

F. Perperopoulou¹, F. Pouliou¹, P. Tsoungas², T. Thireou³, V. Rintas⁴, E. Eliopoulos³, E. Douni^{3,4}, N. Labrou¹, Y. Clonis¹

¹Laboratory of Enzyme Technology and ³Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, GREECE;

²Department of Biochemistry, Hellenic Pasteur Institute, Athens, GREECE; ⁴Division of Immunology, Biomedical Sciences Research Center 'Alexander Fleming', Vari, Greece

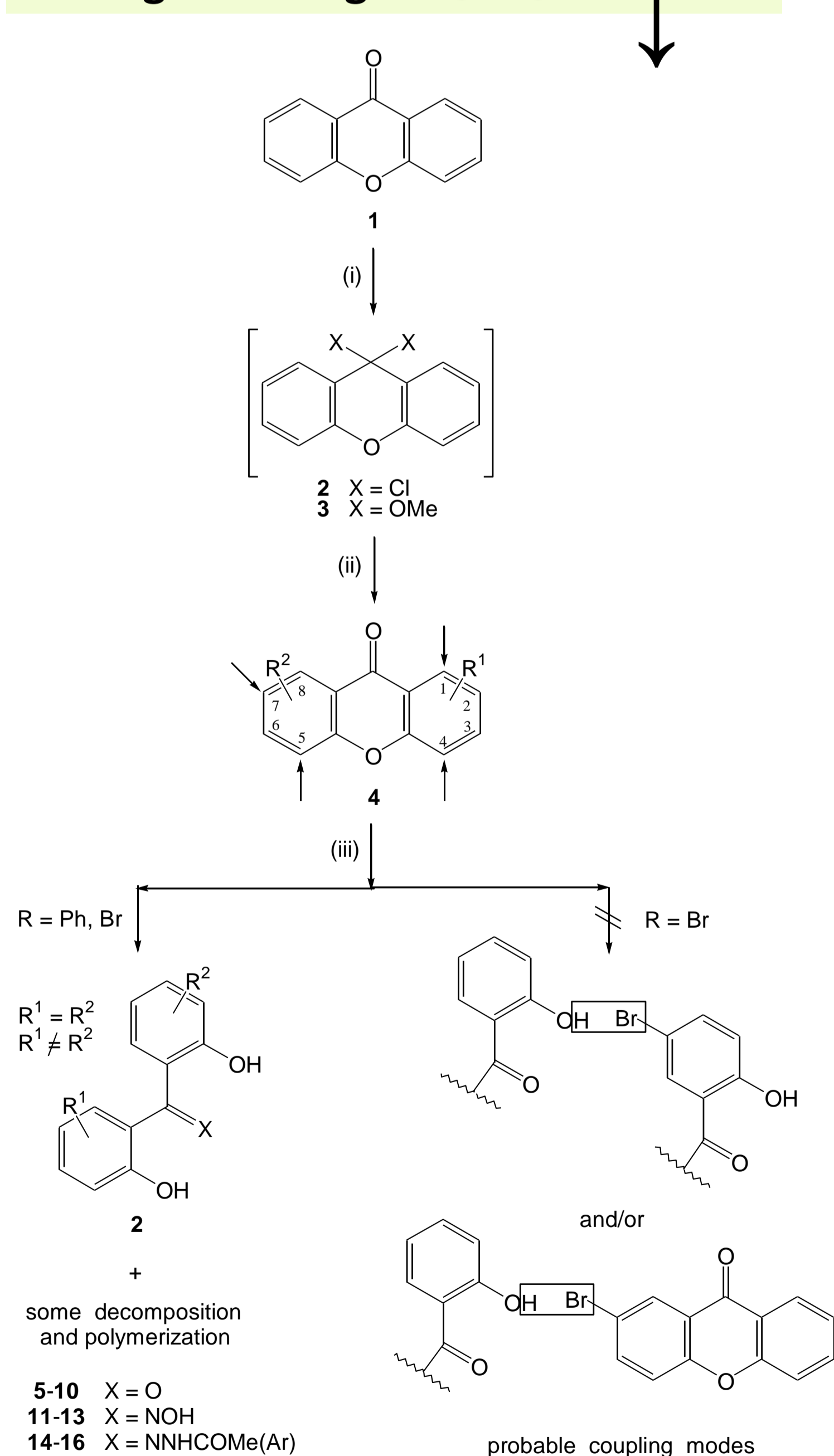
ABSTRACT. Over expression of human GSTA1-1 in tumour cells is part of MDR mechanisms. Substituted 2-hydroxybenzophenones are ubiquitous in naturally occurring and synthetic compounds, exhibiting important biological activities. 2,2'-Dihydroxybenzophenones and N-carbonyl analogues, structurally, are ring-opened 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 ($IC_{50} = 0.18-1.8 \mu M$) and modest cytotoxic activity for Caco2 cell line ($LC_{50} = 35$ to $> 400 \mu M$), 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 (°)	IC_{50} against hGSTA1-1 (μM)	LC_{50} against Caco2 cells (μM)
5 	-	-	> 400
6 	Competitive, linear	$1,77 \pm 0.10$	31.4 ± 0.4
8 	Mixed, linear	$0,24 \pm 0.04$	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.

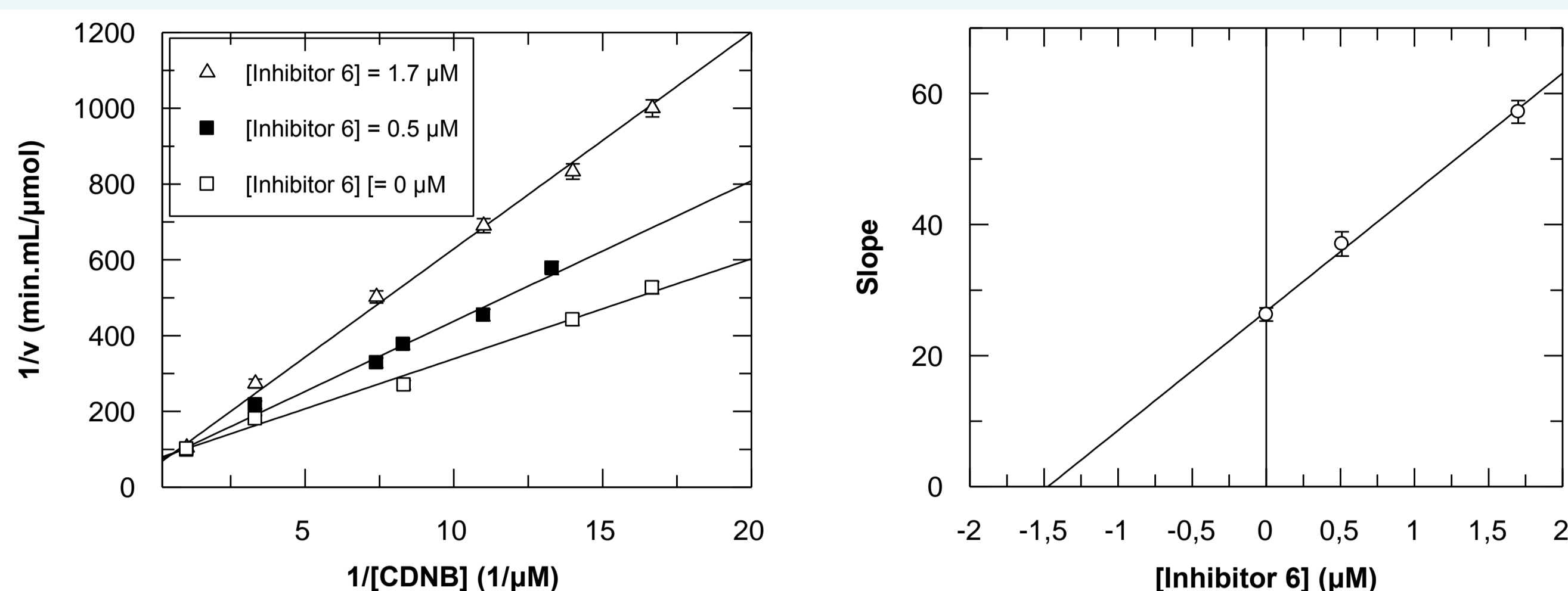


Figure 1. Purely competitive inhibition kinetics of hGSTA1-1 with inhibitor 6 using CDNB as a variable substrate. **Left:** Lineweaver-Burk (primary) plot of initial velocities vs [CDNB] at different [inhibitor 6]. **Right:** secondary plot derived from data of the primary plot.

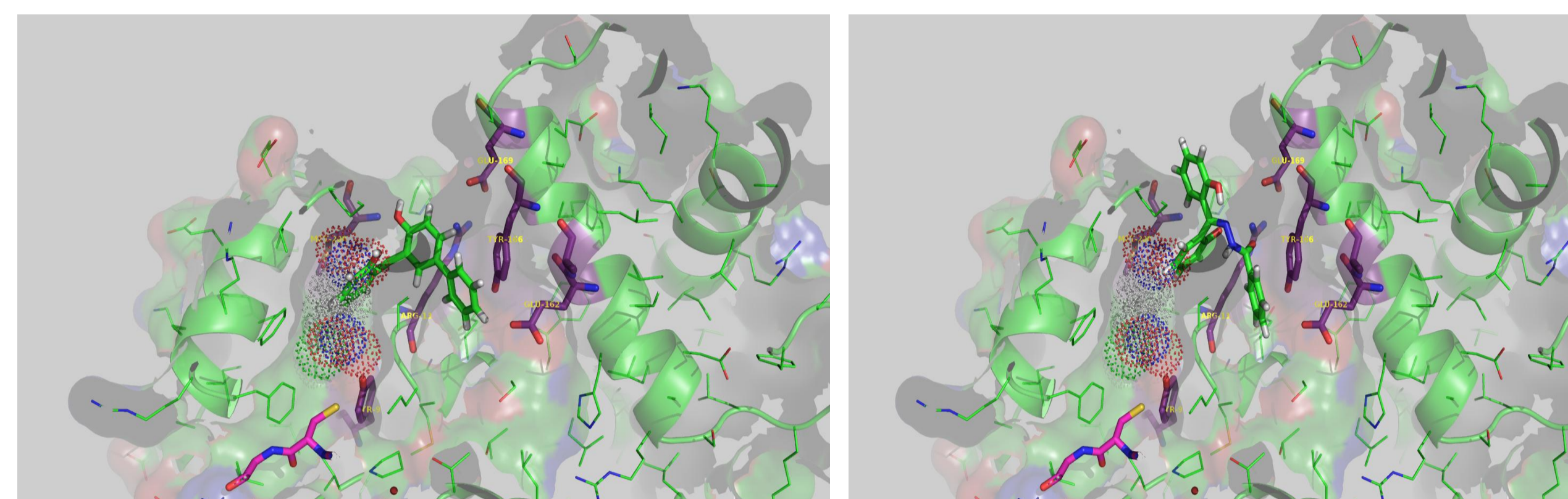


Figure 2. Substrates CDNB, GSH and inhibitors 6 (left) and 14 (right) at the most probable binding sites of hGSTA1-1. All ligands are shown as balls-and-sticks, except for CDNB which is shown as space filling dot models. Both inhibitors (green ligands) partly occupy the catalytic site and clash with 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.

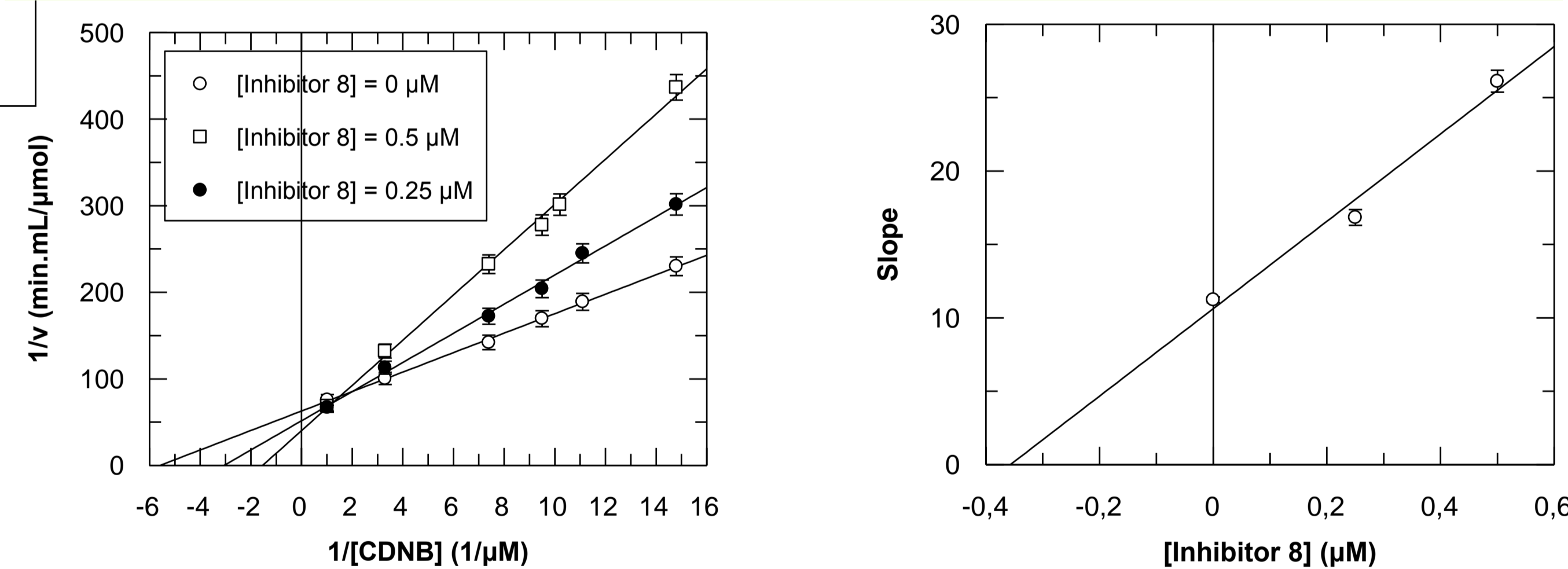


Figure 3. Mixed inhibition kinetics of hGSTA1-1 with inhibitor 8 using CDNB as a variable substrate. **Left:** Lineweaver-Burk (primary) plot of initial velocities vs [CDNB] at different [inhibitor 8]. **Right:** secondary plot derived from data of the primary plot. Points are average of three enzyme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

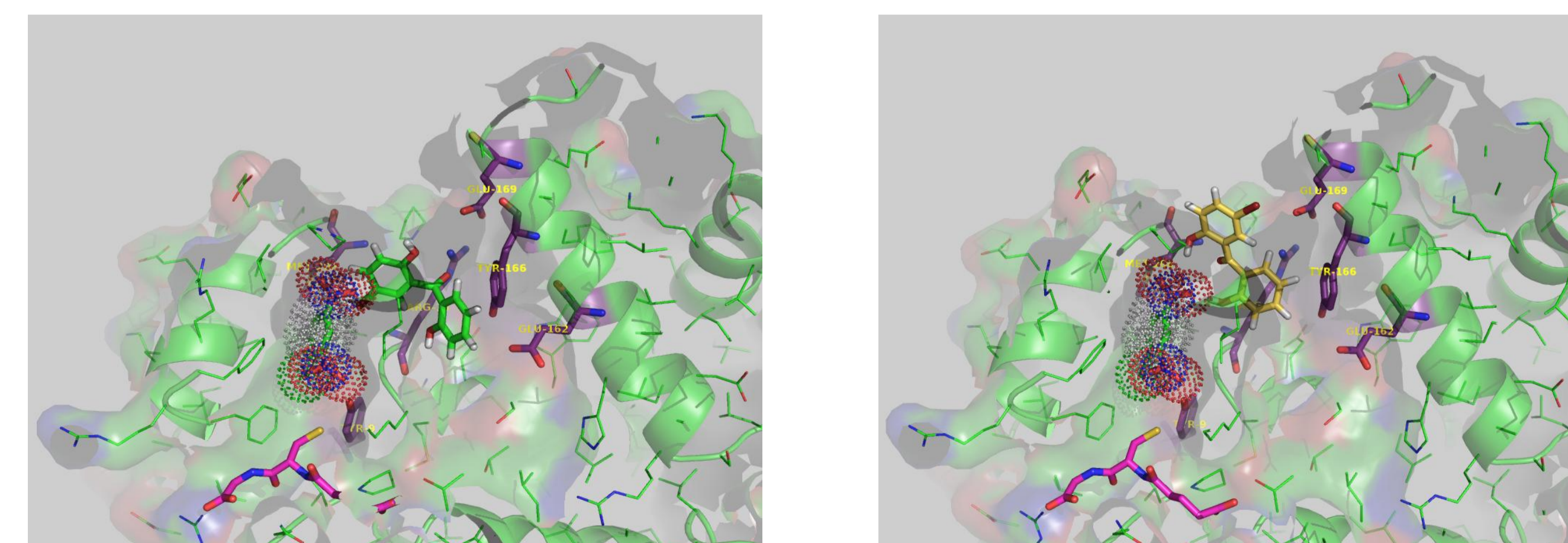


Figure 4. Substrates CDNB, GSH and inhibitor 8 at the most probable binding sites of hGSTA1-1. All ligands are shown as balls-and-sticks, except for CDNB which is shown as space filling dot models. **Left:** in the absence of CDNB, inhibitor 8 (green ligand) is bound close to CDNB-binding region. **Right:**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