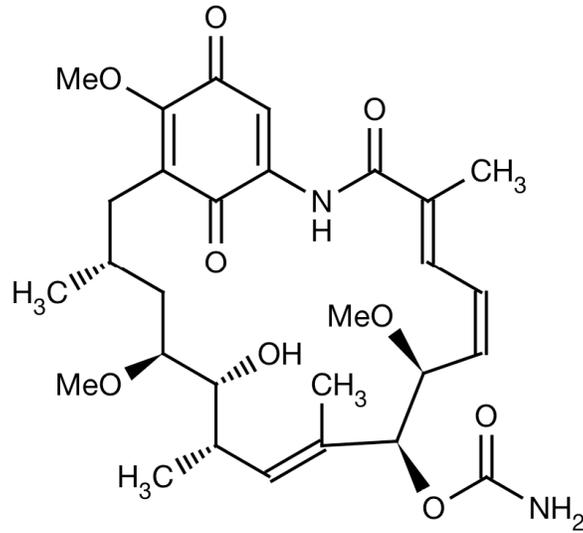


CINAPS Compound Dossier

Geldanamycin



3/29/2009

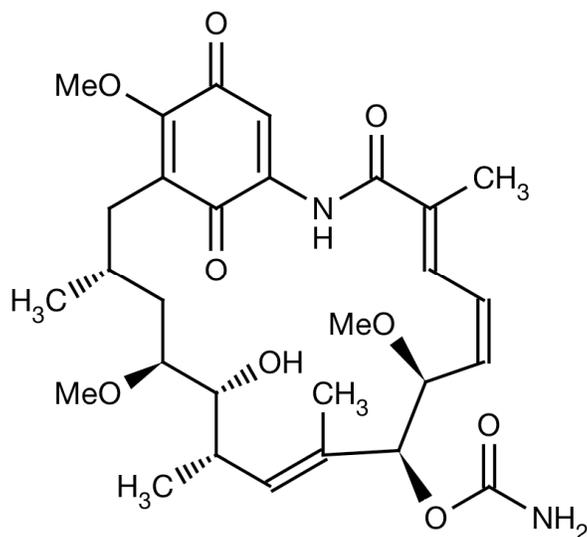
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I. Compound Information

Common name: Geldanamycin

Structure:



PubChem ID: 5288382

Mol. Formula: C₂₉H₄₀N₂O₉

FW: 560.64

CASRN: 30562-34-6

Polar surface area: 163.48

logP: 2.15

IUPAC name: (4E,6Z,8S,9S,10E,12S,13R,14S,16R)-13-Hydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate

Other names: –

Drug class: Antibiotic

Medicinal chemistry development potential: Moderate

II. Rationale

Ila. Scientific Rationale / Mechanism

Geldanamycin (GA) is a selective inhibitor of the heat shock protein (Hsp) Hsp90, which is constitutively expressed in tissues. GA binds at the N-terminal ATP/ADP binding domain of Hsp 90, locking it into its ADP-bound conformation and disrupting its interactions with proteins, including its multiple co-chaperone partners such as other Hsp's and p23 (**Batulan, 2006; Roe, 1999**). Mechanistically key, this primary event precipitates a cascade of biochemical sequelae that confer the protective effect of that drug in each of the models of disease states in the literature reviewed. Hsp 90 and its co-chaperones bind to proteins to (1) facilitate folding into their native conformations, (2) refold abnormally folded proteins, (3) rescue previously aggregated proteins, and (4) guide ubiquitination of proteins for subsequent recognition and digestion by the 26S proteasome (**Ouyang, 2005**). As such, induction of heat shock pathways can remediate damage associated with the progression of Parkinson disease, related diseases involving aberrant amyloidal scaffolds, and other damage caused by protein aggregation in neurons.

Complexation of the heat shock transcription factor Hsf1 with Hsp 90 keeps Hsf1 in an inactive state (**Batulan, 2006**). Hsf1 is the major transcription factor mediating upregulation of heat shock proteins in response to stress. Activation of Hsf1 is a multi-step process that requires its release from Hsp90, immunophilins, and p23. When Hsp's are diverted to chaperone misfolded proteins during stress, released Hsf1 translocates to the nucleus and binds as trimers to heat shock elements (HSE). This alone is not sufficient for activation of heat shock genes, and conversion to the active form, Hsf1act, requires further steps including hyperphosphorylation of Hsf1. Hsf1act induces expression of key Hsp's including Hsp90, Hsp70, and Hsp40, the latter two of which are strongly upregulated by that co-chaperone.

Hsp70 is inducible by stressors and by GA. Hsp70 requires the co-chaperone Hsp40, which is constitutively expressed, for optimal function (**Fan, 2003**). Hsp40 both mediates delivery and ATP-dependent polypeptide binding by Hsp70, and stabilizes Hsp70/polypeptide complex for efficient refolding (**Cheetham, 1998; Cyr, 1994**).

Expression of these heat shock chaperones is manifested in biological responses that can be generally categorized as the interrelated phenomena of protection against (1) protein aggregation/amyloid protein/ α -synuclein toxicity, (2) damage caused by ischemia/reperfusion injury/stroke/oxygen-glucose deprivation, (3) apoptotic pathways, and 4) damage to the dopaminergic neurons of the substantia nigra.

Protein aggregates, particularly those involving α -synuclein are commonly implicated in the etiology of Parkinson disease (PD). α -Synuclein is a major component of Lewey bodies and Lewey neurites, which are pathological protein aggregates characteristic of PD, and polymerizes to form β -sheet amyloids that comprise fibrils (**Auluck, 2002; Auluck, 2005**). Overexpression of Hsp70 protects neural cells from various stressors (**Latchman, 2004; Muchowski, 2005; Yenari, 2002**), and has a major role in processing α -synuclein. It binds and sequesters α -synuclein (**Flower, 2005**), and

II. Rationale (cont.)

shuttles the soluble, toxic, form of the protein into inclusion bodies. The co-chaperone Hdj-2 functions alone or in concert with Hsp70 to prevent protein aggregation, reducing, in astrocytes, both apoptosis and necrosis induced by glucose and/or oxygen deprivation (**Giffard, 2004**). High expression of Hsp's by GA treatment was also neuroprotective in a primary culture cell model of familial amyotrophic lateral sclerosis linked to mutant forms of super oxide dismutase that aggregate into inclusions and cause loss of motor neuron viability (**Batulan, 2003; Durham, 1997; Roy, 1998; Taylor, 2004**). The antiapoptotic action of GA has also been demonstrated in the protection of cultured neurons challenged with the neurotoxic anti-cancer drugs taxol, cisplatin, and vincristine (**Sano, 2001**). GA also protects against MPTP-induced dopaminergic neurotoxicity in mice in a process coincident with induction of Hsp70 (**Shen, 2005a**).

IIb. Consistency

n/a

III. Efficacy (Animal Models of Parkinson's Disease)

IIIa. Animal Models: Rodent

GA is poorly soluble in water and routes of administration are limited to intravenous (i.v.) and intracerebroventricular (i.c.v.), which may have discouraged experimentation in animals and consequently limited the amount of literature available (see also Section V1a, General ADME). Administered i.c.v. 24 h prior to a 2-h middle cerebral artery occlusion, GA protects rat brain from focal ischemia (**Lu, 2002**). GA (1 $\mu\text{g}/\text{kg}$) decreased infarct volumes by 56%, improved behavioral outcomes, and reduced brain edema. This treatment regimen markedly upregulated heat shock proteins and co-chaperones (Hsp70, Hsp25, etc.) described in the Scientific Rationale/Mechanism section for the sequelae of dissociation of Hsf1 from Hsp90 that results from GA binding.

Similarly, GA also protects against MPTP-induced dopaminergic neurotoxicity in mice in a process coincident with induction of Hsp70 (**Shen, 2005c**). An i.c.v. injection of GA (1 and 10 $\mu\text{g}/\text{kg}$) 24 h prior to administration of MPTP increased residual dopamine content and tyrosine hydroxylase immunoreactivity in the striatum in a process initiated by inhibition of Hsp90.

Drosophila have been used as a convenient model for the pathology of Huntington's disease and of α -synuclein toxicity. GA protected against the neuronal toxicity of α -synuclein in flies in which there was directed expression of the protein (**Auluck, 2005**). Similarly, GA protected against Huntington disease-like pathology in flies expressing mutant human *huntingtin* protein (**Agrawal, 2005**).

IIIb. Animal Models: Non-human primates

n/a

IV. Efficacy (Clinical and Epidemiological Evidence)

IVa. Clinical Studies

n/a

IVb. Epidemiological Evidence

n/a

V. Relevance to Other Neurodegenerative Diseases

Parkinson disease shares with Huntington's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease an etiology that involves aggregation of neurotoxic forms of abnormal proteins to form fibrils and inclusion bodies that lead to the death of neurons (**Taylor, 2002**). The therapeutic mechanism of GA in mediating the damage by these proteins has in common induction of Hsp's and other cochaperones secondary to binding of GA to Hsp90. These protein chaperones guide repair, degradation, and shuttling of these proteins into matrices not associated with neuropathology.

In Parkinson disease loss of dopaminergic neurons in the substantia nigra is associated with Lewy bodies, a major component of which is the neurotoxic protein α -synuclein. α -Synuclein polymerizes to form β -sheet amyloid fibers characteristic of the disease. The therapeutic mechanism of GA in Parkinson disease has been attributed to induction of Hsp70, and its shuttling of potentially toxic, soluble forms of α -synuclein into nonpathological inclusions.

In the case of Huntington's disease, an aberrant protein, huntingtin, forms insoluble aggregates with fibrillar amyloid-type morphology that are associated with the progressive neuropathology of the disease (**Sittler, 2001**). GA induces Hsp forms that inhibit this aggregation.

In a familial form of ALS caused by a mutant form of superoxide dismutase (SOD), GA protected cultured motor neurons by reducing aggregation of the mutant protein into inclusions (**Batulan, 2006**).

Hyperstimulation of glutamate receptors was also described as a key mechanism in the neurodegeneration in Parkinson disease, as well as Huntington's disease and Alzheimer's disease (**Gorbacheva, 2008**). GA prevents thrombin-induced changes in the astrocyte skeleton in a process that requires Hsp90 (**Pai, 2001**).

GA attenuates damage due to oxidative stress such as that associated with the related processes involved in ischemia, reperfusion injury, stroke, oxygen-glucose deprivation, and reactive oxygen species mediated by α -synuclein (**Giffard, 2004; Lu, 2002; Ouyang, 2005**). Although the mechanism is not well understood (**Giffard, 2004**), repair and/or catabolism of denatured proteins by Hsp are likely mechanisms.

VI. Pharmacokinetics

Via. General ADME

Only one comprehensive investigation of the pharmacokinetics of GA in mammals was found (**Supko, 1995**). In that study, GA was administered intravenously to mice at 9 and 21 mg/kg, and to dogs at 2 and 4 mg/kg. The authors noted that pharmacokinetics and toxicology of GA in preclinical animal models had not been previously reported. Not unexpected for such a narrow dosing range, the pharmacokinetics were linear for the two dose levels in mice and dogs. The half-life in mouse was longer (78 min) than that of dog (58 min), but mean residence time (MRT; a measure of the average time a molecule of GA is in the central compartment) was twice as long in dog (47 min) than in mouse (21 min). GA was cleared from plasma faster in dog (49 mL/min per Kg) than mouse (31 mL/min per kg). The volume of distribution (V_{ss}) in mouse was unusually low (0.68 L/kg) given the lipophilicity of GA, indicating minimal redistribution into peripheral tissues; the authors attributed this to high plasma protein binding. V_{ss} in dog was higher (2.3 L/kg). In both dog and mouse, plasma concentrations remained above the concentration (0.1 μ g/mL) which is typically effective against neoplastic cell lines responsive to GA *in vitro* (**Supko, 1995**).

There was a paucity of data regarding general ADME of GA, likely due to the limited means of administering the drug (i.v. and i.c.v.). Although there were detailed reports of the disposition of GA analogues, including tissue distribution, after administration of analogues of GA possessed of better “drug-like properties” (**Xiong, 2008**), equivalent data for GA was not found. The pharmacokinetics of GA in mice and dogs following i.v. dosing was comprehensively covered in (**Supko, 1995**) as described above. The significant activity of GA as an inhibitor and substrate of P-glycoprotein (**Huang, 2007**) would indicate potential for facilitating uptake of drugs with which it is coadministered, and for which efflux transport by that carrier has appreciable impact.

Novel approaches to delivery (e.g., formulation of a lipophilic GA prodrug in micelles) are under investigation in animal models and could eventually lead to improved tolerability and pharmacokinetics (**Xiong, 2008**).

Vib. CNS Penetration

There was no indication in the literature reviewed that GA crosses the blood-brain barrier. The contrary is inferred by the use of the i.v.c. route of administration of GA in rats (**Lu, 2002**) and mice (**Shen, 2005b**), the authors of the latter work noting that this was to “circumvent poor central nervous system permeability”. GA is a substrate of the efflux transporter MDR1 (P-glycoprotein), which is a key transporter limiting penetration of the blood-brain barrier (**Huang, 2007**). This may be a significant mechanism for diminished penetration of GA into the CNS.

Vic. Calculated $\log([brain]/[blood])$ (Clark Model): 0.22

VII. Safety, Tolerability, and Drug Interaction Potential

VIIa. Safety and Tolerability

While there were frequent references to the “severe hepatotoxicity” of GA in the literature, there was only one comprehensive investigation of its toxicity in mammals (**Supko, 1995**). In that study, GA was administered intravenously to mice at 9 and 21 mg/kg, and dogs at 2 and 4 mg/kg. The authors noted that liver appeared to be the principal target of acute drug toxicity in the dog. Typical markers of hepatotoxicity were measured in serum, and the highest ALT and AST values (measured 4-7 days postdosing) were 30-40 times control, with a more modest increase in LDH and BUN; alkaline phosphatase increased about 20-fold. Additional toxic effects were limited to symptoms of minor gastrointestinal aggravation and less severe indications of nephrotoxicity (modest increases in BUN). All clinical values returned to baseline within 1 month, suggesting that the toxicity was reversible. A maximum tolerated dose (MTD) of 4 mg/kg for dog, and 20 mg/kg for mice was estimated in these studies.

VIIb. Drug Interaction Potential

Aside from the ancillary action of GA in inducing heat shock proteins and other chaperones that may modulate the biological responses of coadministered drugs, there were no drug interaction studies reported in the literature. However, a recent report (**Huang, 2007**) concluded that GA is both a substrate and inhibitor of the efflux transporter MDR1 (P-glycoprotein), which is a key transporter limiting penetration of the blood-brain barrier. Therefore, drugs coadministered with GA that are also substrates for this efflux transporter may have increased uptake into the CNS.

VIII. Bibliography

- Agrawal, N.; Pallos, J.; Slepko, N.; Apostol, B. L.; Bodai, L.; Chang, L. W.; Chiang, A. S.; Thompson, L. M.; Marsh, J. L. Identification of combinatorial drug regimens for treatment of Huntington's disease using *Drosophila*. *Proc Natl Acad Sci U S A* **2005**, 102, 3777-81.
- Auluck, P. K.; Bonini, N. M. Pharmacological prevention of Parkinson disease in *Drosophila*. *Nat Med* **2002**, 8, 1185-6.
- Auluck, P. K.; Meulener, M. C.; Bonini, N. M. Mechanisms of Suppression of {alpha}-Synuclein Neurotoxicity by Geldanamycin in *Drosophila*. *J Biol Chem* **2005**, 280, 2873-8.
- Batulan, Z.; Shinder, G. A.; Minotti, S.; He, B. P.; Doroudchi, M. M.; Nalbantoglu, J.; Strong, M. J.; Durham, H. D. High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. *J Neurosci* **2003**, 23, 5789-98.
- Batulan, Z.; Taylor, D. M.; Aarons, R. J.; Minotti, S.; Doroudchi, M. M.; Nalbantoglu, J.; Durham, H. D. Induction of multiple heat shock proteins and neuroprotection in a primary culture model of familial amyotrophic lateral sclerosis. *Neurobiol Dis* **2006**, 24, 213-25.
- Cheetham, M. E.; Caplan, A. J. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones* **1998**, 3, 28-36.
- Cyr, D. M.; Langer, T.; Douglas, M. G. DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. *Trends Biochem Sci* **1994**, 19, 176-81.
- Durham, H. D.; Roy, J.; Dong, L.; Figlewicz, D. A. Aggregation of mutant Cu/Zn superoxide dismutase proteins in a culture model of ALS. *J Neuropathol Exp Neurol* **1997**, 56, 523-30.
- Fan, C. Y.; Lee, S.; Cyr, D. M. Mechanisms for regulation of Hsp70 function by Hsp40. *Cell Stress Chaperones* **2003**, 8, 309-16.
- Flower, T. R.; Chesnokova, L. S.; Froelich, C. A.; Dixon, C.; Witt, S. N. Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. *J Mol Biol* **2005**, 351, 1081-100.
- Giffard, R. G.; Xu, L.; Zhao, H.; Carrico, W.; Ouyang, Y.; Qiao, Y.; Sapolsky, R.; Steinberg, G.; Hu, B.; Yenari, M. A. Chaperones, protein aggregation, and brain protection from hypoxic/ischemic injury. *J Exp Biol* **2004**, 207, 3213-20.
- Gorbacheva, L. R.; Storozhevyykh, T. P.; Pinelis, V. G.; Davydova, O. N.; Ishiwata, S.; Strukova, S. M. Activated protein C via PAR1 receptor regulates survival of neurons under conditions of glutamate excitotoxicity. *Biochemistry (Mosc)* **2008**, 73, 717-24.
- Huang, Y.; Blower, P. E.; Liu, R.; Dai, Z.; Pham, A. N.; Moon, H.; Fang, J.; Sadee, W. Chemogenomic analysis identifies geldanamycins as substrates and inhibitors of ABCB1. *Pharm Res* **2007**, 24, 1702-12.

VIII. Bibliography (cont.)

- Latchman, D. S. Protective effect of heat shock proteins in the nervous system. *Curr Neurovasc Res* **2004**, 1, 21-7.
- Lu, A.; Ran, R.; Parmentier-Batteur, S.; Nee, A.; Sharp, F. R. Geldanamycin induces heat shock proteins in brain and protects against focal cerebral ischemia. *J Neurochem* **2002**, 81, 355-64.
- Muchowski, P. J.; Wacker, J. L. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* **2005**, 6, 11-22.
- Ouyang, Y. B.; Xu, L.; Giffard, R. G. Geldanamycin treatment reduces delayed CA1 damage in mouse hippocampal organotypic cultures subjected to oxygen glucose deprivation. *Neurosci Lett* **2005**, 380, 229-33.
- Pai, K. S.; Mahajan, V. B.; Lau, A.; Cunningham, D. D. Thrombin receptor signaling to cytoskeleton requires Hsp90. *J Biol Chem* **2001**, 276, 32642-7.
- Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J Med Chem* **1999**, 42, 260-6.
- Roy, J.; Minotti, S.; Dong, L.; Figlewicz, D. A.; Durham, H. D. Glutamate potentiates the toxicity of mutant Cu/Zn-superoxide dismutase in motor neurons by postsynaptic calcium-dependent mechanisms. *J Neurosci* **1998**, 18, 9673-84.
- Sano, M. Radicicol and geldanamycin prevent neurotoxic effects of anti-cancer drugs on cultured embryonic sensory neurons. *Neuropharmacology* **2001**, 40, 947-53.
- Shen, G.; Blagg, B. S. Radester, a novel inhibitor of the Hsp90 protein folding machinery. *Org Lett* **2005a**, 7, 2157-60.
- Shen, H. Y.; He, J. C.; Wang, Y.; Huang, Q. Y.; Chen, J. F. Geldanamycin induces heat shock protein 70 and protects against MPTP-induced dopaminergic neurotoxicity in mice. *J Biol Chem* **2005b**, 280, 39962-9.
- Shen, Y.; Xie, Q.; Norberg, M.; Sausville, E.; Woude, G. V.; Wenkert, D. Geldanamycin derivative inhibition of HGF/SF-mediated Met tyrosine kinase receptor-dependent urokinase-plasminogen activation. *Bioorg Med Chem* **2005c**, 13, 4960-71.
- Sittler, A.; Lurz, R.; Lueder, G.; Priller, J.; Lehrach, H.; Hayer-Hartl, M. K.; Hartl, F. U.; Wanker, E. E. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum Mol Genet* **2001**, 10, 1307-15.
- Supko, J. G.; Hickman, R. L.; Grever, M. R.; Malspeis, L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* **1995**, 36, 305-15.
- Taylor, D. M.; Minotti, S.; Agar, J. N.; Durham, H. D. Overexpression of metallothionein protects cultured motor neurons against oxidative stress, but not mutant Cu/Zn-superoxide dismutase toxicity. *Neurotoxicology* **2004**, 25, 779-92.
- Taylor, J. P.; Hardy, J.; Fischbeck, K. H. Toxic proteins in neurodegenerative disease. *Science* **2002**, 296, 1991-5.

VIII. Bibliography (cont.)

Xiong, M. P.; Yanez, J. A.; Remsberg, C. M.; Ohgami, Y.; Kwon, G. S.; Davies, N. M.; Forrest, M. L. Formulation of a geldanamycin prodrug in mPEG-b-PCL micelles greatly enhances tolerability and pharmacokinetics in rats. *J Control Release* **2008**, 129, 33-40.

Yenari, M. A. Heat shock proteins and neuroprotection. *Adv Exp Med Biol* **2002**, 513, 281-99.