Cytotoxic Thiol Alkylators

Hari N. Pati[a], Umashankar Das[a], Rajendra K. Sharma[b] and Jonathan R. Dimmock[a,*]

[a]*College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, S7N 5C9, Canada and* [b]*Department of Pathology and Laboratory Medicine, College of Medicine and Health Research Division, Saskatchewan Cancer Agency, University of Saskatchewan, 20 Campus Drive, Saskatoon, Saskatchewan, S7N 4H4, Canada*

Abstract: Various classes of cytotoxic compounds which alkylate cellular thiols are described namely α,β-unsaturated ketones, α-methylene-γ-lactones, azines of Mannich bases, imexon, isothiocyanates, a benzoacronycine as well as activation by thiols prior to alkylation. The mechanisms of action of some of the molecules, such as the formation of reactive oxygen species, are presented. The cytotoxicity of a number of drugs can be influenced by modulation of the concentration of thiols including the observation that potencies can be increased by thiol activation. The ability of certain thiol reagents to reverse multidrug resistance as well as some miscellaneous actions of thiol alkylators are described.

Key Words: Thiols, alkylating agents, α,β-unsaturated ketones, α-methylene-γ-lactones, azines, imexon, isothiocyanates, benzoacronycine, tumour sensitization, drug resistance.

INTRODUCTION

Various groups of cytotoxic agents which alkylate cellular thiols are described with particular emphasis placed on α,β-unsaturated ketones. Other groups of compounds having this capacity are outlined, namely α-methylene-γ-lactones, azines of Mannich bases, imexon, isothiocyanates and a benzoacronycine as well as certain prodrugs which are activated by thiols. Emphasis is placed on the versatility of these compounds, i.e., the varied way in which their cytotoxicity is mediated, and in particular their value in exerting preferential toxicity to malignant cells in contrast to normal tissues on occasions. A number of thiol alkylators find use in sensitizing tumour cells to antineoplastic agents while others are able to reverse drug resistance.

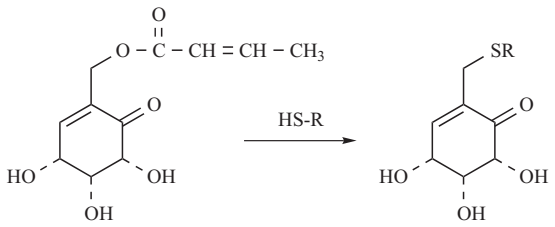
GROUPS OF THIOL ALKYLATORS

A number of different clusters of organic compounds alkylate thiols.

1) α,β-Unsaturated Ketones

Various enones alkylate thiols but do not react with amino or hydroxy groups [1, 2]. Thus, the utilization of such compounds in cancer chemotherapy may avoid the problems of mutagenicity and carcinogenicity associated with a number of anticancer drugs [3]. A number of examples will illustrate the usefulness of these interactions.

COTC **1** exerts its cytotoxic action by at least two mechanisms. First, there is a facile displacement of the acyl group by thiols such as glutathione (GSH) and cysteine [4]. Second, COTC inhibits the enzyme glyoxalase I which is involved in the metabolism of the cytotoxin methylglyoxal [5]. These two processes share a common theme since GSH is an essential cofactor in the glyoxalase system; hence depletion of GSH will reduce the rate of metabolism of methylglyoxal. Recently GSH has been shown to react much more extensively with COTC than melphalan [6]. Subsequently the effect on apoptosis-resistant human pancreatic adenocarcinoma cells of COTC and the alkylating agents cyclophosphamide, cisplatin or melphalan increased the percentage of dead cells markedly. This effect was eliminated if GSH was added to the culture medium. There was little or no synergism with various non-alkylating drugs such as adriamycin and etoposide. Thus COTC in having the dual roles of depleting GSH and inhibiting glyoxalase I exacerbates the cytotoxic properties of certain alkylating agents suggesting its use in an important strategy in cancer chemotherapy.



1

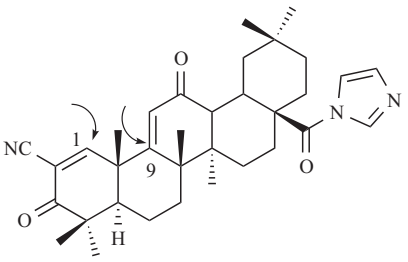
Fig. (1). Reaction of COTC(**1**) with thiols.

The polycyclic enone **2** is a thiol alkylator with antiproliferating and differentiating properties which reacts with mitochondrial GSH in pancreatic cancer cells leading to a number of pathological effects including accumulation of reactive oxygen species, oxidation of the cellular GSH pool and apoptosis [7]. The apoptotic effect was prevented by cotreatment of **2** with the thiols 2-mercaptoethanol, dithiothreitol and GSH indicating that intracellular thiols are an important target of **2**.

The theory of sequential cytotoxicity originated a number of years ago [8]. This hypothesis states that the successive

*Address correspondence to this author at the College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, S7N 5C9, Canada; Tel: (306) 966-6331; Fax: (306) 966-6377; E-mail: jr.dimmock@usask.ca

1389-5575/07 $50.00+.00

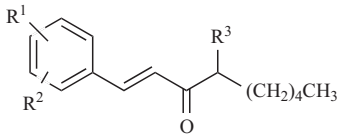


2

Fig. (2). Structure of **2**. The arrows indicate the site of electrophilic attack on GSH.

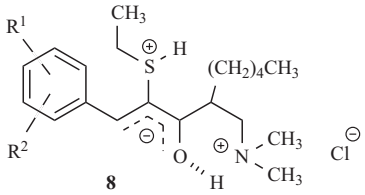
release of two or more cytotoxic compounds may cause greater damage to tumours than the corresponding normal tissues. Thus in Fig. **3**, compound **3** can undergo deamination to yield the initial alkylating species **4** which after an addition – deamination sequence, can liberate a second alkylator **5**. This theory was based on the following observations. First, the lowering of concentrations of cellular thiols prior to an attack by an antineoplastic agent may be more detrimental to tumour cells than normal tissues *vide infra*. Second, variations in the nature and amounts of different forms of glutathione S-transferase (GST) between tumours and normal cells is exploitable. For example, if a tumour had an unusually low quantity of GSTπ and the initial alkylator **4** had a specific affinity for this isozyme, then the release of a second alkylator could be more detrimental to the neoplastic tissue. Third, a number of Mannich bases inhibit respiration in mitochondria [9, 10] and lonidamine, which is an inhibitor of energy metabolism, potentiated the cell killing caused by different antineoplastic agents [11, 12]. Hence if the administered compound inhibited energy initially, the release of a second cytotoxic species may be more lethal to tumours.

The chemical reactivity of α,β-unsaturated ketones may be related to their bioactivities. Thus a series of conjugated



6 $R^1 = R^2 = H, Cl; R^3 = H$

7 $R^1 = R^2 = H, Cl; R^3 = CH_2N(CH_3)_2$. HCl



8

Fig. (4). Structures of **6** and **7** and the intermediate **8** originating from the reaction between **7** and ethanethiol.

styryl ketones **6** have modest cytotoxic properties and were inactive in certain *in vivo* anticancer screens while being well tolerated in mice. On the other hand, the corresponding Mannich bases **7** are potent cytotoxins, some of which extended the life spans of mice with P388 leukemia but generally at doses close to causing mortalities [13]. The second order rate constants of representative compounds in series **6** and **7** revealed that when there were the same substituents in the aryl ring, the Mannich bases reacted approximately 240 times more rapidly with a thiol ethanethiol than the corresponding enones [14]. This effect was attributed to the stabalising influence of the intermediate **8** although solvent effects could also contribute to this effect.

Additional quantitative data on the electrophilicity of different agents that react with thiols was also provided in this study [14]. Thus the second order rate constants for the reaction between ethanethiol and enones in series **6** were dependent on the magnitude of the Hammett σ and Taft σ* values of the aryl substituents R^1 and R^2. Furthermore when

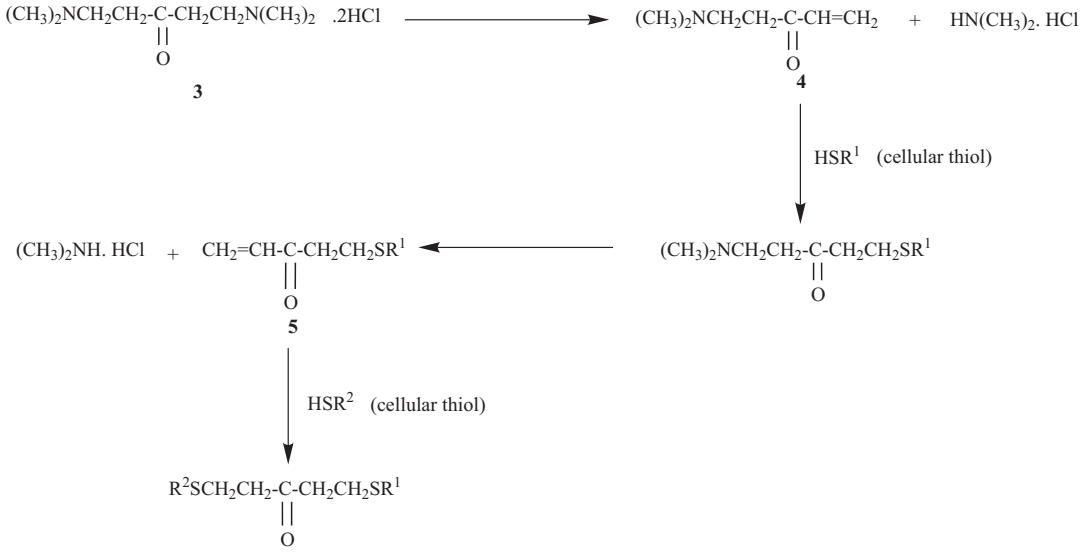


Fig. (3). An illustration of the sequential cytotoxicity hypothesis.

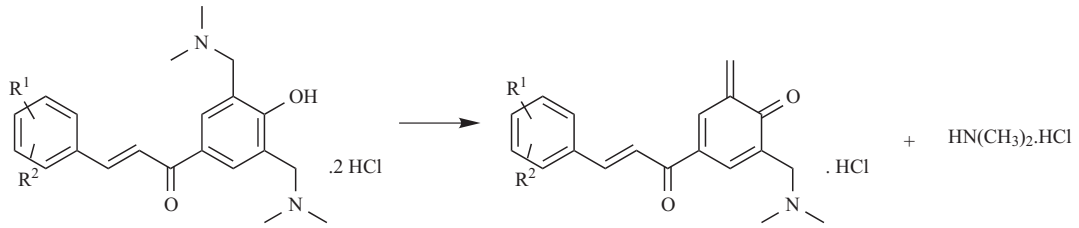
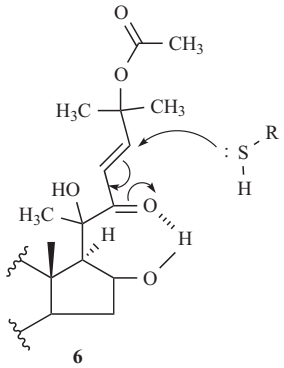


Fig. (5). Possible conversion of series **9** into the cyclohexadienones **10**.

the n-hexyl group in **6** was replaced by either a 1-methylpentyl or a methyl group, the rates of reaction decreased and increased, respectively. Similarly, the rates of reaction between ethanethiol and the Mannich bases **7** were controlled by the σ and σ* figures of the aryl substituents R^1 and R^2 while the replacement of the 1-methylpentyl group lowered the electrophilicity towards ethanethiol. Thus the rates of reaction of α,β-unsaturated ketones with thiols can be controlled by the nature of the groups adjacent to the enone functionality.

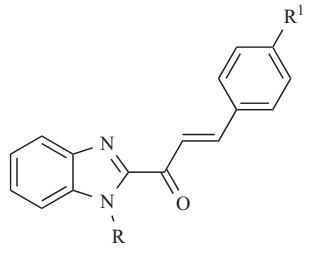
Various chalcones display cytotoxic and anticancer properties [15]. Several years ago, various Mannich bases of chalcones **9** were prepared as putative prodrugs in which deamination to the corresponding cyclohexadienones **10** was envisaged (Fig. **5**) [16]. In the presence of the π isozyme of GST, representative compounds in series **9** reacted with GSH revealing at least one biochemical mechanisms whereby cytotoxicity was achieved. Those members of series **9** which were evaluated in an intrachromosomal recombination assay did not display any mutagenic properties suggesting that interaction with nucleic acids did not take place.



6

Fig. (6). Hydrogen bonding in fabacein which enhances interaction with thiols.

Certain chalcones containing an ortho hydroxyl substituent in the aroyl ring displayed greater cytotoxicity towards Ehrlich's ascitic sarcoma in mice than the structural isomers in which the hydroxyl groups were located in the meta and para positions [17]. A possible explanation for this observation was provided by Kupchan and Tsou in their study of the steroid fabacein and related analogs [18]. In this investigation, interaction with cellular nucleophiles was considered to be enhanced by hydrogen bonding between the hydroxyl and keto functionalities as illustrated in Fig. (**6**). Acetylation of the 16-hydroxy group abolished hydrogen bonding and the cytotoxicity of this ester was lower than noted in fabacein. Thus the insertion of one or more hydroxyl groups in close proximity to the α,β-unsaturated keto function is likely one way to enhance the chemical reactivity of thiol reagents which may be associated with increased cytotoxicity.



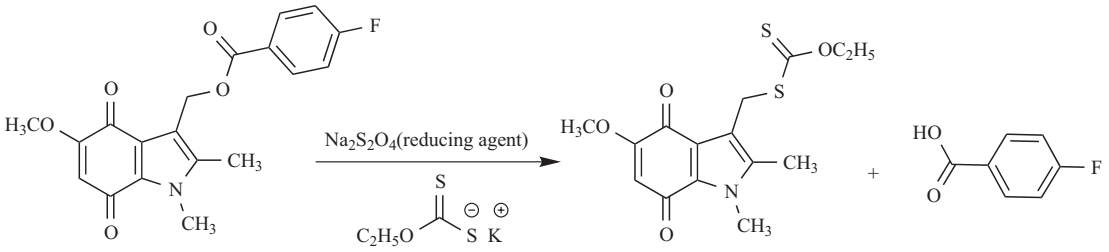
11

R = H, CH_3; R^1= H, OCH_3, Cl, NO_2

Fig. (7). Structure of series **11**.

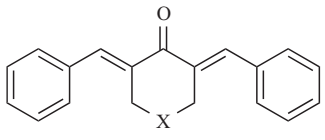
The observations that cytotoxicity has been observed in compounds containing the conjugated styryl keto group [13] as well as in certain benzimidazoles [19] led to the decision to prepare series **11** (Fig. **7**) as candidate cytotoxins [20]. These compounds reacted with L-cysteine under pseudo first order conditions and positive correlations were noted between the rates of thiolation and the magnitude of both the Hammett σ and Taft E_s constants. One of the compounds, namely **11**, R=H; R^1=NO_2, displayed a selective toxicity to leukemic, renal and breast cancers when assayed against a panel of approximately 60 human tumor cell lines from nine different neoplastic diseases.

After reductive activation, certain indolequinones such as **12** display significant cytotoxicity as alkylating agents as illustrated in Fig. (**8**) with a model thiol namely potassium O-ethylxanthate [21]. It is likely that similar reactions with cellular scavengers such as GSH take place under biological conditions. Compound **12** and related analogues demonstrated a hypoxia-selective cytotoxicity *in vitro*. The importance of this group of compounds, as well as other series of molecules which undergo bioactivation to cytotoxic agents under hypoxic conditions, is their ability in treating solid tumours which have loci of anoxia as well as malignant cells which contain a higher percentage of reductases than the corresponding normal cells.



12

Fig. 8). Reductive activation of an indolequinone followed by thiol alkylation.

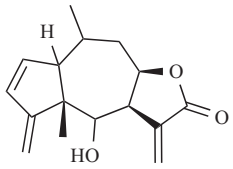


13

a: X = NH. HCl
b: X = N-CO-CH=CH-COOH

Fig. 9). Structure of the 4-piperidones **13a** and **13b**.

In order to determine if compounds bearing multiple sites for nucleophilic attack by cellular thiols would prove to be potent cytotoxins, **13a**,**b** and related compounds were prepared [22]. The IC_{50} values of **13a** and **13b** towards P388/MRI cells were 16 and 134 pM, respectively, which is 5825 and 698 times the potency of N,N-bis(2-chloroethyl)-N-nitrosourea (BCNU), respectively. A dose of 0.87 mmol/kg of **13a** and **13b** lowered hepatic GSH concentrations in mice by 19 and 29%, respectively, suggesting that the mode of action of these compounds, at least in part, is by interaction with cellular thiols.



14

Fig. 10). Structure of helenalin **14**.

2) α-Methylene-γ-Lactones

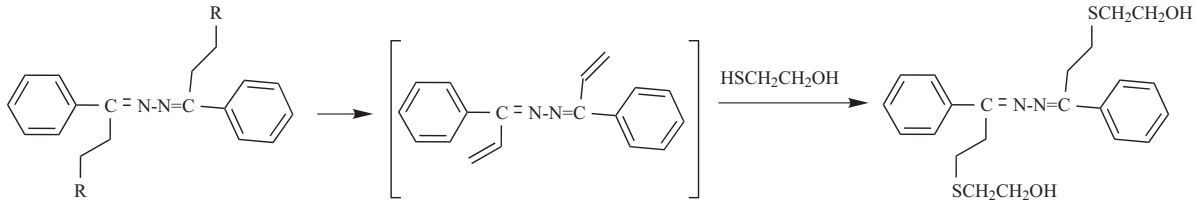
Various compounds containing the α-methylene-γ-lactone moiety are thiol alkylators with cytotoxic and anticancer properties. Thus helenalin **14** (Fig. **10**) reacted with L-cysteine and GSH to yield the corresponding thiol adducts [23]. No reaction occurred between **14** and histidine, DNA, dGMP, dGTP, dAMP or dATP providing further evidence of the specificity of compounds containing the $O=C-C=CH_2$ group for thiols.

The enone group in a number of sesquiterpenoid lactones reacts with GSH and the sulfhydryl portion of the cysteine residues in proteins [24]. These reactions give rise to a variety of biochemical disturbances including enzyme inhibition. In addition, the redox potential in the cell is altered when thiol concentrations are lowered which causes oxidative stress leading to the formation of reactive oxygen species (ROS) which initiates apoptosis *via* the mitochondrial-dependent pathway [25, 26].

One should also note that the α-methylene-γ-lactone group has been reported to alkylate sulfhydryl-bearing enzymes such as phosphofructokinase [27], glycogen synthetase [28] and DNA polymerase [23].

3) Azines of Mannich Bases

Compounds **15a**,**b** (Fig. **11**) were designed as candidate prodrugs of the putative bifunctional alkylating agent **16** which was considered to be capable of reacting with cellular thiols [29]. Incubation of **15a** with 2-mercaptoethanol led to the isolation of the corresponding thiol adduct **17** in 54% yield. Thus the formation of the intermediate **16** seems likely although the thiol could have reacted at the electron-deficient



15 **16** **17**

a: R = $N(CH_3)_3\ I^{\ominus}$
b: R = $N(CH_3)_2$ .HCl

Fig. 11). Formation of the thiol adduct **17** from the azine **15a**.

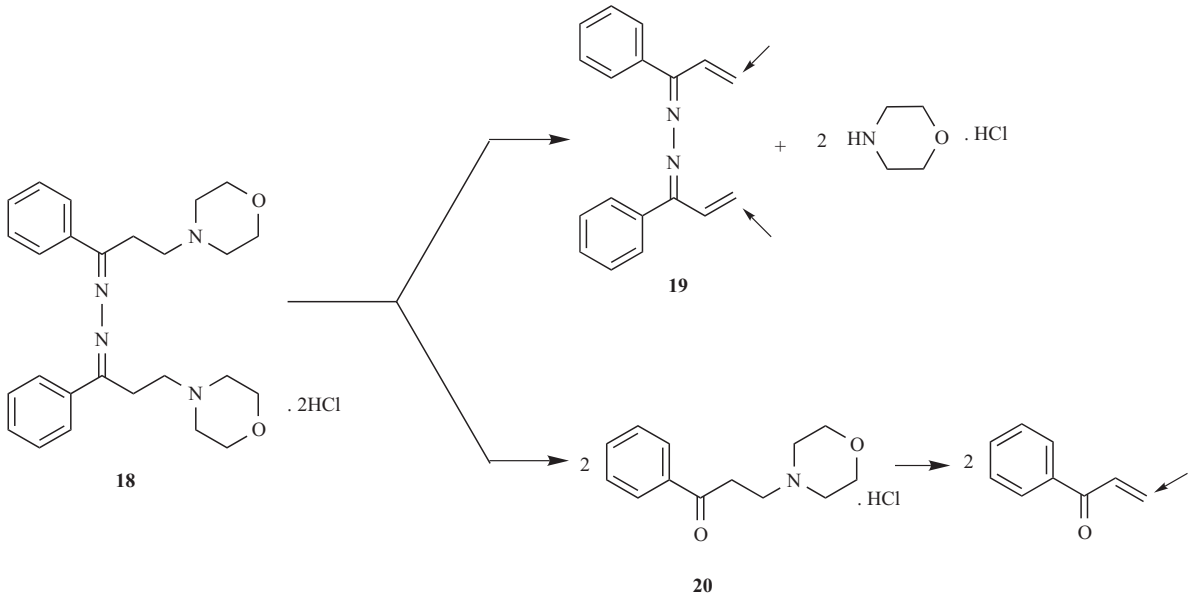
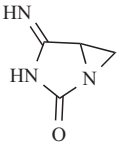


Fig. (12). Possible mechanisms whereby **18** alkylates cellular thiols. The arrows indicate the locations where nucleophilic attack likely occurs.

methylene carbon atom adjacent to the quaternary ammonium nitrogen atom. One the other hand, incubation of **15b** with 2-mercaptoethanol led to the isolation of the free base of **15b** principally, along with very small quantities of **17** and other products which were not identified. The bis quaternary ammonium azine **15a** represents a further example of a thiol alkylator. Both **15a**,**b** exhibited modest cytotoxic properties towards EMT6/Rw cells *in vitro*.

Recently a novel cytotoxic azine **18** lowered glutathione levels in Jurkat cells in a concentration-dependent manner [30]. While several explanations may be proferred for this result, it is possible that deamination of **18** occurred giving rise to the unsaturated azine **19**. Alternatively, hydrolysis of the azine is possible thereby liberating the corresponding Mannich base **20** which on deamination forms the related α,β-unsaturated ketone; this class of compounds are known to be thiol alkylators. These possibilities are presented in Fig. (**12**).



21

Fig. (13). Structure of imexon.

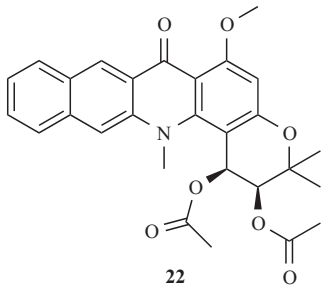
4) Imexon

21 has antitumour properties towards multiple myeloma cells [31] and causes a reduction in the concentrations of cysteine and glutathione [32]. Mass spectrometry revealed that the aziridine ring of imexon opened leading to thiol alkylation. The cytotoxicity of **21** to myeloma cells was abolished when cultured in the presence of N-acetylcysteine; conversely pretreatment of cells with buthionine sulfoximine (BSO) increased imexon cytotoxicity. Reduction of thiol concentrations in cells can lead to oxidative damage due to the formation of ROS and in this study, oxidized nucleosides including 8-hydroxydeoxyguanosine were detected using a monoclonal antibody against this nucleoside [32]. Oxidative stress as well as GSH depletion often induces apoptosis [33, 34] and this phenomenon was observed in treating cells with imexon. *In vitro* experimentation revealed that this compound did not bind to DNA [32]. However one should note that when substantial reduction of thiol concentrations takes place, excess ROS formation may result which in turn can lead to the induction of cancer by mutation of DNA. A balance is therefore required between thiol depletion leading to apoptosis and the prevention of the formation of excess ROS.

5) Isothiocyanates

A different aspect of thiol alkylation that may be considered is the induction of chemopreventative properties. This phenomenon has been observed with certain isothiocyanates which alkylate cellular thiols, initially leading to stress which contributes to their chemoprotective properties. In fact a lowering of the risk of certain tumours developing was noted by the dietary consumption of various isothiocyanates [35, 36]. The mechanism whereby such chemoprotection is achieved likely involves the following sequence of events. The nuclear erythroid 2-related factor 2 (Nrf2) is normally bound to Kelch-like ECH-associated protein 1 (Keap 1) in the cells. Interaction of isothiocyanates with the thiol groups of cysteine residues of Keap 1 liberates Nrf2 which passes to the cell nucleus which then activates the production of a number of antioxidant proteins such as NAD(P)H:quinone oxidoreductase 1 and thioredoxin reductase [37].

A further reason why development of cytotoxic isothiocyanates should be considered is based on reports of their displaying greater growth-inhibitory properties towards malignant cells rather than the corresponding normal cells. For example, the IC_{50} value of allyl isothiocyanate towards two human prostate cancer cells was 16 μM approximately while little effect on normal human prostate cells was noted at 40 μM [38].

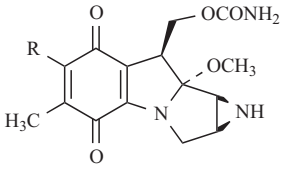


22

Fig. 14). Structure of S23906-1.

6) A Benzoacronycine

On occasions, the potencies of antineoplastic agents are related to the concentrations of cellular thiols. Thus the benzoacronycine derivative **22** (Fig. **14**) displays impressive antitumour properties towards a number of human xenografts in mice [39]. Its mode of action is by alkylation of the N2 position of the guanine residue of DNA [40]. However the efficacy is inversely proportional to the GSH concentrations since **22** reacts readily with GSH rendering less of the drug available to interact with DNA and hence potency is lowered. By reducing GSH concentrations with the γ-glutamylcysteine synthetase inhibitor BSO, cytotoxicity was enhanced markedly [41]. Similarly the IC_{50} values of mechlorethamine hydrochloride to KB-3-1 neoplasms rose when the concentration of GSH in the culture media was elevated [41].



23

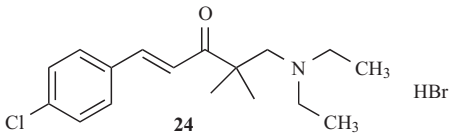
a: R = OCH_3; **b**: R = NH_2

Fig. 15). Structures of mitomycin A (**23a**) and mitomycin C (**23b**).

7) Activation by Thiols

So far examples have been given of cytotoxicity being caused by alkylation of critical thiols in cells. A related concept is when alkylation takes place after activation by thiols. In the case of certain mitomycins, the greater antitumour potencies and toxicity of mitomycin A **23a** over mitomycin C **23b** has been attributed to the rapid and exclusive reaction of **23a** but not **23b** with thiols (Fig. **15**) [42]. Thus thiol activation of **23a** and not **23b** by GSH and dithiothreitol led to cross-linking of DNA due to reduction of the quinone ring in mitomycin A by thiols to the corresponding hydroquinone. Thus tumour cells possessing elevated concentrations of GSH are likely to be more sensitive to mitomycin A than the corresponding normal cells. This observation is of particular relevance in those cases of drug-resistant tumours which have increased concentrations of GSH. Other cytotoxins which are prodrugs activated by thiols include dynemicin [43], irofulven [44], brostacillin [45] and leinomycin [46].

Before leaving the topic of the interactions of a number of different classes of compounds with cellular thiols, mention may be made briefly of the chemical nature of the thiols *per se* which will influence the rate and extent of the interactions. First, the relative acidity of the individual thiol should be considered. For example, the pk_a values of cysteine and glutathione are 8.33 and 8.66, respectively [47], i.e., the acidity of the critical thiol group is influenced by its chemical environment. Second, another feature of the structure of thiols which impinges on the extent of the interaction with different compounds is whether a thiol has a low molecular weight or whether it is present in a protein. This phenomenon was observed with CDDP **24** (Fig. **16**) which reacts reversibly with low molecular weight thiols but irreversibly with protein thiols [1]; it is likely that structurally related analogs of CDDP behave in the same way.



24

Fig. 16). Structure of 1-(4-chlorophenyl)-5-diethylamino-4,4-dimethyl-1-penten-3-one hydrobromide (CDDP).

SENSITIZATION OF TUMOURS TO ANTICANCER DRUGS BY THIOL ALKYLATORS

Various endeavours have been utilized with a view to increasing the sensitivity of tumours to different anticancer drugs by reducing the concentrations of cellular thiols. One approach is by the successive release of alkylating agents as described in the theory of sequential cytotoxicity [8]. A second method is the use of BSO. For example, depletion of GSH in human breast cancer MCF-7 cells by BSO increased the sensitivity of this neoplasm to melphalan, 4-hydroperoxycyclophosphamide (a bioactive metabolite from cyclophosphamide), cisplatin and BCNU by 139, 121, 64 and 27%, respectively [48].

An important aspect of lowering thiol concentrations is the possibility that the rate and extent of thiol depletion may differ between tumours and normal cells [49]. In a study using the KHT sarcoma in mice, a single dose of 2.5 mmol/kg of BSO led to a maximum lowering of the GSH concentration to about 40% of the control which occurred after approximately 14 hours [50]. There was only 80% recovery of the GSH concentration at the end of 24 hours. On the other hand, the same dose of BSO administered to mice revealed that the greatest lowering of the GSH concentration in bone marrow was 45% approximately after about 6 hours and complete recovery of the GSH concentration was noted after 16 hours.

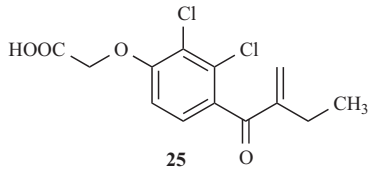
Another approach to obtaining greater alkylating agent toxicity to tumours rather than normal cells by modulation of GSH is the use of L-2-oxothiazolidine-4-carboxylate (OTZ). This prodrug is converted to S– carboxy-L-cysteine by 5-oxo-L-prolinase which undergoes decarboxylation to L- cysteine which is the rate-limiting substrate in GSH synthesis. This compound raises GSH concentrations in normal cells and although the effect on tumours is variable [51, 52], on occasions combinations of an alkylating agent and OTZ have led to a significant selective toxicity for malignant cells. Thus OTZ actually decreased GSH concentrations in B16 melanoma cells while increasing their sensitivity to acrolein which is a bioactive metabolite of cyclophosphamide [53]. *In vivo* experiments revealed a substantial enhancement of the anticancer efficacy of cyclophosphamide using OTC. Furthermore increased protection of peripheral blood mononuclear cells to the toxicity of cyclophosphamide was achieved by OTC. These experiments are important revealing an approach whereby increases in therapeutic indices may be achieved.

REVERSAL OF DRUG RESISTANCE

One of the problems associated with the use of alkylating agents in cancer chemotherapy is the development of drug resistance. This phenomenon has been attributed *inter alia* to increases in GSH concentrations. In the case of murine L1210 cells which were resistant to the bifunctional alkylating agent L-PAM, elevated activities of γ-glutamyl transpeptidase (γ-GT) paralleled the increased GSH concentrations compared to the drug-sensitive cells [54]. The enzyme γ-GT degrades GSH to give an increased supply of cysteine which may be incorporated into the GSH pool.

In a study using human ovary tumour samples, the GSH concentrations were measured prior to a combined treatment of cyclophosphamide and cisplatin [55]. After drug resistance had taken place in the patients, the GSH concentrations in the tumours were elevated tenfold.

GSH concentrations may be reduced by nutritional deprivation of L- cysteine [56] and by using BSO [57]. In both studies, reversal of resistance to melphalan was achieved. Hence the use of thiol-alkylators which are structurally and mechanistically unrelated to current anticancer therapy may bring about reversal of drug resistance as well as exerting antineoplastic activity in their own right.



25

Fig. 17). Structure of ethacrynic acid **25**.

Another way that resistance to certain bifunctional alkylating drugs takes place is by increasing the activities of glutathione-S-transferases (GSTs) in malignant cells [58, 59]. Ethacrynic acid, **25** (Fig. **17**) which contains an α,β-unsaturated keto moiety, inhibits GST-catalyzed thiol alkylations by reacting directly with GSTs as well as lowering thiol concentrations in the cells [60]. One of the values of this result is that **25** can sensitize cancer cells to drugs acting by alkylation such as the enhancement of the cytotoxic action of the nitrogen mustard chlorambucil in rat breast cells which were resistant to nitrogen mustards [61]. Furthermore **25** sensitizes certain human cancer cells to radiation [60].

MISCELLANEOUS ASPECTS OF THIOL ALKYLATORS

In addition to GSH, another important group of cellular thiols are the metallothioneins [62]. These compounds are low molecular weight intracellular proteins characterized by an abundance of thiol groups principally cysteine residues [63]. Modulating metallothionein concentrations has the potential to be beneficial in cancer chemotherapy. For example, the induction of metallothioneins reduced the toxicity of cisplatin while its efficacy was not impaired [64].

One of the characteristics of Michael reaction electrophiles, such as olefines conjugated to electron-withdrawing groups, is their ability to induce Phase II enzymes such as GSTs, quinone reductase and catalase [65]. This induction is perceived to be due to interaction of the ligands with chemically reactive mercapto groups of a primary cellular sensor. Structure-activity relationships obtained from a series of compounds containing one or two styryl keto groups and related compounds revealed that the presence of a 3-(2-hydroxyphenyl)-2-propenoyl group enhanced the induction of a Phase 2 enzymes quinone reductase, presumably acting by thiol alkylation [65]. Thus in addition to depleting thiol concentrations, a number of Michael reaction acceptors may cause an elevation of the quantities of cellular thiols. Specifically, submicromolar concentrations of a number of bis (arylidene) cycloalkanones elevated the GSH content by 50% in PE murine papilloma cells even though representative compounds reacted with various sulfhydryl reagents [65].

The reaction between cellular thiols and cisplatin is complex as exemplified by the following investigations. A study involving parental L1210 cells and a number of cell lines which were resistant to cisplatin and related anticancer platinum agents, revealed that drug sensitivities were related to the cellular GSH but not metallothionein concentrations nor to GST activities [66]. This investigation also showed that the L1210 cells could be sensitized to cisplatin by depletion of the GSH concentration using BSO. Inhibition of DNA repair by aphidicholin and caffeine restored the sensitivity of these cells to cisplatin. In a different study, the cytotoxicity of cisplatin was increased after reducing the GSH concentrations in human melanoma SK-MEL-2 cells [67]. However there was no reduction of the GSH concentration when the IC_{50} and IC_{90} values of cisplatin were employed suggesting that GSH modulations may cause multiple effects on DNA repair, free radical scavenging, apoptosis or other biochemical mechanisms. Other studies have demonstrated that GSH can play a dual action in affecting cytotoxic potencies. Thus administration of cisplatin causes a rise in the concentration of cellular GSH which may lower the cytotoxicity of the drug [68]. On the other hand, the formation of a cisplatin-GSH complex arrests protein synthesis [69] and the co-

administration of both cisplatin and GSH has been shown to be more effective clinically than cisplatin alone [70].

A further feature which affects the reactivity of different compounds with cellular thiols is catalysis by glutathione S-transferases (GST). There are six classes of GST namely alpha, mu, pi, theta, zeta and omega which are further divided into a number of different isoforms [71]. These enzymes catalyze the raction between glutathione and a number of drugs including cisplatin and acetaminophen [72]. Hence the capacity of GST to form a complex with a substrate and GSH will be dependent, to some extent at least, on the structure of the thiol reagent. For example, the reaction between GSH and CDDP **24** is catalyzed rapidly by the alpha class of GST but **24** has low specific activities towards the mu and pi forms of GST [73]. Since the concentrations of different isoforms of GST are claimed to be tissue specific [74], the reactions between substrates and GSH may vary in different tissues which possibly may lead to a greater lowering of thiol concentrations in tumors than in normal tissues.

CONCLUSIONS

This review has presented evidence that a number of different clusters of compounds exert their cytotoxic action by alkylation of cellular thiols. Since the mercapto group is present in a number of cellular constituents such as GSH, cysteine and the metallothioneins as well as part of the cysteinyl group in different enzymes, interactions with different molecular targets likely occur. This pleiotropy should be a distinct advantage since there are many dysregulated processes in cancer. Hence the binding of a ligand at multiple sites may well be beneficial, a viewpoint which has been recently articulated [75].

ACKNOWLEDGEMENTS

The authors thank the Canadian Institutes of Health Research for financial support and Ms. Sandy Knowles for typing various drafts of the manuscript.

REFERENCES

[1] Mutus, B.; Wagner, J.D.; Talpas, C.J.; Dimmock, J.R.; Phillips, O.A.; Reid, R.S. *Anal. Biochem.*, **1989**, *177*, 237.
[2] Baluja, G.; Municio, A.M.; Vega, S. *Chem. Ind.*, **1964**, 2053.
[3] Benvenuto, J.A.; Connor, T.H.; Monteith, D.K.; Laidlaw, J.L.; Adams, S.C.; Matney, T.S.; Theiss, J.C. *J. Pharm. Sci.*, **1993**, *82*, 988.
[4] Chimura, H.; Nakamura, H.; Takita, T.; Takeuchi, T.; Umezawa, H.; Kato, K.; Saito, S.; Tomisawa, T.; Iitaka, Y. *J. Antibiot.*, **1975**, *28*, 743.
[5] Takeuchi, T.; Chimura, H.; Hamada, M.; Umezawa, H.; Yoshioka, O.; Oguchi, N.; Takahashi, Y.; Matsuda, A. *J. Antibiot.*, **1975**, *28*, 737.
[6] Kamiya, D; Uchihata, Y.; Ichikawa, E.; Kato, K; Umezawa, K. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 1111.
[7] Samudio, I.; Konopleva, M.; Hail, N. Jr.; Shi, Y.-X.; McQueen, T.; Hsu, T.; Evans, R.; Honda, T.; Gribble, G.W.; Sporn, M.; Gilbert, H.F.; Safe, S.; Andreeff, M. *J. Biol. Chem.*, **2005**, *280*, 36273.
[8] Dimmock, J.R.; Sidhu, K.K.; Chen, M.; Reid, R.S.; Allen, T.M.; Kao, G.Y.; Truitt, G.A. *Eur. J. Med. Chem.*, **1993**, *28*, 313.
[9] Dimmock, J.R.; Shyam, K.; Hamon, N.W.; Logan, B.M.; Raghavan, S.K.; Harwood, D.J.; Smith, P.J. *J. Pharm. Sci.*, **1983**, *72*, 887.
[10] Dimmock, J.R.; Hamon, N.W.; Waslen, T.A.; Patil, S.A.; Phillips, O.A.; Jonnalagadda, S.S.; Hancock, D.S. *Pharmazie*, **1986**, *41*, 441.
[11] Teicher, B.A.; Herman, T.S.; Holden, S.A.; Epelbaum, R.; Liu, S.D.; Frei, E. III. *Cancer Res.*, **1991**, *51*, 780.
[12] Ning, S.; Hahn, G.M. *Cancer Res.*, **1990**, *50*, 7867.
[13] Dimmock, J.R.; Taylor, W.G. *J. Pharm. Sci.*, **1975**, *64*, 241.
[14] Dimmock, J.R.; Smith, L.M.; Smith, P.J. *Can. J. Chem.*, **1980**, *58*, 984.
[15] Dimmock, J.R.; Elias, D.W.; Beazely, M.A.; Kandepu, N.M. *Curr. Med. Chem.*, **1999**, *6*, 1125.
[16] Dimmock, J.R.; Kandepu, N.M.; Hetherington, M.; Quail, J.W.; Pugazhenthi, U.; Sudom, A.M.; Chamankhah, M.; Rose, P.; Pass, E.; Allen, T.M.; Halleran, S.; Szydlowski, J.; Mutus, B.; Tannous, M.; Manavathu, E.K.; Myers, T.G.; De Clercq, E.; Balzarini, J. *J. Med. Chem.*, **1998**, *41*, 1014.
[17] Kabiev, O.K.; Vermenichev, S.M. *Izv. Akad. Nauk. Kaz. SSR. Ser. Biol.*, **1971**, *9*, 72; *Chem. Abstr.*, **1971**, *75*, 47091.
[18] Kupchan, S.M.; Tsou, G. *J. Org. Chem.*, **1973**, *38*, 1055.
[19] Badeway, E.; Kappe, T. *Eur. J. Med. Chem.*, **1995**, *30*, 327.
[20] Aboul-Fadl, T.; El-Shorbagi, A.-N.; Hozien, Z.A.; Sarhan, A.-W. A.O. *Boll. Chim. Farm.*, **2000**, *139*, 228.
[21] Naylor, M.A.; Swann, E.; Everett, S.A.; Jaffar, M.; Nolan, J.; Robertson, N.; Lockyer, S.D.; Patel, K.B.; Dennis, M.F.; Stratford, M.R.L.; Wardman, P.; Adams, G.E.; Moody, C.J.; Stratford, I.J. *J. Med. Chem.*, **1998**, *41*, 2720.
[22] Dimmock, J.R.; Arora, V.K.; Wonko, S.L.; Hamon, N.W.; Quail, J.W.; Zia, J.; Warrington, R.C.; Fang, W.D.; Lee, J.S. *Drug Des. Deliv.*, **1990**, *6*, 183.
[23] Hall, I.H.; Lee, K.-H.; Mar, E.C.; Starnes, C.O.; Waddell, T.G. *J. Med. Chem.*, **1977**, *20*, 333.
[24] Zhang, S.; Won, Y.-K.; Ong, C.-N.; Shen, H.-M. *Curr. Med. Chem.*, **2005**, *5*, 239.
[25] Lee, M.G.; Lee, K.T.; Chi, S.G.; Park, J.H. *Biol. Pharm. Bull.*, **2001**, *24*, 303.
[26] Dirsch, V.M.; Stuppner, H.; Vollmar, A.M. *Cancer Res.*, **2001**, *61*, 5817.
[27] Hanson, R.L., Lardy, H.A.; Kupchan, S.M. *Science*, **1970**, *168*, 378.
[28] Waddell, T.G.; Geissman, T.A. *Phytochemistry*, **1969**, *8*, 2371.
[29] Dimmock, J.R.; Erciyas, E.; Kirkpatrick, D.L.; King, K.M. *Pharmazie*, **1988**, *43*, 614.
[30] Gul, H.I.; Gul, M.; Vepsälainen, J.; Erciyas, E.; Hänninen, O. *Biol. Pharm. Bull.*, **2003**, *26*, 631.
[31] Salmon, S.E.; Hersh, E.M. *J. Natl. Cancer Res.*, **1994**, *86*, 228.
[32] Dvorakova, K.; Payne, C.M.; Tome, M.E.; Briehl, M.M.; McClure, T.; Dorr, R.T. *Biochem. Pharmacol.*, **2000**, *60*, 749.
[33] Sato, M.; Sasaki, M.; Oguro, T.; Kuroiwa, Y.; Yoshida, T. *Chem. Biol. Interact.*, **1995**, *98*, 15.
[34] Marchette, P.; Decaudin, D.; Macho, A.; Zamzami, N.; Hirsch, T.; Susin, S.A.; Kroemer, G. *Eur. J. Immun.*, **1997**, *27*, 289.
[35] Zhao, B.; Seow, A.; Lee, E.J.D.; Poh, W.-T.; Teh, M.; Eng, P.; Wang, Y.-T.; Tan, W.-C.; Yu, M.C.; Lee, H.P. *Cancer Epidemiol. Biomark. Prev.*, **2001**, *10*, 1063.
[36] Seow, A.; Yuan, J.-M.; Sun, C.-L.; Van den Berg, D.; Lee, H.-P.; Yu, M.C. *Carcinogenesis*, **2002**, *23*, 2055.
[37] Zhang, Y.; Li, J.; Tang, L. *Free Radic. Biol. Med.*, **2005**, *38*, 70.
[38] Xiao, D.; Srivastava, S.K.; Lew, K.L.; Zeng, Y.; Hershberger, P.; Johnson, C.S.; Trump, D.L.; Singh, S.V. *Carcinogenesis*, **2003**, *24*, 891.
[39] Guilbaud, N.; Kraus-Berthier, L.; Meyer-Losic, F.; Malivet, V.; Chacun, C.; Jan, M.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Atassi, G.; Hickman, J.; Pierré, A. *Clin. Cancer Res.*, **2001**, *7*, 2573.
[40] David-Cordonnier, M.H.; Laine, W.; Lansiaux, A.; Kouach, M.; Briand, G.; Pierré, A.; Hickman, J.A.; Bailly, C. *Biochemistry*, **2002**, *41*, 9911.
[41] David-Cordonnier, M.H.; Laine, W.; Joubert, A.; Tardy, C.; Goossens, J.-F.; Kouach, M.; Briand, G.; Mai, H.D.T.; Michel, S.; Tillequin, F.; Koch, M.; Leonce, S.; Pierre, A.; Bailly, C. *Eur. J. Biochem.*, **2003**, *270*, 2848.
[42] Paz, M.M.; Das, A.; Palom, Y.; He, Q.-Y.; Tomasz, M. *J. Med. Chem.*, **2001**, *44*, 2834.
[43] Magnus, P.; Eisenbeis, S.A. *J. Am. Chem. Soc.*, **1993**, *115*, 12627.
[44] Wolkenberg, S.E.; Boger, D.L. *Chem. Rev.*, **2002**, *102*, 2477.
[45] Geroni, C.; Marchini, S.; Cozzi, P.; Galliera, E.; Ragg, E.; Colombo, T.; Battaglia, R.; Howard, M.; D'Incalci, M.; Broggini, M. *Cancer Res.*, **2002**, *62*, 2332.
[46] Asai, A.; Hara, M.; Kakita, S.; Kanda, Y.; Yoshida, M.; Saito, H.; Saitoh, Y. *J. Am. Chem. Soc.*, **1996**, *118*, 6802.

[47] The Merck Index, Thirteenth Edition, Merck and Co., Inc., Whitehouse Station, NY, USA, **2001**; pp. 2807, 4489.
[48] Chen, G.; Waxman, D.J. *Biochem. Pharmacol.*, **1994**, *47*, 1079.
[49] Lee, F.Y. F.; Allalunis-Turner, M.J.; Siemann, D.W. *Br. J. Cancer*, **1987**, *56*, 33.
[50] Siemann, D.W.; Beyers, K. L. *Br. J. Cancer*, **1993**, *68*, 1071.
[51] Sugimoto, C.; Matsukawa, S.; Fujieda, S.; Noda, I.; Tanaka, N.; Tsuzuki, H.; Saito, H. *Anticancer Res.*, **1996**, *16*, 675.
[52] Chen, X.; Batist, G. *Biochem. Pharmacol.*, **1998**, *56*, 743.
[53] del Olmo, M.; Alouso-Varona, A.; Castro, B.; Calle, Y.; Bilbao, P.; Palomares, T. *Melanoma Res.*, **2000**, *10*, 103.
[54] Ahmad, S.; Okine, L.; Wood, R.; Aljian, J.; Vistica, D.T. *J. Cell. Physiol.*, **1987**, *131*, 240.
[55] Britten, R. A.; Green, J. A.; Warenius, H. M. *Int. J. Radiat. Oncol. Biol. Phys.*, **1992**, *24*, 527.
[56] Suzukake, K.; Petro, B.J.; Vistica, D.T. *Biochem. Pharmacol.*, **1982**, *31*, 121.
[57] Somfai-Relle, S.; Suzukake, K.; Vistica, B. P.; Vistica, D.T. *Biochem. Pharmacol.*, **1984**, *33*, 485.
[58] Buller, A.; Clapper, M.L.; Tew, K.D. *Mol. Pharmacol.*, **1987**, *31*, 575.
[59] Batist, G.; Tulpule, A.; Sinha, B.K.; Katki, A.G.; Myers, C.E.; Cowan, K.H. *J. Biol. Chem.,* **1986**, *261*, 15544.
[60] Khil, M.S.; Kim, S.H.; Pinto, J.T.; Kini, J.H. *Int. J. Radiat. Oncol. Biol. Phys.,* **1996**, *34*, 375.
[61] Tew, K.D.; Bomber, A.M.; Hoffman, S.J. *Cancer Res.,* **1988**, *48*, 3622.
[62] Doz, F.; Roosen, N.; Rosenblum, M.L. *J. Neurooncol.,* **1993**, *17*, 123.
[63] Kägi, J.H.R.; Schaffer, A. *Biochemistry,* **1988**, *27*, 8509.
[64] Naganuma, A.; Satoh, M.; Imura, N. *Cancer Res.,* **1987**, *47*, 983.
[65] Dinkova-Kostova, A.T.; Massiash, M.A.; Bozak, R.E.; Hicks, R.J.; Talalay, P. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*, 3404.
[66] Hrubisko, M.; McGown, A.T.; Fox, B.W. *Biochem. Pharmacol.,* **1993**, *45*, 253.
[67] Pendyala, L.; Perez, R.; Weinstein, A.; Zdanowicz, J.; Creaven, P.J. *Cancer Chemother. Pharmacol.,* **1997**, *40*, 38.
[68] Godwin, A.K.; Meister, A.; O'Dwyer, P.J.; Huang, C.S.; Hamilton, T.C.; Anderson, M.E. *Proc. Natl. Acad. Sci. USA,* **1992**, *89*, 3070.
[69] Ishikawa, T.; Ali-Osman, F. *J. Biol. Chem.,* **1993**, *268*, 20116.
[70] Bose, R.N. *Mini Rev. Med. Chem.,* **2002**, *2*, 103.
[71] Balendiran, G.K.; Dabur, R.; Fraser, D. *Cell. Biochem. Funct.,* **2004**, *22*, 343.
[72] Eaton, D.L.; Bammler, T.K. *Toxicol. Sci.,* **1999**, *49*, 156.
[73] Sexton, D.J.; Dimmock, J.R.; Mutus, B. *Biochem. Cell. Biol.,* **1993**, *71*, 98.
[74] Salinas, A.E.; Wong, M.G. *Curr. Med. Chem.,* **1999**, *6*, 279.
[75] Espinoza-Fonseca, L.M. *Bioorg. Med. Chem.,* **2006**, *14*, 896.

Received: 03 October, 2006 Revised: 17 November, 2006 Accepted: 27 November, 2006