MDR-involved human glutathione transferases (hGSTs) are targets for inhibition by 2,2'-dihydroxybenzophenones and N-carbonyl analogues

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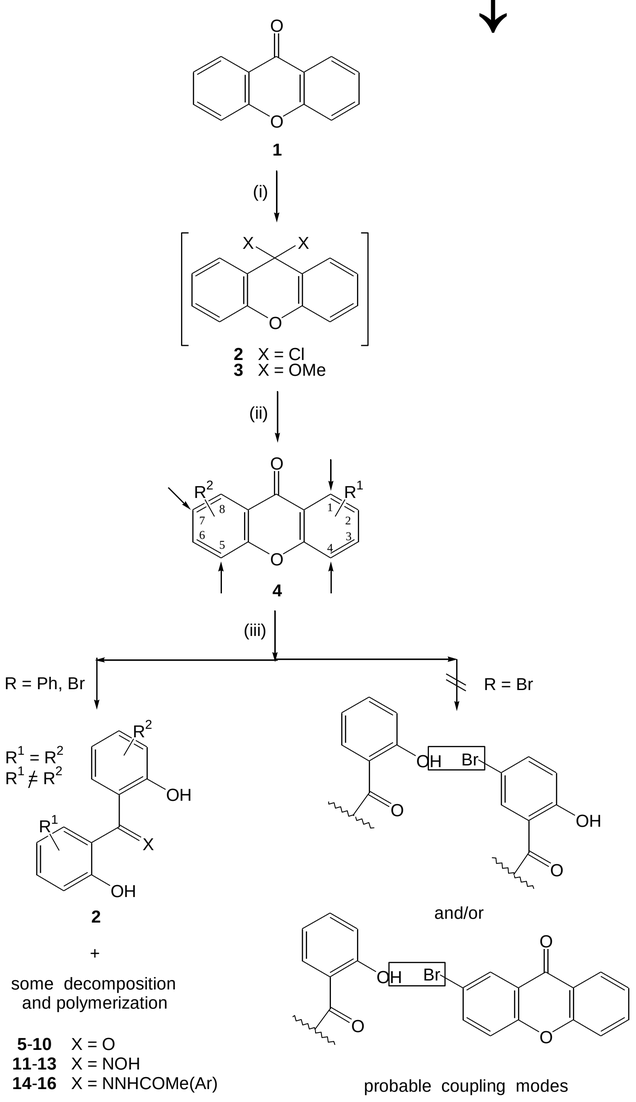
ABSTRACT. Over expression of human GSTA1-1 in tumour cells is part of MDR mechanisms. Substituted 2-hydroxybenzophenones are ubiquitous in naturally occurring and synthetic compounds, exhibiting important biological activities. 2,2'-Dihydroxybenzophenones and N-carbonyl analogues, structurally, are ring-opened forms of xanthone analogues which we reported recently as hGSTA1-1 inhibitors. The present study combined GST inhibition screening, *in silico* molecular docking and enzyme inhibition kinetics, revealing four analogues with strong inhibitory potency (IC_{50} = 0.18-1.8 μM) and modest cytotoxic activity for Caco2 cell line (LC_{50} = 35 to > 400 μM), thus being useful as lead structures for the design of new inhibitors against hGSTs.

INTRODUCTION

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of isoenzymes that differ in their tissue-specificity expression and distribution. They catalyse the conjugation of glutathione (GSH) to a variety of hydrophobic endogenous and exogenous substrates, rendering them hydrophilicity and facilitating their metabolic processing and eventual secretion from the cell [1,2]. Cancer cells may acquire resistance by overexpressing GST activities, hampering the effectiveness of certain chemotherapeutic drugs [3,4]. Several synthetic drugs and prodrugs exhibiting inhibition potency against GSTs have been proposed as strategies to overcoming MDR attributed to GST overexpression [5-9]. We report on the synthesis and enzymological study of twelve 2,2'-dihydroxybenzophenone and N-carbonyl analogues, **5-16**, and their inhibitory profile vs. hGSTA1-1.

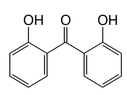
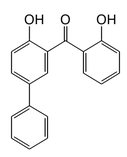
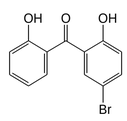
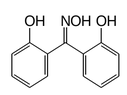
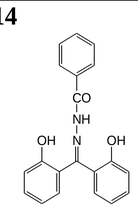
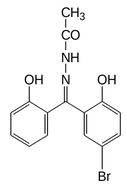
RESULTS

CHEMISTRY. The synthetic routes leading to analogues 5–16 :



'CHERRY-PICKING' FROM THE LIBRARY OF 2,2'-DIHYDROXYBENZOPHENONES & N-CARBONYL ANALOGUES.

Table 1. Inhibition properties for compounds selected from screening experiments ('cherry-picking') against hGSTA1-1 activity (IC_{50}) and Caco2 cell viability (LC_{50}).

Compound number and structure	Modality of inhibition ([a])	IC_{50} against hGSTA1-1 (μM)	LC_{50} against Caco2 cells (μM)
5 	-	-	> 400
6 	Competitive, linear	1,77 ± 0.10	31.4 ± 0.4
8 	Mixed, linear	0,24 ± 0.04	120 ± 1.9
11 	-	-	315 ± 1.4
14 	Competitive, linear	0.33 ± 0.05	87 ± 1.9
16 	Mixed, hyperbolic	0.18 ± 0.02	> 400

[a]Compounds **6**, **8**, **14** and **16** showed mixed inhibition modality against the co-substrate GSH.

ENZYME INHIBITION STUDIES.

Enzyme inhibition screening revealed two 2,2'-dihydroxybenzophenones (6 & 8) and two N-carbonyl analogues (14 & 16) as strong inhibitors against hGSTA1-1 (86-96% inhibition).

• Inhibitors 6 and 14 bind at the CDNB-binding (catalytic) site, showing a purely competitive modality of inhibition (Figure 1 for inhibitor 6). This is in concert with *in silico* molecular docking, predicting that both inhibitors (Figure 2), in their low energy most favored position, clash with CDNB if trying to be accommodated at the site of hGSTA1-1 where CDNB binds.

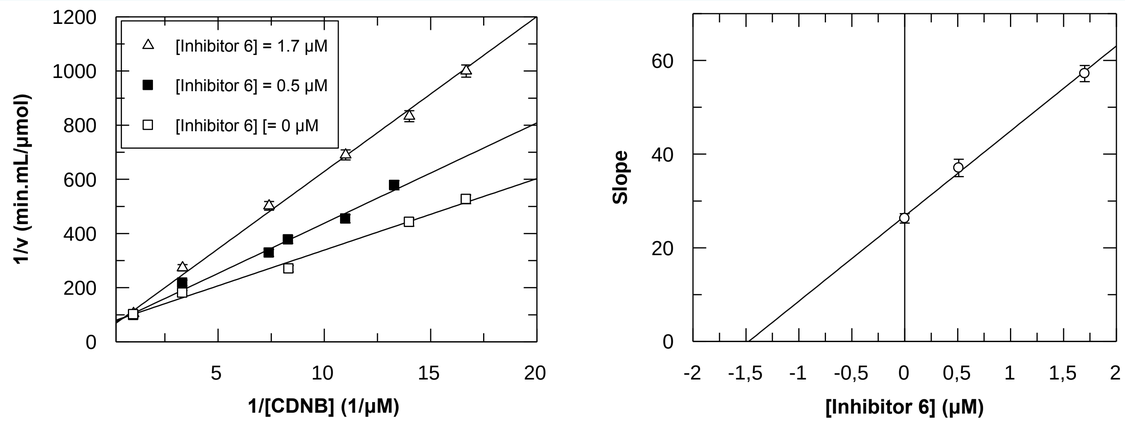


Figure 1. Purely competitive inhibition kinetics of hGSTA1-1 with inhibitor 6 using CDNB as a variable substrate. *Left*: Lineweaver-Burk (primary) plot of initial velocities *vs* [CDNB] at different [inhibitor 6]. *Right*: secondary plot derived from data of the primary plot.

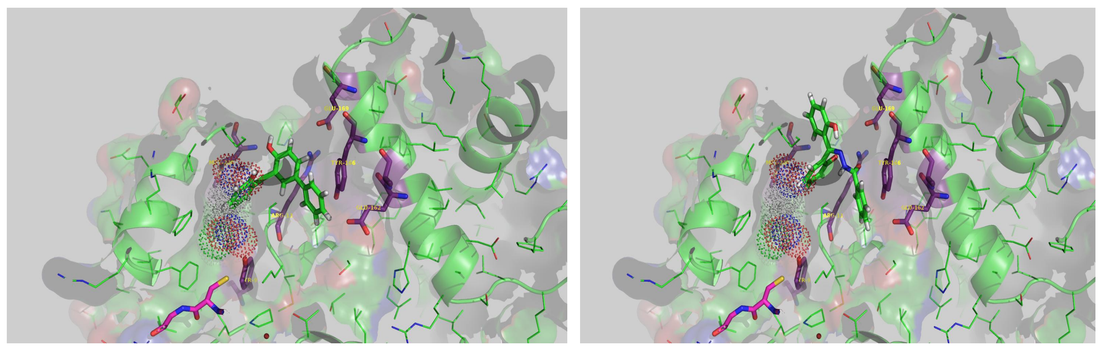


Figure 2. Substrates CDNB, GSH and inhibitors 6 (*left*) and 14 (*right*) at the most probable binding sites of hGSTA1-1. All ligands are shown as *balls-and-sticks*, except for CDNB which is shown as *space filling dot models*. Both inhibitors (green ligands) partly occupy the catalytic site and clash with CDNB when bound at the same site. GSH is depicted in magenta, the S atom in yellow, N atoms in blue and O atoms in red. The figure is created using the PYMOL v1.4 program.

Inhibitors 8 and 16 bind at a site different that the CDNB-binding (catalytic) site, thus showing a mixed modality of inhibition (Figure 3 for inhibitor 8). This is in concert with *in silico* molecular docking, predicting that both inhibitors (Figure 4 for inhibitor 8), in their low energy most favored position, do not bind to the CDNB-binding site.

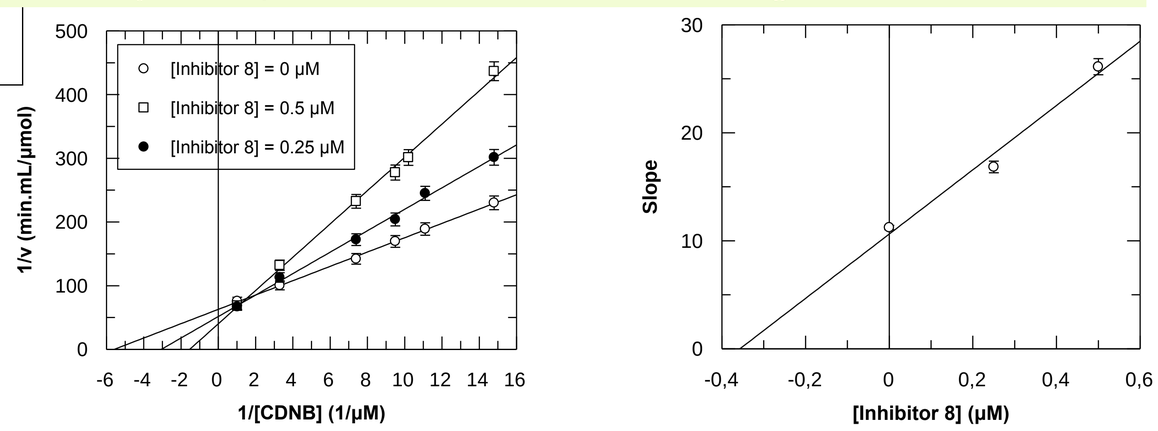


Figure 3. Mixed inhibition kinetics of hGSTA1-1 with inhibitor 8 using CDNB as a variable substrate. *Left*: Lineweaver-Burk (primary) plot of initial velocities *vs* [CDNB] at different [inhibitor 8]. *Right*: secondary plot derived from data of the primary plot. Points are average of three enzyme assays.

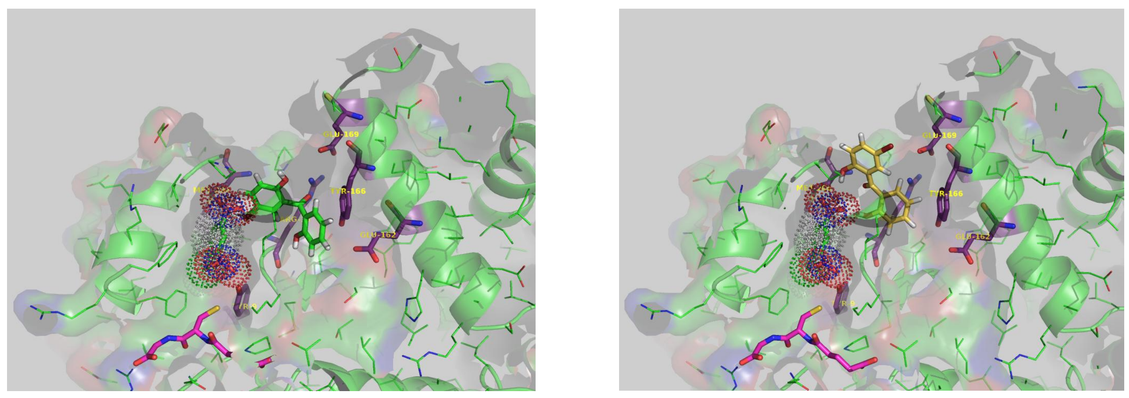


Figure 4. Substrates CDNB, GSH and inhibitor 8 at the most probable binding sites of hGSTA1-1. All ligands are shown as *balls-and-sticks*, except for CDNB which is shown as *space filling dot models*. *Left*: in the absence of CDNB, inhibitor 8 (green ligand) is bound close to CDNB-binding region. *Right*: in the presence of CDNB, inhibitor 8 (yellow ligand) is bound close to CDNB, developing H-bonds (2.56 and 2.76 Å).

CONCLUSIONS: We identified analogues with high inhibitory potency (IC_{50} 0.18-1.8 μM) and modest cytotoxic activity (LC_{50} 35-400 μM), useful as 'lead' structures in designing new inhibitors and prodrugs for human GSTs.

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