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Discovery of XEN445: A potent and selective endothelial lipase inhibitor raises plasma HDL-cholesterol concentration in mice

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ABSTRACT

Endothelial lipase (EL) activity has been implicated in HDL metabolism and in atherosclerotic plaque development; inhibitors are proposed to be efficacious in the treatment of dyslipidemia related cardiovascular disease. We describe here the discovery of a novel class of anthranilic acids EL inhibitors. XEN445 (compound **13**) was identified as a potent and selective EL inhibitor, that showed good ADME and PK properties, and demonstrated in vivo efficacy in raising plasma HDLc concentrations in mice.

1. Introduction

Despite the high rate of adoption of therapeutics, improved diagnostics and the understanding of the role of preventative measures, coronary artery disease (CAD) arising from atherosclerosis remains the leading cause of death in the Western world and CAD has one of the highest disease burdens on health globally.[1] Elevated levels of plasma low density lipoprotein cholesterol (LDLc) are associated with increased atherosclerotic plaque formation and CAD, and as such, therapies to reduce LDLc have become a cornerstone of medicine. However, current therapies only reduce CAD rates by 25–30%.[2] Plasma high density cholesterol (HDLc) is mainly known for its role in reverse cholesterol transport, in which excess cholesterol supplied from the liver and gut to the tissues via LDL, is returned to the liver for excretion via the bile and feces. As plasma HDLc levels are negatively correlated with CAD,[3] therapeutics which raise HDLc have become a focus for development.[2,4,5] HDLc concentration was long believed to be a surrogate for the efficiency of the reverse cholesterol transport process. This concept is being challenged, as ex vivo cholesterol efflux from macrophages to HDL is a better predictor of CAD than HDLc concentration itself.[6] Recently, other roles of HDL particles that may contribute to its atheroprotective functionality have been identified. These include anti-oxidant, anti-inflammatory, nitric oxide-inducing and anti-microbial effects amongst others.[7] Thus, the importance of HDL functionality, beyond its ability to promote the efflux of cholesterol from peripheral cells and to return it to the liver has gained recent prominence.[8,9]

Endothelial lipase (EL) is a member of the triglyceride lipase family which also includes hepatic lipase (HL), lipoprotein lipase (LPL) and pancreatic lipase. Although these enzymes share considerable homologies, including the catalytic triad common to serine hydrolases, their structures do differ in the lid region that plays a role in controlling substrate specificity.[10,11] EL is secreted from vascular endothelial cells and hepatocytes and preferentially hydrolyses the sn-1 (PLA_1) ester bond of phosphatidylcholine (PC) present in HDL particles, releasing Lyso-PC and resulting in a reduction in HDL particle size. LPL predominantly hydrolyses triglycerides in ApoB-containing particles and HL hydrolyses both triglycerides and phospholipids in all classes of lipoproteins.[12,13] The potential role of EL in HDL metabolism has been delineated by a number of mouse genetic studies showing a gene-dose effect, in which EL knockout mice have HDLc concentrations which are 51–140% higher than in wild-type mice.[14–17] Transgenic mice expressing human EL have reduced plasma HDLc, and treatment of wild-type mice with an anti-EL antibody increased plasma HDLc.[18,19] As plasma EL mass and activity both correlate with CAD risk in humans and human loss of function EL variants are associated with increased plasma HDLc,[20] EL has been highlighted

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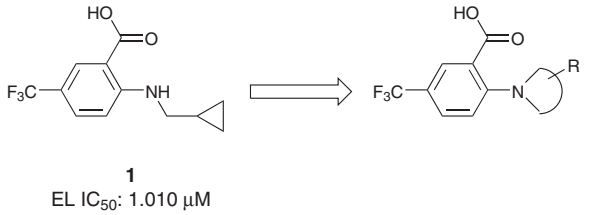
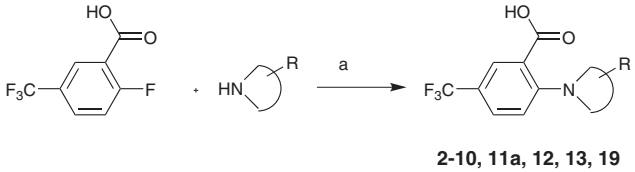


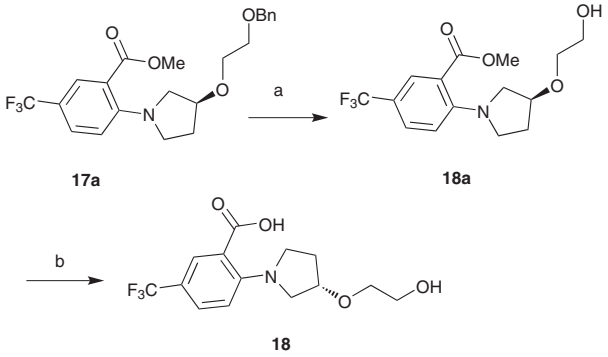
Figure 1. Design strategy.



Scheme 1. Reagents: (a) 4-methylmorpholine (NMM), 1,4-dioxane.

as a therapeutic target to increase HDLc and prevent and treat CAD.[19,21,13,22–24]

Despite the important role of EL in HDLc metabolism, only a few EL inhibitors have been reported[25–27] with no published selectivity or in vivo efficacy. Our efforts to identify novel EL inhibitors utilized a high-throughput screening campaign against EL that identified compound **1** (Fig. 1) as an attractive inhibitor with moderate inhibitory activity against human EL. Inhibitor **1** has high target selectivity over LPL and good physical properties (MW = 259 Da, *c*log*P* = 2.73). This profile led us to consider this hit as the starting point for our discovery program. The 4-(trifluoromethyl) aniline moiety present in **1** raised a safety concern due to the possible formation of reactive metabolites[28], although leflunomide carrying this motif is used as a pyrimidine synthesis inhibitor for rheumatoid arthritis. Our initial SAR focused on replacing this motif which proved to be critical for EL inhibitory activity. Replacement of CF_3 group with other groups such as $-OCF_3$, –Cl, –F, $-CH_3$, –CN, $-SO_2CH_3$, and 4′-CF_3-Ph or replacement –NH– with –O– abolishes activity. Towards this end, we considered substituting the aniline proton and evaluating tertiary anilines which are expected to be less susceptible to potential degradation. This strategy eventually led to the introduction of various nitrogen containing heterocycles such as functionalized pyrrolidines, piperidines or azetidines at the *para* position to the CF_3 on the phenyl ring (Fig. 1). These efforts resulted in the discovery of a class of novel, potent and selective EL inhibitors, exemplified by compound **13** (XEN445), which is a potent and highly selective inhibitor that raises plasma HDLc concentrations in vivo in wild-type mice.



Scheme 3. Reagents: (a) H_2, 10% Pd/C; (b) NaOH, THF, H_2O.

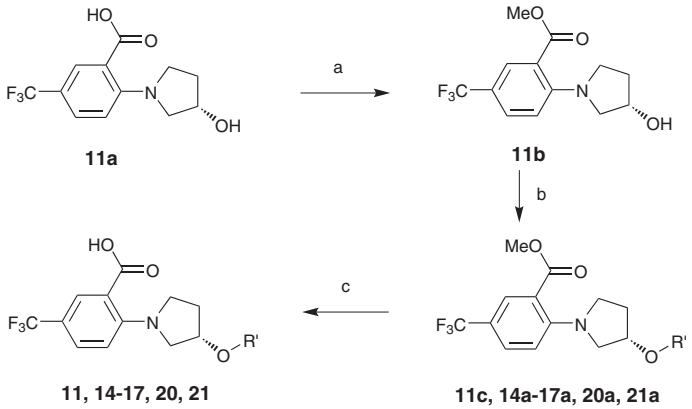
2. Chemistry

The synthesis of these anthranilic acids are outlined in Schemes 1–3. For the synthesis of analogues **2**–**10**, **11a**, **12**, **13** and **19** a simple and efficient aromatic nucleophilic substitution of 2-fluoro-5-(trifluoromethyl)benzoic acid with an appropriate amine was applied (Scheme 1). This method was general, and a wide range of amines could be used in a library fashion to prepare the desired molecules.

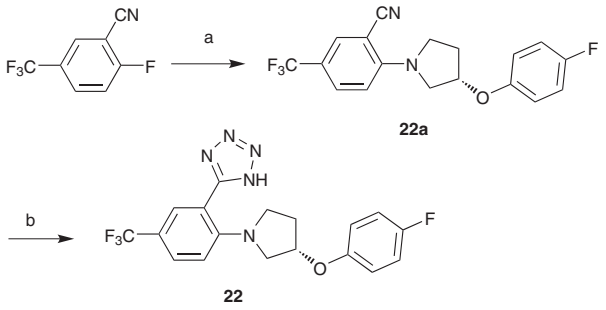
The commercial availability of functionalized pyrrolidines is limited; therefore, a short sequence starting from intermediate **11a** was used to prepare analogs **11**, **14**–**17**, **20** and **21**. As shown in Scheme 2, the carboxylic acid group in **11a** was masked as methyl ester **11b** with K_2CO_3 and MeI; subsequent treatment of **11b** with NaH and 2-cyclopropylethyl 4-methylbenzenesulfonate (for **11c**) or alkyl bromides (for **14a**–**17a**, **20a** and **21a**) led to the corresponding ether. Finally, hydrolysis of the methyl ester with NaOH in aqueous THF under reflux conditions led to the desired compound.

Compound **18** was prepared from intermediate **17a** (Scheme 3). Deprotection of the benzyl group gave the alcohol **18a** and subsequent saponification of the methyl ester gave the desired compound **18**.

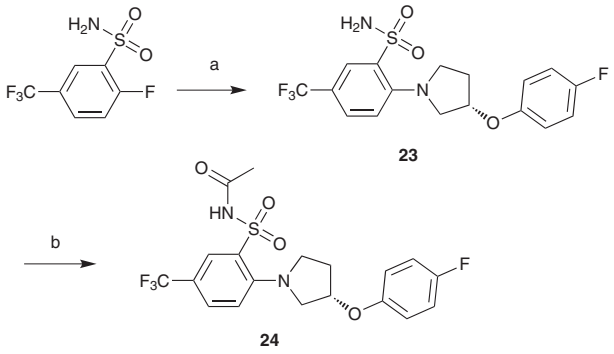
To prepare analogs **22**–**25**, a similar aromatic nucleophlic substitution was applied. Treatment of nitrile **22a** with NaN_3 and NH_4Cl generated tetrazole **22** (Scheme 4). Acylation of sulfonamide **23** with acetyl chloride gave acylsulfonamide **24** (Scheme 5). Reduction of ketone **25a** with $NaBH_4$ led to alcohol **25** (Scheme 6).



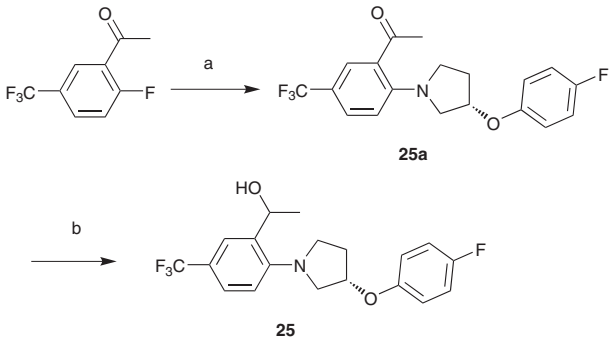
Scheme 2. Reagents: (a) MeI, K_2CO_3, DMF; (b) NaH, XR′; (c) NaOH, THF, H_2O.



Scheme 4. Reagents: (a) (*S*)-3-(4-fluorophenoxy)pyrrolidine, Cs_2CO_3; (b) NaN_3, NH_4Cl, DMF.



Scheme 5. Reagents: (a) (*S*)-3-(4-fluorophenoxy)pyrrolidine, NMM; (b) AcCl, pyridine.



Scheme 6. Reagents: (a) (*S*)-3-(4-fluorophenoxy)pyrrolidine, NMM; (b) $NaBH_4$, MeOH.

3. Results and discussion

Compounds prepared in this study were evaluated in a mixed micelle fluorogenic phosphatidylcholine substrate (FELA, bis-BODIPY-FL C_{11}-PC) assay against isolated human and mouse EL; selected inhibitors were also tested against other members of the triglyceride lipase family, mainly human LPL and HL. Taking **1** as the starting point, our first set of compounds examined the effect of a change of ring size by the synthesis of piperidinyl analog **2**, azidinyl analog **3** and pyrrolidinyl analog **4**; The structure–activity relationship (SAR) indicated that the pyrrolidine ring played a significant role in both EL potency and selectivity (Table 1). Compound **4** had an almost fivefold increase in potency compared to **1** and importantly demonstrated more than 500-fold and 40-fold selectivity over LPL and HL, respectively. Switching pyrrolidinyl to azidinyl (**3**) resulted in a decrease in potency; while the complete lack of activity of the piperidinyl analog **2** was quite striking. To determine if there was any stereoisomeric preference at position 3 of the pyrrolidine, the *R* isomer **5** was prepared according to scheme 1. The potency of **5** suggested no strong preference for this chirality difference. Replacement of the oxygen linker with a carbon linker (compound **6**, racemic) led to a sixfold increase of potency relative to **4**. However, further profiling of **6** revealed an unwanted inhibitory effect against cytochrome P450 2C9 with 86% inhibiton at 10 μM. Thus, compound **4** was taken as a lead compound for further optimization. Exploration of the ether substitution at the 3*S*-position of the pyrrolidine by replacement of the 4-fluorobenzene group of **4** revealed broad tolerances for potency on EL and good selectivity versus LPL and HL. This modification resulted in compounds **9** and **17** with modest improvement in inhibitory potencies relative to **4**.

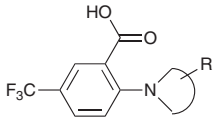
EL hydrolyses HDL phospholipids both as a free plasma enzyme and associated with the surface of vascular endothelial cells via proteoglycan binding. Therefore, the in vivo activities of EL inhibitors are determined by free, un-bound drug plasma levels as well as intrinsic inhibitory potency. Not surprisingly, like most acidic drugs, compounds **4**–**7**, **9**–**12** and **17** displayed high in vitro affinities for rat plasma proteins. We sought to identify new molecules, which maintained or improved in vitro inhibitory potency versus EL were less bound to plasma protein. Therefore, new designs were focused on molecules with less hydrophobic groups or basic groups as exemplified by **13**–**16** and **18**–**21**. These compounds were expected to have less interaction with plasma protein. In general, the inhibitory activity in the FELA assay was closely correlated with the lipophilicity of molecules, decreasing lipophilicity had a negative effect on potency and compounds **18**–**21** were more than fivefold less potent than **17**; however, the high selectivity was maintained and plasma protein binding profile was significantly improved.

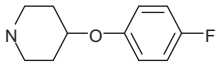
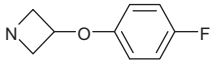
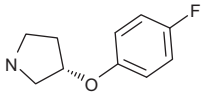
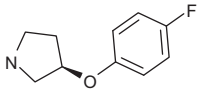
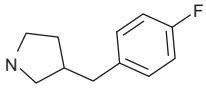
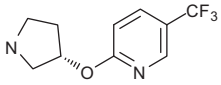
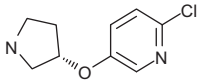
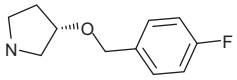
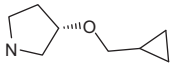
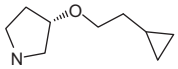
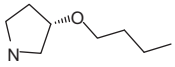
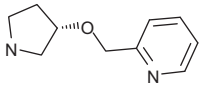
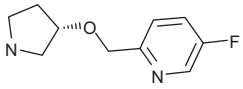
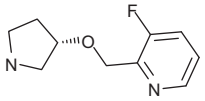
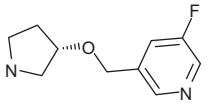
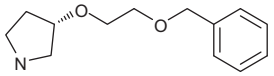
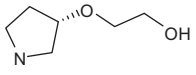
To examine the effects of the carboxylic acid group, we investigated a variety of replacements of the carboxylic acid moiety. As indicated in Table 2, the acidic group proved to be important for activity, as a more than 10-fold drop in potency was observed when the carboxylic acid moiety was replaced by an alcohol as indicated in **25**; while the tetrazole analogue **22** maintained the activity with a slightly improvement in plasma protein binding. In addition, replacement of the carboxylic acid group with sulfonamide group (**23**) and an acylsulfonamide group (**24**) resulted in a dramatic loss in inhibitory activity.

Based on their good inhibitory activity, selectivity and plasma protein binding, the pyridinyl derivatives **13**–**16** were evaluated in the functional HDL particle assay (Table 3), which uses purified human HDL particles as a more physiologically relevant substrate, with the release of free fatty acids as the readout of lipase activity. The potency observed in this assay confirmed that **13**, **14** and **15** were equally potent, while **16** was about twofold less active. **13** (XEN445) can be readily prepared in good yield from commercial available chemicals, which was selected for further evaluation of this series of EL inhibitors.

The relative importance of cell-associated EL and plasma free EL to HDL particle cleavage in vivo is not clear and furthermore, EL is believed to be active as a dimer. EL catalyses the sn-1 cleavage of phosphatidylcholine at the lipid interface, and as such fluorogenic and other EL substrate assays generally employ mixed micelle or particle-based formats. To mimic the in vivo environment in an in vitro assay as closely as possible, we used the recently-described soluble EL fluorogenic substrate PED-A_1 to determine the potency of XEN445 using EL-transfected HEK cells.[29] After a 30 min preincubation of EL-expressing HEK cells with XEN445, the IC_{50} value for XEN445 of 0.25 μM was obtained. This value was very similar to that determined in the cell-free assay (Table 1) and provides more evidence that XEN445 will have in vivo activity, provided

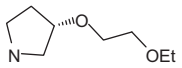
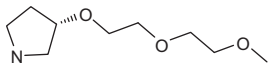
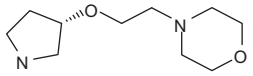
Table 1
SAR of anthranilic acids



Compound		*h*EL IC_{50}[a] (μM)	*h*LPL IC_{50}[a] (μM)	*h*HL IC_{50}[a] (μM)	PPB[b] (%)	*c*log *P*[e]
1	cyclopropanemethylamine	1.010	>10	6.4	99.5	2.73
2		>10	nd	nd	nd	4.57
3		1.96	nd	nd	nd	4.35
4		0.184	>100	7.6	99.8/99.0[c]/97.9[d]	4.46
5		0.259	>33	nd	99.5	4.46
6		0.025	2.56	nd	99.9	5.27
7		0.372	>20	nd	99.7	4.60
8		0.236	31.59	nd	98.7	3.87
9		0.131	>33	nd	99.4	4.37
10		0.236	49.90	nd	99.8	3.21
11		0.156	>33	nd	99.6	3.56
12		0.366	>100	24.11	99.5	3.72
13 (XEN445)		0.237	20	9.5	97.4/88.8[c]/95.4[d]	3.30
14		0.219	76.3	16.92	98.0	3.46
15		0.252	77.2	19.5	97.9	3.46
16		0.214	45.2	23.3	93.5	3.04
17		0.088	>33	nd	99.3	4.06
18		0.57	nd	nd	nd	1.97

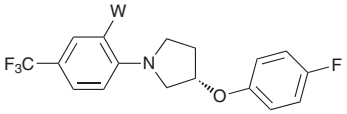
(*continued on next page*)

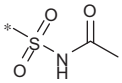
Table 1 (*continued*)

Compound		*h*EL IC_{50}[a] (μM)	*h*LPL IC_{50}[a] (μM)	*h*HL IC_{50}[a] (μM)	PPB[b] (%)	*c*log *P*[e]
19		0.499	>100	nd	85.5	2.67
20		0.551	>100	88.31	85.3	2.17
21		0.659	nd	nd	nd	2.08

[a] IC_{50}s are an average of at least two independent determinations.
[b] PPB refers to rat plasma protein binding assessed by equilibrium dialysis using 10% rat plasma, 90% PBS and 2.5 μM compound for 4 h at 37 °C, unless otherwise indicated.
[c] Mouse plasma protein binding.
[d] Human plasma protein binding.
[e] Calculated by ChemBioDraw Ultra 12.0 (CambridgeSoft, Cambridge, MA) with the option of 'Log *P*'. nd: not determined.

Table 2
SAR on replacement of carboxylic acid group



Compound	W	*h*EL IC_{50}[a] (μM)	Rat PPB (%)
22		0.209	99.0
23		>10	nd[b]
24		>10	nd[b]
25		1.946	nd[b]

[a] IC_{50} values are an average of at least two independent determinations.
[b] nd: not determined.

sufficient plasma drug levels are achieved. The pharmacokinetic parameters of XEN445 in mice are shown in Table 4. XEN445 has sufficiently high oral bioavailability and low clearance rates for it to be an appropriate candidate for testing in a mouse HDLc raising efficacy model.

Since the pharmacokinetic parameters (e.g., inhibitor AUC or trough plasma concentration) associated with the HDLc raising efficacy of EL inhibition are not known, male C57BL6 mice where administered XEN445 by oral gavage at a dose that would provide a high degree of EL inhibition throughout the duration of the experiment. Wild-type mice were orally dosed with XEN445 at 30 mg/kg *b.i.d.* for 3 days and blood was taken on the morning of day 4, 16 h post final dose. At termination, the average plasma levels of XEN445 was 9.9 μM and the drug caused an 18% and 16% increase in total plasma cholesterol and HDLc, respectively (Table 5, Fig. 2).

An HDLc increase of ~10% was observed when mice were treated for the same length of time with XEN445 at a dose of 10 mg/kg *b.i.d.* (data not shown). To determine whether the HDL-raising efficacy was due to on-target inhibition, XEN445, as well as vehicle, were dosed to EL knockout mice using the same paradigm. In agreement with previously published studies using the same founder mice,[17] the plasma HDLc concentration from vehicle-treated EL knockout mice was increased (121% in this study) compared to vehicle-treated wild-type mice (Table 5). However, XEN445 treatment for 3 days did not significantly alter the total plasma cholesterol or HDLc concentrations in EL knockout mice compared to vehicle alone (Table 5, Fig. 3). To determine whether a longer drug treatment paradigm would result in further increase in plasma

Table 3
In vitro potency as determined in functional HDL particle assay

Compound	**13**	**14**	**15**	**16**
*h*EL IC_{50}[a,b] (μM)	0.11	0.13	0.12	0.33

[a] IC_{50}s are an average of at least two independent determinations.
[b] HDL particle assay.

Table 4
Mouse Pharmacokinetics of XEN445[a]

C_{max} (μM)	T_{max} (h)	$C_{min24\ h}$ (μM)	AUC_{last} (μM h)	AUC_{last} (μM h)	$T_{1/2}$ (h)	CL (L/h/kg)	F (%)
62[b]	0.5[b]	4.5[b]	47[c]	253[b]	11.9[b]	0.06[c]	54

[a] All data from non-fasted, male, C57BL/6 mice ($n = 3$).
[b] Per oral (po), dose 10 mg/kg, 50% PEG 400 and 50% D5W.
[c] Intravenous (iv), dose 1 mg/kg, 10% ethanol 25% PEG 400 and 65% D5W.

Table 5
In vivo efficacy of XEN445 in wild-type and EL knockout mice

Strain	Treatment	Plasma total cholesterol		Plasma HDLc	
		Concentration (SEM) (mg/dL)	Percentage change (%)	Concentration (SEM) (mg/dL)	Percentage change (%)
Wild-type	3 d Vehicle	106.1 (3.9)	–	74.7 (2.2)	–
Wild-type	3 d XEN445	125.2 (7.2)*	+18	86.5 (4.2)*	+16
Wild-type	3 d Vehicle	103.5 (4.4)	–	57.1 (4.8)	–
EL KO	3 d Vehicle	219.6 (5.9)*	+112	126.4 (4.5)*	+121
EL KO	3 d XEN445	212.0 (11.7)‡	−3	117.0 (8.5)‡	−7
Wild-type	9 d Vehicle	108.3 (3.9)	–	68.2 (3.9)	–
Wild-type	9 d XEN445	131.2 (3.4)*	+21	88.7 (4.3)*	+30

* $p < 0.05$ versus vehicle-treated wild-type mice within each experiment.
‡ p = not significant versus vehicle-treated EL knockout mice.

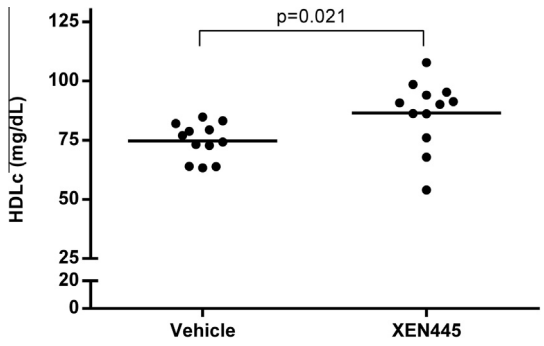


Figure 2. In vivo efficacy of XEN445 in wild-type mice dosed for 3 days at 30 mg/kg *b.i.d.*

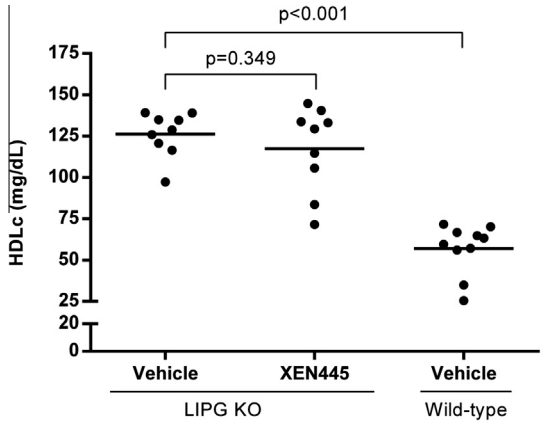


Figure 3. In vivo efficacy of XEN445 in LIPG knockout mice dosed for 3 days at 30 mg/kg *b.i.d.*

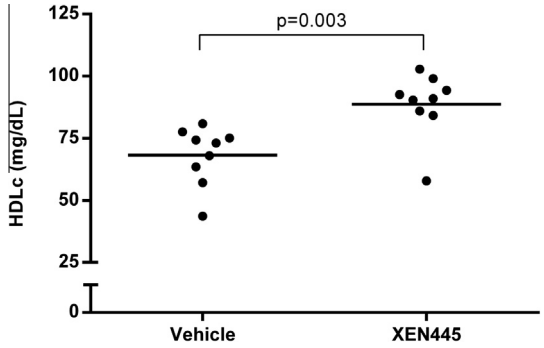


Figure 4. In vivo efficacy of XEN445 in wild-type mice dosed for 9 days at 30 mg/kg *b.i.d.*

HDL levels, XEN445 was dosed to wild-type mice for 9 days at a dose of 30 mg/kg *b.i.d.* Longer treatment with XEN445 resulted in a numerically greater plasma HDLc increase (+30%, Table 5, Fig. 4) than that observed with 3 days dosing.

4. Conclusion

We discovered a class of novel, potent and selective endothelial lipase (EL) inhibitors through the optimization of anthranilic acid hit **1** with focus on safety, potency, selectivity and protein binding properties. XEN445 demonstrated good in vitro potency against EL with high selectivity over LPL and HL. Most importantly, XEN445 demonstrated in vivo efficacy in raising plasma HDLc in wild-type mice. This class of compounds will be useful for further defining the catalytic and non-catalytic roles of EL in HDL metabolism and are promising starting points for the development of EL-directed therapeutics. Further optimization is needed to identify a more potent EL inhibitor suitable for potential therapeutic evaluation in humans.

5. Experimental section

5.1. General method

All chemicals, reagents and solvents were purchased from commercial sources and were either used as supplied or purified using reported methods. All final compounds reported herein exhibited spectral data consistent with their proposed structure by nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) and mass spectra data. NMR spectra were recorded on a Bruker Avance 300 spectrometer with chemical shifts (δ) reported in parts-per-million (ppm) relative to the residual signal of the deuterated solvent. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), coupling constants in Hertz and number of protons. Mass spectra were obtained using a Waters 2795/ZQ LC/MS system (Waters Corporation, Milford, MA). All final compounds were greater than 95% a/a pure as determined by analytical HPLC on Agilent 1200 systems (Agilent Technologies, Santa Clara, CA) using an EMD Chromolith SpeedROD RP-18e column (4.6 mm i.d. × 50 mm length) (Merck KGaA, Darmstadt, Germany). The mobile phase consisted of a gradient of component 'A' (0.1% v/v aqueous trifluoroacetic acid) and component 'B' (acetonitrile) at a flow rate of 1 mL/min. The gradient program used was as follows: initial conditions 5% B, hold at 5% B for 1 min., linear ramp from 5% to 95% B over 5 min, 100% B for 3 min., return to initial conditions for 1 min. Peaks were detected at a wavelength of 254 nm with an Agilent photodiode array detector. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Chemical names were generated using ChemBioDraw version 12.0 (CambridgeSoft, Cambridge,

MA.) The fluorogenic substrates bis-BODIPY-FL C_{11}-PC and PED-A_1 were purchased from Invitrogen. The EL knockout mice (on a pure C57BL6 background) were obtained from the laboratory of Dr. T. Quertermous (Standford University). The wild-type male C57BL6 mice were obtained from Taconic Farms Inc.

5.1.1. (*S*)-2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (4)

To a solution of 2-fluoro-5-(trifluoromethyl)benzoic acid (6.26 g, 30.0 mmol) and (*S*)-3-(4-fluorophenoxy)pyrrolidine (7.0 g, 38.62 mmol) in 1,4-dioxane (100 mL) was added 4-methylmorpholine (10.0 mL, 91.04 mmol). The resulting mixture was heated at 110 °C for 18 h, concentrated in vacuo, and the residue cooled in an ice bath and acidified with 5% hydrochloric acid solution to pH~3; extracted with EtOAc (100 mL × 3) and washed with water and brine. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography eluting with a gradient of 20–50% ethyl acetate in hexanes to give **4** as a colorless solid (6.84 g, 61%). mp 98–99 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36–8.21 (m, 1H), 7.72–7.65 (m, 1H), 7.25–7.20 (m, 1H), 6.99–6.92 (m, 2H), 6.85–6.77 (m, 2H), 4.98 (br s, 1H), 3.82–3.73 (m, 1H), 3.66–3.54 (m, 1H), 3.44–3.28 (m, 2H), 2.37–2.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 157.6 (d, J_{CF} = 238.0 Hz), 152.8, 150.8, 130.0, 129.7, 123.8 (q, J_{CF} = 269.9 Hz), 123.4 (q, J_{CF} = 31.1 Hz), 120.1, 118.9, 116.9, 116.8, 116.3, 116.0, 76.6, 58.7, 51.3, 31.4; MS (ES+) *m*/*z* 370.0 (M+1). Anal. Calcd for $C_{18}H_{15}F_4NO_3$: C, 58.54; H, 4.09; N, 3.79. Found: C, 58.52; H, 4.06; N, 3.89.

5.1.2. 2-(4-(4-Fluorophenoxy)piperidin-1-yl)-5-(trifluoromethyl)benzoic acid (2)

By a similar procedure described for **4**, **2** was obtained as a colorless solid (0.07 g, 10%). mp 146–147 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63–8.59 (m, 1H), 7.91–7.83 (m, 1H), 7.68–7.59 (m, 1H), 7.08–6.87 (m, 4H), 4.58 (br s, 1H), 3.51–3.26 (m, 2H), 3.09–2.90 (m, 2H), 2.27–2.12 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 157.6 (d, J_{CF} = 239.9 Hz), 153.7, 152.7 (d, J_{CF} = 2.2 Hz), 130.5 (q, J_{CF} = 3.2 Hz), 130.1 (q, J_{CF} = 33.2 Hz), 129.7 (q, J_{CF} = 3.5 Hz), 126.0, 123.2 (q, J_{CF} = 272.6 Hz), 123.1, 117.5 (d, J_{CF} = 8.0 Hz), 116.2 (d, J_{CF} = 23.1 Hz), 69.6, 50.0, 30.3; MS (ES+) *m*/*z* 384.1 (M+1).

5.1.3. 2-(3-(4-Fluorophenoxy)azetidin-1-yl)-5-(trifluoromethyl)benzoic acid (3)

By a similar procedure described for **4**, **3** was obtained as a colorless solid (1.00 g, 39%). mp 175–177 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.02 (s, 1H), 7.83–7.82 (m, 1H), 7.65–7.61 (m, 1H), 7.18–7.13 (m, 2H), 6.92–6.89 (m, 2H), 6.74–6.72 (m, 1H), 5.11–5.04 (m, 1H), 4.40–4.34 (m, 2H), 3.98–3.93 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.0, 156.9 (d, J_{CF} = 236.6 Hz), 152.7 (d, J_{CF} = 1.9 Hz), 151.8, 128.3 (q, J_{CF} = 3.3 Hz), 128.0 (q, J_{CF} = 3.6 Hz), 124.7 (q, J_{CF} = 270.3 Hz), 116.3 (d, J_{CF} = 23.1 Hz), 116.2 (q, J_{CF} = 32.7 Hz), 115.9 (d, J_{CF} = 8.6 Hz), 115.3, 114.5, 66.4, 60.0; MS (ES+) *m*/*z* 355.7 (M+1).

5.1.4. (*R*)-2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (5)

By a similar procedure described for **4**, **5** was obtained as a colorless solid (0.57 g, 60%). mp 97–99 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36–8.21 (m, 1H), 7.72–7.65 (m, 1H), 7.25–7.20 (m, 1H), 6.99–6.92 (m, 2H), 6.85–6.77 (m, 2H), 4.98 (br s, 1H), 3.82–3.73 (m, 1H), 3.66–3.54 (m, 1H), 3.44–3.28 (m, 2H), 2.37–2.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 159.2, 156.1, 152.7, 151.8 (d, J_{CF} = 140.2 Hz), 130.1, 129.7, 123.8 (q, J_{CF} = 269.9 Hz), 123.4 (q, J_{CF} = 31.1 Hz), 120.1, 118.9, 116.9, 116.8, 116.3, 116.0, 58.7, 51.3, 31.4; MS (ES+) *m*/*z* 370.0 (M+1).

5.1.5. 2-(3-(4-Fluorobenzyl)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (6)

By a similar procedure described for **4**, **6** was obtained as a colorless solid (0.76 g, 43%). mp 111–113 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.7 (br s, 1H), 8.23 (s, 1H), 7.66–7.58 (m, 1H), 7.20–6.83 (m, 5H), 3.46–3.05 (m, 4H), 2.82–3.53 (m, 3H), 2.22–2.08 (m, 1H), 1.85–1.67 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 161.5 (d, J_{CF} = 242.7 Hz), 150.7, 135.4, 130.0, 129.9, 129.7 (q, J_{CF} = 3.9 Hz), 129.6 (q, J_{CF} = 3.8 Hz), 124.0 (q, J_{CF} = 260.9 Hz), 117.5, 115.5, 115.2, 58.2, 52.6, 40.6, 38.5, 31.2; MS (ES+) *m*/*z* 368.0 (M+1).

5.1.6. (*S*)-5-(Trifluoromethyl)-2-(3-(5-(trifluoromethyl)-pyridin-2-yloxy)pyrrolidin-1-yl)benzoic acid (7)

By a similar procedure described for **4**, **7** was obtained as a colorless solid (0.37 g, 87%). mp 46–47 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.27 (s, 1H), 7.80–7.73 (m, 1H), 7.70–7.64 (m, 1H), 7.21–7.14 (m, 1H), 6.85–6.80 (m, 1H), 5.75 (s, 1H), 3.89–3.80 (m, 1H), 3.69–3.60 (m, 1H), 3.43–3.29 (m, 2H), 2.48–2.31 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.6, 150.7, 144.7, 136.1, 130.1, 129.7, 122.0 (q, J_{CF} = 269.4 Hz), 120.5 (q, J_{CF} = 32.8 Hz), 111.8, 75.0, 58.8, 51.2, 31.6; MS (ES+) *m*/*z* 420.9 (M+1).

5.1.7. (*S*)-2-(3-(6-Chloropyridin-3-yloxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (8)

By a similar procedure described for **4**, **8** was obtained as a colorless solid (0.79 g, 50%). mp 42–43 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.28 (br s, 1H), 8.23 (s, 1H), 8.09–8.04 (m, 1H), 7.69–7.62 (m, 1H), 7.27–7.10 (m, 3H), 5.10–5.04 (m, 1H), 3.89–3.82 (m, 1H), 3.68–3.57 (m, 1H), 3.46–3.37 (m, 1H), 3.32–3.25 (m, 1H), 2.38–2.32 (m, 2H); MS (ES+) *m*/*z* 386.9 (M+1).

5.1.8. (*S*)-2-(3-((4-Fluorobenzyl)oxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (9)

By a similar procedure described for **4**, **9** was obtained as a colorless foam (0.23 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.9 (br s, 1H), 8.24 (s, 1H), 7.68–7.60 (m, 1H), 7.31–7.22 (m, 2H), 7.19–7.13 (m, 1H), 7.03–6.95 (m, 2H), 4.48 (ABq, 2H), 4.33–4.24 (m, 1H), 3.61–3.45 (m, 2H), 3.39–3.28 (m, 1H), 3.25–3.18 (m, 1H), 2.30–2.08 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 162.3 (d, J_{CF} = 244.5 Hz), 151.0, 133.5, 129.9, 129.4, 124.3 (q, J_{CF} = 269.9 Hz), 122.7 (q, J_{CF} = 32.1 Hz), 119.7, 118.6, 115.5, 115.2, 77.6, 70.3, 58.6, 51.2, 31.3; MS (ES+) *m*/*z* 384.0 (M+1).

5.1.9. (*S*)-2-(3-(Cyclopropylmethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (10)

By a similar procedure described for **4**, **10** was obtained as a colorless foam (0.32 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33–8.30 (m, 1H), 7.72–7.65 (m, 1H), 7.34–7.27 (m, 1H), 4.27–4.20 (m, 1H), 3.59–3.14 (m, 6H), 2.26–2.07 (m, 2H), 1.10–0.96 (m, 1H), 0.58–0.49 (m, 2H), 0.23–0.14 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 151.5, 130.1, 129.4, 124.7, 123.8 (q, J_{CF} = 269.9 Hz), 121.6, 120.2, 77.6, 73.8, 59.3, 52.1, 31.7, 10.6, 3.2, 3.1; MS (ES+) *m*/*z* 330.0 (M+1).

5.1.10. (*S*)-2-(3-Butoxypyrrolidin-1-yl)-5-(trifluoromethyl)-benzoic acid (12)

By a similar procedure described for **4**, **12** was obtained as a colorless oil (1.0 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38–8.37 (m, 1H), 7.74–7.72 (m, 1H), 7.38–7.35 (m, 1H), 4.20 (br s, 1H), 3.57–3.31 (m, 5H), 3.20–3.17 (m, 1H), 2.21–2.14 (m, 2H), 1.62–1.53 (m, 2H), 1.44–1.32 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 151.6, 130.2 (q, J_{CF} = 3.3 Hz), 129.3 (q, J_{CF} = 3.8 Hz), 125.0, 123.7 (q, J_{CF} = 271.8 Hz), 122.3, 120.8, 77.9,

68.9, 59.5, 52.5, 31.8, 31.7, 19.3, 13.8; MS (ES+) *m*/*z* 332.1 (M+1), MS (ES-) *m*/*z* 330.2.

5.1.11. (*S*)-2-(3-(Pyridin-2-ylmethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (13)

By a similar procedure described for **4**, **13** was obtained as a colorless foam (1.20 g, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.75 (br s, 1H), 8.60–8.55 (m, 1H), 8.15 (s, 1H), 7.75–7.66 (m, 1H), 7.61–7.54 (m, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.28–7.20 (m, 1H), 7.07 (d, J = 8.7 Hz, 1H), 4.73 (s, 2H), 4.39–4.34 (m, 1H), 3.67–3.49 (m, 2H), 3.39–3.30 (m, 1H), 3.28–3.20 (m, 1H), 2.34–2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 157.4, 150.5, 147.9, 137.9, 129.2, 123.8 (q, J_{CF} = 269.4 Hz), 123.0, 122.1, 121.6, 120.5 (q, J_{CF} = 33.4 - Hz), 117.9, 117.6, 78.6, 70.7, 58.2, 50.5, 31.4; MS (ES+) *m*/*z* 366.9 (M+1). Anal. Calcd for $C_{18}H_{17}F_3N_2O_3$: C, 59.02; H, 4.68; N, 7.65. Found C, 58.97; H, 5.01; N, 7.62.

5.1.12. (*S*)-2-(3-(2-Ethoxyethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (19)

By a similar procedure described for **4**, **19** was obtained as a colorless oil (0.32 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.84 (br s, 1H), 8.14 (s, 1H), 7.58–7.54 (m, 1H), 7.14–7.09 (m, 1H), 4.20 (br s, 1H), 3.59–3.14 (m, 10H), 2.17–2.05 (m, 2H), 1.12 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 150.8, 129.6, 129.2, 124.0 (q, J_{CF} = 269.6 Hz), 121.9 (q, J_{CF} = 34.0 Hz), 119.5, 118.2, 78.4, 69.7, 68.3, 66.7, 58.4, 51.0, 31.2, 15.0; MS (ES+) *m*/*z* 348.0 (M+1).

5.1.13. (*S*)-2-(3-Hydroxypyrrolidin-1-yl)-5-(trifluoromethyl) benzoic acid (11a)

By a similar procedure described for **4**, **11a** was obtained as a colorless foam (10.70 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.76–7.70 (m, 1H), 7.45–7.39 (m, 1H), 4.69 (s, 1H), 3.63–3.50 (m, 2H), 3.79–3.28 (m, 1H), 3.24–3.16 (m, 1H), 2.35–2.10 (m, 2H); MS (ES+) *m*/*z* 276.1 (M+1).

5.1.14. (*S*)-Methyl 2-(3-hydroxypyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (11b)

To a solution of **11a** (8.00 g, 29.06 mol) in DMF (30 mL) was added K_2CO_3 (4.40 g, 31.83 mol). The mixture was stirred at ambient temperature for 0.5 h, MeI (2.40 mL, 38.55 mmol) was added, and stirring was continued for 18 h at ambient temperature, diluted with EtOAc (300 mL), washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo to give **11b** as a colorless oil (8.37 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (m, 1H), 7.51–7.46 (m, 1H), 6.79–6.75 (m, 1H), 4.52 (br s, 1H), 3.87 (s, 3H), 3.65–3.53 (m, 2H), 3.31–3.23 (m, 1H), 2.99–2.92 (m, 1H), 2.11–1.95 (m, 2H).

5.1.15. (*S*)-Methyl 2-(3-(2-cyclopropylethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)-benzoate (11c)

To a solution of **11b** (1.50 g, 5.18 mmol) in DMF (20 mL) was added NaH (60% in mineral oil w/w, 0.23 g, 5.75 mmol) in small portions at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, 2-cyclopropylethyl 4-methylbenzenesulfonate, (1.60 g, 6.65 mmol) was added; stirring was continued for 18 h and then heated at 50 °C for 4 h. The reaction mixture was quenched by addition of aqueous saturated NH_4Cl solution (10 mL) at 0 °C and extracted with ethyl acetate (100 mL × 3), washed with water and brine' dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography with a gradient of 10–30% EtOAc in hexanes to give **11c** as a colorless oil (1.76 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.79 (m, 1H), 7.50–7.45 (m, 1H), 6.80–6.75 (m, 1H), 4.14–4.08 (m, 1H), 3.88 (s, 3H), 3.58–3.38 (m, 4H), 3.36–3.28 (m, 1H), 3.08–3.00 (m, 1H), 2.16–2.00 (m, 2H), 1.43–1.35 (m, 2H), 0.69–0.59 (m, 1H), 0.41–0.35 (m, 2H), 0.19–0.03 (m, 2H); MS (ES+) *m*/*z* 358.1 (M+1).

5.1.16. (*S*)-2-(3-(2-Cyclopropylethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl) benzoic acid (11)

To a solution of **11c** (1.70 g, 4.75 mmol) in THF (30 mL) and water (10 mL) was added NaOH (1.90 g, 11.0 mmol) at ambient temperature. The reaction mixture was heated to reflux for 18 h. The solvent was removed in vacuo, and the residue was neutralized to pH ~3 with 5% hydrochloric acid solution' extracted with EtOAc (50 mL × 3) and washed with water and brine. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and the residue was subjected to column chromatography eluting with a gradient of 10–50% ethyl acetate in hexanes to give **11** as a colorless oil (1.28 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36–8.22 (m, 1H), 7.70–7.63 (m, 1H), 7.28–7.23 (m, 1H), 4.22–4.17 (m, 1H), 3.59–3.29 (m, 5H), 3.20–3.14 (m, 1H), 2.26–2.05 (m, 2H), 1.44 (q, J = 6.9 Hz, 2H), 0.77–0.63 (m, 1H), 0.43–0.35 (m, 2H), 0.45–0.37 (m, 2H); MS (ES+) *m*/*z* 344.1 (M+1).

Compounds **14**–**17** and **20**–**21** were prepared following the general procedure for **11**.

5.1.17. (*S*)-Methyl 2-(3-((5-fluoropyridin-2-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (14a)

By a similar procedure described for **11c**, **14a** was obtained as a colorless oil (0.74 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36–8.34 (m, 1H), 7.82 (s, 1H), 7.51–7.46 (m, 1H), 7.34–7.30 (m, 2H), 6.81–6.77 (m, 1H), 4.66–4.52 (m, 2H), 4.28 (br s, 1H), 3.87 (s, 3H), 3.63–3.49 (m, 2H), 3.38–3.30 (m, 1H), 3.11–3.06 (m, 1H), 2.26–2.03 (m, 2H).

5.1.18. (*S*)-2-(3-((5-Fluoropyridin-2-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (14)

By a similar procedure described for **11**, **14** was obtained as a colorless foam (0.52 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.16 (s, 1H), 7.62–7.57 (m, 1H), 7.41–7.32 (m, 2H), 7.10–7.06 (m, 1H), 4.65 (s, 2H), 4.37–4.33 (m, 1H), 3.63–3.49 (m, 2H), 3.37–3.27 (m, 1H), 3.25–3.18 (m, 1H), 2.32–2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 158.8 (d, J_{CF} = 253.8 Hz), 153.7, 150.6, 137.0, 136.7, 129.4, 124.1 (q, J_{CF} = 269.7 Hz), 124.0 (d, J_{CF} = 18.2 Hz), 123.9, 122.9 (d, J_{CF} = 4.4 Hz), 121.7 (q, J_{CF} = 35.6 Hz), 119.5, 117.6, 78.4, 70.7, 60.4, 50.5, 31.3; MS (ES+) *m*/*z* 384.9 (M+1).

5.1.19. (*S*)-Methyl 2-(3-((3-fluoropyridin-2-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (15a)

By a similar procedure described for **11c**, **15a** was obtained as a colorless oil (0.44 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28–8.25 (m, 1H), 7.74–7.73 (m, 1H), 7.40–7.36 (m, 1H), 7.29–7.22 (m, 1H), 7.17–7.10 (m, 1H), 6.70–6.66 (m, 1H), 4.67–4.53 (m, 2H), 4.24 (br s, 1H), 3.78 (s, 3H), 3.54–3.38 (m, 2H), 3.28–3.20 (m, 1H), 3.05–3.01 (m, 1H), 2.18–1.94 (m, 2H).

5.1.20. (*S*)-2-(3-((3-Fluoropyridin-2-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (15)

By a similar procedure described for **11**, **15** was obtained as a colorless foam (0.41 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43–8.40 (m, 1H), 8.21–8.19 (m, 1H), 7.62–7.57 (m, 1H), 7.45–7.37 (m, 1H), 7.33–7.27 (m, 1H), 7.20–7.14 (m, 1H), 4.75 (ABq, 2H), 4.41–4.38 (m, 1H), 3.65–3.59 (m, 1H), 3.52–3.42 (m, 1H), 3.39–3.31 (m, 1H), 3.24–3.19 (m, 1H), 2.34–2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 158.2 (d, J_{CF} = 257.5 Hz), 151.1, 145.1, 144.9, 129.7, 129.2, 125.1, 124.0, 123.7, 119.0, 78.5, 66.4, 58.8, 51.3, 31.4; (ES+) *m*/*z* 384.9 (M+1).

5.1.21. (*S*)-Methyl 2-(3-((5-fluoropyridin-3-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (16a)

By a similar procedure described for **11c**, **16a** was obtained as a colorless oil (0.08 g, 6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35–8.30 (m,

2H), 7.82 (s, 1H), 7.50–7.46 (m, 1H), 7.35–7.31 (m, 1H), 6.80–676 (m, 1H), 4.51 (ABq, 2H), 4.22 (br s, 1H), 3.87 (s, 3H), 3.63–3.48 (m, 2H), 3.37–3.29 (m, 1H), 3.11–3.00 (m, 1H), 2.22–2.05 (m, 2H).

5.1.22. (*S*)-2-(3-((5-Fluoropyridin-3-yl)methoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (16)

By a similar procedure described for **11**, **16** was obtained as a colorless foam (0.07 g, 93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46–8.44 (m, 1H), 8.38 (s, 1H), 7.64–7.57 (m, 1H), 7.29–7.27 (m, 1H), 7.22–7.17 (m, 1H), 6.57–6.53 (m, 1H), 4.54 (ABq, 2H), 4.22–4.16 (m, 1H), 3.71–3.64 (m, 1H), 3.44–3.32 (m, 3H), 2.10–1.89 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 159.5 (d, J_{CF} = 252.7 Hz), 147.3, 145.3, 137.2, 136.8, 131.6, 126.1 (q, J_{CF} = 269.0 Hz), 125.6, 123.6, 122.5, 122.3, 114.6 (q, J_{CF} = 29.7 Hz), 112.4, 78.3, 66.9, 55.0, 47.2, 30.8; MS (ES-) *m*/*z* 383.1 (M−1).

5.1.23. (*S*)-Methyl 2-(3-(2-(benzyloxy)ethoxy)-pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (17a)

By a similar procedure described for **11c**, **17a** was obtained as a colorless oil (3.40 g, 29%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.50–7.46 (m, 1H), 7.35–7.22 (m, 5H), 6.79–6.75 (m, 1H), 4.51 (s, 2H), 4.15–4.20 (m, 1H), 3.87 s, 3H), 3.66–3.43 (m, 6H), 3.38–3.28 (m, 1H), 3.09–3.03 (m, 1H), 2.09–1.98 (m, 2H).

5.1.24. (*S*)-2-(3-(2-(Benzyloxy)ethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (17)

By a similar procedure described for **11**, **17** was obtained as a colorless oil (0.27 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.53–7.50 (m, 1H), 7.38–7.22 (m, 6H), 4.53 (s, 2H), 4.23 (s, 1H), 3.64–3.15 (m, 8H), 2.27–2.03 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 151.3, 137.9, 130.0, 129.2, 128.4, 127.8, 124.0, 123.9 (q, J_{CF} = 265.7 Hz), 123.5, 121.0, 119.9, 78.5, 73.3, 69.5, 68.4, 59.0, 52.0, 31.7; MS (ES+) *m*/*z* 410.0 (M+1).

5.1.25. (*S*)-Methyl 2-(3-(2-hydroxyethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (18a)

A solution of **17a** (2.90 g, 6.84 mmol) in methanol (100 mL) was hydrogenated with 10% Pd/C (0.5 g) under atmosphere hydrogen at ambient temperature for 20 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo, the residue was subjected to column chromatography eluating with 30% ethyl acetate in hexanes to give **18a** as a colorless oil (2.00 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.82 (m, 1H), 7.51–7.46 (m, 1H), 6.80–6.76 (m, 1H), 4.17–4.14 (m, 1H), 3.88 (s, 3H), 3.68–3.44 (m, 6H), 3.33–3.27 (m, 1H), 3.08–3.03 (m, 1H), 2.18–1.94 (m, 3H); MS (ES+) *m*/*z* 333.9 (M+1).

5.1.26. (*S*)-2-(3-(2-Hydroxyethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (18)

By a similar procedure described for **11**, **18** was obtained as a colorless oil (0.26 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.61–7.57 (m, 1H), 7.15–7.11 (m, 1H), 4.20 (br s, 1H), 3.72–3.66 (m, 2H), 3.61–3.43 (m, 4H), 3.30–3.18 (m, 2H), 2.21–2.04 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 150.3, 129.7, 129.2, 123.9 (q, J_{CF} = 269.7 Hz), 122.7 (q, J_{CF} = 33.7 Hz), 120.3, 118.5, 78.3, 70.2, 61.6, 58.5, 51.0, 31.0; (ES+) *m*/*z* 320.0 (M+1).

5.1.27. (*S*)-Methyl 2-(3-(2-(2-methoxyethoxy)ethoxy)-pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (20a)

By a similar procedure described for **11c**, **20a** was obtained as a colorless oil (0.70 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.82 (m, 1H), 7.50–7.45 (m, 1H), 6.79–6.76 (m, 1H), 4.20–4.15 (m, 1H), 3.88 (s, 1H), 3.64–3.42 (m, 10H), 3.40–3.26 (m, 4H), 3.07–3.02 (m, 1H), 2.19–2.00 (m, 2H); (ES+) *m*/*z* 392.0 (M+1).

5.1.28. (*S*)-2-(3-(2-(2-Methoxyethoxy)ethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (20)

By a similar procedure described for **11**, **20** was obtained as a colorless oil (0.40 g, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31–8.29 (m, 1H), 7.71–7.66 (m, 1H), 7.34–7.30 (m, 1H), 4.28–4.20 (m, 1H), 3.68–3.58 (m, 6H), 3.54–3.47 (m, 3H), 3.45–3.38 (m, 1H), 3.34–3.26 (m, 4H), 3.25–3.19 (m, 1H), 2.27–2.06 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 151.5, 130.0, 129.2, 124.9, 124.5, 123.8 (q, J_{CF} = 266.3 Hz), 122.1, 120.6, 78.5, 71.8, 70.6, 70.5, 68.4, 59.2, 58.9, 52.6, 31.7; MS (ES+) *m*/*z* 378.1 (M+1).

5.1.29. (*S*)-Methyl 2-(3-(2-morpholinoethoxy)-pyrrolidin-1-yl)-5-(trifluoromethyl)benzoate (21a)

By a similar procedure described for **11c**, **21a** was obtained as a colorless oil (0.24 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.75 (m, 1H), 7.47–7.39 (m, 1H), 6.74–6.70 (m, 1H), 4.07–4.02 (m, 1H), 3.82 (s, 3H), 3.64–3.36 (m, 8H), 3.27–3.19 (m, 1H), 3.02–3.97 (m, 1H), 2.48–2.35 (m, 6H), 2.09–1.95 (m, 2H); MS (ES+) *m*/*z* 403.1 (M+1).

5.1.30. (*S*)-2-(3-(2-Morpholinoethoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzoic acid (21)

By a similar procedure described for **11**, **21** was obtained as a colorless solid (0.13 g, 55%). mp 77–79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.44–7.40 (m, 1H), 6.78–6.74 (m, 1H), 4.09 (br s, 1H), 3.94–3.67 (m, 6H), 3.54–3.43 (m, 2H), 3.32–3.19 (m, 2H), 3.03–2.82 (m, 6H), 2.17–1.99 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 149.0, 128.1, 127.7, 124.6 (q, J_{CF} = 269.1 Hz), 121.7, 118.9 (q, J_{CF} = 34.9 Hz), 114.5, 78.6, 64.5, 64.2, 57.4, 56.0, 53.1, 48.5, 31.0; MS (ES+) *m*/*z* 389.1 (M+1).

5.1.31. (*S*)-2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzonitrile (22a)

To a suspension of 2-fluoro-5-(trifluoromethyl)benzonitrile (3.80 g, 20.00 mmol) and (*S*)-3-(4-fluorophenoxy)pyrrolidine (3.80 g, 20.97 mmol) in 1,4-dioxane (100 mL) was added cesium carbonate (9.82, 30.13 mmol). The reaction mixture was heated at reflux for 19 h. The reaction mixture was colded to ambient temperature and filtered. The filtrate was concentrated *in vacuo*, the residue was subjected to column chromatography (10% EtOAc in hexanes) to give **22a** as a pale yellow oil (4.70 g, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.52–7.47 (m, 1H), 7.00–6.93 (m, 2H), 6.83–6.78 (m, 2H), 6.70–6.66 (m, 1H), 4.91–4.98 (m, 1H), 4.05–4.01 (m, 1H), 3.90–3.72 (m, 3H), 2.39–2.12 (m, 2H); (ES+) *m*/*z* 351.0 (M+1).

5.1.32. (*S*)-5-(2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-1*H*-tetrazole (22)

To a solution of **22a** (0.49 g, 1.39 mmol) in DMF (20 mL) was added NaN_3 (1.80 g, 27.68 mmol) and NH_4Cl (1.48 g, 27.66 mmol). The resulting mixture was heated at 120 °C for 72 h. The reaction was cooled to ambient temperature, followed by addition of water (10.0 mL) and extraction with EtOAc (50 mL × 3). The combined organic phase was washed with water and brine, dried over anhydrous sodium sulfate and concentrated in vacuo, the residue was subjected to column chromatography (50% EtOAc in hexanes) to give **22** as a colorless solid (0.14 g, 25%). mp 97–99 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68–7.23 (m, 1H), 7.59–7.57 (m, 1H), 7.11–7.00 (m, 3H), 6.91–6.84 (m, 2H), 4.94 (br s, 1H), 3.33–3.15 (m, 2H), 3.07–3.00 (m, 1H), 2.86–2.81 (m, 1H), 2.15–1.94 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6)) δ 158.7, 155.5, 155.0, 151.7 (d, J_{CF} = 259.5 Hz), 130.0, 128.5, 125.1 (q, J_{CF} = 268.6 - Hz), 117.3, 117.2, 116.5, 116.2, 115.2, 109.1, 76.6, 55.9, 48.3, 30.6; MS (ES+) *m*/*z* 394.0 (M+1).

5.1.33. (*S*)-2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)benzenesulfonamide (23)

To a solution of 2-fluoro-5-(trifluoromethyl)benzenesulfonamide (3.80 g, 14.63 mmol) and (*S*)-3-(4-fluorophenoxy)pyrrolidine (3.00 g, 16.55 mmol) in 1,4-dioxane (40 mL) was added 4-methylmorpholine (8.0 mL, 72.76 mmol). The resulting mixture was heated at 110 °C for 44 h, concentrated in vacuo, and the residue cooled in an ice bath and acidified with 5% hydrochloric acid solution to pH ~3; extracted with EtOAc (100 mL × 3) and washed with water and brine. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography (30% ethyl acetate in hexanes) and recrystalization from EtOAc to give **23** as a colorless solid (5.35 g, 90%). mp 122–124 °C (EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15–8.13 (m, 1H), 7.64–7.59 (m, 3H), 7.12–7.06 (m, 3H), 6.96–6.91 (m, 2H), 5.06 (br s, 1H), 4.08–4.02 (m, 1H), 3.73–3.51 (m, 3H), 2.26–2.03 (m, 2H); MS (ES+) *m*/*z* 405.0 (M+1).

5.1.34. (*S*)-*N*-((2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)-sulfonyl)acetamide (24)

To a solution of (*S*)-2-(3-(4-fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)-benzenesulfonamide **23** (0.70 g, 1.73 mmol) in pyridine (20 mL) was added acetyl chloride (0.15 mL, 2.11 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 19 h and then concentrated in vacuo. To the residue was added aqueous saturated NH_4Cl solution (50 mL) and extracted with EtOAc (50 mL × 3). The combined organics were washed brine' dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography (50% EtOAc in hexanes) to give **24** as a colorless solid (0.41 g, 54%). mp 52–54 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.57 (br s, 1H), 8.31 (s, 1H), 7.32–7.28 (m, 1H), 6.99–6.93 (m, 2H), 6.86–6.80 (m, 2H), 4.91 (br s, 1H), 3.76–3.55 (m, 3H), 3.32–3.24 (m, 1H), 2.40–2.12 (m, 2H), 2.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 157.7 (d, J_{CF} = 238.2 Hz), 152.7, 150.5, 131.2, 129.0, 124.0 (q, J_{CF} = 34.2 Hz), 123.4 (q, J_{CF} = 270.5 Hz), 121.6, 116.8, 116.7, 116.4, 116.1, 77.6, 59.1, 51.2, 31.3, 23.4; MS (ES+) *m*/*z* 447.0 (M+1).

5.1.35. (*S*)-1-(2-(3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ethanone (25a)

To a solution of 1-(2-fluoro-5-(trifluoromethyl)phenyl)ethanone (4.99 g, 24.0 mmol) and (*S*)-3-(4-fluorophenoxy)pyrrolidine (4.4 g, 24.28 mmol) in 1,4-dioxane (60 mL) was added 4-methylmorpholine (3.5 mL, 31.83 mmol). The reaction mixture was heated at 110 °C for 23 h, concentrated in vacuo, and the residue was dissolved in EtOAc (250 mL) and washed with water and brine. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography (15% EtOAc in hexanes) to give **25a** as a pale yellow oil (5.70 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76–7.75 (m, 1H), 7.56–7.51 (m, 1H), 6.98–6.91 (m, 2H), 6.88–6.84 (m, 1H), 6.79–6.74 (m, 2H), 4.93–4.90 (m, 1H), 3.70–3.52 (m, 2H), 3.39–3.31 (m, 1H), 2.92–2.87 (m, 1H), 2.60 (s, 3H), 2.31–2.18 (m, 2H); MS (ES+) *m*/*z* 368.0 (M+1).

5.1.36. 1-(2-((*S*)-3-(4-Fluorophenoxy)pyrrolidin-1-yl)-5-(trifluoromethyl)phenyl)ethanol (25)

To a solution of **25a** (3.67 g, 10.0 mmol) in methanol (80 mL) was added sodium borohydride (0.5 g, 13.20 mmol) in small portions at 0 °C. The reaction mixture was stirred at ambient temperature for 30 min, brine (20 mL) was added and stirred for another 30 min. The mixture was extracted with EtOAc (50 mL × 3) and washed with water and brine. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated in vacuo, the residue was subjected to column chromatography (30% EtOAc in hexanes) to give **25** as a colorless oil (3.65 g, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64–7.59 (m, 1H), 7.48–7.43 (m, 1H), 7.18–7.12 (m, 1H), 7.02–6.89 (m, 2H), 6.85–6.77 (m, 2H), 5.22–5.15 (m, 1H), 4.95–4.91 (m, 1H), 4.21 (s, 0.6H), 3.93 (s, 0.4H), 3.70–3.37 (m, 2.4H), 3.31–3.24 (m, 1H), 3.15–3.07 (m, 0.6H), 2.38–2.15 (m, 2H), 1.61–1.50 (m, 3H); MS (ES+) *m*/*z* 370.0 (M+1).

5.1.37. Preparation of recombinant EL, HL and LPL

Recombinant human and mouse EL, human LPL and human HL were each obtained by transient transfection of HEK-293 cells with full length codon-optimised sequences in pcDNA3.1 plasmid vectors. Cells were grown in 225 cm^2 flasks in DMEM plus 10% FBS to 50–80% confluency and transfected with 95 μg of plasmid DNA using Lipofectamine (Invitrogen). After 5 h, the media was replaced by Opti-MEM media (invitrogen) and the cells cultured for 72 h before the cell-associated lipase was displaced by the addition of heparin to a final concentration of 50 U/mL. After 30 min incubation with cells, the media was removed and concentrated 100-fold using Amicon Ultra-15 concentrators (30 kDa cutoff) and frozen at −80 °C until used. For each assay of enzyme activity (below), concentrated media from HEK-293 cells following mock-transfection was used to determine the background activity. In each case, this was less-than 5% that using media from lipase-transfected cells. The active site concentration of each lipase in the concentrated media was determined using the irreversible inhibitor orlistat. A serial dilution of orlistat was added to the concentrated media and incubated for 1 h. The mixture was then diluted into assay buffer containing substrate (bis-BODIPY-FL C_{11}-PC) as described below and the initial reaction rate monitored. The lipase active site concentrations for EL, HL and LPL were 137, 207 and 700 nM, respectively, and represent the lowest concentrations of orlistat in the preincubation mix that resulted in complete inhibition.

5.1.38. Measurement of lipase activities

A mixed micelle fluorogenic phosphatidylcholine/Triton X-100 assay was used to titrate inhibitors against human and mouse EL, LPL and HL activities.[30] EL was diluted in PBS (1.4 nM final concentration) containing 14% DMEM, plus 2% glycerol and 40 μL was added to each well of a clear bottom 96-well plate containing inhibitors or vehicle alone (5 μL in 10% DMSO). The enzyme-inhibitor mix was incubated at room temperature for 30 min prior to the addition of substrate (5 μL of 10 μM bis-BODIPY-FL C_{11}-PC in 0.1% Triton X-100) and enzyme activity determined using a fluorescence plate reader at excitation and emission wavelengths of 490 and 520 nm, respectively. The activity of LPL and HL were determined in the same manner, except that the LPL assay (4 nM final concentration) required addition of ApoCII (MJS Biolynx Inc, 1 μg/uL final concentration). The HL assay (6 nM final concentration) employed Tris buffer (50 mM Tris, pH 8.5, 1 M NaCl, no DMDM or glycerol).

The potencies of EL inhibitors were also determined using a more physiological human HDL particle substrate in an assay similar to the method reported by Keller.[31] In brief, 35 μL human EL (diluted to 3.7 nM in PBS) in 96-well plates, in the presence or absence of test compounds (diluted from 100× stocks in DMSO), was preincubated at room temperature for 30 min. Orlistat, at 10 μM final concentration, was used as a 100% inhibition control. HDL substrate (10 μL) isolated from fresh human donor plasma (Intracel Frederick, MD) was added to the enzyme mix and incubated at 37 °C for 2 h. The final concentration of phospholipids in the assay was 1 mM which was determined using a Phospholipids C kit (Wako Diagnostics, Richmond VA). EL-mediated HDL hydrolysis was determined by measuring released free fatty acids in the final reaction using a NEFA-HR(2) kit (Wako Diagnostics, Richmond, VA) according to the manufacturer's instructions.

For the cell-associated EL activity assay, HEK-293 cells were transfected with 2 μg full length human EL plasmid in 35 mm^2 wells containing DMEM plus 10% FBS as described above, except that fugene (Roche) replaced lipofectamine. At 24 h post transfection, the cells were harvested and seeded in 96-well plates (1×10^6 cells/well) and cultured in DMEM for a further 24 h. The media was removed and replaced with HBSS containing 25 mM HEPES, pH 7.4 and inhibitors added as 200-fold stocks in DMSO. After an additional 30 min at 37 °C, substrate PED-A1 was added to a final concentration of 4 μM and the reaction monitored continuously using a FlexStation 3 fluorescence plate reader (Molecular Devices) at 37 °C to obtain the initial velocity. The titrations were performed twice and IC_{50} values obtained differed by a factor of 2 or less.

5.1.39. In vivo models

Male EL knockout mice (12–16 weeks of age) and age-matched wild-type male C57BL6 mice were acclimatized for 1 week before experimentation, during which they were fed 7012 Teklad LM-485 Mouse diet (Harlan). During the drug efficacy studies the mice were fed the same diet and given *b.i.d.* oral doses ($t = 8$ h) of vehicle, 30 mg/kg XEN455 (as a suspension in 0.2% Tween-20/1% carboxymethyl-cellulose) for either 3 d or for 9 d. At 16 h post the final dose, approximately 150 μL blood was removed by sub-mandibular vein bleed and EDTA-plasma prepared for analysis of total cholesterol, HDLc and drug concentrations. Further blood (~400 μL) was then immediately obtained by cardiac puncture at euthanasia and serum prepared following the 9 d study. Total plasma cholesterol and plasma HDLc concentrations were determined using the HDL-cholesterol E kit (Wako Diagnostics, Richmond VA).

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