Chapter 7
Designing Drugs Against Hsp90 for Cancer Therapy

Stefan O. Ochiana, Tony Taldone and Gabriela Chiosis

Abstract Heat shock protein 90 (Hsp90) is a molecular chaperone exploited by cancer cells to aid the function of numerous oncoproteins. The recognition of Hsp90 as a critical facilitator for oncogene addiction and survival of the cancer cell has opened a promising new niche for cancer treatment. The serendipitous discovery that the broad spectrum anticancer activity of the natural products geldanamycin (GM) and radicicol (RD) was a result of inhibition of Hsp90 resulted in the development of improved derivatives of these natural products. One of these was 17-allylaminogeldanamycin (17-AAG), a closely related analog of GM, and was in fact the first Hsp90 inhibitor to enter the clinic. However, GM and its analogs suffer from poor "drug-like" properties and this served as a strong impetus for the development of novel synthetic Hsp90 inhibitors. These efforts resulted in the development of numerous potent synthetic small molecule inhibitors with significant scaffold diversity as well as superior pharmacokinetic and toxicity profile to have entered clinical trials. This review highlights the drug discovery efforts pertaining to the development of the first and second-generation Hsp90 inhibitors, and also gleans over their individual promise as clinical agents for anticancer therapy.

1 Introduction

Heat shock proteins were first reported in the early 1960s when they were serendipitously discovered by the Italian scientist Ferruccio Ritossa [1] who observed that cells could mount very strong transcriptional activity upon exposure to heat. Subsequent work undertaken by Tissieres et al. led to the discovery of heat shock proteins by means of electrophoretically studying the newly synthesized proteins

S. O. Ochiana (✉) · T. Taldone · G. Chiosis
Department of Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
e-mail: ochianas@mskcc.org

T. Taldone
e-mail: taldonet@mskcc.org

G. Chiosis
e-mail: chiosisg@mskcc.org

W. A. Houry (ed.), *The Molecular Chaperones Interaction Networks in Protein Folding and Degradation,* Interactomics and Systems Biology, DOI 10.1007/978-1-4939-1130-1_7,

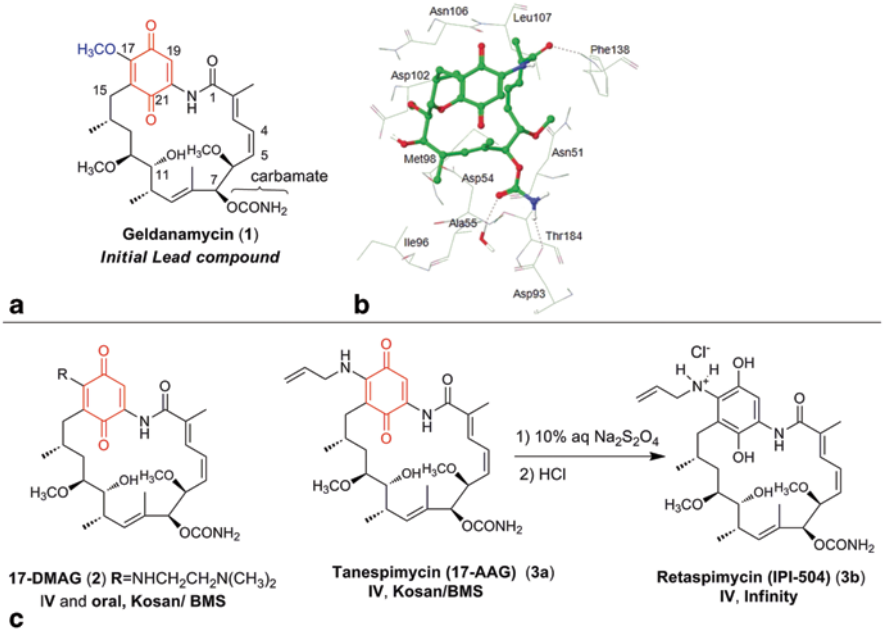

Fig. 7.1 **a** Structure of Geldanamycin, the first reported Hsp90 inhibitor. **b** Structure of human Hsp90α bound to (**1**; PDB ID: 1YET), H-bonds are shown by *dotted lines*. **c** Ansamycin inhibitors of Hsp90; in *red* is shown the *benzoquinone* (i.e., *quinone ring*) moiety and in *blue* the methoxy group at C17

in heat-shocked *Drosophila* larvae [2]. Over the course of a few decades, various research groups have shown that the vast majority of these proteins are molecular chaperones that aid the correct folding of proteins and guard the proteome from the threat of misfolding and aggregation [3, 4]. A noteworthy member of this family is heat shock protein 90 (Hsp90), a chaperone that employs a complex cycle of client protein binding and hydrolysis of adenosine triphosphate (ATP) to regulate both the stability and activity of its client proteins, including kinases, steroid hormone receptors, and transcription factors [5, 6]. Furthermore, when the Hsp90 chaperone cycle is inhibited, its client proteins become destabilized, are ubiquitinated, and consequently degraded by the proteasome [7–9]. The knowledge that many Hsp90 client proteins (e.g., human epidermal growth factor receptor 2, HER2; AKT; met proto-oncogene (MET); epidermal growth factor receptor, EGFR; estrogen receptor (ER); Raf-1) are known to play significant roles in the development and promotion of cancer has sparked a strong interest in this chaperone as a viable target for cancer therapy [10–12].

As is often the case in drug discovery, natural products played an important role in the discovery of Hsp90 inhibitors. In fact, the very concept of inhibition of Hsp90 as a viable anticancer strategy was demonstrated with two seminal natural products, geldanamycin (GM; Fig. 7.1a) and radicicol (RD; Fig. 7.2a). GM is a benzoquinone ansamycin which was first isolated in 1970 from a fermentation broth

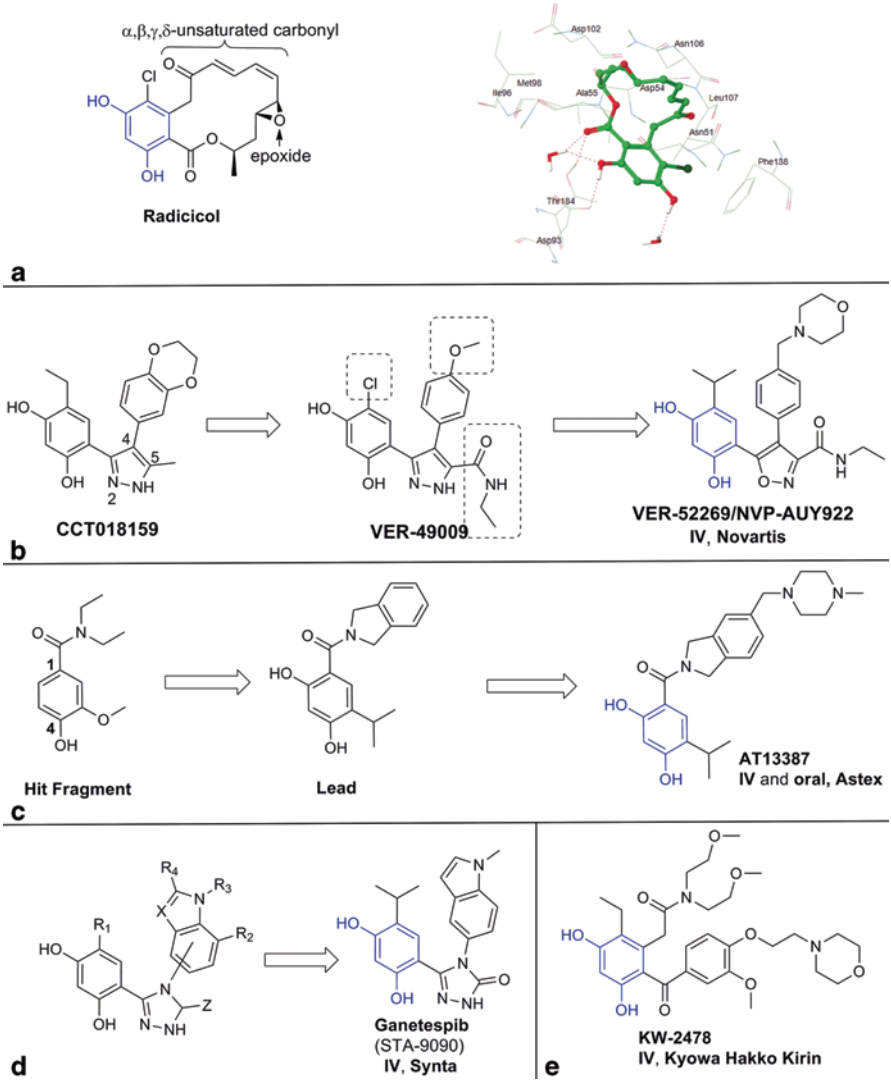

Fig. 7.2 **a** Resorcinol inhibitor of Hsp90, radicicol (in *blue* is shown the resorcinol moiety), and the structure of human Hsp90α bound to RD (Radicicol) (PDB ID: 4EGK), H-bonds are shown by *dotted lines*. **b** Scaffold modifications of CCT018159 leading first to VER-49009, and then to the clinical candidate NVP-AUY922. **c** From fragment to lead to clinical candidate AT13387. **d** Regions explored leading to Ganetespib. **e** KW2478 a resorcinol type inhibitor of Hsp90

of *Streptomyces hygroscopicus* [13]. Though GM was originally pursued as an antibiotic, its anticancer properties were discovered later from a phenotypic screen of compounds able to reverse the phenotype of *v-src* transformed cells [14], and these screening efforts also identified other structurally related benzoquinone ansamycins including herbimycin and macbecin. A landmark publication by Whitesell et al. in

1994 showed that GM bound to Hsp90 [15]. Subsequently, it was demonstrated that GM binds to the N-terminal nucleotide-binding pocket [16, 17] and inhibits the adenosine triphosphatase (ATPase) activity of Hsp90 [18]. The binding of GM to Hsp90 disrupts formation of the "closed" Hsp90 complex, inhibiting progression of the chaperone cycle, resulting in client protein degradation by the ubiquitin proteasome system [7, 19].

The crystal structures of the natural products GM and RD with the Hsp90 N-terminal domain provide essential aspects of their nucleotide mimicry [20]. In the case of GM, the crystal structure showed that the macrocyclic ansa ring and pendant carbamate are directed towards the bottom of the binding pocket (Fig. 7.1b), whereas the benzoquinone ring (depicted in red; Fig. 7.1a) was oriented towards the top of the pocket. On the other hand, the orientation of RD is opposite to that of GM since the resorcinol ring (depicted in blue, Fig. 7.2a) directed towards the bottom of the pocket and the macrocycle, with its conjugated bond system and epoxide, is facing the top of the pocket. The adenosine triphosphate/adenosine diphosphate (ATP/ADP) adenine's NH_2 group is mimicked by the carbamate functionality of GM and the resorcinol moiety of RD, which make direct and indirect (water mediated) hydrogen interactions with the protein (Figs. 7.1b and 7.2a). Additionally, hydrophobic interactions are also observed in this pocket for both natural products. As this chapter will show, the motifs of GM and RD are an occurring theme in many Hsp90 inhibitors that have reached the clinic.

Initially, there was considerable hesitancy towards targeting a housekeeping protein that is abundantly expressed in normal cells due to the perceived risk that inhibition might lead to undesirable toxicity. However, numerous groups were undeterred by this, buoyed in the knowledge that GM had previously been shown to be selectively toxic towards cancer cells, though the reasons for the observed therapeutic index for Hsp90 inhibitors was not yet known. The first major insight into the reasons underlying this was presented in a seminal paper by Kamal et al. which showed that cancer cells are composed of a distinct pool of Hsp90 compared to normal cells [21]. This work showed that the Hsp90 inhibitor 17-allylaminogeldanamycin (17-AAG; Fig. 7.1c) bound Hsp90 in tumor cells with a 100-fold greater affinity than Hsp90 in normal cells. Furthermore, Hsp90 in cancer cells was shown to be entirely in multi-chaperone complexes with co-chaperones (i.e., Hsp70/Hsp90 organizing protein, HOP, p23, Hsp70, etc.) with high ATPase activity, whereas Hsp90 in normal cells was determined to be in a latent uncomplexed state. This work provided a solid rationale for the observed selectivity of GM for cancer cells over normal cells, and served as a powerful impetus for the development of Hsp90 inhibitors by multiple institutions throughout the world. Later on, Moulick et al. revised this view and showed that Hsp90 forms biochemically distinct complexes in malignant cells where a major fraction of cancer cell Hsp90 retained "housekeeping" chaperone functions similar to normal cells, whereas a functionally distinct Hsp90 pool enriched or expanded in cancer cells and referred to as "oncogenic Hsp90" executed functions necessary to maintain the malignant phenotype [22]. This work also indicated that Hsp90 inhibitors have distinct selectivity profiles for the two Hsp90 species, providing perhaps a rationale for the non-overlapping thera-

peutic index and tumor retention profiles noted for the several Hsp90 chemotypes currently in clinical evaluation.

While the early stages in the development of Hsp90 inhibitors was undertaken by only a select group of research institutions and small biotechnology companies, our increased understanding of tumor Hsp90 biology has changed this landscape dramatically. Hsp90 has currently emerged as one of the most highly pursued drug targets for the treatment of cancer with 17 agents already in various stages of clinical trials [23]. Hsp90 is a very attractive target for a number of reasons. Hsp90 regulates multiple oncogenic signaling pathways and its inhibition enables these pathways to be targeted simultaneously [8, 24]. Additionally, it provides a potential advantage over other anticancer strategies (i.e., kinase inhibitors) in that it may be less susceptible to resistance mechanisms [25]. For this reason, the use of Hsp90 inhibitor can be envisioned not only as a monotherapy but also as combination therapy to combat the developing of resistance observed with current anti-cancer drugs. Finally, as we shall show, from a drug discovery perspective Hsp90 has turned out to be a highly druggable target.

It is significant that each of the compounds currently in the clinic target the N-terminal ATP pocket of Hsp90 [11, 23, 26]. There is also future promise for other clinical agents which disrupt Hsp90 chaperone cycle by alternative mechanisms such as targeting co-chaperone-Hsp90 interactions (e.g., Cdc37, HOP), targeting client-Hsp90 interactions or developing inhibitors that bind to the Hsp90 C-terminal ATP-binding site [12, 27, 28]. To date, there are no approved Hsp90 targeted agents, but the fact that clinical activity has been obtained with multiple drugs in various tumors is promising to change this status quo.

The great amount of work that has been undertaken on developing Hsp90 inhibitors is evinced by the sheer number of compounds reported in the literature to modulate Hsp90 function through a variety of mechanisms and constitute a broad array of structural classes [11, 12, 23, 29, 30]. Indeed, the complex and dynamic nature of Hsp90 function presents multiple opportunities for the design of drugs that target it and is a major reason why so many compounds have been identified. The dynamic nature of Hsp90 is exemplified by the significant structural changes it undergoes throughout the various stages of the chaperone cycle that ultimately result in client protein folding. What is more, it is becoming increasingly clear that even if these clinical agents share a similar binding site, their precise mode of binding may be different resulting in wide-ranging effects on biology [22, 31]. Conformational dynamism of Hsp90 enables an inhibitor to potentially target multiple diverse conformations and it is becoming apparent that the ability of an inhibitor to sample a variety of conformations can have dramatic effects on the observed biology. While a better understanding of the observed biological effects to the precise binding mode of inhibitor is clearly necessary, it is obviously clear that the plasticity of Hsp90 has not prevented the discovery and development of inhibitors using standard approaches in drug discovery.

Herein, we would like to review the strategies used to design some of the major classes of Hsp90 inhibitors that are being evaluated in clinical trials. In this chapter, we focus on the medicinal chemistry efforts pertaining to select scaffolds

and describe progress from initial discovery to their advancement into clinical agents. While various methods have been used to discover the initial lead molecules (i.e., phenotypic screening, structure-based drug design (SBDD) [32], biochemical and cell-based screening, and fragment-based drug discovery (FBDD) [33]), in each case standard medicinal chemistry concepts were utilized to optimize the initial leads into clinical candidates. This was done with strong absorption, distribution, metabolism excretion, and toxicity (ADMET) considerations and with an increasing focus on tumor Pharmacokinetics (PK) properties. The focus on tumor PK is derived from the peculiar PK profile that Hsp90 inhibitors display, being selectively retained in tumor rather than normal tissues, often at pharmacologically relevant concentrations for an extended period of time. This effect was first noted by Vilenchik et al. [34]. This profile augurs well for development of these compounds into drugs as one can envision a molecule which is rapidly distributed to the tumor tissues where the drug is retained in sufficient concentration to cause marked anticancer activity and at the same time is rapidly cleared from non-cancerous tissues, thus decreasing the probability of observing toxic side effects.

2 Hsp90 Chemotypes: From Discovery to Development

2.1 Ansamycin Natural Product Class as the First Hsp90 Inhibitors

The discovery of lead agents for Hsp90 inhibition was initially paved by the thorough investigation of the ansamycin natural product class (Fig. 7.1). This was also the first class to have been evaluated in clinical trials. GM demonstrated both in vitro and in vivo antitumor activity in preclinical models, but it never reached clinical trials as a result of liver toxicity as well as poor solubility and metabolic and chemical instability [35, 36]. The hepatotoxicity observed with GM was attributed to the chemical instability of the quinone ring, which is known to be reactive towards nucleophiles that are typically present in biological molecules [37, 38], and is exacerbated by the C-17 methoxy group (Fig. 7.1a, methoxy group shown in blue, quinone shown in red). However, analogs of GM that contain alkylamino groups instead of the C-17 methoxy group retain their biological activity and show decreased hepatotoxicity [39]. Reflective of its high reactivity, the methoxy group of GM is easily substituted by reaction with amines and at least two significant derivatives have been prepared by this approach. Substitution with an allylamino group resulted in 17-allylamino-17-demethoxygeldanamycin (17-AAG; Fig. 7.1c), a compound that shared important biological features with GM but displayed a more favorable toxicity profile [40]. 17-AAG was the first Hsp90 inhibitor to be evaluated in clinical trials in 1999, and subsequent published clinical data for 17-AAG demonstrated in tumors molecular signatures (i.e., c-Raf-1 and CDK4 depletion and Hsp70 induction) indicative of target inhibition [41]. This information combined with the

knowledge that clinical activity has been seen for 17-AAG in melanoma, multiple myeloma, prostate, and breast cancer [8] provided proof of concept for Hsp90 inhibition in patients. The further advancement of this compound was impeded as a result of its poor pharmaceutical and toxicity profile. For example, because of its low solubility, 17-AAG was administered intravenously using a dimethyl sulfoxide (DMSO) and egg phospholipid vehicle, which, at doses higher than 100 mg/m^2 was itself toxic (i.e., bad odor, nausea, anorexia), and these vehicle-related toxicities, attributed to the DMSO formulation, were as major as the ones resulting from the drug itself [23]. Additionally, 17-AAG is a substrate of the P-glycoprotein-mediated efflux and the polymorphic cytochrome P450 CYP3A4 [42]. Finally, the delayed hepatotoxicity observed for 17-AAG in the clinic with the twice a week continuous dosing schedule [43] further supported ongoing endeavors to develop non-ansamycin Hsp90 inhibitors with an improved toxicity profile.

Kosan Biosciences initiated a program aimed at finding potent, water soluble inhibitors of Hsp90 based on the GM scaffold, and thus synthesized more than 60 17-alkylamino-17-demethoxygeldanamycin analogs [11, 39]. Their efforts resulted in 17-(2-dimethylaminoethyl)-amino-17-demethoxygeldanamycin (17-DMAG; Fig. 7.1c), an analog which contains the ionizable N,N-dimethylethylamine group instead of the methoxy group at C-17. The ionizable amino group results in an increased solubility profile as well as higher oral bioavailability and equal if not better antitumor activity than 17-AAG [44]. Thus, in 2004, 17-DMAG was advanced into clinical trials where it was evaluated both orally and as an intravenous (IV) agent. Clinical benefit was reported in 42% (i.e., 8 out of 19) of patients with refractory HER2+ metastatic breast cancer and in patients with refractory ovarian cancer who were progressing on standard chemotherapy, and also promising results were seen in patients with chemotherapy refractory acute myelogenous leukemia since 3 out of 17 patients showed complete response to the Hsp90 therapy [26]. Nonetheless, in 2008, the clinical development of 17-DMAG was discontinued owing to unacceptable toxicity, which was target unrelated [26].

Infinity Pharmaceuticals attempted to address the pharmaceutical deficiencies characteristic to the ansamycin inhibitors through IPI-504 (Retaspimycin), the hydroquinone hydrochloride salt of 17-AAG (Fig. 7.1c). This was obtained by reducing 17-AAG to the corresponding hydroquinone using $Na_2S_2O_4$ which was subsequently treated with hydrochloric acid (HCl; Fig. 7.1c). The hydrochloride salt was chemically stable to oxidation and improved the aqueous solubility >4000-fold when compared to 17-AAG [45, 46]. Moreover, the hydroquinone compound (IPI-504) was shown to be the more potent Hsp90 inhibitor when compared to the quinone (17-AAG) and this was suggested to be due to the various hydrogen bonding now possible with the hydroquinone derivative [45, 46]. In the clinic, IPI-504 has shown promising activity in patients with non-small cell lung cancer (NSCLC) and oncogenic anaplastic lymphoma kinase (ALK) gene rearrangements [47]. In a Phase II trial, 28% of patients with NSCLC were able to see a stabilization in their condition as well as tumor reduction [29]. In the clinic, the evaluation of this agent against gastrointestinal stromal tumor (GIST) was terminated due to unexpectedly high hepatic toxicity. The exact mechanism of the liver toxicity is still elusive but it

may perhaps relate to the potential to form a quinone through oxidation in vivo. The quinone moiety can be seen as a liability since it has potential for adduct formation and redox metabolism, and this most likely contributes to the liver toxicity [48]. On the whole, IPI-504 is a clinical agent with improved PK and toxicity properties and this profile makes it the most promising candidate from the ansamycin class.

Overall, the natural product-based Hsp90 inhibitors have pharmaceutical limitations related to their ADME properties. The pioneering work with GM served as a proof-of-concept for this cancer target. However, the combined knowledge that GM and related derivatives show dose-dependent liver toxicity, and that RD derivatives suffer from severe toxicities in rats and mice [49] opened the landscape to the discovery and development of synthetic inhibitors. These are compounds based on novel chemical scaffolds that sought to overcome the limitations observed with GM, RD, and their derivatives.

2.2 Resorcinol Derivatives

A number of compounds which have been advanced into the clinic contain a resorcinol moiety, and in this regard can be related to RD (Fig. 7.2a). RD is a resorcylic acid lactone first isolated in 1953 from *Monosporium bonorden* [50] and later shown to be a potent Hsp90 inhibitor where it affects key components of the molecular chaperone in a manner similar to GM [51]. The resorcinol core of RD binds deep in the ATP-binding pocket and provides key interactions which are essential for its potent inhibitory activity [20, 51]. Although RD has potent activity in vitro, this did not translate to in vivo activity [52]. The in vivo inactivity is attributed to the highly reactive functionalities in its structure such as α, β, γ, δ-unsaturated carbonyl group and an epoxide (Fig. 7.2a) which are readily metabolized [53, 54]. These liabilities were partially addressed through synthetic modifications. In one approach, the electrophilic epoxide was replaced with a relatively unreactive cyclopropyl group [53], which resulted in an analog that retained potency when tested in the MCF-7 breast cancer cell line with an IC_{50} of 54 nM vs. 23 nM for RD [53] and also showed activity in vivo in a MX-1 adenocarcinoma mouse model [55]. In another approach, the biologically unstable carbonyl was converted to oxime derivatives [52, 56]. One of the most potent of these oxime derivatives was shown to have significant antitumor activity in MX-1 and A431 epidermoid carcinoma xenograft models [57]. Despite these results, any attempts, this far, to directly modify RD have failed to deliver clinically viable compounds. Nonetheless, the resorcinol core of RD is an essential motif distinguishing several small molecule Hsp90 clinical agents which are described below.

2.2.1 NVP-AUY922/VER52296

A high-throughput screen of approximately 56,000 compounds was initiated to identify novel small molecule inhibitors of Hsp90. The assay used in this screen

measured inhibition of yeast Hsp90 ATPase activity using the malachite green detection of inorganic phosphate [58] and identified the resorcinolic pyrazole compound CCT018159 as the most potent hit (Fig. 7.2b). Crystallographic data revealed that CCT018159 bound to the ATP pocket in the N-terminal domain of yeast Hsp90 in a manner similar to RD [59]. The two phenolic hydroxyls and the adjacent pyrazole nitrogen of CCT018159 form a network of hydrogen bonds, similar to RD, with Asp79, Gly83, and the hydroxyl side chain of Thr171, and water molecules found at the base of the pocket. From the crystal structure, it was deduced that the 5-methyl group (Fig. 7.2b, CCT018159) was only 4 Å away from the carbonyl oxygen of Gly97 and this hydrogen bond acceptor could potentially be exploited to increase activity by the installation of a donor group. Replacement of the C5-methyl group by an amide group allowed the formation of a hydrogen bond with Gly97 as predicted by modeling, and this is supported by the increase in potency against the target and functional assays resulting in compound VER-49009 (Fig. 7.2b) which showed inhibition of cell proliferation comparable to the clinical agent 17-AAG [60].

Further medicinal chemistry efforts were focused on varying the pyrazole, introduction of a solubilizing group on the 4-arylpyrazole substituents, and also optimization of the 5'-substituent on the resorcinol ring [61]. Replacement of the more polar pyrazole with the isoxazole was well tolerated and bound Hsp90 with similar affinity, and also showed that isoxazole derivatives were more potent in cell growth inhibition assays [61]. Prior knowledge gained from other work [60, 62–64] suggested the importance of a solubilizing group on the 4-aryl ring, and thus their subsequent efforts focused on substitutions on the para-position of the aromatic ring. The morpholine moiety was found to be an optimal choice, one that also had additional hydrophobic interactions with Thr109 and Gly135 [61]. From the 4,5-diarylisoxazole series, VER-52296/NVP-AUY922 (Fig. 7.2b) emerged as a potent Hsp90 inhibitor which also showed antitumor activity in colon and breast cancer xenograft models [61, 65]. This lead candidate subsequently advanced to clinical trials where it is evaluated not only as a single agent but also as a combination therapy in various cancers. The first clinical results with NVP-AUY922 in patients with advanced solid tumors were disclosed in mid 2013 and showed that the drug was well tolerated, though among other common toxicities (e.g., diarrhea, nausea, fatigue) visual side effects were noticed at higher doses in some patients, but these visual symptoms were typically reversed upon interruption or discontinuation of the treatment [66]. The drug also provided evidence of Hsp90 inhibition in both peripheral blood mononuclear cells (Hsp70 induction) and tumor (reduction in levels of AKT and decrease of metabolic activity by ^{18}F-FDG PET) [66]. Following these results, Phase II single-agent and combination studies are in progress in patients with HER2+ breast, gastric and non-small cell lung cancers [66].

2.2.2 AT-13387

Scientists at Astex Pharmaceuticals used a FBDD approach to identify AT-13387. They used nuclear magnetic resonance (NMR) and high throughput X-ray crys-

tallography to discover fragments with Hsp90-binding affinity. These efforts resulted in the discovery of two fragments, one of which was a phenolic chemotype (Fig. 7.2c, **Hit Fragment**) with affinity in the high micromolar range as determined by isothermal titration calorimetry [67].

The follow-up on this hit fragment used crystallographic information which indicated that the methoxy group could be substituted to fill a proximal lipophilic pocket [67]. Analogs substituted with chloro, ethyl, isopropyl, and *tert*-butyl were initially prepared, and it was shown that a 100-fold improvement in potency was obtained for isopropyl and *tert*-butyl analogs, which are able to fill the lipophilic pocket. Then, two analogs containing the hydroxyl (OH) at the 2-position of the phenol instead of the 4-OH were pursued given the knowledge that 2-OH group in RD forms a direct hydrogen bond to Asp93 (Fig. 7.2c, **Hit Fragment**). However, these analogs were tenfold less potent than the 4-OH substituted compounds, and this implied that the 4-OH group must be an important contributor for affinity in the series. Their next synthetic iteration was aimed at modifying the diethylamide in the original hit fragment, and guided by X-ray crystallography, they pursued tertiary amides substitutions. The isoindoline moiety emerged as the best choice, one that provided a significant improvement in affinity of several-hundred-fold. Further modification of the phenol into a resorcinol resulted in a compound (Fig. 7.2c, **Lead**) with subnanomolar affinity, very good ligand efficiency and cell activity [67]. This initial lead was further modified first by the introduction of halogens at the 4, 5, and 7 positions of the isoindoline which were all tolerated. The subsequent structure-activity relationship (SAR) was primarily focused on substitutions at the 5-position of the isoindoline ring system shown to be best overall for activity, though the 4-position was also explored, with a focus on basic and other solubilizing functionalities. Among the substitutions explored at the 5-position were N-methylpiperazine, N,N-dimethylethanolamine, 2-methoxyethoxy, and 4-morpholinylmethyl, and all these compounds had good enzyme and cell activity [68]. Additionally, analogs containing N-methylpiperazine or N,N-dimethylethanolamine had high tumor retention and efficacy in HCT116 human colon carcinoma xenografts when compared to their original lead [68]. In order to increase the pKa of the basic center and also lower lipophilicity, a methylene linker was introduced at the 5-position linking the isoindoline with N-methylpiperazine or N,N-dimethyl. A compound preclinical selection process then ensued where the key parameters evaluated were in vivo target modulation, predicted human dose, selectivity, solubility, stability, formulation, and ease of synthesis [68]. From this triage process emerged the lead candidate AT-13387 (Fig. 1.2c) [69]. Same study also showed that the suppression of client proteins can last up to 72 h in NCI-H1975 xenograft tumors in mice when given as a single dose. The drug has subsequently advanced to the clinic where it is evaluated in patients with metastatic solid tumors.

2.2.3 STA-9090 (Ganetespib)

Ganetespib (Fig. 7.2d) is a novel resorcinol-containing triazole compound that is being developed by Synta Pharmaceuticals. The medicinal chemistry efforts re-

sulting in the identification of STA-9090 have not yet been described. However, the patent literature does provide the main regions explored for the resorcinol-containing triazole series, and these are shown in Fig. 7.2d (i.e., R_1, R_2, R_3, R_4, Z, and X). Briefly, the Hsp90 inhibitory compounds disclosed explored at R_1, R_2=H, OH, SH, alkyl, etc.; R_3=H, alkyl, alkenyl, cycloalkyl, heteroaryl, etc.; R_4=H, OH, alkyl, alkenyl, cycloalkyl, etc.; Z=OH, SH, NH_2; and X=N, alkyl, H, OH, etc. [70]. Ganetespib is among the Hsp90 triazole-containing inhibitors disclosed in the patent. This compound has a molecular weight (MW=364.4) that is considerably lower than most of the newer second generation Hsp90 inhibitors and is relatively hydrophobic (cLogP=3.3) [71]. X-ray studies of STA-9090 bound to Hsp90 not only confirmed the critical hydrogen interactions of the resorcinol hydroxyl group with Asp93 and the carbonyl of triazolone with Lys58 but also showed that the hydrazinecarboxamide moiety of triazolone makes a distinctive hydrogen bond with Gly97 that is not observed with ansamycin analogs [71]. Additionally, there are water bridge bonds between the 4-hydroxyl of resorcinol and Leu48 and Ser52 that are vital for binding efficiency [71].

STA-9090 was shown to have potent in vitro cytotoxicity when tested in various solid and hematologic tumor cell lines [71]. Additionally, ganetespib demonstrated antitumor efficacy in solid and hematologic xenograft models. Ganetespib subsequently advanced to clinical trials both as single agent and in combination therapy for a variety of cancers. In the clinic, ganetespib showed manageable side effects [72] and also clinical activity, for example, in previously heavily pretreated patients with NSCLCs [73]. This drug candidate is the most advanced Hsp90 inhibitor currently in the clinic where it is undergoing evaluation in several Phase II trials as well as a Phase III clinical trial for various human cancers.

2.2.4 KW-2478

The focus of Kyowa Hakko Kirin Pharma was initially on the development of oxime derivatives of RD, but their progress into the clinic was discontinued due to severe toxicities seen in rats and mice. As a result Kyowa Hakko Kirin Pharma decided to shift its focus towards the development of new small molecule Hsp90 inhibitors with an improved safety profile [49]. To achieve their goal, a unique screening system was established which consisted of a binding assay where Hsp90 was fixed onto plates, and to these wells, the compounds to be screened were added. The plates were washed a few times, after which biotin-labeled RD was added and poly-HRP streptavidin solution was added to verify the competitive inhibition with RD [49]. These efforts resulted in the identification of a number of leads containing a resorcinol moiety. Although detailed SAR leading to the identification of KW-2478 (Fig. 7.2e) has yet to be disclosed, lead optimization utilized X-ray crystallography, cell-based assays, and in vivo models. KW-2478-inhibited biotin-RD binding to Hsp90α with an IC_{50} value of 3.8 nM [74] and was found to have good solubility in saline (>30 mg/mL), no hepatotoxicity in animal studies, good PK profile, and also no metabolism by CYP3A4 enzyme [49]. Hence, it was subsequently advanced into Phase I clinical trials in patients with multiple myeloma and non-Hodgkin's lym-

phoma where no dose limiting toxicities (DLTs) or retinal toxicity was noticed at doses up to 176 mg/m^2 [49]. This clinical agent is also currently being evaluated as a combination therapy with bortezomib in a Phase I/II trial in patients with multiple myeloma.

2.3 Purine and Purine-Like Analogs

Structure-based drug design of Hsp90 inhibitors was made possible with the advent of crystal structures of GM [16, 17], RD [20], and ADP [16] bound to the N-terminal nucleotide-binding domain (NBD) of Hsp90. These structures show the ATP-binding pocket to have a distinctive Bergerat fold that is observed only in the GHKL ATPase protein family [75, 76]. The unique "bent" conformation adopted by ATP when bound to Hsp90 was incorporated into the design of the synthetic class of inhibitors. Chiosis et al. utilized co-crystal structures of Hsp90 with its ligands (i.e., GM, RD, and ADP) to rationally design the first synthetic Hsp90 inhibitor, purine-based PU3 (Fig. 7.3a) [77]. PU3 was shown to inhibit the binding of purified Hsp90 to immobilized GM with an $EC_{50} = 15-20$ μM. Furthermore, PU3 was the first synthetic molecule to show similar phenotypic properties to GM, including degradation of HER2 and inhibition of cancer cell growth [77]. In the design of PU3, the purine core of the endogenous ligands were linked to an aryl moiety via the 8-position through a methylene linker (i.e., Purine-CH_2-Aryl, Fig. 7.3a). This structural arrangement allows PU3 to adopt a bent shape even in the unbound state. Although previous crystal structures aided in the design of this class, they could not predict the precise mode of binding for these molecules. Interestingly, a crystal structure of PU3 bound to the NBD of Hsp90α showed that it induces a conformational shift that exposes a pocket not observed in any of the previous structures [78].

As discussed below, the essential motif exemplified by PU3, purine-linker-aryl, would be maintained for essentially all of the inhibitors of this class, with only slight variations. The SAR that was constructed on the purine scaffold can be divided into four parts: (1) the purine ring, (2) the linker, (3) the 8-aryl ring, and (4) the 9-alkyl substituent. The purine core is not very amenable to structural modifications given its overall tight fit in the pocket and important binding interactions made by the exocyclic amino group. The same holds true for the linker which is critically important for imparting an acceptable angle between the purine and aryl rings. The best results in advancing this class of compounds into clinical candidates came through modifications of the 8-aryl ring and the N9-alkyl substituent. Affinity has generally been improved through modifications of the 8-aryl ring that impart strong binding interactions within the newly created binding pocket. Additionally, PK properties have been improved largely through modifications to the 9-substituent which is oriented towards the solvent exposed region. This strategy was indeed successful as changes made to the aryl fragment resulted in compounds that went from micromolar potency (e.g., PU3, bound Hsp90 with moderate activity and showed biological activity in the 50 μM concentration range or PU24FCl which exhibited biological effects in the 2–6 μM range) through to the nanomolar level (e.g., PU-

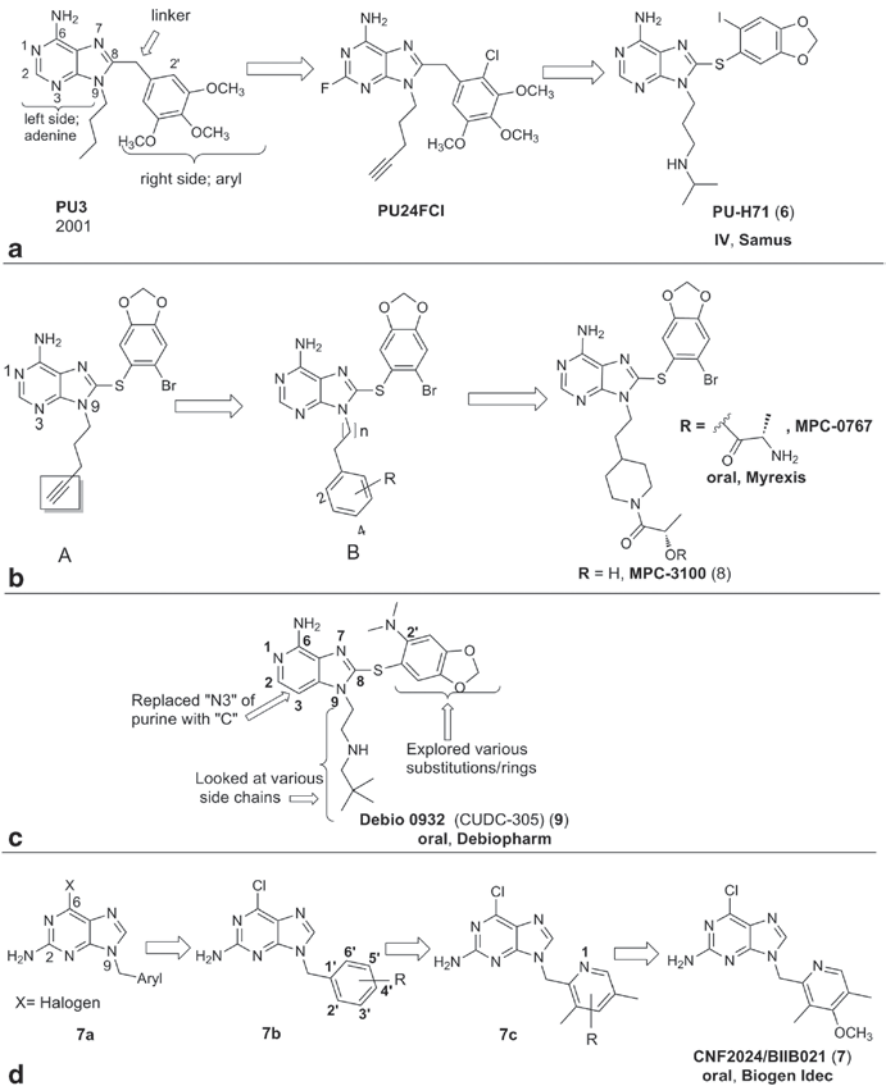

Fig. 7.3 **a** PU-H71, from discovery to clinic. **b** Development of MPC-3100 and MPC-0767 from the initial lead A, following extensive SAR. **c** Imidazopyridine 9 (purine-like) SAR regions. **d** BIIB021, from lead to clinic

H71) [79], and this is schematically depicted in Fig. 7.3a. The initial lead PU3 was subsequently picked up by numerous research groups and optimized via various strategies to lead to potent drug candidates that display a much improved PK and toxicity profile compared to the ansamycin analogs. Combined these efforts have resulted in the advancement to clinical trials of PU-H71, MPC-3100, CNF2024/BIIB021, and Debio 0932 (CUDC-305; Fig. 7.3).

2.3.1 Early SAR of the Purine-Scaffold Class

The discovery of PU3 triggered an interest in the purine-scaffold class demonstrated by early publications aimed at studying the main pharmacophore requirements for this class of inhibitor [78, 80, 81]. In the first reported SAR, extensive knowledge was obtained from a careful study of the almost 70 derivatives synthesized [80]. These analogs were evaluated for Hsp90 binding via a solid phase competition assay for their ability to compete with immobilized GM and their cellular activity was tested by the ability of the drugs to arrest the growth of MCF-7 breast cancer cells and to induce the degradation of HER2 tyrosine kinase [80]. The advent of multiple co-crystal structures of a series of purine-based inhibitors bound to Hsp90 [81] have allowed to explain the earlier observed SAR and subsequent ones. For example, it was previously noticed that when the butyl chain of PU3 is replaced with pent-4-ynyl the activity increases, and this was attributed to an increase interaction between the alkyne group and hydrophobic lid of the pocket. The crystal structure also helped to explain why functionalities on the 9N moiety could not have any substituent on C1 where a steric clash with Leu107 would occur. In addition, at position 2 of the purine only fluorine was tolerated which increased potency and water solubility in this series whereas larger groups like cyano, vinyl, iodo, methoxy, ethoxy, or amino decreased activity [80]. Modification of the methylene linker with -O-, -NH-, $-CH_2CH_2-$, $-OCH_2-$ were each detrimental to activity, which, hinted at the importance of the dihedral angle between the purine and the aryl moiety. With regards to the 8-aryl moiety, the addition of chlorine to the 2'-position increases activity while the addition of bromine was found to have little to no effect compared to PU3. The combination of all the favorable functionalities provided an additive effect and resulted in the most potent compound, PU24FCl, which had an $IC_{50} = 1 - 2$ μM in degradation of HER2 in MCF-7cells and was shown to be 30-fold more potent than PU3 (Fig. 7.3a). Notably, PU24FCl displayed antiproliferative effects against various cancer cell lines with $IC_{50} = 2 - 7$ μM and caused the degradation of Hsp90 client proteins at similar concentrations [34]. Importantly, PU24FCl showed 10- to 50-fold higher affinity for tumor vs. normal tissue Hsp90. This compound also showed antitumor effects in vivo when administered at 200 mg/Kg ip on alternate days in mice bearing MCF-7 breast cancer xenografted tumors where it resulted in significant depletion of HER2, AKT, and Raf-1 in tumors and also a 72% decrease in tumor burden vs. untreated control mice [34]. Various groups took on the prospect of further developing the purine class of inhibitors by performing extensive SAR, and included the testing strategy in the preclinical development of their Hsp90 inhibitors. These combined efforts have culminated in four compounds being evaluated in the clinic for cancer.

2.3.2 PU-H71

PU-H71 was discovered by researchers at Memorial Sloan-Kettering Cancer Center through the further development of PU24FCl (Fig. 7.3a). These efforts were greatly

assisted by improvements in binding assays designed to measure affinity to Hsp90. Development of fluorescent polarization (FP) assays enabled for the accurate evaluation of the potency of compounds in a more high-throughput manner. These assays were first developed for purified protein and then later for cancer cell lysates which more accurately define the affinity of the compound for the oncogenic Hsp90 complex [82–84]. A significant development within the purine-scaffold series came from the finding that the sulfur linker showed activity similar to the methylene [79, 85]. This modification enabled for more extensive SAR evaluation of the 8-aryl moiety through the advent of suitable chemical methods as well as the broader availability of aryl iodides required in the synthesis of the requisite 8-arylsulfanyl adenine derivatives, since the rather limited availability of aryl acetic acids needed for the synthesis of methylene-linked compounds would have limited development [80, 83, 86]. Through these efforts, it was found that the 2-halo-4,5-methylenedioxy series was the best aryl moiety to fit into the Hsp90 hydrophobic pocket [79, 83]. As can be seen from PU-H71, CUDC305, and MPC-3100, the methylenedioxy moiety is indeed a recurring theme in 3 of the 4 clinical candidates within the series.

Orientation of the N9-substituent towards solvent-exposed region makes this part of the molecule an ideal place to introduce moieties that would improve PK properties. Thus, the pent-4-ynyl previously determined to be favored by the Hsp90 pocket [80] was subsequently replaced with ionizable amino groups that enabled the formation of salt, and hence dramatically enhanced solubility while retaining biological activity of these compounds. PU-H71, a potent water soluble compound with an IC_{50} ~ 50 nM in cellular models of cancer, contains a 3-isopropylaminopropyl group at N9, sulfur as the linker, and 2-iodo-4,5-methylenedioxy for the aryl group [79]. This inhibitor was shown to have potent activity in various preclinical models of triple-negative breast cancer (TNBC) [87], small-cell lung carcinoma (SCLC) [88], hepatocellular carcinoma [89], diffuse large B-cell lymphomas [90], and myeloproliferative disorders [91]. In addition, PU-H71 was also shown to have extended tumor retention and prolonged PD effects; one such example is the retention of this compound at pharmacological doses and the associated suppression of Hsp90 oncoclients such as AKT for over 48 h in TNBC tumors. Drug concentrations in normal tissues and plasma declined quickly being almost undetectable by 6 h [87]. This drug candidate has subsequently advanced to Phase I clinical trials where it is being evaluated in patients with solid tumors, lymphoma, and myeloproliferative disorders [23]. The first results from the two Phase I clinical trials with PU-H71 are anticipated in mid 2014.

2.3.3 MPC-3100

MPC-3100 was discovered by scientists at Myrexis Inc. who decided to pursue their Hsp90 discovery program using the intrinsic activity of the purine scaffold as a starting point [92]. Their first SAR efforts entailed the replacement of the propargyl group of **A**, a derivative previously reported by the Chiosis group [79] (Fig. 7.3b), with the phenyl bioisostere linked to the N9 via alkyl chains of different lengths

(Fig. 7.3b; **B**). The two carbon linker was determined to be optimal and various substitutions on the aromatic ring (i.e., at position 2, R = F, Cl, Br, compound **B;** Fig. 7.3b) were probed for best activity using FP. Unfortunately, the limiting factor of these analogs was their decreased metabolic stability, therefore, further structural changes to the pendant aryl group were explored. Introduction of heteroaromatic ring systems (e.g., pyridine, pyrole, imidazole, etc.) or replacement of the phenyl ring with the thiophene bioisostere generally resulted in a significant loss of potency [92]. Since these modifications were not successful and some were even detrimental, other strategies were pursued. To this end, their first attempt was substitution of the phenylethyl with an aminomethyl- cyclopropyl group, still this choice did not bear fruit as the potency was not improved and more this analog suffered from high clearance rate and very low oral bioavailability [92]. Thus, their subsequent endeavors were towards improving oral bioavailability while preserving potency, and they opted for the introduction of the piperidine moiety which is frequently used by medicinal chemists as it provides an expedient platform for drug discovery. Next, variations were made to the piperidine N-substituent in order to improve both potency as well as ADME properties consisting of N-alkyl (e.g., *i*-Pr, *i*-Bu, CH_2CF_3, CH_2-cyclo-hexyl) and N-acylpiperidine analogs. From these efforts, it was discovered that the optimal piperidine N-substituents were N-hydroxyacyl moieties which provided both potency and metabolic stability.

Further extensive SAR studies were carried out for the 8-aryl region in an effort to replace the 6-bromo-1,3-benzodioxole moiety with other functionalities such as dihydrobenzofuran, benzofuran, benzodioxane, benzoxazine. However, none had more favorable PK profiles than the 1,3-benzodioxole analogs [92]. It should also be noted that these derivatives containing a bromine on the 2′-position of the 8-aryl group are less potent than their iodine derivatives at the same position. Overall, the SAR of the pendant N-9 piperidine moiety and of the 1,3-benzodioxole ring, complemented by in vitro profiles and PK properties led to the selection of MPC-3100 (Fig. 7.3b) as the most suitable drug for advancement. MPC-3100 was administered orally and demonstrated significant antitumor activity in the NCI-N87 gastric cancer xenograft model [92]. MPC-3100 has recently completed Phase I clinical trials in refractory or relapsed cancer. This study revealed the safety and tolerability when administered orally of MPC-3100 at doses lower than 600 mg/day with the most common side effects reported having gastrointestinal origin (i.e., diarrhea, nausea, and vomiting) [92].

As a result of the relatively poor solubility and bioavailability of MPC-3100, Myrexis developed MPC-0767 (Fig. 7.3b) [93], a novel L-alanine ester pro-drug of MPC-3100 which has completed all requirements for Investigational New Drug (IND) filing. Nonetheless, in February 2012, the development of activities in the preclinical and clinical oncology programs of Myrexis were suspended [94], and currently, there are no ongoing clinical trials indicative, perhaps of the uncertain future of these two potential drugs.

2.3.4 Debio 0932 (CUDC-305)

The structure of CUDC-305, though technically an imidazopyridine, bears strong resemblance to other members of the purine class (Fig. 7.3c). To date, the only report regarding the design and synthesis of CUDC-305 was provided in a poster presented at the American Association for Cancer Research (AACR) 102nd Annual Meeting that took place in 2011 at Orlando, Florida [95]. Upon analysis of the existing SAR for the purine class [79, 81, 83], efforts at optimization were focused primarily on the regions indicated by arrows in Fig. 7.3c. The impetus to develop the imidazopyridine scaffold was likely the result of available crystal structures of purine-scaffold compounds which showed that the purine N3 interaction with Hsp90 is less critical for activity. Hence, their research team concluded that replacement of nitrogen with carbon should not result in diminished potency and could provide novelty and potentially unique properties [95]. Their SAR for the solvent exposed region examined various side chains such as pentynyl, butynyl, hexynyl, pentyl, replacement of terminal alkynyl with cyano, ether, and amide chains, as well as amino containing chains. This screen provided as the best side chain the neopentylamine with an ethyl linker connected to N9. The 8-aryl region was also subject to SAR exploration, and again the methylenedioxy analogs, similar to PU-H71 and MPC-3100, was determined to be best. Finally, they also examined substituents at position 2′ of the phenyl ring and found the dimethylamine moiety to give a favorable Hsp90 inhibitor [95]. Their most potent analog was CUDC-305 (IC_{50} = 100 nM) [95]. This compound, later renamed Debio 0932, is orally bioavailable, blood-brain barrier-permeable, and can achieve therapeutic levels in brain tissue (suggesting perhaps its potential role to treat brain tumors). Additionally, in tumor tissues the drug displays high concentrations and prolonged half-life ($t_{1/2}$ = 20.4 h) [95, 96]. It was subsequently advanced to the clinic where Debio 0932 went through the dose escalation portion of a Phase I study where it was evaluated for safety and tolerability at various doses given orally in patients with advanced solid tumors or lymphoma [97]. The results of the Phase Ib study are expected in the near term, and currently the drug is the subject of a Phase I/II clinical study in patients with advanced lung cancer [97].

2.3.5 BIIB021

BIIB021 (Fig. 7.3d) was discovered by scientists at Conforma Therapeutics [98] and is unique from the other compounds in this class in that the aryl moiety is attached to the N9 position of the purine instead of the C8 position (Fig. 7.3d; **7a**). They showed that potent inhibition of Hsp90 was possible when the NH_2 group was also moved from the 6- to the 2-position. These changes maintained the optimal six bond distance between the NH_2 and the aryl group required for potent binding by the pharmacophore (purine-linker-aryl). Their first efforts were on exploring

the effect of the halogen (i.e., Cl, Br, and I) at the 2′-position of the benzyl ring of compound **7b** (Fig. 7.3d), and their expectation to see an increase in potency was confirmed. Then, the importance of the chlorine atom at the C6 position of the purine ring was studied. Attempts to modify the chlorine with other functionalities (NH_2, OCH_3, OH, SH, H, or CH_3) resulted in a decrease of activity suggesting that the halogen is important not only for hydrophobic contacts but also for its electron withdrawing characteristics [98]. Their next optimization steps were aimed at improving the aqueous solubility and to achieve this they replaced the phenyl ring with a pyridine. The subsequent SAR on the 9-heteroaryl moiety (Fig. 7.3d; **7c**) determined 3,5-dimethyl-4-methoxypyridine as ideal, and from this emerged the potent inhibitor 6-chloro-9-((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)-9H-purin-2-amine (Fig. 7.3d; **BIIB021**) which had a better profile based on the in vitro potency, pharmaceutical properties, and in vivo oral efficacy. The antitumor activity of BIIB021 was demonstrated in a number of human tumor xenograft models where the drug was given orally on both daily and intermittent dosing schedules [99]. BIIB021 was advanced in the clinic by Biogen Idec where it has been evaluated in several Phase I and II clinical trials in patients with gastrointestinal stromal tumors (GIST) and hormone receptor positive metastatic breast cancer [11]. Additionally, a Phase II study of BIIB021 in patients with GIST refractory to imatinib and sunitinib was published in 2013 and showed that treatment with BIIB021 led to metabolic responses in >20% of patients with no significant hepatotoxicity when compared to IPI-504 [100]. However, the further advancement of BIIB021 in the clinic by Biogen Idec seems unlikely given the desire of the company to shift away from the oncology development program [101], and currently the company is seeking to out-license BIIB021.

2.4 Other Chemotypes

2.4.1 SNX-5422/PF-04929113, a Dihydroindazolone Derivative

SNX-5422 (Fig. 7.4a) is based on a unique dihydroindazolone scaffold and was discovered by scientists at Serenex. They used a chemoproteomics-based strategy in screening a focused chemical library against a diverse set of potential targets [102]. In this unbiased approach both the hit compound and target were identified concomitantly, so that the screen enabled for the identification of the most optimal starting point at the level of both biology and chemistry. This was achieved using an ATP-affinity column to which ATP-binding proteins were first loaded, and this was then challenged with a diverse set of 8000 compounds for their ability to displace unspecified proteins from this column. Some of the compound-protein interactions found from this screen involved Hsp90. The decision to pursue Hsp90 stemmed from the potential of it as a target and by the novelty and diversity of the chemical leads identified by the screen.

The researchers at Serenex were inspired to pursue a synthetically tractable benzamide hit (Fig. 7.4a; **compound A**) which conferred structural novelty, chemi-

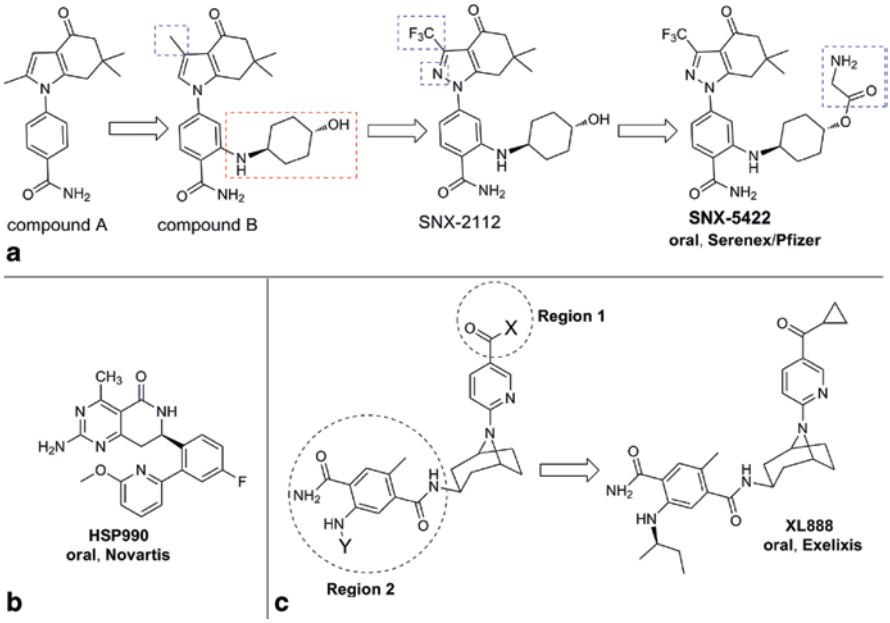

Fig. 7.4 **a** Discovery of SNX-5422, from hit to clinical candidate. **b** Clinical candidate HSP990. **c** Important SAR optimization regions leading to XL888

cal tractability, high selectivity for Hsp90, low molecular weight, and heteroatom count [102]. The screen was further validated by X-ray crystallography analysis. Their follow-up synthetic design complemented by structural information resulted in **compound B** (Fig. 7.4a) which met their requirements of strong affinity to Hsp90 and cell activity. The Serenex efforts extended further since precautions were taken to replace the possible metabolic liability of the methyl at C2 of the pyrrole moiety of **B** with a trifluoromethyl, a group known to enhance metabolic stability. Additionally, the pyrrole moiety of **compound B** was substituted with a pyrazole designed to confer an increase in polarity and solubility [103]. These changes resulted in the new lead candidate SNX-2112 which exceeded the potency of 17-AAG [102]. The last optimization step was focused on improving the oral bioavailability of SNX-2112, and this was achieved via the introduction of the glycine ester prodrug moiety to give their clinical candidate SNX-5422 (Fig. 7.4a) [102]. The prodrug is rapidly hydrolyzed to the parent SNX-2112 in vivo following oral dosing given that the levels of SNX-5422 were below the lower limit of quantitation at all time points. Additionally, SNX-5422 showed significant antitumor activity in different human tumor xenograft models in mice when administered orally [102].

In 2007, Serenex began a Phase I trial of SNX-5422 in patients with solid tumor and lymphoma. Later in 2008, Pfizer Inc. had reached an agreement to acquire Serenex [23] and thus to continue development of SNX-5422. However, the advancement of SNX-5422 in the clinic was halted in 2010 by Pfizer as a result of

reports of ocular toxicity in a Phase I study [104]. At that time, Pfizer decided that comprehensive ocular assessments are needed for the drug before any further development. The drug was subsequently picked up by Esanex Inc. which is presently evaluating the drug in the clinic in patients with resistant lung adenocarcinoma and selected HER2+ cancers.

2.4.2 HSP990 (NVP-HSP990)

HSP990 (Fig. 7.4b) is a potent and selective Hsp90 inhibitor that was discovered by Novartis. The discovery process for this compound relied on high throughput screening which was complemented by structure based lead optimization [105], though the detailed SAR is yet to be published. Potency against the various Hsp90 isoforms was evaluated in a binding assay using biotinylated-GM and in ATPase activity assays [105]. The 2-amino-4-methyl-7,8-dihydropyrido[4,3-d]pyrimidin-5(6H)-one scaffold is structurally diverse from the other Hsp90 inhibitors, and crystallographic work shows that NVP-HSP990 binds to the N-terminal ATP-binding domain of Hsp90 [105]. Though the crystal structure is yet to be published, it is likely that the 2-NH_2 group of NVP-HSP990 interacts with Asp93 similarly to NH_2 of purine and the resorcinol moiety.

This drug candidate was shown to have potent antiproliferative activity in various cell lines as well as efficacy in vivo when tested in the GLT-16 xenograft model [105]. NVP-HSP990 was under investigation as an oral agent in two Phase I trials in patients with advanced solid tumors. However, the further development of NVP-HSP990 has been stopped due to its failure to achieve clinically meaningful responses at the maximum tolerated dose [106].

2.4.3 XL888, A 2-amino Terephthalamide Derivative

XL888 (Fig. 7.4c) is a novel tropane-derived inhibitor of Hsp90 discovered by Exelixis. The strategy used to obtain some hits involved an HTS campaign of 4.1 million compounds from the company's in-house chemical library [107]. The percentage displacement of biotinylated-GM from the Hsp90 chaperone complex was the method used to screen compounds and to identify initial leads. The most promising scaffold was a 3-amidotropane which provided sub-micromolar IC_{50}s in the Hsp90 displacement assay, and this was subsequently optimized through a structure-guided approach. Some problems encountered during optimization related to high molecular weights (>650 amu) and polar surface areas (>135 $Å^2$) which were outside of the desired range for orally bioavailable drugs [107]. This observation was further confirmed by rat PK which indicated high clearance and no calculable oral bioavailability. SAR efforts were focused on reducing the polarity and molecular weight of the nicotinamide portion (Region 1; Fig. 7.4c) of the scaffold. Thus, the amide functionality of the nicotinamide portion was replaced with ketone derivatives (Region 1; Fig. 7.4c) which had lower molecular weights (<520 amu)

and PSA (< 120 Å^2) and improved rat PK when compared to the amides. There was little tolerability for ketone steric bulk (i.e., progressing from methyl ketone to ethyl, isopropyl, or cyclopropylketone) since Hsp90 inhibition decreased with increasing size, but noticeably the cyclopropylketone derivative showed encouraging results in the cell assay measuring HER2 degradation in NCI-N87 cells [107]. Next, efforts were directed towards introducing substituents on the benzamide 5-position (Region 2; Fig. 7.4c) portion of the molecule. A screen of a varied set of small alkyl amines was performed and these showed similar activity in the biochemical assay, still they did show variation in the NCI-N87 HER2 degradation assay. The most potent compound in cells was the R-*sec*-butyl compound (Fig. 7.4c; XL888) with an $IC_{50} = 56$ nM and an attractive rat PK. As a whole, the extensive medicinal chemistry efforts coupled with biochemical and X-ray crystallographic methods were successful in the optimization process of the 3-amidotropane hit leading to XL888. The efficacy experiments performed in NCI-87 mouse xenograft model showed tumor regression, and further preclinical investigations coupled with more toxicity assessment resulted in the selection and advancement of XL888 into clinical trials [107, 108].

The first Phase I safety study of XL888 was terminated by the sponsor Exelixis [23] with no particular reason being disclosed. Another Phase I study of XL888 in combination with vemurafenib for patients with unresectable Serine/threonine-protein kinase B-raf (BRAF) mutated stage III/IV melanoma is underway [108, 109]. This study is supported by a report showing that signaling proteins involved in intrinsic and acquired resistance to BRAF inhibitors are clients of Hsp90 and inhibiting Hsp90 restored sensitivity to vemurafenib [108].

3 Future Drug-Discovery Efforts

3.1 Selective Inhibition of Hsp90 Isoforms

Hsp90 has four different paralogs, Hsp90α and Hsp90β in the cytoplasm, Grp94 in the endoplasmatic reticulum (ER), and tumor necrosis factor receptor-associated protein 1 (Trap-1) in the mitochondria. The Hsp90 clinical candidates described in this chapter are reported to be pan-Hsp90 inhibitors. Nevertheless, there is a growing interest in the development of isoform-selective inhibitors. A better understanding of the biological activity of each isoform would potentially allow the development of better and more selective therapeutics. The advent of new tools to study the contribution of the four Hsp90 paralogs [110, 111] has already set the ground for the design of paralog-selective inhibitors which will not only provide a better understanding of the role of each isoform in disease, but could potentially improve the toxicity profile of the pan-inhibitors. There are currently significant efforts underway and some of these directed towards Trap-1 and Grp94 selective inhibitors are discussed below.

3.1.1 Targeting Trap-1 for Cancer Therapy

Recent studies have demonstrated that Trap-1 levels are highly expressed in mitochondria of tumor cells, but are either very low or undetectable in normal tissues [112, 113]. As a result, selective targeting of Trap-1 has become of interest as a potential cancer-specific target. The first reported class of small molecule to selectively target the Hsp90 network in tumor mitochondria is represented by Gamitrinibs (GA mitochondrial matrix inhibitors) [112, 114]. Gamitrinibs are analogs of GM containing a mitochondrial targeting moiety connected through a linker at the C17 position. The gamitrinibs are distinct from current Hsp90 inhibitors since they display a "mitochondriotoxic" mechanism of action, resulting in very fast and complete tumor cell killing by apoptosis [114]. This property combined with the information that gamitrinibs have no effect on overall Hsp90 homeostasis outside of mitochondria make these anticancer agents novel and potentially attractive for human testing [114]. Extensive preclinical evaluation of gamitrinib for prostate cancer revealed that it induces prostate cancer cell death, and this is achieved mechanistically via acute mitochondrial dysfunction including loss of membrane potential and release of cytochrome c [115]. Overall, it has been demonstrated in vivo that gamitrinibs show preclinical efficacy combined with a good safety profile in models of drug-resistant and bone metastatic prostate cancer [115].

3.1.2 Targeting Grp94 for Cancer Therapy

Glucose-regulated protein 94 (Grp94) expression is associated with advanced stage and low survival in various cancers and correlates well with cancer growth and metastasis [116–119]. Most cancer-related studies on this chaperone looked at the immunogenic activity of Grp94-peptide complexes [117] and its role in the regulation of EGFR and HER2 in the ER, secretion of IGF-I and -II, and the regulation of Toll-like receptors (i.e., TLR1, TLR2, TLR4, and TLR9) and integrins [116–118, 120]. This was complemented by another report which used library screening (i.e., screened >130 purine-scaffold compounds) in a fluorescence polarization assay to test the compounds for all four Hsp90 paralogs, and were able to find derivatives that had selectivity for Grp94 [111]. The library screen was further analyzed by structural and computational analysis which indicated the presence of a new allosteric pocket where these Grp94 selective compounds insert. These paralog selective inhibitors also inhibited Grp94-mediated cellular events such as IGF-II secretion and TLR9 trafficking [111]. Importantly, Hsp70 induction, which is a hallmark of cytosolic Hsp90 inhibition, was not observed. Additionally, this work also provided proof for the role of Grp94 in preserving the architecture of high-density HER2 formations at plasma membrane, specifically in cancer cells where HER2 has to channel the amplified signal through the receptor [111]. The new mechanistic insights reveal the importance of Grp94 inhibition in certain breast cancers (i.e., HER2 overexpressing), and the newly uncovered allosteric-binding site in Grp94 is an avenue to be explored by future drug discovery efforts.

3.2 Alternative Modes of Modulating Hsp90 Activity in Cancer

As already mentioned, all of the Hsp90 inhibitors that have entered clinical trials target the N-terminal ATP pocket of Hsp90. One drawback of these agents is that they induce a heat shock response which in turn increases the cellular levels of prosurvival chaperones (i.e., Hsp27 and Hsp70). Therefore, alternative approaches to inhibit Hsp90 function without inducing a heat shock response would be highly desirable. Efforts in this regard are increasingly being pursued and include developing inhibitors that bind to the C-terminal domain, molecules that target the binding of Hsp90 to either co-chaperones, or to client protein. Though none of these approaches have yet provided a clinical candidate they offer a potential for further future drug development that may result in one.

3.2.1 Inhibitors that Target the C-terminus of Hsp90

Novobiocin (NB), a coumarin antibiotic, was the first compound shown to bind weakly to the C-terminal nucleotide-binding site (~700 μM in SKBr3 cells) [27] and was shown to affect the association of Hsp90 with its co-chaperones Hsc70 and p23 [121, 122]. Subsequent work aimed at improving the observed low affinity of NB for Hsp90 has resulted in more potent analogs such as KU174 (K_d = 94 μM) which has a 12-fold higher affinity than NB (K_d = 1.1 mM) when analyzed by surface plasmon resonance (SPR) [123]. Importantly, KU174 does not induce a heat shock response and displays antitumor activity in prostate cancer cells [123, 124]. However, evaluation of KU174 in mouse was prevented by the extensive metabolism and clearance observed when pilot PK studies were performed with this species [123].

Epigallocatechin-3-gallate (EGCG) has also been shown to inhibit Hsp90 by binding to the C-terminus [125]. Recently, a report on SAR study was disclosed [126] and the best analog displayed anti-proliferative activity against the MCF-7 breast cancer cell line with an IC_{50} = 4 μM, an 18-fold improvement over EGCG (MCF-7 IC_{50} = 74 μM) [126]. Other C-terminus inhibitors reported in the literature are cisplatin [127], taxol [128], and withaferin A [129]. It is interesting to speculate how much of the anticancer activity of cisplatin and taxol, two popular clinical agents, is as a result of affecting Hsp90 function. Despite an undeniable interest in Hsp90 inhibitors for cancers, the further advancement of C-terminal inhibitors in the clinic is sluggish perhaps due to their observed weak biological activity and potential pleiotropic mechanisms of action.

3.2.2 Targeting Co-chaperone–Hsp90 Interactions

Hsp90 is assisted in its chaperone activity by a cohort of co-chaperones (e.g., Hsp70, Cdc37, Aha1, HOP, etc.) which modulate Hsp90 ATPase and determine the rate of chaperone cycling [10]. Therefore, Hsp90 activity can be indirectly modu-

lated by targeting co-chaperone function, and some initial efforts towards targeting co-chaperone-Hsp90 interactions are indeed described in the literature [12].

3.2.3 Targeting Client Protein Binding to Hsp90

Strategies which target the binding of client proteins to Hsp90 offer an exciting opportunity to develop novel anticancer drugs. Developing such molecules which target-specific protein-protein interactions is notoriously difficult; however, this has already been attempted with some success for the client protein survivin. Survivin is an inhibitor of apoptosis protein that is overexpressed in almost all human tumors and has critical roles in tumor cell proliferation and cell viability [130, 131]. Thus targeting the survivin-Hsp90 complex may be a suitable strategy for cancer therapy. To this end, a peptide sequence of survivin called shepherdin was designed via structure-based mimicry as a high affinity ($K_d \sim 80$ nM) inhibitor of the survivin-Hsp90 interaction, but subsequent data suggested that shepherdin could perhaps function as a more global antagonist of Hsp90 activity [132]. Later work screened a database of nonpeptidic structures using combined structure and dynamics based computational design, and this resulted in the identification of the small compound 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) which binds to the Hsp90 N-domain, in vivo destabilizes numerous Hsp90 client proteins (counting survivin), and also displays selective anticancer activity in several tumor cell lines [131]. Overall, the experimental structural data (i.e., docking studies, molecular dynamics, and NMR) for these inhibitors provide insights for the future design of better compounds.

4 Conclusions

Undoubtedly, there has been considerable progress in the development of Hsp90 inhibitors over the past decade. While the first generation of compounds, natural products GM and RD, served as proof of concept for Hsp90 as an anticancer target, their further advancement in the clinic has met many obstacles as a result of their poor drug like properties. The challenge to develop better therapeutics that would overcome most of the limitations observed with the first generation drugs resulted in the second generation synthetic Hsp90 inhibitors. This chapter summarized the main medicinal chemistry SAR and drug design strategies performed by various research groups for a number of candidates being evaluated in clinical trials. All the clinical candidates are indeed a testimony to the arduous efforts of both academia and industry towards the development of better Hsp90 therapeutics for the treatment of cancer. Furthermore, herein we also touched upon other chemotypes with alternative binding modes that are currently in preclinical development.

To date, no Hsp90 inhibitor has yet been approved by the Food and Drug Administration (FDA), a reality which may reflect our little understanding about the best

use of these compounds in the clinic, and also our limited knowledge in selecting those patients who will likely benefit most from Hsp90 inhibitor therapy. This may soon change as results from the numerous clinical trials become available and are carefully scrutinized. To conclude, this chapter highlighted potent and pharmaceutically promising Hsp90 clinical agents that have populated the field, some of which are credibly poised to ultimately reach market approval in the future. Understandably, progress in this field will be aided by a better comprehension of the interaction of the inhibitor with the target thus leading to better clinical response.

References

1. Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. Cell Mol Life Sci 18(12):571–573
2. Tissieres A, Mitchell HK, Tracy UM (1974) Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. J Mol Biol 84(3):389–398
3. Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5(10):761–772. doi:10.1038/nrc1716
4. Wegele H, Muller L, Buchner J (2004) Hsp70 and Hsp90–a relay team for protein folding. Rev Physiol Biochem Pharmacol 151:1–44. doi:10.1007/s10254-003-0021-1
5. Pearl LH, Prodromou C (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem 75:271–294. doi:10.1146/annurev.biochem.75.103004.142738
6. Wandinger SK, Richter K, Buchner J (2008) The Hsp90 chaperone machinery. J Biol Chem 283(27):18473–18477. doi:10.1074/jbc.R800007200
7. Zuehlke A, Johnson JL (2010) Hsp90 and co-chaperones twist the functions of diverse client proteins. Biopolymers 93 (3):211–217. doi:10.1002/bip.21292
8. Workman P, Burrows F, Neckers L, Rosen N (2007) Drugging the cancer chaperone HSP90: combinatorial therapeutic exploitation of oncogene addiction and tumor stress. Ann N Y Acad Sci 1113:202–216. doi: 10.1196/annals.1391.012
9. Neckers L (2002) Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol Med 8(4 Suppl):S55–S61
10. Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 10(8):537–549. doi:10.1038/nrc2887
11. Porter JR, Fritz CC, Depew KM (2010) Discovery and development of Hsp90 inhibitors: a promising pathway for cancer therapy. Curr Opin Chem Biol 14(3):412–420. doi:10.1016/j.cbpa.2010.03.019
12. Patel HJ, Modi S, Chiosis G, Taldone T (2011) Advances in the discovery and development of heat-shock protein 90 inhibitors for cancer treatment. Expert Opin Drug Discov 6(5):559–587. doi:10.1517/17460441.2011.563296
13. DeBoer C, Meulman PA, Wnuk RJ, Peterson DH (1970) Geldanamycin, a new antibiotic. J Antibiot (Tokyo) 23(9):442–447
14. Uehara Y, Hori M, Takeuchi T, Umezawa H (1986) Phenotypic change from transformed to normal induced by benzoquinonoid ansamycins accompanies inactivation of p60src in rat kidney cells infected with Rous sarcoma virus. Mol Cell Biol 6(6):2198–2206
15. 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(18):8324–8328
16. 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

17. Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP (1997) Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 89(2):239–250. doi:S0092-8674(00)80203-2
18. Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, Krutzsch H, Ochel HJ, Schulte TW, Sausville E, Neckers LM, Toft DO (1997) The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. J Biol Chem 272(38):23843–23850
19. Pratt WB (1998) The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. Proc Soc Exp Biol Med 217(4):420–434
20. Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1999) Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem 42(2):260–266. doi:10.1021/jm980403y
21. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, Burrows FJ (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425(6956):407–410. doi:10.1038/nature01913
22. Moulick K, Ahn JH, Zong H, Rodina A, Cerchietti L, Gomes DGEM, Caldas-Lopes E, Beebe K, Perna F, Hatzi K, Vu LP, Zhao X, Zatorska D, Taldone T, Smith-Jones P, Alpaugh M, Gross SS, Pillarsetty N, Ku T, Lewis JS, Larson SM, Levine R, Erdjument-Bromage H, Guzman ML, Nimer SD, Melnick A, Neckers L, Chiosis G (2011) Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. Nat Chem Biol 7(11):818–826. doi:10.1038/nchembio.670
23. Jhaveri K, Taldone T, Modi S, Chiosis G (2012) Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. Biochim Biophys Acta 1823(3):742–755. doi:10.1016/j.bbamcr.2011.10.008
24. Kamal A, Burrows FJ (2004) Hsp90 inhibitors as selective anticancer drugs. Discov Med 4(23):277–280
25. Ardini E, Galvani A (2012) ALK inhibitors: a pharmaceutical perspective. Front Oncol 2:17. doi:10.3389/fonc.2012.00017
26. Kim YS, Alarcon SV, Lee S, Lee MJ, Giaccone G, Neckers L, Trepel JB (2009) Update on Hsp90 inhibitors in clinical trial. Curr Top Med Chem 9(15):1479–1492. doi:CTMC-Abs-031-9-15
27. Donnelly A, Blagg BS (2008) Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. Curr Med Chem 15(26):2702–2717
28. Brandt GE, Blagg BS (2009) Alternate strategies of Hsp90 modulation for the treatment of cancer and other diseases. Curr Topics Med Chem 9(15):1447–1461
29. Gao Z, Garcia-Echeverria C, Jensen MR (2010) Hsp90 inhibitors: clinical development and future opportunities in oncology therapy. Curr Opin Drug Discov Dev 13(2):193–202
30. Janin YL (2010) ATPase inhibitors of heat-shock protein 90, second season. Drug Discov Today 15(9-10):342–353. doi:10.1016/j.drudis.2010.03.002
31. Beebe K, Mollapour M, Scroggins B, Prodromou C, Xu W, Tokita M, Taldone T, Pullen L, Zierer BK, Lee MJ, Trepel J, Buchner J, Bolon D, Chiosis G, Neckers L (2013) Posttranslational modification and conformational state of Heat Shock Protein 90 differentially affect binding of chemically diverse small molecule inhibitors. Oncotarget 4(7):1065–1074
32. Anderson AC (2003) The process of structure-based drug design. Chem Biol 10(9):787–797. doi:10.1016/j.chembiol.20 03.09.002
33. Erlanson DA, McDowell RS, O'Brien T (2004) Fragment-based drug discovery. J Med Chem 47(14):3463–3482. doi:10.1021/jm040031v
34. Vilenchik M, Solit D, Basso A, Huezo H, Lucas B, He H, Rosen N, Spampinato C, Modrich P, Chiosis G (2004) Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90. Chem Biol 11(6):787–797. doi:10.1016/j.chembiol.2004.04.008
35. Supko JG, Hickman RL, Grever MR, Malspeis L (1995) Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. Cancer Chemother Pharmacol 36(4):305–315

36. Neckers L (2006) Chaperoning oncogenes: Hsp90 as a target of geldanamycin. Handb Exp Pharmacol 172:259–277
37. Samuni A, Goldstein S (2012) Redox properties and thiol reactivity of geldanamycin and its analogues in aqueous solutions. J Phys Chem B 116(22):6404–6410. doi:10.1021/jp304206n
38. Samuni Y, Ishii H, Hyodo F, Samuni U, Krishna MC, Goldstein S, Mitchell JB (2010) Reactive oxygen species mediate hepatotoxicity induced by the Hsp90 inhibitor geldanamycin and its analogs. Free Radic Biol Med 48(11):1559–1563. doi:10.1016/j.freeradbiomed.2010.03.001
39. Tian ZQ, Liu Y, Zhang D, Wang Z, Dong SD, Carreras CW, Zhou Y, Rastelli G, Santi DV, Myles DC (2004) Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. Bioorg Med Chem 12(20):5317–5329. doi:10.1016/j.bmc.2004.07.053
40. Schulte TW, Neckers LM (1998) The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 42(4):273–279
41. Banerji U, O'Donnell A, Scurr M, Pacey S, Stapleton S, Asad Y, Simmons L, Maloney A, Raynaud F, Campbell M, Walton M, Lakhani S, Kaye S, Workman P, Judson I (2005) Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino, 17-demethoxygeldanamycin in patients with advanced malignancies. J Clin Oncol 23(18):4152–4161. doi:10.1200/JCO.2005.00.612
42. Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P (1999) DT-diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst 91(22):1940–1949
43. Solit DB, Ivy SP, Kopil C, Sikorski R, Morris MJ, Slovin SF, Kelly WK, DeLaCruz A, Curley T, Heller G, Larson S, Schwartz L, Egorin MJ, Rosen N, Scher HI (2007) Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. Clin Cancer Res 13(6):1775–1782. doi:10.1158/1078-0432.CCR-06-1863
44. Hollingshead M, Alley M, Burger AM, Borgel S, Pacula-Cox C, Fiebig HH, Sausville EA (2005) In vivo antitumor efficacy of 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride), a water-soluble geldanamycin derivative. Cancer Chemother Pharmacol 56(2):115–125. doi:10.1007/s00280-004-0939-2
45. Ge J, Normant E, Porter JR, Ali JA, Dembski MS, Gao Y, Georges AT, Grenier L, Pak RH, Patterson J, Sydor JR, Tibbitts TT, Tong JK, Adams J, Palombella VJ (2006) Design, synthesis, and biological evaluation of hydroquinone derivatives of 17-amino-17-demethoxygeldanamycin as potent, water-soluble inhibitors of Hsp90. J Med Chem 49(15):4606–4615. doi:10.1021/jm0603116
46. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L, Pak RH, Ali JA, Dembski MS, Hudak J, Patterson J, Penders C, Pink M, Read MA, Sang J, Woodward C, Zhang Y, Grayzel DS, Wright J, Barrett JA, Palombella VJ, Adams J, Tong JK (2006) Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. Proc Natl Acad Sci U S A 103(46):17408–17413. doi:10.1073/pnas.0608372103
47. Sequist LV, Gettinger S, Senzer NN, Martins RG, Janne PA, Lilenbaum R, Gray JE, Iafrate AJ, Katayama R, Hafeez N, Sweeney J, Walker JR, Fritz C, Ross RW, Grayzel D, Engelman JA, Borger DR, Paez G, Natale R (2010) Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol 28(33):4953–4960. doi:10.1200/JCO.2010.30.8338
48. Floris G, Debiec-Rychter M, Wozniak A, Stefan C, Normant E, Faa G, Machiels K, Vanleeuw U, Sciot R, Schoffski P (2011) The heat shock protein 90 inhibitor IPI-504 induces KIT degradation, tumor shrinkage, and cell proliferation arrest in xenograft models of gastrointestinal stromal tumors. Mol Cancer Ther 10(10):1897–1908. doi:10.1158/1535-7163.MCT-11-0148
49. Soga S, Akinaga S, Shiotsu Y (2013) Hsp90 inhibitors as anti-cancer agents, from basic discoveries to clinical development. Curr Pharm Des 19(3):366–376
50. Delmotte P, Delmotte-Plaque J (1953) A new antifungal substance of fungal origin. Nature 171(4347):344

51. Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, Neckers LM (1998) Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones 3(2):100–108
52. Soga S, Neckers LM, Schulte TW, Shiotsu Y, Akasaka K, Narumi H, Agatsuma T, Ikuina Y, Murakata C, Tamaoki T, Akinaga S (1999) KF25706, a novel oxime derivative of radicicol, exhibits in vivo antitumor activity via selective depletion of Hsp90 binding signaling molecules. Cancer Res 59(12):2931–2938
53. Yang ZQ, Geng X, Solit D, Pratilas CA, Rosen N, Danishefsky SJ (2004) New efficient synthesis of resorcinylic macrolides via ynolides: establishment of cycloproparadicicol as synthetically feasible preclinical anticancer agent based on Hsp90 as the target. J Am Chem Soc 126(25):7881–7889. doi:10.1021/ja0484348
54. Soga S, Shiotsu Y, Akinaga S, Sharma SV (2003) Development of radicicol analogues. Curr Cancer Drug Targets 3(5):359–369
55. Blagg BS, Kerr TD (2006) Hsp90 inhibitors: small molecules that transform the Hsp90 protein folding machinery into a catalyst for protein degradation. Med Res Rev 26(3):310–338. doi:10.1002/med.20052
56. Agatsuma T, Ogawa H, Akasaka K, Asai A, Yamashita Y, Mizukami T, Akinaga S, Saitoh Y (2002) Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities. Bioorg Med Chem 10(11):3445–3454
57. Ikuina Y, Amishiro N, Miyata M, Narumi H, Ogawa H, Akiyama T, Shiotsu Y, Akinaga S, Murakata C (2003) Synthesis and antitumor activity of novel O-carbamoylmethyloxime derivatives of radicicol. J Med Chem 46(12):2534–2541. doi:10.1021/jm030110r
58. Rowlands MG, Newbatt YM, Prodromou C, Pearl LH, Workman P, Aherne W (2004) High-throughput screening assay for inhibitors of heat-shock protein 90 ATPase activity. Anal Biochem 327(2):176–183. doi:10.1016/j.ab.2003.10.038
59. Cheung KM, Matthews TP, James K, Rowlands MG, Boxall KJ, Sharp SY, Maloney A, Roe SM, Prodromou C, Pearl LH, Aherne GW, McDonald E, Workman P (2005) The identification, synthesis, protein crystal structure and in vitro biochemical evaluation of a new 3,4-diarylpyrazole class of Hsp90 inhibitors. Bioorg Med Chem Lett 15(14):3338–3343. doi:10.1016/j.bmcl.2005.05.046
60. Dymock BW, Barril X, Brough PA, Cansfield JE, Massey A, McDonald E, Hubbard RE, Surgenor A, Roughley SD, Webb P, Workman P, Wright L, Drysdale MJ (2005) Novel, potent small-molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design. J Med Chem 48(13):4212–4215. doi:10.1021/jm050355z
61. Brough PA, Aherne W, Barril X, Borgognoni J, Boxall K, Cansfield JE, Cheung KM, Collins I, Davies NG, Drysdale MJ, Dymock B, Eccles SA, Finch H, Fink A, Hayes A, Howes R, Hubbard RE, James K, Jordan AM, Lockie A, Martins V, Massey A, Matthews TP, McDonald E, Northfield CJ, Pearl LH, Prodromou C, Ray S, Raynaud FI, Roughley SD, Sharp SY, Surgenor A, Walmsley DL, Webb P, Wood M, Workman P, Wright L (2008) 4,5-diarylisoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. J Med Chem 51(2):196–218. doi:10.1021/jm701018h
62. Brough PA, Barril X, Beswick M, Dymock BW, Drysdale MJ, Wright L, Grant K, Massey A, Surgenor A, Workman P (2005) 3-(5-Chloro-2,4-dihydroxyphenyl)-pyrazole-4-carboxamides as inhibitors of the Hsp90 molecular chaperone. Bioorg Med Chem Lett 15(23):5197–5201. doi:10.1016/j.bmcl.2005.08.091
63. Barril X, Beswick MC, Collier A, Drysdale MJ, Dymock BW, Fink A, Grant K, Howes R, Jordan AM, Massey A, Surgenor A, Wayne J, Workman P, Wright L (2006) 4-Amino derivatives of the Hsp90 inhibitor CCT018159. Bioorg Med Chem Lett 16(9):2543–2548. doi:10.1016/j.bmcl.2006.01.099
64. Sharp SY, Prodromou C, Boxall K, Powers MV, Holmes JL, Box G, Matthews TP, Cheung KM, Kalusa A, James K, Hayes A, Hardcastle A, Dymock B, Brough PA, Barril X, Cansfield JE, Wright L, Surgenor A, Foloppe N, Hubbard RE, Aherne W, Pearl L, Jones K, McDonald E, Raynaud F, Eccles S, Drysdale M, Workman P (2007) Inhibition of the heat shock protein 90 molecular chaperone in vitro and in vivo by novel, synthetic, potent resorcinylic pyrazole/

isoxazole amide analogues. Mol Cancer Ther 6(4):1198–1211. doi:10.1158/1535-7163.MCT-07-0149
65. Jensen MR, Schoepfer J, Radimerski T, Massey A, Guy CT, Brueggen J, Quadt C, Buckler A, Cozens R, Drysdale MJ, Garcia-Echeverria C, Chene P (2008) NVP-AUY922: a small molecule HSP90 inhibitor with potent antitumor activity in preclinical breast cancer models. Breast Cancer Res 10(2):R33. doi:10.1186/bcr1996
66. Sessa C, Shapiro GI, Bhalla KN, Britten C, Jacks KS, Mita M, Papadimitrakopoulou V, Pluard T, Samuel TA, Akimov M, Quadt C, Fernandez-Ibarra C, Lu H, Bailey S, Chica S, Banerji U (2013) First-in-human phase I dose-escalation study of the HSP90 inhibitor AUY922 in patients with advanced solid tumors. Clin Cancer Res 19(13):3671–3680. doi:10.1158/1078-0432.CCR-12-3404
67. Murray CW, Carr MG, Callaghan O, Chessari G, Congreve M, Cowan S, Coyle JE, Downham R, Figueroa E, Frederickson M, Graham B, McMenamin R, O'Brien MA, Patel S, Phillips TR, Williams G, Woodhead AJ, Woolford AJ (2010) Fragment-based drug discovery applied to Hsp90. Discovery of two lead series with high ligand efficiency. J Med Chem 53(16):5942–5955. doi:10.1021/jm100059d
68. Woodhead AJ, Angove H, Carr MG, Chessari G, Congreve M, Coyle JE, Cosme J, Graham B, Day PJ, Downham R, Fazal L, Feltell R, Figueroa E, Frederickson M, Lewis J, McMenamin R, Murray CW, O'Brien MA, Parra L, Patel S, Phillips T, Rees DC, Rich S, Smith DM, Trewartha G, Vinkovic M, Williams B, Woolford AJ (2010) Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-dihydrois oindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone Hsp90 by fragment based drug design. J Med Chem 53(16):5956–5969. doi:10.1021/jm100060b
69. Graham B, Curry J, Smyth T, Fazal L, Feltell R, Harada I, Coyle J, Williams B, Reule M, Angove H, Cross DM, Lyons J, Wallis NG, Thompson NT (2012) The heat shock protein 90 inhibitor, AT13387, displays a long duration of action in vitro and in vivo in non-small cell lung cancer. Cancer Sci 103(3):522–527. doi:10.1111/j.1349-7006.2011.02191.x
70. El-Hariry I, Proia D, Vukovic V (2013) Treating cancer with heat-shock protein-90 (HSP90) inhibitory compounds such as ganetespib. WO2013006864A2
71. Ying W, Du Z, Sun L, Foley KP, Proia DA, Blackman RK, Zhou D, Inoue T, Tatsuta N, Sang J, Ye S, Acquaviva J, Ogawa LS, Wada Y, Barsoum J, Koya K (2012) Ganetespib, a unique triazolone-containing Hsp90 inhibitor, exhibits potent antitumor activity and a superior safety profile for cancer therapy. Mol Cancer Ther 11(2):475–484. doi:10.1158/1535-7163.MCT-11-0755
72. Goldman JW, Raju RN, Gordon GA, El-Hariry I, Teofilivici F, Vukovic VM, Bradley R, Karol MD, Chen Y, Guo W, Inoue T, Rosen LS (2013) A first in human, safety, pharmacokinetics, and clinical activity phase I study of once weekly administration of the Hsp90 inhibitor ganetespib (STA-9090) in patients with solid malignancies. BMC Cancer 13:152. doi:10.1186/1471-2407-13-152
73. Socinski MA, Goldman J, El-Hariry I, Koczywas M, Vukovic V, Horn L, Paschold E, Salgia R, West H, Sequist LV, Bonomi P, Brahmer J, Chen LC, Sandler A, Belani CP, Webb T, Harper H, Huberman M, Ramalingam S, Wong KK, Teofilovici F, Guo W, Shapiro GI (2013) A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. Clin Cancer Res 19(11):3068–3077. doi:10.1158/1078-0432.CCR-12-3381
74. Nakashima T, Ishii T, Tagaya H, Seike T, Nakagawa H, Kanda Y, Akinaga S, Soga S, Shiotsu Y (2010) New molecular and biological mechanism of antitumor activities of KW-2478, a novel nonansamycin heat shock protein 90 inhibitor, in multiple myeloma cells. Clin Cancer Res 16(10):2792–2802. doi:10.1158/1078-0432.CCR-09-3112
75. Dutta R, Inouye M (2000) GHKL, an emergent ATPase/kinase superfamily. Trends Biochem Sci 25(1):24–28
76. Chene P (2002) ATPases as drug targets: learning from their structure. Nat Rev Drug Discov 1(9):665–673. doi:10.1038/nrd894

77. Chiosis G, Timaul MN, Lucas B, Munster PN, Zheng FF, Sepp-Lorenzino L, Rosen N (2001) A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. Chem Biol 8(3):289–299. doi:S1074-5521(01)00015-1
78. Wright L, Barril X, Dymock B, Sheridan L, Surgenor A, Beswick M, Drysdale M, Collier A, Massey A, Davies N, Fink A, Fromont C, Aherne W, Boxall K, Sharp S, Workman P, Hubbard RE (2004) Structure-activity relationships in purine-based inhibitor binding to HSP90 isoforms. Chem Biol 11(6):775–785. doi:10.1016/j.chembiol.2004.03.033
79. He H, Zatorska D, Kim J, Aguirre J, Llauger L, She Y, Wu N, Immormino RM, Gewirth DT, Chiosis G (2006) Identification of potent water soluble purine-scaffold inhibitors of the heat shock protein 90. J Med Chem 49(1):381–390. doi:10.1021/jm0508078
80. 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
81. Dymock B, Barril X, Beswick M, Collier A, Davies N, Drysdale M, Fink A, Fromont C, Hubbard RE, Massey A, Surgenor A, Wright L (2004) Adenine derived inhibitors of the molecular chaperone HSP90-SAR explained through multiple X-ray structures. Bioorg Med Chem Lett 14(2):325–328
82. Kim J, Felts S, Llauger L, He H, Huezo H, Rosen N, Chiosis G (2004) Development of a fluorescence polarization assay for the molecular chaperone Hsp90. J Biomol Screen 9(5):375–381. doi:10.1177/1087057104265995
83. Llauger L, He H, Kim J, Aguirre J, Rosen N, Peters U, Davies P, Chiosis G (2005) Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90. J Med Chem 48(8):2892–2905. doi:10.1021/jm049012b
84. Llauger-Bufi L, Felts SJ, Huezo H, Rosen N, Chiosis G (2003) Synthesis of novel fluorescent probes for the molecular chaperone Hsp90. Bioorg Med Chem Lett 13(22):3975–3978
85. Biamonte MA, Shi J, Hong K, Hurst DC, Zhang L, Fan J, Busch DJ, Karjian PL, Maldonado AA, Sensintaffar JL, Yang YC, Kamal A, Lough RE, Lundgren K, Burrows FJ, Timony GA, Boehm MF, Kasibhatla SR (2006) Orally active purine-based inhibitors of the heat shock protein 90. J Med Chem 49(2):817–828. doi:10.1021/jm0503087
86. He H, Llauger L, Rosen N, Chiosis G (2004) General method for the synthesis of 8-arylsulfanyl adenine derivatives. J Org Chem 69(9):3230–3232. doi:10.1021/jo049875c
87. Caldas-Lopes E, Cerchietti L, Ahn JH, Clement CC, Robles AI, Rodina A, Moulick K, Taldone T, Gozman A, Guo Y, Wu N, de Stanchina E, White J, Gross SS, Ma Y, Varticovski L, Melnick A, Chiosis G (2009) Hsp90 inhibitor PU-H71, a multimodal inhibitor of malignancy, induces complete responses in triple-negative breast cancer models. Proc Natl Acad Sci U S A 106(20):8368–8373. doi:10.1073/pnas.0903392106
88. Rodina A, Vilenchik M, Moulick K, Aguirre J, Kim J, Chiang A, Litz J, Clement CC, Kang Y, She Y, Wu N, Felts S, Wipf P, Massague J, Jiang X, Brodsky JL, Krystal GW, Chiosis G (2007) Selective compounds define Hsp90 as a major inhibitor of apoptosis in small-cell lung cancer. Nat Chem Biol 3(8):498–507. doi:10.1038/nchembio.2007.10
89. Breinig M, Caldas-Lopes E, Goeppert B, Malz M, Rieker R, Bergmann F, Schirmacher P, Mayer M, Chiosis G, Kern MA (2009) Targeting heat shock protein 90 with non-quinone inhibitors: a novel chemotherapeutic approach in human hepatocellular carcinoma. Hepatology 50 (1):102–112. doi:10.1002/hep. 22912
90. Cerchietti LC, Lopes EC, Yang SN, Hatzi K, Bunting KL, Tsikitas LA, Mallik A, Robles AI, Walling J, Varticovski L, Shaknovich R, Bhalla KN, Chiosis G, Melnick A (2009) A purine scaffold Hsp90 inhibitor destabilizes BCL-6 and has specific antitumor activity in BCL-6-dependent B cell lymphomas. Nat Med 15(12):1369–1376. doi:10.1038/nm.2059
91. Marubayashi S, Koppikar P, Taldone T, Abdel-Wahab O, West N, Bhagwat N, Caldas-Lopes E, Ross KN, Gonen M, Gozman A, Ahn JH, Rodina A, Ouerfelli O, Yang G, Hedvat C, Bradner JE, Chiosis G, Levine RL (2010) HSP90 is a therapeutic target in JAK2-dependent myeloproliferative neoplasms in mice and humans. J Clin Invest 120(10):3578–3593. doi:10.1172/JCI42442

92. Kim SH, Bajji A, Tangallapally R, Markovitz B, Trovato R, Shenderovich M, Baichwal V, Bartel P, Cimbora D, McKinnon R, Robinson R, Papac D, Wettstein D, Carlson R, Yager KM (2012) Discovery of (2 S)-1-[4-(2-{6-amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-9 H-purin-9-yl}ethyl)piperidin-1-yl]-2-hydroxypropan-1-one (MPC-3100), a purine-based Hsp90 inhibitor. J Med Chem 55(17):7480–7501. doi:10.1021/jm3004619
93. Bajji AC, Tangallapally RP, Kim IC, Kim SH, Parker DP, Trovato R, Papac DI, Yager K, Baichwal VR (2012) Preparation of 6-amino-9 H-purine prodrugs as HSP90 inhibitors for the treatment of cancer. WO2012148550A1
94. 10-Q: MYREXIS, INC (2012). http://www.marketwatch.com/story/10-q-myrexis-inc-2012-11-09. Accessed 8 Oct 2013
95. Cai X, Zhai H-X, Wang J, Samson M, Atoyan R, Forrester J, Qu H, Yin L, Wang D, Zifcak B, DellaRocca S, Xu G-X, Lai C-J, Bao R, Simonin M-P, Keegan M, Pepicelli CV, Changgeng Q (2011) Design and synthesis of imidazopyridine derivatives as novel HSP90 inhibitors for the treatment of cancer. Proceedings: AACR 102nd Annual Meeting 2011, Apr 2–6, Orlando, Florida. doi:10.1158/1538-7445.AM2011-3249
96. Bao R, Lai CJ, Qu H, Wang D, Yin L, Zifcak B, Atoyan R, Wang J, Samson M, Forrester J, DellaRocca S, Xu GX, Tao X, Zhai HX, Cai X, Qian C (2009) CUDC-305, a novel synthetic HSP90 inhibitor with unique pharmacologic properties for cancer therapy. Clin Cancer Res 15(12):4046–4057. doi:10.1158/1078-0432.CCR-09-0152
97. Curis (2013) Debio 0932. http://www.curis.com/pipeline_detail.php?id=15. Accessed 21 Sept 2013
98. Kasibhatla SR, Hong K, Biamonte MA, Busch DJ, Karjian PL, Sensintaffar JL, Kamal A, Lough RE, Brekken J, Lundgren K, Grecko R, Timony GA, Ran Y, Mansfield R, Fritz LC, Ulm E, Burrows FJ, Boehm MF (2007) Rationally designed high-affinity 2-amino-6-halopurine heat shock protein 90 inhibitors that exhibit potent antitumor activity. J Med Chem 50(12):2767–2778. doi:10.1021/jm050752+
99. Lundgren K, Zhang H, Brekken J, Huser N, Powell RE, Timple N, Busch DJ, Neely L, Sensintaffar JL, Yang YC, McKenzie A, Friedman J, Scannevin R, Kamal A, Hong K, Kasibhatla SR, Boehm MF, Burrows FJ (2009) BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. Mol Cancer Ther 8(4):921–929. doi:10.1158/1535-7163.MCT-08-0758
100. Dickson MA, Okuno SH, Keohan ML, Maki RG, D'Adamo DR, Akhurst TJ, Antonescu CR, Schwartz GK (2013) Phase II study of the HSP90-inhibitor BIIB021 in gastrointestinal stromal tumors. Ann Oncol 24(1):252–257. doi:10.1093/annonc/mds275
101. Mitchell P (2011) Biogen Idec restructures, sharpens neurology focus. Nat Biotechnol 29(1):7–8. doi:10.1038/nbt0111-7
102. Fadden P, Huang KH, Veal JM, Steed PM, Barabasz AF, Foley B, Hu M, Partridge JM, Rice J, Scott A, Dubois LG, Freed TA, Silinski MA, Barta TE, Hughes PF, Ommen A, Ma W, Smith ED, Spangenberg AW, Eaves J, Hanson GJ, Hinkley L, Jenks M, Lewis M, Otto J, Pronk GJ, Verleysen K, Haystead TA, Hall SE (2010) Application of chemoproteomics to drug discovery: identification of a clinical candidate targeting hsp90. Chem Biol 17(7):686–694. doi:10.1016/j.chembiol.2010.04.015
103. Huang KH, Veal JM, Fadden RP, Rice JW, Eaves J, Strachan JP, Barabasz AF, Foley BE, Barta TE, Ma W, Silinski MA, Hu M, Partridge JM, Scott A, DuBois LG, Freed T, Steed PM, Ommen AJ, Smith ED, Hughes PF, Woodward AR, Hanson GJ, McCall WS, Markworth CJ, Hinkley L, Jenks M, Geng L, Lewis M, Otto J, Pronk B, Verleysen K, Hall SE (2009) Discovery of novel 2-aminobenzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents. J Med Chem 52(14):4288–4305. doi:10.1021/jm900230j
104. Rajan A, Kelly RJ, Trepel JB, Kim YS, Alarcon SV, Kummar S, Gutierrez M, Crandon S, Zein WM, Jain L, Mannargudi B, Figg WD, Houk BE, Shnaidman M, Brega N, Giaccone G (2011) A phase I study of PF-04929113 (SNX-5422), an orally bioavailable heat shock protein 90 inhibitor, in patients with refractory solid tumor malignancies and lymphomas. Clin Cancer Res 17(21):6831–6839. doi:10.1158/1078-0432.CCR-11-0821

105. Menezes DL, Taverna P, Jensen MR, Abrams T, Stuart D, Yu GK, Duhl D, Machajewski T, Sellers WR, Pryer NK, Gao Z (2012) The novel oral Hsp90 inhibitor NVP-HSP990 exhibits potent and broad-spectrum antitumor activities in vitro and in vivo. Mol Cancer Ther 11(3):730–739. doi:10.1158/1535-7163.MCT-11-0667
106. Vernalis (2012) Interim results for the six months ended 30 June 2012. http://www.vernalis.com/media-centre/latest-releases/2012-releases/642-interim-results-for-the-six-months-ended-30-june-2012. Accessed 21 Sept 2013
107. Bussenius J, Blazey CM, Aay N, Anand NK, Arcalas A, Baik T, Bowles OJ, Buhr CA, Costanzo S, Curtis JK, DeFina SC, Dubenko L, Heuer TS, Huang P, Jaeger C, Joshi A, Kennedy AR, Kim AI, Lara K, Lee J, Li J, Lougheed JC, Ma S, Malek S, Manalo JC, Martini JF, McGrath G, Nicoll M, Nuss JM, Pack M, Peto CJ, Tsang TH, Wang L, Womble SW, Yakes M, Zhang W, Rice KD (2012) Discovery of XL888: a novel tropane-derived small molecule inhibitor of HSP90. Bioorg Med Chem Lett 22(17):5396–5404. doi:10.1016/j.bmcl.2012.07.052
108. Paraiso KH, Haarberg HE, Wood E, Rebecca VW, Chen YA, Xiang Y, Ribas A, Lo RS, Weber JS, Sondak VK, John JK, Sarnaik AA, Koomen JM, Smalley KS (2012) The HSP90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin Cancer Res 18(9):2502–2514. doi:10.1158/1078-0432.CCR-11-2612
109. Study of XL888 With Vemurafenib for patients with unresectable BRAF mutated stage III/IV melanoma. U.S. National Institutes of Health. http://clinicaltrials.gov/ct2/show/NCT01657591?term=XL888&rank=2. Accessed 5 July 2013
110. Taldone T, Patel PD, Patel M, Patel HJ, Evans CE, Rodina A, Ochiana S, Shah SK, Uddin M, Gewirth D, Chiosis G (2013) Experimental and structural testing module to analyze paralogue-specificity and affinity in the Hsp90 inhibitors series. J Med Chem 56(17):6803–6818. doi:10.1021/jm400619b
111. Patel PD, Yan P, Seidler PM, Patel HJ, Sun W, Yang C, Que NS, Taldone T, Finotti P, Stephani RA, Gewirth DT, Chiosis G (2013) Paralog-selective Hsp90 inhibitors define tumor-specific regulation of HER2. Nat Chem Biol 9(11):677−684.doi:10.1038/nchembio.1335
112. Kang BH, Plescia J, Dohi T, Rosa J, Doxsey SJ, Altieri DC (2007) Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. Cell 131(2):257–270. doi:10.1016/j.cell.2007.08.028
113. Altieri DC, Stein GS, Lian JB, Languino LR (2012) TRAP-1, the mitochondrial Hsp90. Biochim Biophys Acta 1823(3):767–773. doi:10.1016/j.bbamcr.2011.08.007
114. Kang BH, Plescia J, Song HY, Meli M, Colombo G, Beebe K, Scroggins B, Neckers L, Altieri DC (2009) Combinatorial drug design targeting multiple cancer signaling networks controlled by mitochondrial Hsp90. J Clin Invest 119(3):454–464. doi:10.1172/JCI37613
115. Kang BH, Siegelin MD, Plescia J, Raskett CM, Garlick DS, Dohi T, Lian JB, Stein GS, Languino LR, Altieri DC (2010) Preclinical characterization of mitochondria-targeted small molecule hsp90 inhibitors, gamitrinibs, in advanced prostate cancer. Clin Cancer Res 16(19):4779–4788. doi:10.1158/1078-0432.CCR-10-1818
116. Marzec M, Eletto D, Argon Y (2012) GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochim Biophys Acta 1823(3):774–787. doi:10.1016/j.bbamcr.2011.10.013
117. Zheng H, Dai J, Stoilova D, Li Z (2001) Cell surface targeting of heat shock protein gp96 induces dendritic cell maturation and antitumor immunity. J Immunol 167(12):6731–6735
118. Ni M, Lee AS (2007) ER chaperones in mammalian development and human diseases. FEBS Lett 581(19):3641–3651. doi:10.1016/j.febslet.2007.04.045
119. Eletto D, Dersh D, Argon Y (2010) GRP94 in ER quality control and stress responses. Semin Cell Dev Biol 21(5):479–485. doi:10.1016/j.semcdb.2010.03.004
120. Duerfeldt AS, Peterson LB, Maynard JC, Ng CL, Eletto D, Ostrovsky O, Shinogle HE, Moore DS, Argon Y, Nicchitta CV, Blagg BS (2012) Development of a Grp94 inhibitor. J Am Chem Soc 134(23):9796–9804. doi:10.1021/ja303477g

121. Marcu MG, Chadli A, Bouhouche I, Catelli M, Neckers LM (2000) The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. J Biol Chem 275(47):37181–37186. doi:10.1074/jbc.M003701200
122. Centenera MM, Fitzpatrick AK, Tilley WD, Butler LM (2013) Hsp90: still a viable target in prostate cancer. Biochim Biophys Acta 1835(2):211–218. doi:10.1016/j.bbcan.2012.12.005
123. Eskew JD, Sadikot T, Morales P, Duren A, Dunwiddie I, Swink M, Zhang X, Hembruff S, Donnelly A, Rajewski RA, Blagg BS, Manjarrez JR, Matts RL, Holzbeierlein JM, Vielhauer GA (2011) Development and characterization of a novel C-terminal inhibitor of Hsp90 in androgen dependent and independent prostate cancer cells. BMC Cancer 11:468. doi:10.1186/1471-2407-11-468
124. Matthews SB, Vielhauer GA, Manthe CA, Chaguturu VK, Szabla K, Matts RL, Donnelly AC, Blagg BS, Holzbeierlein JM (2010) Characterization of a novel novobiocin analogue as a putative C-terminal inhibitor of heat shock protein 90 in prostate cancer cells. Prostate 70(1):27–36. doi:10.1002/pros.21035
125. 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(13):5041–5052. doi:10.1021/bi047433p
126. Khandelwal A, Hall J, Blagg BS (2013) Synthesis and structure-activity relationships of egcg analogues, a recently identified Hsp90 inhibitor. J Org Chem 78(16):7859−7884. doi:10.1021/jo401027r
127. Rosenhagen MC, Soti C, Schmidt U, Wochnik GM, Hartl FU, Holsboer F, Young JC, Rein T (2003) The heat shock protein 90-targeting drug cisplatin selectively inhibits steroid receptor activation. Mol Endocrinol 17(10):1991–2001. doi:10.1210/me.2003-0141
128. Chaudhury S, Welch TR, Blagg BS (2006) Hsp90 as a target for drug development. ChemMedChem 1(12):1331–1340. doi:10.1002/cmdc.200600112
129. Yu Y, Hamza A, Zhang T, Gu M, Zou P, Newman B, Li Y, Gunatilaka AA, Zhan CG, Sun D (2010) Withaferin A targets heat shock protein 90 in pancreatic cancer cells. Biochem Pharmacol 79 (4):542–551. doi:10.1016/j.bcp. 2009.09.017
130. Fortugno P, Beltrami E, Plescia J, Fontana J, Pradhan D, Marchisio PC, Sessa WC, Altieri DC (2003) Regulation of survivin function by Hsp90. Proc Natl Acad Sci U S A 100(24):13791–13796. doi:10.1073/pnas.2434345100
131. Meli M, Pennati M, Curto M, Daidone MG, Plescia J, Toba S, Altieri DC, Zaffaroni N, Colombo G (2006) Small-molecule targeting of heat shock protein 90 chaperone function: rational identification of a new anticancer lead. J Med Chem 49(26):7721–7730. doi:10.1021/jm060836y
132. Plescia J, Salz W, Xia F, Pennati M, Zaffaroni N, Daidone MG, Meli M, Dohi T, Fortugno P, Nefedova Y, Gabrilovich DI, Colombo G, Altieri DC (2005) Rational design of shepherdin, a novel anticancer agent. Cancer Cell 7(5):457–468. doi:10.1016/j.ccr.2005.03.035