Hsp90 Activity Modulation by Plant Secondary Metabolites

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Abstract

Hsp90 is an evolutionarily conserved adenosine triphosphate-dependent molecular chaperone and is one of the most abundant proteins in the cells (1–3%). Hsp90 is induced when a cell undergoes various types of environmental stresses such as heat, cold, or oxygen deprivation. It is involved in the turnover, trafficking, and activity of client proteins, including apoptotic factors, protein kinases, transcription factors, signaling proteins, and a number of oncoproteins. Most of the Hsp90 client proteins are involved in cell growth, differentiation, and survival, and include kinases, nuclear hormone receptors, transcription factors, and other proteins associated with almost all the hallmarks of cancer. Consistent with these diverse activities, genetic and biochemical studies have demonstrated the implication of Hsp90 in a range of diseases, including cancer, making this chaperone an interesting target for drug research.

During the last few decades, plant secondary metabolites have been studied as a major source for lead compounds in drug discovery. Recently, several plant-derived small molecules have been discovered exhibiting inhibitory activity towards Hsp90, such as epigallocatechin gallate, gedunin, lentiginosine, celastrol, and deguelin. In this work, an overview of plant secondary metabolites interfering with Hsp90 activities is provided.

Abbreviations

17-AAG:	17-allylamino-17-demethoxygeldanamycin
AhR:	aryl hydrocarbon receptor
Akt:	protein kinase B
BBM:	berbamine
CEP:	cepharanthine
CL:	celastrol
17-DMAG:	17-dimethylaminoethylamino-17-demethoxygeldanamycin
EGCG:	(−)-epigallocatechin-3-gallate
EGFR:	epidermal growth factor receptor 1
eNOS:	endothelial nitric oxide synthase
ERα:	estrogen receptor α
GDA:	geldanamycin
Her2:	human epidermal growth factor receptor 2
HIF-1α:	hypoxia-inducible factor-1α
HRI:	heme-regulated eIF2α kinase
HSF1:	heat shock factor-1
Hsp90:	heat shock protein 90
LPS:	lipopolysaccharide
Met:	mesenchymal-epithelial transition factor
NF-κB:	nuclear factor-kappaB
NSCLC:	non-small cell lung cancer
p-Akt:	phosphorylated protein kinase B
PAH:	polycyclic aromatic hydrocarbon
PI3K:	phosphotidylinositol-3-kinase
Raf-1:	proto-oncogene serine/threonine-protein kinase
SPR:	surface plasmon resonance
STAT3:	signal transducer and activators of transcription 3
TPH:	triterpenes from *Patrinia heterophylla*
VEGF:	vascular endothelial growth factor

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Introduction

Presently, the effective therapeutic interventions in cancer diseases still remain elusive, thus the identification of new possible molecular target(s) becomes crucial. In the last two decades, attention has been focused on the role of Hsp90, an evolutionary conserved molecular chaperone involved in the regulation, folding, stabilization, activation, and assembly of more than 200 "client" proteins, including apoptosis-related protein kinases, transcription factors, and signaling proteins, directly associated with most of the cancer hallmarks [1,2]. Structurally, Hsp90 is a flexible homodimer including three distinct domains: an *N*-terminal nucleotide-binding domain where the ATPase activity is mainly located, a middle client protein binding/ATP-hydrolysis regulating domain, and a *C*-terminus domain responsible for dimerization of the protein [3]. Hsp90 exists in different isoforms: two in the cytoplasm, Hsp90*α* (the heat shock-induced form) and Hsp90*β* (the constitutively active form), one localized at the endoplasmic reticulum (94KDa glucose-regulated protein, Grp94), one at the mitochondria (tumor necrosis factor receptor-associated protein 1, TRAP1), and finally the cell surface-bound, cell-released and cell-secreted Hsp90 collectively known as "extracellular Hsp90" (eHsp90). Several data reported in the literature suggest the eukaryotic cytosol Hsp90*α* to be the major form involved in cancer, also demonstrating that this isoform may have more diverse action than Hsp90*β* [4]. In the presence of stressed microenvironments, cancer cells undergo adaptive mutations that lead to an increment of Hsp90 activity. Therefore, in order to obtain a simultaneous disruption of multiple signaling pathways important for the growth and viability of cancer cells, and to induce cellular apoptosis and/or necrosis, the inhibition of the Hsp90 protein folding machinery could represent a multifaceted approach toward the tumor treatment [5–8]. Hsp90 inhibitors are grouped into numerous classes based on individual modes of inhibition which are as follows: i) obstructing the binding of ATP at the ATPase catalytic site, ii) interference in co-chaperone/Hsp90 interactions, iii) blocking the receptor interactions of client/Hsp90 alliances, and iv) intervention in the routes of post-translational modification in Hsp90 [9,10]. Regardless of the mechanism, a hallmark of Hsp90 inhibition is the degradation of its client proteins; the inhibition of this chaperone indeed leads Hsp90 client proteins to adopt an aberrant conformation, which triggers their ubiquitination, followed by proteasome-dependent degradation. Therefore, compounds able to downregulate the levels of Hsp90 client proteins are often considered promising Hsp90 inhibitor candidates.

Consistent with the notion that small molecules synthesized by the plant kingdom can be considered evolutionary chosen "privileged structures" since they have evolved in a natural selection process to achieve optimal interactions with biological macromolecules, natural molecules have shown an extraordinary potential as modulators of proteins functions and have been subjected to biological studies aimed at discovering their specific molecular targets and elucidating important signaling pathways [11]. Besides, many plant molecules have a crucial role as pharmaceuticals, according to the current number of drugs based on plant molecules in clinical trials or present in the market, and these molecules depict a source of new leads for drug discovery. Since Hsp90 is situated at the critical intersection of genotypes, environment, and development, it's reasonable to expect that natural products represent a fertile territory for the identification of new Hsp90 inhibitors. Most natural products active as Hsp90*α* inhibitors, such as radicicol, GDA, and 17-DMAG, are derived from fungi metabolism and possess the benzoquinone ansamycin (GA) or the resorcinol scaffold (radicicol). They are currently being evaluated in over 80 ongoing or completed clinical trials, offering a promising approach for the treatment of different cancer diseases [12,13].

However, there is still a wide requirement for new Hsp90 inhibitors, and the plant kingdom has been explored in order to find new promising compounds. This review provides a brief excursion of plant secondary metabolites interfering with Hsp90 activities and highlights findings regarding their possible molecular mechanism of action.

Flavonoids

Flavones and flavonols

Apigenin, chemically known as 4′,5,7,-trihydroxyflavone, is a common flavone widespread in plants of the Asteraceae family and in many fruits and vegetables. This flavonoid possesses various clinically relevant properties, such as antitumor and anti-inflammatory activities [14]. Among its anticancer effects, it has been reported to suppress metastasis, to inhibit cancer and cell proliferation, and to have antiangiogenic properties mediated by the alteration of different pathways [15]. Anyway, it has to be considered that the metabolic activity of the gut microflora on apigenin is often responsible for the modulation of its biological activity as a dietary compound, since it's catabolized into smaller phenolic compounds [16,17]. In a study focused on the investigation of the antiangiogenic effect of apigenin, it was found that it suppresses the expression of VEGF in endothelial cells via the degradation of HIF-1α protein mediated by interference with the Hsp90 chaperoning function [18]. Recently, the *in vitro* therapeutic potential role of apigenin for the treatment of multiple myeloma has emerged. Different biological techniques reveled that apigenin manifested antiproliferative activity in human multiple myeloma through CK2-mediated phosphorylation of Cdc37, disrupting the Hsp90/Cdc37/client complex via the proteasome pathway [19]. Moreover, its modulation of the chaperone Hsp90 induced the degradation of many kinase client proteins, including RIP1, Src, Raf-1, Cdk4, and Akt.

Chrysin (5,7-dihydroxyflavone) is a naturally occurring flavone found in the plant kingdom (*Passiflora caerulea* L. and *Passiflora incarnata* L., Passifloraceae) [20–22] endowed with many biological activities, such as anti-inflammatory, anticancer, and antioxidant [23,24]. Although chrysin is a promising antitumor agent, little information is known about its molecular mechanism. It was described that chrysin inhibits the expression of HIF-1α and VEGF in human prostate cancer DU145 cells [25]. In this study, it was reported that chrysin inhibits the expression of HIF-1α by multiple pathways, including Hsp90 modulation. In particular, it was able to inhibit the binding of HIF-1α to Hsp90, resulting in the suppression of HIF-1α expression [25].

Eupatilin, or 5,7-dihydroxy-3′,4′,6-trimethoxyflavone, is one of the bioactive components of *Artemisia asiatica* Nakai (Asteraceae) extracts, which are known to have anti-inflammatory, antioxidant, and antitumor effects [26]. In 2008, it was reported that it has anti-inflammatory activity by targeting the NF-κB pathway. The aim of this study was to gain a better understanding on the molecular mechanism of eupatilin-induced attenuation of intestinal inflammation induced by enterotoxigenic *Bacteroides fragilis* (ETBF) stimulation in an intestinal epithelial cell culture. In

particular, it was observed that this flavonoid reduces the activity of NF-κB and the expression of proinflammatory mediators through the dissociation of the complex Hsp90/IKK-γ [27].

Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a natural flavonoid contained in many medical herbs and vegetables (e.g., parsley, artichoke, celery, green pepper, and perilla leaf). In recent years, experimental evidences have shown that luteolin has multiple and interesting biological profiles: antitumor, anti-inflammatory, antioxidant, and radical scavenging properties, although the problem of its bioavailability should be considered. In light of the metabolic pathways of luteolin, *in vitro* studies should be carefully assessed [28–31]. The antitumor activity of this flavonoid has been reported *in vitro* against different kinds of tumor cells and also confirmed *in vivo* [30–32]. A recent study ascribed luteolin's proapoptotic effect on carcinoma cells to Hsp90 modulation [33]. In particular, it induces apoptosis of HeLa cells by preventing the association between Hsp90 and STAT3. The binding of luteolin to Hsp90 results in the degradation of Tyr^{705}- and Ser^{727}-phosphorylated STAT3 through the proteasome-dependent pathway as well as in the degradation of other Hsp90 client proteins such as Akt and IKK. Molecular modeling and SPR approaches suggested the interaction of luteolin with the *N*-terminal ATP-binding site, showing that it is able to prevent ATP-Hsp90 binding and to inhibit ATPase activity of this molecular chaperone [33]. In 2014, Hong et al. showed luteolin's efficacy as an antitumor agent in NSCLC and this effect is ascribed to the lysosome-dependent degradation of the EGF by inhibiting its interaction with Hsp90 [34]. Luteolin showed a potent anti-inflammatory property *in vitro,* and it reduced LPS-induced lethality in a mouse endotoxin shock model [35]. In addition, it was found to significantly reduce the LPS-induced release of high-mobility group B-1 (HMGB), a nonhistone chromosomal DNA-binding protein involved in the pathogenesis of several inflammatory diseases, both from mouse peritoneal macrophages and RAW 264.7 cells. Moreover, luteolin also inhibited high-mobility group B-1 translocation from the nucleus to the cytoplasma. This effect was correlated to the destabilization of c-Jun and Akt through the suppression of Hsp90 chaperone activity [35].

The naturally occurring flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a component of many common fruits and vegetables with multiple medicinal properties [36]. However, the actual bioavailability of this compound *in vivo* remains a controversial point in making an assessment of its biological importance [37]. Anti-inflammatory, antitumor, and antiproliferative activities were reported for quercetin [38–40]. Quercetin is able to prevent or retard tumor growth probably thanks to multifunctional effects, in particular, at the molecular level it, inhibits many ATP binding enzymes, especially kinases such as CDK-4 and PI3K [41]. Treatment with quercetin arrests the growth of cancer cells both *in vitro* and *in vivo*, and also exhibits tumor cell selectivity [42]. To date, it is well established that this flavonoid inhibits the heat-induced expression of heat shock proteins in many different cell types (such as human breast carcinoma cells and prostate cancer cells) by affecting HSF1 hyperphosphorylation, DNA binding, and transcriptional activity [43]. Furthermore, quercetin induces cancer cell apoptosis in three different prostate cancer cell lines by downregulating the levels of Hsp90 [44]. Further evidence of quercetin's ability to interact with this chaperone was furnished by a proteomic approach that revealed Hsp90 as a possible target of this flavonoid [45]. Finally, a downregulation of the level of Her-2/neu protein in Her-2/neu overexpressing human breast cancer SK-Br3 cells, in time- and dose-dependent manners, was induced by quercetin treatment. In particular, this effect was ascribed to the polyubiquitination of Her-2/neu and to an increase in interaction between Hsp90 and Her-2/neu [46].

Isoflavones

Derrubone, a prenylated isoflavone originally isolated from the Indian tree *Derris robusta* (Roxb ex DC) Benth. (Fabaceae) [47], was identified in 2007 as a new potent *C*-terminal Hsp90 inhibitor through a high-throughput screening assay of a large chemical library. This screening was based on the measure of Hsp90-dependent refolding of thermally denatured firefly luciferase which is catalyzed by the Hsp90 present in rabbit reticulocyte lysate [48]. Biological studies revealed that derrubone inhibits Hsp90 with an IC_{50} value of 0.23 ± 0.04 μM and possesses potent antiproliferative activity against two human breast cancer cell lines, MCF-7 and SkBr3, with IC_{50} values of 9 ± 0.70 and 12 ± 0.30 μM, respectively [48]. Cell-based assays showed that this natural compound causes degradation of Hsp90 client proteins, including Raf-1, Akt, Her2, and ERα, in a concentration-dependent manner and does not inhibit the ATPase activity of Hsp70. In addition, derrubone inhibits the interaction of Hsp90 and the oncogenic client protein Cdc37 (cell division cycle protein 37) with HRI in breast cancer cell lines, suggesting, therefore, a potential chemotherapeutic use in human breast cancer [49–51]. The effect of derrubone on the Hsp90 protein folding machinery is due to its ability to stabilize Hsp90 client interactions and to prevent the progression of the Hsp90/co-chaperone complex, containing bound client proteins through its cycle [48]. Soon after its discovery, a small collection of selected analogues was synthesized and evaluated for antiproliferative activity, exhibiting only a modest improvement in the biological activity over the natural product [52]. Recently, to further investigate the crucial structural features for Hsp90 inhibition, Mays and coworkers reported the design, synthesis, and biological evaluation of flavones and isoflavone chimeras of novobiocin and derrubone [53]. These studies revealed that the functionality at the 3-position of the isoflavone is essential for the modulation of Hsp90 and, moreover, suggest a different binding mode for the bicyclic ring system present in both natural compounds. In 2014, molecular docking studies performed on four different Hsp90 inhibitors, novobiocin, clorobiocin, EGCG, and derrubone, led to the identification of the specific ATP-binding residues of the *C*-terminal domain (Leu 665, Leu 666, and Leu 694) as the key amino acids involved in the ligand binding. Among these four natural inhibitors, derrubone showed the highest binding energy for the Hsp90 *C*-terminal domain [54].

Chalcones

Butein (3,4,2′,4′-tetrahydroxychalcone) is a chalcone found in the stem bark of cashews (*Semecarpus anacardium* L., Anacardiaceae), the heartwood of *Dalbergia odorifera* T. Chen (Fabaceae), and the traditional medicinal herbs *Caragana jubata* Pall. (Anacardiaceae) and *Rhus verniciflua* Stokes (Anacardiaceae) [55]. Butein exhibited pleiotropic properties, such as anti-inflammatory effects, in macrophages and adipocytes, probably by inhibiting the NF-κB and mitogen-activated protein kinase signaling pathways [56,57] and anti-fibrogenic [58] and anticancer activity [59]. Furthermore, it influences cell proliferation, apoptosis, and the cell cycle in many human tumors, including liver, breast, and pancreatic cancers, suggesting a significant antineoplastic therapeutic potential [60–62]. In a recent study, the antiproliferative activity of this chalcone was evaluated against two different

drug-resistant cancer cell lines, cisplatin-resistant ovarian cancer cells (A2780*cis*) and gefitinib-resistant NSCLC cells (H1975). However, even if butein moderately inhibited cell proliferation in a concentration-dependent manner, the Western blot analyses revealed a robust dose-dependent degradation of oncogenic Hsp90 client proteins, Her2, Met, Akt, EGFR, and a small induction of Hsp70 [63]. These results suggested that 3,4,2′,4′-tetrahydroxychalcone inhibits the Hsp90 chaperoning function and may represent a therapeutic agent to overcome drug resistance in cancer therapy. In this context, in 2014, the synthesis of some butein analogues and their biological evaluation against gefitinib-resistant NSCLC cells (H1975) provided a new bioactive compound with a more potent antiproliferative effect on H1975 cancer cells through Hsp90 inhibition [64].

Flavokawain B is a naturally occurring chalcone isolated from the extract of kava-kava [*Piper methisticum* (L.) G. Forst, Piperaceae], a native plant to the Pacific Islands used as a social drink and as a traditional remedy, and from *Alpinia pricei* Hayata (Zingiberaceae), a perennial rhizomatous native to Taiwan commonly used in traditional Chinese food and folk medicine [65]. The antitumor potential of the kava extract chalcones has been referred to epidemiological studies that correlate the consumption of kava root extracts in the Pacific Islands with a lower incidence of cancer [66,67]. Different studies have shown that flavokawains (flavokawains A, B and C) are apoptotic inducers and anticarcinogenic agents. Moreover, flavokawain B has been reported to induce apoptosis and to have potent antitumor effects both *in vitro* and *in vivo* against several carcinoma cell lines, including prostate, colon, bladder, oral, human osteosarcoma, lung cancer cells, and mesenchymal tumors such as synovial sarcoma and uterine leiomyosarcoma [68–72]. Taken together these data suggest that multiple pathways are involved in flavokawain B-mediated antiproliferative activitity. In particular, recent findings have demonstrated that this promising chalcone inhibits the growth of geftinib-resistant NSCLC, H1975, with an IC_{50} value of 33.5 µM and its antiproliferative activity is correlated to Hsp90 inhibition [73]. Flavokawain B induces the downregulation of EGFR, Met, Her2, Akt, Cdk4, and Hsp90 client proteins and upregulates the cellular level of Hsp70 in a concentration-dependent manner, disrupting the Hsp90 protein folding machinery.

Licorice root has been used in traditional medicine for the treatment of diverse pathological conditions such as bronchial asthma, gastric ulcer, and inflammation [74]. One of the major and biogenetically characteristic chalcones isolated from the root of Xinjiang liquorice, *Glycyrrhiza inflate* Batalin (Fabaceae), is licochalcone A, which is well known for its biological properties [75]. The antitumor activity of licochalcone A has been discovered against gastric cancer cells and in androgen-independent PC-3 prostate cancer cells by cell cycle arrest and apoptosis induction [76,77]. Furthermore, licochalcone A exerted potent anti-inflammatory effects in *in vitro* and *in vivo* models induced by LPS [78]. In 2013, from a screening program of natural compounds for the identification of new potential Hsp90 inhibitors, licochalcone A was found to inhibit, with a modest potency (IC_{50} = 50 µM), the growth of the H1975 cancer cell line and to induce the degradation of Hsp90 client proteins such as the signal-transducing proteins Akt, Her2, EGFR, and Met [78]. In this study, a docking pose of licochalcone A bound to the *N*-terminal ATP binding site of human Hsp90 was also proposed.

Catechins

Catechins are the major constituents in green tea [*Camellia sinensis* (L.) Kuntze, Theaceae] (30–42% of dried weight). EGCG particularly is one of the most abundant polyphenols (50% of total catechins) and is well known for its antioxidant properties, which is crucial for its preventing activity in cancer and cardiovascular diseases. EGCG has been extensively studied for its capability to inhibit cell proliferation and induce apoptosis in several human cancer cells [79]. In the last decades, many different target and signaling pathways have been demonstrated as a partner of EGCG in various cell lines. The first report on EGCG binding to Hsp90 was in 2005 when Palermo et al., studying the AhR gene transcription, demonstrated that it directly binds Hsp90 to the *C*-terminus of the chaperone, resulting in a conformational change responsible for a modification of the Hsp90-AhR interaction, leading to the inhibition of AhR transcriptional activation. EGCG seems to interact with the XAP2-bound Hsp90 complex [80]. In 2009, Yin and coworkers studying the binding site of EGCG with Hsp90. Using ATP and novobiocin as reference compounds, they demonstrated that EGCG protects a *C*-terminus Hsp90 fragment from trypsine-catalized lysis [81]. They also stated that EGCG binds at/or near the *C*-terminal ATP binding site on Hsp90 producing the block of chaperone dimerization [81]. In this study, it was also established that EGCG stabilizes the association of Hsp70, Cyp40, and XAP-2 to Hsp90. In this way, EGCG could stabilize the Hsp90 co-chaperones complex. In the same year, Li and coworkers, using a proteolytic fingerprint assay, confirmed that EGCG protected the *C*-terminus from cleavage by high concentrations of trypsin [82]. Thus, EGCG impairs the association of Hsp90/Hsp70 and Hsp90/p23 by directly binding to the *C*-terminal region, inhibiting the Hsp90 chaperoning function. One year later, other authors carrying out an EGCG-conjugated Sepharose 4B beads pull-down assay confirmed that Hsp90 interacts efficiently with the molecule, stating also that EGCG competes with ATP for binding of the ATPase domain of Hsp90 in MCF7 cell lines. They also studied the expression of Hsp90 both *in vitro* and *in vivo*. The immunohistochemistry assay showed that the levels of Hsp90 were decreased in EGCG-treated mice compared with the control [83]. Therefore, evidences on the Hsp90 EGCG binding site are in disagreement. The ability of the EGCG Hsp90 function may not be the only mechanism whereby EGCG exerts its antitumoral activity, as many EGCG targets have been reported [84,85]. Its interaction with Hsp90 may be a common mechanism for several of the proposed targets. Many of the proteins reported to bind to EGCG are Hsp90 client proteins or closely related to this chaperone.

Another polyphenol constituent of green tea, (−)-epicatechin, was reported to induce the activation of eNOS in calcium depleted HCAE cells in an Akt- and Hsp90-dependent manner [86]. Black tea polyphenol phytocomplex, theaflavins, and thearubigins, also extracted from tea, downregulated the expression level of Hsp90 in human leukemic U937 and K562 cells. Following the treatment of these cells with the Hsp90 inhibitor 17-AAG alone or in combination with theaflavins and/or thearubigins, the authors observed the downregulation of the expression of p-Akt, cD1, and cDK2. These data supported the hypothesis that the inhibition of Hsp90 by these polyphenols caused the downregulation of its client proteins [87].

Biflavonoids

SPR analysis was used to evaluate the ability of the biflavonoids isolated from *Daphne linearifolia* Hart. (Thymelaceae) to interact with Hsp90. These compounds interacted with the immobilized protein with a K_D in the μM range, and sensible differences in their affinity towards Hsp90 were observed. Among biflavonoids, 2″-hydroxygenkwanol A showed the best affinity towards Hsp90 (K_D 0.5 ± 0.10 μM) compared to the other tested compounds. The presence of a hydroxyl group on C-2″ increases the stability of the biflavonoid/Hsp90 complex about three times [88].

Coumarins

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Since 2000, when the fungal aminocoumarins novobiocin was identified as a new type of Hsp90 inhibitor acting with a mechanism clearly different from that of classical anti-ATPase inhibitors such as GDA and radicicol [89], plant coumarins were screened in order to identify further Hsp90 modulators. However, such a large effort produced poor results, and actually only one plant coumarin can be undoubtedly considered an Hsp90 inhibitor: GUT-70. It is a tricyclic coumarin extracted from the stem bark of *Calophyllum brasiliense* Britton (Calophyllaceae) showing significant and specific cytotoxicity towards human leukemic cells [90] and proapoptotic effects on mantle cell lymphoma cell lines. These anticancer activities can be, almost partially, ascribed to GUT-70's ability to interact with Hsp90, as inferred by specific competition studies, and to induce intracellular level reductions for some Hp90 client proteins, such mt-p53, Raf-1, cyclin D1, and Akt [91].

The only other report on plant isolated coumarin interaction with Hsp90 is the K_D (~ 0.20 μM) of the dicoumarinyl ether glycoside-Hsp90 interaction obtained through SPR analysis by Malafronte et al. in 2012. This result was expected on the basis of the well-know affinity of novobiocin and its derivatives towards Hsp90 [88].

Anthraquinones

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Anthraquinones are well known for their action on the gastrointestinal system and for their estrogenic activities. However, some interesting findings have been reported also on the antiproliferative and antiangiogenic effects, which could be related to Hsp90 inhibition [92]. Aloe-emodin, a natural anthraquinone purified from different aloe latex, was shown to have antiproliferative effects on breast cancer cell proliferation by downregulating ERα protein levels. This downregulation was due to the inhibition of interaction of ER with Hsp90, leading to a significant increase of ER ubiquitination and proteasomal degradation [93]. Interestingly, the related compound emodin had no effect on Hsp90/ER binding. On the other hand, the semisynthetic derivative emodin azide methyl anthraquinone strongly inhibits the interaction of Hsp90 with several client proteins. In particular, it induces proteasomal degradation of the Her2/neu protein, a transmembrane tyrosine kinase well known to be associated with a poor prognosis in breast cancer, preventing its interaction with Hsp90 [94].

Also rhein, the primary anthraquinone in the roots of *Cassia alata* L. (Fabaceae), was described as a promising antitumoral agent, but its effect seems to be mainly related to the inhibition of tumor-induced angiogenesis by suppressing the activation of PI3K, p-Akt, and phosphorylated extracellular signal-regulated kinase. However, an in-depth investigation on its mechanism of action in breast cancer cell lines revealed that rhein induces proteosomal degradation of some Hsp90 client proteins, such as NF-κB and Her-2, thus suggesting this anthraquinone as a direct inhibitor of Hsp90 [95].

Tannins

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The ellagitannin geraniin, firstly isolated from the Japanese medicinal plant *Geranium thunbergii* Siebold ex Lindl. & Paxton (Geraniaceae), has emerged from an SPR-based screening of a small library, including different plant polyphenols, as an efficient ligand of Hsp90 (K_D 415 ± 2.70 nM) [96]. On the basis of this first evidence, the authors carried out several biological and biochemical assays, demonstrating that geraniin dose-dependently inhibits ATPase and chaperone activities of Hsp90. Moreover, the level of the client proteins c-Raf, pAkt, and EGFR was strongly downregulated in two cancer cell lines (HeLa and Jurkat) treated with different doses of geraniin (from 0.5 to 10 μM). Preliminary, a structural investigation performed on the Hsp90/geraniin complex suggested the *N*-terminal region of the protein to be involved in geraniin binding [96]. Geraniin has been shown by different authors to possess several biological activities: it suppresses A549 cancer cells proliferation, arresting the cell cycle in the S phase [97], negatively modulates the expression of carbonic anhydrase II mRNA in osteoclasts [98,99], attenuates radiation-induced damage in mice splenocytes by reducing DNA breakage and apoptosis [100], and inhibits human enterovirus 71 replication [101]. Interestingly, most of these activities could depend on geraniin's ability to inhibit Hsp90 function. However, the biological effects of tannins usually depend on their grade of polymerization and solubility. Highly polymerized tannins exhibit low bioaccessibility in the small intestine and low fermentability by colonic microflora. Therefore, the low bioavailability of this class of compounds should be considered [102].

Terpenes

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Monoterpenes

Picrocrocin is a monoterpene glycoside constituent of saffron (*Crocus sativus* L., Iridaceae) stigmas, and it's a precursor of safranal. In a study to attempt the identification of the antitumor therapeutic target of saffron, picrocrocin was subjected to a comparative analysis based on two different reverse screening approaches, i.e., reverse docking system based on an id Target and the reverse pharmacophore mapping strategy based on a PharmMapper system. Results suggested that picrocrocin acts as a competitive inhibitor in the ATPase site of Hsp90α, revealing that an electrostatic interaction and hydrogen bonds facilitate the binding of the molecule in the ATPase catalytic site of the enzyme and can possibly act as a competitive inhibitor and a potential antitumor drug [103].

Iridoids

In a target-oriented screening on a plant molecule library for putative Hsp90 inhibitors, the class of iridoids was selected as one chemical scaffold. Iridoids, secoiridoids, and C_9-type iridoids were screened, and only the latter showed a promising inhibitory activity. These results motivated the authors to isolate C_9-type iridoids from two plants belonging to the Bignoniaceae family,

Tabebuia argentea Britt. and *Catalpa bignonioides* Walter. A SPR-based binding assay was performed to study the binding of C_9-type isolated iridoids to Hsp90. Four monomers, catalposide, specioside, 3,4-dihydrocatalposide, and 6-*O*-*p*-hydroxybenzoyl-5,7-bisdeoxycynanchoside, and two dimers, named argenteoside A and B, were demonstrated to bind the chaperone [104]. Argenteoside A and B showed a good affinity towards Hsp90, having K_D values measured for the interaction with the chaperone of K_D 14 ± 4.00 nM and 50 ± 9.00 nM, respectively, comparable to that of radicicol (4.20 nM), used as a reference compound. Moreover, these compounds showed a kinetic dissociation constant (k_d) notably lower than those measured for the monomers, indicating that the Hsp90/dimeric iridoids complexes were extremely stable. Thus, a panel of chemical and biological approaches was used to characterize the most active iridoid argenteoside A inhibitory activity. ATPase assay, citrate synthase aggregation assay, and cell-based studies were carried out to deepen the study of the interaction between argenteoside A and the enzyme, suggesting that it efficiently inhibited Hsp90 ATPase and chaperone activity, downregulating Hsp90α client proteins without inducing any increase in Hsp70 levels. The structural characterization of the Hsp90-argenteoside A complex was obtained by limited proteolysis mass spectrometry-based strategy analysis using trypsin and chemotrypsin as proteolytic probes. A comparison between the cleavage sites of Hsp90 and the Hsp90-argenteoside A complex demonstrated that the *N*-terminal domain and a middle domain of the chaperone were involved in the molecule binding. To obtain more details about a possible binding mode of argenteoside A on Hsp90, a molecular modeling analysis was also performed. Achieved results were refined taking into account the limited proteolysis experimental data. This last study supported the view that argenteoside A interacts with the *N*-terminal domain as disclosed by limited proteolysis analysis. C_9-types iridoids may be considered a new promising chemical scaffold for an Hsp90 inhibitors drug discovery program [104].

Recently, oleocanthal, an olive oil phenolic component, has attracted increasing interest for its biological effects. This molecule was reported to interfere with a number of pathways related to inflammation and Alzheimer's disease [105]. Margarucci and coauthors, to obtain a comprehensive identification of oleocanthal interactome, applied a mass spectrometry proteomic approach. The study of oleocanthal putative partner in HeLa and U937 cell lysates revealed Hsp90 as a unique shared target. Oleocanthal was found to strongly inhibit the Hsp90 ATPase activity in a concentration-dependent manner, similar to radicicol. A molecular docking analysis suggested a potential covalent reactivity between the compound and the chaperone. This interaction was also analyzed by detailed MS analysis. The authors suggested that the correct positioning of oleocanthal in the Hsp90 binding site was induced by van der Waals and hydrophobic interactions, and is followed by covalent modifications at Lys112 and Lys58. Moreover, oleocanthal influenced the Hsp90 oligomeric state by inducing a chaperone conformational change with no covalent oleocanthal cross-linked species between Hsp90 monomers. Anyway, oleocanthal did not influence the regulation of Hsp90 and Hsp70 [106].

Sesquiterpenes

Among sesquiterpenes, terpenes with a C_{15} carbon atom skeleton, two compounds isolated from plants of the Zingiberaceae family, were found to interact with Hps90. *β*-Elemene is an active component of the medicinal plant *Curcuma wenyujin* Y. H. Chen & C. Ling (Zingiberaceae) and antagonizes glioblastoma cells by inducing apoptosis. Zhao et al. found that *β*-elemene disrupted the Hsp90/Raf-1 complex, hypothesizing that an alkylating agent could antagonize the molecular chaperone function of Hsp90 (that is dependent on its conserved spatial conformation), causing its misfolding and unbinding to Raf-1 [107]. Deactivation of Raf-1 consequently inhibited the Raf/MEK/ERK pathway, leading to apoptosis of glioblastoma cells.

Zerumbone, isolated from *Zingiber zerumbet* Smith (Zingiberaceae), was reported to exert many bioactivities such as cancer preventive, anti-inflammatory, and detoxifying actions. Ohnishi et al. studying zerumbone's possible mechanism of action in hepa1c1c7 mouse hepatoma cells found that this compound bound to proteins through its *α, β*-unsaturated carbonyl group. The authors hypothesized that zerumbone-modified proteins were recognized by Hsp90 for heat shock response induction [108].

Diterpenes

Andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide are the major labdane diterpenoids isolated from *Andrographis paniculata* (Burm.f.) Wall ex Nees, an herbaceous plant belonging to the Acanthaceae family, that have exhibited a variety of pharmacological activities [109]. Andrographolide possesses interesting *in vitro* and *in vivo* anti-inflammatory (asthma, stroke, and arthritis) and anticancer effects [110, 111]. Indeed, it suppresses the secretion of cytokines, chemokines, and inflammatory enzymes (iNOS and COX) from macrophages [112]. In these years, many studies have reported andrographolide as a very promising compound for cancer treatment, and several molecular mechanisms and potential molecular targets have been proposed for its antitumor effect [111]. A number of reports showed that andrographolide and its analogues induced cell cycle arrest and promoted apoptosis in human cancer cells by interfering with various cell signaling pathways. In a recent study, Liang and coworkers demonstrated that this natural compound suppresses viral sarcoma-induced epithelial cellular transformation by promoting viral sarcoma protein degradation [113]. These findings provide evidence that andrographolide exerts its anticancer action by a novel mechanism involving the degradation of sarcoma oncoprotein. In 2014, to further investigate its biological activity, using proteomics and Western blot approaches, they proposed a novel mechanism for the suppression of cancer cell malignancy induced by andrographolide through the inhibition of the Hsp90 chaperone machinery and depletion of Hsp90-dependent client proteins. Finally, a treatment with andrographolide induced Hsp90 cleavage, downregulated the client protein Bcr-Abl, and inducted apoptosis in K562 cells [114].

Teucrin A, a furan-containing diterpenoid, is a major constituent of the neo-clerodane diterpenoid fraction of the hydroalcoholic extract of germander (*Teucrium chamaedrys* L., Lamiaceae). Among the broad range of protein targets affected by teucrin A treatment in the rat liver, the cytosolic Hsp90 was found as a target of the activated diterpenoid [115].

Rosmarinic acid, carnosic acid, and carnosol are the three major bioactive constituents in rosemary leaves (*Rosmarinus officinalis*, L. Lamiaceae) responsible for the antioxidant, anti-inflammatory, and anticancer properties. Carnosol is contained in zyflamend, a commonly used dietary supplement that contains extracts of ten common herbs including rosemary (*R. officinalis*), turmeric (*Curcuma longa* L., Zingiberaceae), ginger (*Zingiber officinale* Roscoe, Zingiberaceae), holy basil (*Ocimum sanctum* L., Lamiaceae), green tea (*C. sinensis*), hu zhang (*Polygonum cuspidatum* Sieb. & Zucc.,

Polygonaceae; a source of resveratrol), Chinese goldthread (*Coptis chinensis* Franch., Ranunculaceae), barberry (*Berberis vulgaris* L., Berberidaceae), oregano (*Origanum vulgare* L., Lamiaceae), and baikal skullcap (*Scutellaria baicalensis* Georgi, Lamiaceae). The inhibition of Hsp90 ATPase activity induced by carnosol and zyflamend has been reported [116]. In this study, both carnosol and zyflamend inhibited Hsp90 function leading to a rapid reduction in AhR levels, an Hsp90 client protein that is strongly involved in PAH-induced carcinogenesis. Tobacco smoke, a source of PAHs, activates the AhR, leading to the improved transcription of CYP1A1 and CYP1B1, which encode proteins that convert PAHs to genotoxic metabolites. Through the inhibition of Hsp90 ATPase activity, carnosol induces a decrease in AhR protein levels, suppresses PAH-mediated activation of CYP1A1 and CYP1B1, and represses mutagenesis [116,117]. These results suggest that the antitumor effects of rosemary herbs may be attributed to the inhibition of Hsp90 mediated by carnosol.

The bioactive diterpene tanshinone IIA, isolated from *Salvia miltiorrhiza* Bunge (Lamiaceae), has been used in the treatment of cardiovascular and metabolic disorders [118]. eNOS uncoupling plays a causal role in endothelial dysfunction in many cardiovascular and metabolic diseases, and it was found that this natural product acts by interacting with the NO pathway. Recently, a study addressed to investigate its role in oxidative stress-related signaling disclosed the effect of tanshinone IIA on the expression of Hsp90 [118].

Limonoids

Gedunin, a tetranortritepenoid (limonoid) isolated from Indian neem (*Azadirachta indica* A. Juss., Meliaceae), is well known for its antimalarial and insecticidal activities, and has demonstrated anticancer activity. This activity was firstly explored through the use of the gene expression signatures, a method applied to connect small molecules, genes, and diseases. With this technique, Lamb et al., in 2006, found that gedunin exhibited its antiproliferative activity through Hsp90 modulation [119]. Later, the same authors found that gedunin inhibited androgen receptor-mediated signaling and a gene expression-based approach was used to predict that it acts as an Hsp90 pathway inhibitor [120]. At that time, the only known mechanism of action of Hsp90 inhibitors was the one demonstrated for 17-AAG and GDA at the *N*-terminal ATP-binding pocket, while a new mechanism outside the *N*-terminal was proposed for gedunin. In fact, this tetranortriterpene was found to promote the Hsp90-dependent client protein similarly to other Hsp90 inhibitors, while, contrary to the other inhibitors, it was unable to displace GDA in a fluorescence polarization assay with Hsp90. In an effort to probe gedunin's mechanism of action, 19 semisynthetic derivatives were prepared and their antiproliferative activity was determined. No compound was found to be more effective than the natural compound [121] and gedunin could be considered an Hsp90 inhibitor that didn't interact directly with the chaperone.

Taking into account Hsp90-gedunin inhibitor activity and that limonoids are a relatively small group of tetranorterpenoids typical of Meliaceae, Dal Piaz et al. started a phytochemical investigation of plants belonging to this family. Extraction and chromatographic separation of *Trichilia emetica* ssp. *suberosa* JJ de Wilde and *Pseudrocedrela kotschyi* (Schweinf.) Harms root extracts led to the isolation of 16 compounds. Two phragmalin limonoids, kotschyin A and kotschyin D from *P. kotschyi*, interacted with the immobilized protein by an SPR study. Among them, kotschyin D exhibited a K_D of 0.36 ± 0.04 µM, comparable to that measured for 17-DMAG (K_D 0.39 ± 0.07 µM). Combining limited proteolysis and molecular docking with biochemical and cellular studies, kotschyin D was demonstrated to be a client-selective inhibitor of the chaperone, binding to the Hsp90 middle domain. Moreover, observations on the effects of cell incubation with kotschyin D on the level of different Hsp90 clients inferred the hypothesis that this molecule reduces the efficiency of the chaperone towards specific client proteins by preventing its interaction with the co-chaperone Aha1 [122].

Recently, the same authors isolated 14 limonoids from *A. indica* leaves collected in Venezuela. An SPR-based study was used to study the interaction between these limonoids and Hsp90. Among them, deacetylsalannin and 1,3-diacetylvilasinin showed a capability to bind Hsp90 with an affinity higher than that obtained for the 17-AAG. The results obtained showed that the Hsp90 inhibitory effect of these limonoids is strongly related to different structural features of the side chains [123].

Triterpenes

The quinone methide triterpene CL belongs to a small category of plant secondary metabolites that possess a broad range of biological activity. It was isolated from *Tripterygium wilfordii* Hook. f. (Celastraceae) root extract (Thunder God Vine), a remedy used for inflammatory and autoimmune diseases in Oriental traditional medicine [124]. Different evidences showed that in the CL structure, carbons C-2 and C-6 on the A and Bring, respectively, exert a high susceptibility towards a nucleophilic attack, forming Michael adducts with nucleophilic groups of proteins. Analogously to many plant molecules, this property seems to be the major mechanism by which CL modulates the activity of a variety of proteins [125]. Over recent years, CL has attracted many researchers attention through its diverse biological activities [126–131]. Hieronymus and coauthors, in order to investigate the molecular target of CL, applied a gene-based expression analysis to connect its activities to other known drugs, demonstrating that it inhibits Hsp90 functions [120]. Several investigations on the effects of CL-Hsp90 interaction have been successively performed. The effect of CL on many Hsp90 clients, including some kinases, was confirmed in many cancer cell line studies by different authors, reporting the downregulation of Hsp90 clients such as Akt-1, NF-κB, Raf-1, Cdks, ERK, EGFR, FcεRI, and PKCδ [131–137]. Zhang and coworkers studied the effect of CL on different transcription factors, one being the subpopulation of Hsp90 clients, in three human cancer cell lines (MCF7, HepG2, THP-1), observing the protein levels and showing that this compound affects many nuclear factors in a cell-type and dose-dependent way. CL showed a capability of blocking the binding of Hsp90 and its co-chaperones (Cdc37, P23, Hop), resulting in antitumoral activity [125,138,139]. The molecular mechanism of the CL-Hsp90 interaction has been extensively investigated. Zhang et al. studied the effect of CL on the Hsp90 interaction and ATPase inhibition, suggesting that it binds to the *N*-terminal domain, and that this is the site of Cdc37 and ATP binding [138,140]. On the other hand, trypsinolysis studies suggested that CL binds to the *C*-terminal domain of the chaperone. Some studies have reported the inhibition of Hsp90 ATPase activity [131], while others demonstrated that CL does not affect the ATPase activity of the chaperone [138,141]. Other authors demonstrated that CL disrupts the Hsp90/Hop complex [142], but others stated that it has no effect on Hop [138]. Recently, Zanphorlin and coworkers, based on these conflictual evidences, performed a characterization of the CL-Hsp90 interaction using both biochemical and biophysical

techniques [143]. These authors suggested a model in which CL binds to the *C*-terminal domain, causing oligomerization of the chaperone. CL may act primarily by inducing specific oligomerization that inhibits some, but not all, of the Hsp90 functions. Definitively, the characterization of the CL-Hsp90 molecular interaction needs further studies.

In a study focused on the observation of protein changes caused by betulin, a representative triterpene of *Betula platyphylla* Sukaczev (Betulaceae), Pyo et al. demonstrated that Hsp90 was downregulated in betulin-treated human lung cancer A549 cells. They also confirmed that betulin induced apoptosis by up/downregulations of different proteins by means of 2D SDS PAGE coupled with nano-HPLC tandem mass spectrometry [144].

Triterpenes have been identified as the major active constituents of *Patrinia heterophylla* Bunge (Valerianaceae), a native herb to China used as an antitumor herb in traditional Chinese medicine. To elucidate the antitumor mechanism of these compounds, a proteomic analysis was carried out by TPH treatment in K562 cells. According to their previous phytochemical analysis, hederagenin, friedelin, ursolic acid, oleanolic acid, canophyllol, oleanolic acid 3-*O*-α-L-arabinopyranoside, α-amyrin, and β-amyrin are the main components of TPH [145]. The authors found that TPH downregulated the expression of Hsp90α in K562 cells, so triterpenes could be responsible of this activity, but further investigations are needed to explain these evidences [146].

Alkaloids

The wide range of biological activities shown by alkaloids encouraged researchers to evaluate their effects on Hsp90 activity and/or expression in tumor cells in order to identify new potent and multitarget compounds. Promising results have been achieved by some *bis*-coclaurine alkaloids such as CEP and BBM. Using different chromatographic approaches, Haginaka and coworkers [147] demonstrated that CEP, an alkaloid mainly extracted from *Stephania cepharantha* Hayata (Menispermaceae) and widely used in Japan to treat several diseases [148], interacts with the Hsp90 middle domain, and a 5.3 µM dissociation constant was measured for this interaction. These authors also obtained a similar result investigating the Hsp90 interaction with BBM, purified from several plants belonging to the *Berberis* genus (Berberidaceae), and reported it as promising anticancer agent [149–152]. Moreover, it was also demonstrated that BBM treatments induce leukemia cell apoptosis, interfering with different metabolic pathways and significantly reducing the Hsp90 intracellular levels [153].

An efficient interaction with the Hsp90 middle domain was also observed for lentiginosine [154], a hydroxylated indolizidine alkaloid originally extracted from the leaves of *Astragalus lentiginosus* Douglas ex Hook (Fabaceae) and described as a potent inhibitor of the fungal α-glucosidase amyloglucosidase [155]. A multidisciplinary study demonstrated that the binding of this iminosugar to the chaperone is enantioselective, with only the (+)-lentiginosine being able to interact with Hsp90 with a high affinity ($K_D = 24.76 \pm 0.60$ nM); besides, it was able to significantly affect chaperone and ATPase activities of the protein *in vitro* [154].

Miscellaneous

Gambogic acid, a component of the exudate of *Garcinia harburyi* Hook. f. (Clusiaceae), has been demonstrated to possess antitumor and anti-inflammatory activities and has entered phase I clinical trials in China as an anticancer agent [156]. This compound was firstly shown to interact with Hsp90 in 2010. In fact, gambogic acid inhibits HeLa cell proliferation with an IC_{50} of 0.69 ± 0.22 µM, which correlates with the downregulatation of the TNF-α/NF-κB signaling pathway [157]. Later, a high-throughput screening of a natural product library for the discovery of new Hsp90 inhibitors identified gambogic acid as an antitumor agent that binds to the *N*-terminal domain of Hsp90 [158]. In this study, gambogic acid emerged for its ability to inhibit the Hsp90-dependent refolding of luciferase. Biological assays on gambogic acid displayed its ability to inhibit the proliferation of different cancer cell lines (HeLa, MCF7, and SK-Br3) in a concentration-dependent manner, and induced degradation of Hsp90-dependent proteins Her2, Akt, and Raf-1 in cultured cells [158]. This promising anticancer agent also disrupted the interaction of Hsp90, Hsp70, and Cdc 37 with the HRI *in vitro*. In addition, by SPR analysis and virtual docking approaches, Davenport and coworkers indicated that gambogic acid binds to the *N*-terminal domain of Hsp90, and it is not able to displace GDA, suggesting its interaction with a distinct binding site on the Hsp90 *N*-terminal pocket [158].

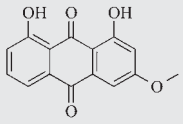
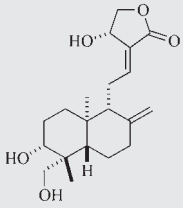
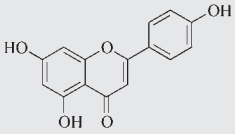
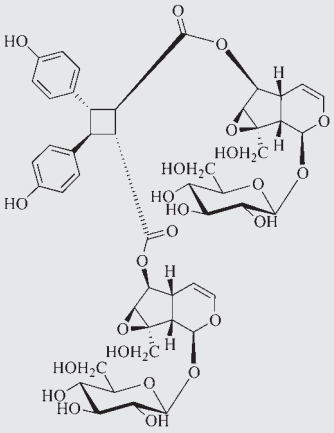
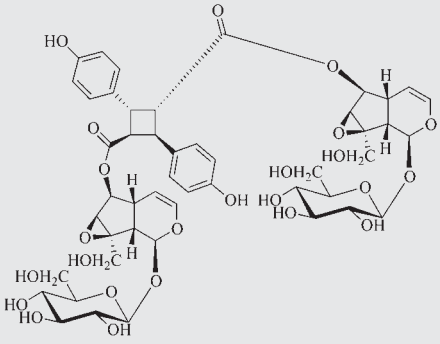
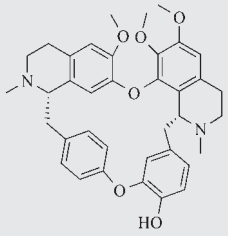
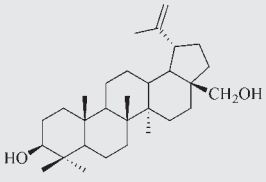
Conclusions

In this review, plant molecules interfering with Hsp90 activities (**Table 1**) and outlining findings on their molecular interaction with the chaperone are presented. The identification of new compounds to modulate Hsp90 activities is still a crucial challenge for biomedical research. In the last ten years, many putative Hsp90 inhibitors have been reported, including natural compounds, but actually only a limited number of plant secondary metabolites showing promising effects have been described. However, many of these plant compounds inhibit Hsp90 by mechanisms definitively different from those demonstrated for the classic inhibitors interacting with the Hsp90 ATP binding site; this is the case of the prenylated isoflavone derrubone, the terpenoids kotshin D and CL, and the alkaloids CEP and lentiginosine. In fact, the first is able to over-stabilize the binding between the chaperone and some of its clients, the second prevent the interaction of Hsp90 with its co-chaperones Aha1 (kotshin D), Cdc37, P23, and Hop (CL), while the third bind the Hsp90 middle domain, thus affecting its interaction with several client proteins, respectively. The peculiarity of the mechanism of action demonstrated by these compounds makes them suitable leads for the design of new therapeutic agents, or chemical probes allowing for the in-depth study of the biochemistry of Hsp90, a chaperone machine which plays a key role in many pathological and physiological processes.

Conflict of Interest

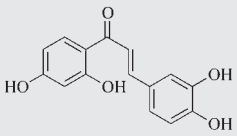
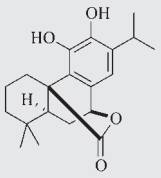
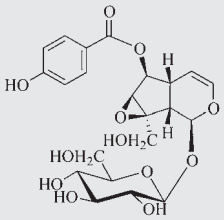
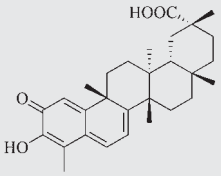
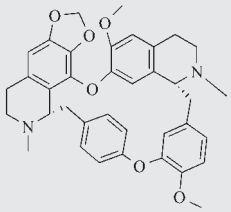
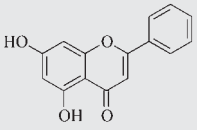
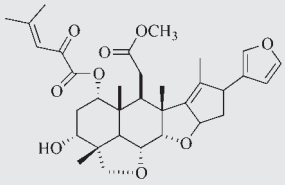
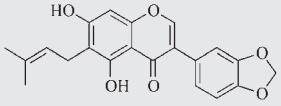
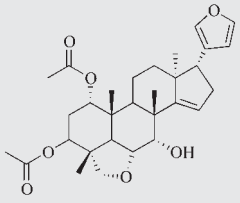
The authors declare no conflict of interest.

Table 1 Plant secondary metabolites interfering with Hsp90 activities.

Compound	Structure	Origin	References
Aloe-emodin		*Aloe vera* (L.) Burm. f. (Aloaceae)	[93, 94]
Andrographolide		*Andrographis paniculata* (Burm. F.) Wall. Ex Nees (Acanthaceae)	[109–114]
Apigenin		Asteraceae family	[14–19]
Argenteoside A		*Tabebuia argentea* Britt. (Bignoniaceae)	[104]
Argenteoside B		*Tabebuia argentea* Britt. (Bignoniaceae)	[104]
Berbamine		*Berberis* ssp. (Berberidaceae)	[149–153]
Betulin		*Betula platyphylla* Sukaczev (Betulaceae)	[144]

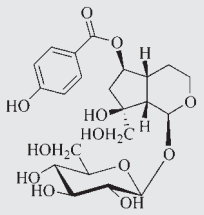
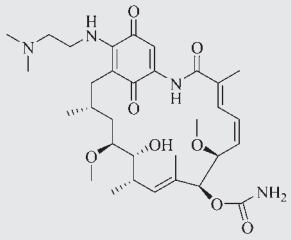
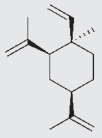
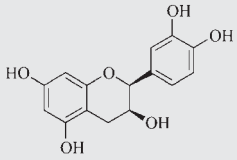
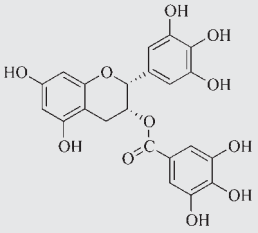
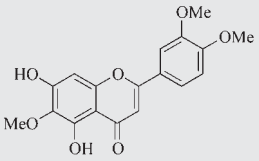
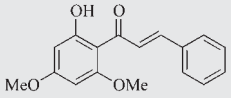
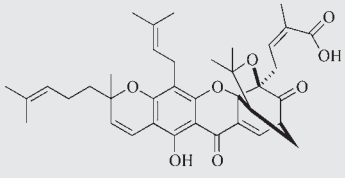
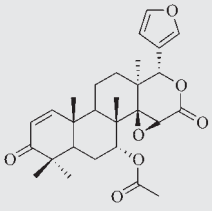
cont.

Table 1 *Continued*

Compound	Structure	Origin	References
Butein		*Semecarpus anacardium* L., *Caragana jubata* Pall., *Rhus verniciflua* Stokes (Anacardiaceae), *Dalbergia odorifera* T. Chen (Fabaceae)	[55–64]
Carnosol		*Rosmarinus officinalis* L. (Lamiaceae)	[116, 117]
Catalposide		*Tabebuia argentea* Britt. (Bignoniaceae)	[104]
Celastrol		*Tripterygium wilfordii* Hook.f. (Celastraceae)	[124–143]
Cepharanthine		*Stephania cepharantha* Hayata (Menispermaceae)	[147, 148]
Chrysin		*Passiflora* ssp. (Passifloraceae)	[20–25]
Deacetylsalannin		*Azadirachta indica* A. Juss. (Meliaceae)	[123]
Derrubone		*Derris robusta* (Roxb ex DC) Benth (Fabaceae)	[47–54]
1,3-Diacetylvilasinin		*Azadirachta indica* A. Juss. (Meliaceae)	[123]

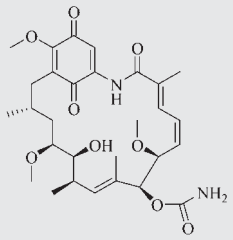
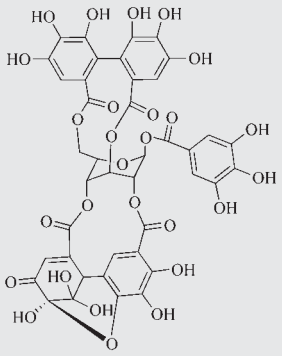
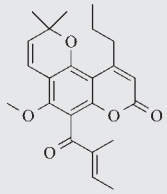
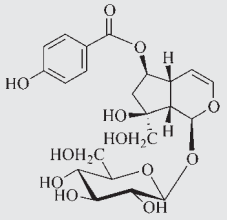
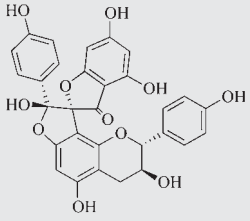
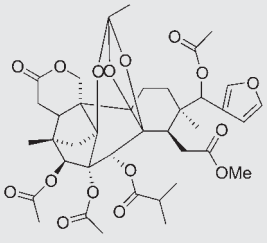
cont.

Table 1 *Continued*

Compound	Structure	Origin	References
3,4-Dihydrocatalposide		*Catalpa bignonioides* Walter (Bignoniaceae)	[104]
17-DMAG		Semisynthetic derivative	[12, 13]
β-Elemene		*Curcuma wenyujin* Y. H. Chen & C. Ling (Zingiberaceae)	[107]
(−)-Epicatechin		*Camellia sinensis* (L.) Kuntze (Theaceae)	[86]
(−)-Epigallocatechin-3-gallate		*Camellia sinensis* (L.) Kuntze (Theaceae)	[54, 79–85]
Eupatilin		*Artemisia asiatica* Nakai (Asteraceae)	[26, 27]
Flavokawain B		*Piper methisticum* (L.) G. Forst (Piperaceae), *Alpinia pricei Hayata* (Zingiberaceae)	[65–73]
Gambogic acid		*Garcinia harburyi* Hook.f. (Clusiaceae)	[156–158]
Gedunin		*Azadirachta indica* A. Juss. (Meliaceae)	[119–121]

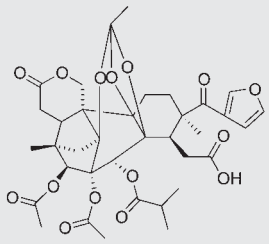
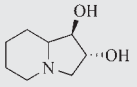
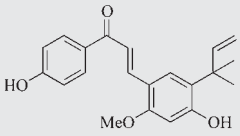
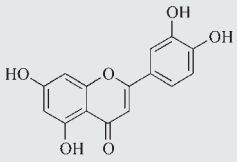
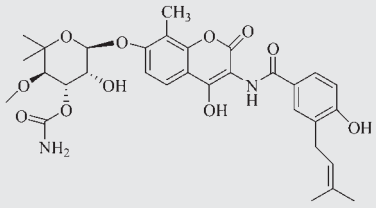
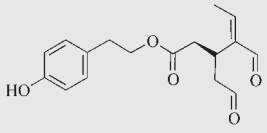
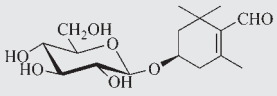
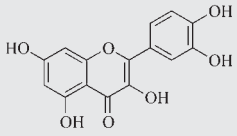
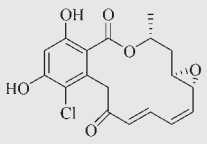
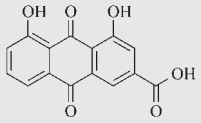
cont.

Table 1 *Continued*

Compound	Structure	Origin	References
GDA		*Streptomyces hygroscopicus*	[12, 13]
Geraniin		*Geranium thunbergii* Siebold ex Lindl. & Paxton (Geraniaceae)	[96–102]
GUT-70		*Calophyllum brasiliense* Britton (Calophyllaceae)	[90, 91]
6-*O*-*p*-Hydroxybenzoyl-5,7-bisdeoxycynanchoside		*Catalpa bignonioides* Walter (Bignoniaceae)	[104]
2′′-Hydroxygenkwanol A		*Daphne linearifolia* (Hart.) (Thymelaeaceae)	[88]
Kotschyin A		*Pseudrocedrela kotschyi* (Schweinf) Harms (Meliaceae)	[122]

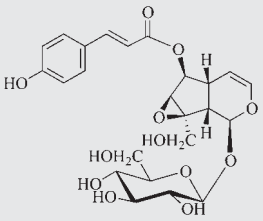
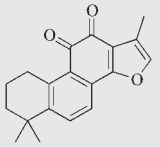
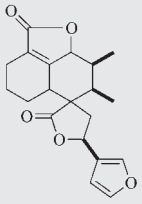
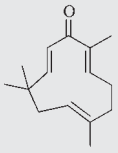
cont.

Table 1 *Continued*

Compound	Structure	Origin	References
Kotschyin D		*Pseudrocedrela kotschyi* (Schweinf) Harms (Meliaceae)	[122]
Lentiginosine		*Astragalus lentiginosus* Douglas ex Hook. (Fabaceae)	[154, 155]
Licochalchone A		*Glycyrrhiza inflate* Batalin (Fabaceae)	[74–78]
Luteolin		Many plants and vegetables	[28–35]
Novobiocin		*Streptomyces niveus*	[89]
Oleocanthal		*Olea europaea* L. (Oleaceae)	[105, 106]
Picrocrocin		*Crocus sativus* L. (Iridaceae)	[103]
Quercetin		Many plants and vegetables	[36–46]
Radicicol		*Penicillium luteo-aurantium*	[12, 13]
Rhein		*Cassia alata* L. (Fabaceae)	[95]

cont.

Table 1 *Continued*

Compound	Structure	Origin	References
Specioside		*Tabebuia argentea* Britt. (Bignoniaceae)	[104]
Tanshinone II A		*Salvia miltiorrhiza* Bunge (Lamiaceae)	[118]
Teucrin A		*Teucrium chamaedrys* L. (Lamiaceae)	[115]
Zerumbone		*Zingiber zerumbet* Smith (Zingiberaceae)	[108]

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