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Design and synthesis of boronic acid inhibitors of endothelial lipase

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ABSTRACT

Endothelial lipase (EL) and lipoprotein lipase (LPL) are homologous lipases that act on plasma lipoproteins. EL is predominantly a phospholipase and appears to be a key regulator of plasma HDL-C. LPL is mainly a triglyceride lipase regulating (V)LDL levels. The existing biological data indicate that inhibitors selective for EL over LPL should have anti-atherogenic activity, mainly through increasing plasma HDL-C levels. We report here the synthesis of alkyl, aryl, or acyl-substituted phenylboronic acids that inhibit EL. Many of the inhibitors evaluated proved to be nearly equally potent against both EL and LPL, but several exhibited moderate to good selectivity for EL.

Heart disease is the most common cause of death in the United States.[1] High density lipoprotein (HDL) cholesterol represents the component among the lipid populations that protects against coronary artery disease (CAD) and cardiac events. Whereas serum concentrations of low density lipoprotein (LDL) cholesterol and triglycerides are positively correlated with risks for developing CAD, concentrations of HDL-cholesterol are negatively correlated. As such, it is now estimated that each 1 mg/dL increase in HDL-C levels is accompanied by a corresponding decrease of cardiovascular risk of 2% in women and 3% in men.[2] Studies have also shown low HDL-C to be an independent risk factor for coronary heart disease, which includes death by cardiac events, as well as myocardial infarction and silent infarct.[3] Additionally, there is evidence that HDL-C may play a direct role in protecting from atherosclerosis. Much of this protection may be the result of HDL-C's role in the reverse cholesterol transport pathway. This generally provides that cholesterol from peripheral tissues may be loaded onto HDL via ABCA1 channels to be transported back to the liver either directly or through CETP-mediated shuttling onto LDL. To this end, factors that affect the circulating levels of HDL-C are important components of the cardiac risk factor mosaic. Included in this list are therapeutic agents such as niacin and torcetrapib, as well as enzymes of the triglyceride lipase gene family.

The triglyceride lipase gene family is a subset of the α/β hydrolase family. Endothelial lipase (EL) is the most recently discovered member of the triglyceride lipase family, sharing 44% sequence identity to human lipoprotein lipase (LPL) and 41% sequence identity to human hepatic lipase (HL).[4,5] The catalytic center, consisting of a classic serine hydrolase aspartate–histidine–serine motif, is conserved among these proteins. Additionally, the putative lipid binding pockets appear conserved as well, indicating that EL likely interacts with lipid substrate in much the same way that HL and LPL do. Before interacting with potential substrate, EL remains in a closed conformation, whereby a 10-amino acid disulfide-linked lid blocks entrance to the active site. Upon binding lipid, this lid domain undergoes significant conformational change, to allow substrate access to the relatively buried catalytic residues.[6] While all three proteins contain a lid, EL appears to have a lid region that is significantly distinct from the other proteins.[7] This suggests that EL may have a different substrate specificity than either HL or LPL, and indeed, EL has considerably greater preference for HDL over other lipoproteins; moreover, HDL is modulated by EL more than by any other lipase.[8] EL preferentially hydrolyses fatty acid esters from the phospholipid components of HDL. Comparing hydrolysis of triglyceride substrates to phospholipid substrates, EL demonstrates a clear preference for phospholipids. In contrast, LPL prefers hydrolysis of triglyceride substrate to phospholipid substrate over 200-fold more than EL.[9]

That HDL-C concentration is greatly influenced by EL is clear in studies that have manipulated its expression levels in vivo. In gene knock-out studies, mice that have an altered EL genetic profile have significantly increased levels of HDL-cholesterol: 57% in the EL −/− model, 25% in the EL +/− model.[8] Infusion of wild-type mice with an anti-EL antibody lead to an increase of HDL-C by 30–50% over 48 h.[10] Perhaps most striking is the effect that EL depletion

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has on the atherosclerotic ApoE −/− mouse model. ApoE −/− mice were bred with EL −/− animals to generate a double knockout strain. In comparison to the ApoE −/− model, the double knockouts had higher levels of HDL-C and (V)LDL, but nonetheless had significantly reduced atherosclerotic lesion areas at the aortic root.[11] In this model, then, the abrogation of EL-dependent depletion and modification of HDL effectively rescued the atherosclerotic profile. These last results have been disputed[12] to the extent that the EL −/− reversed the atherosclerotic lesion; both studies, however, are in agreement with respect to the increased HDL-C effect.

Lipoprotein lipase (LPL) is another member of the lipase gene family involved in lipoprotein metabolism, particularly of VLDL and chylomicrons. It is primarily responsible for the liberation of fatty acids to be utilized as energy by muscle or stored by adipose. There is a positive correlation between HDL-C levels and LPL activity.[13,14] The hydrolytic action of LPL releases lipids that are transferred to immature HDL particles. The CETP-mediated exchange between (V)LDL triglycerides and HDL cholesterol esters is in part regulated by the relative concentrations of each of these lipid components. As such, increased LPL activity extends the half-life of HDL particles by diminishing the concentration of triglyceride-rich particles available for a CETP-mediated interaction.[15] For these reasons, LPL is viewed as a powerful protector against both development of atherosclerosis and decreasing levels of HDL-C. LPL shares considerable sequence, and presumably structural, homology with other members of this family, such as EL and HL.[16] Therefore, cross-inhibition of LPL would be detrimental to the goals of increasing levels of HDL-C or decreasing risks of CAD.

Two assay systems were used to explore the inhibition of EL and LPL. A vesicle-based system[9] allowed for the exploration of the inhibition of these enzymes as they would undergo interfacial catalysis, as would be expected under physiological conditions. The micelle assay,[17] on the other hand, permitted the exploration of effects of inhibition directly at the active sites, as the mechanism of interfacial catalysis is effectively removed under such conditions.[18] Derivatives of palmitic acid containing various electrophilic traps known to inhibit hydrolases that utilize Asp-His-Ser triads in their active sites were synthesized according to literature precedents. Table 1 illustrates that among these compounds, the boronic acid **1f** demonstrated the greatest potency at our screening concentration of 50 μM, inhibiting 90% of EL activity. The trifluoromethyl ketone **1e** was the second most potent, at 56% EL inhibition. No other electrophile demonstrated significant inhibition. While not selective, these data substantiated an exploration of boronic acids as a class of inhibitory molecules. Indeed, boronic acids have been shown to be reversible active site inhibitors of many serine hydrolases.[19]

Phenylboronic acid derivatives were then tested as inhibitors. Derivatization was accomplished with Suzuki coupling reactions using *O*-triflate nitrobenzene (Scheme 1, compounds **2–4**) as starting materials. Coupling of the triflate with an alkylboronic acid using $Pd(OAc)_2$ with the S-PHOS ligand[20] produced the corresponding alkylnitrobenzene (**5c–5q**) in quantitative yield by GC–MS. These were reduced with H_2 over Pd/C to produce the corresponding aniline (**6c–6q**) in high yield.[21] The amino group was converted to an iodide through diazonium formation with $NaNO_2$ and H_2SO_4, followed by displacement with KI,[22] forming iodobenzene compounds **7c–7q**.

Synthesis of the pinacol esters was explored to capitalize on their increased stability relative to free boronic acids. Recent work has suggested that the pinacol boronate group can be directly inserted onto an aryliodide via a transition metal catalyzed reaction. One reported system (CuI/NaH)[23] failed to produce any product in our hands, but a palladium catalyzed system[24] showed much more potential. Since the S-PHOS ligand proved to be so versatile in earlier synthetic steps, we examined the possibility of using the same ligand system to couple pinacolborane here. Indeed, $Pd(OAc)_2$/S-PHOS proved very adept at inserting pinacolborane, often in crude yields of near 80% (**8c–8q**). The remaining 20% resulted from addition of hydride at the aryliodide bond, as determined by GC–MS. Interestingly, this result was not observed for the synthesis of pinacol (2-nonylphenyl)boronate. Repeated attempts failed to produce any boron-containing product. Instead, only unreacted starting aryliodide **7j** was collected. The free boronic acid **9j** could only be synthesized through more traditional lithium-halogen exchange, although the yield was very low (10%). For the remaining alkyl-substituted boronate compounds **8c–8i**, removal of the pinacol protection group was effected by a two step process whereby the ester was first converted to the potassium trifluoroborate salt.[25] This salt was recrystallized from warm acetone and then converted to the free boronic acid by use of a fluorophile, chlorotrimethylsilane. Generally, a 40% overall recovery of free boronic acid from the pinacol ester was obtained via this unoptimized methodology. This was not the case for biarylboronate esters, **8k–8q**, as these compounds showed very low solubility in methanol during the conversion to the trifluoroborate salt. As such, these compounds were not converted to free acids, but rather examined in assays as the pinacol esters.

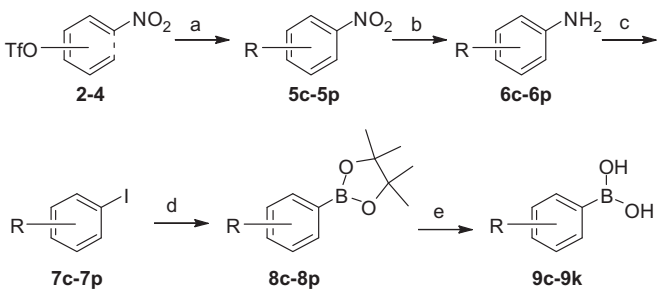
A carboxylate-containing boronate compound was synthesized in the hopes of capitalizing on the different substrate preferences between phospholipid-hydrolyzing EL and triglyceride-hydrolyzing LPL. For this compound, the order of synthetic manipulation was changed to insert the aryliodide before benzylic bromination as illustrated in Scheme 2. Once the enolate condensation reaction had been accomplished, insertion of the pinacol boronate proceeded without loss of the *tert*-butyl ester protection group, effecting compound **15**. This proved a highly fruitful strategy as the last several steps of this synthetic scheme occurred in very satisfactory yields (60% for pinacolboronate insertion, compound **15**, and 70%

Table 1
Inhibition of EL and LPL by electrophilic derivatives of palmitic acid

$CH_3-(CH_2)_{14}-X$

Compound	X	% EL inhibition[a]	%LPL inhibition
1a	$C(O)CH_2Cl$	10	−10
1b	CN	4	14
1c	CHO	7	−1
1d	$C(O)CHN_2$	−22	20
1e	$C(O)CF_3$	56	14
1f	$B(OH)_2$	90	63

[a] Percent inhibition of EL or LPL at 50 μM concentration in the vesicle assay. Values are expressed as a percent of activity of each enzyme incubated with DMSO control.



Scheme 1. Reagents: (a) R-$B(OH)_2$, $Pd(OAc)_2$, S-PHOS, K_3PO_4–H_2O, $PhCH_3$. (b) H_2, Pd/C, EtOH. (c) (1) $NaNO_2$, HCl, ice, H_2O. (2) KI, H_2O. (d) Pinacolborane, $Pd(OAc)_2$, S-PHOS, NEt_3, Dioxane. (e) (1) KHF_2, MeOH. (2) TMSCl, H_2O, CH_3CN.