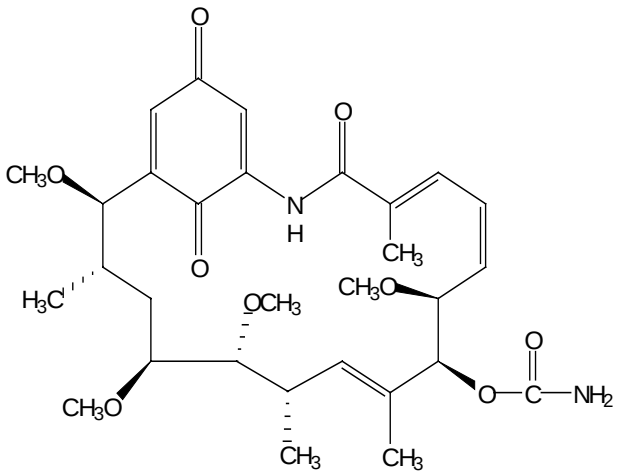
ProductInformation

HERBIMYCIN A

Sigma Prod. No. H6649



CAS NUMBER: 70563-58-5
SYNONYMS: Antibiotic Tan 420F[1]; 17-Demethoxy-15-Methoxy-11-O-Methyl-Geldanamycin[2]

PHYSICAL DESCRIPTION:

Appearance: Film in vial or yellow powder.[3]
Molecular Formula: $C_{30}H_{42}N_2O_9$
Molecular Weight: 574.7

METHOD OF PREPARATION:

Herbimycin A (HA) is synthetically prepared.[4] A method of isolation, the NMR characteristics and structure-activity relationships have been reported.[5,6]

STABILITY / STORAGE AS SUPPLIED:

HA should be stable for at least one year when stored at -20°C and protected from light.[3]

SOLUBILITY / SOLUTION STABILITY:

Stock solutions, i.e. 1 mg/ml (1.75 mM), can be prepared in 100% dimethyl sulfoxide (DMSO) then diluted to the required working concentrations with water, aqueous buffer or culture medium before addition to cells.[4,7] Honma reported the preparation of a 7.5 mg/ml solution in DMSO followed by a 500-fold dilution in PBS for i.p. injections into mice.[8] Stock solutions can also be prepared in methanol.[9] Stock solutions of HA in DMSO are relatively stable and can be aliquoted and stored at -20°C, protected from light for several months.[9]

To determine the optimum inhibitor concentrations for the system under study it is recommended that a range of inhibitor concentrations be tested. Published literature (i.e. see "References") describing treatment of whole cells and cell extracts reported concentration ranges from 0.5-875 nM (0.3-500 ng/ml) and 2.0-175 nM (1.2-100 ng/ml), respectively.[4]

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GENERAL NOTES:

HA is a benzoquinoid ansamycin antibiotic isolated from Streptomyces hygroscopicus and exerting herbicidal, anti-tobacco mosaic virus and cytotoxic activities.[5] Both in intact cells and in cell free systems, HA is a specific tyrosine kinase inhibitor. Tyrosine phosphorylation is an essential event in many membrane signal transduction systems, including growth factor receptor-associated protein tyrosine kinases, i.e. platelet derived growth factor (PDGF) and epidermal growth factor (EGFR). HA inactivates tyrosine kinases by binding directly to the kinase domain, probably to thiol groups, and preventing access to ATP.[9,10] HA specifically acts on cells expressing the src oncogenes, i.e., expressing protein kinase activity of p60*src* (=p60*v-src*), and reverses various transformed characteristics to the normal ones.[11] Concentrations required to reverse the morphology varies among the cells.[9] The results of inhibition of the intracellular p60*src* kinase activity includes reducing the elevation of glucose uptake, increasing fibronectin gene expression of transformed cells and increasing serum requirement and anchorage dependency for cell growth.[12] HA did not decrease activity of the serine/threonine kinases, protein kinase A and protein kinase C.[9,13] HA should be valuable in examining the role of tyrosine phosphorylation in cell transformation, growth and differentiation and for its potential antineoplastic properties.[9]

USAGE:

HA reduced the intracellular phosphorylation of p60*src* in a Rous Sarcoma Virus (RSV) infected rat kidney cell (presumably by reduction of p60*src* tyrosine kinase activity) and induced the phenotypic change from transformed to normal cell morphology.[14] HA irreversibly inhibited p60*v-src*,(IC_{50}=7 µg/ml), possibly by binding of the benzoquinone moiety to the reactive thiol groups of the enzyme, i.e., HA's ability to inactivate the enzyme and transform cells to normal cell morphology was lost in the presence of thiol groups.[9,10,13,15] HA also inactivated other cytoplasmic tyrosine kinases such as p120*v-abl*,p130*v-fps*,p210*bcr-abl*, and an activated form of p60*c-src* of human colon cancer cells.[9,13] HA also reversed the morphologies of transformed chicken and mammalian cells by tyrosine kinase oncogenes src, yes, fps, ros, abl, erbB and decreased the phosphotyrosine content of the total cellular proteins.[16] The addition of HA (0.5 µg/ml) to cultures of RSV infected rat kidney cells increased the fibronectin expression and converted the morphology of the transformed neoplastic cells to that of normal cells.[17] HA inhibited the proliferation of breast cancer cells through inhibition of tyrosine phosphorylation of a ras regulating protein, Shc, and MAP kinase activity.[18]

USAGE: (continued)

HA (0.1 µM) decreased thrombin-induced mitogenesis (in the signal transduction cascade) by 90% and inhibited tyrosine phosphorylation of phospholipase C (PLC gamma-1).[19] HA induced differentiation of K562 human leukemic cells.[20] HA exhibited anti-angiogenesis effects inhibiting the proliferation of cultured capillary endothelial cells and also proliferation of fibroblasts and HeLa S_3 cells (50% inhibition, 0.013, 0.20 and 0.43 µg/ml, respectively).[21,22] HA inhibited T-cell receptor-mediated inositol phospholipid hydrolysis in signal transduction when used in studies of T-cell antigen receptor.[23] HA (IC_{50}=8µg/ml) inhibited platelet derived growth factor (PDGF)-induced phospholipase D activation possibly through reduction in tyrosine phosphorylation of proteins in the PDGF-stimulated signalling pathway.[24] HA inhibited (up to 0.5 µg/ml) anti-CD3 monoclonal antibody induced thymic apoptosis.[25] HA specifically induced the 70-kD heat stress protein in rat neonatal cardiomyocytes and protected cells against severe stress presumably through a tyrosine kinase-independent mechanism.[26] HA also induced the heat stress protein Hsp 72 in a range of cells.[27] HA decreased the level of expression of EGF-receptor protein in A431 cells and converted EGF into a stimulatory ligand for cell growth. HA had no direct inhibitory effect on EGF-receptor kinase (receptor-type tyrosine kinase) activity.[28] HA (0.2-2 µM) inhibited the activation of nuclear factor kappa B (NFkB), a transcription factor, by Interleukin 1 and PMA in cells.[29,30] HA inhibited osteoclastic bone resorption and hypercalcemia in mouse cultures and mice, respectively and decreased the activity of pp60*c-src* in mouse bone marrow cells.[7] The increase of Ras GTP induced by Interleukin 3 and granulocyte-macrophage colony stimulating factor was lowered in cells treated with HA (0.5 to about 1 µg/ml).[31] HA prolonged the survival of mice inoculated with leukemia cells with high expression of v-abl tyrosine kinase.[8] HA inhibited retinol-induced epidermal mucous metaplasia in chick embryonic cultured dermal cells.[32] HA inhibited the expression of c-myc gene expression in L5178Y cells, the gene product of which is involved in the initiation of cellular DNA replication.[33]

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