

**Aminoflavone Analog, L-Lysyl Prodrug, Dimethanesulfonate Salt
(NSC 710464, AFP 464)**

ADME-PK and Biochemical Pharmacology Summary

Division of Cancer Treatment and Diagnosis
National Cancer Institute

The lysyl prodrug of aminoflavone is rapidly converted to the parent compound after administration to animals as either the HBr (NSC 702295) or the dimethanesulfonate (NSC 710464; AFP464) salt. The elimination of aminoflavone (NSC 686288) is rapid after administration of the parent compound or the prodrug (terminal half-life ranged from 18 min to 4.8 hours after i.v. administration to rodents, dogs, and non-human primates). The clearance was similar in mice and rats on a body surface area basis (9.7 - 14 L/hr/m²) but more rapid in dogs (74 - 127 L/hr/m²) and cynomolgus monkeys (39 - 79 L/hr/m²). Clearance of the drug was increased upon repeated administration - modestly in the rat and markedly in the dog, but not significantly in the monkey. It is likely that NSC 686288 induces its own metabolism through an AhR-CYP1A1-dependent pathway.

NSC 686288 is extensively metabolized *in vitro* by CYP1A enzymes, producing multiple hydroxylated compounds. In the rat, N-acetylation is also a prominent pathway for metabolism of NSC 686288. The oxidative metabolism of NSC 686288 is postulated to produce reactive products that covalently bind DNA and other macromolecules. Recent studies suggest that N-hydroxyl species (hydroxylamines) produced by CYP1A1 are substrates for further bioactivation by sulfotransferases (e.g., *SULT1A1*). It is postulated that the resulting N-sulfoxy-groups can be further converted to nitrenium ions that form adducts with DNA and proteins.

The covalent binding of NSC 686288 metabolites to macromolecules induces DNA damage and apoptosis through a p53/p21 dependent mechanism as evidenced by the presence of DNA-protein crosslinks, γ -H2AX foci, and S-phase arrest in treated cells. These effects were highest in tumor cell lines that were most sensitive to NSC 686288 and much lower in resistant cell lines. Further studies demonstrated that NSC 686288 induced CYP1A1, CYP1B1 and *SULT1A1* expression in sensitive cells subsequent to translocation of AhR to the nucleus. Studies using renal cancer cell lines showed that NSC 686288 increased CYP1A1 mRNA expression in sensitive cell lines. Experimental data from renal cell isolates from 13 patients with renal cell carcinoma found that drug-sensitive isolates had increased binding of aminoflavone metabolites when compared with resistant isolates.

Thus it is postulated that sensitivity of tumor cells to NSC 686288 is dependent upon an interaction of the compound with Ah receptors, followed by induction of *CYP1A* and *SULT1A1* gene expression and subsequent activation of the compound to DNA-interactive metabolites. Assessment of appropriate endpoints along this pharmacodynamic-metabolic cascade (e.g., *CYP1A1* and *SULT1A1* induction, DNA binding, apoptosis) may provide suitable biomarkers for correlation with AFP 464 dose/exposure, toxicity, and clinical response. Further validation of a responsive biomarker may ultimately allow pre-selection of patients most likely to benefit from treatment with AFP 464.

**Aminoflavone Analog, L-Lysyl Prodrug, Dimethanesulfonate Salt
(NSC 710464, AFP 464)**

ADME-PK and Biochemical Pharmacology Summary

Division of Cancer Treatment and Diagnosis
National Cancer Institute

References

Meng, LH; Shankavaram, U; Chen, C; et al. Activation of aminoflavone (NSC 686288) by a sulfotransferase is required for the antiproliferative effect of the drug and for induction of histone gamma-H2AX. *Cancer Research*, 66 (19): 9656-9664, 2006.

Chen, C; Meng, LH; Ma, XC; et al. Urinary metabolite profiling reveals CYP1A2-mediated metabolism of NSC686288 (aminoflavone). *Journal of Pharmacology and Experimental Therapeutics*, 318 (3): 1330-1342, 2006.

Pobst, LJ; Ames, MM. CYP1A1 activation of aminoflavone leads to DNA damage in human tumor cell lines. *Cancer Chemotherapy and Pharmacology*, 57 (5): 569-576, 2006.

Meng, LH; Shankavaram, U; Chen, C; et al. Selective activity of aminoflavone (NSC 626288), a novel drug in phase I clinical trials is determined by cellular expression of sulfotransferase. *Clinical Cancer Research*, 11 (24): 9086s-9087s Part 2 Suppl., 2005.

Meng, LH; Kohlhagen, G; Liao, ZY; et al. DNA-protein cross-links and replication-dependent histone H2AX phosphorylation induced by aminoflavone (NSC 686288), a novel anticancer agent active against human breast cancer cells. *Cancer Research*, 65 (12): 5337-5343, 2005.

Loaiza-Perez, AI; Kenney, S; Boswell, J; et al. Aryl hydrocarbon receptor activation of an antitumor aminoflavone: Basis of selective toxicity for MCF-7 breast tumor cells. *Molecular Cancer Therapeutics*, 3 (6): 715-725, 2004.

Loaiza-Perez, AI; Kenney, S; Hose, C; et al. CYP1A1 and CYP1B1 induction and high covalent binding of metabolites are markers to predict sensitivity to aminoflavone in breast and renal cancer cells. *Cancer Epidemiology Biomarkers & Prevention*, 11 (10): A224 Part 2, 2002.

Kuffel, MJ; Schroeder, JC; Pobst, LJ; et al. Activation of the antitumor agent aminoflavone (NSC 686288) is mediated by induction of tumor cell cytochrome P450 1A1/1A2. *Molecular Pharmacology*, 62 (1): 143-153, 2002.

Loaiza-Perez, AI; Vistica, D; Kenney, S; et al. The aryl hydrocarbon receptor mediates sensitivity of MCF-7 breast cancer cells to the antitumor agent aminoflavone NSC 686288. *Clinical Cancer Research*, 7 (11): 413 Suppl., 2001.

Ames, MM; Schroeder, JC; Pobst, LJ; et al. Aminoflavone (NSC 686288) induction of cytochrome P450 1A1 in sensitive human tumor cell lines is associated with metabolic activation, DNA damage and antiproliferative activity. *Clinical Cancer Research*, 7 (11): 712 Suppl., 2001.

**Aminoflavone Analog, L-Lysyl Prodrug, Dimethanesulfonate Salt
(NSC 710464, AFP 464)**

ADME-PK and Biochemical Pharmacology Summary

Division of Cancer Treatment and Diagnosis
National Cancer Institute

Kuffel, MJ; Bowman, SL; Browne, JA; Reid, JM; Naylor, S; Ames, M.M. Tumor Cell and Tissue-Selective Cytochromes P450 Oxidation of Aminoflavone Analog NSC-686288. Proc. Amer. Assoc. Cancer Res., 41: 370, 2000.

Kuffel, MJ; Squillace, DP; Reid, JM; Ames, M.M. Cytochrome P450-catalyzed Metabolism of the Aminoflavone Analog NSC 686288 in Human and Rat Liver Microsomes: the Role of CYP1A Subfamily Enzymes. Proc. Amer. Assoc. Cancer Res., 40: 384, 1999.

Brown, AP; Morrissey, RL; Rodvold, KA; Tolhurst, TA; Donohue, SJ; Tomaszewski, JE; Levine, BS. Intravenous Plasma Elimination Kinetics and Toxicity of an Aminoflavone in the Dog. Proc. Amer. Assoc. Cancer Res., 40: 390, 1999.