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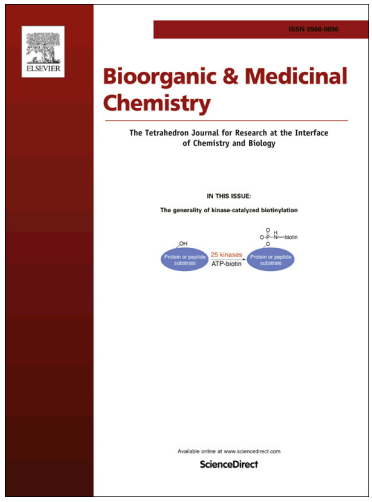

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Discovery of anti-cancer activity for benzo[1,2,4]triazin-7-ones: Very strong correlation to pleurotin and thioredoxin reductase inhibition

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ABSTRACT

The thioredoxin (Trx)-thioredoxin reductase (TrxR) system plays a key role in maintaining the cellular redox balance with Trx being over-expressed in a number of cancers. Inhibition of TrxR is an important strategy for anti-cancer drug discovery. The natural product pleurotin is a well-known irreversible inhibitor of TrxR. The cytotoxicity data for benzo[1,2,4]triazin-7-ones showed very strong correlation (Pearson correlation coefficients ~ 0.8) to pleurotin using National Cancer Institute COMPARE analysis. A new 3-CF_3 substituted benzo[1,2,4]triazin-7-one gave submicromolar inhibition of TrxR, although the parent compound 1,3-diphenylbenzo[1,2,4]triazin-7-one was more cytotoxic against cancer cell lines. Benzo[1,2,4]triazin-7-ones exhibited different types of reversible inhibition of TrxR, and cyclic voltammetry showed characteristic quasi-reversible redox processes. Cell viability studies indicated strong dependence of cytotoxicity on substitution at the 6-position of the 1,3-diphenylbenzo[1,2,4]triazin-7-one ring.

1. Introduction

First identified in the late 1960s,[1] and isolated in 1980,[2] the rich chemistry of 1,3-diphenylbenzo[1,2,4]triazin-7-ones **1** (Figure 1) has more recently been explored.[3-6] Benzotriazinone **1a** (R = H) and derivatives have been implicated as multi-target inhibitors in Alzheimer's disease of beta-amyloid (Aβ) aggregation and acetyl-(AChE)/butyryl- (BChE) cholinesterase.[7] Scaffold **1** contains a highly conjugated iminoquinone motif and an iminoquinone derivative of imidazo[5,4-*f*]benzimidazoles was shown to have good specificity (Pearson correlation coefficient of 0.51) towards NAD(P)H:quinone oxidoreductase 1 (NQO1) expression using COMPARE analysis of toxicity towards the 60 cell lines at the National Cancer Institute (NCI) Development Therapeutics Program (DTP).[8]

We now report COMPARE analysis of the toxicity of benzotriazinones leading to the discovery of very strong correlations to pleurotin. The latter naturally occurring antibiotic is a *para*-quinone with a perhydroanthracene core, which was first isolated in the 1940s from the basidiomycete, *Pleurotus griseus*.[9] Though pleurotin has been synthesized,[10] a multi-gram fermentation process using *Hohenbuehelia atrocaerulea* for supply of pleurotin to the NCI has been reported.[11] Pleurotin possesses antibacterial,[9] antifungal,[12] and anti-cancer activity, including inhibiting the hypoxia-induced factor (HIF-1α), a transcription factor associated with many aspects of tumor growth.[13] The underlying pathway to much of this biological activity is pleurotin's ability to act as a potent inhibitor (IC_{50} 0.17 μM) of the thioredoxin (Trx)-thioredoxin reductase (TrxR) system.[14]

Earlier reports more specifically describe pleurotin as an irreversible inhibitor of TrxR with a K_i of 0.28 μM.[13,15] TrxR is a flavoprotein homodimer with each monomer containing a FAD prosthetic group, NADPH binding domain, and a redox-active selenothiol active site.[16,17] TrxR is the only known enzyme to reduce Trx protein, which in turn provides reducing equivalents for a

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