CHAPTER IV

INHIBITION STUDY OF SELENIUM-GLUTATHIONE PEROXIDASE WITH MERCAPTANS USING *IN SILCO* APPROACH

4. INTRODUCTION

The concept of the chemoprevention of cancer as originally proposed refers to prevention of cancer by use of pharmacological agents to inhibit or reverse the process of carcinogenesis. Experimental and clinical studies have shown that a major mechanism for cytotoxic activity of the numerous chemotherapeutic agents is through increased formation of the reactive oxygen species (ROS), including hydroxyl radicals, hydrogen peroxide and superoxide anion (Clemens *et al.*, 1989; Sangeetha *et al.*, 1990; Weijl *et al*., 1997). The reactive oxygen species play an effective role in pathogenesis of different pathological diseases including cancer. Free radical induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and functions of cell membrane.

Glutathione peroxidases (GPXs) are the major enzymes in the antioxidative defense mechanism depending on glutathione. At least six types of GPXs have been identified (Rotruck *et al*., 1973; Papp *et al.*, 2007), and divided into the following types: cytosolic- (cGPX or GPX1), gastrointestinal- (GI-GPX or GPX2), plasma- (pGPX or GPX3), phospholipid hydroperoxide- (PHGPX or GPX4) glutathione peroxidase, GPX5 and GPX6. Glutathione peroxidase reduces hydrogen peroxide to water (Mills, 1957) and organic

hydroperoxides to the corresponding alcohols (Little *et al*., 1970). Intracellular and tissue levels of GPX1 activity affect apoptotic signaling pathway, protein kinase phosphorylation and oxidant-mediated activation of NFκB. Data are accumulating to link alteration or abnormality of GPX1 expression to etiology of cancer, cardiovascular disease, neurodegeneration, autoimmune disease and diabetes. Future research should focus on the mechanism of GPX1 in the pathogeneses and potential applications of GPX1 manipulation in the treatment of these disorders.

Among the most widely found selenoproteins in mammals are thioredoxin reductase and glutathione peroxidase. The enzyme glutathione peroxidase contains stoichiometric amounts of selenium and has been thought to account for the essentiality of selenium as a nutrient (Papp *et al.*, 2007). Selenium (*Se*) is a very important component of antioxidative protective mechanism which belongs to every cell, and there is evidence that this essential trace element has anti-cancerous properties. *Se* exerts its chemoprevention effect in different ways, such as a protective effect against oxidative damage by decreasing the amount of free radicals and increasing the synthesis of glutathione peroxidase (GPX) (Clement, 1998; Rayman, 2000; Patricle, 2004). Understanding the chemistry of selenium at the active site of glutathione peroxidase may help to elucidate some general features of seleno-cysteine-mediated catalysis. Steady state kinetic data do not separate the bimolecular rate constants for the two consecutive reactions of the oxidized forms of glutathione peroxidase with GSH in the absence of a specific effector of the enzyme.

Inhibitors that interact with a given form of the enzyme may prove to be a useful tool to characterize the steps of the enzyme cycle. *Se*-Glutathione Peroxidase has been found to be

inhibited by mercaptans (Chaudiere *et al*., 1984). In practice, the Mercaptans have not afforded great insights into the catalytic cycle of glutathione peroxidase. The studies of these compounds strongly suggest that a search for specific and reversible effectors of the active site of glutathione peroxidase should focus on bi or polyfunctional mercaptans (Chaudiere *et al*, 1984). Thus this specific study was undertaken in view of exploring the possible role of cysteine concerning biomolecules as well as alike synthetic compounds.

4.1 MATERIALS AND METHODS

4.1.1 Receptor X-ray Structure

The 3D coordinates of the crystal structure of the seleno-cysteine to glycine mutant of human glutathione peroxidase 1 (PDB Id.: 2F8A) was retrieved from PDB and taken as the receptor model in flexible docking program. Before docking heteroatom Malonic acid (MLA) was removed from .PDB file of glutathione peroxidase 1 by charge method AMI-BCC using chimera. After removing the water molecule, polar hydrogen atoms were added to protein.

4.1.2 Inhibitors Dataset

The data regarding the experimentally known seven Mercaptans inhibitors, found to be strong and specific inhibitors of the enzyme glutathione peroxidase, was obtained from the literature (Chaudiere *et al*., 1984). The 3D structures of known 7 inhibitors were downloaded in .sdf format from pub-chem compound database. They were later converted in .pdb format by the

help of open babel (O'Boyle *et al*., 2011) software. Babel is a cross-platform program designed to converts chemical objects (currently molecules) from one file format to another.

4.1.3 Molecular Docking

Docking of the 7 inhibitors screened from literature (Chaudiere *et al.*, 1984) against GPX1 structure was done using molecular docking program AutoDock 3.0.5 (Morris *et al*., 1998). Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool (http://autodock.scripps.edu/resources/adt). Kollman charges and the solvation term were then added to the protein structure using the same. The Lamarckian genetic algorithm implemented in Autodock was used. Docking parameters were as follows: 30 docking trials, population size of 150, and maximum number of energy evaluation ranges of 2,50,000, maximum number of generations is 27,000, mutation rate of 0.02, cross-over rate of 0.8. Other docking parameters were set to the software's default values. After docking, the ligands were ranked according to their docked energy as implemented in the AutoDock 3.0.5 program.

4.2 RESULTS AND DISCUSSION

In this work, *in silico* approach has been applied to study the binding of 2-Mercaptoethanol, Cysteine, 2-Mercaptoacetate, 3-Mercaptopropionate, Methyl mercaptoacetate, Methyl (3-mercaptopropionate) and Beta-mercaptopyruvate as potent inhibitors of glutathione peroxidase (GPX1). Molecular docking study was performed to find specific binding mode using AutoDock. From the docking results it was observed that cysteine showed strong

inhibition with GPX1with docking energy of -6.87 kcal/mol. 2-Mercaptoethanol, Methyl mercaptoacetate and methyl 3-mercaptopropionate caused only moderate inhibition. The inhibition by cysteine may be explained by a preferential interaction of the sulfhydryl group with the amino group of GLN78. Besides, a sulfhydryl group likely to be essential for inhibition, is supported by two lines of evidence. First, analogs of mercapto-succinate that do not bear sulfur-containing group, such as malate and succinate, are not inhibitor. Second, substitution of an S-alkyl group for a sulfhydryl group also relieves the inhibition (Chaudiere *et al.*., 1984; Table 4.1, 4.2 and Fig. 4.1).

Table 4.1: List of inhibitors known to be active against Glutathione Peroxidase.

Sl. No	CID No.	Name	MWT (g/mol)	Molecular formula	X Log P	HBD	HBA
1	1567	2-Mercaptoethanol	78.13344	C_2H_6OS	-0.2	2	2
2	5862	Cysteine	121.15818	$C_3H_7NO_2S$	-2.5	3	4
3	5086465	2-Mercaptoacetate	91.10902	$C_2H_3O_2S^-$	.8	1	3
4	7057961	3-Mercaptopropionate	105.1356	$C_3H_5O_2S^-$	1.1	1	3
5	16907	Methyl mercaptoacetate	106.14354	$C_3H_6O_2S$	.4	1	3
6	18050	methyl 3-mercaptopropionate	120.17012	$C_4H_8O_2S$	0.3	1	3
7	98	Beta-mercaptopyruvate	120.12706	$C_3H_4O_3S$	0	2	4

Table 4.2: The docking results of Mercaptocarboxylic acids and related compounds with human Glutathione Peroxidase.

Sl. No	Name	Binding Energy (Kcal/mol)	Docking Energy (Kcal/mol)	Intermol Energy (Kcal/mol)	Torsional Energy (Kcal/mol)	Internal Energy (Kcal/mol)
1	2-Mercaptoethanol	-3.25	-3.52	-3.57	0.31	0.04
2	Cysteine	-6.16	-6.87	-6.79	0.62	-0.09
3	2-Mercaptoacetate	0.56	0.23	0.25	0.31	-0.02
4	3-Mercaptopropionate	0.42	-0.29	-0.2	0.62	-0.09
5	Methyl mercaptoacetate	-3.72	-4.2	-4.34	0.62	0.14
6	methyl 3-mercaptopropionate	-3.9	-4.8	-4.83	0.93	0.03
7	Beta-mercaptopyruvate	-0.23	-0.81	-0.86	0.62	0.04

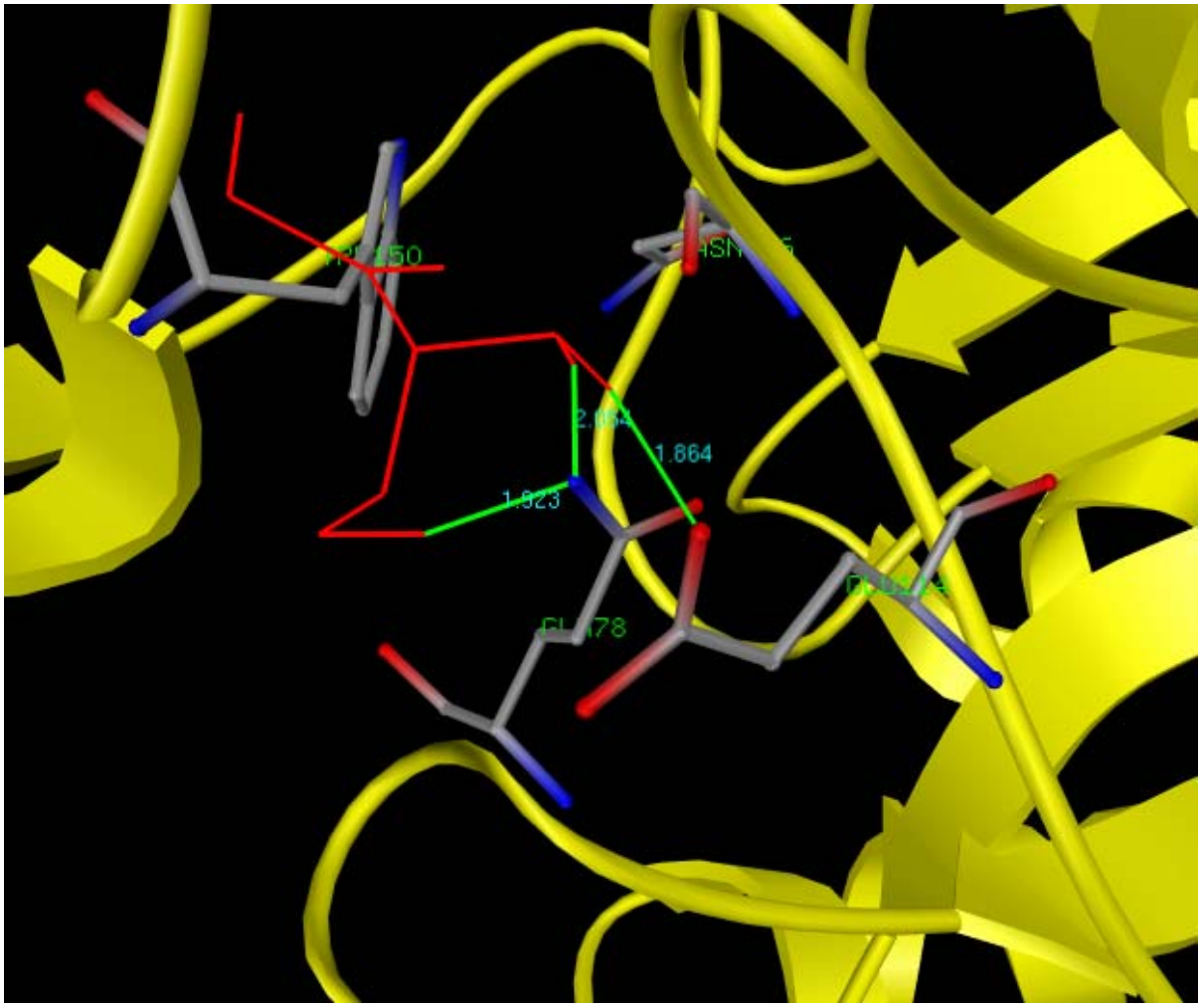

Fig 4.1: Three H-bonds are formed between amino acid GLN78 (NE2), GLU114 (OE1) and GLN78 (NE2) of GPX1 (Pdb Id.: 2F8A) with Cysteine (H1), (H2), (HG), respectively. Inhibitor Cysteine is shown in line representation and is colored with red using Python Molecular Viewer. Amino acid residues GLN78, GLU114, TRP150 and ASN156 are represented in the pattern of stick model. Hydrogen bond is represented by green line.

Glutathione is widely found in all forms of life and plays an essential role in the health of organisms, particularly aerobic ones. In humans, animals, and plants, glutathione is the predominant non-protein sulfhydryl group and functions most especially as an antioxidant, keeping its own -SH groups and related proteins in a reduced (non-oxidized) condition (Sies, 1999). Though there are undoubtedly multiple functions for glutathione yet to be appreciated it is known that glutathione is: (a) a co-factor for the glutathione peroxidases, which are crucial selenium-containing antioxidant enzymes; (b) a co-factor for glutathione S-transferases, enzymes which are involved in the detoxification of xenobiotics, including carcinogens; (c) involved in the regeneration of ascorbate (Vitamin C) from its oxidized form, dehydro-ascorbate (Exner *at al*., 2000). Glutathione itself is a non-essential nutrient composed of three amino acids: glutamic acid, glycine and cysteine, or more exactly the tripeptide L-gamma-glutamyl-Lcysteinyl-glycine. Availability of cysteine is a limiting factor in the liver's synthesis of glutathione. Chronic functional glutathione deficiency is associated with immune disorders, an increased incidence of cancer and in the case of HIV disease, probably accelerated pathogenesis of the disease. (Novi, 1981; Palamara *et al*., 1995). Acute manifestations of functional glutathione deficiency can be seen in those who have taken an over-dosage of acetaminophen (Tylenol). A vital role of glutathione is the maintenance of a normal redox state of the liver. An overdose of acetaminophen leads to its metabolism into large quantities of N-acetyl-benzo-quinoneimine (NABQI) in the liver. NABQI depletes hepatic glutathione stores, placing an enormous oxidative stress on the liver, leading to liver failure (Exner *at al*., 2000).

4.3 CONCLUSION

Molecular docking study was performed in order to find specific binding mode using AutoDock 3.0.5. From the docking results it was observed that Cysteine showed strong inhibition with GPX1 with docking energy of -6.87 kcal/mol. Taken together the previous available results, the findings of this chapter provide new insight into the mechanism of glutathione peroxidase with reference to oral cancer diagnostics.