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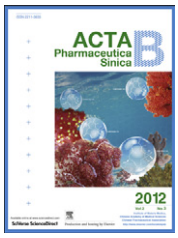

REVIEW

# Discovery and development of natural heat shock protein 90 inhibitors in cancer treatment

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**Abstract** Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone that plays a vital role in the signal transduction of cancers. Hsp90 inhibitors are able to inhibit Hsp90 or the complex of Hsp90 and co-chaperones resulting in the degradation of Hsp90-dependent client proteins through the ubiquitination-proteasome pathway, thereby leading to the growth inhibition of tumor cells. This review will briefly discuss the molecular structure and biological function of Hsp90, and focus on a summary of recent progress in the development and testing of natural Hsp90 inhibitors and their different means by which they interact with Hsp90.



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## 1. Introduction

Heat shock proteins (Hsps) are a family of highly conserved stress proteins which can be induced by environmental stress such as heat, hypoxia, DNA damage or UV radiation to regulate cell metabolism and protect prokaryotic and eukaryotic cells from harmful exogenous stimulation. They are also known as molecular chaperones due to their ability to maintain proper protein folding, and facilitate the assembly of protein polymers and the formation of mature proteins.

The highly conserved molecular chaperone Hsp90 is one of the most abundant of proteins in almost all eukaryotic cells, accounting for 1–2% of total cellular proteins[1]. Hsp90 has two major cytoplasmic isoforms Hsp90α (inducible form) and Hsp90$\beta$ (constitutive form), as well as a Raf-associated protein Hsp90N which is associated with cellular transformation[2]. Other Hsp90 analogues include glucose regulated protein 94 (Grp94) in the endoplasmic reticulum and tumor necrosis factor receptor-associated protein 1 (TRAP1) in the mitochondrial matrix[1,2].

Hsp90, in general, exists as a homodimer (α/α or $\beta/\beta$) in cells. The structure of Hsp90 is composed of three domains: the highly conserved $NH_2$-terminal nucleotide binding domain (25 kDa, Hsp90N), the middle domain (35 kDa, Hsp90M) and the C-terminal domain (10 kDa, Hsp90C). Hsp90N is an important binding site for ATP/ADP, which is associated with forming the complex of Hsp90 and co-chaperones *via* conformational transition[3]. Hsp90M is the binding site for nuclear localization signal and client proteins, and is involved in the specific recognition of client proteins and regulating molecular chaperones for appropriate substrate activation including ATP hydrolysis[4]. The C-terminal of Hsp90 is the site of dimerization. This region contains a pentapeptide domain (MEEVD) which is also the binding site of co-chaperones of Hsp90 such as Hop and Sti 1[5].

In eukaryotic plasma, Hsp90 is considered to mediate the folding, stabilization, activation and assembly of its client proteins, including steroid receptors, protein kinases and transcription factors, through the formation of complexes with other co-chaperones such as Hsp70, Hop and p23[6]. A client protein is bound to Hsp70/Hsp40 to form an early complex, and then Hsp90 along with the co-chaperone Hop binds to this early complex to form an intermediate complex. When ADP is replaced by ATP, Hsp90 undergoes a conformational change which releases Hsp70/Hsp40 and Hop, thus allowing the ATP-dependent association of other co-chaperones, including p23 and the immunophilins to form a mature complex. When the ATP-binding site of Hsp90 is occupied by competitive inhibitors, the Hsp90 complex is disrupted and the client proteins can be degraded through the ubiquitination-proteasome pathway[6,7].

Cancer is a disease associated with genetic instability which allows cancer cells to acquire six distinguishing characteristics, including self-sufficiency in growth signaling, resistance to apoptosis, insensitivity to growth inhibitory signaling, sustained angiogenesis, tissue invasion and metastasis, and limitless proliferative potential. Many signaling proteins related to these hallmarks of cancer are client proteins of Hsp90, such as Akt, Her-2, p53, CDK4, Raf-1, IKK, HIF-1α, Src (Table 1). In addition, the rapid growth of cancer cells requires the participation of large quantities of cytosolic heat shock proteins. Thus, it is useful to inhibit cancer growth and metastasis by suppressing the formation of complexes of Hsp90 and its client proteins, which not only reduces the tolerance of cancer cells to chemotherapeutics, but also synergistically enhances the efficacy of multiple anticancer drugs. Hsp90 has been considered as a potent molecular target in the development of new anticancer agents. Since the first natural anticancer drug vinblastine was applied in clinic, many chemotherapeutic agents from natural products have been discovered and developed, including paclitaxel, camptothecin, epothilone, bleomycin and cantharidin, *etc*. Current Hsp90 inhibitors are derived from natural products, such as geldanamycin, radicicol, novobicon, epigallocatechin-3-gallate, celastrol and hypericin (Fig. 1). This review will briefly describe the discovery and development of natural product Hsp90 inhibitors.

**Table 1** Relationship between Hsp90 client proteins and hallmarks of cancer.

| Hallmark of cancer | Hsp90 client protein |
|---|---|
| Evasion of apoptosis | Akt,Rip,P53,Survivin, Apaf-1,Bcl-2,IGF-IR |
| Sustained angiogenesis | VEGFR, HIF1,Akt, Fit-3,FAK,Src |
| Limitless replicative potential | n-TERT, telomerase |
| Tissue invasion and metastasis | c-MET,MMP2 |
| Self-sufficiency in growth signals | EGFR/Her-2,Raf, Bcr-Abl,ErbB-2, Src,Akt,MEK |
| Insensitivity to anti-growth signals | Plk-1,Cdk4,Cdk6, Myt-1,cyclin D |

## 2. Inhibitors of Hsp90 N-terminal ATP binding domain

### 2.1. Geldanamycin (GA) and derivatives

Geldanamycin(**1**) (Fig. 1), originally isolated from the culture filtrates of *Streptomyces hygroscopicus*, inhibits the growth of protozoa, bacteria and fungus[8]. GA was initially found to arrest cancer cell proliferation by inhibiting Src tyrosine kinase, but it was unable to directly inhibit the activity of purified kinase[9]. Further studies revealed that the anti-proliferative activity of GA resulted from the binding of GA to Hsp90. GA inhibits client protein binding to Hsp90 through competitive coupling with ATP/ADP binding "pocket" of the Hsp90 N-terminal domain, leading to the degradation of the client proteins *via* the ubiquitination-proteasome pathway (Fig. 2). The selectivity of GA for Hsp90 is due to the special ATP binding "pocket" of Hsp90 as compared to other nucleotide-binding proteins. Structure–activity analysis has revealed that the 2,3-double bond of GA was indispensible for stable conformation of Hsp90; 11-hydroxy and 17-methoxy moieties contribute to formation of hydrogen bonds and increase the binding affinity of GA or its derivatives for Hsp90, thereby leading to an enhancement of anticancer activities[10]. These results suggest that the effectiveness of Hsp90 inhibitors is closely related to their binding ability with Hsp90.

The structure of geldanamycin has been modified to reduce its hepatotoxicity and increase its water-solubility. The site of focus is the 17 position of the benzoquinone group to generate more potent derivatives, including 17-allylamino-17-demethoxy geldanamycin (17-AAG, **2**) (Fig. 1) and 17-*N*,*N*-dimethylaminoethylamino-17-demethoxy geldanamycin

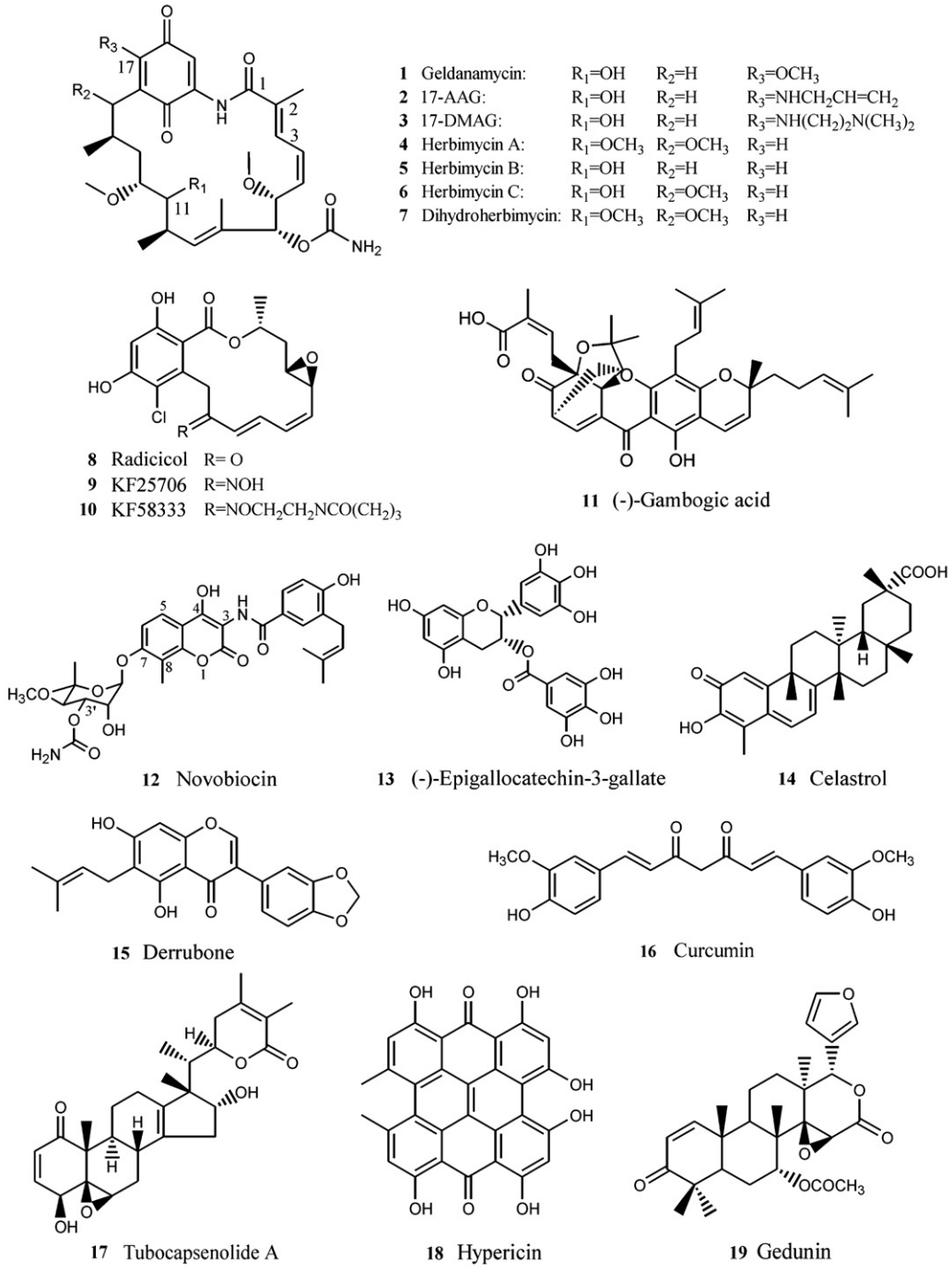


**Figure 1** Chemical structures of Hsp90 inhibitors.

(17-DMAG, **3**) (Fig. 1) which are in phase II/III or I clinical trials respectively (Table 2).

17-AAG (17-allyamino-17-demethoxygeldanamycin), in which methoxymoiety of GA is replaced by an ally amino group, is the first Hsp90 inhibitor used in a clinical study with hepatotoxicity is apparently less than that of geldanamycin. 17-AAG can be metabolized by cytochrome P450 CYP3A4 to 17-amino-demethoxygeldanamycin (17-AG) *in vivo* which maintains Hsp90 inhibitory activity and with higher stability than 17-AAG[11]. 17-AAG binds to purified full-length dimeric Hsp90 in the low micromolar range, whereas it inhibits tumor cell growth in the low nanomolar range. This difference could be explained by the finding that benzoquinone ansamycins largely accumulated in tumor cells with high micromolar concentration[12]. Further studies revealed that 17-AAG had a higher affinity for Hsp90 derived from the breast carcinoma cell line BT474 ($IC_{50}=6$ nM) than Hsp90 from primary human renal epithelial cells ($IC_{50}=600$ nM and 400 nM) and normal fibroblasts ($IC_{50}=400$ nM). This indicated that Hsp90 in tumor cells existed in the form of active multi-chaperone complexes with Hsp70, Hsp40, Hop or p23, whereas Hsp90 in normal cells was in an uncomplexed inactive form[13]. In contrast to this suggestion, a recent study proposed that the affinity between GA and Hsp90 alone or the Hsp90 complex was similar, and that GA and its derivatives inhibited

Hsp90 through slow but tight binding interaction with high affinity and low dissociation[14]. An alternative explanation was also provided by Maroney et al.[15], as they revealed that the benzoquinone ring of ansamycins could be reduced to dihydroquinone by NAD(P)H: Quinone oxidoreductase 1. The reduced product had approximately 40-fold greater affinity for Hsp90 *in vitro* than its oxidized form and exhibited enhanced anticancer activity, which contributed to the accumulation of ansamycin dihydroquinones in cancer cells. Additionally, quantum chemical calculations and mutation analysis demonstrated that geldanamycin could undergo keto-enoltautomerization catalyzed by Hsp90 which enhanced its binding to Hsp90[16]. It was also found that Hsp90 did not catalyze the *trans–cis* isomerisation of GA and confirmed that the binding capability of the dihydroquinone form of GA and its analogues to Hsp90 was more potent than the affinity of their oxidized quinone forms[17].

17-AGG (oxidized form) entered clinical trials in 1999, and its reduced form entered clinical trials in 2005. Further clinical research and application of 17-AAG was limited due to its poor solubility. 17-DMAG, a new generation of Hsp90 inhibitor developed by Kosan Biosciences, has better water solubility, tissue dispersibility and bio-availability[18]. In comparison with 17-AAG, 17-DMAG possesses more potent anticancer activity against many cell lines *in vitro* and *in vivo*, and some scholars have suggested that this difference might be attributed to the greater affinity of Hsp90 for the benzoquinone ansamycins or their metabolites[19]. Unfortunately, 17-DMAG was also revealed to have gastrointestinal, liver, bone marrow, renal and gallbladder toxicity in dogs[20]. Thus, Kosan Biosciences stopped the clinical development of 17-DMAG in the beginning of 2008. However, the further clinical tests are still being carried out by other research groups because of its favorable water solubility and various antitumor activities against different tumor types.

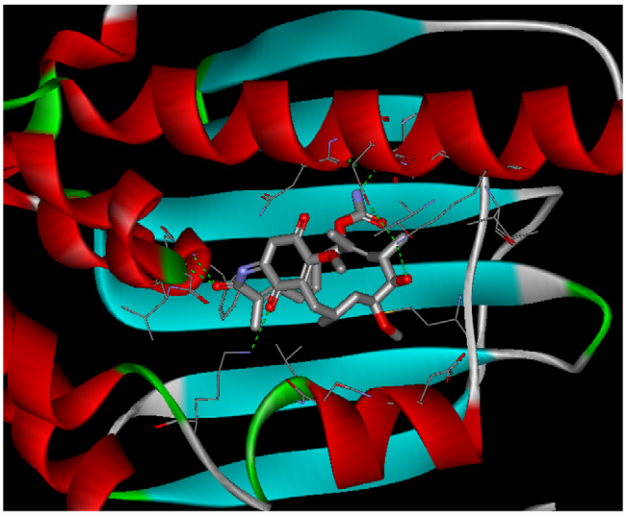

**Figure 2** Binding mode of GA to Hsp90.

### 2.2. *Herbimycin and its analogues*

Herbimycin is also a member of benzoquinone ansamycin antibiotics isolated from the culture filtrates of *Streptomyces hygroscopicus*. Herbimycin A (**4**) (Fig. 1) was primarily isolated from the fermentation broth of *Streptomyces hygroscopicus* (No. AM-3672)[21], and later herbimycin B (**5**), C (**6**) (Fig. 1) and dihydroherbimycin were obtained. Herbimycin was a selective inhibitor of a tyrosine kinase, and had an inhibitory effect on cancer angiogenesis and proliferation[22,23]. Yang et al.[24] have compared the anti-proliferative activities of herbimycin analogues on Vero and B16-F10 cells as follows: herbimycin C > herbimycin A > dihydroherbimycin A (**7**) (Fig. 1). Further studies suggested that the underlying anti-tumor mechanism was also competitive binding to the N-terminal domain of Hsp90 to arrest the ATPase cycle[25]. To date there is no clinical report on herbimycin and its analogues.

### 2.3. *Radicicol (RA) and its analogues*

Radicicol (**8**) (Fig. 1) belongs to the macrocyclic lactone antibiotics originally extracted from the filtrate of fungus *Monosporiumbonorden*. It can inhibit cancer cell proliferation and

**Table 2** Hsp90 inhibitors in clinical trials.

| | Phase | Indication | NCT ID |
|---|---|---|---|
| 17-AAG | II/III | Multiple myeloma | NCT00514371, NCT00546780 |
| | II | Pancreatic cancer | NCT00577889 |
| | II | Breast cancer | NCT00096109, NCT00118092 |
| | II | Melanoma | NCT00087386 NCT00088374 |
| | II | Kidney tumor | |
| 17-DMAG | I | Solid tumor | NCT00088868, NCT00089362 |
| | I | Leukemia lymphoma | NCT01126502[a] |
| Gambogic acid | IIa | Cancers | 2004L00333(SFDA) |
| EGCG | I | Small cell lung cancer | NCT01317953[a] |
| | II | Breast cancer | NCT00917735[a] |
| | II | Prostate cancer | NCT00676780, NCT00596011[a] |
| Curcumin | II | Pancreatic cancer | NCT00192842 |
| | II | Breast cancer | NCT01042938 |
| | I/II | Osteosarcoma | NCT00689195[a] |

[a]This study is currently recruiting participants.

angiogenesis *in vitro* and *in vivo* through depletion of $p60^{v\text{-}src}$ protein kinase and disruption of K-ras-activated aberrant signaling pathways[26–28]. Similar to benzoquinone ansamycins, it can also competitively bind to the N-terminal domain of Hsp90 to disrupt Hsp90 complex formation[29]. Results from isothermal titration calorimetry suggested that the affinity of radicicol to Hsp90N was about 50-fold greater than that of geldenamycins[10]. Radicicol showed more potent antitumor activity than geldenamycins *in vitro*, but weaker activity *in vivo*, which could be explained by the fact that the presence of epoxy and $\alpha$, $\beta$, $\gamma$, $\delta$-unsaturated carbonyl groups reduced the stability of radicicol[30].

Structure–activity relationship analysis indicated that the benzene hydroxyl and macrocyclic ring were required for biological activity of radicicol[31]. A series of stable radicicoloxime derivatives have been synthesized by means of reducing the nucleophilicity of unsaturated carbonyl groups. Soga et al.[32,33] have indicated that KF25706 (**9**) (Fig. 1) could selectively deplete the client proteins of Hsp90 *via* inhibition of v-Src and K-ras-activated signaling pathways and KF58333 (**10**) (Fig. 1) could suppress VEGF expression and tumor angiogenesis through an HIF-1$\alpha$-dependent pathway. These results indicate that the oxime moiety plays a significant role in sustaining the stability and enhancing the biological activity of radicicoloxime analogues. In contrast to geldanamycin and its derivatives, radicicol has no hepatotoxicity, which implies that hepatotoxicity of benzoquinone ansamycins is not a common characteristic of all Hsp90 inhibitors[34]. Thus, radicicol and its analogues are promising drug candidates for further development as non-benzoquinone ansamycin Hsp90 inhibitors. Unfortunately, there is no clinical report of these compounds to date.

### 2.4. Gambogic acid

Gambogic acid **(11)** (Fig. 1) is the main component of gamboge used for treating tumors or infections. It has been reported that gambogic acid was an inhibitor of Hsp90 through binding to the N-terminal domain of Hsp90 in a non-competitive manner similar to that of geldanamycin[35]. A phase IIa clinical trial to explore tolerability and safety of gambogic acid in cancer patients *via* different intravenous administration methods was completed in 2008 in China (Table 2).

## 3. Inhibitors of Hsp90 C-terminal ATP-binding domain

### 3.1. Novobiocin and analogues

Novobiocin (**12**) (Fig. 1), a coumarin antibiotic isolated from several *Streptomyces* strains, exhibited anti-bacterial activity through binding with topoisomerase II to inhibit ATP hydrolysis[36]. Given that Hsp90 is also an ATPase-dependent protein, novobiocin might be also an inhibitor of Hsp90. Novobiocin interacted with the Hsp90C ATP-binding domain to disrupt Hsp90 dimerization and induce conformational changes of the Hsp90 complex, finally leading to the degradation of client proteins *via* the ubiquitination-proteasome pathway. However, novobiocin at a concentration of 700 μM induced depletion of Hsp90 clients in SKBr3 cells, which was much weaker than Hsp90N inhibitors including benzoquinone ansamycins and radicicol analogues[37]. Further reasonable modifications should be carried out to enhance the bioactivity of novobiocin.

The structure of novobiocin is composed of three domains, including benzamide moiety, a coumarin moiety and noviose moiety. Blagg et al.[38] have synthesized a series of analogues and elucidated their structure–activity relationships: 3-secondary amine and 7-noviose moieties were indispensable for their bioactivities; 8-methoxy was useful to increase the bioactivity but 5-substitution may decrease the bioactivity; and the coumarinlactone moiety was not critical for Hsp90 inhibition. 4-Hydroxyl and 3'-carbamate of noviose moieties could impair the inhibitory activity towards Hsp90 but were essential for inhibition of DNA topoisomerase[39]. In contrast to Blagg's reports, Renoir et al.[40] found that antitumor activities of these derivatives were significantly enhanced through substituting a tosyl moiety at C-4 or C-7 position of coumarin rings and highlighted that C-4 and/or C-7 positions of coumarin may be required for degradation of Hsp90 client proteins. Currently, novibiocon has mainly been used to treat bacterial infection in the clinic.

### 3.2. (−)-Epigallocatechin-3-gallate (EGCG)

(−)-Epigallocatechin-3-gallate (EGCG) (**13**) (Fig. 1) is a major polyphenolcatechin of green tea and possesses excellent antitumor activity, although its mechanism has not yet been clarified. Palermo et al.[41] first reported EGCG could inhibit aryl hydrocarbon receptor binding of Hsp90. Furthermore, Li et al.[42] proposed that EGCG may disrupt the association of Hsp90 with co-chaperone Hsc70 and p23 *via* direct binding to the Hsp90 C-terminal domain, and degrade Hsp90-related client proteins including Akt, Cdk4, Raf-1, Her-2 and pERK. Additionally, EGCG was also proven to be able to inhibit the growth and development of tumors *in vitro* and *in vivo* by suppressing the expression of Hsp70 and Hsp90[43]. Gasiewicz et al.[44] suggested that EGCG directly inhibited the dimerization and modulated the conformation of Hsp90 by binding at or adjacent to the C-terminal ATP-binding site, just not as N-terminal Hsp90 inhibitors with destabilization of the Hsp90 complex. These reports indicate that EGCG could treat various tumors and enhance the anti-cancer activity of chemotherapeutics. Recently, a phase I clinical trial is testing the safety of EGCG for extensive-stage small lung cancer in those who achieved the objective tumor response after first-line therapy (Table 2).

## 4. Inhibitors of Hsp90 and interaction with co-chaperones

Co-chaperones play a significant role in modulating the formation and maturation of Hsp90 and its client proteins. The cell division cycle protein 37 (CDC37) is an important co-chaperone, which helps the efficient recruitment of client proteins (mainly protein kinases) to the chaperone machinery and facilitates the formation of mature client proteins. Therefore, inhibition of CDC37/Hsp90 complex may efficiently decrease the expression of client proteins in cancer cells.

Celastrol (**14**) (Fig. 1), also called tripterine, was isolated from the root extracts of Thunder God Vine which is used to treat inflammatory and autoimmune diseases (*e.g.*, rheumatoid arthritis, sclerosis and systemic lupus erythematosus) in traditional Chinese medicine. It was recently reported to

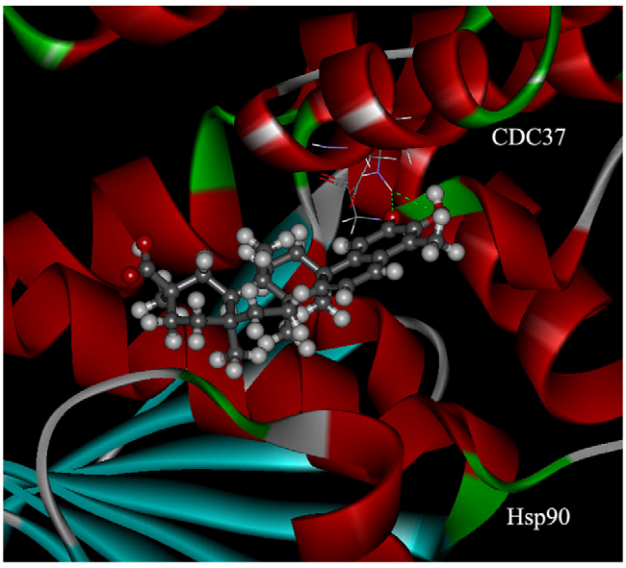


**Figure 3** Binding mode of celastrol to Hsp90/CDC37.

possess antitumor activity[45]. Zhang et al.[46] have found that celastrol showed an anti-pancreatic cancer effect *in vitro* and *in vivo* through disruption of the interaction of CDC37 with Hsp90 without affecting ATP binding to Hsp90, thereby inducing the degradation of Hsp90 client proteins (Fig. 3). According to results from $^{1}H$, $^{15}N$-HSQC NMR, the thiol group of CDC37 N-terminal may react with the quinone-methide of celastrol through a Michanel addition at the C6 position, resulting in a change of CDC37 conformation and disruption of the Hsp90 complex formation. In addition, there is a large hydrophobic core at the interface of CDC37/Hsp90 complex and celastrol could disrupt the hydrophobic core at C293 and block the formation of Hsp90/CDC37 complex. Thus, the N-terminal of CDC37 and the middle domain may be the main interaction sites of celastol[47]. Extracts of Thunder God Vineis used to treat rheumatoid arthritis, Crohn's disease and immunological rejection of kidney transplantation in IIb clinical trials, whereas the anti-tumor effect of celastrol has been studied only in preclinical tests.

Derrubone (**15**) (Fig. 1) is a natural prenylated isoflavone originally isolated from *Derris robusta*. In high-throughput screening, Blagg et al.[48] discovered that it induced potent cell growth inhibition and Her2 degradation in human breast cells (MCF-7 and SkBr3) without describing its specific mechanism. Furthermore, they synthesized chimeric analogues of novibiocin and derrubone sharing several key structural features, and evaluated their bioactivities. They also found that the isoflavone motif of derrubone and the coumarin moiety of vovibiocin exerted different modes of action on binding to Hsp90, however, the C-3 position of the bicyclic ring of both was required for inhibiting Hsp90[49]. Derrubone, as a lead Hsp90 inhibitor, is expected to have a promising development.

Curcumin (**16**) was isolated from Zingiberaceae family plants, including *Curcuma aromatica*, *Curcuma longa* and *Curcuma phaeocaulis*. It possesses anti-oxidant, anti-inflammatory and anti-tumor activities. Curcumin can also induce apoptosis in cancer cells by different signaling pathways and markedly enhance the sensitization of cells to other anticancer drugs[50]. Jung et al.[51] found that curcumin induced degradation of ErbB2 (a client of Hsp90), suggesting that Hsp90 or an Hsp90 complex may be a target of curcumin. Lee et al.[52] indicated that curcumin treatment resulted in dissociation of the Hsp90/p23 complex from human telomerase reverse transcriptase (hTERT), down-regulation of hTERT expression and inhibition of nuclear localization of telomerase to modulate the growth and development of cancer. Giommarelli et al.[53] observed that curcumin could directly inhibit Hsp90 and facilitate dissociation of client proteins (EGFR, Akt, surviving and Raf-1) from Hsp90 complexes. But given that curcumin had a low affinity for Hsp90 and led to the down-regulation of Hsp70, it was indicated that the mechanism behind its Hsp90 inhibitory effect may be not identical to that of 17-AAG. In addition, it could also enhance the sensitivity of tumor cells to HDAC inhibitors and synergistically induce cellular growth inhibition and apoptosis. These results suggest that curcumin may be a promising agent to treat cancers in clinic. Currently, several clinical trials have been reported in the treatment of colorectal cancer, rectal cancer and pancreatic cancer (Table 2). Unfortunately, the application of curcumin in the clinic was limited owing to its poor bioavailability. It is anticipated that structural modifications will markedly improve its bioavailability and antitumor activity.

## 5. Other Hsp90 inhibitors

Tubocapsenolide A (**17**) (Fig. 1), a member of withanolide compounds, was isolated from *Tubocapsicum anomalum*. It could selectively induce thiol oxidation of Hsp90 and Hsp70 to destabilize and degrade Hsp90 client proteins, leading to cell cycle arrest and apoptosis in breast cancer MDA-MB-231 cells[54].

Hypericin (**18**) (Fig. 1) was isolated from the European plant *Hypericum perforatum*, which is used in anti-virus and anti-tumor treatments. Blank et al.[55] proposed that hypericin enhanced ubiquitinylation of Hsp90 to degrade Hsp90 client proteins. Phase I/II or II clinical trials have been conducted to test the effectiveness, safety, and tolerance of hypericin for patients with recurrent malignant gliomas or cutaneous T-cell lymphaomas, as well as psoriasis.

Gedunin (**19**) (Fig. 1) was derived from the Indian neem tree (*Azadirachta indica*). Hieronymus et al.[56] reported that gedunin modulated Hsp90 to inhibit proliferation of cancer cells, but it did not competitively bind to the Hsp90 ATP-binding pocket as seen with geldanamycin. Blagg et al.[57] synthesized 19 gedunin analogues in an effort to elucidate the structure–activity relationships of the gedunin–Hsp90 interaction. Unfortunately, anti-proliferative activities of these derivatives were much lower than that of gedunin in breast cancer cell lines MCF-7 and SkBr3. Further study is underway to obtain more gedunin analogues with potent chemotherapeutic properties.

## 6. Conclusions

A significant hallmark of cancer cells in the process of growth and development is infinite proliferation and immortalization, which requires associated chaperones for regulating protein synthesis. Studies also find Hsp90 and co-chaperones are abnormally expressed in cancer cells, thus it is expected that the identification and development of Hsp90 inhibitors will have a promising future. However, whether Hsp90 inhibitors

also influence Hsp90 function in normal cells while they inhibit Hsp90 and Hsp90 complexes of tumor cells remains to be determined. Some studies have shown that 17-DMAG is widely distributed in different tissues, but it is unclear why it is metabolized much more slowly in cancer cells than normal cells[58]. The affinity of ansamycins for Hsp90 in cancer cells is apparently 20–50 times stronger than it is in normal cells. This may be explained by the idea that Hsp90 in cancer cells is in the form of multi-chaperone complexes that modulate client protein maturation, while Hsp90 in normal cells is in an uncoupled inactive form. Another explanation is that the ATPase activity of Hsp90 in tumor cells is markedly higher than that in normal cells[13]. Nevertheless, the molecular mechanisms of interaction of Hsp90 inhibitors with Hsp90 warrants further study.

Most chemotherapeutics have serious side effects, such as geldanamycin and its derivatives demonstrating hepatotoxicity, poor solubility, nausea and vomiting in clinical use. In China, traditional Chinese medicines in cancer treatment offer unique advantages and are widely accepted and used in the clinic. The multiple effects of Hsp90 inhibitors on the treatment of tumor are consistent with the characteristics of traditional Chinese medicines in which multi-level, multi-target approaches are valued. Therefore, natural Hsp90 inhibitors can be useful in the enhancement of antitumor activity and decreased toxicity and resistance to drugs when combined with other chemotherapeutic agents. Hsp90 inhibitors have been isolated from Chinese herbs and yielded multiple bioactive constituents, including curcumin, hypericin, celastrol, *etc*. Although the biological activities of these compounds are less than those of geldanamycin and its analogues, their toxicities are also lower. Furthermore, these compounds not only enrich the chemical structure library of Hsp90 inhibitors, but also provide lead compounds for further synthesis of more selective and less toxic Hsp90 inhibitors. It is anticipated that the focus on discovery and development of Hsp90 inhibitors from traditional Chinese medicines through chemical separation and computer-aided drug design combined with high-throughput screening technology will yield further success in the development of Hsp90 inhibitors for use in cancer.

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