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Synthesis and anti-tumor evaluation of novel organoselenocyanates and symmetrical diselenides dyestuffs

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ABSTRACT

*A convenient procedure for the synthesis of novel organoselenium compounds was developed from simple aniline selenocyanate **1** and its corresponding diselenide **2**. Their chemical reactivity was investigated towards azo coupling type reactions and their corresponding diazo derivatives **3a,b** and **5a,b** were sequentially obtained. Besides, heterocyclic selenocyanates **7-9** and diselenocyanate **10** were obtained by the subsequent reaction of hydrazines diazo **3a,b** and **5b** compounds, respectively. Furthermore, condensation of **1** and **2** with phthalic anhydride afforded the phthalimide selenocyanates **4a,b** and **6**, alternately. Moreover, some representatives of the new synthesized compounds were evaluated for their antitumor activity and were found to be more cytotoxic compared to their corresponding analogues without selenium. It is worth mentioning that the dying characteristic of these compounds was also evaluated and found to exhibit good dying properties.*

Keywords: Organoselenium, selenocyanate, diselenides, azo coupling, antitumor activity (*in vitro*).

INTRODUCTION

Selenium is one of the substantial micronutrients which are vital to the proper functioning of all of the body's systems. Its role has been referred to its presence in selenoproteins which in turn have diverse biological functions including antioxidant defense, redox signaling, oxido-reduction, and immune responses [1]. This phenomenon has been established by mount laboratory experiments and clinical trials. As such, much attention for selenium to control and prevent many degenerative diseases including cancer, inflammatory, cardiovascular, neurological, and infectious diseases has been paid [2,3].

Given the similarity of the chemical properties between sulfur and selenium, heterocyclic compounds of selenium have more biological importance [4-6]. In this context, organoselenium compounds have emerged as an exceptional class of structures in recent years not only as synthetic reagents or intermediates in organic synthesis but also due to their pivotal role in the synthesis of a large number of biological compounds (e.g., selenocarbohydrates, selenoamino acids, and selenopeptides) [5, 7-8]. Moreover, organic selenides are recently accepted as important therapeutic compounds ranging from antiviral and anticancer agents to naturally occurring food supplements [9-12].

MATERIALS AND METHODS

General

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Elemental analyses were carried out at Micro analytical Center, Faculty of Science, Cairo University. IR spectra were recorded (KBr), (ύ cm^{-1}) on a Mattson 5000 FTIR Spectrophotometer at Micro analytical Center Faculty of Science, Mansoura University. The ^{1}H-NMR Spectra were measured on a Varian Spectrophotometer at

300, 500 MHz, using TMS as an internal reference and DMSO-d_6 or $CDCl_3$ as solvent at Chemistry Department, Faculty of Science, Cairo University. The chemical shifts (δ) are reported in parts per million and where referenced to the residual solvent peak. ^{13}C NMR (100MHz) was recorded in DMSO-d_6 using a Bruker AV 400 spectrometer at Chemistry Department, Faculty of Science, Assiut University. Mass spectra were recorded on (Kratos, 70 eV) MS equipment and/or a Varian MAT 311A Spectrometer, at Microanalytical Center, Faculty of Science, Cairo University. Reaction mixtures were monitored by thin layer chromatography (TLC) using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp. Biological Testing was carried out at Drug Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. Ehrlich cells (Ehrlich ascites carcinoma, EAC) were derived from ascetic fluid from diseased mice (the cells were purchased from the National Cancer institute, Cairo, Egypt).

*General Procedure for Preparation of **3a,b** and **5a,b***: 4-aminophenylselenocyanate (**1**) (1.97g, 0.01 mol) was dissolved in aqueous hydrochloric acid (8 ml, 1:1), cooled to 0-5°C, then added a cold solution of sodium nitrite (0.7g in 3 ml water), while maintaining the temperature at 0-5°C. The formed diazonium salt solution was added dropwise to a cooled and stirred mixture of active methylene compounds (e.g. **a**- malononitrile or **b**- ethyl cyanoacetate) (0.01 mol) and sodium acetate (2.0g), dissolved in (10 ml of 50% aqueous ethanol). Stirring was continued for 1.5 h. The resulting crystals were collected, washed with water, and recrystallized from ethanol to give **3a** and **3b**, respectively. However, replacing the selenocyanate derivative **1** by the diselenide derivative **2** in acetic acid instead of hydrochloric acid and using 2 mmol equivalents of the diazonium salt solution in the above procedure afforded **5a,b.**

General procedure for preparation of phthalimide derivatives **4a,b** and **6**: The appropriate amine and phthalic anhydride derivative were stirred under reflux in acetic acid for 6–10 h. The solution was poured into water, and the resulting precipitate filtered off and recrystallized from DMSO to give **4a,b** and **6**.

Pyrazole Ring Formation via Cyclocondensation of Compounds **3a,b** *and* **5b** *with Hydrazine Derivatives.*

General Procedure: A mixture of selenocyanatodiazines **3a,b** or diselenodiazine derivative **5b** (0.01 mol) and the appropriate hydrazine derivatives (0.01 mol except using 0.02 mol for **5b**) in ethanol (30 ml) was refluxed for 3-12 h., then allowed to cool. The formed solid products were collected and recrystallized from (EtOH/DMF) to give the corresponding pyrazole derivatives **7a,b** , **8a,b** , **9** and **10**.

2-((4-Selenocyanatophenyl)diazenyl)malononitrile (**3a**): Yield 73%. Yellow crystals (EtOH). M.p. 180-181°C. R_f = 0.19 [pet. ether (60-80)/ethyl acetate (4:1)]. IR (KBr): 3224, 2226, 2153, 1634 , 1604. ^1H NMR (DMSO-d_6- 300 MHz,) 7.51 (2H, d, H-Ar), 7.74 (2H, d, H-Ar), 11.77 (1H, br, NH). EIMS *m/z* 275 $[M+H]^+$, (34), 197 (33.8), 80 (100.0, base peak). Anal. Calcd. for $C_{10}H_5N_5Se$ (274.14): C, 43.81; H, 1.84 %. Found: C, 43.52; H, 1.99 %.

Ethyl 2-cyano-2-((4-selenocyanatophenyl)diazenyl)acetate (**3b**): Yield 62 %;.Yellow crystals (EtOH). M.p. 200°C. R_f = 0.425 [pet. ether (60-80)/ethyl acetate (4:1)]. IR (KBr): 2213, 2149, 1725, 1637, 1601. ^1H NMR (DMSO-d6, 300 MHz,): 12.3 (1H, br, NH), 7.76-7.44 (4H, m, H-Ar); 4.31 (2H, q, CH_2), 1.32 (t, 3H, CH_3); ^{13}C NMR (DMSO-d6, 100 MHz,): 160.7, 147.3, 143.3, 135.3, 118.7, 117.6, 111.2, 105.5, 62.1, 14.1. EIMS m/z 321 M^+ (83), 52 (100.0). Anal. Calcd. for $C_{12}H_{10}N_4O_2Se$ (321.19): C, 44.87; H, 3.14 %. Found: C, 45.08; H, 3.31 %.

2,2'-(4,4'-Diselanediylbis(4,1-phenylene)bis(diazene-2,1-diyl))dimalononitrile (**5a**): Yield 72 %. Brown crystals (EtOH); M.p. 160 °C. R_f = 0.3 [pet. ether (60-80)/ethyl acetate (4:0.5)]; IR (KBr): 3227, 2225, 1602, 819. ^1H NMR (DMSO-d6, 300 MHz,): (1H, br, NH); 7.48-7.25 (8H, m, H-Ar). EIMS *m/z* 496 M^+ (23), 76(100). Anal. Calcd. for $C_{18}H_{10}N_8Se_2$ (496.25): C, 43.57; H, 2.03 %. Found: C, 43.28; H, 2.15 %.

Diethyl 2,2'-dicyano-2,2'-(4,4'-diselanediylbis(4,1-phenylene)bis(diazene-2,1-diyl)) diacetate (**5b**): Yield 68 %. Orange crystals (EtOH). M.p. 130-131°C. R_f = 0.35 [pet. ether (60-80)/ethyl acetate (4:0.5)]. IR (KBr): 3222, 2209, 1719 , 1593, 820. ^1H NMR (DMSO-d_6, 300 MHz,): 12.3 (2H, br, 2NH), 7.66-7.30 (8H, m, H-Ar), 4.29 (4H, q, $2CH_2$), 1.29 (6H, t, $2CH_3$). EIMS *m/z* 590 $[M]^+$ (12), 76 (100) ; Anal. Calcd. for $C_{22}H_{20}N_6O_4Se_2$ (590.35): C, 44.76; H, 3.41%. Found: C, 44.52; H, 3.62.%.

*2-(4-Selenocyanatophenyl)isoindoline-1,3-dione (**4a**)*:. Yield 68%. Yellow crystals (DMSO). M.p. 260-262 °C. R_f = 0.257 [pet. ether (60-80)/ethyl acetate (4:1)]. IR (KBr): 2221, 1709, 1593. ^1H NMR (DMSO-d_6, 500 MHz,): 7.94 (2H, d, *J* = 7.7 *Hz*, H-4'+ H-7'); 7.80 (4H, m, H-2+H-6+H-5'+H-6'), 7.42 (2H, dd, $J_{3-2} = J_{5-6}$ = 7.6 *Hz*, H-3'+ H-5'). EIMS *m/z* 327$[M]^+$, (5), 301 (100). Anal. Calcd. for $C_{15}H_8N_2O_2Se$ (327.20): C, 55.06; H, 2.46 %. Found: C, 55.13; H, 2.64 %.

5-Nitro-2-(4-selenocyanatophenyl)isoindoline-1,3-dione **(4b)**: Yield 68%. Yellow crystals (DMSO). M.p. 240-241 ^{o}C. R_f = 0.2 [pet. ether (60-80)/ethyl acetate (4:1)].IR (KBr): 2221, 1709, 1593. EIMS *m/z* 374 $[M+H]^+$ (5), 50 (100). Anal. Calcd. for $C_{15}H_7N_3O_4Se$ (372.19): C, 48.41; H,1.90 %. Found: C, 48.63; H, 2.13%.

2,2'-(4,4'-diselanediylbis(4,1-phenylene))bisisoindoline-1,3-dione (**6**): Yield 70 %. Yellow crystals (DMSO). M.p. 220 ^{o}C. R_f = 0.15 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): 1710, 1593, 816. ^{1}H NMR (DMSO-d_6, 300MHz,): 7.98-7.44 (16H, m, , H-Ar). EIMS *m/z* 602 $[M]^+$ (5), 76 (100); Anal. Calcd. For $C_{28}H_{16}N_2O_4Se_2$ (602.36): C, 55.83; H, 2.68 %. Found: C, 55.67; H, 2. 54 %.

4-((4-Selenocyanatophenyl)diazenyl)-1H-pyrazole-3,5-diamine (**7a**): Yield 68 %. Brown crystals (EtOH/DMF). M.p. 290-292 ^{o}C. R_f = 0.125 [ethyl acetate]; IR (KBr): 454, 3193, 2222 , 1600, 1482. EIMS *m/z* 306 $[M]^+$ (5), 66 (100). Anal. Calcd. for $C_{10}H_9N_7Se$ (306.19): C, 39.23; H, 2.96 %. Found: C, 39.01; H, 2.85.%.

1-phenyl-4-((4-selenocyanatophenyl)diazenyl)-1H-pyrazole-3,5-diamine (**7b**): Yield 65%. Brick red crystals (EtOH/DMF). M.p. 260- 262 ^{o}C. R_f = 0.1 [pet. ether (60-80)/ethyl acetate (4:3)]. IR (KBr) 3368, 3433, 2211, 1604 , 1494. ^{1}H NMR (DMSO-d_6, 500MHz) 7.88-7.27 (9H, m, H-Ar); 6.20 (2H, br, NH_2), 5.6 (2H, br, NH_2). EIMS *m/z* 383 $[M+H]^+$ (6), 76 (100). Anal. Calcd. for $C_{16}H_{13}N_7Se$ (382.28): C, 50.27; H, 3.43 %. Found: C, 50.11; H, 3.66%.

-amino-4-((4'-selenocyanatophenyl)diazenyl)-1H-pyrazol-3(2H)-one (**8a**): Yield 72%. Reddish brown crystals (EtOH/DMF). M.p. >300 ^{o}C. R_f = 0.175 [ethyl acetate]. IR (KBr) 3444, 3224, 2221, 1689, 1623, 1478. ^{1}H NMR (DMSO-d_6, 300MHz): 12.86 (1H, br, NH), 10.53 (1H, br, NH), 7.59 (2H, d, $J_{2\text{-}3}$=$J_{6\text{-}5}$ = 9.0 *Hz*, H-2'H-6';),7.52 (2H, d, $J_{3\text{-}2}$=$J_{5\text{-}6}$ = 9.0 *Hz*, H-3'+H-5'),5.83 (2H, br, NH_2). EIMS *m/z* 307 $[M]^+$ (7), 99 (100). Anal. Calcd. for $C_{10}H_8N_6OSe$ (307.17): C, 39.10; H, 2.63 %. Found: C, 39.28; H, 2.85%.

5-amino-1-phenyl4-((4'-selenocyanatophenyl)diazenyl)-1H-pyrazol-3(2H)-one (**8b**). Yield 69%. Brown crystals (EtOH/DMF). M.p. 250-252 ^{o}C. R_f = 0.1 [pet. ether (60-80)/ethyl acetate (4:2)]. IR (KBr) 3416, 3311, 2221, 1661 1622, 1482. EIMS *m/z* 383 $[M]^+$, (3), 77 (100.0, base peak). Anal. Calcd. for $C_{16}H_{12}N_6OSe$ (383.27): C, 50.14; H, 3.16%. Found: C, 49.96; H, 3.01%.

5-amino-1-thioamide-4-((4'-selenocyanatophenyl)diazenyl)-1H-pyrazol-3(2H)-one (**9**): Yield 71%. Orange crystals (EtOH). M.p. 230^{o}C. R_f = 0.5 [pet. ether (60-80)/ethyl acetate (4:2)]. IR (KBr) 3460, 3220, 2222, 1685, 1623, 1478. ^{1}H-NMR (DMSO-d_6, 300 MHz) 12.27 (1H, br, NH); 7.66-7.41 (4H, m, H-Ar), 6.41 (2H, br, NH_2), 5.93 (2H, br, NH_2), EIMS *m/z* 367 $[M+H]^+$ (4), 169 (100), Anal. Calcd. for $C_{11}H_9N_7OSSe$ (366. 26): C, 36.07; H, 2.48 %. Found: C, 36.07; H, 2.54 %.

4,4'-(4,4'-diselanediylbis(4,1-phenylene)bis(diazene-2,1-diyl))bis(5-amino-1H-pyrazol-3(2H)-one (**10**): Yield 69 %. Reddish brown crystals. M.p. >300^{o}C (EtOH/DMF). R_f = 0.10 [ethyl acetate]. IR (KBr) 3412 , 3224 , 1661,1624, 811. ^{1}H-NMR (DMSO-d_6-300 MHz) 12.56 (2H, br, 2NH); 10.44 (2H, br,2 NH), 7.59 (4H, d, *J* = 9.9 Hz, H-Ar), 7.52 (4H, d, *J*= 9.3 Hz, H-Ar), 5.62 (4H, br, $2NH_2$), EIMS *m/z* 563 $[M+H]^+$, (14), 562 (M^+, 35.7), 561 (M^+-H 25.4), 98 (100), 84 (96.6), 76 (69.0). Anal. Calcd. for $C_{18}H_{16}N_{10}O_2Se_2$ (562.31): C, 38.45; H, 2.87 %. Found: C, 38.19; H, 2.93 %.

Antitumor activity using Ehrlich ascites in vitro assay. Different concentrations of the tested compounds were prepared (100, 50 and 25 g/mL DMSO). Ascites fluid from the peritoneal cavity of the donor animal (contains Ehrlich cells) was aseptically aspirated. The cells were grown partly floating and partly attached in a suspension culture in RPMI 1640 medium, supplemented with 10% fetal bovine serum. They were maintained at 37 ^{o}C in a humidified atmosphere with 5% CO_2 for 2 hrs. The viability of the cells determined by the microscopical examination using a hemocytometer and using trypan blue stain (stains only the dead cells) [30].

Dyeing procedure

Dyeing at 130-135ºC and high pressure (24-30psi) is a convenient method for dyeing polyester fabrics in the laboratory. A laboratory model glycerin-bath high-temperature beaker dyeing machine was used. A dispersion of the dye was produced by dissolving the appropriate amount of dye (2% shade) in 1 ml acetone and then added dropwise with stirring to the dye bath (liquor ration 20:1) containing 1% Setamol WS as anionic dispersing agent of BASF. The pH of the dyebath was adjusted to 5.5 using aqueous acetic acid and the wetted-out polyester fibers were added. Dyeing was performed by raising the dyebath temperature to 130ºC at a rate of 3ºC/min, holding at this temperature for 60 min, under pressure. After cooling to 50ºC, the dyed fibers were rinsed with cold water and reduction cleared (1 g/l sodium hydroxide, 1 g/l sodium hydrosulfite, 10 min, 80ºC). The samples were rinsed with hot and cold water and finally air-dried.

Color assessment

The colorimetric parameters (Table 2) of the dyed polyester fibers were determined on a reflectance spectrophotometer (Gretag-Macbeth CE 7000a), equipped with a D65/10° source and barium sulphate as standard blank, UV excluded, specular component included and three repeated measurements average settings. The following CIELAB coordinates are measured, lightness (L*), chroma (C*), hue angle from 0º to 360º (H), (a*) value represents the degree of redness (positive) and greenness (negative) and (b*) represents the degree of yellowness (positive) and blueness (negative). A reflectance spectrophotometer (Gretag Macbeth CE 7000a) was used for the colorimetric measurements on the dyed samples.

Color fastness tests

Fastness to washing, perspiration, rubbing, sublimation, and light was tested according to the reported methods [28, 29]. Fastness to washing: A specimen of dyed polyester sample was stitched between two pieces of undyed cotton and polyester fabrics (10 cm × 4 cm), all of equal weight and then washed at 50ºC for 30 min. The staining of adjacent fabrics was assessed using the grey scale: 1-poor, 2-fair, 3-moderate, 4-good, 5-excellent. Fastness to Perspiration: A composite sample was sandwiched on each side by the undyed cotton, all of equal length, and then immersed in the acid or alkaline solution for 30 min. The staining on the undyed adjacent fabric was assessed according to the grey scale: 1-poor, 2-fair, 3-moderate, 4-good, 5-excellent. The acid solution (pH = 3.5) contained sodium chloride (10 g/l), lactic acid (1 g/l), disodium orthophosphate (1 g/l) and histidine monohydrochloride (0.25 g/l). The alkaline solution (pH = 8) contained sodium chloride (10 g/l), ammonium chloride (4 g/l), disodium orthophosphate (1 g/l) and histidine monohydrochloride (0.25 g/l). Rubbing fastness: Dyed polyester fibers were placed on the base of Crockmeter, so that they rested flat on the abrasive cloth with its long dimension in the direction of rubbing. A square of white testing cloth was mounted over the end of the finger which protects downward on the dry specimen sliding back and forth twenty times by making ten complete turns of the crank at the rate of one turn per second. For wet rubbing test, the testing squares were thoroughly wet in distilled water and squeezed between filter papers through hand wringer under standard conditions. The rest of the procedure is the same as the dry crocking test. Sublimation fastness: Fastness to sublimation was measured with an iron tester (Yasuda no. 138). The dyes samples were stitched between two pieces of un dyed polyester, all of equal length, and then treated at 180 and 210ºC each for 1 min. Light Fastness: Fastness light to was determined using a Xenon test 150 (Original Hanau, chamber temperature 25–30ºC, black panel temperature 60ºC, relative humidity 50-60%, dark glass (UV filter system) for 40 hours. The changes in color were assessed according to the blue scale: 1-poor, 3-moderate, 5-good, 8-very good.

RESULTS AND DISCUSSION

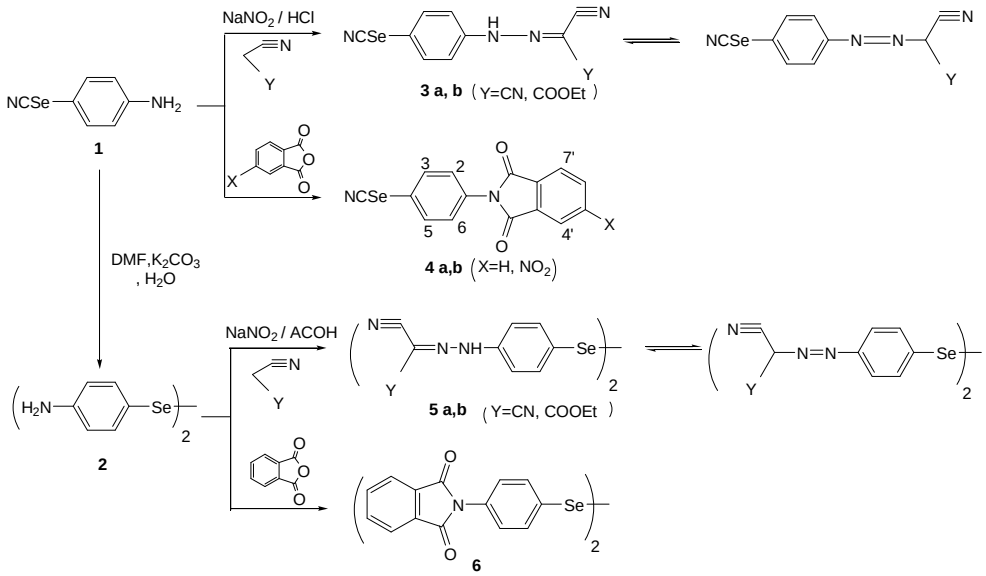
Organoselenium chemistry is not always straight forward. It's often marred by decomposition of the products and difficulties to generate compounds with sufficient purities. Although numerous reports on the synthesis of organoselenium compounds have already been published, it generally requires the handling of unstable reagents, strongly basic or acidic reaction conditions, and mostly two-step procedures. Therefore, simple and new approaches for the synthesis of novel selenium compounds by using more stable, less toxic, and easily accessible selenium reagents are also required.

We have recently reported and evaluated the synthesis and cytotoxic effect of novel peptidomimetic compounds containing selenium [13, 14a, 14b, 14c]. In cancer cells, these agents inhibit proliferation and induce cell death via multifactorial mechanisms such as induction of oxidative stress, cell cycle delay, and apoptosis induction [15, 16a, 16b]. Interestingly, some of these compounds showed apparent lower reduction in cell survival when incubated with primary cells [13, 14, 16]. The aforementioned observations point to a selective anticancer activity which in turn needs to be further studied. Based on these findings, we report here the synthesis of new organoselenium compounds conjugated to a nitrogen-containing heterocycles starting from stable organoselenocyanates as a selenium sources. Their respective antitumor activity and dying characteristic were evaluated. The selenocyanates were chosen as they are less-toxic, safe to handle, easy to prepare and store. Indeed, selenocyanates have latterly emerged as a powerful key for the synthesis of selenium-containing heterocycles.

There are several methods known for the synthesis of selenocyanates, however selenium diselenocyanate was mainly used to introduce the selenocyanate group into the scaffold of arenes with a free para positions. In this context, 4-aminophenylselenocyanate **(1)** was prepared from the reaction of aniline with triselenium dicyanide[17].

Compound **1** is considered to be bifunctionalized; i.e. it contains two active cites, a free amino and a selenocyanate groups. Under neutral or acidic conditions, compound **1** would be reacting only as a primary aromatic amine since the selenocyante group is quite unreactive under these conditions. On the other hand, alkaline hydrolysis leads to the formation of the corresponding diselenide **2** which alternatively could be obtained through the usual performed

reduction procedure [18]. The chemical reactivity of **1** and **2** was investigated by condensation with acid anhydrides and azo coupling with different active methylenes (Scheme 1).



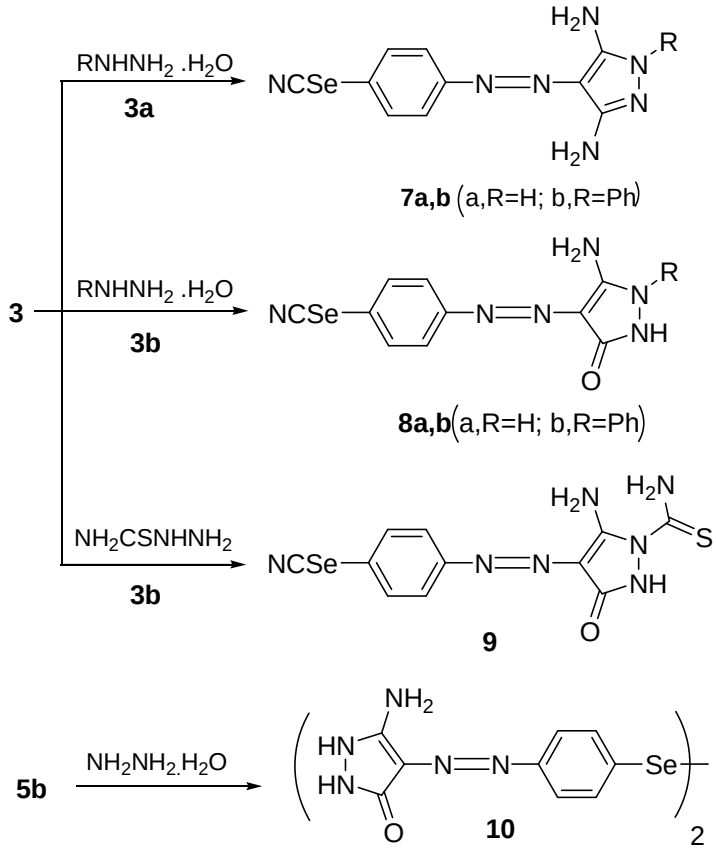
Scheme (1) Azo coupling of aniline selenocyanate and diselenide with different active methylene and their respective condensation with phthalic anhydride

Azo compounds, containing alternate nitrile and azo groups, have been used also in analytical chemistry as pH indicators, complexometric or redox titrations. From view of the biological importance of azo compounds, is well known as they have been demonstrated to possess antimicrobial, anticonvulsant, analgesic, antiinflammatory, antitubercular activities and other useful chemotherapeutic agents [19]. In this context, the mono and diazo derivatives **3a,b**, **5a,b** were synthesized by diazotization of **1** and **2** at 0-5 ^{0}C either in hydrochloric acid or in acetic acid. Coupling with active methylene compounds such as malononitrile and ethyl cyanoacetate in sodium acetate buffered solution afforded azobenzenemalononitrile **3a** and **5a** and azobenzene ethyl cyanoacetate derivatives **3b** and **5b** in a good yield (62 % and 68 %), respectively.

Once more, the diazo compounds **3a,b** and **5b** are consider to be a versatile intermediates which may used as important synthons for several transformations [20]. Indeed, they were used as starting building blocks for the synthesis of potential adenine, aminopyrimidine and pyrazolone antitumor drugs [21]. Within this context, the reactivity of **3a,b** and **5b** were investigated by the reaction with different hydrazines (namely, hydrazine , phenyl hydrazine and thiosemicarbazide). Thus, the diazo compounds **3a,b** were allowed to react with hydrazines in ethanol to furnish 3,5-diamino-pyrazole derivative **7a**, 3,5-diamino-1-phenylpyrazole derivative **7b**, 3-aminopyrazol-5-one derivative **8a**, 3-amino-1-phenylpyrazol-5-one derivative **8b** and 1-carbothioamide-3-aminopyrazole-5-one derivative **9**, respectively (Scheme 2). Besides, the new synthesized selenoheterocycles containing pyrazole are usually anticipated to be associated with enhanced biological activity.

Furthermore, selenium containing thalidomide analogues **4a, 4b** and **6** were also prepared by condensation of **1** and **2** with phthalic anhydrides under acidic conditions. Thalidomide compounds have high antitumor activity against a variety of murine and human tumor cells [22]. These compounds bind to DNA by intercalation of the chromophore and can photoinduce DNA cleavage [23, 24]. Studies have shown that such cleavage relies on the substituent's electronic effect on the naphthalimide moiety [25]. Interestingly, phthaloyl compounds **4a**, **4b** and **6** are considered to be amino-protected compounds since they can be introduced and removed under milder conditions or by using an appropriate nucleophile, most typically by hydrazinolysis in boiling alcohol [26, 27]. On the other hand, the reaction of compound **5b** with hydrazine in ethanol under reflux furnished, diselenide-3,5-diaminopyrazole **10** .

As far as the chemical synthesis is concerned, a preliminary in vitro antitumor screening was performed using Ehrlich ascites cells (EAC). This cell line was chosen, as it allows a basic evaluation of the activity of the compounds [30]. In the first step, cytotoxicity of the compounds was determined using trypan blue assay and the results were summarized in Table 1. The biological results showed clearly that compound **8b** proved to have the highest cytotoxic activity (98.6 %) followed by compounds **10**, **7b**, **5a** and **8a**. On the other hand, compounds **4a** and **6** showed the lowest cytotoxicity. The rest of the tested compounds (**3a**, **3b** and **5b**) exhibited moderate activities.



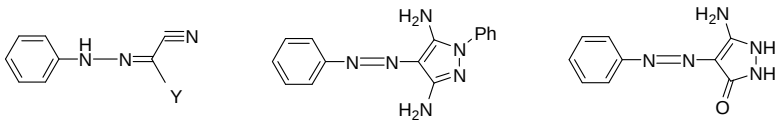
Scheme (2) pyrazole ring formation through transformations with hydrazine derivatives

Table 1. In vitro potential antitumor activity of seleno analogues using EAC assay.The dead % refers to the % of the dead tumor cells; 5-Fluorouracil(5-FU) is well known cytotoxic agent

Entry	Compound	% Death		
		100 µg/ml	50 µg/ml	25 µg/ml
1	5-FU	99.0%	54.7%	29.8%
2	3a	77.6%	36.4%	17.2%
3	3a`	65.2%	32.3%	11.5%
4	3b	75.3%	37.1%	18.8%
5	3b`	68.0%	32.4%	15.9%
6	4a	48.0%	25.3%	11.7%
7	5a	85.0%	43.1%	21.9%
8	5b	75.9%	37.0%	19.1%
9	6	55.9%	27.1%	12.6%
10	7b	95.3%	49.8%	27.3%
11	7b`	83.5%	34.9%	18.6%
12	8a	84.6%	42.7%	21.0%
13	8a`	72.3%	31.4%	14.8%
14	8b	98.6%	56.1%	30.4%
14	10	97.2%	53.5%	30.0%

In general, pyrazol-containing compounds **8b**, **10**, **7b**, and **8a** were more cytotoxic than the other tested compounds. Additionally, diselenides **10, 5a**, **5b** and **6** were also more toxic than their corresponding selenocyanates **8a**, **3a, 3b** and **4a**, respectively. Contrary to our expectations, phthaloyl compounds **4a** and **6** showed the lowest cytotoxic effect in the tested concentration range. This may be attributed to its poor solubility.

To further ensure that incorporation of selenium is accompanied by cytotoxicity enhancement, compounds **3a`**, **3b`**, **7b`** and **8a`** were synthesized and their cytotoxic effects were evaluated using EAC viability assay.



3 a',b' (Y=CN, COOEt) **7b'** **8a'**

Figure (1) Compounds 3a', 3b', 7b' and 8a' without Selenium

It was found that selenium containing analogues **3a**, **3b**, **7b** and **8a** have higher cytotoxic effect than compounds **3a`**, **3b`**, **7b`** and **8a`** without selenium, respectively (Figure 1). This further proves our hypothesis and opens the door for more investigations on the molecular mechanisms underlying selenium's anticancer action.

From the obtained results, the following structural activity relationship's (SAR's) were postulated: **(a)** Organoseleno compounds have higher anticancer activities than their corresponding analogues (without selenium) **(b)** The presence of pyrazol-ring system enhances the antitumor activity **(c)** Also, compounds with two selenium atoms (diselenides) are usually more toxic than those containing one selenium atoms (selenocyanates) **(d)** Contrary to our expectations, thalidomide compounds were the less cytotoxic.

Furthermore, the synthesized disperse dyes **5a, 5b** and **3b** were applied to polyester fabrics at 2% shade by high-temperature pressure technique (130ºC) and range of color shades has been obtained as the visual color shades varied from red to reddish violet. The dyeing on polyester fabrics were evaluated in terms of their fastness properties (*e.g.* fastness to washing, perspiration, rubbing, sublimation, and light) using a standard method [28]. The results given in Table **2** reveal that these dyes have good fastness properties.

Moreover, the colorimetric parameters of dyed polyester fabrics were assessed by colorimetric measurements using reflectance spectrophotometer (Gretag Macbeth CE 7000a) (Table 3).

Table (2) Color assessment of the dyes

Dye	Absorption [λ_{max}(nm)]	K/S	L*	a*	b*
5a	395	16.46	69.60	6.18	61.86
5b	375	13.22	80.6	4.32	44.03
3b	380	5.46	83.06	4.23	19.65

K/S values given by the reflectance spectrometer are calculated at λ_{max} and are directly correlated with the dye concentration on the dye substrate according to the Kubelka–Munk equation [29]: $K/S = (1-R)^2/2R$, where K = absorbance coefficient, S = scattering coefficient, R = reflectance ratio. Generally, Increasing of K/S values for the **5a** and **5b** than **3b** (which are substituted with more electron withdrawing groups as nitriles and ester. Also, The positive values of b* (yellow-blue axis) indicate that the color hues of the selenium dyes **5a, 5b and 3b** on polyester fabric are shifted to the yellowish directions.

Table (3) Fastness properties of synthetic dyes on polyester fabric

Dye	Shade	Sample dyed	K/S	Fastness to rubbing		Fastness to perspiration						Wash Fastness			light
				Wet	Dry	Acidic			Alkaline			Sw	Sc	Alt	
						sw	sc	Alt	sw	sc	Alt				
5a	2%	P.E	16.46	4-5	4-5	3-4	4-5	4-5	4	4-5	4-5	4-5	4-5	4-5	3
5b	2%	P.E	13.22	4-5	4-5	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	3-4
3b	2%	P.E	5.46	4-5		4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4

CONCLUSION

The objective of the present study was to synthesize and evaluate the cytotoxic activity of some novel selenium containing compounds with the hope of discovering new structure serving as antitumor agent. The data showed clearly those compounds **3a, 3b, 7b** and **8a** have high cytotoxic activity than the corresponding analogues without selenium **3a'**, **3b'**, **7b'** and **8a'** which may be attributed to the presence of selenium as selenocyanate. Also, the data showed clearly those compounds **8b**, **10, 7b, 5a** and **8a** displayed promising in vitro cytotoxic activities. The biological results showed clearly that compound **8b** proved to have the highest cytotoxic activity (98.6 %). Additional research, including mode of action studies, is planned to accurately establish relative activity for (SAR's) and rational design.

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