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A thiocarbamate inhibitor of endothelial lipase raises HDL cholesterol levels in mice

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ABSTRACT

By screening directed libraries of serine hydrolase inhibitors using the cell surface form of endothelial lipase (**EL**), we identified a series of carbamate-derived (**EL**) inhibitors. Compound **3** raised plasma HDL-C levels in the mouse, and a correlation was found between HDL-C and plasma compound levels. Spectroscopic and kinetic studies support a covalent mechanism of inhibition. Our findings represent the first report of **EL** inhibition as an effective means for increasing HDL-C in an in vivo model.

EL is a serine phospholipase that functions in the metabolism of lipoproteins.[1] Although **EL** is a member of the triglyceride lipase family, it differs from other triglyceride lipases, such as lipoprotein lipase (LPL) and hepatic lipase (HL), in its substrate preference for phospholipids rather than triglycerides.[1] Studies in mice have shown that **EL**−/− (knockout) mice have a significant elevation in HDL cholesterol relative to wild type mice, thus establishing that **EL** plays a role in regulating HDL cholesterol in mice.[2] Epidemiological studies have shown that plasma HDL-C levels and cardiovascular disease are inversely related.[3] In addition, **EL** is upregulated under high inflammatory conditions and has been shown to have negative effects at the vessel wall that further contribute to atherosclerosis.[4] Therefore, **EL** inhibitors may represent potential therapeutic agents for the treatment of cardiovascular disease.[5]

A key strategic component of our drug discovery approach was the identification of a tool compound suitable for assessment of in vivo target validation, prior to commencement of lead optimization.

Carbamate **1** (IC_{50} = 0.47 ± 0.70 μM) was identified in a directed high-throughput, cell-based screen of a serine hydrolase inhibitor library using cell surface bound mouse **EL** (Fig. 1). We addressed the solution instability of **1** by focusing on analogs containing replacements for the labile phenylcarbamate moiety. Although the corresponding benzylcarbamate **2** was inactive, thiobenzylcarbamate **3** (mouse **EL** IC_{50} = 0.68 ± 0.45 μM) displayed sufficient potency and stability to serve as a tool compound for in vivo evaluation.

Although it was not potent or selective enough to serve as a lead compound (human HL IC_{50} = 1.36 ± 1.26 μM; human LPL IC_{50} = 1.69 ± 1.86 μM), we used **3** as a tool to get an early read on the potential efficacy of an **EL** inhibitor and evaluated its effect on plasma HDL-C levels in an in vivo model. Vehicle or **3** was administered to C57BL/6J mice by the intraperitoneal route (five doses over three days) and plasma HDL-C levels measured 1 h after the last dose. Compound **3** at 100 mg/kg produced an increase in plasma HDL-C of 37% (from 27 ± 2 to 37 ± 1 mg/dL) compared to vehicle controls (Fig. 2). Because the HDL raising effect was not robust, we sought to identify a different animal model that might produce a more robust HDL response with an **EL** inhibitor. Treatment of C57BL/6 HL−/− (knockout) mice with an **EL**-specific antibody was reported to produce a larger increase in plasma HDL-C than was observed in C57BL/6 mice.[6] Therefore, C57BL/6J HL−/− mice were treated intraperitoneally with **3** or vehicle for three days and the levels of plasma lipoproteins measured 1 hour after the last dose. In HL−/− mice, **3** produced a robust increase in HDL-C levels at the highest dose (Fig. 3A). Plasma HDL-C increased 23% and 77% over vehicle controls at the 30 and 100 mg/kg dose, respectively, although the changes at the 10 and 30 mg/kg doses were not statistically significant. The plasma compound levels increased in a dose related fashion (Fig. 3B). Further analysis of this experiment and repeat experiments suggested that the plasma compound concentration needed to be greater than 3.0 μM in order to obtain a statistically significant increase in HDL-C. In

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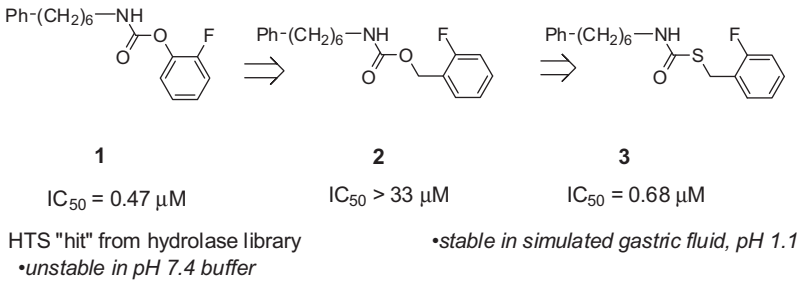


Figure 1. Progression of a screening hit **1** to a lead **3**.

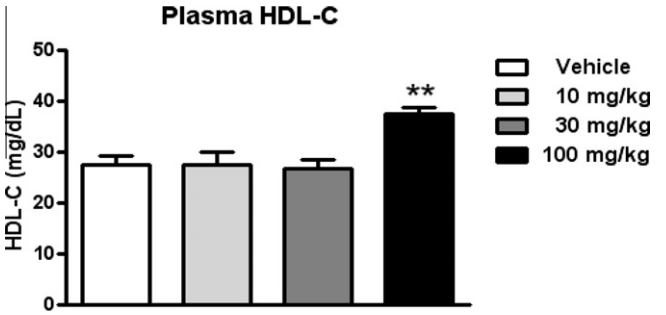
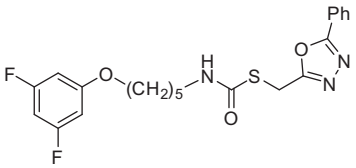


Figure 2. Effects of compound **3** on HDL-C in C57BL/6 mice after intraperitoneal administration. Bars represent means ± SD ($n = 8$ per group; $^{**}p < 0.01$ relative to vehicle group).

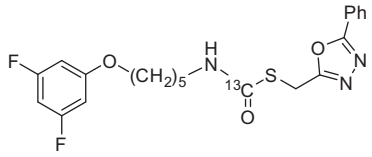
individual animals, plasma levels of compound **3** correlated with elevated plasma HDL-C and increased plasma phospholipids (Fig. 3C and D), both of which are pharmacodynamic markers of **EL** inhibition in vivo. Because **3** inhibited both **EL** and LPL, triglyceride levels also increased significantly at the 30 and 100 mg/kg doses (data not shown). Given that we used HL−/− mice, and inhibition of LPL would most likely result in a reduction in HDL-C,[7] we were fairly confident that the resulting increase in HDL-C in these studies was primarily due to inhibition of **EL**. Therefore, these in vivo results were promising enough to prompt us to continue optimizing this series in search of a more drug-like molecule.

We next examined the mechanism of **EL** inhibition by **3**. The kinetics of the reaction were consistent with time-dependent onset of inhibition (Fig. S1, Supplementary data).[8] However, recovery of enzyme activity did not occur via a rapid-reversible mechanism and a k_{off}-value could not be determined (Fig. S2, Supplementary data).[9] In the course of optimizing **3**, we identified a series of oxadiazole derivatives represented by **4**, which proved to be a potent **EL** inhibitor ($IC_{50} = 0.023 \pm 0.006$ μM). Because **4** was more potent than **3**, we thought it might have a higher affinity for the enzyme and serve as a better tool for unequivocally determining the binding mode of this series. Therefore we conducted additional spectroscopic studies using **4**.

We prepared the ^{13}C labeled carbonyl analog **5** and examined its NMR spectrum in the presence of **EL**.



4 EL IC_{50} = 23 nM



5

A stable tetrahedral enzyme–inhibitor complex such as **6** (Fig. 4) would be expected to cause a large upfield shift of the

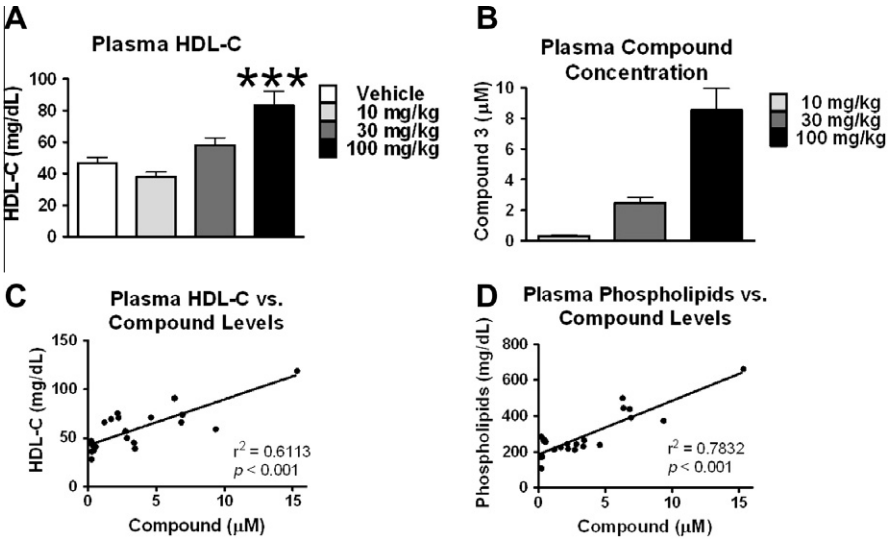


Figure 3. Effects of compound **3** in C57BL/6 HL−/− mice after intraperitoneal administration, (A) plasma HDL-C, (B) plasma compound levels, (C) correlation between plasma HDL-C and compound levels and (D) correlation between plasma phospholipids and compound levels. Bars represent means ± SD ($n = 8$ per group; $^{***}p < 0.001$ relative to vehicle group).

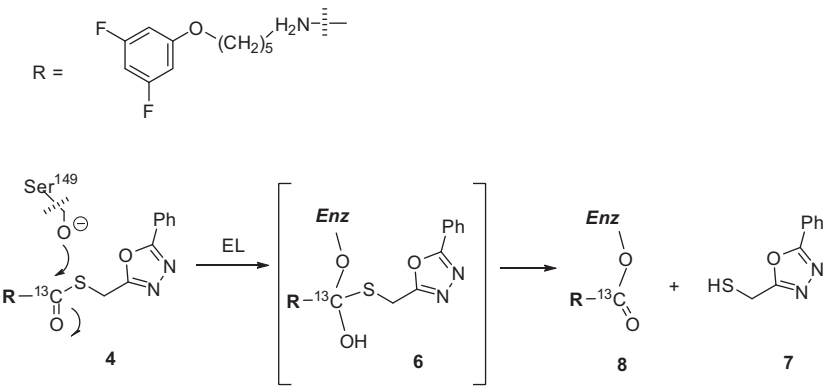


Figure 4. Proposed pathway for **EL** inhibition.

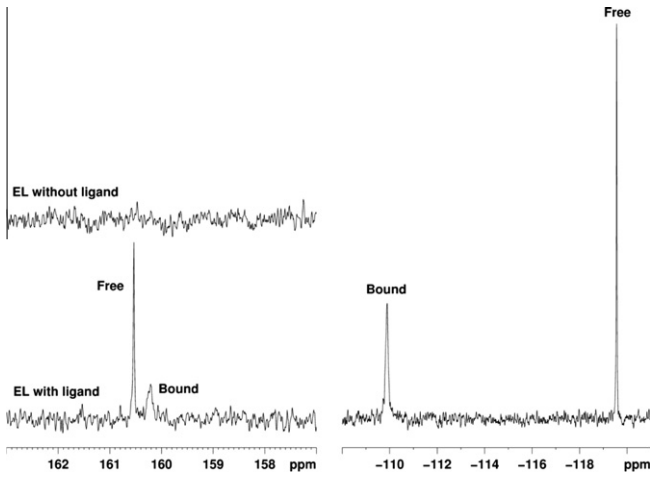


Figure 5. Left: 125 MHz ^{13}C NMR spectra of **EL** with and without **5** obtained using a carbon detect cryoprobe. Right: 470 MHz ^{19}F NMR of bound **EL**:**5** complex and free **5**.

^{13}C labeled carbon relative to unbound **5**. The ^{13}C NMR of a solution of **5** and **EL** (2:1 ratio of **5** to **EL**) showed a sharp signal at 160.5 ppm corresponding to the unbound inhibitor and a line broadened signal at 160.2 ppm corresponding to the bound ligand. However, a carbon signal indicative of a stable tetrahedral enzyme–inhibitor complex such as **6** was not observed (Fig. 5). Similarly, the ^{19}F NMR of this solution displayed a line broadened signal at −110 ppm consistent with the **EL** bound inhibitor **5**, and a sharp signal for the unbound inhibitor at −120 ppm (Fig. 5); only one adduct was detected. Thus, NMR analysis does support formation of a covalent enzyme–inhibitor complex. Furthermore, LCMS–MS analysis of this sample detected the thiol leaving group **7**, which is the expected by-product of formation of the covalent acyl-enzyme complex **8** (Fig. S3, Supplementary data). These results support a proposed pathway (Fig. 4) in which **8** is the most likely covalent adduct formed. Overall, our findings are consistent with the covalent mode of inhibition observed for carbamate-based inhibitors of fatty acid amide hydrolase (FAAH).[10]

In summary, we have identified a potent series of **EL** inhibitors based on a thiocarbamate scaffold. Kinetic and spectroscopic studies indicate a covalent mechanism of inhibition for the series. The tool compound **3** raised HDL-C plasma levels in both C57Bl/6J and C57Bl/6 HL−/− mice. This work is the first report to demonstrate that an **EL** inhibitor raises HDL-C and phospholipid plasma levels in vivo. The results of our studies support the potential for **EL** inhibitors as effective treatment for raising plasma HDL-C in the treatment of atherosclerosis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.113.

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