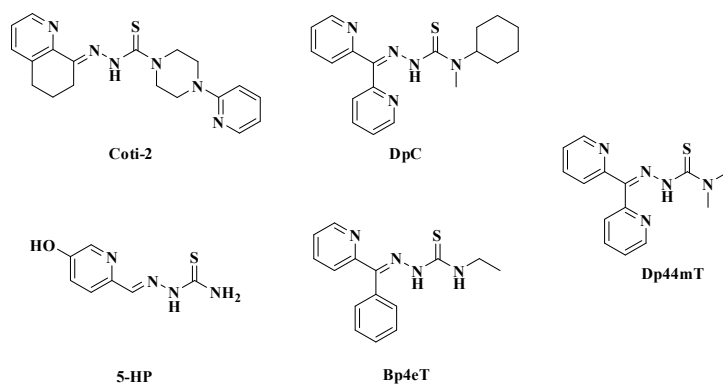


Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Comparison of metabolic pathways of different α -*N*-heterocyclic thiosemicarbazones

Karla Pelivan, Lisa M. Frensemeier, Uwe Karst, Gunda Koellensperger, Petra Heffeter, Bernhard K. Keppler, Christian R. Kowol



Scheme S1 Molecular structures of Triapine, Coti-2, DpC, 5-HP, Dp44mT and Bp4eT

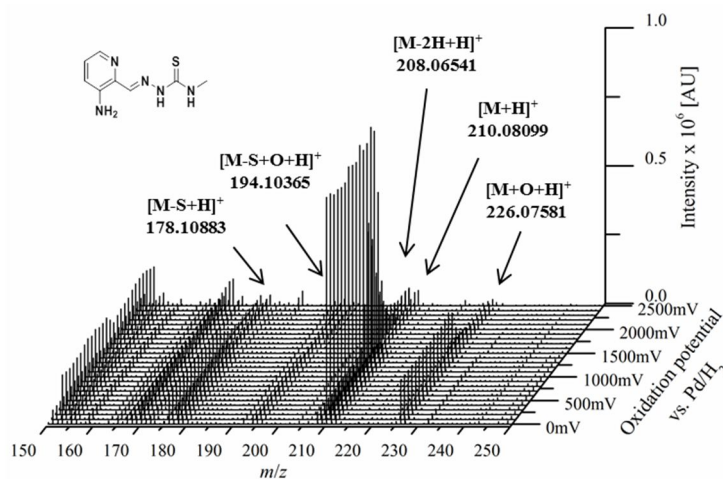


Fig. S1 Mass voltammogram of thiosemicarbazone 1

Table S1 Metabolites of compound 1 detected by EC/MS

Modification	Sum formula	Detected <i>m/z</i>	Theoretical <i>m/z</i>	Rel. deviation [ppm]	Metabolic reaction
[M+H] ⁺	C ₈ H ₁₂ N ₅ S	210.08099	210.08079	0.94	–
[M–NH–CH ₃] ⁺	C ₇ H ₇ N ₄ S	179.03859	179.03873	0.76	–
[M–2H+H] ⁺	C ₈ H ₁₀ N ₅ S	208.06541	208.06514	1.29	Dehydrogenation
[M–4H+H] ⁺	C ₈ H ₈ N ₅ S	206.04944	206.04949	0.26	Dehydrogenation
[M+O+H] ⁺	C ₈ H ₁₂ N ₅ SO	226.07581	226.07571	0.45	Hydroxylation
[M+O–2H+H] ⁺	C ₈ H ₁₀ N ₅ SO	224.06027	224.06006	0.95	Hydroxylation/ Dehydrogenation
[M–S+O+H] ⁺	C ₈ H ₁₂ N ₅ O	194.10365	194.10363	0.07	Desulfuration
[M–S+O–2H+H] ⁺	C ₈ H ₁₀ N ₅ O	192.08795	192.08799	0.19	Desulfuration/ Dehydrogenation
[M–S+H] ⁺	C ₈ H ₁₂ N ₅	178.10883	178.10872	0.61	Desulfuration
[M–S–2H+H] ⁺	C ₈ H ₁₀ N ₅	176.09323	176.09307	0.90	Desulfuration/ Dehydrogenation
[2M–2H+H] ⁺	C ₁₆ H ₂₁ N ₁₀ S ₂	417.13864	417.13866	0.05	Disulfide formation
[2M–4H+H] ⁺	C ₁₆ H ₁₉ N ₁₀ S ₂	415.12299	415.12301	0.05	Disulfide formation/ Dehydrogenation
[2M–6H+H] ⁺	C ₁₆ H ₁₇ N ₁₀ S ₂	413.10745	413.10736	0.22	Disulfide formation/ Dehydrogenation

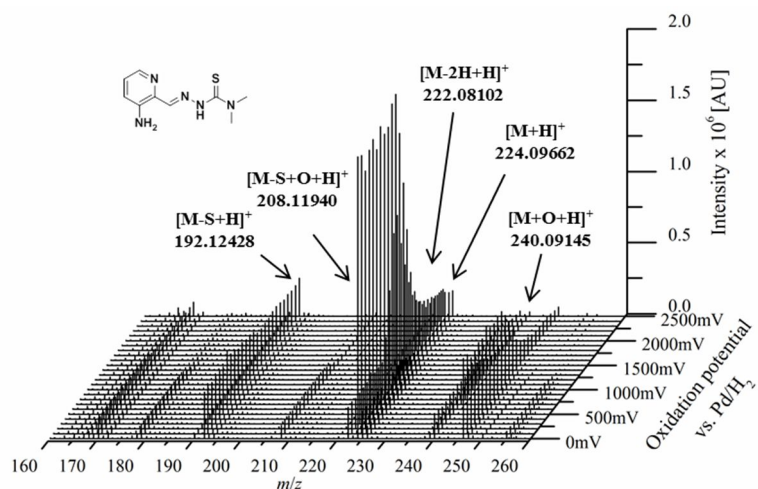


Fig. S2 Mass voltammogram of thiosemicarbazone **2**

Table S2 Metabolites of compound **2** detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_9H_{14}N_5S$	224.09662	224.09644	0.79	—
$[M-N-2xCH_3]^+$	$C_7H_7N_4S$	179.03865	179.03873	0.32	—
$[M-2H+H]^+$	$C_9H_{12}N_5S$	222.08102	222.08079	1.02	Dehydrogenation
$[M-4H+H]^+$	$C_9H_{10}N_5S$	220.06538	220.06514	1.08	Dehydrogenation
$[M+O+H]^+$	$C_9H_{14}N_5SO$	240.09145	240.09136	0.39	Hydroxylation
$[M+O-2H+H]^+$	$C_9H_{12}N_5SO$	238.07587	238.07571	0.68	Hydroxylation/ Dehydrogenation
$[M+O-4H+H]^+$	$C_9H_{10}N_5SO$	236.06026	236.06006	0.86	Hydroxylation/ Dehydrogenation
$[M-S+O+H]^+$	$C_9H_{14}N_5O$	208.11940	208.11929	0.55	Desulfuration
$[M-S+O-2H+H]^+$	$C_9H_{12}N_5O$	206.10374	206.10364	0.50	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_9H_{14}N_5$	192.12428	192.12437	0.48	Desulfuration
$[M-S-2H+H]^+$	$C_9H_{12}N_5$	190.10878	190.10872	0.31	Desulfuration/ Dehydrogenation
$[M-CH_2+H]^+$	$C_8H_{12}N_5S$	210.08044	210.08079	1.68	Demethylation*
$[M-CH_2-2H+H]^+$	$C_8H_{10}N_5S$	208.06487	208.06514	1.31	Demethylation/ Dehydrogenation*
$[2M-2H+H]^+$	$C_{18}H_{25}N_{10}S_2$	445.17007	445.16996	0.25	Disulfide formation
$[2M-4H+H]^+$	$C_{18}H_{23}N_{10}S_2$	443.15433	443.15431	0.05	Disulfide formation/ Dehydrogenation
$[2M-6H+H]^+$	$C_{18}H_{21}N_{10}S_2$	441.13870	441.13866	0.09	Disulfide formation/ Dehydrogenation

*observed after electrochemical oxidation only via LC-HRMS, but not via EC-HRMS

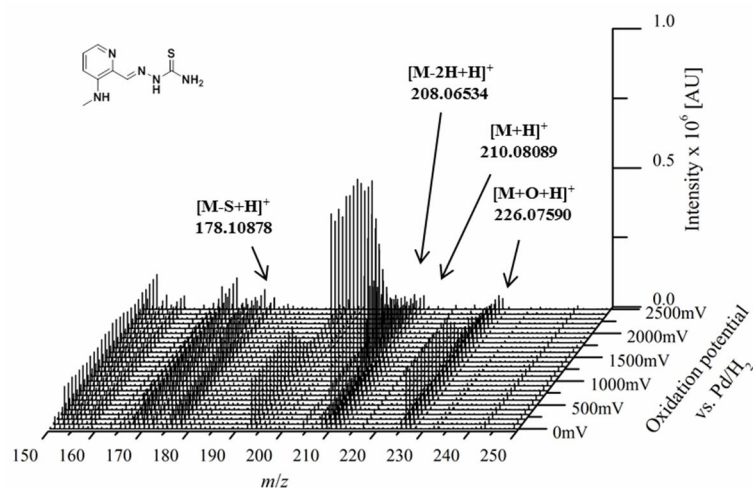


Fig. S3 Mass voltammogram of thiosemicarbazone 3

Table S3 Metabolites of compound 3 detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_8H_{12}N_5S$	210.08089	210.08079	0.46	–
$[M-NH_2]^+$	$C_8H_9N_4S$	193.05434	193.05424	0.50	–
$[M-2H+H]^+$	$C_8H_{10}N_5S$	208.06534	208.06514	0.05	Dehydrogenation
$[M-4H+H]^+$	$C_8H_8N_5S$	206.04982	206.04949	1.59	Dehydrogenation
$[M+O+H]^+$	$C_8H_{12}N_5SO$	226.07590	226.07571	0.85	Hydroxylation
$[M+O-2H+H]^+$	$C_8H_{10}N_5SO$	224.06024	224.06006	0.82	Hydroxylation/ Dehydrogenation
$[M+O-4H+H]^+$	$C_8H_8N_5SO$	222.04442	222.04441	0.06	Hydroxylation/ Dehydrogenation
$[M-S+H]^+$	$C_8H_{12}N_5$	178.10878	178.10872	0.33	Desulfuration
$[M-S-2H+H]^+$	$C_8H_{10}N_5$	176.09320	176.09307	0.73	Desulfuration/ Dehydrogenation
$[M-S-4H+H]^+$	$C_8H_8N_5$	174.07756	174.07742	0.79	Desulfuration/ Dehydrogenation
$[M-CH_2-2H+H]^+$	$C_7H_8N_5S$	194.04926	194.04949	1.20	Demethylation/ Dehydrogenation*
$[2M-2H+H]^+$	$C_{16}H_{21}N_{10}S_2$	417.13898	417.13866	0.77	Disulfide formation
$[2M-4H+H]^+$	$C_{16}H_{19}N_{10}S_2$	415.12317	415.12301	0.39	Disulfide formation/ Dehydrogenation
$[2M-6H+H]^+$	$C_{16}H_{17}N_{10}S_2$	413.10748	413.10736	0.29	Disulfide formation/ Dehydrogenation

*observed after electrochemical oxidation only via LC-HRMS, but not via EC-HRMS

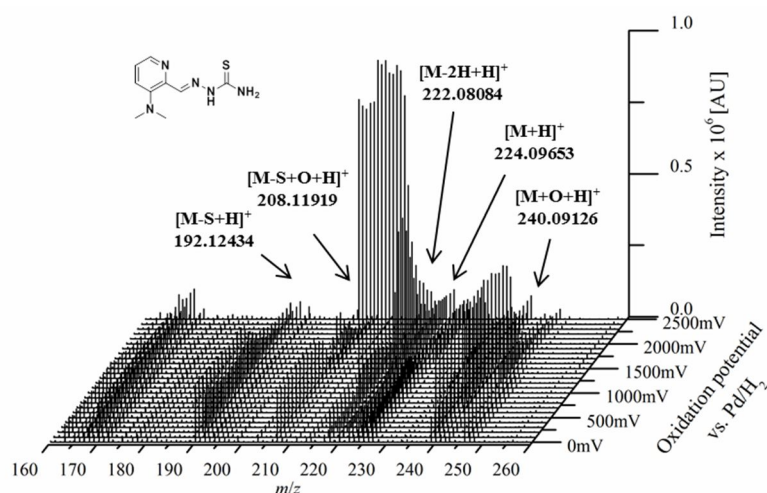


Fig. S4 Mass voltammogram of thiosemicarbazone **4**

Table S4 Metabolites of compound **4** detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_9H_{14}N_5S$	224.09653	224.09644	0.39	–
$[M-NH_2]^+$	$C_9H_{11}N_4S$	207.06984	207.06989	0.26	
$[M-2H+H]^+$	$C_9H_{12}N_5S$	222.08084	222.08079	0.21	Dehydrogenation
$[M-4H+H]^+$	$C_9H_{10}N_5S$	220.06526	220.06514	0.53	Dehydrogenation
$[M+O+H]^+$	$C_9H_{14}N_5SO$	240.09126	240.09136	0.41	Hydroxylation
$[M+O-2H+H]^+$	$C_9H_{12}N_5SO$	238.07567	238.07571	0.16	Hydroxylation/ Dehydrogenation
$[M+O-4H+H]^+$	$C_9H_{10}N_5SO$	236.06000	236.06006	0.24	Hydroxylation/ Dehydrogenation
$[M-S+O+H]^+$	$C_9H_{14}N_5O$	208.11919	208.11929	0.46	Desulfuration
$[M-S+O-2H+H]^+$	$C_9H_{12}N_5O$	206.10382	206.10364	0.89	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_9H_{14}N_5$	192.12434	192.12437	0.17	Desulfuration
$[M-S-2H+H]^+$	$C_9H_{12}N_5$	190.10870	190.10872	0.12	Desulfuration/ Dehydrogenation
$[M-CH_2+H]^+$	$C_8H_{12}N_5S$	210.08064	210.08079	0.73	Demethylation
$[M-CH_2-2H+H]^+$	$C_8H_{10}N_5S$	208.06523	208.06514	0.42	Demethylation/ Dehydrogenation
$[M-CH_2-4H+H]^+$	$C_8H_8N_5S$	206.04961	206.04949	0.57	Demethylation/ Dehydrogenation
$[M-2xCH_2-2H+H]^+$	$C_7H_8N_5S$	194.04929	194.04949	1.04	Demethylation/ Dehydrogenation*
$[2M-2H+H]^+$	$C_{18}H_{25}N_{10}S_2$	445.16980	445.16996	0.36	Disulfide formation
$[2M-4H+H]^+$	$C_{18}H_{23}N_{10}S_2$	443.15448	443.15431	0.39	Disulfide formation/ Dehydrogenation

*observed after electrochemical oxidation only via LC-HRMS, but not via EC-HRMS

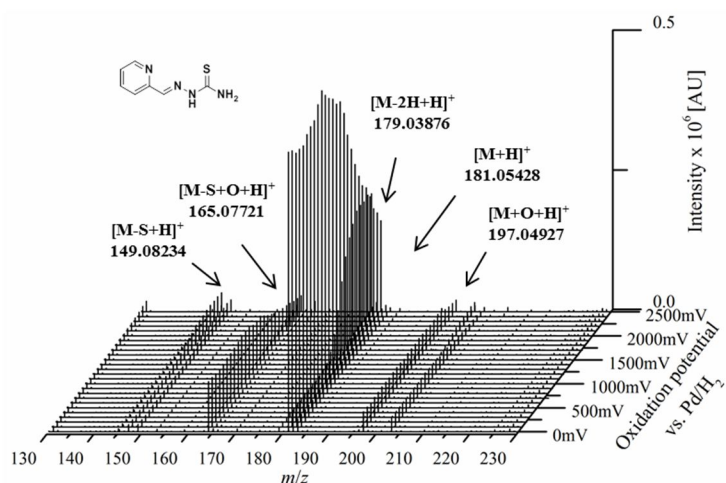


Fig. S5 Mass voltammogram of thiosemicarbazone **6**

Table S5 Metabolites of compound **6** detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_7H_9N_4S$	181.05428	181.05424	0.20	–
$[M-NH_2]^+$	$C_7H_8N_3S$	164.02782	164.02769	0.77	
$[M-2H+H]^+$	$C_7H_7N_4S$	179.03876	179.03859	0.93	Dehydrogenation
$[M+O+H]^+$	$C_7H_9N_4SO$	197.04927	197.04916	0.57	Hydroxylation
$[M-S+O+H]^+$	$C_7H_9N_4O$	165.07721	165.07709	0.74	Desulfuration
$[M-S+O-2H+H]^+$	$C_7H_7N_4O$	163.06158	163.06144	0.87	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_7H_9N_4$	149.08234	149.08217	1.12	Desulfuration
$[M-S-2H+H]^+$	$C_7H_7N_4$	147.06664	147.06652	0.80	Desulfuration/ Dehydrogenation
$[2M-2H+H]^+$	$C_{14}H_{15}N_8S_2$	359.08566	359.08556	0.28	Disulfide formation
$[2M-4H+H]^+$	$C_{14}H_{13}N_8S_2$	357.07013	357.06991	0.62	Disulfide formation/ Dehydrogenation

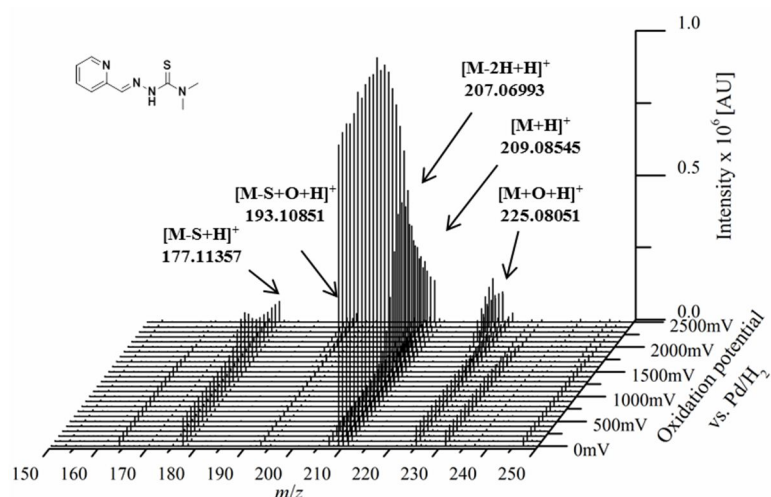


Fig. S6 Mass voltammogram of thiosemicarbazone **8**

Table S6 Metabolites of compound **8** detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_9H_{13}N_4S$	209.08545	209.08554	0.49	–
$[M-N-2xCH_3]^+$	$C_7H_6N_3S$	164.02774	164.02769	0.28	
$[M-2H+H]^+$	$C_9H_{11}N_4S$	207.06993	207.06989	0.18	Dehydrogenation
$[M-4H+H]^+$	$C_9H_9N_4S$	205.05449	205.05424	1.20	Dehydrogenation
$[M+O+H]^+$	$C_9H_{13}N_4SO$	225.08051	225.08046	0.23	Hydroxylation
$[M+O-2H+H]^+$	$C_9H_{11}N_4SO$	223.06491	223.06481	0.46	Hydroxylation/ Dehydrogenation
$[M+O-4H+H]^+$	$C_9H_9N_4SO$	221.04927	221.04916	0.51	Hydroxylation/ Dehydrogenation
$[M-S+O+H]^+$	$C_9H_{13}N_4O$	193.10851	193.10839	0.63	Desulfuration
$[M-S+O-2H+H]^+$	$C_9H_{11}N_4O$	191.09264	191.09274	0.51	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_9H_{13}N_4$	177.11357	177.11347	0.55	Desulfuration
$[M-S-2H+H]^+$	$C_9H_{11}N_4$	175.09785	175.09782	0.16	Desulfuration/ Dehydrogenation
$[M-CH_2-2H+H]^+$	$C_8H_9N_4S$	193.05426	193.05424	0.09	Demethylation/ Dehydrogenation
$[M-2xCH_2-2H+H]^+$	$C_7H_7N_4S$	179.03838	179.03859	1.19	Demethylation/ Dehydrogenation*
$[2M-2H+H]^+$	$C_{18}H_{23}N_8S_2$	415.14828	415.14816	0.29	Disulfide formation
$[2M-4H+H]^+$	$C_{18}H_{21}N_8S_2$	413.13266	413.13251	0.36	Disulfide formation/ Dehydrogenation

*observed after electrochemical oxidation only via LC-HRMS, but not via EC-HRMS

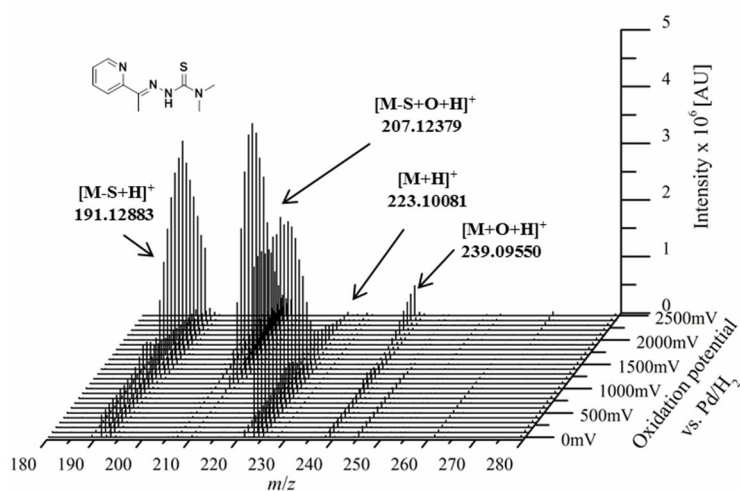


Fig. S7 Mass voltammogram of thiosemicarbazone **9**

Table S7 Metabolites of compound **9** detected by EC/MS

Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_{10}H_{15}N_4S$	223.10081	223.10119	1.72	–
$[M-N-2xCH_3]^+$	$C_8H_8N_3S$	178.04292	178.04334	2.39	
$[M+O+H]^+$	$C_{10}H_{15}N_4SO$	239.09550	239.09611	2.54	Hydroxylation
$[M+O-2H+H]^+$	$C_{10}H_{13}N_4SO$	237.07994	237.08046	2.19	Hydroxylation/ Dehydrogenation
$[M-S+O+H]^+$	$C_{10}H_{15}N_4O$	207.12379	207.12404	1.20	Desulfuration
$[M-S+O-2H+H]^+$	$C_{10}H_{13}N_4O$	205.10796	205.10839	2.09	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_{10}H_{15}N_4$	191.12883	191.12912	1.53	Desulfuration
$[M-S-2H+H]^+$	$C_{10}H_{13}N_4$	189.11314	189.11347	1.76	Desulfuration/ Dehydrogenation
$[M-CH_2-2H+H]^+$	$C_9H_{11}N_4S$	207.06976	207.06989	0.65	Demethylation/ Dehydrogenation*
$[M-2xCH_2-2H+H]^+$	$C_8H_9N_4S$	193.05423	193.05424	0.07	Demethylation/ Dehydrogenation*
$[M-3xCH_2-2H+H]^+$	$C_7H_7N_4S$	179.03842	179.03859	0.97	Demethylation/ Dehydrogenation*
$[2M-2H+H]^+$	$C_{20}H_{25}N_8S_2$	441.16302	441.16381	1.79	Disulfide formation
$[2M-4H+H]^+$	$C_{20}H_{23}N_8S_2$	439.14792	439.14816	0.55	Disulfide formation/ Dehydrogenation

*observed after electrochemical oxidation only via LC-HRMS, but not via EC-HRMS

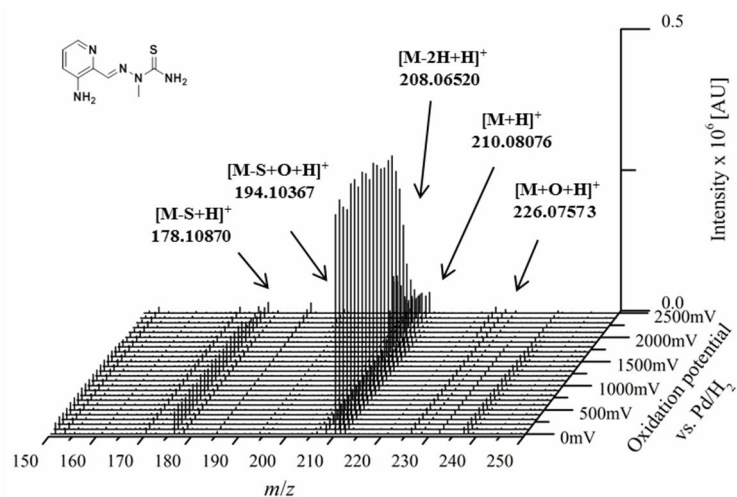
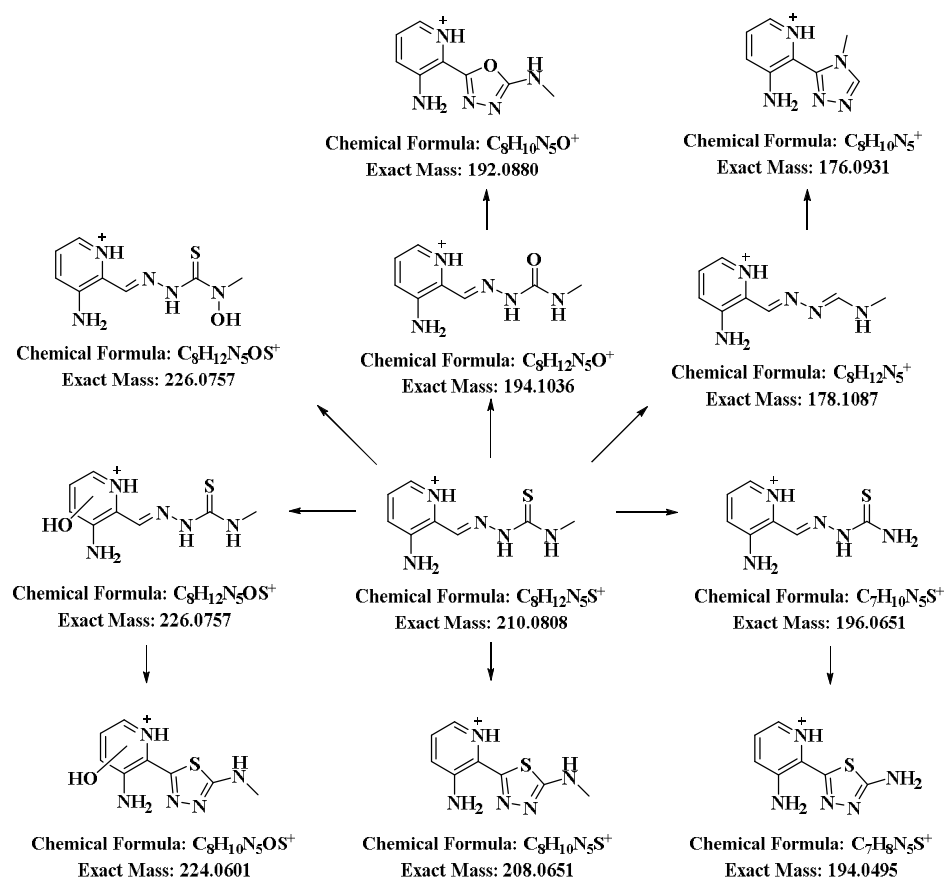


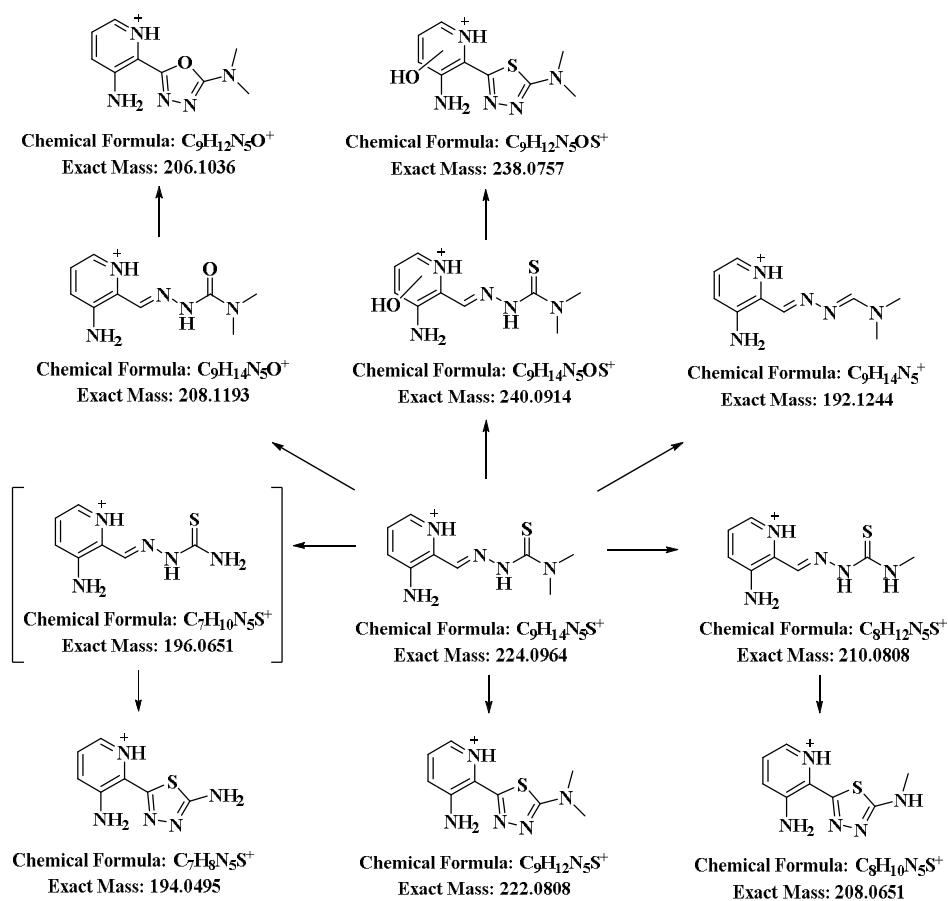
Fig. S8 Mass voltammogram of thiosemicarbazone **10**

Table S8 Metabolites of compound **10** detected by EC/MS

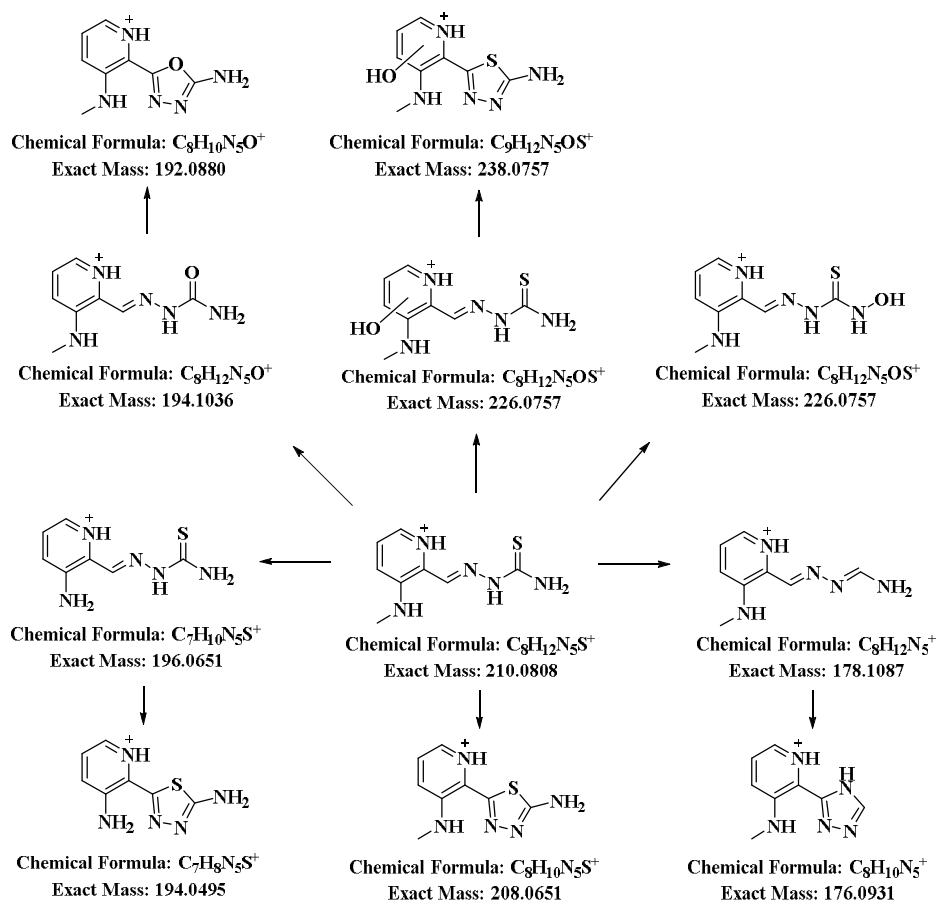
Modification	Sum formula	Detected m/z	Theoretical m/z	Rel. deviation [ppm]	Metabolic reaction
$[M+H]^+$	$C_8H_{12}N_5S$	210.08076	210.08079	0.16	–
$[M-2H+H]^+$	$C_8H_{10}N_5S$	208.06520	208.06514	0.28	Dehydrogenation
$[M-4H+H]^+$	$C_8H_8N_5S$	206.04945	206.04949	0.21	Dehydrogenation
$[M+O+H]^+$	$C_8H_{12}N_5SO$	226.07573	226.07571	0.10	Hydroxylation
$[M+O-2H+H]^+$	$C_8H_{10}N_5SO$	224.06000	224.06006	0.26	Hydroxylation/ Dehydrogenation
$[M+O-4H+H]^+$	$C_8H_8N_5SO$	222.04443	222.04441	0.10	Hydroxylation/ Dehydrogenation
$[M-S+O+H]^+$	$C_8H_{12}N_5O$	194.10367	194.10364	0.17	Desulfuration
$[M-S+O-2H+H]^+$	$C_8H_{10}N_5O$	192.08794	192.08799	0.24	Desulfuration/ Dehydrogenation
$[M-S+H]^+$	$C_8H_{12}N_5$	178.10870	178.10872	0.12	Desulfuration
$[M-S-2H+H]^+$	$C_8H_{10}N_5$	176.09303	176.09307	0.24	Desulfuration/ Dehydrogenation
$[M-S-4H+H]^+$	$C_8H_8N_5$	174.07747	174.07742	0.28	Desulfuration/ Dehydrogenation
$[2M-2H+H]^+$	$C_{16}H_{21}N_{10}S_2$	417.13843	417.13866	0.55	Disulfide formation
$[2M-4H+H]^+$	$C_{16}H_{19}N_{10}S_2$	415.12292	415.12301	0.21	Disulfide formation/ Dehydrogenation



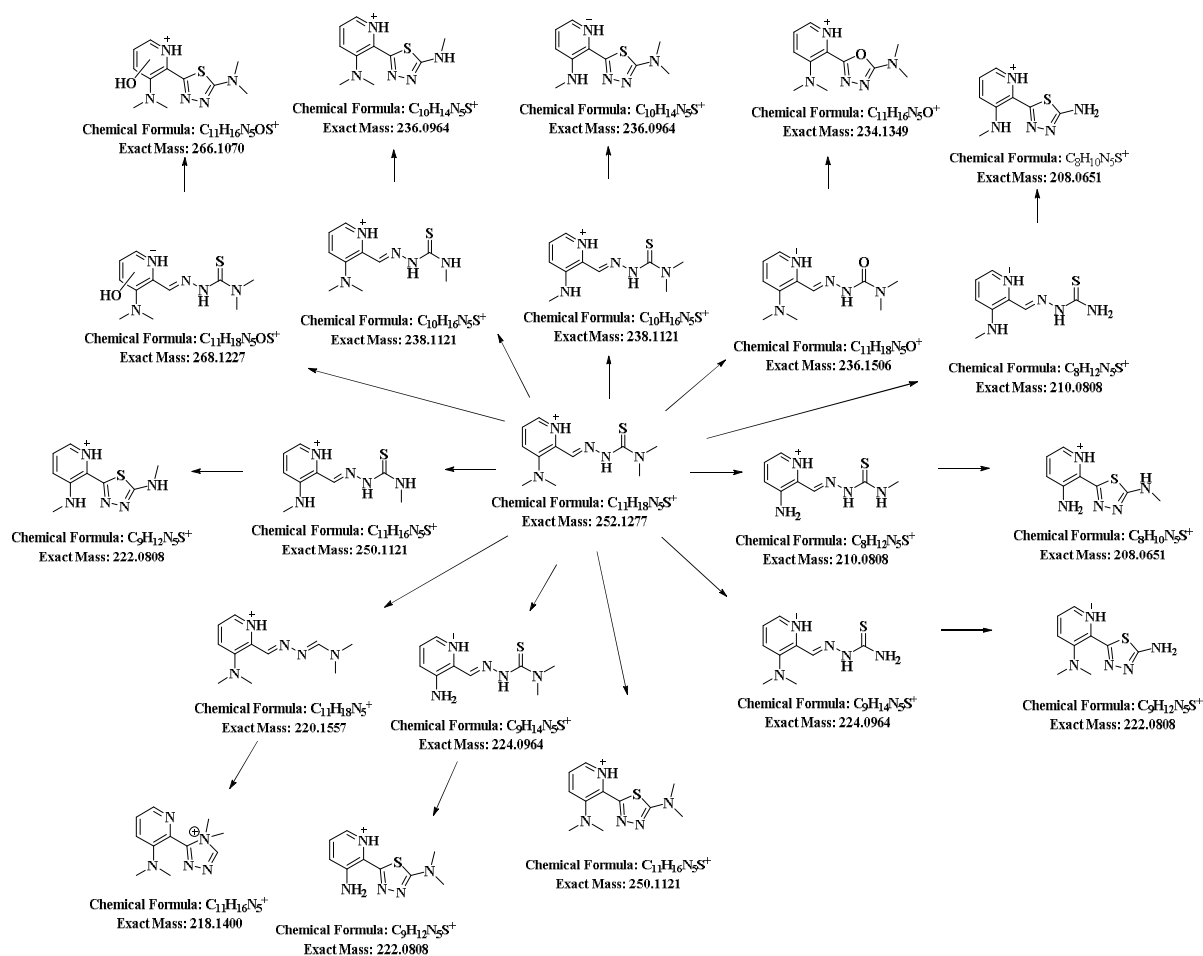
Scheme S2 Metabolic pathways of **1** elucidated by LC-HRMS after incubation with cell-free microsomes



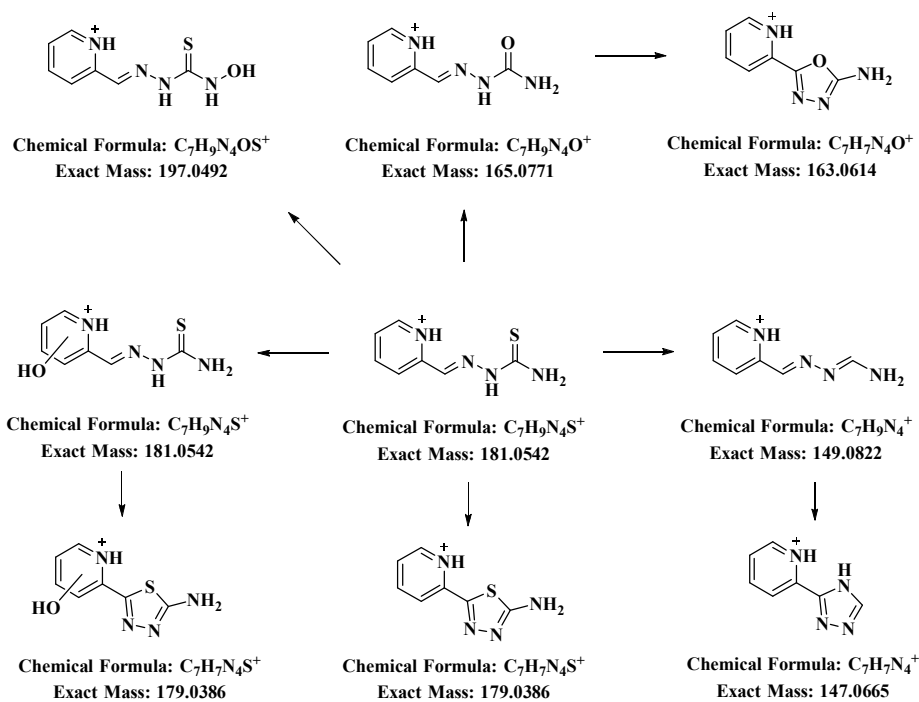
Scheme S3 Metabolic pathways of **2** elucidated by LC-HRMS after incubation with cell-free microsomes



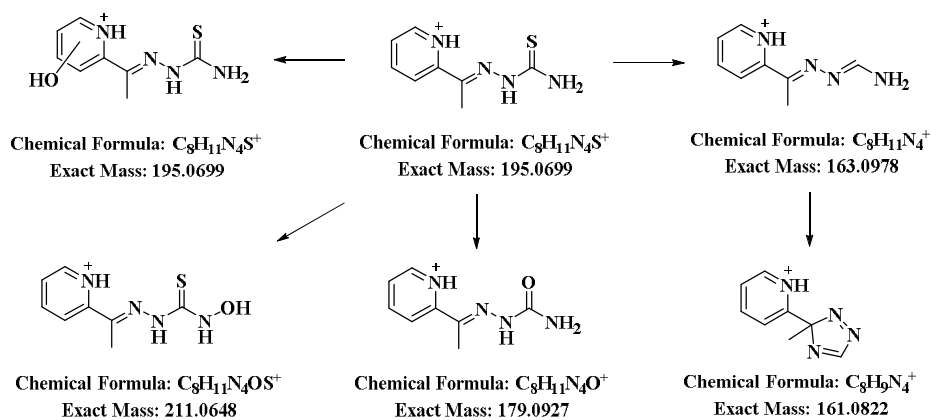
Scheme S4 Metabolic pathways of **3** elucidated by LC-HRMS after incubation with cell-free microsomes



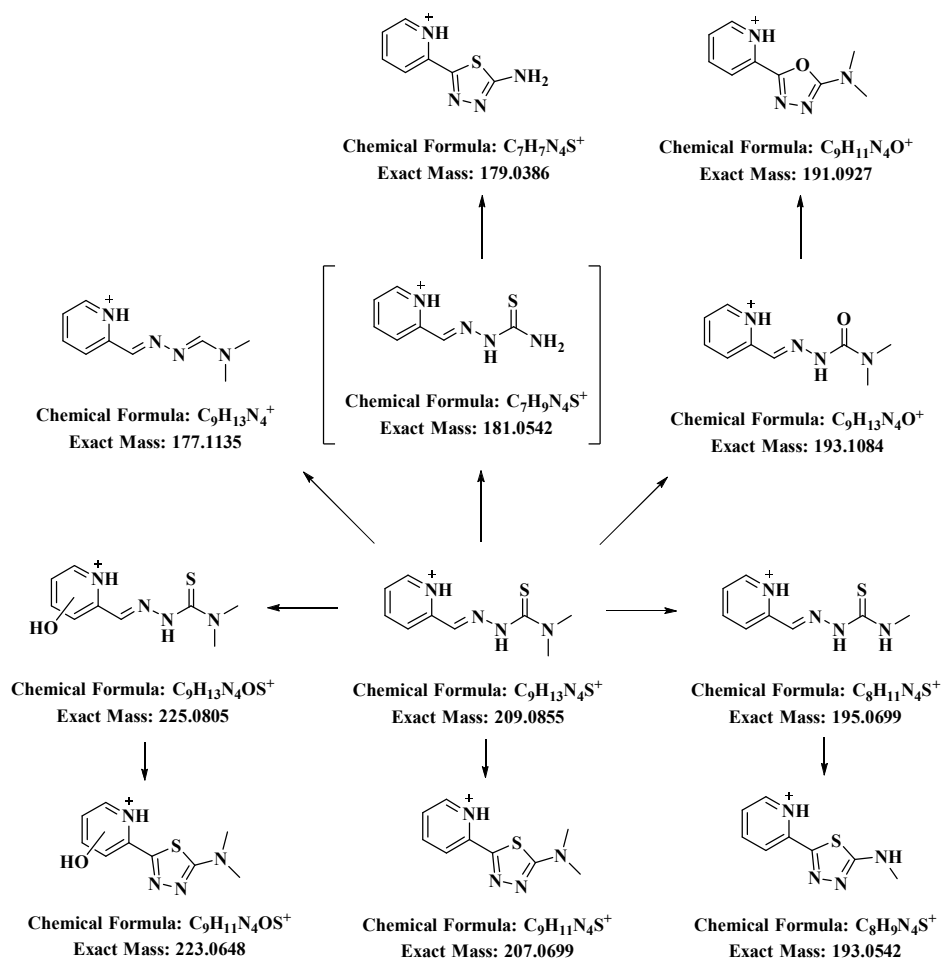
Scheme S5 Metabolic pathways of **5** elucidated by LC-HRMS after incubation with cell-free microsomes



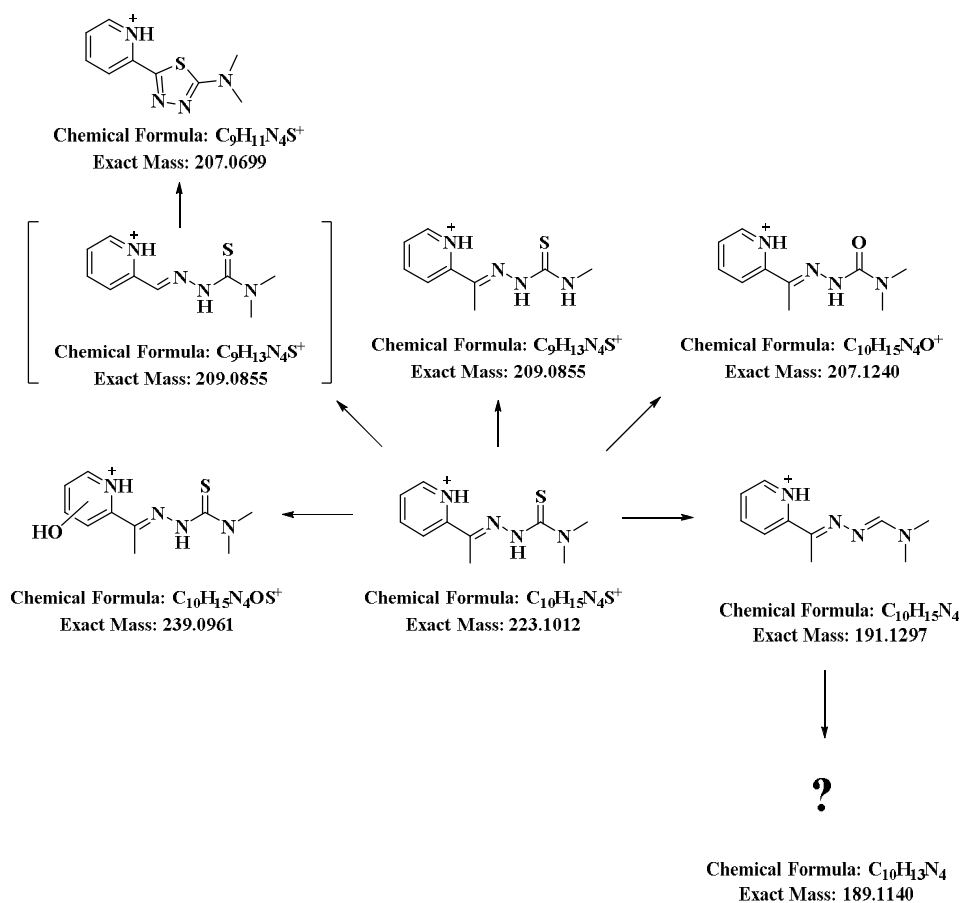
Scheme S6 Metabolic pathways of **6** elucidated by LC-HRMS after incubation with cell-free microsomes



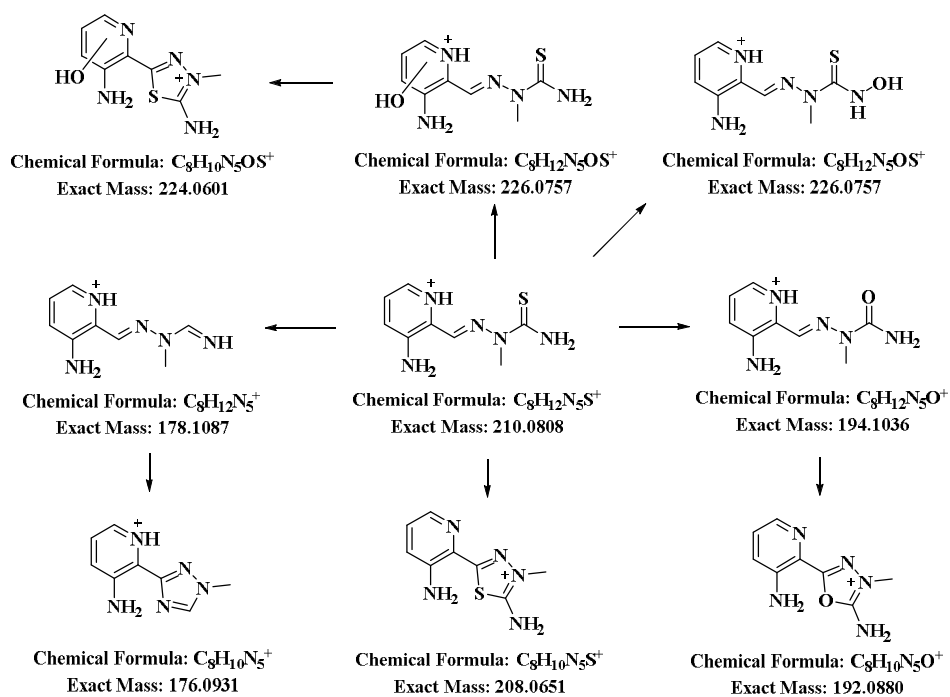
Scheme S7 Metabolic pathways of **7** elucidated by LC-HRMS after incubation with cell-free microsomes



Scheme S8 Metabolic pathways of **8** elucidated by LC-HRMS after incubation with cell-free microsomes



Scheme S9 Metabolic pathways of **9** elucidated by LC-HRMS after incubation with cell-free microsomes



Scheme S10 Metabolic pathways of **10** elucidated by LC-HRMS after incubation with cell-free microsomes

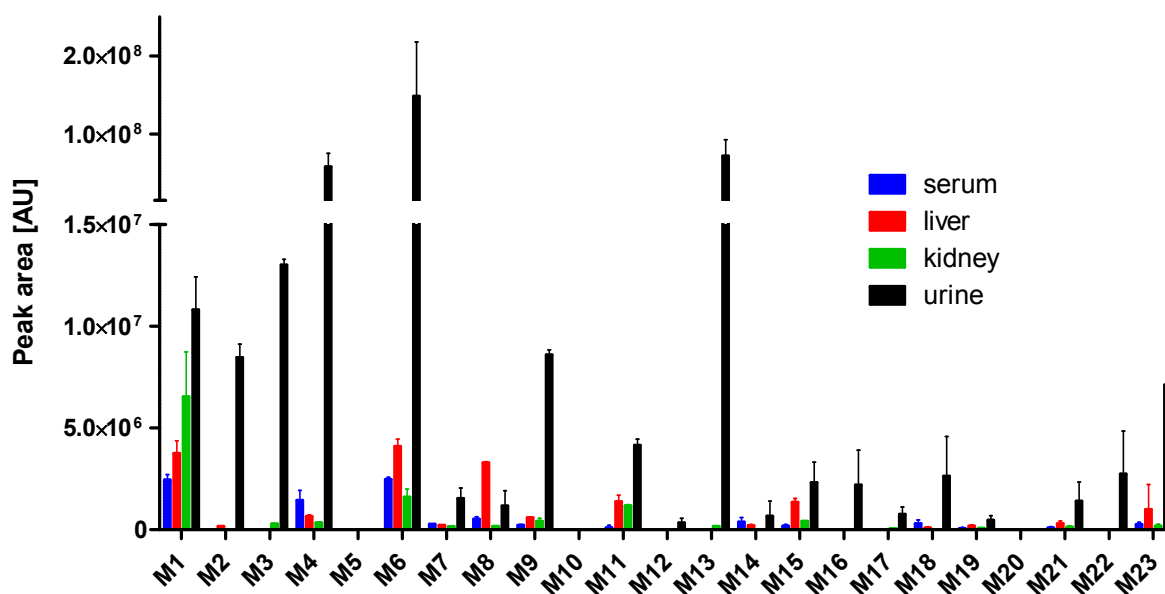


Fig. S9 Relative quantification of metabolites 1–23 of compound **5** in serum/liver/kidney/urine samples of mice (zoom of the data of Figure 8)