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ORIGINAL ARTICLE

The evaluation of novel natural products as inhibitors of human glutathione transferase P1-1

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Abstract
Glutathione transferase P1-1 is over expressed in some cancer cells and contributes to detoxification of anticancer drugs, leading to drug-resistant tumors. The inhibition of human recombinant GSTP1-1 by natural plant products was investigated using 10 compounds isolated from plants indigenous to Southern and Central Africa. Monochlorobimane and 1-chloro-2,4-dinitrobenzene were used to determine GST activity. Each test compound was screened at 33 and 100 µM. Isofuranonapthoquinone (**1**) (from *Bulbine frutescens*) showed 68% inhibition at 33 µM, and sesquiterpene lactone (**2**) (from *Dicoma anomala*) showed 75% inhibition at 33 µM. The IC_{50} value of **1** was 6.8 µM. The mode of inhibition was mixed, partial (G site) and noncompetitive (H site) with K_i values of 8.8 and 0.21 µM, respectively. Sesquiterpene **2** did not inhibit the CDNB reaction. Therefore, isofuranonapthoquinone **1** needs further investigations *in vivo* because of its potent inhibition of GSTP1-1 *in vitro*.

Keywords: Glutathione transferases, cancer, multidrug resistance, GST P1-1, natural products

Introduction

Natural products have been in use since ancient times as medicines and spices, and the use of herbal remedies and dietary supplements is ever increasing[1,2]. With this resurgence of natural products, the focus on the interaction of the latter with xenobiotic metabolizing enzymes has received increased attention. For instance, cytochrome P450 enzymes have been found to interact with commonly used herbs[3], and flavonoids have been shown to inhibit glutathione *S*-transferases in blood platelets[4]. These characteristics suggest that phytochemicals may have important pharmacological and toxicological consequences[5].

During treatment of many cancers, there is often a development of drug resistance in a tumor that was originally sensitive to treatment resulting in a phenomenon known as multidrug resistance (MDR)[6]. Many mechanisms are involved in MDR, and these include alterations in drug transport resulting in impaired entry or enhanced efflux of the drug from the tumor cell, enhanced DNA repair, alterations in target proteins, and alterations in drug metabolism[7]. The glutathione transferases (EC 2.5.1.18: GST) are a unique family of detoxification enzymes comprising a large group of cytosolic, mitochondrial, and microsomal proteins which are capable of multiple reactions with a multitude of substrates, both endogenous and xenobiotic[8]. These enzymes can constitute up to 10% of cytosolic protein in some mammalian organs and play an important role in the detoxification of electrophilic xenobiotics such as, drugs, toxins, and carcinogens allowing the products to be exported from the cell through the GS-X pump in an ATP-dependent manner[9]. Besides catalysing the inactivation of various electrophile-producing anticancer agents via conjugation to the tripeptide glutathione, some cytosolic proteins belonging to the glutathione

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Abbreviations

CDNB	1-chloro-2,4-dinitrobenzene	GST	Glutathione transferase, isopropylthiogalactoside
DTT	dithiothreitol; GSH, glutathione	JNK	c-jun *N*-terminal kinase-1; PM

transferase superfamily are emerging as negative modulators of stress/drug-induced cell apoptosis through the interaction with specific signalling kinases[10]. GST P1 is over expressed in cancer cells; hence, it is regarded as a prognostic factor in cancer treatment[11]. Cancer cells and normal cells are known to respond differently to nutrients and drugs that affect glutathione status. Numerous studies have shown that tumor cells have elevated levels of glutathione levels, which confers resistance to chemotherapeutic drugs[12]. The genes coding for GST Pi are up regulated during early stage oncogenesis and significantly over expressed in human tumors. The high levels of GST Pi that result are associated with anti-cancer drug resistance and poor cancer patient survival[13].

This study focuses on the interaction of glutathione transferase Pi (GST Pi) and natural products isolated from *Dicoma anomala* (Asteraceae), *Bulbine frutescens* (Asphodelaceae), *Plumeria rubra* (Apocynaceae), *Dorstenia elliptica*, *Treculia africana* (Moraceae), *Garcinia smeathmannii*, and *Mammea africana* ((Clusiaceae or Guttiferae). *Dicoma anomala* is a small shrub whose common English names are fever bush and stomach bush. It is widely distributed in sub-Saharan Africa. Root decoctions are administered orally to children believed to be suffering from blood disorders[14]. The aerial parts of this plant collected from Namibia have been shown to contain several sesquiterpene lactones[15]. *G. smeathmannii* is an evergreen tree commonly found in southern and central Africa. It is used to treat eye inflammation, scabies, wounds, and stomach pain[16]. The isolation of xanthones and poly-prenylated benzophenone derivatives from *G. smeathmannii* as well as the antimicrobial and antioxidant properties of these compounds have been reported recently[17,18]. *Bulbine frutescens* is an ornamental herb that grows widely in Africa. It is also used medicinally to enhance the healing of wounds. The roots of *B. frutescens* are good sources of phenylanthraquinones and isofuranonaphthoquiones which are reported to possess anti-plasmodial and antimicrobial agents[19]. *T. africana*, commonly known as African bread fruit, is used in folk medicine against skin diseases and dental allergies. Two flavonol derivatives were isolated from the leaves of *T. Africana*. One of these (**6**) is shown to possess antimicrobial properties[20]. *Dorstenia elliptica* Bureau, an undergrowth perennial plant, is used in the treatment of many diseases, especially, for eye infections. Phytochemical investigation of the twigs of *D. ellipitca* resulted in the isolation of several coumarins including compound **4**. The crude extracts as well as compound 4 are reported to have antimicrobial properties[21]. *P. rubra*, commonly known as Red Frangipani, is a spreading shrub or small tree to a height of 7–8 m and wide flushed with fragrant flowers. It is widely cultivated in subtropical and tropical climates worldwide. It is reported to contain triterpenes[22] alkaloids[23], and other cytotoxic compounds[24].

Mammea africana is a large forest tree commonly known as the African apple, African apricot, and African mammey apple. Extracts from this plant consist mainly of coumarin derivatives which are known to exhibit a number of bioactivities such as insecticidal, antioxidant, anticancer, antibacterial, antimicrobial, and antibiotic activities[25].

Multidrug resistance is often associated with decreased intracellular drug accumulation in a patient's tumor cells due to enhanced drug efflux or enhanced metabolism via GSTs[26]. Therefore, there is an urgent need to find replacement for drugs previously used or to find suitable chemo-modulators in order to reverse drug resistance[27]. Several natural products have been identified as possible anticancer agents that exhibit antimutagenic and anti-proliferative characteristics[28]. Seventy percent of all present antileukemia drugs have been derived from natural products or their derivatives[29]. Doxorubicin, vinblastine, and vincristine represent some of the current standard chemotherapeutic drugs that have been isolated from plants and used in the treatment of solid and blood cancers[30]. The use of GST inhibitors as therapeutic agents has been proved to be useful in endeavours to modulate anticancer drug resistance[31]. Natural products that are potent GST P1-1 inhibitors may have possible uses in chemo-modulation and cancer therapy given the role that the elevated GST P1-1 levels play in cancer proliferation and progression. The aim of this study was to evaluate novel natural products as inhibitors of glutathione transferase P1-1 from the plants *Dicoma anomala*, *B. frutescens*, *P. rubra, Dorstenia elliptica, T. africana, G. smeathmannii*, and *M. africana*. This evaluation was aimed at searching for potential effective inhibitors of GSTs that could augment the cytotoxic effects of alkylating anticancer drugs in the case where these enzymes are involved in alkylating anticancer drug resistance.

Materials and methods

Reagents and chemicals

The substrates 1-chloro-2,4 dinitrobenzene (CDNB), monochlorobimane (MCB), and other chemicals and reagents were obtained from Sigma Chemical Company and Aldrich Chemical Company (St Louis, MO, USA). The natural product compounds used in this study were obtained from Professor Berhanu Abegaz (University of Botswana, Botswana). The compounds and the plants from which they are isolated are as follows. The isofuranonaphthoquinone (**1**) was extracted from *B. frutescens*[32], the sesquiterpene lactone **2** was extracted from *Dicoma anomala*[33], iridoid **3** was extracted from *P. rubra*[34], furocoumarin (**4**) was obtained from *Dorstenia elliptica*[35], and benzophenone derivative (**5**) was extracted from *G. smeathmannii*[36]. Flavanol 6 was isolated from *T. africana*[37]. The xanthones (**7** and **9**) and coumarin (**8**) were obtained from *M. africana*[38–40]. The natural products were extracted from the above-mentioned plants using the following general protocol. The sun-dried plant material (ca 1 kg) was soaked in a mixture of dichloromethane–methanol (1:1) and pure methanol for 24 h and 2 h, respectively, at room temperature. Concentration of the combined organic extract gave a residue (ca 50–65 g). Part of this residue was chromatographed on a silica gel column eluting with hexane–ethyl acetate mixtures, to give fractions of 250 ml each. The fractions were concentrated and monitored by TLC and ^{1}H NMR, and similar fractions were combined. The first fractions examined by TLC (hexane–ethyl acetate; 9:1) contained mainly mixtures of hydrocarbons and phytosterols, which were not investigated further. More polar fractions were passed through Sephadex LH-20 column ($CHCl_3$–methanol, 2:1). The post-chlorophyll fractions were subjected to repeated silica gel CC and PTLC to yield the various metabolites. Pure metabolites' molecular structures were established by spectroscopic techniques such as NMR, MS, and IR. The structures of the chemicals used are shown in Figure 1. All the other chemicals used were of the highest purity obtained from different sources. The structures of the compounds used are shown in Figure 1. *Escherichia coli* cells with the gene for human GST P1-1 were obtained from Professor Bengt Mannervik (Department of Biochemistry, Uppsala University, Sweden).

Expression and purification of recombinant glutathione S-transferases Pi

Recombinant human P1-1 were expressed in *E. coli* and purified as described by Mukanganyama *et al.* (35). A 100 ml portion of 2TYA medium (54 g tryptone, 40.5 g yeast extract, 13.5 g NaC1 and 27 g glycerol in 2 700 ml water) containing 13.5 µl ampicillin (1 M stock) was inoculated with 20 µl of the *E. coli* cells. The culture was incubated in a shaking incubator (Labcon, Labotec, South Africa) operating at 170 rpm and 37°C for 20 h. Three 2000-ml conical flasks containing 500-ml 2TYA medium and 67.5 µl of 1-M ampicillin were inoculated with 5 ml of the culture and incubated in a shaking incubator at the same settings for 22 h. The bacteria were sedimented, lysed and GSTs purified, affinity chromatography on an *S*-hexylglutathione Sepharose 6B (Pharmacia, Uppsala, Sweden) affinity gel. The activity of the enzyme was determined using CDNB as substrate[40] and protein content was determined using the Lowry procedure[41].

The purity of the enzyme purification fractions was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE), carried out on 15% slab gels using a Hoeffer SE Mighty Small II electrophoresis system (Hoeffer Scientific Instruments, CA, USA). Protein bands were stained with Coomasie Blue-G.

Screening for Inhibition by Natural Products

Compounds **1–10** (Figure 1) were screened for inhibition of the major human cytosolic P1-1. First, the effects of NPs were determined on GSTs using monochlorobimane as a substrate for GST. A Shimadzu UV-1501 spectrophotofluorometer (Shimadzu Corporation, Kyoto, Japan) in the kinetics mode was used for the assay. The excitation wavelength was set at 390 nm and the emission wavelength was 478 nm. For all the readings, a concentration of 0.24 µg/ml of GST P1-1 was used and the final concentrations of MCB and GSH were 4 µM and 0.5 mM, respectively. Inhibition by the compounds was tested at 100 µM and 33.3 µM final concentrations from stock concentrations of 2.5 mM and 0.833 mM prepared in dimethyl sulfoxide (DMSO). Compounds that were found to be potent inhibitors using the MCB assay were also tested for inhibitory activity using CDNB as a substrate (Figure 2). The assay with CDNB was adapted

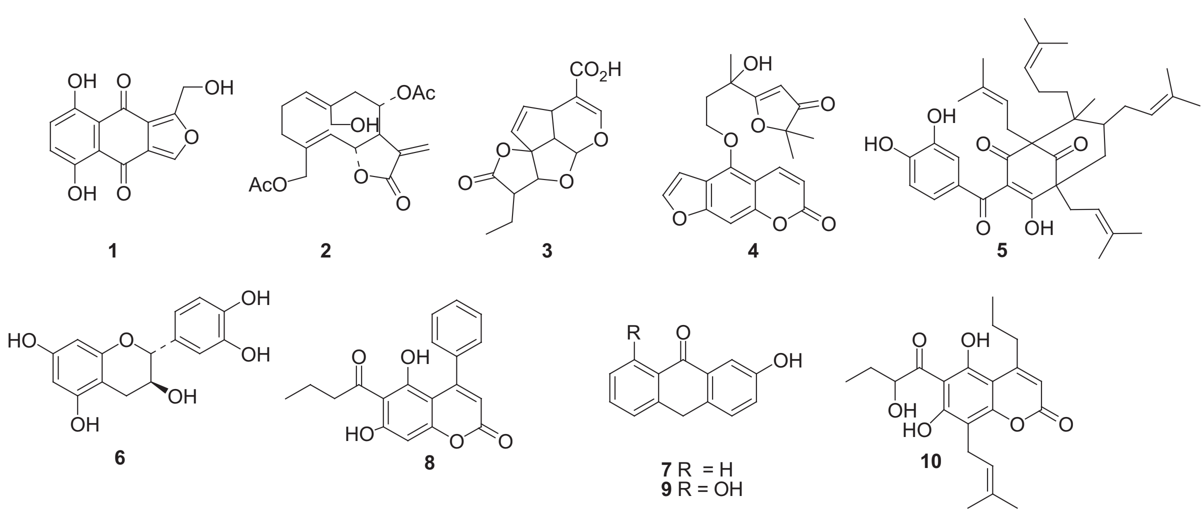

Figure 1. Chemical structures of the natural plant compounds used in this study.

for measurement of absorbance with a SpectraMax 340 microplate spectrophotometer equipped with a kinetics mode (Molecular Devices, Sunnyvale, CA, USA). All compounds were used in the concentration range of 0–100 μM and were dissolved in 95% ethanol or dimethylsulfoxide. The final concentration of each solvent in the inhibition assays was 2.5%, and this concentration had no effect on activity of the GSTs (data not shown). The concentration of natural product required to bring about 50% inhibition of GST activity, the IC_{50} value, was determined by plotting sigmoidal dose–response curves of enzyme activity *vs.* log natural product concentration using GraphPad Prism version 4.00 for Windows (GraphPad™ Software Inc., San Diego, CA, USA).

The effects of isofurano-napthoquinone **1** and sesquiterpene **2** on the kinetics of GSTs were determined as described by Mukanganyama *et al.*,[35]. The $K_{m(app)}$ and $V_{max(app)}$ were determined using GraphPad Prism™ version 4.00 for Windows. The K_i values with respect to GSH and CDNB, as well as the type of inhibition, were determined. The type of inhibition was deduced by determination of trends of K_m and V_{max} values with an increase in natural product concentration. To determine the trend, the means of the K_m (or V_{max}) values with increase in inhibitor concentration were compared by performing a one-way ANOVA with Dunnett's post-test using GraphPad InStat™ version 3.00 for Windows 95 (GraphPad™ Software, Inc.). The inhibition constant, K_i, was determined by means of re-plots[35]. The type of re-plot depends on the type of inhibition, for example, plotting $1/V_{max}$ versus inhibitor concentration for noncompetitive inhibition, will give K_i as the intercept on the baseline[43].

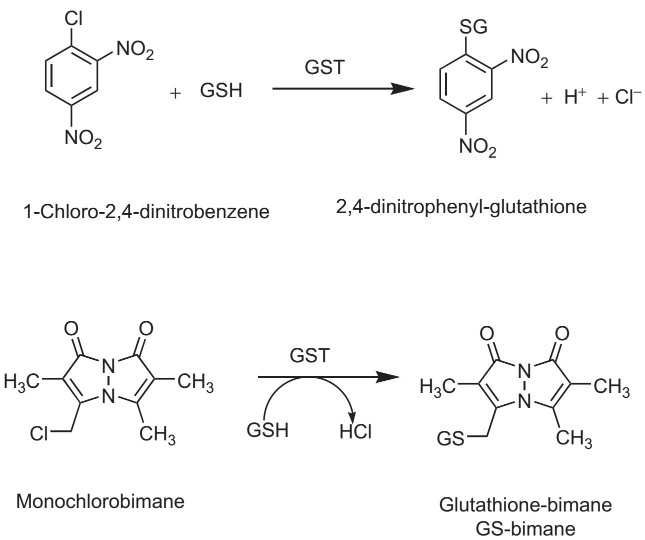

Figure 2. The chemical reactions of CDNB and MCB catalysed by GST P1-1.The monochlorobimane fluorescence assay for GST activity was used to measure the product of conjugation at the excitation wavelength 390 nm and the emission wavelength of 478 nm. The CDNB spectrophotometric assay for GST activity was used to determine the conjugation product 2, at a wavelength of 340 nm.

Results

Purification of heterologously expressed GSTs

Human GST P1-1 was expressed heterologously in *E. coli* and was purified by affinity chromatography. The GSTs were purified to homogeneity and a single band was obtained on SDS–PAGE analyses (data not shown) and the specific activity was 160 μmol/min/mg protein. This value is comparable to the value of 129 μmol/min/mg obtained by Hayeshi *et al.*[44]

Effects of the natural products on GST activity

The effects of compounds **1**–**10** on the activity of human recombinant GST activity were assessed by measuring the conjugation activity with MCB and CDNB. The inhibition profile when using MCB as a substrate is shown in Table 1. Isofuranonaphthoquinone **1**, sesquiterpene lactone **2**, and xanthone **7** were potent inhibitors of the enzyme. The other compounds, the monoterpene-substituted furocoumarin **4**, the benzophenone derivative **5**, the 4-phenyl coumarin derivative **8**, and xanthone **9** inhibited the enzyme but to a lesser extent even at the highest concentration of 100 μM. Iridoid **3** was, however, shown to activate the enzyme at 100 μM. Thus, the compounds **1** and **2** were found to be potent GST P1-1 inhibitors. For this reason, their potential inhibitory activity was also assessed using CDNB as a substrate to confirm if there was any substrate-dependent

Table 1. Percentage inhibition of GST P1-1 using natural plant compounds at 33 and 100 μM concentration.

Compound	Class of compound	Source	Percentage inhibition at 33 μM final concentration[a]	Percentage inhibition at 100 μM final concentration[a]
1	Isofurano-naphthoquinone	*Bulbine frutescens*	68 (87)[b]	91 (100)[b]
2	Sesquiterpene	*Dicoma anomala*	75 (+19)[b]	84 (+5)[b]
3	Iridoid	*Plumeeria rubra*	51	+29 (activation)
4	Furocoumarin	*Dorstenia elliptica*	34	62
5	Benzophenone	*Garcinia smeathmannii*	41	62
6	coumaroflavan	*Treculia africana*	26	26
7	Xanthone	*Mammea africana*	38	52
8	Coumarin	*Mammea africana*	23	50
9	Xanthone	*Mammea africana*	21	36
10	Coumarin	*Mammea africana*	33	98

[a]Values obtained with MCB as a substrate.
[b]Values obtained with CDNB as substrate.

inhibition. The results showed that **2** did not inhibit the CDNB reaction, as it did for the MCB conjugation but activation at 19 and 5% at 33 and 100 μM, respectively was shown (Table 1). Isofuranophthoquinone **1** displayed a high level of potency with both CDNB and with MCB as substrates. The reaction was inhibited by 87% and 100% at 33 and 100 μM, respectively. The IC_{50} for **1** (6.8 μM) was determined photometrically using CDNB as the substrate. Figure 3 shows the sigmoidal dose–response curve for the determination of the IC_{50} for **1** using CDNB as a substrate.

Effect of the natural products on GST kinetics

On the basis of the results for the inhibitory effects of isofuranonaphthoquinone **1**, its effects on the kinetics of the GSTs were determined. The trend in changes of $K_m^{GSH/CDNB}$ and $V_{max}^{GSH/CDNB}$ values with increase in natural product concentration was used to determine the type of inhibition. The predominant type of inhibitions with respect to the G site (GSH) and H site (CDNB) was noncompetitive and mixed type of inhibition. Figure 4 shows the secondary plot for determination of K_i values for **1** on GST P1-1. The data for **1** are summarized in Table 2.

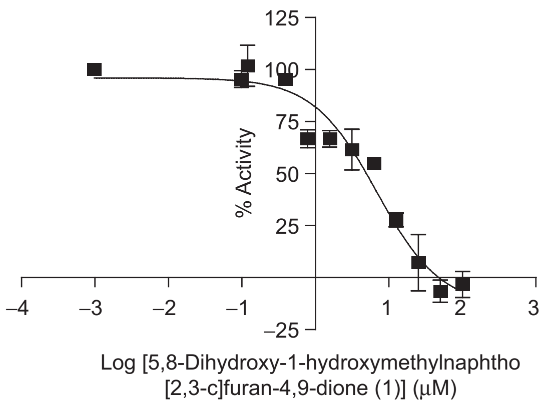

Figure 3. Replot of slope (K_m/V_{max}) and $1/V_{max}$ versus [I] to determine K_i^{GSH} and Ki'^{GSH} values of 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-*c*]furan-4,9-dione (**1**), which showed mixed type inhibition for GST P1-1 with respect to GSH.

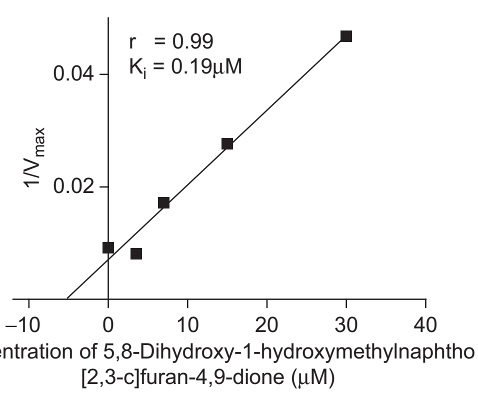

Figure 4. Inhibition of GST P1-1 by 5,8-Dihydroxy-1-hydroxymethylnaphtho[2,3-*c*]furan-4,9-dione (**1**). The IC_{50} value is the concentration of inhibitor giving 50% inhibition of enzyme activity. Data are the mean ± standard deviation of quadruplicate experiments each performed twice.

Discussion

During cancer development, synthesis of the enzyme GST P1-1 is greatly increased so that tumors over express this enzyme[45]. GST P1-1 detoxifies drugs, particularly anti-cancer drugs, and, therefore, the elevated GST P1-1 levels could greatly inhibit the effectiveness of chemotherapy due to the inactivation of drugs. Therefore, increased levels of GST P1-1 found in tumor cells promote the growth and spread of cancer[13]. These observations have led for the need of compounds that can inhibit GST P1-1 as this inhibition could be a useful cancer treatment strategy.

The mono- and di-hydroxyxanthones **7** and **9** have almost the same level of potency, with the slight difference in favour of the mono-hydroxy xanthone probably attributed to the preferred point of attachment specified by the only hydroxyl group at the second position. The coumarins **8** and **10** are both pentasubstituted and belong to a small group of unusual coumarins that are also alkylated at position 4. The 5-hydroxygroup is hydrogen-bonded to the carbonyl of the side chain carbonyl in both compounds. Furthermore **8** is substituted with the bulky phenyl group at the fourth position. However, the 7-hydroxy group is relatively less hindered in **8** than in **10**. This may explain the greater potency of **8** in inhibiting GST Pi than that of **10**. Xanthone **9** appears to be slightly weaker than either of the coumarins at the low concentration of 33 μM, but has a comparable activity to **8** at higher concentration.

The iridoid **3** seemed to inhibit GST P1-1 at low concentration but activate the enzyme at high concentration. These observations indicate that there could be other molecular interactions occurring between **3** and either the substrates or the enzyme. The compounds **1** and **2** were the two inhibitors that were found to be potent since they displayed more than 60% inhibition of the enzyme at the lower concentration of 33 μM.

Isofuranonaphthoquinone **1** displayed potent inhibition properties when using both CDNB and MCB as substrates. However, **2** was not effective as an inhibitor of GSTP1-1 when CDNB was used a substrate although inhibition was observed when MCB was used a substrate. The differences in the results obtained in the interaction of **2** with MCB and CDNB can be explained by the different interactions that some enzymes display with different substrates. For example, in the study of the effect of the antimalarial drugs on GST activity, it was noticed that artemisinin inhibited GSTs when the substrate CDNB was used but when the substrate ethacrynic acid was used instead, the drug showed no inhibition[35]. It has also been suggested that GSTs may have two other substrate-binding sites that are distinct from the H site[46]. These are the benzyl isothiocyanate (BITC) and monobromobimane (MBB) sites. MBB is an analogue of MCB where the chlorine atom in the latter is replaced by bromine, and it may be reasonable to suppose that both MBB and MCB would likely bind to the same site. The MBB site

Table 2. The effects of 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-*c*]furan-4,9-dione (**1**) on the kinetic properties of GST P1-1 with 1-chloro-2,4 dinitrobenzene as electrophilic substrate.

Compound **1** Concentration (μM)	K_{cat}^{CDNB} (S^{-1})	K_m^{CDNB} (mM)	K_{cat}/K_m^{CDNB} (S^{-1} mM^{-1})	K_{cat}^{GSH} (S^{-1})	K_m^{GSH} (mM)	K_{cat}/K_m^{CDNB} (S^{-1} mM^{-1})
0	45	0.3558	126.48	26.63	0.04591	580
3.5	51.29	0.9333	54.96	25.48	0.8519	29.9
7.0	24.26	0.2276	106.59	14.11	0.1477	95.53
15	15.08	0.383	39.37	14.33	0.4627	30.97
30	8.91	0.3569	24.96	14.43	0.211	68.39

has been found in pig GST Pi and rat GST Mu classes[46]. A study on these sites found an MBB derivative to be a competitive inhibitor of rat GST M1-1 and pig GST Pi using MBB as a substrate but not CDNB[46]. These findings imply that MBB and CDNB have separate binding sites. An earlier study using affinity labelled MBB showed that the MBB- and CDNB-binding sites were independent[47]. It may, thus, be that human GST P1-1 contains a site similar to the MBB site that can bind MCB, and that **2** is an inhibitor at that MBB site and not the CDNB site.

The IC_{50} value for **1** of 6.8 μM is only slightly higher than those of other potent GST inhibitors, such as the natural plant phenolic compound curcumin whose IC_{50} value was found to be 5 μM using GST P1-1 and ellagic acid which has a 1.6 μM IC_{50} value using GST A2-2[44]. Cibacron blue, a known GST inhibitor, has an IC_{50} value of approximately 2.0 μM. A study by Van Haaften *et al.*[43] showed that α-tocopherol can inhibit GST P1-1 activity. α-Tocopherol was found to have an IC_{50} value 0.5 μM and had a K_m and V_{max} of 1.11 mM and 18.83 μmol/min/mg protein, respectively, at the H site and 1.0 mM and 18.11 μmol/min/mg protein, respectively, at the G site[48]. These findings show that natural products can modify the activity of drug metabolizing enzymes and, thus, have potential use as chemomodulators[48]. Low IC_{50} values are desirable if inhibition is to occur in cells since very high levels of exogenous compounds can be toxic to a cell because these xenobiotics may interfere with certain biological pathways. Also, these high levels can be difficult to achieve *in vivo* since cells actively efflux xenobiotics, and so the desired inhibitions might not occur[6]. However, since **1** is potent even at low concentrations, it may possibly inhibit GSTs *in vivo*.

Isofuranonaphthoquinone **1** was found to display noncompetitive inhibition at the H site. In this type of inhibition, the inhibitor binds to both the enzyme and the enzyme substrate complex. This type of inhibition cannot be overcome by large amounts of substrate[49]. However, **1** showed mixed inhibition at the G site. Mixed inhibition is similar to noncompetitive inhibition in that the inhibitor binds to both the free enzyme and the enzyme–ubstrate complex. Mixed inhibition may come up as a result of reversible binding of the inhibitor at a site other than the active site or reversible binding to the enzyme–substrate complex[49].

The results indicate that **1** is a more potent inhibitor of the H site than the G site. The structure of GSH and **1** is very different: the former being a linear flexible aliphatic chain which may engage in inter- or intra-molecular attractions due to hydrogen bonds between carbonyl oxygen and the N–H and O–H bonds. On the other hand, isofuranonaphthoquinone is a tricyclic aromatic molecule, which can easily form an alternative quinone–quinol structure through tautomerism. It is, therefore, possible to conclude that **1** and GSH are not likely to bind to the same site. K_{cat}/K_m, the catalytic efficiency of the reaction, would decrease as inhibitor concentration increased due to the reduced activity of the enzyme. This trend was noted for **1** in the inhibition of both the G- and H sites, and these findings were consistent with those found in the literature[35]. The K_i value of **1** at the H site was low, and this shows that the inhibitor had a high affinity for the H site. The K_i value is comparable to that of other natural compounds that inhibit GST P1-1. Curcumin has a much higher K_i of 9.6 and ellagic acid has a K_i of 11[44]. Thus, **1** is more potent as an inhibitor than these compounds.

Flavonoids and isoflavonoids, such as eriodictyol, quercetin, and genistein, found in dietary agents such as soy foods have been found to reduce the risk of cancer through many mechanisms, including the inhibition of drug-metabolizing enzymes[45]. Other inhibitors of GSTs include other phenolic compounds such as epigallocatechin galate[50], ellagic acid, and curcumin[44]. Ethacrynic acid modulates the cytotoxicity of doxorubicin, an anti-cancer agent, by inhibiting GSTs and reducing efflux of the drug from the cell, thereby, increasing the therapeutic efficiency of the drug[51]. It is postulated that **1** may inhibit GST P1-1 via two ways. It may react with GSH via its quinone moiety. The conjugate formed then inhibits GSTP1-1 as glutathione analogue. Alternatively **1** may react directly with the protein. The major target in proteins is the thiol group of cysteine residues. GST P1-1 has a cysteine at the active site and this may be susceptible to reaction with the quinone. Both proposed schemes are shown in Figure 5. Compounds with quinone groups have been shown to react with glutathione *in vitro*[10]. The activity of **2** may be different from all the above compounds which contain at least one aromatic ring and phenolic hydroxyl groups. The compound **2** is a sesquiterpene lactone with a characteristic α,β-unsaturated double bond, which may be responsible for the observed high level of GSTP1-1 inhibition. Indeed, van Iersel *et al.*[52] have found that naturally occurring α and β-unsaturated aldehydes and ketones, such as acrolein and cinnamaldehyde, can inhibit GST P1-1.

In conclusion, The isofuranonapthoquinone **1** from *Bulbine frutescens* is a potent inhibitor of human recombinant glutathione transferase P1-1 *in vitro* using both

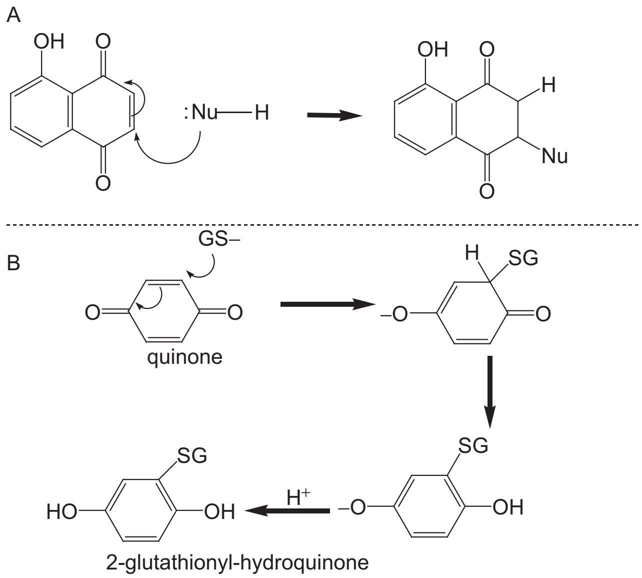

Figure 5. Reaction of proteins or GSH with quinones. In A, the reaction is with any nucleophile, whilst in B, the reaction is with reduced glutathione (GSH).

the fluorescent substrate monochlorobimane and the photometric substrate CDNB. The compound may inhibit GST P1-1 *in vivo* and could, therefore, be of importance in its potential use as a chemomodulator in situations where GST P1-1 is over expressed and is involved in alkylating anticancer drug resistance. However, care should be taken in interpolating data from *in vitro* to *in vivo* situations, as one needs to know about the metabolism of this compound as well as its bioavailability.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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