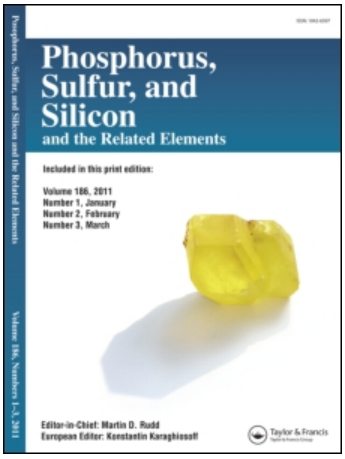

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Synthesis of Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Esters: Cytogenetic Studies on Human Peripheral Blood Leucocyte Cultures, and Anti-Bacterial Studies, and Anti-Fungal Studies of Their Effects

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The compounds selenolo(2,3-b)quinoline-2-carboxylic ethyl esters were synthesized in good yields by the reaction of 3-(2-chloro-3-quinolyl)acrylic ethyl esters, with the nucleophilic reagent sodium diselenide in ethanol medium under a nitrogen atmosphere. Cytogenetic studies on human blood leucocytes in vitro *were evaluated for some of the synthesized compounds. Most of the synthesized compounds were tested for their antibacterial and antifungal activities.*

Keywords Condensation; cyclisation using sodium diselenide; dehydroxychlorination; esterification; hydrolysis; Vilsmeier-Haack Reaction

INTRODUCTION

(i). Selenium

It is known to be an important dietary antioxidant and essential component of the active sites of a number of enzymes and several

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Presented as a poster presentation entitled "Synthesis of Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Esters and Their Cytogenetic Studies on Human Chromosomes, Antibacterial Studies, Antifungal Studies" in the IXth International Conference on the Chemistry of Selenium and Tellurium held on 23–27th of February 2004.

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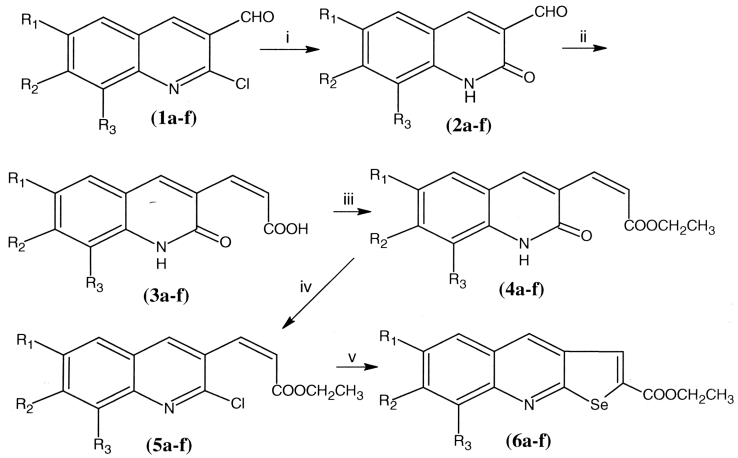
additional mammalian selenoproteins. Dietary selenium deficiency has been linked to diseases as diverse as cancer,[1] heart disease,[2] arthritis,[3] and AIDS.[4] Organoselenium compounds have been extensively investigated due to their close association with various type of activities such as antifungal and antibacterial activities;[5–7] cardiovascular and antihistaminic actions;[8] antiadrenalin, antiradiation,[9] antileukemic activity;[10] antitumour activity,[11] analgesic, antirheumatic, and antipyretic activities;[12] and parathyroid scanning for the deduction of lymphomas,[13] etc.

Early workers from our laboratory have synthesized selenolo(2,3-b)quinolines[14–16] using 2-quinolone-3-ethanols and 2-chloro-3-vinyl quinolines as the starting compounds. In view of their remarkable activities, we pursued our investigation on selenium heterocycles and we report herein an expedient and convenient procedure for the synthesis of selenolo(2,3-b)quinoline-2-carboxylic ethyl esters (**6**) in very good yields from 3-(2-chloro-3-quinolyl)acrylic ethyl esters (**5**) (Scheme 1).



SCHEME 1 (i) 4M HCl (ii) Malonic acid, pyridine, piperidine, (iii) abs. ethanol, conc. H_2SO_4 (iv) $POCl_3$ (v) Na_2Se_2. (**a**) R_1=R_2=R_3=H, (**b**) R_1=CH_3, R_2=R_3=H, (**c**) R_1=R_3=H, R_2=CH_3, (**d**) R_1=R_2=H, R_3=CH_3, (**e**) R_1=OCH_3, R_2=R_3=H, (**f**) R_1=R_3=H, R_2=OCH_3.

(ii). Cytogenetic Studies

DNA molecular assay is extensively used in *in-vitro* and *in-vivo* genetic drug toxicology. Different types of cell lines and practically any type of cell from any target organs can be tested on molecular assay.

DNA molecular assay can be used to study the damage in cells in different cell cycle phases. The rationale of this application is proportional to the DNA content in cells. Alternatively, it is possible to use this approach for an unknown cell line on the basis of distribution, the comet fluorescence as measured by the DNA molecular assay.

Chemicals or drugs capable of interacting with DNA have a high probability of being carcinogenic or otherwise hazardous. Genetic effects of various diseases and drugs can be detected by their interactions with DNA and by the consequences of these interactions such as chromosome alterations, mutagenesis, and the inhibition of DNA synthesis and DNA repair. The carcinogenesis or mutagens of drugs involve an alteration in DNA that must be stable and permanent so that it can be transmitted in subsequent genetic copies.

The SCG/Comet analysis is becoming more significant because of its simplicity, speed, and requirement of only a few cells. Blood cells embedded in low-melting agrarose, when treated with high-salt lysis solution, retain only high mol.-wt. supercoiled DNA. When placed in an alkaline solution, it commences to unwind from sites of strand breakage. When an electric current is then applied, DNA fragments stream towards the anode giving the appearance of a 'Comet tail.' Undamaged DNA remains trapped within the nucleolus. The length of these comet tails is indicative of the extent of DNA damage. DNA damage is likely to happen under oxidative stress. Direct binding of drug compounds could be the possible reason for the DNA single damage.

EXPERIMENTAL

Melting points were determined using Raaga melting point apparatus and were uncorrected. The IR spectra were recorded on an FTIR 8201 (PC)S spectrometer as KBr pellets and the absorption frequencies are expressed in reciprocal centimeters (cm^{-1}). Proton NMR spectra were recorded on a Gemini-200 MHz or on a Varian AMX 400 spectrometer in $CDCl_3$. The chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. Elemental analysis was performed by a Perkin-Elmer model 240B CHN analyzer and the values are within the permissible limits (± 0.5). The mass spectra were recorded by an EIMS technique on an Autospec mass spectrometer. The crude products were checked by thin-layer chromatography and purified by column chromatography using silica gel (60–120 mesh).

(i). In our present work selenium heterocycles were synthesized from the potential precursor 2-chloro-3-formyl quinolines, which were prepared by following the O. Meth-cohn procedure.[17] 2-chloro-3-formyl quinolines **(1)** were converted to the oxo compounds **(2)** by refluxing

with 4 MHCl. These were then condensed with malonic acid under the conditions of the Knoevenagel reaction to furnish 3-(2-oxo-1, 2-dihydro-3-quinolyl)acrylic acids[18] **(3)**.

Esterification of 3-(2-Oxo-1,2-dihydro-3-quinolyl)acrylic Acid (4)

The ethyl ester was obtained in an almost quantitative yield by boiling the acrylic acid **(3)** (3 g) with concentrated sulphuric acid (4.5 mL) and absolute ethanol (150 mL). The acid gradually passed into the solution after boiling for 5–6 h on a steam bath. The solution was then allowed to cool, and a mass of needles of ester formed. A considerable quantity was further precipitated by dilution with two volumes of water. The whole solid was collected, washed with water, dried, recrystallised from ethanol, and was ready for further use.

Preparation of 3-(2-Chloro-3-quinolyl)acrylic Ethyl Ester (5)

The ester **(4)** (0. 0228 mole) was treated with freshly distilled phosphorus oxychloride (13.6 mL, 0. 148 mole) and kept on a steam bath for 5–6 hrs. On cooling and pouring into crushed ice, the compound separated as a creamy white solid. It was then recrystallised from petroleum ether:benzene (4:1 v/v) and obtained as colourless needles (Table I).

Preparation of Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6)

To a freshly prepared solution of sodium diselenide[19] [selenium powder (0. 0120 mole, 0.955 g) and sodium borohydride (0. 00846 mole, 0.319 g) in ethanol], chloroester (**5**) (1 g, 0.00404 mole) was added and heated under reflux temperature for 15–16 h on a steam bath. Thereafter the

TABLE I Physical and IR Data of 5a–f*

Compound	Yield %	M.p°C	IR(cm^{-1})	Mass m/e
5a	85	120–122	1710, 1267, 1064	261, 263
5b	87	90–92	1701, 1263, 1041	275, 277
5c	89	135–137	1708, 1265, 1039	275, 277
5d	92	144–146	1711, 1274, 1031	275, 277
5e	80	96–98	1709, 1269, 1049	291, 293
5f	85	115–117	1712, 1261, 1026	291, 293

*Recrystallised from petroleum ether:benzene (4:1 v/v).

solution was evaporated and the residue was dissolved in chloroform followed by washing with water. The organic extract was dried and evaporated. The residue was then chromatographed over silicagel with pet. ether (60–80°C) as the eluent.

PHYSICAL AND SPECTROSCOPIC DATA OF (6A–F)

Selenolo(2,3-b)-quinoline-2-carboxylic Ethyl Ester (6a)

Light yellow needle-shaped crystals, Yield = 65%, Melting point = 194–196°C, (Cal: C, 55.20; H, 3.64; N, 4.62; Found: C, 55.4; H, 3.50; N, 4.59), IR (KBr, cm^{-1}) = 1726 (—C=O of ester), 1192 (—C—O of ester), ^{1}H NMR ($CDCl_3$) δppm: 1.40 (t, J = 7 Hz, $-CH_3$ of ester), 4.30 (q, J = 7 Hz, $-OCH_2$ of ester), 7.28–8.01 (m, 6H, C_3, C_4, C_5, C_6, C_7, C_8—H), M.F. = $C_{14}H_{11}NO_2Se$, Mass (m/z)M^+ = 304.

6-Methyl Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6b)

Light yellow needle shaped crystals, Yield = 60%, Melting point = 159–161°C, (Cal: —C, 56.59; H, 4.12; N, 4.42; Found: —C, 56.50; H, 4.10; N, 4.40), IR (KBr, cm^{-1}) = 1709 (C=O of ester), 1251 (—C—O of ester), ^{1}H NMR ($CDCl_3$) δppm: 1.43 (t, J = 7 Hz, $-CH_3$ of ester), 4.42 (q, J = 7 Hz, $-OCH_2$ of ester), 2.57 (s, 3H, $-CH_3$), 8.26 (s, 1H, C_4—H), 8.53 (s, C_3—H), 8.13 (d, J = 2 Hz, C_5—H), 8.03 (s, C_7—H),7.64 (d, C_8—H, J = 8.2 Hz). M.F. = $C_{15}H_{13}NO_2Se$, Mass (m/z)M^+ = 318.

7-Methyl Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6c)

Light yellow needle-shaped crystals, Yield = 66%, Melting point = 178–180°C, (Cal: —C, 56.59; H, 4.12; N, 4.42; Found: —C, 56.60; H, 4.12; N, 4.50), IR (KBr, cm^{-1}) = 1712 (C=O of ester), 1251 (—C—O of ester), ^{1}H NMR ($CDCl_3$) δppm: 1.42 (t, J = 6.6 Hz, $-CH^3$ of ester), 4.44 (q, J = 6.8 Hz $-OCH_2$ of ester), 2.61 (s, 3H, $-CH_3$), 8.25 (s, 1H, C_4—H), 8.56 (s, C_3—H) 7.39–7.92 (m, 3H, C_5, C_6, C_8—H). M.F. = $C_{15}H_{13}NO_2Se$, Mass (m/z)M^+ = 318.

8-Methyl Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6d)

Light yellow needle-shaped crystals, Yield = 75%, Melting point = 164–166°C, (Cal: —C, 56.59; H, 4.12; N, 4.42; Found: —C, 56.60; H, 4.12; N,

4.50), IR (KBr, cm^{-1}) = 1724 (C=O of ester), 1254 (—C—O of ester), ^1H NMR ($CDCl_3$) δppm: 8.25 (s, C_4—H), 8.56 (s, C_3—H), 7.45 (t, J = 7.4 Hz, C_6—H), 7.79 (d, J = 8 Hz, C_5—H), 7.63 (d, J = 7 Hz, C_7—H), 2.86 (s, 3H, CH_3), 1.44 (t, J = 7 Hz, 3H, CH_3 (of ester)), 4.43 (q, J = 7 Hz, 2H, —OCH_2). M.E. = C_{15} H $_{13}NO_2Se$, Mass (m/z)M^+ = 318.

6-Methoxy Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6e)

Light yellow needle-shaped crystals, Yield = 66%, Melting point = 145–147°C, (Cal: —C, 53.88; H, 3.92; N, 4.21; Found: —C, 53.80; H, 3.99; N, 4.21), IR (KBr, cm^{-1}) = 1718 (C=O of ester), 1242 (—C—O of ester), ^1H NMR ($CDCl_3$) δppm: 8.28 (s, C_4—H), 8.54 (s, C_3—H), 7.51–7.93 (m, 3H, C_5, C_7, C_8—H), 4.47 (q, J = 7.6 Hz, 2H, —OCH_2), 3.99 (s, 3H, —OCH_3) 1.46 (t, J = 6.4 Hz, 3H, —CH_3) M.F. = C_{15} H_{13} NO_3Se, Mass (m/z)M^+ = 334.

7-Methoxy Selenolo(2,3-b)quinoline-2-carboxylic Ethyl Ester (6f)

Light yellow needle-shaped crystals, Yield = 58%, Melting point = 154–156°C, (Cal: —C, 53.88; H, 3.92; N, 4.21; Found: —C, 53.89; H, 3.99; N, 4.15), IR (KBr, cm^{-1}) = 1700 (C=O of ester), 1245 (—C—O of ester), ^1H NMR ($CDCl_3$) δppm: 8.23 (s, C_4—H), 8.50 (s, C_3—H), 7.24–7.84 (m, 3H, C_5, C_6, C_8—H), 3.97 (s, 3H, —OCH_3), 1.45 (t, 3H, —CH_3), 4.40 (q, J = 6.2 Hz, —OCH_2) M.F. = $C_{15}H_{13}NO_3Se$, Mass (m/z)M^+ = 334.

(ii). Cytogenetic Studies

DNA molecular assay was carried out for some of the synthesized compounds 7-methoxy-selenolo(2,3-b)quinoline-2-carboxylic ethyl ester **(6f)**, 8-methyl-selenolo(2,3-b)quinoline-2-carboxylic ethyl ester **(6d)**, and 6-methyl-selenolo(2,3-b)quinoline-2-carboxylic ethyl ester **(6b)**.

Cell Culture from Peripheral Blood Leucocytes

Cultures of leucocytes obtained from peripheral blood were set up following the method of Hungerford.[20] The chromosomal preparation obtained were strained with Giemsa and were also processed to obtain G-bands.

Leucocyte Cultures In-Vitro

The experiments were carried out as outlined by Hungerford.[20] Three experiments were carried out using three nonsmoking healthy male

donors, ages 23, 24, and 25 years. Intravenous blood was collected aseptically and whole blood was cultured with McCoy's medium containing 25% human AB serum, 0.5% phytohemagglutinin, and 0.25% Dicrystin-S. The test compounds were dissolved in 1% $CHCl_3$. Four different concentrations of 0.02, 0.2, 2, and 20 μg/mL were added to the culture medium (0.1 mL solution of the compounds (**6f**), (**6d**), and (**6b**) 8 mL of the medium) at 0, 24, and 48 h after culture initiation, indicated as treatment for 72, 48, and 24 hr, respectively. Triplicate cultures for each dose from three donors were maintained for the study of chromosomal aberrations. Control-I was given an equal volume of distilled water. Control-II was given an equal volume of 1% $CHCl_3$. 0.1 mL $CHCl_3$ was added to 8 mL of medium at 0, 24, and 48 h after culture initiation indicated as treatment for 72, 48, and 24 h respectively. All cultures were incubated at 37°C for the 72-h slides, which were prepared using the method of Moorhead et al.[21]

Analysis of Chromosomal Aberrations

All the prepared slides were strained with Giemsa. Three hundred and fifty well-spread metaphase plates were screened for chromosome aberrations. The aberrations include chromatid gaps, chromatid breaks, isochromatic breaks, and chromatid interchanges. Polyploid cells were also screened in all the slides.

Preparation of Slides

A test slide was prepared by placing a drop of the cells suspended on a clean, chilled slide, immediately drying it on a slide by keeping the slide at 40°C for few seconds. Thereafter, the dried slides were strained with giemsa for 10 min and air dried. Three hundred and fifty well-banded metaphases were analysed under an oil-immersion lens by Olympus microscope.

The clastogenic properties of the compounds **(6f)**, **(6d)**, and **(6b)** were studied by investigating the effects of the compounds on human chromosomes *in-vitro* in leukocyte cultures.

(iii). Antibacterial Activity

Methodology

Antibacterial activity of selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6a**) and its derivatives: 7-CH_3 **(6c)**, 6-CH_3 **(6b)**, 7-Ome **(6f)**, and 8-CH_3 (**6d**) were screened for the *in-vitro* growth inhibitory activity against *Escherichia coli, Aeromonas hydrophila, Salmonella typhi*, and *Pseudomonas aeurginosa*.

Anti-bacterial activity of the synthesized compounds were determined by Disc diffusion technique.[22] About 10^5 concentration of bacterial cells were enriched in 5 mL of the nutrient broth (NaCl-5.0 g, peptone-5 g, beef extract-3 g, yeast extract-3 g in 1000 mL of distilled water, pH = 7.3 ± 0.2).

Enriched bacterial cells were swabbed onto a Muller Hinton Agar medium (Himedia India). The test compounds were dissolved in $CHCl_3$ to a final concentration of 0.25%, 0.5%, and 1% and soaked in filter paper discs of (5 mm diameters and 1 mm thickness). These discs were placed on the seeded plates and incubated at 37°C for 24 h. The zones of inhibition around the discs were measured after 24 h. Norfloxacin(Nx)-10 mcg and Amoxycillin(Ac)-30 mcg were used as the standard to compare the antibacterial activity of the compounds.

(iv). Antifungal Activity

Antifungal activity of selenolo(2,3-b)quinoline-2-carboxylic ethyl ester **(6a)** and its derivatives: 7-CH_3 **(6c)**, 6-CH_3 **(6b)**, 7-Ome **(6f)**, and 8-CH_3 **(6d)** were screened for *in-vitro* growth inhibitory activity against *Aspergillus niger, Aspergillus flavus*, and *Fusarium spp* using the disc diffusion method.[5] The above fungal spores were enriched in sabouraud dextrose broath (dextrose-10 g, peptone-10 g, chloramphenicol-0.002 g, distilled water-1000 mL, pH-5.6 ± 0.2) and incubated at room temperature for 4–6 days. The enriched fungal mycelium were cultured in a sabouraud dextrose agar medium (dextrose-10 g, peptone-10 g, chloramphenicol-0.002 g, distilled water-1000 mL, pH-5.6 ± 0.2, agar-20 g. The test compounds were dissolved in $CHCl_3$ to a final concentration of 0.25%, 0.5%, and 1% and soaked in filter-paper discs. These discs were placed on the seeded plates and incubated at room temperature for 4–6 days. After 4 days the inhibition zones appearing around the discs in each plate were measured and tabulated using Carbendazim and Fluconazole (Himedia, India) and were used as the standard.

RESULTS AND DISCUSSION

(i). Even though there are many inorganic selenium reagents and organoselenium reagents, the role of Na_2Se_2 is excellent when it is used for the introduction of selenium into organic molecules. The ethanolic solution of sodium diselenide is advantageous, as it provides a superior solvent for nucleophilic displacement reactions in water insoluble or hydrolysis-sensitive organic compounds. In this context it is relevant to mention the synthesis of selenolo(2,3-b)quinolines by reacting 2-chloro-3-vinyl quinolines with Na_2Se_2 prepared *in situ* by Shanmugam

et al.[15] The present work was started with the synthesis of 8-methyl derivatives.

The compound (**5d**) was synthesized by dehydroxychlorination of 8-methyl-3-(2-oxo-1,2-dihydro-3-quinolyl)acrylic acid **(4d)** with freshly distilled phosphorus oxychloride on a steam bath for 5–6 h. This was followed by recrystallisation from pet. ether: benzene (4:1 v/v), giving rise to colorless needle-shaped crystals melting at 144°C. The structure of **(5d)** was attested through the following spectral values.

IR(KBr)Cm^{-1}: 1711 (—C=O of ester), 1031 (—C—Cl), ^{1}H-NMR ($CDCl_3$) δ(ppm):8.33 (s, C_4—H), 8.15, 6.56 (d, 1H each —CH=CH_{trans}, J = 16 Hz), 7.69 (d, J = 8 Hz, C_7—H), 7.60 (d, J = 7.4 Hz, C_5—H), 7.47 (t, J = 7.4 Hz, C_6—H), 1.49 (t, J = 7 Hz, 3H, —CH_3 (of ester)), 4.44 (q, J = 7 Hz, 2H,—OCH_2), Mass spectra: m/e = 275(M^+), 277 (M+2) which is one third the intensity of the parent peak, attested to the structure of the compound as 8-methyl-3-(2-chloro-3-quinolyl)acrylic ethyl ester **(5d)**.

The compound (**5d**) formed a very good intermediate for the synthesis of the selenium heterocycles in good yields by reacting it with a freshly prepared solution of sodium diselenide in absolute ethanol on a steam bath. The starting compound **(5d)** completely disappeared during a course of 15 h as revealed by the TLC analysis. Thereafter the solution was evaporated and the residue obtained was taken up with chloroform and washed with water. The chloroform extract was dried and evaporated. The obtained residue was purified through column chromatography using petroleum ether as the eluent. The product was obtained as light yellow needle-shaped crystals upon recrystallisation from petroleum ether with m.p 164–166°C in 75% yield.

Strong peaks at 1724 cm^{-1} (—C=O of ester) and 1254 cm^{-1} (—C—O of ester) in IR spectrum, three singlets at δ 8.25, δ 8.56, and δ 2.86, two triplets at δ 7.45 and δ 1.44, two doublets at δ 7.79, δ 7.63, one quartet at δ 4.43 in ^{1}H NMR spectrum, molecular ion peak at 318(M^+) with characteristic isotopic abundance pattern expected for selenium containing compounds in mass spectrum, clearly indicated the structure as 8-methyl selenolo(2,-3-b)quinoline-2-carboxylic ethyl ester (**6d**).

The above reaction sequences were extended to synthesize **6a**, **6b**, **6c**, **6e**, and **6f** derivatives, respectively.

(ii). Cytogenetic Studies

The data of the compounds (**6b**), (**6d**), and (**6f**) were pooled. It was noted from Table II that the compound showed chromosomal aberrations as a dose dependant increase. An increase was seen only with the concentration of 20 μg/mL, when the compound (**6f**) was added at 24 h, 48 h

TABLE II Number of Chromosomal Aberrations *In Vitro* Following Addition of the Compound (6f) at 0, 24, and 48 h After Culture Initiation

Concentration of the compound (**6f**) (μg/mL)	Average no of metaphases/replicate (n = 3)	Gaps		Breaks			
		Chromatid	Isochromatid	Chromatid	Isochromatid	Chromatid interchange	Total
0 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0
24 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	10(2.85)	—	8(2.28)	—	—	18 ± 1.00
48 h							
Control—I	350/3	—	—	—	—	—	0.0
Control—II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	5(1.42)	—	5(1.42)	—	—	10 ± 0.00

Mean values are the number of aberrations from three replicate donors (on average 350 cells) values in parentheses are percentages.

Control-I was given an equal volume of distilled water.

Control-II was given an equal volume of 1% $CHCl_3$.

after culture initiation. Chromosomal aberrations like chromatid gaps and chromatid breaks were observed. Polypolid cells were absent in all the examined slides. However, it was inferred from Tables III and IV that compounds (**6d**) and (**6b**) caused no chromosomal aberrations at all concentrations.

In the present study, it was inferred that the compound (**6f**) was a very mild mutagen and at higher concentration it might cause genotoxicity in humans. However, compounds (**6d**) and (**6b**) did not cause genotoxicity in humans.

(iii). Anti-Bacterial Activity

6-CH_3 selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6b**) was sensitive against *E. coli* and A. *hydrophila* at 1% $CHCl_3$, *S. typhi* at

TABLE III Number of Chromosomal Aberrations *In Vitro* Following Addition of the Compound (6d) at 0, 24, and 48 h After Culture Initiation

Concentration of the compound (**6d**) (μg/mL)	Average no of metaphases/replicate (n = 3)	Gaps		Breaks			
		Chromatid	Isochromatid	Chromatid	Isochromatid	Chromatid interchange	Total
0 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0
24 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0
48 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0

Mean values are the number of aberrations from three replicate donors (on average 350 cells) values in parentheses are percentages.

Control-I was given an equal volume of distilled water.

Control-II was given an equal volume of 1% $CHCl_3$.

0.25% $CHCl_3$. Intermediate against *Ps. aeurginosa* at 0.25% $CHCl_3$. The pathogens *E. coli*, *S. typhi*, *Ps. aeurginosa* and *A. hydrophila* were resistant at 0.5% chloroform.

7-OCH_3 selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6f**) was intermediate against all the test organisms and resistant against *A. hydrophila* at all concentrations. With an increase in concentration the test compound (**6f**) was resistant against all the pathogens at all concentrations. 8-CH_3 selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6d**) was sensitive against *S. typhi* at 0.5% $CHCl_3$, intermediate against *E. coli* and *Ps. aeurginosa*, and resistant against. *A. hydrophila* at all concentrations. Selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6a**) was resistant against all the pathogens at all concentrations. 7-CH_3 selenolo(2,3-b)quinoline-2-carboxylic ethyl ester (**6c**) was intermediate

TABLE IV Number of Chromosomal Aberrations *In Vitro* Following Addition of the Compound (6b) at 0, 24, and 48 h After Culture Initiation

Concentration of the compound (**6b**) (μg/mL)	Average no of metaphases/replicate (n = 3)	Gaps		Breaks			
		Chromatid	Isochromatid	Chromatid	Isochromatid	Chromatid interchange	Total
0 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0
24 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0
48 h							
Control-I	350/3	—	—	—	—	—	0.0
Control-II	350/3	—	—	—	—	—	0.0
0.02	350/3	—	—	—	—	—	0.0
0.2	350/3	—	—	—	—	—	0.0
2	350/3	—	—	—	—	—	0.0
20	350/3	—	—	—	—	—	0.0

Mean values are the number of aberrations from three replicate donors (on average 350 cells) values in parentheses are percentages.

Control-I was given an equal volume of distilled water.

Control-II was given an equal volume of 1% $CHCl_3$.

TABLE V Antibacterial Activity

	Diameter of inhibition zone in mm											
	Escherichia coli			*Aeromonas hydrophila*			*Salmonella typhi*			*Pseudomonas aeurginosa*		
Compounds	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%
6a	12	0	0	11	0	0	13	0	0	13	0	0
6c	14	0	0	16	12	0	0	0	0	17	12	11
6b	15	12	19	15	12	18	18	12	12	16	12	11
6f	17	12	11	0	0	0	17	12	12	16	12	11
6d	12	14	17	12	0	0	14	19	11	16	12	11
Nx	28	28	28	21	21	21	24	24	24	24	24	24
Ac	21	21	21	28	28	28	24	24	24	—	—	—

TABLE VI Antifungal Activity

	Diameter of inhibition zone in mm								
	Aspergillus niger			*Aspergillus flavus*			*Fusarium spp*		
Compounds	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%
6a	—	—	—	11	13	12	11	11	10
6c	11	15	14	14	14	13	—	—	—
6b	12	13	12	17	12	12	—	—	—
6f	11	11	10	—	—	—	—	—	—
6d	16	15	15	11	16	15	—	—	—

against *A. hydrophila* and *Ps. aeurginosa* at 0.5% $CHCl_3$ and resistant against *E. coli* and *S. typhi*.

Generally 6-CH_3 derivative was more sensitive to the test organisms than the other derivatives. The overall results showed that the derivatives could not reach the effectiveness of the conventional bactericide, Norfloxacin and Amoxycillin (Table V).

(iv). Antifungal Activity

All the test compounds were resistant to *fusarium spp* at all concentrations. **(6b)** and **(6d)** showed intermediate activity against *Aspergillus flavus* at 0.25% and 0.5% $CHCl_3$, but was resistant at other concentration. **(6a)**, **(6c)**, **(6b)**, and **(6f)** were resistant to *Aspergillus niger* but **(6d)** showed intermediate activity at 0.25% $CHCl_3$. The results gave a clear picture with regard to the inhibition zone, which decreased with an increase in concentration of the test solution containing the above compounds. The overall results showed that the derivatives could not reach the effectiveness of the conventional fungicide, Carbendazim and Fluconazole (Himedia, India) (Table VI).

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