

Geldanamycin and its derivatives as Hsp90 inhibitors

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1. ABSTRACT

The Hsp90 molecule, one of the most abundant heat shock proteins in mammalian cells, maintains homeostasis and prevents stress-induced cellular damage. Hsp90 is expressed under normal conditions at a level of about 1-2% of total proteins, while its expression increases 2-10 fold in cancer cells. The two main constitutively expressed isoforms of Hsp90 are known as Hsp90-alpha and Hsp90-beta, and their upregulation is associated with tumor progression, invasion and formation of metastases, as well as development of drug resistance. The Hsp90 is a key target for many newly established, potent anticancer agents containing Hsp90 N-terminal ATP binding inhibitors, such as geldanamycin, and its analogues 17AAG and 17DMAG. The therapeutic usage of geldanamycin has been limited due to its poor water solubility and severe hepatotoxicity. Therefore, its analogues, including 17AAG, 17DMAG, Tanespimycin and Retaspimycin hydrochloride, with improved pharmacokinetic profiles, have been developed.

2. INTRODUCTION

Heat shock proteins (Hsps) belong to chaperones that are responsible for maintaining homeostasis of the organisms and promoting cell survival induced by increased temperature as well as various chemical and physical factors. One of the most abundant eukaryotic HSPs is Hsp90, ubiquitously expressed protein of a molecular weight of 90kDa, whose expression level is estimated at 1-2% of total proteins under normal conditions (1, 2).

In humans, there have been identified two cytoplasmic isoforms of Hsp90 - Hsp90-alpha and Hsp90-beta, the endoplasmic reticulum homolog Glucose-regulated Protein 94 (GRP94), mitochondrial matrix TRAP1 and Hsp90N while in mice Hsp84 and Hsp86 are found. Although both Hsp90-alpha and Hsp90-beta are constitutively expressed isoforms, the first one seems to be more inducible (3). Researches typically associate Hsp90-alpha expression with tumor progression and sustained

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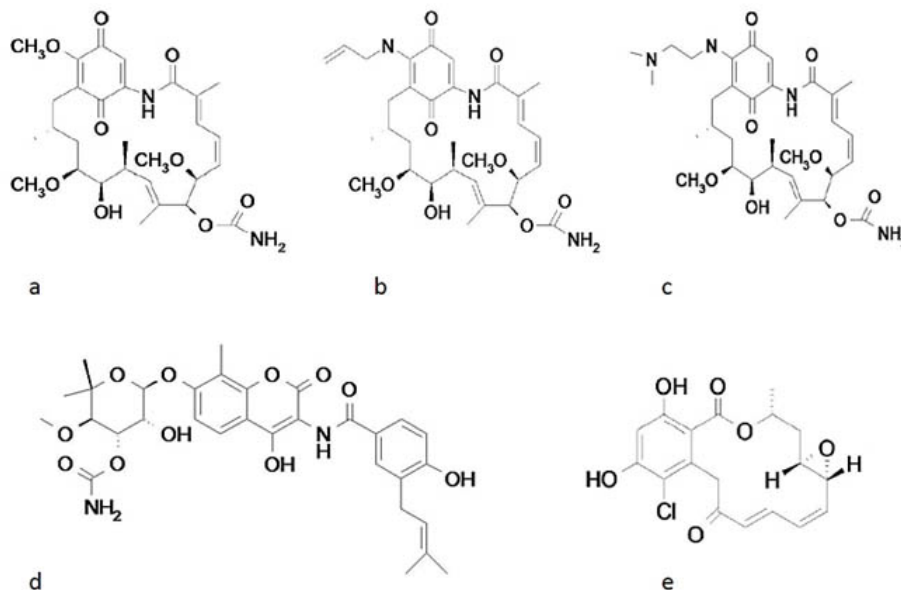


Figure 1. Chemical structures of Hsp90 inhibitors, *a.* Geldanamycin, *b.* 17AAG, *c.* 17-DMAG, *d.* Novobiocin, *e.* Radicol.

proliferation of cancer cells (4-6) while the Hsp90-beta isoform seems to be responsible for development of drug resistance (7, 8). Hsp90 exists as dimer, whereas homodimers of Hsp90 are more common than heterodimers. In stressed or injured cells, the Hsp90 protein accumulates in the nucleus and is involved in guarding the so called cytosolic molecular chaperone complex (9, 10). The molecule maintains solubility, stability, intracellular location and function of Hsp90 client proteins involved, among others, in promoting cell proliferation and survival. Amongst the Hsp90 client proteins there can be distinguished factors such as a mutated form of p53 (11), tyrosine kinases Src kinase family (12), Wee1 kinase (13), serine/threonine kinases like Raf1 (14), and enzymes including nitric oxide synthases involved in oxidative and nitrative stress (15, 16). Hanahan and Weinberg have characterized some typical capabilities shared by almost all cancer types. Fascinatingly, Hsp90 and its client proteins take part in maintaining six of these capabilities mentioned in the article by influencing the regulation of key factors and proteins:

1. Self-sufficiency in growth signaling (Human Epidermal Growth Factor Receptor- HER2 also known as ErbB2)
2. Insensitivity to anti-growth signaling (Cyclin dependent kinases Cdc4, Cdc6, Cyclin D)
3. Capacity to avoid programmed cell death like apoptosis (AKT kinase)
4. Chronic angiogenesis (Hypoxia-induced factor1alpha HIF1-alpha, Vascular Endothelial Growth Factor VEGF)
5. Metastasis (metalloproteases MMP2, urokinase)
6. Infinite proliferative potential (telomerase) (17)

Interestingly, the Hsp90 protein is expressed at a level 2-10 fold higher in cancer cells compared to unstressed, healthy ones, suggesting that the protein is one of the major components involved in cancer cell survival and/or in tumor growth (18).

The mammalian isoforms of Hsp90 have a conserved structure containing: a C-terminal domain (11-15 kDa), that provides constitutive dimerization of protein, a Hsp90 client protein binding domain (38-44 kDa), and an N-terminal ATP binding site (24-28 kDa), responsible for impermanent dimerization. ATP binding is essential for forming a mature dimer of the Hsp90 capable to bind, chaperone and leave its client proteins. Primarily, a Hsp90 client protein is bound to a Hsp40/Hsp70 chaperone complex, that subsequently binds to the Hsp90 thanks to HOP (Hsp90/Hsp70 Organizing Protein). Finally, the Hsp90 binds ATP molecule thus resulting in a dissociation of the HOP and Hsp40/Hsp70 complex, and in an association of Hsp90 with its co-chaperones and client protein. Co-chaperones e.g. Hsp70, Hsp40, p23 and CHIP accompany Hsp90 in nucleotide exchange, ATP hydrolysis, chaperoning and degradation of its client proteins. Non-chaperoned Hsp90 client proteins undergo proteasomal degradation by E3 ubiquitin ligases, such as CHIP (Carboxy-terminus of Hsp70 Interacting Protein) (19-21).

3. HSP90 INHIBITORS

3.1. Geldanamycin and its derivatives

Geldanamycin (GA) and its derivatives (Figure 1) have been reported to possess multiple pharmacological properties- antitumoral properties, inhibition of angiogenesis and metastasis of diseases such as multiple myeloma (22), as well as breast (23) or prostate cancer (24). GA have been identified from *Streptomyces hygroscopicus* in 1970 as tyrosine kinase inhibitor (25, 26). Nowadays it is

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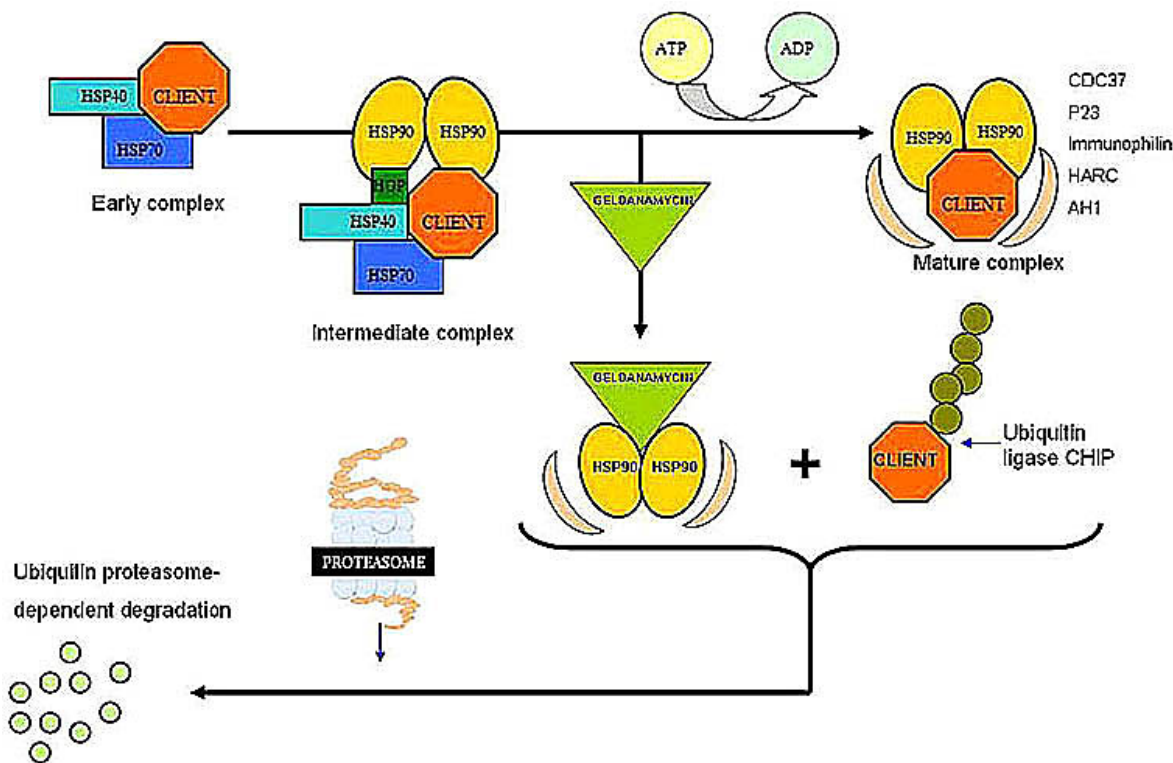


Figure 2. Geldanamycin as depletor of Hsp90. Primarily Hsp90 client protein binds to Hsp40/Hsp70 early chaperone complex, subsequently undergoes Hsp90 dimer-binding thanks to HOP- Hsp90/Hsp70 Organizing Protein forming intermediate complex. ATP molecule hydrolysis results in dissociation of HOP and Hsp40/Hsp70, and successively in association of Hsp90 with its co-chaperones, client protein. Geldanamycin binds to N-terminal ATP-binding site of Hsp90, perturbs formation of mature complex and leads to proteasomal degradation by E3 ubiquitin ligase like CHIP (Carboxy-terminus of Hsp70 Interacting Protein) (based on 76).

known as potent small molecule inhibitor of Hsp90. GA binds to Hsp90 N-terminal ATP binding site, similarly to radicicol, another Hsp90 inhibitor (27). GA inhibits Hsp90 ATPase activity, affecting the dissociation of mature chaperone Hsp90 complex and the degradation of Hsp90 client proteins in proteasomes by E3 ligase (28) (Figure 2). The mechanisms involved in proteasomal degradation of Hsp90 client proteins and destabilization of Hsp90 complexes are relatively well understood, but little is known about effects of GA on the modulation of Hsp90 genes at the transcriptional level. Expression of Hsps is regulated by the heat-shock transcription factors (HSFs), that bind to HSE after activation and conduce to the transactivation of heat shock genes. In mammalian cells, three isoforms of HSFs can be distinguished: stress-activated HSF1, developmentally regulated HSF2, tissue-specifically regulated HSF4 (29, 30). Interestingly, it has been revealed that HSF1 is a client protein of Hsp90 and that GA leads to transactivation of the heat-shock genes involving the Hsp90 gene. GA acts as an inhibitor of the Hsp90 function, but also as an inducer of heat-shock genes, including the Hsp90 gene. Moreover, GA can induce expression of many stress proteins, such as GRPs and Hsp90s, in a concentration-dependent and cell-specific manner (31, 32). Despite its influence on the Hsp90 function and expression of heat-shock genes and proteins,

the distinct mechanism of action of GA is associated with the induction of oxidative stress and production of reactive oxygen species (ROS) related to its quinone group (33). For that reason GA provokes not only TNF-alpha-mediated or intrinsic apoptotic pathways, but also oxidative-stress induced apoptosis (34). Unfortunately, the stress response induced by treatment with this compound can also cause resistance to therapy with GA, and seems to be associated not only with P-glycoprotein expression but, predominantly, with the stress-induced expression of Hsp27 and Hsp70 proteins. Andrea K. McCollum, Cynthia J. TenEyck and colleagues have stated that a knockdown of Hsp27 or Hsp70 is sufficient to reverse resistance to 17-AAG and EC78 (analogues of GA) in a A549 cell line (selected for GA resistance), while inhibition of P-glycoprotein by verapamil is ineffective (35).

Regardless of the pleiotropic activities of GA involving its potent anticancer properties, this compound cannot be evaluated in clinical trials because of its hepatotoxicity, poor water solubility and limited oral bioavailability. Thankfully, modified GA derivatives have been developed as potential drug candidates. 17AAG (17-(Allylamino)-17-demethoxygeldanamycin, NSC 330507, KOS 953, Tanespimycin), a geldanamycin analogue has been evaluated in Phase II/III clinical trials, and has been

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found to possess a lower toxicity, a better stability than GA and to retain a potent anticancer activity even at nanomolar concentrations, although its binding to Hsp90 has resulted weak. It has been evaluated in phase II/ III clinical trials for treatment of multiple myeloma, metastatic melanoma and breast cancer. (36-39). There is evidence of its high activity toward cancers cells both in monotherapy and in combination of therapies with drugs such as bortezomib, sorafenib or trastuzumab (40-42). Due to the fact that tanespimycin is water insoluble, the most appropriate drug formulations for this compound are still being researched. In the "Phase II trial of the Hsp90 inhibitor tanespimycin (Tan) + trastuzumab (T) in patients (pts) with HER2-positive metastatic breast cancer (MBC)" the patients were given cremophor-based 17AAG combined with antihistamine/steroid premeds and tanespimycin in form of a suspension without premeds. The toxicity after drug administration was found to be very controllable; the main side effects were fatigue (39%), diarrhea (33%), dizziness (24%) and headache (19%). Both used forms of 17AAG have been shown to have similar safety profiles, and trial outcomes have been encouraging as well: among others, a 75% decrease in liver metastases, a 57% decrease in lymph node, liver, and breast lesions were reported (43).

Subsequently a second-generation GA analogue has been developed: 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, Alvespimycin, KOS-1022, Kosan), characterized by improved properties. Studies *in vitro* have revealed 17-DMAG is more specific for Hsp90 complexes in cancer cells compared with healthy cells. In addition, it is water-soluble, and it has been reported to have a better oral bioavailability or even a marginally superior activity comparing to 17AAG. Moreover, 17-DMAG has been conveyed to be widely distributed to tissues, and quantitatively much less metabolized than 17-AAG (44-46). Another GA analogue, IPI-504 (Retaspimycin hydrochloride, 18, 21-Didehydro-17-demethoxy-18,21-dideoxo-18,21-dihydroxy-17-(2-propenylamino)geldanamycin) is a novel potent water-soluble Hsp90 inhibitor. It has been reported that it IPI-504 is an active form of 17AAG, as tanespimycin is metabolized to retaspimycin. IPI-504 is especially effective in the treatment of Non-Small Cell Lung Cancer (NSCLC), Gastrointestinal Stromal tumors (GIST) and Soft Tissue Sarcomas (STS) (47-49); moreover, it is also effective both as a single agent as well as acting synergistically with bortezomib and docetaxel (Taxotere) in solid tumors (41, 50).

Since Hsp90 acts as a dimer, Hong Zhang and colleagues compared the pharmacological properties of 17AAG and dimer ansamycins with prolonged inhibitory activity CF237 and CF483. The dimers were characterized by extremely high Hsp90 binding affinity, forming extraordinary stable complexes with the target protein, thus resulting in improved activity and in an increased efficacy and period of action of the dimers in comparison to monomer 17AAG. There are also disadvantages of dimer ansamycin use in therapy, firstly owing to their high reactivity with water: they are in fact poorly water-soluble, due to their high affinity to target proteins and irreversible

inhibition (their action cannot be reversed by ceasing their administration); moreover, they can cause tolerability issues as a result of forming complexes also with non-target proteins (51).

3.2. Influence of geldanamycin on the major Hsp90 client proteins- possible anti-cancer targets

3.2.1. Src family kinases

P60v-src is the transforming protein of Rous sarcoma virus and client protein of Hsp90, complexed with the p50Cdc37 protein (52, 53). One of the major target proteins of p60v-src is Focal Adhesion Kinase (FAK), a nonreceptor protein-tyrosine kinase. P60v-src phosphorylates FAK, but, moreover, induces autophosphorylation and protects against the action of tyrosine phosphatases (54). High levels of both p60v-src and FAK protein conduce to a dysregulation of the transmission of cell growth signaling and anchor-independent proliferation of the cells. Researches uncovered the presence of diminished levels of p60v-src and tyrosine phosphorylated proteins in mutant yeast with a lowered Hsp90 expression, with the consequent restoration of a normal cell cycle. GA and other ansamycin antibiotics were originally discovered as p60v-src inhibitors, while today it is known that this inhibition is indirect and affected by the destabilization of the Hsp90-p60v-src complex, leading to diminished levels of the tyrosine kinase (55, 56).

Conversely, the association between Hsp90 and cellular c-Src still seems to be unclear. Experiments have failed to detect Hsp90-c-Src complexes, in contrast to Hsp90-p60v-src, which are very stable and abundant (57). In yeast with a lowered Hsp90 level no differences were found in c-Src levels. Interestingly, GA leads to disruption of Hsp90-c-Src complexes and other Hsp90 complexes with Src kinases sharing homology in their C-termini with c-Src like Lck and Fgr kinases (58).

3.2.2. Raf1 kinase

Raf 1 protooncogene belongs to the serine/threonine kinases responsible for the regulation of cell proliferation, differentiation and apoptosis. It is a component of the MAPK (Mitogen-activated Protein Kinases)/ERK (Extracellular Signal-regulated Protein Kinases) signaling transduction pathway recruited by the activated Ras protein, a membrane-associated GTPase, after activation Raf1 induces phosphorylation of kinases such as ERK1, ERK2 or MAP kinase kinase MEK1 and MEK2 (59). Relatively to p60v-src, Raf1 forms complexes with Hsp90 and p50Cdc37. MEK1 kinase has likewise been found to associate with both Hsp90 and Raf1 (60, 61).

3.2.3. Cyclin-dependent kinases Cdk4, Cdk6

Cyclin-dependent kinases take part in the regulation of cell cycle in eukaryotic cells; one of the mechanisms of their action is phosphorylation/dephosphorylation. Cdc4 is the kinase responsible for triggering mitosis and the factor stimulating cells to transition from G2 to M phase by phosphorylation of the retinoblastoma protein. Cdc4 that is regulated partly by its association with cyclin B, forms complexes with cyclinD, active in the nucleus. Cdc4 also forms complexes

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with Hsp90/p50Cdc37 in primed conformation, and after dissociation from the chaperone complex, it connects with cyclin D (53, 62). Through inhibition of the Hsp90 function, GA decreases the level of Cdc4 by post-translational destabilization similarly to Raf1 kinase. Cdk6, comparably to Cdk4, induces transition of cells through the G1 phase of cell cycle, being activated by interaction with cyclin D protein, and forms complexes with Hsp90/p50Cdc37 (53). It has been revealed that GA lowers the expression of Cdk4, Cdk6 and cyclin B, as well as inducing mostly G2 or M arrest in cancer cells like the glioblastoma U87MG cell line with the consequence of inhibiting cell cycle progression.

3.2.4. Transforming growth factor TGF-beta

TGF-beta, a member of the polypeptide growth factor family, is a cytokine involved in the regulation of cell proliferation, differentiation and survival. It is known well dichotomy of TGF-beta, which in normal epithelial cells acts as tumor suppressor and inducer of apoptosis, while in malignant cells, it works as a tumor promoter, stimulating proliferation, angiogenesis and metastases (63). There are different isoforms of TGF-beta, with a 70-80% homology in amino acids. One can distinguish two transmembrane protein receptors for TGF-beta with serine/threonine activity- TGF-beta type 1 receptor (TGF-betaRI) and TGF-beta type 2 receptor (TGF-beta RII); downstream effectors of TGF-beta are the Smads proteins that regulate the expression of target genes. GA has been reported to inhibit TGF-beta signaling through increased receptor ubiquitination, partly by increasing expression of inducible Hsp70, co-chaperone of Hsp90 expressed in the majority of cancer cells (64).

3.2.5. Mutated p53

Wild type p53 is a well known tumor suppressor protein that is involved in the adjustment of cell cycle progression, apoptosis. It is referred to as "the guardian of the genome", and characterized by very short half-life, with its mutated form being fairly stable; the latter interferes in the activity of wild type p53 by perturbing its activity and increasing drug resistance according to cells with mutations of p53 protein (65). Blagosklonny, Toretsky and Neckers (66) exposed the potential of GA to decrease mutated p53 level and half-life, in contrast to the wild type protein that is unaffected by drug therapy.

3.3. Another Hsp90 inhibitors

3.3.1. Radicicol

Radicicol is a macrocyclic lactone antibiotic possessing epoxy α , β , γ - unsaturated carbonyl groups; it was isolated from the fungus *Monosporium bonorden* (67). Radicicol is a potent Hsp90 inhibitor that acts by interacting with its N-terminal binding domain and triggering Hsp90 client proteins proteasomal degradation, similarly to GA and its analogues (27). Radicicol has been found to induce apoptosis even in 17AAG-resistant cancer cells. Possibly because of its unstable epoxy group, radicicol has failed to be effective in animal models, although it has been found to possess strong anti-cancer properties *in vitro* (68). Oxime derivatives of radicicol with enhanced stability, synthesized by Kyowa Hakko Kirin, are known as KF55823 and KF58333. Interestingly, despite of these two derivatives

achieving the same level of plasma concentrations, KF58333 showed significant antiproliferative and antitumor properties through depletion of Hsp90 in KPL-4 human breast cancer xenografts, unlike its stereoisomer KF58332; this finding suggests that oxime derivatives of radicicol may possess stereospecific antitumor properties (69).

3.3.2. Novobiocin

Novobiocin (Albamycin, Cathomycin) is an aminocoumarin antibiotic produced by *Actinobacteria Streptomyces niveus* (*Streptomyces spheroides*). In contrast with geldanamycin and radicicol, novobiocin binds to the C-terminal ATP binding site of Hsp90 (70); moreover, it also acts as an inhibitor of bacterial DNA gyrase (71). Similarly to ansamycin antibiotics, novobiocin affects the destabilization and degradation of Hsp90 client proteins involving Raf1, p60v-src, mutated p53 protein or AKT kinase (72, 73). An analogue of novobiocin, F-4, has been found to be more potent and efficient in comparison with its parental compound and even with the N terminal inhibitor 17AAG in prostate cancer cells (74).

3.3.3. STA-9090 and STA-1474

Novel Hsp90 inhibitors have been developed, that are not related to geldanamycin- derivatives of resorcinol, containing triazol ring in chain. STA-9090 and its pro-drug STA-1474 seem to be effective *in vitro*, at nanomolar concentrations, against multiple tumor cell lines including osteosarcoma. Both drugs bind to the ATP-binding domain at the N-terminus of Hsp90 and induce a degradation of Hsp90 client proteins (75).

4. SUMMARY AND PERSPECTIVE

Due to the fact that isoforms of HSP90 are implicated in cancer development, progression and formation of metastases, Hsp90 inhibitors are at the center of interest of oncologists and researches. One type of Hsp90 inhibitors are the benzoquinone ansamycin antibiotics, including herbimycin A, geldanamycin and its derivatives with enhanced pharmacokinetics and pharmacodynamics profile, while another one, dissimilar to benzoquinone antibiotics, is the Hsp90-binding small molecule-natural product, radicicol, and newly analogues of resorcinol with triazol ring in chain- STA-9090 and STA-1474. All of the aforementioned inhibitors possess a high affinity to binding to the N-terminal ATP binding site of Hsp90 and affect its function. Novobiocin is an antibiotic and a potent Hsp90 inhibitor that, in contrast to the abovementioned chemicals, interacts with the C-terminal ATP binding pocket. These anti-cancer agents or their derivatives have been developed in preclinical studies or already evaluated in clinical trials involving different kinds of malignancies such as multiple myeloma, breast or prostate cancer.

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Abbreviations: 17AAG: 17- (Allylamino)-17-demethoxygeldanamycin, Cdc: cyclin dependent kinases, CHIP: carboxy-terminus of Hsp70 interacting protein, 17-DMAG: 17-dimethylaminoethylamino-17-demethoxygeldanamycin, GA: geldanamycin, Hsps: heat

shock proteins, IPI-504: 18, 21-Didehydro-17-demethoxy-18,21-dideoxo-18,21-dihydroxy-17- (2-propenylamino)geldanamycin, TGF-beta: transforming growth factor beta

Key Words: Anticancer drugs, Geldanamycin, 17AAG, 17-DMAG, IPI-504, STA-9090, STA-1474, Radicicol, Novobiocin, Hsp90, Hsp90 inhibitors, Hsp90 client proteins, Review

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