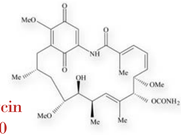
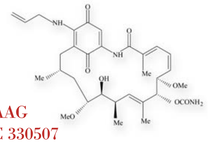
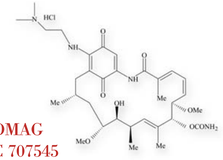
Geldanamycin Analogs



Geldanamycin
NSC 122750



17-AAG
NSC 330507



17-DMAG
NSC 707545

SUCCESS STORY

NSC 330507.....17-AAG RECEIVED 1980.....DN2A APRIL 1992.....DN2B OCTOBER 1997.....CLINICAL TRIAL APRIL 1999

NSC 707545.....17-DMAG RECEIVED OCTOBER 1998.....DN1B 2001.....DN2B 2002.....CLINICAL TRIAL 2004

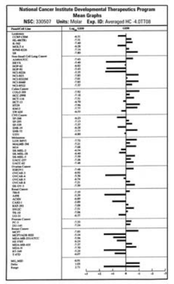
17-AAG (NSC 330507)

Background

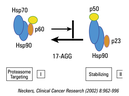
- The parent compound, geldanamycin (NSC 122750), was first isolated as the fermentation product of *Streptomyces hygroscopicus*.
- This general class of benzoquinone ansamycins first became of interest in the 1980s as potential tyrosine kinase inhibitors.
- Inhibition is through heat shock protein (HSP) 90 chaperon function.
- Geldanamycin proved to be a poor candidate for clinical trials due to liver toxicity and instability in biological fluids.
- 17-AAG was selected for development as a less toxic alternative to the parent compound.

In Vitro Studies

- Mechanism of anticancer activity was elucidated with contributions from Dr. Len Neckers, CCR, NCI; Dr. Luke Whitesell, University of Arizona, and Dr. Pavletich, Memorial Sloan-Kettering Cancer Center; and their respective co-workers.
- 17-AAG exhibits a multilog differential pattern of activity in the DTP 60 cell line screen at an average growth inhibitory potency of 0.123 uM.

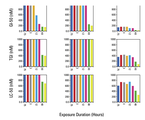


17-AAG Destabilizes HSP90-Client Protein Complex



Neckers, Clinical Cancer Research (2002) 8:962-896

Time Course Assay



- Time course assays against sensitive cell lines showed that brief exposures (< 1 hr) at a drug concentration of less than 1 μM were sufficient to cause growth inhibition.

In Vivo Studies

Hollow Fiber (HF) Assay

- 17-AAG was active in the HF assay when given i.p. at a dose of 50 mg/kg/day for 4 days. IP score 28 + SC score 10 for a total score of 38.

Xenograft Studies

- Agent was highly active in a LOX melanoma model, yielding 5 out of 6 tumor-free mice at 33.5 mg/kg/dose given for 5 days.
- Other tumor models include MDA-MB-231 (breast), NCI-H522 (non-small cell lung), and PC-3 (prostate). No activity was observed in these models.

Pharmacokinetic (PK) Studies

Murine Studies

- Mice bearing MCF-7 breast tumors were treated with 17-AAG at various dose levels.
- Modulation of HSP90 was measurable in the tumor tissue.
- Maximum half-life in plasma was 4.4 hours after i.v. administration of 40 mg/kg.
- Maximum bioavailability was 99% using i.p. dosing.
- 17-AAG was not detectable following oral administration.

Dog Studies

- Half-life was found to be between 60 and 90 minutes after i.v. dosing.

Toxicology Studies

Rat Studies

- Doses in range-finding studies ranged between 5 and 25 mg/kg/day given as an i.v. bolus.
- Maximum tolerated dose (MTD) was 25 mg/kg/day given i.v.
- Dose-limiting toxicities (DLT) were gastrointestinal and hepatic.

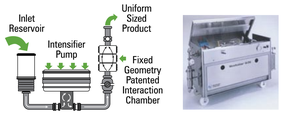
Dog Studies

- Doses were 2–7.5 mg/kg/day given as a 1 hour infusion daily for 5 days.
- MTD was 100 mg/m^2/day.
- DLTs were gastrointestinal and gall bladder.
- Recommended phase I starting dose was 10mg/m^2/day (1/10 the MTD in dogs) given as a 1-hour infusion daily for 5 days.

Bulk Production and Formulation

- Geldanamycin is produced using a 3,000-gallon fermentor at the SAIC facility in Frederick, Maryland.
- Isolation of pure geldanamycin has produced approximately 1.2 kg of product.
  - If purchased commercially, 1.2 kg parent would cost more than $383 million.
- 17-AAG is synthesized from the parent geldanamycin.
- This compound possesses limited aqueous solubility (ca. 0.01 mg/mL) and precipitates upon dilution with aqueous liquids almost at any concentration. Therefore, preparing a therapeutically suitable formulation of 17-AAG has been a challenge.
- A reproducible, sterile, and stable nanodispersed lipid containing formulation suitable for i.v. administration has been developed.
- The drug concentration, volume of organic solvent, and EPL concentration are the parameters that play a significant role in the preparation of suitable formulation.
- Use of the Microfluidics® processing technology (a large manufacturing unit is depicted to the right) allowed us problem-free production of this product under the cGMP condition for clinical trials.

Microfluidizer® M-210 for Nano-Particulate Processing



Clinical Trials Experience

Phase I and II Trials Are Underway

17-DMAG (NSC 707545)

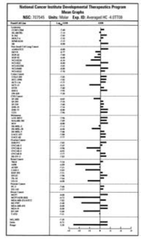
Background

Although less toxic than the parent, 17-AAG still poses challenges:
- Formulation is complicated and not well-received by patients (garlic-like odor).
- Pharmaceutical properties are not optimal.

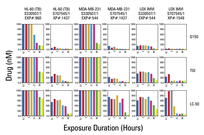
The search for a better analog therefore continued. 17-DMAG advantages over 17-AAG are as follows:
- Water soluble.
- More potent *in vitro* and *in vivo*.
- Less protein bound than 17-AAG.
- Its metabolism is less extensive.

In Vitro Advantages over 17-AAG

A COMPARE analysis indicates that the 60 cell line screen pattern of 17-DMAG most closely correlates with that of 17-AAG with a Pearson correlation co-efficient (PCC) of 0.783 (0.6 is considered significant).



In Vitro Time Course Assay



Comparative *in vitro* cytotoxicity profiles of 17-AAG and 17-DMAG in three sensitive tumor cell lines. The drug concentrations and times of exposure to achieve 50% growth inhibition (GI50), total growth inhibition (TGI), and 50% cell kill (LC50) are shown for each cell line. HL-60 (TB) promyelocytic leukemia, MDA-MB-231 (breast), and LOX IMVI (melanoma).

In Vivo Advantages over 17-AAG

HF Studies

- Although both agents are active in the assay when dosed i.p., only 17-DMAG is active when dosed orally (total score 6 for 17-AAG; total score 48 for 17-DMAG).

Xenograft Studies

- Although both 17-AAG and 17-DMAG exhibit activity in various tumor models, only 17-DMAG is active when given orally.
- In the AsPC-1 pancreatic tumor model, 17-DMAG significantly reduced the metastatic activity of the tumor, shown by decreased weight of the livers (organ of metastatic activity) at the end of the study.

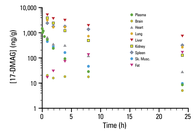
Bulk Production, Formulation, and Clinical Batch Production

- The clinical formulation consists of a freeze-dried powder containing 10 mg/vial, which can be readily reconstituted with water.
- A clinical batch of 4,000 vials was produced.
- Currently undergoing phase I evaluation as a single agent.

Pharmacokinetic (PK) and Pharmacodynamic (PD) Summary

- Plasma kinetics are linear with dose in mouse and rat.
- Agent is extensively distributed to well-perfused tissues.
- Clearance is similar across species on a mg/m^2 basis.
- Oral bioavailability: mouse/rat/dog = 50%/39%/37%.
- No major plasma or urinary metabolites, but many found in the bile.
- Human protein binding is 15%–25% vs. 70% for 17-AAG.
- Time-dependent modulation of PD markers in tissues.

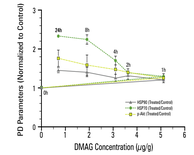
Concentrations of 17-DMAG in Plasma and Tissues of Rats Given 5 mg/kg i.v.



Toxicology Summary

- Dose-dependent, dose-limiting gastrointestinal and hepatic toxicity and bone marrow suppression were present by day 6 (dogs) and day 8 (rats).
- Evidence of renal toxicity was also observed in dogs.
- Adverse effects in surviving animals were minimal or absent by day 22.
- MTD was approximately 24 mg/m^2 in rats and 15 mg/m^2 in dogs.
- Highest non-toxic dose was 2.4 mg/m^2 in rats and < 8 mg/m^2 in dogs.
- A clinical starting dose of not more than 2.4 mg/m^2 is recommended.
- Dose response curve appears to be very steep; therefore, a slow dose escalation regimen is recommended.

Changes in PD Parameters With DMAG Concentrations In CDF Male Rat Liver After 5 mg/kg DMAG i.v.



An IND to Begin Clinical Trials Was Approved and Trials Began in May 2004

17-AAG and 17-DMAG Have Been Licensed to Kosan

Developmental Therapeutics Program of the National Cancer Institute

TURNING MOLECULES INTO medicines FOR THE public health