Design of allosteric stimulators of the HSP90 ATPase as novel anticancer leads

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Heat Shock Protein 90 (Hsp90) is a ATP-dependent chaperone that controls the folding of more than 200 client proteins and constitutes a central node in many signaling pathways[1]. Overexpression and dysregulation of Hsp90 have been linked with cancer and neurodegeneration. It is thus not surprising that this chaperone has become an important drug-target: in principle its inhibition can result in the simultaneous degradation of multiple clients associated with different pathological hallmarks[2]. A viable way to interfering with Hsp90 is represented by allosteric ligands, which perturb the chaperone by targeting sites alternative to the ATP-site.

In this context, we have developed a method for the identification of allosteric pockets via the analysis of residue-pair distance fluctuations in the structural ensemble around the active state of the chaperone. The protien works as a homodimer and the site we identified is located at the interface between the C-terminus of the two protomers (Figure 1). Based on this, we designed modulators characterized by a *O*-aryl rhamnoside benzofuran scaffold, showing promising anticancer activities and a novel molecular mechanism of perturbation of Hsp90 functions: the ligands in fact were experimentally proved to be *activators* of closure kinetics and ATPase of the chaperone *in vitro*, induce cancer cell death, and interfere with client maturation[2]. We developed a first Quantitative-Structure-Dynamics-Activity-Relationship (QSDAR) model correlating the structures of an initial set of modulators to observed activation effects[3].

Based on this initial model, we report the rational design of new allosteric ligands, reaching low micromolar to nanomolar anticancer activities, which support their potential in the development of anticancer therapeutics (Figure 2). On the computational side, we further develop a model to evaluate the potency of allosteric modulators by taking into account the dynamic cross-talk that exists between the protein and the ligand. We calculated Dynamic Ligand Efficiency (DLE). i.e. the Ligand Efficiency in a multiconformational protein ensemble[3], to correlate predicted docking scores to ATPase stimulation and cellular effects. The model provides a good correlation between DLEs and measured ATPase stimulations (R = -0.66 considering all compounds discussed here and in [3]; R = -0.71 when considering only co-generic amino-derivatives). This finding supports the validity of our model for the design of allosteric activators of Hsp90. Next, we assessed the capacity of our new DLE descriptor to evaluate the potency of the designed compounds in antiproliferative assays. Importantly, the calculated DLE shows a significant correlation with measured cytotoxicities against the cancer STO cell line, with correlation value of 0.62 when considering the whole series, which raises to 0.67 when considering only amines indicating the ability of this very simple model to quantitatively capture the main determinants of cytotoxic activities.

To the best of our knowledge, these results are the first that show the actual feasibility of pushing integrated knowledge of dynamic protein-ligand cross-talk into the design of new Hsp90 allosteric compounds, with novel functional impacts as well as improved antiproliferative activities. We have shown before [2] that these compounds stimulate Hsp90 ATPase activity by accelerating the protein conformational cycle and favoring the catalytically active state: we hypothesize that this reverberates in a modification of the population of the chaperone structural ensembles and of the timing with which Hsp90 conformational families are presented to interaction with co-chaperones and clients. Consistent with recent findings based on mutational studies [3, 4], this novel way of perturbing chaperone populations and kinetics can expectedly be detrimental to cell viability.

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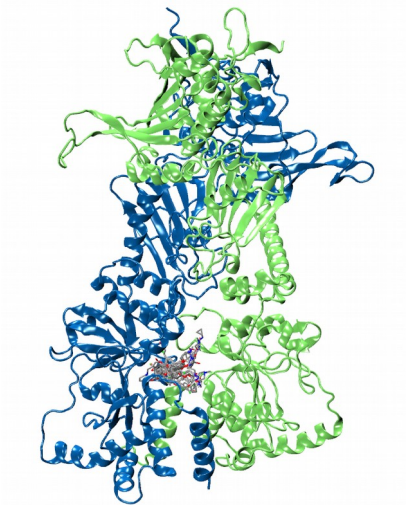

Figure 1.Overall view and detailed superposition of the best pose for each compound in the allosteric site. Color code: Protomers A and B, light blue and green respectively; compounds: C=grey, O=red, N=blue, Cl=green, F=dark yellow.

Table 1. Structures, stimulatory potencies, and cytotoxic activities of designed compounds.[a]

Compound	Normalized ATPase rate	Toxicity in STO (IC_{50} [μM])
1	1.1[b]	57.0±2.1[b]
18	4.8[b]	8.9±1.1[b]
19	6[b]	9.9±1.1[b]
20	1.5	16±1
21	2.2	8.2±2.7
22	1.9	11.7±2.4
23	2.4	3.9±1.8
24	4.1	8.6±2.1
25	4.5	22.1±1.1
26	4.2	4.1±0.4
27	1.4	not soluble
28	1.3	36±6
29	1.17	>75
30	0.98	>100
31	–	0.57±0.04

Figure 2. Structures, stimulatory potencies and cytotoxic activities of designed compounds.