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PHOSPHONATE-CONTAINING ANALOGS OF CHOLESTERYL ESTER AS NOVEL INHIBITORS OF CHOLESTERYL ESTER TRANSFER PROTEIN

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Abstract: A series of phosphorus-containing analogs of cholesteryl ester have been synthesized as potential inhibitors of cholesteryl ester transfer protein (CETP). The most potent inhibitor, phosphonate **7,** represents a novel inhibitor of CETP. Copyright © 1996 Elsevier Science Ltd

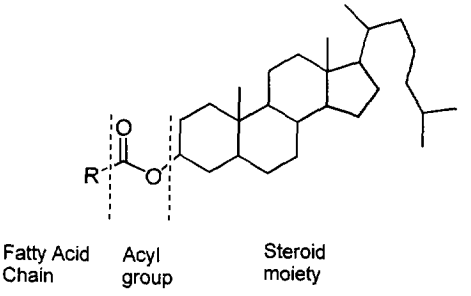
The plasma cholesteryl ester transfer protein (CETP) transfers cholesterol ester from high density lipoprotein (HDL) to triglyceride-rich lipoproteins and plays a major role in HDL catabolism.[1] Evidence accumulated from animal models[2] and human genetic deficiency states[3] indicates that elevation in CETP activity lowers HDL cholesterol and elevates low density lipoprotein (LDL) cholesterol levels, resulting in a lipoprotein profile that contributes to the development of coronary heart disease (CHD). The potential proatherogenic effect of CETP activity has prompted intense interest in the discovery of CETP inhibitors as a therapeutic strategy for the prevention and treatment of CHD.[4-6] In this communication we describe the results of studies into the design and synthesis of new inhibitors of CETP.

CETP is a 74 kDa plasma glycoprotein that contains a binding site for cholesteryl ester. The cholesteryl ester transfer mechanism of this transfer protein is, however, unknown. Our focus on the inhibitor design, therefore, has been directed to the competitive inhibition of CETP by analogs of the natural substrate. In this substrate analog program, we envisioned that proper replacements of the fatty acid chain, acyl group, and steroid moiety in the substrate (Figure 1) should lead to an analog with enhanced CETP binding through potential hydrophobic and electrostatic interactions.[7] In particular, we expected that a replacement of the acyl group in the substrate, with a more strongly basic moiety, should greatly improve the electrostatic interaction with CETP. Various derivatives of cholesteryl ester were prepared to profile the structural requirements associated with the CETP inhibitory activity.

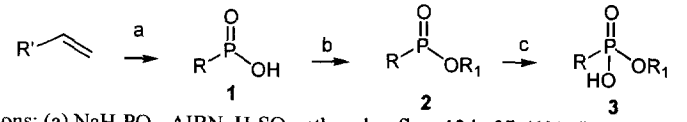
Chemistry

As an initial evaluation of this approach, the synthetically more accessible saturated fatty acid chain analogs of cholesteryl ester were used. The synthesis of phosphorus containing analogs (**7, 9-23**) was achieved as outlined in Scheme 1. Hydrophosphorylation of the terminal olefin with sodium hypophosphite under free-radical conditions gave the phosphonous acid **1**.[8] Condensation with the appropriate alcohol and oxidation with sodium periodate in aqueous dioxane gave the phosphonate **3**.[9] In the route to the compounds **4-6** (Scheme 2), alkylation[10a] of dimethyl phosphite with octadecyl iodide and deprotection[10b] with thiophenol gave the phosphonic acid **5**. Reaction of **5** with oxalyl chloride followed by condensation with benzyl alcohol gave the phosphonate **6**. The synthesis of the phosphinic acid **8** is shown in Scheme 3. The benzyl phosphonate **7** was treated with oxalyl chloride and the resulting phosphonyl chloride was reacted with phenethylmagnesium bromide.[11] Deprotection of the benzyl ester then produced the free acid **8**.

Figure 1

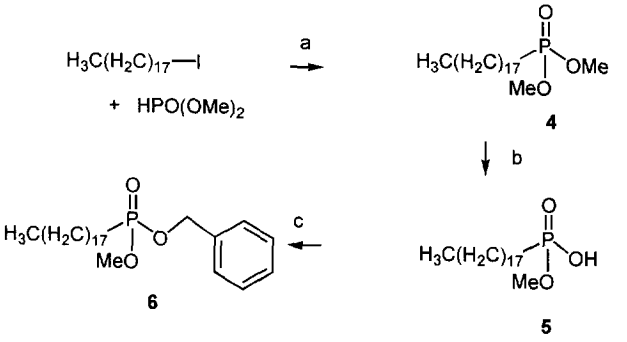


SCHEME 1



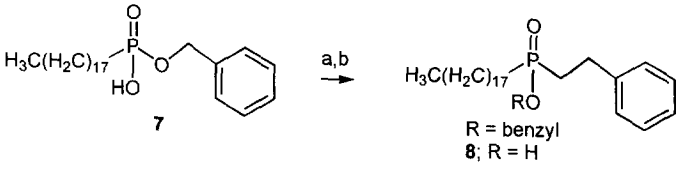
Reagents and conditions: (a) NaH_2PO_2, AIBN, H_2SO_4, ethanol, reflux, 12 h, 37-41%; (b) R_1OH, DCC, DMAP, THF, rt, 12 h, 60-70%; (c) $NaIO_4$, 1,4-dioxane, water, rt, 12 h, 66-91%.

SCHEME 2



Reagents and conditions: (a) NaH, THF, rt, 4 h, 52%; (b) Et_3N, thiophenol, THF, rt, 20 h, 99% ; (c) oxalyl chloride, 60 °C, 1h; then benzyl alcohol, diisopropylethylamine, THF, rt, 12 h, 80%.

SCHEME 3



Reagents and conditions: (a) oxalyl chloride, 60 °C, 1 h; then phenethylmagnesium bromide, THF, rt, 8 h, 60%; (b) H_2, 10 % Pd/C, ethyl acetate, rt, 12 h, 73%.

Results and Discussion

Compounds were evaluated for inhibition of CETP transfer activity (Table).[12] The in vitro assay

TABLE

Compound		IC_{50} (μM)
5		46% (30 μM)
6		inactive[a]
7		2
8		14
9	**2**, R = n-octadecyl; R_1= cholesteryl	inactive[a]
10	**3**, R = n-octadecyl; R_1= cholesteryl	30% (30 μM)
11	**1**, R = n-octadecyl	28% (30 μM)
12	**3**, R = n-octadecyl; R_1= 9-fluorenyl	inactive[a]
13	**3**, R = n-octadecyl; R_1= 2-fluorenyl	inactive[a]
14	**3**, R = n-octadecyl; R_1= 9-phenanthrene	inactive[a]
15	**3**, R = n-tetradecyl; R_1= 3-phenoxybenzyl	6
16	**3**, R = n-tetradecyl; R_1= 2-naphthyl	24
17	**3**, R = n-tetradecyl; R_1= 3-furylmethyl	15
18	**3**, R = n-octadecyl; R_1= 3-picolyl	inactive[a]
19	**2**, R = n-octadecyl; R_1= benzyl	inactive[a]
20	**3**, R = n-tetradecyl; R_1= benzyl	5
21	**3**, R = n-pentadecyl; R_1= benzyl	4
22	**2**, R = n-decyl; R_1= benzyl	inactive[a]
23	**2**, R = n-hexyl; R_1= benzyl	inactive[a]

a less than 10% inhibition at 30 μM.

identifies inhibitors of CETP-catalyzed transfer of cholesteryl ester from HDL to LDL. The inhibition of CETP by these synthetic compounds ranged from no inhibition to an IC_{50} of 2 μM. The cholesteryl phosphonous ester **9** was inactive but the phosphonate **10** showed moderate CETP inhibition.[13] Replacement of the steroid moiety with smaller hydrophobic groups has led to the benzyl derivative **7** with an IC_{50} of 2 μM. The corresponding methyl ester **5** and the phosphonous acid **11** were less active, suggesting a hydrophobic requirement in this site. The tricyclic analogs (**12**, **13**, and **14**) displayed little or no CETP inhibition at concentrations up to 30 μM. Smaller analogs (**15**, **16**, and **17**) possessed good inhibitory activity. The inactivity of the 3-picolyl derivative (**18**) may suggest a non-basic tolerance in this potential steroid binding site. As anticipated, the negatively ionizable charged group of the phosphonate (**6**, **7**, and **19**) is crucial for the inhibition. [14] The more basic phosphinate **8**, however, was less active than the phosphonate **7**.[15] Concurrently with our studies on the effects of modifying the acyl and steroid moieties we have investigated the fatty acid chain length requirement. A chain length of approximately fourteen atoms (**20** and **21**) is needed for good inhibition. Slight improvement was seen with a chain length of eighteen (**7**). Analogs with chain lengths of ten (**22**) and six (**23**) were inactive. The most potent inhibitor in this work, compound **7**, is comparable in activity to PD 140195.[4,12] Further optimization of this in vitro active compound may furnish a useful CETP inhibitor.[16] In conclusion, our efforts based on the substrate analog of CETP have led to the identification of phosphonate-containing compound **7** as a novel synthetic inhibitor of CETP.[17]

Acknowledgments

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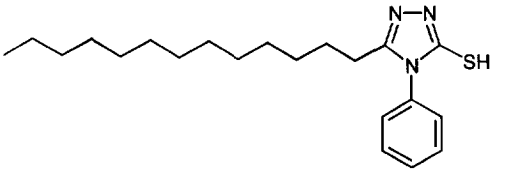
12. The assay measures the transfer of [^{3}H]-cholesteryl linoleate (CE) from donor HDL to acceptor LDL driven by recombinant human CETP. To test for inhibition, the compound was preincubated with recombinant human CETP overnight at 4°C, followed by a one hour incubation in the presence of donor and acceptor. The donor and acceptor lipoproteins were then separated by selectively immuno-precipitating HDL using anti-human apolipoprotein A-I antibody and Staphylococcal protein A. The appearance of [^{3}H]-CE in the LDL fraction yielded a direct measure of CETP activity. Unless otherwise stated, the concentration of compound necessary to inhibit 50% of the CE transfer (IC_{50}) was calculated and is reported as an average of at least duplicate determinations using several concentrations. Under these assay conditions, PD 140195 showed an IC_{50} of 1.5 μM.

PD 140195



13. Replacement of the ester group in cholesteryl oleate with the corresponding amide group showed no effect in CETP inhibitory activity.
14 The corresponding amide, urea, and acyl ester of **7** showed no CETP inhibitory activity.
15. For the discussion on binding energies of phosphorus-containing groups, see: (a) Bartlett, P. A.; Marlowe, C. *Science* **1987**, *235*, 569.; (b) Grobelny, D.; Goli, U. B.; Galardy, R. E. *Biochemistry* **1989**, *28*, 4948.
16. Since desirable compounds must inhibit CETP activity in the plasma components, secondary assays have been established to test these compounds for CETP inhibition in the presence of plasma components. Preliminary results showed that compound **7** inhibited CETP activity in the presence of plasma components.
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