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Anticancer Inhibitors of Hsp90 Function: Beyond the Usual Suspects

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Abstract

The 90-kDa heat-shock protein (Hsp90) is a molecular chaperone responsible for the stability and function of a wide variety of client proteins that are critical for cell growth and survival. Many of these client proteins are frequently mutated and/or overexpressed in cancer cells and are therefore being actively pursued as individual therapeutic targets. Consequently, Hsp90 inhibition offers a promising strategy for simultaneous degradation of several anticancer targets. Currently, most Hsp90 inhibitors under clinical evaluation act by blocking the binding of ATP to the Hsp90 N-terminal domain and thereby, induce the degradation of many Hsp90-dependent oncoproteins. Although, they have shown some promising initial results, clinical challenges such as induction of the heat-shock response, retinopathy, and gastrointestinal tract toxicity are emerging from human trials, which constantly raise concerns about the future development of these inhibitors. Novobiocin derivatives, which do not bind the chaperone's N-terminal ATPase pocket, have emerged over the past decade as an alternative strategy to inhibit Hsp90, but to date, no derivative has been investigated in the clinical setting. In recent years, a number of natural or synthetic compounds have been identified that modulate Hsp90 function via various mechanisms. These compounds not only offer new chemotypes for the development of future Hsp90 inhibitors but can also serve as chemical probes to unravel the biology of Hsp90. This chapter presents a synopsis of inhibitors that directly, allosterically, or even indirectly alters Hsp90 function, and highlights their proposed mechanisms of action.

1. INTRODUCTION

An efficient protein quality control system is fundamental to all cellular processes and is critical for protein homeostasis within the crowded cellular environment (Taipale, Jarosz, & Lindquist, 2010). Since the cellular environment undergoes rapid change, numerous adaptive mechanisms have evolved to manage protein folding and quality control. Upon exposure to environmental stresses, such as high temperature, oxidative stress, hypoxia, acidosis, or malignant transformation, cells induce the expression of a diverse set of proteins, including molecular chaperones, which maintain the dynamic equilibrium between protein folding and degradation (Caplan, Mandal, & Theodoraki, 2007). Molecular chaperones are a highly conserved class of proteins that modulate the folding, intracellular disposition, and degradation of client protein substrates (Whitesell & Lindquist, 2005). The heat-shock proteins (Hsp's) represent a class of molecular chaperones that are constitutively expressed

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under normal physiological conditions, but are upregulated in response to cellular stress to sustain cell viability by maintaining the structural and functional integrity of key regulators of cell growth, differentiation, and survival (Jolly & Morimoto, 2000).

The 90-kDa heat-shock protein (Hsp90) is a highly abundant molecular chaperone that is responsible for the maintenance of protein homeostasis under basal conditions and during the stress response (Young, Agashe, Siegers, & Hartl, 2004). Hsp90 comprises ~1–2% of total cell protein in unstressed cells, but is overexpressed (~4–6%) under hostile conditions to buffer proteotoxic stresses (Donnelly & Blagg, 2008). In humans, Hsp90 exists as four isoforms: Hsp90α (inducible form) and Hsp90β (constitutive form) are mainly found in the cytosol, while the 94-kDa glucose-regulated protein (GRP94) and Hsp75/tumor necrosis factor receptor associated protein 1 (TRAP-1) are localized in the endoplasmic reticulum and mitochondria, respectively (Blagg & Kerr, 2006). In addition, a fraction of Hsp90 is found on the cell surface of cancer cells as well as in the extracellular milieu (Sidera & Patsavoudi, 2008; Trepel, Mollapour, Giaccone, & Neckers, 2010). Hsp90 plays a central role in the conformational maturation, activation, cellular trafficking, and proteolytic turnover of a wide range of substrates, referred to as client proteins (Neckers & Workman, 2012; Taipale et al., 2010). In fact, recent studies indicate that there are ~400 client proteins that depend upon the Hsp90 protein folding machinery to achieve and maintain their active conformations (Taipale et al., 2012). Hsp90 client proteins regulate a vast array of cellular functions, including signal transduction, protein trafficking, chromatin remodeling, autophagy, cell proliferation, and survival (Zuehlke & Johnson, 2010). However, many Hsp90 client proteins are frequently mutated and/or overexpressed in cancer cells and are consequently pursued as individual therapeutic targets for cancer treatment (Whitesell & Lindquist, 2005). As a result, Hsp90 inhibition can provide a unique opportunity to simultaneous deplete multiple anticancer targets (Koga, Kihara, & Neckers, 2009). Therefore, current Hsp90 research has focused on its therapeutic potential as a target for the development of cancer chemotherapeutics. In contrast to its role in driving oncoprotein degradation, Hsp90 inhibition has been shown to induce the prosurvival heat-shock response, which increases molecular chaperone levels (Luo, Sun, Taldone, Rodina, & Chiosis, 2010; Whitesell, Bagatell, & Falsey, 2003). The upregulation of molecular chaperones appears beneficial for neurodegenerative disorders, such as Alzheimer's and Parkinson disease, where they protect cells from the accumulation of neurotoxic proteins (Paul & Mahanta, 2014). As a result of its broad participation in cell biology, Hsp90 has emerged as a promising therapeutic target for the treatment of multiple disease states, including cancer. To date, however, there is no FDA-approved Hsp90 inhibitor. Given the essential role played by Hsp90 in multiple cellular processes, unanticipated adverse effects resulting from Hsp90 inhibition cannot be ruled out in future.

2. HSP90 STRUCTURE, FUNCTION, CHAPERONE CYCLE, AND POINTS OF DISRUPTION BY INHIBITORS

Hsp90 belongs to the GHKL (*G*yrase, *H*sp90, Histidine *K*inase, and Mut*L*) superfamily of ATPases that contain a Bergerat ATP-binding fold. GHKL family members feature an ATP-binding pocket in which ATP is bound in a unique, bent conformation that is distinct from

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the typical extended conformation exhibited by protein kinases (Dutta & Inouye, 2000). In humans, Hsp90 exists as a homodimer with each monomer consisting of a highly conserved N-terminal ATP-binding domain (NTD) connected to a middle domain (MD) and a C-terminal dimerization domain (CTD) (Donnelly & Blagg, 2008). The NTD of Hsp90 contains an ATP-binding site that is responsible for its ATPase activity and provides the requisite energy for the chaperone cycle (Dutta & Inouye, 2000; Panaretou et al., 1998). Historically, the NTD has been the major binding site for the development of Hsp90 inhibitors; e.g., natural products geldanamycin (GDA) and radicicol (RDC) compete with ATP for N-terminal ATP-binding and block Hsp90 function. The NTD is connected to the MD of Hsp90 by a flexible, highly charged linker. This domain plays a key role in modulating Hsp90 ATPase activity by binding the γ-phosphate of ATP when bound to the N-terminus (Meyer et al., 2003). Structural and mutagenesis studies indicate that this site serves for the recognition and binding of client proteins and cochaperones (e.g., Aha1) (Huai et al., 2005). The CTD is important for the homodimerization of Hsp90 into its biologically active conformation (Pearl & Prodromou, 2006). The CTD contains a second nucleotide-binding site that allosterically regulates N-terminal ATPase activity (Prodromou et al., 1999; S ti, Vermes, Haystead, & Csermely, 2003). This domain also features a conserved MEEVD sequence that is responsible for recruiting TPR-domain (tetratricopeptide-containing repeats) containing cochaperones, such as Hsp70–Hsp90 organizing protein (HOP) and immunophilins. Natural products such as novobiocin (NB) and epigallocatechin-3-gallate (EGCG) bind the CTD and modulate Hsp90 function (Marcu, Chadli, Bouhouche, Catelli, & Neckers, 2000; Yin, Henry, & Gasiewicz, 2009).

The Hsp90-mediated protein folding process is complex and has been reviewed extensively (Blagg & Kerr, 2006; Donnelly & Blagg, 2008; Hall, Forsberg, & Blagg, 2014; Li, Soroka, & Buchner, 2012; Wandinger, Richter, & Buchner, 2008). Although the complete mechanism of the Hsp90-mediated protein folding cycle is still not fully understood, accumulating evidence indicates that this cycle requires the interaction of Hsp90 with a number of cochaperones, partner proteins, and immunophilins to form the multiprotein complexes that enable proper function of the machinery (Fig. 1; Peterson & Blagg, 2009).

The chaperone cycle begins with the binding of nascent polypeptides to the Hsp70/Hsp40/ADP complex (**I**) to prevent aggregation (Walter & Buchner, 2002). This complex can be stabilized by the Hsp70-interacting protein (HIP) or, alternatively, Bcl2-associated athanogene homologs that bind and stimulate the exchange of ATP for ADP (Chaudhury, Welch, & Blagg, 2006). The Hsp70/Hsp40/client complex (**II**) then associates with Hsp90 (**III**) to deliver the unfolded protein. The association between Hsp70 and Hsp90 is mediated by HOP/Sti1 (Hsp90–Hsp70 organizing protein), which serves as an adaptor protein (Murphy, Kanelakis, Galigniana, Morishima, & Pratt, 2001). In the case of protein kinases, the cochaperone Cdc37 (cell-division-cycle 37 homologue, also known as p50) is often recruited to the Hsp70/Hsp40/client complex, which promotes the loading of client kinases onto Hsp90 with the aid of HOP (Caplan et al., 2007). Following client substrate loading, various immunophilins (FKBP51, FKBP52), cochaperones, and partner proteins bind the Hsp90 homodimer (**IV**) to form an activated heteroprotein complex (**V**) with concomitant release of Hsp70, HIP, and HOP (Kosano, Stensgard, Charlesworth, McMahon, & Toft,

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1998). ATP binds to the "open" heteroprotein complex (**V**) at the N-terminus of Hsp90 and promotes a structural reorganization of Hsp90 that results in a "closed" conformation (**VIII**) (Prodromou et al., 2000). At this stage, Hsp90 inhibitors can compete with ATP for the N-terminal binding site, which prevents formation of the closed conformation, and ultimately, leads to the degradation of many clients via the ubiquitin–proteasome pathway (Donnelly & Blagg, 2008). In the absence of an inhibitor, this multiprotein assembly is stabilized by the association of cochaperone p23, followed by the recruitment of Aha1 (activator of Hsp90 ATPase homologue 1) to the MD of each Hsp90 monomer. Binding of Aha1 stimulates the hydrolysis of ATP and promotes folding of the bound client, followed by the dissociation of immunophilins and cochaperones (Ali et al., 2006).

As depicted in Fig. 1, the chaperone cycle is a multistage process that requires the participation of various cochaperones and coactivators that work in conjunction with Hsp90 to modulate the activity of the machinery (Table 1; Peterson & Blagg, 2009; Zuehlke & Johnson, 2010). These cochaperones and partner proteins enter the chaperone cycle at different stages and assist in the conformational maturation of specific client protein classes. For example, Cdc37 is required for the recruitment of kinase clients to the Hsp90 machinery and is overexpressed in some kinase-driven cancers. In addition, numerous posttranslational modifications, including phosphorylation, S-nitrosylation, and SUMOylation, regulate Hsp90 function by modulating its affinity for cochaperones and/or client proteins (see Impact of Posttranslational Modifications on the Anticancer Activity of Hsp90 Inhibitors by Woodford et al.) (Hall, Forsberg, et al., 2014; Trepel et al., 2010).

3. THE ROLES OF HSP90 IN CANCER

As the field of cancer research has progressed, new approaches to cancer chemotherapy have emerged. Molecularly targeted therapeutic strategies initially focused on the inhibition of specific enzymes and/or receptors associated with cell signaling, but off-target effects and/or resistance have limited their efficacy against most advanced solid tumors. Through better understanding of cancer biology, it has become increasingly evident that clinical cancers result from dysregulation of multiple interconnected pathways (Logue & Morrison, 2012). In 2000 and 2011, Hanahan and Weinberg proposed 10 hallmarks of cancer that result from genetic and epigenetic alterations of key regulatory proteins, enzymes, and receptors (Hanahan & Weinberg, 2000, 2011). These hallmarks include (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) activating invasion and metastasis, (7) deregulated cellular energetics, (8) avoiding immune destruction, (9) tumor-promoting inflammation, and (10) genome instability and mutation. In light of this understanding, Hsp90 inhibition is particularly appealing because it has the potential to simultaneously disrupt multiple pathways by acting on a single target and thereby exerting a multipronged attack on malignant cells (Xu & Neckers, 2007). Hsp90 is essential for the stability and function of a wide range of oncogenic proteins, such as signaling kinases, steroid hormone receptors, telomerase, and many others that contribute directly to the hallmarks of cancer (Table 2) (and chapter by Vartholomaiou et al., 2016) (Blagg & Kerr, 2006; Koga et al., 2009). Therefore, inhibition of Hsp90 by a small molecule represents an exciting strategy for development of new cancer chemotherapeutics (Fig. 2).

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The ubiquitous and essential nature of Hsp90 function raised concerns about the potential for selective use of Hsp90 inhibitors. However, studies have indicated that Hsp90 inhibitors are more toxic to malignant cells than to normal tissues, and therefore the potential does exist for selective cytotoxicity (Chiosis & Neckers, 2006; Chiosis et al., 2003; Kamal et al., 2003; Workman, 2004). Moreover, at least some Hsp90 inhibitors accumulate in malignant cells to a greater extent than surrounding tissue (Chiosis et al., 2003). In support of this observation, Kamal and coworkers demonstrated that the apparent accumulation of Hsp90 inhibitors in tumor cells following systemic administration could result from the increased affinity of Hsp90 present in cancer cells as compared to normal cells (Kamal et al., 2003). Although the concept remains debated, it has been suggested that Hsp90 in cancer cells is engaged in an activated heteroprotein complex that exhibits both enhanced ATPase activity and higher affinity for Hsp90 inhibitors, compared to the inactivated, homodimeric complex found in normal cells. Moreover, a number of Hsp90 inhibitors have shown promising results in clinical trials and have been relatively well tolerated at drug exposures that clearly impair Hsp90 function as judged by induction of a systemic heat-shock response and depletion of client proteins in both tumor and normal tissues (Jhaveri, Taldone, Modi, & Chiosis, 2012; Neckers & Workman, 2012). Taken together, preclinical studies and a decade of clinical experience with various Hsp90 inhibitor chemotypes indicate that Hsp90 can be safely targeted for the development of cancer chemotherapeutics.

Classical Hsp90 inhibitors act by competitive binding to the ATP-binding site at the N-terminal domain of Hsp90, and consequently halt progression of the protein folding machinery, which leads to the degradation of most client proteins (Khandelwal, Crowley, & Blagg, 2016). The natural products, GDA and RDC, were the first Hsp90 inhibitors identified in the early 1990s. Upon their identification, both GDA and RDC served as starting points for various drug discovery programs (Bagatell & Whitesell, 2004), which ultimately led to the investigation of 17 distinct chemical entities in clinical trials (Neckers & Workman, 2012). Some of these investigational new drugs that inhibit the Hsp90 N-terminus are shown in Fig. 3. Clinical experience to date has provided a proof of concept for the use of Hsp90 inhibitors in cancer patients as a novel approach to inhibit multiple cancer pathways via Hsp90 modulation. Although there have been some encouraging clinical responses, concerns regarding concomitant induction of the prosurvival response, disruption of apoptotic mechanisms, impairment of antitumor immune mechanisms, cardiac arrhythmia, and hepatotoxicity have emerged from clinical trials (Whitesell & Lindquist, 2005). While isoform-selective N-terminal inhibitors may address some of the toxic liabilities, induction of the prosurvival heat-shock response (Fig. 4) by inhibitors when given at or near their maximally tolerated dose may represent a fundamental impediment to their clinical efficacy (Whitesell & Lindquist, 2005). Therefore, Hsp90 inhibitors that do not induce the heat-shock response represent a promising new direction for the Hsp90 field of research.

In 2000, Neckers and coworkers discovered that NB, a coumarin antibiotic, bound to a previously unrecognized Hsp90 C-terminal nucleotide-binding site (IC_{50} ~ 700 μM in SKBr3 cells) and induced the degradation of Hsp90-dependent client proteins, v-src, Raf-1, and Erb2 (Marcu, Chadli, et al., 2000; Marcu, Schulte, & Neckers, 2000). Interestingly, NB did not induce a prosurvival heat-shock response, one of the major drawbacks associated

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with N-terminal inhibition. Moreover, it was observed that Hsp90 C-terminal inhibitors allosterically modulate the N-terminal ATPase activity. Encouraged by the initial findings, attempts were made to improve the selectivity and potency of NB. In 2005, initial structure–activity relationship studies were performed to reveal several key structural features of NB that are required for Hsp90 inhibitory activity (Burlison, Neckers, Smith, Maxwell, & Blagg, 2006; Yu et al., 2005). It was observed that both the coumarin 4-hydroxy and the 3′-carbamoyl group on NB are detrimental to Hsp90 inhibition and upon their removal, the first Hsp90 C-terminal inhibitors (DHN1 and DHN2) with improved potency and selectivity were reported by our group.

Subsequent SAR studies explored the coumarin core and benzamide side chain of NB and revealed optimal appendages for these moieties (Burlison et al., 2008; Donnelly et al., 2008; Garg, Zhao, & Blagg, 2015; Zhao et al., 2011, 2015). It was observed that the coumarin core of NB serves as a backbone for orientation of the sugar and benzamide side chains within the binding pocket and could be replaced with other aromatic/heteroaromatic cores (Burlison et al., 2008; Donnelly et al., 2008). In fact, introduction of a biphenyl or quinolinone ring system in lieu of the coumarin core not only improved efficacy but also provided insights into the nature of the C-terminal binding pocket (Zhao, Moroni, Colombo, & Blagg, 2013; Zhao et al., 2015). In addition, it was discovered that replacement of benzamide side chain with biaryl and triazole moieties led to analogs that manifested improved antiproliferative activities (Burlison et al., 2008; Zhao et al., 2014). Furthermore, recent studies indicate that the sugar moiety, although important for enhancing solubility (and hence activity), could be replaced with other sugars or sugar surrogates without compromising inhibitory activity (Donnelly, Zhao, Reddy Kusuma, & Blagg, 2010; Shelton et al., 2009; Zhao, Reddy Kusuma, & Blagg, 2010; Zhao et al., 2011). SAR studies on the NB scaffold have led to the development of several promising lead molecules such as KU135, KU174, and KU675, which manifest potent antiproliferative activity against multiple cancer cell lines (Donnelly et al., 2008; Eskew et al., 2011; Ghosh et al., 2015; Liu et al., 2015; Samadi et al., 2011; Zhao et al., 2010, 2011). A summary of SAR for NB and its derivatives based on their cytotoxicity is presented in Fig. 5.

4. NOVEL HSP90 INHIBITORS: BEYOND THE USUAL SUSPECTS

Current Hsp90 inhibitors derived from the natural products, GDA, RDC, or a purine scaffold have been reviewed extensively in literature (Hong et al., 2013; Khandelwal et al., 2016; Trepel et al., 2010). Although significant progress has been made toward the development of highly active N-terminal inhibitors, their clinical application has been hampered by undesired side effects in many cases. Several NB analogs have been developed during the past decade (Burlison et al., 2006; Donnelly & Blagg, 2008; Zhao et al., 2011), and these have shown promising results in preclinical studies. Their evaluation in clinical trials, however, has not yet been undertaken. More recently, new compounds have been identified that disrupt Hsp90 chaperone activity via yet other mechanisms (Brandt & Blagg, 2009; Piaz, Terracciano, De Tommasi, & Braca, 2015). These compounds can be broadly divided into two main categories: (1) direct Hsp90 inhibitors or (2) disruptors of Hsp90/cochaperone interactions, both of which will be discussed in detail below.

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4.1 Compounds that Bind Directly to Hsp90

4.1.1 Epigallocatechin-3-Gallate—EGCG is a polyphenolic compound found in green tea and is well known for its antioxidant, antimicrobial, and anticancer activities (Zaveri, 2006). Previous studies have shown that EGCG inhibits the activity of a wide range of proteins at 70 μM, including telomerase, the aryl hydrocarbon receptor (AhR), several kinases, and transcription factors, all of which are well-known Hsp90 client proteins (Yin, Henry, & Gasiewicz, 2008). In 2005, using affinity purification experiments, Palermo and coworkers revealed that EGCG exhibits its inhibitory activity against AhR, at least in part via Hsp90 inhibition (Palermo, Westlake, & Gasiewicz, 2005). Subsequent studies by Yin and coworkers demonstrated that EGCG binds near the C-terminal ATP-binding site (residues 538–738) of Hsp90 and unlike NB and other inhibitors, stabilizes the association of cochaperones Hsp70, Cyp40, and XAP-2 to Hsp90 (Yin et al., 2008). Furthermore, it was found that EGCG induces concentration-dependent degradation of the Hsp90-dependent oncoproteins ErbB2, Raf-1, and pAkt along with a slight increase in Hsp70 levels.

Recently, Khandelwal and coworkers published the first structure–activity relationships studies on EGCG using cytotoxicity and the depletion of several Hsp90 client proteins as endpoints (Khandelwal, Hall, & Blagg, 2013). Results are summarized in Fig. 6. In these studies, they observed that the phenols on the B- and the D-rings are detrimental to inhibitory activity, while syn-stereochemistry of the linker that connects the B- and D-rings with the benzopyran core is beneficial. The prenylated benzamide present in NB was shown to represent an ideal replacement for the gallic acid moiety of EGCG and resulted in ~15-fold improvement in antiproliferative activity and ultimately led to the development of compound **21** (MCF-7, IC_{50} = 4 μM). Further studies by Bhat and coworkers confirmed the non-essential nature of the B- and D-ring phenols and established the ester linker connecting the C- and D-rings could be replaced with an amide or sulfonamide without compromising anticancer activity (Bhat et al., 2014). How these modifications affect interactions with Hsp90 remain unknown.

4.1.2 Silybin—Silybin is the major component of the flavonolignan extract isolated from the seed of milk thistle plants (*Silybum marianum*) and has demonstrated hepato-protective effects and growth inhibitory activity against various cancer cells (Gazak, Walterova, & Kren, 2007). Early studies demonstrated that silybin induced cell cycle arrest and caused the depletion of CDK2, CDK4, cyclin E, and cyclin D1 proteins in colon cancer cells (Agarwal et al., 2003). CDK2 and CDK4 are well-known Hsp90-dependent clients, which led to speculation that Hsp90 could be the primary target of silybin. In an effort to determine whether silybin can bind Hsp90, Zhao and coworkers performed a luciferase-refolding assay with silybin and demonstrated that silybin inhibited the renaturation of heat-denatured luciferase, suggesting that Hsp90 could be a biochemical target for silybin (Zhao, Brandt, Galam, Matts, & Blagg, 2011). Subsequent studies demonstrated that silybin induced a concentration-dependent degradation of the Hsp90-dependent client proteins Her2, Raf-1, and Akt, without affecting Hsp70 or Hsp90 levels (Zhao, Brandt, et al., 2011). SAR studies by the same research team identified key structural features required for the scaffold's cytotoxic activity in which Hsp90 inhibition could play a part (Fig. 7; Zhao, Brandt, et al., 2011). Their studies showed that the C-3 and C-23 hydroxyl groups were not required for

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activity; however, at least one substitution (preferably 4-hydoxyl) on the E-ring was important for activity. Furthermore, SAR studies suggested that the A-ring phenol was not required and its removal led to the development of compounds **25** and **26**, which manifest IC_{50}s of 13 and 16 μM against MCF-7 cell line, respectively, versus an IC_{50} of ~200 μM for silybin. Recently, Riebold and coworkers demonstrated that silybin binds to the C-terminal domain of Hsp90 and releases mature glucocorticoid receptors from the Hsp90 complex as demonstrated by NMR analysis (Riebold et al., 2015).

4.1.3 Cisplatin and LA-12—Cisplatin (Fig. 8) is a platinum-containing chemotherapeutic agent that is widely used for the treatment of ovarian, testicular, bladder, cervical, and other solid tumors (Galanski, 2006). The anticancer activity of cisplatin has been ascribed to its ability to form intrastrand and/or interstrand DNA adducts which are particularly lethal in several cancer cell types (Jordan & Carmo-Fonseca, 2000). However, it has also been shown that due to its chemical reactivity, cisplatin interacts with various proteins, phospholipids, and RNA (Sreedhar, Soti, & Csermely, 2004). In 1999, Itoh and coworkers reported that cisplatin inhibits Hsp90 chaperone activity (Itoh et al., 1999). Affinity purification and protein fingerprinting studies were used to demonstrate that cisplatin binds to the Hsp90 C-terminal domain. Subsequently, Csermely and coworkers demonstrated that cisplatin is a C-terminal inhibitor that binds near the previously identified C-terminal nucleotide-binding site (Söt, Rácz, & Csermely, 2002). Studies by Rosenhagen and colleagues indicated that the administration of cisplatin to neuroblastoma cells resulted in the degradation of steroid hormone receptors (androgen and glucocorticoid receptors), but no other Hsp90-dependent clients, such as Raf-1, lck, and c-rac (Rosenhagen et al., 2003). Moreover, by use of a heat-shock factor (HSF)-dependent luciferase reporter assay, they showed that cisplatin does not induce the heat-shock response. These results suggest that unlike compounds that bind Hsp90, cisplatin selectively inhibits some Hsp90 functions and thus, could provide insights into novel ways to modulate its chaperone activity. Recently, it was shown that LA-12 (Fig. 8), an optimized derivative of cisplatin, exhibits higher affinity for Hsp90 than cisplatin and moreover, induces the degradation of additional Hsp90 client proteins, such as mutant p53, Cyclin D1, and estrogen receptors (Kvardova et al., 2010). In addition, LA-12 exhibits a more favorable pharmacokinetic profile as compared to cisplatin and demonstrates enhanced cytotoxicity against multiple cancer cell lines, including those that are cisplatin resistant (Kvardova et al., 2010; Zak et al., 2004).

4.1.4 Taxol—Taxol (Fig. 8) is a frequently used chemotherapeutic agent for the treatment of various cancers and its anticancer activity has been attributed to the inhibition of mitosis via stabilization of microtubules (Wani, Taylor, Wall, Coggon, & McPhail, 1971). In addition, taxol produces many lipopolysaccharide (LPS)-like cellular responses, such as induction of cytokines, activation of kinases, and transcription factors, suggesting that it exhibits a multifaceted effect on cancer cells (Ding, Porteu, Sanchez, & Nathan, 1990; Ding, Sanchez, & Nathan, 1993). Byrd and coworkers performed affinity purification experiments with biotinylated taxol and identified Hsp90 and Hsp70, as potential mediators of its LPS-mimicking activity (Byrd et al., 1999). Surprisingly, unlike classical Hsp90 inhibitors (e.g., GDA), taxol appears to stimulate Hsp90 function and induces macrophage activation (Byrd et al., 1999). No study describing the region in which taxol binds Hsp90 has been reported.

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Further work is needed to investigate the effect of taxol on Hsp90-dependent client proteins and to determine whether its binding to Hsp90 causes disruption of cochaperone interactions. Interestingly, a follow-up study showed that taxol and 17-AAG act synergistically in breast cancer xenografts and that 17-AAG sensitizes cancer cells to taxol-induced apoptosis through suppression of the Hsp90 client, Akt kinase (Solit, Basso, Olshen, Scher, & Rosen, 2003). Combination therapies with taxol and other Hsp90 inhibitors may represent new avenues for cancer chemotherapy, but benefit has yet to be demonstrated in the clinic. A recent phase-3 study of ganetespib in combination with the taxane, docetaxel, in recurrent lung cancer (GALAXY 2) failed to show any significant clinical benefit and was terminated.

4.1.5 Sansalvamide A-Amide—Sansalvamide A (San A) is a depsipeptide isolated from a marine fungus of the genus *Fusarium* and exhibits moderate antitumor activity (IC_{50} 45 μg/mL against HCT-29 colon cancer cells) (Belofsky, Jensen, & Fenical, 1999). San A is a cyclic pentapeptide containing a lactone moiety (Fig. 9), which is susceptible to ring opening by the esterases present in plasma and in cells. In an effort to improve stability, Silvermann and coworkers synthesized a peptide derivative of San A, sansalvamide A-amide (San A-amide), which was found to be 10-fold more potent (IC_{50} 4.5 μg/mL) than the natural product (Gu, Liu, & Silverman, 2002; Sellers et al., 2010). Biochemical studies by Vasko and coworkers revealed that San A-amide binds the N-MD of Hsp90 and disrupts Hsp90 chaperone activity (Vasko et al., 2010). Interestingly, like 17-AAG, San A-amide induces Hsp70 levels (Ardi, Alexander, Johnson, & McAlpine, 2011); however, it shows no effect on the binding of the client protein Her2, suggesting a unique mechanism of action for this compound (Vasko et al., 2010). In addition, it was found that San A-amide disrupts interactions between Hsp90 and various C-terminal domain-binding cochaperones, including IP6K2, FKBP52, and HOP, suggesting an allosteric mechanism for its modulation of Hsp90 function (Kunicki et al., 2011; Vasko et al., 2010). Moreover, San A-amide shows no effect on Hsp90 ATPase activity and preferentially binds to the closed conformation of Hsp90, further supporting the notion that San A-amide, among many other biological activities might act as an allosteric modulator of Hsp90 function (Alexander, Partridge, Agard, & McAlpine, 2011).

Over the past decade, McAlpine and coworkers have conducted several structure–activity relationship studies on San A-amide and have developed a number of analogs that manifest potent cytotoxicity against several cancer cell lines, including pancreatic, breast, prostate, and colon (Ardi et al., 2011; Carroll et al., 2005; Davis et al., 2012; Rodriguez et al., 2007; Sellers et al., 2010). Like San A-amide, these compounds have been reported to allosterically inhibit interaction between Hsp90 and multiple TPR-containing proteins, and also, induce caspase-dependent apoptosis in cancer cells. Interestingly, some of these analogs (**32** and **33**) manifest anti-proliferative activities without inducing the heat-shock response, a major drawback associated with the parent compounds (Koay et al., 2014; McConnell, Alexander, & McAlpine, 2014). Recently, Ramsey and coworkers reported a novel San A-amide derivative, **34**, which induces apoptosis and interacts with Hsp90 in biochemical pull-down assays, but has no effect on interaction between Hsp90 and C-

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terminal domain proteins, suggesting a novel mechanism by which it might modulate Hsp90 function (Ramsey et al., 2012).

4.1.6 Deguelin and L80—

Deguelin is a naturally occurring flavonoid isolated from *Derris trifoliata* Lour. or *Mundulea sericea* (Leguminosae). It has demonstrated potent antiproliferative, antimetastatic, and apoptotic activity against several cancers both *in vitro* and *in vivo* (Chang et al., 2012; Hastings, Hadden, & Blagg, 2008; Lee et al., 2015). A number of reports indicate deguelin induces cell death by inhibiting several cell signaling pathways, such as PI3K-Akt, IKK-IκBα-NF-κB, AMPK-mTOR-survivin, and HIF-1α-VEGF. In 2007, Oh and coworkers reported that deguelin disrupts interactions between Hsp90 and its client protein, HIF-α (Oh et al., 2007). Subsequent biochemical analysis and molecular docking studies suggested that deguelin binds the C-terminal ATP-binding pocket of Hsp90 and suppresses Hsp90 function, which leads to proteasome-mediated degradation of Hsp90 client proteins, without inducing Hsp90 expression (Lee et al., 2015). In preclinical studies, administration of deguelin significantly reduced tumor growth by inducing apoptosis. However, it was observed that deguelin produces Parkinson's disease-like syndrome in rats at high doses, which may limit its therapeutic application (Caboni et al., 2004). In an attempt to circumvent this detrimental feature and to develop simpler analogs, Chang and coworkers reported structure–activity relationships studies for deguelin using HIF-1α reduction and cytotoxicity as endpoints (Fig. 10; Chang et al., 2012). Their studies revealed that the 2,2-dimethyl-2*H*-chromene moiety and both methoxy groups at the C9 and C10 positions of deguelin are critical for these activities. The SAR insights reported led to the development of compounds **36** and **37**, which have cytotoxicity IC_{50}s of 0.14 and 0.49 μM, against H1299 cell line, respectively. The extent to which these effects result from Hsp90 inhibition remain unclear. Recently, Lee and coworkers reported a novel deguelin derivative, L80 that manifests antiproliferative and apoptotic activities both *in vitro* and *in vivo* without systemic toxicity (Lee et al., 2015). Consistent with earlier observations for this scaffold, L80 was found to bind directly to Hsp90 in biochemical assays and disrupt the Hsp90 chaperone cycle. Computational studies suggest L80 might form key interactions with Ser677 and Lys615 within the Hsp90 C-terminal domain. However, the exact mechanism remains unclear in cells.

4.2 Disruptors of Hsp90 Interaction with Cochaperones and Client Proteins

Currently, Hsp90 inhibitors undergoing clinical evaluation are pan-inhibitors and induce the degradation of many Hsp90-dependent client proteins (Jhaveri et al., 2012; Patel et al., 2013). Although pan-Hsp90 inhibition could be beneficial by providing a multifaceted attack on cancer cells, it may also produce detrimental side effects. For example, it has been found that inhibition of the Hsp90-dependent trafficking of cardiac potassium channel hERG could be responsible for the cardiac arrhythmias seen in clinical trials of some Hsp90 inhibitors (Peterson, Eskew, Vielhauer, & Blagg, 2012). Consequently, there is growing interest in identifying new Hsp90 modulators that could provide alternative mechanisms of action without the negative effects of pan-inhibition. Over the past decade, new compounds have emerged that disrupt interactions between Hsp90 and its cochaperones (Brandt & Blagg, 2009; Hall, Forsberg, et al., 2014; Piaz et al., 2015). These compounds not only represent new chemotypes for the development of future Hsp90 inhibitors but also appear to block the

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maturation of more restricted subsets of Hsp90 client proteins, which could limit target-related toxicities induced by pan-inhibitors. Several natural products, such as celastrol, gedunin, and cruentaren A, have been shown, among a plethora of other biochemical and biological effects to disrupt interactions between Hsp90 and its partner proteins.

4.2.1 Celastrol, a Disruptor of Cdc37–Hsp90 Interaction—Celastrol (Fig. 11) is a pentacyclic triterpenoid isolated from the root extract of *Tripterygium wilfordii* Hook F. (as known as Thunder of God Vine) and has been used for centuries in oriental traditional medicine to treat inflammatory and autoimmune disorders, such as rheumatoid arthritis (Allison, Cacabelos, Lombardi, Álvarez, & Vigo, 2001; Duan et al., 2000; Salminen, Lehtonen, Paimela, & Kaarniranta, 2010; Tao & Lipsky, 2000). In recent years, a renewed interest in the therapeutic application of celastrol has increased due to its diverse biological activities, especially for the treatment of various inflammatory diseases and cancer (Allison et al., 2001; Duan et al., 2000; Kannaiyan et al., 2011). Studies have indicated that celastrol manifests cytotoxicity against different cancer cell lines, including prostate, multiple myeloma, lung, gliomas, pancreatic, and cervical cancers (Liu, Ma, & Zhou, 2011; Liu et al., 2010; Yang, Chen, Cui, Yuan, & Dou, 2006; Zhou & Huang, 2009). In addition, it suppresses metastatic invasion, induces apoptosis (Kannaiyan et al., 2011; Sethi, Ahn, Pandey, & Aggarwal, 2007), and sensitizes drug-resistant cancer cells to combination therapy (Chen, Rose, Doudican, Osman, & Orlow, 2009). Celastrol is a quinone methide triterpene and exhibits a high propensity to form covalent Michael adducts with cysteine residues (Salminen et al., 2010). In fact, studies with celastrol have identified numerous intracellular targets with relevance to cancer, such as NF-κB/IKKβ (Sethi et al., 2007), the proteasome (Yang et al., 2006), topoisomerase II (Nagase et al., 2003), and a variety of signaling pathways that are essential to the survival of cancer cells (Liu et al., 2011).

In 2006, Lamb and coworkers developed a novel chemical genomic approach called connectivity maps to discover and predict the biological pathways targeted by various anticancer agents (Lamb et al., 2006). Briefly, these researchers generated a database of gene expression changes resulting from the exposure of tumor cells to drugs with well-characterized modes of action. Using this database to identify compounds with similar modes of action, Hieronymus and coworkers reported that celastrol exerts its antiproliferative activity, at least in part via disruption of Hsp90-related pathways (Hieronymus et al., 2006). Since this initial work, extensive research has been conducted to investigate the effect of celastrol on Hsp90. Using molecular docking studies and coimmunoprecipitation assays, Zhao and coworkers revealed that celastrol disrupts the association between Hsp90 and Cdc37, which leads to the degradation of Hsp90-dependent client kinases, such as Akt and Cdk4 (Zhang et al., 2008). It was also observed that celastrol induces the heat-shock response by activation of HSF-1 (Westerheide et al., 2004). In another study, Chadli and coworkers showed that celastrol can inactivate the cochaperone p23 and cause amyloid-like fibril formation, which in turn halts the chaperoning of steroid hormone receptors (Chadli et al., 2010). Early studies indicated that celastrol binds the Hsp90 C-terminal domain and allosterically modulates its chaperoning activity (Zhang et al., 2009). However, HSQC NMR-based studies by Sreeramulu and coworkers suggested that celastrol disrupts Hsp90/Cdc37 interactions by covalently binding to cysteine residues on

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Cdc37, without direct interactions with Hsp90 (Sreeramulu, Gande, Göbel, & Schwalbe, 2009). Recently, Zanphorlin and coworkers conducted detailed studies on celastrol and proposed a new model for celastrol/Hsp90 binding, suggesting that celastrol binds the C-terminal domain of Hsp90 and interferes with Hsp90 function by induction of its oligomerization (Zanphorlin, Alves, & Ramos, 2014). Taken together, these studies suggest that celastrol may modulate Hsp90 function through multiple mechanisms. Additional studies are needed to further clarify the exact nature of its effects on Hsp90, but promiscuity limits its utility as a probe for studying Hsp90 function specifically in whole cells.

In 2014, Wei and coworkers performed a limited structure–activity relationship study on the celastrol scaffold using cytotoxicity and depletion of kinase levels as endpoints (summarized in Fig. 11; Wei et al., 2014). Seven celastrol derivatives were prepared and their antitumor activity evaluated against human hepatocellular carcinoma (HCC) cell line *in vitro* and in HCC patient-derived xenografts. These celastrol derivatives were shown to deplete cellular levels of protein kinases involved in the Raf/MEK/ERK and PI3K/AKT/mTOR signaling pathways and induce apoptosis. Although no derivative was found more active than the natural product (cytotoxicity IC_{50}, 0.30 μM against Hep3B HCC cell line), it was revealed that modifications to the carboxylic acid moiety of celastrol were tolerated.

4.2.2 Gedunin, a Disruptor of Hsp90–p23 Interaction—Gedunin (Fig. 12) is a tetranortriterpenoid isolated from the Indian neem tree (*Azadirachta indica*, Meliacae) and has been used for the treatment of malaria and other infectious diseases in traditional Indian medicine (Patwardhan et al., 2013). In addition, gedunin has demonstrated antiproliferative activity against various cancer cell line including prostate, colon, and ovarian (Hieronymus et al., 2006; Kamath et al., 2009; Uddin et al., 2007). Like celastrol, gedunin is a strong, thiol-reactive electrophile that activates the heat-shock response. In 2006, Hieronymus and coworkers used connectivity map analysis to report that gedunin exerts its antiproliferative activity at least in part via modulation of Hsp90-dependent pathways, which results in the depletion of cellular levels of Hsp90-dependent client proteins (Hieronymus et al., 2006; Lamb et al., 2006). Subsequent biochemical studies showed that gedunin inhibits Hsp90 ATPase activity and disrupts the Hsp90 chaperone cycle. However, unlike most Hsp90 inhibitors, gedunin was unable to compete with GDA for binding to the N-terminal ATP-binding pocket in florescence polarization assays, suggesting a novel mechanism for Hsp90 modulation (Hieronymus et al., 2006). Recent studies by Patwardhan and coworkers revealed that gedunin binds to the cochaperone p23 and blocks its interaction with Hsp90, which leads to deactivation of the Hsp90 folding machinery (Patwardhan et al., 2013). Interestingly, unlike GDA, gedunin induced relatively modest overexpression of Hsp70. Furthermore, it was observed that gedunin selectively destabilizes steroid receptors such as GR and induces apoptotic cell death through the activation of caspase 7. In 2008, Brandt and coworkers synthesized a series of compounds with chemical modifications to the gedunin scaffold and revealed key structural features that are required for cytotoxic activity (Brandt, Schmidt, Prisinzano, & Blagg, 2008). Nineteen semisynthetic derivatives of gedunin were prepared and their antiproliferative activity evaluated against MCF-7 and SKBr3 breast cancer cells. No analog was found to be more active than the natural product (MCF-7, IC_{50} = 8.84 μM). Further-more, it was shown that the α,β-unsaturated ketone within the A-ring,

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although important for antiproliferative activity, did not necessarily serve as a Michael acceptor and therefore, may avoid potential toxicities associated with such motifs.

4.2.3 Withaferin A, a Disruptor of Hsp90–Cdc37 Interaction—Withaferin A (WA, Fig. 13) is a withanolide isolated from the Indian medicinal plant of *Withania somnifera* (commonly known as "Ashwagandha" or "Indian Winter Cherry" in Ayurvedic medicine) and possesses diverse biological activities, such as anti-inflammatory (Kaileh et al., 2007), antistress, antioxidant, immunomodulatory (Mishra, Singh, & Dagenais, 2000), anti-angiogenesis (Mohan et al., 2004), and anticancer activities (Yang, Shi, & Dou, 2007). Since its discovery in the late 1960s, withaferin A has been extensively studied for its anticancer activity, and numerous mechanisms and molecular targets proposed (Falsey et al., 2006; Kaileh et al., 2007; Shohat, Gitter, Abraham, & Lavie, 1967; Srinivasan, Ranga, Burikhanov, Han, & Chendil, 2007; Yang et al., 2007; Yokota, Bargagna-Mohan, Ravindranath, Kim, & Mohan, 2006). It has been reported that withaferin A inhibits nuclear factor-κB (NF-κB) activation of IκB kinase via a thioalkylation-sensitive redox mechanism (Kaileh et al., 2007) induces apoptosis in prostate cancer cells through Par-4 induction (Srinivasan et al., 2007), targets β5 subunit of tumor proteasome (Yang et al., 2007), and covalently binds to Annexin II to alter cytoskeletal architecture (Falsey et al., 2006). In 2010, Yu and coworkers demonstrated that withaferin A exhibits antiproliferative activity and inhibits Hsp90 in pancreatic cells where it was reported to deplete cellular levels of Hsp90-dependent client proteins (Akt, Cdk4, and GR) (Yu et al., 2010). In addition, it was observed that withaferin A induces Hsp70 expression, without affecting Hsp90 levels. Moreover, these researchers found that withaferin A binds Hsp90 and halts the Hsp90 chaperone cycle through a novel ATP-independent mechanism. To identify the domain to which withaferin A binds in Hsp90, a pull-down assay using WA-biotin was used, which suggested interaction with the chaperone's C-terminal domain (Yu et al., 2010). Coimmunoprecipitation studies showed that withaferin A disrupts formation of the Hsp90/Cdc37 complex in pancreatic cancer cells (Yu et al., 2010). Structure–activity relationship studies have identified a pharmacophore of WA that involves the 4-hydroxy-5,6-epoxy-22-*en*-1-one moiety and its unsaturated lactone as critical for cytotoxic activity (Mohan et al., 2004; Yousuf et al., 2011). Recent studies with the withanolides indicate that the 5,6-epoxide may react with reactive cysteine residues in Hsp90 and induce aggregation, leading to disruption of Hsp90 function (Gu et al., 2014).

4.2.4 Derrubone, a Disruptor of Hsp90–Cdc37 Interaction—Derrubone is a prenylated isoflavone that was originally isolated from the Indian tree *Debrris robusta* in 1969 (East, Ollis, & Wheeler, 1969). However, its biological activities remained uncharacterized until recently. In 2007, high-throughput screening of a library of natural products identified derrubone as a potential Hsp90 inhibitor (Hadden, Galam, Gestwicki, Matts, & Blagg, 2007). The screening was based on the ability of natural products to inhibit the Hsp90-dependent refolding of thermally denatured firefly luciferase (Galam et al., 2007). In this study, derrubone potently inhibited refolding with an IC_{50} value of 0.23 ± 0.04 μM. Subsequent cellular studies revealed that it exhibits antiproliferative activity against various cancer cell lines (MCF-7 IC_{50} = 11.9 μM; HCT116 IC_{50} = 13.7 μM), and depletes cellular levels of Hsp90-dependent client proteins, including Her2, Raf-1, Akt, and ERα, without altering Hsp90 levels (Hadden et al., 2007; Mays, Hill, Moyers, & Blagg, 2010). Using

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purified recombinant Hsp70, Hadden and coworkers demonstrated that derrubone has no effect on Hsp70 ATPase activity, suggesting Hsp90 inhibition as a plausible mechanism for its inhibitory activity on refolding (Hadden et al., 2007). Moreover, it was observed that derrubone inhibits Hsp90 function by stabilizing the Hsp90 hetero-complex (Hsp90/cochaperone/client complex) formed between Hsp90, Cdc37, and heme-regulated eIF2α kinase (HRI), and consequently, halts progression of the chaperone cycle. In follow-up, a small library of derrubone analogs was prepared and evaluated to elucidate the critical structural features of derrubone for cytotoxicity and Hsp90 client protein depletion (Hastings et al., 2008; Mays et al., 2010). These structure–activity relationship studies revealed the importance of the 6-prenyl and 3-aryl side chains for activity (Fig. 14). Recent molecular docking studies by Khalid and coworkers suggest derrubone binds to the Hsp90 C-terminal domain and interacts with Leu665, Leu666, and Leu694 (Khalid & Paul, 2014).

4.2.5 Gambogic Acid, a Disruptor of Hsp90–Cdc37 Interaction—Gambogic acid (Fig. 15) is a xanthonoid isolated from the exudate of *Garcinia hanburyi* Hook f. (Clusiaceae) and has been used for centuries to treat infections and tumors (Ren et al., 2011). In recent decades, interest in gambogic acid as a potential anticancer agent has increased because it demonstrates antitumor, antiangiogenic, and antimetastatic activities against multiple cancer cell lines (Ren et al., 2011). Recently, it entered phase II clinical trials in China for metastatic cancers (Chi et al., 2013). Several studies have shown that gambogic acid exerts its anticancer activity via numerous targets and signaling pathways, such as apoptosis induction (Pandey et al., 2007), antiangiogenesis (Yi et al., 2008), inhibition of human topoisomerase-Iiα (Qin et al., 2007), and telomerase (Zhao et al., 2008). In 2010, Zhang and coworkers ascribed the antiproliferative activity of gambogic acid to Hsp90 inhibition in HeLa cells (Zhang et al., 2010). Using fluorescence-quenching assays and spectroscopic tools, they demonstrated that gambogic acid binds the Hsp90 N-terminal domain and inhibits its ATPase activity. In addition, it was found that gambogic acid causes down-regulation of the TNF-α/NF-κB signaling pathway, which in turn induces apoptosis in HeLa cells. Their findings were further supported by a contemporaneous study by Davenport and coworkers, which demonstrated that gambogic acid inhibits Hsp90-dependent refolding of thermally denatured luciferase in a high-throughput screening assay previously developed by the same group (Davenport et al., 2011; Galam et al., 2007). In these studies, it was found that gambogic acid inhibits the proliferation and survival of two breast cancer cell lines (MCF-7, IC_{50} 2.0 μM and SKBr3, IC_{50} 0.8 μM), and depletes cellular levels of the Hsp90-dependent client proteins Her2, Akt, and Raf-1 in a concentration-dependent manner. Importantly, it was observed that gambogic acid induces Hsp90 and Hsp70 expression, a hallmark of Hsp90 N-terminal inhibition, but also a general feature of many thiol-reactive compounds. Like celastrol, gambogic acid was found to block the association of Hsp90, Hsp70, and Cdc37 with HRI. Recent surface plasmon resonance (SPR) analysis and virtual docking studies suggest gambogic acid binds the Hsp90 N-terminal domain; however, it does not compete with GDA for binding, suggesting a site of interaction distinct from the ATP-binding pocket (Davenport et al., 2011).

4.2.6 Cruentaren A, a Hsp90/F1F0 ATP Synthase Disruptor—Cruentaren A (Fig. 16) is a macrolide isolated from the myxobacterium *Byssovorax cruenta* which is highly

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cytotoxic to various cancer cell lines (Kunze, Sasse, Wieczorek, & Huss, 2007). It has been reported that cruentaren A exerts its cytotoxicity through selective inhibition of F_1F_0-ATP synthase, the enzyme responsible for the mitochondrial production of ATP (Kunze et al., 2007, 2006). In 2006, Papathanassiu and coworkers discovered that F_1F_0-ATP synthase can act as an Hsp90 cochaperone that provides the energy required for the maturation of client proteins (Papathanassiu, MacDonald, Bencsura, & Vu, 2006). They also demonstrated that fungal peptides, known as efrapeptins, disrupt interaction between Hsp90 and F_1F_0-ATPase synthase, which leads to the depletion of Hsp90-dependent client proteins, including ERα, mutated p53, and caspase-3, along with downregulation of Hsp27, Hsp70, and even Hsp90 levels. However, the complex peptide structure and promiscuous nature of efrapeptins render them unsuitable for further development. Recently, Hall and coworkers demonstrated that incubation of cruentaren A, a more selective F_1F_0-ATP synthase inhibitor, disrupts interactions between Hsp90α and F_1F_0-ATPase synthase (Hall, Kusuma, Brandt, & Blagg, 2014). Interestingly, it was observed that inhibition of F_1F_0-ATPase synthase via cruentaren A reduces cellular levels of select Hsp90 client proteins without induction of the heat-shock response and thus, could provide a novel approach to modulating Hsp90 function. However, limited synthetic accessibility to cruentaren A represents an obstacle that has yet to be overcome.

5. CONCLUSIONS AND FUTURE PROSPECTIVE

Since recognition of Hsp90 as a critical mediator of oncogenic survival and proliferation decades ago, significant progress toward the development of N-terminal Hsp90 inhibitors has been made and numerous compounds have undergone clinical evaluation. Although these inhibitors have shown promising results in a limited number of disease settings, problems, such as induction of prosurvival responses, and a range of dose-limiting systemic toxicities have become apparent. In contrast, C-terminal Hsp90 inhibitors derived from NB have shown promising results in preclinical studies, but their therapeutic potential has yet to be tested in humans. In recent years, many new compounds that modulate Hsp90 function by a variety of mechanisms have been reported. Natural products, such as EGCG, taxol, and silybin, have been identified as exhibiting the potential to inhibit Hsp90, but the extent to which such activity contributes to their broad-spectrum of antitumor activities remains largely unknown. In addition, small molecules have been reported that disrupt the protein–protein interactions that occur between Hsp90 and its cochaperones and client proteins. These may provide useful insights for the development of compounds that can alter Hsp90 function in more subtle ways than direct inhibition of its N-terminal ATP-binding pocket, such as selective disruption of only certain client proteins which might lead to fewer undesirable effects. Interestingly, many of these natural products, including celastrol and withaferin, feature thiol-reactive motifs, which react with cysteine residues in Hsp90 or its cochaperones. Such compounds represent “soft spots” for Hsp90 manipulation, and suggest a potential role for Hsp90 in global protein homeostasis as a sensor of redox stress. This chapter has highlighted the broad range of compounds emerging in recent times that impact Hsp90 function and are now being used to probe more deeply into the biology of Hsp90. The insights gained from such studies should enable the development of new Hsp90 inhibitors with improved properties for clinical applications in the near future.

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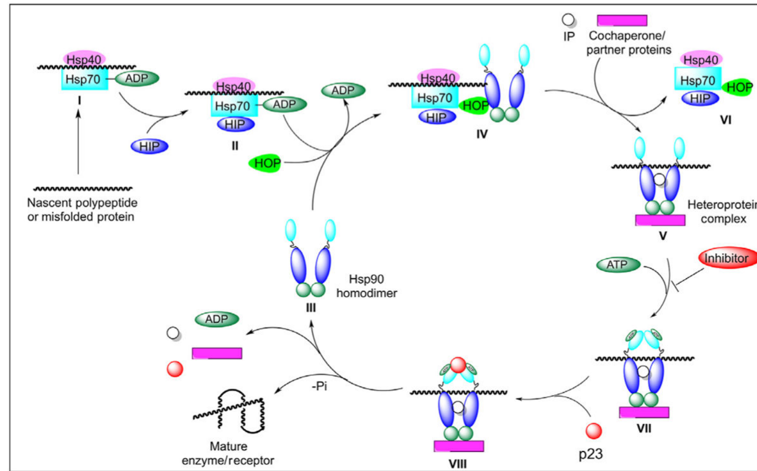

Figure 1.
The Hsp90-mediated protein folding process.

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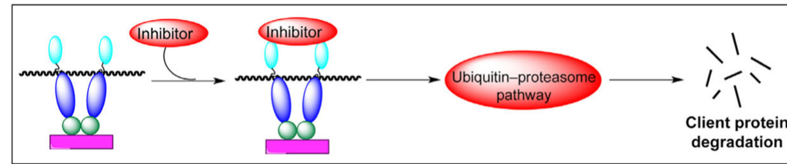

Figure 2.

Rationale for Hsp90 inhibition as an anticancer strategy. Classical Hsp90 inhibitors compete with ATP for the nucleotide-binding domain of Hsp90 and halt the progression of the chaperone cycle. Consequently, the client protein is often directed to the ubiquitin-mediated degradation pathway.

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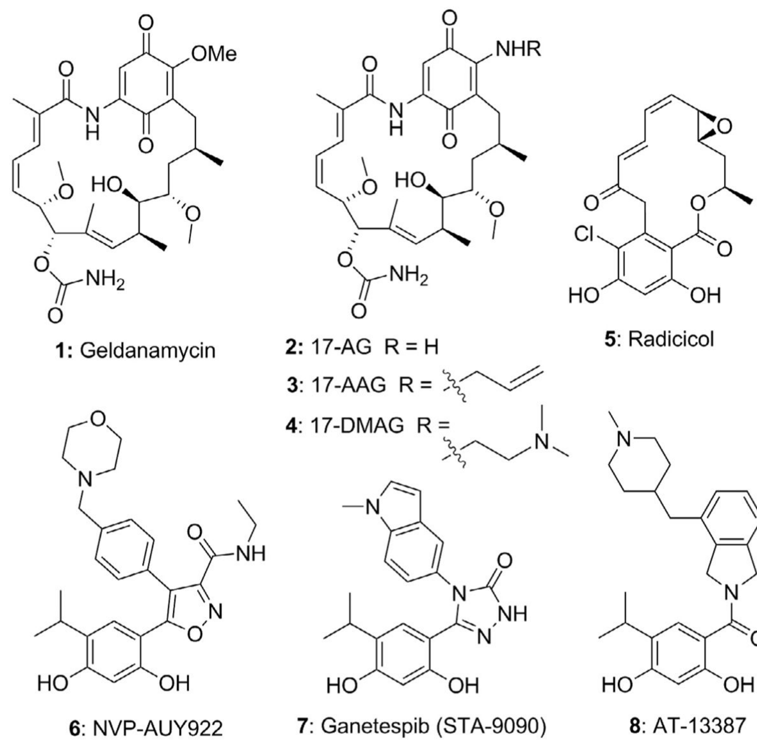

Figure 3.

Structure of representative examples of Hsp90 N-terminal inhibitors.

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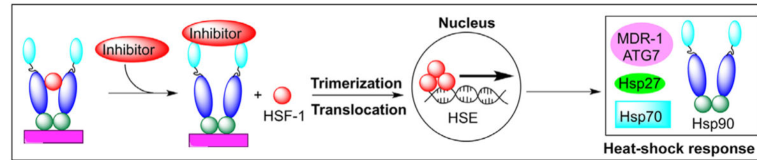

Figure 4.
A proposed mechanism for induction of heat-shock protein expression by Hsp90 N-terminal inhibitors. Hsp90 inhibitors bind to the Hsp90 N-terminus, which result in the release of a transcription factor, HSF-1. Upon release, HSF-1 becomes trimerized, phosphorylated, and translocated to nucleus, wherein it binds to consensus sequences and upregulates many prosurvival mechanisms, including overexpression of prosurvival chaperones such as Hsp27, Hsp40, Hsp70, and Hsp90.

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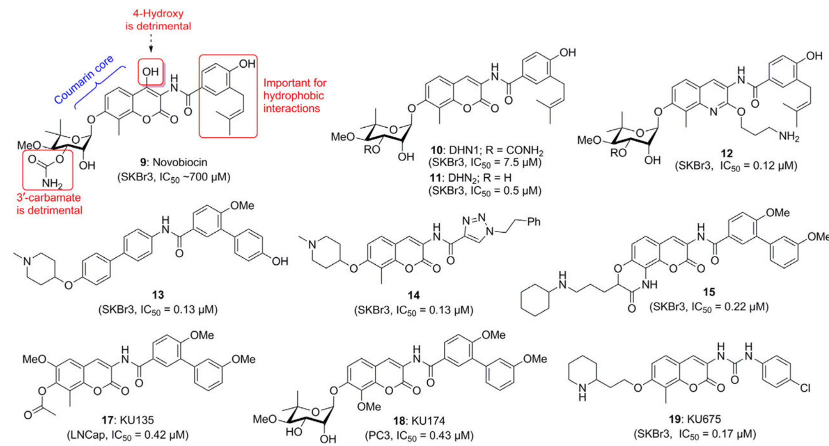

Figure 5.

Structure–activity relationships for novobiocin and its derivatives.

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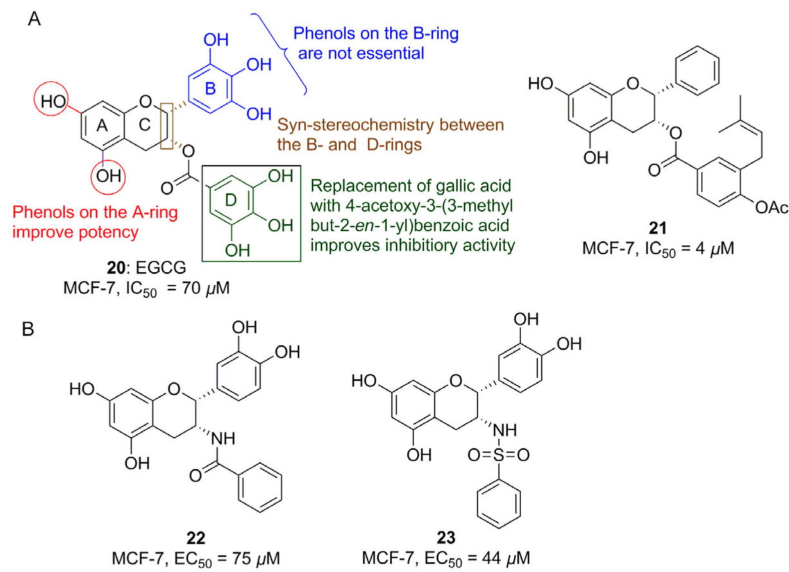

Figure 6.
Summary of cytotoxicity structure–activity relationships for EGCG and its analogs.

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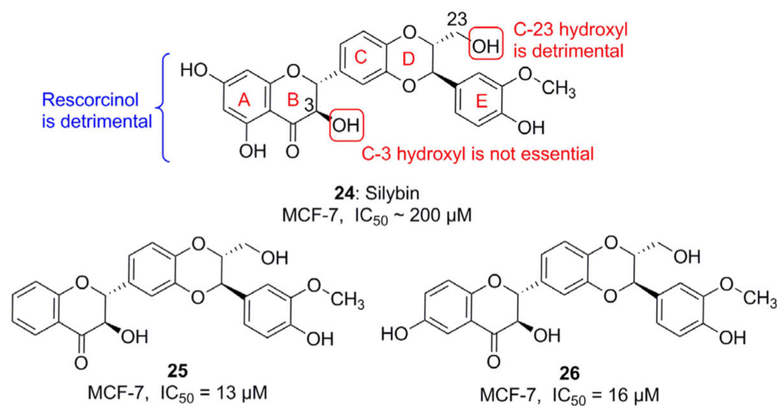

Figure 7.

Summary of SAR for silybin and its analogs.

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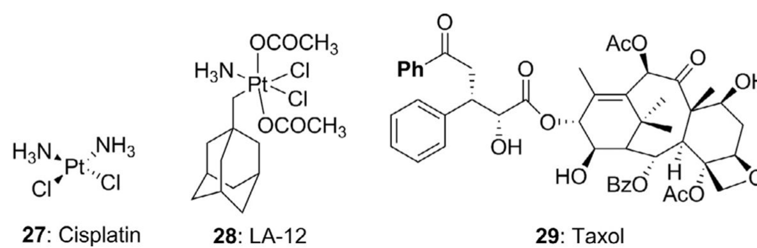

Figure 8.

Structures of cisplatin, LA-12, and taxol.

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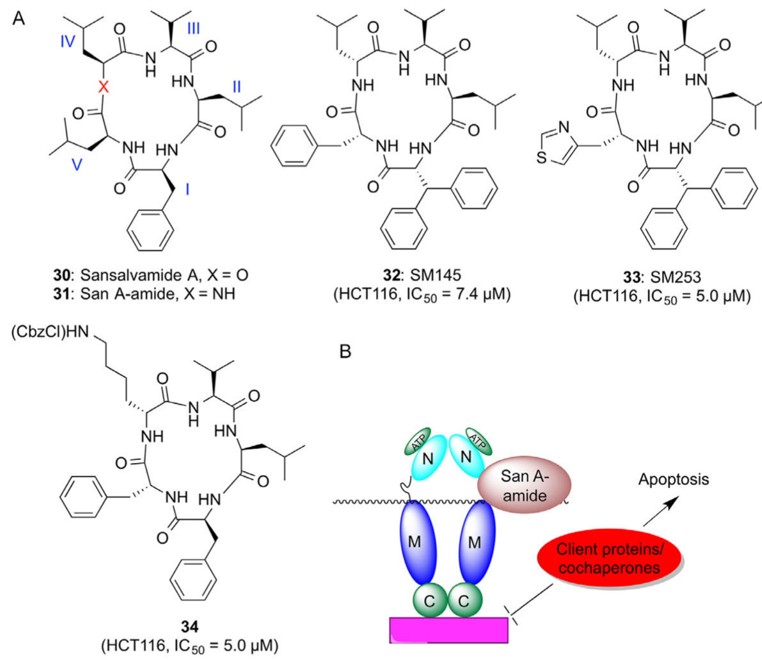

Figure 9.
(A) Structures of sansalvamide A and its analogs. (B) Proposed mechanism of action of sansalvamide A derivatives.

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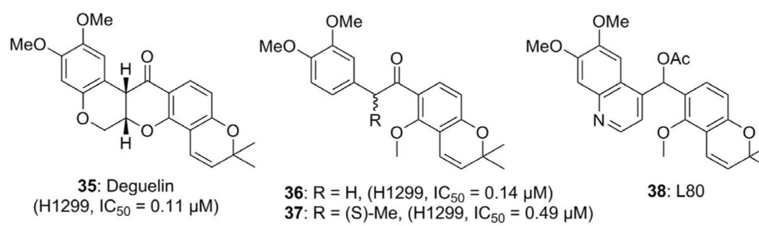

Figure 10.
Structures of deguelin, L80, and its derivatives.

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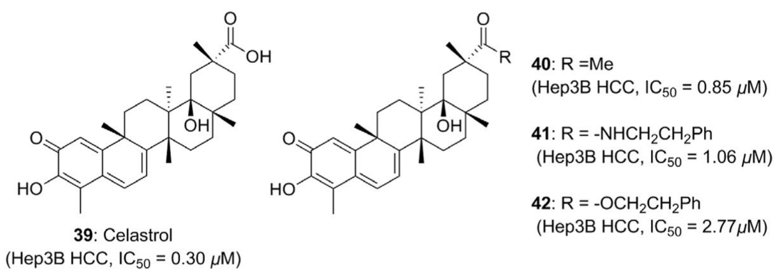

Figure 11.
Structures of celastrol and its derivatives.

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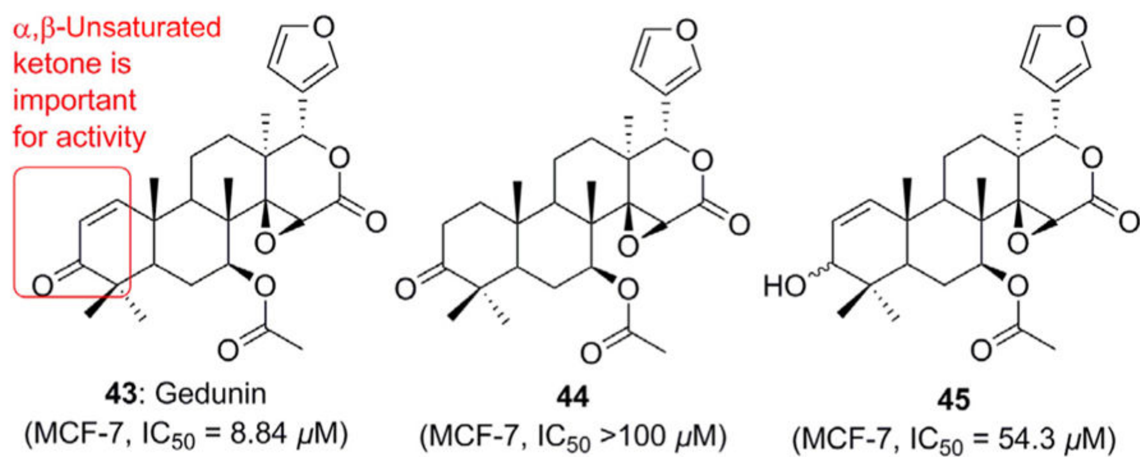

Figure 12.
Summary of SAR for gedunin and derivatives.

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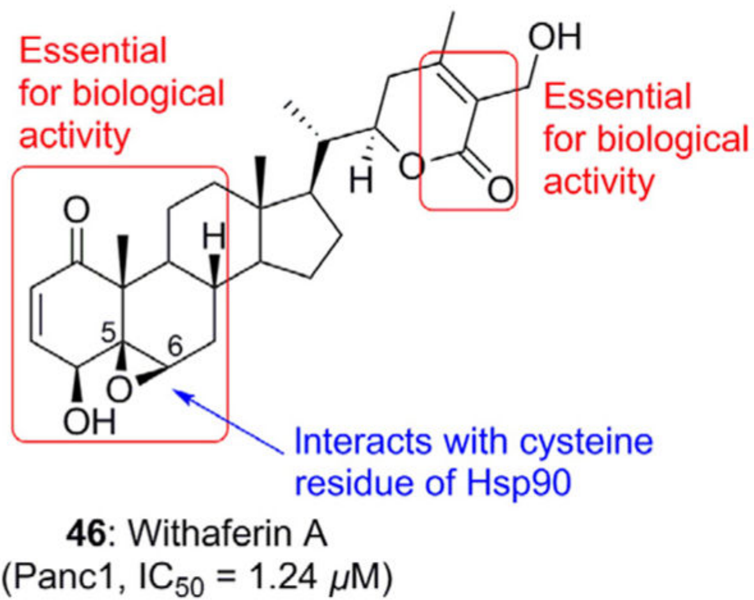

Figure 13.
Summary of SAR for withaferin A.

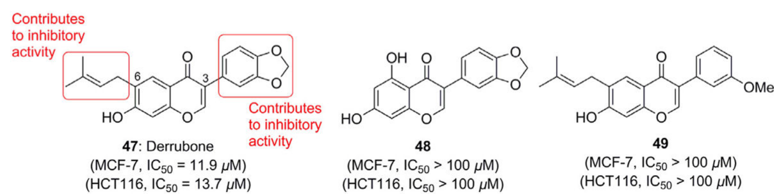

Figure 14.

Summary of cytotoxicity SAR for derrubone and its derivatives.

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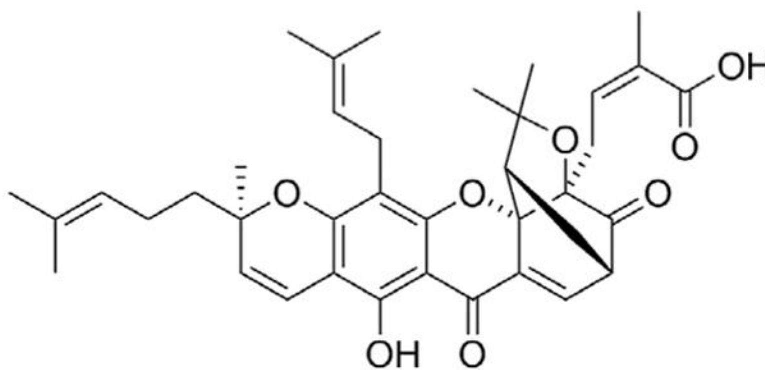

Figure 15.
Structure of gambogic acid.

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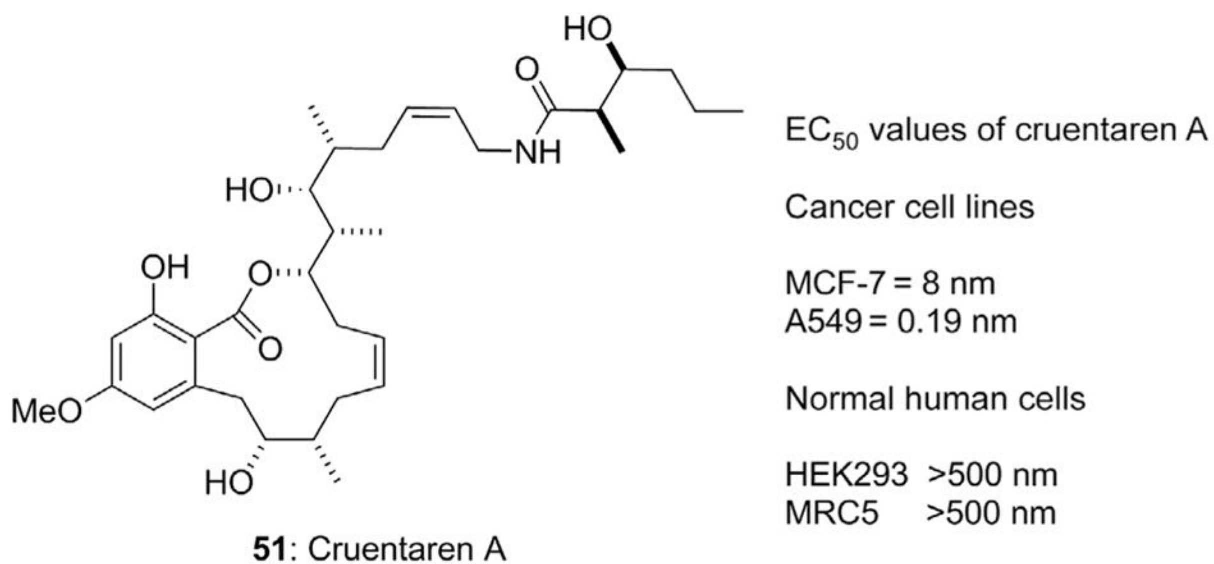

Figure 16.
Structure and cytotoxic activity of cruentaren A.

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Table 1

Cochaperones and Partner Proteins That Participate in the Hsp90 Protein Folding Cycle (Peterson & Blagg, 2009; Zuehlke & Johnson, 2010)

Cochaperone or Partner Proteins	Description
Aha1	Stimulates ATPase activity
Cdc37	Mediates activation of protein kinase substrates
CHIP	Involved in degradation of unfolded client proteins
Cyclophilin-40	Peptidyl propyl isomerase
FKBP51 and 52	Peptidyl propyl isomerase
HOP	Mediates interaction between Hsp90 and Hsp70
Hsp40	Stabilizes and delivers client proteins to Hsp90 complex
Hsp70	Stabilizes and delivers client proteins to Hsp90 complex
p23	Stabilizes closed, clamped substrate bound conformation
HIP	Inhibits ATPase activity of Hsp70
PP5	Protein phosphatase 5
Sgt1	Client adaptor, involved in client recruitment
Tom70	Facilitates translocation of pre-proteins into mitochondrial matrix
WISp39	Regulates p21 stability

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Table 2

Hsp90 Client Proteins Associated with the Hallmarks of Cancer (Blagg & Kerr, 2006; Vartholomaiou et al., 2016)

Hallmarks of Cancer	Hsp90 Client Protein(s)
1. Sustaining proliferative signaling	Raf-1, AKT, Her2, MEK, Bcr-Abl
2. Evading growth suppressors	Plk, Wee1, Myc1, CDK4, CDK6, Myt1
3. Resisting cell death	NF-κ, AKT, p53, c-MET, Apaf-1, Survivin
4. Enabling replicative immortality	Telomerase (h-Tert)
5. Inducing angiogenesis	HIF-1α, VEGFR, PI3K/AKT, RTKs, flt-3
6. Activating invasion and metastasis	c-MET, SSDF-1, MMP-2
7. Deregulated cellular energetics	ARNT, ARRB1, HIF-1α, HMG1, SREBF1
8. Avoiding immune destruction	IRAK3
9. Tumor-promoting inflammation	IL-6, IL-8, IRAK1, IRAK2, IRAK3
10. Genome instability and mutation	FANCA, MAFG, NEK8, NEK9, NEK11