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

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Synthesis and identification of novel phenylalkyl isoselenocyanates (ISCs), isosteric selenium analogues of naturally occurring phenylalkyl isothiocyanates (ITCs), as effective cytotoxic and antitumor agents are described. The structure–activity relationship comparison of ISCs with ITCs and effect of the increasing alkyl chain length in inhibiting cancer cell growth were evaluated on melanoma, prostate, breast, glioblastoma, sarcoma, and colon cancer cell lines. IC_{50} values for ISC compounds were generally lower than their corresponding ITC analogues. Similarly, in UACC 903 human melanoma cells, the inhibition of cell proliferation and induction of apoptosis were more pronounced with ISCs 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 3 times lower doses of ISCs 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] that inhibit carcinogenicity in animal models.[5] In addition, epidemiological studies have demonstrated that the human consumption of isothiocyanates in vegetables decreases cancer risk.[6,7] This fact is supported by strong literature data that suggest 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 postinitiation and progression stages.[12−16]

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 six 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 analogues 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 analogues, 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 past 3 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 postinitiation stages of chemical carcinogenesis.[27] Mechanistically, organoselenium compounds have been suggested to activate certain proapoptotic 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 controls. Thus, in view of the known anticancer properties of ITCs and oraganoselenium coumpounds, we have developed isosteric selenium analogues [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

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

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

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 to 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 to inhibit tumor development in preclinical melanoma xenograft.

Results

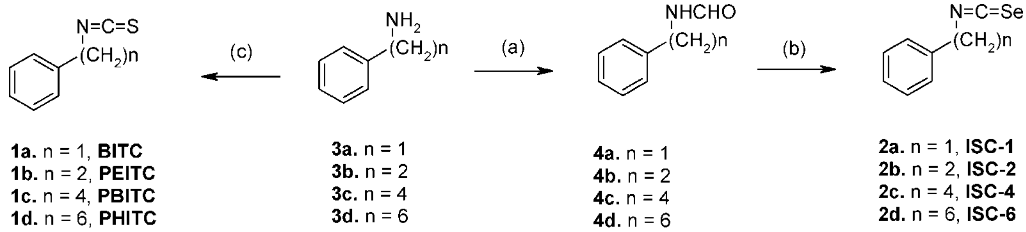
Synthesis of Isoselenocyanates. The first synthesis of isoselenocyanates was reported by Barton et al.[29] starting from the corresponding formamides. Several modifications[30] in the procedure have been made since then to improve yields and avoid polymerization[31] of intermediate compounds.[29,32] 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 ^{1}H 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 IC_{50} values for compounds **1** and **2** are depicted in Table 1. The IC_{50} values consistently decreased with increasing alkyl chain length of ITCs in the cases of glioblastoma, breast, and prostate cancer cell lines but showed no particular trend in fibrosarcoma, colon, and melanoma cells. In the case of the melanoma cell line, the IC_{50} values remained essentially the same for all ITCs while in the case of the colon cancer cell line the IC_{50} 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 analogues 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 IC_{50} values than corresponding ITCs. This suggested superiority of ISC compounds over ITCs and that lower concentrations of selenium analogues would be required for similar therapeutic efficacy.

In Vitro Studies on Melanoma Cells. For melanoma cells (UACC 903), an increase in chain length or a change from ITCs to ISCs by replacement of sulfur with selenium led to an insignificant change in IC_{50} 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 h later exposed to DMSO or increasing concentrations (5, 10, and 15 μM) of ITCs (**1**) or ISCs (**2**) for 24 h. 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 analogues ISC-4 (at 10 and 15 μM) and ISC-6 (at 10 μM) were found to be a little more effective than the corresponding ITC derivatives. To establish the potency of ISCs in comparison to ITCs and to quantify the effect of increasing chain length of these agents, cell proliferation and apoptosis were 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 homogeneous 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 was evaluated in preclinical mouse model of melanoma. Nude mice were subcutaneously injected with UACC 903 cells, and tumor development was allowed to occur for 6 days, by which time tumors have undergone vascularization (angiogenesis). Mice were then treated ip 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 (Figure 5C). No evidence of systemic toxicity was observed at the doses used for any of the ISC or ITC derivatives (parts B and D of Figure 5). 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 a study where they observed BITC to be more effective compared to PEITC in breast cancer cell lines.

Scheme 1. Synthesis of ITCs **1** and ISCs **2**[a]



[a] Reagents and conditions: (a) C_2H_5OCHO, −20 °C to reflux; (b) Et_3N, triphosgene, Se powder, CH_2Cl_2, reflux; (c) $CSCl_2$, NaOH.

Table 1. IC_{50} (μM) of ITC and ISC Derivatives on Different Cancer Cells

compd	cancer cell line, IC_{50} (μM)[a] breast MDA-MB-231	glioblastoma T98G	prostate PC-3	fibrosarcoma HT-1080	colon[b] 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

[a] Values are mean values ± SE. [b] Drug treatment for 72 h.

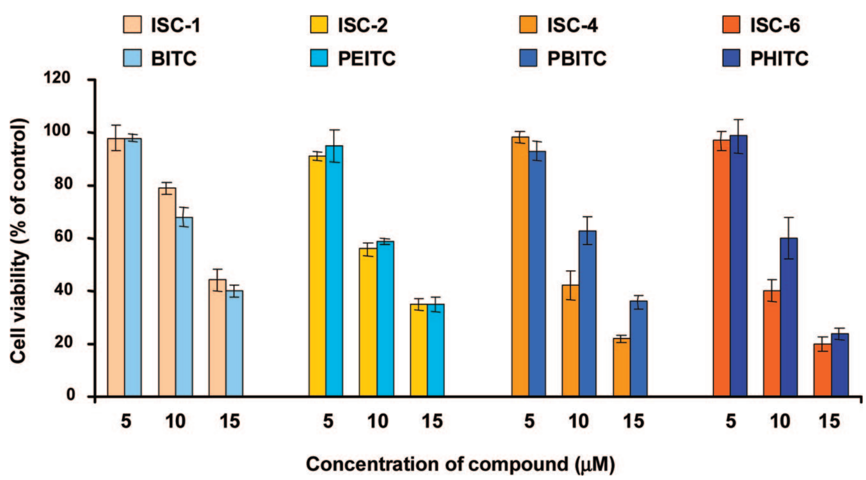


Figure 2. MTS assay showing the viability of UACC 903 cells after treatment with ISC and ITC derivatives. Both ITCs (BITC, PEITC, PBITC, and PHITC) and ISCs (ISC-1, ISC-2, ISC-4, and ISC-6) effectively reduced cell viability compared to DMSO control. The average value is represented as the percentage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

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 conjugates 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 IC_{50} value of 17 ± 0.5 μM for the ISC-4−NAC conjugate (**6**) compared to 10 ± 3 μM for ISC-4 and a value of 24 ± 1 μM for the PBITC−NAC conjugate (**5**) compared to 16 ± 1 μM for PBITC.

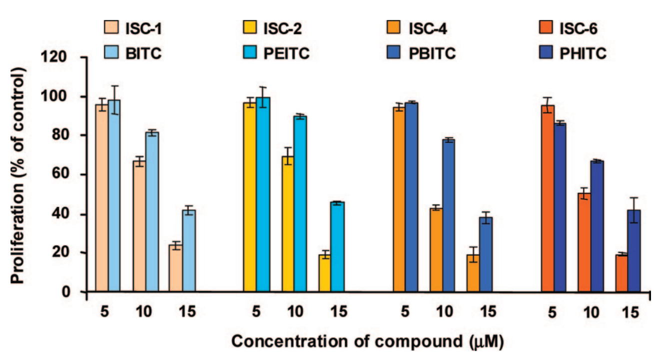


Figure 3. Proliferation profile of UACC 903 cells after treatment with ISC and ITC derivatives. The 5×10^3 cells were treated with DMSO or increasing concentrations of ITCs or ISCs for 24 h and proliferating cells measured using BrdU labeling. The average value is represented as the percentage of control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

Discussion

ITCs (R−N=C=S) are electrophilic compounds and are known to react predominantly with thiols and to a much lesser extent with NH_2 and OH groups.[35] Therefore, the major route

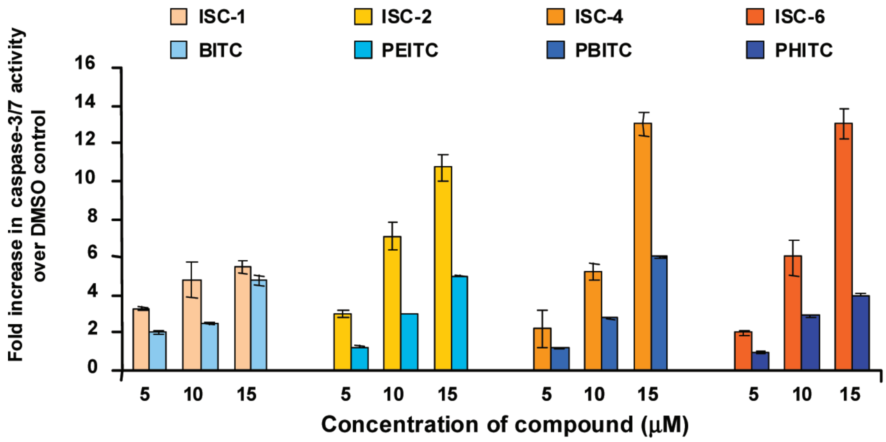


Figure 4. Levels of caspase-3/7 activity (an indicator of apoptosis) in cells exposed to ITCs or ISCs were measured using the Apo-ONE homogeneous caspase-3/7 assay kit. Results show fold increase in caspase-3/7 activity relative to DMSO vehicle treated cells. Results represent the average of three independent experiments; bars represent 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.

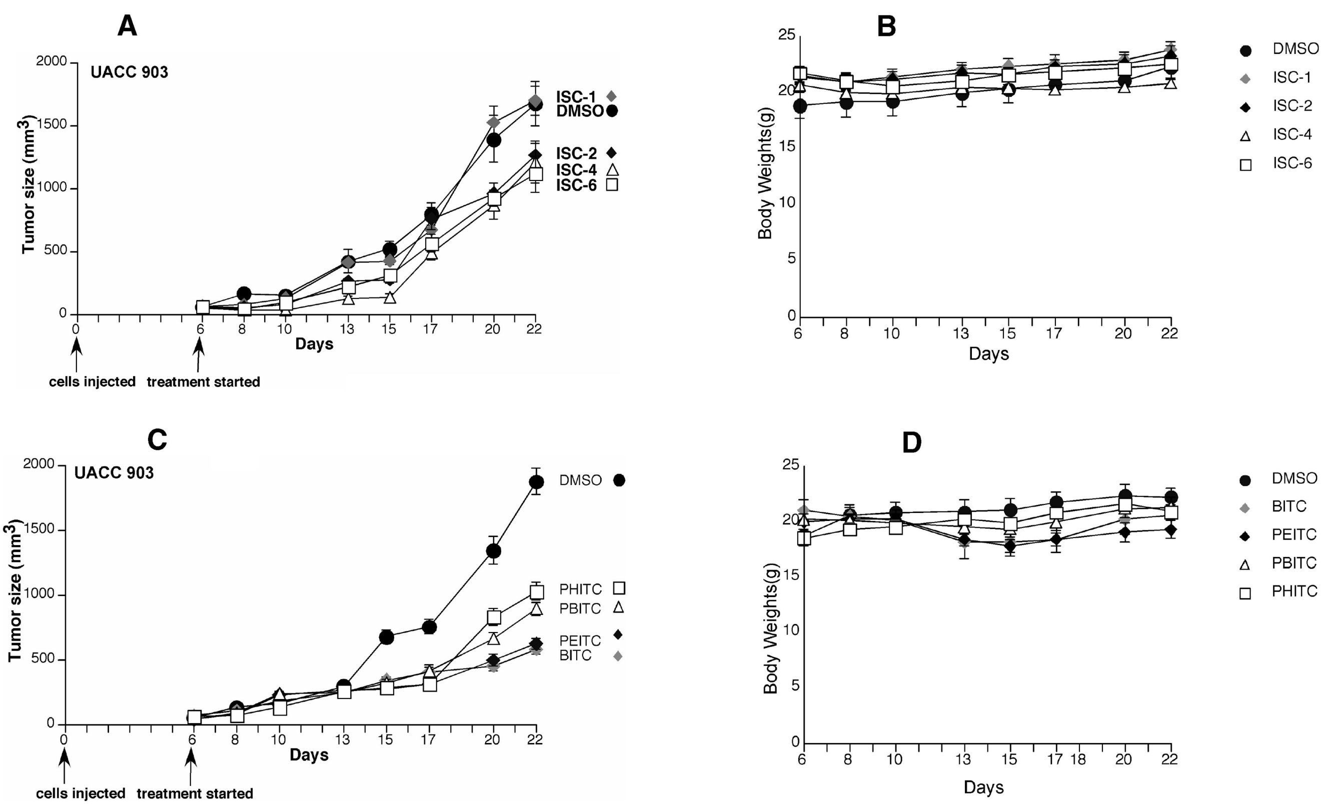


Figure 5. In vivo melanoma tumor inhibition using ISC and ITC derivatives. Six days after subcutaneous injection of UACC 903 cells, mice were treated ip with ISC or ITC derivatives thrice per week. Both ISC and ITC derivatives significantly reduced tumor development; however, the concentration of ISC (0.76 μmol) was 3-fold less than ITCs (2.5 μmol) (A, C). At a dose of 0.76 μmol, ISC-2, ISC-4, and ISC-6 showed about 30−45% reduction in tumor size (A). ISC-1 failed to show any effect at this concentration. ITC derivatives were also effective in reducing the tumor size at 2.5 μmol (~3 times higher than ISC compound) (C). Parts B and D 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.

of metabolism and elimination of ITCs[36] 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 conjugates 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-lives 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 that the IC_{50} value of the ISC-4−NAC conjugate (**6**) (17 ± 0.5 μM) compared to that of the ISC-4 (10 ± 3 μM) and

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

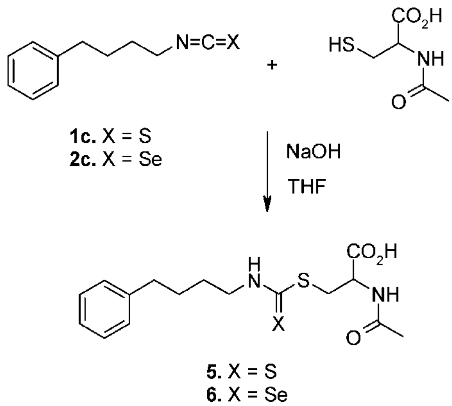


Table 2. Calculated log P [a] (CLogP) Values of ITC and ISC Compounds

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

[a] Calculated log P values were estimated using ChemDraw Ultra 9.0.

the IC_{50} value of the PBITC−NAC conjugate (**5**) (24 ± 1 μM) compared to that of the PBITC (16 ± 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 a slowing 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 log P value of both ITC and ISC series of compounds using ChemDraw 9.0 Ultra. The log P values increased with increasing chain length in both series, with ISC compounds in general having a slightly higher value than the corresponding ITC analogues (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 the case of ITCs. This reverse trend is in agreement with the Xiao et al. results in breast cancer cells.[20] ISC compounds, however, showed 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 (log P = 5.472), just like PHITC, makes it less suitable as a probable chemopreventive/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 most suitable agent for evaluation as a drug.

Conclusion

In summary, ISCs, the isosteric selenium analogues 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 compared to corresponding ITC analogues. Furthermore, in general, the efficacy increased with increasing alkyl chain length in the cases of ITC and ISC compounds. In the preclinical melanoma xenograft model, the ISC compounds showed similar tumor inhibition at 3 times lower doses 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, on the basis of both in vitro and in vivo experiments, ISC-4 proved to be the most effective agent among the series of ISC and ITC analogues 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 results were determined at the Chemistry Instrumentation Center, State University of New York at Buffalo, NY. Thin-layer chromatography (TLC) was developed on aluminum-supported precoated silica gel plates (EM industries, Gibbstown, NJ). Column chromatography was conducted on silica gel (60−200 mesh). Benzyl isothiocyanate (BITC, **1a**) and 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 Phenylalkylformamides. 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 phenylakylformamide as colorless viscous oils.

Phenylethylformamide (4b). Yield, 96%; viscous oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.84 (t, 2H, J = 6.9 Hz), 3.57 (dt, 2H, J = 6.9 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 $C_9H_{11}NO$, 149.0835; found, 149.0839.

Phenylbutylformamide (4c). Yield, 98%; viscous oil; ^{1}H NMR ($CDCl_3$, 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 $C_{11}H_{15}NO$, 177.1148; found, 177.1149.

Phenylhexylformamide (4d). Yield, 91%; viscous oil; ^{1}H NMR ($CDCl_3$, 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 $C_{13}H_{19}NO$, 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 CH_2Cl_2 (35 mL), and 4 Å molecular sieves was added dropwise a solution of triphosgene (1.48 g, 5.0 mmol) in CH_2Cl_2 (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 an additional 7 h. The mixture was cooled and 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. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.81 (s, 2H, CH_2), 7.30−7.44 (m, 5H). HRMS (EI) calcd for C_8H_7NSe, 196.9738; found, 196.9741.

Phenylethyl Isoselenocyanate (ISC-2, 2b). A mixture of phenethylformamide (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 CH_2Cl_2 (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. ^{1}H NMR ($CDCl_3$, 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 C_9H_9NSe, 210.9895; found, 210.9892.

Phenylbutyl Isoselenocyanate (ISC-4, 2c). A mixture of phenylbutylformamide (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 CH_2Cl_2 (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. ^{1}H NMR ($CDCl_3$, 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 $C_{11}H_{13}NSe$, 239.0208; found, 239.0211.

Phenylhexyl Isoselenocyanate (ISC-6, 2d). A mixture of phenylbutylformamide (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 CH_2Cl_2 (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. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.37−1.43 (m, 2H), 1.45−1.51 (m, 2H), 1.64−170 (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 $C_{13}H_{17}NSe$, 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), and the aqueous layer was acidified with 2 N HCl and extracted with ethyl acetate. The organic layer was separated, dried ($MgSO_4$), and concentrated in vaccuo to give **5** as a viscous oil in quantitative yield. ^{1}H NMR (CD_3OD, 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 $C_{16}H_{22}N_2O_3S_2 \cdot H^+$, 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 workup as mentioned for **5** gave **6** as a viscous oil in quantitative yield. ^{1}H NMR (CD_3OD, 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 $C_{16}H_{22}N_2O_3SSe \cdot H$, 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 min) 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)-2*H*-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, (2.5−5) × 10^3 cells per well in 100 μL of 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 h. The percentages of viable cells compared to control DMSO treated cells were determined using MTS assay and IC_{50} values calculated using GraphPad Prism, version 4.01 (GraphPad software, San Diego, CA). The IC_{50} 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 a 96-well plate, followed by treatment for 24 h with ITCs or ISCs. Proliferation and apoptosis rates were measured using a BrdU ELISA kit (Roche Applied Sciences, Indianapolis, IN) or Apo-ONE homogeneous 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 into 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 ip with ITC (2.5 μmol) or ISC (0.76 μmol, equivalent to 3 ppm selenium) three times per week (Mondays, Wednesdays, and Fridays). 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 (Mondays, Wednesdays, and Fridays), and the size was 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.

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Supporting Information Available: ^{1}H NMR spectra for compounds **1c**, **1d**, **2a**−**d**, **4b**−**d**, **5**, and **6** and ^{1}H NMR data for compounds **1c** and **1d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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