

Human 90 kDa Heat Shock Protein Hsp90 as a Target for Cancer Therapeutics

Lisandra M. Gava^{1,2} and Carlos H.I. Ramos^{*,2}

¹Institute of Biology and ²Institute of Chemistry, University of Campinas-UNICAMP, P.O. Box 6154, 13083-970, Campinas, SP, Brazil

Abstract: Protein misfolding causes a phenotype of disorders that is modulated by the action of multi-complexes formed by molecular chaperones and the proteasome machine. Hsp90 is a molecular chaperone involved in maintaining folding, stability and function of many proteins involved in apoptosis, signal-transduction pathways and cell-cycle regulation. Many of these proteins are usually deregulated in cancers and by keeping them active Hsp90 helps the stabilization of tumorigenic cells. Therefore, inhibition of Hsp90 will result in degradation of its client proteins *via* the proteasome followed by a down regulation of several properties of the malignant phenotype. As a consequence, Hsp90 has been considered to be an appealing target for cancer therapeutics because its inhibition can affect multiple oncogenic pathways simultaneously. Major efforts have generated Hsp90 inhibitors that passed Phase I clinical trials and have entered Phase II trials. Furthermore, other compounds are in development to improve efficacy as antitumor agents. In conclusion, the development of Hsp90 inhibitors is considered to be a good example of medicinal chemistry. Specific important aspects of Hsp90 structure and function, the role of the chaperone in cancer and the development of Hsp90 inhibitors that causes growth arrest and apoptosis in cancer cells are discussed.

Keywords: Hsp90 inhibitors, molecular chaperone, cancer therapeutics, geldanamycin, antitumor drugs, medicinal chemistry.

1. CHAPERONES AND PATHOLOGY

The necessary information for a protein to fold to its native conformation lies in the amino-acid sequence (for a specific review see [1]). However, efficient reversible folding and unfolding has been usually observed only for small proteins whereas for many others, refolding experiments result in partially unfolded, or even completely misfolded molecules, and in aggregation. Aggregation is unproductive since the function of most proteins is ordinarily related to its native conformation, although Intrinsically Unfolded Proteins, or IUP, seem to have cellular functions [2]. Protein aggregation has become a general problem because any protein sequence has the potential to form amyloid fibrils given the appropriate conditions [3,4]. In agreement with the aforementioned, the deleterious effect of aggregates has been connected to major pathological effects and tissue deposition of protein aggregates *in vivo* causes degenerative diseases and organ damage [5]. There are two aspects of protein folding in the cellular context that further aggravate the pathological effects of protein misfolding. Protein aggregation is enhanced by crowding effects inside the cell [6] and aggregates may become infections by promoting aggregation of native conformers as is the case with prions [7].

Inside the cell the folding of many proteins is assisted by molecular chaperones that are also referred to as heat-shock proteins (Hsp) for being first described as proteins induced during thermal stress [8-10]. In a general way, molecular chaperones help other proteins to fold and are considered cellular housekeepers due to their capability of activating

cellular processes by regulating the folding/function of key proteins. For instance, when cells are submitted to an increase in temperature, a heat-shock response (HSR) is activated and mRNAs related to Hsps are detected subsequently. The importance of molecular chaperones for the organism can also be evaluated by the high number of genes belonging to molecular chaperones present in the genome and the high expression of these genes. Take plants for instance, their cells trigger the expression of molecular chaperones to increase their chance of survival in the extreme environmental stress to which they are usually exposed. The genome of the model plant *Arabidopsis thaliana* has been sequenced and the gene expression profile, measured by RNA expression, of many plants, among them sugarcane and eucalyptus, have been investigated. The data provided by these works can be mined specifically for molecular chaperones and used to generate new information about them (The TIGR *Arabidopsis thaliana* Database: <http://www.tigr.org/tdb/e2k1/ath1/>; [11, 12, 13]). The general picture from the mined data is that about 20% of the expressed molecular chaperones belong to the Hsp70 family, about 20% to Hsp70 co-chaperones and about 10% to the Hsp90 family. This result emphasizes the importance of Hsp70 and Hsp90 as regulators of many cellular processes.

Molecular chaperones also have a prophylactic action because they prevent proteins with aggregative potential to form amyloid fibrils. In addition to that, some specialized molecular chaperones have the ability to dissolubilize aggregates, a powerful characteristic that opens the possibility to use them as therapeutics against diseases caused by misfolded proteins. It is conceivable that an accumulation of misfolded proteins with time would overload the chaperone system accounting for the increase in the number of diseases verified in the elderly [14]. Consequently, some researchers

*Address correspondence to this author at the Institute of Chemistry, University of Campinas-UNICAMP. P.O. Box 6154, 13083-970, Campinas, SP, Brazil; Tel: 55-19-3521-3144; Fax: 55-19-3521-3023; E-mail: cramos@iqm.unicamp.br

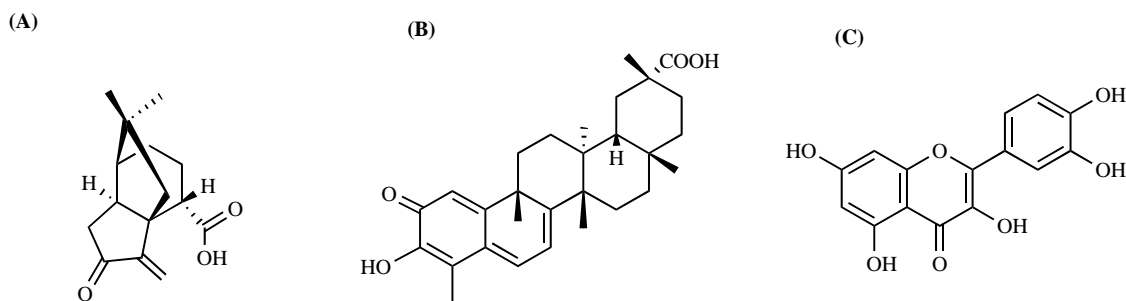


Fig. (1). (A) Terrecyclic acid A, (B) celastrol and (C) quercetin.

have pointed out that misfolded proteins would play a role in the manifestation of as much as half of the human diseases (see Reference [15] and references therein). We may conclude that a complex formed by molecular chaperones and the proteasome (responsible for protein degradation) constitute the quality control system that deals with proper protein folding and degradation. This complex system would then modulate the phenotype of disorders caused by protein misfolding.

Protein misfolding causes both the loss of function, as in the case of some cancer diseases, and again of damaging function, as in the case of Alzheimer and Parkinson diseases. These deleterious actions motivate developing small molecules capable of manipulating chaperones and protein homeostasis. As an example, compounds that could induce a high expression of molecular chaperones would be important as therapeutic agents to increase the yield of correctly folded proteins and to decrease the number of aggregates in pathological conditions.

The heat shock response (HSR) is transcriptionally regulated by heat shock factors (HSFs) that triggers the expression of molecular chaperones. There are three HSFs in humans: HSF1, HSF2 and HSF4. Several chaperones, most importantly Hsp70 and Hsp90, were shown to bind to HSF1 and keep it in an inactive form. In a situation of stress mis-

folded proteins compete with HSF for the binding site consequently releasing HSF that migrates to the cell nucleus [16]. HSF1 is activated by proteasome inhibitors, by molecules that regulate inflammation and by Hsp90 inhibitors (see below). Some compounds that increase the action of HSF are in clinical trials and had positive impact on oxidative stress and on some cancers [17,18]: terrecyclic acid A [19], celastrol [20], and quercetin [21,22] (Fig. 1). The aforementioned compounds have also critical importance when considering targeting Hsp90 for chemotherapy [18].

2. STRUCTURE, INTERACTION AND FUNCTION OF HUMAN HSP90

Hsp90 is a specialized ATP-dependent protein folding tool, which is essential for the growth of eukaryotic cells [23]. This chaperone is a homodimer and each monomer consists of a highly conserved N-terminal domain (~30 kDa) that contains an ATP-binding site followed by a charged region of unknown function, a flexible domain located in the middle (~35 kDa) and a C-terminal domain (~20 kDa) that is responsible for dimerization (Fig. 2). The N-terminal ATPase domain has a regulatory pocket that binds and hydrolyzes ATP to mediate a series of association-dissociation cycles between Hsp90 and client proteins. This domain contains the Bergerat-fold, in which the nucleotide adopts a bent shape, and was previously found in bacterial gyrases and

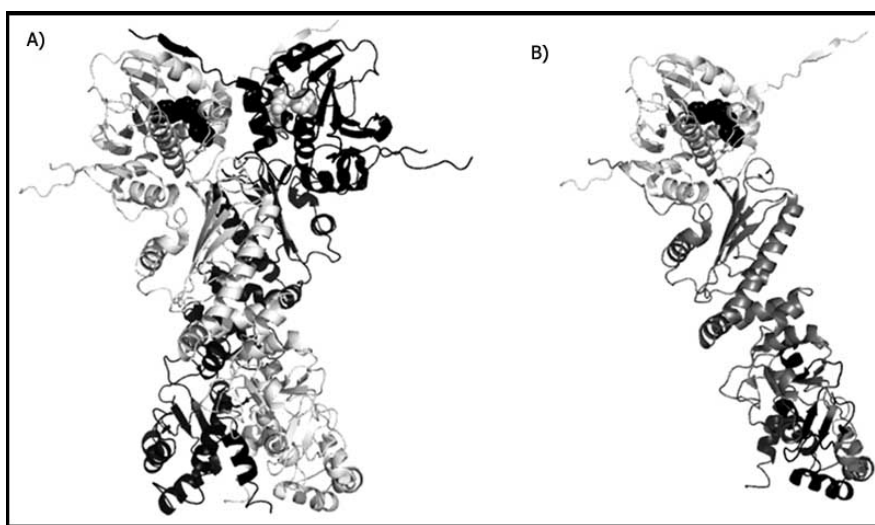


Fig. (2). Tertiary structure of Hsp90 from *Saccharomyces cerevisiae* which is 82% similar to human Hsp90 α . A) One monomer is in black and the other in white. B) Top white: N-terminal domain residues (2-216), middle gray: central domain residues (262-560) and bottom black: C-terminal domain residues (561-677). AMP-PNP is represented by spheres. PDB accession number 2CG9. All molecular graphics were produced with PyMOL (<http://pymol.sourceforge.net/>).

co-chaperones in modulation, acceleration, potentiation, and stabilization of folding (see Table 1 for a list of important co-chaperones involved with Hsp90). A list of K_D values for binding for selected Hsp90 factors can be found in Reference [35]. Hsp90 co-chaperones promote the interconversion of the ATP- and ADP-bound states and modulate the formation of client specific complexes [36].

In eukaryotes, the Hsp70-Hsp90 machinery is formed by Hsp70, Hsp90, and co-chaperones HOP, Hsp40 and p23. As a direct support to this hypothesis functional Hsp90-heterocomplex can be assembled *in vitro* by mixing the purified chaperones and co-chaperones listed above. The function of Hsp90 is driven by a dynamic association with its client proteins and their co-chaperones to form protein complexes. The tetratricopeptide repeat (TPR) is a degenerate 34-residue motif present in a subset of co-chaperones that binds Hsp90. TPR is formed by a compact helical domain of six consecutive α -helices arranged in a regular right-handed superhelix, generating an amphipathic groove that is capable of interacting with 7-12 residues of the polypeptide. The binding site for TPR is in the C-terminus where a domain formed by a MEEVD motif is present [37].

The Hsp90 chaperone is highly regulated. For instance, its ATPase activity regulates the substrate binding cycle and HOP inhibits this activity by preventing nucleotide binding. The high affinity state of Hsp90 for client proteins is switched to the lower affinity state by ATP binding, which hydrolyze the affinity back to high. The p23 co-chaperone recognizes Hsp90 bound to ATP and stimulates dissociation of the complex. In addition, Aha1 stimulates the ATPase activity of Hsp90 by about tenfold [38] and CHIP (C-terminus of Hsp70-interacting protein) connects Hsp90 to the ubiquitin complex, helping to control the degradation of proteins by the proteasome. As a matter of fact, there is evidence that in several tumor cell lines, Hsp90 might be exclusively bound to co-chaperones in a state of high affinity for nucleotide [39] (see below).

3. HSP90 AND CANCER

A recent and large-scale study using yeast as model [40] showed that at least 200 client proteins make physical interactions with Hsp90. One of the most important conclusions of this work is that Hsp90 is connected with a wide range of cellular functions. As examples of its diversity Hsp90 is involved in epigenetic gene regulation by interacting with proteins that are chromatin remodeling factors and Hsp90 participates in inherent genetic variation [41].

New client proteins for Hsp90 seem to be discovered continuously and an up to date list of Hsp90 'interactors' can be found at <http://www.picard.ch/DP/downloads/Hsp90interactors.pdf>. Important to the cancer therapeutics field is that signal transduction proteins including many kinases and steroid hormones are also clients of Hsp90, which maintains their conformation, stability and function [41-43]. These structurally labile signal transducers have a crucial role in cell cycle, growth control, apoptosis and developmental processes. In fact, the largest single group of Hsp90 client proteins are protein kinases such as oncogenic kinases c-Src, b-Raf, PKB/Akt1, ErbB2 and Cdk4 [44,45]. Many of them, like ErbB2, Src, Raf, and cyclin-dependent serine kinases are key players in malignant transformation [46,47]. Hsp90 is also necessary for the maturation of the nuclear hormone receptors and the hypoxia-inducible factor-1, and is associated with nitric oxide synthases and the antiapoptotic protein. The chaperone acts in regulating proteins involved in both the intrinsic and extrinsic apoptotic pathways [36] and the accumulation of mutant forms of the tumor suppressor transcription factor p53 [46,47].

Increased chaperone expression favors oncogenesis because they increase the chance that cancer cells survive in extreme environmental stress. For instance, free radicals generated by hypoxia and acidosis can cause significant physical damage to cellular proteins, which will be protected by the expression of molecular chaperones. Therefore, the fact that Hsp90 maintain its client proteins in an activated

Table 1. Important Hsp90 Co-Chaperones

Co-Chaperone	Class	Main Function	Ref.
p23	<i>Other</i>	Essential for assembly of stable steroid receptor heterocomplexes; coupling factor (ATPase inhibitor)	[118]
Cdc37	<i>Other</i>	Kinase-specific and co-chaperone	[119]
Aha1	<i>Other</i>	ATPase activator	[120]
HOP	<i>TPR</i>	Essential for assembly of stable steroid receptor heterocomplexes; Hsp70/Hsp90 adaptor protein	[121,122]
Tom70	<i>TPR</i>	Mitochondrial protein import	[123]
Sgt1	<i>TPR</i>	Binding partner (nucleotide-dependent)	[124]
Unc45	<i>TPR</i>	Myosin folding and assembly	[125]
FKBP51 FKBP52	<i>PPIases</i>	Essential for assembly of stable steroid receptor heterocomplexes	[126]
Cyp40	<i>PPIases</i>	Essential for assembly of stable steroid receptor heterocomplexes	[126,127]
PP5	<i>Hsp90 phosphatase</i>	Modulation of Hsp90 substrate maturation	[128]
CHIP	<i>Ubiquitin ligase</i>	Protein quality control system – protein labeling for degradation	[129,130]

state combined with the protective role of molecular chaperones toward damaged proteins help to stabilize tumorigenic cells. For that, Hsp90 has to have highly ATPase activity, which is usually potentiated by co-chaperones, thus it is worth noting that Hsp90 complexes from tumor cells are almost entirely bound to HOP and p23 [39].

Hanahan and Weinberg [48] proposed six essential alterations in cell physiology that are characteristic of most if not all cancers: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Hsp90 is essential for the stability and function of many oncogenic client proteins which are usually deregulated in cancers and contribute to the hallmark traits of malignance aforementioned [49-51] (a small list of Hsp90 client proteins involved with malignance are summarized in Table 2). Oncogenic mutations increase the instability of the client proteins therefore requiring increased amounts of Hsp90. Thus, the Hsp90 action stabilizes mutated oncogenic proteins required for the transformed phenotype that would otherwise be lethal [41]. In conclusion, Hsp90 has the ability to maintain the functional conformations of mutant and aberrant oncoproteins and grants survival advantage to tumor cells.

Another important finding is that survivin, an apoptosis inhibitor and essential regulator of mitosis, is maintained by Hsp90. Disruption of the survivin-Hsp90 complex results in proteasomal degradation of survivin, mitochondrial-dependent apoptosis, and cell cycle arrest [52]. Therefore, the association between survivin and Hsp90 reduces the chance of apoptosis and promotes the proliferation of tumor cells. Hsp90 is also an important determinant of tumor cell invasion and metastasis because it is expressed in the surface of melanoma metastases and cell surface Hsp90 seems to be crucial for the invasiveness of sarcoma cells *in vitro* [33,53,54].

The information discussed above forms the basis for using Hsp90 as a target for cancer therapeutics because inhibition of Hsp90 will significantly weaken a cancer cell and will cause regression of tumor growth. Client proteins are degraded in the absence of the chaperoning activity of Hsp90 and consequently inhibition of the chaperone results in degradation of its client proteins *via* the ubiquitin-proteasome

Table 2. Important Hsp90 Client-Proteins Involved with Cancer

Client Protein	Function
ErbB2	Proliferation, differentiation, and oncogenesis
Bcr-Abl	Pathogenesis of chronic myelogenous leukemia
Akt/PKB	Anti-apoptosis
C-RAF	Growth factor independence
CDK4	Resistance to anti-growth signals
PLK-1	Mitotic regulator kinase
Mutant p53	Tumor suppressor
HIF-1 α	Angiogenesis stability
hTERT	Unlimited replicative potential

pathway. As a consequence, the down-regulation of signal being propagated via numerous signaling pathways and modulation of all aspects of the malignant phenotype will follow [50]. Therefore, Hsp90 provides a broader target for anticancer therapies than single, oncogenically activated signaling pathways. As a matter of fact, Hsp90 inhibition *in vitro* leads to growth arrest and apoptosis in cancer cells [51,55] and causes a defect in a number of proliferative signals, including the Akt-dependent survival pathway [56-58]. Hsp90 inhibition may also sensitize tumor cells against various attacks by helping their lysis under hypoxia and complement attack [59,60].

Hsp90 inhibitors, by interacting specifically with a single molecular target, the Hsp90 chaperone, causes the inactivation, destabilization, and eventual degradation of Hsp90 client proteins. As mentioned below, inhibitors of Hsp90 have shown promising antitumor activity in preclinical model systems [41,51]. The genetic knockout of Hsp90 in eukaryotes is lethal and therefore it was not obvious that the chaperone may be a target in disease. Nonetheless, compounds that target Hsp90 have the potential to treat cancers (see below).

4. THERAPY: INHIBITORS AND CLINICAL TRIALS

The Hsp90 inhibitors currently in clinical trial share the property of displacing nucleotide from their binding pocket located at the N-terminus of Hsp90. Therefore, these inhibitors promote a significant decrease in the activity of oncogenic kinases and disrupt the activity of numerous receptors and transcription factors that are known to be involved in oncogenesis. For such reasons, compounds that target Hsp90 have been identified as potential anticancer agents. In contrast to most direct inhibitors, which are often fairly specific for a given protein, Hsp90 inhibitors can affect multiple oncogenic pathways simultaneously. The ability to diminish the level of many protein targets in parallel is therapeutically attractive because they behave as typical multi-target drugs, a feature potentially more efficient than highly selective single-target drugs [61,62].

Important classes of compounds found to be inhibitors against Hsp90 mediated oncogenic in the last decade are summarized in Table 3. They are geldanamycin, its less toxic analogs, 17AAG and 17DMAG, radicicol and its more stable oxime derivatives, purine-scaffold inhibitors and novobiocin [41,49-51]. These compounds target the ATP-binding pocket and since ATP binds to the Bergerat fold in Hsp90, its inhibitors are likely to adopt a bent conformation as well to achieve high affinity binding. Fig. (5) shows the pocket that forms the ADP/ATP-binding site located at the N-terminus of Hsp90 in the apo form, in complex with ADP, with geldanamycin and with its analog 17-DMAG.

Now that the proof-of-principle regarding Hsp90 inhibitors has been established by several phase I trials, there are several efforts are underway to synthesize drug candidates with higher affinity for Hsp90 and in developing clinical assays to test these inhibitors. There is also an increase in the study of the crystal structure of Hsp90 with inhibitors to understand their mode of action and to screen potential ligands. For all these reasons and others mentioned elsewhere, the development of Hsp90 inhibitors is considered to be a good example of medicinal chemistry [50,51,63]. For a recent list

Table 3. Important Hsp90 Inhibitors

Inhibitor	Class	Binding
Geldanamycin	<i>ansamycin</i>	N-terminus
17-AAG	<i>ansamycin</i>	N-terminus
17-DMAG	<i>ansamycin</i>	N-terminus
Radicicol	<i>Macrocyclic antibiotic</i>	N-terminus
KF58333	<i>Oxime derivatives</i>	N-terminus
PU3 and analogs	<i>Purine scaffold</i>	N-terminus
Novobiocin	<i>Coumarins</i>	N- and C-termini
Cisplatin	<i>Platinum complex</i>	C-terminus

of Hsp90 drugs which are on clinical trials and their present status see Reference [64].

4.1. Geldanamycin

Geldanamycin (Fig. 6) is a benzoquinone microbial product classified as ansamycin antibiotic that competes with ATP for the N-terminal binding site of Hsp90 [65,66]. Although the antitumor property of this molecule has been known for a long time [67], its association with Hsp90 was discovered several years later [68]. Geldanamycin was shown to activate HSF [69] and consequently the expression of Hsp40, Hsp70 and Hsp90 [70].

Geldanamycin interferes with the Hsp90 function and generates proteosomal degradation of several key regulatory proteins, including tyrosine kinases and steroid receptors, many of which are involved in promoting malignancy [45,71]. This natural ligand also causes differentiation and

apoptosis in a cell-line-dependent manner [56] and its affinity for Hsp90 complexes from tumor cells is 100-fold higher than for Hsp90 from non-tumor cells. However, geldanamycin does not have acceptable pharmacological properties for clinical application because its solubility is low and it appears broadly cytotoxic. Geldanamycin causes acute hepatic necrosis and nephrotoxicity in dogs [72] and is also toxic for rats [73,74]. These results precluded testing in humans.

4.2. Geldanamycin Analogs

Stimulated by the low solubility and high cytotoxicity of geldanamycin additional natural products and natural product derivatives have been identified and developed to inhibit the Hsp90 protein folding machinery. An important review published in 2005 [63] informed that more than 500 compounds related to geldanamycin were reported at that time, some of them able to inhibit Hsp90 at femtomol levels [75].

One of the first Hsp90 inhibitors developed with lower toxicity was the geldanamycin analog 17-allylamino, 17-demethoxygeldanamycin (17-AAG) (Fig. 6). This analog has all the Hsp90-related characteristics of geldanamycin but shows lower toxicity [76-79]. 17AAG also activates HSF [69] and the expression of Hsp40, Hsp70 and Hsp90 [70] and downregulates several client proteins including Raf-1, cdk4 and Akt [80]. The National Cancer Institute initiated Phase I clinical trials with 17-AAG in 1999 that are now complete and several Phase II trials are in progress [62,71,79]. Also, the latest results with 17-AAG in breast cancer and melanoma are especially encouraging [81-83]. These trials demonstrate that 17-AAG inhibits the biological function of Hsp90 in patients with breast cancer, multiple myeloma and other cancers. Several preclinical studies have shown that 17-AAG may enhance the efficacy of a variety of chemotherapeutic agents. 17-AAG was combined with taxol to enhance cytotoxic effects on taxol-resistant ErbB2-

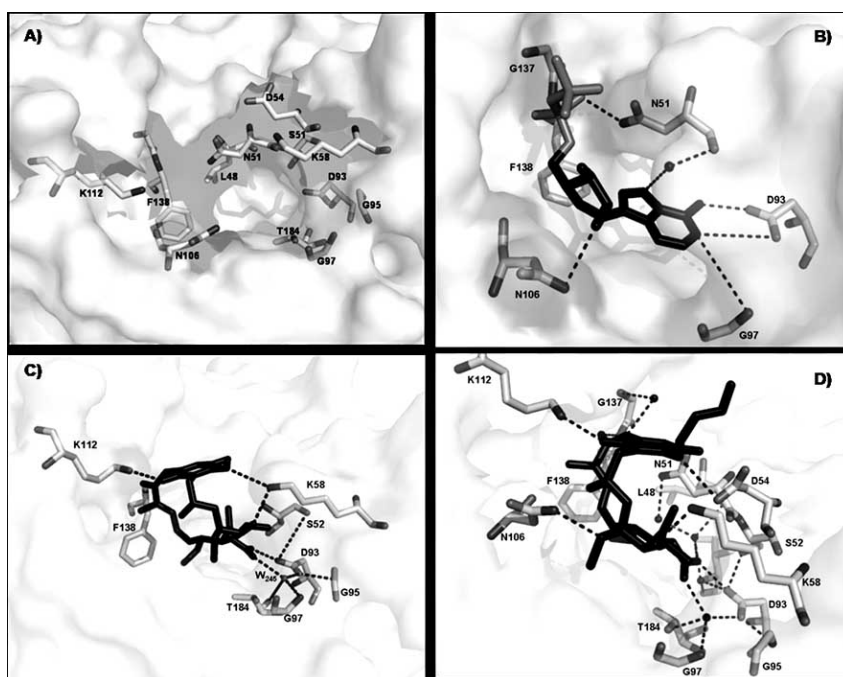


Fig. (5). Figure shows the pocket that forms the ADP/ATP-binding site located at the N-terminus of Hsp90 in the apo form (A), in complex with ADP (B), with geldanamycin (C) and with its analog 17-DMAG (D). PDB accession numbers: 1YES, 1BYQ, 1YET and 1OSF, respectively.

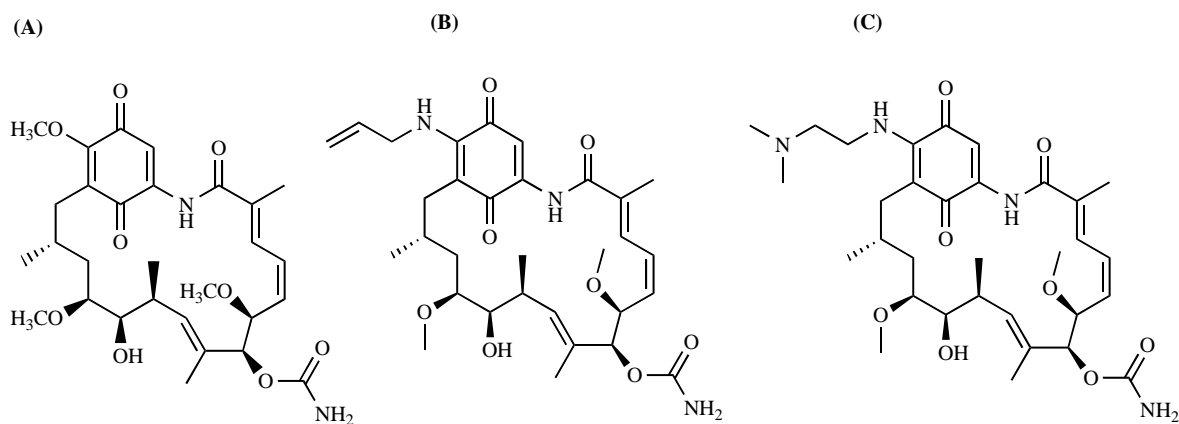


Fig. (6). (A) Geldanamycin and its analogs (B) 17-AAG and (C) 17-DMAG.

overexpressing breast cancer cells [56] and with angiogenesis inhibitors in breast tumors with encouraging results [84]. 17-AAG is also capable to potentiate both the *in vitro* and *in vivo* radiation response of cervical carcinoma cells [85]. However, there are several limitations with 17-AAG such as restricted solubility, hepatotoxicity, and its formulation, which is difficult to handle [50]. However, the positive clinical results with 17-AAG show that the Hsp90 target can be inhibited without causing unacceptable toxicity.

The development of another geldanamycin analog, 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG) (Fig. 6) was made in an effort to improve the solubility and bioavailability of 17-AAG [51,63,82,86]. The depletion of Hsp90 was more pronounced in cells exposed to 17-DMAG than treated with 17-AAG, 17-DMAG is easier to formulate and since it is water soluble it has the potential to be orally bioavailable [86,87]. 17-DMAG has entered clinical trials.

The binding of 17-DMAG with human Hsp90 β has been studied in solution by a combination of Hydrogen/Deuterium techniques analyzed by mass spectrometry [88]. The results showed that peptide comprising residues 123-133, 201-208 and 620-635 of Hsp90 (Fig. 4) are protected in the presence of 17-DMAG. The results point to conformational changes in all three domains of Hsp90 caused by binding of 17-DMAG demonstrating that long-range effects are present. To sum up, the binding of the inhibitor induces a change both in the interaction between the C-terminal and middle domains and in the interface between the N-terminal and middle domains [88].

4.3. Radicicol, Purine Scaffold Inhibitors and Novobiocin

Radicicol (Fig. 7) is a 14-membered macrocyclic antibiotic isolated as an antibiotic of fungal origin [89] that has effects on tumor cells similar to those of ansamycins [90]. However, radicicol, as geldanamycin, is inactive *in vivo* due to its instability in serum and does not have acceptable pharmacological properties for clinical application. On the other hand, oxime derivatives of radicicol have potent anti-tumor activity *in vivo* via destabilization of the binding of Hsp90 with specific client proteins [91,92]. Oxime derivatives are more stable than radicicol, do not cause serious liver toxicity and work both *in vivo* and *in vitro* [93].

The binding of radicicol with human Hsp90 β has also been studied in solution by a combination of Hydrogen/Deuterium techniques analyzed by mass spectrometry [88]. The results showed that peptides comprising residues 123-133, 584-589, and 620-635 of Hsp90 (Fig. 4) are protected in the presence of radicicol and the Phenyl ring of Phe133 makes hydrophobic contact with the coplanar aromatic ring of radicicol. Also, the results for radicicol are in good agreement with those for 17-DMAG (see above) indicating that the different classes of inhibitors induce equivalent changes in the conformation of Hsp90.

The discovery of the antibacteriotoxic novobiocin (Fig. 7) as an Hsp90 inhibitor involved two important findings. One was the screening of a structurally distinct small molecule with affinity for Hsp90 and the second was the identification of an uncharacterized ATP binding site at the C-terminal domain of Hsp90. Novobiocin binds both to the N- and to

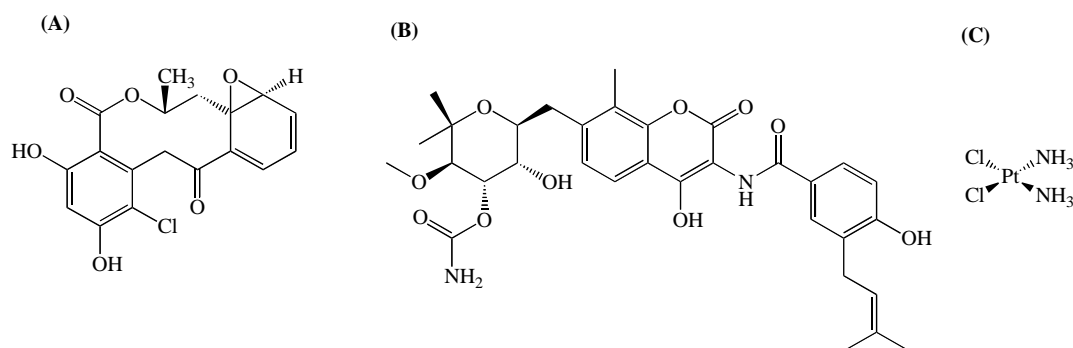


Fig. (7). (A) Radicicol, (B) novobiocin and (C) cisplatin.

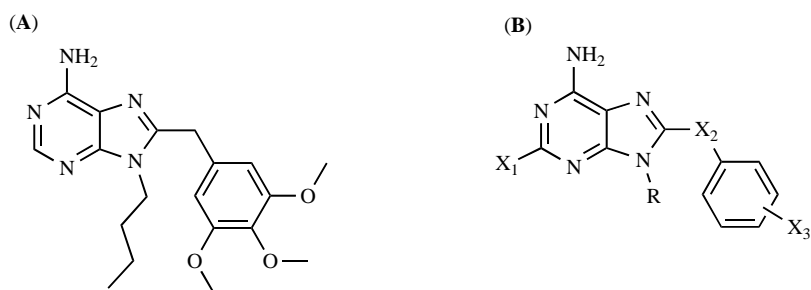


Fig. (8). Purine scaffold molecules. **(A)** PU3 and **(B)** analogs.

the C-termini of Hsp90 [26,28,94]. These findings were obtained using both competitive assays with geldanamycin and radicicol and deletion mutants consisting of either the N-terminal domain or the C-terminal domain. Novobiocin reduces the cellular level of several oncogenic protein kinases, including HER-2 and Raf-1, and inhibited the association of Hsp70 and p23 with Hsp90 [26,94]. Although the inhibitory action of novobiocin has been identified as very low, the development of novobiocin analogs for Hsp90 inhibition allowed the identification of compounds with higher potency that caused the degradation of several Hsp90 client proteins in both breast and prostate cancer cell lines [95]. Another compound, cisplatin (Fig. 7), is a selective C-terminal nucleotide competitor that strengthens the Hsp90-Hsp70 complex and has potential to be used as a model target to the development of new drugs against cancer using Hsp90 as target [28].

Chiosis and colleagues have designed small molecules that bind to Hsp90 by making use of existent crystallographic data [36,96,97]. These small-molecules use purine as scaffold and are highly potent Hsp90 inhibitors with improved drug-like properties (Fig. 8).

5. FUTURE DIRECTIONS

There are several new strategies and recently discovered compounds that have inhibitory action against Hsp90 and

potential to enter preclinical trials for antitumor characterization. A structure-based design approach has been used to generate potent resorcinolic pyrazole/isoxazole amide analogs as Hsp90 inhibitors with therapeutic potential that have entered Phase I clinical trials. These molecules have antiproliferative effects and cause induction of Hsp70 and Hsp27, depletion of client proteins, statistically significant growth inhibition and regressions in human tumor xenografts [98,99]. A new compound, macbecin (Fig. 9), compares favorably to geldanamycin, being more soluble, stable, and more potent. This quinone-containing Hsp90 inhibitor induces both tumor cell growth inhibition and the degradation of Hsp90 client proteins [100]. Tanespimycin, a 17-AAG derivative, in combination with trastuzumab [101] and IPI-504 [102] (Fig. 9) are improved formulations which are being used as Hsp90 inhibitors with relative success in clinical trials.

Although many new screening have been used to select new compounds with inhibitory action against Hsp90, the improvement of pharmacological properties and potency of the natural pharmacophores remains important. It is worth mentioning the use of phenolic derivatives of geldanamycin isolated from *Streptomyces sp* [103], the combination of 17AAG with carboplatin [104] (Fig. 9), and SNX-2112, which was selected by a purine-based affinity resin [105].

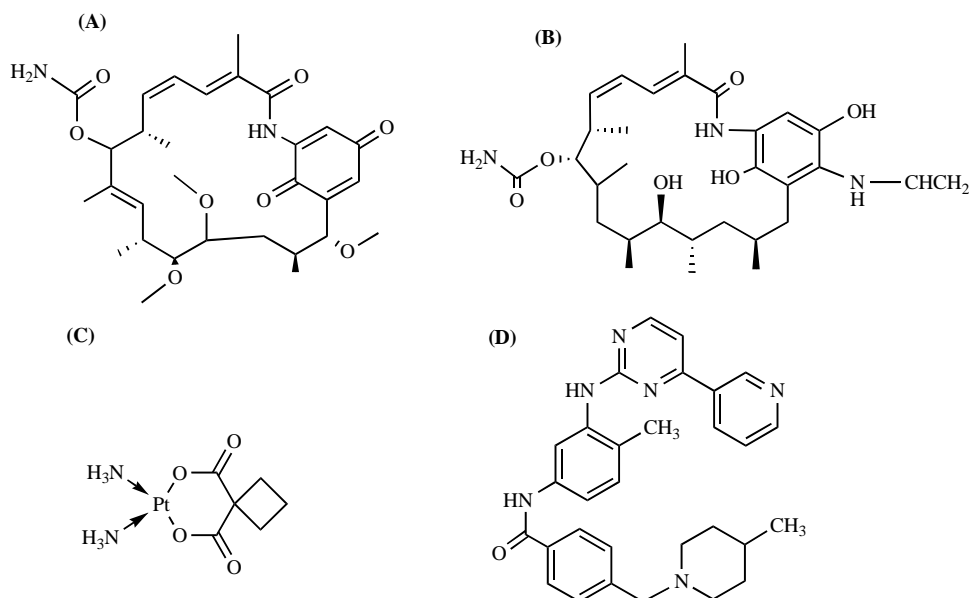


Fig. (9). **(A)** Macbecin, **(B)** IPI-504, **(C)** carboplatin and **(D)** imatinib.

Furthermore, the importance of posttranslational modifications in regulating Hsp90 function has not been entirely elucidated yet. Hsp90 is regulated by co-chaperones and by post-translational modification, such as phosphorylation, acetylation, and ubiquitinylation. Hyperacetylation of Hsp90 disrupts assembly with co-chaperones, in particular, the affinity of co-chaperone p23 for Hsp90 was dramatically reduced. Since the association with p23 requires ATP binding activity it is likely that hyperacetylation of Hsp90 lowers this activity. In fact, hyperacetylation of Hsp90 seems to inhibit its ATP-binding and decreases association of Hsp90 with its client proteins [106]. Inhibitors of acetylation have effects similar to those caused by compounds targeting the ATP-pocket at the N-terminus of Hsp90 that decrease the ATPase activity. These effects induce growth arrest and apoptosis in a variety of human cancer cells, reduced expression of mutant, but not wild-type, p53, depletion of Her1, Her2, and Raf-1 proteins, lower ERK1/2 activity, and Hsp70 induction in both Her2 overexpressing breast cancer cells and lymphocytes. These potential epigenetic regulatory signals have consequences for various biological processes and potentially important implications for the development of cancer therapeutics [107].

S-nitrosylation is also another important regulator of Hsp90 activity. The nitrosylating agent NO modifies and inhibits both human Hsp90 ATPase activity and its positive effect on the substrate eNOS [108]. Heat shock increases the turnover of Hsp90 phosphate groups [109] and apparently phosphorylation decreases the ATPase activity of Hsp90. A previous work [110] has found that Hsp90 phosphorylation leads to the release of the chaperone from the target protein and can be inhibited by geldanamycin. Another importance of post-translational modifications for the function of Hsp90 in cancer is that of the action of phosphorylation on the interaction of Hsp90 and apoptosome [111]. Hsp90 inhibits the apoptosome [112] and failure of apoptosis contributes to oncogenesis [48]. Kurokawa *et al.* [111] showed that hypophosphorylation of Hsp90 β at Ser 226 and Ser 255 promotes apoptosome inhibition suggesting that kinases and phosphatases regulating Hsp90 β phosphorylation are potential therapeutic targets and that tyrosine kinase inhibitors such as imatinib [113] (Fig. 9) could be used in combination with specific inhibitors of Hsp90.

Curiously, Hsp90 inhibitors have little effect on normal cells but have high affinity for recombinant Hsp90 proteins produced by bacteria, likely because they target Hsp90 that do not have post-translation modifications [50,51]. These observations are likely to be the focus of future studies with significant biological and clinical ramifications.

Another very promising area of research in this field is the association of Hsp90 with peptides which are specific in cancers [114]. Ishii *et al.* [115] showed that a restricted cytotoxic T lymphocyte epitope of a mouse leukemia was associated with Hsp90 in the cytosol. Recently, Callahan and co-authors [116] have shown that Hsp90 plays a global role in direct antigen presentation to MHC I molecules which are responsible for presenting antigens of cancers and viruses to CD8⁺ T cells. Therefore, the association of cancer specific antigenic peptides to HSP90 may serve as an indication to the immune system. To sum up, post-translation modifica-

tions that affect properly Hsp90 function (see above) would also affect the presentation of cancer specific antigens.

Since general increasing in chaperone expression favors oncogenesis, the early promise of Hsp90 inhibitors is stimulating interest in additional chaperone targets. For instance, Hsp70 has been considered a promising target because it limits response to Hsp90 inhibition [117]. One way or the other, it has been exciting to the study cancer therapeutics using Hsp90 as a target. Important research has yet to be done such as having high resolution structure of the entire human Hsp90 in complex with many ligands and co-chaperones, defining the exactly function of each Hsp90 isoform and screening compounds with better solubility and less toxic effects. However, an important part of the effort has already been accomplished and encouraging recent results with compounds already in clinical trial are raising positive hope.

ACKNOWLEDGEMENTS

Research in the laboratory of CHIR is supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Ministério da Ciência e Tecnologia/Conselho Nacional de Pesquisa e Desenvolvimento (MCT/CNPq), and NIH-R03TW007437 funded by the Fogarty International Center. LMG has a doctoral fellowship from FAPESP.

ABBREVIATIONS

17-AAG	=	17-(allylamino)-17-demethoxygeldanamycin
17-DMAG	=	17-dimethylaminoethylamino-17-demethoxygeldanamycin
CHIP	=	C-terminus of Hsp70 interacting protein
EST	=	Expression sequence tag
HOP	=	Hsp70-Hsp90 organizing protein
HSF	=	Heat-shock factor
HSR	=	Heat-shock response
Hsp70	=	70 kDa heat-shock protein
Hsp90	=	90 kDa heat-shock protein
TPR	=	Tetratricopeptide repeat

REFERENCES

- [1] Ramos CHI, Ferreira ST. Protein folding, misfolding and aggregation: evolving concepts and conformational diseases. *Protein Pept Lett* 2005; 12: 213-22.
- [2] Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol* 2005; 6:197-208.
- [3] Fandrich M, Fletcher MA, Dobson CM. Amyloid fibrils from muscle myoglobin. *Nature* 2001; 410: 165-66.
- [4] Fandrich M, Dobson CM. The behaviour of polyamino acids reveals an inverse side chain effect in amyloid structure formation. *EMBO J* 2002; 21: 5682-90.
- [5] Dobson CM. Experimental investigation of protein folding and misfolding. *Methods* 2004; 34: 4-14.
- [6] Minton AP. Implications of macromolecular crowding for protein assembly. *Curr Op Struct Biol* 2000; 10:34-9.
- [7] Prusiner SB. Shattuck lecture--neurodegenerative diseases and prions. *N Engl J Med* 2001; 344:1516-26.
- [8] Beissinger M, Buchner J. How chaperones fold proteins. *Biol Chem* 1998; 379: 245-59.

- [9] Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol, from nascent chain to folded protein. *Science* 2002; 295: 1852-8.
- [10] Borges JC, Ramos CHI. Protein folding assisted by chaperones. *Protein Pept Lett* 2008; 12: 256-61.
- [11] Borges JC, Peroto MC, Ramos CHI. Molecular Chaperone Genes in the SugarCane Expressed Sequence Database (SUCEST). *Gen Mol Biol* 2001; 24: 85-92.
- [12] Borges JC, Cagliari TC, Ramos CHI. Expression and variability of molecular chaperones in the sugarcane expressome. *J Plant Physiol* 2007; 164: 505-513.
- [13] Cagliari TC, Tiroli AO, Borges JC, Ramos CHI. Identification and in silico expression analysis of eucalyptus expressed sequencing tags (ESTs) encoding molecular chaperones. *Gen Mol Biol* 2005; 28: 520-8.
- [14] Csermely P. Chaperone overload is a possible contributor to 'civilization diseases'. *Trends Genet* 2001; 17: 701-4.
- [15] Chaduri TK, Paul S. Protein-misfolding diseases and chaperone-base therapeutic approaches. *FEBS J* 2006; 273: 1331-49.
- [16] Söti C, Nagy E, Giricz Z, Végli L, Csermely P, Ferdinandy P. Heat shock proteins as emerging therapeutic targets. *Br J Pharmacol* 2005; 146: 769-80.
- [17] Macario AJL, de Macario EC. Chaperonopathies and chaperonotherapy. *FEBS Lett* 2007; 581: 3681-8.
- [18] Powers MV, Workman P. Inhibitors of the heat shock response: Biology and pharmacology. *FEBS Lett* 2007; 581: 3758-69.
- [19] Turbyville TJ, Wijeratne EM, Whitesell L, Gunatilaka AA. The anticancer activity of the fungal metabolite terrecyclin acid A is associated with modulation of multiple cellular stress response pathways. *Mol Cancer Ther* 2005; 4: 1569-76.
- [20] Westerheide SD, Bosman JD, Mbadugha BN, *et al.* Celastrols as inducers of the heat shock response and cytoprotection. *J Biol Chem* 2004; 279: 56053-60.
- [21] Nagai N, Nakai A, Nagata K. Quercetin suppresses heat shock response by down regulation of HSF1. *Biochem Biophys Res Commun* 1995; 208: 1099-1105.
- [22] Dechsupa S, Kothan S, Vergote J, *et al.* Quercetin, Siamois 1 and Siamois 2 induce apoptosis in human breast cancer MDA-MB-435 cells xenograft *in vivo*. *Cancer Biol Ther* 2007; 6: 56-61.
- [23] Prodromou C, Pearl LH. Structure and functional relationships of Hsp90. *Curr Cancer Drug Targets* 2003; 3: 301-23.
- [24] Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 1997; 90: 65-75.
- [25] Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* 1997; 89: 239-50.
- [26] Marcu MG, Chadli A, Bouhouche I, Catelli M, Neckers LM. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. *J Biol Chem* 2000; 275: 37181-6.
- [27] Garnier C, Lafitte D, Tsvetkov PO, *et al.* Binding of ATP to heat shock protein 90: evidence for an ATP-binding site in the C-terminal domain. *J Biol Chem* 2002; 277: 12208-14.
- [28] Soti C, Racz A, Csermely P. A nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90. N-terminal nucleotide binding unmasks a C-terminal binding pocket. *J Biol Chem* 2002; 277: 7066-75.
- [29] Obermann WM, Sondermann H, Russo AA, Pavletich NP, Hartl FU. *In vivo* function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J Cell Biol* 1998; 143: 901-10.
- [30] Jez JM, Chen JC, Rastelli G, Stroud RM, Santi DV. Crystal Structure and Molecular Modeling of 17-DMAG in Complex with Human Hsp90. *Chem Biol* 2003; 10: 361-8.
- [31] Boston RS, Viitanen PV, Vierling E. Molecular chaperones and protein folding in plants. *Plant Mol Biol* 1996; 32: 191-222.
- [32] Sreedhar AS, Kalmár E, Csermely P, Shen YF. Hsp90 isoforms: functions, expression and clinical importance. *FEBS Lett* 2004; 562: 11-5.
- [33] Eustace BK, Sakurai T, Stewart JK, *et al.* Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. *Nat Cell Biol* 2004; 6: 507-14.
- [34] Wegele H, Müller L, Buchner J. Hsp70 and Hsp90 – a relay team for protein folding. *Rev Physiol Biochem Pharmacol* 2004; 151: 1-44.
- [35] Wandinger SK, Richter K, Buchner J. The Hsp90 chaperone machinery. *J Biol Chem* 2008; 283: 18473-7.
- [36] Chiosis G, Vilenchik M, Kim J, Solit D. Hsp90: the vulnerable chaperone. *Drug Discov Today* 2004; 9: 881-8.
- [37] Russell LC, Whitt SR, Chen MS, Chinkers M. Identification of conserved residues required for the binding of a tetratricopeptide repeat domain to heat shock protein 90. *J Biol Chem* 1999; 274: 20060-3.
- [38] Panaretou B, Siligardi G, Meyer P, *et al.* Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. *Mol Cell* 2002; 10: 1307-18.
- [39] Kamal A, Thao L, Sensintaffar J, *et al.* A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* 2003; 425: 407-10.
- [40] Zhao R, Davey M, Hsu YC, *et al.* Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* 2005; 120: 715-27.
- [41] Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. *Nat Rev Cancer* 2005; 5: 761-72.
- [42] Pratt WB. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc Soc Exp Biol Med* 1998; 217: 420-34.
- [43] Nardai G, Végli EM, Prohászka Z, Csermely P. Chaperone-related immune dysfunction: an emergent property of distorted chaperone networks. *Trends Immunol* 2006; 27: 74-9.
- [44] Citri A, Harari D, Shohat G, *et al.* Hsp90 recognizes a common surface on client kinases. *J Biol Chem* 2006; 281: 14361-9.
- [45] Pearl LH, Prodromou C, Workman P. The Hsp90 molecular chaperone: an open and shut case for treatment. *Biochem J* 2008; 410: 439-53.
- [46] Csermely P, Schnaider T, Soti C, Prohászka Z, Nardai G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol Ther* 1998; 79: 129-68.
- [47] Buchner J. Hsp90 & Co. a holding for folding. *Trends Biochem Sci* 1999; 24: 136-41.
- [48] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100: 57-70.
- [49] Workman P. Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett* 2004; 206: 149-57.
- [50] Sharp S, Workman P. Inhibitors of the HSP90 molecular chaperone: current status. *Adv Cancer Res* 2006; 95: 323-48.
- [51] Neckers L. Heat shock protein 90: the cancer chaperone. *J Biosci* 2007; 32: 517-30.
- [52] Fortugno P, Beltrami E, Plescia J, *et al.* Regulation of survivin function by Hsp90. *Proc Natl Acad Sci* 2003; 100: 13791-6.
- [53] Becker B, Multhoff G, Farkas B, *et al.* Induction of Hsp90 protein expression in malignant melanomas and melanoma metastases. *Exp Dermatol* 2004; 13: 27-32.
- [54] Eustace BK, Jay DG. Extracellular roles for the molecular chaperone, hsp90. *Cell Cycle* 2004; 3: 1098-100.
- [55] Sreedhar AS, Csermely P. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. *Pharmacol Ther* 2004; 101: 227-57.
- [56] Munster PN, Basso A, Solit D, Norton L, Rosen N. Modulation of Hsp90 function by ansamycins sensitizes breast cancer cells to chemotherapy-induced apoptosis in an RB- and schedule-dependent manner. *Clin Cancer Res* 2001; 7: 2228-36.
- [57] Munster PN, Marchion DC, Basso AD, Rosen N. Degradation of HER2 by ansamycins induces growth arrest and apoptosis in cells with HER2 overexpression *via* a HER3, phosphatidylinositol 3'-kinase-AKT-dependent pathway. *Cancer Res* 2002; 62: 3132-7.
- [58] Basso AD, Solit DB, Chiosis G, Giri B, Tschlis P, Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. *J Biol Chem* 2002; 277: 39858-66.
- [59] Sreedhar AS, Mihaly K, Pato B, *et al.* Hsp90 inhibition accelerates cell lysis. Anti-Hsp90 ribozyme reveals a complex mechanism of Hsp90 inhibitors involving both superoxide- and Hsp90-dependent events. *J Biol Chem* 2003; 278: 35231-40.
- [60] Sreedhar AS, Nardai G, Csermely P. Enhancement of complement-induced cell lysis: a novel mechanism for the anticancer effects of Hsp90 inhibitors. *Immunol Lett* 2004; 92: 157-61.

- [61] Csermely P, Agoston V, Pongor S. The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol Sci* 2005; 26: 178-82.
- [62] Neckers L, Neckers K. Heat-shock protein 90 inhibitors as novel cancer chemotherapeutics – an update. *Expert Opin Emerg Drugs* 2005; 10: 137-49.
- [63] Janin YL. Heat shock protein 90 inhibitors. A text book example of medicinal chemistry? *J Med Chem* 2005; 48: 7503-12.
- [64] Solit DB, Chiosis G. Development and application of Hsp90 inhibitors. *Drug Discov Today* 2008; 13: 38-43.
- [65] Wehrli W. Ansamycins. Chemistry, biosynthesis and biological activity. *Top Curr Chem* 1977; 72: 21-49.
- [66] Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 1997; 90: 65-75.
- [67] Sasaki K, Yasuda H, Onodera K. Growth inhibition of virus transformed cells *in vitro* and antitumor activity *in vivo* of geldanamycin and its derivatives. *J Antibiot* 1979; 32: 849-51.
- [68] Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc Natl Acad Sci* 1994; 91: 8324-8.
- [69] Bagatell R, Paine-Murrieta GD, Taylor CW, *et al.* Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of hsp90-binding agents. *Clin Cancer Res* 2000; 6: 3312-8.
- [70] Sittler A, Lurz R, Lueder G, *et al.* Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum Mol Genet* 2001; 10: 1307-15.
- [71] Neckers L, Ivy SP. Heat shock protein 90. *Curr Opin Oncol* 2003; 15: 419-24.
- [72] Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* 1995; 36: 305-15.
- [73] Egorin MJ, Zuhowski EG, Rosen DM, Sentz DL, Covey JM, Eiseaman JL. Plasma pharmacokinetics and tissue distribution of 17-(allylamino)-17-demethoxygeldanamycin (NSC 330507) in CD2F1 mice. *Cancer Chemother Pharmacol* 2001; 47: 291-302.
- [74] Patel K, Piagentini M, Rascher A, *et al.* Engineered biosynthesis of geldanamycin analogs for Hsp90 inhibition. *Chem Biol* 2004; 11: 1625-33.
- [75] Xie Q, Gao CF, Shinomiya N, *et al.* Geldanamycins exquisitely inhibit HGF/SF-mediated tumor cell invasion. *Oncogene* 2005; 24: 3697-707.
- [76] Schulte TW, Neckers LM. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother Pharmacol* 1998; 42: 273-279.
- [77] Chiosis G, Huezio H, Rosen N, Mimnaugh E, Whitesell L, Neckers L. 17AAG: low target binding affinity and potent cell activity – finding an explanation. *Mol Cancer Ther* 2003; 2: 123-9.
- [78] Workman P. Auditing the pharmacological accounts for Hsp90 molecular chaperone inhibitors: unfolding the relationship between pharmacokinetics and pharmacodynamics. *Mol Cancer Ther* 2003; 2: 131-8.
- [79] Banerji U, O'donnell A, Scurr M, *et al.* Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino,17-demethoxygeldanamycin in patients with advanced malignancies. *J Clin Oncol* 2005; 23: 4152-61.
- [80] Sausville EA, Tomaszewski JE, Ivy P. Clinical development of 17-allylamino, 17-demethoxygeldanamycin. *Curr Cancer Drug Targets* 2003; 3: 377-83.
- [81] Modi S, Stopeck AT, Gordon MS, *et al.* Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. *J Clin Oncol* 2007; 25: 5410-7.
- [82] Powers MV, Workman P. Inhibitors of the heat shock response: biology and pharmacology. *FEBS Lett* 2007; 581: 3758-69.
- [83] Banerji U, Affolter A, Judson I, Marais R, Workman P. BRAF and NRAS mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors. *Mol Cancer Ther* 2008; 7: 737-9.
- [84] De Candia P, Solit DB, Giri D, *et al.* Angiogenesis impairment in Id-deficient mice cooperates with an Hsp90 inhibitor to completely suppress HER2/neu-dependent breast tumors. *Proc Natl Acad Sci* 2003; 100: 12337-42.
- [85] Bisht KS, Bradbury CM, Mattson D. Geldanamycin and 17-allylamino-17-demethoxygeldanamycin potentiate the *in vitro* and *in vivo* radiation response of cervical tumor cells via the heat shock protein 90-mediated intracellular signalling and cytotoxicity. *Cancer Res* 2003; 63: 8984-95.
- [86] Smith V, Sausville EA, Camalier RF, Fiebig HH, Burger AM. Comparison of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17DMAG) and 17-allylamino-17-demethoxygeldanamycin (17AAG) *in vitro*: effects on Hsp90 and client proteins in melanoma models. *Cancer Chemother Pharmacol* 2005; 56(2): 126-37.
- [87] Egorin MJ, Lagattuta TF, Hamburger DR, *et al.* Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. *Cancer Chemother Pharmacol* 2002; 49(1): 7-19.
- [88] Phillips JJ, Yao ZP, Zhang W, *et al.* Conformational dynamics of the molecular chaperone Hsp90 in complexes with a co-chaperone and anticancer drugs. *J Mol Biol* 2007; 372(5): 1189-203.
- [89] Delmotte P, Delmotte-Plaque J. A new antifungal substance of fungal origin. *Nature* 1953; 171: 344.
- [90] Kwon HJ, Yoshida M, Abe K, Horinouchi S, Beppu T. Radicol, an agent inducing the reversal of transformed phenotypes of src-transformed fibroblasts. *Biosci Biotechnol Biochem* 1992; 56(3): 538-9.
- [91] Soga S, Neckers LM, Schulte TW, *et al.* KF25706, a novel oxime derivative of radicol, exhibits *in vivo* antitumor activity via selective depletion of Hsp90 binding signaling molecules. *Cancer Res* 1999; 59(12): 2931-8.
- [92] Agatsuma T, Ogawa H, Akasaka K, *et al.* Halohydrin and oxime derivatives of radicol: synthesis and antitumor activities. *Bioorg Med Chem* 2002; 10(11): 3445-54.
- [93] Soga S, Shiotsu Y, Akinaga S, Sharma SV. Development of radicol analogues. *Curr Cancer Drug Targets* 2003; 3(5): 359-69.
- [94] Marcu MG, Schulte TW, Neckers L. Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. *J Natl Cancer Inst* 2000; 92(3): 242-8.
- [95] Yu XM, Shen G, Neckers L, *et al.* Hsp90 inhibitors identified from a library of novobiocin analogues. *J Am Chem Soc* 2005; 127(37): 12778-9.
- [96] Llauger L, He H, Kim J, *et al.* Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90. *J Med Chem* 2005; 48(8): 2892-905.
- [97] He H, Zatorska D, Kim J, *et al.* Identification of potent water soluble purine-scaffold inhibitors of the heat shock protein 90. *J Med Chem* 2006; 49(1): 381-90.
- [98] Sharp SY, Prodromou C, Boxall K, *et al.* Inhibition of the heat shock protein 90 molecular chaperone *in vitro* and *in vivo* by novel, synthetic, potent resorcinolic pyrazole/isoxazole amide analogues. *Mol Cancer Ther* 2007; 6(4): 1198-211.
- [99] Eccles SA, Massey A, Raynaud FI, *et al.* NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Res* 2008; 68(8): 2850-60.
- [100] Martin CJ, Gaisser S, Challis IR, *et al.* Molecular characterization of macbecin as an hsp90 inhibitor. *J Med Chem* 2008; 51(9): 2853-7.
- [101] Modi S, Stopeck AT, Gordon MS, *et al.* Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. *J Clin Oncol* 2007; 25(34): 5410-7.
- [102] Sydor JR, Normant E, Pien CS, *et al.* Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. *Proc Natl Acad Sci* 2006; 103(46): 17408-13.
- [103] Onodera H, Kaneko M, Takahashi Y, *et al.* Conformational significance of EH21A1-A4, phenolic derivatives of geldanamycin, for Hsp90 inhibitory activity. *Bioorg Med Chem Lett* 2008; 18(5): 1577-80.
- [104] Banerji U, Sain N, Sharp SY, *et al.* An *in vitro* and *in vivo* study of the combination of the heat shock protein inhibitor 17-allylamino-17-demethoxygeldanamycin and carboplatin in human ovarian cancer models. *Cancer Chemother Pharmacol* 2008; 62(5): 769-78.
- [105] Chandralapaty S, Sawai A, Ye Q, *et al.* SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against

- HER kinase-dependent cancers. *Clin Cancer Res* 2008; 14(1): 240-48.
- [106] Yu X, Guo ZS, Marcu MG, *et al.* Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. *J Natl Cancer Inst* 2002; 94(7): 504-13.
- [107] Aoyagi S, Trevor K. Archer Modulating molecular chaperone Hsp90 functions through reversible acetylation. *Trends in Cell Biology* 2005; 15(11): 565-7.
- [108] Martínez-Ruiz A, Villanueva L, González de Orduña C, *et al.* S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. *Proc Natl Acad Sci* 2005; 102(24):8525-30.
- [109] Legagneux V, Morange M, Bensaude O. Heat shock increases turnover of 90 kDa heat shock protein phosphate groups in HeLa cells. *FEBS Lett* 1991; 291(2): 359-62.
- [110] Zhao YG, Gilmore R, Leone G, Coffey MC, Weber B, Lee PW. Hsp90 phosphorylation is linked to its chaperoning function. Assembly of the reovirus cell attachment protein. *J Biol Chem* 2001; 276(35): 32822-7.
- [111] Kurokawa M, Zhao C, Reya T, Kornbluth S. Inhibition of apoptosome formation by suppression of Hsp90 β phosphorylation in tyrosine kinase-induced leukemias. *Mol Cell Biol* 2008; 28(17): 5494-506.
- [112] Beere H. Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest* 2005; 115: 2633-9.
- [113] Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCRABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood* 2002; 100: 3041-4.
- [114] Srivastava PK, Maki RG. Stress-induced proteins in immune response to cancer. *Curr Top Microbiol Immunol* 1991; 167: 109-23.
- [115] Ishii T, Udono H, Yamano T, *et al.* Isolation of MHC class I-restricted tumor antigen peptide and its precursors associated with heat shock proteins hsp70, hsp90, and gp96. *J Immunol* 1999; 162(3):1303-9.
- [116] Callahan MK, Garg M, Srivastava PK. Heat-shock protein 90 associates with N-terminal extended peptides and is required for direct and indirect antigen presentation. *Proc Natl Acad Sci* 2008; 105(5):1662-7.
- [117] Brodsky JL, Chiosis G. Hsp70 molecular chaperones: emerging roles in human disease and identification of small molecule modulators. *Curr Top Med Chem* 2006; 6: 1215-25.
- [118] Johnson JL, Beito TG, Krco CJ, Toft DO. Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes. *Mol Cell Biol* 1994; 14: 1956-63.
- [119] Kimura Y, Rutherford SL, Miyata Y. Cdc37 is a molecular chaperone with specific functions in signal transduction. *Genes Dev* 1997; 14: 1775-85.
- [120] Mayer MP, Nikolay R, Bukau B. Aha, another regulator for hsp90 chaperones. *Mol Cell* 2002; 6: 1255-6.
- [121] Perdeu GH, Whitelaw ML. Evidence that the 90-kDa heat shock protein (HSP90) exists in cytosol in heteromeric complexes containing HSP70 and three other proteins with Mr of 63,000,56,000, and 50,000. *J Biol Chem* 1991; 266: 6708-13.
- [122] Johnson BD, Schumacher RJ, Ross ED, Toft DO. Hop modulates Hsp70/Hsp90 interactions in protein folding. *J Biol Chem* 1998; 6: 3679-86.
- [123] Young JC, Hoogenraad NJ, Hartl FU. Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. *Cell* 2003; 1: 41-50.
- [124] Catlett MG, Kaplan KB. Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *J Biol Chem* 2006; 44: 33739-48.
- [125] Liu L, Srikakulam R, Winkelmann DA. Unc45 activates Hsp90-dependent folding of the myosin motor domain. *J Biol Chem* 2008; 19: 13185-93.
- [126] Pirkel F, Buchner J. Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. *J Mol Biol* 2001; 4: 795-806.
- [127] Warth R, Briand PA, Picard D. Functional analysis of the yeast 40 kDa cyclophilin Cyp40 and its role for viability and steroid receptor regulation. *Biol Chem* 1997; 5: 381-91.
- [128] Silverstein AM, Galigniana MD, Chen MS, Owens-Grillo JK, Chinkens M, Pratt WB. Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin. *J Biol Chem* 1997; 26: 16224-30.
- [129] Connell P, Ballinger CA, Jiang J, *et al.* The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat Cell Biol* 2001; 3: 93-6.
- [130] Rosser MF, Washburn E, Muchowski PJ, Patterson C, Cyr DM. Chaperone functions of the E3 ubiquitin ligase CHIP. *J Biol Chem* 2007; 31: 22267-77.