Supporting Information

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Speciation Analysis of the Antirheumatic Agent Auranofin and its Thiol Adducts by LC/ESI-MS and LC/ICP-MS

Supporting information is presented on LC/ESI-Orbitrap-MS, ESI-IT-MS and LC/ICP-MS experimental conditions for the analysis of Auranofin, glutathione (GSH), and human serum albumin (HAS). Additional information on the fragmentation experiments of Auranofin with mass spectra and fragmentation pathway is provided.

Table S-1. ESI-Orbitrap-MS parameters for the analysis of Auranofin, GSH and HAS.

Parameters \ Analyte	Auranofin, GSH positive ion mode	Auranofin, GSH negative ion mode	HSA
Scan Parameters			
Scan Range	100-1500	100-1500	750-3000
Fragmentation	HCD Gas On	HCD Gas On	HCD Gas On
Resolution	High	High	Medium
Polarity	Positive	Negative	Positive
Microscans	1	1	5
Lock Masses	Off	Off	Off
AGC Target	Balanced	Balanced	Balanced
Maximum Injection Time	10 ms	10 ms	10 ms
ESI Source			
Sheath Gas Flow Rate	40	40	40
Aux Gas Flow Rate	15	15	15
Sweep Gas Flow Rate	0	0	0
Spray Voltage (|kV|)	3.5	3.0	3.5
Capillary Temperature (°C)	350	350	350
Capillary Voltage (V)	37.50	-57.50	37.50
Tube Lens Voltage (V)	115.00	-190.00	190.00
Skimmer Voltage (V)	22.00	-34.00	44.00

Table S-2. ESI-IT-MS parameters for the fragmentation experiments of Auranofin.

Ion Source	
Tune Mode	smart
Nebulizer Pressure [psi]	10
Drying Gas [L/min]	4
Dryingt Temp. [°C]	330
Ion Transfer	
Target Mass [m/z]	679 (+), 923 (–)
Compound Stability [%]	100
Trap Drive Level [%]	100
Optimize	wide
Polarity	positive, negative
Trap ICC	
Target	15000
Max. Accu T [ms]	10

Table S-3. LC/ICP-MS parameters for the analysis of Auranofin, GSH and HSA.

Nebilizer Type	PFA µflow
Sampling Gas [L/min]	0.780
Oxygen Flow [L/min]	0.100
ICP RF Power [W]	1500
Scan Type	E-Scan
Isotope	Au197
Resolution	Low
Mass Window	10
Samples per Peak	200
Sample Time	0.01
Integration Window	10
Acquisition Points	10

Figure S-1. Fragmentation experiment of the Auranofin precursor ion at *m/z* 586 by ESI-IT-MS. Mass spectra are shown of the fragmented precursor ion at *m/z* 586. See table S-1 for structural information.

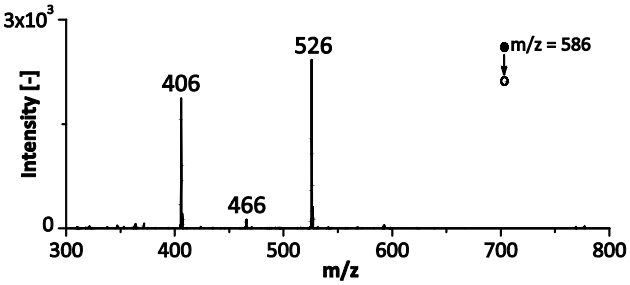
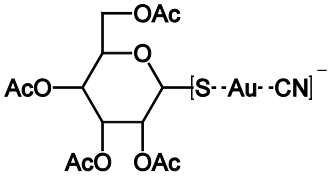
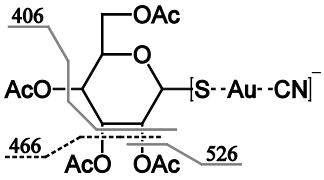


Table S-4. Fragmentation pathway of the Auranofin precursor ion at *m/z* 586.

Experiment	Structure and *m/z*
MS^1	 586
m/z = 586	

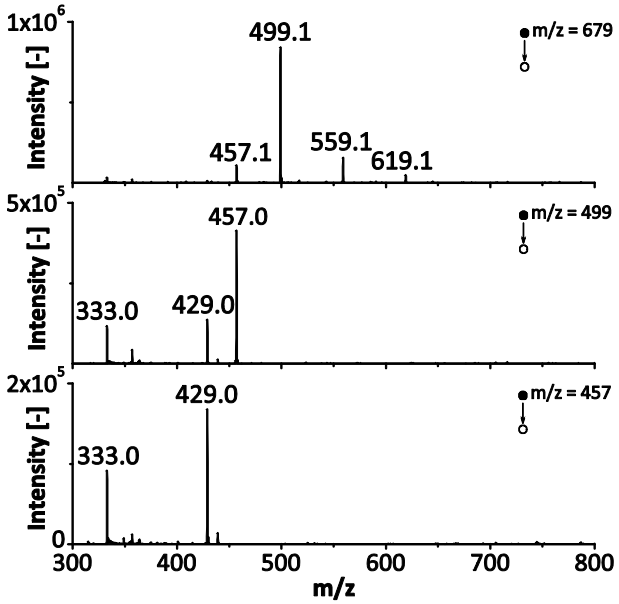
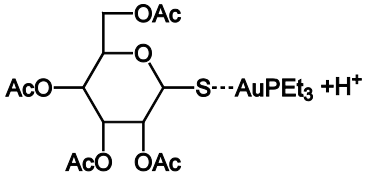
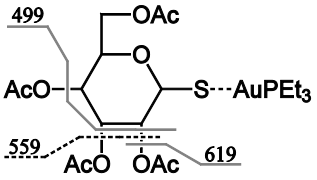
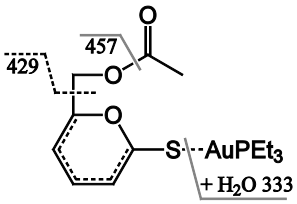


Figure S-2. Fragmentation experiment of the Auranofin precursor ion at *m/z* 679 by ESI-IT-MS. Mass spectra are shown of the fragmented a) precursor ion at *m/z* 679, b) fragment ion at *m/z* 499 and c) fragment ion at *m/z* 457. See table S-5 for structural information.

Table S-5. Fragmentation pathway of the Auranofin precursor ion at *m/z* 679.

Experiment	Structure and *m/z*
MS^1	 679
● m/z = 679 ↓ ○	
● m/z = 499 ↓ ○	

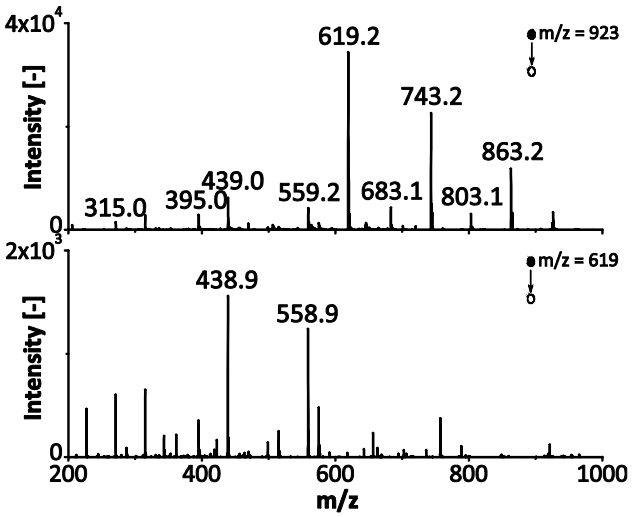
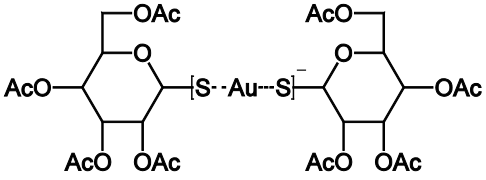
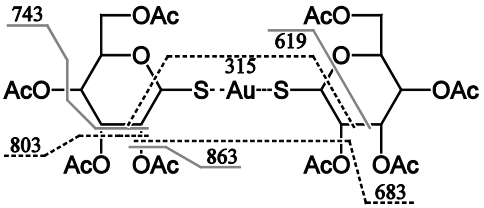
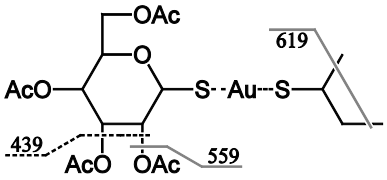
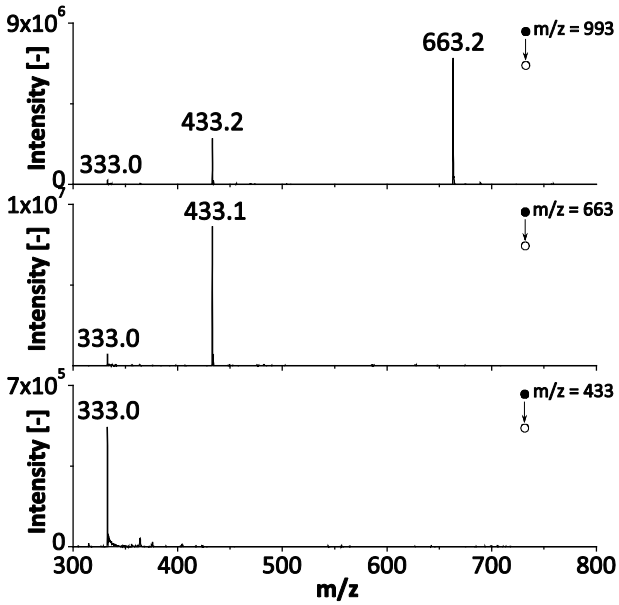


Figure S-3. Fragmentation experiment of the Auranofin precursor ion at *m/z* 923 by ESI-IT-MS. Mass spectra are shown of fragmented a) precursor ion at *m/z* 923 and b) fragment ion at *m/z* 619. See table S-6 for structural information.

Table S-6. Fragmentation pathway of the Auranofin precursor ion at *m/z* 923.

Experiment	Structure and *m/z*
MS^1	
m/z = 923 →	
m/z = 619 →	



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154 **Figure S-4.** Fragmentation experiment of the Auranofin precursor ion at *m/z* 993 by ESI-IT-MS. Mass
155 spectra are shown of fragmented a) precursor ion at *m/z* 993, b) fragment ion at *m/z* 663 and c) fragment
156 ion at *m/z* 433. See table S-7 for structural information.

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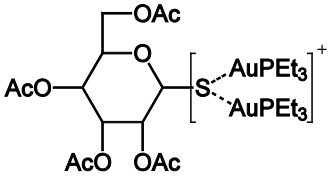
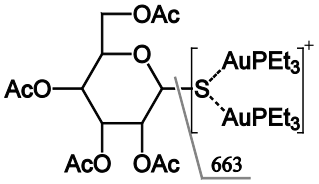
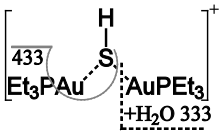
167 **Table S-7.** Fragmentation pathway of the Auranofin precursor ion at *m/z* 993.

Experiment	Structure and *m/z*
MS^1	
● m/z = 993 ↓ ○	
● m/z = 663 ↓ ○	

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