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Short communication

Cancer chemopreventive activity of sulforamate derivatives

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Abstract

Chemoprevention can be defined as an intervention in the carcinogenic process by use of natural or synthetic substances. Induction of Phase II enzyme is an important mechanism of chemoprevention. In the present studies we have synthesized several derivatives of (+)(–) 4-methylsulfinyl-1-(*S*-methyldithiocarbamyl)-butane (sulforamate) and evaluated their effectiveness as monofunctional inducer of the NAD(P)H Quinone oxidoreductase [quinone reductase (QR)] a phase II enzyme in cultured Hepa1c1c7 murine hepatoma cells. The cytotoxicity of some of the derivatives was strongly reduced in comparison to [(–)-1-isothiocyanato-4(R)-(methylsulfinyl)butane] (sulforaphane). However, the induction potential of these compounds was comparable to sulforaphane. On the basis of these results sulforamate derivatives can be regarded as simple, inexpensive and readily available chemopreventive agents.
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Keywords: Chemoprevention; Phase II enzymes; Sulforamate; Quinone oxidoreductase (QR); Monofunctional inducer

The term "cancer chemoprevention" coined by Sporn [1,2] and coworkers in 1976 has been defined as a strategy for reducing cancer mortality by the prevention, delay or reversal of cancer by ingestion of dietary or pharmaceutical agents capable of mediating the process of carcinogenesis [3]. In recent years cancer prevention by natural products has received considerable attention. Much emphasis has been placed on dietary intake of certain fruits and vegetables that have shown to reduce the risk of developing cancer. Numerous epidemiological studies have shown an association between reduced cancer risk and increased intake of green yellow fresh vegetables [4–6]. Particularly noteworthy is the group of food plant generally falls in the family Cruciferae and genus brassica, i.e. cauliflower, cabbage, brussel sprouts and broccoli [7].

It has already been established that three dietary components brassinin (**1,** indole based dithiocarbamate), cyclobrassinin (**2**) spirobrassinin (**3**) sulforaphane (**4,** aliphatic isothiocyanate) and 1.2-dithiol-3-thione (**5**) found in the Cruciferous plants are capable of mediating chemopreventive activity in animal models [8–10]. Feeding of vegetables induces enzymes of xenobiotic metabolism and thereby accelerates the metabolic disposal of the xenobiotics. The human body's first lines of defense against cancer are the Phase I and Phase II enzymes. The metabolic activation and deactivation of carcinogens is catalyzed by xenobiotic Phase I and II enzymes. By measurement of induction of Phase II detoxifying enzymes, such as quinone reductase (QR), glutathione S-transferase, UDP-glucoronosyl transferase and epoxide hydrolase, compounds may be screened for anticarcinogenic activity[10a]. These enzymes can convert electrophiles (ultimate carcinogens) to less reactive compounds, which can in turn conjugate to sugars, glutathione and sulfates resulting in less toxic and more excretable compounds. Phase I enzymes are promoted by cytochromes P-450 that are mediated by the cytosolic Ah receptor (Aryl hydrocarbon). When ligands bind to the Ah receptors the complex is transported to the nucleus and binds to the xenobiotic responsive elements (XRE) upstream for P-450 genes[10b]. Phase I enzymes typically carry out oxidation and reduction reactions that make carcinogens more water soluble, however they are capable of activating compounds to electrophilic species, which can damage DNA. These two families of enzyme help

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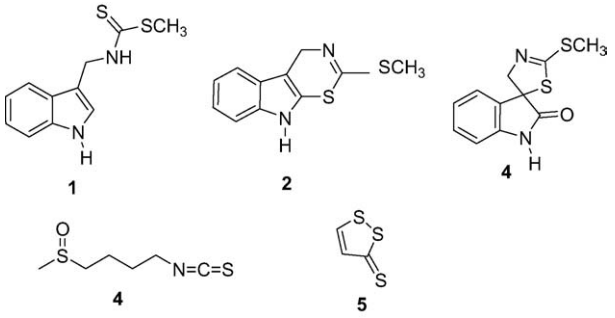
body protect itself from all types of carcinogens that routinely enter the human body through the diet and the environment.

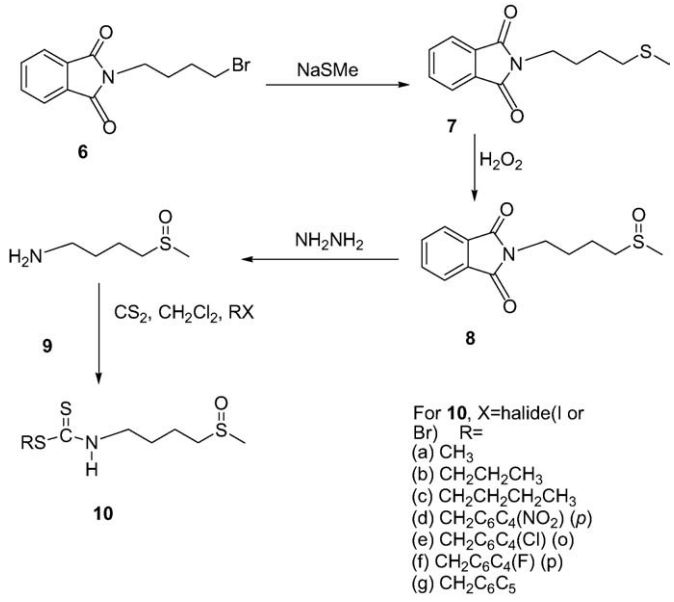
A key component in the understanding the initial events of carcinogenesis is the reorganization of the fact that many of the carcinogens are not chemically reactive per se but undergo metabolic activation to form electrophilic reactants [10c].

Amount of carcinogens available represents a balance between activating and detoxifying reactions of Phase I and Phase II enzymes, respectively [11–13]. This balance under normal circumstances is genetically controlled but gets modulated by variety of factors like age, hormone, and exposure to drugs.



On the basis of structural similarities of brassinin to compounds like indole-3-carbinol and indole-3-acetonitrile, it was found to induce drug-metabolizing enzymes in cell culture. It was found that Brassinin also induced the activity of P-450 isoenzymes. This particular induction is a risk factor in the activation of carcinogens. Infact indole-3-carbinol was found to induce liver carcinogenesis in rainbow trout when applied to the post initiation stage [14]. Although indole-3-carbinol and related compound demonstrate significant chemopreventive activity, induction of Phase I metabolizing enzymes, is considered as a risk factor in the activation of carcinogens. On the basis of this potential cancer risk of the indole based compounds and relative toxicity of the isothiocyanates a hybrid molecule was conceptualized. Isothiocyanate was replaced by the dithiocarbamate moiety of brassinin thus exchanging the indole ring with methyl sulfinyl butane side chain.

In a program directed towards discovery of the novel chemo preventive agents and to evaluate the potential of test agents to enhance the activity of the QR we currently report the synthesis and activity of six novel sulforamate derivatives. These compounds are aliphatic analogs of Brassinin and show structural similarity to sulforaphane. The methyl group of the



Scheme 1.

dithiocarbamate has been replaced by different groups (aliphatic chain, substituted benzyl) Scheme 1.

We anticipated that during metabolism the ability of these moieties to act as a good leaving group would have effect on the induction potential. Compound **10f** (having *para*-fluoro benzyl group instead of methyl in sulforamate) **10b** and **10c** (having propyl and butyl group, respectively, in place of methyl in sulforamate) have shown excellent activity. The CD (concentration required to double QR induction) and CQ (concentration required to quadruple QR induction) of these compounds are comparable to sulforaphane. In addition to this the reduced toxicity of these compounds make it far better chemopreventive agent than sulforaphane. The chemopreventive index (CI) of these compounds is three to four times that of Sulforaphane (Table 1).

Synthesis of **10** (Scheme 1) was carried out by addition of sodium thiomethoxide to N-(4-bromobutyl)pthalimide that in turn was obtained by treating phtalimide with 1,4, dibromobutane. Subsequent oxidation with hydrogen peroxide resulted in the formation of sulfoxide **8,** which upon addition of hydrazines afforded the amines (**9**).

The amine (**9**) was later treated with triethylamine, carbon disulfide and then iodomethane to yield **10** in good yields.

Table 1

Compound number	Concentration required to double QR induction (CD) (μg/M)	Concentration for 50% inhibition of cell viability (IC50) (μg/M)	CQ concentration required to quadruple QR induction (μg/M)	CI
Sulforaphane	0.06	1.7	0.55	28
10a	0.08	9.9	0.94	123
10b	0.07	7.9	0.24	112
10c	0.2	17.9	1.5	90
10d	0.07	1.5	0.6	21
10e	0.1	2.5	1.4	25
10f	0.05	6.1	0.5	122
10g	0.06	0.8	0.4	13

1. QR assay

To evaluate the potency of the compounds (**10a**–**10g**) as inducers of QR, murine Hepa1c1c7 cells were used as described previously. In brief Hepa1c1c7 were plated at a density of 1×10^4 cells per ml in 96 well plates. After preincubation of for 24 hours the medium was changed Samples were added in final concentration of 0.15–20 μg/ml. The cells were incubated for 48 h. QR was determined by measuring NAD(P)H dependent menadiol mediated reduction of the MTT [3(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide]. Induction of the QR activity was calculated by comparing the QR specific activity of compound treated cells with that of solvent treated cells Enzyme activity was expressed as CD, concentration required to double QR induction. CI is obtained by dividing IC values (Concentration for 50% inhibition of cell viability) with CD values. The results have been tabulated in Table 1.

On the basis of these encouraging results and in particular reduced cytotoxicity of the sulforamate derivatives these can be regarded as novel chemopreventive agents that require a detailed further investigation.

2. Experimental

Cell cultures and supplements were purchased from Life Technologies (Grand Island, New York). ^{1}H NMR spectra were taken on 400-MHz Bruker NMR spectrometer. All the melting points have been taken in open capillaries and are uncorrected.

3. General procedure

The sulforamate derivatives were synthesized as outlined in Scheme 1. Using modification of the literature procedure [15] (+)(–)1-amino-4-(methylsulfinyl)butane (**9**) was synthesized from N-(4-bromobutyl)phthalimide (**6**). Carbon disulfide (0.3 g, 4 mmol,) was added dropwise to the dichloromethane (40 ml) solution of **9** (0.5 g, 4 mmol) and imidiazole (0.8 g, 12 mmol) under Argon (0 °C). The mixture was stirred for 1 hour followed by dropwise addition of iodomethane. It may be noted that alkyl iodide was used in preparation compounds **10a**–**10c** while arylnenzyl bromides were used for the synthesis of compounds **10d**–**10g**. The resulting mixture was stirred for 2 hours. The bulk of the solution was removed in vacuo and the residue taken in diethylether. The resulting mixture was washed with 1.2 N HCl extracted with diethyl ether and dried with $MgSO_4$. The excess of solvent was removed and the residue was purified on silica flash gel. The fractions were concentrated and recrystallized from ether/petrol ether to yield **10a** as white solid. Other compounds **10b**–**10g** were synthesized in similar manner.

10(a) Methyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 56% (0.50 g), m.p. 94–96 °C, Ref. [7], m.p. 95–96 °C.

10(b) Propyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 51% (0.516 g), m.p. 76–77 °C, ^{1}H NMR ($CDCl_3$): δ 0.95–1.01 (m, 3H, CH_3), 1.62–1.69(m,2H,CH_2) 1.85(m,4H, CH_2CH_2), 2.57(s,3H,CH_3), 2.70–2.76 (m,2H,CH_2), 3.17–3.26 (m,2H,CH_2), 3.74–3.76 (m,2H,CH_2), 8.46(brs,1H,NH). Anal. Calc. for $C_9H_{19}NOS_3$ (253): C, 42.6; H, 7.50; N, 5.53, Found C, 42.56; H, 7.51; N, 5.44.

10(c) Butyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 54%(0.57 g), m.p. 68–70 °C ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 0.86–0.92 (m,3H,CH_3), 1.35–1.40 (m, 2H, CH_2) 1.56–1.65 (m, 2H, CH_2) 1.82 (m, 4H, CH_2CH_2), 2.57(s,3H, CH_3), 2.70–2.76 (m, 2H, CH_2), 3.17–3.26 (m, 2H, CH_2), 3.74–3.76 (m,2H,CH_2), 8.46(brs,1H). Anal. Calc. for $C_{10}H_{21}NOS_3$ (267) C, 44.9; H, 7.80; N, 5.24, Found C, 44.76; H, 7.65; N, 5.97.

10(d) *p*-Nitro-benzyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 58% (0.802 g), m.p. 132–135 °C ^{1}H NMR ($CDCl_3$): δ 1.86–1.91 (m, 4H,CH_2CH_2), 2.59 (s, 3H, CH_3) 2.72–2.78 (m, 2H, CH_2), 3.78–3.81(d,2H, CH_2) 4.63 (s, 2H, benzylic CH_2), 7.53 (d, 2H, Ar–H), 8.15 (d, 2 h, Ar–H) 8.06 (brs, 1H). Anal. Calc. for $C_{13}H_{18}N_2O_3S_3$ (346) C, 44.82; H, 5.2; N, 8.0, Found C, 44.49; H, 5.14; N, 7.98.

10(e) *p*-Chloro-benzyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 54% (0.72 g), m.p. 92–94 °C ^{1}H NMR ($CDCl_3$): δ 1.84 (m, 4H), 2.56 (s, 3H, CH_3) 2.71–2.77 (m, 2H), 3.75–3.77 (d, 2H, CH_2), 4.66–4.72 (s, 2H, benzylic CH_2), 7.56–7.9 (m, 4H, –ArH), 8.07 (brs, 1H, NH). Anal. Calc. for $C_{13}H_{18}ClNOS_3$ (335) C, 46.5; H, 5.37; N, 4.17, Found C, 46.45; H, 5.41; N, 4.22.

10(f) *p*-Fluoro-benzyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 50% (0.638 g), m.p. 72–73 °C ^{1}H NMR ($CDCl_3$): δ 1.87 (m, 4H, CH_2), 2.57(s, 3H, CH_3) 2.72–2.75 (m, 2H), 3.76–3.79 (d, 2H, CH_2), 4.52 (s, 2H, CH_2), 7.53(d, 2H, Ar–H), 8.10 (d, 2H, Ar–H) 8.07 (brs, 1H). Anal. Calc. for $C_{13}H_{18}FNOS_3$ (319) C, 48.90; H, 5.66; N, 4.40, Found C, 48.40; H, 5.57; N, 5.03.

10(g) Benzyl-4-(methylsulfinyl)butylcarbamodithioate, yield: 53% (0.612 g), m.p. 88–89 °C, ^{1}H NMR ($CDCl_3$): δ 1.83–1.85 (m, 4H), 2.56 (s, 3H) 2.72–2.73 (m, 2H), 3.76–3.79 (d, 2H, CH_2), 4.52 (s, 2H, CH_2), 7.23–7.37 (m, 5H, Ar–H) 8.46 (brs, 1H, NH). Anal. Calc. for $C_{13}H_{19}NOS_3$ (289) C, 51.8; H, 6.3; N, 4.65, Found C, 49.21, H 5.97; N, 4.72.

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