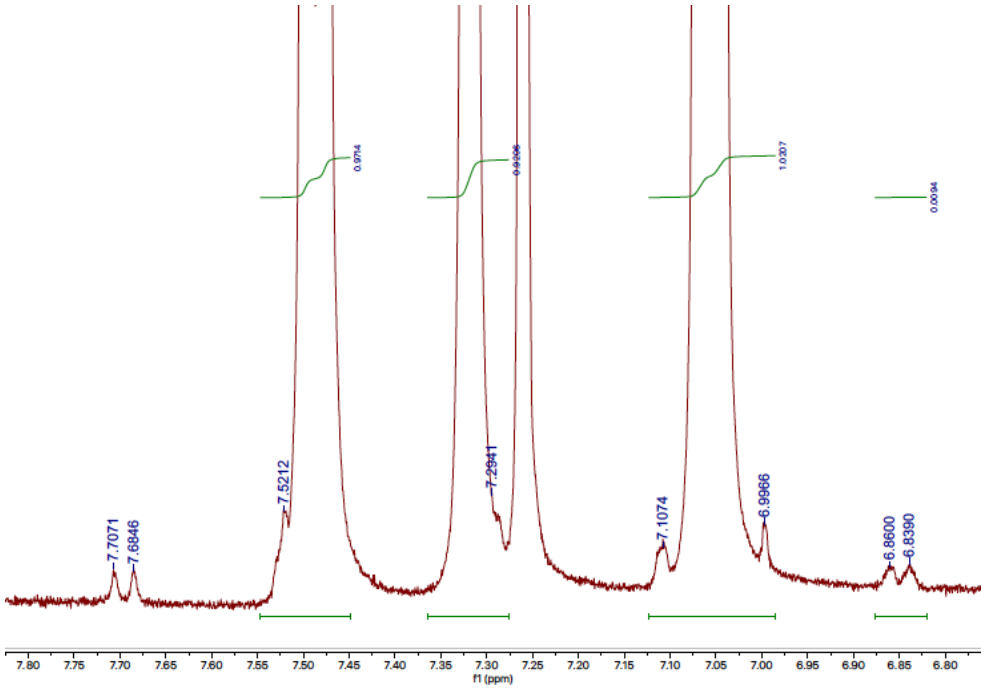
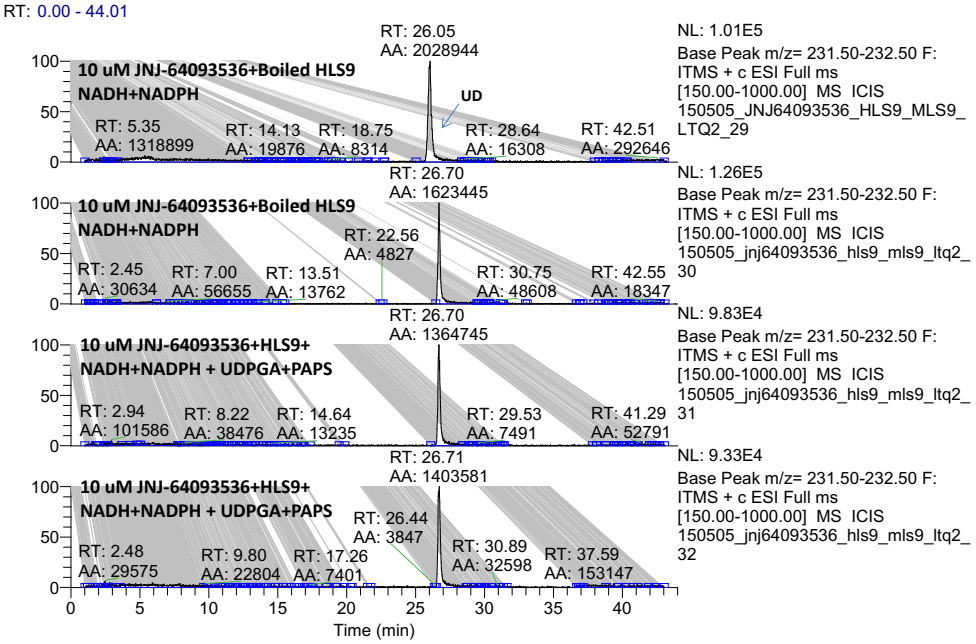


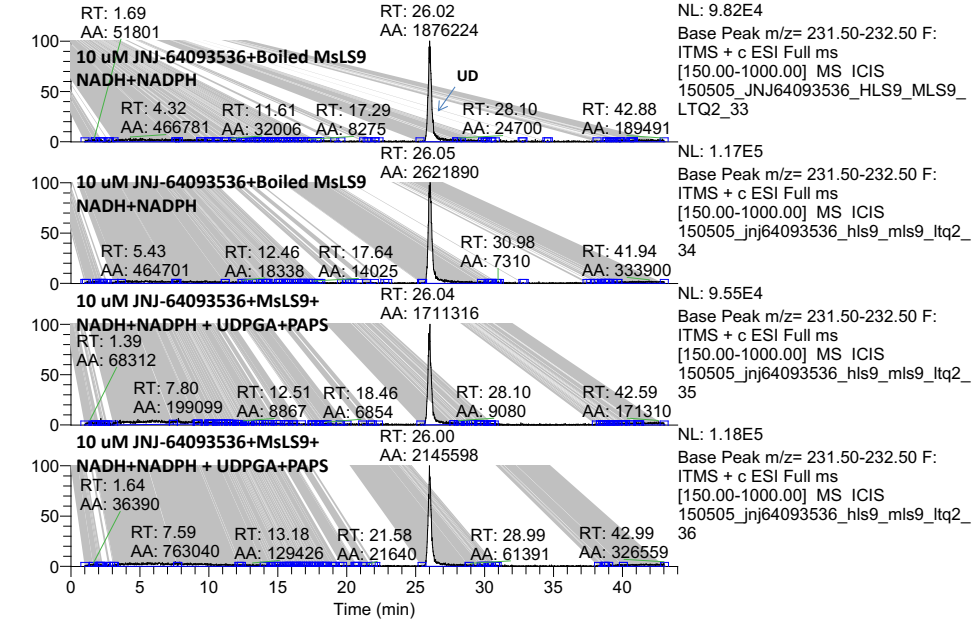
Fig. S1. Expanded aromatic region of the ^{1}H NMR (400 MHz, $CDCl_3$) of ELP-004 showing the chemical shifts of resonances belonging to protons attached to ^{13}C atoms. The ^{13}C resonances are observed at ~1% making the sample >99% pure since no impurity resonance can be observed in the spectrum.

A.



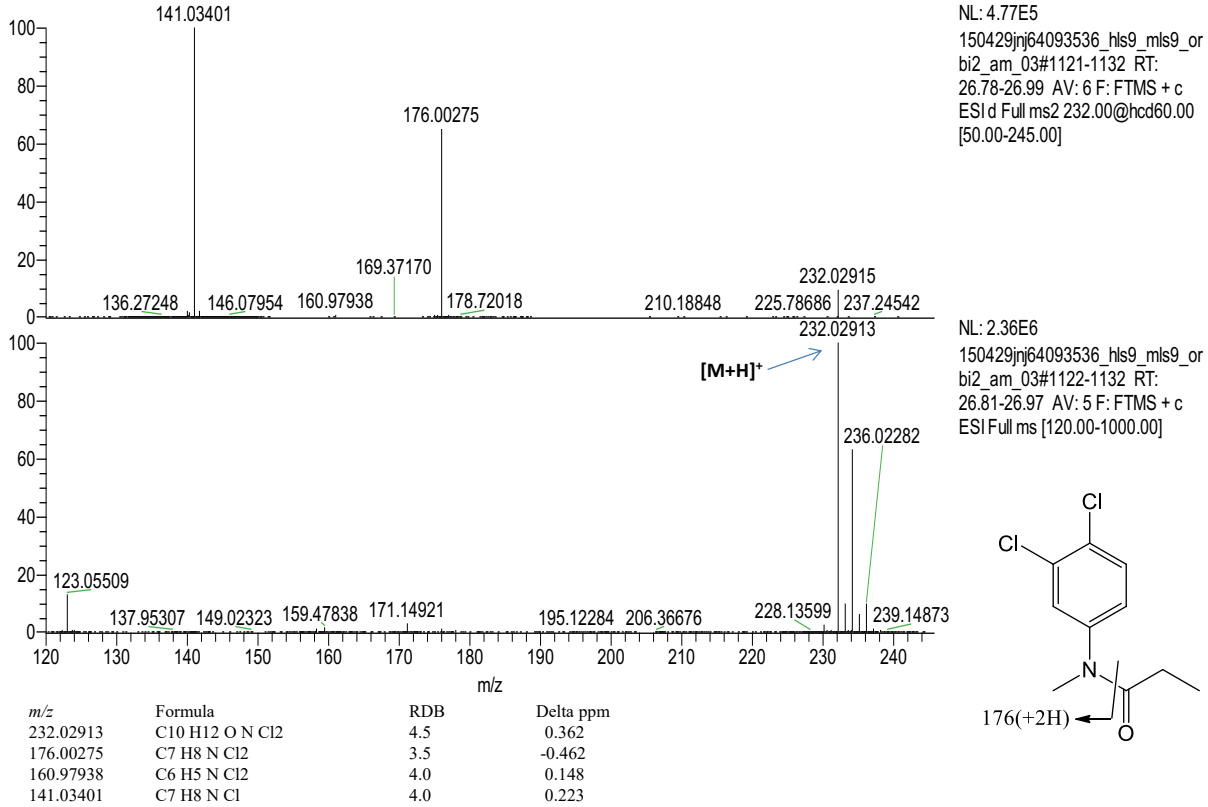
Avg Turnover : 24.2%

B.



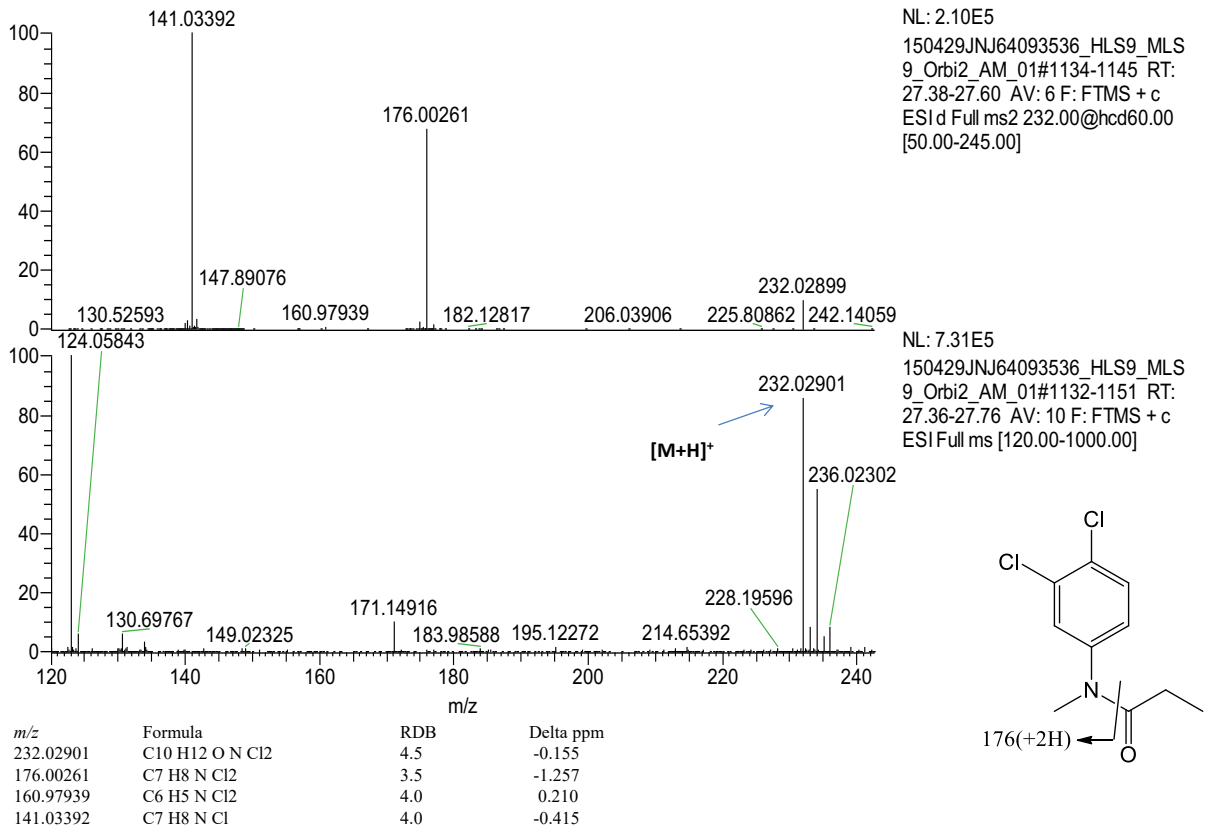
Avg Turnover : 14.3%

Fig. S2. Reconstructed ion chromatograms displaying the unchanged drug (UD) in **(A)** human and **(B)** mouse liver S9 fractions following 1 hour incubation of 10 µM of ELP-004 with boiled liver S9 (top 2) or fortified with NADPH, NADH, UDPGA, DTT, PAPS (bottom 2).



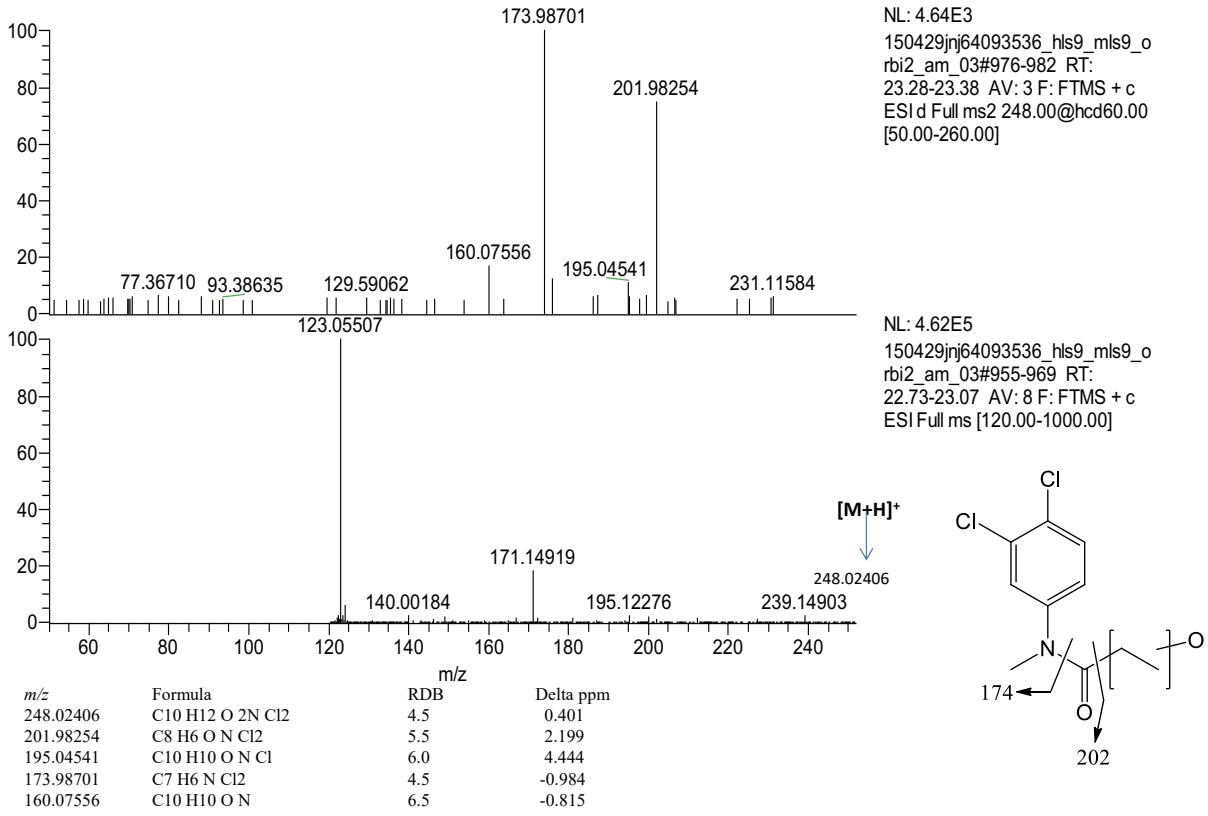
m/z	Formula	RDB	Delta ppm
232.02913	C10 H12 O N Cl2	4.5	0.362
176.00275	C7 H8 N Cl2	3.5	-0.462
160.97938	C6 H5 N Cl2	4.0	0.148
141.03401	C7 H8 N Cl	4.0	0.223

Fig. S3. Full scan and product ion mass spectra of ELP-004 from LC-MS/MS analysis of an hour incubation of 10 µM of ELP-004 in fortified human liver S9. Top = Product ion spectrum obtained by collision-induced dissociation (CID) of the respective protonated monoisotopic MH^+ species. Bottom = Full scan



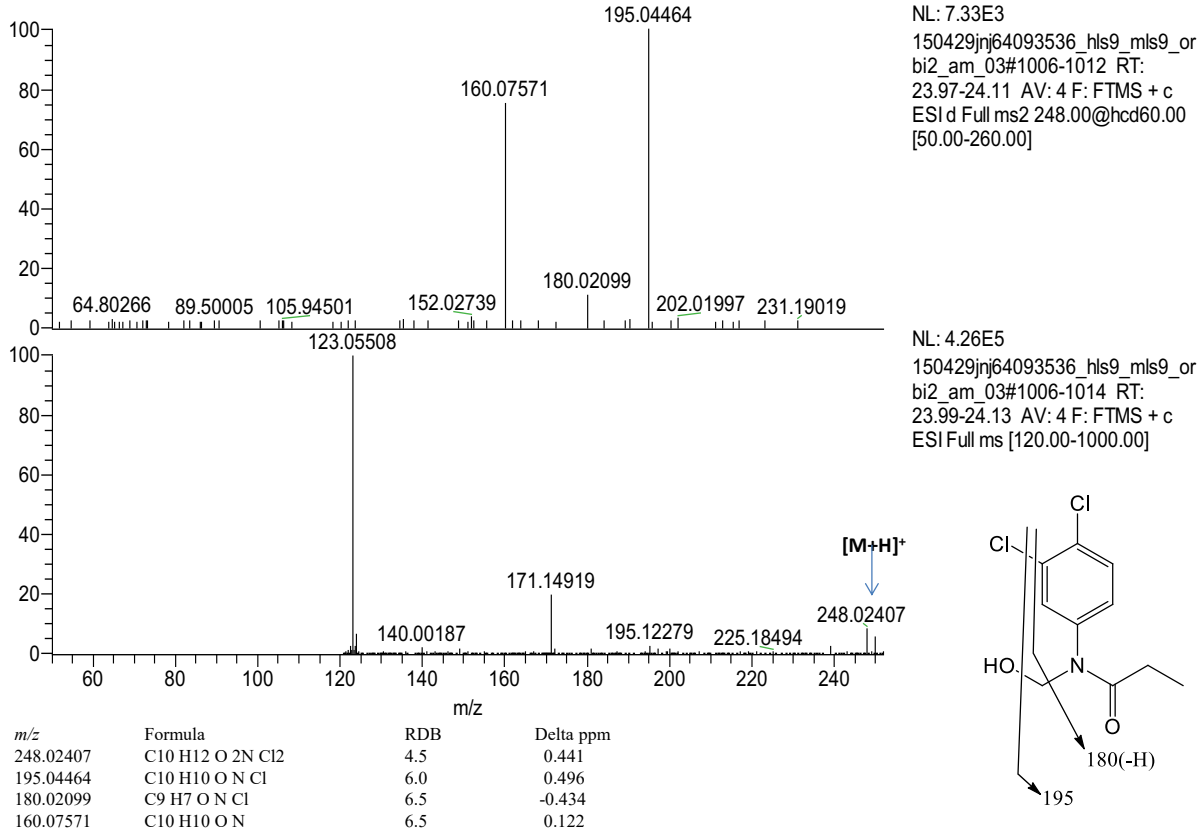
m/z	Formula	RDB	Delta ppm
232.02901	C10 H12 O N Cl2	4.5	-0.155
176.00261	C7 H8 N Cl2	3.5	-1.257
160.97939	C6 H5 N Cl2	4.0	0.210
141.03392	C7 H8 N Cl	4.0	-0.415

Fig. S4. Full scan and product ion mass spectra from LC-MS/MS analysis of ELP-004 standard. Top = Product ion spectrum obtained by collision-induced dissociation (CID) of the respective protonated monoisotopic MH^+ species. Bottom = Full scan



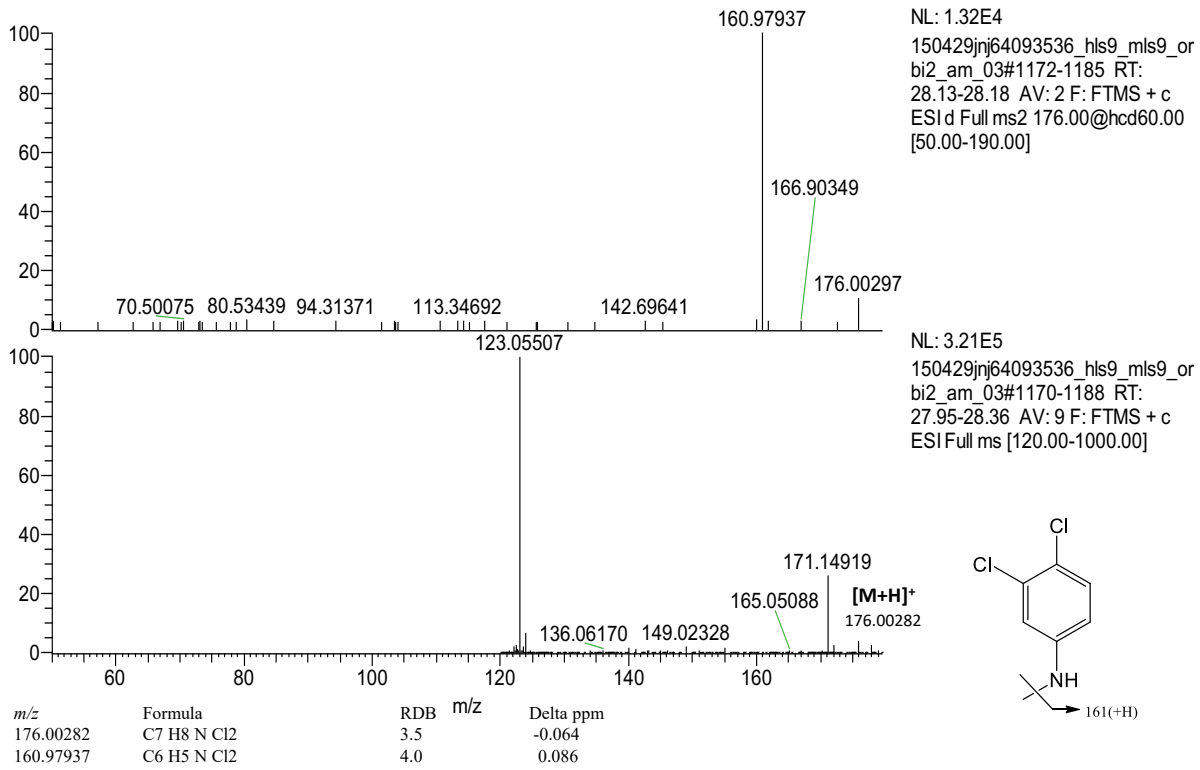
m/z	Formula	RDB	Delta ppm
248.02406	C10 H12 O 2N Cl2	4.5	0.401
201.98254	C8 H6 O N Cl2	5.5	2.199
195.04541	C10 H10 O N Cl	6.0	4.444
173.98701	C7 H6 N Cl2	4.5	-0.984
160.07556	C10 H10 O N	6.5	-0.815

Fig. S5. Full scan and product ion mass spectra of M1 (monooxy UD) from LC-MS/MS analysis of an hour incubation of 10 µM of ELP-004 in UDPGA, PAPS, NADPH and NADH fortified human liver S9. Top = Product ion spectrum obtained by collision-induced dissociation (CID) of the respective protonated monoisotopic MH^+ species. Bottom = Full scan



m/z	Formula	RDB	Delta ppm
248.02407	C10 H12 O 2N Cl2	4.5	0.441
195.04464	C10 H10 O N Cl	6.0	0.496
180.02099	C9 H7 O N Cl	6.5	-0.434
160.07571	C10 H10 O N	6.5	0.122

Fig. S6. Full scan and product ion mass spectra of M2 (monooxy UD) from LC-MS/MS analysis of an hour incubation of 10 µM of ELP-004 in UDPGA, PAPS, NADPH and NADH fortified human liver S9. Top = Product ion spectrum obtained by collision-induced dissociation (CID) of the respective protonated monoisotopic MH^+ species. Bottom = Full scan



m/z	Formula	RDB	Delta ppm
176.00282	C7 H8 N Cl2	3.5	-0.064
160.97937	C6 H5 N Cl2	4.0	0.086

Fig. S7. Full scan and product ion mass spectra of M3 (amide hydrolyzed UD) from LC-MS/MS analysis of an hour incubation of 10 μM of ELP-004 in fortified human liver S9. Top = Product ion spectrum obtained by collision-induced dissociation (CID) of the respective protonated monoisotopic MH^+ species. Bottom = Full scan

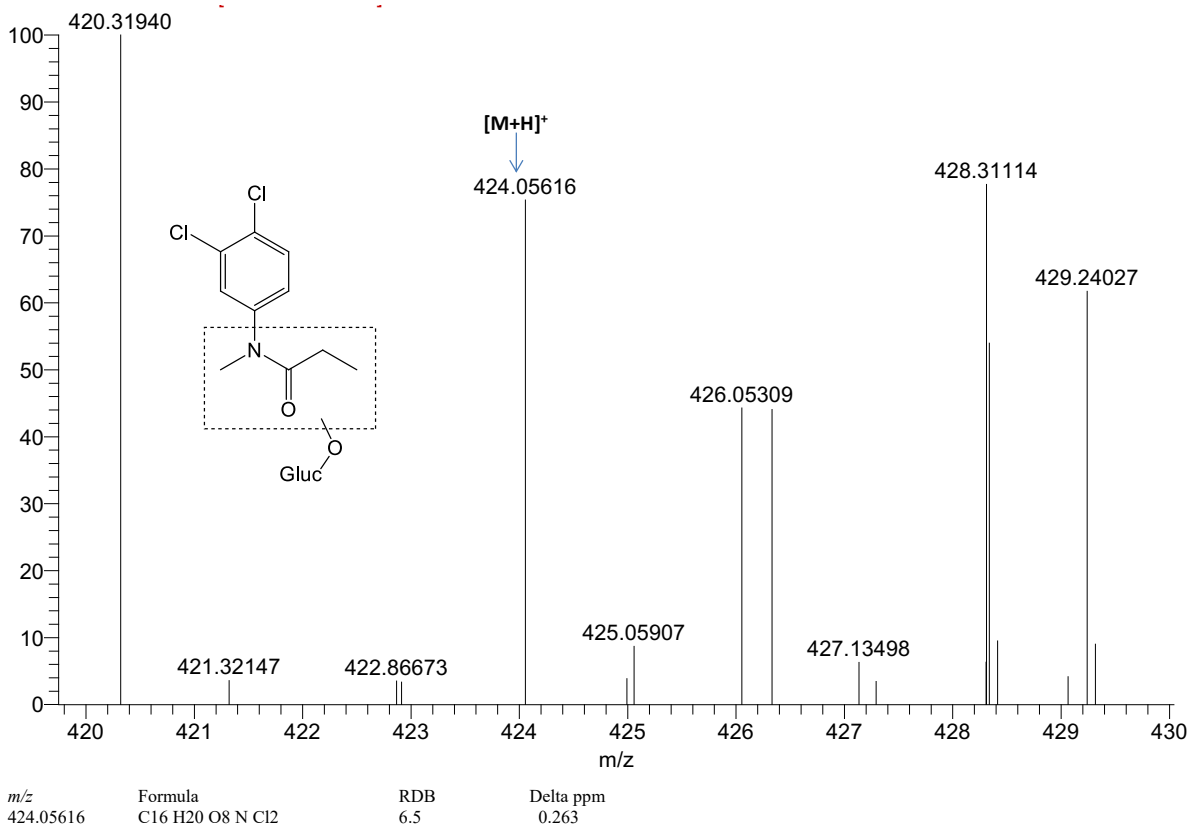


Fig. S8. Full scan spectra of M4 (glucuronide of monooxygenated UD) from LC-MS/MS analysis of an hour incubation of 10 µM of ELP-004 in UDPGA, PAPS, NADPH and NADH fortified human liver S9

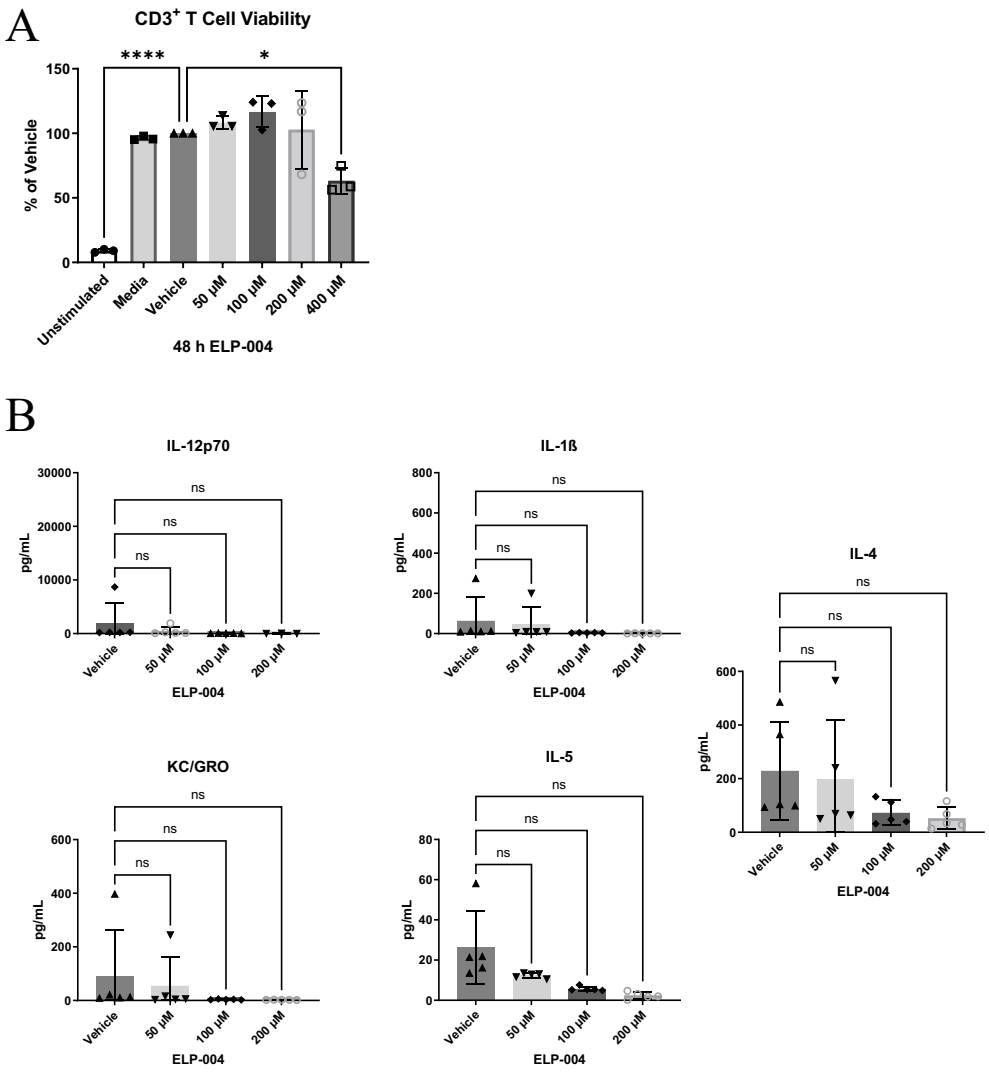


Fig. S9. T cells were stimulated *ex vivo* with anti-CD3/CD28 and treated with increasing doses of ELP-004. **(A)** After 48 h, supernatant was collected, remaining cells were stained with anti-CD3 (FITC). Live $CD3^+$ cells were quantified by flow using PI exclusion. Viability is shown as percentage of vehicle-treated live cells. Groups were compared to vehicle using a one-way ANOVA with a Dunnett's post hoc test for multiple comparisons * $p<0.05$, **** $p<0.0001$. **(B)** Secreted cytokines were assessed in the supernatant using a multi-analyte ELISA (MSD plate). Cytokine concentrations were using compared using repeated measures one-way ANOVA with a Dunnett's post hoc test for multiple comparisons * $p<0.05$, ** $p<0.01$.

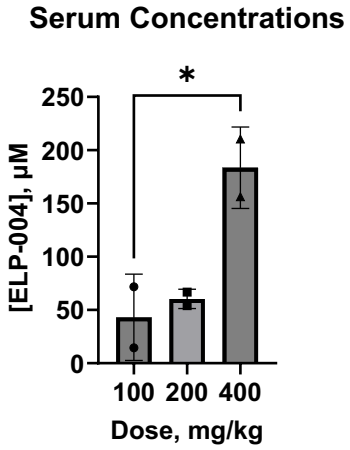


Fig. S10. Serum concentrations of ELP-004. ELP-004 was dissolved in 5% DMSO and peanut oil. Male DBA1J (n=2 per group) were administered a single, high dose (100 mg/kg, 200 mg/kg, or 400 mg/kg) of ELP-004 via i.p. injection. Serum was isolated from cardiac blood after 1 hour and ELP-004 concentrations were quantified by mass spectrometry.

Table S1. cDNA-expressed human P450 enzyme preparations from baculovirus-infected insect cells

Isoform	Source	Supplier	Batch
CYP1A1	Supersomes™	Corning	2081004
CYP2B6	Supersomes™	Corning	2125161
CYP2C8	Supersomes™	Corning	8080001
CYP2C9	Supersomes™	Corning	1306003
CYP2C19	Supersomes™	Corning	1055002
CYP2D6	Supersomes™	Corning	9140003
CYP3A4	Supersomes™	Corning	1349003

Table S2. CYP Phenotyping Conditions

Isoform	Protein conc (pMol/mL)	Control compound	Control compound conc (µM)
CYP1A2	25	Ethoxycoumarin	1
CYP2B6	100	Efavirenz	1
CYP2C8	25	Amodiaquine	1
CYP2C9	25	Diclofenac	1
CYP2C19	25	Omeprazole	1
CYP2D6	25	Dextromethorphan	1
CYP3A4	25	Midazolam	1

Table S3. Incubation conditions for CYP Inhibition Assays

Isoform	Probe Substrate	Protein Conc (mg/mL)	Incubation Time (min)	Positive Control Inhibitor
CYP1A2	Tacrine 5 µM	0.25	5	alpha-naphthoflavone (0-1 µM)
CYP2B6	Bupropion 100 µM	0.25	10	Ticlopidine (0-50 µM)
CYP2C8	Amodiaquine 5 µM	0.25	10	Quercetin (0-50 µM)
CYP2C9	Tolbutamide 100 µM	0.5	5	Sulphaphenazole (0-10 µM)
CYP2C19	Mephenytoin 100 µM	0.25	60	Ticlopidine (0-50 µM)
CYP2D6	Dextromethorphan 5 µM	0.5	10	Quinidine (0-10 µM)
CYP3A4	Midazolam 2.5 µM	0.25	10	Ketoconazole (0-10 µM)
CYP3A4	Testosterone 50 µM	0.25	10	Ketoconazole (0-10 µM)

Table S4. LC-MS/MS Conditions for CYP Analyses

LC Gradient

Column: Acquity™ C18 Reverse Phase UPLC HSS T3 (1.8 µm) 2.1 x 50 mm column
Column Temp.: 50 °C
Injection Vol.: 10 µL
Mobile Phase A: 0.1 % v/v formic acid in water
Mobile Phase B: 0.1 % v/v formic acid in acetonitrile
Gradient Profile:

Time (min)	Flow Rate (µL/min)	% Mobile Phase A	% Mobile Phase B
0.10	1000	98	2
0.35	1000	5	95
0.70	1000	5	95
0.80	1000	98	2
0.90	1000	98	2

MS Optimization data for ELP-004

Sought Mass (amu)	Optimized Mass (amu)	Parent Daughter Transition	Declustering Potential (V)	Collision Energy (V)	Ionization Mode
232.11	232.00	232.00 > 141.00	120	35	ESI+

Table S5. LC gradient for chromatographic separation of ELP-004 and its metabolites (Flow Rate: 200 μL/min)

Time (min)	B%
0	5
2	5
4	6
6	8
8	11
10	14
12	18
14	23
16	29
18	35
20	42
22	50
24	60
26	70
28	80
30	95
35	95
35.1	5
45	5

Column: BETASIL PhenylHexyl (150 x 2.1 mm i.d., 3.0 μm); ThermoScientific, Inc
LC gradient: A - 10 mM ammonium acetate; B - acetonitrile

Table S6. Individual data points shown in Fig. 1B and quantified in Table 1.

i.v. administration **2** **mg/kg**

	Conc (ng/mL)					
Time (h)	Mouse7	Mouse8	Mouse9	Mouse10	Mouse11	Mouse12
0.083	771	846	866			
0.25				366	437	330
0.5	148	214	184			
1				49.4	138	62.2
2	23.4	19.3	22.4			
4				BQL	BQL	BQL
7	BQL	BQL	BQL			
24	BQL	BQL	BQL	BQL	BQL	BQL

p.o. administration **10** **mg/kg**

	Conc (ng/mL)					
Time (h)	Mouse1	Mouse2	Mouse3	Mouse4	Mouse5	Mouse6
0.25	250	257	125			
0.5				131	215	219
1	62.8	57.4	61.4			
2				44.6	26.2	24.6
4	BQL	10.4	BQL			
7				BQL	BQL	BQL
24	BQL	BQL	BQL	BQL	BQL	BQL

*value is based on single value only

s.c. administration **2** **mg/kg**

	Conc (ng/mL)					
Time (h)	Mouse13	Mouse14	Mouse15	Mouse16	Mouse17	Mouse18
0.083	370	404	246			
0.25				500	BQL	422
0.5	285	261	209			
1				147	14.6	89.2
2	18.1	20.7	29.6			
4				BQL	BQL	BQL
7	BQL	BQL	BQL			
24	BQL	BQL	BQL	BQL	BQL	BQL

NA - Not available
BQL - Below limit of quantitation LOQ (ng/mL) 2

Table S7. Off-target pharmacological profiling of ELP-004 [10 μM] – Targets with <10% inhibition detected.

Assays	Concentration (M)	Reference Compound
A2A (h) (agonist radioligand)	1.0E-05	NECA
A3 (h) (agonist radioligand)	1.0E-05	IB-MECA
alpha 1 (non-selective) (antagonist radioligand)	1.0E-05	prazosin
alpha 2 (non-selective) (antagonist radioligand)	1.0E-05	yohimbine
beta 1 (h) (agonist radioligand)	1.0E-05	atenolol
AT1 (h) (antagonist radioligand)	1.0E-05	saralasin
BZD (central) (agonist radioligand)	1.0E-05	diazepam
B2 (h) (agonist radioligand)	1.0E-05	NPC 567
D1 (h) (antagonist radioligand)	1.0E-05	SCH 23390
D2S (h) (antagonist radioligand)	1.0E-05	(+)butaclamol
ETA (h) (agonist radioligand)	1.0E-05	endothelin-1
GABA (non-selective) (agonist radioligand)	1.0E-05	GABA
GAL2 (h) (agonist radioligand)	1.0E-05	galanin
CXCR2 (IL-8B) (h) (agonist radioligand)	1.0E-05	IL-8
H1 (h) (antagonist radioligand)	1.0E-05	pyrilamine
H2 (h) (antagonist radioligand)	1.0E-05	cimetidine
MC4 (h) (agonist radioligand)	1.0E-05	NDP-alpha -MSH
M1 (h) (antagonist radioligand)	1.0E-05	pirenzepine
M2 (h) (antagonist radioligand)	1.0E-05	methoctramine
M3 (h) (antagonist radioligand)	1.0E-05	4-DAMP
NK2 (h) (agonist radioligand)	1.0E-05	[Nleu10]-NKA (4-10)
NK3 (h) (antagonist radioligand)	1.0E-05	SB 222200
Y1 (h) (agonist radioligand)	1.0E-05	NPY
Y2 (h) (agonist radioligand)	1.0E-05	NPY
NTS1 (NT1) (h) (agonist radioligand)	1.0E-05	neurotensin
delta 2 (DOP) (h) (agonist radioligand)	1.0E-05	DPDPE
mu (MOP) (h) (agonist radioligand)	1.0E-05	DAMGO
NOP (ORL1) (h) (agonist radioligand)	1.0E-05	nociceptin
5-HT1B (antagonist radioligand)	1.0E-05	serotonin
5-HT2A (h) (antagonist radioligand)	1.0E-05	ketanserin
5-HT5a (h) (agonist radioligand)	1.0E-05	serotonin
5-HT6 (h) (agonist radioligand)	1.0E-05	serotonin
5-HT7 (h) (agonist radioligand)	1.0E-05	serotonin
sst (non-selective) (agonist radioligand)	1.0E-05	somatostatin-14
VPAC1 (VIP1) (h) (agonist radioligand)	1.0E-05	VIP
V1a (h) (agonist radioligand)	1.0E-05	[d(CH2)51,Tyr(Me)2]-AVP
Ca2+ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	1.0E-05	D 600
KV channel (antagonist radioligand)	1.0E-05	alpha -dendrotoxin
SKCa channel (antagonist radioligand)	1.0E-05	apamin
Na+ channel (site 2) (antagonist radioligand)	1.0E-05	veratridine
Cl- channel (GABA-gated) (antagonist radioligand)	1.0E-05	picrotoxinin
norepinephrine transporter (h) (antagonist radioligand)	1.0E-05	protriptyline
5-HT transporter (h) (antagonist radioligand)	1.0E-05	imipramine