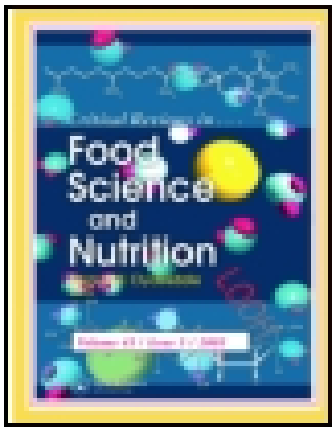

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An Update on Potential Perspectives of Glucosinolates on Protection Against Microbial Pathogens and Endocrine Dysfunctions in Humans

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An Update on Potential Perspectives of Glucosinolates on Protection Against Microbial Pathogens and Endocrine Dysfunctions in Humans

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Abstract

Glucosinolates are the major bioactive secondary metabolites found in the Brassicaceae family and studied extensively in biosynthetic and application perspectives. Because of their potential applications in the welfare of plants (protection against plant pathogens) and human life (prevention of cancer and other diseases), these compounds attracted much interest in the scientific community. In this review, we presented updates on glucosinolate derivatives in protection against microbial pathogens and endocrine related diseases in human. Further, the mechanism of action of glucosinolate derivatives and the strategies to improve their efficiency through modern approaches were discussed. Finally, the genetic enrichment of their contents in plant systems has also been discussed.

Keywords: Glucosinolates, *Brassicaceae*, pathogens, endocrine, secondary metabolites

INTRODUCTION

Glucosinolates (GSLs) are found in 16 families of dicotyledonous angiosperms, but are predominantly present in the *Brassicaceae* family (Fahey et al., 2001). GSLs are the major sulfur-enriched plant secondary metabolites of the *Brassicaceae* family that includes 350 genera and 3200 species (Hall et al., 2002) and produce various beneficial functions in plants and human beings. Sinalbin, the first GSL identified from the seeds of white mustard (*Sinapis alba*), was reported by Robiquet and Boutron in 1831, followed by 132 natural GSLs, which have been structurally reported till date (Fahey et al., 2001; Dinkova-Kostova and Kostov, 2012). GSLs are stored in the plant vacuoles away from the ß-thioglucosidase enzymes known as myrosinases (EC 3.2.1.147). Various biologically active derivatives of GSLs such as isothiocyanates (ITCs), indoles, nitriles, and epithionitriles are produced from the direct contact between the substrate (GSL) and enzyme (myrosinases) (Shapiro et al., 2006). These bioactive hydrolysis products of GSLs have been shown as chemopreventive molecules (anti-cancerous and antimicrobial agents) (Fahey et al., 1997 and 2002). Furthermore, the chemopreventive effect of these molecules depends on the bioavailable concentrations of the GSL hydrolysis products (GSL HPs), and the inactivation of plant myrosinase while cooking decreases its bioavailability. Apart from plants, myrosinases have been reported in microorganisms (bacteria, fungi) and aphids (reviewed in the report by Baskar et al., 2012). In mammals, microorganisms reside in the intestinal tract assisting the hydrolysis of GSLs, and this conversion is decreased to a great extent through the reduction of intestinal microflora by mechanical cleansing and antibiotic treatment. Other possible factors

that influence the bioavailability between individuals are polymorphisms in glutathione transferases, biotransformation, and excretion (Dinkova-Kostova and Kostov, 2012). GSL HPs, such as allyl isothiocyanate (AITC; from sinigrin), phenylethyl isothiocyanate (PEITC; from gluconasturtin), benzyl isothiocyanate (BITC; from glucotropaeolin), sulforaphane (SF; from glucoraphanin), Indole-3-carbinol (I3C; from glucobrassicin), and its condensation products 3,3'-diindolylmethane (DIM), 1-(3-hydroxymethyl) indolyl-3-indolylmethane, and indolo (3,2-b) carbazole were shown to be effective in preventing endocrine-related cancer diseases and protecting against microbial pathogens (Aires et al., 2009b; Firestone and Sundar, 2009; Kang et al., 2009; Alumkal et al., 2013; Yu et al., 2013). The schematic structural representation of different isothiocyanates and indoles and their natural sources are shown in Figure 1. The antimicrobial activities of various GSL HPs have been briefly discussed. The molecular mechanisms of the chemopreventive action of GSL HPs against hormone-related cancer diseases in humans have also been discussed. The overview of the chemopreventive activities of GSL HPs is schematically presented in Figure 2. In addition, we have briefly discussed the chemopreventive mechanism of action of the GSL HPs and the current methods of increasing their contents in plants, as well as their efficiency in clinical research using modern approaches.

ANTIMICROBIAL ACTIVITIES

Effects of GSL HPs against fungi

Although there are 250,000–500,000 reported plant species on earth, only a small fraction has been evaluated for the presence of antimicrobial compounds and only 1–10% of the plants are used by humans (Cowan, 1999). Previous studies have demonstrated the antagonizing activities of plant-based antimicrobials known as GSL HPs against pathogenic fungi. Some

studies have illustrated that the GSL HPs have the potential to degrade powerful toxins produced by pathogenic fungi in food industries (Azaiez et al., 2013). Drobnica et al. (1967) examined the effects of 57 substituted derivatives of phenylisothiocyanate (PITC) against a group of fungi, including *Aspergillus niger*, *Penicillium cyclopium*, and *Rhizopus oryzae*, as well as against saprophytic and parasitic fungi. The results revealed that all the three tested fungi were equally affected by these compounds. Later, Johns et al. (1982) reported the antifungal activity of benzyl and p-methoxybenzyl isothiocyanates against *Candida albicans*, whereas Al-Bagieh et al. (1998) demonstrated the antifungal effects of *Salvadora persica* against *C. albicans* and found that BITC is the major ITC in *S. persica*. Furthermore, based on the concentrations tested, BITC was found to act as a fungistatic agent (0.05, 0.1, and 0.5 µg/ml), a fungicidal agent (1.0 µg/ml), and an inhibitor of acid production (10, 50, and 100 µg/ml) (Al-Bagieh et al., 1998). On the other hand, the fungicidal activity of AITC has been proven against several fungi and yeasts, including *Aspergillus flavus*, *Endomyces fibuliger*, *Penicillium commune*, *Penicillium corylophilum*, *Penicillium discolor*, *Penicillium palitans*, *Penicillium polonicum*, *Penicillium roqueforti*, *Penicillium solitum*, and *Pichia anomala* (Nielsen and Rios, 2000). Mustard oil is one of the major sources of AITC (99%), and has been reported to display strong antifungal activity (Suhr and Nielsen, 2003). Goralska et al. (2009) examined the antifungal activity of GSL extracted from the seeds of four cruciferous plants, namely, broccoli, small radish, white cabbage, and white mustard against *C. albicans*, and found that the broccoli seed extracts showed significant antifungal activity, when compared with the commercial antifungal drug fluconazole. Furthermore, the antifungal activity of white mustard seed extract was similar to that of fluconazole. Radulovic et al. (2011) identified a new ITC from *Erysimum diffusum* Ehrh.

(*Brassicaceae*), namely, 4-isothiocyanatobutanoic acid, and studied its effect against various pathogenic bacterial and fungal strains. Their results showed that this ITC exhibited fungicidal activity against *C. albicans* with a minimum fungicidal concentration (MFC) of 20 μg/ml, when compared with the standard antifungal agent nystatine with a MFC of 6.25 μg/ml. Moreover, Kaur et al. (2011) investigated the effects of the antifungal activity of the seeds of GSL-producing plants such as rocket salad, Indian rape, cabbage, cauliflower, knol-khol, and radish against *Alternaria alternata*, and found a dose-dependent inhibition of the fungal growth and a significant level of inhibition at higher concentrations of GSL HPs. Furthermore, the antimicrobial efficacy of *Hornungia petraea* GSL derivatives (BITC as well as 3- and 4-methoxybenzyl isothiocyanate) was tested against *A. fumigatus* and *C. albicans*, and the results indicated that BITC was effective against *A. fumigatus*, whereas 4-methoxybenzyl isothiocyanate was effective against both the fungal species (Radulovic et al., 2012). Fumonisins (FBs) are the mycotoxins primarily produced by molds such as *Fusarium verticillioides* (also known as *Fusarium moniliforme*) and *Fusarium proliferatum* contaminating wheat, maize, maize-based foods, and other grains. In a previous study, the role of ITC activity against the growth of three mycotoxin-producing strains (*Gibberella moniliformis* 2983, 5847, and 5850) as well as on the reduction of FB2 toxin (class 2B carcinogen) produced by *G. moniliformis* was examined. It was found that ITCs such as PITC, AITC, and BITC were active against the three mycotoxigenic strains of *G. moniliformis* and resulted in the reduction of 2.1−89.7% of mycelial size. Moreover, these ITCs effectively reduced the FB2 mycotoxin synthesized by *G. moniliformis* to a great extent (73−100%) (Azaiez et al., 2013). Nevertheless, as most of these reports involved only a

few groups of fungal species, further studies are warranted to evaluate the potential of these GSL-derived products against various fungal pathogens harmful to humans.

Antibacterial activities of GSL HPs

Recently, the prevalence of decreased susceptibility of bacteria to antibiotics owing to the emergence of drug resistance has necessitated the scientific community to find alternative ways to control bacterial growth and their virulence. Several antibiotic-resistant bacteria have been reported, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* O157:H7, *Pseudomonas aeruginosa,* and multi-drug resistance *Mycobacterium tuberculosis* (Alekshun and Levy, 2007). ß-Lactamases are the bacterial enzymes produced by Gram-negative bacterial pathogens that inactivate the ß-lactam antibiotics by hydrolysis, resulting in ineffective compounds. One group of ß-lactamases, known as extended-spectrum ß-lactamases (ESBLs), has the ability to hydrolyze ß-lactam antibiotics and cause resistance to various types of newer ß-lactam antibiotics (Paterson and Bonomo, 2005).

Several studies have shown the anticancer activity of GSL HPs, whereas only a few reports are available on their activity against human pathogenic bacterial species. Recently, the bactericidal activity of AITC against several pathogenic bacteria such as *Helicobacter pylori, E. coli, Salmonella typhimurium, S. aureus, Streptococcus mutans, Bacillus cereus,* and *Vibrio parahaemolyticus* was reported (Shin et al., 2004; Luciano and Holley, 2009; Zhang, 2010). It was found that PITC exhibited bactericidal activity against three strains of *H. pylori,* with an efficiency that was 7.8–20.5 folds higher than that of AITC (Shin et al., 2004; Zhang, 2010). Furthermore, Kim and Lee (2009) identified aromatic ITC (PEITC) as the active compound found in *Sinapis alba* L. seeds, and examined its antibacterial effects against a range of intestinal

bacteria, including *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Clostridium difficile*, *Clostridium perfringens*, *E. coli*, *Lactobacillus acidophilus*, and *Lactobacillus casei.* Among these bacteria, *C. difficile, C. perfringens*, and *E. coli* showed higher and moderate sensitivity to PEITC, respectively, whereas the growth of *Bifidobacteria* and *Lactobacilli* was found to be unaffected. Furthermore, the clinical isolate *S. aureus* MJS241 was reported to be highly susceptible to PEITC, when compared with that to commercial antibiotics such as gentamicin and vancomycin (Aires et al., 2013b). In another study, the synergistic activity between the phytochemicals (phenolics, ITCs (AITC, BITC, and PEITC) and antibiotics (ciprofloxacin, gentamicin, and streptomycin) was demonstrated, and it was suggested that ITCs, which worked synergistically with antibiotics, were the most potent inhibitors of bacterial growth than phenolics (Saavedra et al., 2010). Moreover, ITCs such as 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanates were analyzed for their antibacterial efficacy against selected Gram-positive (*B. cereus, Bacillus subtilis, Listeria monocytogenes*, and *S. aureus*) and Gram-negative (*Aeromonas hydrophila, P. aeruginosa, Salmonella choleaesuis, Salmonella enterica, Serratia marcescens, Shigella sonnei*, and *V. parahaemolyticus*) bacteria and the effective inhibition rate of *B. cereus* was achieved with BITC, followed by PEITC. Furthermore, *A. hydrophila* showed higher susceptibility to 3-butenyl and 4-pentenyl ITCs. Among the ITCs tested, most of the bacterial species were severely inhibited by BITC and PEITC than by 3-butenyl and 4-pentenyl ITCs; in particular, Gram-positive bacteria were more inhibited by these ITCs than Gram-negative bacteria (Jang et al., 2010).

In chronic obstructive pulmonary disease (COPD) patients suffering from frequent bacterial attacks owing to defective bacterial clearance, defective macrophage phagocytosis

system has been observed. Furthermore, the cytoprotective regulatory gene NRF-2 expression has been noted to decrease in COPD patients, thus rendering them susceptible to oxidative stress. Administration of SF has been reported to lead to the induction of NRF-2 expression, stimulates the scavenger receptor "MARCO" in the alveolar macrophages of COPD patients, resulting in an improved bacterial clearance (*Haemophilus influenzae* (NTHI) and *P. aeruginosa*) (Harvey et al., 2011). Radulovic et al. (2011) isolated a new ITC from *Erysimum E. diffusum* Ehrh. (*Brassicaceae*), referred to as 4-isothiocyanatobutanoic acid, and examined its activity against various pathogenic bacterial strains. Their results showed that this ITC could severely inhibit the growth of Gram-positive bacteria (*S. aureus, Sarcina lutea*), while being ineffective against Gram-negative bacteria (*P. aeruginosa*). Furthermore, Sofrata et al. (2011) examined the antimicrobial effects of *S. persica* root extracts against the oral pathogens and identified BITC as the major antimicrobial compound in this extract. Subsequently, the *S. persica* root extracts as well as the commercial BITC were studied for their antibacterial efficiency, and the results revealed that both these agents killed Gram-negative oral pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, as well as other clinically important Gram-negative bacterial species, such as *H. influenzae* and *S. enterica*. Particularly, the bactericidal effect of these agents was rapid and, within minutes, a 1000-fold reduction in the bacterial population was observed. However, the Gram-positive oral bacterial pathogens such as *S. mutans* and *L. acidophilus* mostly remained unaffected. The strong protrusions observed on the bacterial membrane following BITC and *S. persica* root extract treatments suggested that they could disturb the membrane potential and integrity. It must be noted that AITC derived from natural sources have been permitted as a food preservative in Japan and as a GRAS flavoring

agent in the USA (Olaimat and Holley, 2013). Several previous studies have reported that AITC effectively controlled food-borne pathogens (*E. coli* O157:H7, *L. monocytogenes, S. typhimurium, B. cereus, S. aureus,* and *C. jejuni*) in vitro or in meat products (Olaimat and Holley, 2013). However, some of the limitations of AITC include high volatility, pungency, poor water solubility, and reactions with the nucleophiles of food restrict its use in food processing industries (Chacon et al., 2006; Olaimat and Holley, 2013). Nevertheless, alternative approaches have been employed to avoid these negative effects, and some studies that have been conducted to solve this problem will be discussed in the section describing the strategies for improved GSL efficiency.

In a recent work, AITC and BITC were tested against the food-borne pathogen, *C. jejuni,* isolated from chicken feces, human infections, and contaminated foods. Both the ITCs were noted to exhibit the strongest inhibition, particularly, bactericidal effects, against the tested *C. jejuni* strains, and among them, BITC performed better than AITC (Dufour et al., 2012). Furthermore, Dias et al. (2012) reported the in vitro synergic activity between ITCs and commercial antibiotics such as gentamicin and vancomycin against various bacterial pathogens belonging to Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*E. coli, Enterococcus faecalis,* and *P. aeruginosa*) groups. They found that both the groups of bacteria were severely affected by BITC. Phytochemicals (ITCs) alone or in combination with antibiotics were effective against Gram-positive bacteria. The best synergistic results were observed between antibiotics and ITCs such as BITC and PEITC. Aires et al. (2013a) investigated the efficiency of eight different GSL HPs against *Aeromonas* spp. such as *Aeromonas allosaccharophila, Aeromonas hydrophila, Aeromonas media,* and *Aeromonas veronii* isolated

from pig intestine, and found that the GSL HPs, especially the ITCs, exhibited antimicrobial activity. Among the ITCs, BITC, SF, and PEITC were observed to be the strongest inhibitors of bacterial growth in vitro. In another study, the extracts of watercress (*Nasturtium officinale* R. Br.), PEITC, and commercial antibiotics were tested against 11 different isolates of ESBL producing *E. coli* strains. An improved antimicrobial performance was exhibited by antibiotic co-treated with natural extracts of watercress or pure PEITC, suggesting that this synergistic approach could be useful in eliminating antibiotic insensitive microorganisms (Freitas et al., 2013).

H. pylori is a bacterium found in the stomach, which causes gastritis and increases the risk of stomach cancer. The higher urease activity has been found to produce ammonia, which neutralizes gastric acidity and enhances inflammation. SF has been reported to show bactericidal activity against both urease-positive and -negative *H. pylori* strains, while the other related natural ITCs of SF such as berteroin, hirsutin, PEITC, alyssin, and erucin have been observed to exhibit bactericidal activity, but not effective urease activity. On the other hand, it has been found that some ITCs, namely, benzoyl-ITC, are not bactericidal, but act as potent inhibitors of urease activity, suggesting that the bactericidal effects of ITCs do not strictly depend on urease inactivation, but may suppress the inflammation induced by *H. pylori* (Fahey et al., 2013). Similarly, oral administration of SF-enriched broccoli sprouts into *H. pylori* Sydney strain 1 infected mice was noted to result in the following pathological events: decreased gastric bacterial colonization, suppression of the expressions of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), reduced corpus inflammation, and reduction in high salt-induced gastric corpus atrophy. Moreover, supplementation of SF-rich broccoli sprouts resulted in a strong reduction in

the biomarkers for *H. pylori* colonization (urease and *H. pylori* stool antigen) as well as gastric inflammation (serum pepsinogens I and II) (Yanaka et al., 2009). Furthermore, Zou et al. (2013) showed the increased synergistic antimicrobial activity of nisin (commercial antibiotic) and AITC against a group of Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*S. typhimurium* and *Salmonella boydii*) food-borne pathogenic bacteria. The combined treatment caused potential loss of permeability and perturbation of membrane potential in the pathogen, leading to cell death. In summary, the above-mentioned studies suggest the promising potential of GSL HPs in effectively controlling bacterial pathogens, including the drug-resistant pathogens that are difficult to eradicate through commercial antibiotics. Therefore, alternative approaches using phytochemicals alone or in combination with commercial antibiotics could be effective in controlling the growth of drug-resistant microorganisms. Nevertheless, further studies are needed to extend the use of GSL-derived products against several bacterial pathogens that are dangerous to human health.

Effects of GSL HPs against viruses

Approximately 60% of the Earth's biomass is occupied by microbial community (Radulovic et al., 2013). Microbial infectious diseases remain the leading cause of morbidity and mortality, and among them, viral diseases are considered to possess a high health risk factor. Recently, more attention has been paid to the use of phytochemicals as alternative antimicrobials against pathogens owing to their lower side effects, when compared with synthetic drugs (Luciano and Holley, 2009). Nevertheless, very few studies have demonstrated the anti-viral effects of GSL HPs. For instance, the main antimicrobial compound found in *S. persica* is BITC, which has been reported to exhibit virucidal activity against *Herpes simplex virus-1* (HSV–1) at

a concentration of 133.3 µg/ml (Al-Bagieh et al., 1992). Similarly, Taha et al. (2008) investigated the effects of ethanolic extract of *S. persica* (Siwak) against HSV virus through in vitro and in vivo experiments, and found that the external skin treatments using *S. persica* significantly decreased cutaneous lesions and viral population in the skin, as well as reduced ganglia formation. Furthermore, it has been reported that the induction and suppression of estradiol C-2 and C-16 hydroxylation, respectively, following DIM treatment, can be used to treat the viral disease caused by human papilloma virus (HPV), which can proliferate in the presence of the estrogen metabolite 16α-hydroxestrone (16α-OHE). Accordingly, HPV-induced cervical intra-epithelial neoplasia (CIN) was efficiently reduced by employing I3C treatments (Bell et al., 2000). A study by Xue et al. (2008) showed that mice supplemented with indole GSL derivatives displayed an increased host response to enteric reovirus infection. Following oral supplementation of DIM, the mice challenged with reovirus serotype 1 exhibited an enhanced host response to infection through increased mucosal IgA response and induction of various cytokines, including granulocyte colony stimulating factor (GCSF), IL-6, IL-12, and interferon-γ (IFN-γ). In addition, an increased viral clearance from the gastrointestinal tract was also found following this treatment. Although all these studies have indicated the beneficial effects of GSL HPs in controlling viruses and combating viral diseases, only a very few of them have reported the antiviral activity of GSL HPs, suggesting the need for extensive research to examine the effects of these natural bioactive metabolites against a diverged group of viral pathogens.

Inhibition of Biofilm formation

To avoid the development of antibiotic resistance in microorganisms, alternative strategies should be employed to control the microbial growth and virulence. Most of the

pathogenic bacteria use quorum sensing (QS) to regulate their virulence, which makes this system an ideal target for antimicrobial therapy (Bjarnsholt et al., 2007). The microbial attachment to abiotic and biotic surfaces for multiplication results in the formation of a slimy matrix of extracellular polymeric substances (mostly proteins and polysaccharides), known as biofilms (Simoes, 2011). Biofilm formation has been correlated with various health risks such as periodontal disease, endocarditis, osteomyelitis, cystic fibrosis, and infections related to surgical implants (Simoes, 2011). The removal of biofilm by using conventional antibiotic treatments is a difficult task, mainly owing to the poor penetration or inactivation of antibiotics in the extracellular polymeric matrix, presence of persister cells, and induction of efflux pumps. However, the disruption of biofilm allows the bacteria to be effectively controlled even at lower doses of antibiotics (Dell'Acqua et al., 2004).

Some studies have shown the potential use of GSL HPs in the control of biofilm formation and QS activity by using different bacterial systems. The plant-derived indole-3-acetonitrile (IAN) was identified as a stable indole to prevent bacterial biofilms formed by *E. coli* and *P. aeruginosa*. Furthermore, the synthesis of various virulence factors, including 2-heptyl-3-hydroxy-4(1H)-quinolone (PQS), pyocyanin, and pyoverdine by *P. aeruginosa* was effectively suppressed by IAN (Lee et al., 2011). Jakobsen et al. (2012) reported that the SF and its analogs, including iberin, strongly inhibited QS activity in *P. aeruginosa*. Furthermore, Ganin et al. (2013) investigated the effect of SF and erucin derived from broccoli against *P. aeruginosa* QS activity and virulence, and observed QS inhibition activity at SF and erucin concentration of 25 mM, and only a slight growth-inhibiting activity at SF concentrations >100 mM. The inhibition of QS activity by SF is shown in Figure 3. In addition, Borges et al. (2012) investigated the

effects of AITC and PEITC on bacterial motility and prevention of biofilm formation by *E. coli, P. aeruginosa, S. aureus,* and *L. monocytogenes*. They found that AITC could inhibit the swimming motility of *P. aeruginosa* and swarming motility of *E. coli* and *P. aeruginosa,* whereas PEITC could inhibit the swimming motility of *E. coli, P. aeruginosa,* and *L. monocytogenes* and swarming motility of *E. coli* and *P. aeruginosa*. Furthermore, for all the biofilms tested, the viability was reduced to > 87% by AITC and PEITC. However, AITC and PEITC did not exhibit biofilm prevention activity against *S. aureus* and *L. monocytogenes,* respectively. In another study, phenyl isothiocyanate was observed to significantly reduce the viability of *E. coli* and *S. aureus,* and effectively prevent their biofilm formation by interrupting the adhesion process and motility of the bacteria (Abreu et al., 2013). All these exciting results indicate that the use of GSL HPs to prevent QS and biofilm formation is certainly promising from clinical and environmental perspectives.

PROTECTION AGAINST ENDOCRINE-RELATED DISEASES

Effects of GSL HPs on estrogen-mediated cancer development by modulation of estrogen metabolites

Numerous studies have shown the positive correlation between consumption of GSL-enriched cruciferous vegetables and lower risk of various cancers, including steroid hormone associated cancers (Firestone and Sundar, 2009). Evidence from the cellular and physiological studies has revealed that DIM and I3C are the IGSL derivatives that selectively alter the synthesis of steroid metabolites, which, in turn, exhibit in vivo endocrine disruption and cancer preventive effects (Firestone and Sundar, 2009). Estrogens are steroid hormones that have a negative role in the development of breast and endometrial cancer and positive role in bone

health. Phytonutrients such as polyphenols, isothiocyanates, and carotenoid derivatives have been noted to suppress the transactivation of estrogen response element (ERE) in breast cancer cells, but induce the ERE activity in bone derived cells (Veprik et al., 2012). The estrogen-induced cell growth and proliferation have been found to be executed through the specific nuclear receptors known as estrogen receptors (ERs). Among the two isoforms of ERs, namely, ER-α and ER-ß, synthesized from different genes (Imamov et al., 2005), ER-α has been observed to be involved in estrogen-activated cell growth, whereas ER-ß has been shown to play a protective role. Furthermore, previous studies have also reported the presence of increased ER-α and decreased ER-ß levels in high-risk precancerous breast lesion expression, indicating the protective role of ER-ß (Shaaban et al., 2003). Baba et al. (2005) demonstrated that aryl hydrocarbon receptor (AhR) plays a central role in female reproduction by controlling the expression of ovarian cytochrome P450 aromatase (CYP19), an enzyme involved in estrogen synthesis. AhR is a ligand-activated transcription factor belonging to the basic helix-loop-helix (bHLH)/Per-Arnt-Sim family (Beischlag et al., 2008). Upon AhR ligand activation, the activated AhR binds to ER, which leads to the prevention of its interaction with ERE (Kharat and Saatcioglu, 1996). This ligand–receptor interaction facilitates the use of AhR ligands (several phytochemicals, including GSL HPs) as selective AhR modulators (sAhRM), indicating their potential role in the treatment of endocrine-related cancer diseases (Medjakovic et al., 2011). It has been previously demonstrated that AhR induces the gene expressions of Phase-I enzyme (CYP1A1) and Phase-II enzymes (glutathione transferases and oxidoreductases) in various cells, such as prostate and breast cancer cells, as well as in rat liver (Weng et al., 2008). Phase-I and Phase-II enzymes are involved in the elimination of carcinogenic metabolites, and Phase-I

enzymes such as CYP1A1, CYP3A4, and CY1PB1 catalyze the formation of 2-hydroxyestrone (2-HE), 16-hydroxyestrone (16-HE), and 4-hydroxyestrone (4-HE) estrogen metabolites, respectively (Martucci and Fishman, 1977). Furthermore, it has been reported that C-2 hydroxyestrone mediates the antiproliferative effects, whereas 16-HE and 4-HE are associated with increased risk of cancer (Weng et al., 2008).

I3C is an important dietary compound found in cruciferous vegetables, which has the ability to induce or suppress carcinogenesis depending on various animal models. It acts as an antiestrogen and promotes apoptosis. For example, I3C treatment has been found to induce CYP1A1, CYP1A2, and CYP1B1 genes both at transcription and translational levels and also upregulate estradiol 2- and 4-hydroxylase activities in the liver; however, it did not affect the 16α-hydoxylase activity (Yoshida et al., 2004). Moreover, I3C-mediated increase in the 2-hydroxyestrone level has been noted to reduce the substrate availability to potent estrogen metabolite, 16-HE, formation and keep the pathway away from the formation of 16-HE (Greenwald et al., 2004). Another indole derivative DIM (condensation product of I3C) acts as a potent activator of CYP1A1, which leads to the stimulation of estradiol C-2 hydroxylation (Sepkovic et al., 2002). DIM has been shown to inhibit the production of carcinogenic estrogen metabolites such as 16-HE and 4-HE, and thus provide protection against cancer (Sepkovic and Bradlow, 2009). Moreover, increased urinary estrogen metabolite, 2-HE, found in postmenopausal women treated with DIM, has been noted to correlate with decreased breast cancer risk (Dalessandri et al., 2004). The schematic representation of the effect of DIM on the regulation of estrogen-mediated carcinogenesis is shown in Figure 4.

The concentrations of GSL HPs used for clinical application also determine its activity. For instance, DIM at low concentrations (10–50 μM) activates AhR without altering CYP1A1 expression and represses estrogen-induced proliferation in MCF-7 cells. On the other hand, at higher concentrations (> 50 μM), DIM may favor the transformation of AhR and activation of cytochrome P450 enzymes. However, at concentrations of 100 μM, DIM stimulates CYP1A1 expression, similar to the effect of 1 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, chemical carcinogen) (Chen et al., 1998). DIM acts as a ligand binding to AhR, which in turn induces the degradation of ER-α protein, and their chemopreventive activity is associated with the upregulation of ER-ß and downregulation of ER-α (Firestone and Sundar, 2009). Furthermore, DIM does not directly bind to ER-β while activating the ER-β target genes (keratin 19, NKG2E, and CECR6) through selective recruitment of ER-β and steroid receptor coactivator-2 to target genes. ER-β represses the actions of ER-α, and high levels of ER-β may block the ability of ER-α, which represses NRF-2 activity (Matthews et al., 2006). Similarly, strong suppression of ER-α at transcription and translational levels has been reported in the ER-sensitive (MCF7 and T47D) breast cancer cell lines treated with I3C (Sundar et al., 2006). Moreover, I3C-mediated induction of AhR has been noted to trigger Rbx-1 E3 ligase mediated ubiquitination and proteosomal degradation of ER-α (Marconett et al., 2010). I3C administration has been observed to interrupt the estrogen responsive gene expression and inhibit estrogen-dependent proliferation. A recent study revealed that I3C blocked the expression of insulin-like growth factor receptor-1 (IGF1R) and insulin receptor substrate-1 (IRS1), the downstream effectors of the IGF1 signaling pathway, in human MCF-7 breast cancer cells. Thus, I3C could arrest the multiplication of estrogen-dependent human breast cancer cells via interruption of ER-α-mediated transcription of cell

signaling components within the IGF1 cascade (Marconett et al., 2012). Indolylfuran, an indole derivative from sauerkraut known to be a potent activator of AhR, when compared with other natural derivatives, has been noted to exhibit antiestrogenic activities through the disruption of estradiol (E2)-induced expression of the cellular proliferating marker Ki67 and suppression of the expressions of ER-α and ER-β (Medjakovic et al., 2011). Recently, Benninghoff and Williams (2013) reported that I3C may prevent or inhibit transplacental carcinogenesis induced by the carcinogen dibenzo [*def, p*] chrysene (DBC) by acting on ER-β receptor. Furthermore, DIM administration on ER-positive and ER-negative breast cancer cell lines has been demonstrated to induce miR-21 associated with the degradation of Cdc25A and suppress the translational level of CDK1, CDK2, CDK4, CDK6, and cyclin B1, leading to cell cycle arrest (Jin, 2011). Similarly, 1-benzyl-I3C, an I3C analog, has been noted to exhibit a 1000-fold increased activity in controlling the growth of human breast cancer cells, and induce G1 cell cycle arrest even at very low concentrations, when compared with I3C. In addition, this compound has been observed to suppress the ER-α protein synthesis and act synergistically with tamoxifen, resulting in increased efficiency in controlling breast cancer cell growth (Nguyen et al., 2010).

Aromatic ITCs such as BITC and PEITC have been shown to suppress the expression of ER-α and estrogen-induced cell growth in ER-α-positive breast cancer cell lines such as MCF-7 and T-47D. Furthermore, the expression of estrogen responsive gene, pS2, has been noted to be blocked owing to the potential disrupting activity of BITC and PEITC on ER-α transcription (Kang et al., 2009). PEITC has also been shown to inhibit the growth of ER-positive and ER-negative breast cancer cells, and suppress the ER-mediated MAPK/ERK1/2 signaling,

maintaining reduced ER-α and ER-α36 level. The suppression of both ER-α and its novel variant, ER-α36, by PEITC suggests that it is more potent than the "pure" anti-estrogen ICI 182,780, which can inhibit only ER-α (Kang et al., 2010). ITCs-induced apoptosis have been shown to be linked to the upregulation of the pro-apoptotic protein (Bcl-2-associated X protein (Bax)) and downregulation of anti-apoptotic proteins (myeloid cell leukemia sequence-1 (Mcl-1), B-cell lymphoma 2 (Bcl-2), and B-cell lymphoma extra large (Bcl-XL)) in various human cancer cells (Brown and Hampton, 2011). For instance, the estrogen-induced Bcl-2 gene expression was diminished by I3C treatment through ER-α and Sp1 interactions at the Bcl-2 gene promoter (Firestone and Sundar, 2009). In addition, previous studies have revealed that the induction of apoptosis by SF is achieved through the regulation of heat shock proteins (HSPs) in breast cancer cells. HSPs such as HSP 27, 70, and 90 play a crucial role in the development of breast cancer, and interestingly, SF has been shown to suppress the expressions of HSP 70, HSP 90, and HSF1 (Sarkar et al., 2012).

In a previous study, the anticancer activity of the mixtures of cyclic tetrameric derivatives (CTet) such as tri- and tetrameric cyclic I3C derivatives (CTr/CTet) against breast cancer was examined. The CTr/CTet mixture showed high solubility, induced the expressions of p21, p27, and GADD45A and nuclear translocation of FOXO3a, inhibited Akt signaling, and suppressed the estrogen receptor (Brandi et al., 2013). In addition, CTet was observed to induce enhanced cytotoxicity in breast cancer cells pretreated with cisplatin and doxorubicin, indicating their synergistic activity with commercial anticancer drugs (De Santi et al., 2013). Thus, all these extensive studies indicate the potential applications of GSL HPs, especially the indole

derivatives such as I3C, and its condensation products in the control of estrogen-mediated cancer development.

Effects of GSL HPs on androgen-mediated cancer diseases by modulation of androgen signaling pathway

Epidemiological studies have shown that increased dietary uptake of cruciferous vegetables is correlated with lower risk of prostate cancer (Gibbs et al., 2009). Androgen receptor (AR) is a member of the steroid receptor superfamily transcription factors activated by suitable ligands, and plays a crucial role in the development of the male reproductive organ function and in prostate cancer progression. The transition from androgen-sensitive to androgen-independent prostate cancer is mainly controlled by AR signaling (Hotte and Saad, 2010). AR is the major signaling pathway in prostate cancer development, and its inhibition is used for both the prevention and treatment of this disease (Gibbs et al., 2009).

It has been reported that I3C and DIM interrupt androgen-sensitive and androgen-insensitive AR signaling in human prostate cancer cells at various cellular levels of regulation (Firestone and Sundar, 2009). In LNCaP human prostate cancer cells, I3C-induced cell cycle arrest has been noted to occur with the suppression of AR-regulated gene known as prostate-specific antigen (PSA) (Zhang et al., 2003). Furthermore, I3C treatment has been found to hasten the ablation of AR transcript as well as decrease the AR promoter driven reporter plasmid activity (Hsu et al., 2005). On the contrary, it has been observed that DIM acts as a powerful phytochemical ligand and is capable of directly binding to AR, leading to effective inhibition of AR transcription (Le et al., 2003). Wang et al. (2012) observed the direct action of DIM on AR in inhibiting the induction of androgen responsive gene (ARG) expression by androgen, and the

indirect action of I3C in effectively inhibiting estradiol-induced upregulation of ARG (Wang et al., 2012). Furthermore, B-DIM (formulated DIM: 3,3'-diindolylmethane by Bio Response, Boulder, CO) treatment has been reported to suppress the AR transcripts in the LNCaP human prostate cancer cells by decreasing the recruitment of the FOXO3A transcription factor to the AR promoter (Li et al., 2007). Wang et al. (2012) investigated the mechanism of actions of DIM and I3C at different concentrations in prostate cancer cell culture. Their results illustrated that at 1–5 μM, both DIM and I3C inhibited androgen- and estrogen-mediated pathways and stimulated xenobiotic metabolism pathway, whereas at concentrations $\geq$ 25 μM, both the compounds activated the cyclin inhibitors, which are indicators of stress/DNA damage. Furthermore, both the ITCs exhibited growth inhibition through inhibition of IGF1R expression.

It has been reported that PEITC inhibits dihydrotestosterone (DHT)-stimulated AR transcriptional activity, and the growth of prostate cancer (PCa) cells has been proven in AR-responsive LNCaP cells. The upregulation of p300/CBP-associated factor (PCAF, co-regulator for AR) in PCa cells has been noted to be mainly through the downregulation of miR-17 gene, and PCAF expression has been found to be downregulated via the induction of miR-17 in PEITC-treated LNCaP cells (Yu et al., 2013). A previous study revealed that PEITC acted synergistically with Docetaxel (Taxotere®), the drug of choice for androgen-independent prostate cancer (AIPC), and enhanced apoptosis in PC-3 and DU145 cells in vitro. Furthermore, increased anticancer activity against PC-3 xenograft was observed in vivo following PEITC and Docetaxel dual combination treatment (Xiao and Singh, 2010). Kong et al. (2012a) reported that overexpression of miR-34a in PCa cell lines resulted in the downregulation of AR, PSA, and Notch-1 expressions, whereas the functional loss of miR-34a in tumor cells resulted in higher

levels of AR. Furthermore, they observed that PCa cells treated with BR-DIM exhibited demethylation of miR-34a promoter (induced the expression of miR-34a), which in turn decreased the expressions of AR and PSA in LNCaP and C4-2B cells. In addition, studies on BR-DIM intervention in PCa patients also revealed the re-expression of miR-34a, leading to the decrease in the expressions of AR, PSA, and Notch-1 in PCa tissue specimens. It has been reported that BR-DIM and CDF (a synthetic analog of curcumin) effectively induced the re-expression of miR-200 family and PTEN and also downregulated the expressions of membrane type-1-matrix metalloproteinase (MT1-MMP) (Soubani et al., 2012). Similarly, another study also indicated that the disregulation of miRNAs is associated with PCa development and progression. The miRNA let-7 family has been found to be a crucial factor in PCa, which regulates cancer stem cells through its target called enhancer of Zeste homolog 2 (EZH2). Kong et al. (2012b) observed that enhanced expression of EZH2 in human PCa tissue specimens, particularly in higher Gleason grade tumors, correlated with the loss of miRNA let-7 family. The upregulation of let-7 and suppression of EZH2, along with the decrease in self-renewal and clonogenic capacity, were found in PCa cells treated with B-DIM both in vitro and in human intervention studies (Kong et al., 2012b). Furthermore, in another study, the antiproliferative and antiandrogenic activities of DIM were observed in the androgen-dependent human prostate cancer cells. DIM suppressed the transcription of PSA and decreased the intracellular and secreted PSA protein levels induced by DHT in LNCaP cells. In addition, it also inhibited the translocation of androgen-induced AR to the nucleus and acted as a powerful competitive inhibitor of DHT binding to the AR, and also showed high similarity to the known AR antagonist with respect to conformational geometry and surface charge distribution (Le et al., 2012).

An earlier study demonstrated that SF could induce repression of AR transcription and inhibit nuclear translocation of AR in LnCaP and C4-2 cells (Kim and Singh, 2009). In addition, SF has been found to inhibit histone deacetylase (HDAC) induced in cancer tissues. HDAC6 has been reported to regulate the acetylation state of HSP90 (androgen receptors chaperone), which prevents proteosomal degradation of AR. However, SF treatment has been noted to cause inactivation of HDAC6-mediated HSP90 deacetylation, resulting in the hyperacetylation of HSP90 and proteosomal degradation of AR, and leading to decreased AR gene expression (Gibbs et al., 2009). Therefore, it can be concluded that SF destabilizes AR protein and disrupts AR signaling through the inactivation of HDAC6. Recently, a clinical trial study (NCT01228084) reported that SF treatment at 200 μmol/day in men with recurrent PCa is suitable, safe, and inhibits HDAC function (Alumkal et al., 2013). Thus, these reports reveal the potential applications of GSL HPs in the regulation of androgen-related cancer diseases in humans.

GSL HPs induced protection against health risks related to insulin hormone dysfunction

Insulin is a hormone responsible for controlling the blood glucose level, and its deficiency results in a metabolic disorder known as "diabetes" (Hoseini et al., 2009). There are two types of diabetes: (a) type 1 diabetes (T1D), which results from autoimmune destruction of pancreatic β-cells (insulin-producing cells) with subsequent absolute insulin deficiency (Atkinson et al., 2011) and (b) type 2 diabetes (T2D), which is characterized by insulin resistance as well as pancreatic β-cell dysfunction associated with relative insulin deficiency (Robertson, 2006). Apart from the main clinical signs such as insulin resistance (IR) and β-cell dysfunction, other pathological features, including enhanced oxidative stress and subclinical inflammation,

play a major role in the development of T2D and advancement of vascular complications (Bahadoran et al., 2013).

Previous reports have indicated the potential usage of *N. officinale* extracts (consisting of the major GSL bioactive compound "gluconasturtin") in treating diabetes (Engelen-Eigles et al., 2006). Hoseini et al. (2009) studied the effects of different types of *N. officinale* extracts (ethyl acetate, methanol, and aqueous) on in vitro streptozotocin induced diabetes in rats, and found that the blood glucose level was effectively decreased in diabetic rats treated with methanol and ethyl acetate extracts of *N. officinale*. As a potent activator of the NRF-2 antioxidant systems, SF restricts oxidative stress induced under hyperglycemic conditions and inhibits the major inflammatory inducer NF-κB. In addition, SF also stimulates some of the peroxisome proliferator-activated receptors (PPAR) that mediate lipid metabolism and glucose homeostasis. The following biochemical changes were observed in T2D patients administered with broccoli sprouts containing high levels of SF: enhanced antioxidant level of plasma and reduced oxidative stress, lipid peroxidation, serum triglycerides, oxidized low-density lipoprotein (LDL)/LDL-cholesterol ratio, serum insulin, insulin resistance, and serum high-sensitive C-reactive protein. Moreover, nephropathy, diabetes-induced fibrosis, and vascular complications were noted to be effectively controlled by SF (Bahadoran et al., 2013). Fu et al. (2013) demonstrated that the induction of antioxidant level by exogenous SF treatment enhanced the cytoprotective effects and diminished reactive oxygen species (ROS) production and glucose-stimulated insulin secretion (GSIS) in β-cells. Furthermore, they observed that treatment of INS-1 (832/13) cell lines with SF at non-cytotoxic concentrations (2–10 μM) for 24 h enhanced protection against oxidative stress through induction of NRF-2-mediated antioxidant response and decreased

insulin secretion induced by 20 mM glucose. A recent study demonstrated that intake of high amount of broccoli sprouts (BSP) (10 g/ d) with high antioxidant potential for 4 weeks reduced the serum insulin level and homeostasis model assessment-estimated insulin resistance (HOMA-IR), leading to protection against IR-mediated metabolic and cardiovascular diseases in T2D patients (Bahadoran et al., 2012). All these above-mentioned studies imply the protective role of GSL HPs against diabetes and the health risks associated with it. Taken together, GSL HPs could be an alternative choice to synthetic drugs for treating diabetes-related health issues in humans, particularly, through the induction of antioxidant response.

Regulation of growth factor and other hormone signaling by GSL HPs

Few studies have reported the role of GSL HPs in the regulation of growth factor and other hormone signaling related diseases in humans. GSL HPs can alter the growth factor and cytokine receptor signaling by suppressing or inducing the expression and activity of the receptor triggered signal transduction components, or by selectively modulating the receptors and their respective ligand expressions (Firestone and Sundar, 2009). The transcription factor NF-κB (regulator of several pro-inflammatory cytokines) has been found to enhance the cell growth through the induction of cyclin-producing genes, inhibit apoptosis by stimulating anti-apoptotic factors, facilitate metastasis, and favor angiogenesis (reviewed in the report by Brown and Hampton, 2011). It has been reported that the indole GSL derivative, I3C, inhibits angiogenesis through the downregulation of vascular endothelial growth factor (VEGF) by inhibiting NF-κB activity, while DIM decreases VEGF receptor signaling by effective downregulation of extracellular signal regulated protein kinases 1 and 2 (ERK1/2) activity (Firestone and Sundar, 2009).

The aromatic ITC (PEITC) has been shown to inhibit the expression of heat inducible factor-1α (HIF-1α) and VEGF secretion in various cancer cell lines. Particularly, PEITC suppresses angiogenesis via the downregulation of phosphorylated Akt and ERK, HIF-1α, and VEGF. Furthermore, the synergic activity of PEITC with LY294002, an Akt inhibitor, or with PD98059, an inhibitor of ERK, has been noted to result in enhanced level of suppression of phosphorylated proteins (Gupta et al., 2013). Similarly, PEITC treatment has been observed to show a concentration-dependent inhibition of epidermal growth factor (EGF)-induced metastasis in human oral squamous carcinoma cells (Chen et al., 2013). In addition, PEITC has also been demonstrated to induce apoptosis in human epidermal growth factor receptor-2 (HER2) expressing tumor cells both in vitro and in vivo, and potentiate doxorubicin efficiency (Gupta et al., 2012).

Lee et al. (2009) reported that BITC treatment at different concentrations decreased the LPS-stimulated secretion of IL-1β, TNF-α, IL-6, and their respective mRNA levels as well as the synthesis of nitric oxide (NO) and prostaglandin E2 (PGE2) in a dose-dependent manner in lipopolysaccharide (LPS)-induced macrophages. Furthermore, BITC has been noted to exert anti-inflammatory effects through the suppression of Akt and ERK, and the consequent downregulation of NF-κB signaling. Similarly, PEITC has also been found to inhibit LPS-stimulated NO production through the inhibition of Akt activation and lead to reduced expression of IFN-γ. Moreover, PEITC has been observed to act as a potent inhibitor of the Janus-activated kinase-2 (JAK2)/STAT1 signaling pathway (Okubo et al., 2010). On the other hand, it has been reported that the treatment of SF reduces the constitutive (DU145 cells) and IL-6-stimulated (DU145 and LNCaP cells) phosphorylation of oncogenic transcription factor STAT3 (Tyr^{705}) as well as its upstream regulator JAK2 ($Tyr^{1007/1008}$). Moreover, SF administration has also been evidenced to reduce the expressions

of STAT3-regulated genes (Bcl-2, cyclin D1, and survivin) (Hahm and Singh, 2010). In a previous study, the dietary supplementation of PEITC in LNCaP human prostate cancer cell xenograft model was shown to diminish the expression of platelet endothelial cell adhesion molecule (PECAM-1) and reduce the tumor cell growth (Hudson et al., 2012).

In human breast cancer cells, I3C has been observed to induce the expression and promote the activity of IFN-γ receptor-1, leading to the prevention of cancer (Chatterji et al., 2004). In addition, DIM has also been noted to induce the IFN-γ receptor-1 in human breast cancer cells through the activation of c-Jun N-terminal kinase (JNK) and p38/MAPK pathways (Xue et al., 2005). The immunoregulatory and pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) is known to be involved in various pathogenic diseases (rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis) and different aspects of cancerous growth, including cell proliferation and angiogenesis. ITCs inhibit the tautomerase activity of MIF catalyzed by the N-terminal proline, and among the ITCs, BITC has been observed to be the most effective inhibitor of MIF (reviewed in the report by Brown and Hampton, 2011). AITC and PEITC have been found to induce the expression of IL-2 and tissue inhibitor of metalloproteinase-1 (TIMP-1), and have been demonstrated to be equally effective in the suppression of VEGF, proinflammatory cytokines (IL-1ß, IL-6, GCSF), and TNF-α (Thejass and Kuttan, 2007).

Prostaglandins are hormone-like substances, among which PGE2 is predominantly present in humans and is involved in several physiological and pathological processes. In addition, it also promotes cancer progression (Zhou et al., 2012). One of the well-known ITC, SF, has been noted to inhibit the biosynthesis of PGE2 through the suppression of PGE2 synthase-1 (mPGES-1) transcription. HIF-1α, present at the mPGES-1 promoter, has been found

to be suppressed by SF, resulting in decreased mPGES-1 transcription and leading to suppression of PGE2 (Zhou et al., 2012). The GSL HPs have also been observed to be involved in protection against thyroid cancer. Rajoria et al. (2011) recently reported that DIM effectively targets estradiol-stimulated thyroid cancer cell proliferation and metastasis process (adhesion, migration, and invasion) by targeting the metastatic modulators such as matrix MMP-2/MMP-9. Thus, all these reports suggest the potential role of GSL HPs in the regulation of various growth factors and other hormone receptor signaling pathways associated with various diseases.

CHEMOPREVENTIVE MECHANISM OF ACTION OF GSL HPs

In the presence of myrosinases or thioglucosidases, GSLs are metabolized into various compounds such as isothiocyanates, nitrites, thiocyanates, epithionitriles, and oxazolidines, depending on the conditions of pH, metal ions, and other proteinic elements (Vaughn and Berhow, 2005). In general, GSL HPs are biologically active (Gimsing and Kirkegaard, 2006), and their antimicrobial activity is influenced by the important chemical groups (ITC, cyanate, and thiocyanate) present in them. Kurepina et al. (2013) suggested that the ITC derivatives are more effective antimicrobials than cyanate or thiocyanate derivatives, which are ineffective. Some previous studies have reported that the aromatic ITCs are more toxic than aliphatic ITCs (Aires et al., 2009a). For instance, the presence of an aromatic ring in the phenethyl and benzyl ITCs have been noted to show strong binding and modification of α-tubulin, when compared with the presence of aliphatic side chain containing the ITC, sulforaphane (Mi et al., 2009a). The ITC groups of GSL HPs can covalently cross-link to the cellular targets, and this cross-linking efficiency can be further enhanced by the adjacent groups found close to the ITC moiety (Tajima et al., 2003). Hence, ITCs with adjacent small chemical groups show less activity than those with

larger chemical groups that display potent activity. In addition to the main ITC group, the variations in their side chain (R groups of ITC) play a major role and significantly affect the chemopreventive activity by altering the electrophilicity of –NCS groups, accessibility to nucleophilic centers, and lipophilicity of ITCs (Zhang, 2012), thus affecting the transporting ability of ITCs across the membranes.

Different modes of action have been proposed for the effects of GSL HPs against infectious pathogens, such as disruption of cell membrane, essential enzymes, and ATP level. It has been reported that the chemopreventive activity of ITCs is achieved through the formation of thiocarbamate adducts by reaction with the thiol groups of the target key proteins/enzymes, whereas the reaction with the amino groups gives rise to thiourea derivatives (Juge et al., 2007). Although the central electrophilic carbon of ITCs (R\NCS) reacts with amine and thiol groups, whose reactivity with the thiol groups is more rapid (1000 times faster) than that with the amino groups, it has been indicated that proteins with the critical cysteine residues are the most sensitive targets for modification (Drobnica et al., 1977). Furthermore, the major mechanism of antimicrobial activity of ITCs (changes in the membrane potential or inactivation of cellular proteins and generation of ROS and other free radicals via the suppression of important thiols such as GSH and L-Cys) has been noted to depend on these two above-mentioned reactions (Aires et al., 2009b). It has been reported that the changes in the cellular redox level cause inactivation of intracellular enzymes by oxidative cleavage of disulfide bonds (Zsolnai, 1966). Moreover, the cytochrome P450 enzymes have been evidenced to readily interact with the ITCs and produce highly potent reactive molecules (Davidson and Botting, 1997), as well as cause membrane leakage and enzyme inhibition (Luciano et al., 2008). Tang et al. (1974) reported that

the ITCs can bind to the sulfhydryl (SH) groups present in the active sites of essential enzymes in the microorganisms to control their growth. Moreover, previous studies have demonstrated that the *E. coli* enzymes, such as thioredoxin reductase and acetate kinase, are strongly inhibited by AITC (Luciano and Holley, 2009). Tajima et al. (1998) observed that the cytolytic toxin streptolysin S, produced by *Streptococci,* was decreased to 50% in the presence of 10 μg/ml of the 2-(4-hydroxyphenyl) ethyl ITC, when compared with the control. Furthermore, the ATP concentrations were decreased to a great extent in *E. coli* treated with 2-(4-hydroxyphenyl) ethyl ITC. However, addition of cysteine (externally added thiol derivatives) reversed the growth inhibition, revealing that sulfhydryl groups are the ideal targets of ITCs. Similarly, mustard oil (a major source of AITC) decreased the intracellular ATP level and pH, enhanced the extracellular ATP concentrations in *E. coli* and *S. typhi,* and affected the bacterial membrane potential (Turgis et al., 2009). Lin et al. (2000) investigated the antibacterial mechanism of AITC, and their results revealed that the mode of action of AITC was similar to that of the antibiotic polymyxin B, with both the compounds displaying antibacterial activity through their effects on the cell membranes. Moreover, it has been reported that the ITCs directly bind to and modify the critical cysteine moieties in α-tubulin, and also disrupt the polymerization of dimers into microtubules, consequently leading to the arrest of cell growth (Mi et al., 2009b). In addition, the ITCs has been observed to bind to multiple cysteine residues in the membrane receptors (Toll-like receptors, TLR), inhibiting NF-κb activation in mouse macrophages (Youn et al., 2010). Similarly, PEITC has been indicated to bind to the N-terminal proline residue of the MIF and produce anti-inflammatory effects (Brown et al., 2009). The schematic representation of the chemoprotective mechanistic actions of the ITCs is shown in Figure 5. Several methods have

been proposed for the mode of action of GSL HPs against the control of microbial pathogens and other diseases. However, detailed molecular studies should be carried out to explore the mechanism of action of GSL HPs, and their target identification could be useful in developing therapeutic drugs.

STRATEGIES TO IMPROVE THE EFFICIENCY OF GSL HPs

The use of phytochemicals in food preservation depends on the stability and bioavailability of the chemopreventive plant compounds. Most of the bioactive phytochemicals are less soluble in nature and display less bioavailability. Moreover, some phytochemicals exhibit strong irritable odor, high volatility, pungency, and poor water solubility. Although reactions with the nucleophiles of food makes phytochemicals unsuitable for direct usage in food preservation, encapsulating them in a matrix using nanoencapsulation increases their stability and bioactivity. Synthesis of nano-size particles at a nano size range (10–1000 nm) and large surface-to-volume ratios are the major objectives of nanotechnology (Santos et al., 2013), and nanoencapsulation is useful for targeting some substances and enhancing their activity as well as bioavailability (Nair et al., 2010). In addition, encapsulation also avoids the interaction of the substance with food components. As high volatility and irritant odor limit the use of AITC in food preservation, in a previous study, microcapsules of AITC were prepared from the gum acacia and tested for antibacterial efficacy against *E. coli* O157:H7 in chopped beef. The results revealed that the AITC microcapsules exhibited bacteriostatic as well as bactericidal properties against *E. coli* O157:H7 in chopped beef in a concentration-dependent manner (Chacon et al., 2006). Furthermore, Piercey et al. (2012) demonstrated that the AITC entrapped in ß-cyclodextrin inclusion complexes was more effective than the unentrapped AITC in the

prevention of microbial growth (*E. coli, L. monocytogenes,* and *P. expansum*) owing to its slow release and sustained activity. Similarly, Seo et al. (2012) developed an antimicrobial packaging system composed of synthetic calcium alginate encapsulated AITC beads for spinach leaves, and found that the AITC beads effectively inhibited the growth of *E. coli* O157:H7, molds, and yeasts. Thus, these alternative approaches avoid the demerits associated with AITC and favor its use in food preservation industries.

Chemotherapy is one of the major approaches in cancer treatment, and has several drawbacks, including limited intratumoral drug disposition, which make this approach less effective. Nanoparticles such as lipid nanoparticles (LNs, i.e., solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been used to achieve improved drug delivery to tumor tissues and also to minimize the side effects. In a previous study, the anticarcinogenic phytochemical PEITC and efflux transporter inhibitors such as tamoxifen, verapamil HCl, or nifedipine were encapsulated in the chitosan SLN microparticle (CSM), and it was found that the PEITC encapsulated in CSM increased the cytotoxic expression of Calu-3 cells in the presence of efflux transporter inhibitors owing to higher accumulation of PEITC (Dharmala et al., 2008). Furthermore, Do et al. (2010) developed SF encapsulated in albumin microspheres and observed that the encapsulated SF exhibited superior chemotherapeutic activity via sustained release as well as enhanced uptake by macrophages. Later, Qhattal et al. (2011) prepared nanoemulsions of BITC and examined its anticancer activity in human cancer cells in vitro (A549 and SKOV-3). The BITC nanoemulsions exhibited increased solubility and dissolution rate, when compared with pure BITC, and enhanced the apical to basolateral transport of BITC in Caco-2 cell monolayers. In addition, the permeability values were higher for the nanoemulsified BITC, when

compared with those for pure BITC. Thus, it can be concluded that nanoencapsulation of bioactive compounds is an ideal method to enhance the physical stability of the bioactive molecules, protecting them from interactions with food ingredients, and their subcellular size makes this approach useful for increasing the bioactivity of these compounds.

GENETIC ASPECTS OF IMPROVED GSL PROFILES

The field of plant biology aims to improve the nutritional and medicinal values of plants through conventional breeding and modern genetic engineering approaches. Several studies have demonstrated the role of phytochemicals in the treatment of various human diseases through normal diet (Wang et al., 2011; Mann and Khanna, 2013). Among the phytochemicals, GSLs have gained more attention owing to the increased accumulation of data regarding their potential applications for plant and human welfare (Baskar et al., 2012).

Genetic engineering of GSLs in various plants by modulating the expression of structural and regulatory genes of GSL biosynthesis has already been reported (Baskar et al., 2012). Three commercial F1 broccoli hybrids producing high level of glucoraphanin were obtained through independent breeding programs via crossing of the wild species *Brassica villosa* and a common Broccoli (*Brassica oleracea*) for several times. These hybrids accumulated 2.5–3-fold higher level of glucoraphanin (Traka et al., 2013). Recently, Liu et al. (2012) developed *Brassica napus* transgenic plants, which could accumulate higher glucoraphanin concentrations (42.6 μmol/g) in seeds, through the RNAi silencing of the GSL-ALK gene family. Moreover, the level of detrimental progoitrin (causes goiter in animals) was reduced to 65% in these transgenic plants. In addition, the content of progoitrin level was also reduced through conventional breeding (Liu et al., 2010). It has been previously reported that the AGSL and IGSL branches are positively

controlled by the R2R3MYB transcription factors. In *Arabidopsis*, MYB28, 29, and 76 were found to be involved in the regulation of AGSL pathway, whereas MYB51, 122, and 34 were responsible for the control of the IGSL branch (reviewed in the report by Gigolashvili et al., 2009). Thus, precise modification of these regulatory genes could provide suitable GSL chemotype. For instance, inactivation of MYB28 led to the absence of long-chain AGSL, whereas inactivation of MYB29 caused reduction in the short-chain AGSL (reviewed in the report by Gigolashvili et al., 2009). Recently, Augustine et al. (2013) developed a low GSL producing *Brassica juncea* through the silencing of *BjMYB28* gene, which accumulated 11.26 μmol/g DW GSL, when compared with the wild-type *B. juncea* seeds (80–120 μmol/g DW GSL). On the other hand, Araki et al. (2013) overexpressed the Kale (*B. oleracea* var. *acephala*) BoMYB29 gene in *Arabidopsis*, which led to higher accumulation of AGSLs as well as increased expressions of AGSL biosynthetic genes. Interestingly, the methylsulfinyl GSL content, including glucoraphanin level, was higher in these plants, suggesting the potential use of this gene to increase the chemopreventive methylsulfinyl GSL content. Apart from these regulatory genes, several studies have indicated the role of biosynthetic genes in controlling specific GSL chemotype (reviewed in the report by Baskar et al., 2012).

The chemopreventive GSLs have been predominantly found in the *Brassicaceae* family. However, some reports have stated the successful production of GSLs in heterologous hosts such as tobacco and yeasts. For instance, previous studies have demonstrated the metabolic engineering of phenylalanine-derived BGSLs, tryptophan-derived IGSLs, and methionine-derived AGSLs (glucoraphanin) in tobacco (Geu-Flores et al., 2009; Mikkelsen et al., 2010; Pfalz et al., 2011). Recently, Mikkelsen et al. (2012) showed the production of IGSLs in a

heterologous yeast host *Saccharomyces cerevisiae,* through the overexpression of *Arabidopsis* plant genes. These results imply that this approach could be promising to achieve large-scale production of specific bioactive GSLs to improve human health. Recently, Nour-Eldin et al. (2012) reported two nitrate/peptide transporter family genes in *Arabidopsis thaliana,* namely, glucosinolate transporter-1 (AtGTR1) and glucosinolate transporter-2 (AtGTR2), which were found to be responsible for the transport of GSLs from the source tissues such as roots and leaves to the sink tissues, namely, seeds. The double mutants (*gtr1/gtr2*) displayed absence of GSLs in the seeds, indicating that these transporters could be used to divide these bioactive compounds restricted to specific tissues, and also to effectively reduce the antinutritive values of GSLs through the elimination of their storage in edible parts. These promising results suggest that enrichment of specific GSLs with high medicinal value in common foods could be an effective way to combat various health issues through their daily intake.

CONCLUSION

Dietary intake of cruciferous vegetables, which are rich in GSL contents, is involved in protection against various types of cancer and other diseases, as it has been demonstrated in various in vitro and in vivo studies. The derivatives of GSL HPs are the biologically significant molecules that exert various functions through the alteration of different signaling pathways associated with several diseases. GSL HPs, especially the indole compounds (I3C and DIM), have been shown to inhibit endocrine-stimulated mitogenesis by changing the circulating levels of major steroids, such as androgens and estrogens, as well as by suppressing the receptor expression and/or regulating receptor activities and intracellular signaling components. Furthermore, GSL HPs have been observed to confer protection against various pathogenic

organisms such as bacteria, virus, and fungi. Interestingly, some reports have stated the potential role of these compounds in the eradication of biofilm and QS activity, which widens the scope of these molecules.

It has been found that GSL HPs such as ITCs achieve their biological effects through direct protein modification or, indirectly, by interrupting the redox homeostasis and enhancing thiol oxidation. Thus, the identification of the target proteins of ITCs could be useful for rational drug design as well as for exploiting the therapeutic potential of isothiocyanates (Brown and Hampton, 2011). Moreover, most of the studies on the antimicrobial effects of GSL HPs had been carried out on a few groups of microorganisms, which warrants further extensive antimicrobial screening studies covering large groups of harmful pathogens. In addition, the molecular targets of the GSL derivatives in various pathogenic diseases should be identified to increase their therapeutic potential. It is known that the bioavailability determines the performance of GSL HPs, and that most of the phytochemicals are slightly soluble and exhibit the least biological availability. Furthermore, the presence of less reactive moieties also contributes to the decreased activity of GSL HPs. Thus, development of new synthetic GSL derivatives with superior biological activity than the parent molecule, and use of nanotechnology to increase their bioavailability and target delivery could be helpful to improve their applications in clinical research.

Conflict of interest statement

The authors report no conflicts of interest.

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Legends to figures

Figure 1. General scheme of structural representation of glucosinolate hydrolysis products and their natural sources. Abbreviation: GSL glucosinolate.

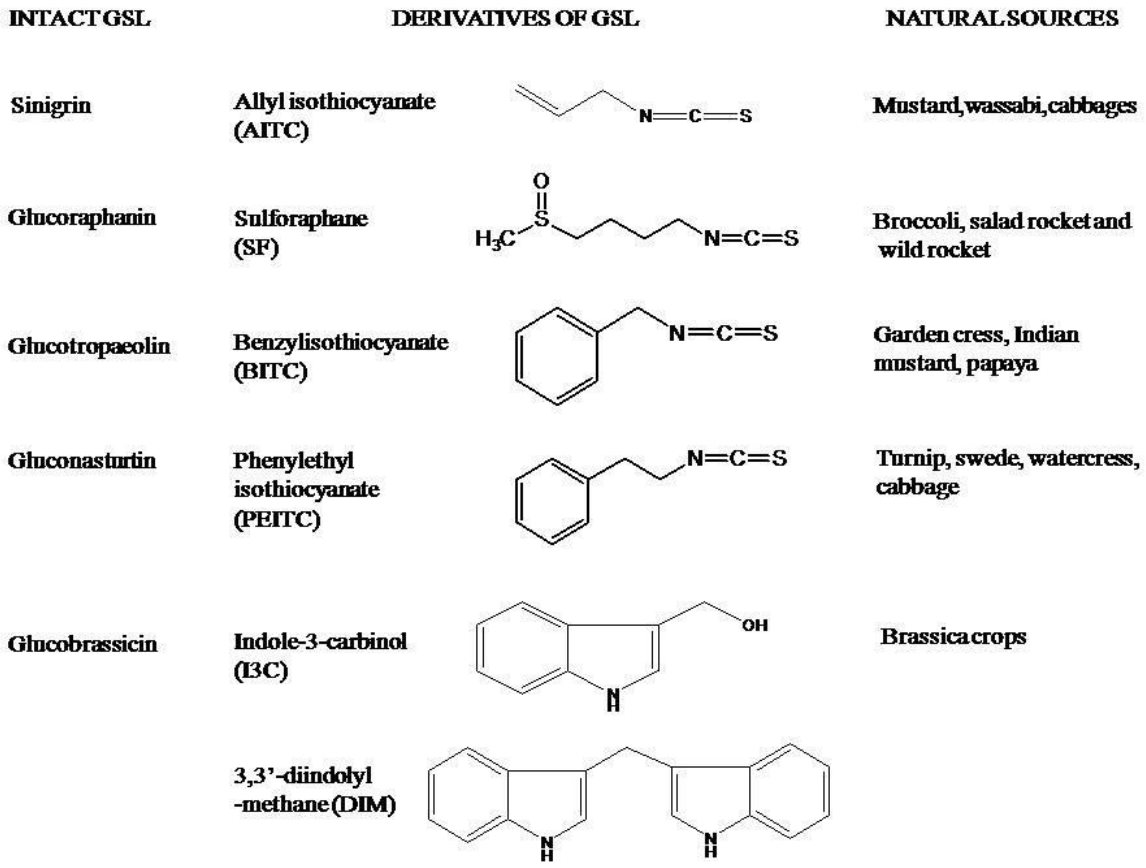

Figure 2. An overview of graphical representation of chemopreventive activities of GSL hydrolysis products. Abbreviation: GSL glucosinolate, GBC glucobrassicin, I3C Indole-3-carbinol, DIM 3,3'-diindolylmethane, ICZ indolo (3,2-b) carbazole, GRA glucoraphanin, SF sulforaphane, GNS gluconasturtin, PEITC phenylethyl isothiocyanate, SIN sinigrin, AITC allyl isothiocyanate, GTR glucotropaeolin, BITC benzyl isothiocyanate, *H. pylori Helicobacter pylori*, Bcl-2 B-cell lymphoma-2, Bcl-XL B-cell lymphoma extra large, Mcl-1 myeloid cell leukemia sequence-1, Bax BCL-2-associated X protein, XIAP X-linked inhibitor of apoptosis protein, IL-2 interleukin-2, TIMP-1 tissue inhibitors of metalloproteinase-1, CDK6 cyclin dependent kinase -6, VEGF vascular endothelial growth factor, VEGFR vascular endothelial growth factor receptor, ERK 1/2 extracellular signal-regulated kinases 1/2, HIF1α heat inducible factor-1-alpha, NF-κB nuclear factor-kappa-b, IL-1β interleukin-1-β, IL-6 interleukin-6, TNF-α tumour necrosis factor-alpha, GCSF granulocyte colony stimulating factor, AR androgen receptor, PSA prostate specific antigen, AP-1 activator protein-1, COX-2 cyclooxygenase-2.

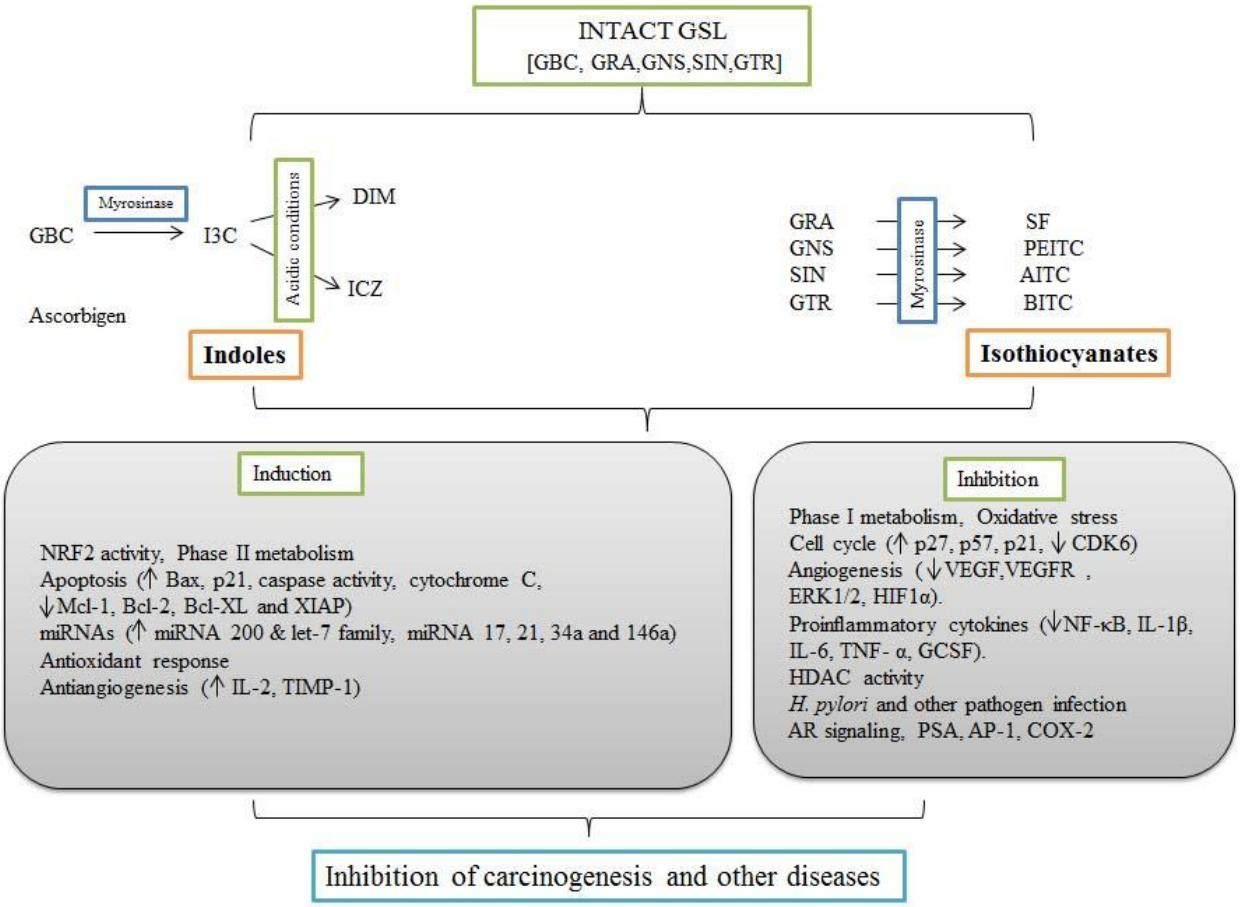

Figure 3. The diagrammatic view of sulforaphane mediated inhibition of quorum sensing activity in *Pseudomonas aeruginosa* through interfering Las system (Las-I/ Las-R). Autoinducer synthase (las-I) interact with the transcriptional activator (las-R) results in the production of a quorum sensing signaling molecule (3-oxo-C12-HSL). Sulforaphane has affinity towards las-R that leads to the disruption of las-I/ las-R interaction, which results in the inhibition of quorum sensing activity produced by *P. aeruginosa*. Abbreviation: *P. aeruginosa Pseudomonas aeruginosa*, SF sulforaphane, 3–oxo- C12-HSL N-3-oxo-dodecanoyl-L-Homoserine lactone**.**

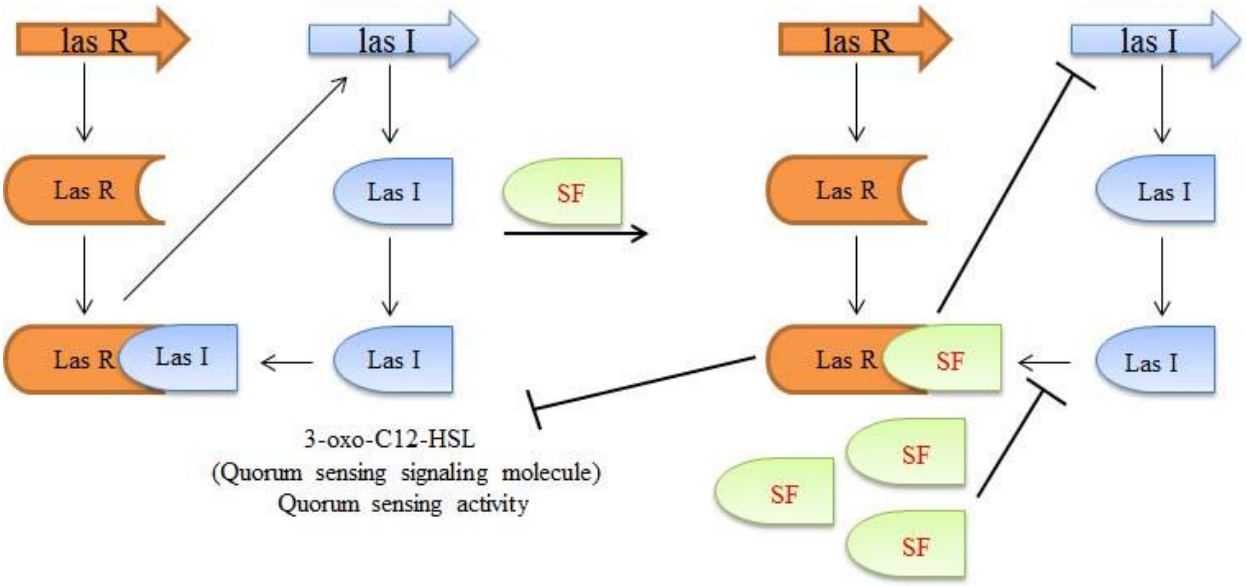

Figure 4. Graphical illustrations of the potential activities of DIM on the regulation of estrogen mediated carcinogenesis. Abbreviation: DIM 3, 3'-diindolylmethane, ER estrogen receptor.

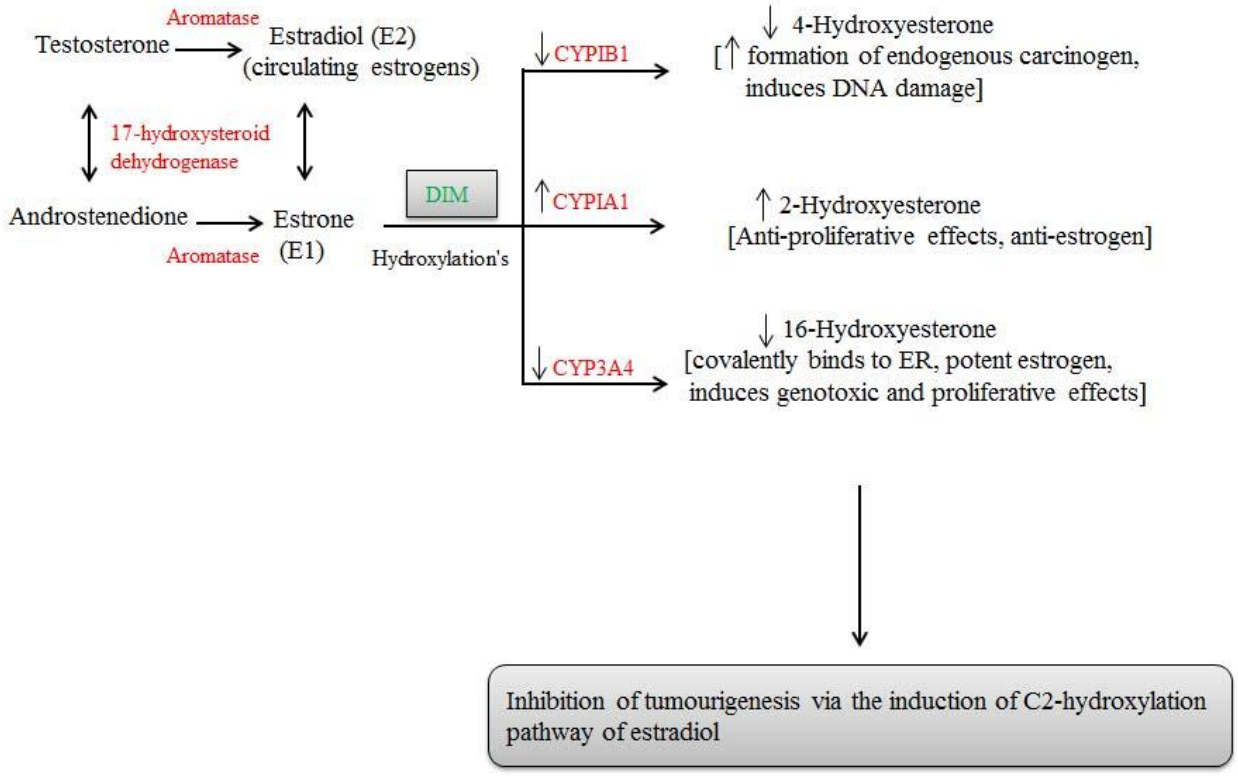

Figure 5. General schematic representation of the mechanism of action of isothiocyanates in chemoprevention against various diseases. Glucosinolate hydrolysis products (i.e., isothiocyanates) interact with the sulfhydral group to form dithiocarbamate which is 1000 times faster than the reaction with the amino group that forms thiourea. The interactions of isothiocyanates with sulfhydral or amino groups of biological molecules were responsible for the various chemopreventive activities. Abbreviation: GSL glucosinolate, ITC isothiocyanate, AP-1 activator protein-1, MEKK-1 mitogen-activated protein kinase kinase kinase-1, TLR-4 toll-like receptor -4.