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Synthesis and Anticancer Activity Comparison of Phenylalkyl Isoselenocyanates with Corresponding Naturally Occurring and Synthetic Isothiocyanates

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Abstract

Synthesis and identification of novel phenylalkyl isoselenocyanates (ISCs), isosteric selenium analogs of naturally occurring phenylalkyl isothiocyanates (ITCs), as effective cytotoxic and anti-tumor agents is described. The structure-activity relationship comparison of ISCs with ITCs and effect of the increasing alkyl chain length in inhibiting cancer cell growth was evaluated on melanoma, prostate, breast, glioblastoma, sarcoma, and colon cancer cell lines. IC₅₀ values for ISC compounds were generally lower than their corresponding ITC analogs. Similarly, in UACC 903 human melanoma cells, the inhibition of cell proliferation and induction of apoptosis were more pronounced with ISCs as compared to ITCs. Further, ISCs and ITCs effectively inhibited melanoma tumor growth in mice following intraperitoneal xenograft. A similar reduction in tumor size was observed at three times lower doses of ISCs as compared to corresponding ITCs.

Introduction

Isothiocyanates (ITCs) are naturally occurring compounds that are stored as thioglucoside conjugates, termed glucosinolates, in plants and cruciferous vegetables such as watercress, brussels sprouts, broccoli, cabbage, cauliflower, radish, turnip etc.^{1–3} They are among the most effective naturally occurring cancer chemopreventive agents,⁴ which inhibit carcinogenicity in animal models.⁵ In addition, epidemiological studies have demonstrated that the human consumption of isothiocyanates in vegetables decrease cancer risk.^{6, 7} This fact is supported by strong literature data that suggests that ITCs are effective chemopreventive agents for specific human cancers. ITCs have been shown to exhibit the anticarcinogenic effects through dual mechanisms occurring at the level of initiation of carcinogenesis by blocking Phase I enzymes (cytochrome P-450) that activate procarcinogens and by inducing Phase II enzymes that detoxify electrophilic metabolites generated by Phase I enzymes.^{8–13} ITCs are also known to block cell-cycle progression and induce apoptosis in human cancer cells suggesting that these agents act also at the post initiation and progression stages.^{12–16}

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The activity of ITCs has been shown to vary with varying alkyl chain length. While some reports show the increase in potency with increasing alkyl chain length,^{17–19} others have even reported a reverse phenomenon.²⁰ Structure-activity studies have also demonstrated that increased lipophilicity or chain length of ITCs increases inhibitory potency against NNK-induced lung tumorigenesis in A/J mice.^{21,22} Conaway *et al.* have shown that synthetic ITCs can be generated with enhanced lipophilicity by increasing alkyl chain length up to 6 carbons.¹⁷ In addition, the elimination half-life (T_{1/2e}) of ITCs with longer alkyl chain length has been shown to be higher than those with shorter alkyl chain length.¹⁷ Collectively, these data indicate that ITCs with a longer alkyl chain persist longer at the target organ site.

The well established chemopreventive properties of ITCs warranted a structure-activity study of synthetically modified ITC analogs to enhance their chemopreventive and chemotherapeutic efficacy. We hypothesized that isosteric replacement of sulfur in ITCs by selenium would result in more effective anticancer agents. The hypothesis was based on the observation that in comparison to the sulfur structural analogs, selenium compounds are much more active in cancer prevention.²³ Furthermore, selenium supplementation is recognized for pharmacological intervention, especially in the clinical domain of cancer chemoprevention, and its use is not limited only to correct nutritional deficiencies. This is evident from the observation that epidemiological studies carried out for last three decades provide evidence of an inverse relationship between selenium intake and cancer mortality, and selenium supplementation in the diet or drinking water has been shown to inhibit neoplasms of the liver, skin, pancreas, colon and mammary glands.^{24, 25} Furthermore, two-thirds of animal studies showed reduced incidence of tumors triggered by chemical carcinogens or viruses following selenium supplementation.²⁶ The organoselenium compounds have also been reported to inhibit initiation and post-initiation stages of chemical carcinogenesis.²⁷ Mechanistically, organoselenium compounds have been suggested to activate certain pro-apoptotic genes linked to p53, NFκB and stress signal pathways thereby preventing tumorigenesis.²⁸ In addition, selenium supplementation could provide significant therapeutic potential since patients with melanoma, colon, breast, ovary, pancreas, as well as head and neck cancers show decreased levels of selenium in whole blood or serum than do healthy control. Thus, in view of the known anti-cancer properties of ITCs and organoselenium compounds, we have developed isosteric selenium analogs [the phenylalkyl isoselenocyanates (ISCs), **2**] of ITCs (**1**) (Figure 1). The objectives were to: (a) determine whether replacing sulfur in ITCs with selenium increases the cancer inhibitory potency; and (b) determine the optimal chain length for maximal anticancer activity. The overall goal was to establish whether altered chemical reactivity and lipophilicity, caused by the outlined structural modifications will affect potency of compounds and hence identify structural requirements necessary for optimal activity against multiple cancers. Thus, we synthesized a variety of selenium isostere of naturally occurring and synthetic ITCs and evaluated their effect on *in vitro* cell viability of melanoma, prostate, colon, glioblastoma, sarcoma, and breast cancers cell lines. The two classes of compounds were also evaluated for their ability to inhibit melanoma cell proliferation and induction of apoptosis *in vitro* and for inhibiting tumor development in preclinical melanoma xenograft.

Results

Synthesis of isoselenocyanates

The first synthesis of isoselenocyanates was reported by Barton *et al.*²⁹ starting from the corresponding formamides. Several modifications³⁰ in the procedure have been made since then to improve yields and avoid polymerization³¹ of intermediate compounds.^{29, 32} We have used the method of Fernandez-Bolanos *et al.*³³ which conveniently uses solid triphosgene, instead of phosgene,²⁹ in a one-pot dehydration of the formamides in refluxing dichloromethane (Scheme 1). The synthetic strategy involved the formylation of

phenylalkylamines, followed by treatment with triphosgene and selenium powder in the presence of triethylamine to furnish the desired phenylalkyl isoselenocyanates (**2**) in good yields. Compounds were purified by silica gel column chromatography and characterized by ¹H NMR and high-resolution MS.

SAR Study on cancer cell lines

The ITCs (**1**) and the corresponding ISCs (**2**) were tested for their ability to inhibit cell growth in six cancer cell lines e.g. melanoma (UACC 903), breast (MDA-MB-231), glioblastoma (T98G), fibrosarcoma (HT-1080), colon (Caco-2) and prostate (PC-3) cancer cell lines. The IC₅₀ values for compounds **1** and **2** are depicted in Table 1. The IC₅₀ values consistently decreased with increasing alkyl chain length of ITCs in case of glioblastoma, breast, and prostate cancer cell lines; but showed no particular trend in fibrosarcoma, colon and melanoma cells. In case of melanoma cell line, the IC₅₀ values remained essentially the same for all ITCs while in case of colon cancer cell line the IC₅₀ values actually increased with increasing carbon chain length of ITCs. Among ISC derivatives, ISC-1 was least effective at killing cancer cells compared to higher alkyl chain analogs ISC-2 to ISC-6. The difference was more striking in glioblastoma, prostate and breast cancer cell lines, while in other cancer cell lines, the difference between ISC-1 and higher alkyl chain ISCs was not significant. Among ISC-2, ISC-4 and ISC-6 there was no particular trend. In almost all the cases (except for comparable values of ISC-1 and ISC-2 with BITC and PEITC in UACC 903 cells) the ISC derivatives had lower IC₅₀ values than corresponding ITCs. This suggested superiority of ISC compounds over ITCs and that lower concentrations of selenium analogs would be required for similar therapeutic efficacy.

In vitro studies on melanoma cells

For melanoma cells (UACC 903), increase in chain length or changing from ITCs to ISCs by replacing sulfur with selenium, led to an insignificant change in IC₅₀ values (Table 1) indicating that both ISCs and ITCs are capable of inhibiting melanoma cell growth equally. Cell viability was measured using the MTS assay (Figure 2) which determines the capacity of mitochondrial dehydrogenase in viable cells to transform tetrazolium salt into colorimetric formazan.³⁴ UACC 903 human melanoma cells (5×10^3) were plated in a 96 well plate and 24 hours later exposed to DMSO or increasing concentrations (5 μM, 10 μM, 15 μM) of ITCs (**1**) or ISCs (**2**) for 24 hours. MTS assay showed a dose dependent decrease in cell viability for both ISC and ITC series of compounds. There was no significant difference in activities between the two series; longer alkyl chain analogs ISC-4 (at 10 and 15 μM concentrations) and ISC-6 (at 10 μM concentration) were found a little more effective than corresponding ITC derivatives. To establish the potency of ISCs in comparison to ITCs and quantify the effect of increasing chain length of these agents, cell proliferation and apoptosis was carried out in UACC 903 human melanoma cells. Cellular proliferation following ISC and ITC derivatives treatment was measured using bromodeoxyuridine (BrdU) incorporation which is a convenient tool to monitor DNA synthesis within the cells. A dose dependent decrease in number of proliferating cells was observed upon treatment with ITC or ISC derivatives (Figure 3). In general, ISC derivatives were more effective compared to corresponding ITCs with similar alkyl chain length, especially at higher concentrations. The caspase 3/7 activity, which is reflective of apoptosis, was determined using Apo-ONE Homogenous caspase-3/7 Assay kit (Promega Corporation, Madison, WI). A dose dependent increase in caspase-3/7 activity was observed for both ITCs and ISCs. In comparison to ITCs, ISC derivatives exhibited much higher caspase activity. While the difference was not significant between BITC and ISC-1, there was a striking difference between longer alkyl chain derivatives of ITCs and ISCs, especially at higher concentrations of 10 and 15 μM (Figure 4). ISC-4 and ISC-6 were found to be the most effective inducer of apoptosis among all other compounds tested.

ISCs reduce melanoma tumor development more effectively than ITCs

The *in vitro* studies in general suggested the superiority of ISC compounds over ITC derivatives. To further establish the effect *in vivo*, the efficacy of ISC and ITC compounds for inhibiting tumor development were evaluated in preclinical mouse model of melanoma. Nude mice were subcutaneously injected with UACC 903 cells and tumor development was allowed to occur for six days, by which time tumors have undergone vascularization (angiogenesis). Mice were then treated i.p with ISC and ITC derivatives three times a week on Mondays, Wednesdays and Fridays. At a dose of 0.76 μM (3 ppm of selenium), ISC-2, ISC-4 and ISC-6 showed a 30–45% reduction in tumor size (Figure 5A). ISC-1 had no effect at this concentration. Notably, none of the ITC derivatives were effective in reducing tumor size at 0.76 μM (data not shown). However, at a dose of 2.5 μM (~3 times that used for ISC compounds) a 40–60% reduction in tumor size was observed. No evidence of systemic toxicity was observed at the doses used for any of the ISC or ITC derivatives (Figure 5B and 5D). Interestingly, a reverse trend of chain length effect was observed i.e. the potency actually decreased with increasing alkyl chain length in ITCs, with BITC being the most effective. A similar trend in behavior was observed by Xiao *et al.*²⁰ in study where they observed BITC to be more effective compared to PEITC in breast cancer cell lines.

Synthesis and cell viability of *N*-acetylcysteine (NAC) conjugates

The activity of ITCs is reported to be through the formation of NAC conjugates, and presumably the ISCs may follow a similar mechanism of action. To determine that we synthesized NAC conjugate of representative ISC-4 and the corresponding PBITC by treating them with NAC in THF in the presence of NaOH (Scheme 2). The conjugates were characterized on the basis of ¹H NMR and high resolution MS spectra. The cell viability measurements on UACC 903 cells using MTS assay revealed an IC₅₀ value of 17 \pm 0.5 μM for the ISC-4-NAC conjugate (**6**) compared to 10 \pm 3 μM for ISC-4, and 24 \pm 1 μM for PBITC-conjugate (**5**) compared to 16 \pm 1 μM for PBITC.

Discussion

ITCs (R-N=C=S) are electrophilic compounds and are known to react predominantly with thiols, and to a much lesser extent with NH₂ and OH groups.³⁵ Therefore, the major route of metabolism and elimination of ITCs³⁶ from the body is the mercapturic acid pathway i.e. by formation of nonenzymatic and enzymatic conjugation with glutathione (GSH) to give thiol conjugates. Stepwise enzymatic hydrolysis of GSH conjugates of ITCs yields L-cysteine (Cys) conjugate (ITC-Cys), which on subsequent acetylation gives *N*-acetyl-L-cysteine (NAC) conjugates of ITCs (NAC-ITCs).^{37, 38} NAC conjugates of ITCs have been detected as the main metabolite in the urine of rodents and humans.³⁸ NAC and thiol conjugate of ITCs have shown a chemopreventive efficacy against TSNA and PAH-induced lung tumorigenesis in rodents.^{39–41} Conaway *et al.* have also shown that ITC conjugates have longer half-life than the parent isothiocyanates.³⁹ ISCs, being structurally similar to ITCs, are expected to follow a similar metabolic and elimination pathway. Thus, like ITCs, the activity of ISCs may also be due to the formation of NAC-ISC conjugate metabolites. Our results have indicated the IC₅₀ value of ISC-4- NAC conjugate (**6**) (17 \pm 0.5 μM) compared to ISC-4 (10 \pm 3 μM) and IC₅₀ value of PBITC-NAC conjugate (**5**) (24 \pm 1 μM) compared to PBITC (16 \pm 1 μM), were consistent with the trend reported in the literature for ITC and their corresponding conjugates,⁴² where NAC conjugates have been shown to be slightly less effective than the parent ITC. However, the comparable activity of ISC-4 and the corresponding NAC conjugate indicates that, similar to ITCs, the main route of metabolism and hence the *in vivo* activity of ISCs may be mediated by metabolic transformation to GSH conjugates.

Literature reports have attributed the increased potency with the increasing chain length of ITCs, to the increase in lipophilicity.¹⁷ It has been suggested that increasing lipophilicity decreases the reactivity of ITCs with glutathione (GSH) resulting in slowing down of excretion and thus enhancing the potency of the compound.⁴³ In an attempt to correlate ITC and ISC data to their lipophilicity, we calculated the LogP value of both ITC and ISC series of compounds using ChemDraw 9.0 Ultra. The LogP values increased with increasing chain length in both the series with ISC compounds in general having a slightly higher value than the corresponding ITC analogs (Table 2). The observed reverse trend of tumor inhibition decreasing with increasing alkyl chain length in melanoma model (Figure 5C) suggests that lipophilicity is not the only determining factor in case of ITCs. This reverse trend is in agreement with the Xiao *et al.* results in breast cancer cells.²⁰ ISC compounds however, showed the increased efficacy both *in vitro* and *in vivo* with increasing lipophilicity. Overall the results suggest the participation of other pharmacokinetic and pharmacodynamic factors that influence the efficacy of these compounds. Both ISC-4 and ISC-6 were equally effective *in vitro* and in inhibiting tumor growth. However, the higher lipophilicity of ISC-6 (LogP, 5.472), just like PHITC, makes it less suitable as a probable chemo-preventive/therapeutic agent. Higher lipophilicity of PHITC has been linked to the observed induction of esophageal carcinogenesis in Fisher 344 rats.⁴⁴ In view of this, ISC-4 is the best suitable agent for evaluation as a drug.

Conclusion

In summary, ISCs, the isosteric selenium analogs of well known naturally occurring and synthetic anticancer agents ITCs, have been developed. We have demonstrated that by isosterically replacing sulfur with selenium, we were able to significantly improve antitumor activity of ITCs. The ISC compounds were more efficient in inhibiting cell growth in melanoma, glioblastoma, fibrosarcoma, colon, breast, and prostate cancers cells as compared to corresponding ITC analogs. Furthermore, in general, the efficacy increased with increasing alkyl chain length both in case of ITC and ISC compounds. In preclinical melanoma xenograft model, the ISC compounds showed similar tumor inhibition at three times lower doses as compared to ITC derivatives without any measurable systemic toxicity. Interestingly, tumor inhibitory effect decreased with increasing chain length in ITCs while it increased with increasing chain length in case of ISCs. Collectively, based on both *in vitro* and *in vivo* experiments, ISC-4 proved to be the most effective agent among the series of ISC and ITC analogs tested. ISC-4 thus holds promise of being an effective chemopreventive/chemotherapeutic agent.

Experimental Section

General

Melting points were recorded on a Fischer-Johns melting point apparatus and are uncorrected. NMR spectra were recorded using a Varian 300 MHz NMR spectrometer or a Bruker Avance 500 MHz spectrometer. Chemical shifts (δ) were reported in parts per million downfield from the internal standard. The signals are quoted as s (singlet), d (doublet), t (triplet), m (multiplet) and dt (doublet of triplet). HRMS were determined at the Chemistry Instrumentation Center, State University of New York at Buffalo, NY. Thin-layer chromatography (TLC) was developed on aluminum-supported pre-coated silica gel plates (EM industries, Gibbstown, NJ). Column chromatography was conducted on silica gel (60–200 mesh). Benzyl isothiocyanate (BITC, **1a**), phenethyl isothiocyanate (PEITC, **1b**) were obtained from commercial sources. Phenylbutyl isothiocyanate (PBITC, **1c**) and phenylhexyl isothiocyanate (PHITC, **1d**) were synthesized according to a literature method.²¹

General method for synthesis of phenylalkyl formamides

Formamides (**4b–d**) were synthesized following a literature method.⁴⁵ Briefly, ethyl formate (120 mmol) was added dropwise to phenylalkylamine (40 mmol) at room temperature and the resulting mixture was refluxed for 4–6 h. The excess ethyl formate was removed under reduced pressure to yield the corresponding phenylalkylformamide as colorless viscous oils.

Phenylethyl formamide (**4b**)

Yield, 96%; viscous oil; ¹H NMR (CDCl₃, 300 MHz) δ 2.84 (t, 2H, *J* = 6.9 Hz), 3.57 (dt, 2H, *J* = 6.9 Hz and 6.6 Hz), 5.68 (br d, 1H, NH), 7.15–7.35 (m, 5H), 8.12 (s, 1H, CHO); HRMS (EI) calcd for C₉H₁₁NO, 149.0835; found, 149.0839.

Phenylbutyl formamide (**4c**)

Yield, 98%; viscous oil; ¹H NMR (CDCl₃, 500 MHz) δ 1.56–1.62 (m, 2H), 1.66–1.72 (m, 2H), 2.66 (t, 2H, *J* = 6.5 Hz), 3.35 (dt, 2H, *J* = 7.0 and 6.5 Hz), 5.92 (br s, 1H), 7.18–7.24 (m, 3H), 7.29–7.33 (m, 2H), 8.19 (s, 1H); HRMS (EI) calcd for C₁₁H₁₅NO, 177.1148; found, 177.1149.

Phenylhexyl formamide (**4d**)

Yield, 91%; viscous oil; ¹H NMR (CDCl₃, 500 MHz) δ 1.36–1.41 (m, 4H), 1.52–1.58 (m, 2H), 1.61–1.67 (m, 2H), 2.63 (t, 2H, *J* = 7.5 Hz), 3.30 (dt, 2H, *J* = 7.0 and 6.5 Hz), 5.58 (br s, 1H), 7.18–7.21 (m, 3H), 7.28–7.31 (m, 2H), 8.19 (s, 1H); HRMS (EI) calcd for C₁₃H₁₉NO, 205.1461; found, 205.1462.

Benzyl isoselenocyanate (ISC-1, **2a**)

To a refluxing mixture of the benzyl formamide (1.35 g, 10.0 mmol), triethylamine (4.35 g, 6.0 mL, 43.0 mmol) in CH₂Cl₂ (35 mL) and 4 Å molecular sieves was added dropwise a solution of triphosgene (1.48 g, 5.0 mmol) in CH₂Cl₂ (15 mL) for a period of 1 h. After the addition was complete, the mixture was refluxed for an additional 2.5 h. Selenium powder (1.58 g, 20 mmol) was then added and the resulting mixture was refluxed for additional 7 h. The mixture was cooled, filtered, and the solvent was evaporated to yield the crude mixture, which was purified by silica gel column chromatography (EtOAc/hexanes 3:97) to afford 1.21 g (62 %) of **2a** as a viscous oil. ¹H NMR (CDCl₃, 300 MHz) δ 4.81 (s, 2H, CH₂), 7.30–7.44 (m, 5H); HRMS (EI) calcd for C₈H₇NSe, 196.9738; found, 196.9741.

Phenylethyl isoselenocyanate (ISC-2, **2b**)

A mixture of phenethyl formamide (0.67 g, 4.5 mmol), triethylamine (1.94 g, 2.67 mL, 19.2 mmol), 4 Å molecular sieves, triphosgene (0.65 g, 2.2 mmol), and selenium powder (0.71 g, 9.0 mmol) in CH₂Cl₂ (35 mL) was refluxed and worked up as mentioned above for **2a**. The crude residue thus obtained was purified by silica gel column chromatography (EtOAc/hexanes 5:95) to give 0.62 g (65%) of **2b** as an oil. ¹H NMR (CDCl₃, 300 MHz) δ 3.03 (t, 2H, *J* = 6.9 Hz), 3.81 (t, 2H, *J* = 6.9 Hz), 7.20–7.38 (m, 5H); HRMS (EI) calcd for C₉H₉NSe, 210.9895; found, 210.9892.

Phenylbutyl isoselenocyanate (ISC-4, **2c**)

A mixture of phenylbutyl formamide (1.77 g, 10.0 mmol), triethylamine (4.35 g, 6.0 mL, 43 mmol), 4 Å molecular sieves, triphosgene (1.48 g, 5.0 mmol), and selenium powder (1.58 g, 20.0 mmol) in CH₂Cl₂ (50 mL) was refluxed and worked up as mentioned above for **2a**. The crude residue thus obtained was purified by silica gel column chromatography (EtOAc/hexanes 5:95) to give 1.7 g (71%) of **2c** as an oil. ¹H NMR (CDCl₃, 300 MHz) δ 1.74–1.76 (m, 4H), 2.66 (t, 2H, *J* = 6.6 Hz), 3.60 (t, 2H, *J* = 5.6 Hz), 7.15–7.32 (m, 5H); HRMS (EI) calcd for C₁₁H₁₃NSe, 239.0208; found, 239.0211.

Phenylhexyl isoselenocyanate (ISC-6, 2d)

A mixture of phenylbutyl formamide (1.64 g, 8.0 mmol), triethylamine (3.49 g, 4.8 mL, 34.5 mmol), 4 Å molecular sieves, triphosgene (1.19 g, 4.0 mmol), and selenium powder (1.26 g, 16.0 mmol) in CH₂Cl₂ (40 mL) was refluxed and worked up as mentioned for **2a**. The crude residue thus obtained was purified by silica gel column chromatography (EtOAc/hexanes 3:97) to give 1.35 g (63%) of **2d** as an oil. ¹H NMR (CDCl₃, 500 MHz) δ 1.37–1.43 (m, 2H), 1.45–1.51 (m, 2H), 1.64–1.70 (m, 2H), 1.72–1.78 (m, 2H), 2.64 (t, 2H, *J* = 7.6 Hz), 3.60 (t, 2H, *J* = 6.7 Hz), 7.19–7.25 (m, 3H), 7.32–7.40 (m, 2H); HRMS (EI) calcd for C₁₃H₁₇NSe, 267.0521; found, 267.0529.

PBITC-NAC conjugate (5)

To a solution of **1c** (382 mg, 2.0 mmol) in THF (25 mL) at 0 °C was added a solution of *N*-acetylcysteine (326 mg, 2.0 mmol) in water (15 mL) and to this mixture was added 2% NaOH (0.1 mL). The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The mixture was washed with hexane (2 × 5 mL), the aqueous layer was acidified 2N HCl and extracted with ethyl acetate. Organic layer was separated, dried (MgSO₄) and concentrated in vacuo to give **5** as a viscous oil in quantitative yield. ¹H NMR (CD₃OD, 500 MHz) δ 1.67–1.70 (m, 4H), 1.96 (s, 3H), 2.01–2.03 (m, 2H), 2.64–2.67 (m, 2H), 3.69–3.74 (m, 2H), 4.67 (dd, 1H, *J* = 8.5 and 4.5 Hz), 7.14–7.22 (m, 3H), 7.25–7.28 (m, 2H); HRMS (ESI) calcd for C₁₆H₂₂N₂O₃S₂.H⁺, 355.1145; found, 354.1144.

ISC-4-NAC conjugate (6)

To a solution of **2c** (238 mg, 1.0 mmol) in THF (15 mL) at 0 °C was added a solution of *N*-acetylcysteine (163 mg, 1.0 mmol) in water (7.0 mL) and to this mixture was added 2% NaOH (50 μL). Following similar reaction conditions and work up as mentioned for **5**, gave **6** as a viscous oil in quantitative yield. ¹H NMR (CD₃OD, 500 MHz) δ 1.73–1.78 (m, 2H), 1.80–1.84 (m, 4H), 1.98 (s, 3H), 2.69 (t, 2H, *J* = 8.0 Hz), 3.63–3.67 (m, 2H), 4.69–4.73 (m, 1H), 7.17–7.30 (m, 5H), 7.67 (d, 1H, *J* = 7.0 Hz), 9.92 (br s, 1H); HRMS (ESI) calcd for C₁₆H₂₂N₂O₃SSe.H, 403.0589; found, 403.0576.

Cell lines and culture conditions

Colon adenocarcinoma cell line (Caco-2, ATCC No. HTB-37) was grown in Advanced DMEM supplemented with 10% heat treated (56°C for 30 minutes) FBS and L-glutamine. Fibrosarcoma (HT-1080; ATCC No. CCL-121), prostate adenocarcinoma (PC-3; ATCC No. CRL-1435), breast adenocarcinoma cell line (MDA-MB-231; ATCC No. HTB-26), glioblastoma cell line (T98G; ATCC No. CRL-1690) and human melanoma cell line UACC 903 were grown in DMEM supplemented with 10% FBS.

Cell viability, proliferation, and apoptosis determination

In vitro inhibitory efficacy of cancer cell lines representing different cancer types following treatment with ITC and ISC was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, 2.5 – 5 × 10³ cells per well in 100 μL DMEM containing 10% FBS were grown in a 96-well plate for 24 h and treated with either control DMSO vehicle or increasing concentrations (2.5 – 100 μM) of ITC and ISC for 24 hours. The percentages of viable cells compared to control DMSO treated cells were determined using MTS assay and IC₅₀ values calculated using GraphPad Prism version 4.01 (GraphPad software, San Diego, CA). IC₅₀ value for each compound was determined by at least three independent experiments and represented with a standard error (Table 1).

Cellular proliferation and apoptosis rates were measured by seeding 5×10^3 human melanoma cell line UACC 903 in 96-well plate, followed by treatment for 24 hours with ITCs or ISCs. Proliferation and apoptosis rates were measured using a BrdU ELISA kit (Roche Applied Sciences, Indianapolis, IN) or Apo-ONE Homogenous caspase-3/7 Assay kit (Promega Corporation, Madison, WI), respectively.

Tumorigenicity assessment

Animal experimentation was performed according to protocols approved by the Institutional Animal Care and Use Committee at The Pennsylvania State University College of Medicine. Tumor kinetics were measured by subcutaneous injection of 5×10^6 UACC 903 melanoma cells in 0.2 ml of DMEM supplemented with 10% FBS above both left and right rib cages of 4–6 week old female athymic nude mice (Harlan Sprague Dawley, Indianapolis, IN). Six days later mice were randomly divided in to control (DMSO) and experimental (ISC-1, ISC-2, ISC-4, ISC-6, BITC, PEITC, PBITC, PHITC) groups (5 mice/group; 2 tumors/mouse). Six days after subcutaneous injection of UACC 903 melanoma cells, mice were treated i. p. with ITC (2.5 μ moles) or ISC (0.76 μ moles, equivalent to 3 ppm selenium) three times per week. (Monday, Wednesday and Friday). Control mice received an equal volume of the vehicle. The Dimensions of the developing tumors (using calipers) and body weight were measured three times a week (Monday, Wednesday and Friday) and the size estimated in cubic millimeters.

Statistical analysis

Statistical analysis was undertaken using the One-way ANOVA followed by an appropriate *post hoc* test. Results were considered significant at a *P*-value of <0.05 .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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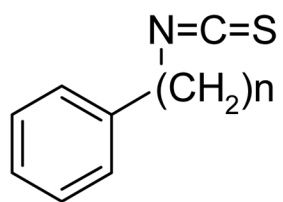
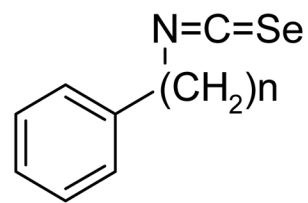
**1****Isothiocyanates****2****Isoselenocyanates**

Figure 1.
General structures of phenylalkyl isothiocyanates (**1**) and phenylalkyl isoselenocyanates (**2**).

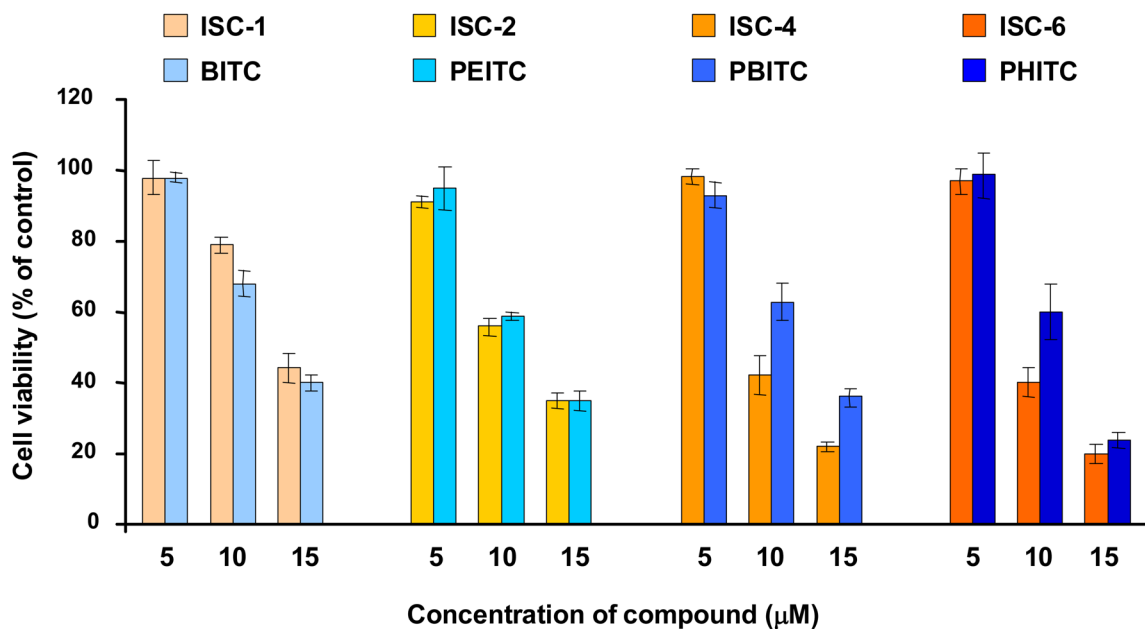


Figure 2. MTS assay showing the viability of UACC 903 cells after treatment with ISC and ITC derivatives. Both ITCs (BITC, PEITC, PBITC and PHITC) and ISCs (ISC-1, ISC-2, ISC-4 and ISC-6) effectively reduced cell viability compared to DMSO control. The average value is represented as the percentage of control DMSO treated cells. The values for all experiments represent mean with bars indicating SEM from three independent experiments.

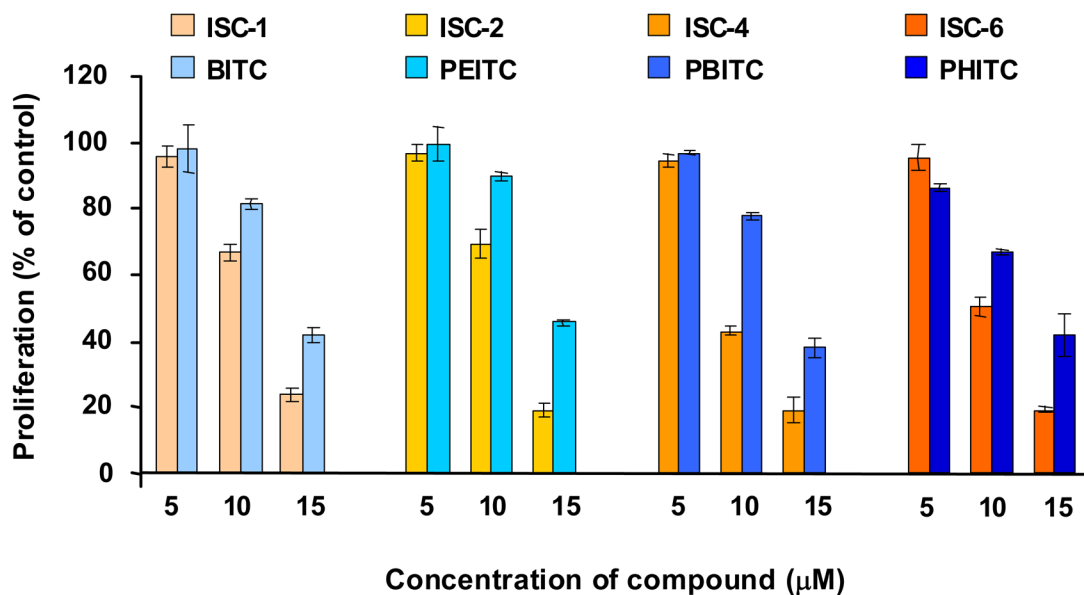


Figure 3.

Proliferation profile of UACC 903 cells after treatment with ISC and ITC derivatives. 5×10^3 cells were treated with DMSO or increasing concentrations of ITCs or ISCs for 24 hours and proliferating cells measured using BrdU labeling. The average value represented as the percentage of control DMSO treated cells. The values for all experiments represent mean with bars indicating SEM from three independent experiments.

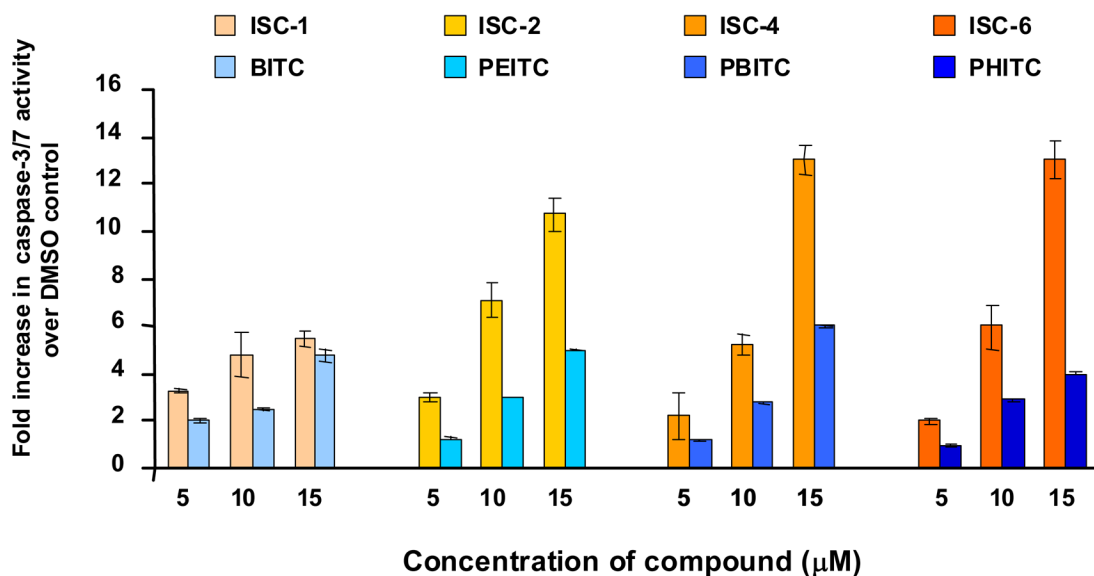


Figure 4.

Levels of caspase-3/7 activity (an indicator of apoptosis) in cells exposed to ITCs or ISCs were measured using the Apo-ONE homogeneous caspase-3/7 assay kit. Results show fold increase in caspase-3/7 activity relative to DMSO vehicle treated cells. Results represent average of three independent experiments; bars, SEM. Compared to DMSO control, increasing concentrations of ISCs or ITCs elevated caspase-3/7 activity in a dose dependent manner. ISC-2, ISC-4 and ISC-6 showed significantly higher caspase-3/7 activity compared to their corresponding ITCs.

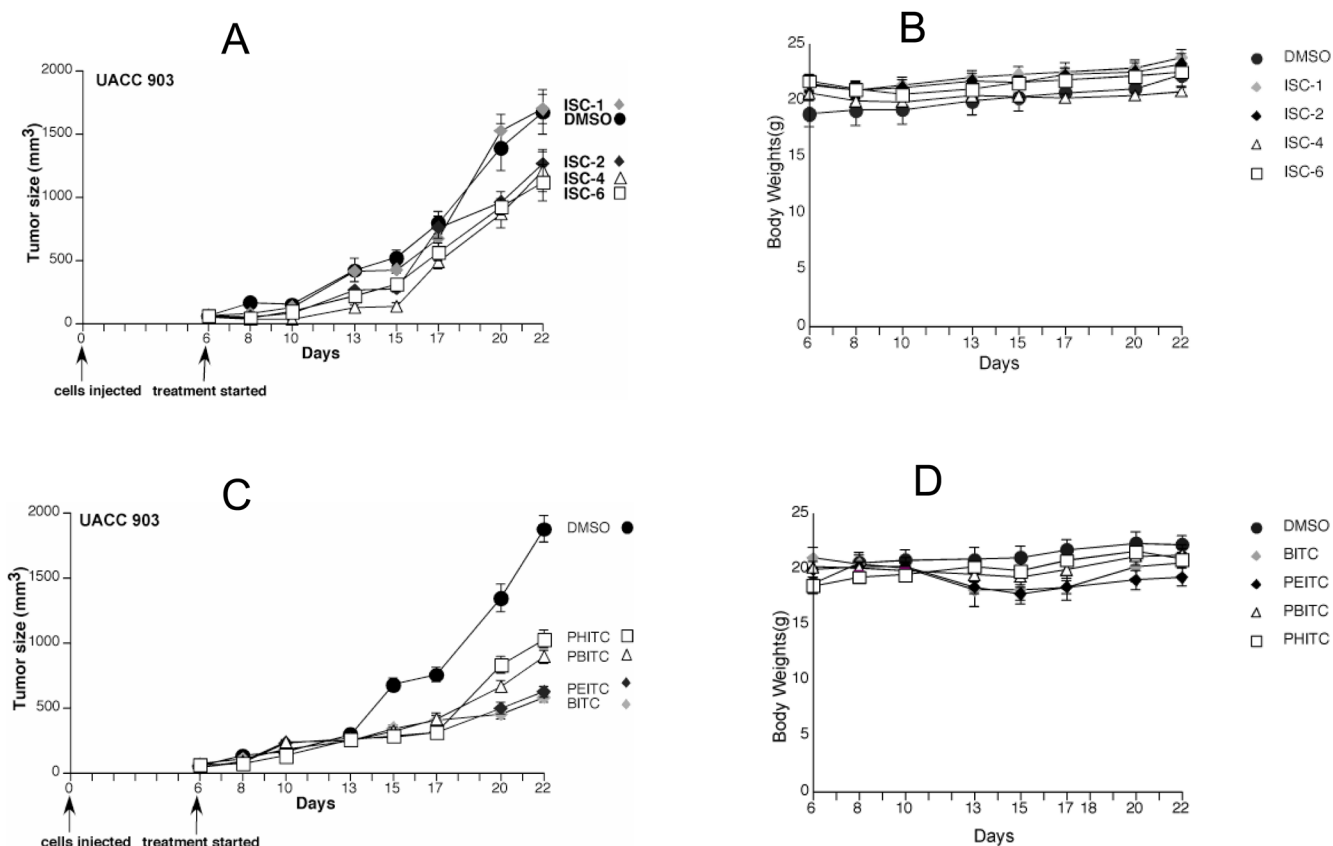
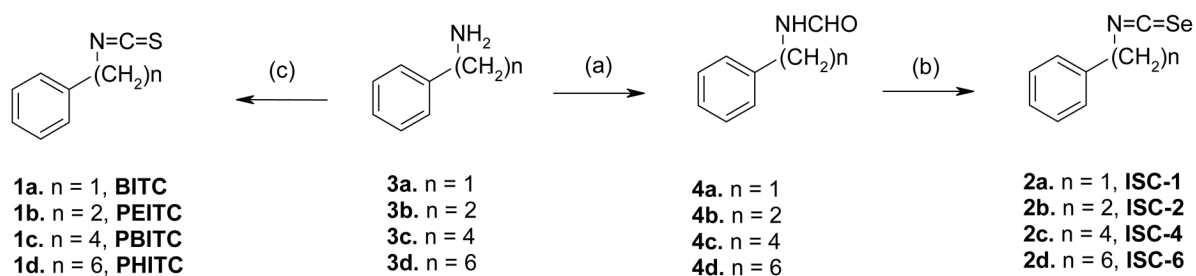
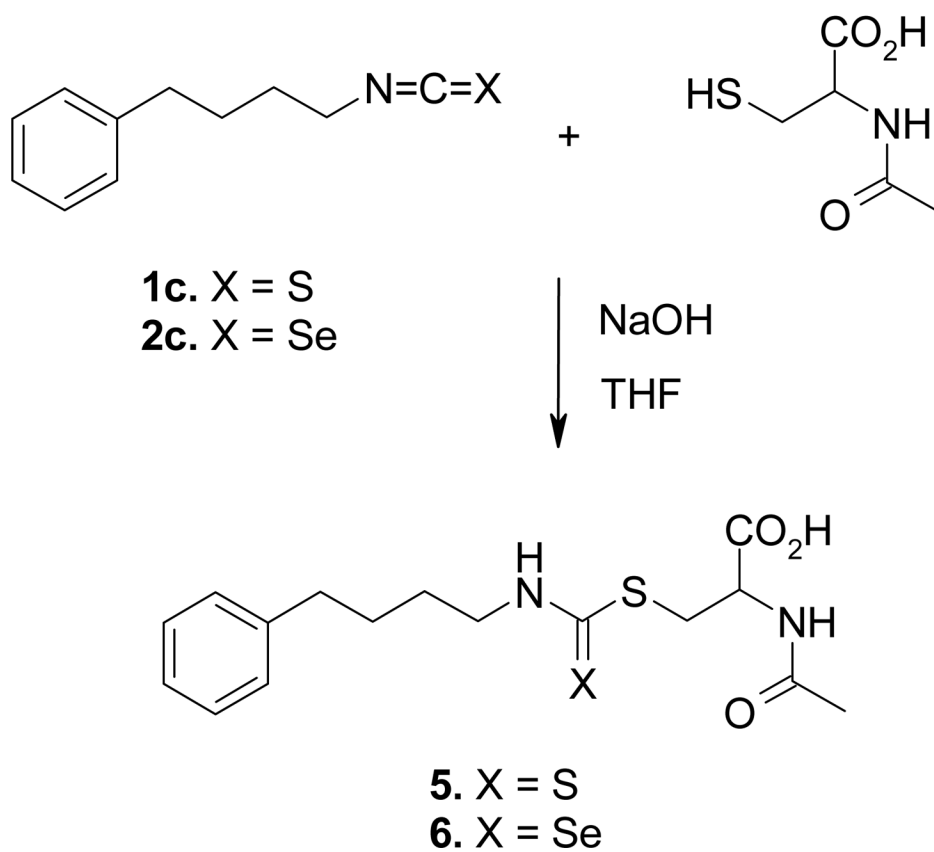


Figure 5. *In vivo* melanoma tumor inhibition using ISC and ITC derivatives

Six days after subcutaneous injection of UACC 903 cells, mice were treated i. p. with ISC or ITC derivatives thrice per week. Both ISC and ITC derivatives significantly reduced tumor development, however, the concentration of ISC (0.76 μmol) was 3 fold less than ITCs (2.5 μmol) (Figures 5A and 5C). At a dose of 0.76 μmoles , ISC-2, ISC-4 and ISC-6 showed about 30–45% reduction in tumor size (Figure 5A). ISC-1 failed to show any effect at this concentration. ITC derivatives were also effective in reducing the tumor size at 2.5 μmoles (~3 times higher than ISC compound) (Figure 5C). Figures 5B and 5D show the body weights of mice treated with ISC and ITC derivatives, respectively, compared to the control DMSO vehicle treated mice. No significant difference in weights was detected between groups, demonstrating negligible systemic toxicity.

**Scheme 1.**

Synthesis of ITCs **1** and ISC-2; Reagents and conditions: (a) C_2H_5OCHO , $-20^\circ C$ to reflux
 (b) Et_3N , triphosgene, Se powder, CH_2Cl_2 , reflux (c) $CSCl_2$, NaOH.



Scheme 2.
Synthesis of NAC conjugates of PBITC and ISC-4.

TABLE 1

IC₅₀ (μM) of ITC and ISC derivatives on different cancer cells.

Compounds	Cancer cell lines IC ₅₀ (μM)					
	Breast MDA-MB-231	Glioblastoma T98G	Prostate PC-3	Fibrosarcoma HT-1080	Colon* Caco-2	Melanoma UACC 903
1a (BITC)	42±3	>100	>50	>50	15±2	15±3
1b (PEITC)	38±6	>100	24±2	15±1	14±2	12±1
1c (PBITC)	27±2	35±1	24±2	15±1	27±2	16±1
1d (PHITC)	24±2	26±2	17±1	29±3	49±9	15±2
2a (ISC-1)	29±2	43±4	24±1	13±3	13±3	16±3
2b (ISC-2)	20±3	24±1	16±1	12±3	11±1	12±4
2c (ISC-4)	21±6	27±1	19±1	11±1	12±3	10±3
2d (ISC-6)	22±2	23±2	14±1	12±1	10±1	10±1

Values are mean ± S.E.

* Drug treatment for 72 h

TABLE 2Calculated LogP^a (CLogP) values of ITC and ISC compounds.

ITCs	CLogP	ISCs	CLogP
1a (BITC)	3.204	2a (ISC-1)	3.177
1b (PEITC)	3.263	2b (ISC-2)	3.506
1c (PBITC)	4.171	2c (ISC-4)	4.414
1d (PHITC)	5.229	2d (ISC-6)	5.472

^aLogP was estimated using ChemDraw 9.0 Ultra