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Letter
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PK/PD Disconnect Observed with a Reversible Endothelial Lipase Inhibitor

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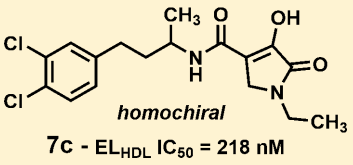
Supporting Information

ABSTRACT: Screening of a small set of nonselective lipase inhibitors against endothelial lipase (EL) identified a potent and reversible inhibitor, *N*-(3-(3,4-dichlorophenyl)propyl)-3-hydroxy-1-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide (**5**; EL IC_{50} = 61 nM, EL_{HDL} IC_{50} = 454 nM). Deck mining identified a related hit, *N*-(3-(3,4-dichlorophenyl)propyl)-4-hydroxy-1-methyl-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxamide (**6a**; EL IC_{50} = 41 nM, EL_{HDL} IC_{50} = 1760 nM). Both compounds were selective against lipoprotein lipase (LPL) but nonselective versus hepatic lipase (HL). Optimization of compound **6a** for EL inhibition using HDL as substrate led to *N*-(4-(3,4-dichlorophenyl)butan-2-yl)-1-ethyl-4-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxamide (**7c**; EL IC_{50} = 148 nM, EL_{HDL} IC_{50} = 218 nM) having improved PK over compound **6a**, providing a tool molecule to test for the ability to increase HDL-cholesterol (HDL-C) levels in vivo using a reversible EL inhibitor. Compound **7c** did not increase HDL-C in vivo despite achieving plasma exposures targeted on the basis of enzyme activity and protein binding demonstrating the need to develop more physiologically relevant in vitro assays to guide compound progression for in vivo evaluation.



KEYWORDS: *Endothelial lipase (EL), high density lipoprotein (HDL), reverse cholesterol transport (RCT), coronary artery disease (CAD)*

Endothelial lipase (EL; gene nomenclature *LIPG*)[1,2] exerts pleiotropic effects on cardiovascular biology through its role in high density lipoprotein (HDL) catabolism,[3−6] vessel wall inflammation,[7−15] and subsequent effects on reverse cholesterol transport (RCT).[4,16−19] Inhibition of EL using neutralizing polyclonal antibodies in mice[20] or pharmacologically using irreversible small molecule inhibitors XEN445 (**1**)[21] and compound **2**[22] (Figure 1) has been reported to raise HDL-C levels in mice, whereas loss of function EL variants in humans (e.g., Asn396Ser) have been associated with increased HDL-C levels.[23] A recently published Mendelian randomization study showed no correlation between increased HDL-C levels resulting from a loss of function EL variant and the risk of myocardial infarction despite an expected 13% reduction of risk estimated from the amount of HDL-C increase associated with the loss of function allele.[24] This study throws doubt into the hypothesis that raising HDL-C levels by inhibiting EL enzymatic activity will decrease coronary artery disease (CAD). To fully investigate the effect of raising HDL-C levels on CAD through pharmacological inhibition of EL will require high quality drug molecules with potent in vivo efficacy.

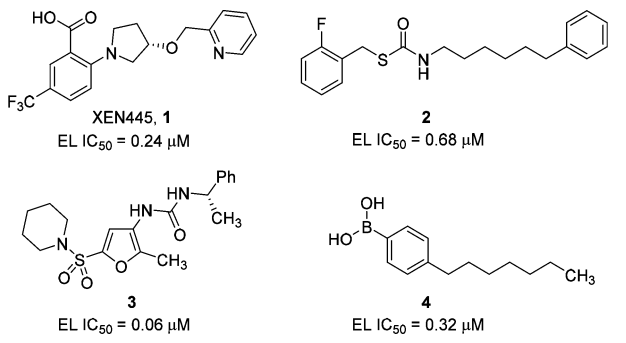


Figure 1. Examples of literature EL inhibitors.

EL is a member of the family of enzymes that includes lipoprotein lipase (LPL), hepatic lipase (HL), and pancreatic lipase (PL). In contrast to LPL and PL, which selectively hydrolyze triglycerides (TGs) and HL that hydrolyzes both TGs and phospholipids, EL shows a preference for the hydrolysis of the sn1 ester of phosphatidylcholines found in HDL producing lyso-phosphatidylcholines (LPCs) and free fatty acids (FFAs), resulting in lipid-depleted HDL particles that are cleared more rapidly from the circulation.[25]

Several small molecule inhibitors of EL have been reported in the literature, e.g., anthranilic acids (XEN445, **1**),[21] thiocarbamates (**2**),[22] sulfanylfuran ureas (**3**),[26,27] and phenyl boronic acids (**4**)[28] (Figure 1). We report herein our initial efforts to identify potent, reversible inhibitors of EL for the purpose of elevating HDL-C blood levels in vivo.

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In conjunction with virtual and high throughput screens, nonselective in-house lipase inhibitors were screened for EL inhibition using a mixed vesicle substrate (EL assay; see Supporting Information). The screen led to the identification of *N*-(3-(3,4-dichlorophenyl)propyl)-3-hydroxy-1-methyl-2-oxo-1,2-dihydropyridine-4-carboxamide (compound **5**, Figure 2) as a potent inhibitor of EL activity (EL IC_{50} = 61 nM)

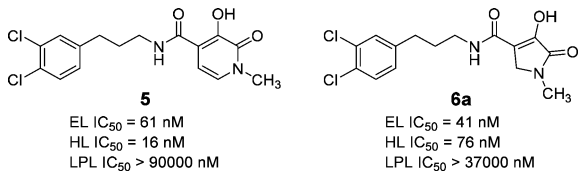


Figure 2. Initial EL hits from focused deck screening.

containing a weakly acidic to neutral heterocycle (measured pK_a = 6.5). Compound **5** was also a potent inhibitor of HL (HL IC_{50} = 16 nM) but was found to be highly selective against LPL (LPL IC_{50} > 90,000 nM). When EL was inhibited with a high concentration of compound **5** (2 μM), enzymatic function was restored by dialyzing away unbound inhibitor from the assay buffer (data not shown), demonstrating the reversibility of inhibition. Further deck mining around compound **5** identified a structurally related EL inhibitor, *N*-(3-(3,4-dichlorophenyl)-propyl)-4-hydroxy-1-methyl-5-oxo-2,5-dihydro-1*H*-pyrole-3-cabox-amide (compound **6a**, Figure 2), with EL IC_{50} = 41 nM that contained a slightly more acidic heterocycle (measured pK_a = 4.8). Compound **6a** was also not selective against HL (HL IC_{50} = 76 nM) but had excellent selectivity versus LPL (LPL IC_{50} > 37,000 nM). Pharmacokinetic (PK) profiling of compounds **5** and **6a** in CD1 mice (Table 1) showed both compounds to have

Table 1. Pharmacokinetic Parameters for Compounds 5 and 6a in CD1 Mice

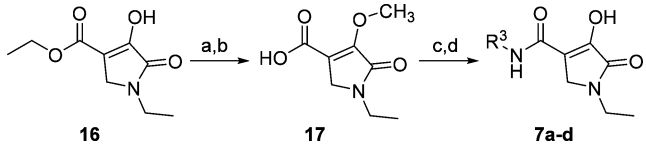
	compound **5**		compound **6a**	
parameter	i.v.	p.o	i.v.	p.o.
dose (mpk)[a]	1.0	1.0	1.0	1.0
C_{max} (μM)	25	2.0	32	8.2
AUC_{total} (μM·h)	19	16	24	18
CL (mL/min/kg)	2.5		2.1	
$t_{1/2}$ (h)	4.9	2.9	3.4	3.2
F (%)		82		76

[a]Bolus administration i.v. and p.o. using 60% PEG400, 30% water, and 10% EtOH as vehicle. mpk = milligram per kilogram.

excellent oral bioavailability (82% and 76%, respectively). Owing to slightly better selectivity against HL and facile synthetic methods available for compound **6a**, we focused our initial SAR efforts on this chemotype, seeking to improve potency, to maintain the favorable PK properties, and to establish a pharmacodynamic (PD) profile and PK/PD relationship so the EL mechanism of action could be evaluated pharmacologically in vivo.

The synthesis of compounds **6a–g**, **8–12**, and **13a–d** is reported in the Supporting Information. Synthesis of the in vivo candidate **7c** and related compounds **7a**, **7b**, and **7d** is shown in Scheme 1. Ethyl 1-ethyl-4-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrole-3- carboxylate (**16**)[29,30] was first *O*-methylated with trimethylsilyldiazomethane in the presence of DIPEA,[31] followed by saponification of the ester. The acid (**17**) was coupled with

Scheme 1. Synthesis of Compounds 7a–d



[a]Reagents and conditions: $TMSCH_2N_2$, DIPEA, Et_2O, rt, 48 h; [b]NaOH, MeOH/H_2O (1:1), 70 °C, 1 h; [c](i) oxalyl chloride, CH_2Cl_2, $DMF_{(cat)}$, (ii) R^3-NH_2, DIPEA, CH_2Cl_2, rt, 3 h; [d]BCl_3, CH_2Cl_2, rt, 5 h. See Supporting Information for individual compound yield.

amines (R^3-NH_2) using the corresponding acid chloride generated with oxalyl chloride. The resulting amides were demethylated with boron trichloride to give the final products **7a** and **7b**. Compounds **7c** and **7d** were obtained by chiral HPLC separation of **7b**. Compound **7c** was also obtained from the homochiral amine synthesized using the method of Ellman.[32] Excellent stereochemical induction was observed (dr = 99:1) when methylmagnesium bromide was combined with the (*R*)-*N*-*tert*-butanesulfinyl imine of 3-(3,4-dichlorophenyl)propanal at 60 °C leading to a high enantiomeric excess of the final product (see Supporting Information).

The compounds were evaluated using a PED-A1/DMPG mixed vesicle assay (EL assay) and an HDL assay using purified HDL particles as enzyme substrate with detection of the product, linoleoyl-lyso-phosphatidylcholine, by LCMS (EL_{HDL} assay; see Supporting Information) in an effort to provide a bridging assay between in vitro and in vivo activity.

We first explored the SAR of the R^1 position of the 1-methyl-5-oxo-2,5-dihydro-1*H*-pyrrole core found on compound **6a**. It was observed that extending the R^1 side chain with lipophilic or polar groups maintained or improved EL potency (see compounds **7a** and **8–10**, Table 2), ranging between 3.6 nM (compound **9**, R^1 = benzyl) and 30 nM (compound **7a**, R^1 = ethyl). Addition of a basic side chain (R^1 = *N*-ethylmorpholine) reduced EL potency by ca. 5-fold, whereas the acidic acyl sulfonamide **12** had similar potency to compound **6a**. When these compounds were tested using the EL_{HDL} assay, a significant right-shift to reduced potency was observed giving EL_{HDL} IC_{50}/EL IC_{50} ratios between 11- and 150-fold.

The SAR for substitution at the R^2 position was examined within the context of R^1 = CH_3 (Table 3). A limited set of analogs was examined (compounds **6b–6d**). All were of similar potency to the original hit. These analogs did not improve EL_{HDL} potency and the ratio of EL_{HDL} to EL potencies ranged from 60- to 100-fold. We concluded that further exploration of this position was not warranted.

Replacing the C-4 hydroxyl substituent with amines (R^4-NH_2) in the context of R^1 = ethyl and R^3 = *N*-(3-(3,4-dichlorophenyl)-prop-1-yl provided compounds **13a–d** (Table 4). Modification at this position provided analogs that were slightly more potent or equipotent to **6a** in the EL assay (IC_{50} range from <10 to 59 nM); however, a significant right-shift in potency in the EL_{HDL} assay was observed, with EL_{HDL}/EL ratios ranging from 50- to >300-fold.

Exploration of the SAR of the amide side chain (R^3) was accomplished in two series where R^1 = CH_3 or ethyl (Table 5). It was generally observed that the most potent compounds in the EL assay contained extended lipophilic R^3 groups and two chlorine atoms located at C-3 and C-4 on the terminal phenyl. Thus, compound **6e** (R^3 = phenyethyl) was 10-fold less potent than the bis-chloro compound **6f**, and compound **6a** was ca. 140-

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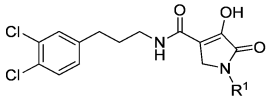
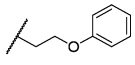
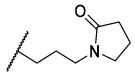
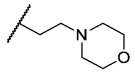
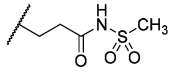
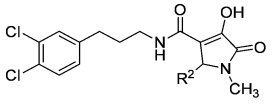
Table 2. R^1 Group SAR of 1-Methyl-5-oxo-2,5-dihydro-1*H*-pyrrole core (compounds 7a and 8−12)



Cmpd	R^1	EL IC_{50} (nM)[a]	EL_{HDL} IC_{50} (nM)[a]
6a		41[b]	1760[b]
7a		30	3520
8		3.6	ND[c]
9		5.4	826
10		19	2440
11		190	ND[c]
12		95	1050

[a]IC_{50} values are an average of at least two independent determinations. [b]See Supporting Information for standard deviations. [c]ND = not determined.

Table 3. R^2 Group SAR of 1-Methyl-5-oxo-2,5-dihydro-1*H*-pyrrole Core (Compounds 6b−d)



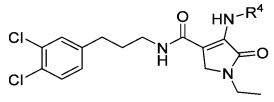
Cmpd	R^2	EL IC_{50} (nM)[a]	EL_{HDL} IC_{50} (nM)[a]
6b		88	5290
6c		29	1770
6d		59	5750

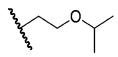
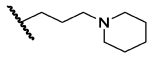
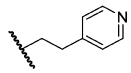
[a]IC_{50} values are an average of at least two independent determinations.

fold more potent than the des-chloro analog compound **6g**. Further SAR development of the R^3 side chain identified a key modification that provided an analog with more balanced EL and EL_{HDL} potency, compound **7b** (EL IC_{50} = 44 nM, EL_{HDL} IC_{50} = 264 nM). When the two enantiomers were obtained in homochiral form, one enantiomer (compound **7c**; EL IC_{50} = 148 nM, EL_{HDL} IC_{50} = 218 nM) was significantly more potent than the other (compound **7d**) and was equally potent for mouse EL (mouse EL_{HDL} IC_{50} = 100 nM). Importantly, the potency of compound **7c** using human or mouse EL was relatively insensitive to increasing concentration of HDL (tested at 40, 100, 300, and 1000 μg/mL HDL; see Supporting Information) suggesting compound **7c** is not competitive with HDL.

When tested against a panel of related enzymes, compound **7c** was found to be >250-fold selective for EL versus LDL,

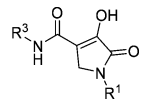
Table 4. R^4 Group SAR of 1-Methyl-5-oxo-2,5-dihydro-1*H*-pyrrole Core (Compounds 13a−d)

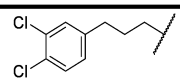
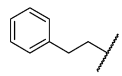
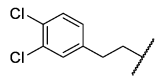
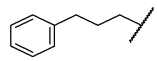
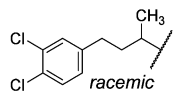
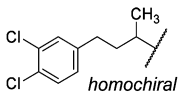
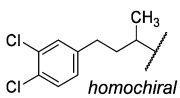


Cmpd	R^4	EL IC_{50} (nM)[a]	EL_{HDL} IC_{50} (nM)[a]
13a		<10	3020
13b		19	945
13c		25	4210
13d		59	5990

[a]IC_{50} values are the average of at least two independent determinations.

Table 5. R^3 Group SAR of 1-Methyl-5-oxo-2,5-dihydro-1*H*-pyrrole Core (Compounds 6a, 6e−g, and 7b−d)



Cmpd	R^3	R^1	EL IC_{50} (nM)[a]	EL_{HDL} IC_{50} (nM)[a]
6a			41[b]	1760[b]
6e			2950	ND[c]
6f			296	ND[c]
6g			5830	ND[c]
7b	 racemic		44	264
7c	 homochiral		148[b]	218[b]
7d	 homochiral		910	835

[a]IC_{50} values are an average of at least two independent determinations. [b]See Supporting Information for standard deviations. [c]ND = not determined.

monoacylglycerol lipase (MAGL), and pancreatic lipase (PL). Using the HDL-based assay, compound **7c** was 12-fold selective for EL vs HL (see Supporting Information).

Pharmacokinetic evaluation of compound **7c** using male CD1 mice at a dose of 1.0 mpk i.v. or p.o. (Table 6 and Chart 1) showed it to have excellent oral exposure (oral AUC_{total} = 292

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μM; 96% bioavailability) and C_{max} (15 μM) with a longer half-life ($t_{1/2}$ = 13 h) than compound **6a** ($t_{1/2}$ = 3.2 h).

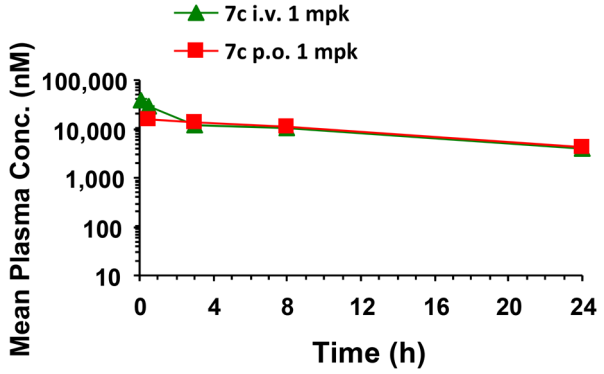
When compound **7c** was dosed at 10 and 50 mpk, systemic exposure was dose related but less than dose proportional for both (7.5-fold and 18-fold increase in AUC_{total}, respectively, vs 1.0 mpk dose; see Supporting Information). Protein binding in mice was 97.7%.

Table 6. Pharmacokinetic Data for Compound 7c in CD1 Mice

parameter	i.v.	p.o.
dose (mpk)[a]	1.0	1.0
C_{max} (μM)	39	15
T_{max} (h)	0.05	0.5
AUC_{total} (μM·h)	304	292
$t_{1/2}$ (h)	13	13
F (%)		96
C 24 h (nM)		4310
C_{free} 24 h (nM)[b]		99

[a]Dosing vehicle for i.v. and p.o. administration was 60% PEG400, 30% water, 10% ethanol. mpk = milligram per kilogram [b]Adjusted for mouse protein binding using 2.3% free fraction.

Chart 1. Mean Plasma Concentrations of Compound 7a in CD1 Mice[a]

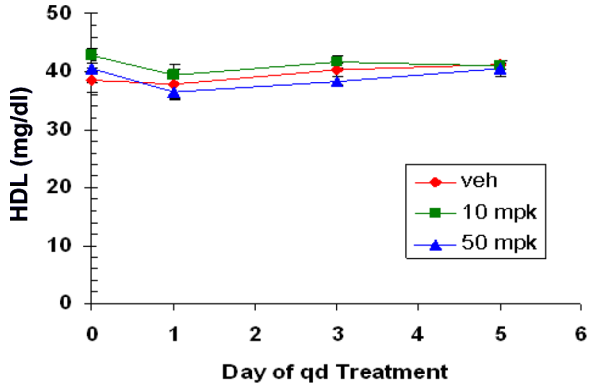


[a]Compound **7c** dosed p.o. at 1 mpk in CD1 mice using 60% PEG400/30% water/10% ethanol as dosing solution.

When compound **7c** was administered to C57BL/6J mice once a day p.o. for 5 days at 10 and 50 mpk, high plasma exposures were observed in the PK arm of the experiment (see Supporting Information). Thus, on day 5 at 50 mpk, C_{max} = 238 μM (6 h postdose) and C_{trough} = 57 μM (24 h postdose). After adjusting for protein binding, $C_{max\ free}$ (5.5 μM) and $C_{trough\ free}$ (1.3 μM) were 55- and 13-fold, respectively, above the mouse EL_{HDL} IC_{50}. Despite the high exposures achieved, there was no effect on plasma HDL-C levels at either dose (Chart 2). A follow-up study in normal hamsters dosed once a day for 7 days at 50 mpk gave the same outcome. In this study, drug exposures were comparable to mouse. Accounting for protein binding in hamster (98.4%), $C_{max\ free}$ = 5.1 μM (day 5, 6 h postdose), ca. 51-fold above the IC_{50} for mouse EL (see Supporting Information).

In summary, optimizing potency of screening hit **6a** using the EL_{HDL} assay resulted in the identification of compound **7c** (EL_{HDL} IC_{50} = 218 nM; mouse EL_{HDL} IC_{50} = 100 nM). The lack of a pharmacodynamic effect in two animal models of increasing plasma HDL-C levels, despite achieving levels of compound exposure that would predict good inhibition of the target enzyme, suggests factors other than protein binding may be involved in the inability of compound **7c** to inhibit plasma EL. In addition, these results point to the need to develop other, more physiologically relevant in vitro assays to guide compound progression for in vivo evaluation. The results of these investigations will be reported separately.

Chart 2. . Plasma HDL Levels in C57BL/6J Mice Dosed with Compound 7c[a]



[a]Compound **7c** was dosed at 10 and 50 mpk once daily p.o. using 60% PEG400/30% water/10% ethanol as dosing solution.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00138.

Reaction scheme, experimental procedures and characterization data for compounds **6a–g**, **7a–d**, **8–12**, and **13a–d**, standard deviations of EL and EL_{HDL} IC_{50} data for compounds **6a** and **7c**, dependence of EL_{HDL} potency on HDL concentration, mouse and hamster PK data, hamster PD data, biochemical assay protocols, and mouse in vivo protocol (PDF)

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The manuscript was written by J.J.H. and through contributions by all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DIPEA, diisopropylethylamine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-[phosphor-*rac*-(1-glycerol)] sodium salt;; dr, diastereo-

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meric ratio; PED-A1, *N*-((6-(2,4-DNP)amino)-hexanoyl)-1-(BODIPY FL C5)-2-hexyl-sn-Gly-cero-3-phosphoethanolamine.

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