Screening of sulfasalazine analogues for inhibition of human glutathione transferase P1-1, a target for anticancer therapy

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Glutathione transferase P1-1 (glutathione *S*-transferase P1-1; GSTP1-1) is overexpressed in a variety of human tumors and several lines of evidence indicate that it plays a role in both natural and acquired tumor resistance to anticancer agents (Ruzza *et al*., 2009). In particular, besides being capable of detoxifying certain cytotoxic drugs by catalysis of their conjugation with the tripeptide glutathione (GSH), GSTP1-1 exhibits antiapoptotic activity based mainly on its interaction and inhibition of c-Jun N-terminal kinase (JNK), a kinase involved in the apoptotic response to various stimuli, including exposure to several chemotherapeutics. In light of the above, GSTP1-1 inhibitors have potential to be developed as novel agents that sensitize tumor cells to conventional anticancer drugs by disrupting the GSTP1-1-JNK complex and/or inhibiting GST-catalyzed drug conjugation to GSH.

Sulfasalazine (*sulfasalazopyridine*; SASP), a drug currently used primarily to treat ulcerative colitis and Crohn's disease, is a non-substrate inhibitor of various human GSTs including GSTP1-1 (Ahmad *et al*., 1992; Awasthi *et al*., 1994), and may therefore serve as a potential structure lead for the development of improved GSTP1-1 inhibitors. In particular, the availability of *crystallographic data* on the structure of human GSTP1-1 complexed with SASP and GSH (Oakley *et al*., 1999) offers the opportunity to rationally design novel analogues endowed with a superior pharmacological profile.

Thirty SASP analogues containing an imidazole ring in substitution of the azo group of SASP (i.e. salycylbenzoimidazole derivatives), have been designed, synthesized and screened for *in vitro* inhibition of the GSH conjugation activity of GST from human placenta (GSTP1-1), using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, and HPLC-UV for quantitation of the reaction product, 1-glutathionyl-2,4-dinitrobenzene. Given that SASP is rapidly inactivated through cleavage of the diazo bond, these compounds may possess a more desirable profile as GSTP1-1 inhibitors for *in vitro* whole cell and *in vivo* studies.

Interestingly, some analogues in which the pyridine ring of SASP was replaced by a thiazole ring or a branched aliphatic chain, displayed higher inhibitory activity towards GSTP1-1 than SASP; these compounds are currently undergoing further analysis for their GST form selectivity using recombinant human GSTP1-1, GSTM1-1 and GSTA1-1. Furthermore, preliminary *in vitro* cytotoxicity experiments using the human melanoma cell line A375, indicate that two of the synthesized SASP analogues (i.e. EML259 and EML339) display a significant cytotoxic activity.

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