Evaluation of inhibitory activity and selectivity of novel sulfasalazine analogues towards human glutathione transferases

L. Calderan[1,2], V. Di Paolo[2], S. Castellano[3], A. L. Balzano[4], D. Saggioro[1], A. Ciancetta[2], G. Deganutti[2], C. Milite[4], G. Sbardella[4], S. Moro[2], L. Quintieri[2]

[1] Veneto Institute of Oncology IOV - IRCCS, Padua, Italy
[2] Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy
[3] Dept. of Medicine and Surgery, University of Salerno, Salerno, Italy
[4] Dept. of Pharmacy, University of Salerno, Salerno, Italy

Glutathione transferases (formerly glutathione *S*-transferases, GSTs) constitute a superfamily of enzymes responsible for the glutathione (GSH)-dependent detoxification of a wide range of chemicals, including some anticancer drugs. Moreover, certain members of this superfamily interact with and inhibit the activity of protein kinases such as c-Jun N-terminal kinase (JNK), a protein involved in the apoptotic response to various stimuli, and are frequently overexpressed in a variety of human tumors where they contribute to conferring an anti-apoptotic phenotype (Ruzza *et al*., 2009). In light of this, several GSTP1-1 inhibitors have been investigated throughout the years as novel agents capable of sensitizing tumor cells to conventional anticancer drugs by inhibiting GST-catalyzed drug conjugation to GSH, and/or disrupting the GSTP1-1-JNK complex.
Sulfasalazine (salicylazosulfapyridine, SASP), a drug currently used to treat rheumatoid arthritis and inflammatory bowel diseases, is a non-substrate inhibitor of various human GSTs, including GSTP1-1 (Ahmad *et al*., 1992). However, its use as an anticancer agent is hampered mainly by poor oral bioavailability and metabolic instability, the latter being linked to the presence of an azo group in its molecule. Based on the availability of data on the structure of human GSTP1-1 complexed with SASP and GSH (Oakley *et al*., 1999), we designed and synthesized over 30 novel SASP analogues containing a 1,3-diazole ring in substitution of the azo group. All of them were screened *in vitro* for inhibition of enzymatic activity of GST from human placenta (mostly GSTP1-1), using 1-chloro-2,4-dinitrobenzene and GSH as substrates, and HPLC-UV for quantitation of the reaction product, 1-glutathionyl-2,4-dinitrobenzene. Interestingly, 7 analogues in which the pyridine ring of SASP was replaced by a thiazole ring (EML340, 277, 357, and 279) or a branched aliphatic chain (EML259, 337, and 338) displayed higher inhibitory activity towards placental GST than SASP. More recently, the most promising compounds that emerged from the placental GST-based screening (EML340, 277, 259, and 337) were advanced to GST form-selectivity studies, which were carried out using human recombinant GSTA1-1, GSTM1-1 and GSTP1-1. Compound EML337, a methyl ester containing a branched aliphatic chain replacing the pyridine ring of SASP, showed a certain grade of selectivity for GSTP1-1, while EML340 and EML277, both of which are carboxylic acids containing a thiazole nucleus, inhibited preferentially the GSH-conjugating activity of GSTM1-1. In addition, preliminary *in vitro* cytotoxicity assays indicated that methyl esters EML259, EML337 and EML339 can inhibit the growth of the GSTP1-positive melanoma cell lines A375 and SK-MEL 23. Finally, molecular docking and molecular dynamics simulations carried out to investigate the interaction pattern between GSTP1-1 and EML339 or EML340 confirmed that the substitution of the azo group with a 1,3-diazole ring allows these compounds to establish with the enzyme the same major interactions observed with SASP.

References:

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