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Review Article

Natural and Synthetic Inhibitors of Heat Shock Protein 90 Chaperone in Cancer Treatment

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ABSTRACT

The heat shock protein 90 (HSP90) plays a crucial role in regulating cellular homeostasis. Importantly, enhanced dependence of cancer-specific proteins or mutants on HSP90 for their stability and activity offers HSP90 as an attractive therapeutic target. But, HSP90 being an important house-keeping protein, raised skepticism towards achieving cancer-specific therapeutic efficacy. Biochemical and functional studies revealed differential activity of HSP90 between healthy and cancer cells indicating the promise of specific chemical inhibitors in treating the disease. The current educational article elaborates on multiple classes of inhibitors that either have pre-clinical activity or being tested in various phases of clinical trials. In addition, recent pre-clinical findings that offer mechanistic insights into the therapeutic efficacy of HSP90 inhibitors are detailed. Thus, the article focuses on the promise of HSP90 as a therapeutic target and recent advances in the development of novel HSP90 inhibitors. Furthermore, we discuss the genetic factors and molecular mechanisms that influence the success of targeting HSP90 chaperone in cancer treatment.

Keywords: Chaperones, Cancer, Drug resistance, HSP90 inhibitors, Natural products.

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INTRODUCTION

Heat shock protein 90 (HSP90) is an important house-keeping chaperone that stabilizes multiple classes of proteins including kinases, hormone receptors and transcription factors.[1] The proteins that require a functional HSP90 for correct folding and stability are called "client" proteins.[1] Many proteins which are known as "co-chaperones" assist the HSP90 in performing the chaperoning activity.[2,3] In addition to chaperoning normal client proteins, HSP90 was found to be crucial for the stability of several mutated proteins that underlie pathologies including cancer and neurodegenerative diseases. Thus, inhibition of HSP90 activity holds promise for the treatment of these disorders.[4] So far, many HSP90 inhibitors (HSP90i) were developed and tested for their efficacy in treating cancer but met with little success.[5] However, new HSP90 inhibitors with better safety and efficacy profiles are being developed and tested in various clinical trials.[5] This article focuses on the biology of HSP90 chaperone and a detailed analysis of several natural and synthetic inhibitors of HSP90 chaperone.

Structure and Function of HSP90 Chaperone

Based on the phylogeny and their resident cellular compartment, the *HSP90* family of genes is classified into five groups: *HSP90A* (cytoplasm), *HSP90B/GRP94* (endoplasmic reticulum), *HSP90C* (chloroplast), *TRAP* (mitochondria) and *HTPG* (bacteria). The cytosolic *HSP90A* group further consists of two isoforms: an inducible *HSP90AA (HSP90Aα)* and a constitutively expressed *HSP90AB (HSP90Aβ)*. The HSP90 monomer consists of an N-Terminal Domain (NTD) connected to a Middle Domain (MD) via a charged linker, followed by a C-Terminal Domain (CTD) (Figure 1A). Protein dimerization and ATP hydrolysis are essential for HSP90 function, which is assisted by interaction with a battery of co-chaperones. Both the NTD and the CTD are involved in the dimerization (Figure 1B). In inactive state, the CTD alone is dimerized while during the activation, both the NTD and the CTD are dimerized. The ATP initially binds to the relaxed or open state of HSP90 leading to the dimerization of N-terminal domains (tense or closed state) (Figure 1B). Following hydrolysis, the N-terminal domains revert back to relaxed or open state for the release of ADP in exchange for the new ATP (Figure 1B).

A conformational rearrangement involving the creation of a hydrophobic patch at the NTD dimer interface together with the folding (closure) of lid over the bound ATP happens during the transition from the apo (open) state to the ATP-bound (closed) state.[6] It is important to note that the time HSP90 spends in a particular conformation determines its interaction

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with client and therefore its function. In addition, several co-chaperones also function in loading client proteins on to HSP90 for their maturation as well as activation.[6] The HSP90-co-chaperone-client interaction and activity is further dependent on specific post-translational modifications on each of these three components.[7] The biochemical details including the activation state as well as the conformation of client proteins were recently elucidated further, improving our understanding of the mechanism of chaperone function.[8]

HSP90 as a therapeutic target

The identification of functionally distinct HSP90 chaperones in cancer cells suggested the possibility to use HSP90 inhibitors as selective cancer chemotherapeutics. Enhanced susceptibility of transformed cells towards HSP90 inhibition when compared to that of non-transformed isogenic cells was also reported.[9] This observed specificity of HSP90 inhibitors towards transformed cells arise from the fact that many oncogenes that are involved in the pathogenesis of multiple cancers are HSP90 client proteins. For example, the leukemia specific BCR-ABL and FLT3-ITD mutants, lymphoma specific NPM-ALK mutant, myeloid disorder specific JAK2-V617F mutant, lung cancer specific EGFR-L858R mutant and melanoma specific BRAF-V600E mutant are stabilized by the HSP90 and are degraded upon HSP90 inhibitor treatment. Apart from mutant oncoproteins, the wild type ERBB2 protein also is a HSP90 client that is overexpressed in nearly one-third of breast cancers. Thus, the efficacy of HSP90 inhibitors is due to indirect targeting (degradation) of oncoproteins from which cancer cells derive their survival and mitogenic signals.

The pre-clinical activity varied among HSP90 inhibitors; several-fold differences in the mean GI_{50} values were reported for HSP90 inhibitors towards the NCI-60 panel of cell lines (Figure 1C).[10] Furthermore, the anti-cancer activity of widely-used HSP90i differed between various commonly used cancer cell lines; for example, among the four commonly used cell lines considered, K562 cells are most sensitive to Alvespimycin (AM) while A549 is most sensitive towards Tanespimycin (TM) (Figure 1D).[11] Apart from inherent differences in binding affinities for various inhibitors towards the HSP90, differences in anti-cancer activity may also arise from the biological availability, off-target inhibition and more importantly altered gene expression upon inhibitor treatment. In addition, paralog-specific HSP90 inhibitors with differential binding affinities towards alpha and beta isoforms hold more promise in overcoming some of the limiting features of traditional HSP90 inhibitors; examples include PU-29F (HSP90α: 5 μM and HSP90β: 25 μM), PU-20F (HSP90α: 8 μM and HSP90β: 34 μM), PU-11 (HSP90α: 18 μM and HSP90β: 90 μM), SNX-0723 (HSP90α: 3 nM and HSP90β: 4 nM) and TAS-116 (HSP90α: 34.7 nM and HSP90β: 21.3 nM).[12] Most of the clinical trials were conducted in breast cancer, multiple myeloma, lung cancer, melanoma and prostate cancer. TM was well tolerated, displayed anti-cancer activity across all tested doses and exhibited better clinical efficacy in combination with standard-of-care agents (sorafenib, bortezomib and trastuzumab) than monotherapy. Both TM and AM in combination with trastuzumab displayed anti-tumor activity against breast cancer.[13] Luminespib (LP), alone or in combination with capecitabine showed activity against advanced solid tumors. Ganetespib (GP) alone or in combination with paclitaxel and trastuzumab showed anti-cancer activity against breast cancer.[14] TM, LP and KW-2468, in combination with bortezomib displayed activity against multiple myeloma.[15] BIIB028, debio0932, Onalespib (OP), HSP990 and LAQ824 showed some anti-cancer activity against solid tumors.[5,16,17] TAS-116 and KW-2468 showed reduced ocular toxicities, a common side effect observed with HSP90 inhibitors.[18] Even though clinical trials with HSP90 inhibitors so far met with little success, recent preclinical developments with novel inhibitors are encouraging and hold promise for their efficacy in certain cancers.

Natural product N-terminal inhibitors of HSP90

Geldanamycin

The first known HSP90 inhibitor is a natural compound Geldanamycin (GA), which was isolated from the bacteria *Streptomyces hygroscopicus* var. *geldanus* var. *nova*.[5,19] The polyketide natural product GA is a 1,4-benzoquinone ansamycin and binds the ATP-binding pocket (NTD) of HSP90 (Figure 2A). The C-7 of GA carbamate interacts directly with Asp79 and indirectly via water molecules with Leu34, Gly83 and Thr171 of HSP90. GA showed good anti-tumor activity against NCI-60 panel of human cancer cell lines.[10] Notably, GA showed activity against cells that expressed inhibitor-resistant FLT3 mutations.[9] Despite the potency *in vitro*, GA displayed poor drug-like properties such as low solubility, hepatotoxicity and less stability, and was found not suitable for systemic administration. The toxicity of GA has been ascribed to a reactive functional group at C17.[5] Thus, GA was never tested in clinical trials.

Radicicol

The resorcylic acid lactone Radicicol (RD) is an HSP90 inhibitor which was first isolated from the fungus *Monosporium bonorden* (Figure 2B).[19] The anti-transformation effect of RD was due to its ability to bind to the ATP-binding pocket of HSP90. The 2-hydroxy and 12'-carbonyl groups interact with Asp79 while the epoxide group interacts with Lys44 of HSP90.[20] RD also interacts with Asp79 via a water molecule.[20] Treatment with RD resulted in the degradation of multiple oncoproteins resulting in selective cytotoxicity towards cancer cells.[19] RD showed significantly less hepatotoxicity than GA plausibly due to the absence of hydroquinone moiety. Despite good pharmacokinetic properties, RD displayed less stability possibly due to the conjugated enone moiety which can react with nucleophiles such as thiols.

Radicicol-related molecules

Monocillin I (6-(7,8-epoxy-10-hydroxy-2-oxo-3,5-undecadi enyl)-beta-resorcylicacid mu-lactone) was first isolated from the fungus *Monocillium nordinii* (Figure 2B) and was identified as a HSP90 inhibitor in an *in vitro* screen.[21,22] Pochonins A and D are resorcyclic macrolide analogs of RD that were first isolated from the fungus *Pochonia chlamydosporia* var. *catenulata* P0297 and were shown to be potent HSP90 inhibitors (Figure 2B).[22] KF25706 (Radicicol 6-oxime) is a radicicol oxime derivative that binds to the N-terminal ATP binding pocket of HSP90 chaperone and inhibits the chaperone activity. It exhibited both *in vitro* activity against 3Y1-B, SR-3Y1, NRK, and KNRK5.2 cell lines and *in vivo* anti-cancer activity against human tumor xenograft models.

Herbimycin

Herbimycin (HB) is a benzoquinone ansamycin, isolated from the bacteria *Streptomyces hygroscopicus* strain no. AM-3672. HB is a C-11,15, dimethoxy 17-desmethoxy analog of GA (Figure 2C).[23] Similar to GA, HB also binds to the N-terminal ATP-binding pocket of the HSP90. HB exhibited activity against both the parental as well as drug-resistant MCF7 cell lines.

Macbecin

The benzoquinone ansamycin, Macbecin (MB) (Figure 2D) was first isolated from the actinomycete bacteria *Nocardia* sp.no. C-14919(N-2001). MB displayed significant activity against HSP90 chaperone and caused disruption of its interaction with client proteins. MB is similar to HB except that a methyl group is present at C-6 of MB while a methoxy group is present in HB. However, MB differs from GA at C-6 (methyl in MB and methoxy in GA), C-11 (methoxy in MB and hydroxy in GA) and a methoxy group at C-15 (instead of at C-17 of GA).[23] Co-crystal structures revealed the hydrogen bonding between carbamate group of MB directly with Asp79, Gly83 and indirectly via water molecules with Leu34 and Thr171 of HSP90.

Gambogic acid

Gambogic Acid (GBA) is a xanthanoid (Figure 2E) derived from the plant *Garcinia hanburyi*.[24] GBA inhibits the ATPase activity of HSP90 by binding to the N-terminal domain in a non-competitive manner; molecular docking analysis further predicted GBA binding to HSP90 at a site distinct from the ATP-binding pocket. However, in a different study, GBA was shown to specifically bind the middle domain of the HSP90β isoform.[25] GBA was shown to exhibit anti-proliferative activity in several wild type as well as drug-resistant cancer cell lines.[24] The alpha, beta unsaturated carbonyl moiety at the C-10 was suggested to cause cytotoxicity of GBA. A phase II randomized trial revealed a favorable safety profile for GBA suggesting its promise as a potential chemotherapeutic agent.[19]

Sansalvamide A

A cyclic pentadepsipeptide derived from mycelia of the fungus Fusarium, Sansalvamide A (SA) was shown to exhibit anti-cancer activity in multiple cell lines (Figure 2F).[26] SA binds preferentially to the closed conformation of HSP90 both to the N-terminal domain and middle domain but allosterically influences the interaction of co-chaperones and clients with the C-terminal domain.[26] Novel derivatives of SA were also shown to be potent selective inhibitors against drug-resistant colon cancer cells indicating SA as a potent drug lead.[26]

Gedunin

The tetranortriterpenoid limonoid Gedunin (GE) was isolated from the plant *Azadirachta indica* and displayed potent anti-cancer activity.[17,19] Using a connectivity map, GE (Figure 2G) was found to modulate HSP90 activity albeit with a molecular mechanism that is different from other ATP-competitive inhibitors.

Hypericin

Hypericin (HR) is a perihydroxylated perylene quinone (Figure 2H) and was first isolated from the plant *Hypericum perforatum*. HR causes poly-ubiquitination and degradation of HSP90 thereby destabilizing client proteins and consequently growth inhibition in cancer cells.[17] A phase I/II study using oral hypericin in glioma patients revealed encouraging results thus indicating its promise as a chemotherapeutic agent.

Cruentaren A

Cruentaren A (CA) belongs to benzolactone class of natural products (Figure 2I) and is isolated from the myxobacterium *Byssovorax cruenta*.[19] CA disrupts the interaction between HSP90 and F_1F_0-ATPase synthase resulting in the degradation of client proteins without inducing heat shock response.[17] CA was shown to exhibit anti-proliferative activity against cancer cells and drug-resistant cell lines.[19]

Synthetic molecule N-terminal inhibitors of HSP90

Geldanamycin derivatives

Tanespimycin (TM) or 17-AAG (17-Allylamino-17 -demethoxygeldanamycin) is the first HSP90 inhibitor that was tested in clinical trials that binds to the N-terminal domain of the chaperone (Figure 2J). TM is the more stable analog of GA wherein the methoxy group at C-17 of GA is replaced with an allylamino group (Figure 2J). Degradation of cancer-related HSP90 client proteins was observed upon TM treatment leading to cell death; in addition, TM was shown to overcome secondary drug resistance by inducing the degradation of kinase inhibitor resistant mutants.[9] Alvespimycin (AM) or 17-DMAG (17-Dimethylaminoethylamino-17-demethoxygeldanamycin), a second-generation HSP90 inhibitor, is a water-soluble analog of geldanamycin with lower hepatotoxicity and higher activity

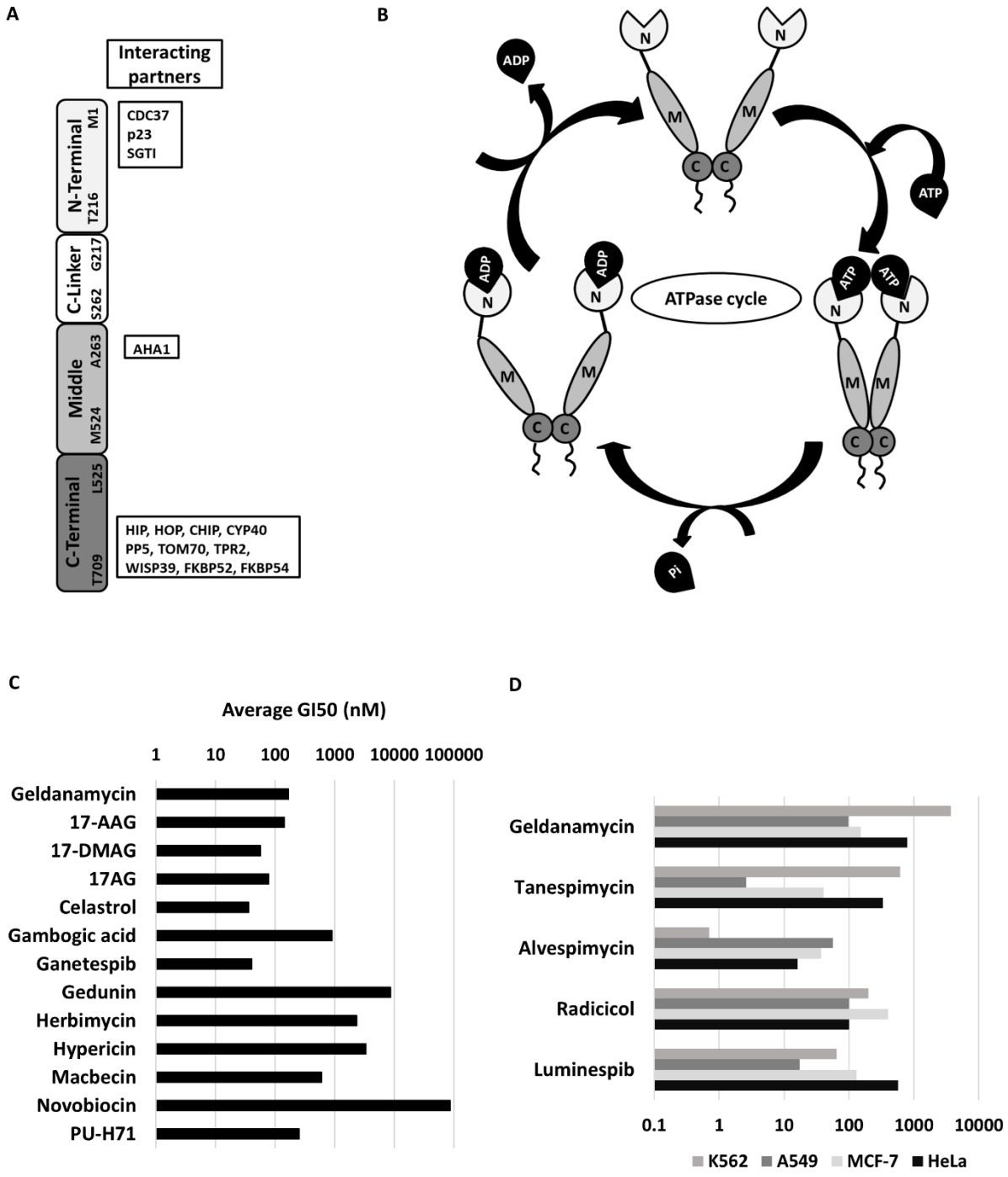

Figure 1: Biochemistry of HSP90 and anti-cancer activity of various HSP90 inhibitors. (A) Structure of HSP90 showing various domains and their interacting proteins. The amino acids are numbered for each domain. (B) ATPase cycle of HSP90 chaperone involving ATP-bound (closed) and ADP-bound (open) conformations. (C) Depiction of variability in average GI_{50} values of various HSP90 inhibitors (shown in logarithmic scale) derived from the screen that was performed with NCI-60 panel of human cancer cell lines. (D) Differential sensitivity of HSP90 inhibitors towards cancer cell lines.

than TM (Figure 2J). AM binds to the N-terminal domain of HSP90 with the ligand conformation slightly different from that of the GA; while the Lys58-C11 interaction is shared between GA and AM, the Lys58-C17 interaction in GA is replaced by the Asp54-C17 interaction in AM. AM was shown to cause HSP90 client oncoprotein degradation leading to cancer cell death and to overcome secondary drug resistance caused by mutant kinases.[9] Retaspimycin (RM) or IPI504 (18,21-Didehydro 17-demethoxy-18,21-dihydroxy-17-(2-propenylamino) geldanamycin) is a water-soluble hydroquinone hydrochloride salt of TA (Figure 2J). RM was shown to cause HSP90 client kinase degradation and consequent cell death both in cancer cells as well as in

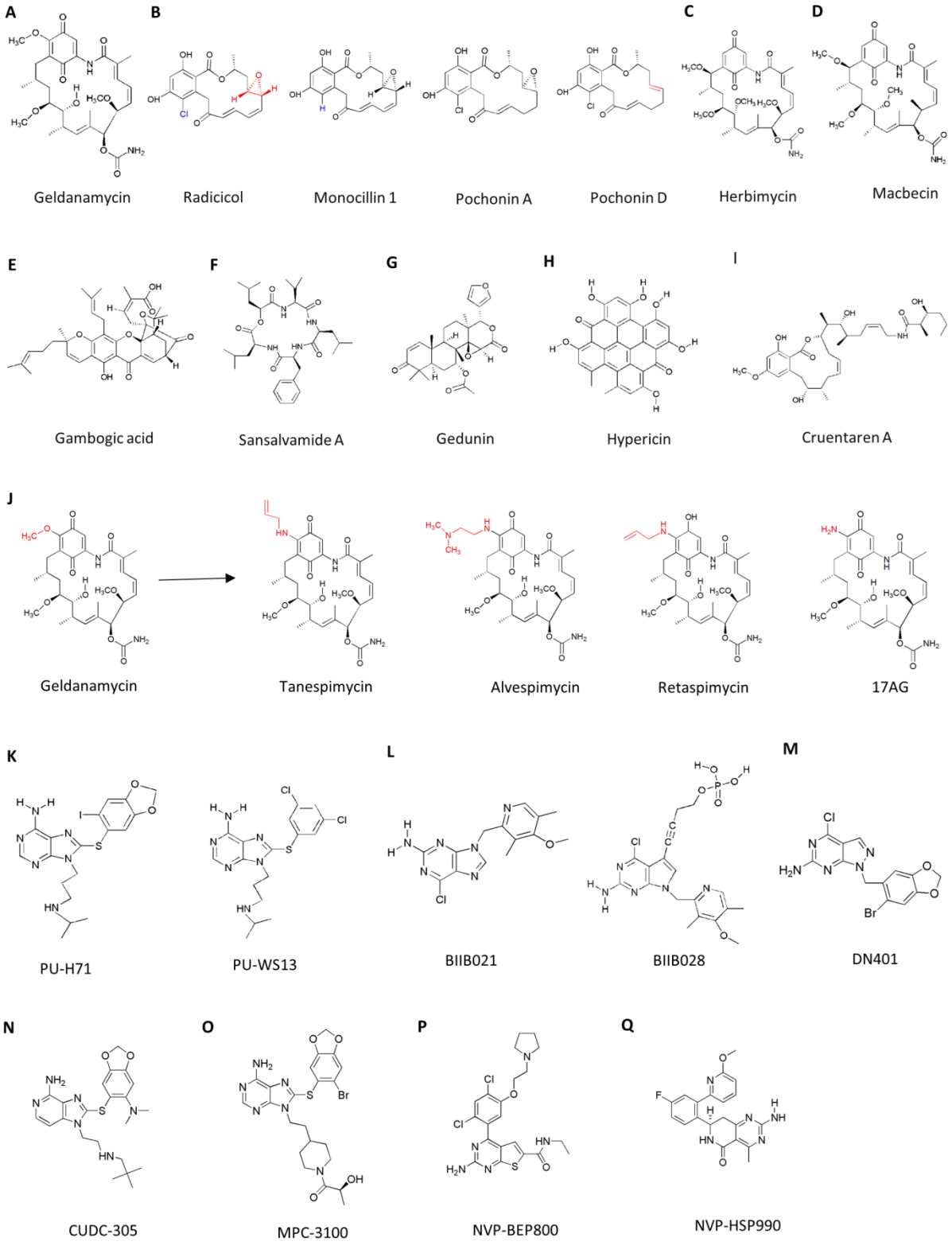

Figure 2: N-terminal natural and synthetic inhibitors of HSP90 chaperone. (A) and (C-I) Natural product inhibitors of HSP90. (B) The radicicol-related molecules are colored to relate their structures to radicicol. (J) Geldanamycin derivatives. (K) PU-series purine-based inhibitors of HSP90. (L) BIIB-series purine-based inhibitors of HSP90. (M-O) Purine-based inhibitors. (P) and (Q) Pyrimidine-based inhibitor. The colored part represents the difference between the parent compound and its derivatives.

drug-resistant mutant cells. Phase I/II trials revealed modest to good tolerability and anti-tumor activity. In addition to RM, another active and stable metabolite of TM, IPI-493 (17-desmethoxy-17-amino-geldanamycin, 17-AG) was also shown to downregulate oncoproteins and possess anti-cancer activity (Figure 2J).[23]

Purine and pyrimidine compounds

The N-terminal binding, ATP-competitive synthetic HSP90 inhibitor PU-H71 (Figure 2K) is a derivative of purine scaffold and was shown to cause oncoprotein degradation and has anti-cancer activity.[5] The efficacy of PU-H71 was further demonstrated in triple-negative breast cancer cells and lymphoma.[5] PU-WS13 is another purine-based selective inhibitor of GRP94 (Figure 2K) with significant activity in breast cancer cells and multiple myeloma.[27,28]

The synthetic purine-based inhibitor BIIB021 (Figure 2L) is an NTD-binding inhibitor of HSP90 with potent growth-inhibitory activity in cancer cells. Significant anti-cancer activity and good safety profiles were also demonstrated in phase I/II clinical studies.[23] The second-generation purine inhibitor BIIB028 (Figure 2L) also is an HSP90 NTD-binding inhibitor and was demonstrated to have anti-tumor activity and good safety profile in a phase I trial.

DN401, a purine scaffold derivative of PU-H71 and BIIB021 is a mitochondria-permeable pan-HSP90 inhibitor (Figure 2M).[29] It inhibited all HSP90 paralogs and degraded the clients of HSP90 (Akt, Chk1 and HER2), GRP94 (HER2) and TRAP1 (SIRT3, Sorcin and SDHB) without inducing HSP70 expression.[29]

CUDC-305 (Debio 0932), an imidazopyridine (Figure 2N) showed high affinity for cytosolic HSP90 isoforms and displayed anti-cancer activity in a wide range of tumor cell lines as well as animal models of glioblastoma, breast cancer and erlotinib-resistant NSCLC.[23] The purine-based MPC-3100 is an orally bioavailable HSP90 inhibitor (Figure 2O) that displayed anti-cancer activity against a broad-range of xenograft models.[30] However, MPC-3100 possesses the properties such as poor solubility and development of prodrug, thereby restricting its clinical development. The purine-based NVP-BEP800 (Figure 2P) and an aminopyrimidine NVP-HSP990 (Figure 2Q) are orally bioavailable HSP90 inhibitors that bind to the N-terminal ATP-binding pocket.[31] Both the inhibitors displayed a broad-range of *in vitro* and *in vivo* anti-cancer activity with well tolerated profile.[15,31]

Resorcinol and benzamide compounds

Ganetespib (GP) or STA-9090 is a triazanole containing second-generation N-terminal inhibitor of HSP90 with activity against several tumor cell lines (Figure 3A).[32] GP induces the degradation of client proteins leading to anti-cancer activity in hematological and solid cancers both *in vitro* and *in vivo*.[32] In addition, GP also displayed very good safety profiles when compared to the TM. Luminespib (LP) or NVP-AUY922 (Figure 3A) is an isoxazole resorcinol derivative and is an N-terminal inhibitor of HSP90 which was shown to have anti-cancer activity in a wide range of tumor types both as a single agent as well as in combination with chemotherapeutics.[33] HSP90 inhibition by LP also resulted in client protein degradation and was shown to overcome secondary resistance towards kinase inhibitors.[34] Phase I/II trials in solid cancers revealed that LP has moderate to good clinical activity and acceptable tolerability when treated in combination with standard anti-cancer drugs.[33] Onalespib (OP) or AT13387 (Figure 3A) is a resorcyclic dihydroxybenzamide, was identified as a second-generation N-terminal HSP90 inhibitor in a fragment-based drug screen.[19,30] OP caused HSP90 client degradation and exhibited anti-tumor activity in cell line and xenograft models of cancer either alone or in combination with other chemotherapeutics.[35] OP also displayed significant anti-cancer activity against multiple drug-resistant tumor models, and phase I/II studies further showed moderate to good anti-tumor activity and acceptable safety profiles.[16]

CCT018159 (3,4-diaryl pyrazole) (Figure 3A) an NTD-binding inhibitor of HSP90, displayed anti-proliferative activity against cancer cells and xenograft mice models.[19] KW-2478 (Figure 3A) is another resorcinol-based HSP90 inhibitor which was identified as part of a lead optimization strategy with pre-clinical activity against multiple myeloma cells.[19] Phase I/II studies revealed both clinical activity and tolerability both in multiple myeloma and B-cell malignancies.[19] VER-49009 (Figure 3B) is a pyrazole amide analogue that binds to the N-terminal ATP-binding pocket of HSP90 and possesses anti-proliferative activity against multiple cancer cell lines.[36] VER-50589 (Figure 3B) is an analogue of VER-49009 that contains isoxazole oxygen in place of pyrazole nitrogen and displayed superior anti-proliferative activity, pharmacodynamic properties and higher binding affinity to HSP90 as compared to VER-49009.[36] XL888 and SNX-5422 (a prodrug of SNX-2112) is orally bioavailable benzamide scaffold containing HSP90 inhibitor (Figure 3C) that bind to the N-terminal ATP-binding pocket. Both the inhibitors displayed potent anti-proliferative activity against various cancer cells and animal models.[5,15]

Natural and synthetic C-Terminal HSP90 inhibitors

Silybin or Silibinin (SY), Epigallocatechingallate (EGCG), Derrubone (DB), Deguelin (DG) and Novobiocin (NB) binds near or to the C-terminal ATP binding site of HSP90. SY is a flavonoid (Figure 3D) isolated from plant Silybum marianum and displayed anti-proliferative activity against colon and urinary bladder cancer cells.[37] EGCG is a polyphenolic catechin (Figure 3E) that is found in green tea, was shown to exhibit anti-cancer activity.[19,30] DB is a natural prenylated isoflavone (Figure 3F) isolated from the root material of *Derris robusta*.[17] DG is a flavonoid (Figure 3G) isolated from the plant Derris trifoliata Lour or Mundulea sericea

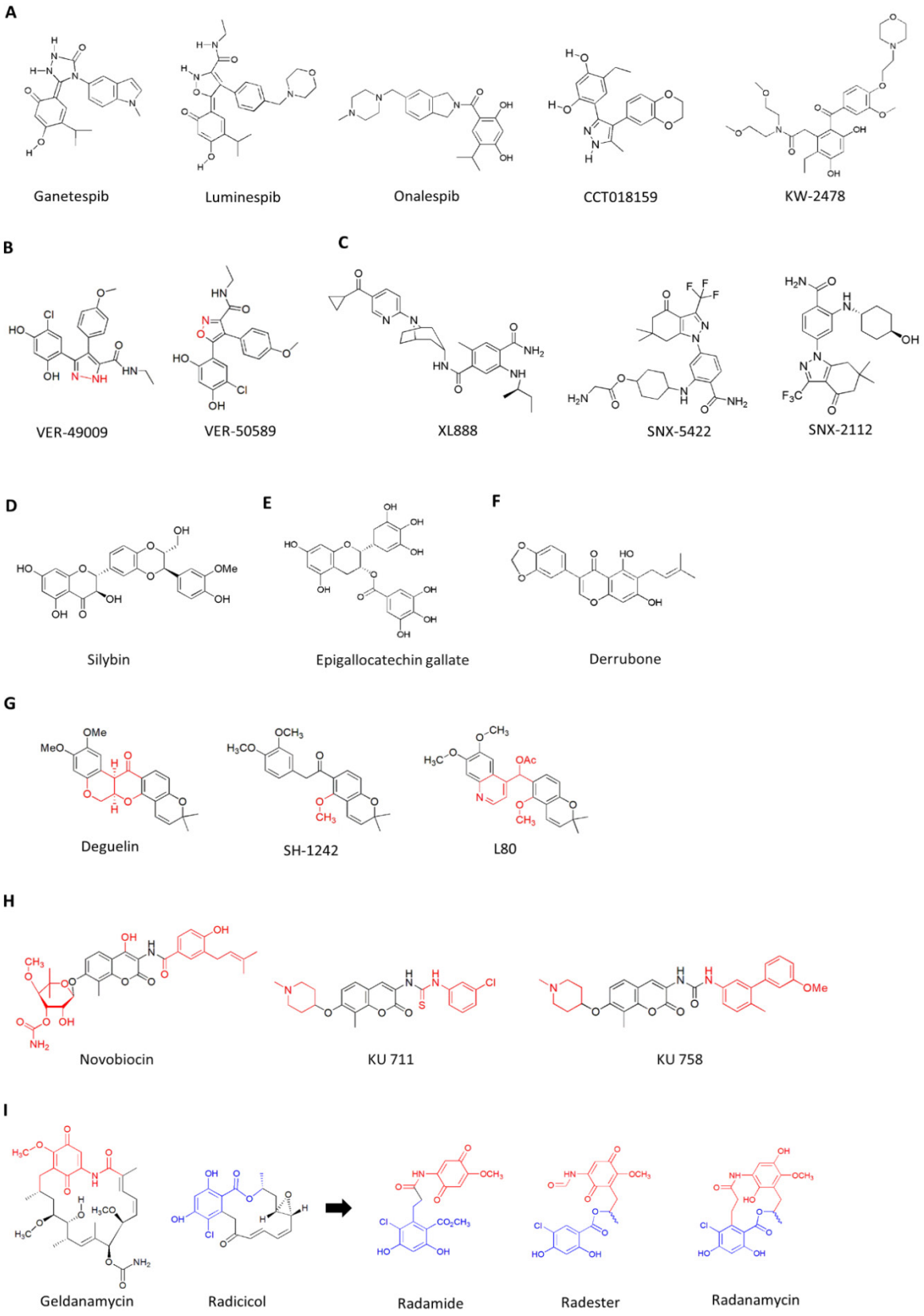

Figure 3: Resorcinol, benzamide based synthetic inhibitors, C-terminal inhibitors and hybrid inhibitors of HSP90 chaperone. (A) and (B) Resorcinol-based inhibitors of HSP90 chaperone. (B) VER-49009 and its analogue VER-50589. (C) Benzamide-based inhibitors of HSP90 chaperone. (D-H) C-terminal HSP90 inhibitors. (D-F) Natural product inhibitors. (G) Degulin (natural inhibitor) and its analogues SH-1242 and L80. (H) Novobiocin (natural inhibitor) and its analogues KU711 and KU758. (I) Hybrid inhibitors derived from geldanamycin and radicicol. The colored part represents the difference between the inhibitor and its analogue.

(Leguminosae), reduced the tumor growth in xenograft-bearing mice by inducing apoptosis.[19,30] In addition, DG analogues SH-1242 and L80 (Figure 3G) showed anti-tumor activity against lung cancer cells.[38] NB is an aminocoumarin antibiotic (Figure 3H) that was isolated from the bacterium *Streptomyces niveus*. Further NB analogues KU711 and KU758 (Figure 3H) displayed anti-metastatic activity against breast cancer cells.[39]

Hybrid Inhibitors

Radamide (RA) is a hybrid molecule that contains resorcinol ring of radicicol and quinone ring of geldanamycin connected via an amide linkage (Figure 3I).[19] RA occupies the ATP-binding pocket of N-terminal domain of HSP90, where the resorcinol ring interacts with the carboxylic group of Asp residue via hydrogen bonding thereby blocking ATP binding. RA displayed potent inhibitory activity against HSP90 ATPase causing degradation of client proteins.

Radester (RE) (Figure 3I) is a chimera formed by connecting the resorcinol ring of RD and the quinone of GA through an isopropyl ester.[19] The carbonyl group of RE is more planar than the carbonyl of RD leading to a high electron density at the carbonyl group and together with increased hydrogen bond interactions resulting in high affinity binding to the HSP90. RE exhibited potent anti-cancer activity against MCF-7 cells by inhibiting HSP90 activity, resulting in a concentration dependent degradation of HSP90 clients.

Radanamycin (RM) (Figure 3I) is another macrocyclic chimera formed by combining two natural products RD and GA.[19] Biological evaluation of RM revealed inhibitory activity against HSP90 resulting in the degradation of client proteins.

HSP90 inhibitors and cancer drug resistance

The major mechanism of resistance towards kinase inhibitor is the presence of point mutations in the inhibitor binding pocket and/or target amplification. In addition, oncogene switching i.e. shift in the dependence of cancer cell survival from one oncogene to the other, was also observed to cause resistance to kinase inhibitor treatment. HSP90 inhibitors were successful in overcoming secondary drug resistance towards kinase inhibitors. However, resistance towards HSP90 inhibitors was also detected in various studies and heat shock response was shown to be the underlying mechanism. Based on several studies using multiple cell lines, HSF1 was shown to be upregulated along with its target genes HSP90α, HSP90β, HSP70 and BAG3 following HSP90i treatment. DNAJA1 and HIP, which are also upregulated along with HSF1 target genes upon HSP90i treatment results in heat shock response and consequent inhibitor resistance. This unique seven-gene signature may thus serve as a marker for resistance towards HSP90 inhibitors. Interestingly, a second-generation C-terminal dimerization inhibitor of HSP90 was recently reported to have significant efficacy in overcoming drug resistance without inducing heat shock response thus reducing toxicity in K562 derived cell lines.[40-42] These results indicate the possibility of developing novel HSP90 inhibitors that can overcome the limitations for the therapeutic utility of currently available HSP90 inhibitors. However, acquired resistance to HSP90 inhibitors were also recently reported due to point mutations in the ATP-binding pocket,[43] due to target amplification during clonal evolution[44] and the activation of key cell signaling pathways,[45] thus posing a new challenge towards HSP90i treatment.

CONCLUSION

HSP90 serves as a hub for homeostasis in cancer cells by stabilizing mutant proteins that drive tumorigenesis. Thus, HSP90 offers a central therapeutic target especially in the case of secondary drug resistance that arises due to either point mutations in target client kinase or switching of the survival dependence from one client oncoprotein to the other. The efficacy of HSP90 inhibitors towards cancer cell lines and its ability to overcome secondary drug resistance was demonstrated as a proof-of-concept in several pre-clinical models. However, the importance of HSP90 in normal cellular functions has initially raised concerns regarding its inhibition and consequent cytotoxicity specifically towards cancer cells. Despite these safety issues concerning selectivity and safety, several HSP90 inhibitors of multiple chemotypes were developed and tested in the clinic. However, resistance towards HSP90i due to multiple mechanisms still remains unresolved. Given the promise of targeting HSP90 in cancer, there is thus an urgent need for the development of novel HSP90 inhibitors with enhanced efficacy (including selectivity, specificity, bioavailability and safety) as well as that can overcome secondary resistance towards first and second-generation HSP90 inhibitors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABL: Abelson; **ADP:** Adenosine diphosphate; **AKT:** Serine/threonine protein kinase; **ALK:** Anaplastic lymphoma kinase; **ATP:** Adenosine triphosphate; **BAG3:** Bcl-2-associated athanogene 3; **BCR:** Breakpoint cluster region; **Chk1:** Checkpoint kinase1; **DNAJA1:** DnaJ heat shock protein family (Hsp40) member A1; **EGFR:** Epidermal growth factor receptor; **ERBB2:** v-erb-b2 avian Erythroblastic leukemia viral oncogene homolog 2; **FLT3:** Fms-like tyrosine kinase; **FLT3-ITD:** FLT3 internal tandem duplication; **GI_{50}:** Growth inhibitory concentration of 50;

GRP94: Glucose-regulated protein 94; **HER2:** Human epidermal growth factor receptor 2; **HIP:** Hsp70-interacting protein; **HSF:** Heat shock factor; **HSP:** Heat shock protein; **HTPG:** High-temperature protein G; **JAK2:** Janus kinase 2; **MCF-7:** Michigan Cancer Foundation-7; **NCI:** National cancer institute; **NPM:** Nucleophosmin; **NRK:** Normal rat kidney epithelial cells; **NSCLC:** Non-small cell lung cancer; **SDHB:** Succinate dehydrogenase B; **SIRT3:** Sirtuin 3; **TRAP:** Tumor necrosis factor receptor- associated protein.

REFERENCES

1. Bhattacharya K, Picard D. The Hsp70–Hsp90 go-between Hop/Stip1/Sti1 is a proteostatic switch and may be a drug target in cancer and neurodegeneration. Cell Mol Life Sci. 2021; 78(23): 7257-73. doi: 10.1007/s00018-021-03962-z, PMID 34677645.
2. Liu M, Xu Z, Zhang C, Yang C, Feng J, Lu Y, *et al.* NudC L279P mutation destabilizes filamin A by inhibiting the Hsp90 chaperoning pathway and suppresses cell migration. Front Cell Dev Biol. 2021; 9: 671233. doi: 10.3389/fcell.2021.671233, PMID 34262899.
3. Baker-Williams AJ, Hashmi F, Budzyński MA, Woodford MR, Gleicher S, Himanen SV, *et al.* Co-chaperones TIMP2 and AHA1 competitively regulate extracellular HSP90:client MMP2 activity and matrix proteolysis. Cell Rep. 2019; 28(7): 1894-1906.e6. doi: 10.1016/j.celrep.2019.07.045, PMID 31412254.
4. Niu M, Zhang B, Li L, Su Z, Pu W, Zhao C, *et al.* Targeting HSP90 inhibits proliferation and induces apoptosis through AKT1/ERK pathway in lung cancer. Front Pharmacol. 2021; 12: 724192. doi: 10.3389/fphar.2021.724192, PMID 35095481.
5. Li ZN, Luo Y. HSP90 inhibitors and cancer: prospects for use in targeted therapies (Review) [review]. Oncol Rep. 2023; 49(1): 6. doi: 10.3892/or.2022.8443, PMID 36367182.
6. Prodromou C, Bjorklund DM. Advances towards understanding the mechanism of action of the Hsp90 complex. Biomolecules. 2022; 12(5): 600. doi: 10.3390/biom12050600, PMID 35625528.
7. Backe SJ, Sager RA, Woodford MR, Makedon AM, Mollapour M. Post-translational modifications of Hsp90 and translating the chaperone code. J Biol Chem. 2020; 295(32): 11099-117. doi: 10.1074/jbc.REV120.011833, PMID 32527727.
8. Kancha RK, Bartosch N, Duyster J. Analysis of conformational determinants underlying HSP90-kinase interaction. PLOS ONE. 2013; 8(7): e68394. doi: 10.1371/journal.pone.0068394, PMID 23844194.
9. Yu C, Kancha RK, Duyster J. Targeting oncoprotein stability overcomes drug resistance caused by FLT3 kinase domain mutations. PLOS ONE. 2014; 9(5): e97116. doi: 10.1371/journal.pone.0097116, PMID 24849514.
10. Holbeck SL, Collins JM, Doroshow JH. Analysis of Food and Drug Administration-approved anticancer agents in the NCI60 panel of human tumor cell lines. Mol Cancer Ther. 2010; 9(5): 1451-60. doi: 10.1158/1535-7163.MCT-10-0106, PMID 20442306.
11. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, *et al.* Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res. 2013;41(D1):D955-61. doi: 10.1093/nar/gks1111, PMID 23180760.
12. Patel PD, Yan P, Seidler PM, Patel HJ, Sun W, Yang C, *et al.* Paralog-selective Hsp90 inhibitors define tumor-specific regulation of HER2. Nat Chem Biol. 2013; 9(11): 677-84. doi: 10.1038/nchembio.1335, PMID 23995768.
13. Jhaveri K, Miller K, Rosen L, Schneider B, Chap L, Hannah A, *et al.* A Phase I dose-escalation trial of trastuzumab and alvespimycin hydrochloride (KOS-1022; 17 DMAG) in the treatment of advanced solid tumors. Clin Cancer Res. 2012; 18(18): 5090-8. doi: 10.1158/1078-0432.CCR-11-3200, PMID 22781552.
14. Jhaveri K, Wang R, Teplinsky E, Chandarlapaty S, Solit D, Cadoo K, *et al.* A phase I trial of ganetespib in combination with paclitaxel and trastuzumab in patients with human epidermal growth factor receptor-2 (HER2)-positive metastatic breast cancer. Breast Cancer Res. 2017; 19(1): 89. doi: 10.1186/s13058-017-0879-5, PMID 28764748.
15. Moscvin M, Ho M, Bianchi G. Overcoming drug resistance by targeting protein homeostasis in multiple myeloma. Cancer Drug Resist. 2021; 4(4): 1028-46. doi: 10.20517/cdr.2021.93, PMID 35265794.
16. Mohajershojai T, Jha P, Boström A, Frejd FY, Yazaki PJ, Nestor M. *In vitro* characterization of 177Lu-DOTA-M5A anti-carcinoembryonic antigen humanized antibody and HSP90 inhibition for potentiated radioimmunotherapy of colorectal cancer. Front Oncol. 2022; 12: 849338. doi: 10.3389/fonc.2022.849338, PMID 35433442.
17. Serwetnyk MA, Blagg BSJ. The disruption of protein–protein interactions with co-chaperones and client substrates as a strategy towards Hsp90 inhibition. Acta Pharm Sin B. 2021; 11(6): 1446-68. doi: 10.1016/j.apsb.2020.11.015, PMID 34221862.
18. Shaaban AA, Abdelhamid AM, Shaker ME, Cavalu S, Maghiar AM, Alsayegh AA, *et al.* Combining the HSP90 inhibitor TAS-116 with metformin effectively degrades the NLRP3 and attenuates inflammasome activation in rats: A new management paradigm for ulcerative colitis. Biomed Pharmacother. 2022; 153: 113247. doi: 10.1016/j.biopha.2022.113247, PMID 35724510.
19. Zhang J, Li H, Liu Y, Zhao K, Wei S, Sugarman ET, *et al.* Targeting HSP90 as a novel therapy for cancer: mechanistic insights and translational relevance. Cells. 2022; 11(18): 2778. doi: 10.3390/cells11182778, PMID 36139353.
20. Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem. 1999; 42(2): 260-6. doi: 10.1021/jm980403y, PMID 9925731.
21. Turbyville TJ, Wijeratne EM, Liu MX, Burns AM, Seliga CJ, Luevano LA, *et al.* Search for Hsp90 inhibitors with potential anticancer activity: isolation and SAR studies of radicicol and monocillin I from two plant-associated fungi of the Sonoran Desert. J Nat Prod. 2006; 69(2): 178-84. doi: 10.1021/np058095b, PMID 16499313.
22. Kuttikrishnan S, Prabhu KS, Al Sharie AH, Al Zu'bi YO, Alali FQ, Oberlies NH, *et al.* Natural resorcylic acid lactones: A chemical biology approach for anticancer activity. Drug Discov Today. 2022; 27(2): 547-57. doi: 10.1016/j.drudis.2021.10.001, PMID 34655796.
23. Ardestani M, Khorsandi Z, Keshavarzipour F, Iravani S, Sadeghi-Aliabadi H, Varma RS. Heterocyclic compounds as Hsp90 inhibitors: A perspective on anticancer applications. Pharmaceutics. 2022; 14(10): 2220. doi: 10.3390/pharmaceutics14102220, PMID 36297655.
24. Pesonen L, Svartsjö S, Bäck V, de Thonel A, Mezger V, Sabéran-Djoneidi D, *et al.* Gambogic acid and gambogenic acid induce a thiol-dependent heat shock response and disrupt the interaction between HSP90 and HSF1 or HSF2. Cell Stress Chaperones. 2021; 26(5): 819-33. doi: 10.1007/s12192-021-01222-4, PMID 34331200.
25. Yim KH, Prince TL, Qu S, Bai F, Jennings PA, Onuchic JN, *et al.* Gambogic acid identifies an isoform-specific druggable pocket in the middle domain of Hsp90β. Proc Natl Acad Sci U S A. 2016; 113(33): E4801-9. doi: 10.1073/pnas.1606655113, PMID 27466407.
26. Wang X, Zhang J, Han Z, Ma L, Li Y. 18F-labeled Dimer-Sansalvamide A Cyclodecapeptide: a Novel Diagnostic Probe to Discriminate Pancreatic Cancer from Inflammation in a Nude Mice Model. J Cancer. 2022; 13(6): 1848-58. doi: 10.7150/jca.69710, PMID 35399736.
27. Mishra SJ, Ghosh S, Stothert AR, Dickey CA, Blagg BS. Transformation of the non-selective Aminocyclohexanol-based Hsp90 inhibitor into a Grp94-Seletive scaffold. ACS Chem Biol. 2017; 12(1): 244-53. doi: 10.1021/acschembio.6b00747, PMID 27959508.
28. Dernovšek J, Tomašič T. Following the design path of isoform-selective Hsp90 inhibitors: small differences, great opportunities. Pharmacol Ther. 2023; 245: 108396. doi: 10.1016/j.pharmthera.2023.108396, PMID 37001734.
29. Park HK, Yoon NG, Lee JE, Hu S, Yoon S, Kim SY, *et al.* Unleashing the full potential of Hsp90 inhibitors as cancer therapeutics through simultaneous inactivation of Hsp90, Grp94, and TRAP1. Exp Mol Med. 2020; 52(1): 79-91. doi: 10.1038/s12276-019-0360-x , PMID 31956271.
30. Li L, Wang L, You QD, Xu XL. Heat shock Protein 90 inhibitors: an update on achievements, challenges, and future directions. J Med Chem. 2020; 63(5): 1798-822. doi: 10.1021/acs.jmedchem.9b00940, PMID 31663736.
31. Mshaik R, Simonet J, Georgievski A, Jamal L, Bechoua S, Ballerini P, *et al.* HSP90 inhibitor NVP-BEP800 affects stability of SRC kinases and growth of T-cell and B-cell acute lymphoblastic leukemias. Blood Cancer J. 2021; 11(3): 61. doi: 10.1038/s41408-021-00450-2, PMID 33737511.
32. Lang JE, Forero-Torres A, Yee D, Yau C, Wolf D, Park J, *et al.* Safety and efficacy of HSP90 inhibitor ganetespib for neoadjuvant treatment of stage II/III breast cancer. npj Breast Cancer. 2022; 8(1): 128. doi: 10.1038/s41523-022-00493-z, PMID 36456573.
33. Epp-Ducharme B, Dunne M, Fan L, Evans JC, Ahmed L, Bannigan P, *et al.* Heat-activated nanomedicine formulation improves the anticancer potential of the HSP90 inhibitor luminespib *in vitro*. Sci Rep. 2021; 11(1): 11103. doi: 10.1038/s41598-021-90585-w, PMID 34045581.
34. Jacobson C, Kopp N, Layer JV, Redd RA, Tschuri S, Haebe S, *et al.* HSP90 inhibition overcomes ibrutinib resistance in mantle cell lymphoma. Blood. 2016; 128(21): 2517-26. doi: 10.1182/blood-2016-04-711176, PMID 27742706.
35. Canella A, Welker AM, Yoo JY, Xu J, Abas FS, Kesanakurti D, *et al.* Efficacy of Onalespib, a long-acting second-generation HSP90 inhibitor, as a single agent and in combination with temozolomide against malignant gliomas. Clin Cancer Res. 2017; 23(20): 6215-26. doi: 10.1158/1078-0432.CCR-16-3151, PMID 28679777.
36. Sharp SY, Prodromou C, Boxall K, Powers MV, Holmes JL, Box G, *et al.* 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. 2007; 6(4): 1198-211. doi: 10.1158/1535-7163.MCT-07-0149, PMID 17431102.
37. Barros TMB, Lima APB, Almeida TC, da Silva GN. Inhibition of urinary bladder cancer cell proliferation by silibinin. Environ Mol Mutagen. 2020; 61(4): 445-55. doi: 10.1002/em.22363, PMID 32078183.
38. Lee SC, Min HY, Choi H, Kim HS, Kim KC, Park SJ, *et al.* Synthesis and evaluation of a novel deguelin derivative, L80, which disrupts ATP binding to the C-terminal domain of heat shock Protein 90. Mol Pharmacol. 2015; 88(2): 245-55. doi: 10.1124/mol.114.096883, PMID 25976766.
39. Subramanian C, Grogan PT, Wang T, Bazzill J, Zuo A, White PT, *et al.* Novel C-terminal heat shock protein 90 inhibitors target breast cancer stem cells and block migration, self-renewal, and epithelial–mesenchymal transition. Mol Oncol. 2020; 14(9): 2058-68. doi: 10.1002/1878-0261.12686, PMID 32255264.

40. Bhatia S, Diedrich D, Frieg B, Ahlert H, Stein S, Bopp B, *et al.* Targeting HSP90 dimerization via the C terminus is effective in imatinib-resistant CML and lacks the heat shock response. Blood. 2018; 132(3): 307-20. doi: 10.1182/blood-2017-10-810986, PMID 29724897.
41. Bhatia S, Spanier L, Bickel D, Dienstbier N, Woloschin V, Vogt M, *et al.* Development of a first-in-class small-molecule inhibitor of the C-terminal Hsp90 dimerization. ACS Cent Sci. 2022; 8(5): 636-55. doi: 10.1021/acscentsci.2c00013, PMID 35647282.
42. Cabaud-Gibouin V, Durand M, Quéré R, Girodon F, Garrido C, Jego G. Heat-shock proteins in leukemia and lymphoma: multitargets for innovative therapeutic approaches. Cancers (Basel). 2023; 15(3): 984. doi: 10.3390/cancers15030984, PMID 36765939.
43. Rouhi A, Miller C, Grasedieck S, Reinhart S, Stolze B, Döhner H, *et al.* Prospective identification of resistance mechanisms to HSP90 inhibition in KRAS mutant cancer cells. Oncotarget. 2017; 8(5): 7678-90. doi: 10.18632/oncotarget.13841, PMID 28032595.
44. Arolla RG, Malladi S, Bhaduri U, Gayatri MB, Pattamshetty P, Mohan V, *et al.* Analysis of cellular models of clonal evolution reveals co-evolution of imatinib and HSP90 inhibitor resistances. Biochem Biophys Res Commun. 2021; 534: 461-7. doi: 10.1016/j.bbrc.2020.11.059, PMID 33246559.
45. Chatterjee S, Huang EH, Christie I, Kurland BF, Burns TF. Acquired resistance to the Hsp90 inhibitor, ganetespib, in *KRAS*-mutant NSCLC is mediated via reactivation of the ERK-p90RSK-mTOR signaling network. Mol Cancer Ther. 2017; 16(5): 793-804. doi: 10.1158/1535-7163.MCT-16-0677, PMID 28167505.

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