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CYTOTOXIC NAPHTHALENE BASED-SYMMETRICAL DISELENIDES WITH INCREASED SELECTIVITY AGAINST MCF-7 BREAST CANCER CELLS

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ABSTRACT

A series of naphthalene based-symmetrical diselenides were synthesized in good-moderate yields and their *in vitro* cytotoxic activity was evaluated against breast adenocarcinoma (MCF-7) and compared with their cytotoxicity in normal fibroblast cells (WI-38). Furthermore, their *in vitro* antimicrobial activities were also evaluated against gram-negative (*Escherichia coli*), gram-positive (*Staphylococcus aureus*) bacteria and the pathogenic yeast *Candida albicans*. A significant difference in toxicity zones between the breast solid tumor MCF-7 cells and normal WI-38 cells was observed indicating that it is not general selenium toxicity. Within this context, compounds **2**, **9, 10**, **12**, **19**, and **20** exhibited therapeutic indices (TI) up to eleven fold and in most cases were higher than the TI of 5-fluorouracil (5-fu) suggesting their effectiveness as anti-cancer agents. Indeed, these ccompounds exhibited also good antibacterial activity against *E. coli* bacteria compared to the known drug, ampicillin. Moreover, compounds **2, 7, 8** and **10** exhibited good antifungal activity against *C. albicans* compared to colitrimazole.

Keywords: Breast cancer, Therapeutic selectivity, Drug resistance, Organoselenium, Naphthalene, Diselenides

INTRODUCTION

Breast cancer is a heterogeneous disease being the second most prevalent solid tumor in the world and, by far the most common diagnosed invasive malignancy in women globally.[1-2] It is a serious global health problem with approximately 1.7 million new cases and half million deaths per year, mostly in Europe. Despite the tremendous progress made over the last decades in understanding the biology of breast cancer, there is no clear, proven and effective single agent that constitutes a systemic regimen recommended for treatment.[1-3] Concerted efforts have therefore made in order to provide patients with suitable treatment; however, high risk of relapse to a broad spectrum of drugs is constantly observed. This is mainly due drug resistance obstacle that is facing the chemotherapy of solid malignancies. Furthermore, the lack of selective drugs that initiate death machinery in breast cancers without executing the same in normal cells makes available treatment options are far from satisfactory. These obstacles necessitate the development of more effective and less cytotoxic therapies in order to expand the available effective drug arsenal to battle the disease.[4-6]

Mount epidemiological studies have related the disorder in selenium-body status with the increased risk of many cancers, including breast cancer.[7] In this context, many organoselenium compounds were developed and have shown favorable chemoprevention profiles in breast carcinogenesis.[7] Besides the organoselenium compounds chemopreventive activities, there are growing evidences demonstrating the potential of these compounds as chemotherapeutic agents (e.g., diphenyl diselenide, dinaphthyl diselenide, ebselen and ethaselen) (Figure 1). These observations were supported by large numbers of epidemiological and preclinical studies and stimulated the initiation of many clinical intervention trials (e.g., SELECT trial in US and PRECISE trial in Europe); however, these findings are also still in need for further investigations and merit further research.[8, 9]

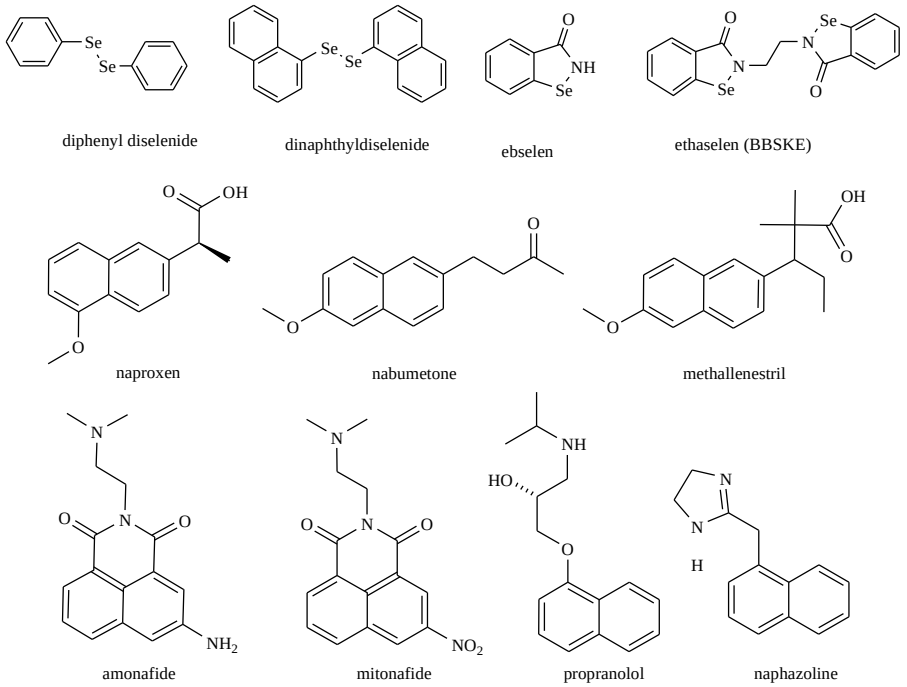


Figure 1. Chemical structures of selected biologically important organoselenium compounds and naphthalene based drugs.

Six years ago, we developed a series of organoselenium redox agents, of which some showed significant cytotoxicity, yet also selectively, against specific type of cancer cells as well as different pathogenic microorganisms.[10,14] Since then, we are involved in the development of more effective cancer therapy based on organoselenium redox modulators and the exploration of their corresponding intracellular diagnostics. Within this context, we have further developed structurally diverse libraries of selenium-containing quinone pseudopeptides which have also shown considerable cytotoxicity (at sub-micromolar concentrations) against different types of cancer cells with minimal cytotoxicity in normal cells. It is worth mentioning that the selectivity was more pronounced in case of breast cancer (MCF-7) cells compared to the other investigated cancer cells at that time.[14-18]

Promoted by these findings, it's likely that a combination of selenium with bioactive pharmacophores (e.g., quinones) is expected to synergistically potentiate the overall cytotoxicity and enhance the chemotherapeutic properties. From this viewpoint, the naphthalene pharmacophore has drawn our attention as it is the core structure of great number of clinical drugs such as propranolol, naphazoline, naproxen, nabumetone, methallenestril, amonafide and mitonafide (Figure 1).[19,20] In continuation of our previous work,[21-25] our aim is to synthesize a new series of naphthalene based-symmetrical diselenides and to evaluate their cytotoxicity against human breast cancer cells (MCF-7) and compare it with their cytotoxicity in normal fibroblast cells (WI-38) employing standard MTT assay.

MATERIAL AND METHODS

All chemical reagents for the synthesis of compounds were purchased from Sigma-Aldrich-Fluka or Merck (AMD) and used without further purification unless stated otherwise. TLC plates (silica gel 60 F_{254}, 0.20 mm) were purchased from Merck. All melting points are in degree Celsius (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 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 (75 MHz) 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 by Mr Ahmed Abbas at Drug Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Synthesis and characterization

Synthesis of 4-selenocyanatonaphthalen-1-amine (1) [26]

To a well stirred solution of malononitrile (0. 2 g, 3 mmol) in DMSO (2 mL), SeO_2 (0.67 gm, 6 mmol) was added. The mixture became reddish after 10 min and an exothermic reaction with vigorous gas evolution began during the next 5 min. When the gas evolution was ceased the reaction mixture was filtered to remove any solids present, then napthyl amine (0.64 g, 4.5 mmol) was added with stirring. Stirring was continued for additional 1 h 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**.

Synthesis of 4,4'-diselanediylbis(naphthalen-1-amine) (2)

Sodium borohydride (113.4 mg, 3 mmol) was gradually added to aminonapthylselenocynate (494.4 mg, 2 mmol) in ethanol (10 ml). The mixture was further stirred for 30 min and then poured on cold water. The resulting yellow precipitate was filtered and recrystallized from ethanol.

Yellow crystals; yield 57%; mp 192-194 ^oC (ethanol); R_f = 0.14 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3363 (NH_2), 1229 (C-N),757 (Se-Se), 493(Se-C); EIMS *m/z* (%) 443 (M^+, 14.3), 444 (M^++1, 27); Anal. Calcd. for $C_{20}H_{16}N_2Se_2$ (443.96): C, 54.31; H, 3.65, N, 6.33. Found: C, 54.23; H, 3.59; N, 6.22.

Formylation of 4,4'-diselanediylbis(naphthalen-1-amine) (2)

Formic acid (150 µL, 3 mmol) was added to 4,4'-di(1-aminonaphthyl)-diselenide (**1**) (0.442 mg, 1 mmol) and the mixture was heated at 50-60 ^{o} C for 1 hr and then poured on cold water. The resulting precipitate was filtered and recrystallized from ethanol.

***N,N'*-(Diselanediylbis(naphthalene-4,1-diyl)) diformamide** (**3**)

Brown yellow crystals; yield 82%; mp 199-201 ^oC (ethanol); R_f = 0.09 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3447 (NH), 2925 (C-H), 1601 (C=O), 1266 (C-N), 751 (Se-Se), 542 (Se-C); EIMS *m/z* (%) 500 (M^+, 54.62); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.27 (s, 2H, 2CHO), 7.71-7.65 (m, 4H, Ar-H), 7.32-7.29 (m, 4H, Ar-H), 7.20-7.18 (m, 2H, Ar-H), 6.50-6.46 (m, 2H, Ar-H); Anal. Calcd. for $C_{22}H_{16}N_2O_2Se_2$ (499.95); C, 53.03; H, 3.24; N, 5.62. Found: C, 53.12; H, 3.12; N, 5.42.

Acylation of 4,4'-diselanediylbis(naphthalen-1-amine) (2)

A mixture of 4,4'-di(1-aminonaphthyl)-diselenide (**2**) (0.442 mg, 1.00 mmol), 0.2 ml acetic anhydride and 0.2 ml glacial acetic acid was heated in an oil bath at 60 – 65 ^{o}C for 1 h. The reaction mixture was allowed to cool at room temperature and then poured on cold water. The resulting precipitate was filtered and recrystallized from ethanol.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl)) diacetamide (**4**)

Brown crystals; yield 73%; mp 196-198 ^oC (ethanol); R_f = 0.11 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3423 (NH), 2967 (C-H), 1654 (C=O), 1264 (C-N), 755 (Se-Se), 506 (Se-C); EIMS *m/z* (%) 528 (M^+, 0.51); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 2H, NH), 8.20-8.11 (m, 2H, Ar-H), 7.85-7.80 (m, 2H, Ar-H), 7.60-7.40 (m, 4H, Ar-H), 6.98-6.92 (m, 2H, Ar-H), 6.82-6.79 (m, 2H, Ar-H), 2.20 (s, 6H, $2CH_3$); Anal. Calcd. for $C_{24}H_{20}N_2O_2Se_2$ (527.99): C, 54.77; H, 3.83; N, 5.32. Found: C, 54.67; H, 3.73; N, 5.22.

Benzoylation of 4,4'-diselanediylbis(naphthalen-1-amine) (2)

Benzoyl chloride (500 µL, 4 mmol) was add to 4,4'-di(1-aminonaphthyl)-diselenide (**2**) (0.88 mg, 2 mmol) in 10 ml dioxane. The mixture was refluxed for 2 hr and then poured on cold water. The resulting precipitate was filtered and recrystallized from ethanol.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl)) dibenzamide (**5**)

Brown crystals; yield 71%; mp 233-235 ^oC (ethanol); R_f = 0.10 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3442 (NH), 2924 (C-H), 1639 (C=O), 1252 (C-N), 752 (Se-Se), 485 (Se-C); EIMS *m/z* (%) 652 (M^+, 71.84), 653 (M^++1, 61.17); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.07 (s, 2H, 2NH), 8.31-7.86 (m, 10H, Ar-H), 7.77-7.24 (m, 12H, Ar-H); Anal. Calcd. For $C_{34}H_{24}N_2O_2Se_2$ (652.02): C, 62.78; H, 3.72; N, 4.31. Found: C, 62.63; H, 3.61; N, 4.22.

General procedure for the synthesis of diazo derivatives 7 and 8

1-4,4'-Di(1-aminonaphthyl)-diselenide (2) (0.442 mg, 1.00 mmol) was dissolved in glacial acetic acid (8 ml), cooled to 0-5 °C, then a cold solution of sodium nitrite (1.4 g in 4 ml water) was added, 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. malononitrile or ethyl cyanoacetate) (0.02 mol) and sodium acetate (2.0 g), 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.

(Diselanediylbis(naphthalene-4,1-diyl))dicarbonohydrazonoyl dicyanide (**7**)

Brown crystals; yield 64%; mp 210-212 ^{o}C (ethanol); R_f =0.10 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3349 (NH), 2958 (C-H), 2261 (CN), 752 (Se-Se), 464 (Se-C); EIMS *m/z* (%) 598 (M^{+}, 5.62); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49-7.81 (m, 4H, Ar-H), 7.70-6.80 (m, 8H, Ar-H); Anal. Calcd. For $C_{26}H_{14}N_8Se_2$ (597.97): C, 52.36; H, 2.37; N, 18.79. Found: C, 52.29; H, 2.20; N, 18.62.

(2E,2'E)-Diethyl-2,2'-(2,2'-(diselanediylbis (naphthalene-4,1-diyl))bis(hydrazin-2-yl-1-ylidene))bis(2-cyanoacetate) (8)

Brown crystals; yield 63%; mp 222-224 ^{o}C (ethanol); R_f = 0.11[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3376 (NH), 2966 (C-H), 2246 (CN), 1619 (C=O), 754 (Se-Se), 455 (Se-C); EIMS *m/z* (%) 692 (M^{+}, 29.94); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59-7.07 (m, 5H, Ar-H), 6.74-6.36 (m, 5H, Ar-H), 6.28-6.10 (m, 2H, Ar-H), 4.33 (q, J = 7.18 Hz, 4H, 2CH$_2$), 1.06 (t, *J* = 7.0 Hz, 6H, 2CH$_3$); Anal. Calcd. For $C_{30}H_{24}N_6O_4Se_2$ (692.02): C, 52.18; H, 3.50; N, 12.17. Found: C, 52.09; H, 3.43; N, 12.03.

General procedure for the synthesis of pyrazoles 9 and 10 *via* cyclocondensation of 7 and 8 with Hydrazine hydrate.

A mixture of diselenodiazine derivatives **7** or **8** (0.01 mol) and the appropriate hydrazine derivatives (0.02 mol) in dioxane (10 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 **9** and **10**.

4,4'-((Diselanediylbis(naphthalene-4,1-diyl))bis(diazene-2,1-diyl))bis(4H-pyrazole-3,5-diamine)(9)

Brown crystals; yield 67%; mp 227-229 ^{o}C (ethanol); R_f =0.11[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3441 (NH), 3373 (NH$_2$), 2956 (C-H), 752 (Se-Se), 501 (Se-C); EIMS *m/z* (%) 662 (M^{+}, 55.00); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37-7.92 (m, 8H, Ar-H), 7.65-7.38 (m, 4H, Ar-H), 6.63 (s, 4H, 2NH$_2$) ppm, 1.37(s, 2H, 2CH) ppm; Anal. Calcd. For $C_{26}H_{22}N_{12}Se_2$ (662.04): C, 47.28; H, 3.36; N, 25.45. Found: C, 47.25; H, 3.34; N, 25.41.

4,4'-((Diselanediylbis(naphthalene-4,1-diyl))bis(diazene-2,1-diyl))bis(5-amino-2,4-dihydro-3H-pyrazol-3-one) (**10**)

Brown crystals; yield 62%; mp 217-219 ^{o}C (ethanol); R_f =0.12[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3447 (NH), 3432 (NH$_2$), 2981 (C-H), 1680 (C=O), 1250 (C-N), 1220 (C-O), 758 (Se-Se), 473 (Se-C); EIMS *m/z* (%) 664 (M^{+}, 10.00); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46-8.03 (m, 8H, Ar-H), 7.91-7.36 (m, 4H, Ar-H), 6.48 (s, 2H, NH$_2$) ppm, 2.38 (s, 2H, 2CH)ppm ; Anal. Calcd. For $C_{26}H_{20}N_{10}O_2Se_2$ (664.01): C, 47.14; H, 3.04; N, 21.14. Found: C, 47.09; H, 3.00; N, 21.12.

Synthesis of α-chloroacetamide 6

To a solution of 1-4,4'-di(1-aminonaphthyl)-diselenide (**2**) (0.44 g, 1.00 mmol) in dry Acetone (15 ml) containing K_2CO_3 (2 g), chloroacetyl chloride (0.32 ml, 4.0 mmol) was added dropwise with stirring at 0-5^{0} C. Stirring was continued for 4 h at room temperature and the reaction mixture was poured into ice cooled water. The resulting precipitate was collected, dried and recrystallized from ethanol.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(2-chloroacetamide) (**6**)

Green yellow crystals; yield 85%; mp 209-211 ^{o}C (ethanol); R_f = 0.11 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3253 (NH), 2951 (C-H), 1667 (C=O), 1244 (C-N), 755 (Se-Se), 733(C-Cl), 470 (Se-C); EIMS *m/z* (%) 596 (M^{+}, 0.80), 597(M^{+}+1, 23.80); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2H, 2NH), 8.13 (dd, *J* = 8.5, 3H, Ar-H), 7.86 (d, *J*=7.9 Hz, 3H, Ar-H), 7.56-7.54 (m, 6H, Ar-H), 4.46 (s, 4H, 2CH$_2$); ^{13}C NMR (75 MHz, DMSO) δ 165.66, 134.81, 133.99, 133.79, 128.09, 128.00, 127.03, 126.47, 126.36, 123.27, 121.24, 43.32; Anal. Calcd. for $C_{24}H_{18}Cl_2O_2Se_2$ (595.91): C, 48.43; H, 3.05; N, 4.71. Found: C, 48.55; H, 3.15 N, 4.55.

General procedure for the reaction of α-chloroacetamide 6 with different nucleophiles (thiophenol, α-naphthol, morpholine and 4-phenyl-5-selenocyanatothiazol-2-amine)

A mixture of α-chloroacetamide **6** (0.59 mg, 1mmol), appropriate nucleophile (2 mmol) and few drops of triethylamine in 10 ml dioxane was refluxed for 10 h and the reaction mixture was then poured into ice

cooled water. The resulting precipitate was collected, dried and recrystallized from ethanol.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(2-(phenylthio)acetamide) (11)

Yellow crystals; yield 71%; mp 229-231 °C (ethanol); R_f =0.13[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3253(NH), 2953 (C-H), 1667 (C=O), 1242 (C-N) , 756 (Se-Se), 688 (C-S-C), 646 (Se-C); EIMS *m/z* (%) 745 (M^++1, 14.55); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2H, 2NH), 8.36-7.99 (m, 4H, Ar-H), 7.57-7.50 (m, 10H, Ar-H), 7.33-7.25 (m, 8H, Ar-H), 4.47 (s, 4H, $2CH_2$); ^{13}C NMR (75 MHz, DMSO) δ 165.65, 135.76, 134.82, 134.48, 133.99, 133.80, 131.30, 130.06, 129.24, 128.08, 127.79, 127.52, 127.18, 126.58, 126.46, 123.26, 121.21, 43.34; Anal. Calcd. For $C_{36}H_{28}N_2O_2S_2Se_2$ (743.99): C, 58.22; H, 3.80; N, 3.77. Found: C, 58.11; H, 3.69; N, 3.65.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(2-(naphthalen-2-yloxy)acetamide) (12)

Yellow crystals; yield 69%; mp 240-242 °C (ethanol); R_f =0.12[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3252 (NH), 2952 (C-H), 1668 (C=O), 1246 (C-N), 1151 (C-O), 755 (Se-Se), 472 (Se-C); EIMS *m/z* (%) 812 (M^+, 81.13), 813 (M^++1, 51.89); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2H, 2NH), 8.13-8.09 (m, 6H, Ar-H), 8.00-7.84 (m, 6H, Ar-H), 7.82-7.47 (m, 10H, Ar-H), 7.43-7.04 (m, 4H, Ar-H), 4.55 (s, 4H, $2CH_2$); Anal. Calcd. For $C_{44}H_{32}N_2O_4Se_2$ (812.07): C, 65.19; H, 3.98; N, 3.46. Found: C, 65.01; H, 4.03; N, 3.52.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(2-morpholinoacetamide) (13)

Brown crystals; yield 69%; mp 229-230 °C (ethanol); R_f =0.13[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3446 (NH), 2955 (C-H), 1681 (C=O), 1294 (C-N), 1114 (C-O), 754 (Se-Se), 516 (Se-C); EIMS *m/z* (%) 698 (M^+, 34.25), 699 (M^++1, 22.59); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 2H, 2NH), 8.73-7.17 (m, 12H, Ar-H), 3.67-3.62 (m, 8H, $4CH_2$), 3.33 (s, 4H, $2CH_2$), 2.60-2.58 (m, 8H, $4CH_2$); ^{13}C NMR (75 MHz, DMSO) δ 168.64, 140.8, 135.07, 134.45, 134.39, 133.84, 128.24, 127.43, 126.92, 126.56, 125.0, 122.39, 119.60, 66.25, 61.77, 53.21; Anal. Calcd. For $C_{32}H_{34}N_4O_4Se_2$ (698.09): C, 55.18; H, 4.92; N, 8.04. Found: C, 55.28; H, 4.85; N, 8.14.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(2-((4-phenyl-5-selenocyanatothiazol-2-yl)amino)acetamide) (14)

Yellow Brown crystals; yield 71%; mp 220-233 °C (ethanol); R_f =0.14[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}= 3440 (NH), 2956 (C-H), 2198 (CN), 1617 (C=O), 1266 (C-N), 770 (Se-Se), 696 (C-S-C), 472 (Se-C); EIMS *m/z* (%) 1085 (M^+, 100.00); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (s, 2H, 2NH), 8.39 -7.96 (m, 6H, Ar-H), 7.92-7.75 (m, 6H, Ar-H), 7.72-7.49 (m, 5H, Ar-H), 7.42-7.21 (m, 7H, Ar-H), 4.45 (s, 4H, $2CH_2$); ^{13}C NMR (75 MHz, DMSO) δ 171.66, 170.09, 165.66, 132.55, 132.01, 128.85, 128.59, 128.49, 128.19, 128.08, 127.98, 127.90, 127.85, 127.71, 127.57, 126.86, 126.57, 123.52, 122.57, 109.09, 101.50, 66.32. Anal. Calcd. For $C_{44}H_{30}N_8O_2S_2Se_4$ (1085.86): C, 48.81; H, 2.79; N, 10.35. Found: C, 48.70; H, 2.62; N, 10.23.

General procedure for the Ugi four component reaction

As a general procedure, a mixture of 4,4'-di(1-aminonaphthyl)-diselenide (**2**) (0.44 g, 1.00 mmol), aldehyde (2 mmol), amine (2 mmol) and isonitrile (2.2 mmol) in 2 mL methanol was stirred at room temperature overnight. Upon completion (monitored by TLC), water was and the aqueous layer was extracted three times with CH_2Cl_2, the organic layers were combined, dried over Na_2SO_4 and concentrated to yield a sticky product which was purified by chromatography on silica gel.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(N-(2-(tert-butylamino)-2-oxoethyl)acetamide) (15)

Yellow crystals; yield 75%; mp 249-251 °C (ethanol); R_f =0.14[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}= 3443 (NH), 2966 (C-H), 1651 (C=O), 1257 (C-N), 762 (Se-Se); EIMS *m/z* (%) 754 (M^+, 34.52), 755 (M^++1, 26.79); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.32 (s, 2NH), 8.15-8.10 (m, 2H, Ar-H), 7.92 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.73-7.53 (m, 4H, Ar-H), 7.44-7.42 (m, 2H, Ar-H), 7.30 (d, *J* = 6.9 Hz, 2H, Ar-H), 4.64 (s, 4H, $2CH_2$), 1.63 (s, 6H, $2CH_3$), 1.20 (s, 18H, $6CH_3$). Anal. Calcd. For $C_{36}H_{42}N_4O_4Se_2$ (754.15): C 57.45; H, 5.62; N, 7.44. Found: C, 57.33; H, 5.52; N, 7.30.

2,2'-((Diselanediylbis(naphthalene-4,1-diyl))bis(acetylazanediyl))bis(N-(tert-butyl)-2-(furan-2-yl)acetamide) (16)

Yellow crystals; yield 64%; mp 267-269 °C (ethanol); R_f =0.12[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3446 (NH), 2969 (C-H) , 1634 (C=O), 1256 (C-N), 756 (Se-Se), 463 (Se-C); EIMS *m/z* (%) 886 (M^+, 82.9); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 – 8.27 (m, 2H, Ar-H), 8.07 (d, *J* = 23.7 Hz, 4H, Ar-H), 7.82 – 7.15 (m, 6H, Ar-H), 6.84-6.65 (m, 2H, Furyl-H), 6.50 (d, *J* = 7.9 Hz, 2H, Furyl-H), 6.27 (d, *J* = 22.9 Hz, 2H, Furyl-H), 1.91 (s, 6H, $2CH_3$), 1.29 (s, 18H, $6CH_3$); Anal. Calcd. For $C_{44}H_{46}N_4O_6Se_2$ (886.17): C, 59.73; H, 5.24; N, 6.33. Found: C, 59.86; H, 5.16; N, 6.29.

N,N'-(Diselanediylbis(naphthalene-4,1-diyl))bis(N-(2-oxo-2-((tosylmethyl)amino)ethyl)acetamide) (**17**)
Brown crystals; yield 77%; mp 275-276 ºC (ethanol); R_f =0.13[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3353(NH), 2921 (C-H), 1623 (C=O), 1280 (C-N), 754 (Se-Se), 670 (C-S-C), 511 (Se-C); EIMS *m/z* (%) 978 (M^+, 72.9); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2H, 2NH), 8.97-8.67 (m, 4H, Ar-H), 8.50-7.94 (m, 4H, Ar-H), 7.90-7.09 (m, 8H, Ar-H), 6.78-6.34 (m, 4H, Ar-H), 5.16 (s, 4H, $2CH_2$), 4.80 (s, 4H, $2CH_2$), 2.39 (s, 6H, $2CH_3$), 2.31 (s, 6H, $2CH_3$). Anal. Calcd. For $C_{44}H_{42}N_4O_8S_2Se_2$ (978.08): C, 54.10; H, 4.33; N, 5.74. Found: C, 54.00; H, 4.29; N, 5.70.

2,2'-((diselanediylbis(naphthalene-4,1-diyl))bis(acetylazanediyl))bis(2-(4-nitrophenyl)-N-(tosylmethyl)acetamide) (**18**)
Orange crystals; yield 75%; mp 280-282 ºC (ethanol); R_f =0.12[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3371 (NH), 2931 (C-H), 1599 (C=O), 1518 (N=O), 1341 (N-O), 1262 (C-N), 758 (Se-Se), 685 (C-S-C), 514 (Se-C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (s, 2H, 2NH), 8.82 (d, *J* = 12.9 Hz, 6H, Ar-H), 8.47-8.04 (m, 8H, Ar-H), 7.80-7.74 (m, 4H, Ar-H), 7.71-7.27 (m, 6H, Ar-H), 7.13-7.09 (m, 7.6 Hz, 4H, Ar-H), 5.15 (s, 2H, 2CH), 4.70 (s, 4H, $2CH_2$), 2.32 (s, 6H, $2CH_3$), 2.25 (s, 6H, $2CH_3$). Anal. Calcd. For $C_{56}H_{48}N_6O_{12}S_2Se_2$ (1220.11): C, 55.17; H, 3.97; N, 6.89. Found: C, 55.25; H, 4.00; N, 7.00.

General procedure for the preparation of maleanilic 19 and glutaranilic 22 acids
To a stirring solution of anhydride (2 mmol) in dry acetone (10 mL), 4,4'-di(1-aminonaphthyl)-diselenide (**2**) (0.44 g, 1.00 mmol) was added at room temperature. The mixture was vigorously refluxed for 3 h. The reaction mixture was poured into ice water and the separated product was filtered, dried and recrystallized from ethanol.

(2Z,2'Z)-4,4'-((Diselanediylbis(naphthalene-4,1-diyl))bis(azanediyl))bis(4-oxobut-2-enoic acid) (**19**)
Brown crystals; yield 66%; mp 205-207 ºC (ethanol); R_f = 0.54 [pet. ether /ethyl acetate (4:3)]; IR (KBr): υ_{max}. cm^{-1}: 3226 (OH), 3039 (NH), 2864 (C-H), 1713 (C=O), 1261 (C-N) , 756 (Se-Se), 487 (Se-C); EIMS *m/z* (%) 639 (M^+, 16.5), 640 (M^++1, 14.25); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.58 (s, 2H, 2COOH), 8.51-8.04 (m, 4H, Ar-H), 8.01-7.80 (m, 4H, Ar-H), 7.54-7.50 (m, 4H, Ar-H), 6.75 (d, *J* = 11.7 Hz, 2H, 2CH), 6.39 (d, *J* = 12.2 Hz, 2H, 2CH); ^{13}C NMR (75 MHz, DMSO) δ 166.83, 164.30, 134.86, 134.01, 133.83, 132.56, 128.15, 127.95, 127.01, 126.38, 123.60, 121.15, 120.79, 106.93; Anal. Calcd. For $C_{28}H_{20}N_2O_6Se_2$ (639.97): C, 52.68; H, 3.16; N, 4.39. Found: C, 52.56; H, 3.27; N, 4.45.

5,5'-((Diselanediylbis(naphthalene-4,1-diyl))bis(azanediyl))bis(5-oxopentanoic acid) (**22**)
Yellow crystals; yield 67%; mp 203-205 ºC (ethanol); R_f = 0.55 [pet. ether /ethyl acetate (4:3)]; IR (KBr): υ_{max}. cm^{-1}: 3442 (OH), 3262 (NH), 2855 (C-H), 1701 (C=O), 1249 (C-N) , 753 (Se-Se), 487 (Se-C); EIMS *m/z* (%) 672 (M^+, 79.41), 673 (M^++1, 50.98); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.02 (s, 2H, 2COOH), 8.13 (dd, *J* = 13.2, 8.3 Hz, 2H, Ar-H), 7.82 (d, *J* = 7.8 Hz, 4H, Ar-H), 7.72 – 7.34 (m, 4H, Ar-H), 7.02-6.41 (m, 2H, Ar-H), 2.36 (t, *J*=7.4 Hz, 8H, $4CH_2$), 1.92 (p, *J*=7.0 Hz, 4H, $2CH_2$); ^{13}C NMR (75 MHz, DMSO) δ 174.23, 171.58, 149.47, 135.69, 134.24, 133.88, 128.05, 128.01, 126.82, 126.16, 125.45, 123.41, 120.93, 33.16, 20.66; Anal. Calcd. For $C_{30}H_{28}N_2O_6Se_2$ (672.03): C, 53.74; H, 4.21; N, 4.18. Found: C, 53.64; H, 4.35; N, 4.38.

General procedure for the preparation of ethyl ester derivatives 20 and 23
To a solution of the corresponding acid (0.1 mmol) in ethanol (10 ml), conc. H_2SO_4 (200 µl) was added and the mixture was stirred at room temperature for 6 h. The mixture was poured into ice water and the separated solid was recrystallized from ethanol.

(2Z,2'Z)-Diethyl-4,4'-((diselanediylbis (naphthalene-4,1-diyl))bis(azanediyl))bis(4-oxobut-2-enoate) (**20**)
Brown crystals; yield 69%; mp 210-212 ºC (ethanol); R_f =0.4[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3063 (NH), 2924 (C-H), 1702 (C=O), 1665 (C=O), 1255 (C-O), 1218 (C-N), 754 (Se-Se), 484 (Se-C); EIMS *m/z* (%) 696 (M^+, 3.59); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36-7.95 (m, 2H, Ar-H), 7.78-7.65 (m, 4H, Ar-H), 7.63-7.23 (m, 4H, Ar-H), 6.96-6.62 (m, 2H, Ar-H), 6.56 (dd, *J* = 7.9, 0.8 Hz, 2H, 2CH), 6.36-6.21 (m, 2H, 2CH), 3.75 (q, *J* = 7.1, 0.9 Hz, 4H, $2CH_2$), 1.11 (t, *J* = 7.1, 0.9 Hz, 6H, $2CH_3$). Anal. Calcd. For $C_{32}H_{28}N_2O_6Se_2$ (696.03): C, 55.34; H, 4.06; N, 4.03. Found: C, 55.44; H, 4.17; N, 4.12.

Diethyl-5,5'-((Diselanediylbis(naphthalene-4,1-diyl))bis(azanediyl))bis(5-oxopentanoate) (**23**)
Brown crystals; yield 61%; mp 216-218 ºC (ethanol); R_f =0.13[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 3063(NH), 1745 (C=O), 1665 (C=O), 1218 (C-N), 1182 (C-O), 715 (Se-Se), 528 (Se-C); EIMS *m/z* (%) 728 (M^+, 4.59); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.56-7.96 (m, 6H, Ar-H), 7.48-6.98 (m, 6H, Ar-H), 4.12 (q, *J* = 7.3, 0.78 Hz, 4H, $2CH_2$), 2.21

(m, 8H, $4CH_2$), 2.01 (m, 4H, $2CH_2$), 1.23 (t, J = 7.3, 0.8 Hz, 6H, $2CH_3$). Anal. Calcd. For $C_{34}H_{36}N_2O_6Se_2$ (728.09): C, 56.20; H, 4.99; N, 3.86. Found: C, 56.37; H, 4.82; N, 3.66.

General procedure for the preparation of cyclic imides 21, 24 and 25

A mixture of appropriate acid (0.1 mmol), freshly fused sodium acetate (100 mg) and acetic anhydride (5 mL) was heated for 2 h at 55 °C. The reaction was cooled and quenched with ice water and the separated solid was recrystallized from ethanol.

1,1'-(Diselanediylbis(naphthalene-4,1-diyl))bis(1H-pyrrole-2,5-dione) (21)

Brown crystals; yield 61%; mp 202-204 °C (ethanol); R_f =0.13[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 1715 (C=O), 1255 (C-N), 758 (Se-Se), 457 (Se-C); EIMS *m/z* (%) 603 (M^+, 37.71), 604 (M^++1, 22.03); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29-7.84 (m, 6H, Ar-H), 7.83-7.33 (m, 4H, Ar-H), 7.21 (d, J = 12.9 Hz, 2H, Ar-H), 6.63 (d, J = 11.4 Hz, 2H, 2CH), 6.36 (d, J = 11.6 Hz, 2H, 2CH); Anal. Calcd. For $C_{28}H_{16}N_2O_4Se_2$ (603.94): C, 55.83; H, 2.68; N, 4.65. Found: C, 55.68; H, 2.78; N, 4.58.

1,1'-(Diselanediylbis(naphthalene-4,1-diyl))bis(piperidine-2,6-dione) (24)

Yellow crystals; yield 63%; mp 206-208 °C (ethanol); R_f =0.12[pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}= 1685 (C=O),1247 (C-N) , 757 (Se-Se), 444 (Se-C); EIMS *m/z* (%) 636 (M^+, 52.82); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 -7.95 (m, 2H, Ar-H), 7.75 (d, J = 7.7 Hz, 4H, Ar-H), 7.42 (dd, J = 69.4, 7.3 Hz, 4H, Ar-H), 7.06-6.36 (m, 2H, Ar-H), 2.88 (dt, J = 35.9, 14.3 Hz, 8H, $4CH_2$), 2.38-1.88 (m, 4H, $2CH_2$). Anal. Calcd. For $C_{30}H_{24}N_2O_4Se_2$ (636.01): C, 56.79; H, 3.81; N, 4.42. Found: C, 56.89; H, 3.71; N, 4.22.

2,2'-(Diselanediylbis(naphthalene-4,1-diyl))bis(isoindoline-1,3-dione) (25)

Brown crystals; yield 80%; mp 203-205 °C (ethanol); R_f = 0.13 [pet. ether /ethyl acetate (4:2)]; IR (KBr): υ_{max}. cm^{-1}: 2971 (C-H), 1602 (C=O), 1267 (C-N), 751 (Se-Se), 500 (Se-C); EIMS *m/z* (%) 703 (M^+, 17.5); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39-8.36 (m, 2H, Ar-H), 8.18-8.14 (m, 4H, Ar-H), 7.85-7.07 (m, 4H, Ar-H), 6.89-6.85 (m, 6H, Ar-H), 6.68 (d, J = 16.9 Hz, 2H, Ar-H), 6.57-6.54 (m, 2H, Ar-H).^{13}C NMR (75 MHz, DMSO) δ 163.05, 144.21, 135.48, 130.33, 129.69, 128.39, 128.34, 128.07, 127.86, 127.41, 125.34, 125.19, 125.14, 124.28, 120.76, 120.72, 114.17, 112.87. Anal. Calcd. for $C_{36}H_{20}N_2O_4Se_2$ (703.98): C, 61.55; H, 2.87; N, 3.99. Found: C, 61.43; H, 2.67; N, 3.87.

Biological assays

Cytotoxicity assay

Breast cancer cells (MCF-7) and human fibroblast cells (WI-38) were obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil were purchased from sigma co., St. Louis, USA. Fetal Bovine serum was purchased from GIBCO, UK. **C**ells were cultured in RPMI-1640 medium supplemented with 10% (v/v) calf serum (Hyclone Laboratories, Ogden, UT), 60 mg/mL penicillin G and 100 mg/mL streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 15% (v/v) CO_2 in air.

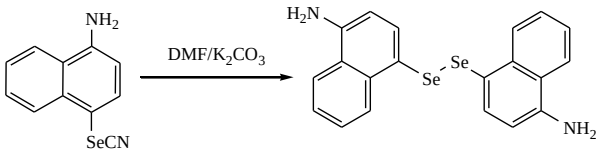
MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide] (Sigma) was used to measure the metabolic activity of cells. Briefly, 120 µL aliquots of a cell suspension (50,000 cells mL^{-1}) in 96-well microplates were incubated at 37 °C and 10% CO_2 and allowed to grow for two days. Then 60 µL of serial dilutions of the test compounds were added. After 48h of incubation at 37 °C and 10% CO_2, 75 µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h the precipitate of formazan crystals was centrifuged and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL DMSO. The resulting colored solution was measured at 590 nm using an ELISA plate reader. All investigations were carried out in two parallel experiments. The IC_{50} values were determined as the concentrations of tested materials, which showed 50% of the absorbance of untreated control cells as estimated from the dose-response curves. 5-Fluorouracil (5-Fu) was used as a positive control.

Antimicrobial activity

Chemical compounds were tested against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterial pathogens as well as *Candida albicans* fungus (yeast) strain. Antimicrobial tests were carried out by the agar well diffusion method using 100 µL of suspension containing $1x10^8$ CFU/mL of pathological tested bacteria and $1x10^4$ spores/mL of fungi spread on nutrient agar (NA), and potato dextrose agar (PDA) medium respectively. After the media had cooled and solidified, paper discs of 6 mm diameter soaked with 20 µl of the test compounds (1mg/ml) were added to the agar plates and incubated at 30 °C. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard.

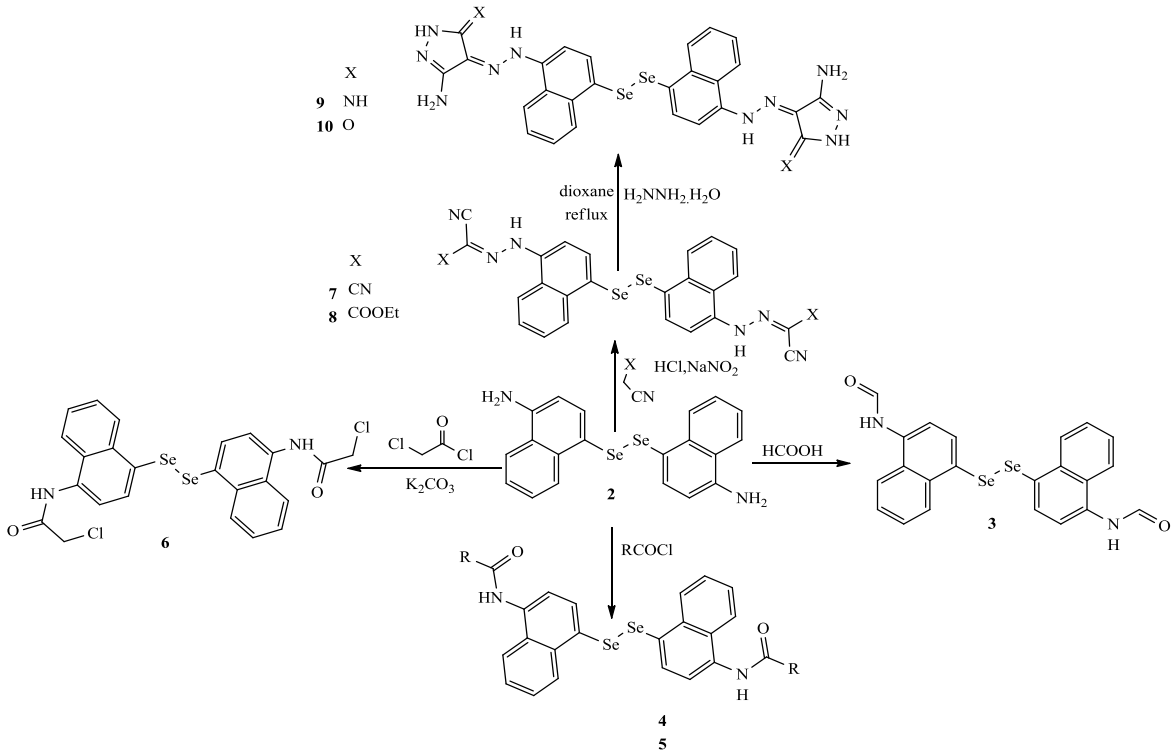
The antibacterial activity of a common standard antibiotic ampicillin and the antifungal coltrimazole were chosen as positive control using the same procedure as above at the same concentration. The relative (%) activity index was calculated as shown below:

% activity index= (inhibition zone of the test compounds/ inhibition zone of the standard drug) ×100.

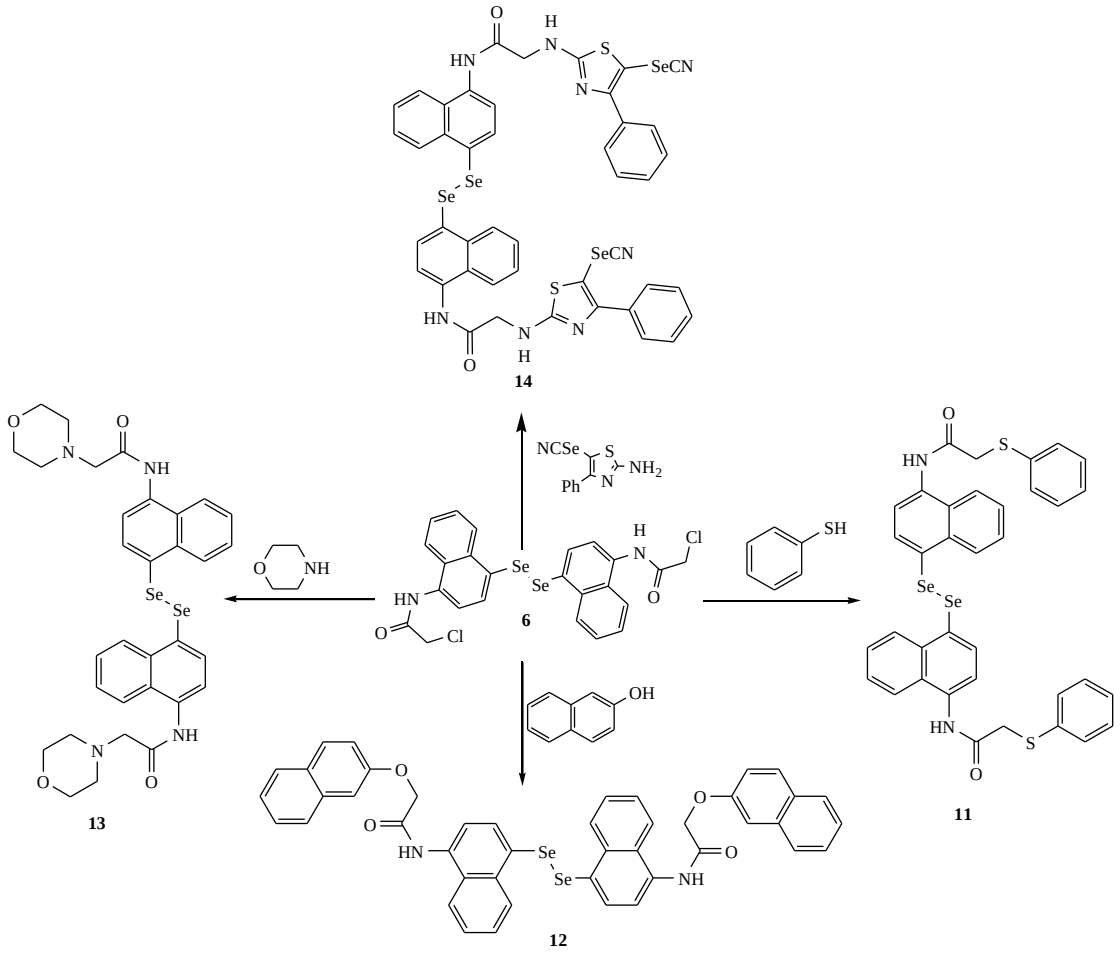


1 **2**

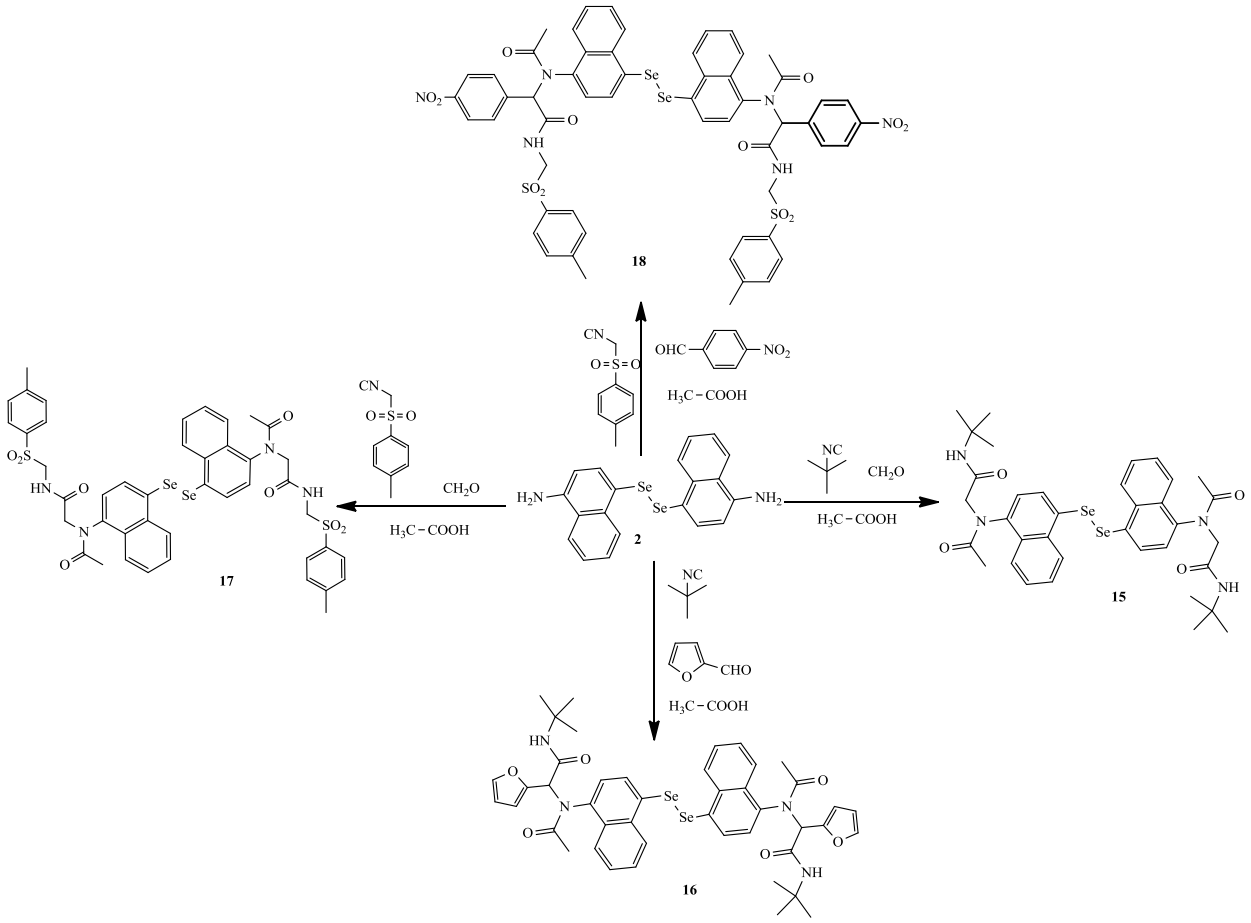
Scheme 1. Synthesis of 4-(2-(1-aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**) either by alkaline hydrolysis or by reduction with $NaBH_4$.



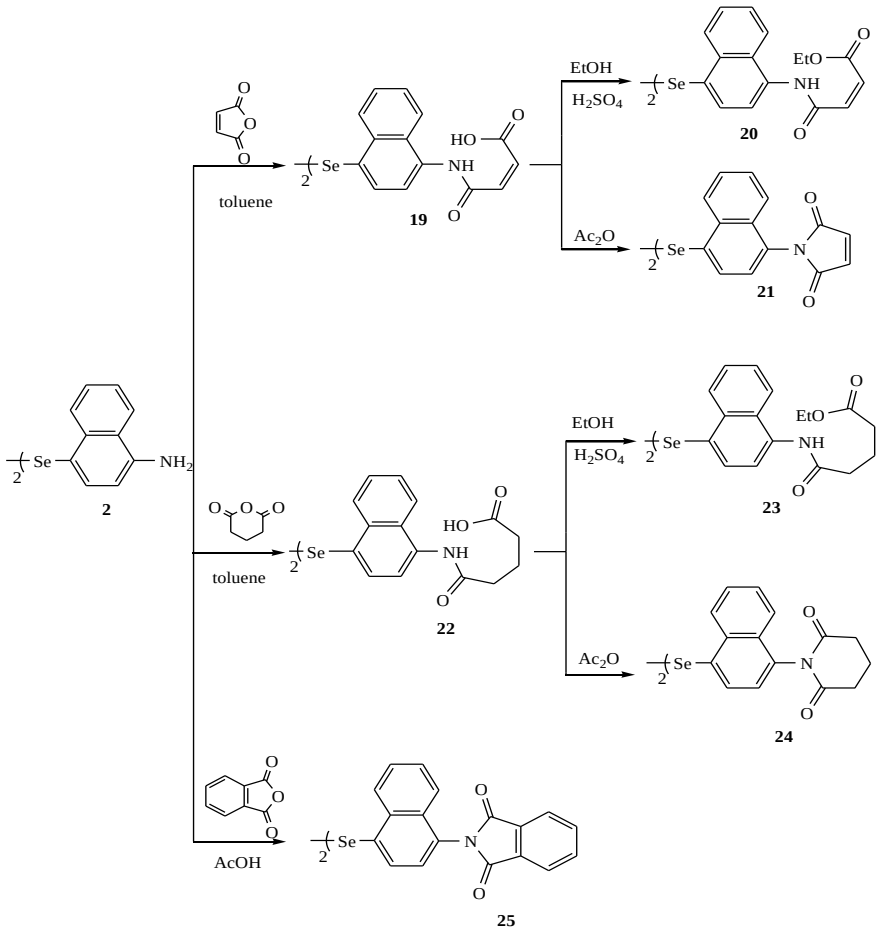
Scheme 2. Formylation, acylation, benzoylation and azo coupling reactions of 4-(2-(1-aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**).



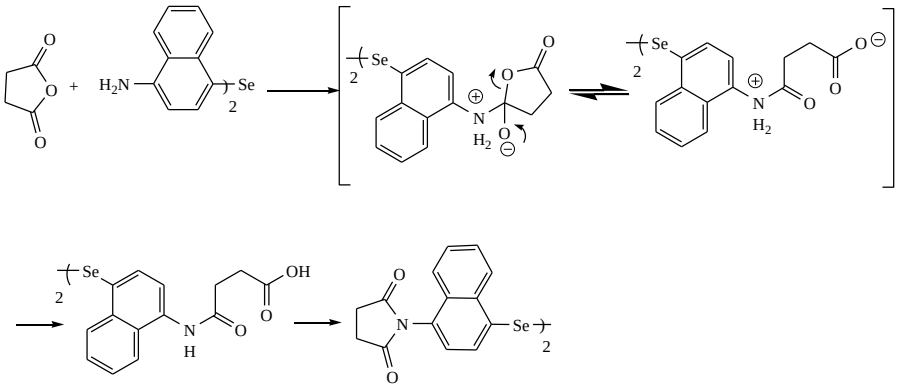
Scheme 3. Nucleophilic displacement reactions of α-chloroacetamide **6**.



Scheme 4. Ugi four component synthesis of functionalized diseleno-peptidomimetic compounds.



Scheme 5. Synthesis of diseleno *N*-amido-acids, *N*-amido-ethyl ester and cyclic imides using 4-(2-(1-aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**).



Scheme 6. Synthesis of diseleno *N*-amido-acids, *N*-amido-ethyl ester and cyclic imides using 4-(2-(1-aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**).

RESULTS & DISCUSSIONS

Chemistry:

To the best of our knowledge, the amine key start 4-(2-(1-aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**) was not synthesized before and we herein report and evaluate its reactivity employing the characteristic reactions of primary aromatic amine (e.g., formylation, acylation and azo coupling as well as Ugi MCR).

4-(2-(1-Aminonaphthalen-4-yl)diselanyl)naphthalen-1-amine (**2**) is synthesized from 4-selenocyanatonaphthalen-1-amine (**1**) either by alkaline hydrolysis (e.g., K_2CO_3 in DMF) or by reduction using alkali metal hydrides (e.g., sodium borohydride ($NaBH_4$)) (Scheme 1).

Formylation, acylation and benzoylation of amines are the most elaborated protocols in synthetic organic and medicinal chemistry. The resulting *N*-formyl-, *N*-acyl and *N*-benzoyl derivatives are considered amino-protecting groups and they are widely used as intermediates in the synthesis of many interesting biological and pharmaceutical compounds (e.g., amino acids, fluoroquinolines) as well as important synthetic reagents (e.g., Vilsmeier).[27-30]

Based on these observations, the reactivity of **2** was preliminary explored *via* reaction with formic acid, acetic anhydride, acyl/aryl chlorides (e.g., benzoyl chloride and chloroacetyl chloride) and azo coupling (Scheme 2).

N-Formylation was performed by the reaction of **2** with formic acid at 50 ºC to give the corresponding formamide **3** in excellent yield (82%). Furthermore, *N*-acetyl **4** and *N*-benzoyl **5** derivatives were also obtained in good yields (up to 73%) by the reaction with acetic anhydride and benzoyl chloride, respectively. Moreover, α-chloroacetamide **6** was also synthesized in 85% yield by reaction with chloroacetyl chloride in dry acetone and anhydrous potassium carbonate.

On the other hand, the diazo derivatives **7** and **8** were obtained in moderate yields (up to 64%) by coupling of the corresponding diazonium salt of **2,** prepared *in situ* at 0-5 ºC using hydrochloric acid and sodium nitrite, with active methylene compounds (*e.g.*, malononitrile and ethyl cyanoacetate) in sodium acetate buffered solution.

The structures of **7** and **8** were confirmed on the basis of their spectral data. The IR spectra of **7** showed a characteristic absorption band of conjugated CN group at 2261 cm^{-1}.

On the other hand, the IR spectra of **8** showed characteristic absorption bands of a conjugated CN group at 2246 cm^{-1} and an ester absorption band at 1619 cm^{-1}. In the ^{1}H-NMR spectra, the ethyl ester signal of **8** was found at 4.33 ppm as a quartet and at 1.06 ppm as a triplet. The spectra for product **7** and **8** are given in the Experimental section.

The diazo compounds **7** and **8** were further used as building blocks for the synthesis of pyrazole selenaheterocycles **9** and **10** *via* reaction with hydrazine in ethanol (Scheme 2). The IR spectra of **10** show the absence of the characteristic absorption band of the conjugated CN group.

The α-chloroacetamide **6** was used for further derivatisation *via* nucleophilic displacement of the chloride by phenols (e.g., α-naphthol), thiophenol, amines (e.g., morpholine, 4-phenyl-5-selenocyanatothiazol-2-amine). The corresponding phenylthio- **11**, naphthalenyloxy- **12**, morpholino- **13** and thiazol-2-ylamino- **14** derivatives were obtained in good-moderate yields (up to 71%). The synthetic strategies adopted for the synthesis of the amide containing heterocyclic rings are depicted in Scheme **3**.

Preliminary experiments made to investigate the reactivity of **2** revealed a typical reactivity of primary aromatic amines. This promoted us to further investigate the reactivity of **2** in Ugi four component reaction (U4CR) which *in turn* would give access to highly functionalized dinapthyldiseleno-peptidomimetic scaffolds (Scheme 4).

The U4CR comprises the one pot synthesis of α-aminoacyl amides from an amine, aldehyde, acid and isonitrile.[31, 32] As the starting carbonyl components, we chose commercially available aldehydes including aliphatic (paraformaldehyde) and aromatic (4-nitrobenzaldehyde) as well as heteroaromatic (furfuraldehyde) aldehydes. For the library validation, *tert*-butylisocyanide and toluenesulfonylmethyl isocyanide (TosMIC) were chosen because of their commercial availability and high reactivity; whereas acetic acid was used as the acid component (Scheme 4). The U-4CR sequence was initiated by the one pot addition of aldehyde to a methanolic solution of **2** followed by the subsequent addition of carboxylic acid and isocyanide. Functionalized diseleno-peptidomimetic scaffolds **15**, **16**, **17** and **18** were obtained in good yields (up to 75%).

Our efforts were then directed to the synthesis of cyclic imides which *in turn* have recently received much attention in drug discovery. These compounds constitute an integral part of various therapeutically and biologically relevant compounds (*e.g.*, the natural alkaloid rebeccamycin, thalidomide, chlorophthalim, isogranulatimide) and many of them are used as antioxidants, neuroprotectives, nootropics, anxiolytics, antinociceptives and antidepressants. Furthermore, their hydrophobic and

neutral nature enables them to be easily taken up by cells (*in vivo*).[33-36]

The reactions of maleic and glutaric anhydrides with **2** in refluxing acetone afforded the corresponding *N*-substituted maleanilic **19** and *N*-substituted glutaranilic **22** acids in moderate yields (up to 67%). Acid-catalysed esterification of the resulting *N*-substituted monoamidic acids afforded the corresponding ethyl ester **20** and **23**, respectively. Furthermore, cyclic imides **21** and **24** were also obtained by dehydration and subsequent ring-closure of the monoamidic acids **19** and **22** up on gentle heating with acetic anhydride and sodium acetate (Scheme 5). The reaction was accomplished in 30 minutes and the products were isolated by ice-water precipitation.

On the other hand, reaction of **2** with phthalic anhydride afforded the corresponding phthaloly derivative **25** in a good yield (80 %). The present reaction might proceed according to the mechanism depicted in Scheme 6.

Cytotoxic activity of compounds in breast cancer cells (MCF-7) and normal cells (WI-38)

Recently, chemotherapy is suffering from a slim therapeutic index, with significant toxicity from effective drug doses or tumor recurrence.[5,37] Consequently, searching for new anticancer agents with lower toxicity to normal cells is of particular interest. A new organoselenium compounds was therefore developed in an attempt to obtain compounds with superior chemotherapeutic index in terms of increased selectivity, higher cytotoxicity and lower side effects than currently known chemotherapeutic drugs (e.g., 5-fu).

The cytotoxic potency of the synthesized compounds was evaluated in breast adenocarcinoma (MCF-7) and compared with their cytotoxicity in normal fibroblast cells (WI-38) employing the standard MTT assay. The IC_{50} values were estimated from the respective dose response curves and the therapeutic index (TI) = IC_{50} non-neoplastic cell line (WI-38)/IC_{50} neoplastic cell line (MCF-7) (Table 1).

Interestingly, the substitution pattern plays a distinct role in the activity of the diselenides, showing that it is not general selenium cytotoxicity. The compounds under investigation could be divided into two classes: 1) cytotoxic compounds (**2**, **9**, **10**, **12**, **19** and **20**) *i.e.*, compounds able to reduce the viability of MCF-7 tumor cells and 2) compounds with mid-low cytotoxicity.*

TI provides a simple index for evaluating the safety and efficacy of a drug and defined as the ratio of the drug concentration that inhibits 50% viability of the normal cells to the concentration that inhibits 50% viability of tumor cells. Compounds with high TI are more selective and often preferred, as they will be more effective in killing cancer cells at a lower concentration than those with lower TI.

Interestingly, significant difference in toxicity zones between breast solid tumor cells and normal WI-38 cells was noticed. In this context, the TI values of cytotoxic compounds **2**, **9**, **10**, **12**, **19** and **20** were ranging from four to eleven fold therapeutic indices in killing MCF-7 cells relative to WI-38 normal cells. These values were even higher than that of 5-fu suggesting their effectiveness as anti-cancer agents. A basic SAR shows a correlation between chemical structures and the cytotoxic/selective activities. *N*-substituted maleanilic **19** and its corresponding ethyl ester **20,** pyrazole selenoheterocycles **9** and **10** and 2-(naphthalen-2-yloxy)-N-(naphthalen-5-yl)acetamide **12** were among the most potent cytotoxic compounds.

Contrary to our expectations, peptidomimetic compounds **15**, **16, 17** and **18** showed low cytotoxicity. The degree of bulkiness besides their comparable high molecular weight and large molecular volumes may affect their action on various levels i.e., cell penetration and localization or even at the receptors/enzymes binding sites.

To this end, the selectivity of these compounds is not solely limited to the cell lines used and these initial results need further investigations using a wider arsenal of cancer and normal cells. Furthermore, we are fully aware that these findings raise wealth of more questions. For example, what are the possible applications and the corresponding pharmacological and pharmacokinetic properties of such compound?

Antimicrobial evaluation

To study the cytotoxic activity beyond a human cell line we also studied the effect on lower organisms i.e. fungi and bacteria. Thus the antimicrobial activity of the compounds was evaluated against gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Staphylococcus aureus* (*S. aureus*) as well as against the pathogenic fungus *Candida albicans* (*C. albicans*). A standard agar diffusion assay was used and the diameters [mm] of inhibition zones are summarized in Table 2.

In general, most compounds exhibited toxicity against gram-negative (*E. coli*) bacteria, more than the gram-positive (*S. aureus*) bacteria. In this context, compounds **2, 8, 9, 10, 12, 19** and **20** were the most active compounds against *E. coli* with 50-83 relative activity (compared to the known drug, ampicillin). The same holds true in the case of *C. albicans*, compounds **2, 7, 8** and **10** exhibited 50-82 relative activity compared to the antifungal drug

* The cytotoxicity threshold is at $IC_{50} \leq 23$ μM.

colitrimazole. These initial promising results point toward a reasonably activity of some of these compounds, which needs to be further investigated by using a considerably wider arsenal humanopathogenic bacteria and fungi.

Table 1 Influence of the synthesized compounds on the viability of MCF-7 and WI-38 cells and their corresponding therapeutic windows.[a]

Compd. No.	*In vitro* Cytotoxicity IC_{50} (µM)		TI
	MCF-7	WI-38	
5-FU	8±0.13	4±0.63	0.50
2	12±0.18	[b]	8
3	35±2.07	82±4.06	2
5	[b]	[b]	-
6	58±3.11	73±3.32	1
7	38±1.22	78±2.64	2
8	31±1.37	[b]	3
9	15±0.36	[b]	7
10	13±0.68	[b]	8
11	[b]	[b]	-
12	10±0.17	100±0.27	10
13	[b]	[b]	-
14	[b]	[b]	-
15	[b]	[b]	-
16	[b]	[b]	-
17	[b]	[b]	-
18	[b]	[b]	-
19	19±0.24	89±3.87	5
20	9±0.15	[b]	11
21	[b]	[b]	-
24	[b]	[b]	-
25	48±2.41	96±2.11	2

[a)]The metabolic activity of the cells was measured after 48h of incubation with different concentrations of the investigated compounds by means of an MTT assay. The IC_{50} was determined from the dose-response curves as the mean of three parallel experiments; therapeutic index (TI) is the ratio of the IC_{50} normal cells (WI-38) to the IC_{50} breast cancer cells (MCF-7); 5-fluorouracil (5-Fu) was used as a positive control; [b)] no growth inhibition was recorded.

Table 2. Diameters (in mm) of inhibition zones of agar diffusion assays against a variety of fungi and bacteria (growth was quantified after 2 days).[a]

Compd. No.	Diameter inhibition zone in mm (% activity index)[a]		
	E. coli	*S. aureus*	*C. albicans*
2	20 (83)	15(68)	23(82)
3	9(38)	[b]	11(39)
4	[b]	[b]	[b]
5	[b]	[b]	[b]
6	[b]	[b]	[b]
7	9(38)	11(50)	15(54)
8	12(50)	11(50)	16(57)
9	13(54)	10(45)	8(29)
10	17(71)	15(68)	20(71)
11	[b]	[b]	[b]
12	14(58)	17(77)	11(39)
13	[b]	[b]	7(25)
14	[b]	[b]	[b]
15	[b]	[b]	[b]
19	14(58)	10(45)	[b]
20	16(67)	9(41)	[b]
21	[b]	[b]	[b]
24	[b]	[b]	[b]
25	7(29)	[b]	[b]
Ampicillin	24 (100)	22 (100)	[b]
Colitrimazole	[b]	[b]	28 (100)

[a] Diameters (mm) of zones of inhibition (agar diffusion assay) are provided. In each case, 6 mm disks with 20 µg of the test compounds were incubated. Ampicillin and colitrimazole were used as the positive control. [b]Values below 6 mm (25 %) are of limited value as they refer either to inactive or non-diffusing compounds.

CONCLUSION

The synthesis of a novel series of naphthalene based-symmetrical diselenides was described. Most of the compounds were easily prepared in one step and in good-moderate yields. The cytotoxicity of the compounds was evaluated against breast adenocarcinoma (MCF-7) and compared with their cytotoxicity in normal fibroblast cells (WI-38) employing standard MTT assay.

Compounds under investigation were divided into cytotoxic and non-cytotoxic compounds showing that it is not general selenium cytotoxicity. Compounds **2**, **9, 10**, **12**, **19**, and **20** showed TI values ranging from four to eleven fold therapeutic indices in killing MCF-7 cells compared to WI-38 normal cells. These values were even higher than the TI of 5-fu suggesting their effectiveness as anti-cancer agents. Furthermore, the cytotoxicity beyond human cell lines were also studied using *E. coli* (gram-negative) and *S. aureus* (gram-positive) bacteria as well as *C. albicans* (pathogenic fungi) using the standard agar diffusion assay. Interestingly, compounds **2**, **9, 10**, **12**, **19**, and **20** were also the most toxic against *E. coli* with 50-83 relative activity (compared to the known drug, ampicillin). The same holds true in the case of *C. albicans*, compounds **2, 7, 8** and **10** exhibited 50-82 relative activity compared to the antifungal drug colitrimazole.

We are fully aware that a clear QSAR will require diverse sets of compounds including sulfur and

tellurium-containing analogues, to screen for further activities and selectivity. Therefore, in order to derive reliable structure–activity relationships and to obtain a better understanding of the mode(s) of action, this library should be expanded to include wider diselenides functionalities as well as structural variants. While it might appear that these compounds are not fantastic in their activity, there is enough evidence to suggest that further study is warranted and this justifies the realization of more in-depth studies and additional experiments to investigate the exact mode(s) of action responsible for the pronounced biological activity apparently exhibited by this compound and to identify possible intracellular targets (such as specific organelles, membranes or proteins)**.**

Eventually, these findings raise wealth of more questions. For example, what are the possible applications and the corresponding pharmacological and pharmacokinetic properties of such compound?

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REFERENCES:

1. Harford JB, Edwards BK, Nandakumar A, Ndom P, Capocaccia R, Coleman M P. Tumori, 2009; 95: 568-578.
2. Gewefel H, Salhia B. Clinic Breast Canc, 2014; 6: 390-395.
3. Bosch A, Eroles P, Zaragoza R, Vina JR, Lluch A. Canc Treat Rev, 2010; 36: 206-215.
4. Goldie JH. Canc Metastasis Rev, 2001; 20: 63-68.
5. Trachootham D, Alexandre J, Huang P. Nat Rev Drug Discov, 2009; 8: 579-591.
6. Fang J, Nakamura H, Iyer AK. J Drug Target, 2007; 15: 475-486.
7. Noord PAV, Maas MJ, Tweel IVD, Collette C. Breast Canc Res Treat, 1993; 25: 11-19.
8. Thompson I, Kristal A, Platz EA. Am Soc Clin Oncol Educ Book, 2014; e76-80.
9. Martinez EE, Darke AK, Tangen CM, Goodman PJ, Fowke JH, Klein EA, Abdulkadir SA. Cancer Prev Res (Phila), 2014; 7: 950-957.
10. Shabaan S, Negm A, Sobh MA, Wessjohann LA. Eur J Med Chem, 2015; 97: 190-201.
11. Shabaan S, Gaffer HE, Alshahd M, Elmorsy SS. Int J Res Devel Pharm & Life Sci, 2015(In press, accepted Manuscript)
12. Shabaan S, Abdel-Wahaba BF. Mol Divers, 2015 (In Press, Accepted Manuscript) doi: 10.1007/s11030-015-9602-61
13. Shabaan S, Ba LA, Abbas M, Burkholz T, Denkert A, Gohr A, Wessjohann LA, Sasse F, Weber W, Jacob C, Chem Commun (Camb), 2009; (31): 4702-4704.
14. Mecklenburg S, Shaaban S, Ba LA, Burkholz T, Schneider T, Diesel B, Kiemer AK, Röseler A, Becker K, Reichrath J, Stark A, Tilgen W, Abbas M, Wessjohann LA, Sasse F, Jacob C. Organ Biomol Chem, 2009; 7: 4753-4762.
15. Shaaban S, Diestel R, Hinkelmann B, Muthukumar Y, Verma RP, Sasse F, Jacob C, Eur J Med Chem, 2012; 58: 192-205.
16. Shaaban S, Sasse F, Burkholz T, Jacob C, Bioorg Med Chem, 2014; 22: 3610-3619.
17. Shaaban S, Arafat MA, Gaffer HE, Hamama WS, Der Pharma Chemica 2014; 6: 186-193.
18. Elmorsy SS, Shaaban S, Eldesoky F, Kandeel E. Int J Org Chem, 2015 (Accepted Manuscript)
19. Tandon VK, Maurya HK, Mishra NN, Shukla PK, Eur J Med Chem, 2009; 44: 3130-3137.
20. Datta J. J Indian Chem Soc, 1952; 29: 394-396.
21. Shaaban S, Negm A, Ibrahim EE, Elrazak AA, Oncology Reviews, 2014; 8: 25-35.
22. Abdel-Wahab BF, Shaaban S, Synthesis, 2014; 46: 1709-1716.
23. Shaaban S, Arafat MA, Hamama WS, ARKIVOC, 2014, i, 470-505.
24. Metwally MA, Shaaban S, Abdel-Wahab BF, El-Hiti GA, Curr Org Chem, 2009; 13: 1475-1496.
25. Hamama WS, Ismail MA, Shaaban S, Zoorob HH, J Heterocycl Chem, 2008; 45: 939-956.
26. Kachanov VA, Slabko YO, Baranova VO, Shilova VE, Kaminskii AV, Tetrahedron letters, 2004; 45: 4461-4463.
27. Gerack C J, McElwee-White L, Molecules, 2014; 19: 7689-7713.
28. Aleiwi BA, Mitachi K, Kurosu M, Tetrahedron Lett, 2013; 54: 2077-2081.
29. Ortega N, Richter C, Glorius F. Org Lett, 2013; 15: 1776-1779.
30. Suchy M, Elmehriki AA, Hudson RH, Org. Lett, 2011; 13: 3952-3955.
31. Domling A. Chem Rev, 2006; 106: 17-89.
32. Domling A. Curr Opin Chem Biol, 2002; 6: 306-313.

33. Brauch S, Henze M, Osswald B, Naumann K, Wessjohann LA, Berkel SSV, Westermann B. Org Biomol Chem, 2012; 10: 958-965.
34. Bruning CA, Prigol M, Luchese C, Pinton S, Nogueira CW, Prog Neuropsychopharmacol Biol Psychiatry, 2012; 38: 168-174.
35. Smith G, Wermuth UD. Acta Crystallogr C, 2012; 68: 253-6.
36. Maaninen T, Chivers T, Laitinen R, Schatte G, Nissinen M. Inorg Chem, 2000; 39: 5341-5347.
37. Giles NM, Giles GI, Holley JE, Gutowski NJ, Jacob C. Biochem Pharmacol, 2003; 66: 2021-2028.