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Synthesis and Antitumor Activity of Some New Symmetrical Diselenide Derivatives.

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ABSTRACT

A convenient method for the synthesis of new organoselenium compounds was described from simple 2-methoxy-4-selenocyanato-aniline **(1)** and its corresponding diselenide **2**. The chemical reactivity of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis-(2-chloro-acetamide) **(3)** was investigated towards several type of carbon and sulfur nucleophiles to furnish various heterocyclic diselenide derivatives. Moreover, the new synthesized compounds were evaluated for their antitumor activity and were found to be more cytotoxic compared to their corresponding analogues without selenium.

Keywords: Organoselenium, selenocyanate, diselenides, thiocarbamoyl, thiophene, antitumor activity (in vitro).

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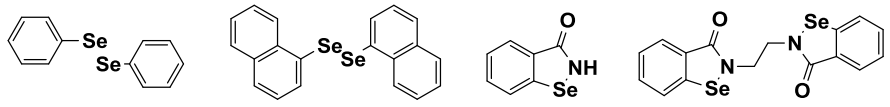
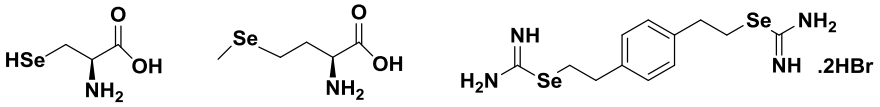
INTRODUCTION

Organoselenium chemistry has been recognized significant progress during the last decade. High selectivity of selenium, both nucleophilic and electrophilic species in different reactions, should be accentuated. Selenium- containing groups can be easily introduced to organic molecules and removed after essential chemical transformations. The removal can be performed by syn-elimination of selenoxide or by scission of the C-Se bond with organolithium or tin hydride compounds. In recent years, organoselenium compounds have developed as an exceptional class of structures not only as synthetic reagents or intermediates in organic synthesis [1] but also due to their pivotal role in the synthesis of a large number of biological compounds (e.g., such as diphenyl diselenide, dinapthyldiselenide, ebselen and ethaselen) and drugs (e.g., PBISe [1,4-phenylenebis(1,2-ethanediyl)bis-isoselenourea).



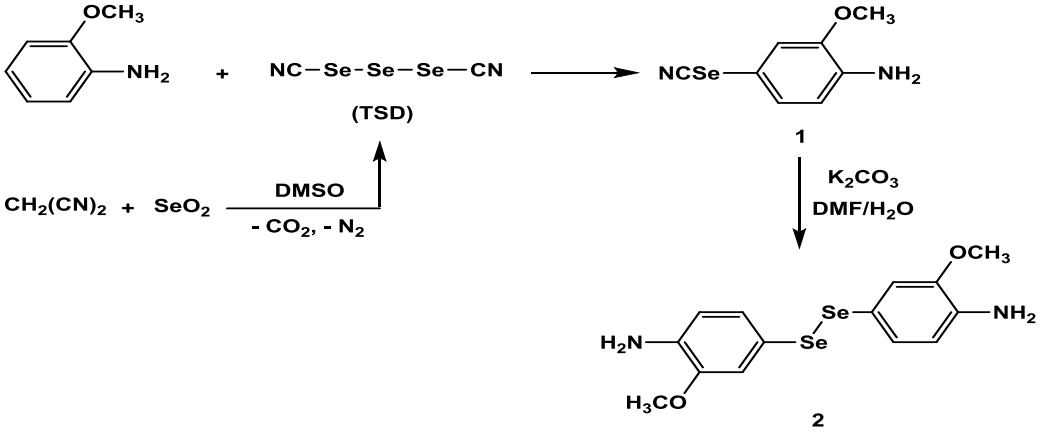
diphenyl diselenide | dinaphthyldiselenide | ebselen | ethaselen (BBSKE)



selenocysteine | selenomethionine | PBISe

RESULTS AND DISCUSSION

Triselenium dicyanide (TSD) has been prepared very easily by treatment of malononitrile (1 mol) with selenium dioxide (1.5–2 mol) in DMSO or DMF [2]. The reaction is exothermic; carbon dioxide and nitrogen were evolved during the oxidative coupling of malononitrile with SeO_2 in the presence of organic bases [3]. However, Kachanov *et*. *al*. [2] discovered that the reaction mixture may be used for selenocyanation of the organic substrates without isolation of the TSD. Aromatic amines with a free para-position, indoles with a free 3-position and some active methylene compounds may be used as substrates. Thus, the 2-methoxy-4-selenocyanato-aniline **(1)** was formed with good yield if *o*-anisidine was added to the reaction mixture after the exothermic reaction between malononitrile and selenium dioxide (Scheme 1). 2-Methoxy-4-selenocyanato-aniline **(1)** was isolated after dilution of the reaction mixtures with water and purified by recrystallization. Alkaline hydrolysis of **1** using K_2CO_3 in DMF afforded the corresponding 4,4'-diselanediyl-bis-(2-methoxyaniline) **(2)** in sufficient degree of purity and quantity. The chemical structure of diselenide **2** was established based on its elemental analysis and spectral data. The IR spectrum revealed the characteristic absorption band of NH_2 at 3406 cm^{-1}. The ^1H NMR spectrum showed singlet signal at 7.27 ppm, doublet signal at 7.05 ppm, doublet at 6.60 ppm for the aromatic protons. The protons of NH_2 group appeared as singlet signal at 7.00 ppm and the protons of methoxy group as singlet signal at 3.80 ppm.



Scheme (1): Synthesis of 4,4'-diselanediyl-bis-(2-methoxyaniline) (2)

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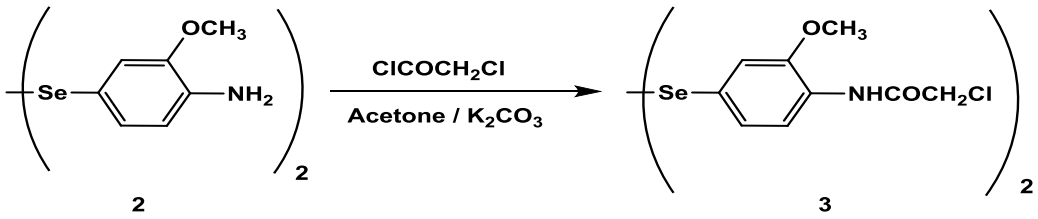
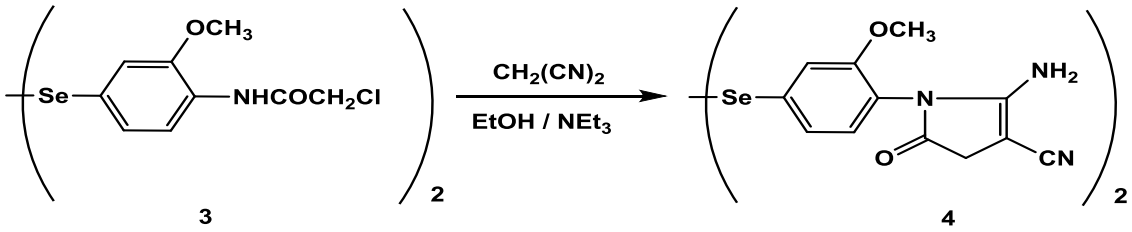
Chloroacetylation of the aromatic amine **2** was achieved by addition of chloroacetyl chloride in acetone as a solvent and anhydrous potassium carbonate to furnish the corresponding chloroacetyl derivative, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis-(2-chloro-acetamide) **(3)**. The structure of the **3** was established based on its elemental analysis and spectral data. The IR spectrum showed a characteristic NH absorption band at 3367 cm^{-1} and C=O absorption band at 1679 cm^{-1}. The ^1H NMR spectrum of **3** showed the proton of NH group as singlet signal at δ 8.90 ppm, the aromatic protons as doublet and multiplet signals at δ 8.25 and 7.08-7.27 ppm, the protons of methylene group (CH_2) as singlet signal at δ 4.21 ppm, in addition to the protons of methoxy group (OCH_3) as singlet signal at δ 3.90 ppm.



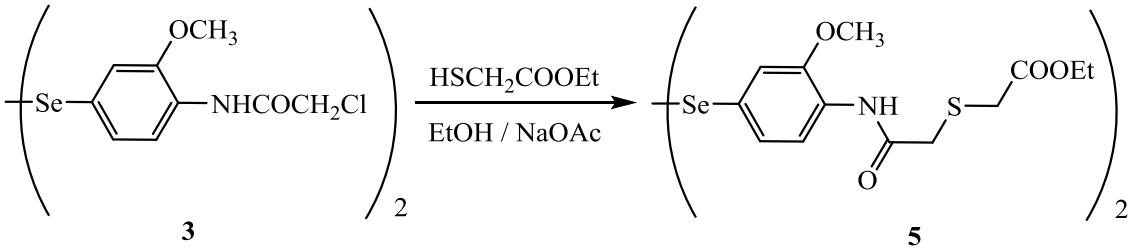
Scheme (2): synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))- bis(2-chloroacetamide) (3)

The chemical behavior of N-chloroacetyl derivative **3** towards several carbon and sulfur nucleophiles was studied. Thus, refluxing equimolar amounts of **3** and malononitrile in dioxane containing catalytic amount of trimethylamine furnished the corresponding pyrrole, 1,1'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(2-amino-3-cyano-5-oxo-4,5-dihydro-1H-pyrrole) **(4)**. The structure of **4** was secured by its correct elemental analysis and spectral data. The IR spectrum showed characteristic absorption band of NH_2 group at 3431 and 3383 cm^{-1}, in addition to the absorption bands of C≡N and C=O functions at 2203 cm^{-1} and 1678 cm^{-1}, respectively. The 1H NMR spectrum showed the doublet and multiplet signals at δ 8.22 and 7.10-7.30 ppm for the aromatic protons, singlet signal at δ 6.84 due to the protons of NH_2 and two singlet signals at δ 4.05 and 3.88 ppm for the protons of cyclic methylene and methoxy groups.



Scheme (3): Synthesis of 1,1'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-amino-3-cyano-5-oxo-4,5-dihydro-1H-pyrrole) (4)

The reaction of chloroacetyl derivative **3** with ethyl thioglycolate in refluxing ethanol and sodium acetae afforded the corresponding sulfide derivative, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-ethoxy-carbonyl-methyl-sulfanyl-acetamide) **(5)**. The structure of the **5** was established based on its elemental analysis and spectral data. The IR spectrum of **5** revealed characteristic absorption band of NH group at 3322 cm^{-1}, the absorption at 1673 cm^{-1} was attributed to the amidic carbonyl (CONH) and that of 1729 cm^{-1} for the carbonyl of ester (COOEt). The mass spectrum of **5** showed a molecular ion peak at m/z = 722 corresponding to the molecular weight of its chemical formula $C_{26}H_{32}N_2O_8S_2Se_2$.



Scheme (4): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-ethoxycarbonyl-methyl-sulfanyl-acetamide) (5)

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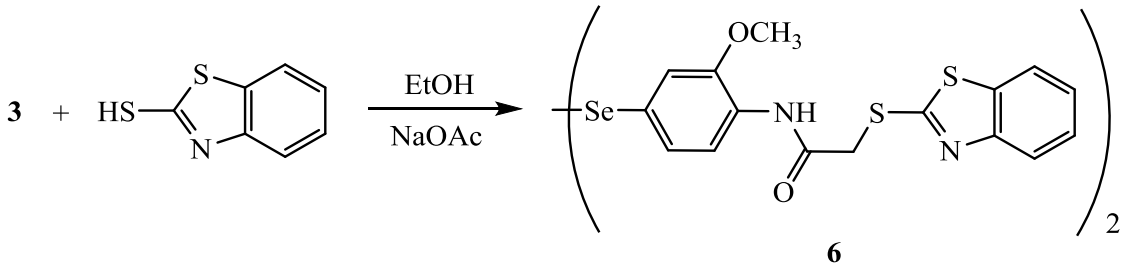
In addition, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis-(2-(benzo[d]thiazol-2-ylthio) acetamide) **(6)** was prepared by the reaction of **3** with 2-mercaptobenzothiazole in hot ethanol and sodium acetate. The structure of **6** was confirmed on the basis of its elemental analysis and spectral data. The IR spectrum showed characteristic stretching frequencies at 3252 and 1763 cm^{-1} for the NH and C=O functions. The mass spectrum showed a molecular ion peak at m/z = 816 corresponding to the molecular weight of the formula $C_{32}H_{26}N_4O_4S_4Se_2$.



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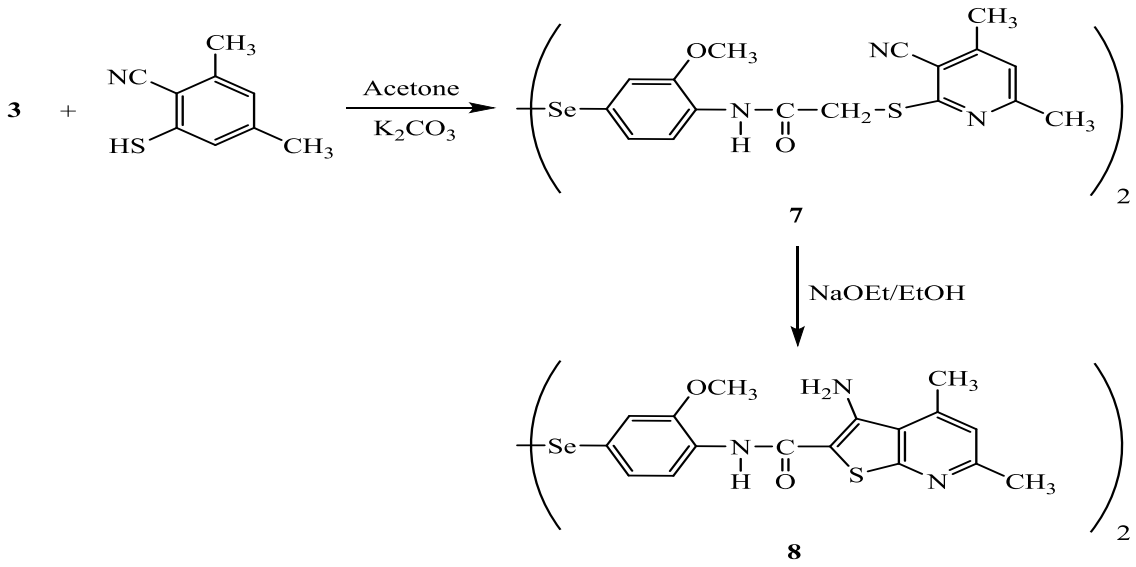
Scheme (5): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-(benzo[d]thiazol-2-ylthio)acetamide) (6)

Moreover, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetamide) **(7)** was obtained by reflux a mixture of **3** and 2-mercapto-4,6-dimethylnicotinonitrile in acetone in the presence of potassium carbonate. The structure of the **7** was established based on its elemental analysis and spectral data. The IR spectra of **7** revealed characteristic absorption band of NH group at 3336 cm^{-1} and absorption band of C≡N group at 2218 cm^{-1} and C=O absorption band at 1675 cm^{-1}. The ^{1}H NMR spectrum of **7** showed singlet signal at δ 9.09 ppm corresponding to NH group, singlet signals at δ 6.90 ppm corresponding to the proton of pyridine C-5, doublet and multiplet signals at δ 8.20 and 7.04-7.62 ppm corresponding to the aromatic protons, singlet signal at δ 4.08 for the protons of methylene group, singlet signal at δ 3.86 ppm corresponding to the protons of OCH_3 group, and two singlet signals at δ 2.56 and 2.49 ppm corresponding to the protons of two methyl groups. Intramolecular hetrocyclization of **7** to furnish the corresponding thieno[2,3-b]-pyridine-2-carboxamide derivative **8** was achieved by heating in sodium ethoxide - ethanol solution. The structure of the **8** was established based on its elemental analysis and spectral data. The IR spectrum of **8** elucidated the absence of any absorption in the region 2150-2250 of the cyano function indicating that the involvement of the cyano group in the reaction. The spectrum revealed characteristic absorption bands of NH_2 and NH group at 3433, 3393, 3321 cm^{-1} while carbonyl C=O absorbed at 1639 cm^{-1}. The mass spectrum showed the molecular ion peak at m/z = 810 corresponding to the molecular weight of the formula $C_{34}H_{32}N_6O_4S_2Se_2$.



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Scheme (6): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(3-amino-4,6-dimethylthieno[2,3-b]pyridine-2-carboxamide) (8)

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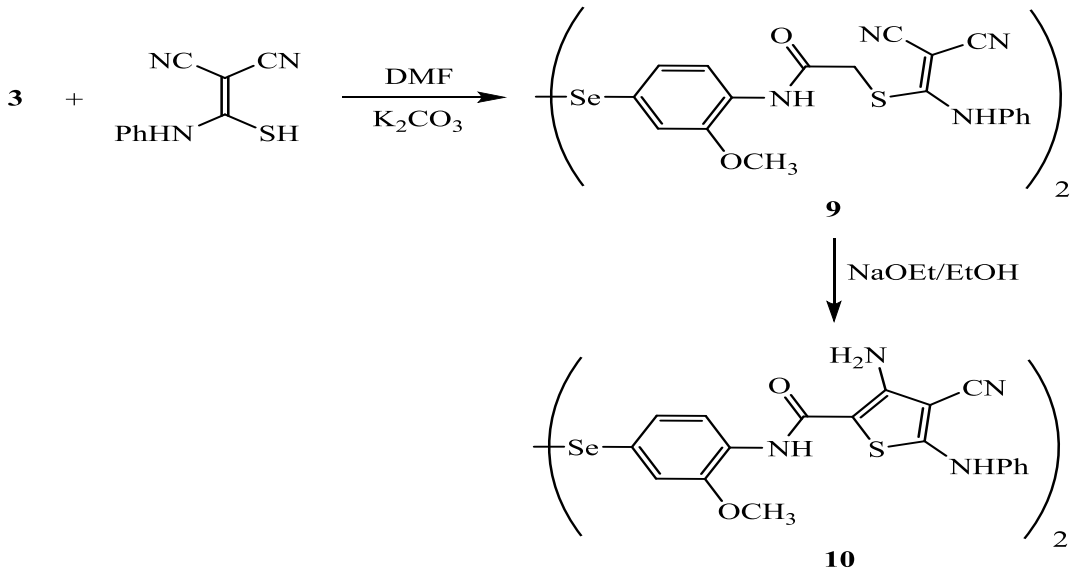
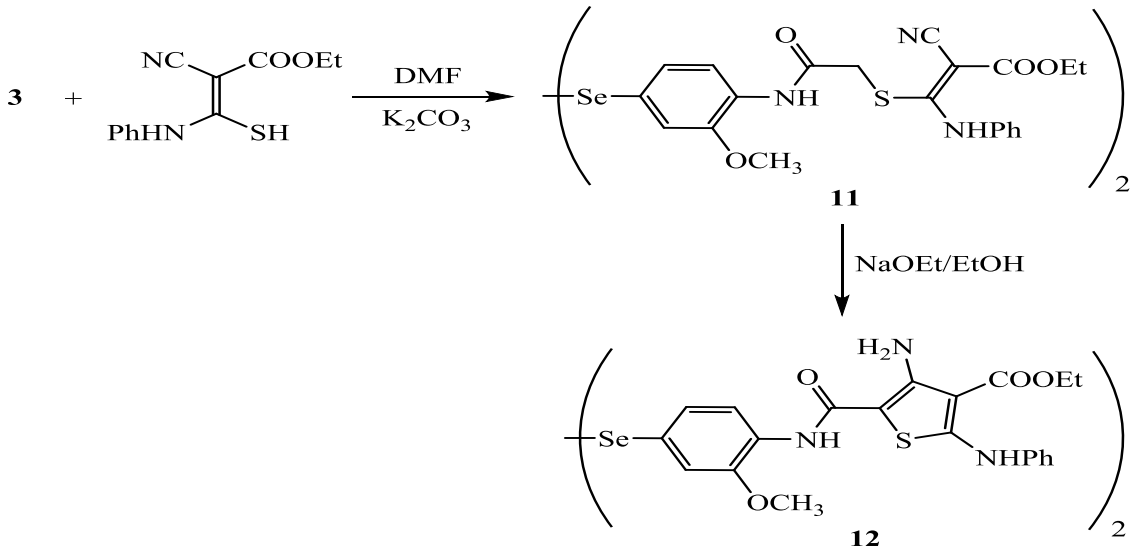
Stirring of chloroacetyl derivative **3** with α-phenylthiocarbamoyl malononitrile [4] in DMF containing anhydrous potassium carbonate afforded the corresponding sulfide derivative **9**, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-((2,2-dicyano-1-(phenylamino)vinyl)thio)-acetamide) **(9)**. Under the previous conditions, the reaction stopped at the state of sulfide formation as a sole product. Heterocyclization of **9** to afford the corresponding 3-amino-4-cyano-5-(phenylamino)-thiophene derivative **10** was achieved by heating in sodium ethoxide – ethanol solution. The chemical structures of **9** and **10** find support from their spectral data. The IR spectrum of **9** revealed the characteristic absorption bands of NH group at 3323 cm^{-1}, cyano group at 2208 cm^{-1} and C =O group at 1674 cm^{-1} . The ^{1}H NMR spectrum of **9** showed two singlet signals at δ 11.82, 10.48 ppm corresponding to NH groups, multiplet signal at δ 7.02-7.82 ppm corresponding to the aromatic protons, singlet signal at δ 4.05 ppm corresponding to the protons of CH_2 group and singlet signal at δ 3.85 ppm corresponding to the methoxy protons. The IR spectrum of **10** revealed characteristic absorption bands of NH_2 group at 3455 and 3367 cm^{-1}, C≡N group at 2196 cm^{-1} and C=O group at 1640 cm^{-1}. The ^{1}H NMR spectrum showed two singlet signals at δ 11.74, 10.11 ppm corresponding to the protons of NH groups, singlet signal at δ 8.35 ppm for the protons of amino group (NH_2), multiplet aromatic signal at δ 7.02-7.76 ppm corresponding to the aromatic protons and singlet signal at δ 3.85 ppm corresponding to the protons of OCH_3.



Scheme (7): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(3-amino-4-cyano-5-(phenylamino)thiophene-2-carboxamide) (10)



Scheme (8): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(3-amino-4-ethoxycarbonyl-5-(phenylamino)thiophene-2-carboxamide) (12)

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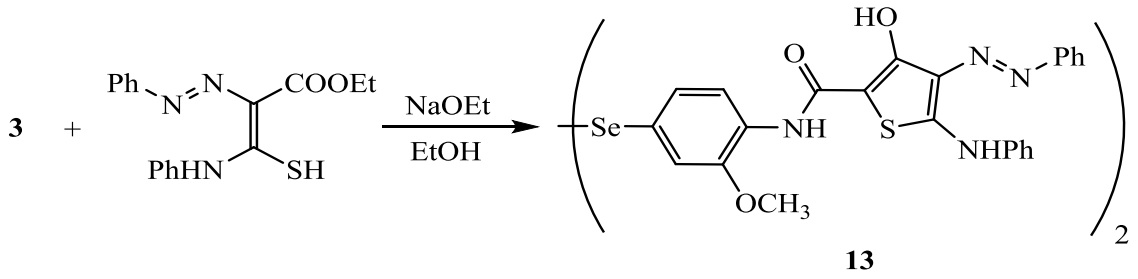
On the other hand, treatment of chloroacetyl derivative **3** with ethyl α-phenylthiocarbamoyl-cyanoacetate [5] in DMF containing anhydrous potassium carbonate furnished the corresponding sulfide derivative, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-((2-cyano-2-ethoxy-carbonyl-1-(phenylamino)vinyl)-thio)acetamide) **(11)**. Intramolecular heterocyclization of **11** was achieved by heating in sodium ethoxide – ethanol solution to afford the corresponding 3-aminothiophene derivative, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(3-amino-4-ethoxy-carbonyl-5 (phenylamino) thiophene-2-carboxamide) **(12)** via nucleophilic addition to the nitrile group. The chemical structures of **11** and **12** were secured based on their spectral data. The IR spectrum of **11** revealed the characteristic absorption band of C≡N group at 2204 cm^{-1} that disappeared from the IR spectrum of **12**. The ^{1}H NMR spectrum of compound **12** showed two singlet signals at δ 11.24, 10.24 ppm for the protons of NH groups, multiplet signal at δ 7.02-7.68 ppm due to the aromatic protons, singlet signal at δ 6.80 ppm for the protons of amino groups, singlet signal at δ 3.85 ppm for the protons of methoxy groups, quartet and triplet signals at δ 4.25 and 1.30 ppm for the protons of methylene and methyl fragments of the ethyl ester ($COOCH_2CH_3$).

Finally, heating of **3** with ethyl α-(p-tolylazo)-α-phenyl-thiocarbamoyl acetate [6] in hot sodium ethoxide – ethanol solution furnished the corresponding 3-hydroxythiophene derivative, N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(3-hydroxy-5-(phenyl-amino)-4-(phenylazo)-thiophene-2-carboxamide) **(13)**. The structure of the **13** was established based on its correct elemental analysis and spectral data. The IR spectrum of **13** revealed a broad absorption band at 3381 cm^{-1} for the OH and NH functions, while the C=O group absorbed at 1675 cm^{-1}. The ^{1}H NMR spectrum showed singlet signal at δ 11.84 ppm for the proton of OH, two singlet signals at δ 8.91 and 8.24 ppm due to two NH groups, multiplet signal at δ 7.13-7.27 ppm for the aromatic protons and singlet signal at δ 3.87 ppm corresponding to the protons of methoxy groups.



Scheme (9): Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(3-hydroxy-5-(phenylamino)-4-(phenylazo)thiophene-2-carboxamide) (30)

***In vitro* Antitumor Activity:**

Cell line: Heptacelluar carcinoma cell line (HepG2) and normal human lung fibroblast cell line (WI-38) were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil was used as a standard anticancer drug for comparison.

Chemical reagents: The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (Sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

MTT assay [7]: The cell line mentioned above was utilized to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric test is based on the transformation of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37 ºC in a 5% CO_2 incubator. The cell lines were seed in a 96-well plate at a density of 1 × 10^4 cells/well [8] at 37 ºC for 48 hours under 5% CO_2. After incubation, the cells were treated with different concentration of compounds and incubated for 24 hours. After 24 hours of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 hours. Dimethyl sulfoxide (DMSO) in volume of 100 µl was added into each well to dissolve the purple formazan formed. The colorimetric assay was measured and recorded at absorbance of 570 nm using a plate reader (EXL 800). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) × 100.

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CONCLUSION

The results obtained in table (1) indicated that compounds **6** and **10** showed the highest activity against Heptacelluar carcinoma cell line (HepG2) compared to the standard anticancer drug 5-fluorouracil. Fortunately, these compounds was also highly effective against normal human lung fibroblast cell line (WI-38). In addition, compounds **9** and **12** have strong activity against Heptacelluar carcinoma cell line (HepG2) but they did not exhibit much harmful against normal human lung fibroblast cell line (WI-38). The rest of obtained compounds exhibit moderate to weak activity against both normal and infected lung fibroblast cells.

Table (1): Cytotoxic activity of the compounds 3-13 on different cell lines against Heptacelluar carcinoma cell line (HepG2)

Compounds	In vitro Cytotoxicity IC50 (µg/ml)	
	HepG2	WI-38
5-FU	7.9±0.16	4.3±0.51
3	24.7±2.11	93.6±4.98
4	36.5±3.20	48.2±3.58
5	84.0±4.24	91.6±5.27
6	8.3±0.46	14.5±0.95
7	52.4±3.40	>100
8	27.1±2.04	40.1±3.61
9	18.9±1.13	31.3±2.67
10	10.5±0.48	18.9±1.06
11	91.2±5.47	>100
12	14.8±0.97	25.7±2.04
13	75.6±4.49	69.4±4.30

IC50 (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic). **5-FU:** 5-Fluorouracil

EXPERIMENTAL

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr) were determined on a Mattson 5000 FTIR spectrometer (not all frequencies are reported). The ^{1}H NMR spectra were acquired using a Bruker WP 300 spectrometer at 300 MHz using TMS as an internal standard. Mass spectra were recorded on a Finnigan MAT 212 instrument. Elemental analyses were carried out at the microanalytical unit, Faculty of Science, Cairo University, Egypt.

Synthesis of 2-methoxy-4-selenocyanato-aniline (1):

To a well stirred solution of malononitrile (2 g, 0.03 mol) in DMSO (15 mL), SeO_2 (6.7 g, 0.06 mol) was added. The mixture became reddish after 10 minutes and an exothermic reaction with vigorous gas evolution began during the next 5 minutes. When the gas evolution was ceased the reaction mixture was filtered to remove any solids present, then *o*-anisidine (3.1 mL, 0.025 mol) was added with stirring. Stirring was continued for additional 1 hour at room temperature. The homogenous solution was diluted with ice-cold water, the precipitate formed was filtered off, air dried and recrystallized from ethanol to give **1**.

Brown powder; yield = 84 %; m.p. = 80-81 °C; R_f = 0.54 [petroleum ether / ethyl acetate (1:1)]. IR ($\bar{\nu}$ /cm^{-1}): 3463, 3348 (NH_2), 2140 (C≡N), 1625 (C=C), 583 (Se-CN).

^{1}H NMR ($CDCl_3$): δ/ppm = 6.90-7.24 (m, 3H, Ar-H), 6.60 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3). Anal. Calcd. for $C_8H_8N_2OSe$ (227.12): C, 42.31; H, 3.55; N, 12.33%; found: C, 42.24; H, 3.58; N, 12.39%.

Synthesis of 4,4'-diselanediyl-bis-(2-methoxyaniline) (2):

DMF (5 mL) was introduced into 2-methoxy-4-selenocyanato-aniline (**1**, 2.27 g, 0.01 mol) in 25 mL two-necked flask fitted with a magnetic stirrer, a septum and a condenser connected to an argon-filled balloon. The solution was heated to 75 ºC and K_2CO_3 (1.38 g, 0.01 mol dissolved in 1 mL of water) was slowly

introduced by a syringe. The resulting mixture was further heated at 75 ºC for 3 hours and then hydrolyzed with ice-cold water and the precipitate formed was filtered off and recrystallized from ethanol to give **2**.

Yellow powder; yield = 72 %; m.p. = 94-96 °C; R_f = 0.32 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3406 (Broad band, NH_2), 819 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 7.27 (s, 2H, Ar-H), 7.05 (d, 2H, Ar-H), 7.00 (s, 4H, $2NH_2$), 6.60 (d, 2H, Ar-H), 3.80 (s, 6H, $2OCH_3$). Anal. Calcd. for $C_{14}H_{16}N_2O_2Se_2$ (402.21): C, 41.81; H, 4.01; N, 6.96%; found: C, 41.70; H, 4.07; N, 6.91 %.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-chloroacetamide) (3):

To a solution of the diselane derivative **2** (4.02 g, 0.01 mol) in dry acetone (25 mL) containing anhydrous K_2CO_3 (2.8 g), chloroacetyl chloride (2.4 mL, 0.03 mol) was added dropwise with stirring at 0-5 °C. Stirring was continued for 4 hours and the reaction mixture was poured into ice cold water. The resulting precipitate was collected, dried and recrystallized from ethanol to afford the corresponding chloroacetamide derivatives **3**.

Yellow powder; yield = 84 %; m.p. = 145 °C; R_f = 0.65 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3367 (NH), 1679 (C=O), 1590 (C=N), 819 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 8.90 (s, 2H, 2NH), 8.25 (d, 2H, Ar-H), 7.08-7.27 (m, 4H, Ar-H), 4.21 (s, 4H, $2CH_2$), 3.90 (s, 6H, $2OCH_3$). Anal. Calcd. for $C_{18}H_{18}C_{l2}N_2O_4Se_2$ (555.17): C, 38.94; H, 3.27; N, 5.05 %; found: C, 38.75; H, 3.37; N, 5.24 %.

Synthesis 1,1'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-amino-3-cyano-5-oxo-4,5-dihydro-1H-pyrrole) (4):

A mixture of **3** (1.11 g, 0.002 mol) and malononitrile (0.26 g, 0.004 mol) in 30 mL dioxane containing trimethylamine (0.5 ml) was heated under reflux for 6 hours and then left to cool. The precipitate that formed on cooling was collected by filtration and recrystallized from ethanol to give compound **4.**

Brown powder; yield = 89 %; m.p. = 190-192 °C; R_f = 0.45 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3383 (NH), 2203 (C≡N), 1678 (C=O), 1591 (C=N), 814 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 8.22 (d, 2H, Ar-H), 7.10-7.30 (m, 4H, Ar-H), 6.84 (s, 4H, $2NH_2$), 4.05 (s, 4H, $2CH_2$), 3.88 (s, 6H, $2OCH_3$). MS (EI): m/z (%) = 614 (24%), 202 (100%). Anal. Calcd. for $C_{24}H_{20}N_6O_4Se_2$ (614.38): C, 46.92; H, 3.28; N, 13.68 %; found: C, 47.09; H, 3.35; N, 13.53 %.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-ethoxycarbonyl-methyl-sulfanyl-acetamide) (5):

A mixture of **3** (1.11 g, 0.002 mol), ethyl thioglycolate (0.48 ml, 0.004 mol) and sodium acetate (0.5 g) in 30 mL absolute ethanol was refluxed for 2 hours. The solid precipitate that formed on cooling was collected by filtration and recrystallized from ethanol to give **5.**

Yellow powder; yield = 80 %; m.p. = 180-182 °C; (ethanol); R_f = 0.45 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3322 (NH), 1729 (C=O), 1673 (C=O) 1589 (C=N), 801 (Se-C). MS (EI): m/z (%) = 722 (5.42%), 80 (100.00%). Anal. Calcd. for $C_{26}H_{32}N_2O_8S_2Se_2$ (722.59): C, 43.22; H, 4.46; N, 3.88%; found: C, 43.08; H, 4.54; N, 3.71%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-(benzo[d]thiazol-2-ylthio)acetamide) (6):

A mixture of **3** (1.11 g, 0.002 mol) and 2-mercaptobenzothiazole (0.67 g, 0.004 mol) was refluxed for 4 hours in absolute ethanol (30 mL) containing sodium acetate (0.5 g). The reaction mixture was allowed to cool at room temperature and poured into cooled water. The solid product that formed was collected by filtration, dried and recrystallized from ethanol.

Yellow powder; yield = 84 %; m.p. = 144-145 °C; R_f = 0.47 [petroleum ether /ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3252 (NH), 1673 (C=O), 831 (Se-C). ^{1}H NMR (DMSO): δ/ppm = 10.75 (s, 2H, 2NH), 7.08-7.72 (m, 14H, Ar-H), 4.15 (s, 4H, $2CH_2$), 3.88 (s, 6H, $2OCH_3$). MS (EI): m/z (%) = 816 (5.82%), 181.00 (100%). Anal. Calcd. for $C_{32}H_{26}N_4O_4S_4Se_2$ (816.74): C, 47.06; H, 3.21; N, 6.86%; found: C, 47.16; H, 3.27; N, 6.71%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(2-((3-cyano-4,6-dimethylpyridin-2-yl)thio)acetamide) (7).

A mixture of **3** (2.77 g, 0.005 mol), 2-mercapto-4,6-dimethylnicotinonitrile (1.64 g, 0.01 mol) and potassium carbonate (2.8 g) in 20 mL acetone was refluxed for 4 hours. The reaction mixture was poured on to crushed ice and the precipitated product was collected by the filtration and washed with water. The crude product was purified by recrystallization from ethanol to give **7**.

Yellow crystals; yield = 83 %; m.p. = 174-176 °C; R_f = 0.44 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3336 (NH), 2218 (C≡N), 1675 (C=O), 1590 (C=N), 806 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 9.09 (s, 2H, NH), 8.20 (d, 2H, Ar-H), 7.04-7.62 (m, 6H, Ar-H), 6.90 (s, 2H, 2pyridine H-5), 4.08 (s, 4H, $2CH_2$), 3.86 (s, 6H, $2OCH_3$), 2.56 (s, 6H, $2CH_3$), 2.49 (s, 6H, $2CH_3$). Anal. Calcd. for $C_{34}H_{32}N_6O_4S_2Se_2$ (810.71): C, 50.37; H, 3.98; N, 10.37%; found: C, 50.25; H, 3.91; N, 10.49%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(3-amino-4,6-dimethylthieno[2,3-b]pyridine-2-carboxamide) (8):

A solution of **7** (1.62 g, 0.002 mol) in sodium ethoxide (prepared from 0.1 g Na and 20 mL absolute ethanol) was refluxed for 2 hours. The reaction mixture was allowed to cool, poured into ice-water. The solid product was filtered off, dried and recrystallized from ethanol.

Yellow powder; yield = 82 %; m.p. = 270-273°C; R_f = 0.63 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3433, 3393, 3321 (NH_2, NH), 1639 (C=O), 1589 (C=N), 810 (Se-C). MS (EI): m/z (%) = 810 (58.52%), 285.10 (100%). Anal. Calcd. for $C_{34}H_{32}N_6O_4S_2Se_2$ (810.71): C, 50.37; H, 3.98; N, 10.37%; found: C, 50.50; H, 3.93; N, 10.52%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-((2,2-dicyano-1-(phenylamino) vinyl)thio)acetamide) (9):

A mixture of **3** (2.77 g, 0.005 mol), α-phenylthiocarbamoyl malononitrile (2.01 g, 0.01 mol) and potassium carbonate (1.4 g, 0.01 mol) was stirred in 20 mL DMF for 8 hours. The reaction mixture was poured into ice-water and neutralized with dilute HCl. The solid product was collected by filtration, dried and recrystallized from ethanol to give **9**.

Yellow powder; yield = 80-82 %; m.p. = 170-172°C; R_f = 0.46 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3323 (NH), 2208 (C≡N), 1674 (C=O), 813 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 11.82 (s, 2H, 2NH), 10.48 (s, 2H, 2NH), 7.02-7.82 (m, 16H, Ar-H), 4.05 (s, 4H, $2CH_2$), 3.85 (s, 6H, $2OCH_3$). MS (EI): m/z (%) = 884 (11.3%), 202.05 (100%). Anal. Calcd. for $C_{38}H_{30}N_8O_4S_2Se_2$ (884.75): C, 51.59; H, 3.42; N, 12.67%; found: C, 51.40; H, 3.31; N, 12.88%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(3-amino-4-cyano-5-(phenylamino) thiophene-2-carboxamide) (10):

Thiocarbamoyl derivative **9** (0.88 g, 0.001 mol) was refluxed for 2 hours in sodium ethoxide – ethanol solution (prepared from 0.05 Na and 15 mL ethanol). The reaction mixture was allowed to cool, and poured into ice-cold water. The separated product was filtered off, dried and recrystallized from ethanol to give **10**.

Brown powder; yield = 75 %; m.p. = 287-288 °C; R_f = 0.71 [petroleum ether / ethyl acetate (4:2)]. IR ($\bar{\nu}$ /cm^{-1}): 3455, 3367 (NH_2), 2196 (C≡N), 1640 (C=O), 815 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 11.74 (s, 2H, 2NH), 10.11 (s, 2H, 2NH), 8.35 (s, 4H, $2NH_2$), 7.02-7.76 (m, 16H, Ar-H), 3.85 (s, 6H, $2OCH_3$). Anal. Calcd. for $C_{38}H_{30}N_8O_4S_2Se_2$ (884.75): C, 51.59; H, 3.42; N, 12.67%; found: C, 51.35; H, 3.52; N, 12.82%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis-(2-((2-cyano-2-ethoxycarbonyl-1-(phenylamino)vinyl)thio)acetamide) (11):

A mixture of **3** (2.77 g, 0.005 mol), ethyl α-phenylthiocarbamoyl cyanoacetate (2.48 g, 0.01 mol) and potassium carbonate (1.4 g, 0.01 mol) was stirred in 15 mL DMF for 8 hours. The reaction mixture was poured into ice-water and neutralized with dilute HCl. The solid product was collected by filtration, dried and recrystallized from ethanol.

Yellow powder; yield = 63 %; m.p. = 192-194 °C; R_f = 0.53 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{\nu}$ /cm^{-1}): 3346 (NH), 2204 (C≡N), 1668 (C=O), 1590 (C=C), 813 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = δ 12.25 (s, 2H, 2NH), 10.42 (s, 2H, 2NH), 6.92-7.58 (m, 16H, Ar-H), 4.25 (q, 4H, 2CH_2), 4.04 (s, 4H, 2CH_2), 3.85 (s, 6H, 2OCH_3), 1.31 (t, 6H, 2CH_3). Anal. Calcd. for $C_{42}H_{40}N_6O_8S_2Se_2$ (978.86): C, 51.54; H, 4.12; N, 8.59%; found: C, 51.66; H, 4.20; N, 8.51%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis(3-amino-4-ethoxycarbonyl-5-(phenylamino)thiophene-2-carboxamide) (12):

Thiocarbamoyl derivative **11** (0.98 g, 0.001 mol) was refluxed for 2 hours in sodium ethoxide – ethanol solution (prepared from 0.05 Na and 15 mL ethanol). The reaction mixture was allowed to cool, and poured into ice-cold water. The separated product was filtered off, dried and recrystallized from ethanol to give **12**.

Brown powder; yield = 100 %; m.p. = 230-231°C; R_f = 0.67 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{\nu}$ /cm^{-1}): 3459, 3393 (NH_2 and NH), 1669 (broad, C=O), 1588 (C=N), 813 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 11.24 (s, 2H, 2NH), 10.24 (s, 2H, 2NH), 7.02-7.68 (m, 16H, Ar-H), 6.80 (s, 4H, 2NH_2), 4.25 (q, 4H, 2$COO\underline{CH_2}CH_3$), 3.85 (s, 6H, 2OCH_3), 1.30 (t, 6H, 2$COOCH_2\underline{CH_3}$). Anal. Calcd. for $C_{42}H_{40}N_6O_8S_2Se_2$ (978.86): C, 51.54; H, 4.12; N, 8.59%; found: C, 51.71; H, 4.20; N, 8.63%.

Synthesis of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))-bis(3-hydroxy-5-(phenylamino)-4 (phenylazo) thiophene-2-carboxamide) (13):

A mixture of **3** (1.11 g, 0.002 mol) and ethyl α-(p-tolylazo)-α-phenylthiocarbamoyl acetate (0.68 g, 0.002 mol) was refluxed for 2 hours in sodium ethoxide – ethanol solution (0.004 mol Na and 20 mL EtOH). The reaction mixture was allowed to cool, poured into ice-water and neutralized with dilute HCl. The precipitate was filtered off, dried and recrystallized from ethanol.

Brown powder; yield = 70 %; m.p. = 177-179 °C; (ethanol); R_f = 0.50 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{\nu}$ /cm^{-1}): 3381 (broad, OH and NH), 1675 (C=O), 1590 (C=C), 813 (Se-C). ^{1}H NMR ($CDCl_3$): δ/ppm = 11.84 (s, 2H, 2OH), 8.91 (s, 2H, 2NH), 8.24 (s, 2H, 2NH), 7.13-7.27 (m, 26H, Ar-H), 3.87 (s, 6H, 2OCH_3). Anal. Calcd. for $C_{48}H_{38}N_8O_6S_2Se_2$ (1044.92): C, 55.17; H, 3.67; N, 10.72%; found: C, 55.03; H, 3.86; N, 10.60%.

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