HDL + CETP, and [^{3}H]CE-HDL + CETP + inhibitor were incubated for 2 h at 37 °C and then fractionated by Superose 12 size-exclusion chromatography to separate the CETP from the HDL. The fractions were assayed for [^{3}H]-CE bound to CETP by liquid scintillation counting.

Direct Binding of Inhibitors to CETP. CETP (1.0 mg/mL) was coupled to 1 g of dried cyanogen bromide-activated Sepharose 4B according to the manufacture's directions (Sigma, St. Louis, MO). To measure the binding of inhibitors, ^{3}H-labeled inhibitor (in 10% v/v 2-propanol) was added to a mixture of HDL (0.4 mg/mL cholesterol) in TSE buffer. A slurry of equal volumes CETP−Sepharose and TSE buffer was added to the inhibitor samples to make a total volume of 0.2 mL. In control experiments using excess unlabeled inhibitor, dilution was from a 10 mM stock in DMSO so that the final inhibitor concentration was 100 μM and the final DMSO concentration was 1% v/v. The samples were mixed end over end for 15 min at 37 °C, then spun in a MicroCentrifuge Model 235C (Fisher Scientific, Pittsburgh, PA) for 90 s. The supernatant was removed and the pellet washed several times with TSE buffer. The pellet was then resuspended with TSE buffer and collected. The pellets were assayed for bound radioactivity by liquid scintillation counting.

Synthesis of N,N-Disubstituted 1,1,1-trifluoro-3-amino-2-propanols. Details of the discovery and synthesis of these *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols, including the preparation of nonlabeled SC-744, its separation by chiral chromatography into (*S*,−)-SC-794 and (*R*,+)-SC-795 and the structural assignment of the (*R*,+) configuration to SC-795 are described elsewhere (*23*).

(2*R*)-3-[(3-phenoxyphenyl)[[3-(1,1,2,2-tetrafluoroethoxy)-phenyl]-^{3}H-methyl]-amino]-1,1,1-trifluoro-2-propanol, [^{3}H]-SC-795, and (2*S*)-3-[(3-phenoxyphenyl)[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-[^{3}H]methyl]amino]-1,1,1-trifluoro-2-propanol, [^{3}H]SC-794.

Step 1: Imine Production. The prerequisite imine was prepared by dissolving 100 mg (0.539 mmol) of (3-phenoxy)-aniline and 122 mg (0.55 mmol, 1.02 eq) of 3-(1,1,2,2-tetrafluoroethoxy)benzaldehyde in 5.0 mL of cyclohexane and then refluxing the reaction mixture at 105 °C using a Dean−Stark trap to remove water. After refluxing for 6.0 h, a sample of the mixture was analyzed by ES/MS, which confirmed the formation of the imine with a parent ion of $m/z = 390$ $[M + H]^+$. The solvent was removed on a rotovap to afford 195 mg of a clear yellow oil. A stock solution of this oil was prepared at a concentration of 0.39 mg/mL by dissolving the residue in 500 mL of absolute ethanol. Aliquots of this stock solution were subsequently used in the following ^{3}H-reduction step.

Step 2: [^{3}H]Sodium Borohydride Reduction. To a 1.0 mL ampule containing 100 mCi (90 Ci/mmol) of solid [^{3}H]-soduim borohydride is added 250 μL (97.5 μg, 0.25 μmol) of the imine in absolute ethanol. A stir bar is added, and the vial is sealed under a nitrogen blanket. After stirring at room temperature for approximately 72 h, a 1.0 μL aliquot of the reaction mixture is removed, diluted in ether, and analyzed with normal phase HPLC/HPLRC. Comparison of retention times with an unlabeled *N*-benzylaniline standard (*23*) con-

firms the preparation of the desired [^{3}H]-*N*-(3-phenoxy-phenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]amine product with only minor ^{3}H-impurities present. The reaction mixture is quenched by adding ~100 μL of 1 N HCl followed by the addition of 10% NaOH to bring the mixture to pH 7.0 by pH paper. The solvent is removed in vacuo resulting in a gray/white residue. The residue is extracted with ether (5 × 1.0 mL), and the extracts are combined and dried over Na_2SO_4. HPLC/HPLRC analysis and liquid scintillation counting of diluted aliquots provides 19.5 mCi of the crude *N*-(3-phenoxyphenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]-amine product with a radiochemical purity of 72%. This material was used without purification in the next step.

Step 3: Preparation and Isolation of [^{3}H]SC-794 and [^{3}H]SC-795. To a dry 1.0 mL vial was added the crude *N*-(3-phenoxyphenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]-amine in ether in portions, and the solvent was removed under a stream of nitrogen. To this residue were added a stir bar and 100 μL of dry acetonitrile. Commercially available 1,1,1-trifluoro-2,3-epoxypropane at 2.0 μL (2.6 mg, 0.023 mmol) is added via a syringe, and a spatula tip of ytterbium(III) triflate is added as a catalyst. The vial is flushed with a stream of nitrogen and then sealed. The mixture is stirred at 45 °C (oil bath), and the progress of the reaction is monitored by normal phase HPLC/HPLRC analysis of diluted reaction aliquots. After 80 h, the reaction is stopped, and the solvent is removed using a stream of nitrogen. HPLC/HPLRC analysis indicated two major radiolabeled peaks corresponding to [^{3}H]*N*-(3-phenoxyphenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]amine starting material (11.5 min) and the desired [^{3}H](2*RS*)-3-[(3-phenoxyphenyl)[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-methyl]amino]-1,1,1-trifluoro-2-propanol product (17.6 min). The crude reaction mixture was dissolved in 2.0 mL of 2-propanol, and the purification/separation of the individual enantiomeric products and the amine starting material was performed with HPLC using a Chiralpak AD 10*v* analytical column. A mobile phase of 10%/90% 2-propanol/heptane, a flow rate of 1.0 mL/min, and detection at 250 nm provided the optimum chromatography conditions for separation. Fractions were collected for the (+) isomer, (2*R*)-3-[(3-phenoxyphenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]amino]-1,1,1-trifluoro-2-propanol, [^{3}H]SC-795 (5.63 min), the (−) isomer, (2*S*)-3-[(3-phenoxy-phenyl)[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]methyl]amino]-1,1,1-trifluoro-2-propanol, [^{3}H]-SC-794 (6.97 min), and the [^{3}H]*N*-benzylaniline starting material at 8.5 min. From collection of these fractions were obtained 350 μCi of [^{3}H]SC-795 (radiochemical purity 98.39%, specific activity 8.52Ci/mmol), 1.5 mCi of [^{3}H]SC-794 (radiochemical purity 99.02%, specific activity 8.52Ci/mmol), and 10.2 mCi of [^{3}H]N-benzylaniline starting material. The resulting [^{3}H]SC-795 and [^{3}H]SC-794 were identical in all respects by chromatographic analyses to the corresponding nonlabeled materials prepared previously by conventional methods (*23*).

1,1′-[Dithiobis[4,1-phenylene[[[3-(1,1,2,2-tetrafluoroethoxy)-phenyl]methyl]imino]]]bis[3,3,3-trifluoro-2-propanol], SC-364. Step 1: Preparation of 3-[[[3-(1,1,2,2-tetrafluoroethoxy)-phenyl]methyl]amino]benzenethiol. 3-Aminobenzenethiol (2.4 mL, 22.5 mmol) and 3-(1,1,2,2-tetra-fluoroethoxy)benzaldehyde (5 g, 22.5 mmol) were dissolved in 40 mL of dichloroethane and acetic acid (1.35 mL, 23.7 mmol), then solid $NaBH(OAc)_3$ (6.2 g, 29.3 mmol) was added. The mixture was stirred at room temperature for 18 h, then quenched with water and diluted with dichloromethane. The organic layer was washed with aqueous saturated sodium bicarbonate, then dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate:hexane 1:10 to give 5.36 g (72%) of the desired 3-[[[3-(1,1,2,2-tetrafluoroethoxy)-phenyl]methyl]amino]benzenethiol product as a brown oil. ^{1}H NMR ($CDCl_3$) δ 3.4 (s, 1H), 4.4 (s, 2H), 5.9 (tt, 1H), 6.4 (dd, 1H), 6.55 (m, 1H), 6.65 (d, 1H), 7.05 (t, 1H), 7.2−7.4 (m, 4H).

Step 2: Preparation of 1,1,1-Trifluoro-3-[(3-mercapto-phenyl)[[3-(1,1,2,2-tetra-fluoroethoxy)phenyl]methyl]amino]-2-propanol. The 3-[[[3-(1,1,2,2-tetrafluoro-ethoxy)phenyl]-methyl]amino]benzenethiol amine (5.36 g, 16.2 mmol) product from step 1 and 1,1,1-trifluoro-2,3-epoxypropane (1 g, 1.6 mmol) were dissolved in 20 mL of acetonitrile. Ytterbium (III) trifluoromethanesulfonate (1 g, 1.6 mmol) was added, and the stirred solution was warmed to 50 °C for 48 h, at which time HPLC analysis indicated that no amine starting material remained. The reaction was quenched with water and extracted with ether. The ether layer was washed with brine, then dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate:hexane 1:10 to give 4.5 g (63%) of the desired 1,1,1-trifluoro-3-[(3-mercaptophenyl)[[3-(1,1,2,2-tetrafluoroethoxy)-phenyl]methyl]amino]-2-propanol product as a yellow oil. HRMS for $C_{36}H_{31}F_{14}N_2O_4S_2$: calcd, 885.1502 [2(M − 1) + H]$^+$; found, 885.1471. ^{1}H NMR ($CDCl_3$) δ 3.0 (s, 1H), 3.6 (dd, 1H), 3.9 (dd, 1H), 4.2 (m, 1H), 4.7 (m, 2H), 5.9 (tt, 1H), 6.5 (dd, 1H), 6.7 (m, 2H), 7.1 (m, 4H), 7.4 (t, 1H).

The disulfide compound SC-364 was isolated as a byproduct from the alkylation reaction of 1,1,1-trifluoro-3-[(3-mercaptophenyl)[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-methyl]-amino]-2-propanol and 4-chloropyridine. 1,1,1-Trifluoro-3-[(3-mercaptophenyl)-[[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-methyl]amino]-2-propanol (150 mg, 0.34 mmol) and 4-chloro-pyridine (hydrochloride salt) (56 mg, 0.37 mmol) were dissolved in 2 mL of acetonitrile. Cesium carbonate (288 mg, 0.88 mmol) was added, and the stirred solution was warmed to 55 °C for 18 h, at which time HPLC analysis indicated that no amine starting material remained. The reaction was quenched with water and filtered through prewetted Celite eluting with ethyl acetate. The solvent was evaporated, and the residue was purified by RPHPLC eluting with 10−90% acetonitrile/water to afford 52 mg (29%) of the alkylation product and 43 mg (29%) of the disulfide SC-364. MS for $C_{36}H_{31}F_{14}N_2O_4S_2$: calcd, 885.15 [M + H]$^+$; found, 885.1. ^{1}H NMR ($CDCl_3$) δ 3.6 (dd, 2H), 3.9 (dd, 2H), 4.2 (m, 2H), 4.6 (m, 4H), 5.9 (tt, 2H), 6.2 (m, 4H), 6.6 (dd, 2H), 7.0−7.2 (m, 8H), 7.3 (m, 2H).

RESULTS

Stereospecific Inhibition of CETP-Mediated Neutral Lipid Transfer by N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols. These newly discovered potent CETP inhibitors (Figure 1) are *N*,*N*-disubstituted analogues of 3-amino-1,1,1-trifluoro-2-propanol (*23*). SC-744, an (*R*,*S*) mixture, was

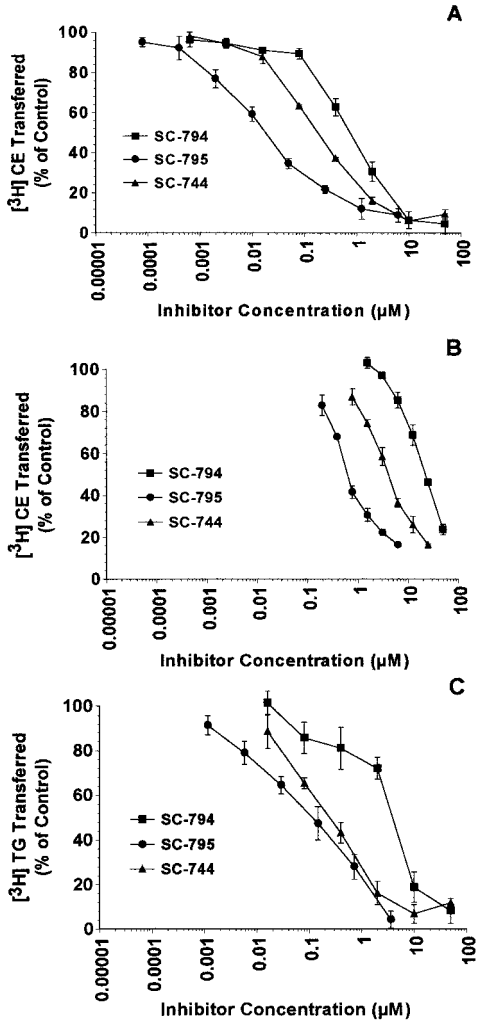

FIGURE 2: Inhibition of CETP-mediated neutral lipid transfer by *N*,*N*-Disubstituted 1,1,1-trifluoro-3-amino-2-propanols. (A) Inhibition of [^{3}H]CE transfer in the reconstituted HDL/LDL assay with purified CETP. different concentrations of inhibitor were included in a [^{3}H]CE transfer assay. The amounts of CE transferred during the 2 h incubation were compared with control samples without added inhibitor. All samples contained donor HDL particles labeled with [^{3}H]CE, acceptor LDL particles and CETP. Data points and error bars represent the mean ± SD of triplicates. (B) Inhibition of [^{3}H]CE transfer in human plasma. [^{3}H]CE-HDL was added to plasma at a final concentration of 25 μg/mL cholesterol. The indicated concentrations of inhibitor were added to plasma containing [^{3}H]CE-HDL and incubated for 4 h at 37 °C. The CETP plasma transfer assay was used to measure the CETP-mediated transfer of [^{3}H]CE from HDL to LDL. (C) Inhibition of [^{3}H]TG transfer in the reconstituted HDL/LDL assay with purified CETP. The experiment was performed as in panel A, except that [^{3}H]TG-HDL was used.

found to be a potent inhibitor of CETP-mediated neutral lipid transfer in vitro. To determine if the configuration at the chiral alcohol center was important for activity, the enantiomers were isolated by chiral chromatography, and their respective optical rotations were determined. The original SC-744 mixture contained both (+) and (−) enantiomers in an unexpected ratio of 1:7. The structures of the minor (*R*,+)-SC-795 and major (*S*,−)SC-794 enantiomers were determined as described (*23*) and are shown in Figure 1. After chromatographic purification, SC-795 was enriched to a stereochemical purity of 96.5% (*R*,+) and SC-794 to 97.9%

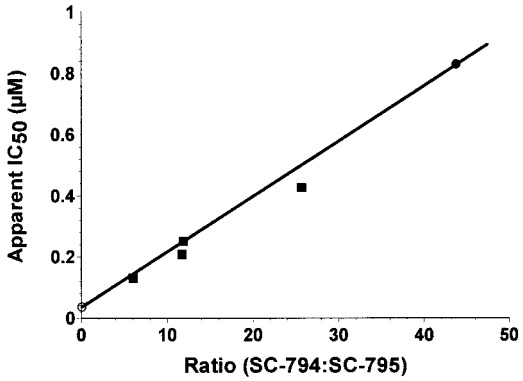

FIGURE 3: Effect of enantiomeric ratio (SC-794:SC-795) on the apparent IC_{50} for inhibition of CETP activity. Stock solutions containing different ratios of SC-794 to SC-795 were prepared. The exact concentrations of each enantiomer were determined using HPLC. IC_{50} curves were determined for each mixture of enantiomers. The extreme points at the end of each line represent undiluted SC-795 and SC-794, respectively. Linear regression analysis produced an $r^2 = 0.989$.

(*S*,−). SC-794 contained about 2% of SC-795 as a contaminant.

Figure 2A shows the effects of varying concentrations of the (*R*,*S*) mixture or of each enriched enantiomer on CETP activity in an assay using purified reconstituted components. In this assay, purified CETP enhances the rate of [^{3}H]CE transfer from HDL particles to LDL particles. (*R*,*S*)SC-744 inhibited [^{3}H]CE transfer with an $IC_{50} = 200$ nM. (*R*)SC-795 was 10-fold more potent, with an $IC_{50} = 20$ nM, and (*S*)SC-794 was 40-fold less potent with and $IC_{50} = 800$ nM.

Similar results were obtained when CETP activity was assayed in purified human plasma, although the inhibitory potencies of the compounds were less than those observed using purified reconstituted CETP and lipoprotein substrates. Figure 2B shows that (*R*,*S*)SC-744 had an $IC_{50} = 6$ μM in plasma, a value that is intermediate between the more potent (*R*) enantiomer SC-795 ($IC_{50} = 600$ nM) and the weaker (*S*) enantiomer SC-794 ($IC_{50} = 20$ μM).

CETP-mediated triglyceride transfer activity was also inhibited in the reconstituted buffer assay by these *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols (Figure 2C). The IC_{50} values were 300 nM for the racemic mixture (SC-744), 100 nM for the (*R*) enantiomer (SC-795), and 4 μM for the (*S*) enantiomer (SC-794).

The total CE concentration in the buffer assay estimated from the total CE present in LDL plus HDL was about 100 μM. Thus, the concentration of (*R*)SC-795 required to inhibit cholesteryl ester transfer by 50% (20 nM) was about 5000-fold lower than the concentration of CE.

The isolation of (*S*)SC-794 from the (*R*,*S*) mixture yielded a preparation that was 97.9% (*S*) enantiomer. To determine if this enantiomer was indeed inhibitory, or if inhibition was due to the small amount of (*R*) enantiomer remaining, mixtures containing different ratios of the compounds were prepared, and the IC_{50} of each mixture determined. A plot of IC_{50} as a function of the ratio of SC-794 to SC-795 is shown in Figure 3. The last point in this plot (closed circle) represents undiluted 97.9% SC-794, whereas the first point represents 96.5% pure SC-795. If SC-794 were inhibitory over this concentration range, a curved plot extrapolating to the IC_{50} of SC-794 would be obtained. Instead, a straight

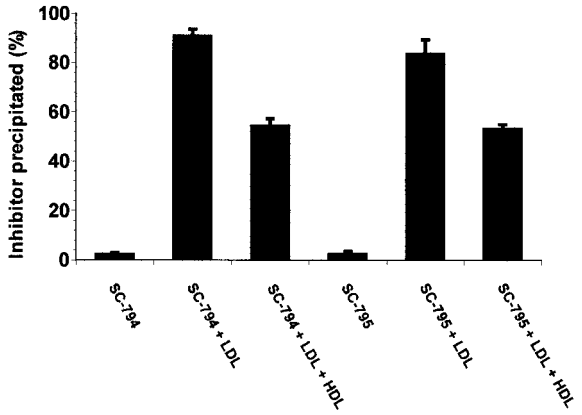

FIGURE 4: Association of *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols with LDL and HDL. [^{3}H]SC-794 and [^{3}H]SC-795 were incubated for 2 h at 37 °C with HDL and LDL or LDL alone. LDL was then differentially precipitated. The LDL pellets and HDL containing supernatants were analyzed for associated radioactivity by liquid scintillation spectrophotometry.

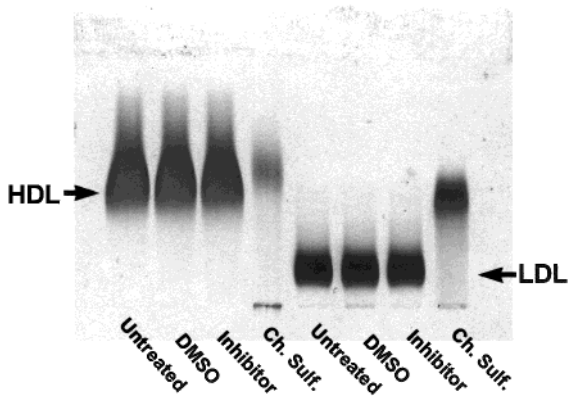

FIGURE 5: Effect of *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols on lipoprotein integrity. SC-744 was incubated with purified lipoproteins at concentrations 1000× its IC_{50} (as determined by in vitro assay) for 2 h at 37 °C prior to subjecting the mixture to buffered agarose gel electrophoresis. Cholesteryl sulfate (Chol. Sulf.), a compound previously shown to alter LDL and HDL structures, was included as a positive control. The gel was fixed to film and stained to visualize the migration of lipoproteins. Lane 1, HDL alone; lane 2, HDL + DMSO (control); lane 3, 150 μM-SC-744; lane 4, HDL + cholesteryl sulfate (100 μM); lane 5, LDL alone; lane 6, LDL + DMSO (control); lane 7, 150 μM-SC-744; lane 8, LDL + cholesteryl sulfate (100 μM).

line is observed ($r = 0.989$), indicating that (*S*)SC-794 does not contribute to inhibition over this concentration range and that the IC_{50} for SC-794 must be greater than 0.8 μM. This was further verified by recalculating the IC_{50} of SC-794 based on the assumption that all of the inhibitory activity was the result of the 2.1% contamination by SC-795. The resulting value, 20 nM, was the same as that of SC-795. We conclude that all of the inhibition observed by SC-794 can be accounted for by the low level of contamination by the more potent SC-795.

Association of N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols with LDL and HDL. The structures of the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanol inhibitors (Figure 1) are quite hydrophobic (measured log $P = 5$), so it might be expected that these compounds could interact with lipoprotein particles. To test this hypothesis, [^{3}H]SC-795 and [^{3}H]SC-794 were incubated with LDL for 2 h at 37 °C. LDL was then differentially precipitated, and the amount of each radiolabeled compound associated with the LDL pellet determined (Figure 4). Greater than 90% of each compound was bound to the LDL. The procedure did not precipitate the compound itself since less than 5% of the compound appeared in the pellet when LDL was omitted. To determine if the compounds also bound to HDL, samples were prepared that contained radiolabeled compound, LDL and HDL. Since HDL does not precipitate under the conditions used to precipitate LDL, the amount of radioactivity in the LDL pellet would be reduced if compound also bound to HDL. Indeed, the amount of radioactivity in the LDL pellet was reduced by about 40% when HDL was included with the LDL, suggesting that both [^{3}H]SC-795 and [^{3}H]SC-794 bound nearly equally well to HDL and LDL under these conditions.

Effect of N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols on Lipoprotein Structure. Modification of the size, charge, or integrity of lipoproteins by inhibitors can affect CETP activity (*5*). To determine if gross lipoprotein structure was altered by these inhibitors, HDL and LDL were incubated with SC-744 for 2 h at 37 °C, and then analyzed by agarose gel electrophoresis. This method has previously been shown to be sensitive to the effects of compounds that modify lipoprotein structure (*5*). Cholesteryl sulfate, which inhibits CETP by altering the charge of both HDL and LDL, was tested as a positive control. As expected, cholesteryl sulfate (100 μM) altered the electrophoretic migration of both types of particles (Figure 5). SC-744 had no detectable effect on migration at a concentration of 150 μM, about 750-fold higher than the CETP IC_{50} for this compound.

Effect of N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols on LCAT and PLTP Activities. To test the selectivity of SC-795, the compound was tested for inhibitory activity against LCAT and PLTP, two other proteins that use plasma lipoproteins as substrates (Figure 6). No inhibition of LCAT or PLTP by SC-795 was observed at concentrations between 50 and 200 μM. These concentrations are well above that required for CETP inhibition. In contrast, cholesteryl sulfate, when added as a positive control for PLTP inhibition, inhibited PLTP with an $IC_{50} = 100$ μM. PLA2−1 served as a positive control for LCAT, with an $IC_{50} = 50$ μM. These experiments demonstrate that SC-795 was selective for CETP.

Binding of CETP to HDL Disks in the Presence or Absence of N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols. To quantitate the binding of CETP to HDL disks in the presence or absence of these *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols, biotinylated HDL was immobilized onto a sensorchip containing avidin. Solutions of CETP were passed through the flow cell, and the binding of CETP to the HDL was monitored by measuring changes in surface plasmon resonance. In experiments measuring the association and dissociation kinetics (data not shown), it was found that CETP associated with HDL disks with a rate constant $k_a = 8 \times 10^5$ M^{-1} s^{-1} and dissociated with a rate constant $k_d = 3.7 \times 10^{-2}$ s^{-1}. Calculation of the equilibrium dissociation constant from these data provided a $K_d = 47$ nM. To determine if SC-744 affects the interaction of CETP with HDL, CETP was preincubated for 2 h with inhibitor before passing the solution through the resonance flow cell. As shown in Figure 7, the sensorgrams were identical in the

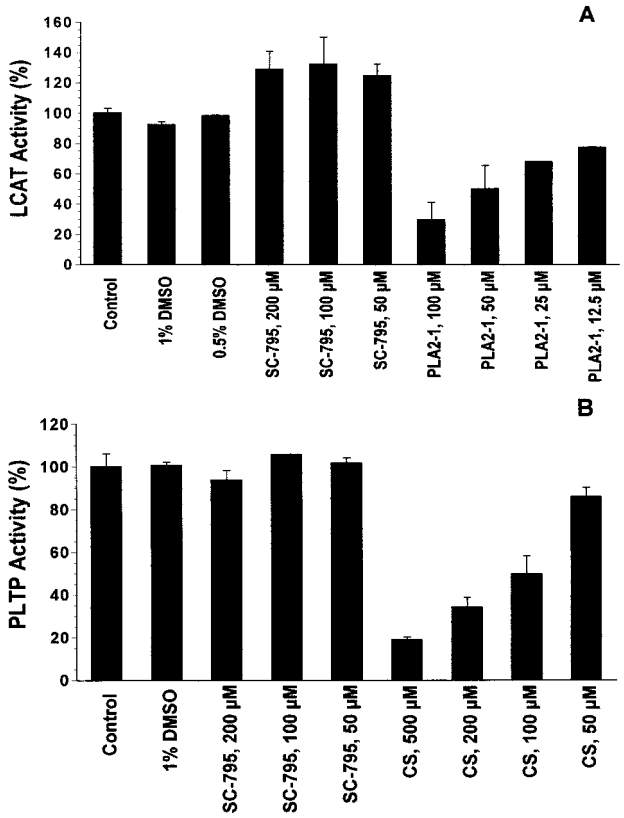

FIGURE 6: Effect of *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols on PLTP and LCAT activities. Different concentrations of SC-795 were added to LCAT assays (panel A) and PLTP assays (panel B). PLA2-1 was included as a positive control for inhibition of PLTP and cholesteryl sulfate (CS) was included as a positive control for of PLTP.

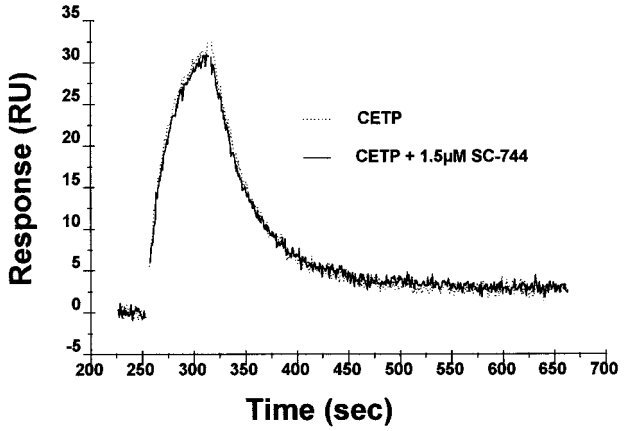

FIGURE 7: Effect of *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols on binding of CETP to HDL. A comparison of SPR sensorgrams of CETP binding to rHDL is shown. CETP in the assay buffer was incubated at 37 °C for 2 h with or without μM SC-744. The CETP samples were then equilibrated to room temperature and passed over control and HDL surfaces in series. The net surface plasmon resonance sensorgrams of each sample were obtained by subtracting the resonance units for the control surface from that of the HDL surface. As shown in the graph, the association region (250−310 s) and the dissociation region (310∼670 s) of the interaction of CETP with HDL of these two samples are identical, an indication that SC-744 does not mediate the interaction of CETP with rHDL.

presence and absence of SC-744, and thus neither the association rate, dissociation rate, nor the overall K_d were affected by SC-744.

Determination of Time-Dependent Inhibition by N,N-Disubstituted 1,1,1-Trifluoro-3-amino-2-propanols and by a Related Bis-Disulfide Analogue. It has previously been shown that certain hydrophobic cysteine-modification reagents can inactivate CETP in a time-dependent manner over a period of hours and that bis-disulfide pyridines were particularly potent CETP inhibitors (*20*, *34*, *35*). Those inhibitors are thought to interact with Cys13 at or near the lipid binding site on CETP. To test if the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanol inhibitors displayed time dependence, the inhibitors were preincubated with CETP for 15 h at 37 °C prior to IC_{50} testing using the reconstituted buffer [^{3}H]-CE transfer assay. There was essentially no difference in the IC_{50} value of SC-744 upon preincubation with CETP (IC_{50} = 100 nM) in comparison with a control sample that had not been preincubated (IC_{50} = 120 nM).

To determine if introduction of a sulfhydryl modifying group into the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanol series of compounds might enhance inhibitory potency, the bis-disulfide analogue SC-364 was synthesized (Figure 1). In contrast to SC-744, the potency of SC-364 was increased from IC_{50} = 42 μM when added directly into the transfer assay, to IC_{50} = 9 μM after 15 h of preincubation with CETP. The bis-disulfide group thus increased the potency 4−5-fold.

Inhibition of Cholesteryl Ester Binding to CETP by N,N-Disubstituted 1,1,1-Trifluoro-3-Amino-2-Propanols. A chromatographic separation assay was employed to determine if these compounds inhibited neutral lipid binding to CETP (*24*). CETP was incubated with [^{3}H]CE HDL for 2 h at 37 °C and then subjected to size-exclusion chromatography to separate the HDL particles from CETP (Figure 8A and 8B). CETP binds approximately 1 mol/mol of [^{3}H]CE under these conditions. When (*R*)SC-795 was included in the incubation, [^{3}H]CE binding to CETP was completely prevented (Figure 8C). In contrast, the inactive enantiomer (*S*)SC-794 did not prevent [^{3}H]CE binding to CETP under these conditions.

Binding of N,N-Disubstituted 1,1,1-Trifluoro-3-Amino-2-Propanols to CETP. ^{3}H-Labeled (*R*)SC-795 and (*S*)SC-794 were prepared to measure direct binding of each compound to CETP. CETP was immobilized onto Sepharose in order to facilitate separation of bound and free ligand. After several trial experiments using different incubation conditions, it was determined that the addition of HDL facilitated binding of (*R*)SC-795 to the immobilized CETP (Figure 9). In these experiments, approximately 770 pmol of CETP was incubated with concentrations of radiolabeled inhibitor ranging from 2 to 6 pmol. The amount of [^{3}H](*R*)SC-795 bound to CETP increased linearly with the amount of compound added (Figure 9), but did not reach saturation, probably because CETP was in vast excess under these conditions. In control experiments, [^{3}H](*R*)SC-795 did not bind to unmodified Sepharose (not shown). Approximately 80% of the binding of [^{3}H](*R*)SC-795 to CETP−Sepharose was blocked by the addition of excess (100 μM) unlabeled (*R*)SC-795, demonstrating that most of the observed binding was specific. In contrast, the amount of [^{3}H](*S*)SC-794 bound to CETP−Sepharose was much less and did not exceed the amount of nonspecific [^{3}H](*R*)SC-795 binding. Furthermore, the low-level binding of [^{3}H](*S*)SC-794 was not prevented by addition of excess unlabeled ligand suggesting that it is nonspecific.

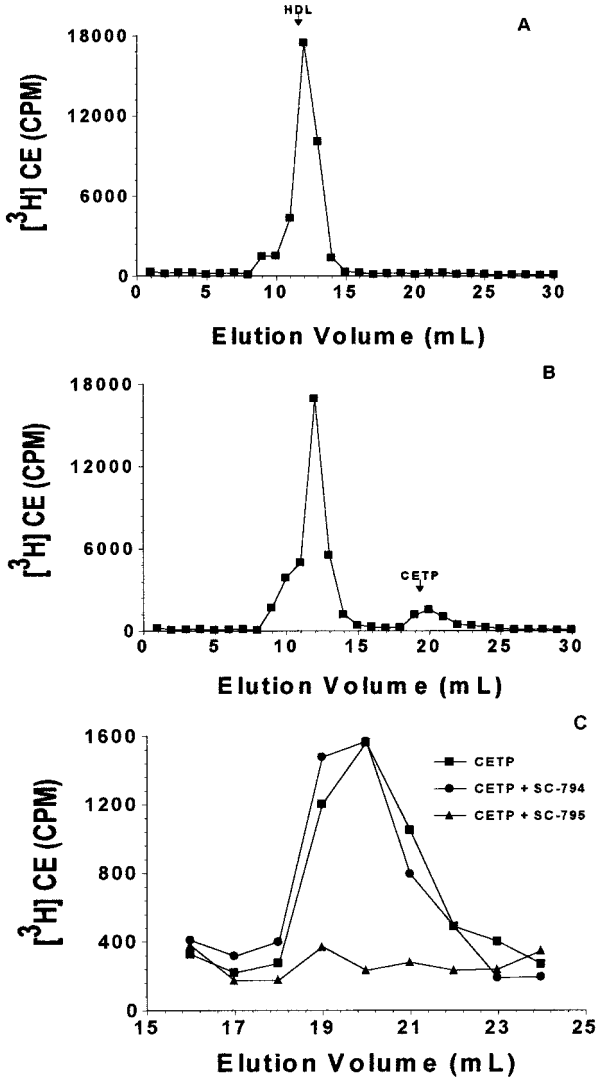

FIGURE 8: Inhibition of cholesteryl ester binding to CETP by *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols. [^{3}H]CE HDL was incubated alone, or with 60 μg of CETP ($\pm$100 μM inhibitor) for 2 h at 37 °C. The samples were applied to a Superose 12 size-exclusion column and the radioactivity in each fraction measured by liquid scintillation spectrometry. The elution of CETP, determined by ELISA, was in fractions 18−22. (A) Control showing elution of [^{3}H]CE HDL alone; (B) transfer of [^{3}H]CE from HDL to CETP. Mixture of CETP and [^{3}H]CE HDL were separated after a 2 h incubation at 37 °C. The presence of the radiolabel in the CETP fractions indicates the association of [^{3}H]CE with CETP. (C) Inhibitors (100 μM) were added to [^{3}H]CE HDL and CETP, incubated for 2 h at 37 °C, separated and CETP fractions analyzed for associated [^{3}H]CE.

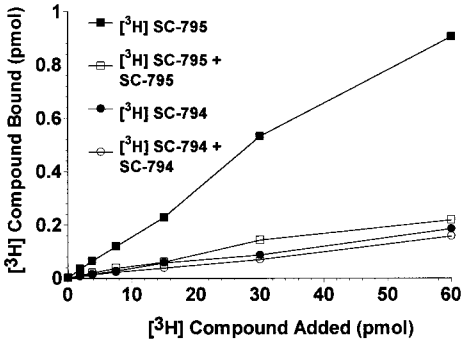

FIGURE 9: Direct binding of *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols to CETP. [^{3}H]SC-794 and [^{3}H]SC-795 were diluted and added to a mixture of HDL in TSE buffer. A slurry of equal volume (100 μL) of CETP−Sepharose and TSE buffer was added to the inhibitor samples. The samples were centrifuged and the pellet collected and assayed for bound radioactivity by liquid scintillation counting. Control samples were prepared containing 100 μM unlabeled SC-794 or SC-795.

DISCUSSION

These *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols represent a highly potent and selective new class of CETP inhibitor that blocks both CE and TG transfer. The original synthesis produced SC-744 as an (*R*,*S*) mixture. Upon separation, it was found that the (*R*,+)SC-795 enantiomer, with an IC_{50} of 20 nM, was about 10-fold more potent than the (*R*,*S*) mixture. In contrast, the (*S*,−)SC-794 enantiomer had significantly less potency, and all of the inhibitory activity in the 97.9% stereochemically pure (*S*,−)SC-794 enantiomer, was the result of the residual 2.1% contamination by the (*R*) enantiomer. Stereospecific inhibition of CE binding to CETP was also observed, suggesting that the inhibitor bound to a specific site on CETP, and that inhibitor binding was competitive with CE binding.

To verify that the inhibitor interacts directly with CETP, binding experiments were conducted using radiolabeled inhibitors and CETP immobilized on Sepharose. The radiolabeled (*R*)SC-795 enantiomer bound to immobilized CETP in a concentration-dependent manner. [^{3}H](*R*)SC-795 binding was blocked by addition of unlabeled (*R*)SC-795 inhibitor. The inactive (*S*)SC-794 enantiomer did not bind specifically under these same conditions. It is important to note that several different conditions were tested for inhibitor binding, and that the addition of HDL was found to facilitate effective binding. These inhibitors are also fairly hydrophobic and have limited solubility in aqueous solution. We have observed that SC-744 partitions efficiently into both LDL and HDL particles. Thus, the inclusion of HDL presumably maintained solubility of the inhibitor and served to deliver the inhibitor to CETP. A recent model of CETP structure places an amphipathic helix over the putative CE binding site (*1*). Upon binding to a lipoprotein surface, the helix is moved away, thereby allowing access to the hydrophobic CETP binding site by hydrophobic neutral lipids within the lipoprotein. It seems plausible that binding of these *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols to CETP occurs by the same mechanism as the natural ligand, CE, but it occurs more efficiently.

The extent of inhibition of CETP by the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols did not increase with time when the inhibitor was preincubated with CETP, consistent with a reversible mode of action. This is in contrast to previously described hydrophobic disulfide modification reagents that were found to display marked time dependence of inhibition (*20*, *34*, *35*). In particular, SC-71952, a substituted bis-pyridine disulfide, was found to bind to CETP and catalyze a disulfide-exchange reaction that inactivated CETP. It was suggested that the target of SC-71952, Cys-13, was near the inhibitor binding site. The inactivation occurred with a $t_{1/2}$ of about 2−4 h of preincubation, and resulted in an increase in potency of the inhibitor of about 20-fold. Similar results were obtained with JTT-705, another cysteine-modifying CETP inhibitor (*35*). SC-744 did not display time dependence under similar conditions of preincubation. However, introduction of a bis-disulfide into the

structure of a related *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanol, SC-364, increased potency 4−5-fold upon preincubation. This result suggests that disulfide-containing *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols may also participate in a disulfide exchange reaction following binding to CETP in the same region near Cys-13 as the pyridine inhibitors and that this site may be near the neutral lipid binding site on CETP. The location of the disulfide moiety in SC-364 may not be optimal relative to the position of cys-13, so other disulfide-based inhibitors in this series may lead to higher inactivation.

Several control experiments were performed to determine if the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols affected other aspects of CETP-mediated lipid transfer. First, lipoprotein integrity and charge properties were monitored by gel electrophoresis of HDL and LDL particles treated with inhibitors. No changes in the lipoprotein structure were detected by this method, although the inhibitors were observed to associate with both LDL and HDL. We next analyzed the binding of CETP to HDL disks since it was conceivable that the binding or recognition of the lipoprotein substrates could be altered by treatment with *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols. Surface plasmon resonance was used to quantitate CETP binding to HDL in the presence or absence of SC-744. No differences in kinetics or affinity were detected by this method. Finally, we tested the specificity of the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols using PLTP and LCAT. PLTP is a related family member that shares about 20% identity with CETP, utilizes identical lipoprotein substrates as CETP, and performs a similar function in transferring phospholipid between lipoproteins. LCAT, an enzyme that utilizes similar lipid substrates as CETP, was also used to assess inhibitor specificity. These *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols inhibitors had no effect on either of these activities. Together, these results support the contention that the primary mechanism of inhibition of CETP by the *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols involves direct interaction of the inhibitor with CETP.

The operational measure of inhibitor potency used in the present study was the concentration of inhibitor required to obtain 50% inhibition of CETP-mediated lipid transfer, that is, IC_{50}. The most potent inhibitor examined here, (*R*)SC-795, had an IC_{50} value of 20 nM using the reconstituted buffer assay in which purified CETP and lipoproteins were used. The CETP concentration in the assay was 8 nM, so (*R*)SC-795 can be regarded as extremely potent. The other assay component to consider is the concentration of substrates relative to inhibitor. The CE concentration used in the reconstituted buffer assay, as contributed by HDL and LDL particles, was approximately 100 μM. The ratio of CE to (*R*)SC-795 at the IC_{50} concentration, therefore, was 5000 to 1, or 0.02 mol % inhibitor relative to CE. By comparison, the K_i of U-95594, a competitive inhibitor for CETP described by Epps et al. (*12*), was 10 mol %.

We have not attempted to obtain traditional binding constants that describe the interaction of CETP and CE or between CETP and the hydrophobic *N*,*N*-disubstituted 1,1,1-trifluoro-3-amino-2-propanols, because these ligands are not evenly distributed in solution, but instead, partition into the lipoprotein particles. The CETP-lipoprotein lipid transfer system is therefore similar in many respects to the well-characterized phospholipase system (*33*), in which the relevant constants are best described in terms of mole fraction within the lipid partition phase. In this unique type of binding system, the concentration of ligand that the lipid binding protein is exposed to is determined by the relative local concentrations of each component within the lipid phase near the contact site of protein and lipid particle. In this context, it is remarkable that inhibitor (*R*,+)SC-795 binds to CETP about 5000-fold more effectively than the natural ligand CE.

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