

Supplementary Information

Paper

Detailed analysis of pro-apoptotic signaling and metabolic adaptation triggered by a *N*-heterocyclic carbene gold(I) complex

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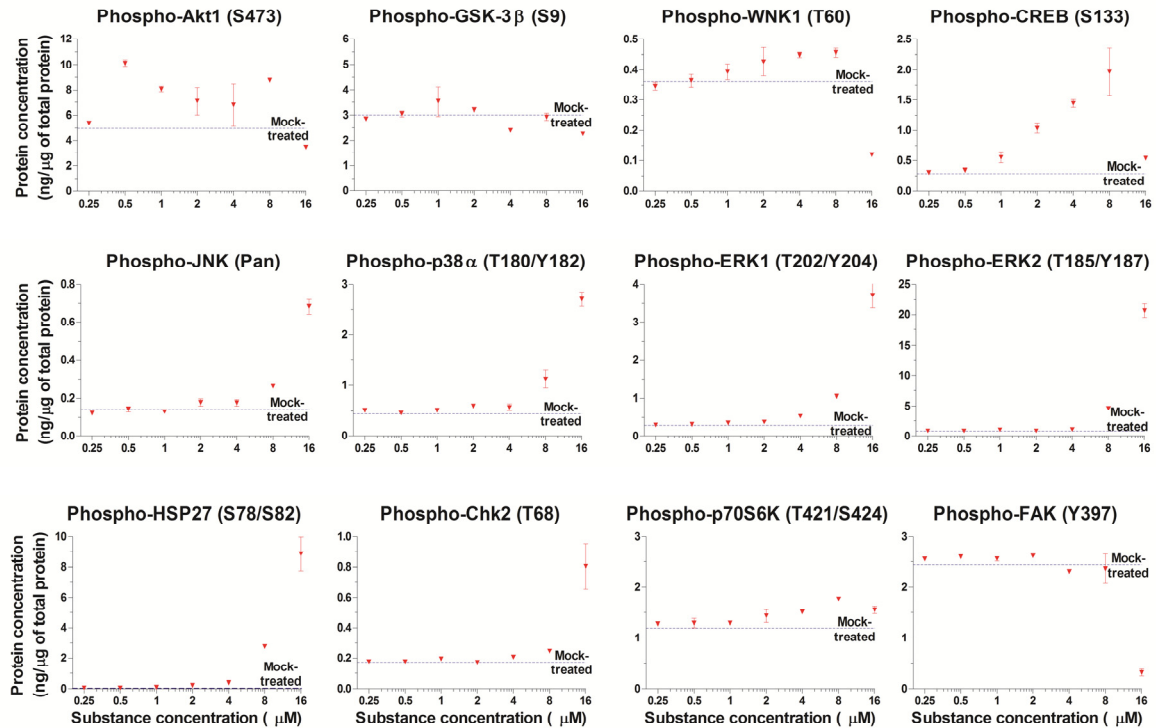
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Supplementary Figure 1 Absolute concentrations of some phosphorylated key regulatory proteins in HT29 cells upon 2 h treatment with serial dilutions of *Au(I)NHC*. Dashed lines indicate protein levels upon mock-treatment. Mock-treated samples contained 0.1 % (v/v) DMF. The protein levels were measured using kinetic ELISA microarrays.

Sample Analyte	Phospho-Akt1 ^{S473}	Phospho-GSK-3 β ^{S9}	Phospho-WNK1 ^{T60}	Phospho-CREB ^{S133}	Phospho-p70S6K ^{T421/S424}	Phospho-TOR ^{S2448}	Phospho-JNK ^(Pan)	Phospho-p38 α ^{T180/Y182}	Phospho-ERK1 ^{T202/Y204}	Phospho-ERK2 ^{T185/Y187}	Phospho-HSP27 ^{S78/S82}	Phospho-Chk2 ^{T68}	Phospho-p53 ^{S15}	Phospho-FAK ^{Y397}	
Non-treated	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	
Mock-treated (0.1% DMF)	1h	0,88	0,99	1,03	1,02	0,92	1,00	1,07	1,03	1,10	1,04	0,98	1,03	1,01	0,98
	2h	0,86	1,11	1,00	1,10	0,98	0,99	1,09	1,04	1,21	1,06	0,88	1,05	1,31	0,97
	3h	1,50	1,13	1,04	1,27	1,01	0,98	1,11	1,04	1,49	1,21	1,04	1,06	1,31	0,97
	4h	0,76	1,08	1,06	1,31	1,00	0,99	0,94	1,04	1,58	1,17	0,92	0,98	1,31	1,00
	5h	0,74	1,06	1,07	1,23	0,99	0,99	0,99	1,00	1,49	1,24	0,83	0,89	1,58	0,93
	6h	0,71	1,14	1,14	1,18	0,99	0,86	0,83	0,94	1,39	1,66	0,90	1,08	1,52	0,87
5 μ M Au(I)NHC	1h	1,44	1,00	1,05	2,03	1,08	1,07	0,91	1,49	1,08	1,18	2,07	1,03	0,82	1,04
	2h	1,50	1,16	1,24	5,47	1,20	1,02	0,95	1,83	2,92	2,85	3,04	1,26	0,36	1,09
	3h	1,56	1,19	1,29	7,66	1,26	1,01	1,02	1,64	3,45	4,22	6,15	1,53	0,19	1,12
	4h	1,50	1,40	1,37	11,11	1,35	1,02	0,96	1,87	3,45	6,47	9,59	1,89	0,13	0,66
	5h	1,51	1,29	1,37	10,61	1,38	1,11	1,05	2,05	3,87	6,90	13,21	1,74	0,25	0,58
	6h	1,47	1,09	1,30	8,68	1,38	1,01	1,07	1,70	3,79	6,66	12,88	1,69	0,21	0,55
10 μ M Au(I)NHC	1h	1,49	0,71	1,28	5,18	1,07	0,97	1,04	1,97	3,01	3,99	6,91	1,07	0,33	0,70
	2h	0,88	0,75	1,04	8,03	1,52	0,94	1,43	2,50	5,79	6,91	32,66	1,38	0,18	0,65
	3h	0,65	0,90	0,69	4,68	1,74	1,22	1,83	2,56	9,29	8,48	40,05	1,85	0,41	0,69
	4h	0,58	0,70	0,50	3,55	1,72	1,18	1,67	2,28	9,36	8,61	40,67	1,94	0,47	0,47
	5h	0,49	0,64	0,42	3,11	1,57	1,41	1,92	3,15	9,90	10,34	48,82	1,83	0,33	0,30
	6h	0,53	0,59	0,35	3,13	1,45	1,34	2,16	2,93	9,10	11,92	56,30	2,36	0,26	0,20

Sample Analyte		Phospho-Akt1 ^{S473}	Phospho-GSK-3 β ^{S9}	Phospho-WNK1 ^{T60}	Phospho-CREB ^{S133}	Phospho-p70S6K ^{T421/S424}
Non-treated		1,00	1,00	1,00	1,00	1,00
Mock-treated (0.1% DMF)	2h	1,01	0,94	0,98	1,18	1,01
	3h	1,04	1,06	1,14	0,89	1,05
	4h	0,99	1,00	1,20	0,92	1,00
	5h	1,15	1,20	1,19	0,94	0,97
	6h	1,30	1,42	1,27	0,95	0,93
4 μ M Auranofin	2h	2,75	1,51	1,18	3,12	1,32
	3h	2,22	1,67	1,43	3,16	1,40
	4h	1,20	1,48	1,28	3,18	1,43
	5h	0,89	1,24	1,26	3,22	1,52
	6h	0,76	1,17	1,22	2,76	1,47

Color scale					
Fold change	1.25-1.50	1.51-1.75	1.76-2.00	2.01-2.50	>2.50

Phospho-JNK ^(Pan)	Phospho-p38 α ^{T180/Y182}	Phospho-ERK1 ^{T202/Y204}	Phospho-ERK2 ^{T185/Y187}	Phospho-HSP27 ^{S78/S82}	Phospho-Chk2 ^{T68}	Phospho-p53 ^{S15}	Phospho-FAK ^{Y397}
1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
1,05	0,95	1,15	1,30	0,97	0,98	0,96	1,00
0,97	0,79	0,96	1,09	0,91	1,03	1,49	0,99
1,01	0,70	0,95	1,02	0,96	1,03	1,49	0,95
0,90	0,93	0,84	0,67	0,95	1,11	1,46	0,88
1,07	0,75	0,93	0,79	0,98	1,36	1,25	0,95
1,00	1,29	1,89	2,97	2,07	1,61	0,43	1,17
1,20	1,50	2,25	4,02	4,45	1,74	0,40	1,30
1,15	1,55	2,65	4,96	5,33	1,60	0,32	1,50
1,07	1,65	2,52	4,44	5,21	1,44	0,32	1,16
1,10	1,78	2,70	4,50	5,55	1,59	0,29	1,30

0.80-0.66	0.65-0.57	0.56-0.50	0.49-0.40	<0.40

Supplementary Figure 2 Comparative ELISA microarray analysis of several key regulatory proteins in HT29 cells upon treatment with *Au(I)NHC* and the specific TrxR inhibitor auranofin. The numbers represent the fold changes in concentration relative to non-treated cells (set to 1,00). Mock-treated samples contained 0.1 % (v/v) DMF. The protein levels were measured using kinetic ELISA microarrays.