

RESEARCH MONOGRAPH . COMPOUND PROFILE

17-DMAG

A water-soluble geldanamycin derivative developed as a second-generation HSP90 inhibitor with improved pharmacokinetic properties relative to tanespimycin, advanced through Phase I oncology trials and subsequently investigated for neuroprotective and anti-inflammatory applications.

DOCUMENT	KDC-MN-1477
ISSUED	2026-05-13
REVISION	1.0
AUTHOR	Kodiac bioLabs Research
COMPOUND CLASS	Semi-synthetic benzoquinone ansamycin and heat shock protein 90 (HSP90) N-terminal ATPase inhibitor derived from geldanamycin

ABSTRACT

17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin; alvespimycin; KOS-1022; NSC 707545) is a semi-synthetic derivative of the benzoquinone ansamycin natural product geldanamycin that binds the N-terminal adenosine triphosphate (ATP) binding pocket of heat shock protein 90 (HSP90) with an IC₅₀ of approximately 24 nanomolar. The compound was developed at the National Cancer Institute (NCI) and by Kosan Biosciences as a water-soluble, orally bioavailable successor to the first-in-class HSP90 inhibitor tanespimycin (17-AAG), from which it is distinguished by the replacement of the C-17 allylamino substituent with a dimethylaminoethylamino group. This substitution confers substantially improved aqueous solubility, reduced hepatic metabolic liability, lower plasma protein binding, and higher oral bioavailability while maintaining or exceeding the antitumor potency of the parent compound in preclinical models. HSP90 is a molecular chaperone essential for the conformational maturation and stabilization of numerous client proteins involved in oncogenic signaling, including HER2/ErbB2, AKT, RAF-1, mutant p53, BCR-ABL, FLT3, KIT, and the inhibitor of nuclear factor kappa-B kinase (IKK) subunits. Binding of 17-DMAG to the HSP90 N-terminal domain displaces the chaperone from its client proteins, targeting them for ubiquitin-proteasome-mediated degradation and simultaneously inducing compensatory heat shock factor 1 (HSF1) activation and upregulation of HSP70 and HSP27 as pharmacodynamic biomarkers of target engagement. The compound preferentially accumulates in tumor tissue relative to normal tissue owing to the higher-affinity, multi-chaperone

HSP90 complex conformation present in malignant cells, resulting in a degree of tumor selectivity that is pharmacologically meaningful despite the ubiquitous expression of HSP90 in normal physiology. In preclinical evaluation, 17-DMAG demonstrated broad-spectrum antitumor activity across the NCI 60-cell-line panel (mean GI50 approximately 53 nanomolar) and in xenograft models of melanoma, non-small cell lung cancer, pancreatic cancer, and pediatric solid tumors, with oral and parenteral routes both producing tumor growth inhibition and client protein degradation at tolerated doses. Pharmacokinetic studies in CD2F1 mice and Fischer 344 rats demonstrated wide tissue distribution, linear pharmacokinetics, predominantly hepatobiliary elimination, and quantitatively less extensive metabolism than tanespimycin. Clinical development encompassed four Phase I trials: a weekly intravenous schedule in advanced solid tumors (maximum tolerated dose 80 mg/m², with dose-limiting hepatic and ocular toxicity at 106 mg/m² including one treatment-related death); a twice-weekly intravenous schedule in advanced malignancies (recommended Phase 2 dose 24 mg/m²); a twice-weekly schedule in acute myeloid leukemia (AML) demonstrating target inhibition and signs of clinical activity including complete responses in combination with chemotherapy; and a weekly combination with trastuzumab in HER2-positive advanced solid tumors (recommended dose 80 mg/m² weekly with trastuzumab, with antitumor activity in refractory HER2-positive metastatic breast cancer). A separate Phase I trial in relapsed chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) demonstrated tolerability without objective responses at the doses studied. Common clinical adverse events included nausea, vomiting, fatigue, hepatic transaminase elevation, and ocular toxicity (blurred vision, dry eye, keratitis). In March 2008, Kosan

Biosciences halted clinical development of alvespimycin on the basis of an unfavorable overall toxicity profile relative to the therapeutic window, and the compound has not been advanced to Phase II or Phase III registration trials as a single agent.

Subsequent non-oncologic research has characterized 17-DMAG as a neuroprotective agent in rodent models of ischemic stroke and intracerebral hemorrhage, operating through suppression of NF-kappaB-mediated neuroinflammation, reduction of blood-brain barrier disruption, and modulation of the PI3K/Akt signaling pathway via SOX5 targeting. This monograph reviews the chemistry, synthesis, and structural pharmacology of 17-DMAG; the HSP90 chaperone biology and client protein degradation mechanism; the preclinical pharmacology across oncologic and neurologic models; the comprehensive human pharmacokinetic record; the clinical evidence base across all studied indications; sourcing and quality verification considerations; reconstitution and handling; stack-interaction implications; adverse-event signal; and a structured comparative assessment of five HSP90 inhibitor candidates against 17-DMAG on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Alvespimycin.

Development codes: 17-DMAG (National Cancer Institute, the canonical identifier in the preclinical and clinical pharmacology literature); KOS-1022 (Kosan Biosciences); NSC 707545 (NCI Developmental Therapeutics Program).

Chemical Abstracts Service registry numbers: 467214-20-6 (free base), 467214-21-7 (hydrochloride salt). The hydrochloride salt is the formulated material used in all clinical studies and the principal commercial research-grade preparation.

International Union of Pure and Applied Chemistry name: 17-(2-dimethylaminoethylamino)-17-demethoxygeldanamycin. The systematic name describes the substitution at C-17 of the geldanamycin macrolactam, where the native methoxy group has been replaced by a 2-(dimethylamino)ethylamino side chain.

Other synonyms: 17-Dimethylaminoethylamino-17-demethoxygeldanamycin; alvespimycin hydrochloride (salt form); DMAG.

1.2 Structural class and chemistry

17-DMAG belongs to the benzoquinone ansamycin class of natural product derivatives, a family of macrolactam antibiotics characterized by a 19-membered ansa bridge connecting two positions of a benzoquinone or hydroquinone aromatic ring. The parent compound geldanamycin was originally isolated from *Streptomyces hygroscopicus* var. *geldanus* by DeBoer et al. in 1970 and subsequently identified as a potent and selective HSP90 inhibitor by Whitesell et al. in 1994, establishing the molecular target that would drive the clinical development of the entire ansamycin class [1, 2].

Molecular formula: C₃₂H₄₈N₄O₈ (free base); C₃₂H₄₉ClN₄O₈ (hydrochloride salt).

Exact mass: 616.3472 g/mol (free base, monoisotopic).

Molecular weight: 616.75 g/mol (free base); 653.21 g/mol (hydrochloride salt).

Physical properties: Purple to dark violet amorphous solid (free base and hydrochloride). Freely soluble in water as the hydrochloride salt, soluble in dimethyl sulfoxide and methanol, sparingly soluble in ethanol. The aqueous solubility of 17-DMAG hydrochloride is approximately 50 mg/mL, a property that differentiates it sharply from the poorly soluble parent compounds geldanamycin (essentially insoluble in water) and tanespimycin (17-AAG; requiring formulation in Cremophor EL or dimethyl sulfoxide for intravenous administration) [3].

Stereochemistry: 17-DMAG retains the stereochemistry of the geldanamycin macrolactam at all chiral centers. The compound contains five stereocenters within the ansa bridge. The absolute configuration at these centers is defined by the biosynthetic origin from the polyketide synthase of *Streptomyces hygroscopicus* and is preserved in the semi-synthetic modification at C-17, which does not introduce new stereocenters.

1.3 Synthesis

17-DMAG is prepared by semi-synthesis from geldanamycin through nucleophilic displacement of the C-17 methoxy group. The synthesis proceeds by reaction of geldanamycin with N,N-dimethylethylenediamine under mild conditions (room temperature to 50 degrees Celsius in chloroform or dichloromethane) to afford the 17-amino-substituted product in moderate to good yield (typically 50 to 70 percent) [3, 4]. The reaction exploits the established reactivity of the C-17 position of geldanamycin toward primary and secondary amines, a transformation first characterized systematically in the NCI structure-activity relationship program that also produced tanespimycin (17-AAG) through reaction with allylamine. The crude product is purified by silica gel column chromatography and the hydrochloride salt is prepared by treatment with hydrogen chloride in diethyl ether or dioxane. The identity and purity of the product are confirmed by proton and carbon-13 nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry, and reversed-phase high-performance liquid chromatography. Geldanamycin itself is produced by fermentation of *Streptomyces hygroscopicus* var. *geldanus* and purified by extraction and chromatography; the supply chain for 17-DMAG therefore depends on the fermentation source material [5].

2. Abstract

17-DMAG (alvespimycin, KOS-1022) is a water-soluble, semi-synthetic geldanamycin derivative that inhibits the N-terminal ATPase domain of heat shock protein 90 (HSP90) with an IC₅₀ of approximately 24 nanomolar and a binding affinity (EC₅₀) of approximately 62 nanomolar in cell-free assays [6, 7]. The compound was developed at the National Cancer Institute and subsequently by Kosan Biosciences as a pharmacologically improved successor to tanespimycin (17-AAG), the first HSP90 inhibitor to enter human clinical trials, and was designed to address the principal limitations of the earlier compound: poor aqueous solubility requiring Cremophor-based formulations, extensive hepatic metabolism by CYP3A4 and NAD(P)H:quinone oxidoreductase 1 (NQO1), high plasma protein binding, and limited oral bioavailability.

The introduction of the dimethylaminoethylamino substituent at C-17 conferred substantially improved aqueous solubility (approximately 50 mg/mL as the hydrochloride salt versus less than 0.01 mg/mL for tanespimycin free base), reduced metabolic liability, lower plasma protein binding (approximately 35 percent versus greater than 90 percent for tanespimycin), and oral bioavailability in rodent species approaching 100 percent by the intraperitoneal route and approximately 40 to 60 percent by the oral route [3, 8]. These pharmacokinetic advantages were accompanied by maintained or superior antitumor activity in preclinical models: 17-DMAG produced growth inhibition across the NCI 60-cell-line panel with a mean GI₅₀ of 53 nanomolar and demonstrated tumor regression in xenograft models of melanoma, non-small cell lung cancer, pancreatic cancer, and several pediatric solid tumors at tolerated oral and parenteral doses [9, 10, 11].

Clinical development spanned four Phase I trials between 2004 and 2012. The weekly intravenous schedule in advanced solid tumors established a maximum tolerated dose of 80 mg/m², with dose-limiting toxicities at 106 mg/m² including hepatic transaminase elevation, ocular adverse events, and one treatment-related death [12]. The twice-weekly schedule established a recommended Phase 2 dose of 24 mg/m², with nausea, fatigue, and hepatic enzyme disturbance as the most common adverse events [13]. The AML trial demonstrated tolerability, linear pharmacokinetics, target inhibition as measured by HSP70 induction, and signs of clinical activity including complete responses in combination with cytarabine [14]. The

trastuzumab combination trial in HER2-positive advanced solid tumors demonstrated antitumor activity in refractory metastatic breast cancer and ovarian cancer at the recommended combination dose of 80 mg/m² weekly [15]. A Phase I trial in relapsed CLL/SLL demonstrated tolerability without objective responses at doses up to 24 mg/m² twice weekly [16].

In March 2008, Kosan Biosciences discontinued clinical development of alvespimycin on the basis of an unfavorable toxicity profile relative to the therapeutic index, particularly the hepatic, ocular, and gastrointestinal adverse events that limited dose escalation to exposures that may have been suboptimal for sustained client protein degradation. The compound has not been advanced to Phase II registration trials as a monotherapy. Subsequent research has extended the pharmacology of 17-DMAG beyond oncology to neuroprotection in experimental stroke and intracerebral hemorrhage, where the compound attenuates neuroinflammation, reduces blood-brain barrier disruption, and improves neurological outcomes through suppression of NF-kappaB signaling and activation of the PI3K/Akt pathway [17, 18].

3. Discovery and Development History

3.1 Origin of geldanamycin and the ansamycin HSP90 inhibitor class

Geldanamycin was first isolated from *Streptomyces hygroscopicus* var. *geldanus* by DeBoer et al. in 1970 and was initially characterized as an antibiotic with antitumor activity in murine leukemia models [1]. The molecular target remained unidentified for over two decades until Whitesell et al. (1994) demonstrated that geldanamycin binds to HSP90 and disrupts its association with oncogenic client proteins, notably the v-Src kinase and the mutant p53 tumor suppressor protein, leading to their proteasomal degradation [2]. This discovery established HSP90 as a druggable target in oncology and initiated the clinical development of the ansamycin class.

Geldanamycin itself was too hepatotoxic for clinical development, with the benzoquinone moiety implicated in the generation of reactive oxygen species and direct mitochondrial toxicity in hepatocytes [19]. The NCI Developmental Therapeutics Program therefore undertook a systematic medicinal chemistry effort to produce C-17 amino-substituted analogs with reduced toxicity and improved pharmaceutical properties. The first product of this effort was tanespimycin (17-AAG, 17-allylamino-17-demethoxygeldanamycin), which entered Phase I clinical trials in 1999 as the first-in-class HSP90 inhibitor [20].

3.2 Development of 17-DMAG

17-DMAG was synthesized by Schnur et al. as part of the NCI structure-activity relationship program exploring C-17 amino substituents with varying physicochemical properties [4]. The dimethylaminoethylamino group was selected to introduce a basic amine that would confer water solubility as a hydrochloride salt while maintaining the pharmacophoric requirements for HSP90 binding. The compound was initially prepared as a potential linker for geldanamycin-antibody conjugates but was rapidly recognized as a standalone pharmacological agent when preclinical evaluation demonstrated superior pharmacokinetic properties relative to tanespimycin.

The NCI designated 17-DMAG as NSC 707545 and advanced it through the standard preclinical evaluation pipeline, including the NCI 60-cell-line panel screen (mean GI50 53 nanomolar), in vivo pharmacokinetic and tissue distribution studies in CD2F1 mice and Fischer 344 rats, and formal preclinical toxicology in rats and dogs [3, 8, 21]. Kosan Biosciences licensed the compound from the NCI and designated it KOS-1022, advancing it through Investigational New Drug (IND)-enabling studies and into Phase I clinical trials beginning in 2004.

3.3 Clinical development and discontinuation

Four Phase I trials were initiated between 2004 and 2008, conducted in collaboration between Kosan Biosciences, the NCI Cancer Therapy Evaluation Program (CTEP), and multiple academic medical centers. The trials evaluated weekly and twice-weekly intravenous schedules in advanced solid tumors [12], advanced malignancies on a twice-weekly schedule [13], AML [14], HER2-positive solid tumors in combination with trastuzumab [15], and relapsed CLL/SLL [16].

Despite evidence of target engagement (HSP70 induction in peripheral blood mononuclear cells), pharmacodynamic client protein degradation (HER2, AKT), and isolated clinical responses (complete response in castration-refractory prostate cancer for 124 weeks; partial response in melanoma for 159 weeks; complete responses in AML in combination), the overall toxicity profile was judged unfavorable. Hepatotoxicity, gastrointestinal adverse events, and ocular toxicity (blurred vision, dry eye, keratitis, conjunctivitis) limited dose escalation and chronic administration. In March 2008, Kosan Biosciences halted clinical development. Kosan was subsequently acquired by Bristol-Myers Squibb in 2008; the alvespimycin program was not resumed [22].

3.4 Post-clinical research

Following the discontinuation of oncologic development, 17-DMAG has continued as a widely used research tool compound for HSP90 biology and as a preclinical pharmacological probe in non-oncologic applications. Published research from 2014 to 2025 has extended its pharmacology to neuroinflammation in experimental stroke [17], intracerebral hemorrhage [18], gastric cancer oxidative stress mechanisms [23], and combination strategies with histone deacetylase inhibitors in hema-

tologic malignancies [24]. The compound remains commercially available from multiple research chemical suppliers and is one of the most extensively characterized HSP90 inhibitors in the published literature.

4. Mechanism of Action

17-DMAG exerts its pharmacological effects through competitive inhibition of the N-terminal ATP-binding pocket of heat shock protein 90 (HSP90), a highly conserved molecular chaperone that is essential for the conformational maturation, stability, and function of a large and diverse set of client proteins involved in cellular signaling, proliferation, survival, and stress response.

4.1 HSP90 chaperone biology

HSP90 is a homodimeric ATPase that functions as the central hub of a multi-chaperone complex including HSP70, HSP40, HOP (HSP70/HSP90 organizing protein), p23, and various immunophilin co-chaperones. The chaperone cycle involves ATP binding to the N-terminal domain, client protein loading through the middle domain, ATP hydrolysis-driven conformational changes, and client protein release in a mature, functionally competent conformation. HSP90 client proteins number over 400 and include many oncogenic kinases (HER2/ErbB2, EGFR, AKT, RAF-1, CDK4, MET, ALK, BCR-ABL, FLT3, KIT), transcription factors (mutant p53, HIF-1alpha, androgen receptor, estrogen receptor), and signaling intermediates (IKK-alpha, IKK-beta, survivin, telomerase) [25, 26].

In malignant cells, HSP90 exists in a high-affinity, multi-chaperone complex conformation that binds N-terminal inhibitors with approximately 100-fold greater affinity than the housekeeping HSP90 homodimer in normal cells [27]. This tumor-selective binding affinity is the molecular basis for the preferential toxicity of 17-DMAG and other ansamycin HSP90 inhibitors toward malignant cells, despite the ubiquitous expression of HSP90 in normal physiology.

4.2 N-terminal ATP pocket binding and client protein degradation

17-DMAG binds competitively to the N-terminal ATP-binding pocket of HSP90, displacing ATP and preventing the conformational cycle required for client protein chaperoning. The IC₅₀ for HSP90 binding in cell-free assays is approximately 24 nanomolar; the cellular EC₅₀ for client protein degradation varies by client and cell

type but is generally in the range of 20 to 200 nanomolar [6, 7]. The binding interaction involves hydrogen bonding between the benzoquinone carbonyl and amide groups of 17-DMAG and conserved residues in the HSP90 N-terminal pocket (Asp93, Lys58, Asp40 in HSP90-alpha numbering), with the ansa bridge and carbamate group occupying the hydrophobic core of the pocket in a conformation similar to the adenine ring of ATP [28].

Upon displacement of HSP90 from its client proteins, the unfolded or partially folded client proteins are recognized by the ubiquitin-proteasome system and targeted for degradation. The kinetics of client protein degradation vary: HER2 and RAF-1 are rapidly degraded (half-life of hours), while AKT and CDK4 show intermediate kinetics, and nuclear receptors such as the androgen receptor and estrogen receptor may show partial degradation or redistribution [25].

4.3 Heat shock factor 1 activation and pharmacodynamic biomarkers

A consistent pharmacodynamic consequence of HSP90 N-terminal inhibition is the compensatory activation of heat shock factor 1 (HSF1), the master transcriptional regulator of the heat shock response. HSF1 activation leads to transcriptional up-regulation of HSP70, HSP27, and HSP90 itself. The induction of HSP70 in peripheral blood mononuclear cells has been used as the principal pharmacodynamic biomarker of HSP90 target engagement in all clinical trials of 17-DMAG and has been validated as a dose-dependent, time-dependent indicator of biologically effective dosing [12, 13, 14].

The HSP70 induction is both a biomarker of target engagement and a potential resistance mechanism: elevated HSP70 can partially compensate for HSP90 inhibition by assuming some chaperone functions, and constitutive HSP70 overexpression has been associated with reduced sensitivity to ansamycin HSP90 inhibitors in cell line models [29].

4.4 NF-kappaB pathway inhibition

A therapeutically significant downstream consequence of HSP90 inhibition by 17-DMAG is the degradation of IKK-alpha and IKK-beta, the catalytic subunits of the inhibitor of NF-kappaB kinase (IKK) complex. IKK-mediated phosphorylation of IkappaB-alpha is required for NF-kappaB nuclear translocation and transcriptional

activation of pro-survival and pro-inflammatory genes. 17-DMAG-mediated IKK degradation therefore suppresses NF-kappaB signaling, which is constitutively activated in many malignancies (including CLL, mantle cell lymphoma, and multiple myeloma) and in neuroinflammatory conditions [16, 17, 30]. This mechanism is the molecular basis for both the anti-leukemic activity in CLL models and the neuro-protective activity in stroke and hemorrhage models.

4.5 Tumor selectivity

The preferential toxicity of 17-DMAG toward malignant cells relative to normal cells is attributable to the higher-affinity HSP90 complex conformation in tumor cells. Kamal et al. (2003) demonstrated that HSP90 in tumor cells exists in multi-chaperone complexes with approximately 100-fold higher affinity for the ansamycin geldanamycin relative to HSP90 from normal tissues, which exists predominantly as uncomplexed homodimers [27]. This difference in binding affinity translates to differential accumulation: 17-DMAG achieves higher and more sustained intratumoral concentrations than concentrations in normal tissues at the same systemic dose, and tumor-to-normal tissue concentration ratios of 3:1 to 10:1 have been reported in preclinical distribution studies [8].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption and bioavailability

17-DMAG hydrochloride is readily absorbed after oral administration in rodent species. Oral bioavailability in CD2F1 mice is approximately 40 to 60 percent, substantially higher than tanespimycin (which has oral bioavailability of less than 5 percent in the same species) [3, 8]. Intraperitoneal bioavailability approaches 100 percent. The improved oral bioavailability relative to tanespimycin is attributable to the combination of increased aqueous solubility (facilitating dissolution in the gastrointestinal tract), reduced first-pass hepatic metabolism (lower CYP3A4 substrate activity and reduced NQO1-mediated reduction), and lower plasma protein binding (allowing greater free drug concentrations for tissue distribution).

In human subjects receiving intravenous alvespimycin at doses of 2.5 to 106 mg/m², pharmacokinetics were linear with both area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) increasing proportionally with dose [12, 13]. Population pharmacokinetic analysis in 63 patients with advanced solid tumors described a two-compartment model with a terminal elimination half-life of approximately 12 to 18 hours, a central volume of distribution of approximately 30 liters, and total clearance of approximately 30 to 40 liters per hour, though substantial interpatient variability was observed (coefficient of variation exceeding 50 percent for several parameters) [31].

5.2 Distribution

17-DMAG distributes widely to tissues after systemic administration. Tissue distribution studies in CD2F1 mice and Fischer 344 rats demonstrated rapid distribution to lung, liver, kidney, spleen, heart, and tumor tissue, with tumor-to-plasma ratios exceeding 3:1 in xenograft-bearing animals [8]. The volume of distribution in humans is approximately 100 to 200 liters (approximately 1.5 to 3 liters per kilogram), consistent with extensive tissue partitioning.

Plasma protein binding of 17-DMAG is approximately 35 percent, substantially lower than tanespimycin (greater than 90 percent) and geldanamycin (greater than 95 percent) [3]. The lower plasma protein binding results in a higher free fraction available for tissue distribution and target engagement, which is pharmacokinetically favorable for an agent whose target (HSP90) is intracellular.

5.3 Metabolism

17-DMAG is metabolized predominantly in the liver but undergoes quantitatively less extensive metabolism than tanespimycin. The principal metabolic pathways include oxidative N-demethylation of the dimethylaminoethyl side chain, hydroxylation at multiple positions on the ansa bridge, and Phase II conjugation (glucuronidation and sulfation) of hydroxylated metabolites [8]. Unlike tanespimycin, 17-DMAG is not a significant substrate for NAD(P)H:quinone oxidoreductase 1 (NQO1), the enzyme that reduces the benzoquinone to the hydroquinone form (17-AAGH2 from 17-AAG). The absence of NQO1 dependence eliminates a source of pharmacogenomic variability that complicated tanespimycin dosing.

Cytochrome P450 involvement in 17-DMAG metabolism has not been characterized as extensively as for tanespimycin, but the compound is not considered a strong CYP3A4 substrate, consistent with its improved metabolic stability.

5.4 Elimination

Elimination of 17-DMAG is predominantly hepatobiliary. In Fischer 344 rats, biliary excretion of 17-DMAG and metabolites accounted for approximately 4.7 percent of the delivered dose over 24 hours, with parent compound representing approximately 50.7 percent of the biliary material [8]. Urinary excretion is minor, with less than 5 percent of an administered dose recovered in urine as parent compound or metabolites.

The terminal elimination half-life in humans is approximately 12 to 18 hours after intravenous administration, supporting both once-weekly and twice-weekly dosing schedules. Plasma concentrations at the 24 mg/m² twice-weekly dose in the CLL/SLL trial reached a median C_{max} of approximately 600 nanomolar, decreasing to approximately 100 nanomolar within 4 hours of administration [16].

5.5 Routes of administration

Two principal routes have been used in clinical development: intravenous infusion (over 1 to 2 hours in normal saline or 5 percent dextrose) and intravenous bolus. Oral administration was evaluated in preclinical species and demonstrated feasibility, but oral formulations were not advanced to human clinical trials before the program was discontinued. Research applications employ intravenous, intraperitoneal, and oral routes in animal models.

6. Preclinical Pharmacology

6.1 In vitro antitumor activity

17-DMAG was evaluated in the NCI 60-cell-line panel, producing growth inhibition across all tumor types with a mean GI50 of approximately 53 nanomolar [9]. Activity was broadly distributed across leukemia, melanoma, non-small cell lung cancer, colon cancer, central nervous system cancer, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines. In direct comparative studies, 17-DMAG demonstrated equal or greater potency relative to tanespimycin (17-AAG) and geldanamycin across the majority of cell lines tested.

In CLL cells, 17-DMAG induced apoptosis through degradation of IKK-alpha, IKK-beta, and AKT, leading to suppression of constitutive NF-kappaB activity and down-regulation of the anti-apoptotic proteins Mcl-1, XIAP, and Bcl-xL [30]. The compound demonstrated greater cytotoxicity and more complete client protein degradation in CLL cells relative to tanespimycin, providing the preclinical rationale for the Phase I CLL/SLL trial.

In gastric cancer cells, 17-DMAG exerted anticancer effects principally through alteration of the cellular oxidant-antioxidant balance, inducing reactive oxygen species generation and depleting glutathione, in addition to the canonical HSP90 client protein degradation mechanism [23].

6.2 In vivo antitumor activity

17-DMAG demonstrated antitumor efficacy in multiple xenograft models. Hollingshead et al. (2005) reported high activity in two lung cancer xenografts and two of four melanoma xenografts in the Freiburg human tumor xenograft panel, with tumor regression at oral doses of 75 mg/kg administered on a 5-day-on/2-day-off schedule [9]. The compound also produced significant tumor growth inhibition in orthotopic and subcutaneous models of pancreatic cancer when administered orally.

In the Pediatric Preclinical Testing Program, Smith et al. (2008) evaluated alvespimycin across 28 solid tumor xenografts representing the major pediatric malignancy types. The compound demonstrated intermediate or high activity against 4 of 28 evaluable xenografts, including 3 of 4 alveolar rhabdomyosarcoma xenografts (one with a partial response), with pharmacodynamic evidence of HSP70 induction and client protein degradation in tumor tissues [11].

6.3 Neuroprotective activity

Yenari and colleagues (2014) demonstrated that 17-DMAG significantly reduced cerebral infarct volume and improved neurological outcomes in a mouse model of transient focal cerebral ischemia (middle cerebral artery occlusion). The neuroprotective mechanism involved suppression of microglial activation, decreased phosphorylation of IkappaB kinase, reduced nuclear translocation of NF-kappaB p65, and downregulation of NF-kappaB-regulated pro-inflammatory genes [17].

Li et al. (2023) extended the neuroprotective characterization to intracerebral hemorrhage (ICH) in rats. 17-DMAG administration decreased neutrophil infiltration into brain tissues, reduced production of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha), and attenuated blood-brain barrier disruption. Mass spectrometry and molecular docking analysis identified SOX5 (sex-determining region Y-box protein 5) as a direct binding target of 17-DMAG, with SOX5 acting as a positive regulator of the PI3K/Akt signaling pathway to mediate the neuroprotective effects [18].

6.4 Preclinical toxicology

Glaze et al. (2005) conducted formal preclinical toxicology studies of 17-DMAG in Sprague-Dawley rats and beagle dogs [21]. In rats, dose-limiting toxicities included gastrointestinal toxicity (diarrhea, mucosal erosion) and bone marrow suppression (leukopenia, thrombocytopenia). In dogs, dose-limiting toxicities included gastrointestinal toxicity, renal toxicity, gallbladder toxicity (mucosal necrosis), and bone marrow suppression. Hepatic enzyme elevation was observed in both species but was generally reversible. The no-observed-adverse-effect level in rats was approximately 2 mg/kg/day on a 5-day schedule; in dogs it was approximately 0.5 mg/kg/day. These findings informed the starting dose selection and dose-escalation strategies for the Phase I clinical trials.

7. Clinical Evidence Base

7.1 Phase I: weekly intravenous schedule in advanced solid tumors

Pacey et al. (2011) reported the results of a Phase I dose-escalation study of alvespimycin administered as a weekly one-hour intravenous infusion to patients with advanced, refractory solid tumors [12]. Twenty-five patients were enrolled at dose levels ranging from 2.5 to 106 mg/m². At 106 mg/m², 2 of 4 patients experienced dose-limiting toxicity (DLT), including one treatment-related death attributed to hepatic and multiorgan failure. No DLT occurred at 80 mg/m². The maximum tolerated dose was therefore established at 80 mg/m² weekly.

Common adverse events were predominantly low grade and included nausea, vomiting, fatigue, and hepatic transaminase elevation. Ocular adverse events were observed in 4 patients at doses at or above 80 mg/m² and comprised blurred vision (3 events), dry eye (3 events), keratitis (2 events), and conjunctivitis or ocular surface disease (2 events); all were grade 2 or lower and reversible. Prophylactic lubricating eye drops were recommended at doses of 80 mg/m² or greater.

Clinical activity included one complete response in castration-refractory prostate cancer (sustained for 124 weeks), one partial response in melanoma (sustained for 159 weeks), and stable disease in chondrosarcoma, castration-refractory prostate cancer, and renal cell cancer (sustained for 28, 59, and 76 weeks, respectively). Pharmacodynamic assessment demonstrated dose-dependent induction of HSP70 in peripheral blood mononuclear cells, confirming target engagement at the recommended dose.

7.2 Phase I: twice-weekly intravenous schedule in advanced malignancies

Kummar et al. (2010) reported a Phase I trial of 17-DMAG administered twice weekly as a one-hour intravenous infusion on days 1 and 4 of each week in patients with advanced malignancies [13]. The recommended Phase 2 dose on this schedule was 24 mg/m² twice weekly. Common adverse events included nausea, vomiting,

fatigue, anorexia, and hepatic enzyme disturbances. Pharmacokinetics were linear, and pharmacodynamic data demonstrated HSP70 induction at the recommended dose.

7.3 Phase I: acute myeloid leukemia

Lancet et al. (2010) reported a Phase I study of alvespimycin administered intravenously twice weekly to patients with relapsed or refractory AML [14]. The compound was evaluated both as a single agent and in combination with cytarabine. The recommended Phase 2 dose as a single agent was 24 mg/m² twice weekly. The study demonstrated tolerability, linear pharmacokinetics, and target inhibition as measured by HSP70 induction and client protein modulation. Signs of clinical activity were observed, including complete responses in combination with cytarabine. At the 2006 Annual Meeting of the American Society of Hematology, it was reported that 17-DMAG in combination with chemotherapy resulted in complete responses in 3 of 17 patients with refractory AML.

7.4 Phase I: combination with trastuzumab in HER2-positive solid tumors

Modi et al. (2013) reported a Phase I dose-escalation trial of trastuzumab and alvespimycin in 28 patients with advanced solid tumors (25 breast cancer, 3 ovarian cancer) [15]. Patients received standard weekly trastuzumab followed by intravenous alvespimycin at 60, 80, or 100 mg/m² weekly. Dose-limiting toxicities were grade 3 left ventricular systolic dysfunction presenting as congestive heart failure in one patient at 100 mg/m², and reversible grade 3 keratitis in two patients at 80 mg/m². The recommended Phase 2 dose was 80 mg/m² weekly in combination with trastuzumab. Antitumor activity was observed in patients with refractory HER2-positive metastatic breast cancer and ovarian cancer.

The scientific rationale for the combination was that HER2 is a well-characterized HSP90 client protein: inhibition of HSP90 degrades HER2 and sensitizes HER2-dependent tumor cells to trastuzumab-mediated antibody-dependent cellular cytotoxicity. The clinical evidence of activity in trastuzumab-refractory disease supported this mechanism.

7.5 Phase I: chronic lymphocytic leukemia and small lymphocytic lymphoma

A Phase I trial (NCT01126502) evaluated alvespimycin in patients with relapsed CLL/SLL after at least one prior nucleoside-analogue-containing regimen [16]. Patients received alvespimycin intravenously twice weekly at escalating doses up to 24 mg/m². No dose-limiting toxicities were observed, suggesting the MTD was not reached in this study. No objective responses were observed at the doses studied, though pharmacokinetic data demonstrated median C_{max} of approximately 600 nanomolar at the 24 mg/m² dose, decreasing rapidly to approximately 100 nanomolar within 4 hours.

7.6 Overall clinical assessment

The clinical development of alvespimycin demonstrated reproducible target engagement (HSP70 induction), evidence of client protein modulation (HER2, AKT degradation), and isolated but durable clinical responses in selected patients with advanced malignancies. The principal limitation was the toxicity profile, particularly hepatic, ocular, and gastrointestinal adverse events, which restricted the therapeutic window and prevented dose escalation to exposures that might have produced more consistent clinical responses. The decision to discontinue development in 2008 reflected a judgment that the benefit-risk ratio was unfavorable for continued investment relative to emerging second-generation and third-generation HSP90 inhibitors with potentially improved therapeutic indices.

8. Sourcing and Quality Verification

17-DMAG hydrochloride is supplied by multiple research chemical suppliers (MedChemExpress, Selleck Chemicals, Cayman Chemical, Santa Cruz Biotechnology, ApexBio, StressMarq Biosciences, Tocris, BPS Bioscience, Focus Biomolecules, and others) at purity of greater than 95 percent (typically greater than 98 percent) by HPLC. Supply is in milligram to gram quantities as the hydrochloride salt. The free base is available from some suppliers but less commonly. Pricing is moderate, in the range of \$50 to \$300 per 5 milligrams at research-grade quality at the time of monograph preparation, reflecting the semi-synthetic supply chain dependent on geldanamycin fermentation.

Pharmaceutical-grade alvespimycin (the clinical trial material manufactured by Kosan Biosciences under cGMP conditions) is no longer commercially available, as the clinical program has been discontinued. Research applications rely on chemical supplier material exclusively.

Investigators should confirm compound identity and purity on every research-grade lot through:

- Reversed-phase high-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- Mass spectrometry confirmation of molecular weight (617.35 plus or minus 0.02 for the protonated free base $[M+H]^+$)
- Proton nuclear magnetic resonance spectroscopy for structural confirmation, with particular attention to the C-17 amine region and the benzoquinone protons
- Karl Fischer or loss-on-drying analysis for residual water content
- Residual solvent analysis (gas chromatography for common synthesis solvents: dichloromethane, chloroform, methanol, ethyl acetate)
- UV-Vis absorbance spectrum confirmation (characteristic purple/violet coloration with absorbance maximum at approximately 330 nanometers)

Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, others) provide independent confirmation of vendor certificates of analysis. The compound's characteristic purple color provides a rapid visual identity check; colorless or off-

white preparations should be treated with suspicion.

9. Reconstitution and Handling

17-DMAG hydrochloride is freely soluble in water at room temperature (approximately 50 mg/mL). For in vitro research applications, the compound is typically dissolved in dimethyl sulfoxide (DMSO) to prepare concentrated stock solutions (10 to 100 millimolar) that are aliquoted and stored at -20 or -80 degrees Celsius. Aqueous stock solutions can be prepared in phosphate-buffered saline or cell culture medium at concentrations up to 50 mg/mL.

For in vivo research applications, the compound is dissolved directly in normal saline, 5 percent dextrose in water, or phosphate-buffered saline without the need for co-solvents or specialized formulation vehicles. This represents a significant practical advantage over tanespimycin, which requires Cremophor EL, egg-phospholipid emulsion (Bind formulation), or DMSO-based vehicles for parenteral administration.

Storage of the solid compound should be at -20 degrees Celsius, protected from light and moisture. The compound is light-sensitive owing to the benzoquinone chromophore and should be handled under reduced lighting or amber glass during prolonged manipulations. Aqueous solutions should be used within 24 hours of preparation if stored at room temperature, or within 7 days if refrigerated at 2 to 8 degrees Celsius. DMSO stock solutions are stable at -20 degrees Celsius for at least 6 months when protected from light and moisture. Repeated freeze-thaw cycles (more than 3) should be avoided; single-use aliquots are recommended.

The compound is an irritant; direct skin and eye contact should be avoided. Standard personal protective equipment (gloves, laboratory coat, eye protection) should be used during handling. The compound has antitumor activity and should be treated as a hazardous pharmaceutical waste material in accordance with institutional guidelines.

10. Stack Interaction Considerations

17-DMAG interacts with several drug classes through its mechanism as an HSP90 inhibitor and through its pharmacokinetic properties. Investigators conducting research with 17-DMAG, especially in combination studies or in the context of other concurrent treatments, should consider:

Other HSP90 inhibitors: Concurrent use with other HSP90 inhibitors (tanespimycin, ganetespib, luminespib, onalespib, pimitespib) is pharmacologically redundant and expected to produce additive toxicity without meaningful pharmacological benefit. The combination is not recommended in research or clinical contexts.

Proteasome inhibitors: HSP90 inhibition degrades client proteins through the ubiquitin-proteasome pathway. Concurrent proteasome inhibitors (bortezomib, carfilzomib, ixazomib) may attenuate the client protein degradation component of the HSP90 inhibitor mechanism by blocking the downstream clearance pathway. However, the combination may produce synergistic cytotoxicity through accumulation of misfolded protein aggregates and activation of the unfolded protein response. The interaction is complex and context-dependent.

HER2-targeted agents: The combination of 17-DMAG with trastuzumab has been evaluated clinically and demonstrates feasibility and preliminary activity [15]. HER2 degradation by HSP90 inhibition may sensitize to antibody-dependent cellular cytotoxicity and reduce HER2-driven signaling resistance. Other HER2-targeted agents (pertuzumab, lapatinib, neratinib, trastuzumab deruxtecan) have not been formally evaluated in combination with 17-DMAG.

Hepatotoxic agents: Given the hepatic toxicity signal observed in clinical trials, concurrent administration with other hepatotoxic compounds (acetaminophen at supratherapeutic doses, methotrexate, azathioprine, isoniazid, and others) should be approached with caution. Liver function monitoring is recommended in combination studies.

CYP3A4 inhibitors and inducers: Although 17-DMAG is not a strong CYP3A4 substrate, some hepatic metabolism does occur through this pathway. Strong CYP3A4 inhibitors (ketoconazole, itraconazole, ritonavir, clarithromycin) may modestly in-

crease exposure, while strong inducers (rifampin, phenytoin, carbamazepine) may reduce it. The magnitude of these interactions has not been formally characterized in clinical drug-drug interaction studies.

Histone deacetylase (HDAC) inhibitors: Preclinical studies have demonstrated synergistic antitumor activity of 17-DMAG in combination with HDAC inhibitors (vorinostat, romidepsin) in mantle cell lymphoma and other hematologic malignancies, through concurrent disruption of HSP90 chaperone function and HDAC-mediated epigenetic regulation [24]. This combination represents a productive research direction but has not been advanced to human studies with alvespimycin specifically.

Cardiotoxic agents: The observation of grade 3 left ventricular systolic dysfunction in one patient in the trastuzumab combination trial warrants caution with concurrent cardiotoxic agents, particularly anthracyclines (doxorubicin, epirubicin) and other HER2-targeted therapies.

11. Adverse Events and Safety

11.1 Common adverse events (clinical)

The principal adverse events observed across Phase I clinical trials at the recommended Phase 2 doses were:

- Nausea (30 to 50 percent of patients, predominantly grade 1 to 2, manageable with standard antiemetics)
- Fatigue (25 to 40 percent, grade 1 to 2)
- Vomiting (15 to 25 percent, grade 1 to 2)
- Hepatic transaminase elevation (alanine aminotransferase and aspartate aminotransferase; 15 to 30 percent, predominantly grade 1 to 2, reversible on dose interruption or reduction)
- Anorexia (10 to 20 percent, grade 1 to 2)
- Diarrhea (10 to 15 percent, grade 1 to 2)
- Headache (5 to 10 percent, grade 1 to 2)

11.2 Ocular adverse events

Ocular toxicity was a distinctive and clinically significant adverse-event class for 17-DMAG and for the ansamycin HSP90 inhibitor class generally. In the weekly solid tumor trial, 4 patients experienced 10 ocular adverse events, comprising blurred vision (3 events), dry eye (3 events), keratitis (2 events), and conjunctivitis or ocular surface disease (2 events). Most (9 of 10) events occurred at doses of 80 mg/m² or greater; all were grade 2 or lower. Two patients required dose reduction [12]. In the trastuzumab combination trial, reversible grade 3 keratitis occurred in 2 patients at 80 mg/m² and was dose-limiting [15].

The mechanism of ocular toxicity is incompletely characterized but is hypothesized to involve HSP90 inhibition in the retinal pigment epithelium and corneal epithelium, where HSP90 client proteins are important for cellular integrity and stress response. Pimipib, a more recent HSP90 inhibitor, has been specifically

characterized for its tumor-to-retina distribution ratio (5.7, compared to 0.7 to 2.3 for alvespimycin and other earlier-generation inhibitors), suggesting that retinal accumulation may contribute to the ocular signal [32].

Prophylactic lubricating eye drops and regular ophthalmologic assessment are recommended in research protocols employing 17-DMAG at doses of 80 mg/m² or greater.

11.3 Hepatotoxicity

Hepatic transaminase elevation is a class effect of the ansamycin HSP90 inhibitors, related to the benzoquinone moiety that was responsible for the severe hepatotoxicity of the parent compound geldanamycin. 17-DMAG demonstrates reduced hepatotoxicity relative to geldanamycin owing to the C-17 amino substitution, but the hepatic signal persists and was dose-limiting at 106 mg/m² in the weekly solid tumor trial, where one treatment-related death included hepatic failure as a contributing factor [12].

11.4 Dose-limiting toxicities

Dose-limiting toxicities across the clinical development program included:

- Hepatic transaminase elevation (grade 3 or higher) at 106 mg/m² weekly
- Treatment-related death (hepatic and multiorgan failure) at 106 mg/m² weekly
- Left ventricular systolic dysfunction (grade 3, congestive heart failure) at 100 mg/m² weekly in combination with trastuzumab
- Keratitis (grade 3, reversible) at 80 mg/m² weekly in combination with trastuzumab

11.5 Preclinical toxicity

In preclinical toxicology studies, dose-limiting toxicities in rats included gastrointestinal mucosal erosion and bone marrow suppression, while in dogs they included gastrointestinal toxicity, renal toxicity, gallbladder mucosal necrosis, and bone marrow suppression [21]. Hepatic enzyme elevation was observed in both species. The preclinical toxicity profile accurately predicted the clinical hepatic and gastrointestinal adverse-event signals.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five HSP90 inhibitor candidates against 17-DMAG, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected to represent the principal structural classes and clinical development stages of the HSP90 inhibitor field, from first-generation ansamycins through second- and third-generation synthetic inhibitors.

12.1 Tanespimycin (17-AAG, KOS-953)

Mechanism: First-generation ansamycin HSP90 N-terminal inhibitor. Binds the same ATP pocket as 17-DMAG with comparable affinity (IC₅₀ approximately 5 to 20 nanomolar in cell-free assays). Requires metabolic activation by NQO1 to the hydroquinone form (17-AAGH₂) for optimal activity in some cellular contexts [20].

Development trajectory: The first HSP90 inhibitor to enter human clinical trials (1999). Developed by the NCI and subsequently by Kosan Biosciences (as KOS-953) and by Bristol-Myers Squibb. Phase I and Phase II trials in multiple solid tumor and hematologic malignancy indications. Phase II trial in HER2-positive metastatic breast cancer in combination with trastuzumab (Modi et al., 2007) demonstrated objective responses [33]. The compound received orphan drug designation for multiple myeloma. Clinical development was discontinued owing to formulation challenges (Cremophor EL-related infusion reactions), hepatotoxicity, and the emergence of more pharmacokinetically favorable candidates including 17-DMAG.

Novelty: Low. The compound is the prototypical ansamycin HSP90 inhibitor and the historical reference compound for the class.

Effect size: Moderate. Phase II data demonstrated objective response rates in HER2-positive breast cancer (in combination with trastuzumab) and in multiple myeloma (in combination with bortezomib), but responses were not sufficient for registration as a single agent.

Promising potential: Low. Clinical development has been discontinued. The compound persists as a widely used research tool.

Side-effect profile: Hepatotoxicity (class effect), infusion reactions related to the Cremophor EL formulation vehicle, gastrointestinal adverse events. The formulation-related toxicity was a principal limitation that 17-DMAG was specifically designed to address.

Overall validation: Moderate. The compound established proof-of-concept for HSP90 inhibition in oncology and generated a substantial clinical and research literature. Limitations in formulation, metabolism, and therapeutic index prevented registration.

Comparison to 17-DMAG: 17-DMAG represents a direct pharmacokinetic improvement over tanespimycin: better aqueous solubility (no Cremophor required), lower plasma protein binding, higher oral bioavailability, and reduced NQO1 metabolic dependence. Antitumor potency is comparable or slightly superior. The toxicity profile of 17-DMAG, while improved relative to geldanamycin, was ultimately judged similarly unfavorable for further clinical development.

12.2 Ganetespib (STA-9090)

Mechanism: Second-generation resorcinol-based HSP90 inhibitor, structurally unrelated to the ansamycins. Binds the N-terminal ATP pocket with high affinity (IC₅₀ approximately 4 to 15 nanomolar). Smaller molecular weight, greater lipophilicity, and longer duration of HSP90 inhibition relative to the ansamycin class [34].

Development trajectory: Developed by Synta Pharmaceuticals (later Madrigal Pharmaceuticals). Advanced through extensive Phase I, Phase II, and Phase III clinical trials. The Phase III GALAXY-2 trial evaluated ganetespib plus docetaxel versus docetaxel alone in advanced non-small cell lung cancer (NSCLC); the trial did not meet its primary endpoint of improved overall survival, and clinical development was subsequently discontinued [35].

Novelty: Moderate to high. The resorcinol chemotype represents a structural departure from the ansamycins and offers improved pharmaceutical properties.

Effect size: Phase II data demonstrated objective responses in ALK-rearranged NSCLC (as a single agent) and preliminary activity in other solid tumors. The Phase III GALAXY-2 trial was negative.

Promising potential: Low. Clinical development has been discontinued following the negative Phase III trial.

Side-effect profile: Gastrointestinal adverse events (diarrhea, nausea, vomiting) were the most common toxicities. Hepatotoxicity was less pronounced than with the ansamycin class. Ocular toxicity was observed but at lower rates than with 17-DMAG. Cardiac toxicity was not prominent. The overall tolerability profile was judged superior to the ansamycin class.

Overall validation: Moderate to high. The compound advanced furthest in clinical development among all HSP90 inhibitors (Phase III), generated the largest clinical dataset, and demonstrated the most mature clinical pharmacology. The negative Phase III outcome has significantly dampened enthusiasm for single-agent HSP90 inhibition in solid tumors.

Comparison to 17-DMAG: Ganetespib represents a structural and pharmacological advance over 17-DMAG, with improved tolerability, a non-ansamycin chemotype that avoids the benzoquinone-associated hepatotoxicity, and more extensive clinical data. 17-DMAG has the advantage of prior characterization in hematologic malignancies (AML, CLL) and in neuroprotection research; ganetespib has been principally evaluated in solid tumors.

12.3 Luminespib (AUY922, NVP-AUY922)

Mechanism: Second-generation isoxazole-resorcinol HSP90 inhibitor. Binds the N-terminal ATP pocket with very high affinity (K_d approximately 1.7 nanomolar). Developed through structure-based drug design using the HSP90 N-terminal crystal structure [36].

Development trajectory: Developed by Novartis. Advanced through Phase I and Phase II clinical trials in multiple solid tumor indications including NSCLC (ALK-rearranged and EGFR-mutant), breast cancer, gastric cancer, and gastrointestinal stromal tumors. Phase II results demonstrated objective responses in ALK-rearranged NSCLC and in HER2-positive breast cancer but did not support advancement to Phase III.

Novelty: Moderate. The isoxazole-resorcinol chemotype is structurally distinct from both the ansamycins and the triazolone ganetespib scaffold.

Effect size: Phase II objective response rates of approximately 20 to 30 percent in ALK-rearranged NSCLC and modest activity in other indications.

Promising potential: Low. Clinical development has been deprioritized. The compound is available for research use.

Side-effect profile: The principal dose-limiting toxicity was ocular: night blindness, visual disturbance, and photopsia were prominent and attributed to inhibition of HSP90 client proteins in retinal photoreceptors. Gastrointestinal adverse events and hyperglycemia were also observed [37]. The ocular toxicity profile was more prominent than with 17-DMAG.

Overall validation: Moderate. Extensive Phase I/II data with pharmacodynamic biomarker validation. The ocular toxicity has been particularly informative for understanding the retinal pharmacology of HSP90 inhibition.

Comparison to 17-DMAG: Luminespib has higher binding affinity and greater potency than 17-DMAG in cell-free assays but shares the limitation of ocular toxicity, which is more pronounced. 17-DMAG has the advantage of a broader research literature in non-oncologic applications and in hematologic malignancies.

12.4 Onalespib (AT13387)

Mechanism: Second-generation resorcinol-based HSP90 inhibitor with a long duration of target binding (sustained HSP90 inhibition for greater than 72 hours after a single dose in preclinical models) [38].

Development trajectory: Developed by Astex Pharmaceuticals (now Otsuka). Advanced through Phase I and Phase II clinical trials in NSCLC (in combination with crizotinib for ALK-rearranged disease), gastrointestinal stromal tumors (in combination with imatinib), and other solid tumors. Phase II trials have not produced registration-quality efficacy data, and the compound is not in active late-stage development.

Novelty: Moderate. The compound is distinguished from ganetespib principally by its binding kinetics (very slow off-rate) rather than by its binding site or mechanism.

Effect size: Phase I and Phase II data demonstrated pharmacodynamic activity and preliminary clinical responses in combination regimens.

Promising potential: Low to moderate. The compound remains in early clinical investigation for selected combination applications.

Side-effect profile: Generally well tolerated. The principal adverse events were gastrointestinal (diarrhea, nausea), fatigue, and hepatic enzyme elevation. Ocular toxicity was less prominent than with luminespib or the ansamycins. The compound was not predicted to inhibit off-target kinases in computational analysis, suggesting a relatively clean pharmacological profile [39].

Overall validation: Low to moderate. The clinical dataset is smaller than for ganetespib or luminespib. The compound is of interest principally for its favorable binding kinetics.

Comparison to 17-DMAG: Onalespib offers potential advantages in tolerability and duration of target engagement. 17-DMAG has the advantage of a more extensive published research literature and characterization in non-oncologic applications.

12.5 Pimitespib (TAS-116)

Mechanism: Third-generation oral HSP90-alpha/beta inhibitor developed through structure-based design. Distinguished from earlier-generation inhibitors by isoform selectivity (preferential inhibition of cytoplasmic HSP90-alpha and HSP90-beta over the endoplasmic reticulum paralog GRP94 and the mitochondrial paralog TRAP1) and by oral bioavailability optimized for chronic dosing [32].

Development trajectory: Developed by Taiho Pharmaceutical. The compound received regulatory approval in Japan in June 2022 for the treatment of gastrointestinal stromal tumors (GIST) that have progressed after chemotherapy, becoming the first and only HSP90 inhibitor to achieve regulatory approval in any jurisdiction. The approval was based on a Phase III randomized controlled trial demonstrating significantly improved progression-free survival compared to placebo [40].

Novelty: High. The compound represents the most advanced iteration of HSP90 inhibitor design, with isoform selectivity and oral administration that distinguish it from all earlier candidates.

Effect size: Phase III data in GIST demonstrated a statistically significant improvement in progression-free survival with a hazard ratio of 0.41, a clinically meaningful effect size in a refractory population.

Promising potential: High. The compound is the only approved HSP90 inhibitor globally. Expansion studies in other tumor types are ongoing.

Side-effect profile: Diarrhea and decreased appetite were the most common adverse events. Importantly, pimitespib demonstrates a substantially improved tumor-to-retina concentration ratio (5.7) compared to alvespimycin and other earlier-generation inhibitors (0.7 to 2.3), which translates to a lower rate of ocular adverse events [32]. Hepatotoxicity was less pronounced than with the ansamycin class.

Overall validation: High. The compound is the only HSP90 inhibitor to achieve regulatory approval and represents the clinical validation of the HSP90 target that the earlier candidates (including 17-DMAG) failed to deliver.

Comparison to 17-DMAG: Pimitespib represents a generational advance over 17-DMAG in virtually all pharmacological dimensions: isoform selectivity, oral bioavailability, tolerability, therapeutic index, and clinical outcome. 17-DMAG retains value as a research tool compound and as a historical reference for the ansamycin class, and its characterization in neuroprotection and hematologic malignancies represents research territory that pimitespib has not entered. The regulatory approval of pimitespib in 2022 validates the HSP90 target and provides retrospective context for the earlier clinical failures including alvespimycin.

12.6 Comparator summary

The five comparators span the full arc of HSP90 inhibitor clinical development. **Tanespimycin** is the first-generation prototype with formulation and metabolic limitations that 17-DMAG was designed to overcome. **Ganetespib** advanced furthest in clinical trials (Phase III) but failed to demonstrate survival benefit in NSCLC. **Luminespib** demonstrated high potency but was limited by ocular toxicity. **Onalespib** offers favorable binding kinetics but limited clinical data. **Pimitespib** is the only approved HSP90 inhibitor, validating the target and demonstrating that the therapeutic index can be achieved with appropriate isoform selectivity and pharmacokinetic optimization.

17-DMAG occupies a transitional position in this landscape: it represented a genuine pharmacokinetic improvement over the first-generation tanespimycin but was overtaken by second- and third-generation synthetic inhibitors that offered better tolerability and ultimately (in the case of pimitespib) clinical registration. The compound retains value as a widely available, well-characterized research tool for

HSP90 biology, as a reference compound for the ansamycin class, and as an agent of interest in non-oncologic applications (neuroinflammation, stroke, hemorrhage) where the newer HSP90 inhibitors have not been extensively characterized.

13. References

1. DeBoer C, Meulman PA, Wnuk RJ, Peterson DH. Geldanamycin, a new antibiotic. *J Antibiot (Tokyo)*. 1970;23(9):442-447.
2. Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. 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*. 1994;91(18):8324-8328.
3. Egorin MJ, Lagattuta TF, Hamburger DR, et al. Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. *Cancer Chemother Pharmacol*. 2002;49(1):7-19.
4. Schnur RC, Corman ML, Gallaschun RJ, et al. Inhibition of the oncogene product p185erbB-2 in vitro and in vivo by geldanamycin and dihydrogeldanamycin derivatives. *J Med Chem*. 1995;38(19):3806-3812.
5. Rascher A, Hu Z, Buchanan GO, Reid R, Hutchinson CR. Insights into the biosynthesis of the benzoquinone ansamycins geldanamycin and herbimycin, obtained by gene sequencing and disruption. *Appl Environ Microbiol*. 2005;71(8):4862-4871.
6. Alvespimycin (17-DMAG) product data. MedChemExpress. Accessed 2026.
7. Alvespimycin HCl (17-DMAG) product data. Selleck Chemicals. Accessed 2026.
8. Eiseman JL, Lan J, Lagattuta TF, et al. Pharmacokinetics and pharmacodynamics of 17-demethoxy 17-[[2-(dimethylamino)ethyl]amino]geldanamycin (17DMAG, NSC 707545) in C.B-17 SCID mice bearing MDA-MB-231 human breast cancer xenografts. *Cancer Chemother Pharmacol*. 2005;55(1):21-32.
9. Hollingshead M, Alley M, Burger AM, et al. In vivo antitumor efficacy of 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride), a water-soluble geldanamycin derivative. *Cancer Chemother Pharmacol*. 2005;56(2):115-125.

10. Smith V, Sausville EA, Camalier RF, Fiebig HH, Burger AM. Comparison of 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17DMAG) and 17-allylamino-17-demethoxygeldanamycin (17AAG) in vitro: effects on Hsp90 and client proteins in melanoma models. *Cancer Chemother Pharmacol*. 2005;56(2):126-137.
11. Smith MA, Morton CL, Phelps DA, et al. Stage 1 testing and pharmacodynamic evaluation of the HSP90 inhibitor alvespimycin (17-DMAG, KOS-1022) by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2008;51(1):34-41.
12. Pacey S, Gore M, Chao D, et al. A phase I study of the heat shock protein 90 inhibitor alvespimycin (17-DMAG) given intravenously to patients with advanced solid tumors. *Clin Cancer Res*. 2011;17(6):1561-1570.
13. Kummar S, Gutierrez ME, Gardner ER, et al. Phase I trial of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), a heat shock protein inhibitor, administered twice weekly in patients with advanced malignancies. *Eur J Cancer*. 2010;46(2):340-347.
14. Lancet JE, Gojo I, Burton M, et al. Phase I study of the heat shock protein 90 inhibitor alvespimycin (KOS-1022, 17-DMAG) administered intravenously twice weekly to patients with acute myeloid leukemia. *Leukemia*. 2010;24(4):699-705.
15. Modi S, Stopeck A, Linden H, 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*. 2013;19(6):E15 (abstract); full publication: *Clin Cancer Res*. 2012;18(18):5090-5098.
16. Ramanathan RK, Egorin MJ, Erlichman C, et al. A phase I trial of the intravenous Hsp90 inhibitor alvespimycin (17-DMAG) in patients with relapsed chronic lymphocytic leukemia/small lymphocytic lymphoma. *Leuk Lymphoma*. 2016;57(10):2463-2466.
17. Kim N, Kim JY, Yenari MA. Anti-inflammatory properties and pharmacological induction of Hsp70 after brain injury. *Inflammopharmacology*. 2012;20(3):177-185. See also: 17-Dimethylaminoethylamino-17-demethoxygeldanamycin attenuates inflammatory responses in experimental stroke. *Mol Cell Neurosci*. 2015;64:59-71.
18. Li XQ, Chen Y, Dai GC, et al. 17-DMAG ameliorates neuroinflammation and BBB disruption via SOX5 mediated PI3K/Akt pathway after intracerebral hemorrhage in rats. *Int Immunopharmacol*. 2023;122:110644.

19. Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol.* 1995;36(4):305-315.
20. Schulte TW, Neckers LM. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother Pharmacol.* 1998;42(4):273-279.
21. Glaze ER, Lambert AL, Smith AC, et al. Preclinical toxicity of a geldanamycin analog, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), in rats and dogs: potential clinical relevance. *Cancer Chemother Pharmacol.* 2005;56(6):637-647.
22. Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? *Clin Cancer Res.* 2012;18(1):64-76.
23. Wu J, Liu T, Rios Z, et al. HSP90 inhibitor 17-DMAG exerts anticancer effects against gastric cancer cells principally by altering oxidant-antioxidant balance. *Oncotarget.* 2017;8(34):56473-56489.
24. Rao R, Fiskus W, Yang Y, et al. Co-treatment with heat shock protein 90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin (DMAG) and vorinostat: a highly active combination against human mantle cell lymphoma (MCL) cells. *Cancer Biol Ther.* 2009;8(13):1237-1245.
25. Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. *Nat Rev Cancer.* 2005;5(10):761-772.
26. Taipale M, Jarosz DF, Lindquist S. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat Rev Mol Cell Biol.* 2010;11(7):515-528.
27. Kamal A, Thao L, Sensintaffar J, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature.* 2003;425(6956):407-410.
28. Stebbins CE, Russo AA, Schneider C, et al. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell.* 1997;89(2):239-250.
29. Powers MV, Clarke PA, Workman P. Dual targeting of HSC70 and HSP72 inhibits HSP90 function and induces tumor-specific apoptosis. *Cancer Cell.* 2008;14(3):250-262.

30. Hertlein E, Wagner AJ, Jones J, et al. 17-DMAG targets the nuclear factor-kappaB family of proteins to induce apoptosis in chronic lymphocytic leukemia: clinical implications of HSP90 inhibition. *Blood*. 2010;116(1):45-53.
31. Zhao Y, Gupta A, Woo S, et al. Population pharmacokinetic analysis of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) in adult patients with solid tumors. *Cancer Chemother Pharmacol*. 2012;70(2):201-209.
32. Ohkubo S, Kodama Y, Muraoka H, et al. TAS-116, a highly selective inhibitor of heat shock protein 90alpha and beta, demonstrates potent antitumor activity and minimal ocular toxicity in preclinical models. *Mol Cancer Ther*. 2015;14(1):14-22.
33. Modi S, Stopeck AT, Gordon MS, et al. Combination of trastuzumab and tane-spimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer. *Clin Cancer Res*. 2011;17(15):5132-5139.
34. Ying W, Du Z, Sun L, et al. Ganetespib, a unique triazolone-containing Hsp90 inhibitor, exhibits potent antitumor activity and a superior safety profile for cancer therapy. *Mol Cancer Ther*. 2012;11(2):475-484.
35. Ramalingam S, Goss G, Rosell R, et al. A randomized phase II study of ganetespib, a heat shock protein 90 inhibitor, in combination with docetaxel in second-line therapy of advanced non-small cell lung cancer (GALAXY-1). *Ann Oncol*. 2015;26(8):1741-1748.
36. Eccles SA, Massey A, Raynaud FI, et al. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Res*. 2008;68(8):2850-2860.
37. Sessa C, Shapiro GI, Bhalla KN, et al. First-in-human phase I dose-escalation study of the HSP90 inhibitor AUY922 in patients with advanced solid tumors. *Clin Cancer Res*. 2013;19(13):3671-3680.
38. Woodhead AJ, Angove H, Sherborne B, et al. Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-dihydroisoindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone Hsp90 by fragment based drug design. *J Med Chem*. 2010;53(16):5956-5969.
39. Klaeger S, Heinzlmeir S, Wilhelm M, et al. The target landscape of clinical kinase drugs. *Science*. 2017;358(6367):eaan4368.

40. Doi T, Kurokawa Y, Sawaki A, et al. Efficacy and safety of TAS-116, an oral inhibitor of heat shock protein 90, in patients with metastatic or unresectable gastrointestinal stromal tumour refractory to imatinib, sunitinib and regorafenib: a phase II, single-arm trial. *Eur J Cancer*. 2019;121:29-37.
-

This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. Compound class generalizations are noted where compound-specific data are sparse; investigators should weight the evidence accordingly. Alvespimycin (17-DMAG) is not approved by any regulatory authority for any therapeutic indication. Clinical development was discontinued in 2008. The compound is available as a research-grade preparation from multiple chemical suppliers; investigators should obtain analytical confirmation of identity and purity on every lot.