

Supplementary Material

Discovery of new molecular entities able to strongly interfere with Hsp90 C-terminal domain

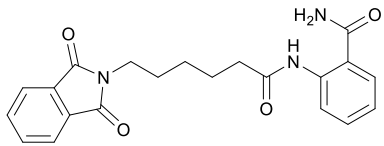
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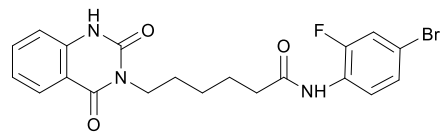
²Department of Science, University of Basilicata, Viale dell'Ateneo Lucano n.10, 85100 Potenza,
Italy

Supplementary Table S1. Otava Codes of compounds **1-48**.

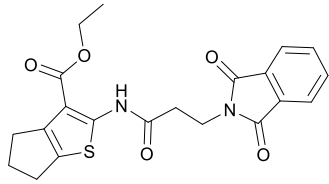
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5	7020141371	29	103490002
6	1678691	30	1098656
7	7715460181	31	7020102417
8	7715460264	32	1089672
9	7715460449	33	4355978
10	1099180	34	2194629
11	1157839	35	4355978
12	1157842	36	7020721800
13	6238190	37	1095656
14	6238227	38	1101808
15	7020470041	39	7510791436
16	7020470326	40	1110852
17	7020470389	41	1101770
18	7020530643	42	6243033
19	7020601381	43	7217221018
20	125180086	44	7010060072
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24	127442333	48	7119461545



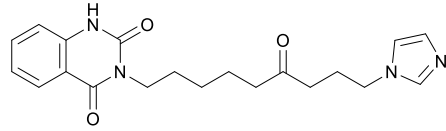
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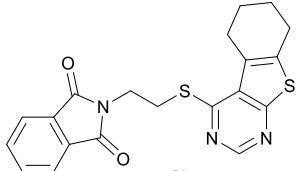
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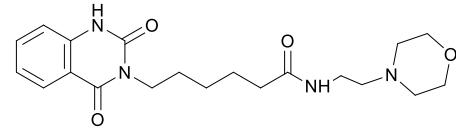
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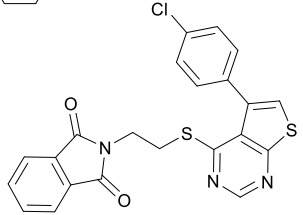
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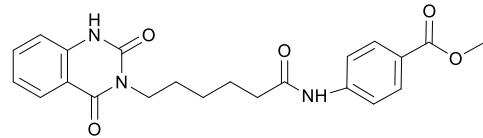
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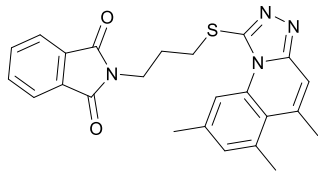
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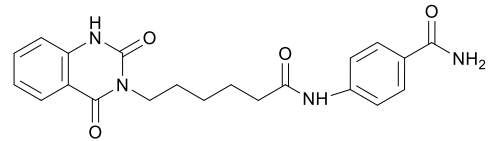
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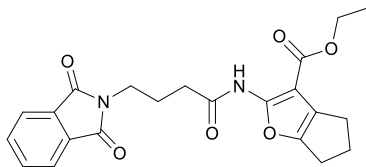
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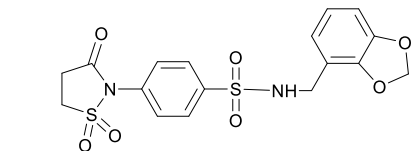
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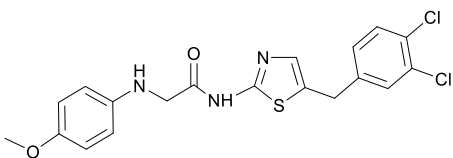
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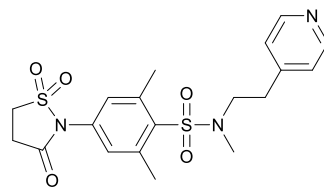
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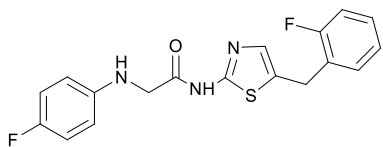
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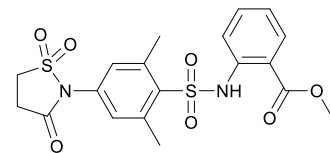
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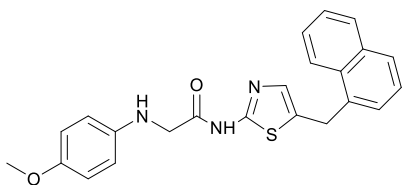
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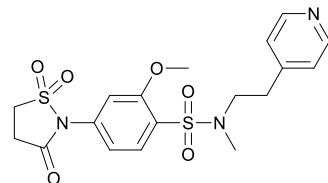
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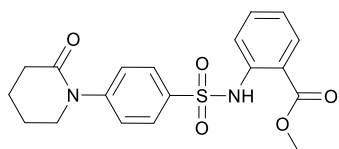
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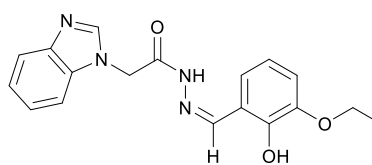
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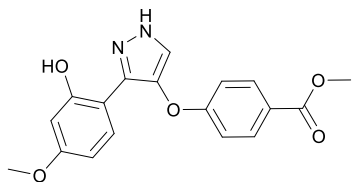
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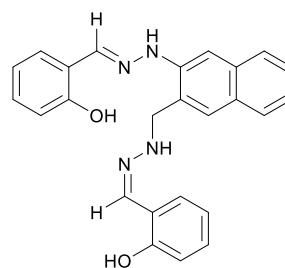
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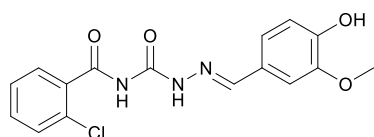
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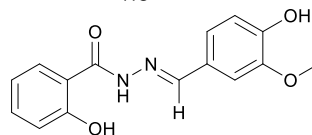
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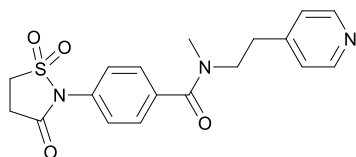
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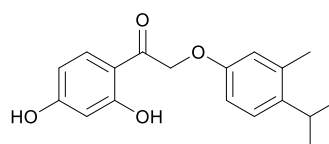
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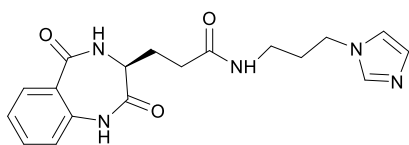
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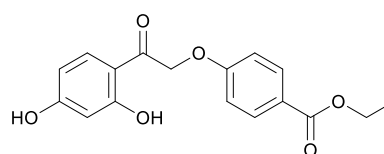
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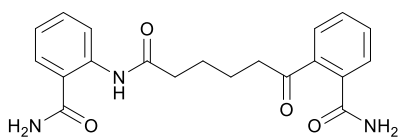
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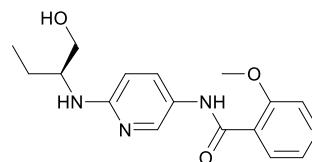
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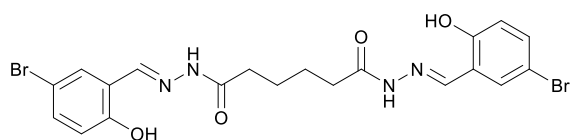
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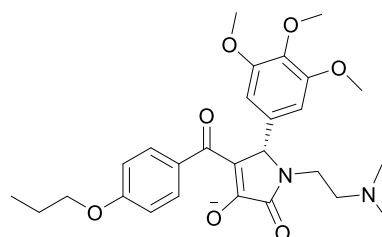
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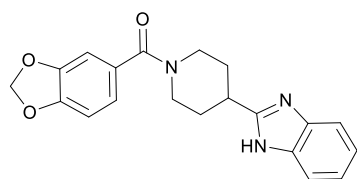
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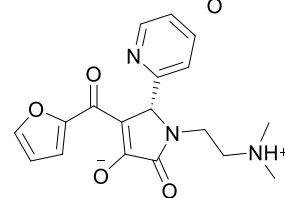
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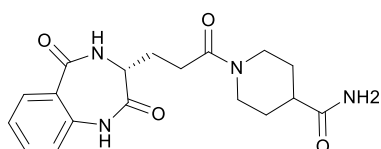
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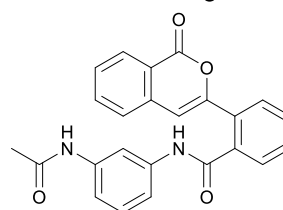
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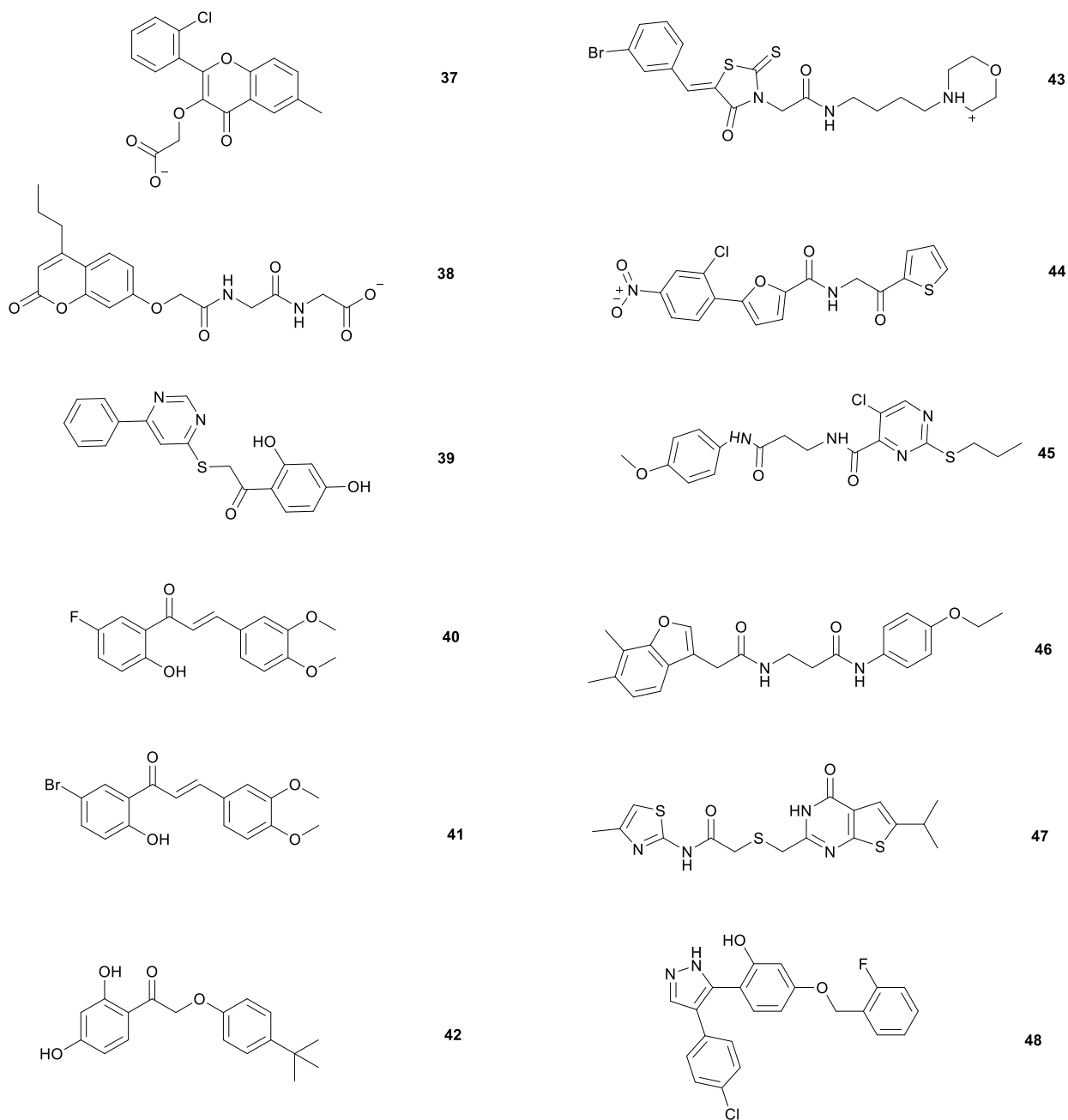
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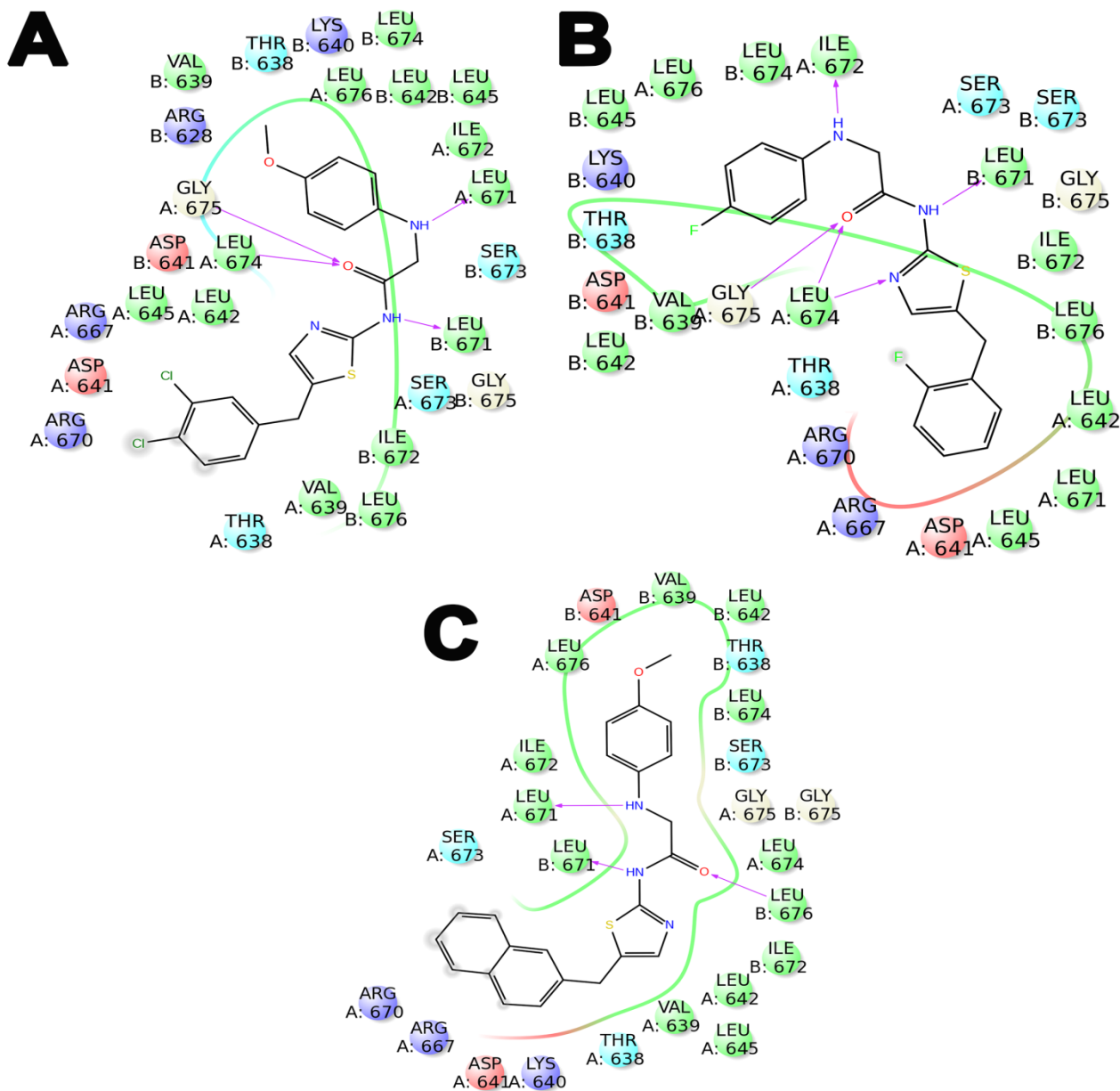
Supplementary Figure S1. Chemical structures of compounds **1-48**.

Supplementary Table S2. IC₅₀ values of **1, 4-5, 7-15, 21-22, 26** on human monocytic cell line U937 from histiocytic lymphoma.

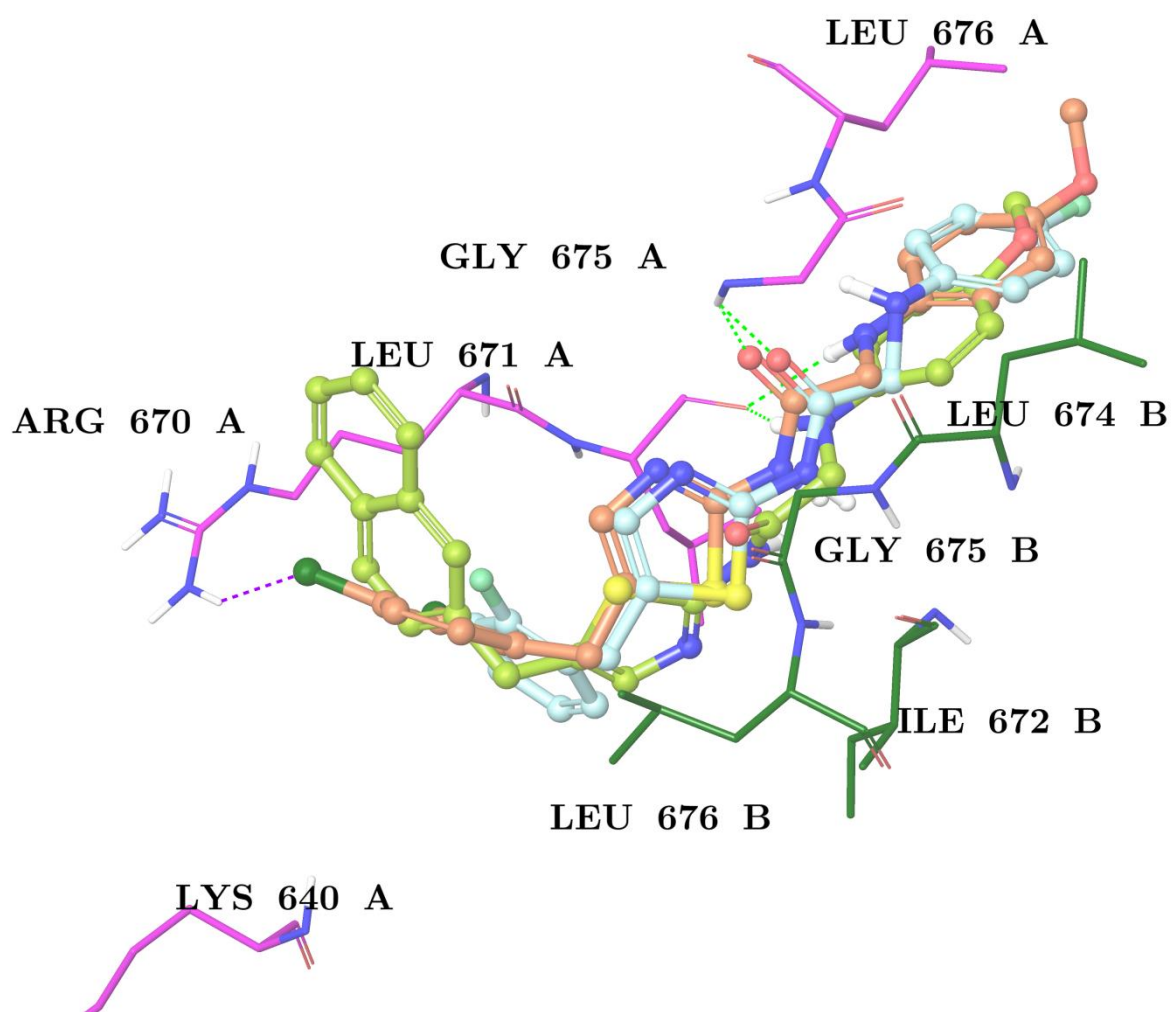
Compound	IC₅₀ (μM) 24h	IC₅₀ (μM) 48h
1	>100	>100
4	>100	>100
5	91.2 ± 1.7	91.2 ± 1.2
7	51.2 ± 0.8	51.0 ± 0.7
8	not active	not active
9	97.3 ± 1.5	95.3 ± 0.9
10	52.0 ± 0.5	50.0 ± 0.7
11	90.1 ± 1.2	90.3 ± 1.3
12	>100	>100
13	98.3 ± 0.9	97.0 ± 1.1
14	>100	>100
15	not active	not active
21	not active	not active
22	not active	not active
26	>100	>100
Novobiocin	232.5± 1.1	190.4± 1.5

Supplementary Table S3. IC₅₀ values of **1, 4-5, 7-15, 21-22, 26** on human leukemic T lymphocyte cell line Jurkat.

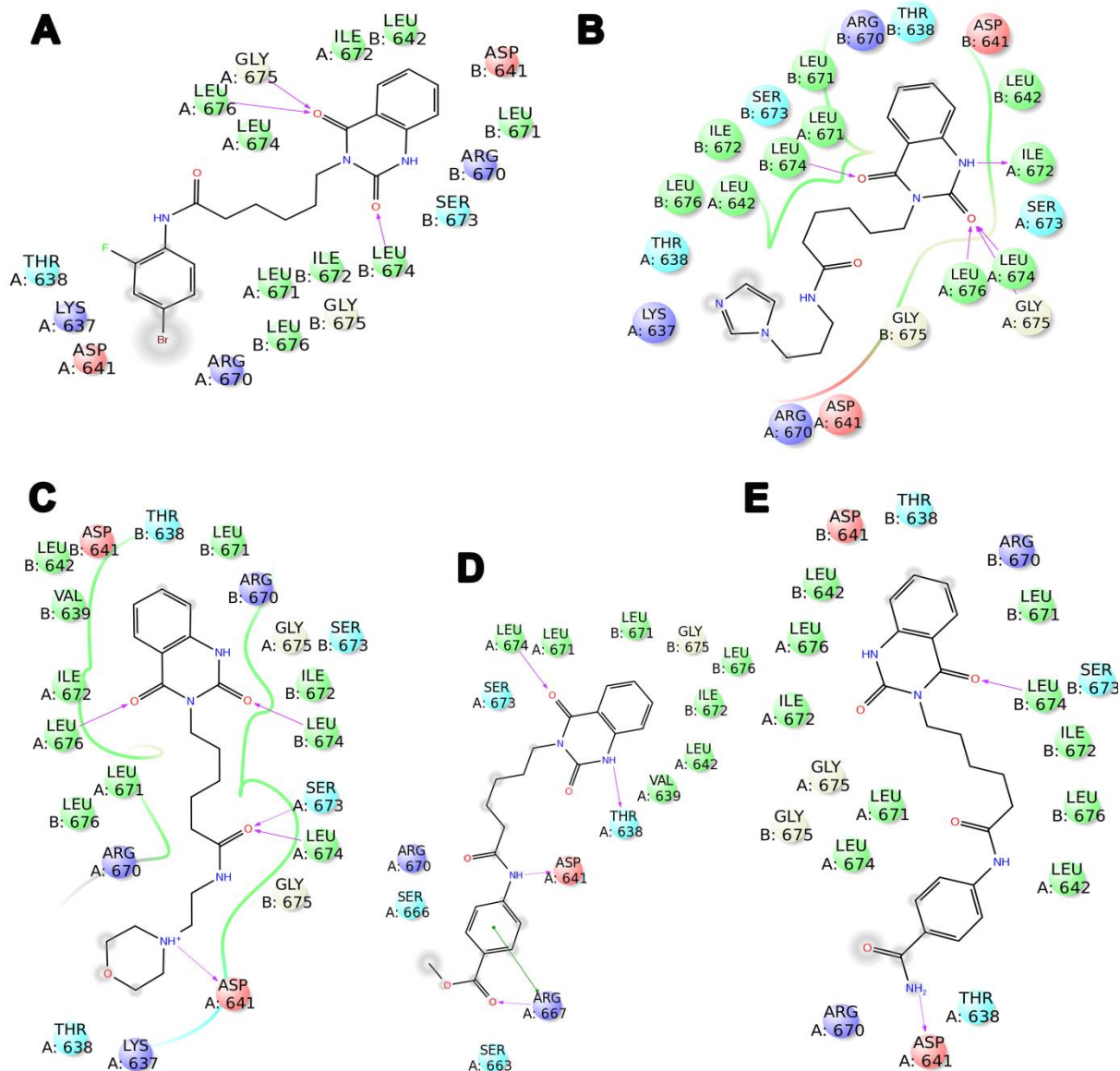
Compound	IC₅₀ (μM) 24h	IC₅₀ (μM) 48h
1	>100	>100
4	>100	>100
5	100.0 ± 1.4	98.3 ± 0.9
7	25.1 ± 0.4	22.0 ± 0.8
8	not active	not active
9	90.2 ± 0.5	85.5 ± 0.7
10	26.1 ± 0.7	23.5 ± 1.2
11	48.2 ± 0.8	42.5 ± 0.5
12	>100	>100
13	56.8 ± 1.3	54.0 ± 0.5
14	>100	>100
15	not active	not active
21	not active	not active
22	not active	not active
26	>100	>100
Novobiocin	170.6 ± 1.1	150.5 ± 0.7



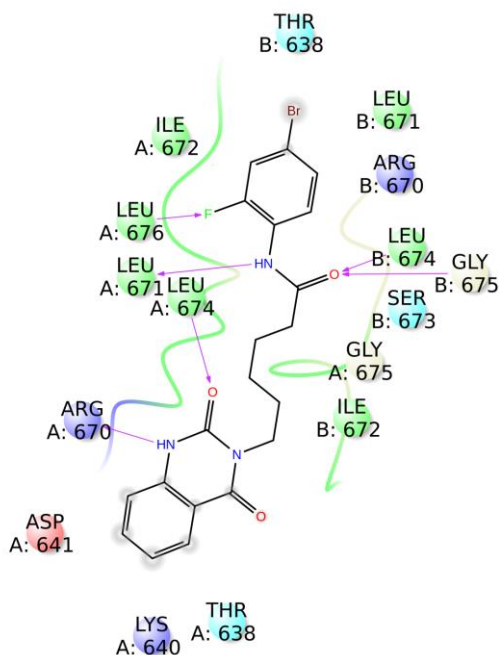
Supplementary Figure S2. 2D diagram interactions (panel A, B and C) of **7**, **8** and **9** with C-terminal domain of HSP82 yeast analogue of Hsp90 α (PDB: 2CG9). Positive charged residues are colored in violet, negative charged residues are colored in red, polar residues are colored in light blue, hydrophobic residues are colored in green. H-bond (side chain) are reported as dotted pink arrows.



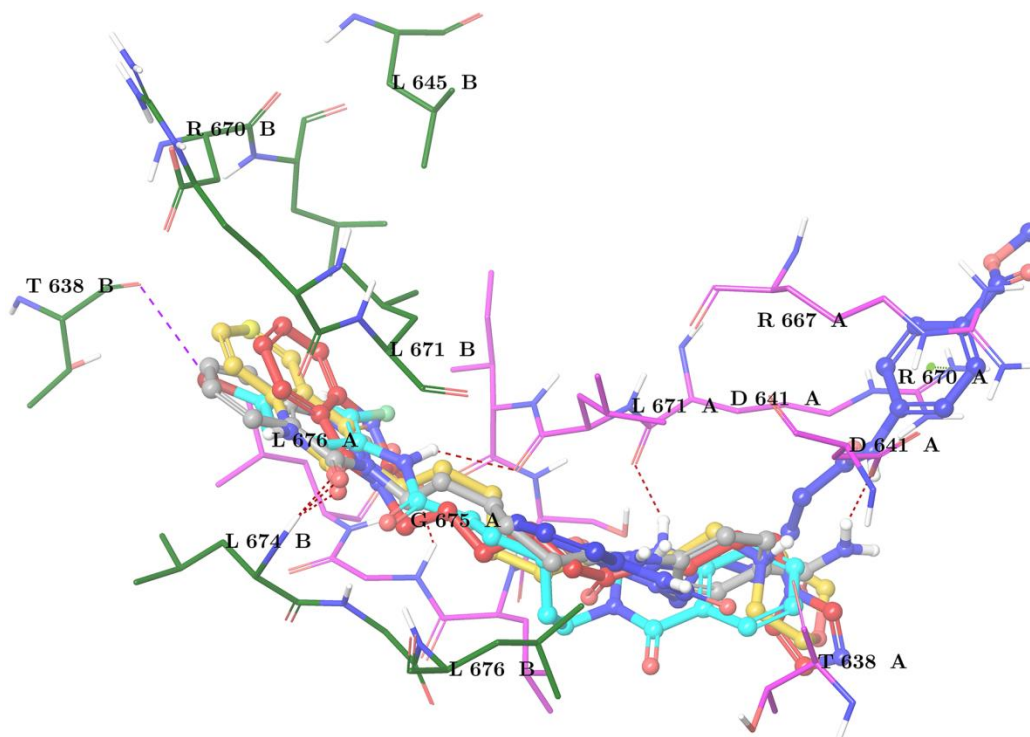
Supplementary Figure S3. Superimposition of **7** (light pink sticks), **8** (sky-blue sticks) and **9** (light green sticks) with C-terminal domain of HSP82 yeast analogue of Hsp90α (PDB: 2CG9). The protein is depicted by purple (chain A) and green (chain B) sticks.



Supplementary Figure S4. 2D diagram interactions (panels A, B, C, D and E) of **10**, **11**, **12**, **13** and **14** with C-terminal domain of HSP82 yeast analogue of Hsp90 α (PDB: 2CG9). Positive charged residues are colored in violet, negative charged residues are colored in red, polar residues are colored in light blue, hydrophobic residues are colored in green. The π - π stacking interactions are indicated as green lines, and H-bond (side chain) are reported as dotted pink arrows.

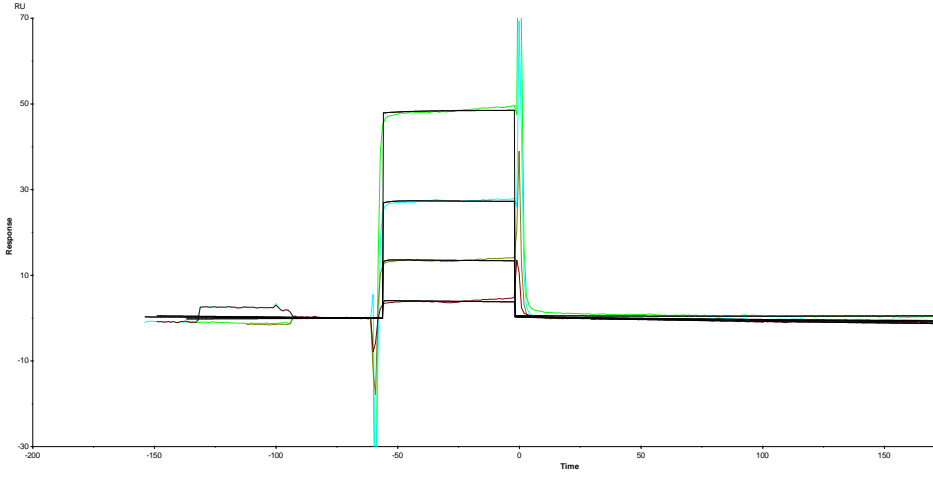


Supplementary Figure S5. 2D diagram interactions of second binding mode of **10** with C-terminal domain of HSP82 yeast analogue of Hsp90 α (PDB: 2CG9). The positive charged residues are colored in violet, negative charged residues are colored in red, polar residues are colored in light blue, hydrophobic residues are colored in green. H-bond (side chain) are reported as dotted pink arrows.

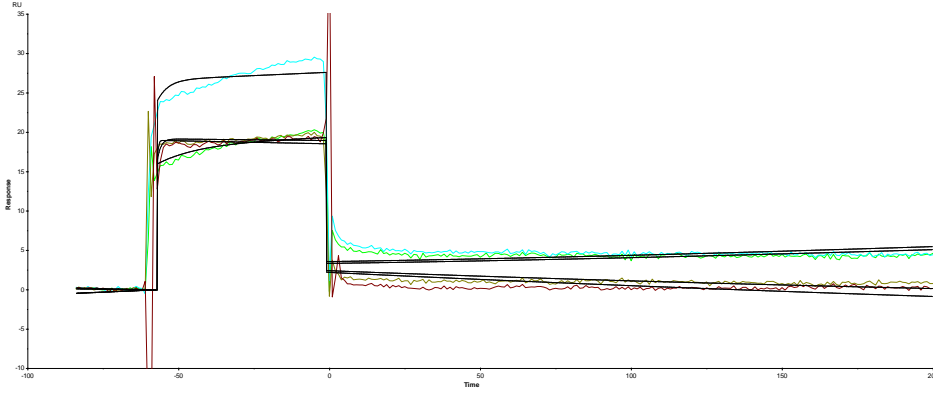


Supplementary Figure S6. Superimposition of **10** (cyan sticks), **11** (red sticks), **12** (yellow sticks), **13** (blue sticks), and **14** (grey sticks) with C-terminal domain of HSP82 yeast analogue of Hsp90 α (PDB: 2CG9). The protein is depicted by purple (chain A) and green (chain B) sticks.

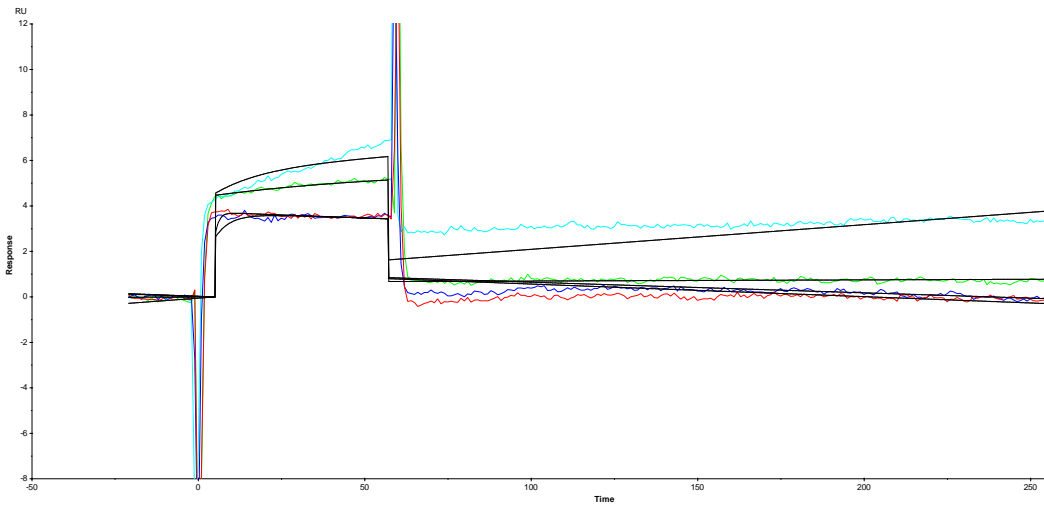
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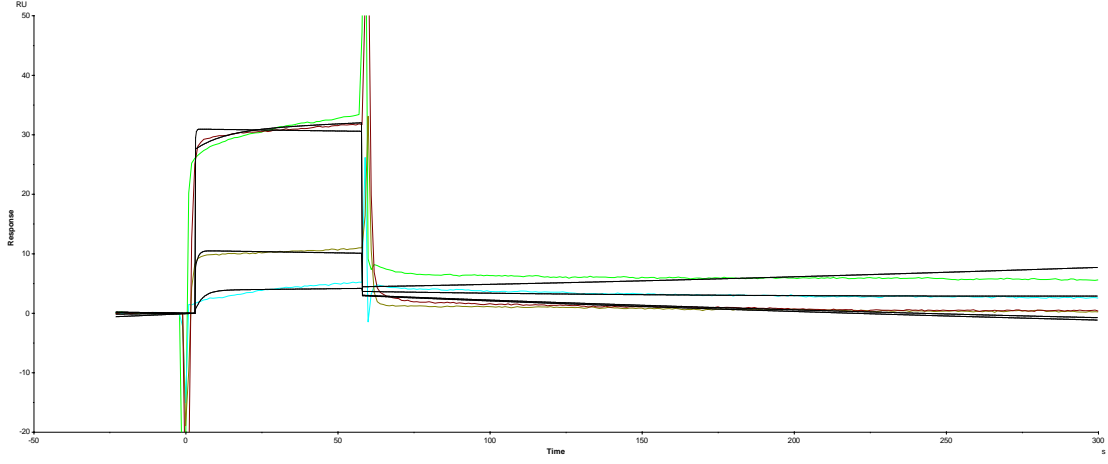
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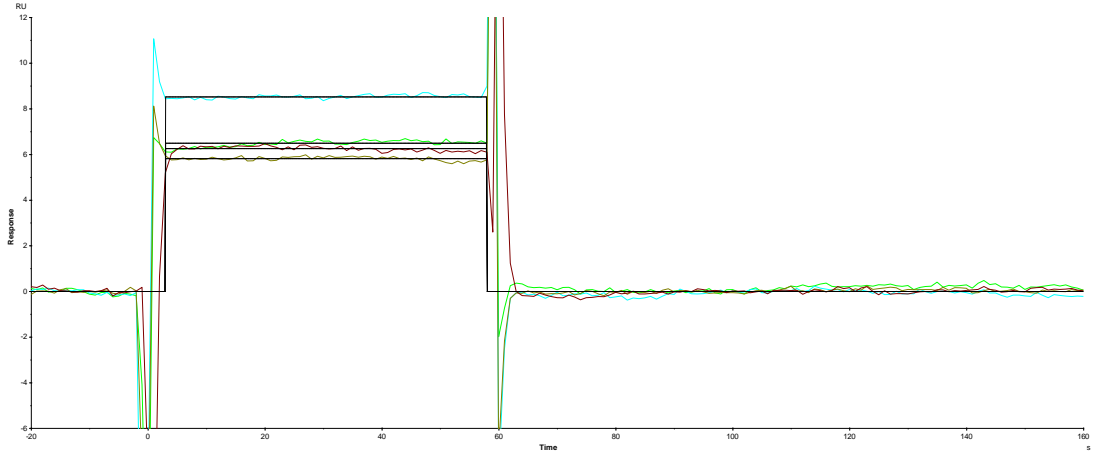
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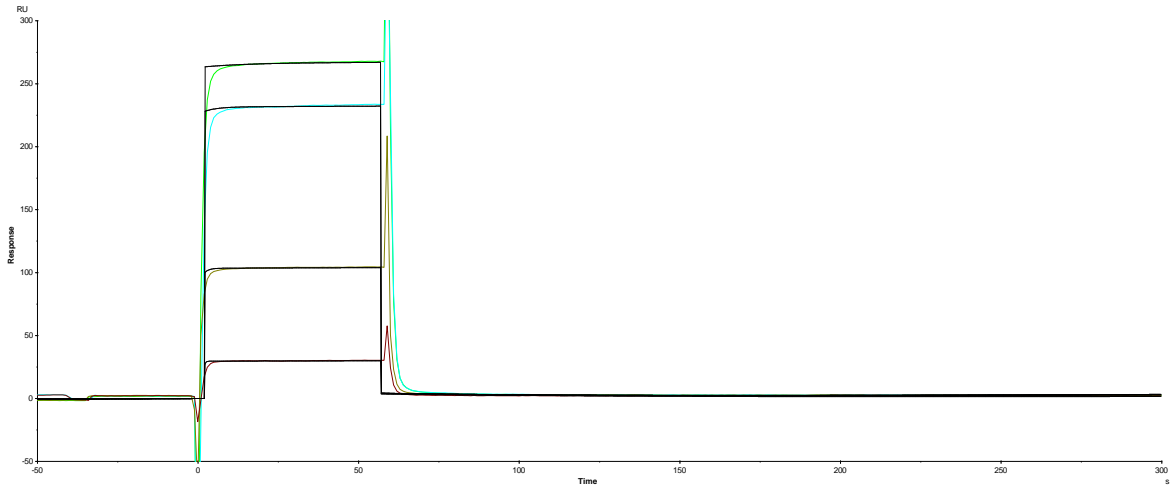
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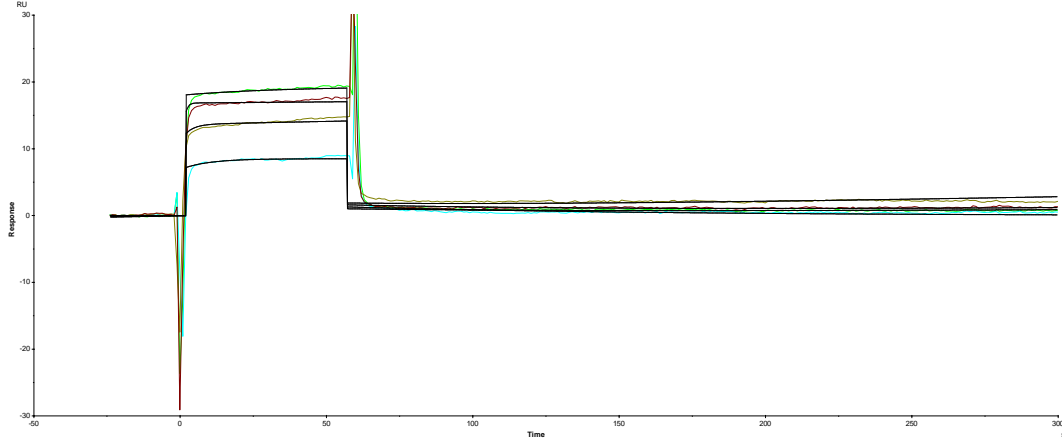
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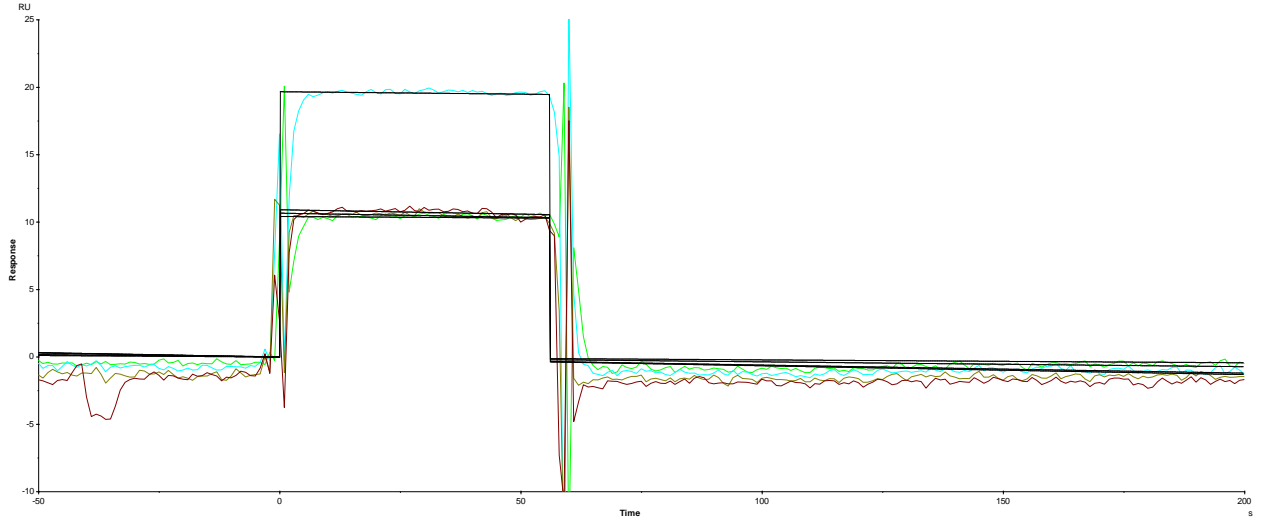
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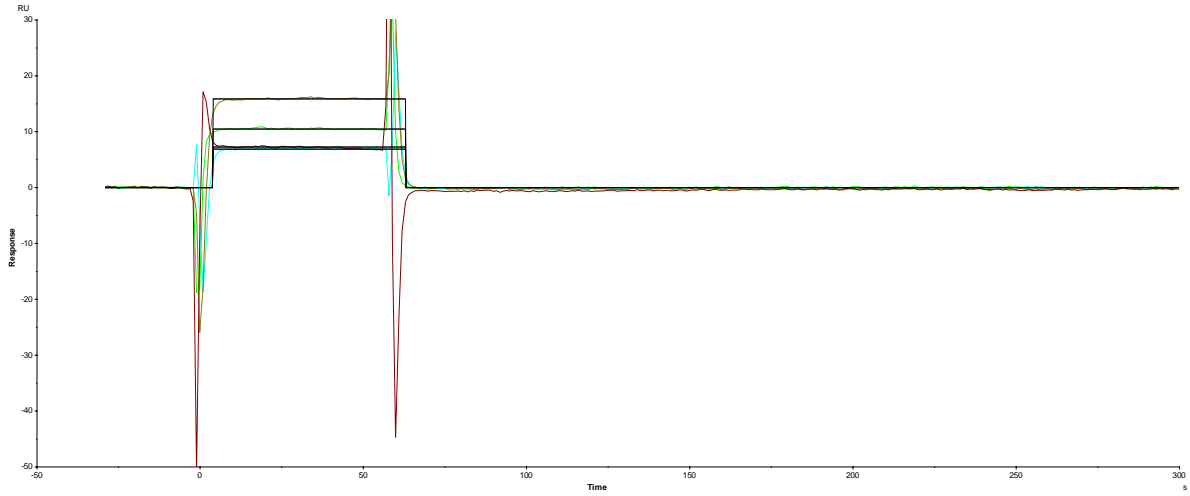
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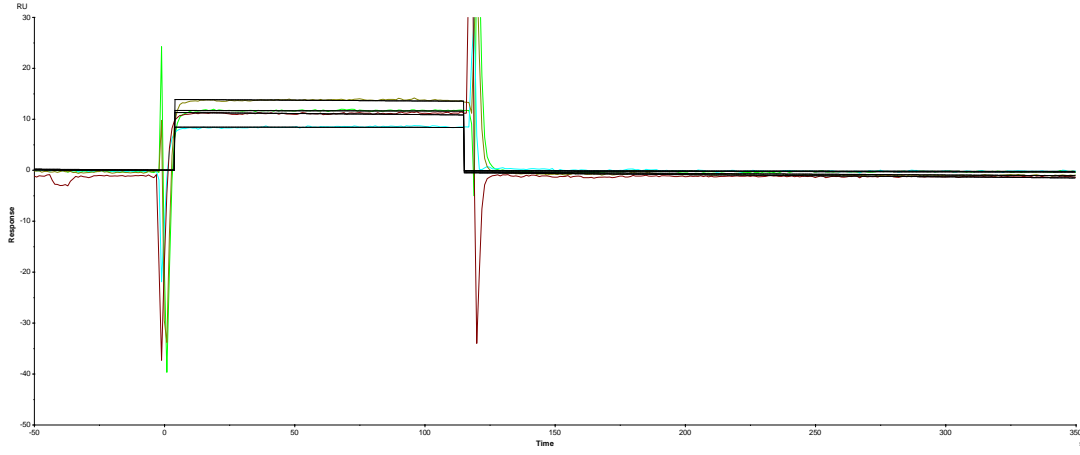
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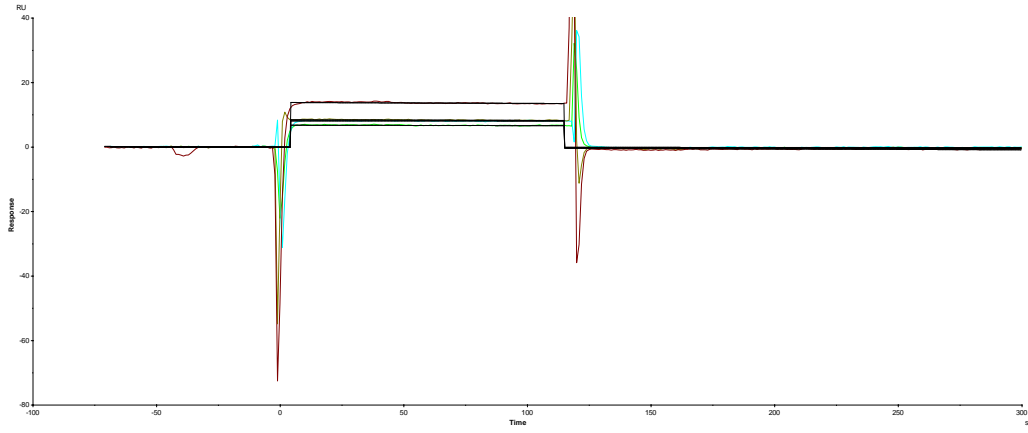
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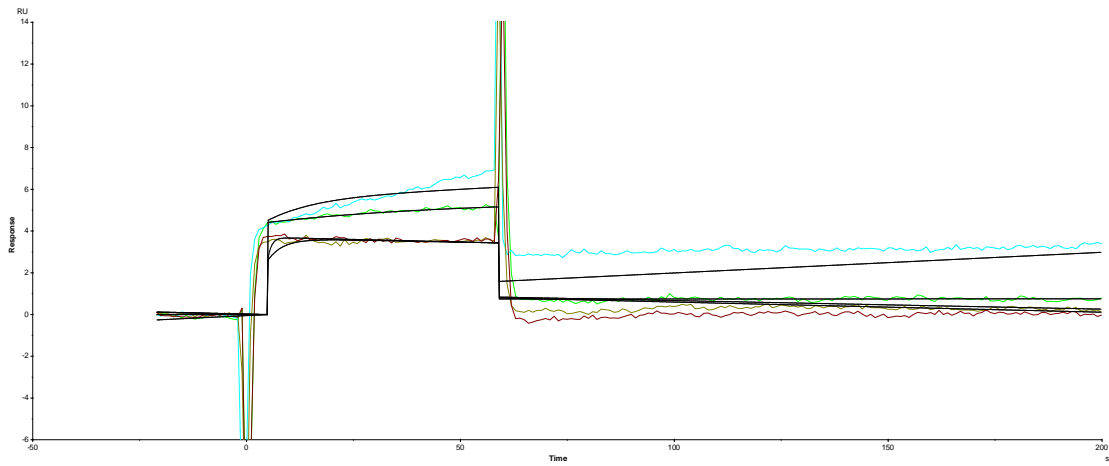
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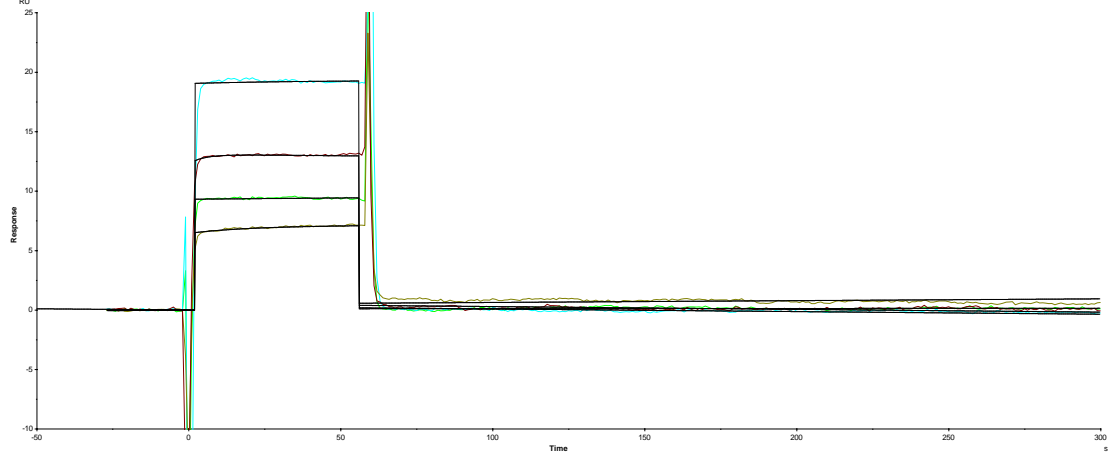
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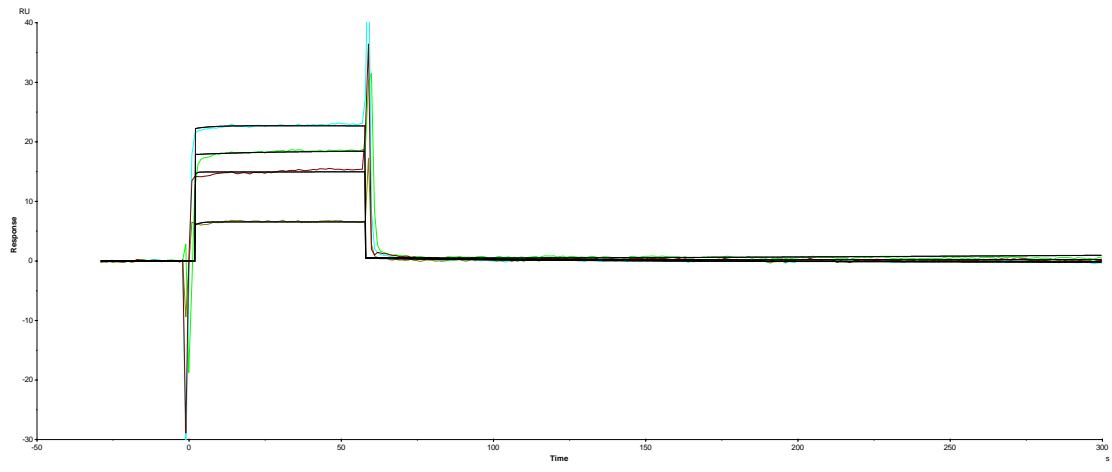
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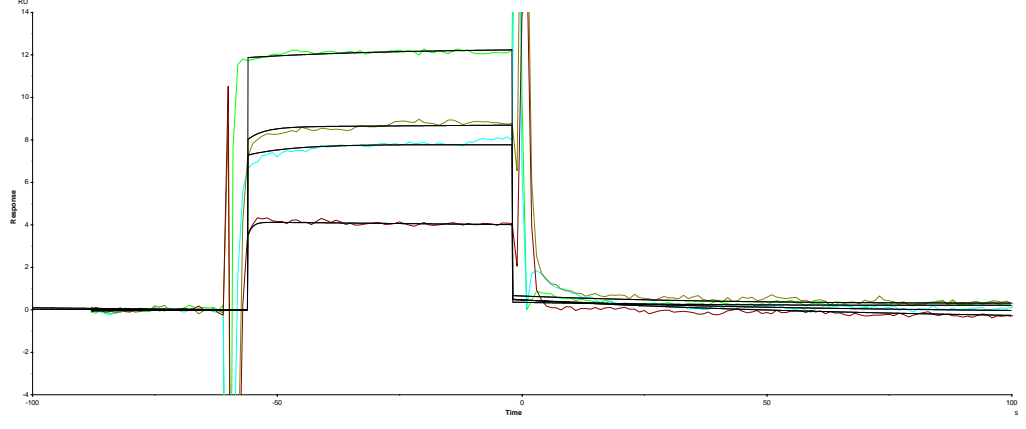
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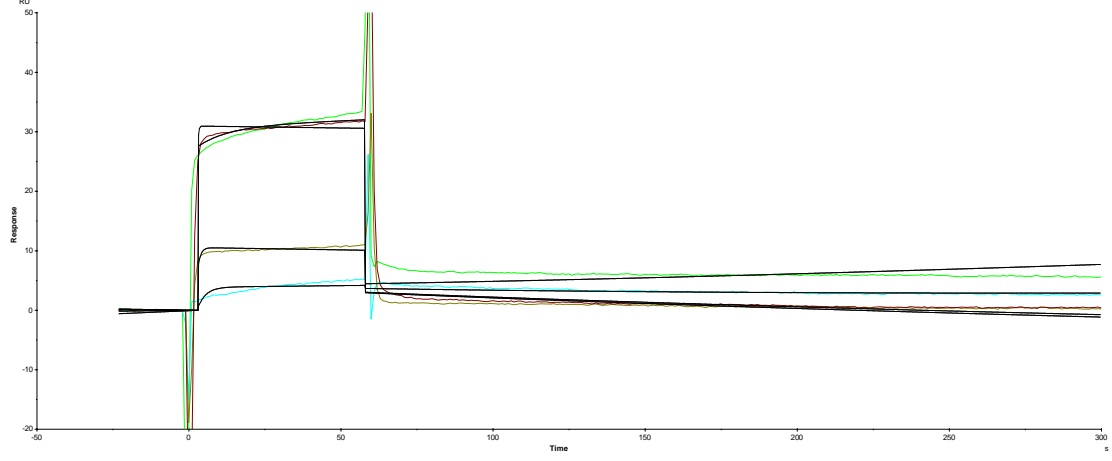
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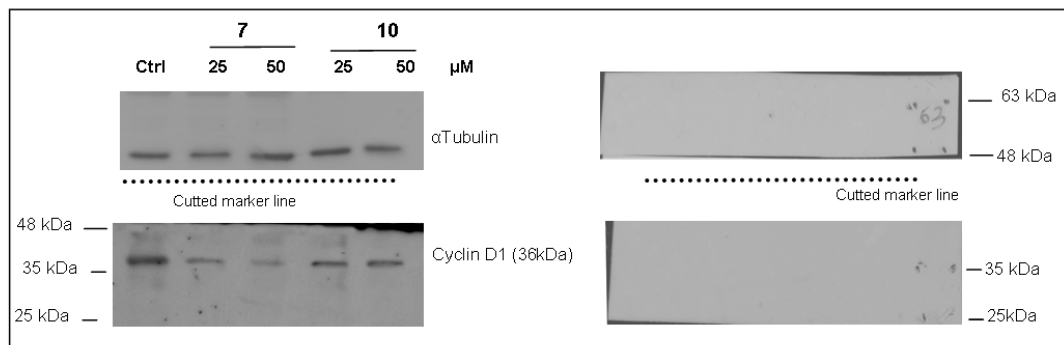
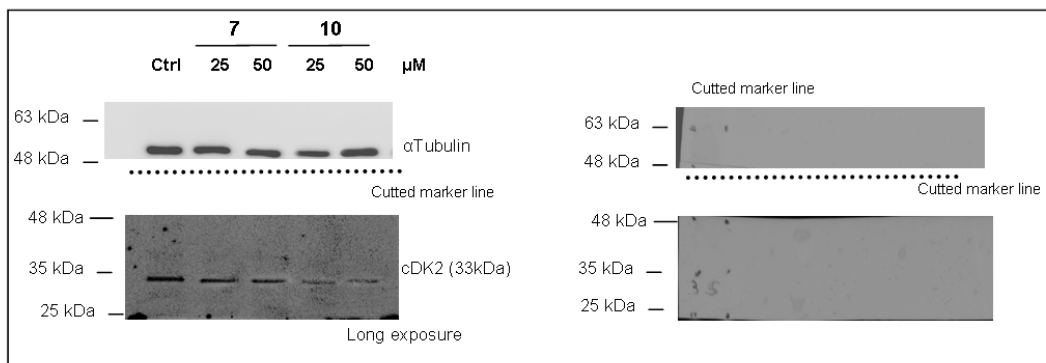
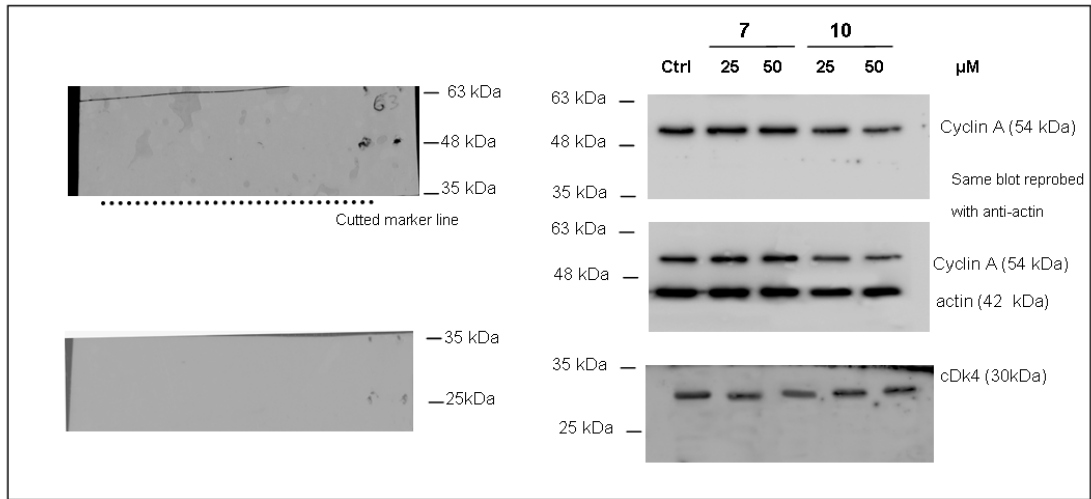
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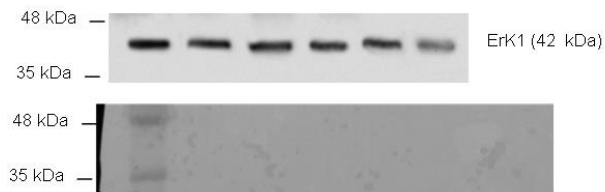
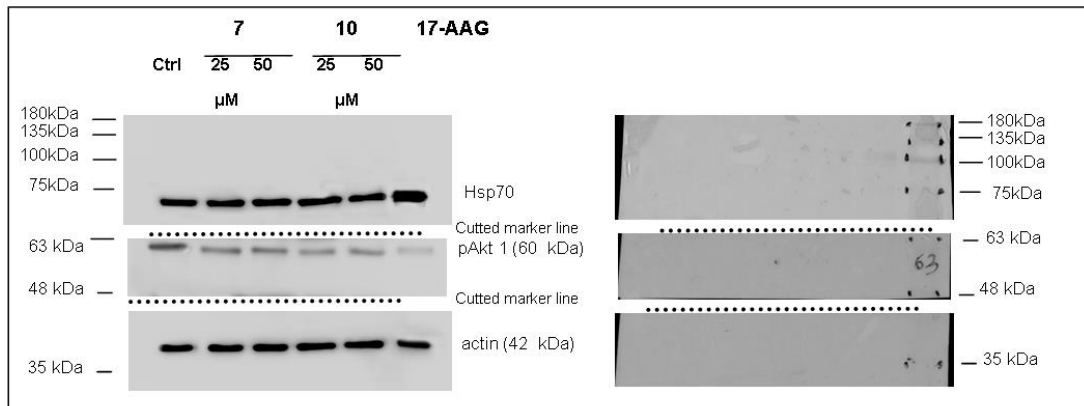
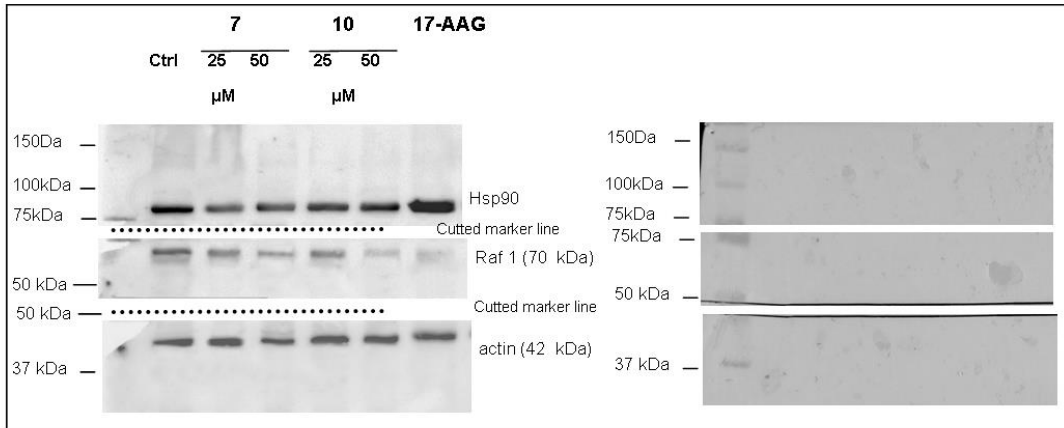
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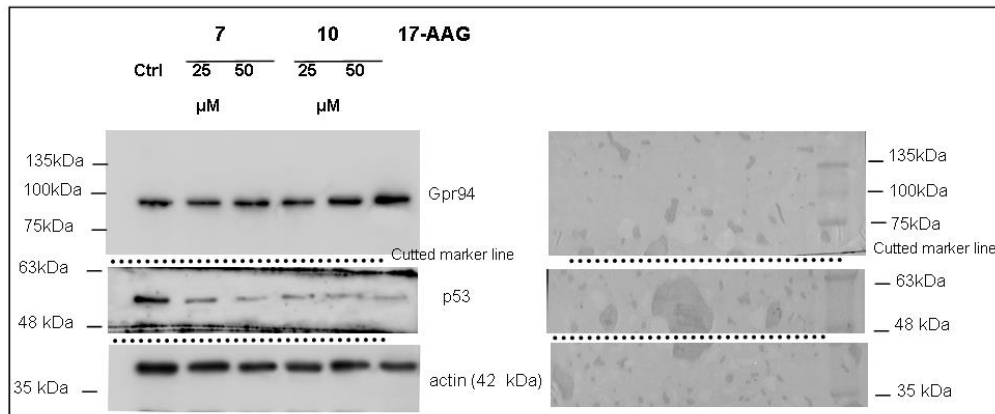
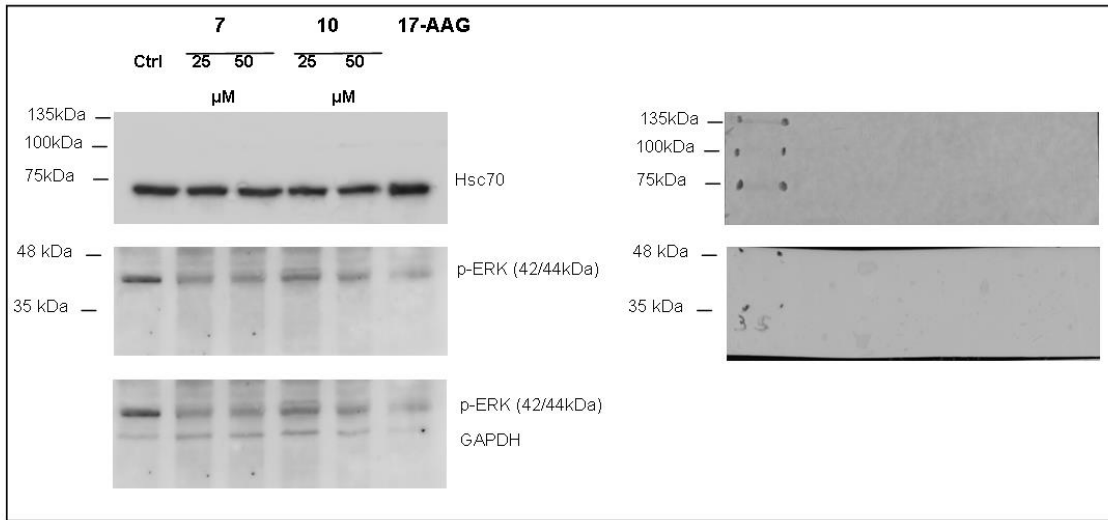


Supplementary Figure S7. SPR sensorgrams documenting the interaction between **1, 4, 5, 7-15, 20-22** and **26** Hsp90.



Supplementary Figure S8. Original images of western blots displayed in Figure 3A of the main text. In the figure was indicate the same filter cropped and probed with different primary antibody (Protein ladder :Opti-Protein XL Marker, Applied Biological Materials Inc., Canada).





Supplementary Figure S9. Original images of western blots displayed in Figure 3B of the main text. The protein ladder was indicated (Opti-Protein XL Marker, Applied Biological Materials Inc., Canada; or Prestained Natural Protein Standard, Bio-Rad.). In the figure was indicate the same filter cropped and probed with different primary antibody.