9 The Right Tool for the Job: An Overview of Hsp90 Inhibitors

John Koren III and Brian S. J. Blagg

Abstract

Molecular chaperones are responsible for maintaining intracellular protein quality control by facilitating the conformational maturation of new proteins as well as the refolding of denatured proteins. While there are several classes of molecular chaperones in the cell, this chapter will focus solely on the small molecule modulation of Hsp90, the 90 kDa heat shock protein. Hsp90 is not only responsible for folding nascent proteins, but it also regulates the triage of numerous client proteins through partnering with the ubiquitin-proteasome pathway. Consequently, Hsp90 plays critical role in maintaining the protein homeostasis (proteostasis) network within the cell and is required for the activation/maturation of more than 300 client protein substrates. Many of the clients that depend upon Hsp90 are overexpressed or mutated during malignant transformation. This often renders the clients thermodynamically unstable and dependent on Hsp90 for stability. This phenomenon results in an oncogenic 'addiction' to the Hsp90 protein folding machinery as Hsp90 maintains onco-client proteins. Furthermore, Hsp90-dependent substrates are associated with all ten hallmarks of cancer, making Hsp90 an attractive target for the development of cancer chemotherapeutics. In fact, 17 small molecule inhibitors of Hsp90 have been developed and clinically evaluated for the treatment of cancer. Unfortunately, most of these molecules have failed for various reasons, necessitating a new approach to modulate the Hsp90 protein folding machine.

Keywords

HSP90 inhibitor · Chaperones · Cancer · Heat shock response · Proteostasis · Translational research · Small molecules · Client proteins

9.1 Introduction

There are at least three distinct mechanisms to modulate Hsp90 with small molecules (Fig. 9.1). The N-terminal domain contains an atypical nucleotide-binding site that is responsible for the hydrolysis of ATP, which serves as the requisite source of energy during the protein folding process. In fact, all four Hsp90 isoforms exhibit >85% identity within this region. Due to the high identity among the ATP-binding sites, the Hsp90 inhibitors

J. Koren III · B. S. J. Blagg (✉)
Department of Chemistry and Biochemistry, The Warren Family Research Center for Drug Discovery and Development, The University of Notre Dame, Notre Dame, IN, USA
e-mail: bblagg@nd.edu

M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_9

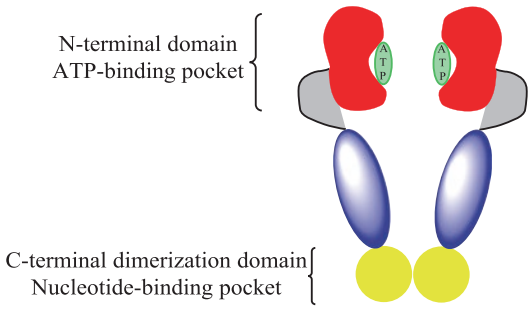


Fig. 9.1 The Hsp90 dimer and points for small molecule modulation

that have undergone clinical evaluation exhibit similar affinity for all four isoforms and manifest pan-inhibitory activity. Upon inhibition of the N-terminal nucleotide binding site, client proteins are unable to achieve their native conformation and instead, become ubiquitinylated and then degraded via the proteasome. At the same concentration of inhibitor needed to induce the degradation of Hsp90-dependent client proteins, the heat shock response (HSR) also occurs. The HSR is a pro-survival response to cellular stress and the accumulation of misfolded proteins, which results in the transcriptional activation of multiple chaperone networks, including Hsp27, Hsp40, Hsp70, and also Hsp90. Thus, inhibitors of the N-terminal domain of Hsp90 not only induce the degradation of oncogenic proteins, but they also induce the levels of Hsp90, which is contraindicated.

Unlike the N-terminal nucleotide binding site, which has been highly sought after for therapeutic development, the C-terminal nucleotide binding site has been less pursued and at present, no co-crystal structure of inhibitors bound to this domain exist. However, multiple studies have now demonstrated that there are several mechanisms to control the Hsp90 protein folding process through modulation of the C-terminal region. In fact, it has been shown that induction of the heat shock response can be segregated from client protein degradation through inhibition of this binding pocket. Thus, compounds targeting this region offer the potential to overcome some of the detriments associated with N-terminal inhibition for the treatment of cancer, in particular, induction of Hsp90 levels. In contrast to the compounds that induce client protein degradation without concomitant induction of the heat shock response, small molecules have also been developed that induce the heat shock response without client protein degradation. Thus, two classes of Hsp90 C-terminal inhibitors have been developed that manifest opposing properties, those that exhibit anti-cancer activity and those that are pro-survival and neuroprotective.

In addition to the N- and C-terminal nucleotide binding sites, natural products have been identified that inhibit the Hsp90-mediated protein folding machinery through disruption of the interactions between co-chaperones and Hsp90, which are required for the maturation of most Hsp90-dependent substrates. Several natural products have been shown to disrupt these protein-protein interactions, and ultimately manifest in distinct inhibitory activities. While none of these molecules are currently undergoing clinical evaluation, they do exhibit properties distinct from N- and C-terminal inhibitors that may be clinically useful. Studies directed toward the development of such inhibitors will be briefly described in this chapter.

9.2 Hsp90 N-Terminal Inhibitors

The N-terminus of Hsp90 contains an ATP-binding pocket. This site, where ATP binds and is hydrolyzed to ADP during the Hsp90-folding process, is the primary location for the binding of numerous Hsp90 inhibitory classes. Kamal et al. were the first to describe an increase in the affinity of Hsp90 for ATP, as well as for ATP-competitive inhibitors, in cancer cells (as compared to normal non-transformed cells) (Kamal et al. 2003). A similar phenomenon has also been described by Dickey et al. following the examination of Hsp90 from Alzheimer's diseased brain (Dickey et al. 2007). Concurrent studies, including the original work by Kamal et al., noted that Hsp90 with altered ATP affinity interacted with a distinct repertoire of co-chaperones (Kamal et al. 2003; Dickey et al. 2007; Moulick et al. 2011; Rodina et al. 2016). In fact, the Chiosis group has published extensively

on this topic, and more recently described an extensive characterization of these complexes in cancer cells, endowing the name 'epichaperome' for these disease-specific Hsp90 complexes (Rodina et al. 2016). These phenomena, although still poorly understood, have served as the impetus for the clinical application of multiple N-terminal Hsp90 inhibitors for potential treatment of cancer and Alzheimer's disease. Ongoing research with existing and novel N-terminal Hsp90 inhibitors has also served to generate valuable chemical tools to investigate Hsp90 function, the disease proteome, as well as probes for detecting and monitoring disease pathogenesis. Herein, we will describe how N-terminal Hsp90 inhibitors have enhanced our understanding of Hsp90 biology in the presence and absence of disease stress.

Geldanamycin (Fig. 9.2), a benzoquinone ansamycin (DeBoer et al. 1970), was the first identified Hsp90 inhibitor, and was initially investigated for its anti-tumor properties. Initially, it was

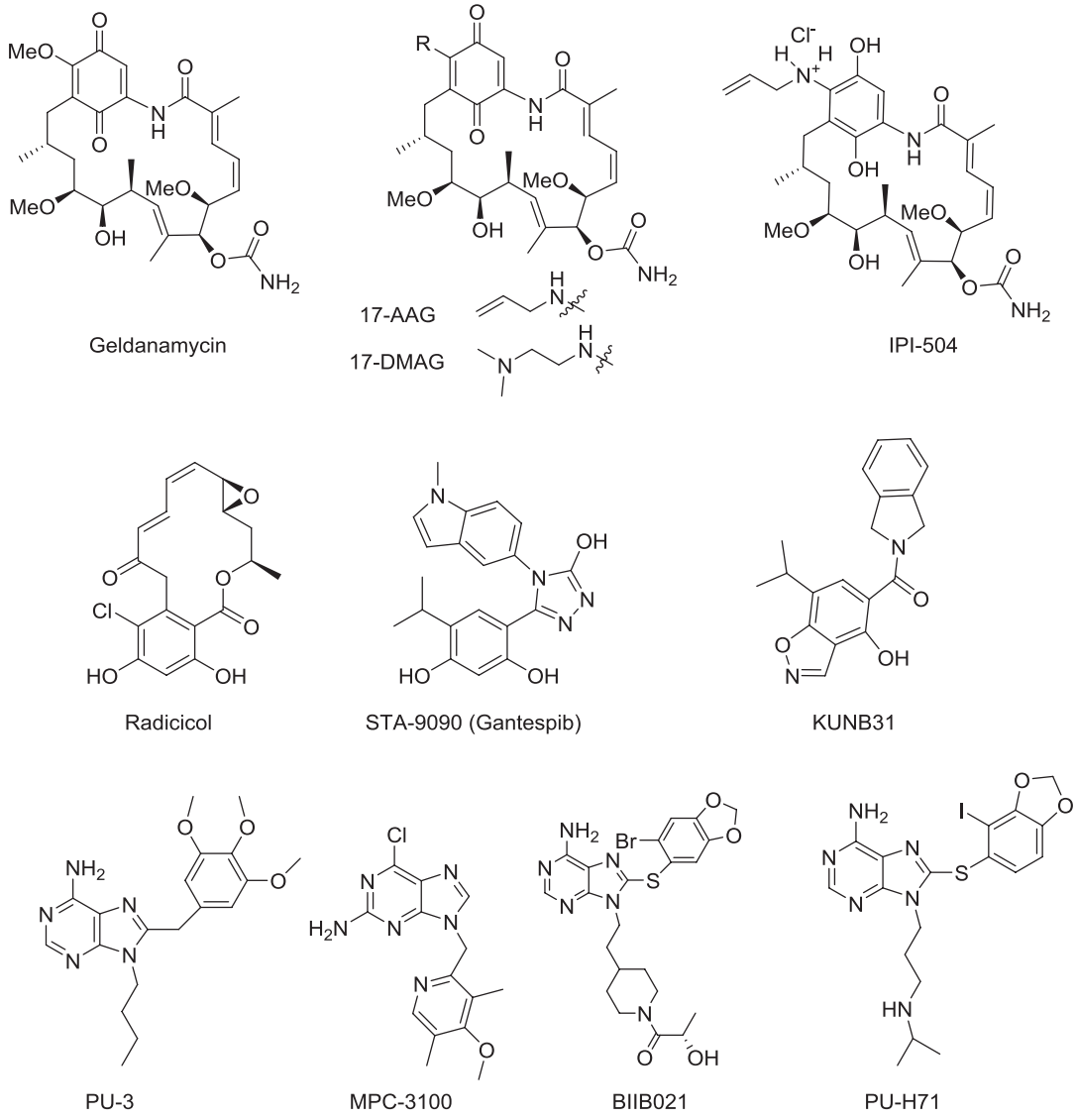


Fig. 9.2 N-terminal Hsp90 inhibitors

thought to inhibit v-src (Whitesell et al. 1992), but was later found to interact with Hsp90 and inhibit formation of the Hsp90/v-src complex (Whitesell et al. 1994). Subsequent studies into the mechanism driving this activity determined geldanamycin to be an ATP-competitive inhibitor (Prodromou et al. 1997). Since then, derivatives of geldanamycin, the macbecins (an additional ansamycin antibiotic) (Bohen 1998; Martin et al. 2008) and several related scaffolds were shown to compete with ATP, and subsequently, inhibit Hsp90 activity. Such selectivity helped propel derivatives of geldanamycin, 17-AAG, 17-DMAG, and IPI-504 (Fig. 9.2) into clinical trials as anti-cancer therapies (reviewed in (Jhaveri et al. 2012)). Due to hepatotoxicity issues associated with this scaffold, which is independent of Hsp90 inhibitory activity, clinical trials have ceased with use of the ansamycin scaffold. However, the use of geldanamycin derivatives have been extensively used outside the clinic to enhance our understanding of Hsp90 biology, particularly in disease. Using this class of Hsp90 inhibitor, it was demonstrated mutant and wild-type proteins have distinct fates following the loss of Hsp90 function. A classic example is the p53 oncogene. Inhibition of Hsp90 promotes the degradation of mutant p53 (Blagosklonny et al. 1996), as mutant p53 is thermodynamically unstable and is therefore more reliant upon the activity of Hsp90 to maintain stability. Wild-type p53, however, remains relatively unaffected following inhibition of Hsp90. As mentioned previously, 17-AAG and geldanamycin were used in seminal studies by Kamal et al. to demonstrate that Hsp90 in cancer cells exhibits higher affinity and distinct co-chaperone complexes as compared to the Hsp90 from non-transformed cells. Although poorly understood, his phenomenon is still a focal point for Hsp90 research and drug development and will be discussed later.

Radicicol (Fig. 9.2), a macrocyclic lactone, is an antibiotic that also acts as an ATP-competitive inhibitor of Hsp90 (reviewed in (Sharp and Workman 2006)). Poor metabolic properties have rendered radicicol inactive in animal studies, and prompted the development of derivatives to improve in vivo activity (Agatsuma et al. 2002; Soga et al. 2001). Radicicol, unlike geldanamycin, does not produce hepatotoxicity (Agatsuma et al. 2002; Soga et al. 2001). Despite this advance over geldanamycin derivatives, no radicicol-based derivatives have advanced into clinical studies. However, the resorcinol ring present in radicicol has been the subject of successful research and clinical advancement and has led to exciting developments in Hsp90-isoform selectivity. Synta Pharmaceuticals compound, STA-9090 (ganetespib) (Fig. 9.2), is one example of this class that is currently under clinical investigation. The use of ganetespib by Taipale in the Lindquist Laboratory has demonstrated several key findings via an elegant screening array (Taipale et al. 2012). These studies demonstrated that there are several dependencies that exist with Hsp90 client proteins. By inhibiting Hsp90 with ganetespib, Taipale identified both 'strong' and 'weak' Hsp90 clients, wherein each class contained client proteins that were degraded or aggregated following the loss of Hsp90 function. These studies concluded that the on-rate of client proteins is the main factor in distinguishing strong and weak clients and found no direct evolutionary correlation between kinase families and Hsp90 client preferences.

Additional modifications to the resorcinol ring have allowed for the generation of small molecules capable of selective-inhibition of the cytosolic Hsp90 family member, Hsp90ß, (KUNB31, Fig. 9.2) (Khandelwal et al. 2018). This advance in the generation of small molecule Hsp90 inhibitors will likely produce effective therapeutics that can avoid some of the problems associated with pan-Hsp90 inhibitors. One example is the dependence of the hERG potassium channel on Hsp90α as well as a lack of induction of the heat shock response (Ficker et al. 2003). Non-selective, or pan, Hsp90 inhibitors can yield cardiotoxicity due to this dependence. Another, and perhaps the most interesting, finding from selective inhibitors was the lack of Hsp70 induction following Hsp90ß-specific inhibition (Soga et al. 2001); Hsp70 induction was long considered an inevitable outcome of Hsp90 inhibition. These findings and continued drug development efforts

will allow researchers to distinguish between the cellular effects of individual Hsp90 family members and produce compounds that may be suitable for translation into the clinic.

The first synthetic Hsp90 inhibitor, PU-3 (Fig. 9.2), is a purine-derived ATP-competitive Hsp90 inhibitor (Chiosis et al. 2002). Following the discovery of PU-3, additional purine-based Hsp90 inhibitors were developed, and include MPC-3100 (Fig. 9.2) by Myriad Pharmaceuticals, BIIB021 (Fig. 9.2) by Biogen, as well as PU-H71 (Fig. 9.2) and PU-AD (structure not disclosed) (reviewed in (Jhaveri et al. 2012); http://www.samustherapeutics.com/; https://www.cancer.gov/publications/dictionaries/cancer-drug/def/iodine-i-124-pu-ad; https://clinicaltrials.gov/ct2/show/NCT03371420). This purine class of Hsp90 inhibitors has been successful thus far during clinical investigation. This success is likely due to their selectivity, pharmacology profiles, and the lack of toxicities that are associated with the natural-product based scaffolds. The purine class, primarily PU-H71 and associated radio-labeled derivatives, have provided a new role for N-terminal Hsp90 inhibitors as imaging agents and diagnostic tools (Rodina et al. 2016). As previously discussed, Kamal et al. performed detailed studies showing Hsp90 in cancer cells to manifest a higher affinity for both ATP and ATP-competitive inhibitors as compared to Hsp90 from non-transformed cells. Rodina and Moulick demonstrated that some, but not all of the Hsp90 in cancer cells present an increased affinity for ATP and ATP-competitive inhibitors (Moulick et al. 2011; Rodina et al. 2016). These properties were further used to identify a network of chaperone complexes with Hsp90, which serves as a central hub for formation of these complexes in cancer. These chaperone hubs, termed the 'epichaperome,' could be evaluated and used as a diagnostic and imaging tool through the use of a radio-labeled purine scaffold Hsp90 inhibitor (Rodina et al. 2016). This discovery allows for enhanced patient selection in the use of Hsp90 inhibitors as anti-cancer agent. In addition, Samus Therapeutics has initiated the clinical evaluation of an epichaperome-targeting agent, named PU-AD, which can serve as an epichaperome biomaker tool for Alzheimer's disease (http://www.samustherapeutics.com/; https://www.cancer.gov/publications/dictionaries/cancer-drug/def/iodine-i-124-pu-ad; https://clinicaltrials.gov/ct2/show/NCT03371420).

Despite the advancement of some Hsp90 N-terminal inhibitors into Phase III clinical trials, clinical advancement has been scarce. Though Hsp90 has demonstrated promise as an anti-cancer target, and perhaps for the treatment of Alzheimer's disease, clinical progress has been slow due to the off-target activities manifested by such compounds.

9.3 Hsp90 C-Terminal Inhibitors

Hsp90-mediated hydrolysis of ATP is required for the folding of bound substrates as well as the release of the folded client. Hsp90 must adopt a closed conformation (Ficker et al. 2003) to undergo ATP hydrolysis. Activator of Hsp90 ATPase activity, Aha1, must first bind Hsp90 before ATP, in an effort to stimulate closure into the closed state. Subsequent structural reorganization ensues, resulting in closure of the N-terminal lid. This reorganization of the conformation represents the rate-limiting step during the protein folding cycle (Prodromou 2012).

It has been demonstrated that the binding of Aha1 to Hsp90 accelerates the conformational change and results in assembly of the closed state. Therefore, small molecules that perturb interactions between Hsp90 and Aha1 are responsible for eliciting unique inhibitory activities that are significantly different than those observed with N-terminal inhibitors.

The coumermycin antibiotics, which include novobiocin (Fig. 9.3), chlorobiocin, and coumermycin A were proposed to bind the N-terminal nucleotide-binding pocket due to structural similarities between the Hsp90 and DNA gyrase ATP-binding sites, both of which contain a unique Bergaret fold. In fact, novobiocin bound competitively versus radicicol and geldanamycin during Hsp90 binding studies. However, neither geldanamycin nor radicicol could displace novobiocin binding to Hsp90 (Marcu et al. 2000).

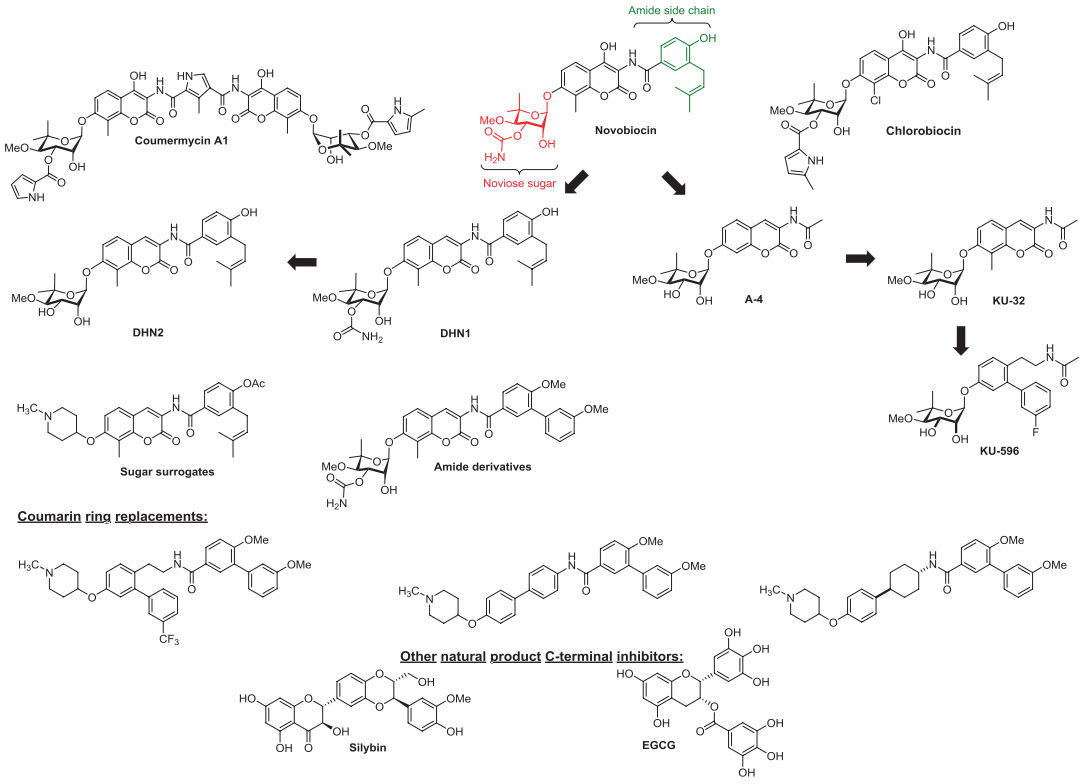


Fig. 9.3 Hsp90 C-terminal inhibitors

Truncated forms of Hsp90 were then evaluated by Neckers and coworkers, who ultimately demonstrated that geldanamycin bound to the N-terminal fragment, but novobiocin bound to a previously unrecognized nucleotide-binding pocket in the C-terminal domain (Marcu et al. 2000). In fact, it was demonstrated that small molecule binding to the C-terminus exhibited allosteric control over the N-terminal binding site.

While novobiocin served as the first Hsp90 C-terminal inhibitor identified, it manifested poor activity in cellular models (Kd ~700 μM in SkBr3 breast cancer cells). Therefore, structure-activity relationship studies were pursued to elucidate the key features of this molecule as well as to improve upon its poor efficacy. The benzamide side chain of novobiocin was replaced with an acetamide, the coumarin ring was modified to remove the 4-hydroxyl, and the 3′-carbamate on the noviose sugar was omitted (Yu et al. 2005). The resulting compound, A4, induced the degradation of Hsp90-dependent client proteins at ~10 μM concentration in the LNCaP prostate cancer cell line (Yu et al. 2005). Remarkably, A4 induced the HSR at concentrations 1000–10,000 fold lower than that needed for client protein degradation (Yu et al. 2005). Prior studies with other classes of Hsp90 inhibitors had never led to segregation of the HSR and client protein degradation. Since A4 exhibited heat-shock induction without the degradation of client proteins, it was evaluated as a neuroprotective agent (Yu et al. 2005). In fact, an analog of KU-32, KU-596, was developed for the treatment of diabetic peripheral neuropathy, and entered clinical trials in 2017. KU-596 is currently awaiting Phase II evaluation.

In contrast to KU-32, which contains the acetamide side chain, 4-deshydroxynovobiocin (DHN1) and 3′-descarbamoyl-4-deshydroxynovobiocin (DHN2) contain the prenylated benzamide side chain, and were prepared to investigate the role of the 4-hydroxyl and

3′-carbamoyl residues (Burlison et al. 2006). Unexpectedly, these compounds did not behave similar to KU-32, and instead induced the degradation of Hsp90-dependent client proteins at concentrations in which no HSR was observed. The most potent compound derived from this series was KU-174, which manifested good inhibitory activity against a large number of cancers in the NCI 60-cell line panel assay (Eskew et al. 2011). Subsequent studies on the coumarin core have been pursued and have led to compounds that exhibit enhanced inhibitory activity and are currently undergoing optimization with the hope of producing an alternative Hsp90 inhibitory class for the treatment of cancer (Kusuma et al. 2014; Donnelly et al. 2008; Bras et al. 2007; Radanyi et al. 2008, 2009).

Based on a number of computational studies, KU-174 was proposed to bind both Hsp90 and Aha1 (Ghosh et al. 2014). Subsequent studies utilizing biotinylated KU-174 demonstrated this molecule indeed binds both Hsp90 and Aha1. However, the aglycone of KU-174 only bound Aha1 (Ghosh et al. 2014), suggesting the noviose sugar is required for binding Hsp90, whereas the aryl amide side chain binds Aha1 (Ghosh et al. 2014). KU-32, which does not contain an aryl amide side chain (acetamide) was biotinylated and also used in affinity purification assays (Eskew et al. 2011). Biotinylated KU-32 was shown to bind both the cytosolic isoform, Hsp90α, and the mitochondria-localized paralog, TRAP-1. Importantly, biotinylated KU-32 did not bind Aha1, supporting the hypothesis that the aryl-containing amide side chain is required for binding Aha1.

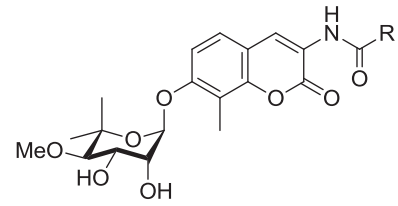
Since KU-32 contains one methyl group on the amide side chain and KU-174 contains a much larger aryl ring on the amide side chain, studies were initiated to identify the point of divergence in which the pro-survival neuroprotective analog was transformed into a compound with anti-cancer activity. Therefore, derivatives of KU-32 were investigated that contained increasingly larger alkyl and cycloalkyl groups on the amide side chain (Table 9.1). The antiproliferative activity manifested by the KU-32 analogs was evaluated against both SkBr3 breast and the androgen independent PC3-MM2 prostate cancer cell lines. Upon evaluation of the results, it became clear that increasing the alkyl chain length resulted in a size-dependent increase in antiproliferative activity as shown in Table 9.1, which was linearly correlative with chain length or bulk.

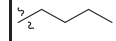
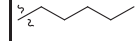
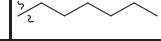
Since the rematuration of firefly luciferase is dependent upon Hsp90, the refolding of denatured firefly luciferase was used to determine whether these analogs affected this process (Matts et al. 2011; Galam et al. 2007; Davenport et al. 2014; Avila et al. 2006). KU-32 did not inhibit the ability of Hsp90 to refold luciferase, but analogs containing the longer alkyl chains or cyclic alkanes did inhibit the re-maturation of firefly luciferase (Fig. 9.4). However, analogs containing shorter alkyl chains failed to inhibit the re-maturation of luciferase, but in contrast to the larger substituents, the smaller alkyl groups increased the re-maturation of firefly luciferase. One explanation that could account for the divergence in activities is that Aha1 remains bound to Hsp90 when the amide side chain is small, however, in the presence of larger side chains, the interaction between Hsp90 and Aha1 is disrupted. Co-immunoprecipitation experiments were performed in the presence of these analogs and it was demonstrated that Hsp90α/Aha1 disruption occurred more readily for the amide side chains that contained larger alkyl groups, which resulted in a linear correlation between activity and chain length (Fig. 9.4).

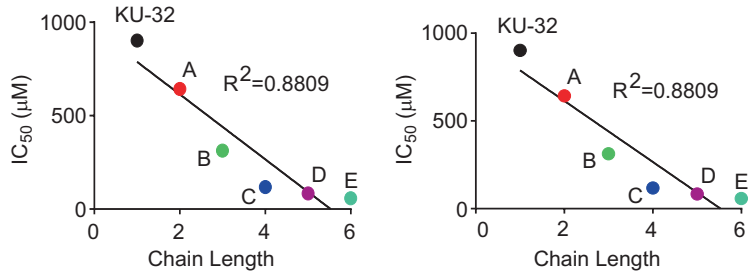
(−)-Epigallocatechin-3-gallate (EGCG) and silybin have been shown to bind the Hsp90 C-terminus and to modulate the Hsp90-mediated protein folding machinery. Gasiewicz and coworkers showed EGCG to bind the same amino acids (538–728) as novobiocin via proteolytic footprinting, immunoprecipitation, and an ATP-argarose pull-down assay (Yin et al. 2009). Inhibition of Hsp90 with EGCG was shown to induce the degradation telomerase, kinases, and the aryl hydrocarbon receptor, and consequently, manifests anti-cancer activity (Palermo et al. 2005; Khandelwal et al. 2013).

Silybin is a traditional medicine that has been used to treat liver and gallbladder disorders (Lu

Table 9.1 Antiproliferative activity resulting from increasing tail length



Entry	R	SKBr3 (IC_{50}, μM)	PC3-MM2 (IC_{50}, μM)
KU32	-Me	902	>500
A		643 ± 50	504
B		313 ± 9	466
C		118 ± 43	317
D		84 ± 38	53.7 ± 0.35
E		58.3 ± 2.1	34.7 ± 1.82
F		517 ± 29	>500
G		220 ± 16	284 ± 99.85
H		67.0 ± 1.4	144.5 ± 10.6
I		46.8 ± 0.7	54.55 ± 4.92



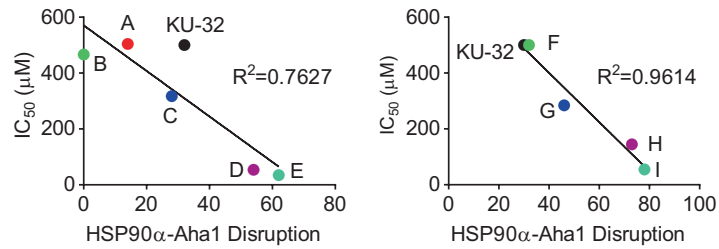


Fig. 9.4 KU-32 analogs disrupt the Hsp90α/Aha1 complex. IC_{50} values report inhibition of refolding of firefly luciferase

et al. 2009). However, silybin was also shown to manifest cytotoxic activity against various cancer cell lines In addition to enhancing the efficacy of chemotherapeutic agents, silybin also manifests anti-cancer activity against a number of cancer cell lines. Silybin has also been shown to possess Hsp90 C-terminal inhibitory activity and cause Hsp90-dependent client proteins degradation without alteration of Hsp90 levels (Zhao et al. 2011). Due to the similar biological properties between novobiocin and silybin, chimeric derivatives of these two natural products were developed, which led to compounds with improved Hsp90 inhibitory activity (Zhao et al. 2012).

9.4 Disruption of Hsp90-co-chaperone Interactions

Hsp90 and its co-chaperones are required for the conformational maturation of the majority of Hsp90-dependent substrates. Below are examples of natural products that have been discovered to disrupt these interactions and consequently, produce activities that are different than those observed with both N- and C-terminal inhibitors (Fig. 9.5). For example, celastrol disrupts interactions between Hsp90 and Cdc37, which is a co-chaperone required for the proper folding of Hsp90-dependent kinases (Zhang et al. 2009). Thus, celastrol manifests its Hsp90

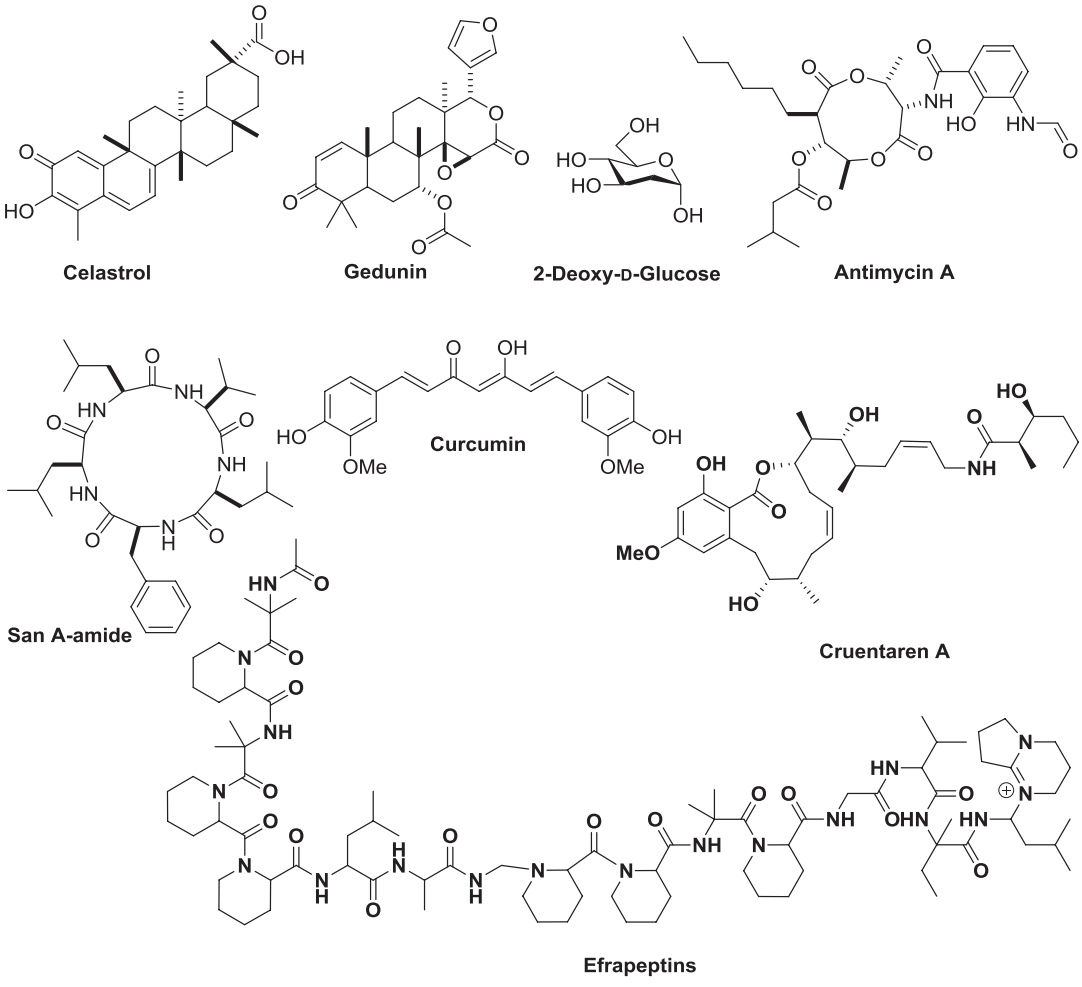


Fig. 9.5 Small molecules that disrupt formation of the Hsp90 heteroprotein complex

inhibitory activity through preventing the maturation of kinases, with little effect on other Hsp90-dependent substrates. The natural product gedunin disrupts Hsp90/p23 interactions, which is a co-chaperone required for the stabilization of Hsp90's closed state, and is required for the maturation of various client proteins (Patwardhan et al. 2013). Interestingly, administration of gedunin to cells does not induce Hsp27 levels, suggesting this approach may not induce the pro-survival HSR.

Since Hsp90 requires ATP for its protein folding activity, the inhibition of ATP synthase with ATP synthase inhibitors including oligomycin A, 2-deoxy-D-glucose, antimycin A and efrapeptins, prevent the Hsp90-dependent maturation of substrates via destabilization of the client-Hsp90 complex, leading to client degradation via the proteosome (Papathanassiu et al. 2011; Hall et al. 2014; Peng et al. 2005). In addition, ATP synthase inhibitors do not induce the HSR, and both oligomycin A and the efrapeptins manifest little to no increase in Hsp90, Hsp70 nor Hsp27 levels. More recently, inhibition of F_1F_0 ATP synthase with the only known selective inhibitor, cruentaren A, was shown to induce client protein degradation through destabilization of the F_1F_0 ATP synthase-Hsp90α interaction (Papathanassiu et al. 2011; Kunze et al. 2007; Jundt et al. 2006). Hsp90 and p23 also form a complex with hTERT, the catalytic subunit of telomerase, a protein whose function contributes to unlimited replicative potential of cancer cells. Curcumin, the active component of Indian curry, was shown to induce the degradation of hTERT through disruption of p23-hTERT interactions as one of its many mechanisms of action (Lee and Chung 2010). Interestingly, curcumin disrupted interactions between p23 and hTERT, but did not alter hTERT's affinity for Hsp90. For comparison, the N-terminal Hsp90 inhibitor, geldanamycin, disrupts both the Hsp90-hTERT and p23-hTERT complexes, leading to the degradation of hTERT.

A derivative of the natural product, sansalvamide A, san A-amide, was demonstrated to induce apoptosis in pancreatic, colon, breast and prostate cancer cell lines (Ardi et al. 2011; Mcconnell et al. 2014). Upon further evaluation, it was shown that San A-amide induced apoptosis in HCT-116 colon cancer cells through displacement of inositol hexakisphosphate kinase-2 (IP6K2) and FKBP52 from the Hsp90 C-terminus (Vasko et al. 2010). It appears as though san A-amide disrupts the structure of the Hsp90 N-terminus, which then alters the substrate binding site. The observed activities appear distinct from other Hsp90 inhibitory classes, as san A-amide exhibits no effect on Her2. In contrast, the Hsp90 N-terminal inhibitor, 17-allylaminogeldanamycin (17-AAG), does not affect IP6K2 and FKBP52 binding, but does inhibit the maturation/activation of Her2.

9.5 Conclusion

While Hsp90 has been seriously sought after as a chemotherapeutic target for the treatment of cancer, all of the inhibitors evaluated for this disease manifested a similar mechanism of action – inhibition of the N-terminal ATPase. Perhaps, alternative approaches toward Hsp90 inhibition that includes disruption of co-chaperone interactions, inhibition of the Hsp90 C-terminus, or isoform-selective inhibition will overcome some of the detriments associated with pan inhibition of the N-terminal ATP-binding site of Hsp90. Alternatively, through modulation of these other domains and partner proteins, modulation of the Hsp90 chaperone machine may be useful for the treatment of other diseases, much like KU-596, which is undergoing clinical evaluation for neuropathy.

Acknowledgements The authors gratefully acknowledge financial support from the National Institutes of Health (CA120458 and CA213566).

References

Agatsuma T, Ogawa H, Akasaka K, Asai A et al (2002) Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities. Bioorg Med Chem 10:3445–3454

Ardi VC, Alexander LD, Johnson VA, Mcalpine SR (2011) Macrocycles that inhibit the binding between

heat shock protein 90 and TPR-containing proteins. ACS Chem Biol 6(12):1357–1366

Avila C, Hadden MK, Ma Z, Kornilayev BA, Ye QZ, Blagg BS (2006) High-throughput screening for Hsp90 ATPase inhibitors. Bioorg Med Chem Lett 16:3005–3008

Blagosklonny MV et al (1996) Mutant conformation of p53 translated in vitro or in vivo requires functional HSP90. Proc Natl Acad Sci U S A 93:8379–8383

Bohen SP (1998) Genetic and biochemical analysis of p23 and ansamycin antibiotics in the function of Hsp90-dependent signaling proteins. Mol Cell Biol 18(6):3330–3339. https://doi.org/10.1128/MCB.18.6.3330

Bras GL, Radanyi C, Peyrat J-F et al (2007) New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90. J Med Chem 50:6189–6200

Burlison JA, Neckers L, Smith AB, Maxwell A, Blagg BSJ (2006) Novobiocin: redesigning a DNA gyrase inhibitor for selective inhibitor of Hsp90. J Am Chem Soc 128:15529–15536

Chiosis G, Lucas B, shtil A, Huezo H, Rosen N (2002) Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. Bioorg Med Chem 10(11):3555–3564

Davenport J, Balch M, Galam L, Girgis A, Hall J, Blagg BS, Matts RL (2014) High-throughput screen of natural product libraries for hsp90 inhibitors. Biology 3:101–138

DeBoer C, Meulman PA, Wnuk RJ, Peterson DH (1970) Geldanamycin, a new antibiotic. J Antibiot (Tokyo) 23:442–447

Dickey CA, Kamal A, Lundgren K et al (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest 117(3):648–658

Donnelly AC, Mays JR, Burlinson JA et al (2008) The design, synthesis and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity. J Org Chem 73:8901–8920

Eskew JD, Sadikot T, Morales P et al (2011) Development and characterization of a novel C-terminal inhibitor of Hsp90 in androgen dependent and independent prostate cancer cells. BMC Cancer 11:468

Ficker E, Dennis AT, Wang L, Brown AM (2003) Role of the cytosolic chaperones Hsp70 and Hsp90 in maturation of the cardiac potassium channel hERG. Circ Res 92(12):e87–e100

Galam L, Hadden MK, Ma Z, Ye QZ, Yun BG, Blagg BS, Matts RL (2007) High-throughput assay for the identification of Hsp90 inhibitors based on Hsp90-dependent refolding of firefly luciferase. Bioorg Med Chem 15:1939–1946

Ghosh S, Shinogle HE, Garg G, Vielhauer GA et al (2014) Hsp90 C-terminal inhibitors exhibit antimigratory activity by disrupting the Hsp90α/Aha1 complex in PC3-MM2 cells. ACS Chem Biol 10(2):577–590

Hall JA, Kusuma BR, Brandt GEL, Blagg BSJ (2014) Cruentaren a binds F1F0 ATP synthase to modulate the Hsp90 protein folding machinery. ACS Chem Biol 9:976–985

Jhaveri K, Taldone T, Modi S, Chiosis G (2012) Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancer. Biochim Biophys Acta 1823(3):742–755

Jundt L, Steinmetz H, Luger P et al (2006) Isolation and structure elucidation of cruentarens A and B – novel members of the benzolactone class of ATPase inhibitors from the myxobacterium Byssovorax cruenta. Eur J Org Chem 2006(22):5036–5044

Kamal A, Thao L, Sensintaffar J et al (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425(6956):407–410

Khandelwal A, Hall JA, Blagg BS (2013) Synthesis and structure-activity relationships of EGCG analogues, a recently identified Hsp90 inhibitor. J Org Chem 78(16):7859–7884

Khandelwal A, Kent CN, Balch M et al (2018) Structure-guided design of an Hsp90ß N-terminal isoform-selective inhibitor. Nat Commun 9(1):425

Kunze B, Sasse F, Wieczorek H, Huss M (2007) Cruentaren a, a highly cytotoxic benzolactone from myxobacteria is a novel selective inhibitor of mitochondrial F1-ATPases. FEBS Lett 581(18):3523–3527

Kusuma BR, Khandelwal A, Gu W et al (2014) Synthesis and biological evaluation of coumarin replacements of novobiocin as Hsp90 inhibitors. Bioorg Med Chem 22(4):1441–1449

Lee JH, Chung IK (2010) Curcumin inhibits nuclear localization of telomerase by dissociating the Hsp90 co-chaperone p23 from hTERT. Cancer Lett 290(1):76–86

Lu P, Mamiya T, Lu LL et al (2009) Silibinin attenuates amyloid beta(25–35) peptide-induced memory impairments: implication of inducible nitric-oxide synthase and tumor necrosis factor-alpha in mice. J Pharmacol Exp Ther 331(1):319–326

Marcu MG, Schulte TW, Neckers L (2000) Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. J Natl Cancer Inst 92(3):242–248

Martin CJ, Gaisser S, Challis IR, Wilkinson B, Gregory M, Prodromou C, Roe SM, Pearl LH, Boyd SM, Zhang MQ (2008) Molecular characterization of macbecin as an Hsp90 inhibitor. J Med Chem 51(9):2853–2857. https://doi.org/10.1021/jm701558c. Epub 2008 Mar 22

Matts RL, Brandt GE, Lu Y et al (2011) A systematic protocol for the characterization of Hsp90 modulators. Bioorg Med Chem 19:684–692

Mcconnell JR, Alexander LA, Mcalpine SR (2014) A heat shock protein 90 inhibitor that modulates the immunophilins and regulates hormone receptors without inducing the heat shock response. Bioorg Med Chem Lett 24(2):661–666

Moulick K, Ahn JH, Zong H, Rodina A et al (2011) Affinity-based proteomics reveal cancer-specific

networks coordinated by Hsp90. Nat Chem Biol 7(11):818–826

Palermo CM, Westlake CA, Gasiewicz TA (2005) Epigallocatechin gallate inhibits aryl hydrocarbon receptor gene transcription through an indirect mechanism involving binding to a 90 kDa heat shock protein. Biochemistry 44:5041–5052

Papathanassiu A, Macdonald N, Emlet D, Vu H (2011) Antitumor activity of efrapeptins, alone or in combination with 2-deoxyglucose, in breast cancer in vitro and in vivo. Cell Stress Chaperones 16(2):181–193

Patwardhan CA, Fauq A, Peterson LB, Miller C, Blagg BSJ, Chadli A (2013) Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. J Biol Chem 288(10):7313–7325

Peng X, Guo X, Borkan SC et al (2005) Heat shock protein 90 stabilization of ErbB2 expression is disrupted by ATP depletion in myocytes. J Biol Chem 280(13):13148–13152

Prodromou C (2012) The 'Active Life' of Hsp90 complexes. Biochim Biophys Acta 1823(3):614–623

Prodromou C, Roe SM, O'brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90(1):65–75

Radanyi C, Bras GL, Messaoudi S et al (2008) Synthesis and biological activity of simplified denoviosecoumarins related to novobiocin as potent inhibitors of heat-shock protein 90 (hsp90). Bioorg Med Chem Lett 18(7):2495–2498

Radanyi C, Bras GL, Marsaud V et al (2009) Antiproliferative and apoptotic activities of tosylcyclonovobiocic acids as potent heat shock protein 90 inhibitors in human cancer cells. Cancer Lett 274(1):88–94

Rodina A, Wang T, Yan P et al (2016) The epichaperome is an integrated chaperome network that facilitates tumor survival. Nature 538(7625):397–410

Sharp S, Workman P (2006) Inhibitors of HSP90 molecular chaperone: current status. Adv Cancer Res 95:323–348

Soga S, Sharma SV, Shiotsu Y et al (2001) Stereospecific antitumor activity of radicicol oxime derivatives. Cancer Chemother Pharmacol 48:435–445

Taipale M, Krykbaeva I, Koeva M et al (2012) Quantitative analysis of Hsp90-client interactions reveals principles of substrate recognition. Cell 150(5):987–1001

Vasko RC, Rodriguez RA, Cunningham CN, Ardi VC, Agard DA, Mcalpine SR (2010) Mechanistic studies of sansalvamide A-amide: an allosteric modulator of Hsp90. ACS Med Chem Lett 1(1):4–8

Whitesell L, Shifrin SD, Schwab G, Neckers LM (1992) Benzoquinonoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res 52:1721–1728

Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM (1994) Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A 91:8324–8328

Yin Z, Henry EC, Gasiewicz TA (2009) (−)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. Biochemistry 48:336–345

Yu XM, Shen G, Neckers L et al (2005) Hsp90 inhibitor identified from a library of novobiocin analogues. J Am Chem Soc 127:12778–12779

Zhang T, Li Y, Yu Y, Zou P, Jiang Y, Sun D (2009) Characterization of celastrol to inhibit Hsp90 and Cdc37 interaction. J Biol Chem 284(51):35381–35389

Zhao H, Brandt GE, Galam L, Matts RL, Blagg BSJ (2011) Identification and initial SAR of silybin: an Hsp90 inhibitor. Bioorg Med Chem Lett 21(9):2659–2664

Zhao H, Yan B, Peterson LB, Blagg BSJ (2012) 3-arylcoumarin derivatives manifest anti-proliferative activity through Hsp90 inhibition. ACS Med Chem Lett 3(4):327–331