

anti-breast cancer activity of some new organoselenium compounds

Abstract

A series of selenazole, (selenazol-nitrone) and selenazine derivatives were synthesized. The reaction of arylselenocarboxamides with substituted α -halo carbonyl compounds, such as 3-chlorobutan-2-one, 1-chloropropan-2-one, 2-bromomalonaldehyde and 2-chloroacetyl chloride, afforded four cyclic compounds of substituted 2-aryl-1, 3-selenazole, while the reaction of arylselenocarboxamides with substituted β -halo carbonyl compounds, such as 3-chloro-1-(4-chlorophenyl) propan-1-one and 3-chloro-1-phenylpropan-1-one, afforded two cyclic compounds of substituted 2-aryl-1, 3-selenazines. The reaction between 2-phenyl-1, 3-selenazole-5-carbaldehyde and N-4-fluorophenylhydroxylamine afforded a compound of selenazole combined with nitrone. All synthesized compounds were characterized by FTIR, ^1H NMR, ^{13}C NMR and MASS spectroscopy. The results verified the expected chemical structures of synthesized compounds. MTT assay used to evaluate 1, 3-selenazine (S_6), namely 2, 4-diphenyl-6H-1, 3-selenazine against human breast cancer MDA-MB231 with 24 and 48 h. Thus, compound S_6 showed significant activity against breast cancer.

Keywords: Arylselenocarboxamides, 1, 3-Selenazoles, (Selenazol-nitrone), 1, 3-Selenazines, MDA-MB231 cancer cells

Introduction

There is wide interest in heterocyclic organic selenium compounds because of their influential biological activities. Whereas the five- and hexa-cyclic organic selenium compounds occupy a distinguished place in the field of organic synthesis^(1,2) Selenazole is one of the most important derivatives containing the five member ring, while selenazine is one of the most important derivatives containing the six member ring⁽³⁾. These compounds have many applications in the field of preparing some pharmaceutical compounds and in the field of agricultural pesticides and as catalysts in fields that include the synthesis of industrial polymers⁽⁴⁻⁶⁾. Where they were considered as raw materials for some polymeric adhesive materials and in some types of coatings, as well as added as stabilizers when synthesising some polymers^(7,8). Research has proven that medicines containing cyclic organic selenium compounds are considered one of the most important types of treatments because It helps to raise the body's immunity, as well as protects the body from oxidative processes that produce free radicals, due to the ability of these compounds to capture these radicals⁽⁹⁾. Selenium is one of the important elements for the completion of many vital reactions naturally, including the metabolism process^(10,11). Nitrone compounds are one of the most important classes in organic chemistry because of their multiple applications, including their use as a drug delivery⁽¹²⁻¹⁴⁾. As well as the antifungal activity of nitrone and as anti-allergic⁽¹⁵⁾.

In this study, three selenazole compounds were synthesized in addition to the synthesis of one compound of nitron as a derivative of selenazole, as well as two compounds of selenazine were synthesized. derivatives and evaluated the anti-proliferation of one derivative (1, 3-selenazine (S_6), namely 2, 4-diphenyl-6H-1, 3-selenazine) as anti-breast cancer via MTT assay for 24 and 48 h.

Materials and Methods

Infrared spectra were recorded using a Bruker type instrument. The NMR spectra were recorded using a Shimadzu device. Using a DMSO solvent at a frequency of three hundred megahertz. Mass spectra were recorded using a Shimadzu type apparatus. Melting points were recorded using a type apparatus IA 9200.

Selenocarboxamide was used in the synthesis of selenazole and selenazine compounds by reacting it with alpha-halo carbonyl and beta-halo carbonyl compounds respectively. Nitron was synthesized as a derivative of selenazole by the reaction of the carbonyl group with the primary hydroxylamine group.

General Procedure of Preparation Arylselenocarboxamide

(0.52 gm, 13.75 mmol) ⁽¹⁶⁾ borohydride in EtOH (25 ml) using argon atmosphere, a solution of (0.83 gm, 12 mmol) Se in EtOH (25 ml), was added to it drop by drop for an hour and a half. After that a mixture of (2.0 ml, 25 mmol) of pyridine, (7 ml, 2M) hydrochloric acid and (6.25 ml, 6.0 mmol) nitrile. Reflux was performed. The reaction was followed up with TLC. The product of the reaction was filtered, then ice water was added to it, filtered again, and recrystallized from benzene. The percentage (93%) and the melting point (123-125) °C.

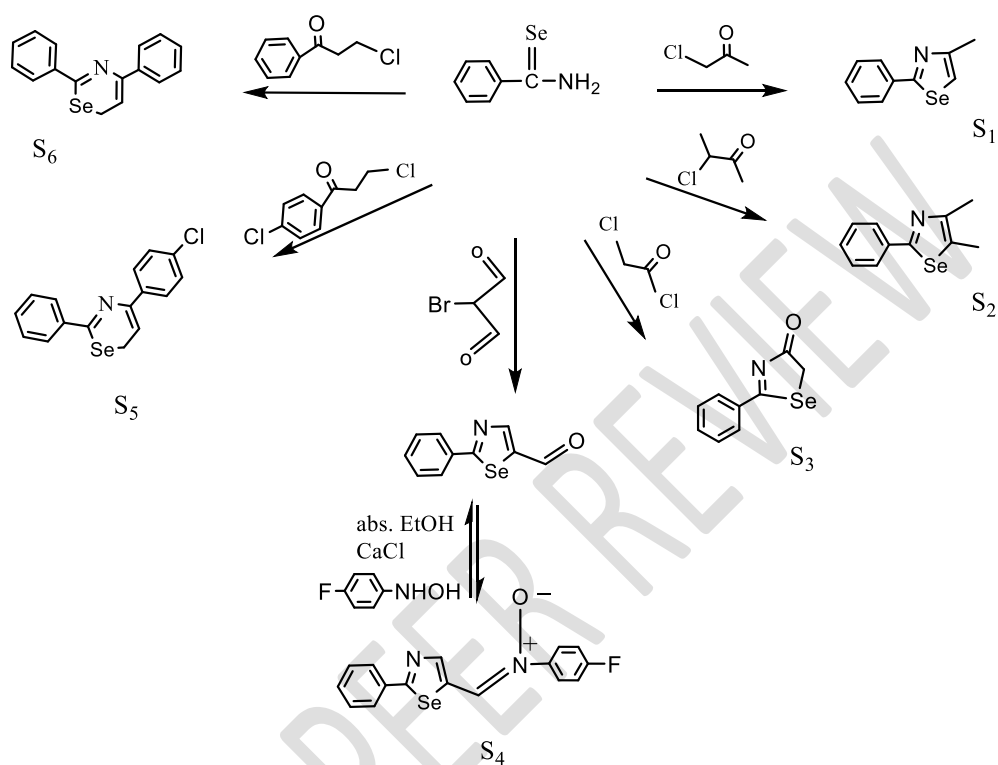
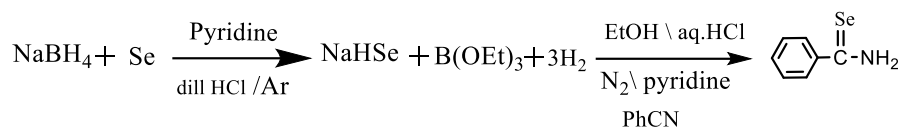
General Procedure of Synthesis Substituted Selenazole and Selenazine

A solution of α -halo and β -halo carbonyl compounds ⁽¹⁷⁾ (10 mmol) in ethanol (10 ml) reacted with selenocarboxamide (10 mmol) in ethanol (20 ml) by using reflux for a quarter of an hour. The solution was neutralized with (10%) (10 ml) ammonia. The reaction was followed up with TLC and the precipitate (selenazole and selenazine), was filtered and recrystallized from benzene, as shown in scheme (1).

General Procedure of Synthesis Nitron:

(selenazole) ⁽¹⁸⁾ (0.01 mole) and anhydrous CaCl₂ (10 gm.) was dissolved in abs. EtOH (35 ml). And then a solution of primary hydroxyl amine (0.01 mole) (30 ml) in abs. EtOH was added and refluxed. The reaction was followed by thin layer chromatography. Filtration was carried out to the product and then recrystallized by using methanol, as shown in "scheme.1".

The spectroscopic results were in agreement with the expected chemical compositions. Some of the properties obtained are listed in "Table 1".



Scheme 1. Reactions synthesis steps of dinitrone compounds.

Table 1. Physical properties of synthesized compounds

Compound symbol	Melting point °C Reaction time (min.)	Percentage (%)	Compound symbol
S1	154-155	1.5	51
S2	96-98	4	60
S3	111-113	3	55
S4	164-165	11	55
S5	108-109	2	61
S6	185-187	3	60

Biological Experiment

MTT Assay

The compound S6 has been sent to the Beatrice Hunter Cancer Research Institute for analysis. The anti-proliferative effects of compound (S₆) on MDA-MB231 cells were examined and followed the method by Batool and Kawthar⁽¹⁹⁾. The cell lines were seeded in 96 plates/well for 24h. Next, exposure the cells to different concentrations of compounds (0.7, 5, 25, 50, 250, 500) μM for 24 h, and 48 h respectively. 50 μM of MTT was added to each well, followed by 4 h incubation. The MTT solution was discarded and the purple formazan crystals formed by the mitochondrial dehydrogenase enzymes were dissolved in 0.1N HCl in isopropanol. The absorbance at 570 nm. The percent of MTT compared to the vehicle control cells was calculated at viability.

Statically analysis

Data were presented with the mean ± standard error. The data were analyzed according to Statistical Package for Social Science (SPSS) by using (ANOVA), one-way analysis of variance. The level of significance was assigned in $\alpha = 0.05$. Statistically significant changes are indicated as shown: *P<0.05, **P<0.01, ***P<0.001, When comparing it to the control

Results and Discussion

(S₁) 4-methyl-2-phenyl-1, 3-selenazole.

Orange powder; Yields: 51 %; Melting point. 157-159 Celsius; IR (centimeter⁻¹): 586 (C-Se), 1606 (C=N), 1586 (C=C), 1310 (C-N), 3110(CH aromatic), 2879-2886 (CH aliphatic); ¹H NMR δ: 6.84 (m, phenyl, 5H), 1.25 (s, 3H, CH₃), 8.02 (s, 1H, CH, selenazole); ¹³C NMR: 59.96, 117.67, 119.82, 120.65, 122.07, 125.67, 127.56, 140.41; MS: m/z: (M⁺) 222.

(S₂) 4, 5-dimethyl-2-phenyl-1, 3-selenazole.

Orange powder; Yields: 60 %; Melting point. 96-98 Celsius; IR (centimeter⁻¹): 580 (C-Se), 1601 (C=N), 1565 (C=C), 1300 (C-N), 3111(CH aromatic), 2887-2898 (CH aliphatic); ¹H NMR δ: 6.84 (m, phenyl, 5H), 1.64 (s, 3H, CH₃), 1.25 (s, 3H, CH₃); ¹³C NMR: 60.54, 113.43, 120.74, 123.67, 128.77, 129.66, 132.78, 129.54, 142.56; MS: m/z: (M⁺) 236.

(S₃) 2-phenyl-1, 3-selenazol-4(5H)-one.

Orange powder; Yields: 55 %; Melting point. 111-113 Celsius; IR (centimeter⁻¹): 575 (C-Se), 1612 (C=N), 1545 (C=C), 1320 (C-N), 3100(CH aromatic), 2874-2881 (CH aliphatic); ¹H NMR δ: 6.84 (m, phenyl, 5H), 3.04 (s, 2H, CH₂); ¹³C NMR: 56.76, 112.75, 120.04, 123.64, 126.86, 129.32, 131.87; MS: m/z: (M⁺) 224.

(S₄) 4-Fluoro-N-((2-phenyl-1,3-selenazole-5-yl)methylene)aniline oxide.

Yellow powder; Yields: 55 %; Melting point. 164-165 Celsius; IR (centimeter⁻¹): 584 (C-Se), 1596 (C=N), 1533 (C=C), 1304 (C-N), 1273 (N-O), 1221 (C-F), 3100(CH aromatic), 2818-2873 (CH aliphatic); ¹H NMR δ: 7.19 (m, phenyl, 4H) 6.82 (m, phenyl, 5H), 8.25 (s, 1H, CH, selenazole), 7.30 (s, 1H, CH, nitrene); ¹³C NMR: 57.66, 112.63, 113.82, 116.94, 119.07, 121.65, 123.93, 126.41, 128.95, 133.96, 144.88, 161.06; MS: m/z: (M⁺) 345.

(S₅) 4-(4-chlorophenyl)-2-phenyl-6H-1, 3-selenazine.

Orange powder; Yields: 61 %; Melting point. 108-109 Celsius; IR (centimeter⁻¹): 578 (C-Se), 1586 (C=N), 1530 (C=C), 1358 (C-N), 874 (C-Cl), 3125 (CH aromatic), 2796-2800 (CH aliphatic); ¹H NMR δ: 7.19 (m, phenyl, 4H) 6.82 (m, phenyl, 5H), 7.01 (m, 1H, CH, selenazine), 2.25 (m, 2H, CH₂, selenazine); ¹³C NMR: 60.64, 111.74, 114.96, 116.08, 118.46, 120.65, 122.76, 125.86, 129.77, 131.97, 135.66, 160.45; MS: m/z: (M⁺) 332.

(S₆) 2, 4-diphenyl-6H-1, 3-selenazine.

Orange powder; Yields: 60 %; M.p. 185-187 Celsius; IR (centimeter⁻¹): 586 (C-Se), 1600 (C=N), 1575 (C=C), 1340 (C-N), 889 (C-Cl), 3132 (CH aromatic), 2987-2890 (CH aliphatic); ¹H NMR δ: 7.23 (m, 5H, ph) 6.99 (m, phenyl, 5H), 7.01 (m, 1H, CH, selenazine), 2.57 (m, 2H, CH₂, selenazine); ¹³C NMR: 65.86, 113.65, 116.98, 117.54, 118.55, 119.97, 121.66, 124.97, 127.96, 130.65, 133.97, 165.75; MS: m/z: (M⁺) 298.

The reaction between aryl nitriles and NaHSe in ethanol resulted in an orange solution of the resulting compound, arylselenocarboxamide and when ice water was added, all selenocarboxamide precipitated in an orange color with a high yield. This method resulted in no need to use the column for purification. The IR spectra of synthesized selenazole and selenazine compounds showed the disappearance of asymmetrical and symmetrical stretching band of NH₂, of arylselenocarboxamide, in the range 3309-3320 cm⁻¹ and 3148-3260 cm⁻¹. The spectra showed strong bands in the range 16134-1626 cm⁻¹ due to the deformation of NH. All the spectra of selenazole selenazol-nitrone and selenazine compounds showed the appearance of strong bands due to (C-Se), (C=C), (C-H) aliphatic, (C-H) aromatic ring and (C=N) groups. The spectrum of synthesized compound S₄ confirmed the disappearance of the carbonyl bond that was appearing in the area (17680-1665) cm⁻¹ and the appearance of a band in region 1237 cm⁻¹ belonging to the N-O group in the nitrone compound. In the nuclear magnetic spectra of proton-one, the presence of a multiple band belonging to the aromatic ring appeared. All the NMR spectra of carbon-13 confirmed the validity of the prepared compounds. Mass spectrometry gave the mother ion band, in addition to many other bands that indicate other molecular ions, and this confirms the validity of the expected structures of the prepared compounds..

Anti-proliferation evaluation of compound S₆

Many studies reported anticancer activities of selenium organo derivatives such as selenoureas, selenoesters, selenium-containing heterocycles, etc.. (20). The chemopreventive of selenium derivatives showed interest research in developing new selenium anticancer agents (21). 1,3- selenazole showed anticancer activity against human breast cancer (MCF-7), prostate cancer (22, 23), melanoma cells (24) and leukemia cells (25). 1,3- selenazine derivatives showed induced cytotoxicity in human HT-1080 fibrosarcoma cells (26), induced apoptosis in human gastric adenocarcinoma cells (27, 28), and did not reported against breast cancer.

In this study, among the six selenium organo derivatives, we selected the compound S₆ which belongs to 1,3- selenazine derivative to evaluate its anti-proliferation against human breast cancer MDA-MB231 with time exposure 24 and 48 h. "Figure. 1", showed that compound S₅ has a significant increase in cell viability at 24 h but not 48 h compared to DMSO. This suggests that a proportion of the cells were affected by the compound or that the compound is not active for the entire 48h. In addition, "Figure 2" showed the regression line has a positive slope. Therefore, increasing concentrations of S₆ increase cell viability. This effect is only significant at 24h, there is no significant difference in the regression line from a horizontal line at 48h, the regression line has a positive slope showing increase concentration of DMSO increased cell viability. Future extensive investigations required to compare and target the anti-breast cancer activity reasons of the six derivatives and develop their structures as anti-breast agents.

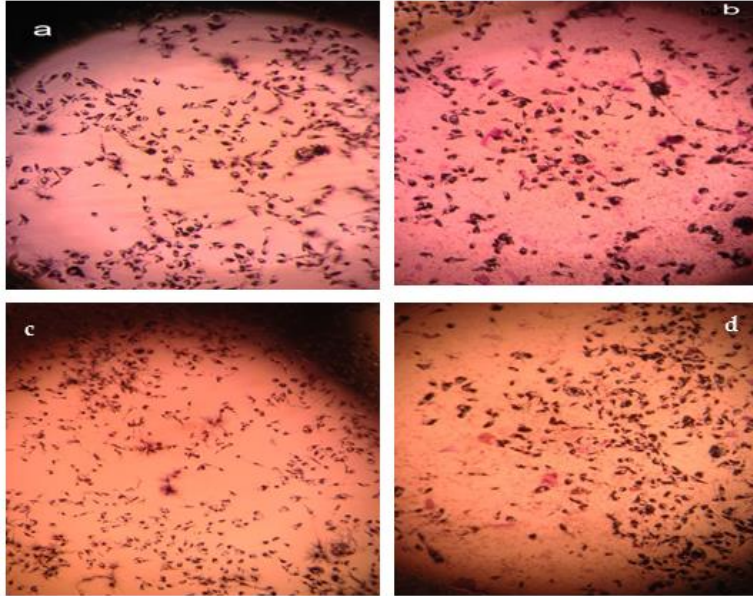


Figure 1. (a) Cells treated with DMSO for 24h. (b) Cells treated with S5 for 24h. (c) Cells treated with DMSO for 48h. (d) Cells treated with S5 for 48h.

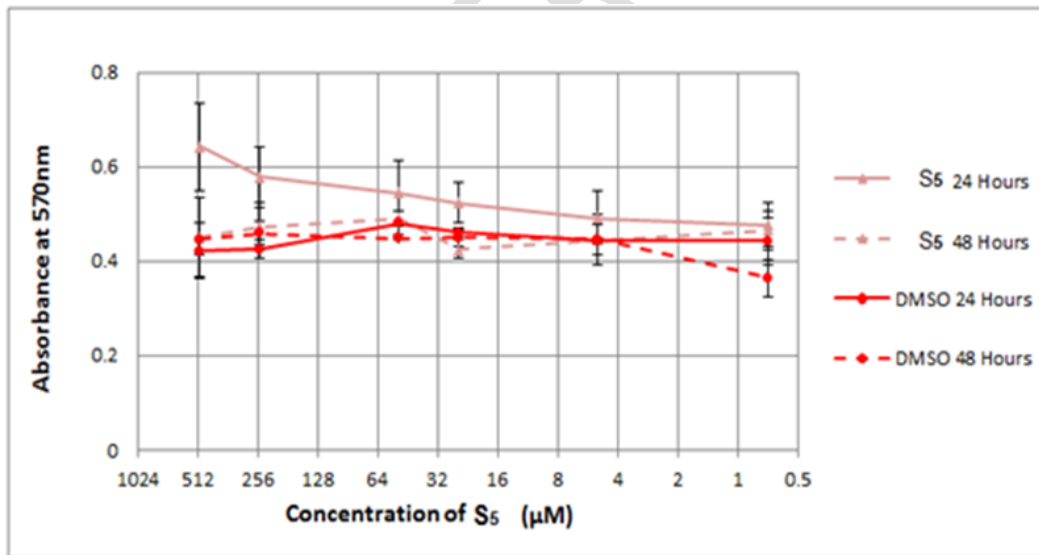


Figure 2. MTT analysis as function of S5 concentration in MDA-MB231 cells incubated for 24 and 48 hours at 570 nm.

Conclusions:

The present study carried out the synthesis of three new derivatives of 1, 3-selenazole and two new derivatives of 1, 3-selenazine compounds by the reaction between selenocarboxamide and (α -halo and β -halo carbonyl compounds). While the derivative of selenazole combined with nitrene has been carried out by the reaction between 2-phenyl-1, 3-selenazole-5-carbaldehyde and N-4-fluorophenylhydroxylamine. The cytotoxicity study demonstrated that the synthesized compound 1, 3-selenazine (S_6) promised as potential compound for anti-breast agents and needed merit studies. The rest of selenium compounds will consider their anti-breast activity in future.

Ethics Statements

The study does not require ethical approval

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