2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Heat Shock Proteins in Targeted Cancer Chemotherapy

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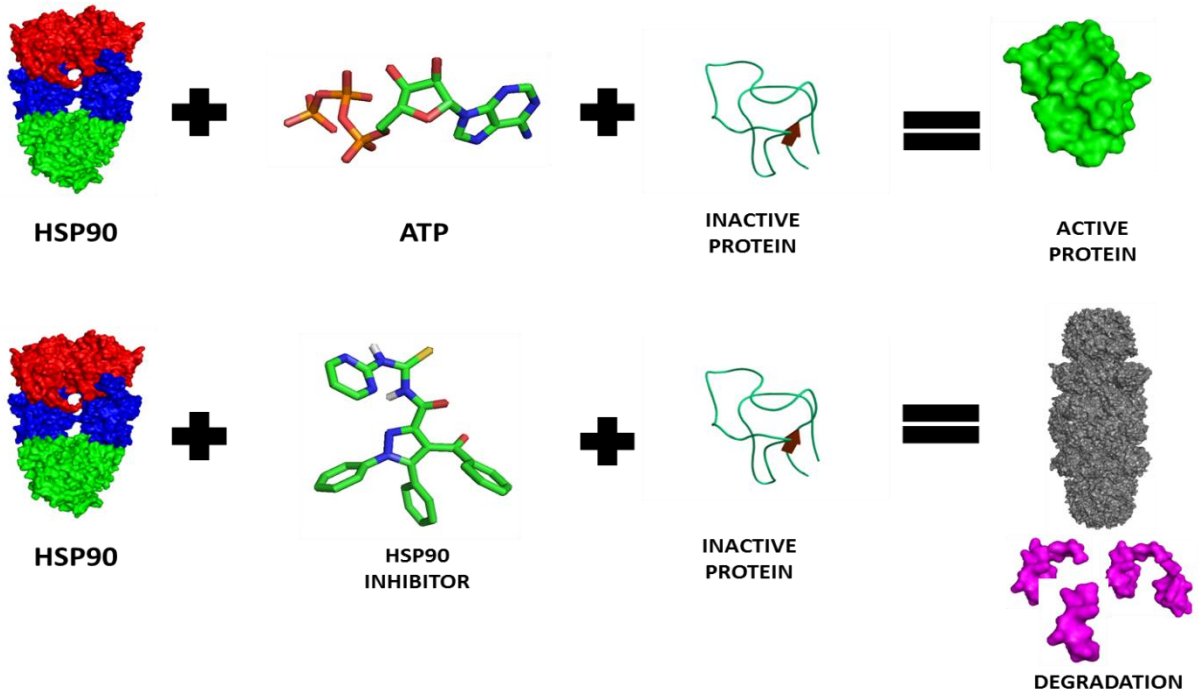
Heat Shock Proteins in Targeted Cancer Chemotherapy

Graphical Abstract



Abstract:

Heat shock proteins (Hsps) are important biological targets in next generation cancer treatment. Hsps play vital roles in protein hemostasis pathways (proper folding and stabilization of nascent proteins, inhibition of protein aggregation, degradation of aggregated proteins, signal transduction and protein translocation). Hsps are found in different cellular compartments and their expression level is increased in response to cellular and external stress factors. Therefore, pathogenesis of diseases is related with expression level of the Hsps. Hsps are over-expressed in cancer cells, and especially, Hsp27, Hsp70 and Hsp90 are involved in all phases of tumorogenesis (apoptosis, metastases, angiogenesis, invasion, and cell differentiation). Hsp27, Hsp70 and Hsp90 ensure stabilization, activation and proper folding of the oncogenic proteins in cancer cells. Therefore, inhibition of Hsps has been significant therapeutic strategy for next generation target specific cancer treatment. Inhibition of Hsp90 chaperone activity has been significant drug target for the past 30 years in cancer treatment. Inhibition of Hsp90 triggers expression of Hsp70 and complements inhibited Hsp90 chaperone activity. Moreover, Hsp27 controls and regulates key points of the apoptotic pathway in cancer cells. Therefore, in addition to Hsp90 inhibition, blocking of Hsp70 and Hsp27 chaperone activities have been remarkable therapeutic strategy for cancer treatment.

Keywords: Hsp90, cancer, drug design, client proteins

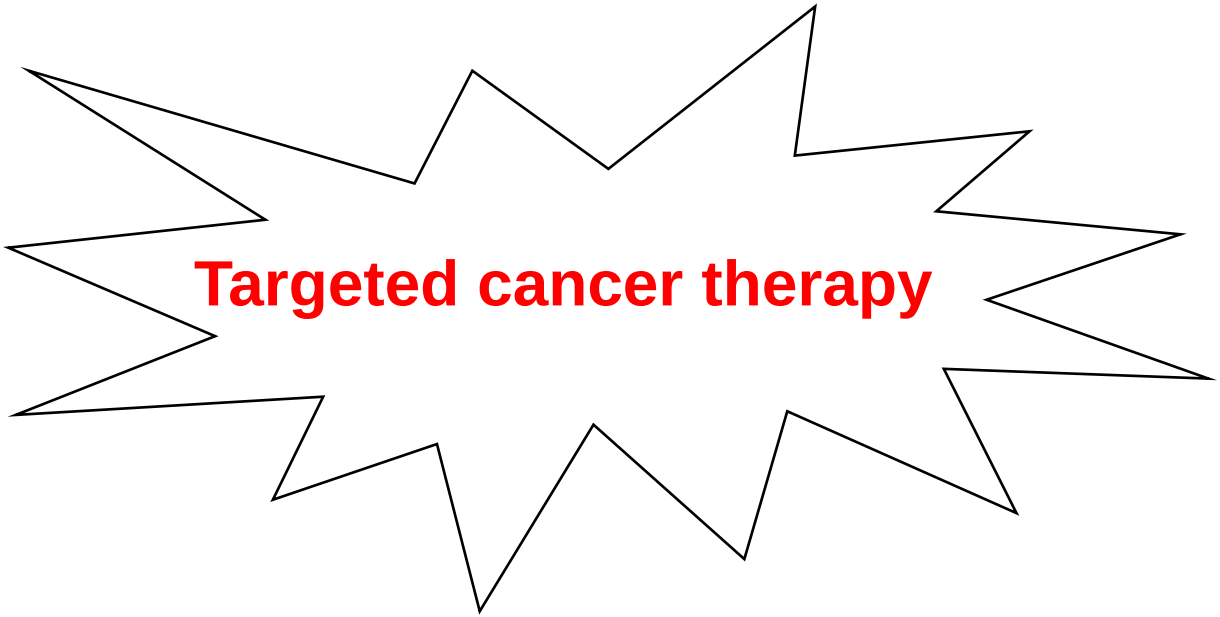
Current Cancer Therapies

Surgery

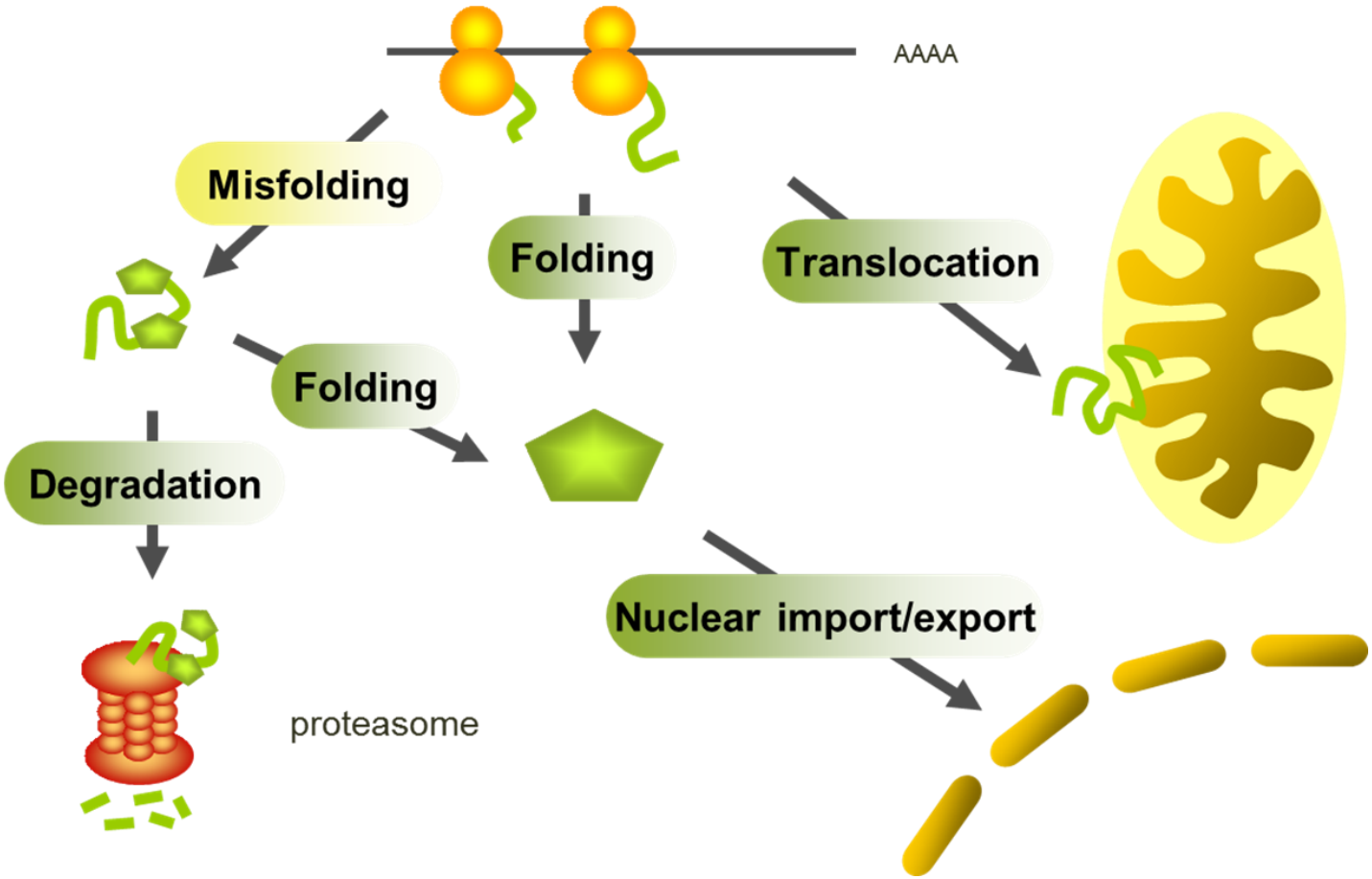
Chemotheraphy

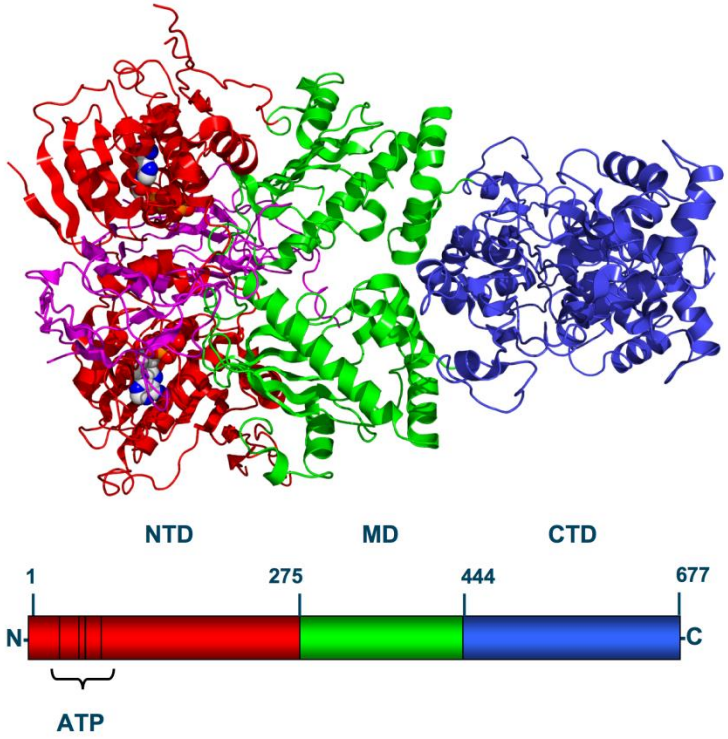
Radiationtherapy

Biological therapy

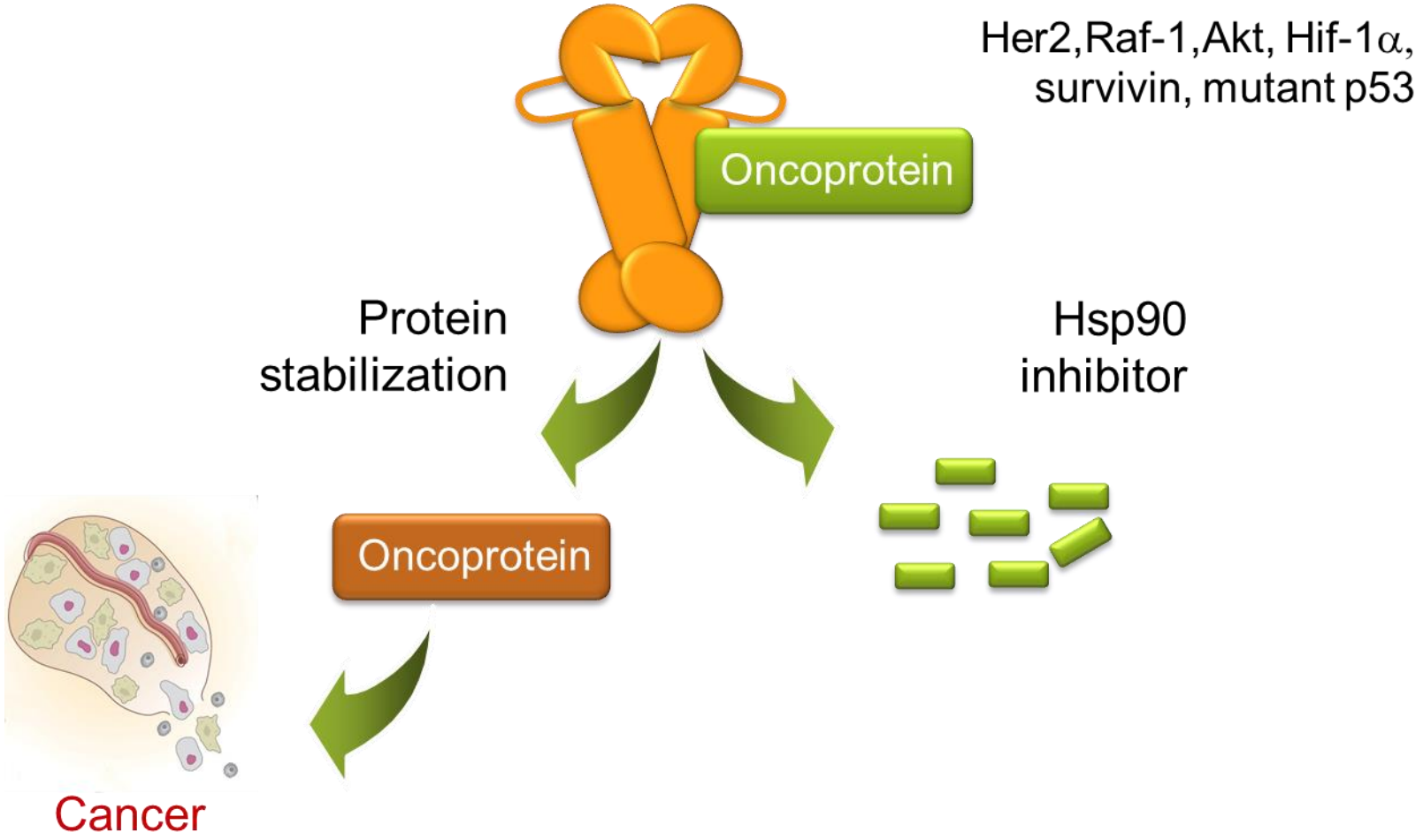


Heat Shock Proteins



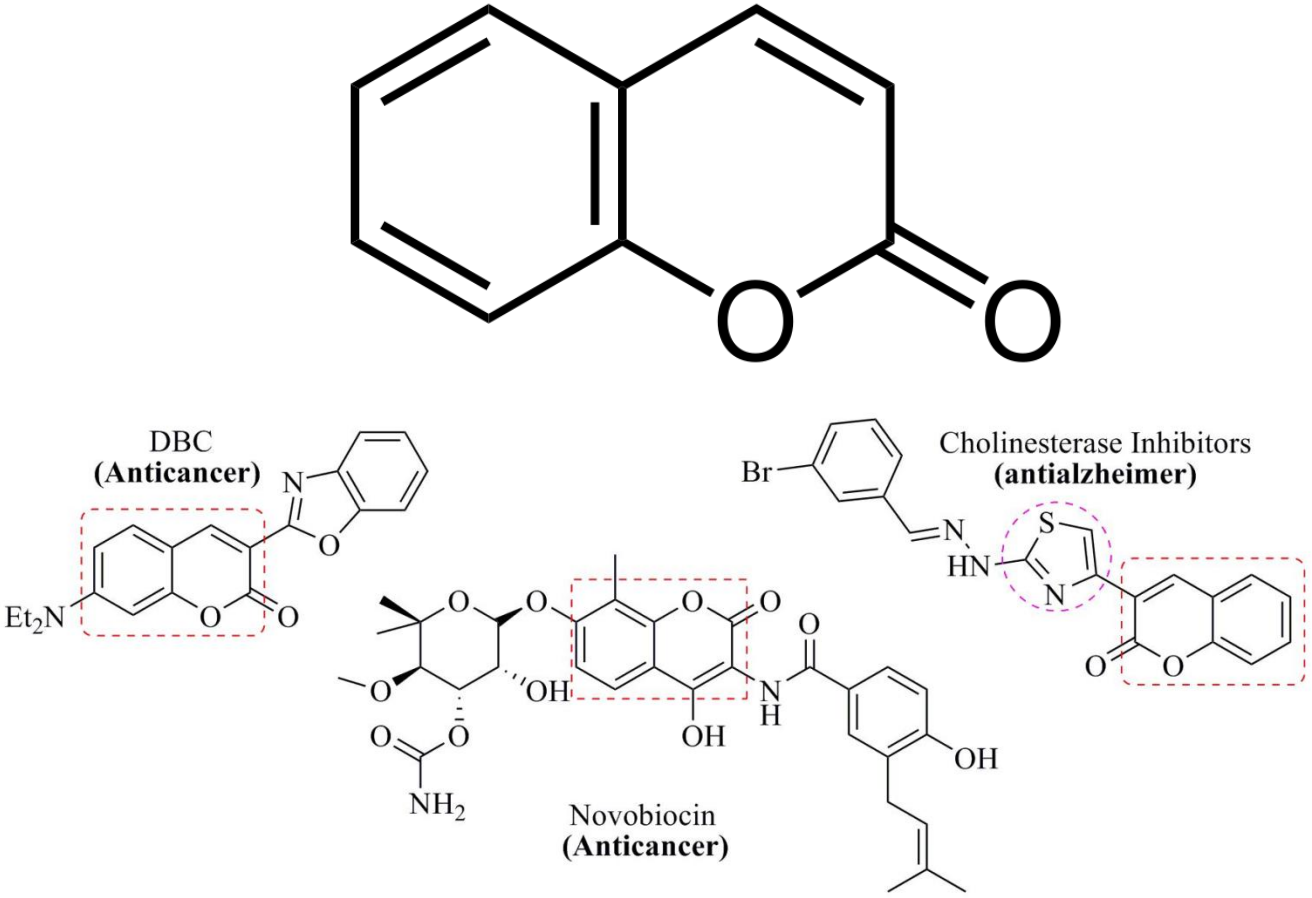


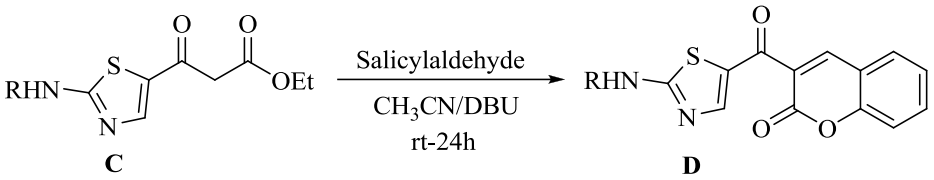
- Heat shock protein 90 represents 1-2% of all cellular proteins
- Facilitates protein-folding and stabilization. Induced under stress, hypoxia and oxidative damage.
- Generally, the expression level of Hsp90 is increased at up to 2- to 10-fold in human cancer cells than in normal cells.

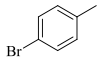
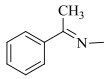
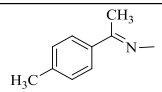
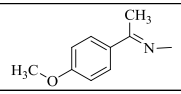

Her2,Raf-1,Akt, Hif-1α,
survivin, mutant p53
Oncoprotein
Protein
stabilization
Hsp90
inhibitor
Oncoprotein
Cancer

COUMARIN COMPOUNDS

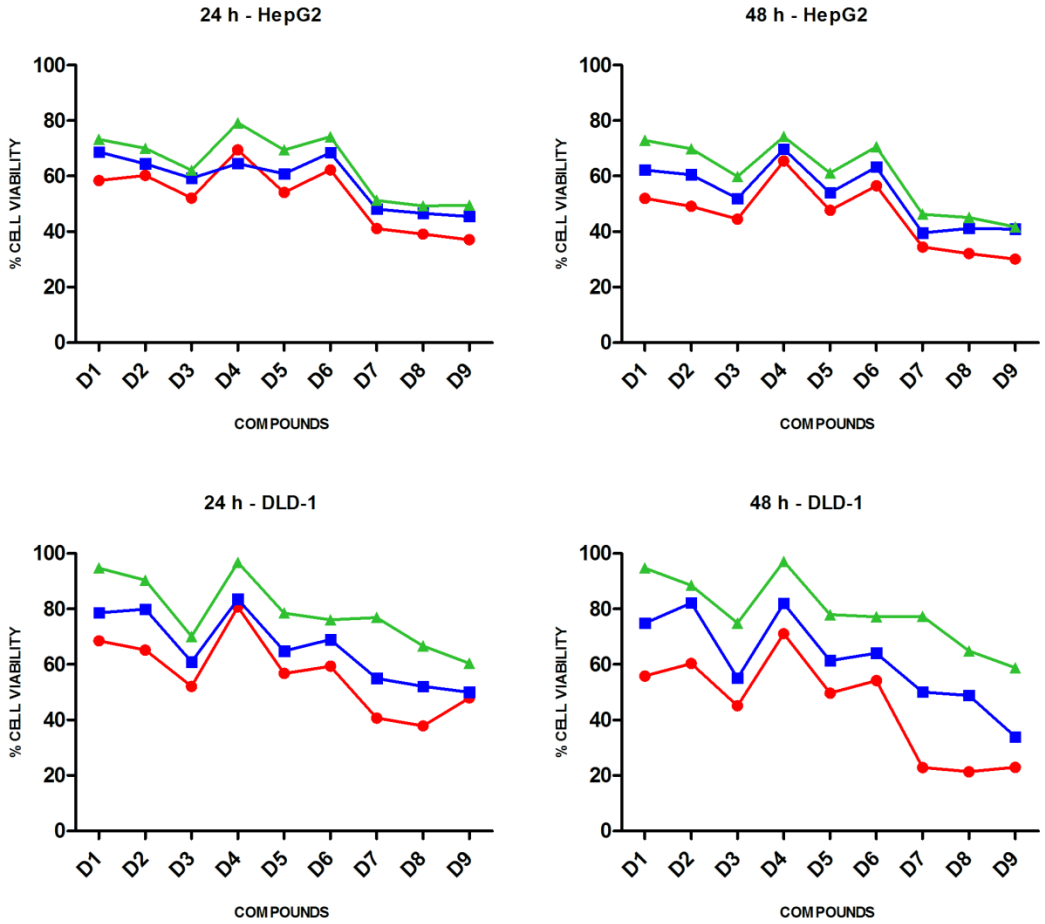
Coumarins (2*H*-1-benzopyran-2-ones) are classified as member of the benzopyrone family of compounds which possess a wide spectrum of biological activity as anticancer, antimicrobial, anti-inflammatory, and analgesic agents





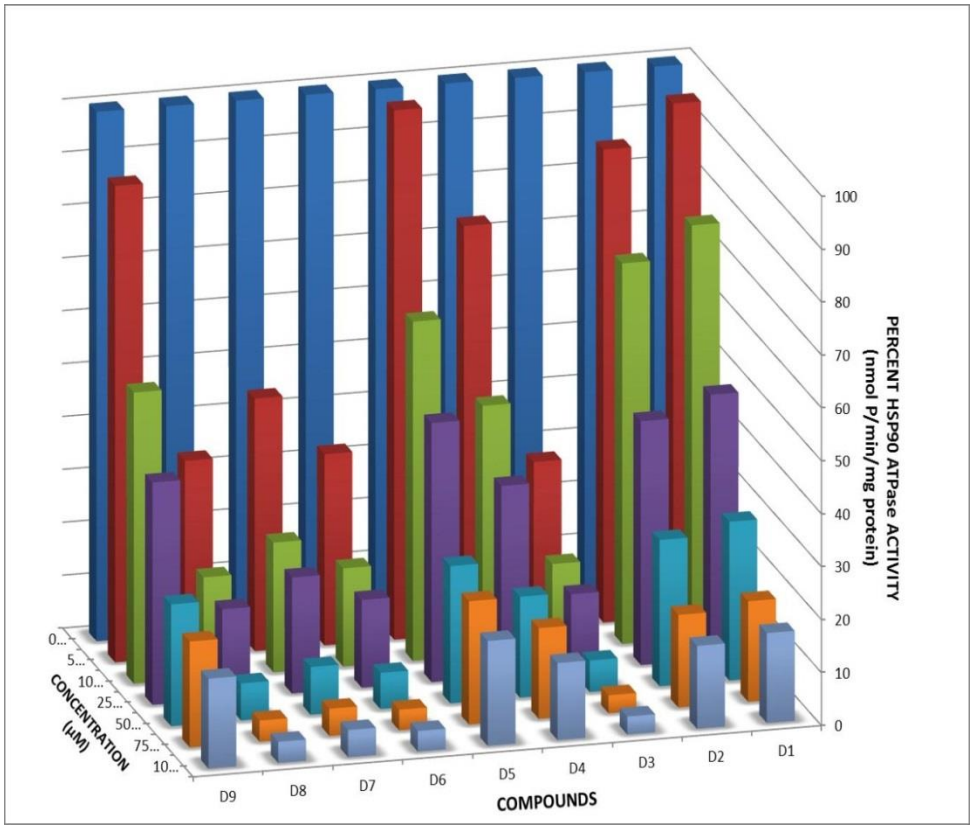
Entry	R-	M_p (°C)	Mol. Formula (Mol. wt.)	Yield	Elemental Analyses	Calcd. %	Found
D1	H_3C—	269	$C_{14}H_{10}N_2O_3S$ 286.31	59	C H N S	58.73 3.52 9.78 11.20	58.50 3.54 10.09 11.48
D2	CH_3CH_2—	268	$C_{15}H_{12}N_2O_3S$ 300.33	56	C H N S	59.99 4.03 9.33 10.68	60.02 4.14 9.20 10.82
D3		329	$C_{19}H_{12}N_2O_3S$ 348.38	61	C H N S	65.50 3.47 8.04 9.20	65.95 3.31 8.48 8.62
D4		357	$C_{19}H_{11}FN_2O_3S$ 366.37	52	C H N S	62.29 3.03 7.65 8.75	62.66 2.94 7.21 8.32
D5	CH_2=$CHCH_2$—	246	$C_{16}H_{12}N_2O_3S$ 312.34	60	C H N S	61.53 3.87 8.97 10.27	61.05 3.96 8.72 9.71
D6		330	$C_{19}H_{11}BrN_2O_3S$ 427.27	64	C H N S	53.41 2.59 6.56 7.50	53.05 2.51 6.19 7.39
D7		317	$C_{21}H_{15}N_3O_3S$ 389.43	67	C H N S	64.77 3.88 10.79 8.23	64.68 3.82 10.61 8.15
D8		308	$C_{22}H_{17}N_3O_3S$ 403.45	43	C H N S	65.49 4.25 10.42 7.95	65.79 4.17 9.99 8.16
D9		298	$C_{22}H_{17}N_3O_4S$ 419.45	39	C H N S	63.00 4.09 10.02 7.64	63.26 4.04 9.80 7.63

Cell proliferation assay (XTT method)



Antitumor properties of thiazolyl coumarin derivatives were tested *in vitro* against human colon (DLD-1) and liver (hepG2) cancer cell lines

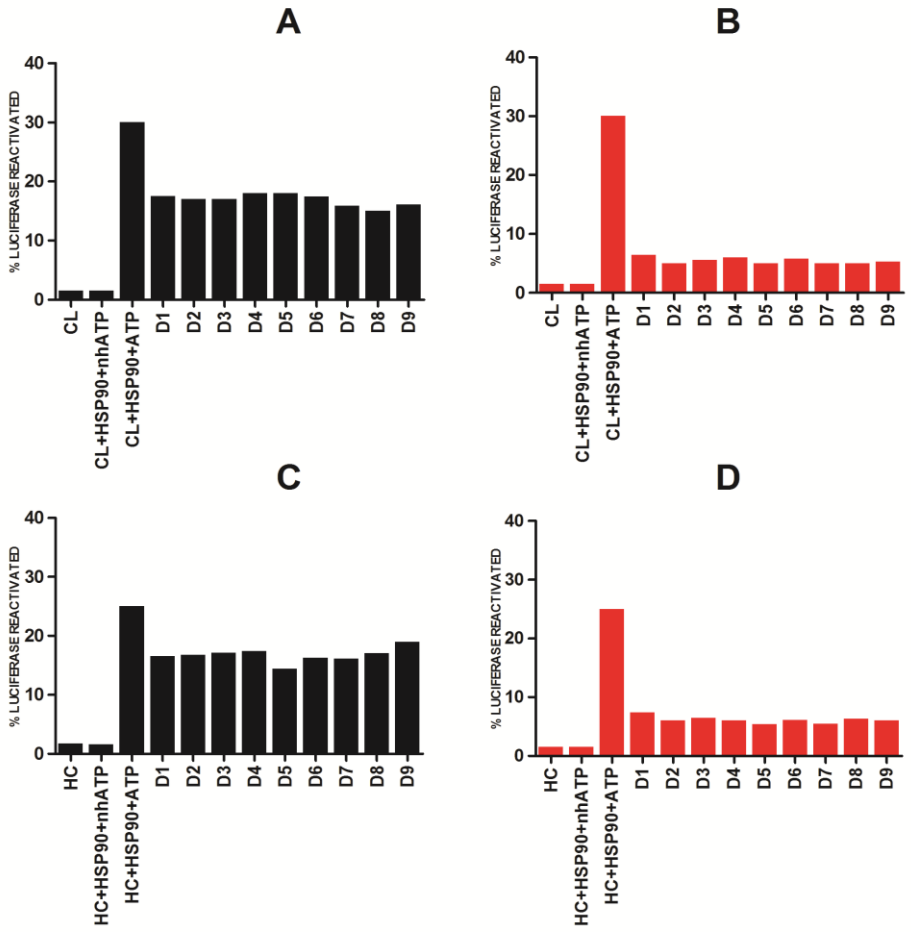
ATP hydrolysis assay



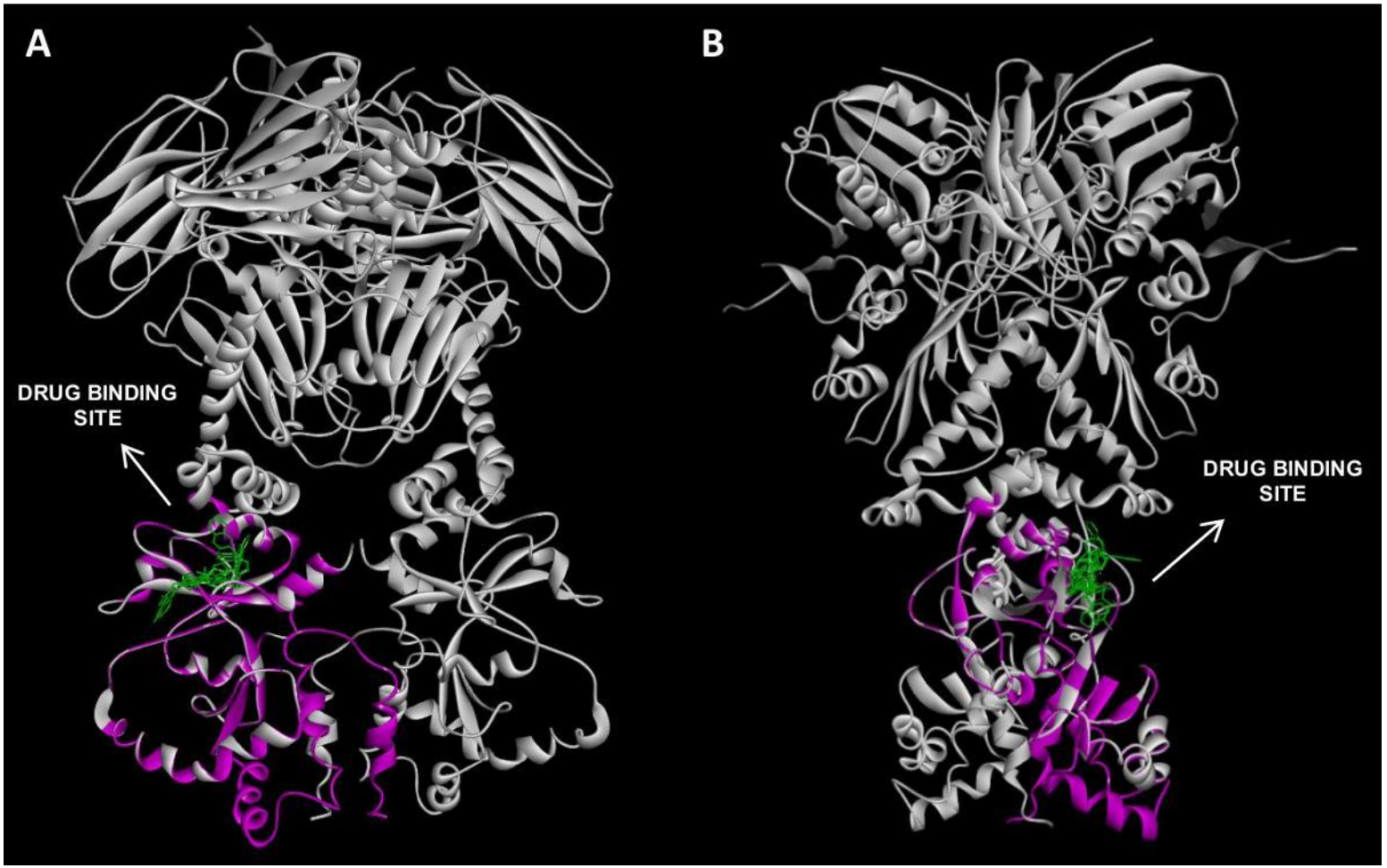
Hsp90 ATPase activity under different inhibitor concentrations.

CTD forms from two sub-domain and D compounds with the ring prevent dynamics of the CTD domain as evidenced by in silico studies. Addition of ATP forms a conformational change at the CTD domain. After hydrolyses CTD sub-domains push each other and this helps dimer formation. However, addition of D compounds with the ring brings these two sub-domains close to each other. This process inhibits proper conformational orientations and blocks dimer formation. In the monomer form Hsp90 may not fold substrate proteins. The two exceptions to D compound behavior are D4 and D9. Fluorine of D3 compound alters the orientation of the compound compared to that of D6 compound which contains Br instead of F. This alteration decreases the effect of D3 inhibition. In a similar fashion CH3O- of D9 compound did not display the effectiveness of D8 compound. Thus, inhibitory compounds exert their effect not only with effective elements but also with proper configuration. And proper configuration of the compound force protein to a conformation in which macromolecule cannot perform its function.

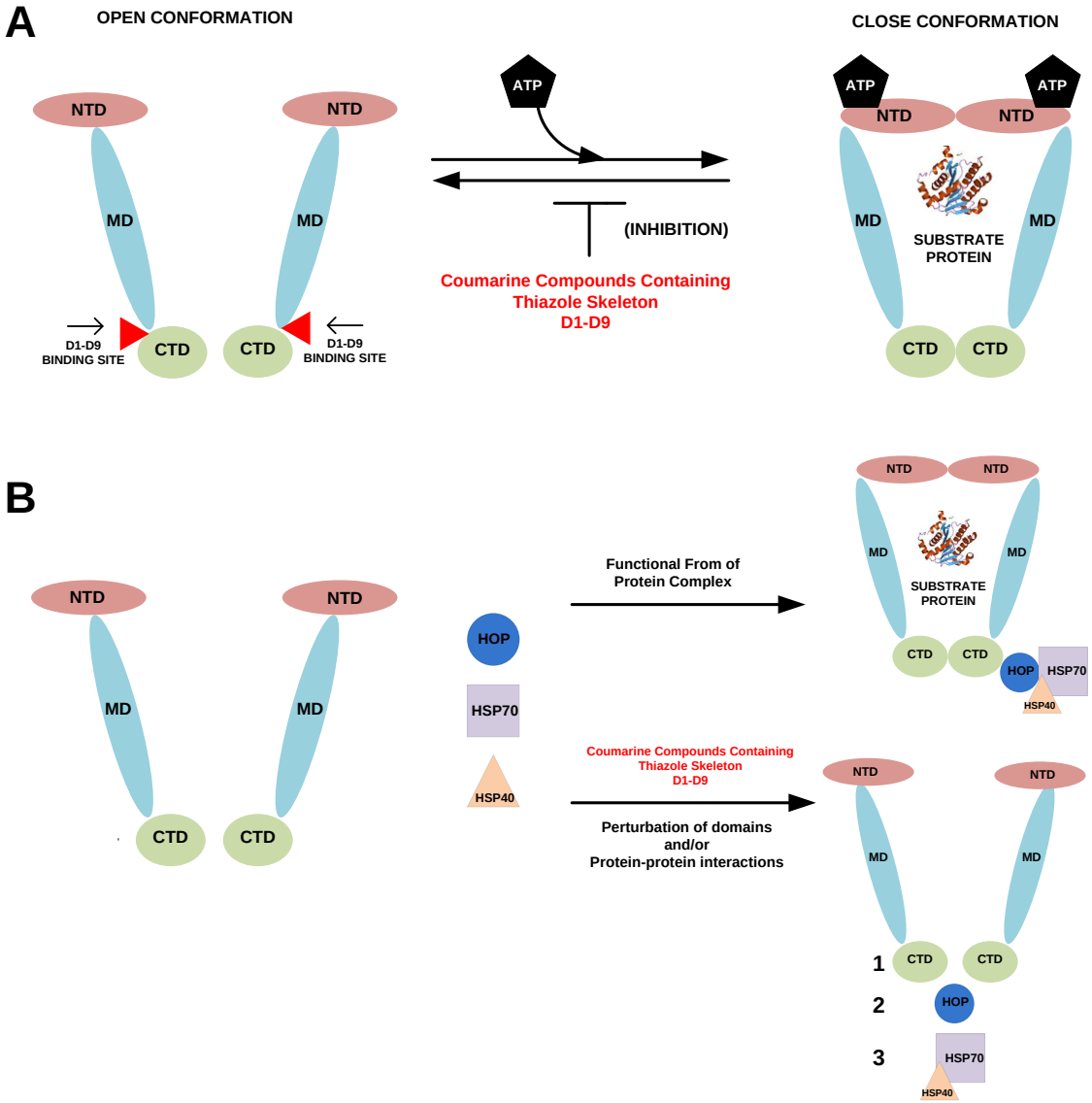
Luciferase aggregation assay



Hsp90 luciferase activity at A: 10 μM (in CL) B: 100 μM (in CL) C: 10 μM (in HC) D: 100 μM (in HC) inhibitor concentrations. CL; cell lysate, HC; Hsp70 + Hsp40 + Hop, nh-ATP; non-hydrolysable ATP (AMP-PNP). D1-D9 were incubated with ATP.



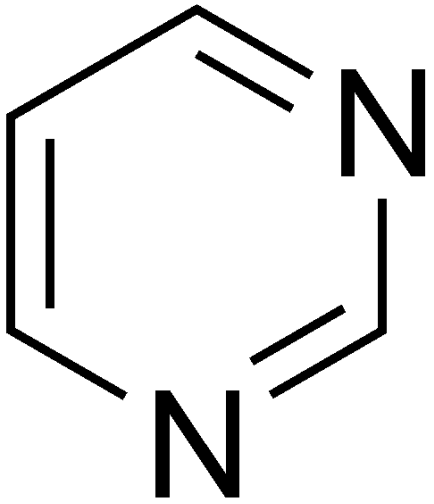
Binding regions of compounds (D1-D9). A. Front view, B. Side view. C terminal domain was shown in magenta and ligands are in green color.

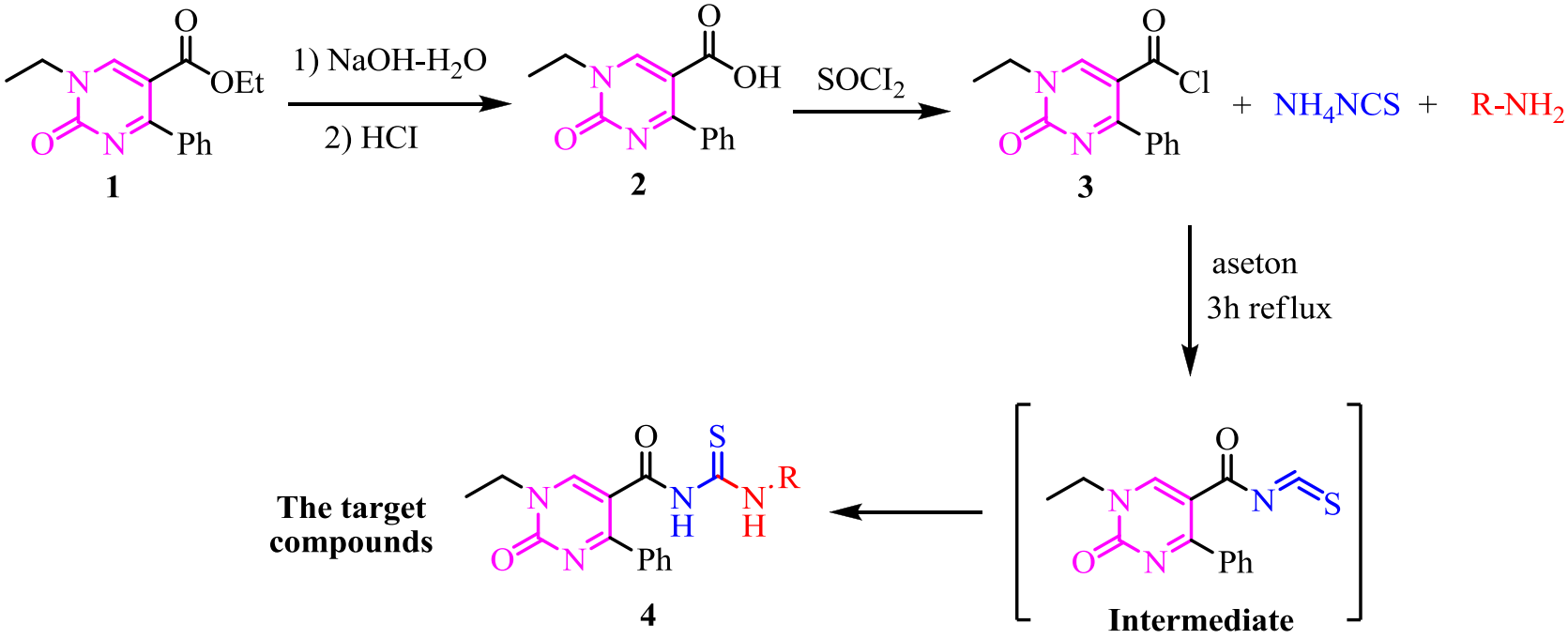


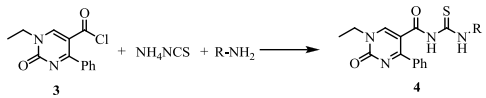
Functional mechanism of Hsp90 (A) and proposed inhibition mechanisms of D1-D9 (B). Hsp90 forms dimer and in the absence of ATP the protein exists in its open conformation. Upon ATP hydrolysis, Hsp90 processes substrate proteins in its closed conformation. Hsp70 interacts with Hsp90 through Hop to process the folding and Hsp40 increase Hsp70 functional properties. Closed conformation provides a hydrophobic environment for proper substrate folding. Presence of D1-D9 disrupts Hsp90 conformation. This may happen by three alternative pathways. 1. Disrupting-weakening dimer formation; 2. Decreasing Hsp90 CTD and Hop interaction; 3. Perturbing interaction with Hsp70-Hsp40 complex. Alternatively any combination of these pathways occur simultaneously during folding process. Thus, substrate peptides may not fold properly.

PYRIMIDINE COMPOUNDS

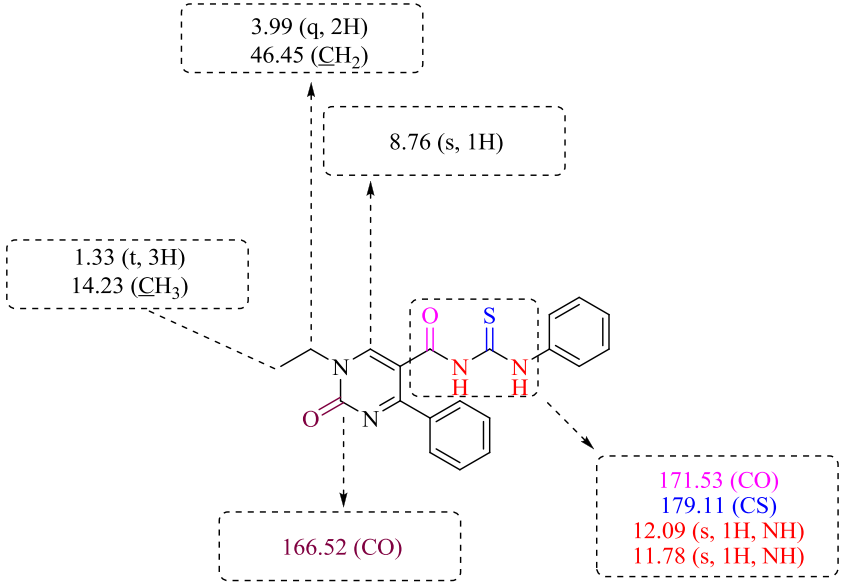
Pyrimidine ring system is one of the most important members of the heterocycles and the compounds containing pyrimidine ring have an increasingly important role in the treatment of cancer (fluorouracil, crizotibib, erlotinib, and cytarabine), diabetes (baloglizatone), gastrointestinal diseases (lansoprazole), cardiovascular diseases (rosuvastatin) and infection diseases (lamivudine). Therefore, pyrimidine derivatives have attracted the attention of synthetic organic chemists and drug designers for many years due to their therapeutic activities. BIIB021 (CNF2024), PU-H71 and Debio 0932 are synthetic pyrimidine ring containing new generation Hsp90 inhibitors and their anticancer activities are currently evaluated in clinical trials




1) NaOH-H2O
2) HCl
SOCl2
+ NH4NCS + R-NH2
aseton
3h reflux
1
2
3
Intermediate
The target compounds
4

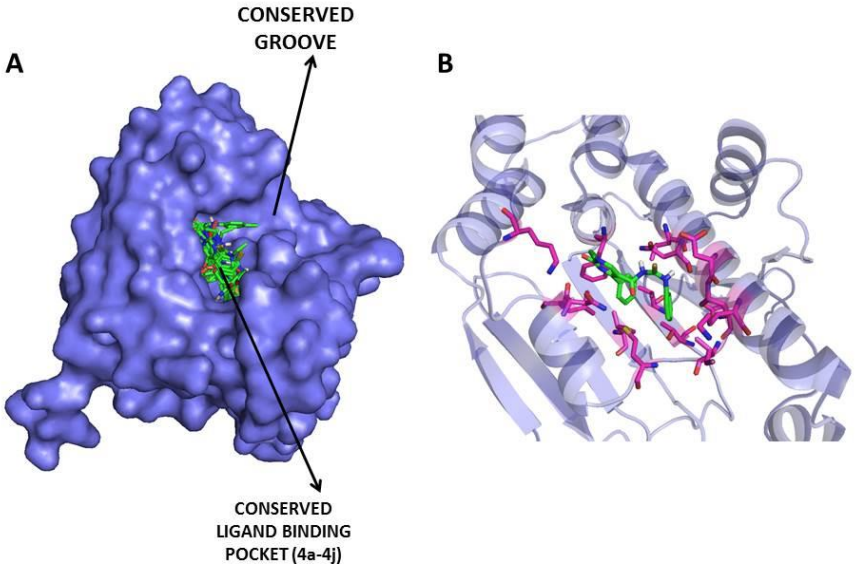


Entry	R	Mp (°C)	Mol. Formula (Mol. wt.)	Yields	Elemental analyses Found (Calcd) %			
					C	H	N	S
4a		223-224	$C_{20}H_{18}N_4O_2S$ 378,45	83	63.04 (63.47)	4.78 (4.79)	13.94 (14.80)	8.53 (8.47)
4b		226	$C_{21}H_{20}N_4O_2S$ 392,47	80	64.71 (64.27)	5.18 (5.14)	13.78 (14.28)	7.73 (8.17)
4c		222	$C_{21}H_{20}N_4O_2S$ 392,47	76	63.72 (64.27)	4.95 (5.14)	13.57 (14.28)	7.34 (8.17)
4d		227	$C_{21}H_{20}N_4O_3S$ 408,47	68	61.34 (61.75)	5.09 (4.94)	13.32 (13.72)	8.00 (7.85)
4e		224	$C_{20}H_{17}FN_4O_2S$ 396,44	75	60.42 (60.59)	4.26 (4.32)	13.96 (14.13)	7.92 (8.09)
4f		226-227	$C_{20}H_{17}ClN_4O_2S$ 412,89	70	58.32 (58.18)	4.00 (4.15)	13.20 (13.57)	7.64 (7.77)
4g		234	$C_{20}H_{17}BrN_4O_2S$ 457,34	71	52.79 (52.52)	3.84 (3.75)	12.04 (12.25)	7.30 (7.01)
4h		219-220	$C_{24}H_{21}N_5O_2S$ 443,52	70	66,83 (67.27)	4.71 (4.70)	13.29 (13.07)	7.76 (7.48)
4i		228	$C_{21}H_{19}N_5O_3S$ 421,47	73	59.50 (59.84)	4.13 (4.54)	16.66 (16.62)	7.33 (7.61)
4j		236	$C_{21}H_{20}N_4O_2S$ 392,47	74	63.96 (64.27)	5.53 (5.14)	13.93 (14.28)	7.87 (8.17)

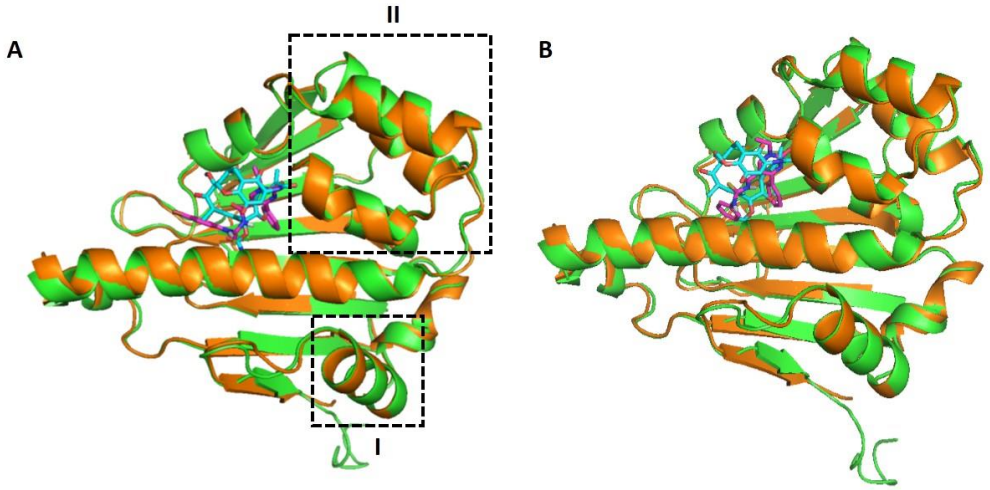


	IC_{50} (μM)	
	MCF-7	**Saos-2**
4a	9,23	10,76
4b	16,21	12,78
4c	16,41	15,91
4d	6,66	5,90
4e	8,51	20,46
4f	73,86	29,77
4g	6,51	7,68
4h	5,24	1,30
4i	36,90	15,32
4j	20,58	59,42

IC_{50} values of compounds against MCF-7 and Saos-2 cell lines.



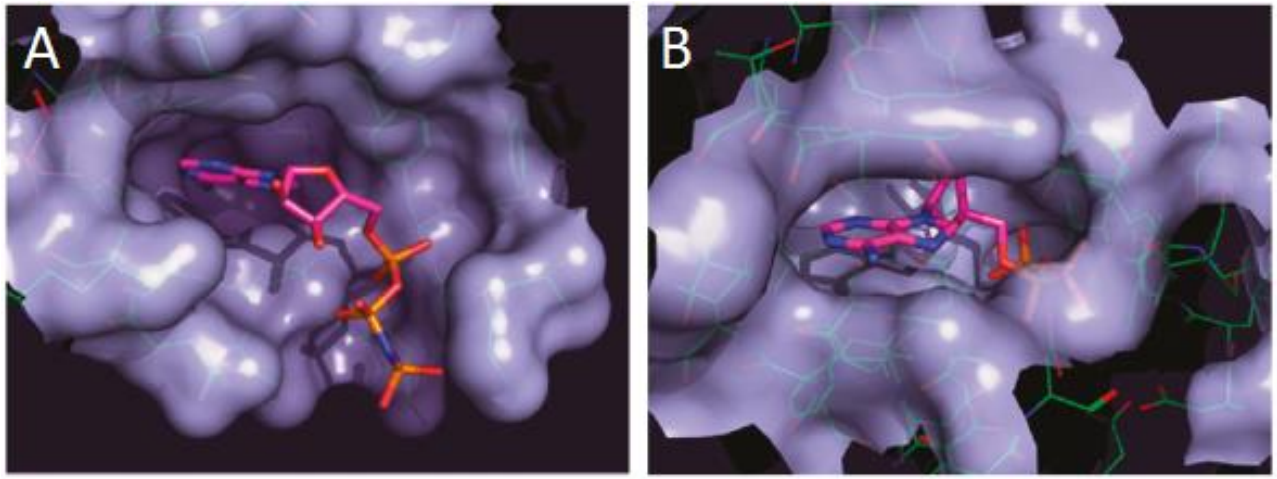
A) Binding regions of the compounds (4a-4j in green color) on human Hsp90 NTD.B) Important residues (pink) of Hsp90 NTD interaction between compounds (green).



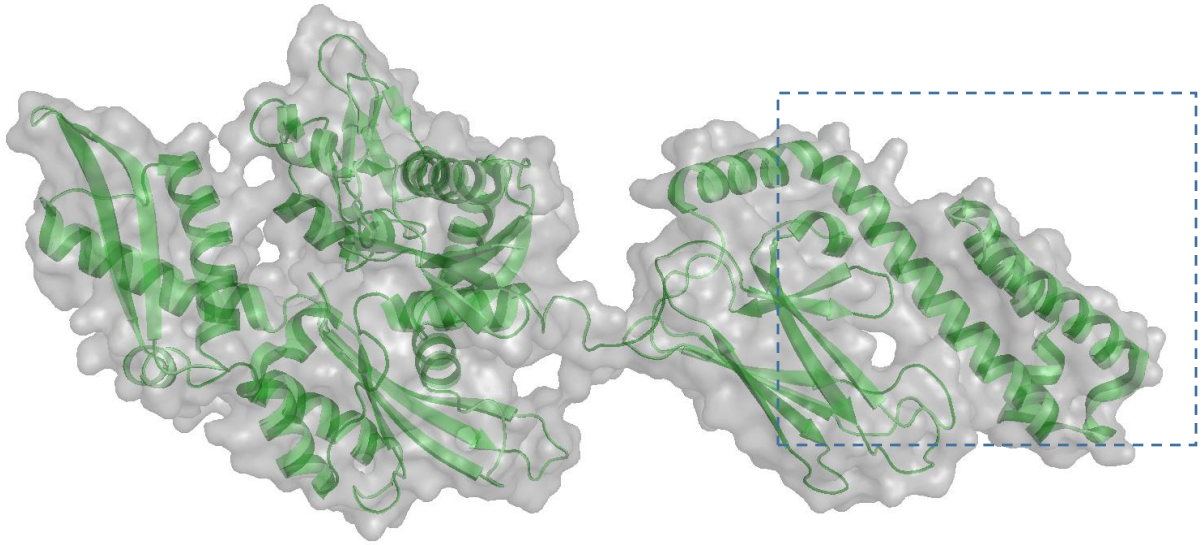
Hsp90 conformational changes in the presence of halogenated compound (4h) and non-halogenated compound (4a). Simulation results were visualized with geldanamycin (Pdb code: 1YET) since geldanamycin perturbs Hsp90 conformation. Green color indicates 4h (A) (magenta), 4a (B) (magenta) and orange indicates geldanamycin (cyan) bound Hsp90 NTD.

Inhibition of Hsp90 triggers expression of Hsp70 and complements inhibited Hsp90 chaperone activity. Moreover, Hsp27 controls and regulates key points of the apoptotic pathway in cancer cells. Therefore, in addition to Hsp90 inhibition, blocking of Hsp70 and Hsp27 chaperone activities have been remarkable therapeutic strategy for cancer treatment.

Similar to Hsp90 inhibition which is an important method; different research groups searched Hsp70 ATP hydrolysis inhibition and designed Hsp70 inhibition with same approach. It was determined that designed inhibitor agents were not suitable for drug development and clinical trials since Hsp70 ATP domain pocket is deep and the nucleotide binds with polar interactions. Orally bioavailable druglikeness compound designing from these polar agents does not meet Lipinski and Veber's criteria.



(Massey 2010)



A model of Hsp70 was made since human Hsp70 protein crystal structure is not elucidated yet. Potential druggable sites were determined on the protein with molecular simulation studies. An inhibitor, YK5, was designed according to a druggable site determined by molecular simulation studies and also it was found that YK5 bound specifically to cytosolic Hsc70. But since this site is on ATP domain it does not meet orally bioavailable drug criteria. At the same time substrate binding domain (SBD) inhibitors designed up till now consist of short peptides. In a similar fashion, these structures are not available for drug design. For these reasons, our work is focused on Hsp70' SBD to determine allosteric changes and design inhibitors by covering the bioavailability criteria.

CONCLUSION

In our lab, we designed and synthesized novel pyrimidine and coumarin derivative compounds as Hsp90 inhibitors for cancer treatment. Pyrimidine analogs interrupt Hsp90 ATP hydrolyses process through disrupting N terminal domain (NTD) conformational change. Coumarin derivative compounds inhibit C terminal domain (CTD) of Hsp90, and block dimerization process.

As an alternative to Hsp90 inhibitors, Hsp70 substrate binding domain (SBD) inhibitors are designed and synthesized for effective cancer treatment by our groups. Results indicated that these inhibitors provide significant opportunities for cancer treatment.

Acknowledgments

The authors gratefully acknowledge the financial support received from the Scientific and Technological Research Council of Turkey, TÜBİTAK (Grant # 114Z365).