Organosulfur Compounds in Cancer Chemoprevention

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Abstract: There has been a renewed interest to the application of natural products derived from cruciferous plants and members of *Allium* genus in chemoprevention of cancer. The potential chemopreventive properties of these vegetables have been attributed to the presence of high level of organosulfur compounds in these plants. Organosulfur compounds have been shown to exert diverse biological effects such as: (a) induction of carcinogen detoxification, (b) inhibition of tumor cell proliferation, (c) antimicrobial effect, (d) free radical scavenging, (e) inhibition of DNA adduct formation, (f) induction of cell cycle arrest and induction of apoptosis etc. It has been suggested that these compounds act as chemopreventive agents through a combination of above mechanisms. Epidemiological and experimental carcinogenesis provides overwhelming evidence to support the claim that individuals consuming diet rich in organosulfur are less susceptible to different types of cancers. The protective effects of OSCs against carcinogenesis have been shown in stomach, esophagus, mammary glands, breast, skin and lungs of experimental animals. Cumulatively all these studies show a strong correlation between cancer prevention and intake of organosulfur compounds in one form or the other. Since the protective effects of all these phytochemicals are as a result of additives and synergistic combination further studies are warranted for complete understanding of chemopreventive action of organosulfur compounds and define the effective dose that has no toxicity in humans. In this review an attempt has been made to summarize the different aspects of organosulfur compounds with relation to their source, chemopreventive mechanistic action, epidemiologic and experimental carcinogenesis.

Key Words: Cancer chemoprevention, organosulfur compounds, combined mechanism, phase II inhibitors, apoptosis, enhanced glutathione synthesis, cruciferous plants, *Allium* genus.

INTRODUCTION

Cancer has been one of the leading causes of death in the entire world. Cancer chemoprevention coined by Sporn [1,2] and coworkers in 1976 has been defined as a strategy for reducing cancer mortality and involves the prevention, delay and reversal of cancer by ingestion of dietary or pharmaceutical agents capable of modulating the process of carcinogenesis [3]. Avoidance of all the cancer causing factors is probably the best way to reduce cancer related mortality. Cancer chemoprevention is a step in this direction with objective of preventing cancer by a combination of various mechanism like inhibition of Phase I transformation enzymes, modulation of Phase II enzymes, induction of cell cycle arrest, induction of apoptosis, antimicrobial activities, inhibition of DNA adduct formation and immunomodulation.

In the recent years cancer prevention by natural products has received considerable attention. A lot of emphasis has been laid on the dietary intake of fresh fruits and vegetables, which have shown to reduce the risk of developing cancer. Literature survey reveals that there are hundreds of natural and synthetic compounds, which have been shown to inhibit the process of cancer carcinogenesis. However there have been a very few compounds which have been thoroughly evaluated. In particular last decade has witnessed a significant leap forward in our ability to study and understand the role of organosulfur compounds in cancer chemoprevention. There is overwhelming epidemiological and experimental evidence that dietary factor rich in organosulfur compounds play an important role in cancer chemoprevention [4-6]. Currently, there is no widely accepted mechanism for classification of cancer chemopreventive agents. On the basis of time period for the activity, Wattenberg classified chemopreventive agent into blocking agents (which prevent carcinogenic agents from reacting with critical site) and suppressing agents (which prevent evolution of neoplastic process in cell that otherwise could become malignant) [7]. In another classification by Boon and Kelloff, the chemopreventive agents can be divided into three classes: (a) antimutagenic (this acts at the activation and DNA adduction of mutagen. (b) antimitogenic reagents (acting at the stimulation of the proliferation signal pathway by mitogens) and (c) antioxidants [8]. On the basis of antiproliferative property, cancer chemopreventive agents also be further differentiated into different groups *in vivo* and *in vitro*. It is hard to assign a particular organosulfur compound acting as chemopreventive agent to a particular class because all these agents are believed to act through combination of two or more mechanisms.

The group of food plant particularly rich in organosulfurs are members of *Allium* genus *i.e*. Onion, garlic, leeks and chives and genus *Brassica* i.e. cauliflower, cabbage, Brussels sprouts and Broccoli. It has been reported that several naturally occurring and related synthetic OSCs exert chemopreventive effects in several target organs in rodent models. There are several studies showing protective effects of these compounds against stomach, colorectal cancers including the breast [9-12]. All this point to overall evidence that OSCs possess promising anti-cancer activity.

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Table 1. Natural Sources of Organosulfur Compounds

S. No.	Type of Compounds	Compounds	Natural Source
1	Sulfides	Dithiolethiones Allyl sulfides	Broccoli, garlic, onion
2	Isothiocyanates	Sulphoraphanes Phenylethyl isothiocyanates	Cauliflower, cabbage, kale, bok choy, brussels sprouts, radish, mustard, water gardencress
3	Glucosinolates	Glucobrassinin	Mustard, turnip, collard greens, wasabi kohlrabi, watercress cauliflower, cabbage, Kale, bok choy, brussels sprouts, radish mustard, arugula, radish, horse radish

In this review different natural and synthetic OSCs have been described along with their source, structure and experimental carcinogenesis studies. The epidemiologic studies, experimental carcinogenesis and cancer chemopreventive mechanistic aspect of OSCs have been discussed in addition to contribution of our laboratory in this area.

Sources of Active Organosulfur Compounds

Sulfur containing compounds are present in all *Cruciferae* family vegetables and plants belonging to *Allium* family (Table **1**).

Cruciferous vegetables, such as broccoli, cabbage and kale are rich sources of sulfur containing compounds called glucosinolates. The chemopreventive properties of cruciferous vegetables are believed to originate from breakdown products of glucosinolates. Glucosinolates are hydrolyzed by thioglucosidase (myrosinase) enzymes to yield an aglycone which undergoes non-enzymatic rearrangements to produce organic isothiocyanates, thiocyanates, nitriles and other products (Fig. **1**).

Although non-enzymatic thermal degradation of glucosinolates may produce chemical species identical to products of the enzymatic hydrolysis [13-14] the chemical structures of the hydrolysis products (breakdown products) of glucosinolates vary depending on the precursors in different plant species, and different endogenous or exogenous factors such as pH and presence of ferrous ions [14-18]. Glucosinolates and their breakdown products have been the focus of many studies because of the possibility of using them as natural pesticides [19]. However, it is considered that glucosinolates themselves possess limited biological activity until they are hydrolyzed.

Different cruciferous vegetables contain variety of glucosinolates each of which give rise to different kind of isothiocyanates. Glucoraphanin derived from broccoli gives rise to sulforaphane while allylisothiocyanate (AITC) is obtained from precursor sinigrin [18-19].

Benzylisothiocyanate (BITC) is derived from glucotropaeolin which in turn is present in garden cress. Watercress is an important source of gluconasturtiin, which yields

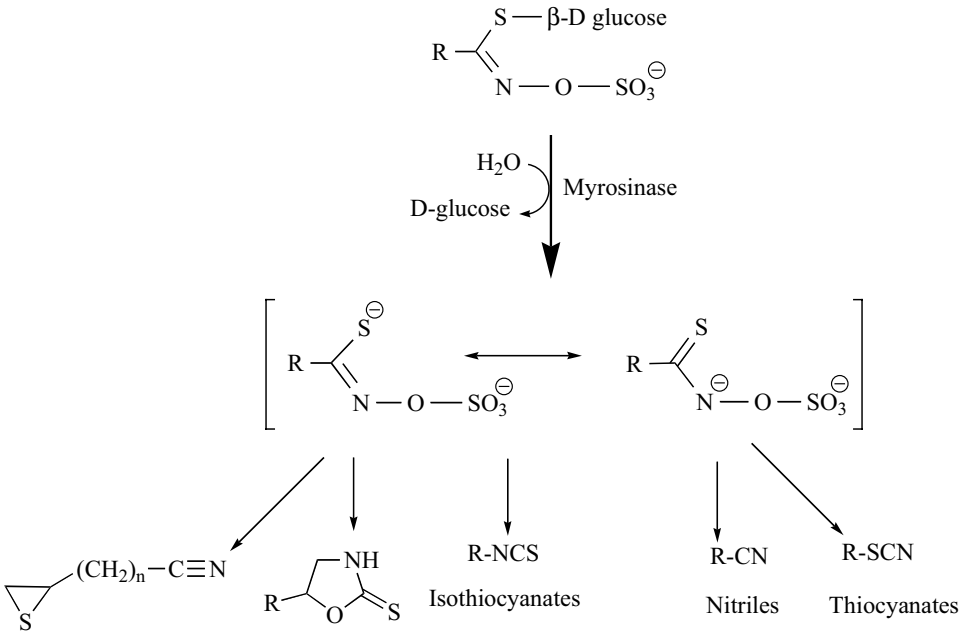


Fig. (1).

phenyl- ethylisothiocyanate (PEITC) [20]. Various compounds (**1-7**) derived from cruciferous are listed below (Fig. **2**).

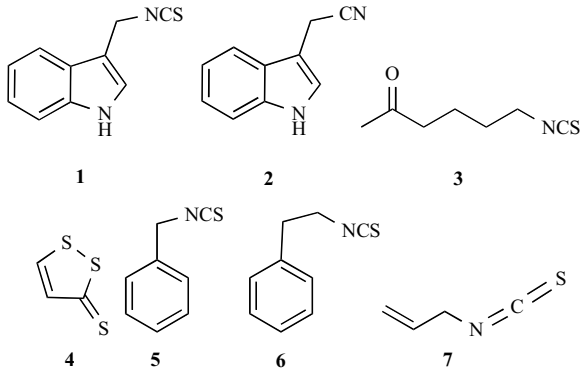


Fig. (2).

In addition to this there is a series of phytolaexins (defense compounds against pathogens having antimicrobial com-pounds). These phytoalexins (**8,9,10**) have a characteristic dithiocarbamate group attached to 3-methyl indolyl moiety [21] (Fig. **3**).

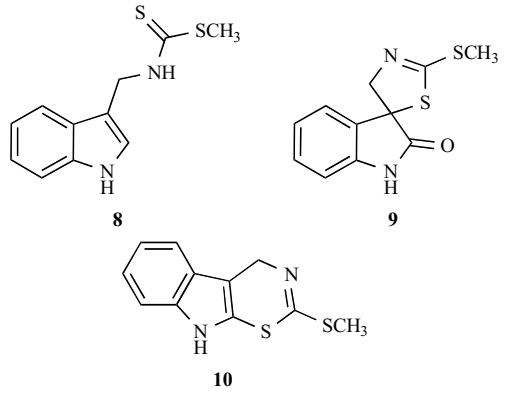


Fig. (3).

Besides crucifers members of genus *Allium* i.e. garlic and onion are rich in OSCs. Garlic upon being chopped or crushed affords an odourless compound called alliin (s-allylcysteine sulfoxide). Alliin accounts for about 80% of the cysteine sulfoxides in garlic [22]. This alliin is acted upon by the enzyme allinase to yield allicin (allyl 2-propenyl thiosulfinate oxide). Since allicin is not chemically stable, its breakdown occurs into various mono-, di-, and trisulfide thioethers [22]. OSCs present in garlic including allyl sulfide, allyl disulfide and allyl methyl, di-, tri-sulfides along with its oil have been found to inhibit carcinogenesis at several organs. The formation of thiosulfinates is very rapid and has been found to complete in 60 seconds. Allicin breaks down into a number of fat soluble OSCs which have been shown in (Fig. **4**).

Water-soluble compounds (Fig. **5**) such as S-allylcysteine are derived during long term incubation of crushed garlic in aqueous solutions.

Onion mainly contains S-propenyl cysteine sulfoxide and S-methyl cystein sulfoxide. The lachrymatory factor of the onion is due to propane thiol-S-oxide. Allyl mercaptan and allyl mercapto cysteine are produced by incubation of cysteine with allyl disulfide and diallylsulfide group [23]. Further transformation of OSCs can occur after interaction with sulfhydrl group including those present in cysteine, glutathione or proteins.

MECHANISMS FOR CANCER CHEMOPREVENTION

Studies have indicated that protecting action of any chemopreventive agents may not be attributed to a single mechanism similarly the chemoprevention activity of OSCs may be explained on the basis of various mechanism as shown in the (Fig. **6**). Application of OSCs to human is advantageous since most of the OSCs are derived from plants and have combined mild effect. The action of organosulfur compounds may be explained on the basis of following probable mechanisms.

Antimicrobial Activity

Bacterial infections markedly increase incidence of cancer at the infection site in humans especially the infection with some strains of *H. pylori* markedly increase risk of gastric cancer. Garlic extract highly rich in OSCs has been found to possess promising antibacterial and anti fungal properties. Thiosulfinates particularly allicin play an important role in the antimicrobial activity [24]. Allicin derived compounds like DATS have been found to have antimicrobial properties against *H. pylori.*

Various garlic presentations along with OSCs have shown to inhibit the growth of these bacteria in laboratory [24,25]. Purified sulforaphane inhibited the growth and killed multiple strains of *H.pylori* infection in tissue culture. These infections are known to cause gastritis and peptic ulcers, and dramatically enhance the risk of gastric cancer. Eradication of this organism is an important medical goal that is complicated by the development of resistance to conventional antimicrobial agents and by the persistence of a low level reservoir of *H. pylori* within gastric epithelial cells. Sulforaphane [1-isothiocyanato-(4*R*)-(methylsulfinyl)butane], an isothiocyanate abundant as its glucosinolate precursor in certain varieties of broccoli and broccoli sprouts, is a potent bacteriostatic agent against three reference strains and 45 clinical isolates of *H. pylori* [25].

Free Radical Scavenging

It has been observed that DAS, DADS, onion oil and garlic oil increase the activity of glutathione reductase and superoxide dismutase [26-29]. Both enzymes are involved in the natural protection by free radicals [29]. Aged garlic extract, SAC, SAMC exhibit radical scavenging activity. DAS and DADS have shown selective action on different markers in tests for their ability to react with carbon tetrachloride free radicals [30]. DADS has been found to inhibit CCl_4 induced lipid per oxidation.

Inhibition of DNA Adduct Formation

Formation of DNA adduct is one of the main reason for causing cancer. It has been found that garlic extract decrease

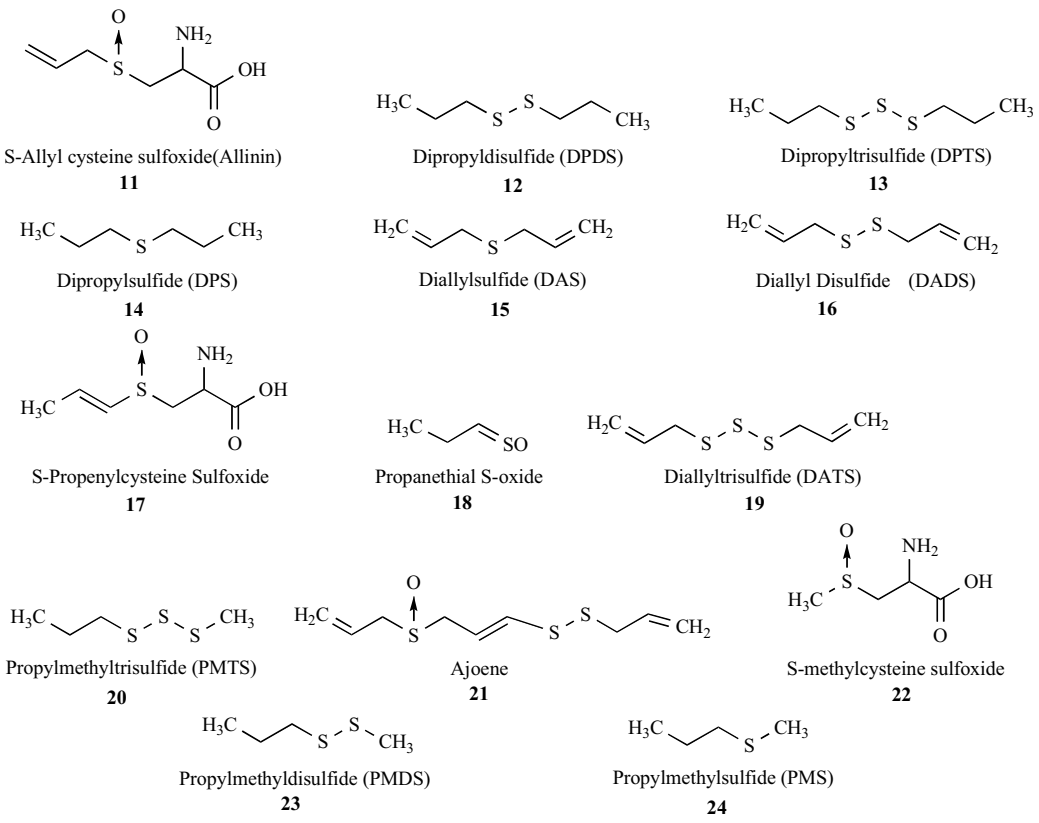


Fig. (4).

the occurrence 7,12-dimethyl benz(a)anthracene (DMBA), DMBA-DNA adducts *in vivo* [31]. SAC was also found to be effective against mammary DMBA-DNA binding [32]. Moroever, DAS and DADS have activity against DNA adducts as a result of incubation of human bladder tumor cells with 2-aminofluorene [33]. In an another study it was shown that onion and garlic compound rich in organosulfur inhibits the notrosation reaction *in vitro* [34]. Harmful effect of aminopyrine and sodium nitrite in diet was minimized in rat liver when garlic powder was added to it [35].

Inhibition of Polyamine Metabolism

The polyamines, putrescence, spermidine and spermine are small polycations. They are known to form covalent complexes with many organic molecules *in vitro* and could thus potentially influence many processes involving DNA and RNA [36]. It has long been implicated that polyamines are involved in the regulation of cell growth and differentiation but their precise role is not clear. It has been well established that one of the earliest events during the transition of many cell types from dormancy to proliferation is an increase in conversion of ornithine to putrescine catalysed by ornithine decarboxylase (ODC), the first and often the rate determining step in the polyamine synthesis [37]. Inhibition of ornithine decarboxylase has been proposed for the anticarcinogenic effect of garlic related compounds such as 1-propenyl sulfide, ajoene and garlic oil [38]. The observations suggest that the inhibition of ODC and suppression of ploymine levels may be rational approach to cancer chemoprevention.

Immunomodulatory Effect

Basically cancer is a result of failure of human immune surveillance and inability of immuno-effective cells (Natural killer cells, lymphokine activated killer cells, macrophages

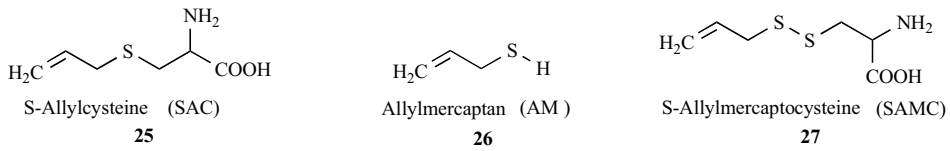


Fig. (5).

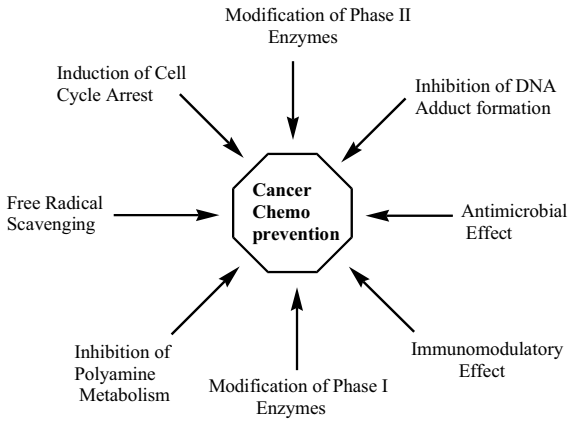


Fig. (6).

etc). A more recent approach has been use of agents, which can be useful in restraining abnormal cell proliferation. Immunomodulatory role of organosulfur compounds has been investigated through a series of studies. Effect of garlic in prevention of oral pre-cancer in Wister rats induced by 4-nitroquinoline-1-oxide by activation of natural killer cells (NK) and T lymphocytes has been reported by Tang *et al*. [39]. DAS was found to be protective against N-nitrosodimethylamine (NDMA) induced immunosupression of humoral and cellular responses in BALB/C mices [40].

A unique garlic preparation, called aged garlic extract (AGE) 3 has been reported to have an array of pharmacological effects, including immunomodulation [41-43]. AGE (garlic cloves (*Allium sativum*) sliced and soaked in a water/ethanol mixture and naturally extracted/aged for at room temperature) used for the studies contained 15% solid materials and 0.1% (calculated on the dried basis) S-allylcysteine, a marker compound for standardization.

Induction of Apoptosis

Human body has a tendency to get rid of the genetically damaged cell as a part of its self- protecting mechanism against cancer. This physiological process called apoptosis is a self- protection mechanism against cancer. Pre-cancerous cells and cancerous cells are resistant to the signals that induce apoptosis [44]. Several compounds such as DAS, DADS, and SMC have apoptosis induction capabilities in various cancer lines cell culture [45]. Oral administration of aqueous garlic extract s-cysteine has been reported to induce apoptosis in various animal models [44, 46].

Induction of Cell Cycle Arrest

Cancer is uncontrolled cellular growth. In normal cells DNA replication follows proper separation of chromosomes followed by cellular division. However, in case of damage to DNA, cell cycle is arrested in order to allow time for DNA repair. Several organosulfur compounds such as DADS, DATS and SAMC have been found to induce cell arrest in cell culture cells [47].

Xenobiotic Mechanism

The human body's first line of defense against cancer involving organosulfur compounds are the Phase I and Phase II enzymes [48-54]. These two families of enzyme help body protect itself from all types of carcinogens that routinely enter the human body through the diet and the environment.

Xenobiotic mechanism may be classically divided into two categories Phase I and Phase II. Over all the most important mechanism of chemoprevention by organosulfur compounds appears to be the induction of phase II enzymes and inhibition of Phase I enzymes. Cancer chemopreventive action is the result of balance between activating and detoxifying reactions of Phase I and Phase II enzymes respectively [49,50,51]. The enzymes most frequently involved in the Phase I reactions are P-450 products of CYP family.

A key component in the understanding the initial events of carcinogenesis is the recognizition of the fact that many of the carcinogens are not chemically reactive per se but undergo metabolic activation to form electrophilic reactants [52]. These reactive species can interact with nucleophilic group in DNA to induce point mutation or other genetic lesions thus leading to the activation of proto-oncogenes and inactivation of their tumour repressor genes. The metabolism of the chemicals to proximate carcinogens usually involves a two electron oxidation and is typically catalysed by cytochrome P-450 system. Collectively the enzymes that catalyze the formation of reactive intermediates are called as Phase I enzymes. The cells also have a chemical protection mechanism against carcinogenesis and mutagenesis and other toxicity by induction of the enzymes involved in the metabolism particularly Phase II enzyme such as NAD(P)H quinone reductase and S-transferases (GSTs). Phase II enzyme inducers can be encountered in our diet. These Phase II enzymes often add large polar groups to the primary metabolite thus limiting further transformation and enhancing elimination thereby leading to detoxification. Thus the amount of the carcinogen available represents a balance between activating and detoxifying reactions of Phase I and Phase II enzymes (Fig. **7**) respectively [52,53]. This balance under normal circumstances is genetically controlled but gets modulated by variety of factors such as age, hormones, exposure to drugs etc.

Quinone Reductase (QR) catalysis is a two electron transfer to a wide variety of the redox cycling species including quinones and transferring them into dehydrodiols thereby preventing the mutation of the DNA and reducing cancer risk. Talalay [51] has coined the term monofunctional or bifunctional inducer. Monofunctional inducers of phase II enzymes decrease the incidence of carcinogenesis through scavenging of electrophilic compounds. The bifunctional inducers are the polycyclic hydrocarbons, dioxins, azo dyes and flavones. They elevate Phase II as well as selectively Phase I. Monofunctional inducer on the other hand elevates Phase II enzymatic activity without any significant elevation of the Phase I. Since the bifunctional inducer can activate procarcinogen to ultimate reactive form monofunctional inducer is always desirable.

Epdemiologic Investigations

There have been numerous epidemiological studies implying that intake of organosulfur may be associated with reduced incidence of cancer. Most of the studies concerned deal with intake of OSCs in forms of green and yellow vege-

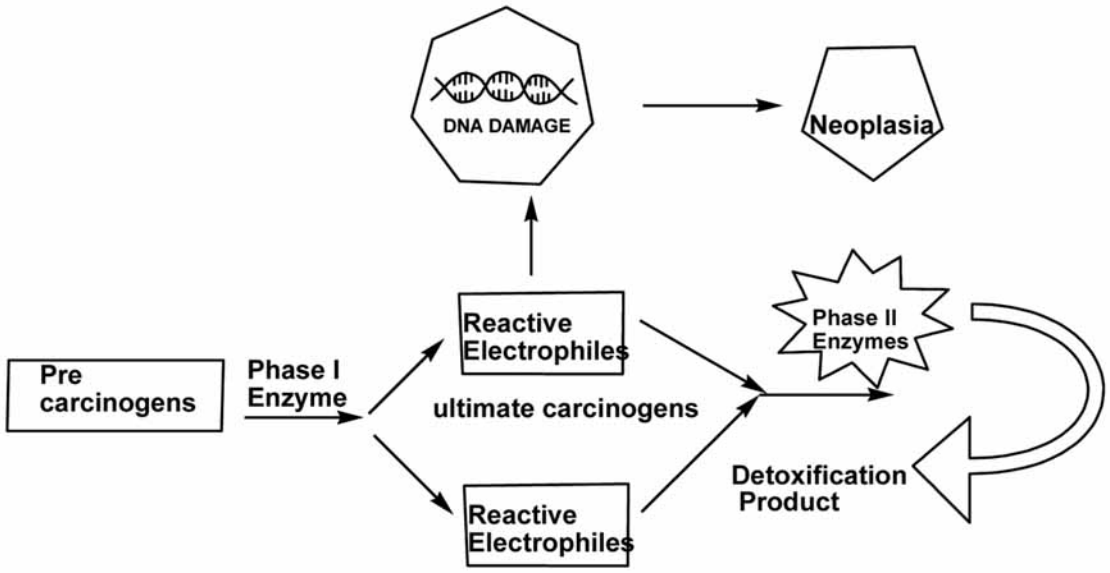


Fig. (7).

tables. There are several epidemiological reports summarizing significant roles of isothiocyanates and glucosinolates derived from crucifers in protection against cancer [53,55]. An inverse relationship was found by Michaud *et al.* between risk of bladder cancer and crucifer intake [54]. A series of epidemiological results have related crucifers intake to significant reduction in the incidence of prostrate, breast and bladder, lung cancer and non Hodgkins's lymphoma [54-55]. Terry has suggested that inclusion of 1-2 servings of *Brassica* vegetables in food reduced the risk of breast cancer by 20-40 % [56]. Verhoeven has reviewed the epidemiological data related to the intake of crucifers and incidence of cancer. Out of the 7 cohort studies 5 showed an inverse association between crucifer intake and cancer occurrence [57]. Overall, the association of consumption of *Brassica* vegetables with a decreased risk for cancer appears to be most consistent for lung, stomach, colon, and rectal cancer and least consistent for prostatic, endometrial, and ovarian cancer.

A considerable number of initial epidemiological investigations have been conducted in China. One of the very first study indicating role of *Allium* vegetables in prevention of stomach cancer was conducted in China by Mei *et al.* [58]. They found strong inverse relation between cancer incidence and *Allium* vegetable intake. Garlic intake was responsible for a 10 fold decrease in the death rate from stomach cancer in two Chinese provinces. Takezaki and his colleagues further supported this report as they found that people having low risk of developing cancer were the ones consuming larger amount of Welsh onions, onions and Chinese Chives [59].

There have been scores of other control studies conducted to evaluate the anticancer activity of OSCs in form of *Allium* vegetables . Most of these studies showed that intake of *Allium* vegetables were associated with considerable decreased risk of cancer [60-64].

In another data gathered by Nurses Health Cohort study it was found that increasing crucifer intake from one to three or more servings per week decreased apparent risk by 41% [65] in men with prostate cancer . Inverse associations bet-ween intake and cancer risk were especially strong for the cruciferous vegetables, cabbage, broccoli, cauliflower, and Brussels sprouts. Cohort studies reported inverse associations between intakes of cabbage, cauliflower, or broccoli and risk for lung cancer, between total crucifer intake and risk for stomach cancer, and between broccoli intake and risk for all cancers [66]. Of >200 case-control and cohort studies, nearly 80% have reported significant inverse relations between consumption of plant foods and the apparent risk of developing prostate cancer. Although conclusions with respect to the overall extent to which diet contributes to cancer incidence, or to be more explicit, the degree to which dietary modification might be expected to reduce cancer risk, vary considerably, a reasonable estimate is 30–40% [67].

Site-specific, case-control studies and cohort studies on garlic (*Allium sativum*) and onion (*Allium cepa*) (highly rich in organosulfur compounds) suggest a preventive effect against stomach, colorectal and prostate cancer, although convincing evidence against cancer at other sites, including the breast, is still to be found [68].

In one of the major, case-control study (a population-based), performed on 238 patients with prostate cancer and 471 male controls, Hsing *et al.* investigated the relationship between intake of OSCs in form of *Allium* vegetables and the risk of prostate cancer [69]. Encouraging results were found in subjects with highest intake of *Allium* vegetables (>10 g day^{-1}). They were found to have statistically significant lower risk of contracting prostate cancer than did those in the category of lowest intake (2.2 g day^{-1}). The notable feature of this study was that the reduced risk of prostate cancer was independent of various other factors such as body weight, consumption of other food and total food intake [69].

You *et al.* [70] studied the association of *Allium* vegetables intake with gastric cancer in Shangdong Province of

Northeastern China, which has high incidence region for stomach cancer than the mainland. It was reported that garlic and other vegetables considerably reduced the risk for stomach cancer. An inhibition of progression of precancerous gastric lesion by garlic preparation was also noted [71]. Regular consumption of garlic was shown to be associated with decreased prevalence of adenomatous polyps in colon and rectum [72].

Hansson *et al.* evaluated association of consumption of OSCs to gastric cancer in a population based case control study of diet in Sweden [73]. The adjusted risk estimate supports protective effect of garlic against gastric cancer. Zheng published population based case control studies from Shanghai to evaluate the effect of *Allium* vegetables on pharyngeal cancer. Significant protective effect against cancer of larynx was observed [74]. A similar report by Gao *et al.* showed significant association between esophageal cancer risk and organosulfur rich *Allium* vegetable intake [75]. A multicentric study in Italy performed by Buiatti and colleagues also revealed that risk of stomach cancer declined with increased intake of onion/garlic as condiments [76]. Reduced risk of gastric cancer was observed by Le Marchand and co-workers reported on a population based case control study in Hawaii [77a]. A weak inverse association was evident with garlic intake and colon cancer. In a population based studies conducted in China it was found that the incidence of stomach cancer decreased inversely with frequency of intake of garlic, onion, welsh onion and chives [77b].

The cohort study in Netherlands suggests a lack of association between garlic supplements and lungs, breast cancer sites. Key *et al.* and Dorant *et al.* have maintained that there is no reliable evidence showing connection between intake of garlic supplement and prevention of cancer in human [78-81].

The published epidemiological evidence strongly suggests that protection from various forms of cancer may be associated to intake of consumption of OSCs rich diet. However, great deal of disparity exists between different reports and thus the minimum amount of OSCs to elicit a protective effect may be unknown. Since, all these studies have been conducted in different hospital settings all around the world it has resulted in lack of uniformity. There is utmost need for additional standardized epidemiological studies before a definite conclusion can be drawn about the role of OSCs in cancer etiology.

Experimental Carcinogenesis

Many studies performed in animal models indicate that the organosulfur micro constituent present in nautural sources have a chemopreventive action against carcinogens. Aromatic isothiocyanates, which arise in plants as a result of enzymatic cleavage of glucosinolates, has been found to be amongst most potent chemopreventive agent. A striking and characteristic chemical property of cruciferous plants is their high contents of glucosinolates and its hydrolysis products, ITCs which are well known for their cancer chemopreventive activity.

As has been discussed earlier the protective mechanism of isothiocyanates includes blockage of N-nitro compound formation, suppression of the bioactivation of several carcinogens enhanced DNA repairs reduced cell proliferation and induction of apoptosis.

Reddy along with his colleagues have reported reduction in azoxymethane (AOM) induced colonic aberrant crypt foci (ACF) in F 344 rats by sulforaphane and PEITC as a result of treatment during initiation and post initiation stages [82]. It should be noted that the dosage of broccoli and brussels sprouts required to increase the enzyme activity was higher than the average daily serving quantity. Inhibitory effect of sulforaphane (SFN) on DNA strand breakage by 2-amino-3-methyl-imidazo[4,5-f]quinoline and N-nitroso dimethyl amine has been reported by Borcelo *et al.* [83].

The effect of isothiocyanate sulforaphane on Phase I and Phase II enzymes on carcinogen metabolism in primary culture of rat and human hepatocytes demonstrated induction of GSTs and inhibition of CYPs [84]. The urinary excretion of 2-amino-1-methyl-6-phenyl imidazo(4,5-b)pyridine and 2-amino 3,8-dimethylimidazo(4,5-f)quinoxaline which have a capability to initiate human colon cancer was significantly decreased in individuals with higher intake of cruciferous products [85]. In one of the recent clinical trials conducted in Qudong China, oltipraz was found to enhance the excretion of Phase II products aflatoxin-mercapturic acid derived from alfatoxin glutathione conjugate [86,87].

Extracts of watercress and brussels containing higher concentration of glucotropeolin and sinigrin have been found to attenuate DNA damage and pre-neoplastic lesion in the colon and liver of rats [88]. As discussed earlier the most important chemopreventive mechanism of cruciferous vegetables appear to be induction of Phase II enzymes and inhibition of Phase I enzymes. Major mechanism for preventing carcinogens appear to be selective inhibition of cytochrome p450 enzymes. ITCs are also inducers of phase II detoxification enzymes such as GSTs, NADPH; Quinone oxidoreductase (QR). Some of the compounds like SFN and PEITC have shown remarkable potential as Phase II inducers [89-91].

In a study to evaluate efficacy of three promising sulfur containing compounds 6-phenyl hexyl isothiocyanate (PHITC), phenyl ethyl isothiocyanate (PEITC) and N-acetyl cysteine (NAC) as protecting agents against lung tumorigenesis it was found that PHITC and PEITC were potent chemopreventive agents for NNK induced lung tumorigenesis in F 344 rats whereas NAC was not at all active [92]. Administration of PEITC showed a 9% incidence of lung tumorigenesis upon treatment with carcinogen 4-(methyl nitroso amino)-1-(3-pyridyl)-1-butanone (NNK) compared to 67% incidence of non PEITC treated control. In another study conducted in F 344 rats dietary phenylethyl isothiocyanate (PEITC) completely inhibited N-nitrosomethylbenzylamine (NMBA) induced esophageal tumor [91].

Apoptosis of cells exposed to genotoxic agent may be viewed as host protective response to pre-cancerous cells. Isothiocyanates inhibited the growth and induced apoptosis of tumor cells *in vitro* [92-93].

Cancer chemoprevention effect of isothiocyanates was also indicated by induction of apoptosis by oral administra-

tion of glucosinolates of aberrant colonic cryptic cells by dimethyl hydrazine induced in rats. Its been demonstrated that dietary supplementation with sinigrin increases the number of colonic crypt cells undergoing apoptosis in rats exposed to dimethylhydrazine (DMH) [94]. Administration of PEITC to rats exposed to cigarette smoke induced apoptosis of bronchial and alveolar epithelial cells and alveolar macrophages [95].

SFN, BEITC and PEITC have been shown to induce the expression of GSTs in cultured cell. Induction of GST-A by SFN in human colon adenocarcinoma cells, BITC induced GST P1 in rat liver epithelial cells and PEITC induced GST activity in esophagus has been reported [96-97].

Phenyl alkyl isothiocyanate cysteine conjugate **28 & 29** (analogs of phenyl alkyl isothiocyanate) were found to possess increased detoxifying activity and less toxicity than the parent compound [98] (Fig. **8**). A survey of literature reveals numerous reports to show Benzyl isothiocyanate as potent inducer of detoxification enzymes QR/GST [98-102].

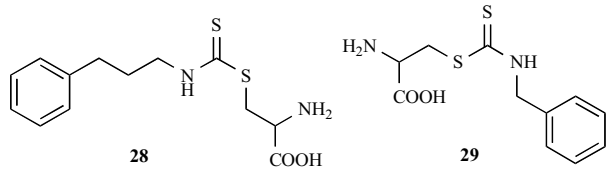


Fig. (8).

Single dose and four dose protocol to test the abilities of phenyl ethyl isocyanate (PEITC) and phenyl hexyl isothiocynanate to inhibit 4-(methylnitrosamino)-1,3-(pyridyl)-1-butanone (NNK) induced lung tumorgenecity revealed significant reduction of tumor multiplicity compared to control irrespective of the administration frequency [103].

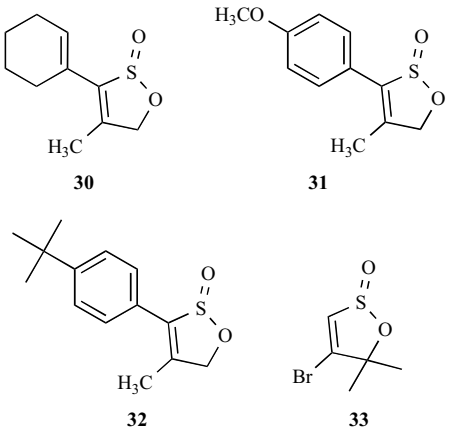
Studies have demonstrated that 6-phenyl hexyl isothiocyanate (PHITC) can inhibit NNK induced lung tomorgenecity in strain A mice by more than 80% when administered at a 50 fold lower dose than NNK. Milner has reviewed the protective effect sulfides against various cancers. This protection of sulfides is as a result of various mechanisms, including blockage of N-nitroso (NOC) compounds formation, suppression of the bio-activation of several carcinogens enhance DNA repairs reduced cell proliferation and induction of apoptosis [104].

In order to provide better understanding of the effect of organosulfur compounds derived from *Allium* S-allylcysteine (SAC), S-allyl mercaptocysteine (SAMC) were examined for their effect on proliferation and cell cycle progession in human colon cancer cells lines SW 480 and HT-29. SAMC inhibited the growth of both cell lines at doses similar to those of sulindac sulfide [105].

Organ specificity and differential efficacy of various OSCs such as DADS, DAS, DATS, DPS, DPDS, in prevention of BP induced tumorigenesis revealed a good correlation between chemopreventive efficacy and induction of QR (an enzyme capable of detoxifying activated quinone metabolite) [106]. Various other derivatives such as allylmethyl disulfides and allyl mercaptans have been found to be effective in lowering the incidence of tumor fomation in lungs and stomach [107, 108]. Structure activity studies to determine the role of allyl group and the disulfide chain in mGST P 1 inducing activity of DADS indicated that allyl group along with oligosulfide chain lengths is equally important for their mGST P 1 activity [109]. Diallyl sulfide have been found to be associated with cancer inhibition in stomach, colon, esophagus, mammary glands.

A comprehensive study to understand the mechanism of differential efficacy of garlic organosulfides in prevention of benzo[a]pyrene (BP) induced cancer in female A/J mice was conducted by Srivastava and his colleagues who investigated, differential activity mechanism of different organosulfides (DAS, DADS, DATS, DPS, DPDS) by their effect on the enzymes of BP activation/inactivation pathways. It was found that except DATS all other sulfides caused a significant increase 37-44% in hepatic ethoxy EROD activity. Further it was found that DAS, DADS, DATS, resulted in the significant increase as compared to control with both hepatic and forestomach glutathione transferase (GST activity) towards anti-7-β-8-alpha-dihydroxy-9α, 10 α oxy 7,8,9,10 tetrahydro-benzo(a)pyrene anti (BPDE) which is carcinogen of B.P [109].

A novel class of OSCs termed as oxathiolene oxides (OTEOS) have been found to be Phase II enzyme inducers (GST, NAD(P)H, Ferritin H and L mRNA) in a normal mouse embryonic liver cell line (Fig. **9**). Structure **30** (3-cyclohexnyl-4-methyl-1,2-oxathiole-3-ene-2-oxide) was found to be strongest inducer. It is of interest to note that **30** failed to induce cytochrome, P 450 1A1 mRNA. This study provides evidence that oxathiolene oxides represent a new series of Phase II inducers that may have activity as chemopreventive agent [110].



Structure of Oxathiolene Compounds

Fig. (9).

Milner and Knowles suggested that early alterations in ERK pathway signaling may be the contributory factor leading to the G2/M phase arrest observed after DADS exposure [111]. Diallyl sulfide has been shown to have anti tumorigenic activity in mice skin tumours. Induction of apoptosis by diallyl sulfide is believed to be the major contributing

factor for the protective action in DMBA induced mouse skin tumours [112]. Arrest of unsynchronized human colon tumor cells (HCT-15) in G2/M phase of the cell cycle has been reported by Milner *et al.* [113] It has been found that the ERK (extracellular signal regulated kinase activity increased by 44 to 66% after treatment with 100 and 500 μmol/mol of DADS.

The protective effect of the various organosulfur compounds appears consistent however it should be borne in mind that over all benefits from the natural sources are from synergistic combination of all the phytochemicals present in the source. One of the major concerns amongst scientists engaged in this area of research has been the impact of heating either by microwave or convection oven on the cancer chemopreventive ability of organosulfur rich vegetables. A study was conducted to evaluate the effect of garlic to reduce the bioactivation of 7,12-dimethyl benz(a)anthracene in female rats. It was found that cooking resulted in immense loss of protecting effect. This study also hilights the role of allinase which is destroyed upto 90% upon cooking [113].

As a part of our ongoing research directed towards synthesis of analogs based having structural similarity to naturally occurring active chemopreventive agents we synthesized and tested a series of brassinin analogs (Fig. **10**). These compounds were tested for quinone induction in hepa1c1c7 murine hepatoma cells [114]. The brassinin analogs tested were, 4-methyl brassinin, 5-methyl brassinin, 6-methyl brassinin, 7-methyl brassinin, 5-chlorobrassinin, 2-methyl brassinin, N-ethyl 2,3-dihydrobrassinin, brassinin, 2,3-dihydrobrassinin, brassinin, methyl(2-indolyl)methyldithiocarbamate, methyl benzyldithiocarbamate, methyl benzyldithio-carabamate, cyclobrassinin, homocyclobrassinin 1-methoxy brassinin and spirobrassinin.

Some of the brassinin analogs tested demonstrated dose dependent induction of quinone reductase activity in hepa1c1c7 murine hepatoma cells Induction of the quinone reductase in hepa1c12c7 cells followed the order, N-ethyl-2,3,-dihydrobrassinin > cyclobrassinin > methyl benzyl dithiocarbamate > spirobrassinin >1-methoxy brassinin> 2,3-dihydrobrassinin, and methyl (2-indolyl) methyl dithiocarbamate. Except for 1-methyl brassinin, which was active in induction and more potent than brassinin and spirobrassinin in preliminary test, other methyl substituted brassinin analogs demonstrated weak activities [114].

Mammary gland organ culture has been successfully employed to evaluate potential chemopreventive agents for their role in inhibiting DMBA induced mammary lesions formation during initiation and promotion phase There appear to be good correlation between the activity of chemopreventive agents in this assay and *in vivo* carcinogenesis experiment. In order to evaluate the potential of brassinin analogs for their role in inhibiting DMBA induced mammary lesions. A series of analogs were screened i.e. 4-methyl brassinin, 5-methyl brassinin, 6- methyl brassinin, 7-methyl brassinin, 5-chloro-

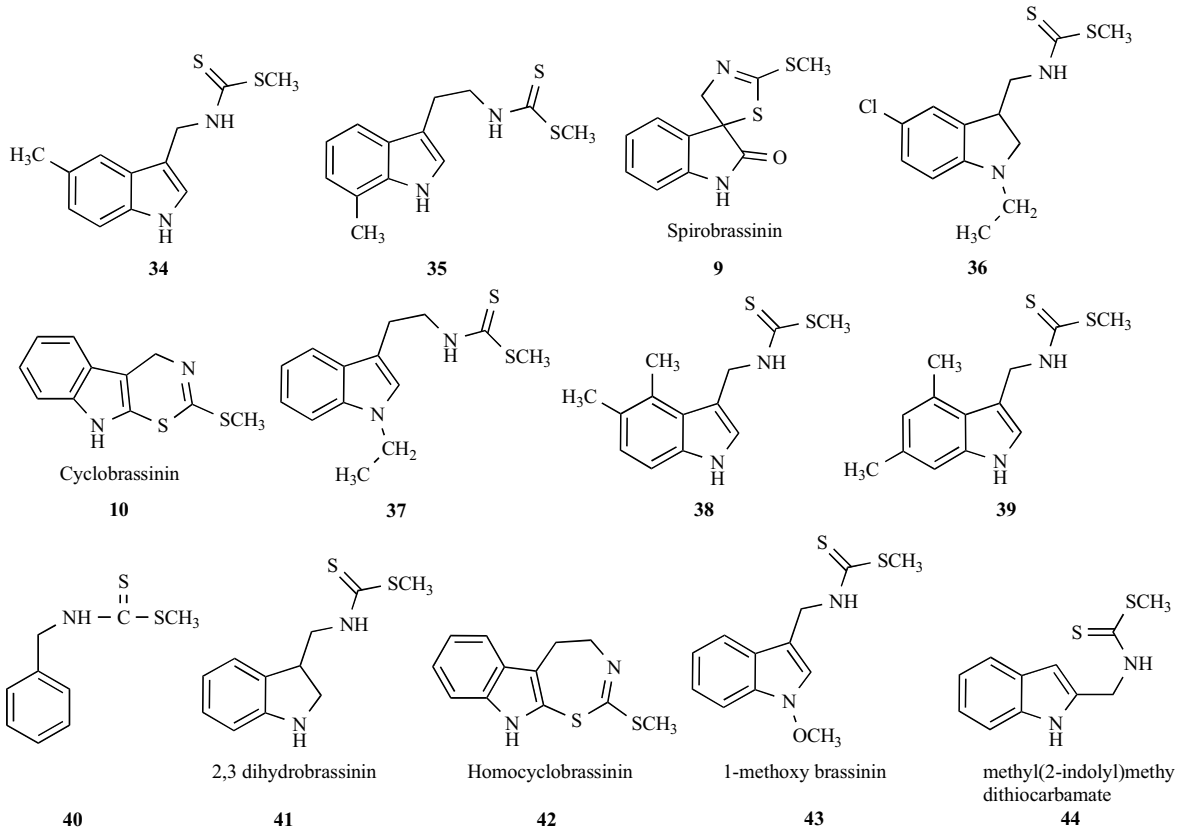


Fig. (10).

brassinin, 2-methyl brassinin, N-ethyl 2,3-dihydrobrassinin, cyclobrassinin and spirobrassinin. Cyclobrassinin and spirobrassinin inhibited mammary lesion development by more than 70% when included in the medium at 10 ug/ml. N-ethyl-2,3- dihydrobrassinin yielded significant suppression in the incidence of mammary lesion formation by 66% in the glands All the rest of the analogs tested did not demonstrate significant chemo preventive activity [115].

In continuation of our efforts dedicated towards discovery of novel chemopreventive agents [116-118] and to evaluate the potential of the test agents to enhance the activity of the QR we synthesized a series of sulforamate derivatives [119]. These compounds were aliphatic analogs of brassinin and show structural similarity to sulforaphane. The methyl group of the dithiocarbamate was replaced by different groups (aliphatic chain and substituted benzyl) in anticipation that the ability of these moieties to act as a good leaving group would affect induction potential.

Three compounds (**46-48**) (Fig. **11**) showed excellent activity as their CD (concentration require to double QR induction) and CQ (concentration required to quadruple QR induction) was comparable to well established chemopreventive agent sulforaphane The encouraging aspect was the reduced toxicity in comparison of sulforaphane [119].

CONCLUSIONS

Over all evidence available in the literature reveals that OSCs have cancer chemo preventive effects. As already discussed a number of epidemiological and experimental carcinogenesis studies have shown that consumption of OSCs in form of vegetables have inverse relationship to cancer risk. There is a huge volume of research findings that strongly support that organosulfur compounds in some measure have preventive action against occurrence of cancer , however there are several issues to be adressed before these phytochemicals can actually be used in treatment of cancer. One of the most difficult problems of chemo preventive drug testing is the lengthy trial due to long development period of majority of cancers.

The utmost need of the hour is to develop surrogate intermediate biomarkers since in case of human trials, the testing is laborious and of course too expensive. The use of these biomarkers would go a long way in speeding up the trials and lowering the experimental cost. Moreover, development of these surrogate measures of efficacy will also lead to the weeding of compounds that are medically not useful. Its worth mentioning here that since carcinogenesis is a multi-step process single biomarkers may appear on only one or a few of carcinogenic pathways thus panel of biomarkers representing series of carcinogens may be useful.

Literature survey reveals that there is no well-documented scientific data on the stability of OSCs derived from plants during cooking and during metabolism. Understanding the relationship between sulfur metabolism and control of neoplastic process and cell proliferation would be useful to know whether tissue retain any metabolites. Additional detailed studies are warranted to specify the minimum quantity of the sulfur compounds to be consumed since the pure organosulfur effective in animals is translated to unrealistic amount when extrapolated keeping in view the body weight.

In spite of the remarkable success and good future prospects it is a well-recognized fact that there is incompleteness of scientific information in the field of cancer chemoprevention in general, thus it is highly critical that this deficiency is overcome by inter disciplinary research and collaboration. As is obvious from the diversity of sulfur action a more comprehensive effort is required to study various aspects of sulfur biochemistry, metabolism and potential toxicity. In addition to that efforts should be made to study comprehensively protective effects of all these phytochemical from additives and synergistic combination point of view rather than focusing on individual agent. Until all these gaps in our knowledge at molecular level are filled OSCs cannot be used as a standard therapy in cure of cancer.

ABBREVIATIONS

OSCs	=	Organosulfur compounds
AITC	=	Allylisothiocyanate
BITC	=	Benzyl isothiocyanate
PEITC	=	Phenyl ethylisothiocyanate
PHITC	=	6-phenylhexyl isothiocyanate
DPDS	=	Dipropyldisulfide
DPTS	=	Dipropyltrisulfide
DPS	=	Dipropylsulfide
DAS	=	Diallylsulfide
DADS	=	Diallyldisulfide
DATS	=	Diallyltrisulfide

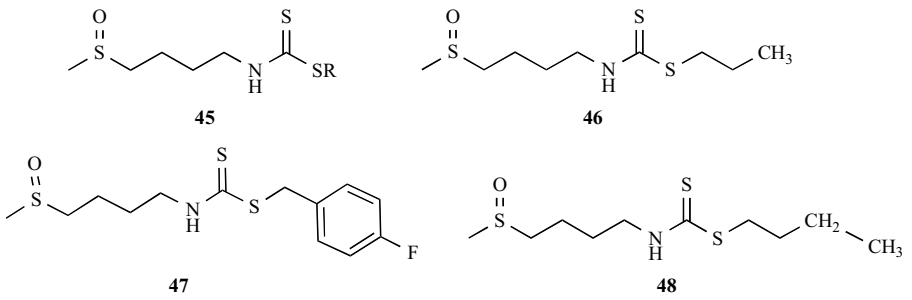


Fig. (11).

PMS = Propylmethylsulfide

PMDS = Propylmethyldisulfide

PMDS = Propylmethyltrisulfide

SAC = S-Allylcysteine

SAMC = S-allylmercaptocysteine

SMC = S-methyl Cysteine

DMBA = 7,12-dimethylbenz[*a*]anthracene

ODC = Ornithine decarboxylase

NDMA = N-Nitrosodimethylamine

AGE = Aged garlic extract

NAD(P)H = Quinone oxidoreductase

GSTs = Glutatathione S-transferases

NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

AOM = Azoxymethane

ITC = Isothiocyanates

SFN = Sulforaphane

BP = Benzo [a] pyrene

OTEOS = Oxathiolene oxide

QR = Quinone reductase

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