TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

INHIBITORY ACTIONS OF GIBBERELLIC ACID AND ABSCISIC ACID ON HUMAN PLACENTAL GLUTATHIONE S-TRANSFERASE P1-1

MOHAMMAD ABU ZAID

MASTER OF SCIENCE THESIS

MEDICAL BIOCHEMISTRY PROGRAM

NICOSIA

2021

TURKISH REPUBLIC OF NORTHERN CYPRUS
NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES

INHIBITORY ACTIONS OF GIBBERELLIC ACID AND ABSCISIC ACID ON HUMAN PLACENTAL GLUTATHIONE S-TRANSFERASE P1-1

MOHAMMAD ABU ZAID

MASTER OF SCIENCE THESIS
MEDICAL BIOCHEMISTRY PROGRAM

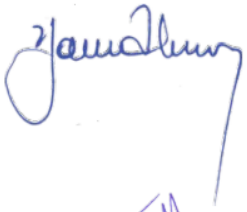
SUPERVISOR
Prof. Dr. ÖZLEM DALMIZRAK

NICOSIA
2021

The Directorate of Institute of Graduate Studies,

This study has been accepted by the thesis committee in Medical Biochemistry program as a Master of Science Thesis.

Thesis committee:

Chair of committee:	Professor Özlem DALMIZRAK Near East University	
Member:	Professor Yasemin AKSOY Hacettepe University	
Member:	Associate Professor Kerem TERALI Near East University	
Supervisor:	Professor Özlem DALMIZRAK Near East University	

Approval:

According to the relevant article of the Near East University Postgraduate Study-Education and Examination Regulation, this thesis has been approved by the above-mentioned members of the thesis committee and the decision of the board of Directors of the institute.

Professor K. Hüsnü Can BAŞER
Director of Institute of Graduate Studies

DECLARATION

I hereby declare that the work in this thesis entitled “Inhibitory Actions of Gibberellic Acid and Abscisic Acid on Human Placental Glutathione S-Transferase P1-1” is the product of my own research efforts undertaken under the supervision of Professor Özlem Dalmızrak. No part of this thesis was previously presented for another degree or diploma in any University elsewhere, and all information in this document has been obtained and presented in accordance with academic ethical conduct and rules. All materials and results that are not original to this work have been duly acknowledged, fully cited and referenced.

Name, Last Name: Mohammad Abu Zaid

Signature:

Date: 07.04.2021

ACKNOWLEDGEMENTS

I would like to thank many supporters who have assisted me with my project and offered me personal support while at the Near East University. First of all, I would like to thank my advisor, Professor Özlem Dalmızrak. She has provided excellent knowledge and guidance during my research and has greatly enriched my experience as a graduate student by her participation with the lab group. I am grateful for all the experiences and lectures I had the fortune to attend during my education.

It is a pleasure to express my deep sense of thanks to Professor Nazmi Özer for his instruction and support during my postgraduate study. I owe a deep sense of gratitude to Associate Professor Kerem Teralı for his support and encouragement during my postgraduate study. I am grateful to Professor Hamdi Öğüş for his contribution during my postgraduate study. I am grateful to all who supported me and to my classmates Pavel Awat, Mohamed Dageem and Umar Ghali who stand by my side during the studies of my thesis.

Finally, I express my profound gratitude to my father and my mother for their support, constant encouragement through all my years of study and through the process of researching and writing the thesis.

TABLE OF CONTENTS

ABBREVIATIONS

ABA	: Abscisic acid
ASK1	: Apoptosis signal-regulating kinase 1
BSA	: Bovine serum albumin
CDNB	: 1-chloro-2,4-dinitrobenzene
CNPSG	: S-(2-Chloro-4-nitro-phenyl)glutathione
DCNB	: 3,4-dichloro-nitrobenzen
DNA	: Deoxyribonucleic acid
DsbA	: Disulfide oxidoreductase
EDTA	: Ethylenediaminetetraacetic acid
G-site	: Glutathione binding site
GA	: Gibberellic acid
GS-DNB	: 1-(S-glutathionyl)-2,4-dinitrobenzene
GSH	: L-glutathione, reduced
GST	: Glutathione S-transferase
H-site	: Electrophilic substrate binding site
*hp*GSTP-P1	: Human placental glutathione S-transferase pi
IC_{50}	: Half maximum inhibitory concentration
JNK-1	: c-Jun-N terminal kinase 1
K_i	: Inhibition constant
K_m	: Michaelis-Menten constant
LANCL2	: Lanthionine synthetase C-like 2
MAPEG	: Membrane-associated Proteins in Eicosanoid and Glutathione metabolism
MAPK	: Mitogen-activated protein kinase
MKK4	: Mitogen-activated protein kinase kinase 4
mPGES	: Microsomal prostaglandin E2 synthase-1 and-2
NBDHEX	: S-2,4-Dinitrobenzylglutathione-(6-(7-nitro-2,1,3-benzoxadiazol -4-ylthio)hexanol
PPARγ	: Peroxisome proliferator-activated receptor gamma
SAPK	: Stress activated protein kinase
SNAr	: Nucleophilic aromatic substitution process

STAT : Signal transducer and activator of transcription

TRAF2 : Tumor necrosis factor receptor-associated factor 2

V_m : Maximum velocity

LIST OF FIGURES

Page No

LIST OF TABLES

Thesis Title: Inhibitory Actions of Gibberellic Acid and Abscisic Acid on Human Placental Glutathione S-transferase P1-1

Name of the Student: Mohammad Abu Zaid

Supervisor: Professor Özlem Dalmızrak

Department: Medical Biochemistry

ABSTRACT

Aim: Gibberellic acid (GA), a member of the gibberellins family, serves as a plant hormone. Abscisic acid (ABA) is a phytohormone with a sesquiterpene structure that plays an important role in cellular processes such as signal transduction. It is the main plant hormone that helps in leaf abscission. Accumulation of ABA leads to various stress responses, and its modulation helps in maintaining the defense mechanism in growth processes in plants. Glutathione S-transferase (GST) is an enzyme with important roles in the detoxification of endo- and xenobiotics by catalyzing their conjugation to reduced glutathione (GSH), and regulation of cell survival and apoptosis by inhibiting c-Jun N-terminal kinase-1 (JNK-1). In this study, the interaction of human placental GSTP1-1 (*hp*GSTP1-1) with ABA and GA was investigated.

Materials and Methods: Different concentrations (0.15625 to 5 mM) of ABA and GA were tested to examine the inhibitory potential of these molecules on *hp*GSTP1-1. Inhibitory kinetic experiments were carried out at four chosen inhibitor concentrations (0.25, 0.5, 1 and 2 mM) while keeping one substrate variable and the other constant, and vice versa. The inhibition types and kinetic parameters were determined from graphs and by using SPSS version 20.

Results: The IC_{50} values of ABA and GA were 5.4 mM and 5.0 mM, respectively. When abscisic acid was used as an inhibitor, competitive type of inhibition was observed with respect to both substrates, GSH and CDNB. At fixed CDNB and variable GSH concentrations V_m, K_m and K_i values were calculated as 205 ± 12 μmol/min-mg protein, 1.32 ± 0.14 mM and 1.95 ± 0.23, respectively. At fixed GSH and variable CDNB concentrations V_m, K_m and K_i values were found to be 166 ± 4 μmol/min-mg protein, 0.76 ± 0.04 mM and 1.79 ± 0.14 mM, respectively. Gibberellic acid also inhibited *hp*GSTP1-1 in a competitive manner. When GSH was

variable and CDNB was fixed, V_m, K_m and K_i values were calculated as 303 ± 10 μmol/min-mg protein, 1.77 ± 0.1 mM and 3.38 ± 0.24 mM, respectively. At fixed GSH and variable CDNB concentrations V_m, K_m and K_i values were found to be 249 ± 7 μmol/min-mg protein, 1.43 ± 0.07 mM and 2.89 ± 0.19 mM, respectively.

Conclusion: However, our *in vitro* study of these phytohormones shows their capacity to inhibit the function of *hp*GSTP1-1, there are also some conditions that should be considered for the development of a new therapeutic drug, including the drug's cytotoxicity and the application of preclinical trials accompanied by clinical trials.

Keywords: Human placental glutathione S-transferase, inhibitory kinetics, abscisic acid, gibberellic acid

Tez Başlığı: Gibberellik Asit ve Absisik Asidin İnsan Plasental Glutatyon S-Transferaz P1-1 Enzimini İnhibe Edici Etkileri

Öğrencinin Adı/Soyadı: Mohammad Abu Zaid

Danışman: Prof. Dr. Özlem Dalmızrak

Anabilim Dalı: Tıbbi Biyokimya

ÖZET

Amaç: Gibberellinler ailesinin bir üyesi olan gibberellik acid (GA) bitki hormonu olarak görev yapmaktadır. Absisik asit (ABA) seskiterpen yapısına sahip bir fitohormondur ve sinyal iletimi gibi hücresel süreçlerde rolü bulunmaktadır. Yaprak dökülmesine yardımcı ana bitki hormonudur. ABA birikimi çok çeşitli stres yanıtı oluşturmakta ve düzenlenmesi bitkilerde büyüme sürecinde koruma mekanizmasının sağlanmasına yardımcı olmaktadır. Glutatyon S-transferaz (GST) endo ve ksenobiyotikleri redükte glutatyona (GSH) konjuge ederek detoksifikasyondan sorumlu bir enzimdir. Ayrıca c-Jun N-terminal kinaz-1 (JNK-1)'i inhibe ederek hücrenin yaşamasını ve apoptozu düzenlemektedir. Çalışmamızda insan plasental GSTP1-1 (*hp*GSTP1-1) enzimi ile ABA ve GA arasındaki etkileşimin araştırılması hedeflenmiştir.

Gereç ve Yöntem: ABA ve GA'nın *hp*GSTP1-1 enzimini inhibisyon potansiyelini araştırmak amacıyla enzim aktivitesi tepkime ortamına artan derişimlerde (0.15625 to 5 mM) ABA ve GA eklenerek ölçülmüştür. Kinetik çalışmalar dört inhibitör derişimi (0.25, 0.5, 1 and 2 mM) belirlenerek ve her ölçümde bir substratı değişken diğerini sabit (ve tam tersi olacak şekilde) tutarak gerçekleştirilmiştir. İnhibisyon türü ve kinetik parametreler farklı grafikler ile ve aynı zamanda SPSS (versiyon 20) kullanılarak hesaplanmıştır.

Bulgular: ABA ve GA için IC_{50} değerleri sırasıyla 5.4 mM ve 5.0 mM olarak bulunmuştur. ABA, enzimi her iki substrata (GSH ve CDNB) karşı kompetitif olarak inhibe etmektedir. Sabit CDNB ve değişken GSH derişimleri kullanılarak yapılan ölçümlerde V_m, K_m ve K_i değerleri sırasıyla 205 ± 12 μmol/dk-mg protein, 1.32 ± 0.14 mM ve 1.95 ± 0.23 olarak hesaplanmıştır. Sabit GSH, değişken CDNB deişimlerinde ise V_m, K_m ve K_i değerleri sırasıyla 166 ± 4 μmol/dk-mg protein, 0.76 ± 0.04 mM ve 1.79 ± 0.14 mM olarak bulunmuştur. Gibberellik asit de *hp*GSTP1-1

enzimini kompetitif olarak inhibe etmektedir. GSH değişken substrat olarak kullanıldığında, V_m, K_m ve K_i değerleri sırasıyla 303 ± 10 μmol/dk-mg protein, 1.77 ± 0.1 mM ve 3.38 ± 0.24 mM olarak; CDNB değişken olduğunda V_m, K_m ve K_i değerleri sırasıyla 249 ± 7 μmol/dk-mg protein, 1.43 ± 0.07 mM ve 2.89 ± 0.19 mM olarak bulunmuştur.

Sonuçlar: Çalışmamız ABA ve GA'nın *in vitro* olarak *hp*GSTP1-1 enzimini inhibe etme kapasitesini gösterse de, bu ve benzeri molekülleri esas alarak tasarlanacak yeni terapötik ilaçların geliştirilmesinde sitotoksisite, preklinik ve klinik çalışmalarda kullanılabilirlik gibi faktörlerin dikkate alınmasında fayda olacaktır.

Anahtar Sözcükler: İnsan plasental glutatyon S-transferazı, inhibitör kinetiği, absisik asit, gibberellik asit

1. INTRODUCTION

Glutathione S-transferase (GST, E.C. 2.5.1.18) is a detoxifying enzyme (phase II) that plays a pivotal role in metabolic pathways. It helps in conjugating antitumor drugs with glutathione through its catalytic activity so as to help smooth excretion via export pumps (Teodori et al., 2006). GST was also found to regulate signal transduction pathways. This involves complex protein–protein interactions, –for example between GSTP1 and stress-activated protein kinase (SAPK), c-jun N-terminal kinase 1 (JNK-1) and between GSTM1 and the apoptosis signal regulating kinase 1 (ASK1) (Singh, 2015). Its activity was also found to be high in tumor cells that are treated with chemotherapy (Dong et al., 2019). Based on localization, GST is divided as: microsomal, cytosolic, and mitochondrial. The cytosolic GST has many structural similarities with the mitochondrial GST (Allocati et al., 2018). There are also non-enzymatic functions of GSTs such as modulating signal transduction pathways e.g in apoptosis and cell survival (Laborde, 2010).

Inhibition of GST enhances the process of cell death by increasing the level of reactive oxygen species that leads to the dissociation of GSTP1 from the JNK (Mohammad et al., 2019). Most of the molecules targeted the tyrosine residues at the active sites of the enzyme. Reversible and irreversible inhibitors have been synthesized to inhibit the activity of the enzyme. The setbacks of some irreversible inhibitors are low permeability to cells. Recently, Shishido et al. has modified an irreversible inhibitor by introducing a sulfonyl fluoride into chloronitrobenzene, which then helps to form sulfonyl ester bonds (Shishido et al., 2019).

GST is evidently a crucial resistance factor for anticancer drugs and has been the subject of intensive pharmaceutical studies in an effort to produce more powerful anticancer agents (Chen et al., 2013). Overexpression of GSTP1-1 in many types of cancer has since been observed, thereby rendering the enzyme as a good target for anticancer therapy as well as anticancer drugs (Laborde, 2010). The use of natural products especially of plant origin has received much attention within the treatment and prevention of cancer and other diseases. Pesticides and antiparasitic agents such as avermectin and its derivatives were found to decrease the activity of GST in a concentration dependent manner (Teralı et al., 2018).

Gibberellic acid (GA) is a plant hormone, but is a diterpenoid carboxylic acid. As a member of the gibberellins family, it serves as a plant regulator hormone. GA was found to stimulate the activity of alpha amylase in bean leaves and down regulate the activity of beta amylase; while in the roots the activity of beta amylase was increased (Dahlstrom and Sfat, 1961). There is a large interaction between abscisic acid (ABA) and GA; as a signal, GA is used for ABA and ethylene-induced production of phenolic acid (Liang et al., 2013).

Abscisic acid (ABA) is a phytohormone with a sesquiterpene (15 carbon atoms) structure that plays an important role in cellular processes such as signal transduction (Cui et al., 2020). It has an essential role in leaf senescence. Accumulation of ABA leads to several stress responses, and its modulation helps in maintaining the defense mechanism in growth processes in plants (Chen et al., 2020). It is synthesized both by plants and phytopathogenic fungi via two different pathways (direct and indirect). Plants uses the indirect pathway (carotenoid pathway) while phytopathogenic fungi use the mevalonate pathway (direct pathway) (Arc et al., 2013; Izquierdo-Bueno et al., 2018; Takino et al., 2018).

Based on literature search, there was no study to explain the interaction of human GSTpi with ABA and GA. Therefore, inhibitory actions of gibberellic acid and abscisic acid were tested against human placental GSTP-1 (*hp*GSTP1-1).

Aims and objectives:

(i) To estimate the IC_{50} values of both ABA and GA

(ii) Investigate the effect of inhibition of ABA and GA on *hp*GSTP1-1.

2. GENERAL INFORMATION

2.1. Glutathione S-Transferase

Glutathione S-transferases (GSTs) are an abundant family of dimeric proteins that have the ability to conjugate glutathione (GSH) with variety of drugs containing electrophilic centers (Croom, 2012). In some mammalian organs, GSTs can constitute up to 10 percent of the cytosolic proteins. To make the molecules more water-soluble, GSTs catalyze the conjugation of compounds with electrophilic centers to GSH via sulfhydryl group (Oakley, 2011). This activity detoxifies endogenous compounds such as peroxidized lipids and makes the degradation of xenobiotics (Leaver and George, 1998). Other functions of GST enzyme include peroxidation and isomerisation, interaction with proteins in signaling pathways, steroid synthesis, synthesis and degradation of eicosanoids, aromatic amino acid degradation and non-catalytical binding to several exogenous and endogenous ligands such as bilirubin, steroid hormones and heme (Oakley, 2011).

2.2. Distribution of Glutathione S-Transferases

Organisms that contain GST enzymes include aerobic bacteria, protozoa and all eukaryotes (plants, insects, reptiles, fish, mammals, etc.). GSTs are not found either in Anaerobic bacteria and Archaea bacteria (Allocati et al., 2009). They are divided into three major families in mammals: cytosolic GSTs, mitochondrial GSTs, and microsomal GSTs. Many distinct gene-independent groups are cytosolic GSTs; each category of GST isoenzymes has common sequences and structural properties (Board and Menon, 2013).

2.3. Classification of Glutathione S-Transferases

The human glutathione transferase superfamily is divided into three major classes, the names of which depend loosely on their intracellular localization. These families are membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) or known as microsomal GSTs, mitochondrial GSTs, and cytosolic GSTs. The human mitochondrial GST family has only one member, GST Kappa (Allocati et al., 2018). Human MAPEG family includes the following

enzymes, mGST1, mGST2, mGST3, microsomal prostaglandin E synthase-1 (mPGES1), leukotriene C4 synthase and 5-lipoxygenase activating protein (Molina et al., 2008). In mammals, the cytosolic GST family is the main GST family. It consists of 7 subclasses per class from 1 to 5 members. These cytosolic GST enzymes are alpha (5 members), pi (1 member), mu (5 member), sigma (1 members), theta (2 members), omega (2 member) and zeta (1 member). There are totally 16 confirmed functioning human GSTs (Nebert and Vasiliou, 2004).

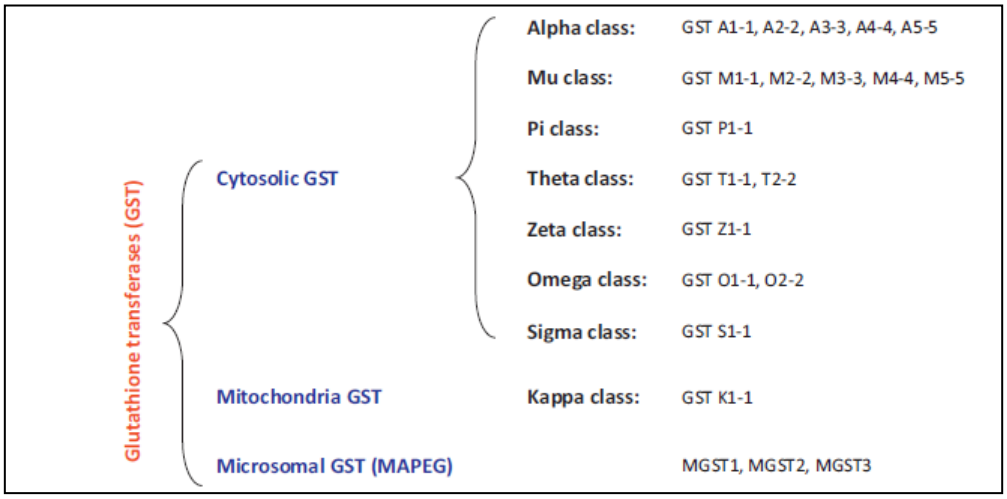


Figure 2.1. Classification of human GSTs (Wu and Dong, 2012)

2.4. The Structure of Glutathione S-Transferases and the Active Site

Due to their role in many important biological processes, such as prostaglandin and steroid biosynthesis, tyrosine catabolism, apoptosis and drug resistance in cancer, there has been increased interest and studies on human GSTs (Mannervik and Danielson, 1988; Wu and Dong, 2012). Cytosolic GSTs have a conserved topology (Figure 2.2) (Bocedi et al., 2019). The cytosolic GST forms a homodimer or heterodimer in order to obtain catalytic activity. Each protomer is approximately 25 kDa in size and has two distinct domains, the N-terminal and the C-terminal domains. A portion of the active site residues is present within each domain. The N-terminus contains a strongly conserved GSH binding site (G-site). Electrophilic second substrate binding occurs at the hydrophobic binding site (H-site) located at the C-terminus. In both the N-terminal and C-terminal regions, monomer dimerization occurs at a dimer interface motif (Allocati, 2018).

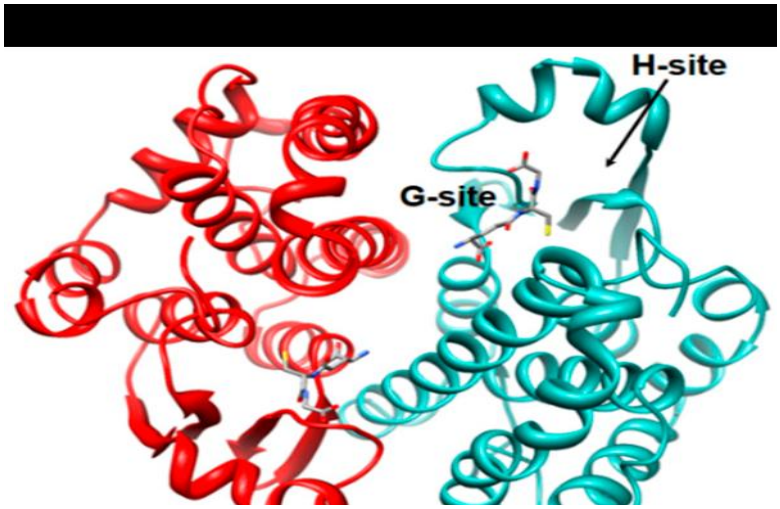


Figure 2.2. Structure of glutathione S-transferase pi (Bocedi et al., 2019).

Dependent on the subunit arrangement, GST isoenzymes are named differently. The GSTA1-2 enzyme, for instance, is in the Alpha class and is composed of heterodimeric subunits 1 and 2. The three dimensional structures of different izoenzymes are shown in Figure 2.3.

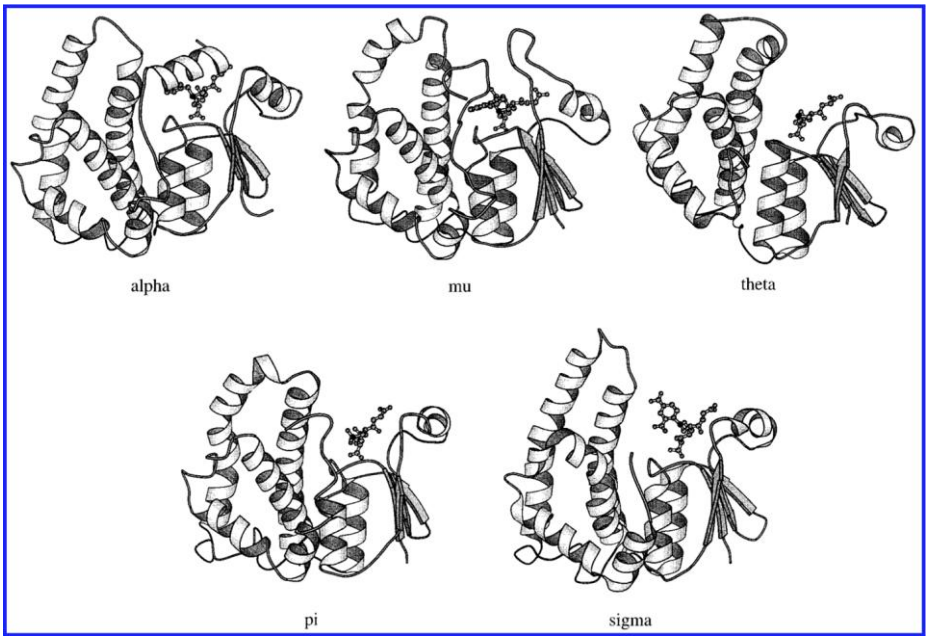


Figure 2.3. A three-dimensional structure of pi, sigma, alpha, mu, theta classes ribbon diagram. These views are from single subunits which are perpendicular toward the two-fold axis of each dimer (Armstrong, 1997).

Glutathione (L-glutamyl-cysteinyl-glycine) is an endogenously synthesized tripeptide. It consists of a glutamate-peptide bound to a cysteinyl glycine dipeptide (Figure 2.4) (Mandal et al., 2017). At the active site of GST, the cysteine thiol is ionized to thiolate and attacks the electrophilic center of the second substrate. GSH conjugation positions the electrophilic center of a co-substrate with a comparatively inert carbon-sulfur bond. As a consequence of GSH conjugation, the conjugate is less reactive and potentially less hazardous than the electrophile. GSH conjugation also decreases transmembrane mobility by adding polarity to the co-substrate. GSH is zwitter ionic at physiological pH and the additional polarity limits the solubility of lipid membranes (Mandal et al., 2017).

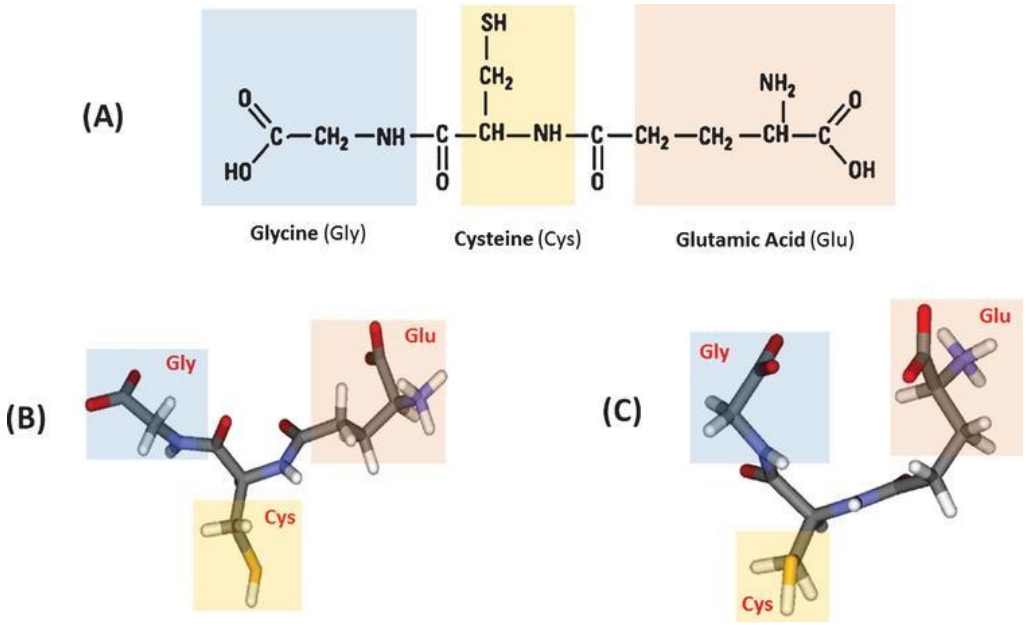


Figure 2.4. Glutathione and its structural conformations (Mandal et al., 2017).

The N-terminal domain of cytosolic GSTs assumes a thioredoxin-like tertiary structure, with a β1α1β2α2β3β4α3 motif (Figure 2.5) (Wu and Dong, 2012). Thioredoxin fold is also described in other GSH-binding enzyme superfamilies, including GSH reductase, GSH peroxidase and disulfide oxidoreductase (DsbA)-like superfamily (Martin, 1995; Wu and Dong, 2012). Within the G-site, the retained sequence of polar residues stabilizes GSH binding by interactions with the polar functional groups of the GSH peptide base (Mannervik et al., 1985).

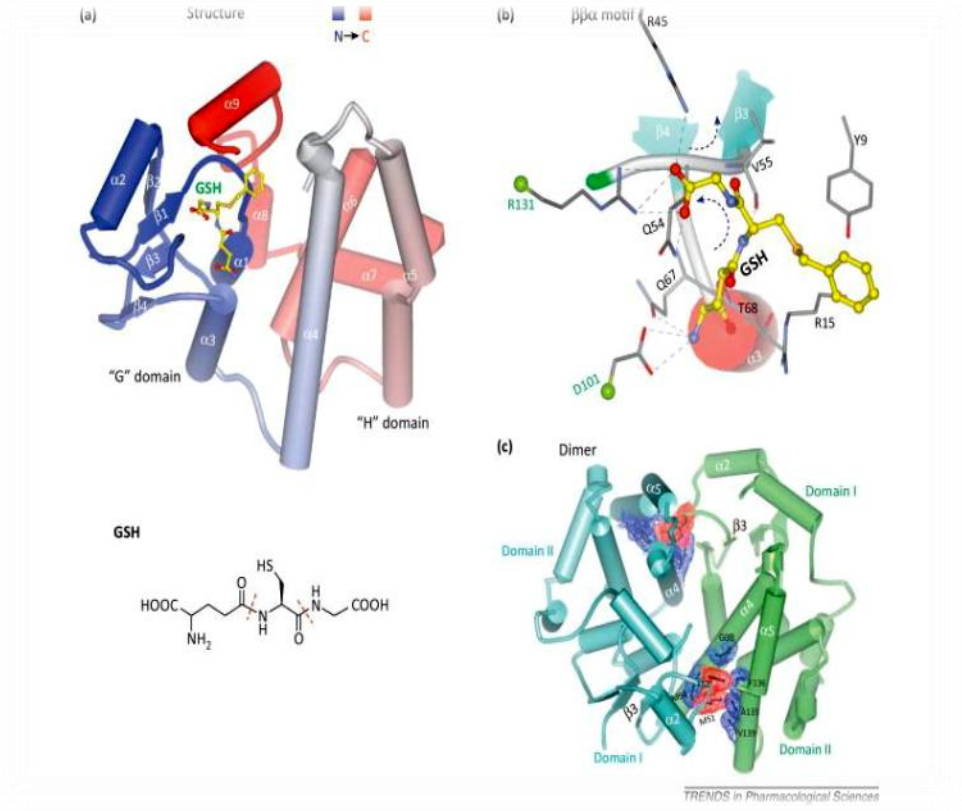


Figure 2.5. The tertiary structure of a GST enzyme using GST A1-1 as an example (PDB code1 GUH) to represent the GSH binding site and the general fold of the GST structure (Wu and Dong, 2012).

Cytosolic GSTs are further divided into two classes on the basis of conserved catalytic residues. The conserved Tyr, Ser, or Cys is found in the N-terminal G-site, which is essential for the ionization of GSH. The catalytic Tyr residue in 'Y-type' GSTs is one of the classes. The retained Tyr residue is found in β-sheet 1. The other class is the 'C/S-type' GSTs containing Cys or Ser residue. Cys/Ser residue is located in α-Helix 1 (Atkinson and Babbitt, 2009; Board and Menon, 2013; Allocati et al., 2018). The primary residue in the G-site (catalytic residue) is recognized as tyrosine in most soluble GST groups, particularly in alpha, mu, pi and sigma, but it is a serine residue in the theta and zeta classes and cysteine residue is in the omega class (Wu and Dong, 2012; Board and Menon, 2013). The tyrosine residue has been shown to stabilize the glutathione thiolate anion (Prade et al., 1997).

The Y type GSTs include alpha, mu, pi, and sigma classes, all of them are present in humans. The most recently diverged cytosolic GSTs are Y-type GSTs;

they are mostly present in mammals, while the C/S-type is common in prokaryotes and eukaryotes. The only enzyme group to contain a thioredoxin-like fold with a catalytic Tyr residue is Y-type GSTs (Atkinson & Babbitt, 2009).

2.5. Catalytic Activity of Cytosolic Glutathione S-Transferases

The catalytic process of GSH conjugation by GSTs causes a reduction of the pKa of the thiol group of cysteine to physiological pH when it is enzyme-bound. UV vis spectroscopy is used to monitor glutathione ionization, since glutathione cysteine thiolate absorbs light at 239 nm. Thiol group of cysteine has a pKa of 9.2. When GSH binds to the active site of the rat GSTM2-2, pKa decreases to 6.6. The pKa of GSTM2-2 bound GSH was measured by titrating the rGSTM2-2-GSH complex over a pH range of 5 to 8 and observing glutathione thiolate formation at 239 nm (Graminski et al., 1989a; Dourado et al., 2008).

A catalytic serine, cysteine or tyrosine residue in the GSH binding site of cytosolic GSTs is necessary for the reduction of pKa. Site-directed mutagenesis was performed in earlier studies to change the catalytically retained Tyr7 residue to phenylalanine in recombinant human GSTpi. The unique behavior of the Y7F form against co-substrate CDNB (1-chloro-2,4-dinitrobenzene) was around 1 percent of the wild form CDNB. The K_m values of wild and mutant enzymes were not affected, but Vm values of latter was decreased, suggesting the essential role of Tyr7 in the function of GSTpi (Kong et al., 1992; Ruzza and Calderan, 2013).

In rats, the catalytic Tyr9 residue of GSTA1-1 also has a decreased pKa (from pH 10 to pH 7) in the active site relative to the solution. Ionized tyrosine deprotonates the cysteine of GSH. The decreased pKa of the Tyr residue also changes the hydrogen-binding balance of the phenol hydrogen, which better stabilizes the GSH thiolate (Atkins et al., 1993). The reduction of the conserved Tyr pKa in rat GSTA1-1 relies on the pressure exerted by the C-terminal helixes of GST enzyme. Atkins et al. examined the pressure-dependent fluorescence emission changes in Tyr9 between phenolic (e.g. max. 305 nm) and tyrosinate (e.g. max. 345 nm) states in the Trp-null version of rat GSTA1-1. Rat GSTA1-1 has one non-essential tryptophan in the protein sequence and less Tyr residues (eight total). This facilitates the monitoring of Tyr ionization fluorescence without intervention from

tryptophan chromophores. The authors proposed that compaction of GST active site and depletion of the solution by second substrate binding co-occur with GSH ionization (Atkins et al., 1993). The human GSTpi enzyme has been found to have two substrate-binding sites in both of its subunits: the −G-site of the GSH-binding site and the −H-site of the electrophilic substrate-binding site (de Oliveira et al., 2014). The G-site is maintained and is very unique to GSH only (Prade et al., 1997; Board et al., 2013). Tyr7 residues have been shown to be a catalytic residue in human GSTpi and to stabilize glutathione thiolate anion (Prade et al., 1997; Oakley et al., 1999). With the exception of the G-site, the H-site is not well established and also has a wide specificity to facilitate the binding of a broad variety of xenobiotics or electrophiles (de Oliveira et al., 2014).

2.6. Glutathione S-Transferase Substrates

Halogenated arenes have been used as common substrates for detecting GST activity. Active chromophores of aryl substrates enable UV visible light to be monitored for product forming, spectrophotometrically. GSH conjugation to an aryl chromophore is followed by the change in absorption to a longer wavelength. This is due to the relocation of electrons from the GSH sulfur atom to the aryl chromophore. Product development can also be distinguished from the absorption of parental compounds. Glutathione S-transferase activity was first examined in partly purified soluble rat liver extract using a substrate of 3,4-dichloro-nitrobenzene (DCNB) (Figure 2.6). DCNB gives an absorption at 270 nm. GSH conjugation yields S-(2-chloro-4-nitro-phenyl-glutathione (CNPSG) which has a maximum absorption peak at 340 nm. 1-chloro-2,4-dinitrobenzene (CDNB) has properties identical to DCNB (Booth et al., 1961).

Previously, it was found that partly purified GST fractions (from rat liver) had a higher specific activity for CDNB. It was observed that CDNB was not a substrate for a few GSTs, including the human GST theta. Despite this, the regular substrate for observing GST activity is still considered to be CDNB (Habig et al., 1974a; Jemth and Mannervik, 1997).

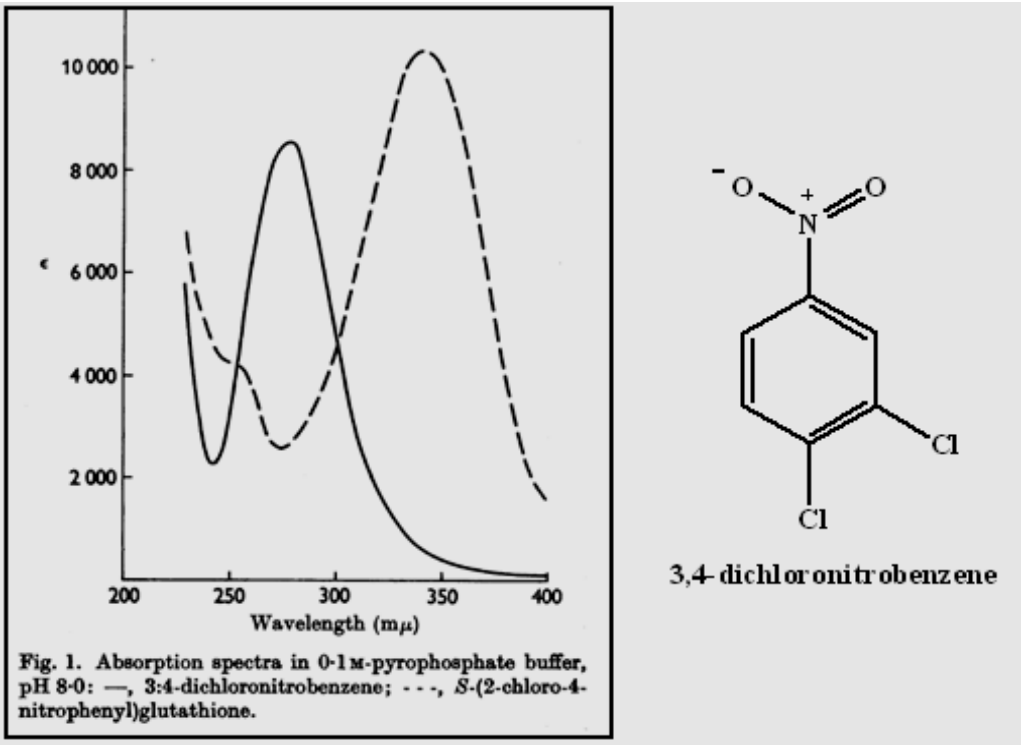


Fig. 1. Absorption spectra in 0·1M-pyrophosphate buffer, pH 8·0: —, 3:4-dichloronitrobenzene; - - -, *S*-(2-chloro-4-nitrophenyl)glutathione.

Figure 2.6. Conjugation of DCNB with GSH to form CNPSG (Booth et al., 1961)

CDNB conjugation to GSH at the active site of GST is accompanied by a nucleophilic aromatic substitution process (SNAr) (Figure 2.7). During the SNAr reaction, the catalytic intermediate 'Meisenheimer complex'/σ-complex forms at the active site. In the case of compounds that lack a leaving group, such as trinitrobenzene, the GST SNAr reaction is not completed, and the dead-end Meisenheimer complex forms at the active site of GST (Figure 2.7). The dead-end Meisenheimer complex remains dormant and can be detected by UV visible spectrophotometry due to their stability at the active site of the enzyme. It also occupies the active site and suppress the catalytic activity of GST in a competitive manner. The Meisenheimer complex has distinct properties from the starting compounds and the GSH conjugation product; thus they can be observable at the active GST site while the reaction is not completed. A competitive GST inhibitor is the TNB-GSH Meisenheimer complex, 1-(S-glutathionyl) - 2, 4, 6-trinitro-cyclohexadiene (Graminski et al., 1989b).

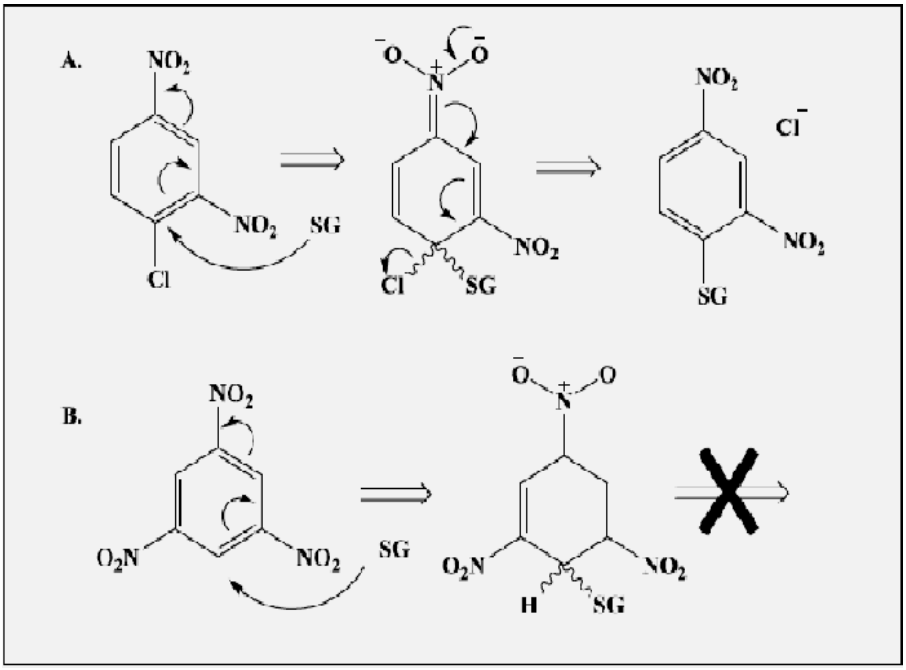


Figure 2.7. A. Reaction of CDNB SNAr. B. 1-(S-glutathionyl)-2,4,6- trinitro-cyclohexadienate dead-end structure Meisenheimer complex (Graminski et al., 1989b).

2.7. Specificity of Human Glutathione S-Transferase Substrates

Early works have attempted to categorize GSTs based on the their specificities for substrates, e.g. glutathione-S-aryl transferase, glutathione-S-alkyl transferase (Boyland and Chasseaud 1969; Ketterer et al., 1983). However, it quickly became apparent that there is no clear functional group specificity for GST enzymes. The promiscuity of substrates varies among GSTs. For some functional groups, certain GST isoenzymes have high unique activity, such as GSTA4-4 and unsaturated carbonyls Michael acceptors, GSTA2-2 and peroxides. There are less distinct characteristic substrate profiles for other GSTs. It is hard to determine substrates for certain GSTs. Identifying GST-specific substrates and inhibitors will be effective in characterizing human GST activity (Hayes et al., 2005). GST aromatic nucleophilic replacement reaction is exemplified mostly by the reaction between 1-chloro-2,4-dinitrobenzene (CDNB) and GSH, in which the chloride is substituted by glutathione to form S-(2,4-dinitrophenyl)glutathione (with detectable Meisenheimer-complex intermediate) as seen in (Figure 2.8) (Bocedi et al., 2019). Water molecules have been found to be absent in the Meisenheimer complex structure attached to GST,

suggesting that cysteine deprotonation occurs during the creation of the ternary complex, which involves the elimination of the inner bound water (Prade et al., 1997).

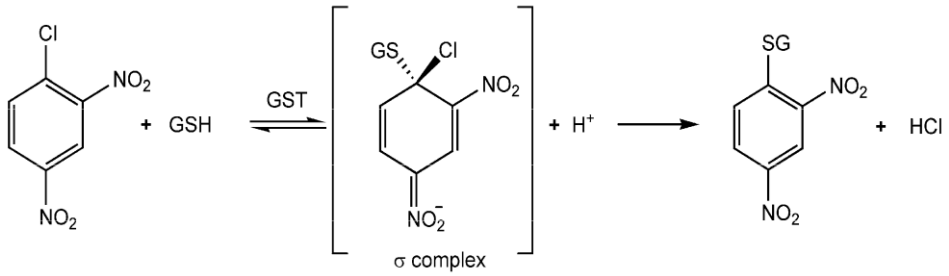


Figure 2.8. Conjugation of glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) catalyzed by GST (Bocedi et al., 2019).

A few other GSTs also catalyze nucleophilic additional reactions by adding GSH to the double bond of α,β-unsaturated carbonyl compounds identified as the Michael acceptor (Zimniak, 2007). This is characterized by the conjugation of ethacrynic acid to GSH catalyzed by pi-and alpha-class of GSTs, however the reaction is much more effective in the pi-class due to the fact that ethacrynic acid is attached to the deep position of the H-site where the corresponding tyrosine is substituted by the valine in alpha-class. This increases the electrophilicity of ethacrynic acid β-alkene carbon and results in a nucleophilic attack on ethacrynic acid β-alkene carbon, which increases efficient Michael addition (Wu and Dong, 2012).

The opening of the stretched oxirane ring is the other important form of reaction, where the glutathione thiolate anion attackes the electrophilic center of the target molecule (Zimniak, 2007). In this category, one of the substrates is (+)-anti-7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene(+)-anti-BPDE, a carcinogen derived from polycyclic aromatic hydrocarbon benzo[a]pyrene, the main clearance mechanism is GSTP1-1 catalyzed conjugation to GSH (Wu & Dong, 2012). This explains how GST reaction plays a vital defensive role against the carcinogenicity of polycyclic aromatic hydrocarbons like benzo[a]pyrene (Zimriak, 2007).

2.8. Noncatalytic Activities of Glutathione S-Transferase

In accordance with their enzymatic function, GSTs have the ability to bind a huge variety of hydrophobic molecules or ligands that may otherwise interfere with the normal cell function (Prade et al., 1997; Zimriak, 2007). This physiological activity is known as ligandin function of GSTs, of which GST acts as a transport protein and binds several ligand molecules, including heme, bilirubin, bile acid, steroids, heme, drugs, a wide variety of organic dyes and other xenobiotics (Wu and Dong, 2012).

Buffering is also an another non-catalytic feature of GSTs. Here, GSTs buffer or provide a form of sequestration or protection for compounds intracellularly, as albumin does in circulation by preventing bioactive ligand or signal molecule activity and modifying cellular response (Zimriak, 2007). This is shown in the ability of some GSTs to attach to 15-deoxy-Δ12,14-prostaglandin J2 or its glutathione conjugate, which also acts as a peroxisome proliferator-activated receptor gamma (PPARγ) ligand in the nucleus, to sequester it in such a manner as to block PPARγ activation and prevent nuclear translocation (Wu and Dong, 2012).

2.9. Glutathione S-Transferase Inhibitors

Cancer cells can develop resistance by overexpressing GST that can improve detoxification and inhibit the cytotoxic activity of antitumor drugs. In fact, it is understood that a variety of chemotherapy agents commonly used in cancer therapy are GST substrates. The wide variety of GST inhibitors indicates the limited substrate specificity of these enzymes. Allosteric inhibition of GST actually occurs by inhibitor binding to the GST dimer interface and binding of an inhibitor to an active site results in competitive inhibition. The GST dimer interface overlaps partly with the active site of GST (Oakley et al., 1999). GSTs are known to bind large aliphatic complexes to the dimer interface. That involves antibiotics, bilirubin, polyaromatic dyes, hormones, steroids and heme. The non-substrate binding property of GSTs was first credited to an unknown protein ligandin. (Habig et al., 1974). Iodocyanine green, 8-anilinonaphthalene sulfonate, bilirubin, 3,6-dibromosulfophthalein and cefalothin are non-competitive GST inhibitors that bind to the dimer interface (Ketley et al., 1975).

Natural GST inhibitors include curcumin, phenolic antioxidants and ellagic acid. Generally these inhibitors are the mixed type or uncompetitive inhibitors of GST (Hayeshi et al., 2007; Appiah-Oppong et al., 2009). GSTs are inhibited by plant-derived flavonoids kaempferol, quercetin and genistein, but the inhibition mechanism is uncharacterized. Some plant secondary metabolites that are GST inhibitors include diosphyrin and geshoidin (Hayeshi et al., 2004).

Synthetic inhibitors specifically designed for the inhibition of GST include the competitive inhibitor NBDHEX (6-(7-nitro-2,1,3-benzoxadiazole-4-ylthio)hexanol), which forms the Meisenheimer complex at the GST active site (Ricci et al., 2005; Federici et al., 2009). Bivalent inhibitors with linkers that bridge the dimer interface and attach to both active sites of the GST dimer have also been developed (Mahajan et al., 2006). Finally, product mimic inhibitors-GSH conjugates have been investigated. This contains GSH conjugates with modified GSH peptides. Since they contain the GSH tripeptide, they are referred to as 'peptidomimetic inhibitors' and they include the γ-glutamyl-S-(benzyl)cysteinyl-R-phenylglycine-diethylester inhibitor (O'Brien et al., 1999).

2.9.1. Applications of glutathione S-transferase inhibitors

GST inhibitors have been tested for their ability to sensitize cancer cells to chemotherapy. Many chemotherapeutics have functional groups that act as GST substrates. Most are DNA alkylating nitrogen mustards that have halogenated alkylating functional groups (Hayes et al., 2005). In human cancer tissues, including breast, colon, lung and stomach tissue, GSTs are usually overexpressed (Chatterjee and Gupta, 2018). The most often overexpressed GST isoenzyme in cancer tissue is GSTpi. Ethacrynic acid was an early investigated chemotherapy adjuvant as GSTpi inhibitor. Ethacrynic acid has entered phase I trials. However, ethacrynic acid is a renal diuretic. Its renal toxicity has since stimulated alternative GST inhibitors to be investigated. Chemotherapeutic resistance also appears in tumors that express GST although the drugs are not GST substrates. Recently, a second mechanism of GST-mediated chemotherapy resistance has been discovered as a regulator of cell cycle progression (O'Dwyer et al., 1991; Dong et al., 2018).

In addition to metabolic detoxification, GSTpi also exhibited ligand-binding characteristics that allow the enzyme to bind covalently and non-covalently with substances (Townsend and Tew, 2003). In the presence of cellular stress, GSTpi can stimulate apoptosis by activating JNK-signal components, MAPK, MKK4 and p38 kinase. Healthy cells have low JNK expression to sustain optimum cell growth conditions. However, in the appearance of oxidative or nitrative stress, GSTpi can form homodimers that modify the reduced levels of cysteine residues within the structure, result in JNK dissociation from the GSTpi–JNK heterocomplex and leading to the subsequent activation of apoptotic pathways (Gate et al., 2004; Sau et al., 2012) (Figure 2.9). Further research shows that GSTpi can affect the MAPK pathway by modulating both JNK and TRAF2. An in-depth study has shown that GSTpi is closely related to the TRAF family. Among the members, TRAF2 has been subject to extensive study (Zhang et al., 2011). Association of TRAF2 with the plasma membrane generates ROS. Subsequently, oxidation of the ASK1 (apoptosis signal-regulating kinase 1)-thioredoxin complex results in the removal of ASK1, followed by the binding of TRAF2 to ASK1 and triggering a cascade of downstream signaling, including the signaling pathways of MKK3/4/6-p38 and MKK4/7-JNK (Sau et al., 2010; D. Dong et al., 2018) (Figure 2.9).

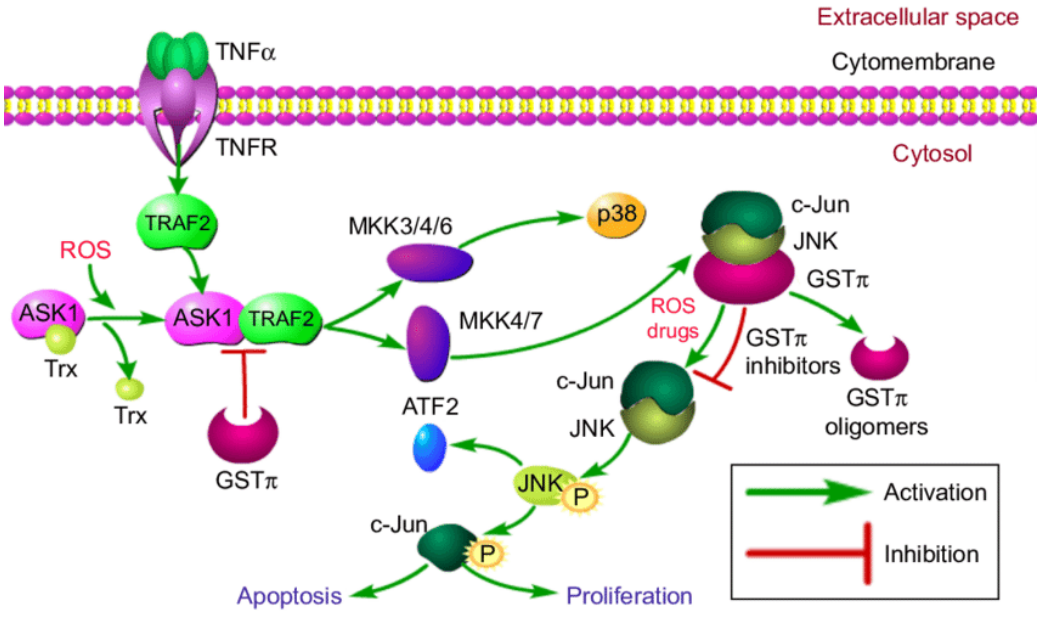


Figure 2.9. Ligand-binding properties of JNK and TRAF2 (Dong et al., 2018).

Additional information from steady-state fluorescence study suggests that direct binding still exists between GSTpi and TRAF2 (Palumbo et al., 2016; Fulci et al., 2017). GSTpi can inhibit JNK activity in tumor cells and block the association of TRAF2 with ASK1 to prevent tumor-cell apoptosis. Consequently, in relation to the production of multidrug resistance in tumor cells, GSTpi can also function as a MAPK-pathway inhibitor to enhance tumor-cell survival, in addition to serving as a detoxifying enzyme for drug excretion to minimize pharmacological efficacy (Lo and Ali-Osman 2007; Bartolini and Galli, 2016).

The relationship of GSTpi with JNK is complicated considering that there are three types of JNKs with separate splice variants whose functions are mutually antagonistic. In GSTpi deficient mice, the overall white blood cell count is increased 2-fold due to increased myeloproliferation (Gate et al., 2004; De Luca et al., 2012). This is partially due to increased phosphorylation/activation of signal transducer and transcription activator (STAT) proteins by JNKs in the absence of GSTPi. In wild type mice expressing GSTpi, the GSTpi inhibitor TLK199 activates bone marrow myeloproliferation. TLK199 has managed to reach phase 3 clinical trials for the treatment of myelodysplastic syndrome, a situation in which inadequate granulocyte precursor cells (eosinophils, neutrophils, basophils and mast cells) are produced in the bone marrow and severe chronic neutropenia, a situation in which inadequate neutrophils are produced (Wang et al., 2001; Raza et al., 2009).

The upstream kinase of JNK is ASK1, also referred to as MAP3K5. The ASK1 activity governs the JNK and p38 signaling pathways. GSTM1-1 has recently been established as the ASK1 activity regulator. In COS7 ASK1 overexpression causes apoptotic cell death, and a dominant-negative ASK1 mutation prevents TNFα-induced apoptosis (Ichijo et al., 1997; Tesch et al., 2020). GSTM1-1 binds with ASK1 in non-stress conditions. GSTM1-1 inhibits ASK1 by blocking ASK1 oligomerization, which is a necessity for ASK1 phosphorylation (Cho et al., 2001). Overexpression of GSTM1-1 prevents the heat-shock activation of ASK1 in Chinese hamster and human HeLa cell lines (Dorion et al., 2002). GSTM1-1 activation inhibition of ASK1 prevents downstream JNK signaling cascades and activation of downstream transcription factors in HEK 293 (Cho et al., 2001).

Few was known about the modulation of ASK1 by GSTM1-1; emphasis was placed on interactions between GSTpi and JNK. It is also interesting to note that in human populations, a prevalent GSTM1 null genotype persists; its relation to ASK1 activity and downstream effects has not been thoroughly studied. What started to emerge from these findings on GST-MAPK interactions is a proposal for a new function for cytosolic GSTs as endogenous stress response regulators – the JNK signaling pathway (Cho et al., 2001). Such newly identified GST functions have received the greatest attention for pharmaceutical applications in developing GST inhibitors. Hence, human placental GSTP1-1 may be a possible marker protein for controlling redox homeostasis. There have been convincing evidence to support this. While other GST enzymes such as GSTA1, GSTA2 and GSTM1 are expressed during developmental stages (Raijmakers et al., 2001).

2.10. Phytohormones

Many medications and chemicals influence the function of the enzyme. A large number of therapeutically active compounds that are currently in use serve as enzyme inhibitors. For drug design trials, the assessment of the effects of biologically active substances on metabolic enzymes is therefore very important.

Plants, the fountain of life on earth, are born, grow up and die. Many chemical events occur in the plant during this life cycle. Plants themselves make vital molecules in order to support their growth and development. In general terms, plants synthesize naturally organic compounds called hormones (phytohormone-plant growth regulators) (Gangwar et al., 2014). The earliest scientific research on phytohormones dates back to the end of the 18th century (Su et al., 2017; Sezgin and Kahya, 2018). Gibberellic acid (GA) was a plant hormone used in Turkey for the first time in 1960 in seedless grapes to enable increased fruit and cluster size. Plant hormones must be capable of occurring in the plant itself, being transferred elsewhere, and directing or regulating various activities, even at very low concentrations (Sezgin and Kahya, 2018). In addition to gibberellin, abscisic acid, ethylene, auxin, and cytokinin, other plant hormones such as salicylic acid, strigolactone, jasmonic acid and brassinosteroid have been detected. These hormones have been identified to play an important role in any stages of the life cycle of the

plant and are grouped into the three major classes according to their biochemical effects (growth, reproduction, stress response) in plants (Sezgin and Kahya, 2018).

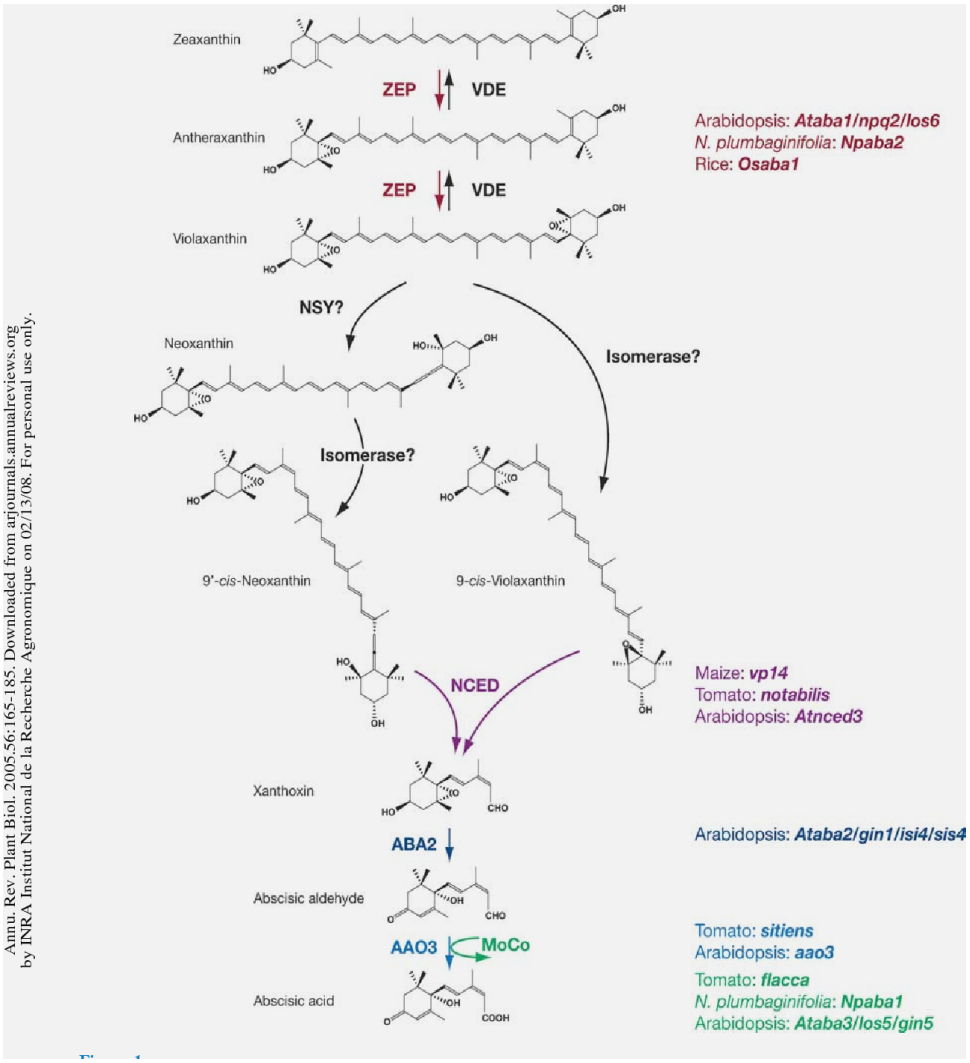
2.10.1. Abscisic acid

Abscisic acid (ABA) is a phytohormone with a chemical formula of $C_{15}H_{20}O_4$. ABA functions in plant growth and development. It is particularly necessary in order to compete with environmental stress such as heat, cold, freezing, metal ions and soil salinity (Finkelstein, 2013). ABA is an isoprenoid phytohormone synthesized in plastids by 2-C-methyl-D-erythritol-4-phosphate pathway; unlike structurally similar sesquiterpenoids formed by the mevalonic acid-derived farnesyl diphosphate precursor, after cleavage of C40 carotenoids into plastidial 2-C-methyl-D-erythritol-4-phosphate, the C15 backbone of ABA is formed. The first ABA precursor is zeaxanthin and is converted to ABA by using the series reactions (Figure 2.10) (Nambara and Marion-Poll, 2005).

Beneficial effects of ABA have been well documented in a variety of pathological and preclinical models of diseases such as colitis, type 2 diabetes, glioma, depression, atherosclerosis, neuroinflammation, hepatitis C virus replication, angiogenesis, malaria, tuberculosis, influenza A virus infection and fungal infection. Over the past few years, the impact of ABA on glucose homeostasis, factors associated with ischaemic retinopathy, and inter-species contact in the dissemination of diseases has been properly examined. Of note, the recorded health gain from homeostasis of glucose and insulin occurs at low doses, 0.5-1 μg/kg body weight, equivalent to typical levels of fruit and vegetables (Kim et al., 2020).

In metazoans, from sponges to mammals, including humans, ABA has also been shown to be present (Li et al., 2011). Its biosynthesis and biological function in animals is not completely understood. In mouse models of influenza virus, atherosclerosis, inflammatory bowel disease, and diabetes/obesity, ABA showed strong anti-inflammatory and anti-diabetic effects (Bassaganya-Riera et al., 2010). Many biological effects have been studied in animals using ABA as a pharmacognostic drug or nutraceutical, but when stimulated, ABA is also endogenously formed by certain cells such as macrophages. Also there are contrasting results from various studies, some of which argue that ABA is important

for pro-inflammatory reactions, while others display anti-inflammatory effects. In naturopathy, ABA has also become common, like several natural substances with medicinal potential. Although ABA definitely has beneficial biological functions and high amounts of ABA can be found in certain naturopathic remedies, some of the health statements made might be exaggerated or unnecessarily positive. ABA targets a protein known as lanthionine synthetase C-like 2 (LANCL2) in mammalian cells, activating an alternative signaling mechanism for peroxisome proliferator-activated gamma-receptor (PPAR gamma) (Bassaganya-Riera et al., 2011).



Annu. Rev. Plant Biol. 2005.56:165-185. Downloaded from arjournals.annualreviews.org
by INRA Institut National de la Recherche Agronomique on 02/13/08. For personal use only.

Figure 2.10. Biosynthesis of abscisic acid (Nambara and Marion-Poll, 2005).

2.10.2. Gibberellins

Gibberellic acid (GA) also called gibberellin A3, and GA3 is a hormone found in plants and fungi. The chemical formula is $C_{19}H_{22}O_6$ (Figure 2.11) (Rodrigues et al., 2012). Plants produce large amounts of GA3 in their original environment. The hormone can be produced industrially by microorganisms. Gibberellic acid is pure gibberellin, a diterpene pentacyclic acid that facilitates cell growth and elongation. It affects plant decomposition and if used in limited quantities, it helps plants to develop, but ultimately plants develop resistance to it. GA activates germinating seeds to generate mRNA of hydrolytic enzymes. In practice, GA is used to minimize the number of fruits in the cluster and to maximize the fruit size of table and dried grapes. Gibberellic acid is a very strong hormone whose production is regulated by natural occurrence in plants. Applications at very low concentrations have a profound effect as GA regulates growth, whereas too much can have the opposite effect. It is commonly used at concentrations ranging from 0.01 to 10 mg/L, In 1926, GA was first described in Japan as a metabolic by-product of *Gibberella fujikuroi*, a plant pathogen that afflicts rice plants (Edwards, 1976; Gupta and Chakrabarty, 2013).

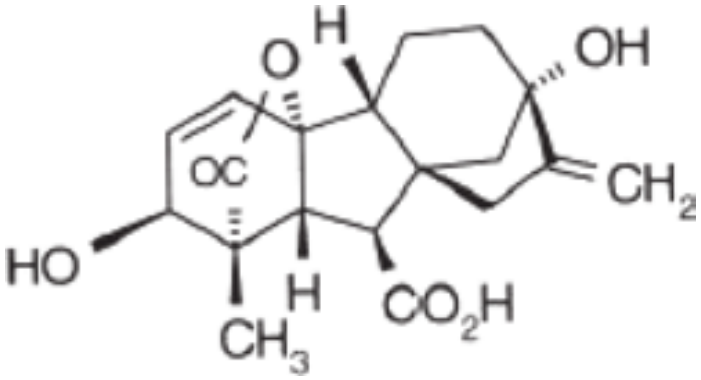


Figure 2.11. Chemical structure of gibberellic acid (Rodrigues et al., 2012).

While it is believed that GAs often behave as paracrine signals, it is still a mystery to understand the biosynthesis of GA and its transportation in plants. Presently, recognizing the appropriate process of GA movement in plant formation, floral production, and sexual expression is a great challenge for the scientific

community. For the survival of plant species and efficient crop production, adequate amount of the GA transport mechanism is necessary (Gupta and Chakrabarty, 2013).

Therefore, the purpose of this research was to explain the interaction of human placental GSTpi with ABA and GA based on the preliminary findings of the inhibition of GSTs by phytohormones. Novel results from a group of compounds that inhibit human GSTpi selectively are described here. This research is expected to include results that will help improving potential molecules that have the ability to suppress the activity of GSTpi to overcome drug resistance in cancer therapy.

3. MATERIALS AND METHODS

3.1. Chemicals

Glutathione S-transferase from human placenta (*hp*GSTP1-1), L-Glutathione reduced (GSH), 1-Chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin (BSA), ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA), sodium azide, sodium phosphate monobasic, sodium phosphate dibasic dodecahydrate, gibberellic acid, 2-cis,4-trans-abscisic acid were all purchased from Sigma-Aldrich, Inc., St. Louis, MO, USA.

3.2. Methods

3.2.1. Preparation of the activity mixture components

Sodium phosphate buffer was used in the activity mixture and for the preparation of the enzyme. For this purpose, 200 mM sodium phosphate buffer was prepared and pH was adjusted to 6.5, then EDTA was added with a final concentration of 2 mM. To avoid bacterial growth, 0.02% sodium azide was added to the buffer solution.

The *hp*GSTP1-1 enzyme was prepared by dissolving 2 mg in 1 mL of 100 mM sodium phosphate buffer, pH 6.5, containing 1 mM EDTA. This stock enzyme was aliquot and kept in -20^{o}C. In all the experiments, the enzyme (2 mg/mL) was diluted by 10 or 35 times with 100 mM sodium phosphate buffer, pH 6.5, containing 1 mM EDTA and 0.05% BSA before use for IC_{50} determination and inhibitory kinetic studies, respectively.

Substrates, GSH and CDNB were dissolved in filtered distilled water and ethanol, respectively to prepare the required concentrations of each.

Gibberellic acid and abscisic acid were dissolved in absolute ethanol.

3.2.2. Determination of the human placental glutathione S-transferase P1-1 enzyme activity

The *hp*GSTP1-1 activity was assayed with minor modifications according to the method of Habig and Jakoby by using Perkin Elmer LAMBDA 25 UV/VIS Spectrophotometer (Habig and Jakoby, 1981). The activity of *hp*GSTP1-1 was

followed by monitoring an absorbance increase due to the conjugation of the substrate molecule, L-glutathione (GSH) to the artificial substrate CDNB at 340 nm for 20 seconds (Habig and Jakoby, 1981). The reaction mechanism is shown below:

$$GSH + CDNB \xrightarrow{GST} GS\text{-}DNB\ Conjugate + HCl$$

The *hp*GSTP1-1 enzyme catalyzes the conjugation of GSH to CDNB via the thiol group of the GSH. The increase in the absorption of GS-DNB conjugate (the product of the reaction) is directly proportional to the GST activity (Habig and Jakoby, 1981). The reaction was followed by the addition of CDNB, which is suitable for the widest range of GST isozymes. A non-enzymatic reaction which contains all constituents of the reaction mixture excluding the *hp*GSTP1-1 enzyme, was run in parallel. From the enzymatic reaction value, the value of the non-enzymatic reaction was deducted. All measurements were performed at 37°C and in triplicates. Average activity (U mL^{-1}) values were converted to a specific activity (U mg^{-1} protein) and were used to depict Michaelis-Menten, Dixon and secondary plots (Segel, 1975). One unit of the *hp*GSTP1-1 enzyme activity was defined as the amount of the enzyme that catalyzes the formation of 1 μmol of product per minute at pH 6.5 and 37^{o}C. The formula used for the calculation of the enzyme activity is given below.

Specific Activity (U mg^{-1} protein):

$$\frac{\Delta Abs_{340/min} \times Vt}{9.6 \times Vs \times [protein]}$$

Where,

$\Delta Abs_{340/min}$ = Absorbance change per minute at 340 nm

Vt = Total volume of the reaction mixture

Vs = Sample volume to measure enzyme activity

9.6 = Extinction coefficient of GS-DNB conjugate.

3.2.3. Effect of abscisic acid and gibberellic acid on human placental glutathione S-transferase P1-1 and the determination of IC_{50}

The inhibition of *hp*GSTP1-1 was calculated by the addition of different concentrations (ranged from 0.15625 to 5 mM) of abscisic acid and gibberellic acid to the reaction mixture. The reaction mixture consisted of 100 mM sodium phosphate buffer, pH 6.5 with 1 mM EDTA, 1 mM GSH, 1 mM CDNB, and the appropriate amount of *hp*GSTP1-1 enzyme (Habig and Jakoby 1981; Dalmizrak et al., 2016). The data was collected in the absence and presence of inhibitors in triplicates. The percentage (%) remaining activity was plotted against the concentration of abscisic acid and gibberellic acid to determine the IC_{50} value (Segel, 1975).

3.2.4. Inhibitory kinetic experiments with abscisic acid and gibberellic acid

Inhibitory kinetic studies were conducted in the absence (control) and presence of abscisic acid and gibberellic acid. For this purpose 0.25, 0.5, 1.0 and 2.0 mM inhibitor concentrations were chosen from percentage remaining activity versus abscisic acid or gibberellic acid plots. Appropriate amounts of abscisic acid and gibberellic acid were dissolved in absolute ethanol and added to the reaction mixture.

The reaction mixture consisted of 100 mM sodium phosphate buffer (pH 6.5) with 1 mM EDTA, different concentrations of abscisic acid or gibberellic acid (as described above), 1 mM fixed [CDNB] or 1 mM fixed [GSH] and appropriate amount of enzyme. In each case, the concentrations of the variable GSH and CDNB were 0.1, 0.2, 0.4, 0.8 and 1.6 mM in the reaction mixture. An increase in the absorbance due to the formation of the GS-DNB conjugate was followed at 340 nm for 20 seconds. The data obtained with and without abscisic acid and gibberellic acid were evaluated with different kinetic models (Segel, 1975). Michaelis-Menten, Dixon and secondary plots were used to estimate inhibition types and kinetic parameters.

3.2.5. Statistical analysis

Non-linear regression module of IBM SPSS Statistics (version 20) (SPSS Inc. Chicago, IL, USA) was also used to confirm the type inhibition and kinetic parameters.

4. RESULTS

4.1. Inhibitory Kinetic Interaction of Human Placental Glutathione S-Transferase P1-1 with Abscisic Acid and Gibberellic Acid

The effect of abscisic acid and gibberellic acid on *hp*GSTP1-1 was examined by applying various concentrations of abscisic acid (0, 0.15625, 0.3125, 0.625, 1.25, 2.5, 5 mM) and gibberellic acid (0, 0.15625, 0.3125, 0.625, 1.25, 2.5, 5 mM) to the reaction mixture. The reaction mixture was made of 100 mM sodium phosphate buffer pH 6.5 with 1 mM EDTA, 1 mM CDNB, 1 mM GSH and appropriate amount of *hp*GSTP1-1 enzyme. The data were obtained in triplicate measurements for each abscisic acid and gibberellic acid concentration. Percentage remaining activity was plotted against different concentrations of abscisic acid and gibberellic acid and the IC_{50} values were determined to be 5.4 mM (Figure 4.1) and 5.0 mM (Figure 4.2), respectively.

For the inhibitory kinetic studies, four selected abscisic acid and gibberellic acid concentrations (0.25, 0.5, 1.0 and 2.0 mM) were used. The inhibitory kinetic studies were conducted to obtain K_m, V_m and K_i in the absence and presence of abscisic acid and gibberellic acid. In the experiments, the reaction mixture comprised of 100 mM phosphate buffer (pH 6.5) with 1 mM EDTA, the various concentrations of abscisic acid and gibberellic acid, 1 mM $[CDNB]_c$ – $[GSH]_v$ or 1 mM $[GSH]_c$ – $[CDNB]_v$ and appropriated amount of enzyme. The concentrations of the varied GSH or CDNB were 0.1, 0.2, 0.4, 0.8 and 1.6 mM within the reaction mixture in each case. The increase in the absorbance due to the formation of the GS-DNB complex at 340 nm was followed for 20 seconds.

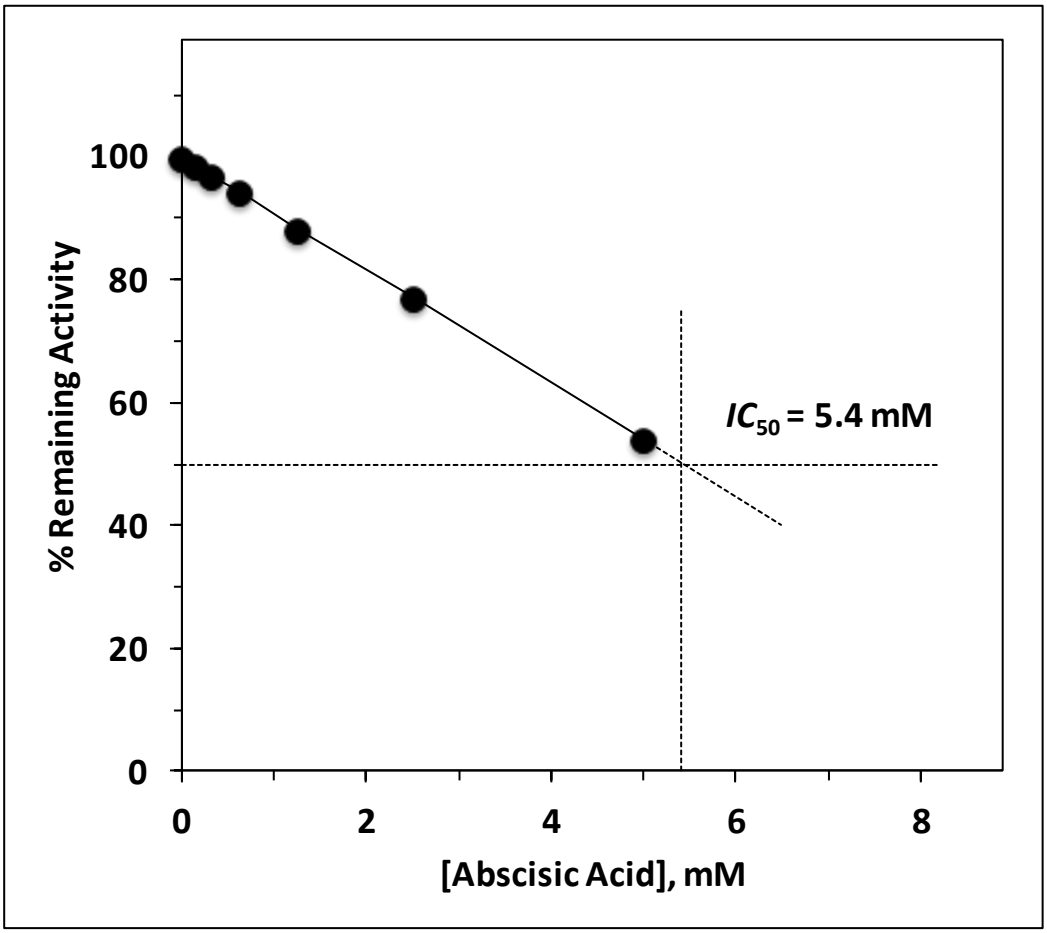


Figure 4.1. Dose-dependent inhibition of *hp*GSTP1-1 by abscisic acid [CDNB] = 1 mM; [GSH] = 1 mM; [Abscisic Acid] = 0, 0.15625, 0.3125, 0.625, 1.25, 2.5 and 5.0 mM.

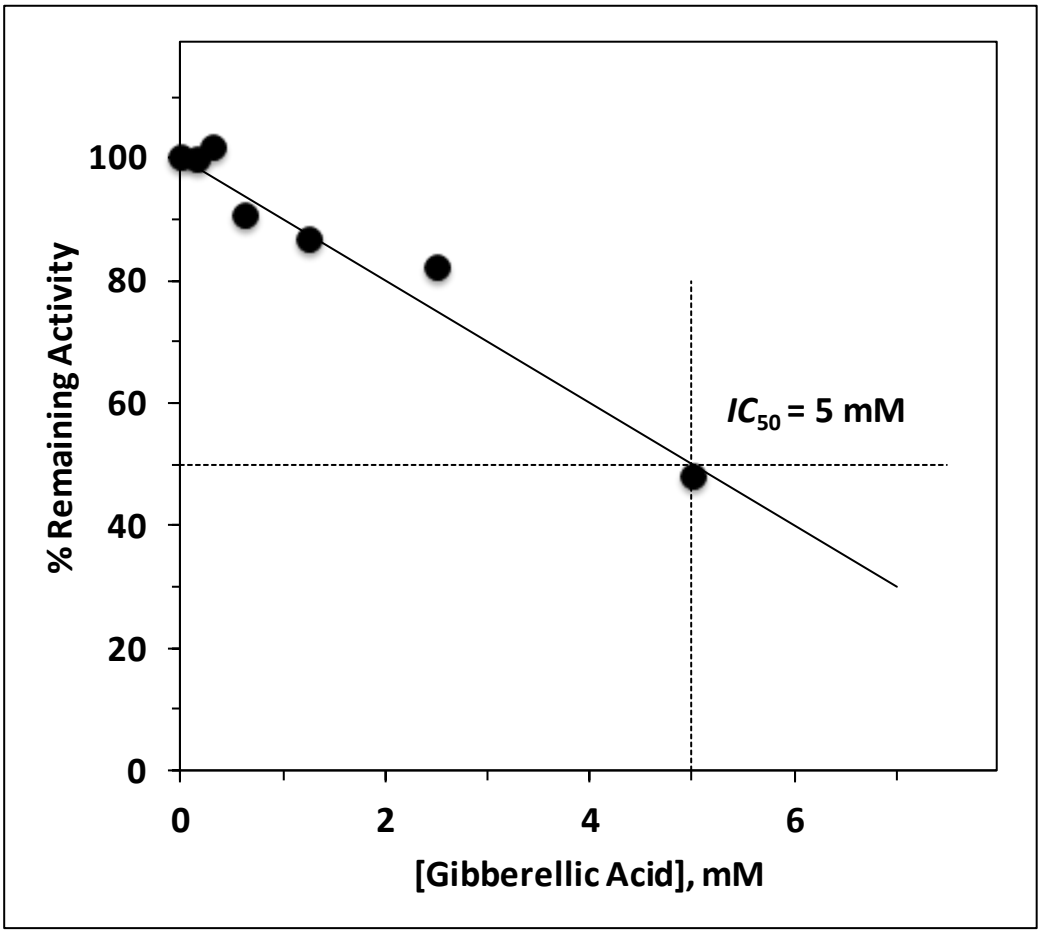


Figure 4.2. Dose-dependent inhibition of *hp*GSTP1-1 by gibberellic acid [CDNB] = 1 mM; [GSH] = 1 mM; [Gibberellic Acid] = 0, 0.15625, 0.3125, 0.625, 1.25, 2.5 and 5.0 mM.

The data obtained in inhibitory kinetic experiments were used to draw Michaelis–Menten, Dixon and secondary plots and also statistical analyses were performed to confirm the type of the inhibition and kinetic parameters.

When abscisic acid was used as an inhibitor, competitive type of inhibition was observed with respect to both substrates (GSH and CDNB). At fixed CDNB and varied GSH concentrations V_m, K_m and K_i were calculated as 205 ± 12 μmol/min-mg protein, 1.32 ± 0.14 mM and 1.95 ± 0.23 mM, respectively (Figures 4.3 – 4.5). At fixed GSH and varied CDNB concentrations V_m, K_m and K_i were found to be 166 ± 4 μmol/min-mg protein, 0.76 ± 0.04 mM and 1.79 ± 0.14 mM, respectively (Figures 4.6 – 4.8).

Gibberellic acid also inhibited *hp*GSTP1-1 in a competitive manner. When GSH was varied and CDNB was fixed, V_m, K_m and K_i were calculated as 303 ± 10 μmol/min-mg protein, 1.77 ± 0.1 mM and 3.38 ± 0.24 mM, respectively (Figures 4.9 – 4.11). At fixed GSH and varied CDNB concentrations V_m, K_m and K_i were found to be 249 ± 7 μmol/min-mg protein, 1.43 ± 0.07 mM and 2.89 ± 0.19 mM, respectively (Figures 4.12 – 4.14).

Table 4.1. Kinetic parameters of *hp*GSTP1-1 inhibition by abscisic acid and gibberellic acid

	Abscisic acid		**Gibberellic acid**	
Parameters	$[GSH]_v$	$[CDNB]_v$	$[GSH]_v$	$[CDNB]_v$
IC_{50}, mM	5.4 mM		5.0 mM	
Inhibition type	Competitive	Competitive	Competitive	Competitive
V_m, μmol/min-mg protein	205 ±12	166 ± 4	303 ± 10	249 ± 7
K_m, mM	1.32 ± 0.14	0.76 ± 0.04	1.77 ± 0.1	1.43 ± 0.07
K_i, mM	1.95 ± 0.23	1.79 ± 0.14	3.38 ± 0.24	2.89 ± 0.19

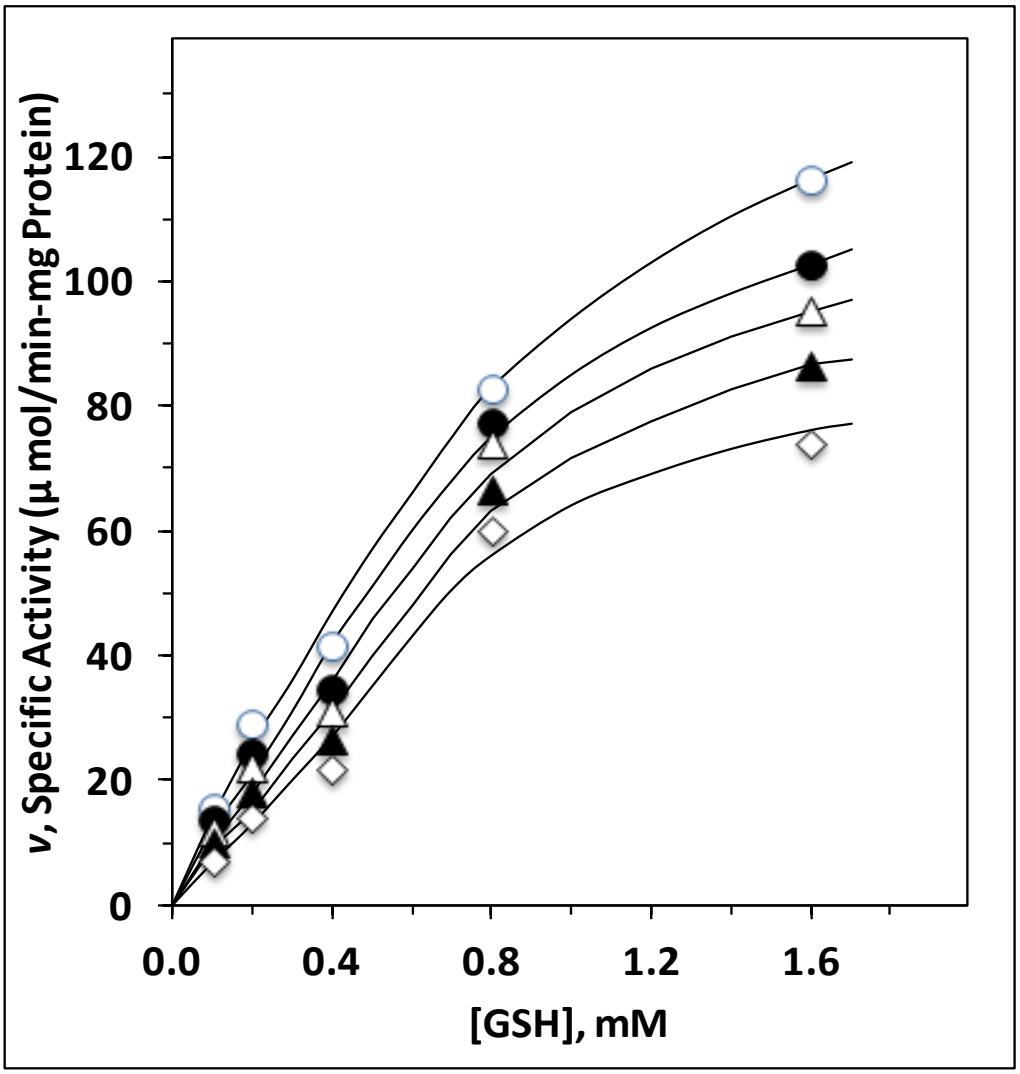


Figure 4.3. Michaelis–Menten plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of abscisic acid at 1 mM fixed [CDNB] and variable [GSH] = 0.1, 0.2, 0.4, 0.8 and 1.6 mM. [Abscisic Acid] = (○), 0; (●), 0.25; (Δ), 0.5; (▲), 1; (□), 2 mM.

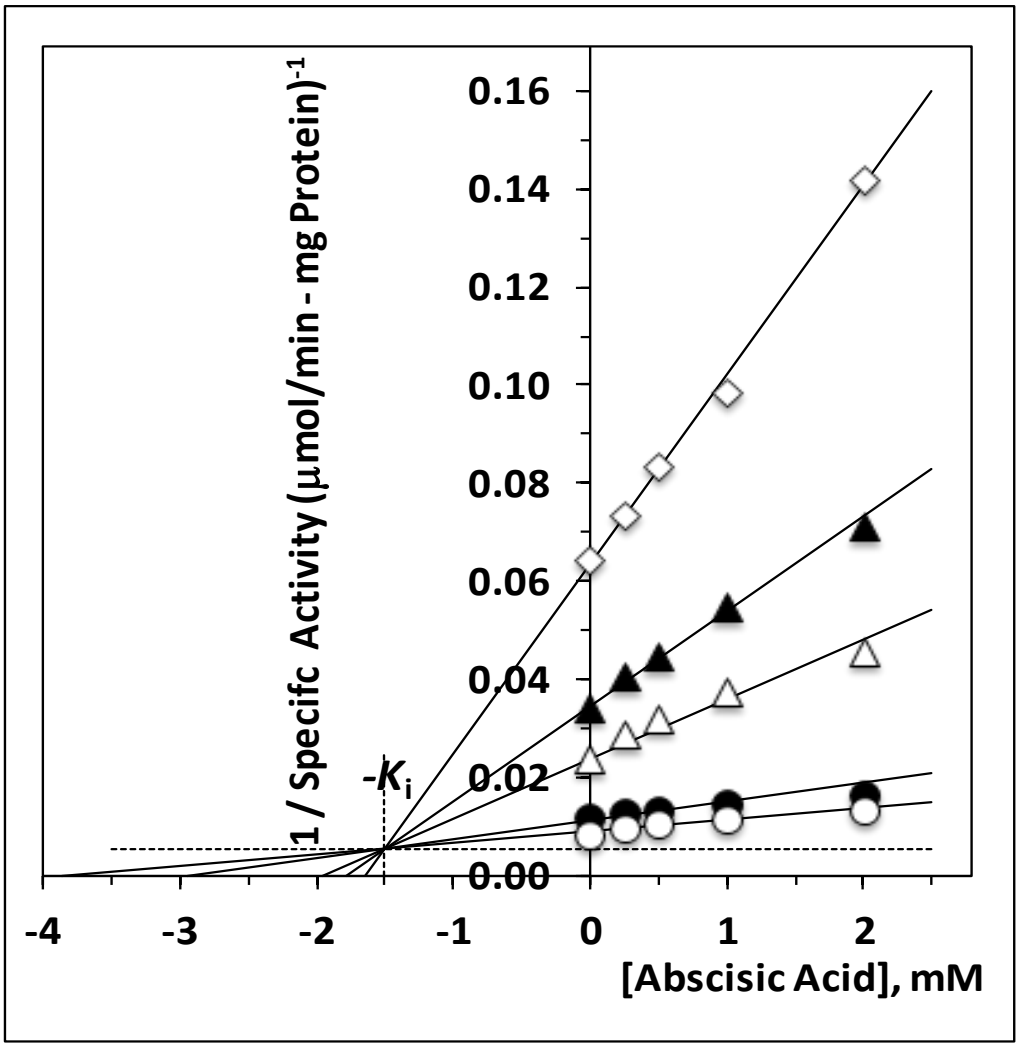


Figure 4.4. Dixon plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of abscisic acid at fixed 1 mM [CDNB] and variable [GSH]. [Abscisic Acid] = 0, 0.25, 0.5, 1 and 2 mM. [GSH] = (□), 0.1; (▲), 0.2; (Δ), 0.4; (●), 0.8; (○), 1.6 mM.

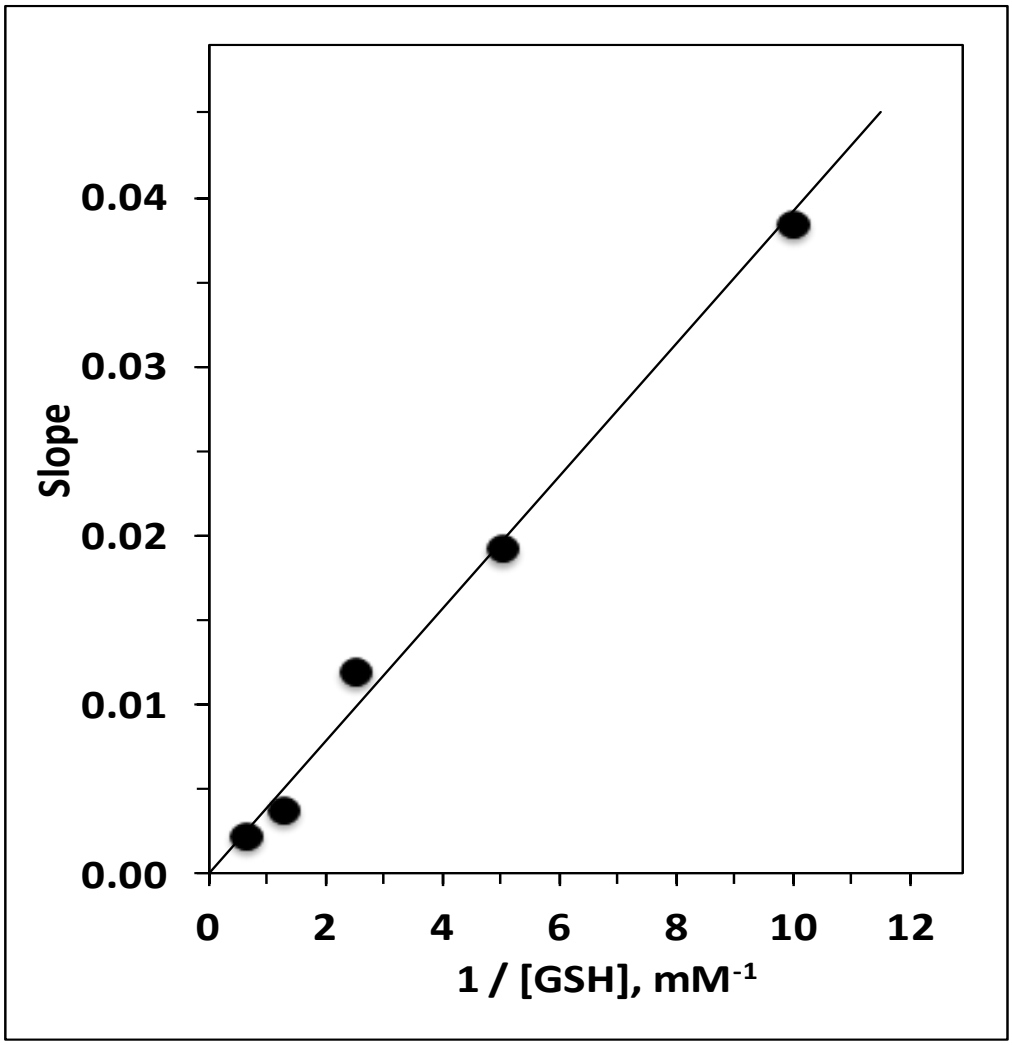


Figure 4.5. Replot of slopes versus 1/ [GSH].

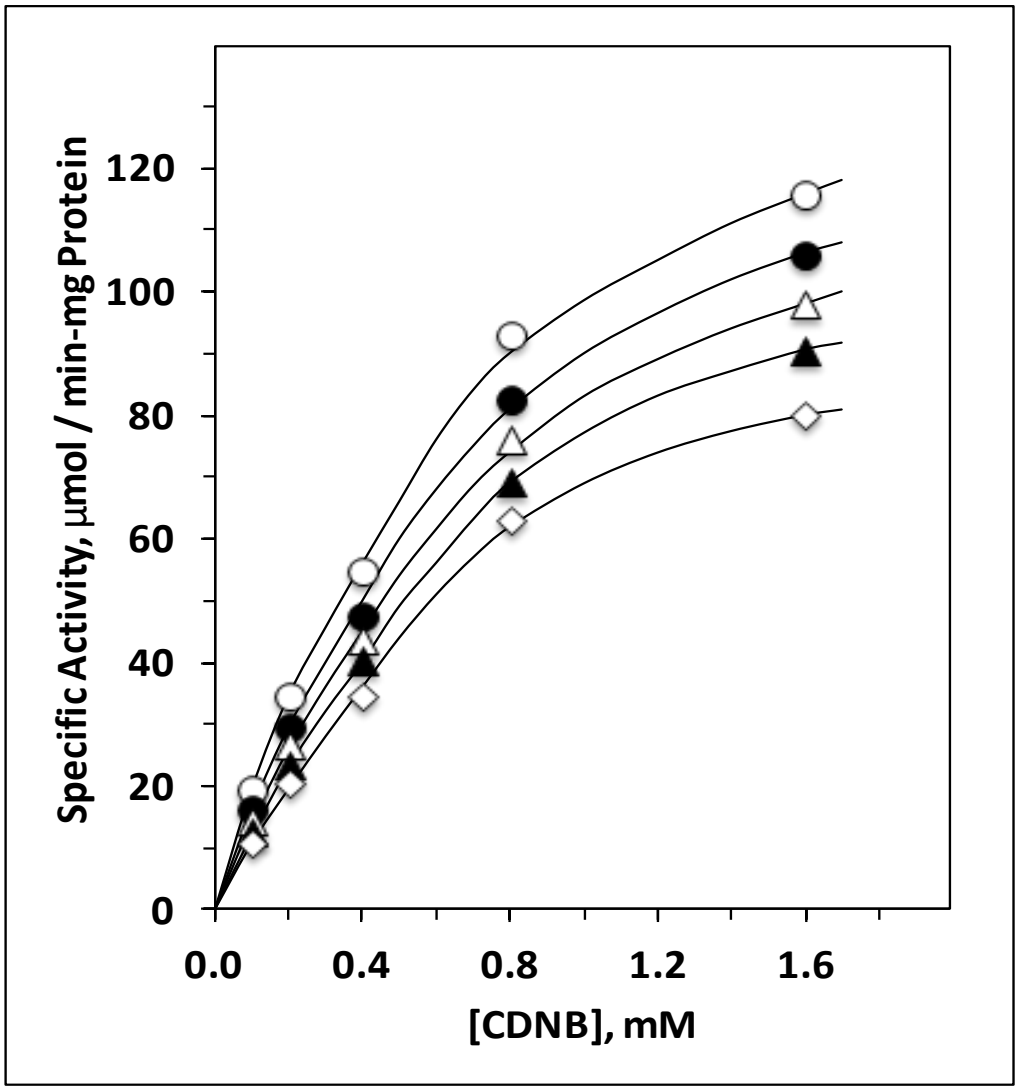


Figure 4.6. Michaelis–Menten plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of abscisic acid at fixed 1 mM [GSH] and variable [CDNB] = 0.1, 0.2, 0.4, 0.8 and 1.6 mM. [Abscisic acid] = (○), 0; (●), 0.25; (Δ), 0.5; (▲), 1; (□), 2 mM.

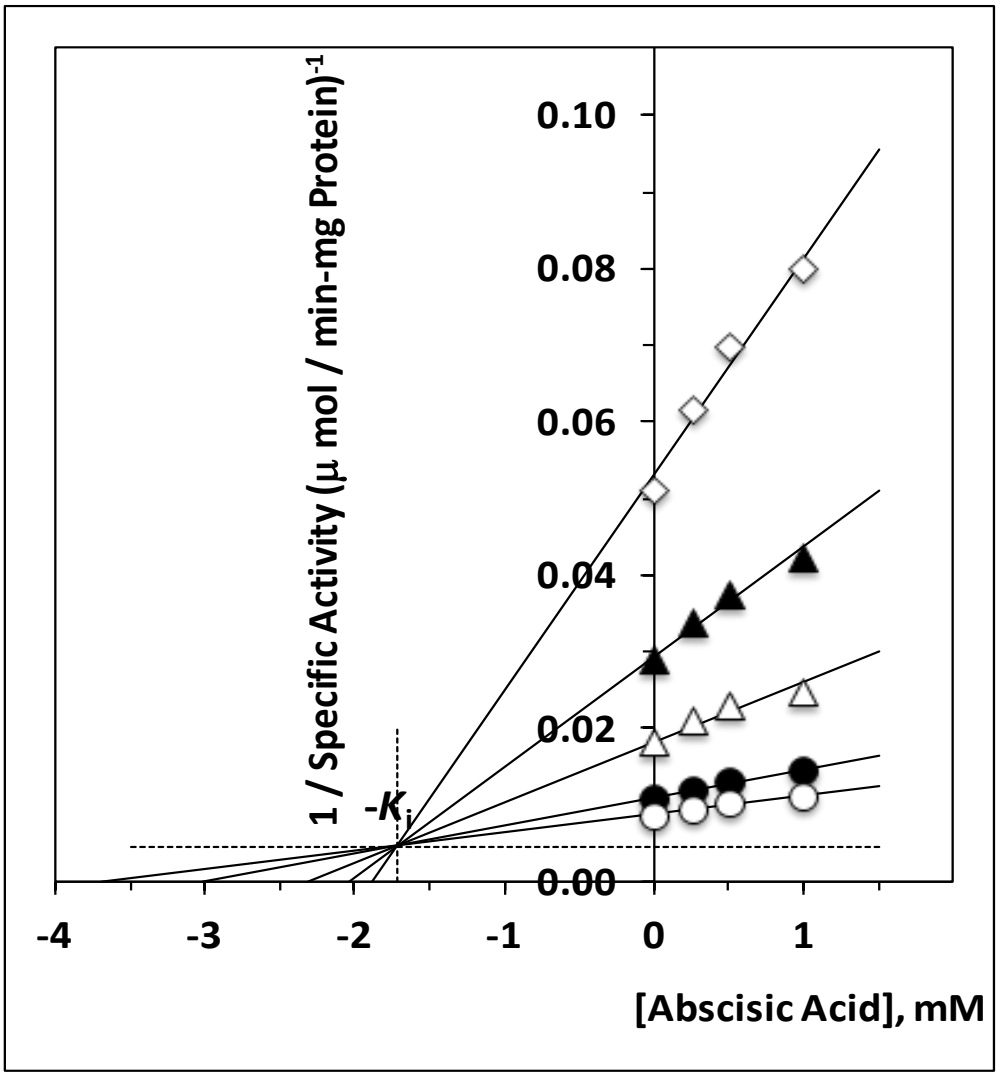


Figure 4.7. Dixon plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of abscisic acid at fixed 1 mM [GSH] and variable [CDNB]. [Abscisic acid] = 0, 0.25, 0.5, 1 and 2 mM. [CDNB] = (□), 0.1; (▲), 0.2; (Δ), 0.4; (●), 0.8; (○), 1.6 mM.

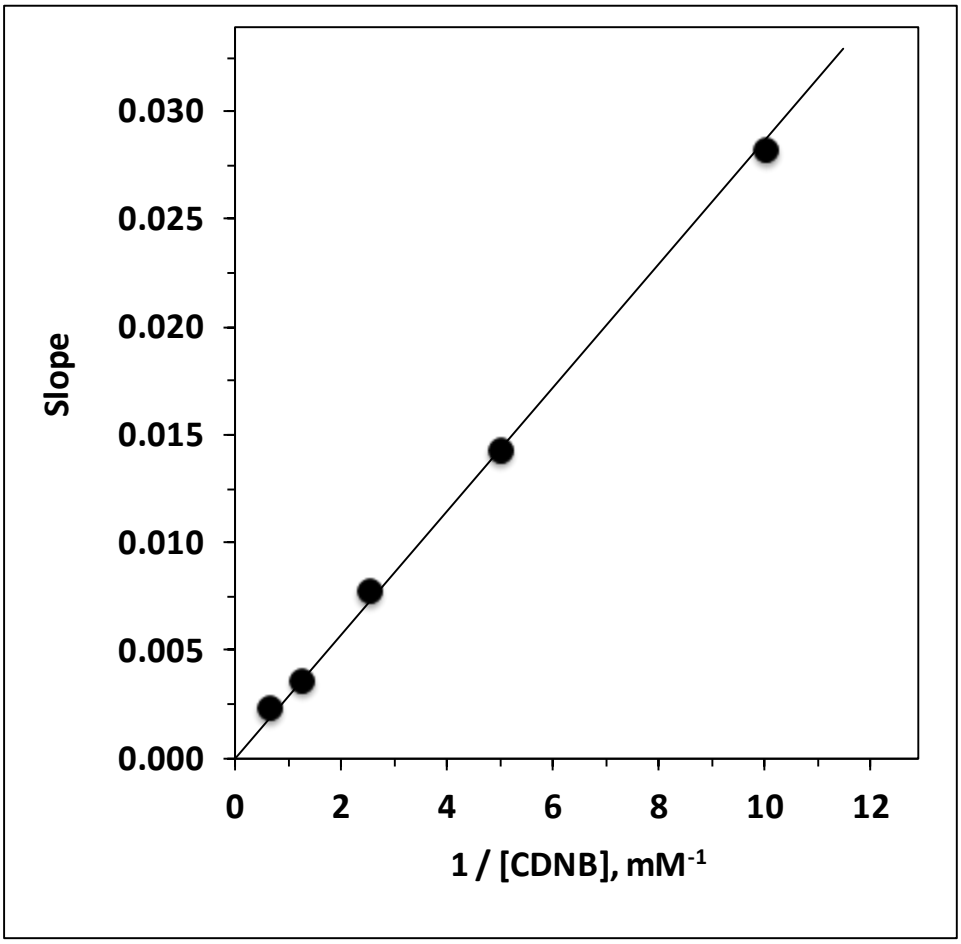


Figure 4.8. Replot of slopes versus 1/ [CDNB].

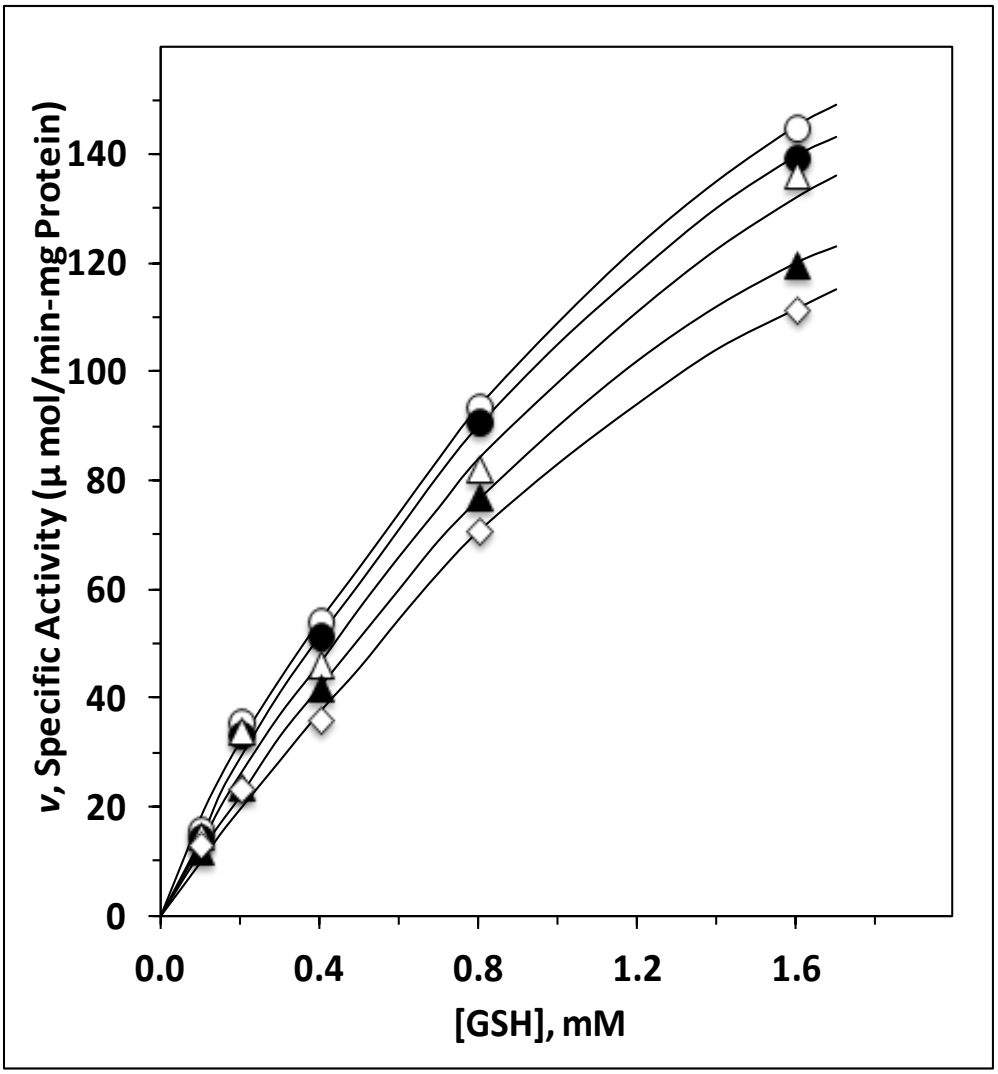


Figure 4.9. Michaelis–Menten plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of gibberellic acid at fixed 1 mM [CDNB] and variable [GSH] = 0.1, 0.2, 0.4, 0.8 and 1.6 mM. [Gibberellic acid] = (○), 0; (●), 0.25; (Δ), 0.5; (▲), 1; (□), 2 mM.

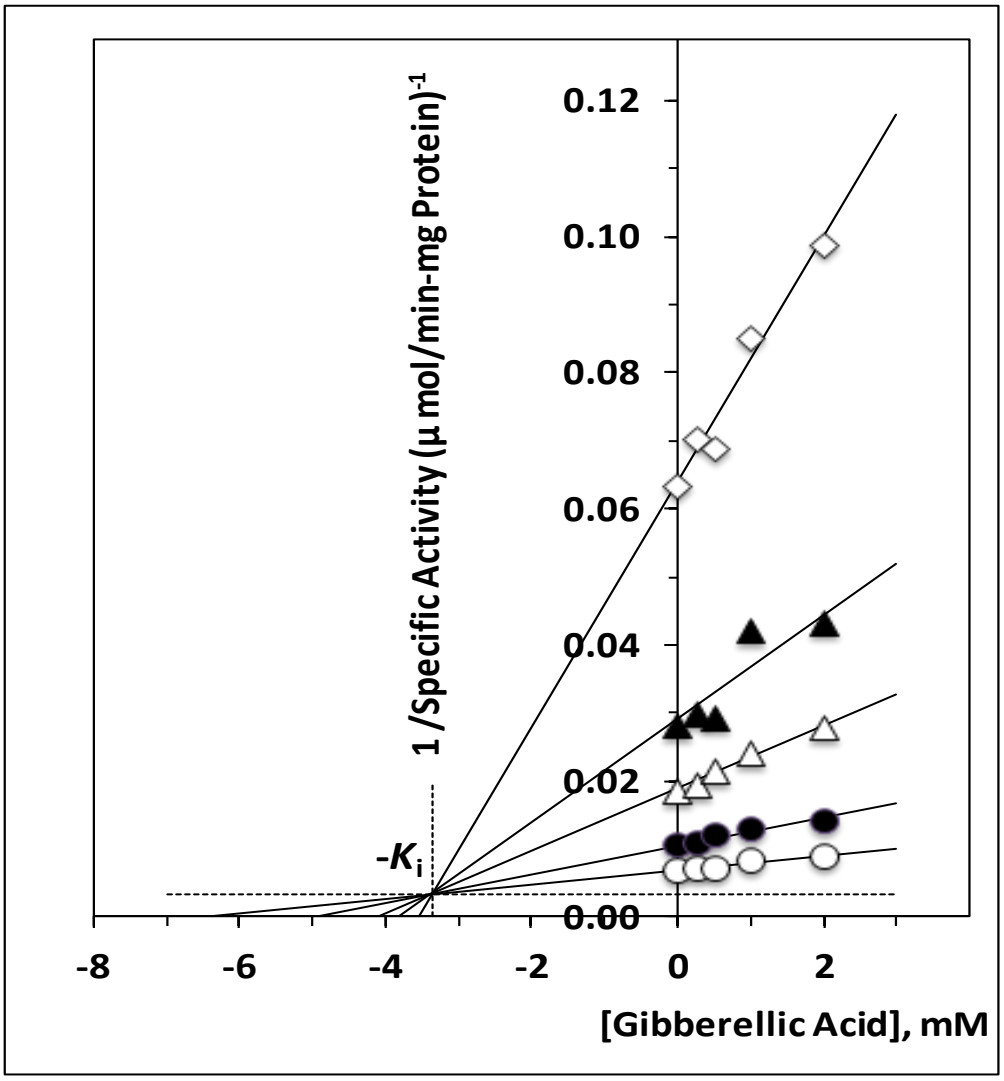


Figure 4.10. Dixon plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of gibberellic acid at fixed 1 mM [CDNB] and variable [GSH]. [Gibberellic acid]: 0, 0.25, 0.5, 1 and 2 mM. [GSH] = (□), 0.1; (▲), 0.2; (Δ), 0.4; (●), 0.8; (○), 1.6 mM.

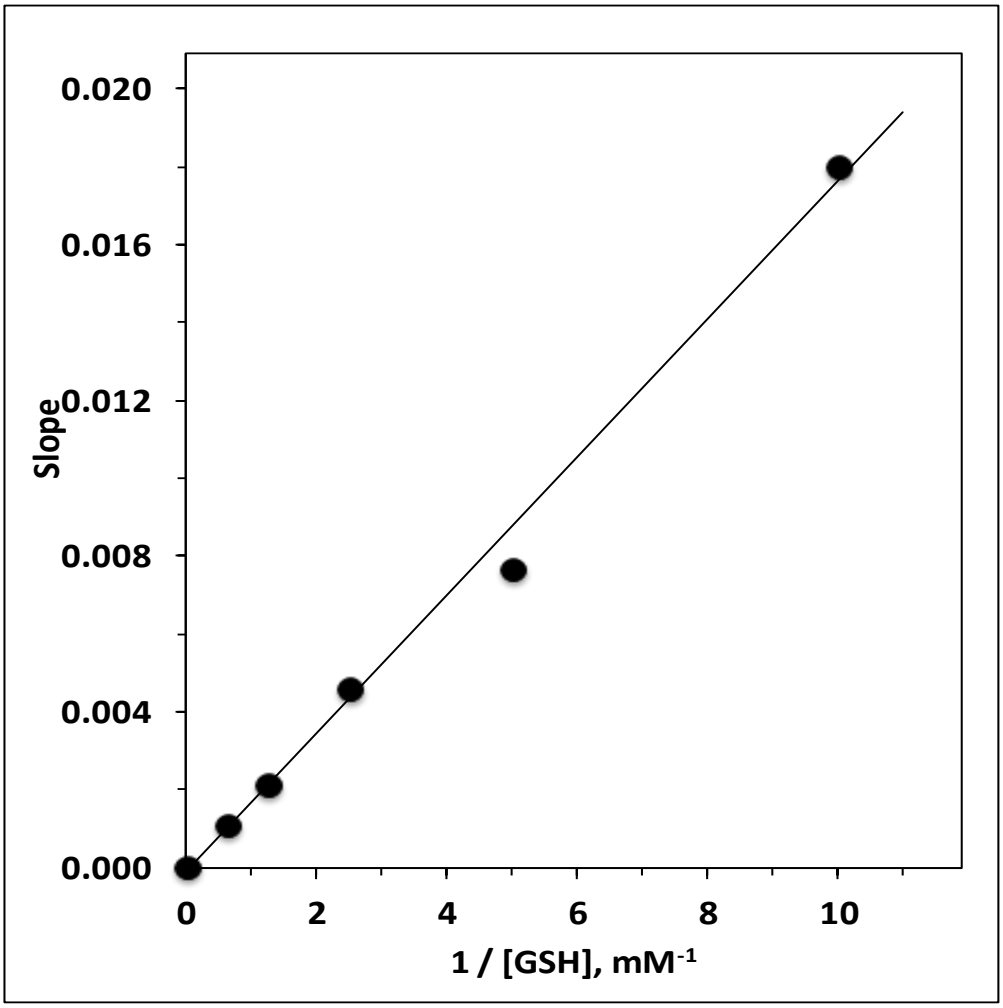


Figure 4.11. Replot of slopes of versus 1/ [GSH].

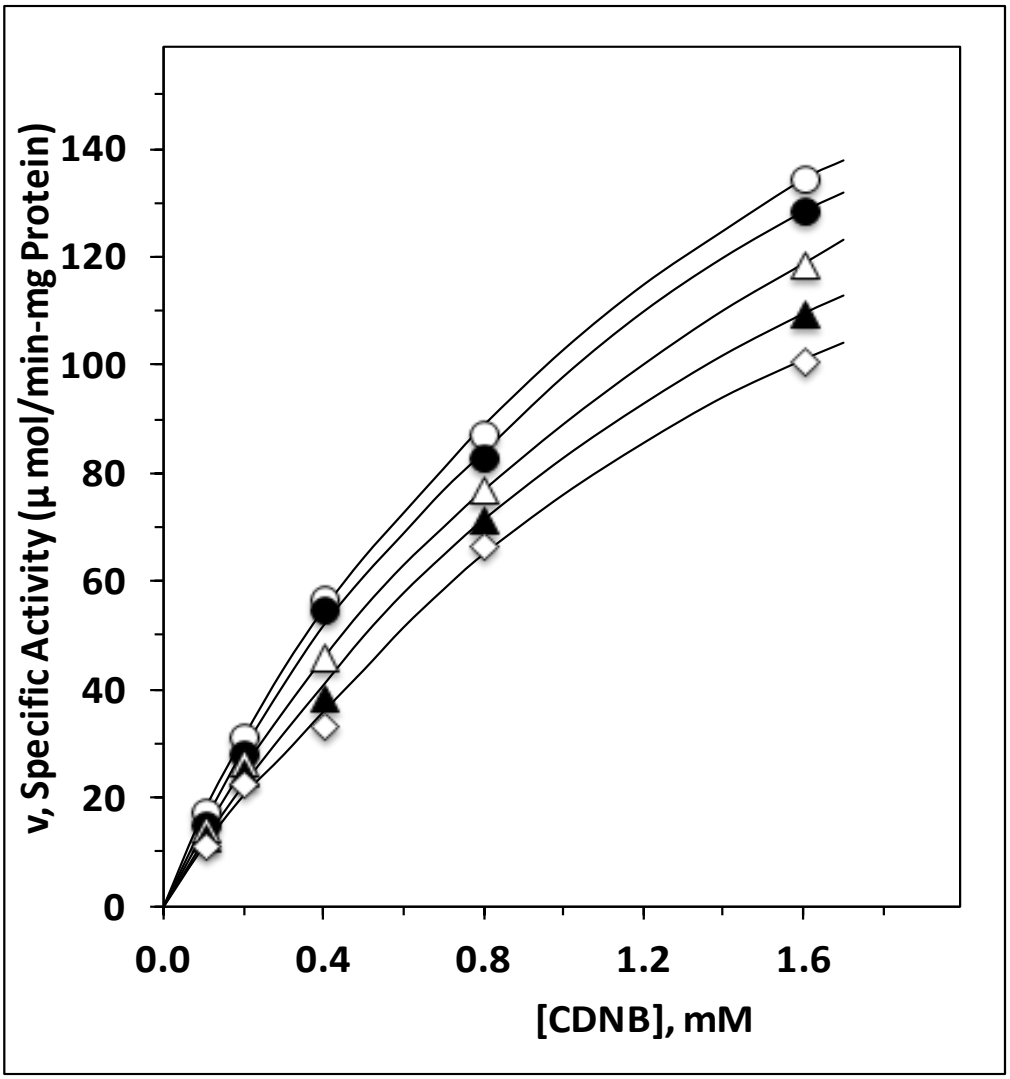


Figure 4.12. Michaelis–Menten plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of gibberellic acid at fixed 1 mM [GSH] and variable [CDNB]= 0.1, 0.2, 0.4, 0.8 and 1.6 mM. [Gibberellic acid] = (○), 0; (●), 0.25; (Δ), 0.5; (▲), 1; (□), 2 mM.

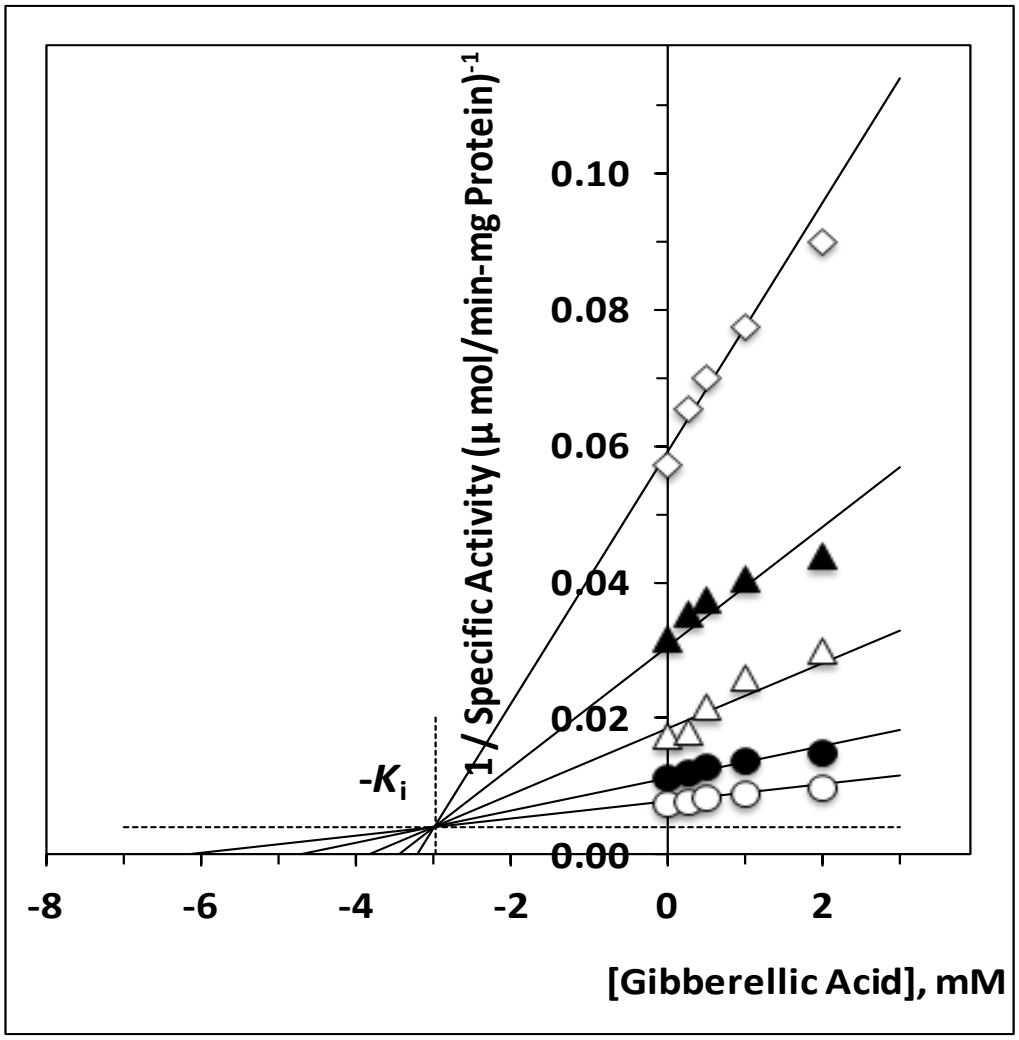


Figure 4.13. Dixon plot for the inhibition of *hp*GSTP1-1 enzyme with different concentrations of gibberellic acid at fixed 1 mM [GSH] and variable [CDNB]. [Gibberellic acid] = [Gibberellic acid]: 0, 0.25, 0.5, 1 and 2 mM. [CDNB] = (□), 0.1; (▲), 0.2; (Δ), 0.4; (●), 0.8; (○), 1.6 mM.

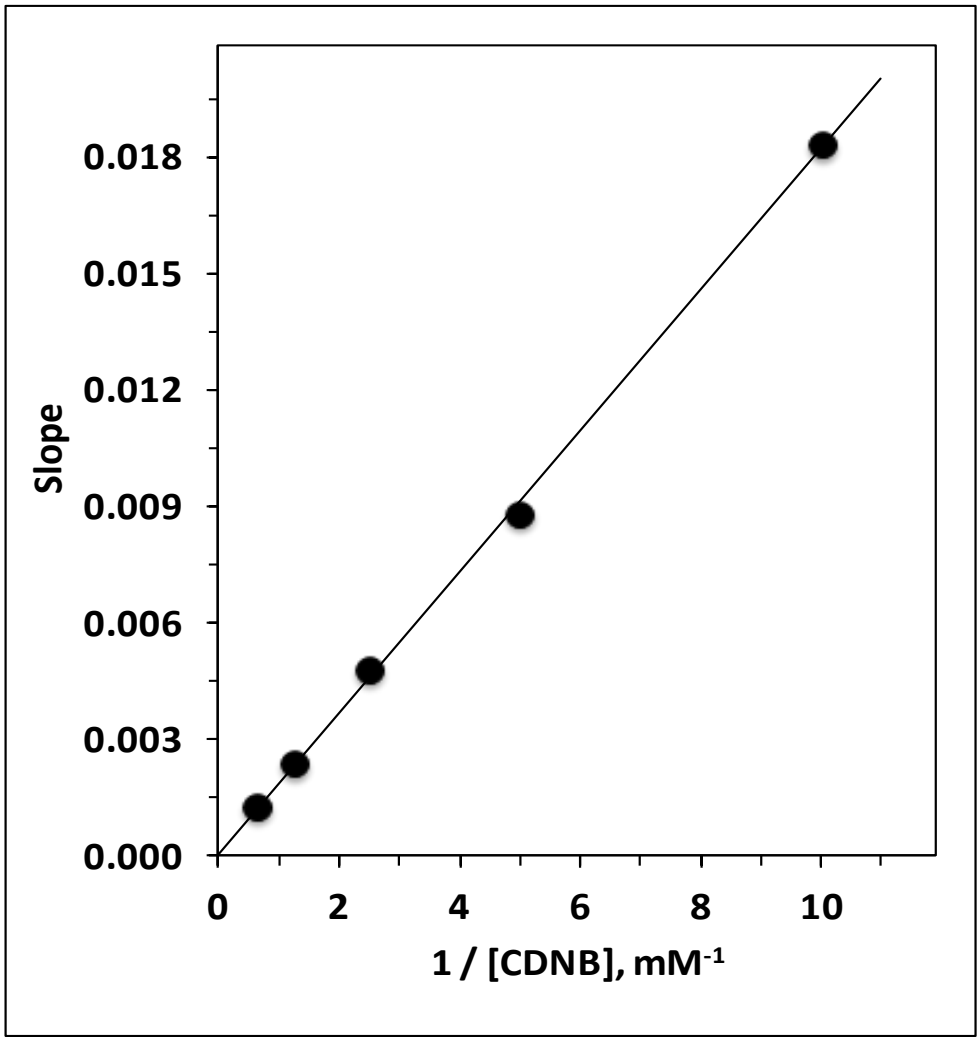


Figure 4.14. Replot of slopes versus 1/ [CDNB].

5. DISCUSSION

Cancer, which is one of the non-communicable diseases currently responsible for most global deaths, is considered to be the leading cause of mortality. In 2015, about 90.5 million people had cancer. Cancer cells can develop resistance by over-expressing GSTs that improves the detoxification of antitumor drugs, therefore inhibits their cytotoxic activity. In fact, it is understood that a variety of chemotherapeutic agents commonly used in cancer therapy are GST substrates. The GST superfamily, especially the pi-class GST (GSTpi), is often over-expressed in numerous human cancers. Over the past 20 years, abnormal GSTP1-1 expression has been consistent with the occurrence of tumor resistance to chemotherapy, suggesting how this enzyme has functions beyond metabolism. This finding reveals promising possibilities in relation to drug discovery, as GSTP1-1 inhibitors and their prodrugs propose a viable method for developing anticancer drugs with the primary aim of overcoming tumor resistance (Dong et al., 2018).

Herein, a graph of percentage remaining activity versus abscisic acid concentration was plotted and an IC_{50} value of 5.4 mM (Figure 4.1) was obtained. Gibberellic acid is slightly more potent than abscisic acid with an IC_{50} value of 5.0 mM (Figure 4.2). All these values are higher than some of the ones obtained using natural compounds such curcumin analogues (IC_{50} ranges 0.4-4.6 μM) (Appiah-Opong et al., 2009), curcumin (5 μM), mitoxantrone (33 μM), resveratrol (100 μM), kaempferol (33 μM), ellagic acid (5 μM) and daidzein (100 μM) (Hayeshi et al., 2007).

The importance of examining the inhibitory effect of plant-derived inhibitors on GSTs has been elucidated by all these observations from previous studies. Gibberellic acid and abscisic acid are the two forms of phytohormones investigated in this study. Inhibitory kinetic studies were also performed with each inhibitor. Different abscisic acid concentrations were tested while keeping [CDNB] constant (1 mM) and [GSH] variable. Michaelis-Menten (Figure 4.3) and Dixon plots (Figure 4.4) were depicted. In Dixon plot, lines were seen to pass through the ordinate and converge at the second quadrant; which is a property of competitive inhibition in which the value of K_m does not change while V_m decreases (Figure 4.4). The K_i was

statistically estimated to be 1.95 ± 0.23 mM, with 1.32 ± 0.14 mM and 205 ± 12 μmol/min-mg protein for K_m and V_m, respectively (Table 4.1). The replot of slopes shows a line passing through the origin, this indicates a pure competitive inhibition for abscisic acid (Figure 4.5).

The Michaelis Menten and Dixon plots were also depicted at variable [CDNB] with constant [GSH] (1 mM) (Figure 4.6 and Figure 4.7). The replot of slopes shows a line passing through the origin to indicate competitive inhibition (Figure 4.8). The K_i of 1.79 ± 0.14 mM was obtained statistically with K_m of 0.76 ± 0.04 mM and V_m of 166 ± 4 μmol/min-mg protein.

Different gibberellic acid concentrations were used while keeping [CDNB] fixed (1 mM) and [GSH] variable. The Michaelis Menten (Figure 4.9), Dixon (Figure 4.10) and secondary plots (Figure 4.11) were depicted. Replots of slopes of versus 1/ [GSH] shows a line passing through the origin, which shows a clear competitive inhibition. The K_m, K_i and V_m values were calculated as 1.77 ± 0.1 mM, 3.38 ± 0.24 mM and 303 ± 10 μmol/min-mg protein, respectively (Table 4.1).

When [GSH] was fixed (1 mM) and [CDNB] was the variable substrate, the inhibition type was also competitive (Figure 4.12, 4.13 and 4.14) with K_m, K_i and V_m values of 1.43 ± 0.07 mM, 2.89 ± 0.19 mM and 249 ± 7 μmol/min-mg protein, respectively (Table 4.1). Both phytohormones examined in this study inactivated *hp*GSTP1-1 in a competitive inhibition mode with respect to both of the substrates.

By interacting with c-Jun-N terminal kinase-1 (JNK-1), GSTP1-1, a member of the cytosolic GSTs, regulates cell survival and apoptosis, retaining in an inactive form, thus defending the cells against hydrogen peroxide-induced cell death (Sheehan et al., 2001; Zimniak, 2007). A recent research found that over-expression of GSTP1-1 suppresses the motility and viability of prostate cancer by interfering with MYC and shutting down the MEK/ERK1/2 pathways (Wang et al., 2017). For this reason, several GST inhibitors have been identified with greater specificity and decreased toxicity that include synthesized and GSH analogues. In addition, various natural inhibitors present in plants were also discovered and studied (Allocati et al., 2018).

Several natural products have been claimed to inhibit GSTP1-1, including flavonoids, plant polyphenols and alkaloids. However, since most of these

compounds often inhibit other GST isoenzymes of comparable potency, this inhibition is also not specific (Laborde, 2010). A study that examined plant derived flavonoids reveals that GSTP1-1 activity can be inhibited by some flavonoids (Galangin IC_{50} = 14.4 μM, Kaempferol IC_{50} = 23.1 μM, Eriodictyol IC_{50} = 22.8 μM and Quercetin IC_{50} = 25.9 μM). Galangin was able to inhibit almost all cellular GSTP1-1 activity, other flavonoids like kaempferol, eriodictyol and quercetin showed moderate GSTP1-1 inhibitory potential (van Zanden et al., 2004). A significant class of inhibitors is GSH analogs that are more specific to GSTs and less toxic to cells. The chemical modification of L-glutamyl-L-cysteinylglycine (GSH) into -glutamyl-S(benzyl)cysteinyl-phenylglycine diethyl ester (i.e. ezatiostat or TLK199) is thus easily absorbed by the cell in which its metabolites bind the G-site (GSH binding site) of GSTP1-1 inducing inhibition (Wu and Batist, 2013).

In this research, the binding of abscisic acid and gibberellic acid to *hp*GSTpi did trigger conformational changes, particularly in the enzyme's substrate-binding site, so the enzyme has not been able to successfully bind substrates and catalyze the reaction. With the knowledge of the inhibition type from the *in vitro* study (i.e. competitive), abscisic acid and gibberellic acid were expected to bind to substrate-binding site rather than other binding sites in the structure of the protein.

6. CONCLUSION

In this research, we demonstrated the inhibition of *hp*GSTP1-1 by two phytohormones, abscisic acid (ABA) and gibberellic acid (GA). It appeared that both phytohormones inhibited the activity of *hp*GSTP1-1 in a competitive manner with respect to both substrates. ABA had an IC_{50} of 5.4 mM. When [GSH] was variable, V_m, K_m and K_i values were calculated as 205 ± 12 μmol/min-mg protein, 1.32 ± 0.14 mM and 1.95 ± 0.23 mM, respectively. At fixed [GSH] and variable [CDNB], V_m, K_m and K_i were found to be 166 ± 4 μmol/min-mg protein, 0.76 ± 0.04 mM and 1.79 ± 0.14 mM, respectively. GA had an IC_{50} value of 5.0 mM. When [GSH] was variable, V_m, K_m and K_i were calculated as 303 ± 10 μmol/min-mg protein, 1.77 ± 0.1 mM and 3.38 ± 0.24 mM, respectively. At fixed [GSH] and variable [CDNB], V_m, K_m and K_i were found to be 249 ± 7 μmol/min-mg protein, 1.43 ± 0.07 mM and 2.89 ± 0.19 mM, respectively. However, the *in vitro* study of these phytohormones shows their capacity to inhibit the function of *hp*GSTP1-1, and there are also some conditions that should be considered for the development of a new therapeutic drug, including the drug's cytotoxicity and the application of preclinical trials accompanied by clinical trials.

REFERENCES

Allocati N, Federici L, Masulli M, Di Ilio C. Glutathione transferases in bacteria. The FEBS Journal. 2009; 276(1): 58-75.

Allocati N, Masulli M, Di Ilio C, Federici L. Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. Oncogenesis. 2018; 7(1): 8.

Appiah-Opong R, Commandeur JNM, Istyastono E, Bogaards JJ, Vermeulen NPE. Inhibition of human glutathione S-transferases by curcumin and analogues. Xenobiotica. 2009; 39: 302-311.

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A. ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci. 2013; 4: 1-19.

Armstrong RN. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol. 1997; 10(1):2-18.

Atkins WM, Wang RW, Bird AW, Newton DJ, Lu AYH. The catalytic mechanism of glutathione S-transferase (GST): Spectroscopic determination of the pK(a) of Tyr-9 in rat α1-1 GST. Journal of Biological Chemistry.1993; 268(26): 19188-19191.

Atkinson HJ, Babbitt PC. Glutathione transferases are structural and functional outliers in the thioredoxin fold. Biochemistry. 2009; 48(46): 11108-11116.

Bartolini D, Galli F. The functional interactome of GSTP: A regulatory biomolecular network at the interface with the Nrf2 adaption response to oxidative stress. J Chromatogr B Analyt Technol Biomed Life Sci 2016; 1019: 29-44.

Bassaganya-Riera J, Skoneczka J, Kingston DG, Krishnan A, Misyak SA, Guri AJ, Pereira A, Carter AB, Minorsky P, Tumarkin R, Hontecillas R. Mechanisms of action and medicinal applications of abscisic Acid. Curr Med Chem. 2010; 17(5):467-78.

Bassaganya-Riera J, Guri AJ, Lu P, Climent M, Carbo A, Sobral BW, Horne WT, Lewis SN, Bevan DR, Hontecillas R. Abscisic acid regulates inflammation via ligand-binding domain-independent activation of peroxisome proliferator-activated receptor γ. The Journal of Biological Chemistry. 2011; 286(4): 2504-2516.

Board PG, Menon D. Glutathione transferases , regulators of cellular metabolism and physiology. Biochim Biophys Acta. 2013; 1830(5): 3267-3288.

Bocedi A, Noce A, Marrone G, Noce G, Cattani G, Gambardella G, Di Lauro M, Di Daniele N, Ricci G. Glutathione transferase P1-1 an enzyme useful in biomedicine and as biomarker in clinical practice and in environmental pollution. Nutrients. 2019; 11(8): 1741.

Booth J, Boyland E, Sims P. An enzyme from rat liver catalysing conjugations with glutathione. Biochem J. 1961; 79(3): 516-524.

Boyland E, Chasseaud LF. Glutathione S-alkyltransferase. Biochem J. 1969; 115(5): 985-991.

Chatterjee A, Gupta S. The multifaceted role of glutathione S-transferases in cancer. Cancer Lett. 2018; 433: 33-42.

Chen C, Wu C, Lu X, Yan Z, Gao J, Zhao H, Li S. Coniferyl ferulate, a strong inhibitor of glutathione S-transferase isolated from radix Angelicae sinensis, reverses multidrug resistance and downregulates P-glycoprotein. Evid Based Complement Alternat Med. 2013; 2013: 639083.

Chen K, Li G-J, Bressan RA, Song C-P, Zhu J-K, Zhao Y. Abscisic acid dynamics, signaling, and functions in plants. Journal of Integrative Plant Biology. 2020; 62(1): 25-54.

Cho SG, Lee YH, Park HS, Ryoo K, Kang KW, Park J, Eom SJ, Kim MJ, Chang TS, Choi SY, Shim J, Kim Y, Dong MS, Lee MJ, Kim SG, Ichijo H, Choi EJ. Glutathione S-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase 1. J Biol Chem. 2001; 276(16): 12749-12755.

Croom E. Metabolism of xenobiotics of human environments. Prog Mol Biol Transl Sci. 2012; 112: 31-88.

Cui YX, Xu ZC, Chen XL, Nie LP, Wu LW, Wang Y, Song JY, Yao H. Genome-wide identification of abscisic acid (ABA) receptor pyrabactin resistance 1-like protein (PYL) family members and expression analysis of PYL genes in response to

different concentrations of ABA stress in Glycyrrhiza uralensis. Chinese Journal of Natural Medicines. 2020; 18(8): 606-611.

Dahlstrom RV, Sfat MR. Relationship of gibberellic acid to enzyme development. Gibberellins. 1961; 28: 59-70.

Dalmizrak O, Kulaksiz-Erkmen G, Ozer N. Fluoxetine-induced toxicity results in human placental glutathione S-transferase-π (GST-π) dysfunction. Drug and Chemical Toxicology. 2016; 39: 439-444.

De Luca A, Federici L, De Canio M, Stella L, Caccuri AM. New insights into the mechanism of JNK1 inhibition by glutathione transferase P1-1. Biochemistry. 2012; 51: 7304-7312.

de Oliveira DM, de Farias MT, Teles AL, Dos Santos Junior MC, de Cerqueira MD, Lima RM, El-Bachá RS. 8-methoxypsoralen is a competitive inhibitor of glutathione S-transferase P1-1. Front Cel Neurosci.2014; 8: 308.

Dong S, Sha H, Xu X, Hu T, Lou R, Li H, Wu J, Dan C, Feng J. Glutathione S-transferase π : a potential role in antitumor therapy. Drug Design, Development and Therapy. 2018; 12: 3535-3547.

Dong X, Yang Y, Zhou Y, Bi X, Zhao N, Zhang Z, Li L, Hang Q, Zhang R, Chen D, Cao P, Yin Z, Luo L. Glutathione S-transferases P1 protects breast cancer cell from adriamycin-induced cell death through promoting autophagy. Cell Death Differ. 2019 ;26(10): 2086-2099.

Dorion S, Lambert H, Landry J. Activation of the p38 signaling pathway by heat shock involves the dissociation of glutathione S-transferase mu from Ask1. The Journal of Biological Chemistry. 2002; 277: 30792-30797.

Dourado D, Fernandes PA, Mannervik B, Ramos MJ. Glutathione transferase : New model for glutathione activation. Chemistry. 2008; 14(31): 9591-9598.

Edwards M. Dormancy in Seeds of Charlock (Sinapis arvensis L.). Plant Physiol. 1976; 58 :626-630.

Federici L, Lo Sterzo C, Pezzola S, Di Matteo A, Scaloni F, Federici G, Caccuri AM. Structural basis for the binding of the anticancer compound 6-(7-Nitro-2,1,3-

benzoxadiazol-4-Ylthio)hexanol to human glutathione S-transferases. Cancer Res. 2009; 69(20): 8025-8035.

Finkelstein R. Abscisic acid synthesis and response. The Arabidopsis Book. 2013; 11:137-155.

Fulci C, Rotili D, De Luca A, Stella L, della Rocca BM, Forgione M, Di Paolo V, Mai A, Falconi M, Quintieri L, Caccuri AM. A new nitrobenzoxadiazole-based GSTP1-1 inhibitor with a previously unheard of mechanism of action and high stability. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017; 32(1): 240-247.

Gangwar S, Singh VP, Tripathi DK, Chauhan DK, Prasad SM, Maurya JN. Chapter 10. Plant Responses to Metal Stress: The Emerging Role of Plant Growth Hormones in Toxicity Alleviation. Second Edi. Elsevier Inc; 2014, pp: 215-248.

Gate L, Majumdar RS, Lunk A, Tew KD. Increased myeloproliferation in glutathione S-transferase π-deficient mice is associated with a deregulation of JNK and Janus Kinase / STAT pathways. The Journal of Biological Chemistry. 2004; 279(10): 8608-8616.

Graminski GF, Kubo Y, Armstrong RN. Spectroscopic and kinetic evidence for the thiolate anion of glutathione at the active site of glutathione S-transferase. Biochemistry. 1989a; 28: 3562-3568.

Graminski GF, Zhang PH, Sesay MA, Ammon HL, Armstrong RN. Formation of the 1-(S-glutathionyl)-2,4,6-trinitrocyclohexadienate anion at the active site of glutathione S-transferase: evidence for enzymic stabilization of sigma-complex intermediates in nucleophilic aromatic substitution reactions. Biochemistry. 1989b; 28(15): 6252-6258.

Gupta R, Chakrabarty SK. Gibberellic acid in plant. Plant Signaling & Behavior. 2013; 8: e25504.

Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. Methods Enzymol. 1981; 77: 398-405.

Habig WH, Pabst MJ, Fleischnert G, Gatmaitant Z, Arias IM, Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver.

Proc Natl Acad Sci USA. 1974; 71(10): 3879-3882.

Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry. 1974a; 249(22): 7130-7140.

Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005; 45: 51-88.

Hayeshi R, Mukanganyama S, Hazra B, Abegaz B, Hasler J. The interaction of selected natural products with human recombinant glutathione transferases. Phytother Res. 2004; 18(11): 877-883.

Hayeshi R, Mutingwende I, Mavengere W, Masiyanise V, Mukanganyama S. The inhibition of human glutathione S-transferases activity by plant polyphenolic compounds ellagic acid and curcumin. Food Chem Toxicol. 2007; 45(2): 286-295.

Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science. 1997; 275: 90-94.

Izquierdo-Bueno I, González-Rodríguez VE, Simon A, Dalmais B, Pradier J-M, Le Pêcheur P, Mercier A, Walker A-S, Garrido C, Collado IG, Viaud M. Biosynthesis of abscisic acid in fungi: identification of a sesquiterpene cyclase as the key enzyme in Botrytis cinerea. Environ Microbiol. 2018; 20(7): 2469-2482.

Jemth P, Mannervik B. Kinetic characterization of recombinant human glutathione transferase T1-1 , a polymorphic detoxication enzyme. Arch Biochem Biophys. 1997; 348: 247-254.

Ketley JN, Habig WH, Jakoby WB. Binding of nonsubstrate ligands to the glutathione S-transferases. The Journal of Biological Chemistry. 1975; 250(22): 8670-8673.

Ketterer B, Coles B, Meyer DJ. The role of glutathione in detoxication. Environ Health Perspect. 1983; 49: 59-69.

Kim SW, Goossens A, Libert C, Van Immerseel F, Staal J, Beyaert R.

Phytohormones: Multifunctional nutraceuticals against metabolic syndrome and comorbid diseases. Biochem Pharmacol. 2020; 175:113866.

Kong KH, Nishida M, Inoue H, Takahashi K. Tyrosine-7 is an essential residue for the catalytic activity of human class pi glutathione S-transferase: Chemical modification and site-directed mutagenesis studies. Biochemical and Biophysical Research Communication. 1992; 182(3): 1122-1129.

Laborde E. Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. Cell Death and Differentiation. 2010; 17: 1373-1380.

Leaver MJ, George SG. A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation. Marine Environmental Research. 1998; 46: 71-74.

Li HH, Hao RL, Wu SS, Guo PC, Chen CJ, Pan LP, Ni H. Occurrence, function and potential medicinal applications of the phytohormone abscisic acid in animals and humans. Biochem Pharmacol. 2011; 82(7):701-12.

Liang Z, Ma Y, Xu T, Cui B, Liu Y, Guo Z, Yang D. Effects of abscisic acid, gibberellin, ethylene and their interactions on production of phenolic acids in salvia miltiorrhiza bunge hairy roots. PloS One. 2013; 8(9): e72806.

Lo H-W, Ali-Osman F. Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Current Opinion in Pharmacology. 2007; 7: 367-374.

Mahajan SS, Hou L, Doneanu C, Paranji R, Maeda D, Zebala J, Atkins WM. Optimization of bivalent glutathione S-transferase inhibitors by combinatorial linker design. Journal of the American Chemical Society. 2006; 128: 8615-8625.

Mandal PK, Shukla D, Govind V, Boulard Y, Ersland L. Glutathione conformations and iIts implications for in vivo magnetic resonance spectroscopy. Journal of Alzheimer's Disease. 2017; 59(2): 537-541.

Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Jörnvall H. Identification of three classes of cytosolic glutathione transferase common to several mammalian species: Correlation between structural data and enzymatic properties.

Proc Natl Acad Sci USA. 1985; 82(21): 7202-7206.

Mannervik B, Danielson UH. Glutathione transferases-structure and catalytic activity. CRC Crit Rev Biochem. 1988; 23(3): 283-337.

Martin JL. Thioredoxin -a fold for all reasons. Structure. 1995; 3: 245-250.

Mohammad J, Singh RR, Riggle C, Haugrud B, Abdalla MY, Reindl KM. JNK inhibition blocks piperlongumine-induced cell death and transcriptional activation of heme oxygenase-1 in pancreatic cancer cells. Apoptosis. 2019; 24(9-10): 730-744.

Molina DM, Eshaghi S, Nordlund P. Catalysis within the lipid bilayer — structure and mechanism of the MAPEG family of integral membrane proteins. Current Opinion in Structural Biology. 2008; 18(4): 442-449.

Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol. 2005; 56: 165-185.

Nebert DW, Vasiliou V. Analysis of the glutathione S-transferase (GST) gene family. Human Genomics. 2004; 1:460-464.

Oakley A. Glutathione transferases: A structural perspective. Drug Metab Rev. 2011; 43(2): 138-151.

Oakley AJ, Lo Bello M, Nuccetelli M, Mazzetti AP, Parker MW. The ligandin (non-substrate) binding site of human pi class glutathione transferase is located in the electrophile binding site (H-site). J Mol Biol. 1999; 291(4): 913-926.

O'Brien ML, Vulevic B, Freer S, Boyd J, Shen H, Tew KD. Glutathione peptidomimetic drug modulator of multidrug resistance-associated protein 1. The Journal of Pharmacology and Experimental Therapeutics. 1999; 291(3): 1348-1355.

O'Dwyer PJ, LaCreta F, Nash S, Tinsley PW, Schilder R, Clapper ML, Tew KD, Panting L, Litwin S, Comis RL, Ozols RF. Phase I study of thiotepa in combination with the glutathione transferase. Cancer Research. 1991; 51: 6059-6065.

Palumbo C, De Luca A, Rosato N, Forgione M, Rotili D, Caccuri AM. C-Jun-N terminal kinase activation by nitrobenzoxadiazoles leads to late stage autophagy inhibition. Journal of Translational Medicine. 2016; 37: 1-12.

Prade L, Huber R, Manoharan TH, Fahl WE, Reuter W. Structures of class pi

glutathione S-transferase from human placenta in complex with substrate , transition-state analogue and inhibitor. Structure. 1997; 5(10): 1287-1295.

Raijmakers MT, Steegers EA, Peters WH. Glutathione S-transferases and thiol concentrations in embryonic and early fetal tissues. Hum Reprod. 2001; 16(11): 2445-2450.

Raza A, Galili N, Callander N, Ochoa L, Piro L, Emanuel P, Williams S, Burris H 3rd, Faderl S, Estrov Z, Curtin P, Larson RA, Keck JG, Jones M, Meng L, Brown GL. Phase 1-2a multicenter dose-escalation study of ezatiostat hydrochloride liposomes for injection (Telintra, TLK199), a novel glutathione analog prodrug in patients with myelodysplastic syndrome. J Hematol Oncol. 2009; 2:20.

Ricci G, De Maria F, Antonini G, Turella P, Bullo A, Stella L, Filomeni G, Federici G, Caccuri AM. 7-Nitro-2,1,3-benzoxadiazole derivatives, a new class of suicide inhibitors for glutathione S-transferases. J Biol Chem. 2005; 280(28): 26397-26405.

Rodrigues C, Porto L, Vandenberghe DS, Oliveira JD. New perspectives of gibberellic acid production: a review. Crit Rev Biotechnol. 2012; 32(3): 263-273.

Ruzza P, Calderan A. Glutathione transferase (GST)-activated prodrugs. Pharmaceutics. 2013; 5(2): 220-231.

Sau A, Pellizzari F, Valentino F, Federici G, Maria A. Glutathione transferases and development of new principles to overcome drug resistance. Archives of Biochemistry and Biophysics. 2010; 500: 116-122.

Sau A, Filomeni G, Pezzola S, D'Aguanno S, Tregno FP, Urbani A, Serra M, Pasello M, Picci P, Federici G, Caccuri AM. Targeting GSTP1-1 induces JNK activation and leads to apoptosis in cisplatin-sensitive and -resistant human osteosarcoma cell lines. Molecular BioSystems. 2012; 8: 994-1006.

Segel IH. Enzyme Kinetics. New York: A Wiley-Interscience Publication; 1975.

Sezgin M, Kahya M. Phytohormones. Journal of Science and Technology. 2018; 8(1): 35-39.

Sheehan D, Meade G, Foley MV, Dowd AC. Structure, function, and evolution of glutathione transferases: implications for classification of non-mammalian members

of an ancient enzyme superfamily. Biochemical Journal.2001; 360, 1-16.

Shishido Y, Tomoike F, Kuwata K, Fujikawa H, Sekido Y, Murakami-Tonami Y, Kameda T, Abe N, Kimura Y, Shuto S, Abe H. A covalent inhibitor for glutathione S-transferase Pi (GSTP 1-1) in human cells. ChemBioChem. 2019; 20(7): 900-905.

Singh S. Cytoprotective and regulatory functions of glutathione S-transferases in cancer cell proliferation and cell death. Cancer Chemother Pharmacol. 2015; 75(1): 1-15.

Su Y, Xia S, Wang R, Xiao L. Phytohormonal quantification based on biological principles. In Hormone Metabolism and Siganling in Plants. 2017; pp: 431-470.

Takino J, Kozaki T, Sato Y, Liu C, Ozaki T, Minami A, Oikawa H. Unveiling biosynthesis of the phytohormone abscisic acid in fungi: Unprecedented mechanism of core scaffold formation catalyzed by an unusual sesquiterpene synthase. Journal of the American Chemical Society. 2018; 140(39): 12392-12395.

Teodori E, Dei S, Martelli C, Scapecchi S, Gualtieri F. The functions and structure of ABC transporters: Implications for the design of new inhibitors of Pgp and MRP1 to control multidrug resistance (MDR). Current Drug Targets. 2006; 7(7): 893-909.

Teralı K, Dalmizrak O, Hoti Q, Ozer N. Evaluation of the inhibitory effect of abamectin on mammalian butyrylcholinesterase: Enzyme kinetic and molecular docking studies. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes. 2018; 53: 713-718.

Tesch GH, Ma FY, Nikolic-Paterson DJ. Targeting apoptosis signal-regulating kinase 1 in acute and chronic kidney disease. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology. 2020; 10: 255

Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene. 2003; 22(47): 7369-7375.

Wang T, Arifoglu P, Ronai Z, Tew KD. Glutathione S-transferase P1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. J Biol Chem. 2001; 276(24): 20999-21003.

Wang XX, Jia HT, Yang H, Luo MH, Sun T. Overexpression of glutathione S-

transferase P1 inhibits the viability and motility of prostate cancer via targeting MYC and inactivating the MEK/ERK1/2 pathways. Oncol Res. 2017; Jun 19.

Wu B, Dong D. Human cytosolic glutathione transferases: structure, function, and drug discovery. Trends in Pharmacological Sciences. 2012; 33(12): 656-668.

Wu JH, Batist G. Glutathione and glutathione analogues; therapeutic potentials. Biochim Biophys Acta. 2013; 1830(5): 3350–3353.

van Zanden JJ, Geraets L, Wortelboer HM, Bladeren PJ, Rietjens IM, Cnubben NH. Structural requirements for the flavonoid-mediated modulation of glutathione S-transferase P1-1 and GS-X pump activity in MCF7 breast cancer cells. Biochem Pharmacol. 2004; 67: 1607–1617.

Zhang L, Blackwell K, Altaeva A, Shi Z, Habelhah H. TRAF2 phosphorylation promotes NF-κB–dependent gene expression and inhibits oxidative stress – induced cell death. Mol Biol Cell. 2011; 22(1): 128-140.

Zimniak, P. Substrate and Reaction Mechanism of Glutathione Transferase: In Awasthi, C.Y. (2007) Toxicology of Glutathione Transferase, CRC Press, USA, pp 71-95.

Turnitin Orijinallik Raporu

İşleme kondu: 06-Nis-2021 16:32 EEST
NUMARA: 1408300882
Kelime Sayısı: 8404
Gönderildi: 5

THESIS Mohammad Abu Zaid tarafından

Benzerlik Endeksi	Kaynağa göre Benzerlik
%17	Internet Sources: %8 Yayınlar: %13 Öğrenci Ödevleri: %2

2% match (yayınlar)
Victor Markus, Kerem Teralı, Ozlem Dalmizrak, Nazmi Ozer. "Assessment of the inhibitory activity of the pyrethroid pesticide deltamethrin against human placental glutathione transferase P1-1: A combined kinetic and docking study", Environmental Toxicology and Pharmacology, 2018

2% match (yayınlar)
Shu-Chen Dong, Huan-Huan Sha, Xiao-Yue Xu, Tian-Mu Hu, Rui Lou, Huizi Li, Jian-Zhong Wu, Chen Dan, Jifeng Feng. "Glutathione S-transferase π: a potential role in antitumor therapy", Drug Design, Development and Therapy, 2018

1% match (27-Oca-2020 tarihli internet)
https://www.mdpi.com/2072-6643/11/8/1741/htm

1% match (yayınlar)
Ozlem Dalmizrak, Gulnihal Kulaksiz-Erkmen, Nazmi Ozer. "The inhibition characteristics of human placental glutathione S-transferase-π by tricyclic antidepressants: amitriptyline and clomipramine", Molecular and Cellular Biochemistry, 2011

1% match (yayınlar)
Baojian Wu, Dong Dong. "Human cytosolic glutathione transferases: structure, function, and drug discovery", Trends in Pharmacological Sciences, 2012

1% match (yayınlar)
Ozlem Dalmizrak, Gulnihal Kulaksiz-Erkmen, Nazmi Ozer. " Fluoxetine-induced toxicity results in human placental glutathione S-transferase- (GST-) dysfunction ", Drug and Chemical Toxicology, 2016

1% match (yayınlar)
Dalmizrak, O., G. Kulaksiz-Erkmen, and N. Ozer. "Possible prenatal impact of sertraline on human placental glutathione S-transferase-Â ", Human & Experimental Toxicology, 2012.

1% match (15-Eki-2019 tarihli internet)
https://en.wikipedia.org/wiki/Abscisic_acid

< 1% match (05-Oca-2017 tarihli öğrenci ödevleri)
Submitted to Yakın Doğu Üniversitesi on 2017-01-05

< 1% match (14-Eyl-2018 tarihli internet)
https://d-nb.info/1139119249/34

< 1% match (25-May-2016 tarihli internet)
http://dmd.aspetjournals.org/content/early/2016/01/12/dmd.115.067397.full.pdf

< 1% match (10-Kas-2007 tarihli öğrenci ödevleri)
Submitted to Brooks Academy of Science and Engineering on 2007-11-10

< 1% match (yayınlar)
Yury Patskovsky, Larysa Patskovska, Steven C. Almo, Irving Listowsky. " Transition State Model and Mechanism of Nucleophilic Aromatic Substitution Reactions Catalyzed by Human Glutathione -Transferase M1a-1a ", Biochemistry, 2006