Identification of putative substrates and inhibitors for Glutathione S-transferases using computational methods

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INTRODUCTION

Glutathione S-transferases (GSTs) include a family of enzymes that utilizes glutathione (GSH) in enzymatic reactions that involves transformation of several compounds including therapeutic drug molecules and carcinogens. Human cytosolic GSTs are classified into seven different classes, namely alpha, zeta, theta, mu, pi, sigma, and omega. GST are globular proteins contain N-terminal mixed helical and beta-strand domain and all-helical C-terminal domain. Hydrophobic H (substrates or ligand binding site) and hydrophilic G (GSH binding site) forms the active site of GST enzyme. GSTs influence cellular survival, by repressing apoptosis signal-regulating kinase 1 (ASK1) thus affecting the activation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) in response to various intra and extracellular stresses. Molecules inhibiting GST activity received attention as an adjuvant therapy to toxic electrophilic molecules to avoid usage of higher doses and toxicity by these agents. The objective of this study is to explore binding patterns of array of substrates and inhibitors for predominantly expressed seven isoforms of GST (Alpha1 or A1, Alpha2 or A2, Pi1 or P1, Mu1 or M1, Mu2 or M2, Mu5 or M5 and Theta1 or T1).

METHODOLOGY

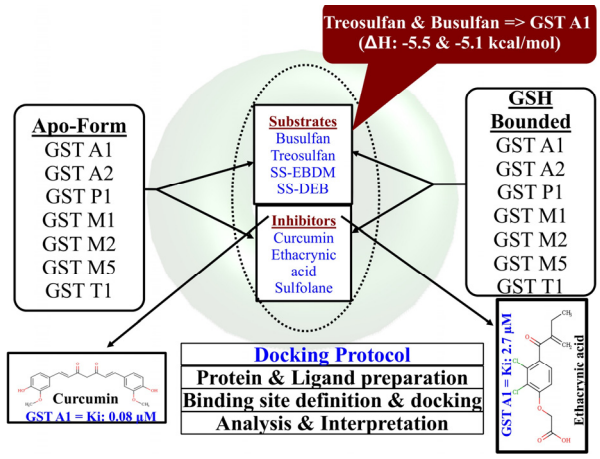


Figure 1: Schematic *work-flow* for the proposed study

1. Retrieval of 3D structures of GSTs and ligand molecules - **Protein Data Bank and PubChem databases**
2. Protein and ligand preparation - **Auto Dock Tools (ADT)**
3. Receptor grid preparation based on a known binding site - **AutoDock/Vina Plugin in PyMOL**
4. Preparation of Auto Dock Vina configuration files
5. Execution of docking calculation - **Auto Dock Vina**
6. Analysis of docking results - **ADT, PyMOL and LigPlus**
7. Calculation of Ki from estimated binding free energies of inhibitor complexes using **DeltaG(inhibition) = R*T*ln(Ki) where R= Constant (J/K.mol), T= Temparature (K)**

RESULTS

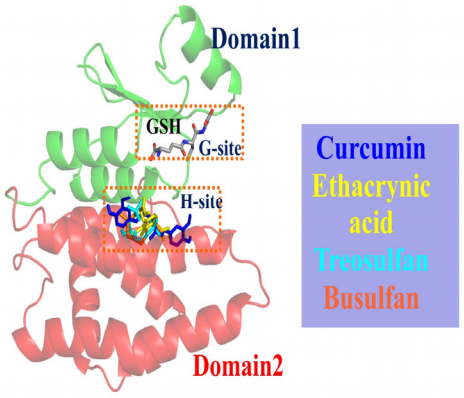


***Figure 3**: Binding orientation of substrates and inhibitors into GST A1*

Table 2: Location of substrates and inhibitors binding in GSTs

Isoforms	Location	Orientation of substrates and inhibitors
GST A1	H-Site	Similar
GST A2	H-Site	Similar
GST P1	H-Site	Similar
GST M1	Closer to H-Site	Similar
GST M2	Closer to H-Site	Similar
GST M5	Closer to H-Site	Similar
GST T1	Closer to H-Site	Similar

RESULTS

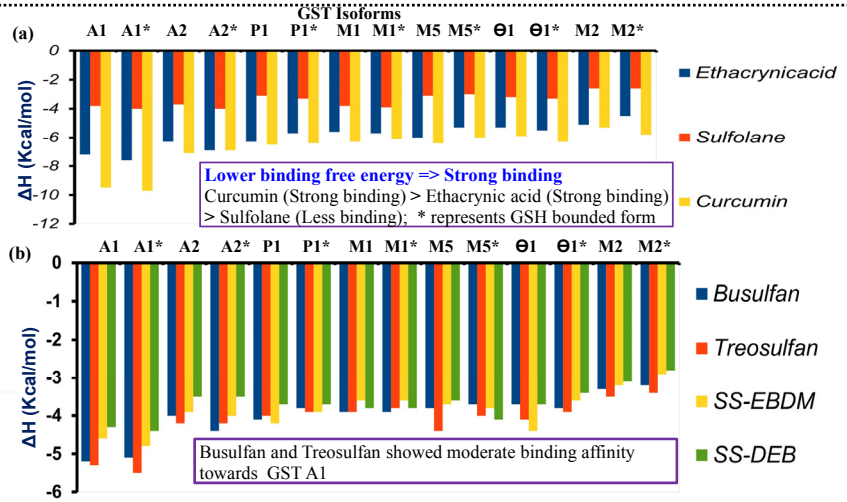


Figure 2: Estimated binding free energy of (a) Inhibitors and (b) Substrates with GST isoforms

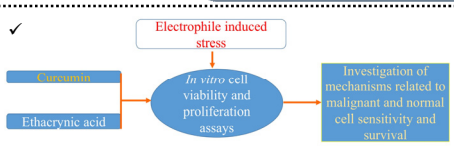
Table 1: Common interacting residues of substrates and inhibitors towards GSTs

GST A1	GST A2	GST P1	GST M1	GST M2	GST T1	GST M5
Ala100	Leu107	Phe8	Leu12	Ile69	Asp8	Trp7
Gly103	Leu108	Tyr108	His107	Thr70	Ile32	Tyr115
Leu107	Phe222	Gly205	Gly111	Gln71	Asp34	
Tyr166			Tyr115	Ala74	Val213	

DISCUSSION & CONCLUSION

- **The proposed methodology is useful to screen new putative GST substrates and inhibitors**.
- **Curcumin showed a significant high binding affinity towards all the classes of GSTs,** particularly GST A1 (ΔH: -9.7 kcal/mol and Ki: 0.08 mM)
- **Order of binding affinity of Curcumin:** GST A1 > GST A2 > GST P1 > GST T1 > GST M1 > GST M5 > GST M2
- **Ethacrynic acid also showed better binding affinity towards GST A1** (ΔH: -7.6 Kcal/mol and Ki: 2.7 µM)
- **Order of binding affinity of Ethacrynic acid:** GST A1 > GST A2 > GST P1 > GST M1 > GST T1 > GST M5 > GST M2
- Sulfolane did not show a stronger affinity towards all the seven GST isoforms.
- **Busulfan and Treosulfan exhibited a reasonable binding affinity towards GST A1** (ΔH: -5.5 and -5.1 kcal/mol) and weakened affinity for the remaining six GST isoforms.
- **Thus, Treosulfan is predicted to be a possible substrate for GST A1.**
- Hypothetical binding site of Substrates/Inhibitors => **H-Site**

ON-GOING AND FUTURE WORK



✓Implementing the methodology to screen other putative substrates and inhibitors of GST isoforms

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