Supplementary Material (ESI) for Molecular BioSystems

Supplementary information

Profiling patterns of glutathione reductase inhibition by the natural product illudin S and its acylfulvene analogues

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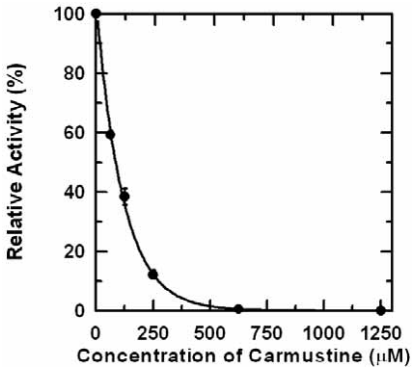

Figure S1. Inhibition of GR by carmustine. GR (5 nM) was incubated with carmustine in the presence of NADPH (150 μM) for 30 min at 25 °C, carmustine, 62.5, 125, 250, 625, 1250 μM.

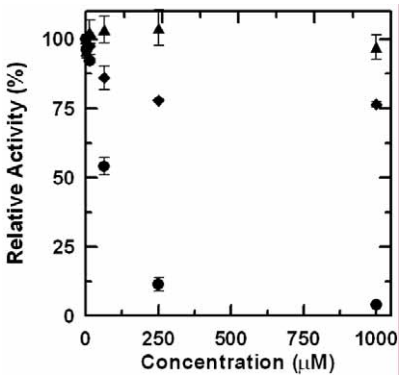

Figure S2. Effect of NADPH and GSSG on the GR Inhibition by carmustine. GR (5 nM) was incubated with carmustine (2.5, 12.5, 62.5, 250, 1000 μM) in the presence (●) and absence (♦) of NADPH (150 μM), or in the presence (▲) of GSSG (0.25 mM) for 30 min at 25 °C, and then the activity was measured.

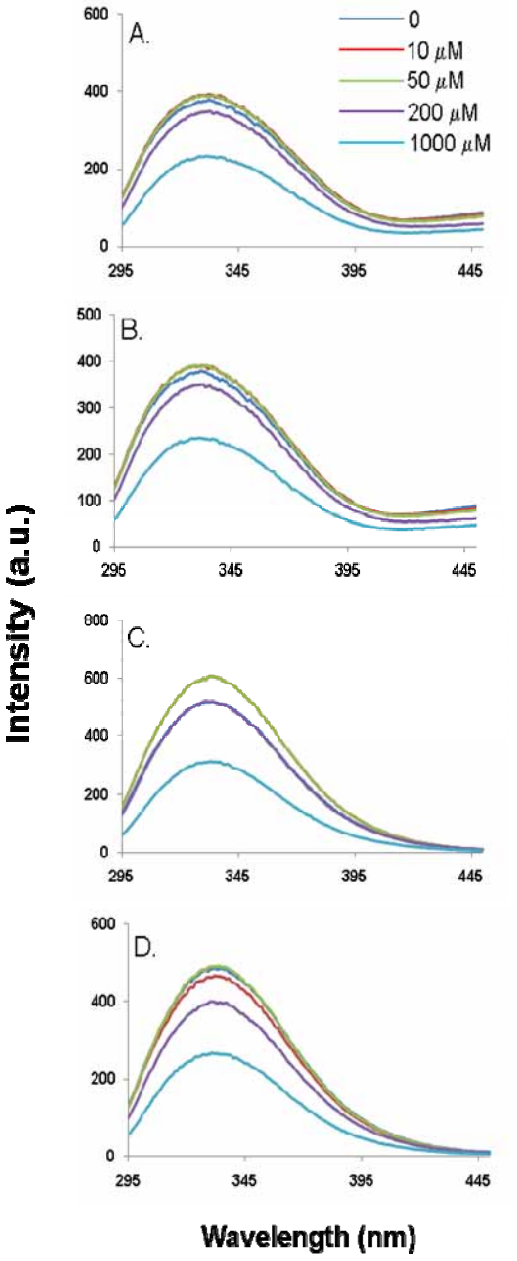

Figure S3. GR fluorescence spectrum changes in the presence of varying concentration of carmustine. Carmustine (0.01, 0.05, 0.20, 1.00 mM) was allowed to react with GR (2 μM) in a total volume of 500 μL TE buffer for 30 min at 25 °C under different conditions: a. the absence of NADPH; b. in the presence of NADPH (100 μM); c. in the absence of NADPH and in the presence of GSSG (400 μM); d. in the presence of NADPH (100 μM) and GSSG (400 μM).

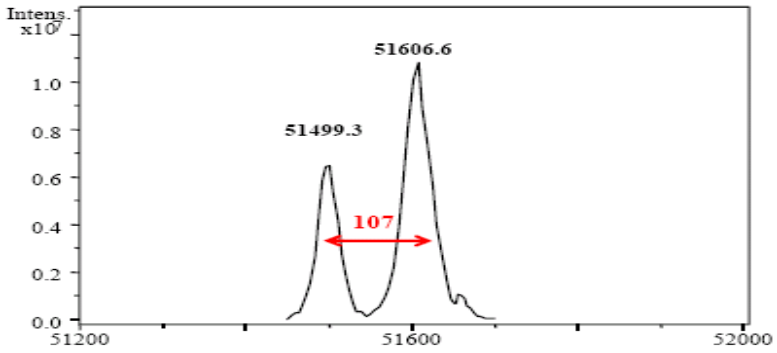

Figure S4. LC/MS spectra derived from GR-carmustine adduct. GR (5 nmol) was allowed to react with carmustine (0.25 mM) in 1 mL TE buffer containing NADPH (1 mM) for 3 h at 25 °C. Unbound compound was removed before LC/MS analysis.

Table 1. Mass of *y* and *b* ions from the IAA- and HMAF- modified GR active site sequence by trypsin digestion.

HMAF	IAA	b		Active site sequence		y	IAA	HMAF
			1	**A**	14			
	185.1	185.1	2	**L**	13	1246.6	1360.7	1738.6
	242.1	242.1	3	**G**	12	1133.5	1247.6	1625.5
	299.2	299.2	4	**G**	11	1076.5	1190.6	1568.5
	400.2	400.2	5	**T**	10	1019.5	1133.5	1511.5
749.2	560.2	503.2	6	**C**	9	918.5	1032.5	1410.5
848.3	659.3	602.3	7	**V**	8	815.4	872.5	1061.4
962.3	773.4	716.3	8	**N**	7	716.4	773.4	962.4
1061.4	872.4	815.4	9	**V**	6	602.3	659.4	848.3
1118.4	929.5	872.4	10	**G**	5	503.3	560.3	749.3
1467.4	1089.5	975.4	11	**C**	4	446.2	503.3	692.2
1566.5	1188.6	1074.5	12	**V**	3	343.2	343.2	
1663.6	1285.6	1171.6	13	**P**	2	244.2	244.2	
			14	**K**	1	147.1	147.1	

*Numbers in red represent corresponding *y* or *b* ion mass plus one molecular mass of IAA or HMAF.

*Number in blue represent corresponding *y* or *b* ion mass plus two molecular masses of IAA or HMAF.