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Synthesis and evaluation of antioxidative properties of a series of organoselenium compounds

Sk. Ugir Hossain, Sumitra Sengupta and Sudin Bhattacharya*

Department of Cancer Chemoprevention, Chittaranjan National Cancer Institute, 37, SP Mukherjee Road, Kolkata 700 026, India

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Abstract—A series of organoselenocyanate compounds **4a–d** were synthesized utilizing 1,8-naphthalic anhydride as the building unit. These compounds were evaluated for their antioxidative activities against DMBA–PMA-induced oxidative stress in a two-stage mouse skin carcinogenic model. Compound **4d** was found to have the maximum antioxidative property in comparison with the other compounds. Also, the pretreatment group showed better results than the concomitant treatment groups.

1. Introduction

Selenium is an essential micronutrient for both animals and humans. Selenium deficiency has been implicated as playing a role in the development of many diseases, including cancer.[1] Epidemiological studies have also suggested an inverse relationship between selenium intake and risk for cancer.[2] It is known from different studies that the formulation of the selenium-containing compound and not the element per se is critical for biological activities.[3] Therefore, it is essential to determine which structural requirements govern and which provide optimal biological activities of selenium compounds. Organoselenium compounds have been found to be less toxic than inorganic selenium compounds; as a result, there has been a growing interest in the synthesis of organoselenium compounds with respect to their use in enzymology and bioorganic chemistry. Organoselenocyanate received wide attention for its better cancer chemopreventive properties. Few organoselenocyanates are reported in the literature for their cancer chemopreventive properties against lung, liver, colon, and mammary gland cancer. These are benzyl selenocyanate,[4] diphenylmethyl selenocyanate,[5,6] phenylenebis(methylene)selenocynate,[7,8] its ortho and meta isomers,[9] and some aliphatic selenocyanates.[10] Naphthalimides are reported for their antitumor activities.[11–13] Two members of this class of compounds, amonifide and mitonafide, are in clinical trials.[14]

Free radicals are formed during normal cellular metabolism and they are known to contribute to healthy functions in human health and development when they are not excessive. Formation of free radicals is not limited to normal cellular process but also occur upon exposure to certain chemicals (polycyclic aromatic hydrocarbon, cadmium, lead, etc.), radiation, cigarette smoke, and high fat diet. Exposure of a healthy cell to free radicals is known to damage structures and consequently interfere with functions of enzymes and critical macromolecule. Mammalian cells possess elaborate defense mechanisms to detoxify free radicals. A balance between formation of free radicals and their detoxification is essential for normal cellular function. When such a balance is disrupted as a result of excessive generation of damaging species or low levels of antioxidants, a cell enters into a state of oxidative stress and is damaged. If the damage persists, the cell will enter into a state of genetic instability that can lead to chronic diseases including cancer.[15]

We report here for the first time the synthesis and characterization of a series of organoselenocyanate using naphthalimide as the carrier of the selenocyanate active group attached to it by 2–5-membered methylene units. In the course of our anticarcinogenesis-drug development program we have evaluated the antioxidative properties of these compounds in a 7,12-dimethylbenz[*a*]anthracene (DMBA)–PMA two-stage mouse skin carcinogenesis model at the initial stage of carcinogenesis.

Keywords: 1,8-Naphthalimide; Organoselenocyanate; Oxidative stress; Phase II enzymes.
* Corresponding author. Tel.: +91 33 2476 5101x316; e-mail: sudinb1957@yahoo.co.in

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2. Chemistry

Several novel organoselenocyanate compounds with 1,8-naphthalimide moiety were designed and synthesized as follows (Scheme 1). Hydroxyl derivatives **2a–d** were formed by refluxing 1,8-naphthalic anhydride with corresponding amino alcohol either in water[16] or in ethanol (see Section 7). Compounds **2a–d** were converted to their corresponding bromo derivatives (**3a–d**) with phosphorous tribromide in ethyl acetate at 70 °C. All the hydroxy compounds (**2a–d**)[17] and bromo compounds (**3a–d**)[12] were reported in the literature but melting points and spectral data were not reported for **2b–d** and **3b–d**. The desired organoselenocyanate compound (**4a**) was obtained by nucleophilic substitution of the bromide group with anhydrous KSeCN in acetone under refluxing conditions. Selenium compounds **4b–d** were formed on treatment of **3b–d** with anhydrous KSeCN in acetone at room temperature (25 °C). Compounds **4a–d** were all purified through column chromatography, over silica gel and further crystallized.

3. Biology

The synthesized compounds were evaluated for their antioxidative activities against DMBA–PMA-induced oxidative stress on Swiss albino mice. Levels of glutathione-*S*-transferase (GST), reduced glutathione, superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation were measured 15 days after the first DMBA application.

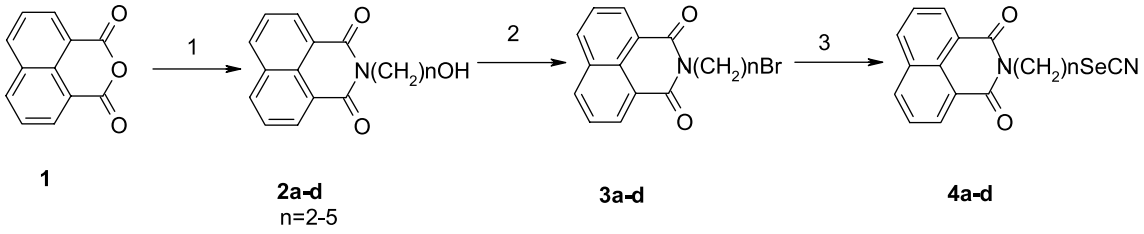
4. Results

All the newly synthesized organoselenocyanate compounds **4a–d** were evaluated for their antioxidative activities against oxidative stress induced by DMBA–PMA in a two-stage mouse skin carcinogenesis model at the preliminary selected treatment dose of 3 mg/kg bw. Results are listed in Table 1.

4.1. Hepatic microsomal lipid peroxidation level

Lipid peroxidation in liver microsomes, expressed in nanomoles of thiobarbaturic acid reactive substances (TBARS) formed per milligram of protein, was found to increase significantly ($p < 0.05$) by 181% in the animals treated with DMBA–TPA (Gr C) in comparison to vehicle-treated animals (Gr N) when measured 15 days after the first DMBA treatment.

The level of lipid peroxidation decreased in all the treated groups (I–VIII) in comparison to the carcinogen-treated Gr C. Lipid peroxidation decreased significantly



Scheme 1. (1) $H_2N(CH_2)n$-OH, EtOH and/or water, reflux; (2) PBr_3, EtOAC; (3) KSeCN, acetone.

Table 1. Modulation of phase II detoxifying enzymes (GST, SOD, CAT, GSH) and lipid peroxidation by organoselenium compounds **4a–d** in DMBA–TPA-induced two stage mouse skin carcinogenesis model after 15 days

Compound	Group	LPO (nm TBARS per milligram of protein)	GST (nmol CDNB–GSH min^{-1} mg^{-1})	GSH (nmol GSH per milligram of protein)	SOD (unit of inhibition per milligram of protein)	CAT (units per milligram of protein)
	N	0.2 ± 0.025*	695.01 ± 49.89*	118.89 ± 8.89*	60.73 ± 5.34*	14.41 ± 1.3*
	C	0.562 ± 0.06	397.17 ± 22.1	62.21 ± 5.64	36.31 ± 1.43	7.55 ± 0.35
4a	I	0.433 ± 0.078**	555.07 ± 56.05**	87.3 ± 4.3**	44.43 ± 4.4*	8.28 ± 0.21**
	II	0.339 ± 0.042**	567.28 ± 39.25**	87.29 ± 4.3**	56.41 ± 5.6*	9.82 ± 0.77*
4b	III	0.379 ± 0.01**	685.46 ± 71.40**	89.17 ± 1.45**	45.72 ± 0.1**	7.98 ± 0.15*
	IV	0.342 ± 0.12**	535.74 ± 20.5**	92.33 ± 9.04**	48.29 ± 0.88*	9.81 ± 0.06*
4c	V	0.356 ± 0.03**	496.63 ± 17.5**	72.23 ± 2.16**	40.06 ± 0.1**	8.41 ± 0.21**
	VI	0.286 ± 0.03**	507.22 ± 10.32**	84.43 ± 16.02**	60.72 ± 0.40*	10.07 ± 0.21*
4d	VII	0.259 ± 0.057**	689.87 ± 28.02**	95.28 ± 8.04**	48.5 ± 0.35**	9.58 ± 0.31**
	VIII	0.24 ± 0.01**	940.89 ± 35.4**	97.85 ± 1.24**	83.84 ± 7.9*	11.19 ± 0.8*

Data represent means ± SD, *$p < 0.05$. **$p < 0.01$ Comparisons are made in the text between group N (vehicle control) and group C (carcinogen control) group C (carcinogen control) and group I: (DMBA–TPA + compound **4a** treatment at the dose of 3 mg/kg bw for 15 days) group C (carcinogen control) and group II: (DMBA–TPA +15 days pretreatment +15 days post-treatment of compound **4a** at a dose of 3 mg/kg bw) group C (carcinogen control) and group III: (DMBA–TPA + compound **4b** treatment at the dose of 3 mg/kg bw for 15 days) group C (carcinogen control) and group IV (DMBA–TPA +15 days pretreatment +15 days post-treatment of compound **4b** at a dose of 3 mg/kg bw) group C (carcinogen control) and group V (DMBA–TPA + compound **4c** treatment at the dose of 3 mg/kg bw for 15 days). Group C (carcinogen control) and group VI (DMBA–TPA +15 days pretreatment +15 days post-treatment of compound **4c** at a dose of 3 mg/kg bw) group C (carcinogen control) and group VII (DMBA–TPA + compound **4d** treatment at the dose of 3 mg/kg bw for 15 days) group C (carcinogen control) and group VIII (DMBA–TPA +15 days pretreatment +15 days post-treatment of Compound **4d** at a dose of 3 mg/kg bw).

($p < 0.01$) by 22.95% in Gr I, 39.68% in Gr II, 32.56% in Gr III, 39.32% in Gr IV, 36.65% in Gr V, 49.11% in Gr VI, 53.91% in Gr VII, and 57.29% in Gr VIII, compared to Gr C (Table 1).

4.2. Effect of compounds 4a–d on GST activity

DMBA–TPA-treated Gr C animal showed a significant decrease of 42.85% ($p < 0.05$) in the total hepatic GST activity expressed in nanomoles of CDNB–GSH formed per minute per milligram of protein in the hepatic cytosol compared to the normal vehicle control Gr N. Whereas all the treated groups (Gr I–VIII) showed a significant ($p < 0.01$) increase of GST activity in comparison to carcinogen-induced Gr C animal.

There was a 39.76% increase in the enzyme activity in Gr I, 42.83% in Gr II, 72.59 % in Gr III, 34.89 % in Gr IV, 25.42% in Gr V, 27.71% in Gr VI, 73.70% in Gr VII, and in Gr VIII the level was increased by 136.90% as compared to the carcinogen control Gr C (Table 1).

4.3. Effects of compounds 4a–d on GSH

There was a significant decrease of 47.67% in GSH level expressed in nanomoles of GSH per milligram of protein, in DMBA–PMA-treated Gr C ($p < 0.05$) mice compared to the normal vehicle control Gr N, measured 15 days after the first DMBA application.

However, treatment with the organoselenium compounds showed a significant ($p < 0.01$) increase in GSH level. There was a 40.19% increase in GSH level in Gr I, 57.08% in Gr II, 43.34% in Gr III, 60.54% in Gr IV, 16.11% in Gr V, 35.72% in Gr VI, 85.82% of GSH level in Gr VII mice, and in Gr VIII there was an increase of 90.89% in GSH level all with respect to carcinogen control Gr C (Table 1).

4.4. Effect of compounds 4a–d on CAT

The CAT enzyme activity measured in the liver cytosols and expressed in units per milligram protein was found to decrease (47.6%) significantly in the DMBA–PMA-treated Gr C ($p < 0.05$) mice with respect to vehicle control mice (Gr N). The selenium compound treated groups, however, showed a significant ($p < 0.05$) rise in the activity by 9.66% in Gr I, 30.07% in Gr II, 8.74% in Gr III, 29.93% in Gr IV, 15.36% in Gr V, 33.38% in Gr VI, 26.88% in Gr VII, and 48.21% in Gr VIII as compared to the carcinogen control Gr C animals (Table 1).

4.5. Effect of compounds 4a–d on SOD

SOD activity expressed in units per milligram of protein present in the liver cytosols was found to decrease significantly ($p < 0.05$) by 40.21% in carcinogen-treated mice (Gr C) as compared to normal vehicle control mice (Gr N). The enzyme level was found to increase significantly ($p < 0.05$) in all the treated groups.

Treatment with the selenium compounds showed a 22.36% increase in SOD activity in Gr I, 55.36% increase in Gr II, 25.91% increase in Gr III, 32.99% increase in Gr IV, 10.33% increase in Gr V, 67.25% increase in Gr VI, 33.57% in Gr VII, and 130.90% increase in Gr VIII as compared to the DMBA–PMA-treated control group (Gr C) (Table 1).

5. Discussion

All the selenium compounds are hitherto unknown and obtained in good yield. Reaction between naphthalic anhydride and 5-amino-1-pentanol in refluxing ethanol did not work in the desired direction, producing a mixture of products. The reaction worked when water was used in place of ethanol, producing desired compound **2d**. Refluxing bromo compound **3b** with KSeCN in acetone for the preparation of **4b** as in **4a** resulted in a product, which did not show any cyanide peak in the IR spectra and could not be yet characterized. Compound **4b** was obtained in good yield when the same reaction was done at 25 °C for 48 h. Compounds **4c** and **4d** were prepared following the modified procedure.

A wide variety of organoselenium compounds have been shown to inhibit chemical carcinogenesis in animal model. Several organic as well as inorganic selenium compounds have protective role against carcinogen-induced covalent DNA adduct[18] formation and retardation of oxidative damage to DNA and in the multistep carcinogenesis process. A number of organoselenium compounds are known to act as antioxidants by reducing H_2O_2, lipid, and phospholipid hydroperoxide level, thereby dampening the propagation of free radical, reactive oxygen, and nitric oxide species mediating cellular damage.[19,20] It has been earlier reported from our laboratory that diphenylmethyl selenocyanate significantly reduced the level of lipid peroxidation by upregulating several phase II detoxifying enzyme.[5,6] Although several organoselenocyanates were known for their cancer chemopreventive properties against lung, liver, colon, and mammary gland cancer, only diphenylmethyl selenocyanate was reported for its antioxidative as well as cancer chemopreventive properties against DMBA–croton oil (which contains phorbol ester PMA)-induced two-stage mouse skin carcinogenesis.

ROS produced in large amounts during the process of any kind of assault to the body initiate various lethal chain reactions that inactivate certain vital enzymes and important subcellular elements and lead to cell death.[20] In the present study, we have synthesized a series of organoselenocyanate compounds and have studied their antioxidative effects on DMBA-induced and phorbol 12-myristate 13-acetate (PMA) promoted skin carcinogenesis in Swiss albino mice because mouse skin carcinogenesis model serves as an useful model for the purpose of screening of antioxidative agents in vivo. Liver is the site of drug metabolism and detoxification in the body; hence the antioxidative enzymes and the lipid peroxidation level were determined in the liver.[21] Formation of free radicals leads to lipid peroxidation,

resulting in the formation of malondialdehyde and other reactive aldehydes as the end products.[22] These products cause chaotic cross-linkage between proteins and nucleic acids that play an important role in the process of carcinogenesis.[23] ROS can also induce DNA strand break by modulating different biochemical pathways and gene expression.[24] In the present study, we found that oral administration of the selenium compounds **4a–d** in 5.5% aqueous propylene glycol produced a remarkable reduction of lipid peroxidation in the DMBA–PMA-treated mice and the extent of reduction increased with the increase of carbon chain bearing the active selenocyanate functional group, with compound **4d** showing the highest activity amongst the concomitant group as well as the pretreatment group. It has been reported in the literature that the length of the aliphatic side chain is a determinant in modulating the cancer chemopreventive efficacy and the activity increased with the increase of chain length in a series of aliphatic selenocyanates.[10] In the present experiment also, the activity as observed increased with increase of methylene carbon chain. The increase in lipophilic character may be one of the reasons for better activity of compound **4d**, which had the maximum number of methylene groups attached to the selenocyanate active group. Also within the individual compound, it was observed that pretreatment with the selenium compound has better effect than with the concomitant treatment (Gr II vs. Gr III and so on). In the pretreatment groups, the level of antioxidant/phase II detoxifying enzymes may already be in an enhanced condition before the DMBA treatment compound to the concomitant group and their enhanced level of antioxidant/phase II detoxifying enzymes plays the part in the better effect on lipid peroxidation.

Compound **4d** was also found to have a better effect than diphenylmethyl selenocyanate. Others (compounds **4a–c**) showed effects comparable to that of diphenylmethyl selenocyanate.

It has been reported in the literature that activities of antioxidative enzymes were lowered in squamous cell carcinoma.[25] It may be noted from the present study that DMBA–PMA treatment significantly lowered the level of phase II enzymes such as GST, CAT, and SOD compared to normal control animals. Treatment with the compounds increased the level of the enzymes. GST plays an important role in detoxifying/transport of many DNA alkylating agents,[26] carcinogens, and environmentally hazardous chemicals[27] by catalyzing the conjugation of GSH with these chemicals or their active metabolites. The cellular GSH, alone as a nucleophile, can also play an important role in the deactivation of the electrophilic compounds.[28,29] Selenium compounds were reported to enhance the level of GST activity in the liver of animals.[30,31] In the present study, it was observed that treatment with the selenium compounds upregulated the activity of GST as well as the GSH level significantly. It showed that these compounds have the potential to improve the host-defense system for providing cellular protection through GST-mediated neutralization of DMBA metabolites, thereby reducing the DMBA-induced oxidative DNA damage. Oxidative DNA damage by DMBA was reported in the literature.[32,33] It has been found that increase in the methylene chain length from two (compound **4a**) to five (compound **4d**) has distinct effects on GST as well as GSH activity. Compound **4d** is the most active in this regard but no structure–activity relationship could be drawn from the results obtained, as compound **4b**, which contains three methylene group attached to selenocyanate, showed better activity than compound **4c**, which contains four methylene groups attached to the selenocyanate active group. The reason is not clear at present. Compound **4d** was also found superior to diphenylmethyl selenocyanate in modulating GST activity with compounds **4a** and **4b**, and also showed comparable or better results than diphenylmethyl selenocyanate.

Superoxide dismutase (SOD) and catalase (CAT) are the enzymes regarded as the first line of antioxidants that protect cells against oxidative stress. SOD scavenges the super oxide radicals whereas CAT catalyzes the breakage of toxic H_2O_2 produced in the cell to O_2 and H_2O.

PMA, a peroxisome proliferator and tumor promoter, stimulates the generation of super oxide anion radicals ($O_2^{\cdot -}$),[34,35] which act as a precursors to the formation of H_2O_2 and the extremely reactive hydroxyl radical (OH), involved in the process of tumor promotion.[34–36] It has been reported that activities of the detoxifying enzymes SOD and CAT were depressed significantly by tumor promoter.[37,38] Increased generation of reactive oxygen radicals coupled with depleted antioxidant protective systems makes the cells more vulnerable to oxidative stress. Similar results were obtained in the present experiments also when the animals were treated with DMBA–PMA. Treatment with the selenium compounds showed a positive effect on the status of both SOD and CAT levels. The depleted level of these enzymes increased significantly compared to carcinogen-treated control animals. However, the effect of increase of the methylene chain length on the SOD activity is more pronounced in the pretreatment group as compared with the concomitant treatment group, with compound **4d** showing the highest activity. No structure–activity relationship could be drawn from the results obtained. Regarding the effect on the CAT activity, the effect of the increase in methylene chain length was pronounced both in the pretreatment group and in the concomitant treatment group, with the pretreatment group showing better effects than the concomitant treatment group. In both the pretreatment and concomitant treatment groups, compounds **4a** and **4b**, which differ in structure by one methylene group, showed some activity, and the activity increase depends on the number of methylene groups attached to the selenocyanate group; thus compound **4d** shows the highest proactive activity. However, the effect of these compounds on both SOD and CAT is less than that showed by diphenylmethyl selenocyanate.

During pretreatment with the selenium compound, the antioxidant/phase II detoxifying enzymes may already be in an enhanced level and this enhanced state

continued to persist even after DMBA–PMA treatment, as the treatment with the selenium compound was continued during the total experimental period, whereas during the concomitant treatment, the levels of enzymes were also enhanced but most of it was utilized to counter the oxidative stress induced by DMBA and PMA. As a result, the pretreatment groups showed better results than the concomitant groups. There may be some other reasons, which are not clear at this moment.

6. Conclusion

The antioxidative potential of the compounds at the preliminary screening dose of 3 mg/kg bw seems clear at this stage of investigation. The compounds act through modulation of the antioxidant enzymes, thereby leading to a favorable shift in the oxidation–reduction balance and resulting in downregulation of lipid peroxidation. So far it has been found that compound **4d** has the highest potential to downregulate the oxidative stress at the preliminary stage of carcinogenesis, and the pretreatment group showed better effects than the concomitant group. However, their cancer chemopreventive efficacy requires further investigation (such as incidence of papilloma formation), which is currently in progress, and its mechanism of action, whether it works through inhibition of DMBA metabolism, induction of apoptosis, inhibition of cell proliferation or inhibition of prostaglandin synthesis, or by the downregulation of protein kinase C, is also to be studied.

7. Experimental

7.1. Chemistry

All reactions were conducted under anhydrous condition except those using water. Solvents were well dried and reactions were performed using oven-dried glassware. Melting points were determined on a capillary melting point apparatus and were uncorrected. NMR spectra were recorded on a 300 and 200 MHz for proton and 75 MHz for carbon and were performed in $CDCl_3$ or DMSO-d_6 solution using tetramethylsilane as the internal reference on Brucker 300-MHz instrument. The coupling constants (J) are reported in Hertz. The mass spectrum was recorded on JEOL JMS600 in FAB ionization mode. Elementary analyses were recorded on Perkin-Elmer auto analyzer 2400 II. The column chromatography was performed using silica gel (60–120 mesh) (Qualigens Fine Chemicals, India), 3-amino-1-propanol, 4-amino-1-butanol, 5-amino-1-pentanol and 1,8-naphthalic anhydride.

7.2. Chemicals

7.2.1. Preparation of 2-(2-hydroxy-ethyl)-benzo[*de*]isoquinoline-1,3-dione (2a). *General procedure:* A mixture of 1,8-naphthalic anhydride (2.5 g, 0.013 mol) and ethanol amine (0.8 g, 0.013 mol) in ethanol (20 mL) was heated under reflux for 1.5 h. The resulting mixture was concentrated by evaporating ethanol under reduced pressure and then cooled at 4 °C. The solid separated was filtered, washed with cold ethanol and dried to afford **2a** (2.5 g, 82.24%) having melting point 172–174 °C (lit. m.p.[16] 175–176 °C). The compound was pure enough to be used directly for the preparation of **3a**.

7.2.2. Preparation of 2-(3-hydroxy-propyl)-benzo[*de*]isoquinoline-1,3-dione (2b). Compound **2b** following the above-described procedure was prepared with 3-amino-1-propanol instead of ethanolamine. Yield: 91.44% m.p.: 121–122 °C. IR (KBr) ν_{max} cm^{-1}: 3437 (–OH), 2944 (aromatic –CH), 1695 and 1645 (amide), 1589 (aromatic ring).

^1H NMR (300 MHz, $CDCl_3$,): 2.0 (q, 2H, $J = 5.8$ Hz), 3.15 (s, –OH), 3.6 (t, 2H, $J = 5.0$ Hz), 4.36 (t, 2H, $J = 6.1$ Hz), 7.77 (t, 2H, Ar-H, $J = 7.4$ Hz), 8.23 (d, 2H, Ar-H, $J = 8.3$ Hz), 8.62 (d, 2H, Ar-H, $J = 7.3$ Hz). Anal. Found: C, 70.21; H, 5.32; N, 5.55. Calcd. for $C_{15}H_{13}O_3N$: C: 70.58; H: 5.13; N: 5.49.

7.2.3. Preparation of 2-(4-hydroxy-butyl)-benzo[*de*]isoquinoline-1,3-dione (2c). *General procedure:* A mixture 1,8-napthalic anhydride (2.5 g, 0.013 mol), and 4-amino-1-butanol (3.6 mL, 0.04 mmol) in water (10 mL) was refluxed for 30 min and then cooled at 4 °C. Then product **2c** formed was filtered, washed with ice cold water and crude product was recrystallized from acetone and dried over P_2O_5 to give the compound **2c** as white crystalline solid.

Yield: 59.6%, m.p.: 111–113 °C. IR (KBr) ν_{max} cm^{-1}: 3323.0 (–OH), 2929.0 (aromatic –CH), 1691.0, 1654.0, 1587. ^1H NMR (300 MHz, $CDCl_3$, TMS = 0.00): 1.77 (m, 4H), 3.74(t, 2H, $J = 9.5$ Hz), 4.24(t, 2H, $J = 10.4$ Hz), 7.76 (t, 2H, Ar-H, $J = 11.12$ Hz), 8.20 (d, 2H, Aro-H, $J = 12.42$ Hz), 8.60 (d, 2H, Aro-H, $J = 10.94$ Hz). Anal. Found: C, 71.06; H, 5.42; N, 5.33. Calcd. for $C_{16}H_{15}O_3N$: C, 71.36; H, 5.61; N, 5.20.

7.2.4. Preparation of 2-(5-hydroxy-pentyl)-benzo[*de*]isoquinoline-1,3-dione (2d). Compound **2d** was prepared using 5-amino-1-pentanol following the above-described procedure.

Yield: 83.91%. m.p.: 94–96 °C. IR (KBr) ν_{max} cm^{-1}: 3403.31, 2943.90, 1712.71, 1663.27, 1589.44. ^1H NMR (200 MHz, $CDCl_3$): 1.50 (q, 2H, $J = 4.6$ and 6.8 Hz), 1.67 (q, 2H, $J = 6.8$ Hz), 1.8 (q, 2H, $J = 7.8$ Hz), 3.7 (t, 2H, $J = 6.3$ Hz), 4.2 (t, 2H, $J = 7.3$ Hz), 7.75 (t, 2H, Ar-H, $J = 7.70$ Hz), 8.2 (d, 2H,Ar-H, $J = 7.6$ Hz), 8.50 (d, 2H, Ar-H, $J = 7.0$ Hz). Anal. Found: C, 71.98; H, 6.21; N, 5.01. Calcd. for $C_{17}H_{15}O_3N$: C, 72.07; H, 6.05; N, 4.94.

7.2.5. Preparation of 2-(2-bromo-ethyl)-benzo[*de*]isoquinoline-1,3-dione (3a). *General procedure*: To a stirred suspension of compound **2a** (1.0 g, 4.149 mmol) in ethyl acetate (5 mL) PBr_3 (0.8 mL) was added at room temperature for 20 min. Then the resulting mixture was heated at 70–75 °C with stirring for 1.5 h. The resulting mixture was cooled, poured into ice water, and extracted with $CHCl_3$ (100 mL) The extract was washed with sat-

urated $NaHCO_3$ and then with water and dried over $CaCl_2$. The solvent was evaporated under reduced pressure to afford a yellow solid which was purified by column chromatography over silica gel (petroleum ether (60–80 °C)/$CHCl_3$ (1:1, v/v)) to give compound **3a**.

Yield: 1.24 g, 79.5%. m.p.: 224–226 °C (lit. m.p.[16] 222–223 °C). Compounds **3b–d** were prepared by following the above-described procedure.

7.2.6. Preparation of 2-(3-bromo-propyl)-benzo[*de*]isoquinoline-1,3-dione (3b). Yield: 64% m.p.: 138–140 °C. IR (KBr) ν_{max} cm^{-1}: 2948, 1696, 1660, 1586. ^1H NMR (300 MHz, $CDCl_3$): 2.34 (q, 2H, $J = 6.8$ Hz), 3.5 (t, 2H, $J = 6.9$ Hz), 4.3 (t, 2H, =N–CH_2–, $J = 7.0$ Hz), 7.76 (t, 2H, Ar-H, $J = 7.7$ Hz), 8.2 (d, 2H, Ar-H, $J = 7.8$ Hz), 8.60 (d, 2H, Ar-H, $J = 7.2$ Hz). Anal. Found: C, 56.32; H, 3.90; N, 4.52. Calcd. for $C_{15}H_{12}BrO_2N$: C, 56.62; H, 3.80; N, 4.40.

7.2.7. Preparation of 2-(4-bromo-butyl)-benzo[*de*]isoquinoline-1,3-dione (3c). Yield: 51.3% m.p: 112–114 °C. IR (KBr) ν_{max} cm^{-1} 2950.9 (aromatic C–H), 1697.2, 1662.5, 1587.3, ^1H NMR (300 MHz, $CDCl_3$): 1.96 (m, 4H,) 3.50 (t, 2H, $J = 6.3$ Hz), 4.2 (t, 2H, $J = 6.8$ Hz), 7.7 (t, 2H, Ar-H, $J = 7.3$ Hz), 8.22 (d, 2H,Ar-H, $J = 8.2$ Hz), 8.61 (d, 2H, Ar-H, $J = 7.2$ Hz). Anal. Found: C, 57.75; H, 4.31; N, 4.15. Calcd. for $C_{16}H_{14}BrO_2N$: C: 57.85; H: 4.25; N: 4.22.

7.2.8. Preparation of 2-(5-bromo-pentyl)-benzo[*de*]isoquinoline-1,3-dione (3d). Yield: 50.22%. m.p.: 121–123 °C. IR (KBr) ν_{max} cm^{-1}. 2932.6, 2156.91 (–CN), 1694.78, 1648.71, 1590.35. ^1H NMR (300 MHz, $CDCl_3$): 1.60 (q, 2H, $J = 6.8$ Hz), 1.8 (q, 2H, $J = 7.4$ Hz), 1.95(q, 2H, $J = 6.9$ Hz), 3.4 (t, 2H, $J = 6.7$ Hz), 4.2 (t, 2H, $J = 7.4$ Hz), 7.7 (t, 2H, Ar-H, $J = 7.75$ Hz), 8.21 (d, 2H, Ar-H, $J = 8.15$ Hz), 8.60 (d, 2H, Ar-H, $J = 7.22$ Hz). Anal. Found: C, 58.77; H, 4.59; N, 4.15. Calcd. for $C_{17}H_{16}BrO_2N$: C: 58.97; H: 4.66; N: 4.05.

7.2.9. Preparation of 2-(2-selenocyanato-ethyl)-benzo[*de*]isoquinoline-1,3-dione (4a). *General procedure*: To a solution of **2a** (1 g, 3.289 mmol) in acetone (25 mL) was added anhydrous KSeCN (660 mg, 4.58 mmol). The mixture was stirred and heated at 65–70 °C for 7 h. Acetone was removed under reduced pressure. The residual mass was extracted with $CHCl_3$ (100 mL). The total extract was washed with water and dried over $CaCl_2$. Chloroform was removed under reduced pressure. The residue obtained was purified by column chromatography over silica gel (petroleum ether (60–80 °C)/$CHCl_3$ (7:3, v/v)) to give the compound (**4a**) as a pale yellowish solid 730 mg, m.p.: 179–181 °C. The compound was further purified by crystallization (acetone–light petroleum 60–80°C).

Yield: 73.9% IR (KBr) ν_{max} cm^{-1}: 2143.6 (–CN), 1697.9, 1651.0 (amide), 1588.7 (Ar-ring). ^1H NMR (300 MHz, DMSO-d_6): 3.37 (t, 2H, $J = 6.9$ Hz), 4.51 (t, 2H, $J = 6.84$ Hz), 7.9 (t, 2H, Ar-H, $J = 7.9$ Hz), 8.47 (d, 2H, Ar-H, $J = 7.7$ Hz), 8.52 (d, 2H, Ar-H, $J = 7.5$ Hz). ^{13}C (75 MHz, $CDCl_3$): 164.16, 134.5, 131.64,131.58, 128.13,127.02, 121.97, 101.46 (–CN), 39.76, 26.76. Mass. FABHRMS m/z 331 (M+1). ^{80}Se correct isotope pattern, 224 (M-SeCN). Anal. Found: C, 54.55; H, 3.21; N, 8.62. Calcd. for $C_{15}H_{10}O_2N_2Se$: C: 54.72; H: 3.06; N: 8.51.

7.2.10. Preparation of 2-(3-selenocyanato-propyl)-benzo[*de*]isoquinoline-1,3-dione (4b). Anhydrous KSeCN (532 mg, 1.46 mmol) in acetone (25 mL) was added dropwise to a stirred suspension of **3b** (800 mg, 2.25 mmol) in acetone (30 mL) over a period of 1 h at 25 °C. The reaction mixture was stirred at room temperature (reaction was monitored by T.L.C.). After 48 h, solvent was evaporated in vacuo and the yellow residue thus formed was extracted with $CHCl_3$ (50 mL), washed with brine, and dried over $CaCl_2$, and the solvent was removed under reduced pressure. The solid obtained was purified by column chromatography [silica gel, 60–120 mesh, light petroleum (60–80 °C)–$CHCl_3$ 4:1, v/v], to furnish **3b** as a white solid (480 mg, 84.8%) m.p.: 152–155 °C. It was crystallized from acetone to light petroleum 60–80 °C to afford **4b** of analytical grade m.p.: 154–155 °C. IR (KBr) ν_{max} cm^{-1}: 2952.8 (CH), 2146.6 (–CN), 1687.9, 1652.9, 1587. ^1H NMR (300 MHz, $CDCl_3$): 2.39 (q, 2H, $J = 6.6$ Hz), 3.13 (t, 2H, $J = 7.0$ Hz), 4.38 (t, 2H, $J = 6.3$ Hz), 7.78 (t, 2H, Ar-H, $J = 8.1$ Hz), 8.24 (d, 2H, Ar-H, $J = 8.2$ and 7.3 Hz), 8.60 (d, 2H, Ar-H, $J = 7.3$ Hz). ^{13}C (75 MHz, $CDCl_3$): 27.695, 29.69, 39.00,102.24, 122.14, 126.97, 128.05, 131.45, 134.3, 164.43. FABHRMS m/z: (M^+ +1, 345). ^{80}Se correct isotope pattern. Anal. Found: C, 55.80; H, 3.12; N, 8.21. Calcd. for $C_{16}H_{12}O_2N_2Se$: C, 55.91; H, 3.52; N, 8.16.

Compounds **3c–d** were prepared by following the above-mentioned procedure.

7.2.11. Preparation of 2-(4-selenocyanato-butyl)-benzo[*de*]isoquinoline-1,3-dione (4c). Yield: 89.34%. m.p.: 107–109 °C. It was crystallized from acetone–hexane to afford **4c** of analytical grade m.p.: 108–109 °C. IR (KBr) ν_{max} cm^{-1}: 2945.86, 2156.91, 1690.85 1655.08, 1588.85. ^1H NMR (300 MHz, $CDCl_3$, TMS = 0.00): 1.9 (q, 2H, $J = 6.5$ Hz), 2.0 (q, 2H, $J = 6.3$ Hz), 3.2 (t, 2H, $J = 7.0$ Hz), 4.2 (t, 2H, $J = 7.1$ Hz), 7.76 (t, 2H, Ar-H, $J = 7.9$ Hz), 8.20 (d, 2H, Ar-H, $J = 8.1$ Hz), 8.60 (d, 2H, Ar-H, $J = 7.2$ Hz). ^{13}C (75 MHz, $CDCl_3$): 27.48, 28.19, 28.93, 38.98, 101.37, 122.34, 126.88, 128.01, 131.23, 131.47, 134.00, 164.08. FABHRMS m/z: (M^+ +1, 359). ^{80}Se correct isotope pattern. Anal. Found: C, 57.00; H, 3.83; N, 7.94. Calcd. for $C_{17}H_{14}O_2N_2Se$: C, 57.15; H, 3.95; N, 7.84.

7.2.12. Preparation of 2-(5-selenocyanato-pentyl)-benzo[*de*]isoquinoline-1,3-dione (4d). Yield: 68%. m.p.: 126–127 °C. It was crystallized from diethyl ether–light petroleum 60–80 °C to afford **4d** of analytical grade m.p.: 154–155 °C IR (KBr) ν_{max} cm^{-1}: 2932.6, 2156.91 (CN), 1694.78, 1648.71, 1590.35. ^1H NMR (300 MHz, $CDCl_3$, TMS = 0.00): 1.61 (q, 2H, $J = 7.8$ Hz), 1.8 (q, 2H, $J = 7.3$ Hz), 2.0 (q, 2H, $J = 7.1$ Hz), 3.1 (t, 2H, $J = 7.3$ Hz), 4.2 (t, 2H, $J = 7.2$ Hz), 7.8 (t, 2H, Ar-H, $J = 7.6$ Hz), 8.22 (d, 2H, Ar-H, $J = 7.9$ Hz), 8.60 (d, 2H, Ar-H, $J = 7.0$ Hz). ^{13}C (75 MHz, $CDCl_3$): 26.45,

27.14, 29.27, 30.33, 39.78, 101.45, 122.46, 126.87, 128.02, 131.17, 131.48, 133.93, 164.1. FABHRMS *m/z*: (M^+ +1, 373). ^{80}Se correct isotope pattern. Anal. Found: C, 58.27; H, 4.3; N, 7.21. Calcd. for $C_{18}H_{16}O_2N_2Se$: C: 58.23; H: 4.34; N: 7.54.

7.3. Biology

7.3.1. Materials. 7,12-Dimethylbenz[*a*]anthracene, PMA, 1-chloro-2,4-dinitrobenzene (CDNB), ethylenediaminetetraacetic acid (EDTA), reduced glutathione, pyrogallol, sodium dodecyl sulfate (SDS), thiobarbituric acid (TBA), bovine serum albumin (BSA) (Sigma, USA) hydrogen peroxide (30%) (Merck India Ltd.).

7.3.2. Animals. Adult (5–6 weeks) Swiss albino female mice bred in the animal colony of Chittaranjan National Cancer Institute, Kolkata, were used for this study. Mice weighing (22 ± 2g.), housed in wire-mesh cages (5 mice/cage). They had free access to water and food under controlled environmental conditions of humidity (60 ± 5%), lighting (12-h light/dark cycle), and temperature (23 ± 2 °C). Standard food pellets and drinking water were provided ad libitum.

7.3.3. Experimental protocol

7.3.3.1. Carcinogen treatment. The backs of the animals were shaved 2 days prior to the start of the experiment. To induce oxidative stress the mice of Gr C and Gr I–VIII were subjected to two topical applications of DMBA at an interval of 72 h at the dose of 0.05 mg/kg bw in acetone (100 μL/mouse) on the shaved area followed by PMA (5 nmol/mouse) twice in a week at an interval of 72 h starting from day 8 of first DMBA application.

7.3.3.2. Drug preparation. Synthetic organoselenocyanate compounds **4a–d** were used as a suspension in 5.5% propylene glycol in water, prepared on the day of experiment, just before treatment.

7.3.3.3. Treatment group

Group N: Animals received topical application of acetone (100 μL/mouse) on the shaved skin and propyleneglycol (5.5% in water, 300 μL/mouse) by oral gavage everyday during the treatment period.

Group C: Two topical applications of DMBA followed by PMA as mentioned above during the period of treatment for 15 days.

Group I: Received DMBA–PMA and compound **4a** at the dose of 3 mg/kg bw from the day of DMBA treatment for 15 days.

Group II: Received compound **4a** at a dose of 3 mg/kg bw 15 days prior to first DMBA treatment and the treatment continued throughout the experimental period for 15 days +DMBA–PMA as in group C.

Group III: Received DMBA–PMA as in group C and compound **4b** at the dose of 3 mg/kg bw from the day of DMBA treatment for 15 days.

Group IV: Received compound **4b** at a dose of 3 mg/kg bw 15 days prior to first DMBA treatment and the treatment continued throughout the experimental period of 15 days +DMBA–PMA as in group C.

Group V: Received DMBA–PMA as in group C and compound **4c** at the dose of 3 mg/kg bw from the day of DMBA treatment for 15 days.

Group VI: Received compound **4c** at a dose of 3 mg/kg bw 15 days prior to first DMBA treatment and the treatment continued throughout the experimental period of 15 days +DMBA–PMA as in group C.

Group VII: Received DMBA–PMA as in group C and compound **4d** at the dose of 3 mg/kg bw from the day of DMBA treatment for 15 days.

Group VIII: Received compound **4d** at a dose of 3 mg/kg bw 15 days prior to first DMBA treatment and the treatment continued throughout the experimental period of 15 days +DMBA–PMA as in group C.

7.3.4. Tissue preparation

7.3.4.1. Preparation of liver microsomes for quantitative estimation of lipid peroxidation. Liver tissues of the animals were collected, washed in 0.9% saline, soaked in filter paper, weighed and 400 mg of liver homogenized in 1.15% KCl. The homogenates were then centrifuged at 12,000*g* at 4 °C for 10 min, and the supernatant were collected and again centrifuged at 10,000*g* at 4 °C for 10 min. This supernatant was then ultracentrifuged at 54,000*g* at 4 °C for 1 h. The supernatant was discarded and the microsomal pellet was dissolved in 0.4 mL of 1.15% KCl to get the sample for assay of lipid peroxidation.

7.3.4.2. Preparation of liver cytosol for GST assay. Liver tissues of experimental animals were collected, washed in 0.9% saline, soaked in filter paper, and weighed. Tissue fragments (200 mg) were homogenized in cold condition in 1 mL homogenizing buffer (250 mM sucrose, 20 mM Tris–HCl, 1 mM dithiothreitol, pH 7.4), using glass/Teflon homogenizers. The homogenates were centrifuged at 75,000*g* at 4 °C for 2 h. Supernatant (cytosolic fraction) was kept in aliquot and used for the assay.[39]

7.3.4.3. Preparation of liver cytosol for assay of reduced glutathione. Liver tissues (200 mg) were homogenized in homogenizing buffer, pH 7.4 (in absence of dithiothreitol). Supernatant was collected as described for GST for quantification[40] of the level of GSH.

7.3.4.4. Preparation of liver cytosol for SOD assay. Liver tissues of the animals were collected, washed in 0.9% saline, soaked in filter paper, and weighed. Then 100 mg liver tissue from each animal was homogenized in 0.5 mL of Tris–HCl buffer and the homogenate was centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant was taken and 0.25 mL of ethanol and 0.15 mL of chloroform were added per milliliter of the supernatant, vortexed for 5–7 min, and centrifuged at 130,000*g* for 15 min, and the supernatant was used as the enzyme source.

7.3.4.5. Preparation of liver cytosol for CAT assay. Liver tissues of the animals were collected, washed in 0.9% saline, soaked in filter paper, and weighed. Then 100 mg liver tissue was homogenized in 500 mL M/150 phosphate buffer in ice and centrifuged at 2000*g* for 10 min at 4 °C. The supernatant was taken for the assay

7.3.5. Biochemical estimation

7.3.5.1. Quantitative estimation of hepatic microsomal lipid peroxidation. Lipid peroxidation was estimated in the liver microsomal fraction. The level of lipid peroxides formed was measured[41] using thiobarbaturic acid and expressed as TBARS formed per milligram of protein using as extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

7.3.5.2. Quantitative estimation of hepatic GST activity. GST activity was measured in the liver cytosol. The enzymatic activity was determined from the increase in absorbance at 340 nm with 1-chloro-2,4-dinitrobenzene as the substrate and specific activity of the enzyme expressed as the formation of 1-chloro-2,4-dinitrobenzene (CDNB–GSH) conjugate per minute per milligram of protein.[42]

7.3.5.3. Quantitative estimation of reduced glutathione. The level of glutathione was estimated from the liver cytosol[40] and expressed in nanomoles of GSH per milligram of protein.

7.3.5.4. Quantitative estimation of SOD activity. SOD activity after partial extraction and purification of SOD[43] was determined[44] by quantification of pyrogallol autooxidation inhibition and expressed in units per milligram of protein. One unit of enzyme activity is defined as the amount of enzyme necessary for inhibiting the reaction by 50%. Autooxidation of pyrogallol in Tris–HCl buffer (50 mM, pH 7.5) was measured by increase in absorbance at 420 nm.

7.3.5.5. Quantitative estimation of CAT activity. Activity of CAT in liver cytosol was determined[45] spectrophotometrically at 250 nm and expressed in units per milligram of protein where unit is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 in 100 s at 25 °C.

7.3.5.6. Estimation of protein. Protein was estimated spectrophotometrically[46] with BSA as standard.

7.3.5.7. Statistical analysis. The data were analyzed by student's *t* test to identify the means of different groups. The p value of <0.05 was considered significant.

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