

STRUCTURE-BASED DESIGN OF THE ANTITUMOR 2-HYDROXYARYLIDENE-4-CYCLOPENTENE-1,3-DIONE TX-1123 AS A PROTEIN TYROSINE KINASE INHIBITOR HAVING LOW MITOCHONDRIAL TOXICITY

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We have developed a new tyrphostin (a protein tyrosine kinase inhibitor) TX-1123 [2-{(3,5-di-*tert*-butyl-4-hydroxyphenyl)-methylene}4-cyclopentene-1,3-dione] having lower mitochondrial toxicity [1]. The protein tyrosine kinases (PTKs) are members of a large family of oncoproteins and proto-oncoproteins that play major roles in signal transduction during normal cell division, terminal cell differentiation, and apoptosis. Because enhanced PTK activity is associated with proliferative disorders such as cancers, PTK inhibitors have been developed as potential therapeutic agents for cancer treatment [2]. Tyrphostins were originally described as a group of low-molecular-weight PTK inhibitors designed to compete with substrate rather than ATP.

In a screening study AG17 was found to be the most potent tumor-cell-growth inhibitor among a series of tyrphostins examined as inhibitors of breast carcinoma cell growth [3]. Prior studies with AG17 had demonstrated that it could function as an inhibitor of the EGFR tyrosine kinase. Studies in intact cells have revealed that AG17 is a potent inhibitor of PDGF-stimulated growth of rabbit vascular smooth muscle cells, as well as of PDGF-mediated tyrosine phosphorylation of several intracellular substrates. Other investigators have further characterized AG17 as a potent inhibitor of epidermal growth factor-stimulated pancreatic carcinoma cell growth and interleukin-2-stimulated human peripheral mononuclear cell proliferation. Recently, we found that AG17 suppressed insulin action in rat white adipocytes [4]. Sausville and co-authors found that AG17 was the most potent tumor cell growth inhibitor among a series of tyrphostins examined as inhibitors of breast carcinoma cell growth [5]. They also noted the possibility that AG17 may act in part by altering mitochondrial function and/or structure, and that impairment of mitochondrial function (mitochondrial toxicity) may be exploitable as a potentially useful mechanism to modulate tumor cell proliferation. Two years later, Levitzki reported that AG17 induces apoptosis and inhibition of cdk2 activity through a mechanism that purportedly does not involve reduction of cellular ATP levels. Shortly thereafter Sausville cautioned against Levitzki's use [6] of AG17 as a pure protein tyrosine kinase inhibitor [7].

In considering potential mechanisms of the anti-proliferative action of AG17, as Sausville cautioned, we should note that SF6847 (an alternate name for AG17) was shown to act as an uncoupler of oxidative phosphorylation in isolated rat liver and heart mitochondria [8]. Then we developed a new tyrphostin analogue of AG17 that had lower mitochondrial toxicity. Thus, we designed a series of 2-

hydroxyarylidene-4-cyclopentene-1,3-diones, synthesized, and evaluated with respect to PTK inhibition, mitochondrial toxicity, and antitumor activity. Our results showed that the cyclopentenedione-derived TX-1123 is a more potent antitumor tyrophostin and also shows lower mitochondrial toxicity than the malononitrile-derived AG17, a potent antitumor tyrophostin. The O-methylation product of TX-1123 retained its tyrophostin-like properties, including mitochondrial toxicity and antitumor activities. However, the methylation product of AG17 retained its tyrophostin-like antitumor activities, but lost its mitochondrial toxicity.

In summary, our comprehensive evaluation of these agents with respect to PTK inhibition, mitochondrial inhibition, antitumor activity, and hepatotoxicity demonstrated that TX-1123 was a more promising candidate for antitumor agents than tyrophostin AG17. We will also discuss our strategies to design tumor hypoxia-targeting 2-nitroimidazole-cyclopentenedione- type tyrophostin TX-1123 analogues [9-11].

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