SUPPLEMENTAL MATERIAL

Drug repurposing for *Leishmania*. Molecular basis of the leishmanicidal activity of the antidepressant sertraline.

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Index for supplemental material

1.-Material and methods for metabolomic studies

1.1- Preparation of samples for metabolomic studies.

Leishmania infantum promastigotes (strain JPC) were grown in RPMI 1640 + HIFCS at 26°C and harvested at a mid-exponential phase (8×10^6 promastigotes/ml). Then, promastigotes were incubated for 12 h under these conditions with 35 μM of SRT, equivalent to its IC_{70} for MTT reduction under these conditions. Once collected, parasites were immediately washed twice with chilled HBSS buffer, and frozen in liquid N_2 (4×10^7 promastigotes per aliquot). Parasites were kept at −80°C until analysis. Six replicates of each group were used for all the analytical techniques employed.

Metabolite extraction was carried out by addition of 350 μl MeOH:H_2O 4:1 (vol:vol) at 4°C to each replicate. Afterwards, disruption of the samples was carried out by shaking in a TissueLyser LT (Qiagen, Germany) (25 mg glass beads acid-water 426-660 μm, 10 min, 50Hz) plus four freeze-thawing cycles in liquid N_2. Insoluble material was removed by centrifugation (15,700 × g, 10 min, 4°C).

1.2- LC-MS fingerprinting.

Leishmania extracts were filtered through 0.22 µm MS® nylon filter and analyzed in an LC system (Agilent 1200 Series HPLC), coupled to a QTOF (quadrupole time-of-flight) (6520, Agilent), controlled by Mass Hunter Workstation Data Acquisition (B.04.00, Agilent), used at both polarities, ESI+ and ESI− to broaden the variety of metabolite ions detected. Briefly, 10 µl of sample were injected into a reverse-phase column at 40°C (Supelco Discovery HS, C18, 150 × 2.1 mm internal diameter, 3.0 µm, Bellefonte, PA, USA), flow rate: 0.6 ml/min; mobile phase: A = 0.1 % (vol:vol) formic acid; B = 0.1 % (vol:vol) formic acid in acetonitrile; gradient: 25 to 95% B in 40 min. The QTOF system was operated in positive and negative Dual Electrospray Ionization mode in full scan from m/z 50 to 1000 at a rate 1.0 spectrum per second. Electrospray conditions were: capillary voltage 3000 V for positive and 4000 V for negative ionization mode; drying gas at 330°C and flow rate of 10.5 ml/min; nebulizer pressure 26 psi; fragmentor 175 V; octopole 750 V; skimmer 65 V. During the analysis two reference masses (purine ($C_5H_4N_4$) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine ($C_{18}H_{18}O_6N_3P_3F_{24}$), (HP-0921))were used to allow constant mass correction: 121.0509 – purine and 922.0098 – (HP-0921) in positive ESI mode; and 119.0363 m/z (proton abstracted purine) and 966.0007 m/z (formate adduct of HP-0921) in negative ion mode. Samples were analyzed in randomized two runs (first for positive and second for negative ion mode) and maintained in an autosampler at 4°C.

1.3- CE-MS analysis.

One hundred and twenty microliters of the metabolic extracts were dried and resuspended in 120 µl Milli-Q H_2O containing 0.2 mM methionine sulfone as internal standard and 0.1 M formic acid. Samples were centrifuged (15,700 × *g*, 10 min, 4°C) and analyzed by CE-

MS in a 7100 Agilent system, coupled to a 6224 Agilent TOF Mass Spectrometer, controlled by ChemStation software (B.04.03, Agilent) and MS mode by Mass Hunter Workstation Data Analysis (B.02.01, Agilent). The separation occurred in a fused-silica capillary (total length, 100 cm; 50 μm internal diameter, Agilent). Separations were carried out in normal polarity with a 0.8 M formic acid in 10 % methanol (vol:vol) as background electrolyte at 20°C. Before each analysis, the capillary was conditioned by flushing the background electrolyte for 5 min (950 mbars). The sheath liquid (6 μl/min) was MeOH/water (1/1, vol:vol) containing 1.0 mM formic acid with two reference masses: 121.0509 – purine and 922.0098 – and HP-0921 ($C_{18}H_{18}O_6N_3P_3F_{24}$,HP-0921). Samples were hydrodynamically injected at 50 mbar for 50 s. Stacking was carried out by applying the background electrolyte at 100 mbar for 10 s. The separation voltage was 30 kV with 25 mbar of internal pressure and the analyses were carried out in 30 min. The optimized MS parameters were: fragmentor, 100 V; skimmer, 65 V; octopole 750 V; nebulizer pressure, 10 psi; drying gas, 200°C, 12.0 ml/min. The capillary voltage was 3500 V. Data were acquired in positive Dual-ESI mode with a full scan from m/z 80 to 1000, at a rate of 1.02 scan/s.

1.4- Quality Controls (QCs).

Regardless of the technique used, QCs were prepared by pooling equal volumes of all the samples, and analyzed throughout the run. The analytical runs were set up starting with minimum of five QCs followed by the samples; a QC sample was injected in between blocks of six samples until the end of the run.

1.5- Data set creation.

Data background noise was cleaned. The final list of features was obtained by the molecular feature extraction (MFE) tool in the Mass Hunter Qualitative Analysis software (B.06.00, Agilent). Data were reprocessed using DA Reprocessor Offline Utilities B.05.00 (Agilent) for ions such as $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$, neutral water loss and double charge for charge state.

1.6- Alignment.

Data were aligned in MPP (Mass Profiler Professional 12.6.1, Agilent) (1). Peaks present in all samples with the same m/z and retention time (RT) were assigned to the same feature. The alignment was performed without prior RT correction. For LC-MS, filtering and alignment were set up between 0.2-36.0 min or 0.2-32.0 min in positive or negative mode respectively, with 1% for RT window and 20 ppm for mass tolerance. For CE-MS, the filtering and alignment were performed into the range 1.26 to 34.0 min applying 10% for RT window and 10 ppm for mass tolerance. Alignment was performed by restricting the number of ions and charge states defined previously during extraction of features. Each compound was described by mass, RT, and abundance.

1.7- Data treatment.

After alignment, the variables were filtered in MS Excel (Microsoft Office, 2010). Data selection required their presence in more than 50% of the QCs, and a relative standard deviation (RSD) < 30% across the QCs. Principal component analysis (PCA) models were built using SIMCA-P1 software (12.0.1.0, Umetrics) to verify analytical reproducibility and stability. Hereafter, data without QCs was further filtered by masses presence in at least 66.6% of the samples in one comparison group (untreated or treated). Any missing values

were replaced by either the mean or zero for variables with a higher or lower 50% percentage in the samples of the group respectively.

1.8- Statistical analysis.

Statistical significance was set at 95% level ($P < 0.05$) using Mann-Whitney U test (program Matlab R2010a, version 7.10.0.499) for Univariate analysis (UVA). Principal component analysis (PCA), partial least squares regression (PLS-DA), jackknife confidence intervals, S plot and VIP scores from OPLS-DA (program Simca-P+ (12.0.1, Umetrics, Umea, Sweden) were also used to select biologically significant variables in multivariate analysis (MVA).

1.9- Identification.

The resulting list of accurate masses with significant difference among groups was searched using METLIN (http://metlin.scripps.edu), KEGG (http://www.genome.jp/kegg/genome.html), and LIPIDMAPS (http://www.lipidmaps.org/) by the in-house developed CEU mass mediator (http://ceumass.eps.uspceu.es/mediator; error 10 ppm). Information from LeishCyc (http://biocyc.org/LEISH/organisms-summary?object=LEISH) was also used. Identification of the compounds was carried out by LC−MS/MS using identical separation conditions of the first analysis coupled to a QTOF (model 6520, Agilent). Ions were targeted by collision-induced dissociation (CID) fragmentation on the fly, according to the previously determined accurate mass and RT. Final confirmation were carried out after study of mass and isotopic distributions for the precursor and product ion of the particular compounds. Confirmation with standards was made by comparison of RT, isotopic distribution, and fragments of commercially available

reagents with those obtained in analyzed samples. For CE-MS, the samples were reanalyzed in presence of commercially available standards for metabolites confirmation.

2.- Supplementary results for metabolomic studies

2.1- Analytical validation of untargeted metabolomics analyses.

In all analytical platforms, tight cluster of the QCs in a well-defined area of plot demonstrates that separation into different groups was based on a real biological variability and techniques and methods possessed stability and reproducibility (Figure S2).

2.2- Data treatment and statistical analyses.

Once the methodology was considered appropriate, the data matrix was further filtered out based on masses present in at least 66.6% of the samples in a specific group (untreated and treated). Any missing values were replaced accordingly to the criteria aforementioned. PLS-DA models were built to discriminate between groups, to adjust the scale of the variables and to statistically validate the difference between groups. Pareto-scaling was the closest scale to the original data ensuring a softer impact and therefore applied in further analyses. Once the biological variability between groups (untreated vs. treated) was statistically supported by cross-validation analysis in PLS-DA models, metabolites responsible for this variation were discriminated by jackknife confidence intervals from OPLS-DA models in MVA analysis. For UVA, Mann-Whitney U test was applied. The full list of identified compounds was compiled in Table S2.

In order to enable comparison between techniques, the metabolite abundances were normalized dividing the mean-centered abundance of each metabolite by the corresponding

standard deviation (2). The normalized data were represented in heatmap (Figure 7, main text) grouped according to their biochemical nature (Figure 8, main text).

REFERENCES

1. Godzien J, Ciborowski M, Angulo S, Barbas C. 2013. From numbers to a biological sense: How the strategy chosen for metabolomics data treatment may affect final results. A practical example based on urine fingerprints obtained by LC-MS. Electrophoresis 34:2812-2826. DOI: 10.1002/elps.201300053
2. Xia J, Sinelnikov IV, Han B, Wishart DS. 2015. MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res 43:W251-W257. DOI: 10.1093/nar/gkv380

3.- Supplementary Figures S1-S6.

Figure S1. Cytofluorometric analysis of Rhodamine 123 accumulation in *Leishmania. pifanoi* axenic amastigotes and its inhibition by sertraline.

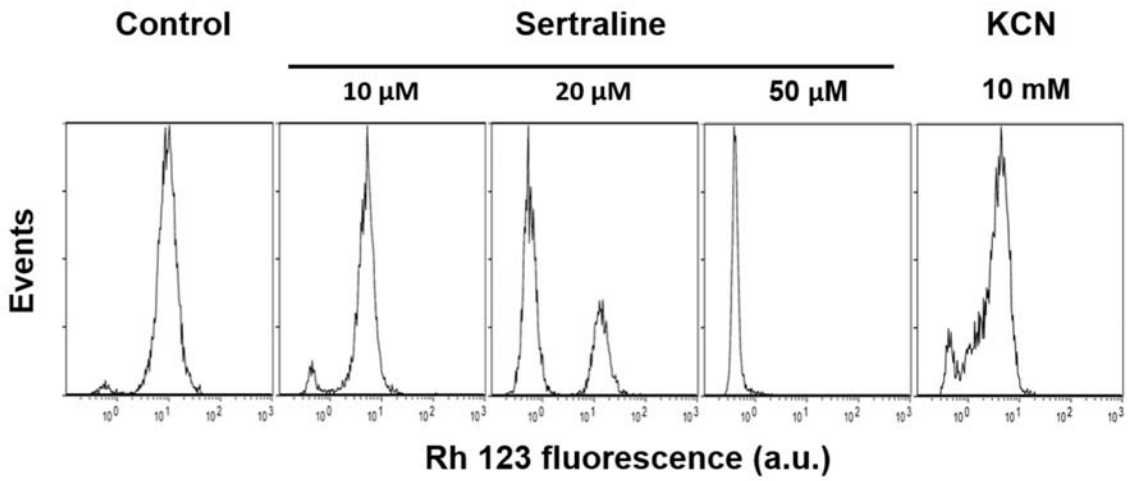


Figure S1- Cytofluorometric analysis of rhodamine 123 (Rh123) accumulation in *L. pifanoi* axenic amastigotes and its inhibition by sertraline. *L. pifanoi* axenic amastigotes were incubated (4h, 32ºC) with different SRT concentrations as stated at the corresponding graphs. After rhodamine 123 (Rh 123) uptake (0.3 µM, 5 min, 32°C), parasites were analyzed in a Beckman Coulter FC500 MPL cytofluorometer (λ_{EXC} =488 nm/λ_{EM} = 520 nm). Parasites incubated with 10 mM KCN were used as control for depolarization.

Figure S2- Morphological damage to *L. infantum* promastigote mitochondrion caused by sertraline.

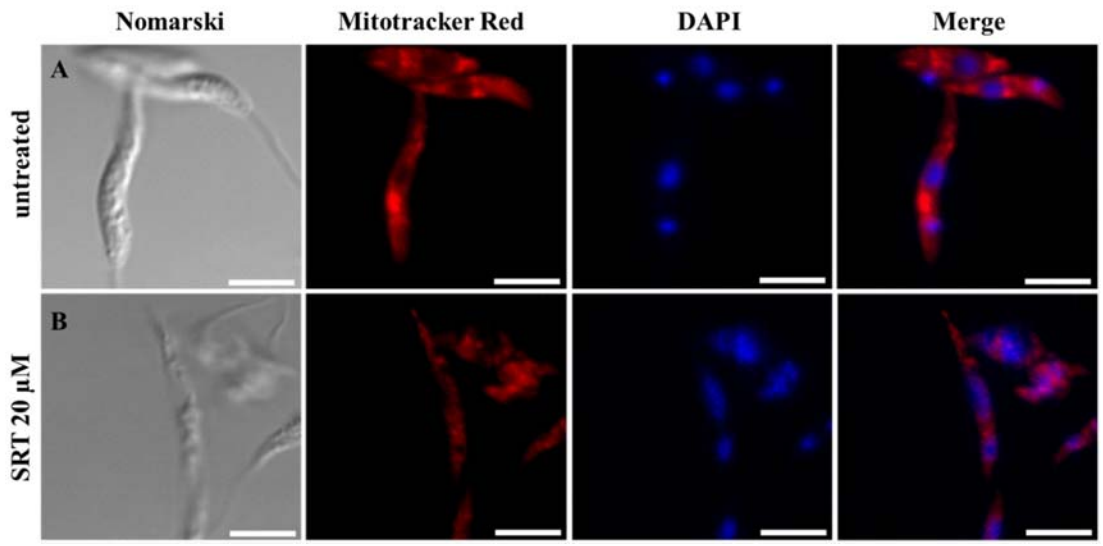


Figure S2. Morphological damage to *Leishmania infantum* promastigote parasite mitochondrion caused by sertraline. *L. infantum* promastigotes were stained with Mitotracker Red prior to their incubation with sertraline (SRT) at its IC_{50} (20 µM, 4 h). Changes in the fluorescence pattern were then assessed by confocal microscopy. **(A)** Untreated promastigotes. **(B)** Promastigotes treated with sertraline. Blue and red fluorescence corresponds to nucleic acids stained with DAPI (5 µg/ml), and to the specific accumulation of Mitotracker Red (0.1 µM) inside the mitochondrion of the parasite. Fluorescence settings: DAPI, λ_{EXC} = 358nm /λ_{EM}= 461nm; Mitotracker Red λ_{EXC} = 578nm /λ_{EM}= 599nm. **Legend**: DAPI (4 ',6-diamidino-2-phenylindol). Magnification bar =5 µm.

Figure S3. Constructions of PCA models.

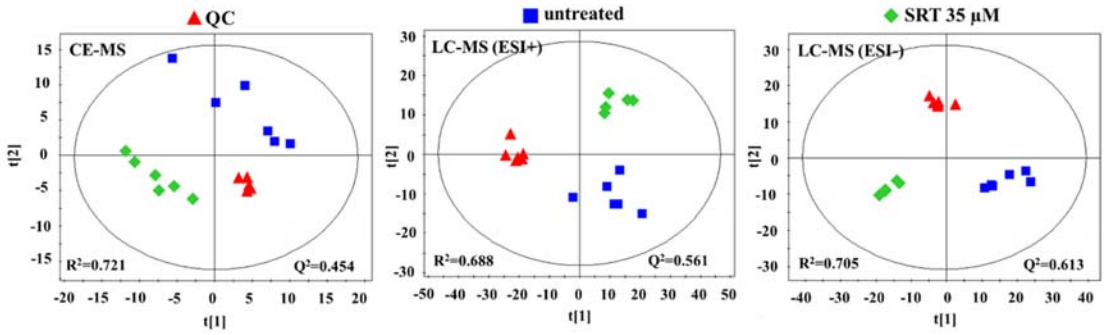


Figure S3. Constructions of PCA models. PCA models were built using the set of filtered data that were present in at least 50% of the QC and RSD lower than 30% across the QCs (component 1 vs. component 2 shown). CE-MS: R^2=0.721, Q^2=0.454, 2 components. LC-MS (ESI+): R^2 = 0.688, Q^2 = 0.561, 2 components. LC-MS (ESI-): R^2 = 0.705, Q^2 = 0.613, 2 components. R^2 = coefficient for variance explained; Q^2 = coefficient for variance predicted.

Figure S4. PLS-DA models built for untreated vs. treated parasites

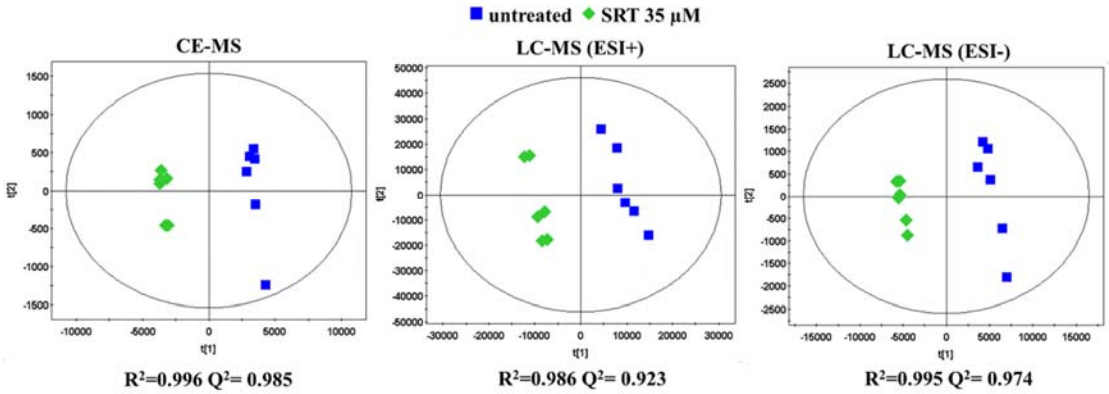
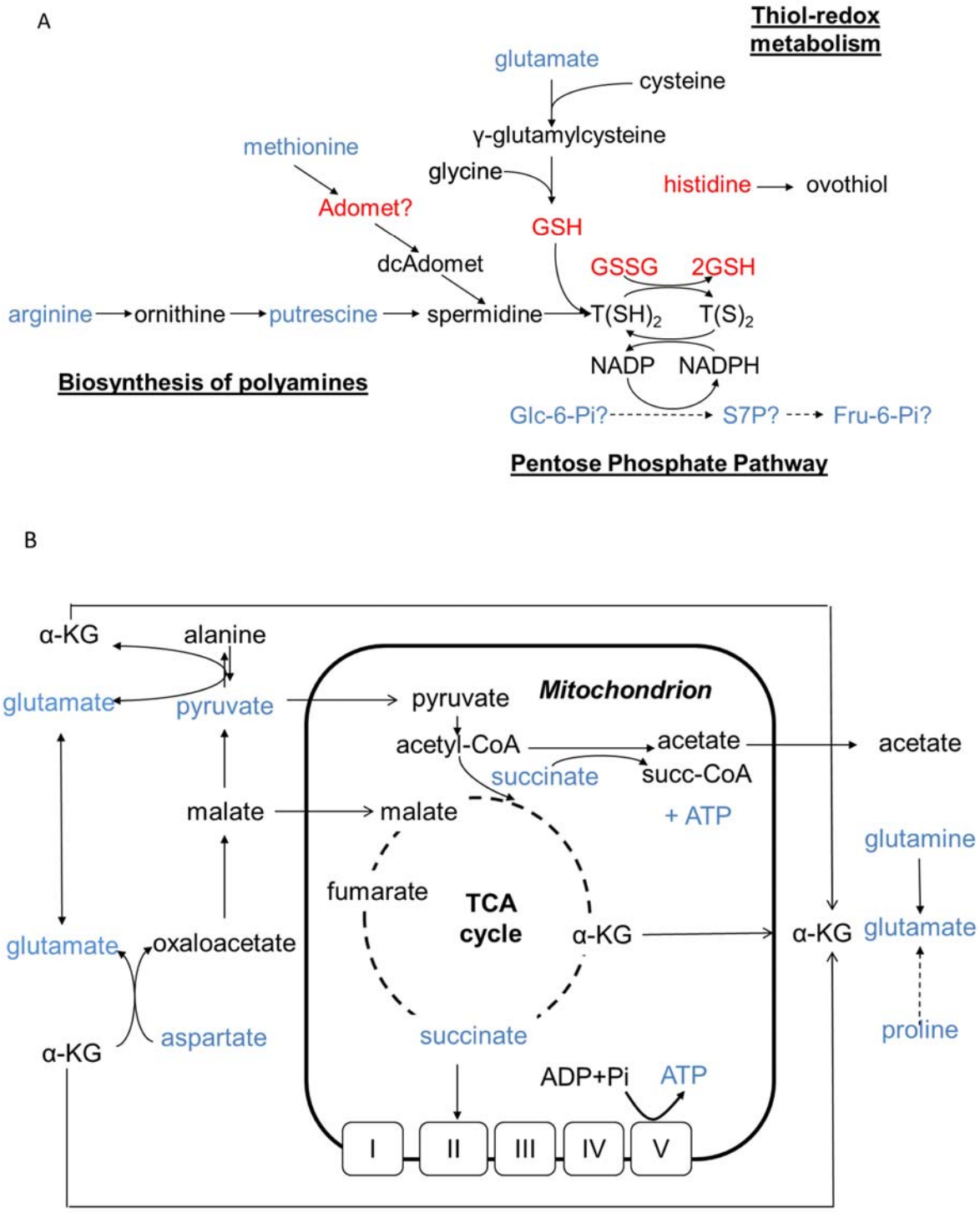


Figure S4. PLS-DA models built for untreated vs. treated. CE-MS: R^2=0.996, Q^2=0.985, 2 components. LC-MS (ESI+): R^2=0.986, Q^2=0.923, 2 components. LC-MS (ESI-): R^2=0.995, Q^2=0.974, 2 components. R^2 = coefficient for variance explained; Q^2 = coefficient for variance predicted.

Figure S5. Main metabolic pathways disturbed in *L. infantum* promastigotes by sertraline treatment.



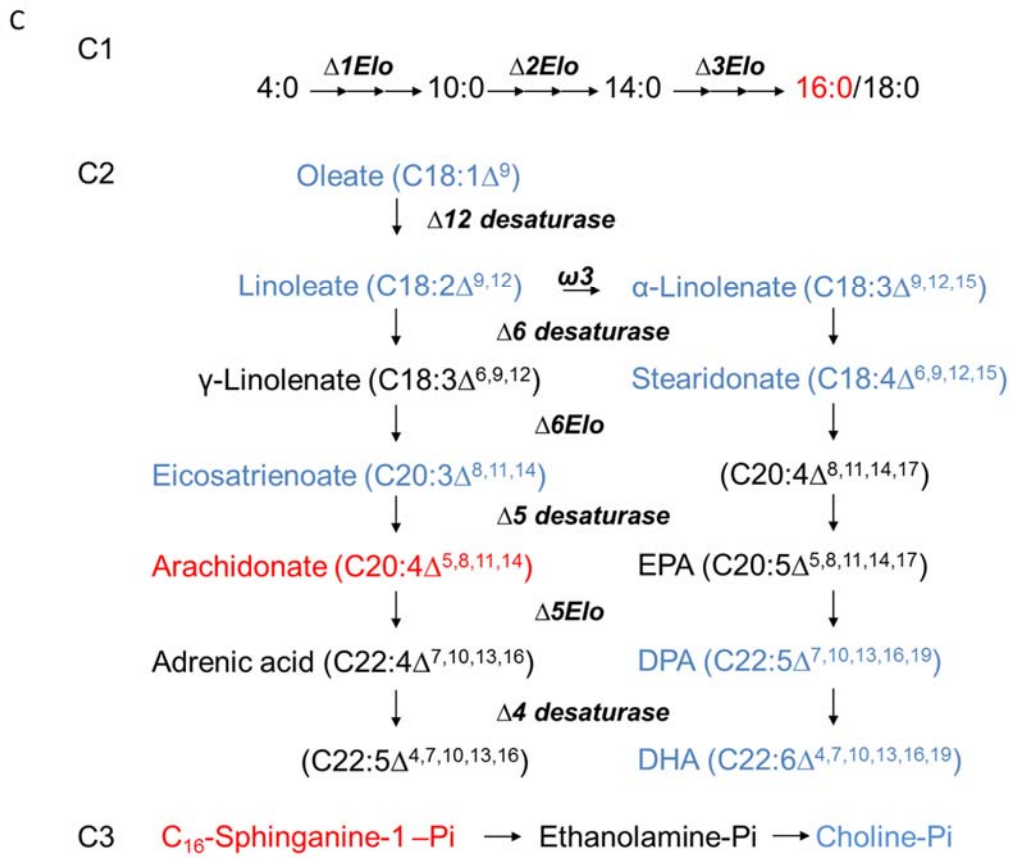


Figure S5. Main metabolic pathways disturbed in *Leishmania infantum* promastigotes by sertraline treatment. **(A).-** Biosynthesis of polyamines and the thiol-redox metabolism of sertraline treated parasites. Metabolites with an interrogation mark were arbitrarily assigned according to their molecular mass. **Legend**: Adomet, S-adenosyl methionine; dcAdomet, decarboxylated S-adenosylmethionine; Fru-6-Pi, fructose-6-phosphate Glc-6-Pi, glucose-6-phosphate, GSH, reduced glutathione; GSSG, oxidized glutathione; S7P, sedoheptulose 7 phosphate; $T(SH)_2$, reduced trypanothione; $T(S)_2$, oxidized trypanothione. **(B).-** Amino acid and bioenergetic metabolism. α-KG, α-ketoglutarate; Succ-CoA, succinyl-CoA. **(C).-** Lipid and sphingolipid metabolism. **C1.-** Elongation of fatty acids. **Legend**: *Elo*, elongase. **C2.-** Unsaturated fatty acids. **Legend**: DPA, docosapentaenoic acid, DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; **C3.-** Sphingolipid metabolism. Metabolites undergoing a rise in their levels in sertraline treated parasites were depicted in red, whereas those that decreased were in blue.

Figure S6.- Schematic representation of the leishmanicidal mechanism of sertraline.

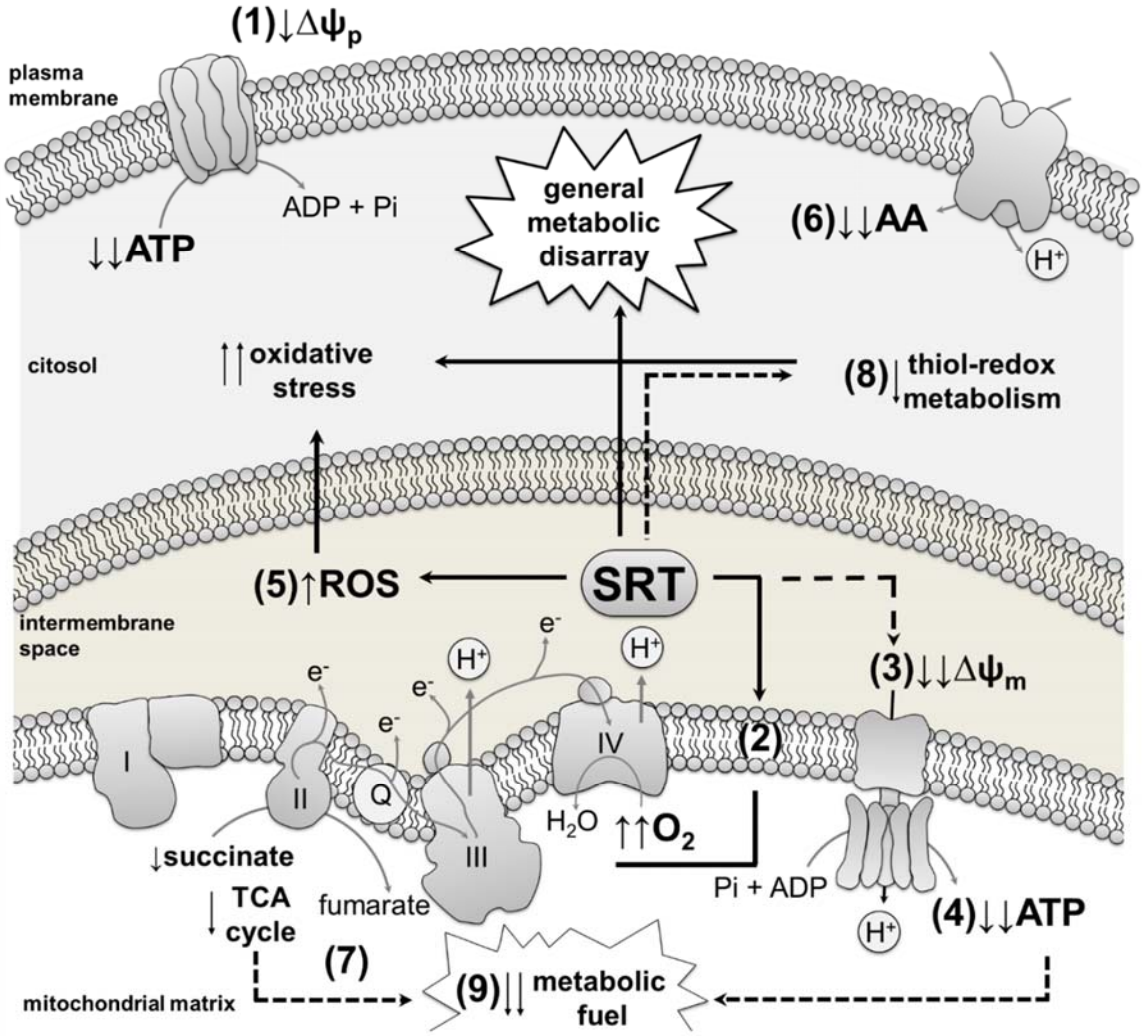


Figure S6.-.Schematic representation of the leishmanicidal mechanism of sertraline. Sertraline (SRT) crosses and depolarizes the plasma membrane causing **(1)** a transient depolarization of the plasma membrane. Once inside the parasite, SRT induces uncoupling of the respiratory chain **(2)**, collapse of $\Delta\psi_m$ **(3)**, with inhibition of ATP synthesis ensuing (**4**), and increase of mitochondrial ROS production **(5)**. These two initial effects were progressively aggravated first, by the decrease of the intracellular pool of amino acids **(6)** and other TCA intermediates pool **(7)**, and secondly by the impairment of the thiol-redox and polyamine metabolism **(8)**. This, together with the crippled energy metabolism of the

parasite **(9)** ended up with a general and irreversible metabolic disarray, lethal to the parasite. Solid and dashed arrows stand for increase or decrease of the respective effect

Table S1. Features detected and identified as changed by SRT treatment of *L. infantum* promastigotes at different steps of the statistical selection.

Analytical technique	After alignment	After filtering	Statistical significant
CE-MS	1128	130	38
LC-MS(ESI+)	7276	543	210
LC-MS(ESI-)	3043	478	337

Table S2. Compounds identified with statistical significance and their variation tendency with sertraline treatment.

#	Name	Formula	MW (Database)	error (ppm)	Fold Change (%)[a]	*P* valor	RSD[b] for QCs (%)	Confimation MS/MS fragments[c] or % of probability	Mode	Technique
Amines										
1	Choline	C5H13NO	103.0997	2.8	1451.4	0.002	7.2	identified	ESI+	CE-MS
2	Phosphocholine	C5H14NO4P	183.0660	-3.5	-74.9	0.002	5.5	60.0814/86.0945/124.9996/184.0726	ESI+	LC-MS
3	Putrescine	C4H12N2	88.1000	4.6	-64.0	0.002	3.0	identified	ESI+	CE-MS
Amino acids, peptides, and analogues (AAs)										
4	Glutathione (GSH)	C10H17N3O6S	307.0838	-1.0	10168.1	0.002	24.7	identified	ESI+	CE-MS
5	Glutathione disulfide (GSSH)	C20H32N6O12S2	612.1520	1.7	108.6	0.002	8.5	identified	ESI+	CE-MS
6	Lysine	C6H14N2O2	146.1055	0.7	57.4	0.002	4.9	identified	ESI+	CE-MS
7	Histidine	C6H9N3O2	155.0695	0.0	35.1	0.002	5.9	identified	ESI+	CE-MS
8	Proline	C5H9NO2	115.0633	2.6	-99.7	0.002	3.2	identified	ESI+	CE-MS
9	Asparagine	C4H8N2O3	132.0535	5.3	-82.9	0.002	4.7	identified	ESI+	CE-MS
10	Arginine	C6H14N4O2	174.1117	0.6	-76.9	0.002	2.5	identified	ESI+	CE-MS
11	Alanine/Sarcosine/βAlanine	C3H7NO2	89.0477	5.6	-71.3	0.002	5.1	Putative 79%	ESI+	CE-MS
12	Glutamate	C5H9NO4	147.0532	2.0	-64.5	0.002	3.1	identified	ESI+	CE-MS
13	Aspartate	C4H7NO4	133.0375	0.0	-63.7	0.002	2.5	identified	ESI+	CE-MS
14	Leucine/ Isoleucine	C6H13NO2	131.0946	0.7	-61.5	0.002	5.1	identified	ESI+	CE-MS
15	Valine	C5H11NO2	117.0790	0.0	-61.2	0.002	4.2	identified	ESI+	CE-MS
16	Serine	C3H7NO3	105.0426	4.7	-59.9	0.002	4.3	identified	ESI+	CE-MS
17	Trypanothione disulfide	C27H47N9O10S2	721.2887	0.3	-55.3	0.065	8.99	identified	ESI+	CE-MS
18	Cystathionine	C7H14N2O4S	222.0674	-8.5	-50.5	0.002	4.3	identified	ESI+	CE-MS
19	Methionine	C5H11NO2S	149.0510	0.7	-48.4	0.002	7.0	identified	ESI+	CE-MS
20	Tyrosine	C9H11NO3	181.0739	-0.6	-29.7	0.002	7.4	identified	ESI+	CE-MS
Carbohydrates (Carbs)										
21	Mannitol	C6H14O6	182.0790	-6.0	150.4	0.002	5.4	43.0165/59.0136/71.0154/89.0269/101.0222/181.0701	ESI-	LC-MS
22	disaccharide-phosphate	C12H23O14P	422.0825	-2.1	106.6	0.002	6.7	78.9573/96.9662/241.0121/421.0892	ESI-	LC-MS
23	glycerol-phosphate monossacarid C6	C9H19O11P	334.0665	-0.9	73.0	0.002	7.0	78.9574/92.9240/94.9256/96.9194/152.9942/241.0136/333.0598	ESI-	LC-MS

continue

Table S2. Compounds identified with statistical significance and their variation tendency with sertraline treatment.

#	Name	Formula	MW (Database)	error (ppm)	Fold Change (%)[a]	p valor	RSD[b] for QCs (%)	Confimation MS/MS fragments[c] or % of probability	Mode	Technique
24	Sedoheptulose 7-phosphate/ glycero-manno-Heptose 7-phosphate	C7H15O10P	290.0403	-3.1	-77.4	0.009	6.7	Putative 85.17	ESI-	LC-MS
25	Hexose-phosphate	C6H13O9P	260.0297	-3.1	-39.4	0.009	10.5	78.9583/96.9689/138.9969/198.9521/ 259.0703	ESI-	LC-MS
Fatty acids (FFAAs)										
26	Octadecenal/Octadecen-11-one/ octadecadien-1-ol	C18H34O	266.2610	-5.5	830.7	0.002	27.6	Putative 72.46	ESI+	LC-MS
27	Arachidonate (C20:4)	C20H32O2	304.2402	-4.6	169.9	0.002	6.2	41.9964/59.0109/112.9954/205.1859/ 259.2409/285.2442/303.2336	ESI-	LC-MS
28	Hexadecanoic acid (palmitic acid) (C16:0)	C16H32O2	256.2402	-1.2	115.2	0.009	7.7	255.231	ESI-	LC-MS
29	12-methyl myristic acid (C14:0 CH3)	C15H30O2	242.2246	-7.4	39.5	0.041	6.3	223.2077/241.214	ESI-	LC-MS
30	Linolenic Acid (C18:3)	C18H30O2	278.2246	-1.1	-72.0	0.002	5.5	59.0132/259.2047/277.2163	ESI-	LC-MS
31	Stearidonate (C18:4)	C18H28O2	276.2089	-4.7	-67.3	0.002	7.4	59.0023/231.1190/257.1843/275.1972	ESI-	LC-MS
32	linoleate (C18:2)	C18H32O2	280.2402	-1.1	-51.2	0.002	6.2	59.0111/71.0119/279.2314*	ESI-	LC-MS
33	Eicosatrienoic Acid (ETA, C20:3)	C20H34O2	306.2559	-5.2	-49.0	0.002	7.8	59.3451/83.0456/287.2246/305.2515	ESI-	LC-MS
34	(4,7,10,13,16,19)-Docosahexaenoic acid (C22:6)	C22H32O2	328.2402	-0.9	-24.4	0.015	5.2	59.0131/67.0533/83.0537/93.0627/107.0826/121.0931/135.1151/149.1313/ 161.1340/175.1448/229.2013/355.2049/283.2364/327.1769	ESI-	LC-MS
35	Oleate (C18:1)	C18H34O2	282.2559	-3.9	-16.7	0.026	6.2	281.2478	ESI-	LC-MS
36	7,10,13,16,19-docosapentaenoic acid (C22:5)	C22H34O2	330.2559	-2.1	-16.0	0.041	4.6	Putative 93.93	ESI-	LC-MS

continue

Table S2. Compounds identified with statistical significance and their variation tendency with sertraline treatment.

#	Name	Formula	MW (Database)	error (ppm)	Fold Change (%)[a]	p valor	RSD[b] for QCs (%)	Confimation MS/MS fragments[c] or % of probability	Mode	Technique
Glycerolipids (GLs)[d]										
37	PA(10:0/10:0)	C23H45O8P	480.2852	1.8	897.4	0.002	20.3	Putative 86.17	ESI+	LC-MS
38	MG(18:1/0:0/0:0)	C21H40O4	356.2927	-6.1	377.9	0.009	24.5	Putative 61.11	ESI+	LC-MS
39	PA(18:4/13:0)	C34H59O8P	626.3948	4.9	-58.8	0.002	11.8	Putative 69.73	ESI-	LC-MS
40	PA(P-16:0/20:5)	C39H67O7P	678.4625	-4.9	-40.7	0.004	6.9	Putative 71.14	ESI-	LC-MS
Glycerophospholipids (PLs)										
41	LPS(O-18:0)	C24H50NO8P	511.3274	-3.7	1790.1	0.002	8.2	Putative 71.11	ESI-	LC-MS
42	LPI(O-16:0)	C25H51O11P	558.3169	-3.8	1768.8	0.002	10.1	78.9564/241.0099/377.2430/557.3109	ESI-	LC-MS
43	LPE(P-16:0)	C21H44NO6P	437.2906	-3.7	608.8	0.002	13.5	78.9586/140.0107/196.0373/239.2359/375.2270/436.2809	ESI-	LC-MS
44	LPC(18:0)/LPE(21:0)	C26H54NO7P	523.3638	-4.7	165.7	0.002	7.9	Putative 87.12	ESI+	LC-MS
45	LPC(16:0)/LPE(19:0)	C24H50NO7P	495.3325	-6.2	88.9	0.002	14.9	Putative 92.93	ESI+	LC-MS
46	LPE-Cer(d14:2/16:0)	C32H63N2O6P	602.4424	7.5	56.2	0.009	7.6	Putative 76.66	ESI-	LC-MS
47	LPE(18:1)	C23H46NO7P	479.3012	-1.7	-84.1	0.002	13.1	78.9609/140.0097/196.0366/214.0465/281.2476/478.2949	ESI-	LC-MS
48	LPS(22:2)	C28H52NO9P	577.3380	-4.8	-83.4	0.002	23.0	Putative 79.71	ESI-	LC-MS
49	LPS(20:3)	C26H46NO9P	547.2910	-7.9	-82.2	0.002	11.3	Putative 72.33	ESI-	LC-MS
50	LPC(18:3)	C26H48NO7P	517.3168	4.0	-81.6	0.002	18.0	60.0813/86.0964/104.1072/124.9993/166.0617/184.0729/258.1081/500.3111/518.3229	ESI+	LC-MS
51	LPC(18:4)	C26H46NO7P	515.3012	0.6	-81.0	0.002	16.0	Putative 97.82	ESI+	LC-MS
52	LPC (17:3)	C25H46NO7P	503.3012	0.0	-80.6	0.002	18.7	60.0825/86.0951/104.1071/184.0716/258.1044/486.2999	ESI+	LC-MS
53	LPC(19:3)	C27H50NO7P	531.3325	-5.9	-78.0	0.002	16.5	60.0793/86.0975/104.1063/124.9928/166.0549/184.0713/514.3202/532.3392	ESI+	LC-MS
54	LPE(18:2)	C23H44NO7P	477.2856	2.8	-73.2	0.002	12.6	62.0611/81.0694/95.0849/216.0532/263.02348/306.2734/337.2719/478.3040	ESI+	LC-MS

continue

Table S2. Compounds identified with statistical significance and their variation tendency with sertraline treatment.

#	Name	Formula	MW (Database)	error (ppm)	Fold Change (%)[a]	p valor	RSD[b] for QCs (%)	Confimation MS/MS fragments[c] or % of probability	Mode	Technique
55	LPC(20:3)	C28H52NO7P	545.3481	-5.4	-73.0	0.002	17.6	Putative 84.76	ESI+	LC-MS
56	LPE(18:3)	C23H42NO7P	475.2699	-2.1	-72.2	0.002	13.7	Putative 95.07	ESI-	LC-MS
57	LPS(20:5)	C26H42NO9P	543.2597	-8.8	-70.9	0.002	11.9	Putative 71.55	ESI-	LC-MS
58	Glycerophosphocholine	C8H20NO6P	257.1028	-0.4	-69.0	0.002	15.2	Putative 94%	ESI+	CE-MS
59	LPC(18:2)	C26H50NO7P	519.3325	3.8	-68.3	0.002	18.7	Putative 94.55	ESI+	LC-MS
60	LPC/LPE	C44H74NO8P	775.5152	2.8	-67.8	0.002	16.4	Putative 73.5	ESI+	LC-MS
61	LPS(22:1)	C28H54NO9P	579.3536	-4.7	-66.3	0.002	21.5	Putative 67.66	ESI-	LC-MS
62	LPC(20:2)	C28H54NO7P	547.3638	0.8	-66.0	0.002	19.3	Putative 90.99	ESI+	LC-MS
63	LPC(20:4)	C28H50NO7P	543.3325	0.3	-61.7	0.002	17.5	Putative 88.23	ESI+	LC-MS
64	LPS(20:1)	C26H50NO9P	551.3223	-3.6	-58.7	0.002	25.8	Putative 75.53	ESI-	LC-MS
65	LPE(22:5)	C27H46NO7P	527.3012	-5.3	-58.3	0.002	8.9	62.0579/81.0677/95.0851/119.0852/133.0908/173.0171/229.1859/320.2473/387.2840/528.3361	ESI+	LC-MS
66	LPC(16:1)	C24H48NO7P	493.3168	0.1	-57.5	0.002	20.0	86.0991/104.1068/184.0716/258.1059/476.3124/494.3225	ESI+	LC-MS
67	LPE(22:6)	C27H44NO7P	525.2855	-3.6	-56.0	0.002	7.2	Putative 90.63	ESI-	LC-MS
68	LPC(20:1)	C28H56NO7P	549.3794	-6.7	-55.9	0.002	2.5	Putative 88.37	ESI+	LC-MS
69	LPC(22:4)	C30H54NO7P	571.3638	1.1	-52.2	0.002	13.9	86.0970/104.1063/124.9961/164.9296/184.0713/554.3669/572.3674	ESI+	LC-MS
70	LPC(14:0)	C22H46NO7P	467.3012	-5.1	-49.2	0.004	15.0	Putative 88.5	ESI+	LC-MS
71	LPC(17:1)	C25H50NO7P	507.3325	-6.5	-49.0	0.002	20.0	86.0936/104.1074/124.9962/184.0713/490.3160/508.3363	ESI+	LC-MS
72	LPC(18:1)	C26H52NO7P	521.3481	-3.9	-47.1	0.002	15.7	86.0971/104.1069/184.0724/258.1089/339.2824/445.2663/504.3114/522.3506	ESI+	LC-MS
73	LPC(22:6)	C30H50NO7P	567.3325	4.2	-44.9	0.002	14.7	Putative 98.3	ESI+	LC-MS
74	LPC(22:5)	C30H52NO7P	569.3481	-2.9	-44.8	0.002	12.9	Putative 90.65	ESI+	LC-MS
Sphingolipids and sphingoid bases (SLs)										
75	Sphinganine 1-phosphate (C18:0)	C18H40NO5P	381.2644	-3.9	PT	0.002	15.5	78.9580/380.2500	ESI-	LC-MS

continue

Table S2. Compounds identified with statistical significance and their variation tendency with sertraline treatment.

#	Name	Formula	MW (Database)	error (ppm)	Fold Change (%)[a]	p valor	RSD[b] for QCs (%)	Confimation MS/MS fragments[c] or % of probability	Mode	Technique
76	C16 Sphinganine-1-phosphate (C16:0)	C16H36NO5P	353.2331	-4.5	2466.4	0.002	11.5	78.9577/352.2214	ESI-	LC-MS
77	Sphingosine (C18:1)	C18H37NO2	299.2824	-8.7	417.2	0.002	12.0	56.0481/69.0672/83.0872/95.0843/121.0978/252.2596/264.2548/282.2811/300.28	ESI+	LC-MS
78	Sphinganine (C18:0)	C18H39NO2	301.2981	-5.0	363.1	0.002	10.9	Putative 93.59	ESI+	LC-MS
79	C16 Sphinganine (C16:0)	C16H35NO2	273.2668	-0.3	163.8	0.002	14.8	Putative 97.97	ESI+	LC-MS
80	C17 Sphinganine (C17:0)	C17H37NO2	287.2824	-4.6	-72.8	0.002	12.5	57.0721/88.0751/106.0860/288.2874	ESI+	LC-MS
Purines/pyrimidines and conjugates (NTs)										
81	S-Adenosyl-L-methionine (Adomet)	C15H22N6O5S	398.1372	4.7	103.4	0.002	3.1	Putative 70%	ESI+	CE-MS
82	Hypoxanthine	C5H4N4O	136.0385	0.0	-11.7	0.041	4.9	identified	ESI+	CE-MS
Others										
83	Pipecolate	C6H11NO2	129.0790	-0.8	543.7	0.002	7.6	identified	ESI+	CE-MS
84	Aminoimidazole ribotide	C8H14N3O7P	295.0569	1.9	-99.3	0.002	17.7	Putative 69.76	ESI+	LC-MS
85	Imidazole lactate	C6H8N2O3	156.0535	1.9	-90.4	0.002	4.3	identified	ESI+	CE-MS
86	Succinate	C4H6O4	118.0266	0.8	-82.4	0.002	16.5	identified	ESI+	CE-MS
87	17β-Hydroxysteroid/Androstan-3α,17β-diol	C19H32O2	292.2402	-6.2	-71.5	0.002	10.9	Putative 82.17	ESI-	LC-MS
88	Phenyllactate	C9H10O3	166.0629	-4.2	PCTL	0.002	7.9	72.9910/91.0491/103.0537/119.0465/147.0411/165.0578	ESI-	LC-MS

[#] Metabolite number assigned for correspondence with Figure 7.
[a] +increase or –decrease in treated related to untreated
[b]Relative standard deviation.
[c]Underlined number refers to the most abundant fragment observed.
dAbbreviations for lipids: LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; LPI: lysophosphatidylinositol; LPS: lysophosphatidylserine MG: monoradylglycerol; PA: phosphatidic acid.