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Naphthalimide based novel organoselenocyanates: Finding less toxic forms of selenium that would retain protective efficacy

Somnath Singha Roy [a], Prosenjit Ghosh [a], Ugir Hossain Sk [b], Pramita Chakraborty [a], Jaydip Biswas [c], Syamsundar Mandal [d], Arin Bhattacharjee [a], Sudin Bhattacharya [a,*]

[a] Chittaranjan National Cancer Institute, Department of Cancer Chemoprevention, 37, S. P. Mukherjee Road, Kolkata 700 026, West Bengal, India
[b] Department of Pharmacology CH72, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA
[c] Director, Chittaranjan National Cancer Institute, 37, S. P. Mukherjee Road, Kolkata 700 026, West Bengal, India
[d] Chittaranjan National Cancer Institute, Department of Epidemiology and Biostatistics, India

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ABSTRACT

A series of naphthalimide based organoselenocyanates were synthesized and screened for their toxicity as well as their ability to modulate several detoxifying/antioxidative enzyme levels at a primary screening dose of 3 mg/kg b.w. in normal Swiss albino mice for 30 days. Compound **4d** showed highest activity in elevating the detoxifying/antioxidant enzymes levels.

It is now well understood that selenium (Se) is an essential micronutrient for normal functioning of the body but can be toxic when present in higher amounts.[1] Se in combination with proteins and amino acids plays a significant role in exerting homeostasis in an organism[2] and its deficiency can cause severe disturbances in the organism. It forms the active center of selenoenzymes such as glutathione peroxidase and thioredoxin reductase that catalyzes essential redox reactions and performs the function of an antioxidant in a number of metabolic and immunologic processes.[3,4] Se compounds show cancer chemopreventive property at doses which are much higher than the nutritional requirement. Due to toxicity of selenium at high doses, Se compounds have limited doses used in chemoprevention study. Literature survey reveals that most of the experimental works for evaluation of Se as cancer chemopreventive agent were carried out using inorganic Se such as sodium selenite (Na_2SeO_3) as the Se source.[5] Studies also indicated that inorganic Se compounds are more toxic than organoselenium compounds.[6,7] Due to high toxicity of inorganic form and easy assimilation of the organic forms[8] the later is more advantageous for an organism,[4] although two naturally occurring organoselenium compounds, selenocysteine, and selenomethionine are found to be toxic and their toxicity is comparable with the toxicity of Na_2SeO_3.[9] Therefore, there has been a growing interest in the synthesis of organoselenium compounds which are less toxic and could be used as antioxidants, enzyme inhibitors, neuroprotective, antiinfectious and cancer chemopreventive agents, cytokine inducers and immunomodulators. On the other hand naphthalimide compounds have high antitumor activity against a variety of murine and human tumor cells.[10] Naphthalimide derivatives bind to DNA by intercalation of the chromophore and can photoinduce DNA cleavage.[11] Studies have shown that the cleaved site may be controlled by the substituent on the naphthalimide moiety.[12] Organoselenocyanates have got wide attention for their better cancer chemopreventive properties. Selenocyanates (RSeCN) were used as the carrier of selenium because they are known to be efficiently metabolized to selenols (RSeH) and therefore represent a convenient precursor compound.[13] Compounds containing selenocyanate functionality showed multiple mode of protection against cancer.[14] It was reported in the literature that replacement of selenium by sulphur in organoselenocyanate compound diminished the potential of the compound to modulate phase II enzymes and GSH.[15]

The objective of this study is to synthesize a series of non toxic organoselenium compounds which have efficient protective activity. Because of the very narrow range between the toxic dose and the adequate dose for the organism, not only the dose volume but also the form of Se supplemented is evenly important. We have previously reported the synthesis and antioxidative properties of a series of unsubstituted naphthalimide based organoselenocyanates where we showed that the antioxidative properties increased with increase in the alkyl chain length containing the selenocyanate

* Corresponding author. Tel.: +91 33 2476 5101x316.
E-mail address: sudinb19572004@yahoo.co.in (S. Bhattacharya).

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active group.[16] In continuation of the previous study, we have synthesized a series of substituted naphthalimide based organoselenocyanates (with the alkyl chain length $n = 5$) and analyzed their systematic toxicity profile in mice by investigating changes in body weight, hepatotoxicity and nephrotoxicity marker such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity, blood urea nitrogen (BUN) and creatinine levels in serum, histopathology of liver and kidney, hepatic lipid peroxidation (LPO) level and chromosomal aberration (CA) in bone marrow cells. We have also evaluated their ability to modulate the levels of phase II detoxifying and antioxidant enzymes such as glutathione-*S*-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin reductase (TRxR) and nonenzymatic antioxidant like reduced glutathione (GSH) levels in liver.

The unsubstituted naphthalimide compound **4a** was synthesized previously[16] and the remaining synthesis of compounds **4b–c** was depicted in Scheme 1. The key precursors in the synthesis that is, the bromoalkyl-naphthalimides **3a–c** were synthesized from **1a–c** by refluxing with 5-amino pentanol followed by bromination with PBr_3 in ethyl acetate at 70 °C, following the literature reported method.[16] The final compounds **4a–c** were synthesized by reacting **3a–c** with KSeCN in acetone at rt.[27–29]

On the other hand, in Scheme 2, naphthalimide-alkylbromo compound **3d** was prepared from 4-amino naphthalimide (**1d**) in the experimental on treatment with 1,5-dibromopentane in presence of base, sodium methoxide in methanol at room temperature. The desired selenocyanate compound **4d** was then obtained by the treatment of **3d** with anhydrous KSeCN in acetone at rt.[30]

All the compound **3a–d** and **4a–d** were purified by column chromatography and characterized by ^1H, ^{13}C and mass spectroscopy. The purity of the compounds was measured by reverse phase HPLC (Waters Analytical System) using methanol/water (in varying proportion) as mobile phase.

Adult (5–6 weeks) Swiss albino female mice (25 ± 1 g) were used for animal experiments.

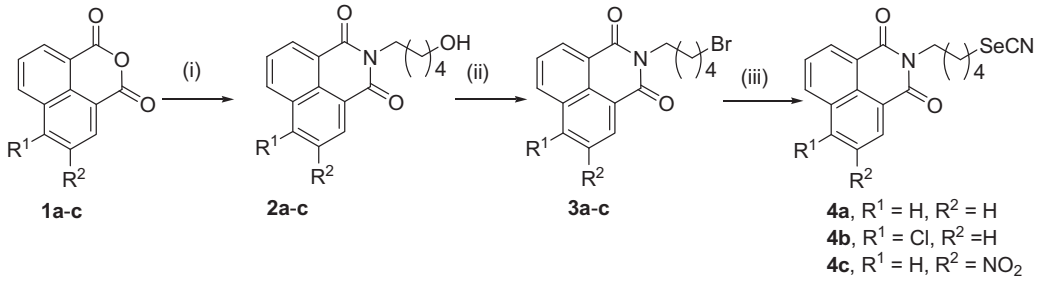
Selenium compounds **(4a–d)** were used as a suspension in 5.5% propylene glycol in water and Na_2SeO_3 was used in water. The compounds were administrated by oral gavage for 30 days. Each animal of the vehicle control group received 5.5% propylene glycol in water during the entire experimental period.

The oral toxicity (LD50) of the synthesized compounds **4a–d** were evaluated and compared to that of Na_2SeO_3. Different doses of compounds **4a–d** as suspension in aqueous solution of 5.5% propylene glycol and sodium selenite in water were made and administered by oral gavage for one time and observed the animals during the experimental period (30 days). The number of animals died each day was noted. LD50 values of **4a–d** were found to be more than 1000 mg/kg as compared to 25 mg/kg for Na_2SeO_3, establishing the fact that organoselenocyanates are relatively much less toxic than Na_2SeO_3. Oral administration of **4a–d** at the primary screening dose of 3 mg/kg (weight of the whole compound) in normal Swiss albino mice for 30 days showed no loss of body weight, whereas a decrease in final body weight (Fig. 1) was observed when the animals were given Na_2SeO_3 orally at a dose of 1.2 mg/kg b.w. (containing comparable amount of Se that is present in **4a–d**) for 30 days. This decrease in the b.w. was not significant for the first three weeks but became statistically significant ($p < 0.05$) at 4th week. Daily food intake was normal and there was no diarrhea or excessive salivation. These observations together with the no loss in the body weight indicated that the synthesized compounds had no negative effects on the growth of mice. Oral administration of compounds **4a–d** did not change the on haemoglobin level significantly ($p > 0.05$) as compared with vehicle control group. Generally high levels of transaminases such as ALT and AST are good indication of hepatic damage.[17] LPO is also used as biomarker to show the index of oxidative stress and causes cell membrane damage resulting in gradual loss of membrane fluidity, decrease membrane potential and increased permeability to ions.[18] Numerical decrease in ALT level ($p > 0.05$) and significant decrease in AST level ($p < 0.05$) was observed when treated with compound **4a–d**. This inhibition indicates the lowering in the release of these enzymes in plasma and characterizes the hepatoprotective effects of these compounds. Hepatic LPO level was numerically decreased when treated with compound **4a–d** but that was not statistically significant ($p > 0.05$) as compared with the vehicle control group. Where as treatment with Na_2SeO_3 increased the AST and LPO level significantly ($p < 0.05$) as compared to the vehicle control group demonstrating the toxic effect of inorganic selenium. Organic forms of selenium are more bioavailable than inorganic forms. This increased bioavailability may be counterbalanced by the lower toxicity of the organic forms.[19]

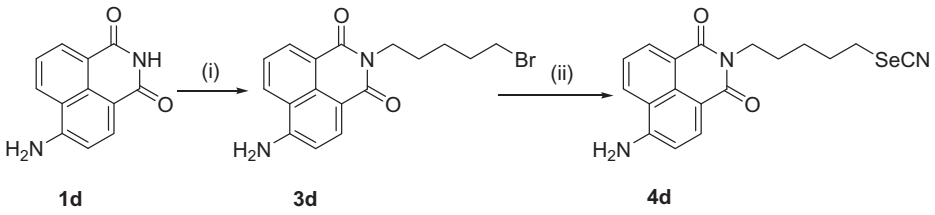
The nephrotoxicity markers BUN and creatinine showed significant decrease in **4a–d** treated groups **(**Table 1**)**. Hence, it is clear from enzyme studies that these organoselenium compounds **4a–d** have no adverse effect on liver and kidney functions and also have some protective efficacy.

Development of total chromosomal aberrations (CA) has often been used as sensitive biological indicator in the mutagenic bioassays of a drug.[20] In this present study no marked chromosomal aberration was observed when treated with **4a–d** with respect to the vehicle control group which supports the non mutagenic character of the compounds. But when treated with Na_2SeO_3, CA was increased numerically in comparison to the vehicle control animals but this increment is not statistically significant ($p > 0.05$) (Table 1).

The organ sparing property of compound **4a–d** is also confirmed by histological assessment. Histopahtological examination of selected organs (liver and kidney) from both and vehicle control and organoselinum treated (only the photomicrograph of compound **4d,** the most active analog**,** treated animals have been shown) animals showed normal architecture, suggesting no detrimental changes or morphological disturbances resulted from the administration of compounds **4a–d** for 30 days [Figs. 2(i), **(**ii) and 3(i), (ii)**]**. The over all cellular structure remains unaltered with no necrosis, inflammation or other significant lesion. Where as



Scheme 1. Reagents and conditions: (i) 5-aminopentanol, ethanol/water, 70 °C, 1 h; (ii) PBr_3, ethyl acetate, 80 °C, 1 h; (iii) KSeCN, 72 h, rt.



Scheme 2. Reagents and conditions: (i) 1,5-dibromopentane, DMF, methanolic sodium methoxide; (ii) KSeCN, 72 h, rt.

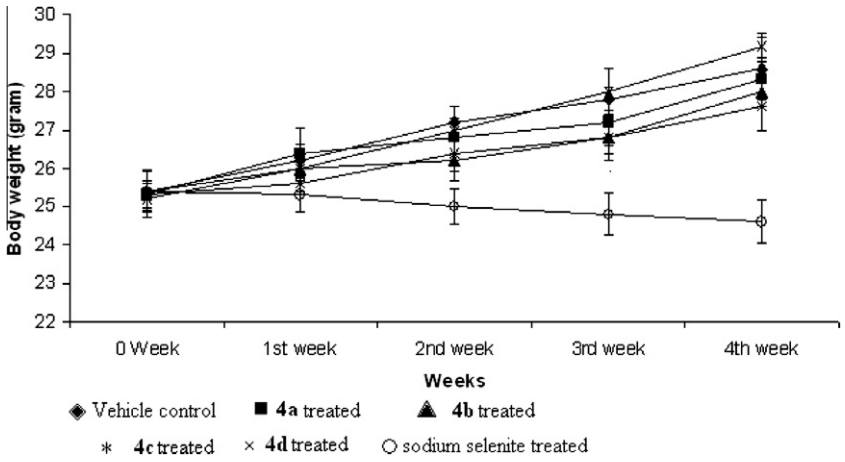


Figure 1. Change in body weight in different groups of mice.

Table 1
Effect of compounds **4a–d** and Na_2SeO_3 on some biochemical parameters along with CA in Swiss albino mice

Entry	Hemoglobin (gm/dL)	LPO (nmTBARS/mg protein)	ALT (U/mL)	AST (U/mL)	BUN (mg/dL)	Creatinine (mg/dL)	% of CA
Vehicle control	12.56 ± 0.43	0.26 ± 0.02	66.00 ± 4.18	179.00 ± 4.18	21.57 ± 0.92	9.40 ± 0.48	14.88 ± 0.99
4a	13.08 ± 0.41	0.24 ± 0.01	61.00 ± 5.47	160.00 ± 3.26*	17.38 ± 0.84*	6.40 ± 0.52*	13.39 ± 0.81
4b	12.86 ± 0.24	0.26 ± 0.01	64.00 ± 5.47	142.00 ± 5.70*	18.25 ± 1.03*	7.45 ± 0.47*	13.97 ± 0.59
4c	12.76 ± 0.43	0.25 ± 0.01	62.00 ± 5.71	154.00 ± 8.21*	17.26 ± 1.25*	7.86 ± 0.53*	13.85 ± 1.00
4d	13.24 ± 0.51	0.22 ± 0.01	59.00 ± 5.47	148.00 ± 13.50*	16.00 ± 0.95*	7.13 ± 0.62*	12.70 ± 0.93
Sodium selenite	11.28 ± 0.46*	0.30 ± 0.03*	72.00 ± 2.73	191.00 ± 6.51*	22.60 ± 1.70	8.47 ± 0.56	16.40 ± 0.95

Blood samples, liver tissue and bone marrow cells were collected 30 days after treatment with compound **4a–d** and Na_2SeO_3.
Results are expressed as means ± SD. Animals ($n = 6$) per group.
* Significantly different from vehicle control group at $p < 0.05$ (one-way ANOVA followed by Tukey test).

Na_2SeO_3 supplementation resulted in infiltration of mononuclear cells in both liver and kidney tissues [Figs. 2(iii) and 3(iii)].

Reactive oxygen species (ROS) such as superoxide anion or hydroxy radicals are known to destroy key biological components and cause damage to cell membranes. These reactive species are involved in the initiation, propagation and maintenance of both acute and chronic inflammatory processes.[21] There is a requirement for cellular defense against reactive oxygen species to protect cell membranes and other cellular component from oxidative damage and living organisms have evolved a number of defense mechanisms to cope with this oxidative stress. Oxygen handling cells have antioxidant and detoxifying enzymes (GST, GPx, SOD, CAT,

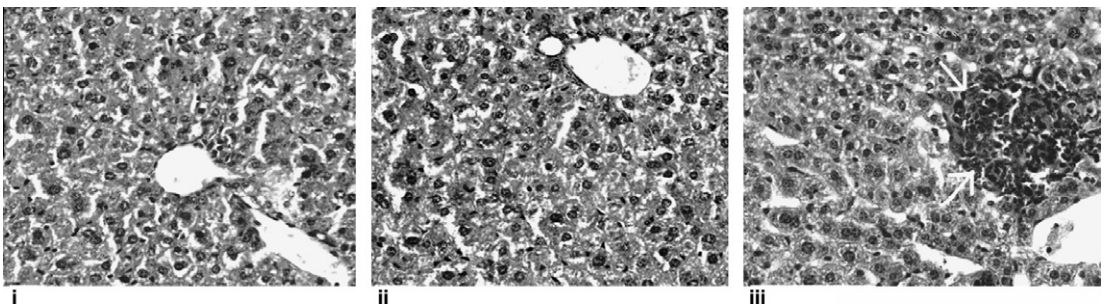


Figure 2. Histopathology of liver (400x); vehicle control group showed normal arrangement of hepatocytes with clear nuclei (i). Supplementation of compound **4d** (3 mg/kg), tissue structure within normal limits (ii). Supplementation of Na_2SeO_3 (1.2 mg/kg), tissue structure showing infiltration of mononuclear cells (iii) indicated by arrow.

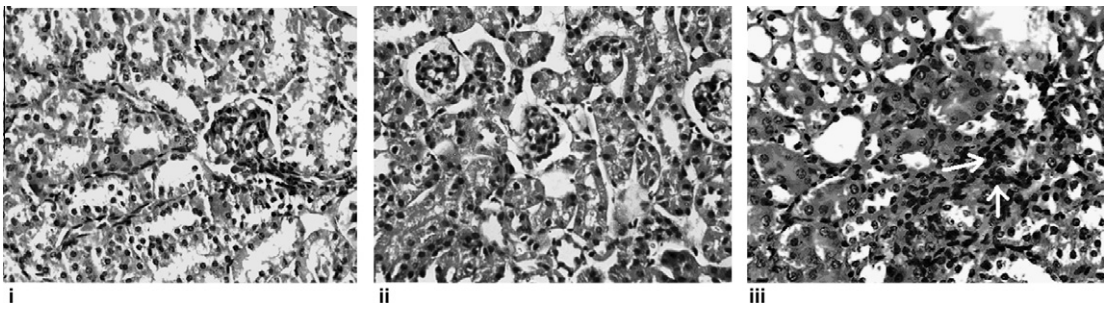


Figure 3. Histopathology of kidney (400x); vehicle control group showed normal arrangement kidney cell with clear nuclei (i). Supplementation of compound **4d** (3 mg/kg), tissue structure within normal limits (ii). Supplementation of Na_2SeO_3 (1.2 mg/kg), tissue structure showing infiltration of mononuclear cells (iii) indicated by arrow.

Table 2
Effect of compounds **4a–d** and Na_2SeO_3 on phase II detoxifying/antioxidant enzyme levels in liver of Swiss albino mice

Entry	GST (nmol CDNB-GSH min^{-1} mg $protein^{-1}$)	GSH (nmol GSH/mg protein)	SOD (unit of inhibition/mg protein)	CAT (units/ mg protein)	Gpx (μ mol NADPH utilized min^{-1} mg $protein^{-1}$)	TRxR (unit/mg protein)
Vehicle control	95.66 ± 4.0	46.87 ± 1.88	250.16 ± 7.46	41.66 ± 1.33	0.646 ± 0.030	3.20 ± 0.134
4a	104.41 ± 3.6*	50.94 ± 2.89*	258.56 ± 10.01*	45.48 ± 3.00	0.864 ± 0.051*	4.61 ± 0.28*
4b	106.58 ± 6.36*	59.34 ± 1.61*	268.71 ± 11.57*	46.60 ± 2.61*	0.995 ± 0.055*	4.21 ± 0.11*
4c	97.29 ± 2.28	55.82 ± 2.51	267.68 ± 21.63*	44.06 ± 2.21	0.938 ± 0.063*	4.15 ± 0.15*
4d	116.19 ± 6.06*	60.40 ± 3.75*	296.03 ± 14.66*	53.04 ± 3.04*	1.058 ± 0.026*	4.91 ± 0.18*
Sodium selenite	85.31 ± 2.64*	44.84 ± 2.45	236.98 ± 6.76*	36.60 ± 2.96*	0.692 ± 0.011*	3.75 ± 0.13*

Liver tissue was collected 30 days after the treatment with compound **4a–d** and Na_2SeO_3.
Results are expressed as means ± SD. Animals ($n = 6$) per group.
* Significantly different from vehicle control group at $p < 0.05$ (one-way ANOVA followed by Tukey test).

TrxR) which are the first line of cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before they interact to form more reactive hydroxide radicals, SOD mainly act by quenching of superoxide and active oxygen free radical, produced in different aerobic metabolism.[22] GPx is the first mammalian enzyme characterized which contains the unusual amino acid selenocysteine in its catalytic center.[23] The role of the enzyme glutathione peroxidase is to reduce hydrogen peroxide via its selenocysteine-containing active site, selenol, through a redox cycle in which two equivalents of glutathione are oxidized to the disulfide and water, while the hydroperoxide is reduced to the corresponding alcohol.[24] Another selenoenzyme, TRxR, which exerts its antioxidant action through catalyzing the reduction of oxidized thioredoxin by using NADPH as the electron donor. It is a key enzyme in Se metabolism, reducing Se compounds and thereby providing selenide to synthesis of all selenoproteins. Additionally, TrxR possesses a number of other antioxidant functions and directly scavenging lipid peroxides and hydrogen peroxide.[25] Compound **4a**, **4b**, **4c** and **4d** were found to be efficient enhancer of host antioxidant defense system such as SOD, CAT, GST, GSH, GPx, and TRxR. Compound **4d** showed better activity. Gpx and TRxR level increased by 63.77% and 53.43%, respectively as compared to the vehicle control group when treated with compound **4d** (Table 2). GPx and TRxR level were also increased when treated with Na_2SeO_3 but the increment was not that much as it was observed in case of organoselenium compounds. Na_2SeO_3 administration down regulated the other antioxidant and detoxifying enzyme levels such as SOD, CAT, and GST significantly ($p < 0.05$) as compare with the vehicle control group (Table 2).

Substitution of $–NH_2$ group at the 6 position on the naphthalimide ring (**4d**) gives an impetus to the antioxidant activity of the compound. The substitution effect on the antioxidative behaviors of the compounds is not clear. Electron donating effect of the $–NH_2$ group may help in the cleavage of selenocyanate functional group and make it more bioavailable for direct incorporation in protein. On the other hand electron withdrawing ability of $–NO_2$ group in naphthalimide ring might reduce the activity of the compound **4c**. Receptor specificity may be another reason for the better activity of **4d**. Further work is required to clarify these interesting phenomenons.

One liability of Se compounds is their toxicity. It is bona fide at this stage of study that these organoselenium compounds were not only nontoxic at the dose used but in addition they showed the ability to modulate the antioxidative defense system by modulating the antioxidant enzyme levels especially GPx and TRxR.

To conclude, these synthesized compounds have the potential to inhibit oxidative stress associated diseases and may help define new avenue for the prevention of such diseases. These compounds have also the potential to be used as chemopreotectors against toxicity imparted by cancer chemotherapeutic drugs.[26] Further structural optimization and detailed biological studies on the molecular mechanism of action about the designed naphthalimide based organoselenocyanate derivatives are under way.

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27. Preparation of 2-(5-selenocyanato-pentyl)-benzo[*de*]isoquinoline-1,3-dione (**4a**): Compound **4a** was prepared as described earlier.[16]
28. Preparation of 2-(5-selenocyanato-pentyl)-6-chloro, benzo[*de*]isoquinoline-1,3-dione (**4b**): A mixture 4-chloro-1,8-naphthalic anhydride (**1b**) (2.5 g, 10.75 mmol), and 5-amino-1-pentanol (3.33 g, 32 mmol) in ethanol (60 mL) was heated at 80–90 °C for 1 h and then cooled at 4 °C. The crystalline solid formed was filtered, washed with ice cold water and dried over P_2O_5 to give the compound **2b** (yield 62.4%) as yellow crystalline solid. Mp 105–106 °C.: The compound was pure enough to be used directly for the preparation of **3b** following the reported procedure[16] (yield 74%, mp 97–98 °C). The desired selenocyanate (**4b**) was obtained by treating the bromo compound **3b** with potassium selenocyanates (1:2) in dry acetone at 25 °C for 48 h. (Yield 65%, purity: 98.4%, mp 102–103 °C.)
Spectral data for **4b**:
^{1}H NMR ($CDCl_3$, TMS = 0.00) δ: 1.586 (q, 2H, J = 7.5 Hz), 1.80 (q, 2H, J = 7.2 Hz), 2.00 (q, 2H, J = 7.5 Hz), 3.08 (t, 2H, J = 7.2 Hz), 4.19 (t, 2H, J = 7.2 Hz), 7.83 (d, 1H, Ar-H, J = 7.8 Hz), 7.86 (t, 1H, Ar-H, J = 7.2 Hz), 8.50 (d, 1H, Ar-H, J = 7.8 Hz), 8.61 (dd, 1H, Ar-H, J = 1.0 Hz, 8.5 Hz), 8.66 (dd, 1H, Ar-H, J = 1.0 Hz, 7.4 Hz). ^{13}C NMR ($CDCl_3$): 26.43, 27.11, 29.24, 30.33. 39.89, 101.41, 121.33, 122.83, 127.29, 127.77, 128.88, 129.16, 130.58, 131.05, 131.94, 139.01, 163.33, 163.58. FABHRMS *m/z*: 405 and 407 $[M+1]^+$.
29. Preparation of 2-(5-selenocyanato-pentyl)-5-nitro benzo[*de*]isoquinoline-1,3-dione (**4c**): A mixture 3-nitro-1,8-naphthalic anhydride (**1c**) (2.5 g, 10.3 mmol) and 5-amino-1-pentanol (3.185 g, 30 mmol) in ethanol (40 mL) was stirred at rt for 10 min and then refluxed for 20 min and the resulting mixture were cooled, the solid product was then poured into ice cold water and again kept at −20 °C for 20 min. The solid formed was then filtered and washed with ice cold water and dried over P_2O_5 to give the compound **2c** (yield 58.7%) as brown solid, mp 115–116 °C. The compound was pure enough to be used directly for the preparation of **3c**. The bromo compound (**3c**) (yield 51%, mp 121–123 °C) and the desired compound **4c** were obtained as described above (yield 71%, purity: 97.4% mp 133–135 °C).
Spectral data for **4c**: ^{1}H NMR ($CDCl_3$, TMS = 0.00) δ: 1.61 (t, 2H, J = 8.1 Hz), 1.82 (q, 2H, J = 7.3 Hz), 2.01 (q, 2H, J = 7.3 Hz), 3.09 (t, 2H, J = 7.3 Hz), 4.23 (t, 2H, J = 7.3 Hz), 7.96 (t, 1H, Ar-H, J = 7.9 Hz), 8.44 (dd, 1H, Ar-H, J = 0.5 Hz, 8.2 Hz), 8.79 (dd, 1H, Ar-H, J = 0.9 Hz, 7.28 Hz), 9.14 (d, 1H, Ar-H, J = 2.1 Hz), 9.31 (d, 1H, Ar-H, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$): 26.39, 27.09, 29.18, 30.34, 40.20, 101.36, 13.00, 124.12, 124.49, 128.92, 129.07, 130.06, 130.93, 134.41, 135.57, 146.27, 162.39, 162.99. FABHRMS *m/z*: 417 $[M]^+$.
30. Preparation of 2-(5-bromo-pentyl)-6-amino benzo[*de*]isoquinoline-1,3-dione (**4d**): A magnetically stirred solution of 4-amino naphthalimide **(1d)** (2.0 g, 9.4 mmol) in dry dimethyl formamide (50 ml) at room temperature was treated with a methanolic solution of sodium methoxide (10 ml, 39.2 mmol). After stirring for 1 h 1,5-dibromo pentane (4.96 ml, 36.8 mmol) was added to the brown solution and stirring continued for another 15 min. The reaction was quenched with ice cold water to yield **3d** as yellow solid. It was purified by column chromatography over silica gel (petroleum ether (60–80 °C)/$CHCl_3$ (1:1, v/v), (yield 69.4%. mp 158–159 °C, lit.[31] mp 157–159 °C). Compound **4d** was obtained as described above (yield 71%, purity: 96.8%, mp 189–190 °C).
Spectral data for **3d**: ^{1}H NMR ($CDCl_3$, TMS = 0.00) δ: 1.565 (m, 2H), 1.765 (q, 2H, J = 7.4 Hz), 1.943 (q, 2H, J = 6.9 Hz), 3.425 (t, 2H, J = 6.3 Hz), 4.168 (t, 2H, J = 7.2 Hz), 4.979 (s, 2H, NH_2), 6.89 (d, 1H, J = 8.1 Hz, Ar-H), 7.659 (t, 1H, J = 7.8 Hz, Ar-H), 8.12 (d, 1H, J = 8.1 Hz, Ar-H), 8.42 (d, 1H, J = 8.1 Hz, 5-H), 8.6 (d, 1H, J = 7.2 Hz, Ar-H). ^{13}C NMR ($CDCl_3$): 25.68, 27.23, 32.45, 33.64, 39.80, 109.57, 112.15, 120.08, 123.11, 124.99, 126.82, 129.79, 131.48, 133.77, 149.08, 163.99, 164.54. ESI-MS *m/z*: 383.04 and 385.04 $[M+Na]^+$.
Spectral data for **4d**: ^{1}H NMR ($CDCl_3$, TMS = 0.00) δ: 1.578 (m, 2H), 1.770 (q, 2H J = 6.6), 1.991 (q, 2H, J = 6.6), 3.3083 (t, 2H, J = 6.9 Hz), 4.180 (t, 2H, J = 6.9 Hz), 4.962 (s, 2H), 6.9 (d, Ar-H, J = 8.1 Hz), 7.672 (t, Ar-H, J = 7.8 Hz), 8.119 (d, Ar-H, J = 8.4 Hz), 8.42 (d, Ar-H, J = 7.5), 8.607 (d, Ar-H, J = 7.2 Hz). ^{13}C NMR (DMSO): 25.90, 26.96, 29.57, 30.40, 39.92, 104.29, 107.54,108.09, 119.30, 121.71, 123.84 129.24, 129.60, 130,88, 133.83,152.59, 162.82, 163.69. ESI-MS *m/z*: 387.81 $[M]^+$.
31. Hodgkiss, R. J.; Jones, G. W.; Long, A., et al *J. Med. Chem.* **1991**, *34*, 2268.