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Fragment-based discovery of hydroxy-indazole-carboxamides as novel small molecule inhibitors of Hsp90

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Dedicated to Professor Christian R. Noe on the occasion of his 65th birthday

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ABSTRACT

Inhibitors of the Hsp90 molecular chaperone are showing considerable promise as potential molecular therapeutic agents for the treatment of cancer. Here we describe the identification of novel small molecular weight inhibitors of Hsp90 using a fragment based approach. Fragments were selected by docking, tested in a biochemical assay and the confirmed hits were crystallized. Information gained from X-ray structures of these fragments and other chemotypes was used to drive the fragment evolution process. Optimization of these high μM binders resulted in 3-benzylindazole derivatives with significantly improved affinity and anti-proliferative effects in different human cancer cell lines.

Molecular chaperones are proteins, which play a key role in the conformational maturation, stability and function of other client protein substrates within the cell.[1,2] Many of the client proteins of Heat shock protein 90 (Hsp90),[3–6] which are involved in signal transduction, cell cycle regulation and apoptosis, are well known oncogenes and are often deregulated (over-expressed and mutated) in tumor cells, and this dysregulation of pathways involving these proteins are commonly associated with cancer pathology.[7] The association of Hsp90 with these client proteins maintains their ability to function in the deregulated state. Therefore, Hsp90 inhibitors target tumor growth by multiple parallel mechanisms. Hsp90 has attracted considerable interest as a therapeutic target for anticancer drugs since it was shown that both geldanamycin[8] and radicicol[9] are able to inhibit Hsp90 function by binding to an ATP binding pocket in the N-terminal domain of the protein. Based on encouraging preclinical data several derivatives of these natural product inhibitors like 17-AAG, 17-DMAG and the prodrug of 17-AAG, IPI-504 entered clinical studies.[10] However, these compounds have several potential limitations, including poor solubility, limited bioavailability and hepatotoxicity. These issues have led to significant efforts to identify small-molecule inhibitors. Indeed, several compounds of different chemical classes have been disclosed so far, like ganetespib (STA-9090),[11] NVP-AUY922,[12] AT-13387,[13] Debio-0932 (CUDC-305)[14] or PU-H71[15] which have entered trials in different phases of clinical development (Fig. 1). A comprehensive overview has been published recently.[16]

Finding novel compounds as starting points for optimization is a major challenge in drug discovery research. Fragment-based approaches have rapidly become a proven technique to identify such starting points in a variety of research programs.[17] Furthermore, the optimization of fragment-like hits using structural information from protein X-ray crystallography has been established as a valuable strategy in the search for new drug molecules of various targets including Hsp90.[18] Herein, we describe the identification of low affinity indazoles and their optimization to potent inhibitors of the N-terminal ATP binding site of Hsp90.

A subset of about 64,000 compounds was preselected from our compound library by filtering with calculated fragment-like properties in adaption to the Astex 'rule of three' (e.g., MW <300).[19] This subset was used for virtual screening by docking in the ATPase binding site of Hsp90. The analysis of the X-ray structures of Hsp90 in complex with reference compounds (phenole and purine

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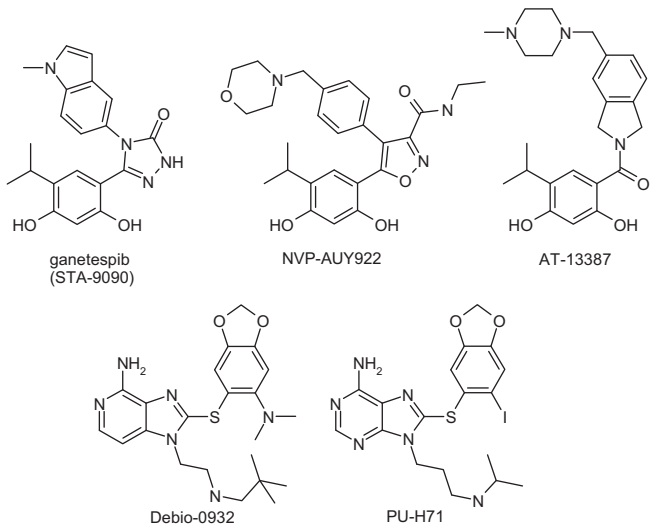

Figure 1. Selected structures of Hsp90 inhibitors in clinical trials.

derivatives) published at the time, when our virtual screening campaign was conducted, revealed crucial interactions within the ATP-site.[20,21] These interactions include hydrogen bonds of the phenolic OH-group and purine N-atoms to Asp93 and an adjacent conserved water-molecule (Fig. 2). The assigned hydrogen bond network of the phenolic OH-group in the Hsp90-complex suggested a donor contact to Asp93 and an acceptor interaction to the interstitial water molecule. Similarly, the purine amino group forms an H-bond donor contact to Asp93 and the N1-atom an acceptor contact to the conserved water-molecule. A large induced fit characterized by the transition of an open, 'apo-like' form as in the geldanamycin complex to a helical form upon binding of purine derivatives was observed in the rear ATP binding site of Hsp90.[22,23] This transition results in the presence of a large lipophilic pocket formed by the side chains of Met98, Leu107, Phe138, Tyr139, Val150 and Trp162 in the helical form. As demonstrated for purine analogs, the optimization of hydrophobic interactions within this lipophilic pocket has resulted in Hsp90 inhibitors with significant biochemical and cellular potency.[24] Consequently, we used a representative structure of the helical form (PDB code 1UYF) as rigid protein model for docking in order to also identify fragments potentially binding into this lipophilic pocket.

The composite inhibitor interactions served as pharmacophore constraints (Fig. 2) for the subsequent docking of a preselected subset of ~64,000 compounds from our corporate library using the program FlexX.[25] The pharmacophore contraints (see Supplementary data) were used as filters to select only fragments with suitable H-bonds to either Asp93 or the conserved water molecule in addition to potential hydrophobic contacts within the lipophilic pocket of the helical form which resulted in 3810 hits. These hits were ranked by a combination of two alternative docking scores (FlexX- and PLP-scoring functions), clustered by chemical substructures and the docking poses of top scored hits from each cluster were finally visually inspected. A subset of 96 fragments was finally selected for testing in a Hsp90 binding assay[26] revealing five hits with an IC_{50}-value below 100 μM (5% hit rate). Three of the hits were structurally similar, low molecular weight indazoles and the remaining two hits contained an aminopyrimidine or quinazoline moiety (Fig. 3a). The binding mode of all hits could be elucidated by X-ray crystallography using the ATPase domain of human Hsp90. A superimposition of the crystal structures revealed, that fragments III and V bind to the helical protein form with their bulky hydrophobic substituents (cyclohexyl and *p*-chlorophenyl respectively) oriented into the same lipophilic pocket as the substituted phenyl ring of Hsp90-purine complexes (e.g., as in PDB code 1UYF). In contrast, the three other fragments I, II and VI bind to the open Hsp90 conformation as present in the geldanamycin complex (Fig. 3b). Interestingly, X-ray structures confirmed that the hydroxy-indazoles bind in two reversed orientations in contact with Asp93 as predicted by our pharmacophore docking protocol (Figs. 4 and 5). Fragment II interacts via H-bonds of both indazole N-atoms to Asp93 and the conserved water molecule, while an identical interaction pattern is found for the phenolic OH-group of fragment III. The reversed orientation might be influenced by the substitution pattern of the hydroxindazole core as the cyclohexyl ring of fragment III is bound in the lipophilic

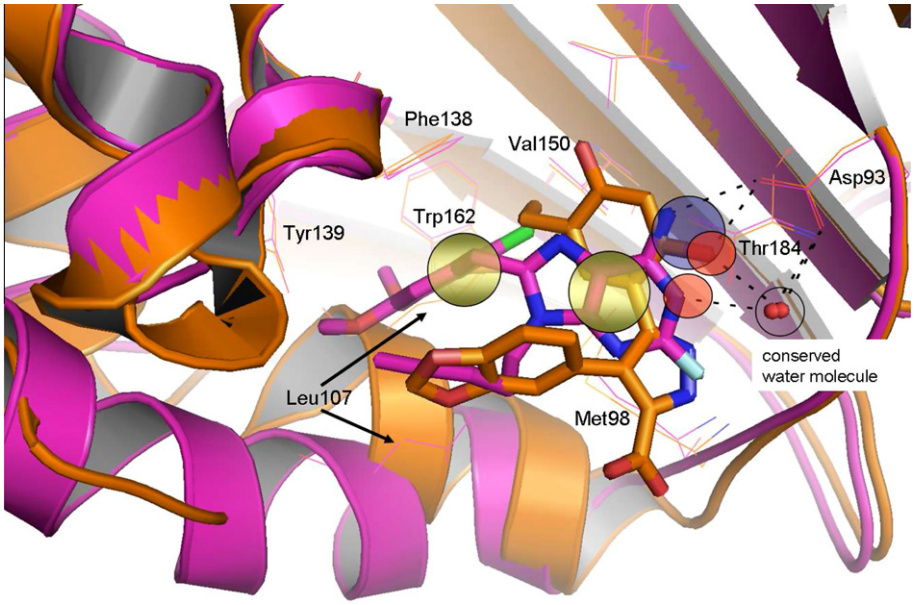

Figure 2. H-bond interactions (dashed lines) of published Hsp90 inhibitors: purine-derivative in 'helical form' (PDB-code 1UYF, magenta) and resorcinol-derivative (PDB-code 1YC1, orange) in 'open form' of Hsp90. Crucial contacts were used as pharmacophore filters (H-donor: blue, H-acceptor: red, lipophilic: golden) for virtual screening by docking.