International Journal of Research and Development in Pharmacy and Life Sciences

Available online at http//www.ijrdpl.com
June - July, 2015, Vol. 4, No.4, pp 1654-1668
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

CYTOTOXIC SYMMETRICAL THIAZOLEDISELENIDES WITH INCREASED SELECTIVITY AGAINST MCF-7 BREAST CANCER CELLS

Saad Shaaban[1*] Hatem E. Gaffer[2], Mohannad Alshahd[1], and Saad S. Elmorsy[1]

1. Department of Chemistry, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt.
2. Textile Research Division, National Research Centre, Dokki, Cairo, Egypt.

***Corresponding Author: Email** dr_saad_chem@mans.edu.eg

(Received: April 24, 2015; **Accepted:** May 18, 2015**)**

ABSTRACT

A novel series of symmetrical thiazolediselenides were synthesized in good to moderate yields and there in vitro cytotoxic activity was evaluated against breast adenocarcinom (MCF-7) and compared with their cytotoxicity in normal fibroblast cells (WI-38) employing standard MTT assay. Additionally, there in vitro antimicrobial activities were also evaluated against gram-negative (Escherichia coli), gram-positive (Staphylococcus aureus) bacteria and a pathogenic yeast (Candida albicans). A significant difference in toxicity zones between breast solid tumor cells and normal WI-38 cells was observed indicating that it is not general selenium toxicity. Within this context, compounds **4b**, **5**, **7**, **18** and **23** exhibited therapeutic indices (TI) up to eleven fold and in most cases were higher than TI of 5-fu suggesting their effectiveness as anti-cancer agents. On the other hand, compounds **4a**, **5**, **7**, **18** and **22** exhibited good antibacterial activity against E. coli and S. aureus bacteria compared to the known drug, ampicillin. Moreover, compounds **4a**, **7**, **11**, **13**, **19**, **22** and **23** exhibited good antifungal activity against C. albicans compared to colitrimazole. These initial promising results point to a reasonable activity of some of these compounds, which needs to be further investigated by using a considerably wider arsenal of human cancer and normal cells as well as humanopathogenic bacteria and fungi.

Keywords: Breast cancer/ Diselenides/Organoselenium/ selectivity/ Thiazole.

INTRODUCTION

Breast cancer has arisen as a global health problem being the second most prevalent solid tumor in the world and, by far the most frequent invasive malignancy in females. It accounts for approximately 1.7 million new cases and half million deaths per year, mostly in Europe [1, 2]. Given such high morbidity and mortality, there continues to be an unmet need for more effective and less cytotoxic therapies. Despite the significant advances in the understanding and diagnosis of breast cancer solid tumors, the available treatment options are far from satisfactory and till today there is no clear, proven and effective single agent that constitutes a systemic regimen recommended for treatment. This is mainly due to limited therapeutic selectivity and drug resistance which constitute major challenges in solid tumors drug discovery [3, 4]. Consequently, this necessitates the developing of more effective and less totoxic therapies to overcome drug resistance and to expand the available drug arsenal to battle the disease.

A growing body of evidences from epidemiologic studies suggests that selenium disorder is implicated with increased risk of many diseases, including cancers [5-7]. In this context, many organoselenium compounds have demonstrated significant chemopreventive activity in vitro as well as in human tumor xenografts [8, 9]. Beside the chemopreventive activities shown by organoselnium compounds, recent studies also showed the potential of organoselenium compounds as chemotherapeutic anticancer agents (Figure 1) [10]. These observations were supported by large numbers of preclinical and clinical studies intervention trials (e.g., SELECT trial in the USA and PRECISE trial in Europe) [11, 12]; however, these findings are still in need for further investigations and merit further research.

On the other hand, thiazole-containing compounds exhibit wide range of pharmacological activities and constitute a crucial part of many potent anticancer drugs such as epothilone (A and B) and tiazofurin (Figure 1). Some thiazoles were found to selectively inhibit the growth of various breast cancer cells in vitro and in xenograft human models at low micromolar concentrations. These compounds are able to inhibit the forkhead transcription factor (FoxM1) which is upregulated in breast cancer while not expressed in normal cells [13].

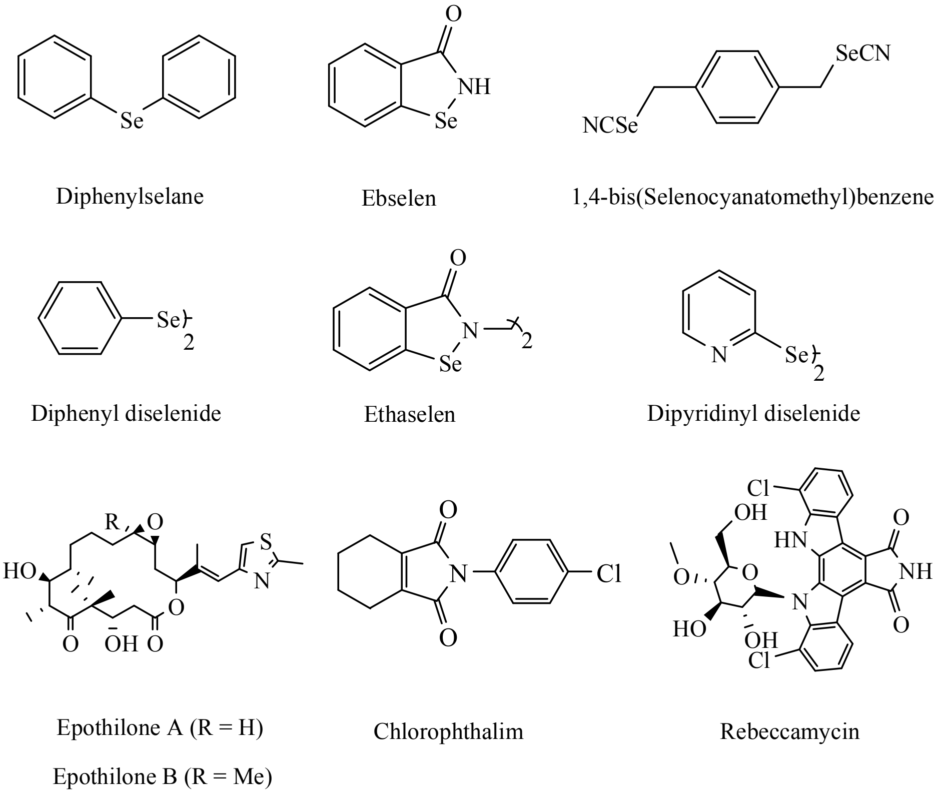

Diphenylselane
Ebselen
1,4-bis(Selenocyanatomethyl)benzene
Diphenyl diselenide
Ethaselen
Dipyridinyl diselenide
Epothilone A (R = H)
Epothilone B (R = Me)
Chlorophthalim
Rebeccamycin

Figure 1. Chemical structures of selected organoselenium, thiazole and cyclic imides of biological and pharmaceutical relevance.

In 2009, we have developed selenium-containing multifunctional redox agents, of which some showed considerable cytotoxicity, yet also selectively, against certain type of cancer cells and range of pathogenic microorganisms [14][15, 16]. Since then, we are involved in the development of novel cancer therapy based on organoselenium redox modulators and the exploration of their corresponding intracellular diagnostics.[17-20] In this regard, we have further developed selenium pseudopeptides with significant cytotoxicity at sub-micromolar concentrations against different cancer cell lines and lower 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 cell lines. In depth analysis of the underlying cytotoxic mechanism(s) revealed to be mainly due to apoptosis induction. These findings were confirmed via estimation of various cellular alterations (e.g., cell morphology and cell cycle arrest) and biochemical changes (e.g., ROS and GSH levels, caspase activity) in addition to hits obtained from chemogenomic assay [21, 22]. Although one can only speculate about the exact mode(s) of action of organoselenium compounds, their cytotoxic mechanisms depend on modulation of the intracellular redox environment.

Promoted by the above mentioned findings, it's likely that a combination of bioactive pharmacophores such as thiazoles with selenium will synergistically not only potentiate the overall cytotoxicity and redox activity but also enhance their corresponding chemotherapeutic properties. Furthermore, such novel combination is expected to have better physico-chemical properties and pharmacological properties. Thus our aim is to synthesize a new series of organoselenium compounds based on the thiazole chromophore. Their corresponding selective cytotoxicity will be evaluated using human breast cancer cells (MCF-7) and compared with their cytotoxicity in normal fibroblast cells (WI-38) employing standard MTT assay.

RESULTS & DISCUSSIONS

Chemistry

The key starting 5-(2-(2-amino-4-phenylthiazol-5-yl)diselanyl)-4-phenylthiazol-2-amine (**2**) used in this study was synthesized by modification of the literature method [23]. Alkaline hydrolysis using K_2CO_3 in DMF offers good yield and superior purity rather than reduction by sodium borohydride ($NaBH_4$).

Owing to the fact that 2-aminothiazoles usually exists in nature in two forms i.e. amino and imino tautomeric forms, it is also expected that 2-aminothiazolediselenide **2** would be acting as as an amine and/or amidine (Figure 2).

This in turn provides an excellent entry point for the construction of bridgehead-nitrogen heterocycles which constitute an important subclass of compounds because of their wide use in medicinal chemistry. Within this context, thiazolo[3,2-a]pyrimidines (**3a, 3b, 4a** and **4b**) were synthesized via one pot cyclocondensation of 2-aminothiazolediselenide 2 either with aromatic aldehydes and malononitrile/ethyl cyanoacetate or with the benzylidene derivatives of malononitrile/ethyl cyanoacetate (Scheme 1).

The reaction is assumed to begin through the condensation of aminothiazoles and aldehyde to afford intermediate A. Subsequent Michael addition of malononitrile to **A** affords intermediate **B** which in turn undergoes intramolecular addition of the NH group to the CN group followed by isomerization to afford **4** in good yields (74-88 %).

The structures of 3a, 3b, 4a and 4b were confirmed on the basis of their spectral data. The IR spectra of thiazolo[3,2-a]pyrimidines 4a shows characteristic absorption bands of a conjugated CN group at 2228 cm-1, and broad absorption

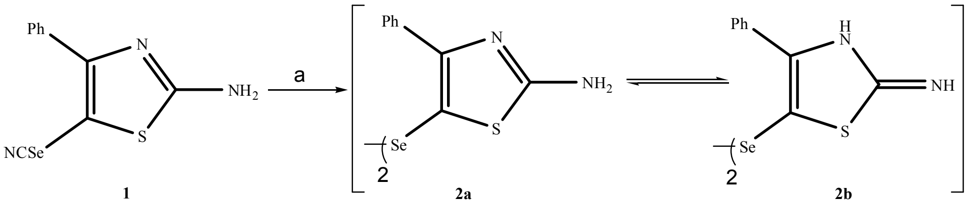
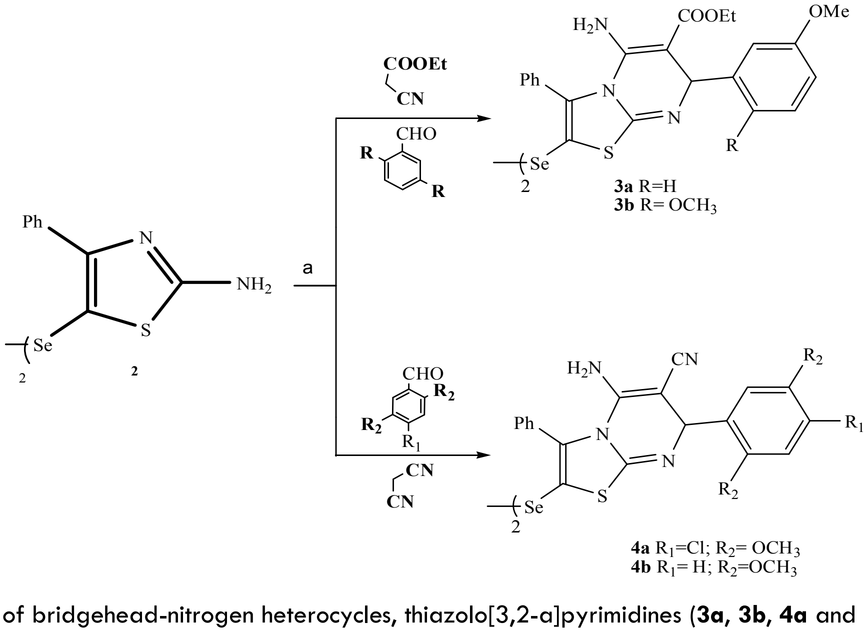


Figure 2. The amino and imino tautomeric forms of 2-aminothiazoles **2**. Reagents and conditions (a) K_2CO_3, DMF, 75 °C, 3 hr.



Scheme 1. The synthesis of bridgehead-nitrogen heterocycles, thiazolo[3,2-a]pyrimidines (**3a, 3b, 4a** and **4b**). Reagents and conditions (a)ethanol, conc. HCl, reflux 6 hr.

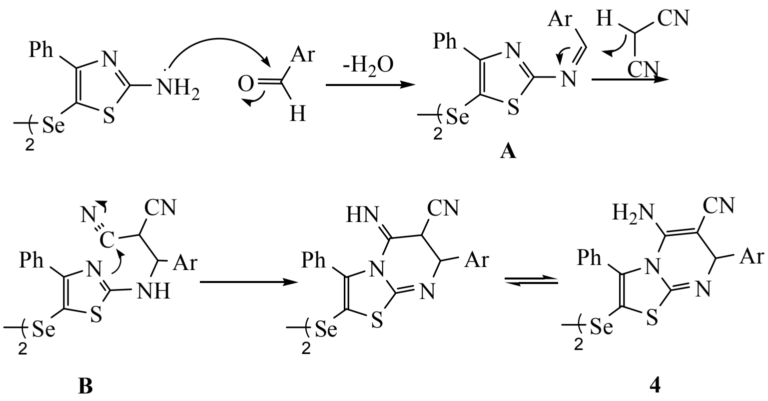
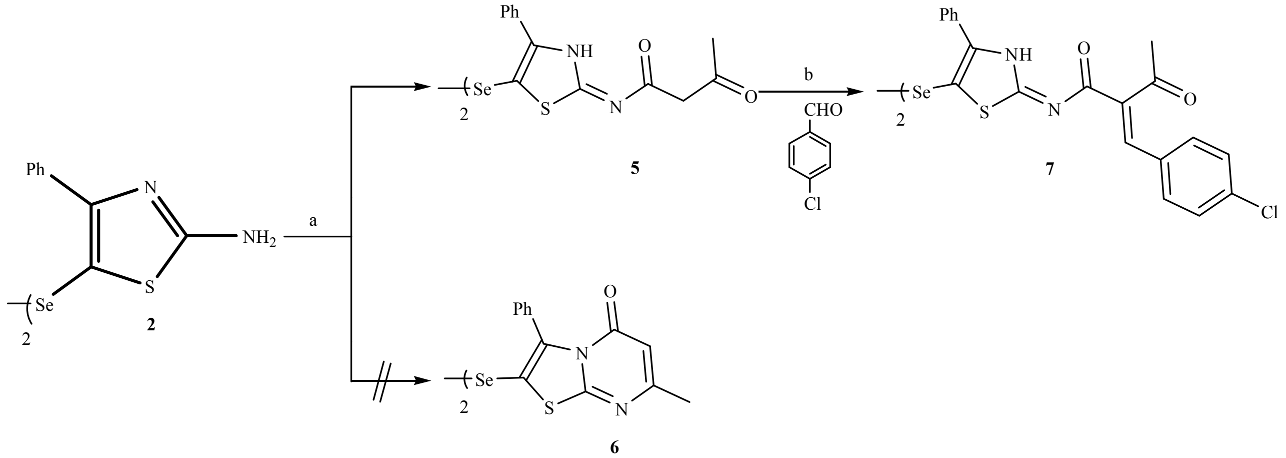


Figure 3. Proposed reaction mechanism of cyclocondensation of aminothiazoles with aromatic aldehydes and malononitrile.



Scheme 2. Reaction of 2-aminothiazolediselenide **2** with ethyl acetoacetate afforded 3-acetoacetyl derivative **5** instead of the anticipated thiazolo[3,2-a]pyrimidine **6**. Reagents and conditions (a) ethyl acetoacetate, reflux, 1 hr; (b) ethanol, reflux, 2 hr.

bands of NH2 at 3375 cm-1. In the 1H-NMR spectra, the NH2 signal was found at 2.5 ppm as singlet signal, and the aromatic protons was found downfield at 7.66 ppm as multiplets. The spectra for product 4a are given in the experimental section.

On the other hand, the reaction of 2-aminothiazolediselenide 2 with ethyl acetoacetate afforded 3-acetoacetyl derivative 5 in good yield (60 %) instead of the anticipated thiazolo[3,2-a]pyrimidine 6. The formation of 5 was further confirmed by the reaction with p-chlorobenzaldehyde which afforded the corresponding benzylidene 7 in a good yield (80 %).

The structure of 5 was confirmed on the basis of their spectral data. The IR spectra of 5 showed characteristic absorption bands of two carbonyl group at 1796 and 1683 cm-1. In the 1H-NMR spectra, the NH signal was found at 2.12 ppm as a singlet, and the aromatic protons was found downfield at 7.10 ppm as multiplets. A singlet signal found at 4.10 ppm corresponding to a CH2 proton. On the other hand, the structure of 7 was confirmed on the basis of their spectral data. The IR spectra of 7 showed characteristic absorption band of C=C at 1421 cm-1 in addition to the two carbonyl group at 1796 and 1683 cm-1. In the 1H-NMR spectra, the benzylidene signal was found at 7.88 ppm as a singlet, and the aromatic protons was found downfield at 7.22 ppm as multiplets. The spectra for product 5 and 7 are given in the Experimental section.

Our efforts were then directed to the synthesis of cyclic imides which 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 (Figure 1) [24][25].

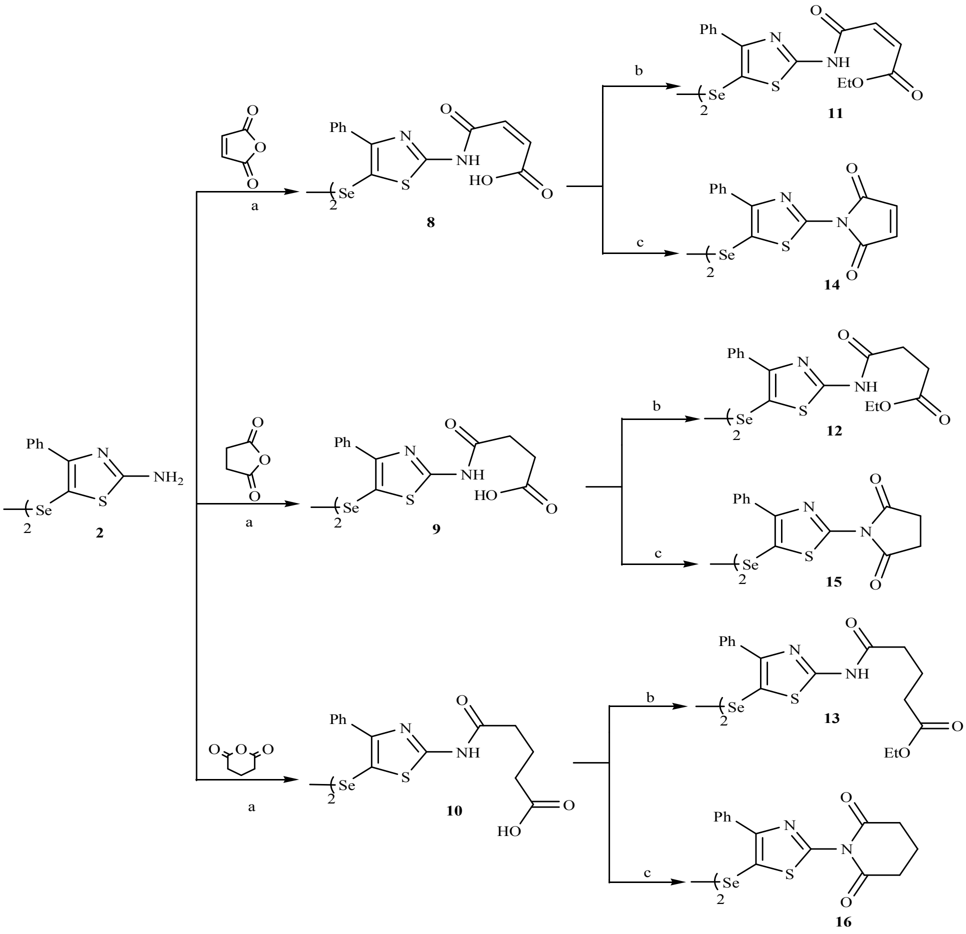
In order to have structurally diversity, two amine-containg selenium were used namely; 2-Aminothiazolediselenide 2 and 4- (2- (4-amino-3-methylphenyl) diselanyl) – 2 - methylbenzenamine (17). The reaction of maleic, succinic and glutaric anhydrides with **2** and **17** in refluxing acetone afforded the corresponding N-substituted maleanilic (**8** and **18**), succinanilic (**9** and **19**) and glutaranilic (**10** and **20**) acids in quantitative yields.

The ethyl esters **11, 12, 13, 21, 22** and **23** were obtained via acid-catalyzed esterification of the corresponding monoamidic acids. On the other hand, cyclic imides **14, 15, 16, 24, 25** and **26** were obtained by dehydration and subsequent ring-closure of the corresponding monoamidic acids up on gentle heating with acetic anhydride and sodium acetate (Scheme 3 and 4). The reaction was accomplished in 30 minutes and the product was easily isolated by ice-water precipitation.

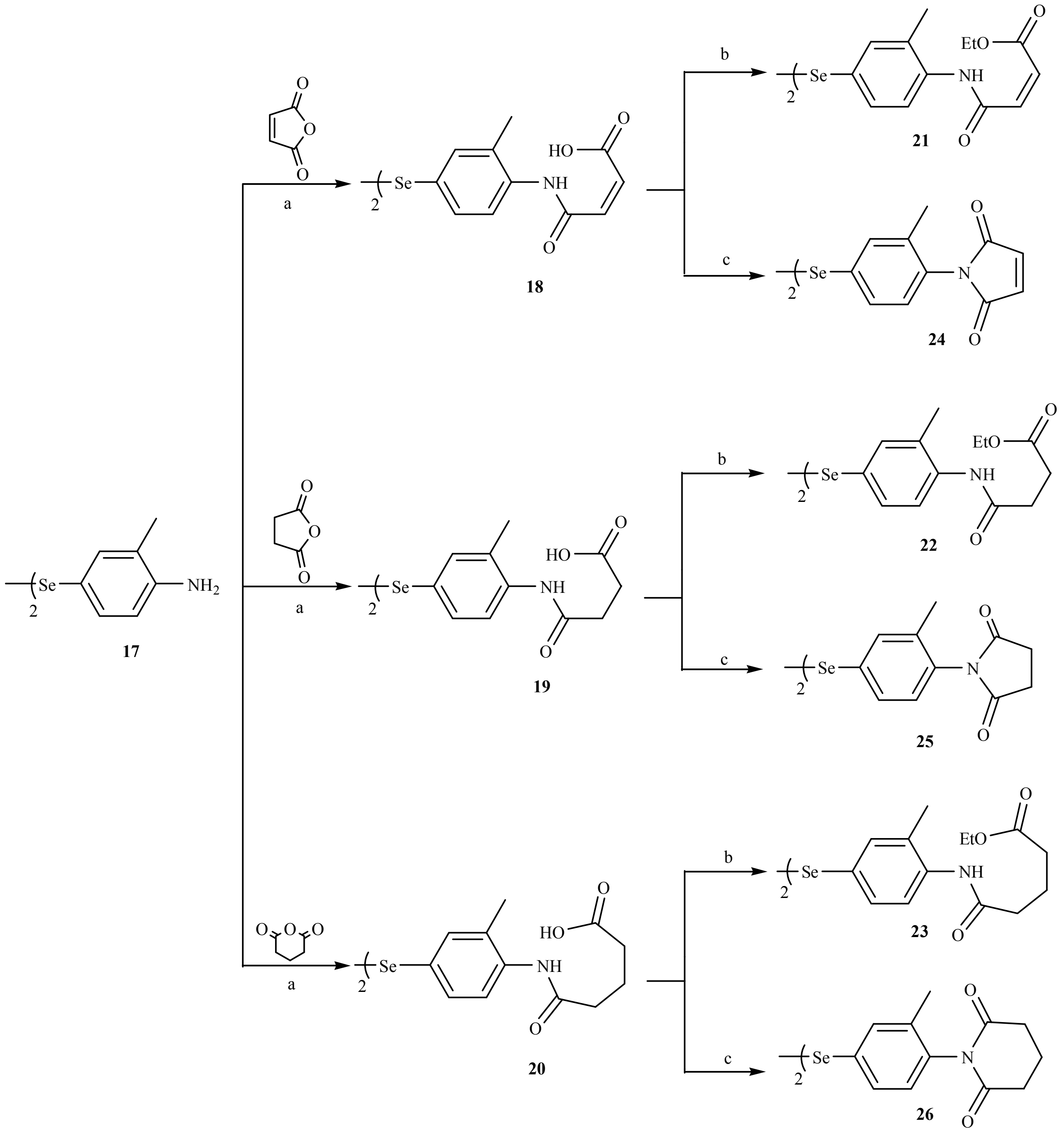
BIOLOGICAL ACTIVITY

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 cytotoxicity from effective drug doses or tumor recurrence. Consequently, searching for new anticancer agents with lower toxicity to normal cells is of particular interest. New organoselenium compounds were therefore developed in an attempt to obtain compounds with superior chemotherapeutic index in terms of increased selectivity, higher cytotoxicity and lower side effects than the currently known chemotherapeutic agent. Within this context, the cytotoxic potency of the synthesized compounds was evaluated in breast adenocarcinoma (MCF-7) and compared with their cytotoxicity in normal lung fibroblast cells (WI-38) employing standard MTT assay using 5-fluorouracil (5-Fu) drug which is extensively used in adjuvant and palliative



Scheme 3. Synthesis of diseleno *N*-amido-acids, *N*-amido-ethyl ester and cyclic imides using 2-aminothiazolediselenide **2** as the amine. Reagents and conditions (a) acetone, reflux, 3 hr; (b) ethanol, conc. H_2SO_4, r.t., 6 hr; (c) acetic anhydride, sodium acetate, 50 °C, 2 hr.



Scheme 4. Synthesis of diseleno *N*-amido-acids, *N*-amido-ethyl ester and cyclic imides using 4-(2-(4-amino-3-methylphenyl)diselanyl)-2-methylbenzenamine (**17**) as the amine. Reagents and conditions (a) acetone, reflux, 3 hr; (b) ethanol, conc. H_2SO_4, r.t., 6 hr; (c) acetic anhydride, sodium acetate, 50 °C, 2 hr.

chemotherapy for cancer. The IC50 values were estimated from the respective dose response curves (Table 1).

The therapeutic index (TI) is 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 (IC50 of WI-38 normal cell line/IC50 of MCF-7 cancer cell line). TI provides a simple index for evaluating the safety and efficacy of drugs. Agents with higher 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.

The compounds under investigation could be divided into two classes: 1) cytotoxic compounds (**4b, 5, 7, 18** and **23**) which are able to reduce the viability of MCF-7 tumor cells and 2) compounds with mid-low cytotoxicity*. The difference in the diselenides cytotoxicity indicates that it is not general

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

Compd. No.	In vitro Cytotoxicity IC50 (μM)		TI
	MCF-7	WI-38	
5-FU	8±0.13	4±0.63	0.5
3a	44±1.38	[b]	2
3b	[b]	[b]	-
4a	48±1.79	87±3.92	1.8
4b	26±0.54	[b]	4
5	21±0.61	[b]	5
7	34±1.06	[b]	3
9	76±2.91	61±3.06	1
10	[b]	[b]	-
11	65±2.41	74±3.22	1
12	[b]	[b]	-
13	53±2.11	85±3.14	2
15	[b]	[b]	-
16	61±1.84	70±2.88	1
18	9±0.20	[b]	11
19	[b]	[b]	-
20	[b]	[b]	-
21	[b]	[b]	-
22	55±3.33	77±2.75	1
23	18±0.96	[b]	6

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 1 and 2 days).a)

Compd. No.	Diameter inhibition zone in mm (% activity index)		
	E. coli	*S. aureus*	*C. albicans*
3a	7(29)	7(32)	[b]
3b	[b]	[b]	[b]
4a	9(38)	7(32)	11(39)
4b	[b]	[b]	[b]
5	10(42)	14(64)	7(25)
7	8(33)	9(41)	9(32)
9	[b]	8(36)	7(25)
10	[b]	[b]	[b]
11	8(33)	[b]	9(32)
12	[b]	[b]	[b]
13	8(33)	[b]	12(43)
16	[b]	[b]	[b]
18	11(46)	12(55)	[b]
19	7(29)	[b]	8(29)
20	[b]	[b]	[b]
21	[b]	[b]	[b]
22	10(42)	9(41)	13(46)
23	15(63)	19(86)	21(75)
Ampicillin	24 (100)	22 (100)	-
Colitrimazole	-	-	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.

selenium cytotoxicity and it further refers to the substitution pattern of the diselenides.

(*The cytotoxicity threshold is at $IC_{50} \leq 34$ μM)

Notably, treatment with test compounds was accompanied with morphological changes in culture. In culture, cells became rounded, cell layer partially condensed forming cell-free areas, and cells were detached from the culture plate. Furthermore, a significant difference in toxicity zones between breast solid tumor cells and normal WI-38 cells was noticed. The TI values of cytotoxic compounds **4b, 5, 7, 18** and **23** were ranging from eleven to three fold therapeutic indices in killing MCF-7 cells relative to WI-38 normal cells. These values were higher than that of 5-fu suggesting their effectiveness as anti-cancer agents. Whilst this may be true, the selectivity is not solely limited to these cell lines used and these initial results need further investigations using a wider arsenal of cancer and normal cells.

Antimicrobial evaluation

To study the cytotoxicity beyond human cell lines, 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 good-moderate toxicity against gram-negative (E. coli) bacteria and the gram-positive (S. aureus) bacteria. In this context, compounds **4a, 5, 7, 18** and **22** were the most active compound against E. coli and S. aureus. On the other hand, compounds **4a, 7, 11, 13, 19, 22** and **23** were the most active against C. albicans. 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.

Conclusions

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

The compounds under investigation were divided into cytotoxic and non-cytotoxic compounds showing that it is not general selenium cytotoxicity. Compounds **4b, 5, 7, 18** and **23** showed TI values ranging from three to eleven fold therapeutic indices in killing MCF-7 cells compared to WI-38 normal cells. These values were even higher than that of 5-fu TI suggesting their effectiveness as anti-cancer agents.

Additionally, 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. Compounds **4a, 5, 7, 18** and **22** were the most toxic against C. albicans. On the other hand, compounds **4a, 7, 11, 13, 19, 22** and **23** were found to be the most active against E. coli and S. aureus bacteria.

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?

EXPERIMENTAL

Chemistry

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 F254, 0.20 mm) were purchased from

Merck. 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), (Ú cm-1) on a Mattson 5000 FTIR Spectrophotometer at Micro analytical Center Faculty of Science, Mansoura University. The 1H-NMR Spectra were measured on a Varian Spectrophotometer at 300 MHz, using TMS as an internal reference and DMSO-d6 or CDCl3 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. 13C NMR (75 MHz) was recorded in DMSO-d6 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-phenyl-5-selenocyanatothiazol-2-amine (1)

To a well stirred solution of malononitrile (0. 2 gm, 3 mmol) in DMSO (2 mL), SeO2 (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 aminothiazole (0.8 gm, 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 5-(2-(2-amino-4-phenylthiazol-5-yl)diselanyl)-4-phenylthiazol-2-amine (2)

DMF (2 mL) was introduced into 2 mmol of 1 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 K2CO3 (2 mmol dissolved in 1 mL of water) was slowly introduced by a syringe. The resulting mixture was further heated at 75 oC for 3 h and then hydrolyzed with ice-cold water and the precipitate formed was filtered off and recrystallized from ethanol to give 2.

Synthesis of 4,4'-diselanediylbis(2-methylaniline)(17)[26, 27]

DMF (2 mL) was introduced into 25-mL two-necked flask fitted with a magnetic stirre, a septum and a condenser connected to an argon-filled balloon and containing 2 mmol of 2-toluidine-4-selenocyanate [27]. The solution was heated to 75 oC and K2CO3 (2 mmol dissolved in 1 mL of water) was slowly introduced by a syringe. The resulting mixture was further heated at 75 oC for 3 h and then hydrolyzed with ice-cold water and the precipitate formed was filtered off and recrystallized from ethanol.

General procedure for the preparation of thiazolo[3,2-a]pyrimidines (3a, 3b, 4a and 4b)

Method A

To a stirred solution of 2 (0.1 gm; 1.96 mmol), aromatic aldehyde (4 mmol), malononitrile/ethyl cyanoacetate (4 mmol) in ethanol (10 mL) and catalytic drops of conc. HCl were added .The reaction mixture was heated under reflux for 6 hr. The reaction mixture was cooled and poured into ice-water beaker, and the separated product was recrystallized from ethanol.

Method B

To a stirred solution of 2 (0.1 gm; 1.96 mmol) and appropriate chalcone (4 mmole) in ethanol (10 mL), catalytic drops of conc. HCl was added .The reaction mixture was heated under reflux for 6 hr. The reaction mixture was cooled and poured into ice-water beaker, and the separated product was recrystallized from ethanol.

Diethyl 2,2'-diselanediylbis(5-amino-7-(3-methoxyphenyl)-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-6-carboxylate) (3a)

Yellow powdery crystals; yield 90%; mp < 300 oC; Rf = 0.95 [Pet. ether (60-80 oC)/ethyl acetate (4:2)]; IR (KBr): υmax. cm-1: 3302(NH2), 1717(C=O), 1560(C=C), 1088(C-O); 1H NMR (300 MHz, DMSO-d6) δ 8.27 (s, 3H, Ar-H), 8.19 – 7.95 (m, 5H, Ar-H), 7.85 – 7.24 (m, 5H, Ar-H), 7.12 (d, J = 8.9 Hz, 5H, Ar-H), 4.29 (q, J = 7.5, 6.8 Hz, 4H, 2CH2), 3.86 (s, 6H, 2OCH3), 3.50 (s, 2H, 2CH), 2.70 (s, 4H, 2NH2), 1.29 (t, J = 7.1, 0.7 Hz, 6H, 2CH3); 13C NMR (75

MHz, DMSO) δ 170.57, 162.33, 162.25, 155.38, 153.15, 134.40, 132.30, 123.88, 116.21, 116.05, 115.91, 113.84, 113.78, 101.43, 98.52, 63.98, 61.98, 58.43, 56.69, 56.44, 54.70, 13.26; EIMS m/z (%) 972 [M+, (11.11)]; Anal. Calcd. for C44H40N6O6S2Se2 (972.08): C, 54.43; H, 4.15; N, 8.66. Found: C, 54.65; H, 4.10; N, 8.59.

Ethyl 5-amino-2-((5-amino-7-(2,5-dimethoxyphenyl)-6-(ethoxycarbonyl)-3-phenyl-7H-thiazolo[3,2-a]pyrimidin-2-yl)diselanyl)-7-(3,5-dimethoxyphenyl)-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-6-carboxylate (3b)

Yellow powdery crystals; yield 85%; mp < 300oC; Rf = 0.95 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3298(NH2), 1704(C=O), 1448(C=C), 1136(C-O); 1H NMR (300 MHz, DMSO-d6) δ 8.55-8.48 (m, 5H, Ar-H), 8.12 – 7.51 (m, 5H, Ar-H), 7.51 – 6.90 (m, 6H, Ar-H), 4.25 (q, J= 7.1, 4H, 2CH2), 3.92 (s, 6H, 2OCH3), 3.79 (s, 2H, 2CH), 2.50 (s, 4H, 2NH2), 1.25 (t, J= 7.1, 0.9 Hz, 6H, 2CH3); EIMS m/z (%) 1032 [M+, (22.18)]; Anal. Calcd. for C46H44N6O8S2Se2 (1032.1): C, 53.59; H, 4.30; N, 8.15. Found: C, 53.60; H, 4.29; N, 8.12.

2,2'-Diselanediylbis(5-amino-7-(4-chloro-2,5-dimethoxy phenyl)-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-6-carbonitrile) (4a)

Yellow powdery crystals; yield 88%; mp < 300oC; Rf = 5.5 [pet. ether (60-80)/ethyl acetate (4:2)]; IR (KBr): υmax. cm-1: 3375(NH2), 2228(CN), 1637(C=O), 1585(C=C), 1096(C-O); 1H NMR (300 MHz, DMSO-d6) δ 8.52–8.50 (m, 5H, Ar-H), 8.10 – 7.86 (m, 5H, Ar-H), 7.69-7.66 (m, 4H, Ar-H), 3.61 (s, 12H, 4OCH3), 3.33 (s, 2H, 2CH), 2.50 (s, 4H, 2NH2); 13C NMR (75 MHz, DMSO) δ 163.3, 161.16, 158.88, 139.04, 133.17, 130.95, 130.85, 130.79, 130.09, 129.99, 129.89, 128.75, 128.53, 128.46, 113.98, 113.91, 112.96, 82.16, 56. 2, 55.3, 40.1; EIMS m/z (%) 1006 [M+, (10.52)]; Anal. Calcd. for C42H32Cl2N8O4S2Se2 (1005.97): C, 50.16; H, 3.21; N, 11.14. Found: C, 50.12; H, 3.42; N, 11.18.

2,2'-Diselanediylbis(5-amino-7-(2,5-dimethoxyphenyl)-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-6-carbonitrile) (4b)

Orange powdery crystals; yield 74%; mp< 300oC; Rf = 4.5 [pet. ether (60-80)/ethyl acetate (4:2)]; IR (KBr): υmax. cm-1: 3381(NH2), 2221(CN), 1632(C=O), 1466(C=C), 1043(C-O); 1H NMR (300 MHz, DMSO-d6) δ 8.55-8.52 (m, 4H, Ar-H), 8.12 – 7.51 (m, 6H, Ar-H), 7.51 – 6.90 (m, 6H, Ar-H), 3.90 (s, 12H, 4OCH3), 3.80 (s, 2H, 2CH), 2.50 (s, 4H, 2NH2); EIMS m/z (%) 939 [M++1, (3.53]; Anal. Calcd. for C42H34N8O4S2Se2 (938.05): C, 53.85; H, 3.66; N, 11.96. Found: C, 53.90; H, 3.63; N, 11.79.

General procedure for the preparation of 5-(2-(2-amino-4-phenylthiazol-5-yl)diselanyl)-4-phenylthiazol-2-oxobutanamide (5)

A suspension of 2 (0.1 gm; 1.96 mmol) in ethyl acetoacetate (10 ml) was heated under reflux for 1 hr. The reaction mixture was cooled and poured into ice-water container then filtered and dried. The separated product was recrystallized from ethanol to give compound 5.

(N,N'E,N,N'E)-N,N'-(5,5'-Diselanediylbis(4-phenylthiazole-5(3H)-yl-2(3H)-ylidene))bis(3-oxobutanamide) (5)

Brown crystals; yield 60%; mp 261oC; Rf = 5.5 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1796(C=O), 1683(C=O), 1566 (C=C), 1160 (C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.95-7.91 (m, 2H, Ar-H), 7.25 – 7.21 (m, 2H, Ar-H), 7.20-7.18 (m, 2H, Ar-H), 7.15-7.10 (m, 4H, Ar-H), 4.10 (s, 4H, 2CH2), 2.21 (s, 6H, 2CH3), 2.12 (s, 2H, 2NH); EIMS m/z (%) 678 (M+, (58.49)]; Anal. Calcd. for C26H22N4O4S2Se2 (677.94): C, 46.16; H, 3.28; N, 8.28. Found: C, 46.20; H, 3.24; N, 8.20.

General procedure for the preparation of (2E,2'E,N,N'E,N,N'E)-N,N'-(5,5'-diselanediylbis(4-phenylthiazole-5(3H)-yl-2(3H)-ylidene))bis(2-(4-chlorobenzylidene)-3-oxobutanamide) (7)

A mixture of 5 (0.25 gm; 0.36 mmol) and 4-chloro-benzaldehyde (0.1 gm; 0.72 mmole) in absolute ethanol (10 ml) was refluxed for 2 hr. The mixture was then cooled to room temperature and poured into ice-water. The resulting solid product was filtered off, washed several times with water, dried and recrystallized from ethanol.

(2E,2'E,N,N'E,N,N'E)-N,N'-(5,5'-Diselanediylbis(4-phenyl thiazole-5(3H)-yl-2(3H)-ylidene))bis(2-(4-chloroben zylidene)-3-oxobutanamide) (7)

Yellow powdery crystals; yield 80%; mp < 300oC; Rf = 1.5 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1796(C=O), 1683(C=O), 1421(C=C), 1325(C-N), 695(C-Cl); 1H NMR (300 MHz, DMSO-d6) δ 7.88 (s, 2H, Ar-CH=), 7.54 – 7.22 (m, 18H, Ar-H), 2.58 (s, 6H, 2CH3), 2.22 (s, 2H, 2NH); 13C NMR (75 MHz, DMSO) δ 198.77, 195.37, 175.86, 172.24, 165.35, 156.21, 148.80, 138.20, 134.21, 134.19, 132.70, 128.65, 128.24, 128.02, 126.4, 125.59, 120.85, 83.74, 80.78, 25.05; EIMS m/z (%) 922 [M++1, (10.21)]; Anal. Calcd. for C40H28Cl2N4O4S2Se2 (921.93): C, 52.13; H, 3.06; N, 6.08. Found: C, 52.08; H, 3.12; N, 6.11.

General procedure for the preparation of maleanilic 8 and 18, succinanilic 9 and 19 and glutaranilic 10 and 20 acids

To a stirring solution of anhydride (2 mmol) in dry acetone (10 mL), the corresponding amine (1 mmol) was added dropwise 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'-((5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis(azanediyl))bis(4-oxobut-2-enoic acid) (8)

Yellow powdery crystals; yield 82%; mp 283oC; Rf = 0.5 [pet. ether (60-80)/ethyl acetate (4:2)]; IR (KBr): Umax. cm-1: 3441(O-H), 1726(C=O), 1670(C=O), 1625(C=C), 1072(C-N); 1H NMR (300 MHz, DMSO-d6) δ 11.12 (s, 2H, COOH), 7.98 – 7.92 (m, 2H, Ar-H), 7.90 – 7.80 (m, 4H, Ar-H), 7.60-7.44 (m, 4H, Ar-CH), 6.97 (dd, J= 11.95 Hz, 4H, 2CH=CH); EIMS m/z (%) 706 [M+, (16.92)]; Anal. Calcd. for C26H18N4O6S2Se2 (705.9): C, 44.33; H, 2.58; N, 7.95. Found: C, 43.29; H, 2.52; N, 7.89.

4,4'-((5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis(azanediyl))bis(4-oxobutanoic acid) (9)

Orange crystals; yield 95%; mp 288oC; Rf = 0.23 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): Umax. cm-1: 3163(O-H), 1717(C=O), 1683(C=O), 1625(C=C), 1072(C-N); 1H NMR (300 MHz, DMSO-d6) δ 11.11 (s, 2H, COOH), 7.68 – 7.22 (m, 5H, Ar-H), 7.13 – 7.01 (m, 2H, Ar-H), 6.97-6.94(m, 1H, Ar-CH), 6.56-6.52 (m, 2H, Ar-CH), 2.26-2.35 (m, 4H, 4CH2); EIMS m/z (%) [710 (0.40)]; Anal. Calcd. for C26H22N4O6S2Se2 (709.93): C, 44.07; H, 3.13; N, 7.91. Found: C, 44.02; H, 3.19; N, 7.83.

5,5'-((5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis(azanediyl))bis(5-oxopentanoic acid) (10)

Orange powdery crystals; yield 92%; mp 281oC; Rf = 0.23 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): Umax. cm-1: 3111(O-H), 1725(C=O), 1670 (C=O), 1616(C=C), 1067(C-N); 1H NMR (300 MHz, DMSO-d6) δ 11.24 (s, 2H), 7.80-7.75 (m, 2H), 7.73 – 7.50 (m, 3H), 7.45 – 7.30 (m, 5H), 2.28 (dt, J = 12.8, 8.4 Hz, 8H), 1.60 (m, 4H); EIMS m/z: 739 (M++1, 14.79) 738 (M+, (21.25)]; Anal. Calcd. for C28H26N4O6S2Se2 (737.96): C, 45.60; H, 3.56; N, 7.61. Found: C, 45.66; H, 3.49; N, 7.58.

(2Z,2'Z)-4,4'-((Diselanediylbis(2-methyl-4,1-phenylene))bis(azanediyl))bis(4-oxobut-2-enoic acid) (18)

Yellow powdery crystals; yield 63%; mp 158oC; Rf = 0.45 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): Umax. cm-1: 3401(O-H), 1711(C=O), 1665(C=O), 1483(C=C), 1083(C-N); 1H NMR (300 MHz, DMSO-d6) δ11.20 (s, 2H), 7.98 – 7.95 (m, 2H, Ar-H), 7.76 – 7.70 (m, 2H, Ar-H), 7.33-7.29 (m, 2H, Ar-H), 6.69 (dd, J= 11.12 Hz, 4H, 2CH=CH), 2.39 (s, 6H, 2CH3-Ar); EIMS m/z: 568 (M+, (33.08)]; Anal. Calcd. for C22H20N2O6Se2 (567.97): C, 46.66; H, 3.56; N, 4.95. Found: C, 46.72; H, 3.48; N, 5.03.

4,4'-((Diselanediylbis(2-methyl-4,1-phenylene))bis(azanediyl))bis(4-oxobutanoic acid) (19)

Yellow powdery crystals; yield 88%; mp 160oC; Rf = 0.45 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): Umax. cm-1: 3272(O-H), 1725(C=O), 1652(C=O), 1419(C=C),1124(C-N); 1H NMR (300 MHz, DMSO-d6) δ 12.11 (s, 2H, 2COOH), 9.31 (s, 2H, 2NH), 7.64-7.49 (m, 6H, Ar-H), 2.69-2.49 (m, 8H), 2.37 (s, 6H, 2CH3-Ar); 13C NMR (75 MHz, DMSO) δ 173.73, 173.53, 136.67, 133.68, 129.64, 128.8, 125.61, 120.85, 30.57, 29.04, 14.3; EIMS m/z: 573 [M++1, (0.05)]; Anal. Calcd. for C22H24N2O6Se2 (572): C, 46.33; H, 4.24; N, 4.91. Found: C, 46.40; H, 4.19; N, 4.88.

5,5'-((Diselanediylbis(2-methyl-4,1-phenylene))bis(azanediyl))bis(5-oxopentanoic acid) (20)

Yellow powdery crystals; yield 88%; mp 160oC; Rf = 0.45 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): Umax. cm-1: 3264(O-H), 1703(C=O), 1655(C=O), 1470(C=C), 1188(C-N); 1H NMR (300 MHz, DMSO-d6) δ 11.93 (s, 2H, 2COOH), 8.26-8.20 (m, 2H, Ar-H), 7.41 (d, J = 10.6 Hz, 4H, Ar-H), 2.36 (s, 6H, 2CH3), 2.30-2.23 (m, 8H, , 4CH2), 1.76-1.72 (m, 4H, 2CH2); 13C NMR (75 MHz, DMSO) δ 174.10, 174.04, 136.63, 133.60, 132.52, 129.59, 125.71, 125.56, 34.83, 32.99, 20.59, 17.59; EIMS m/z (%) 603 [M+, (39.90)]; Anal. Calcd. for C24H28N2O6Se2 (600): C, 48.17; H, 4.72; N, 4.68. Found: C, 48.08; H, 4.80; N, 4.66.

General procedure for the preparation of ethyl ester derivatives 11, 12, 13, 21, 22 and 23

To a solution of the corresponding acid (0.1 mmol) in ethanol (10 ml), conc. H2SO4 (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'-((5,5'-diselanediylbis(4-phenylthiazole-5,2-diyl))bis(azanediyl))bis(4-oxobut-2-enoate) (11)

Yellow powdery crystals; yield 66%; mp 298oC; Rf = 4.8 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3170(O-H), 1724(C=O), 1624(C=C), 1683(C=O), 1094(C-N); 1H NMR (300 MHz, DMSO-d6) δ 8.07-7.89 (m, 5H, Ar-H), 7.46- 7.03 (m, 5H, Ar-H), 6.76 (dd, J= 16.6 Hz, 4H, 2CH=CH), 4.41 (q, J=7.11 Hz, 4H, 2CH2), 1.19 (t, J=7.18 Hz, 6H, 2CH3); 13C NMR (75 MHz, DMSO) δ 177.64, 172.14, 162.52, 131.08, 129.68, 129.3, 128.67, 128.45, 128.23, 127.88, 127.5, 125.62, 108.49, 60.42, 13.79; EIMS m/z (%) 762 [M+, 7.52)]; Anal. Calcd. for C30H26N4O6S2Se2 (761.96): C, 47.37; H, 3.45; N, 7.37. Found: C, 47.47; H, 3.32; N, 7.30.

Diethyl 4,4'-((5,5'-diselanediylbis(4-phenylthiazole-5,2-diyl))bis(azanediyl))bis(4-oxobutanoate) (12)

Orange powdery crystals; yield 90%; mp 265oC; Rf = 0.8 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1803(C=O), 1680(C=C), 1630(C=O), 1072(C-N), 1030(C-O); 1H NMR (300 MHz, DMSO-d6) δ 7.77 – 7.47 (m, 5H, Ar-H), 7.44 – 7.20 (m, 5H, Ar-H), 3.39 (q, J = 1.4 Hz, 4H, 2CH2), 2.51-2.48 (m, 8H, 4CH2), 2.23 (t, J= 7.12 Hz, 6H, 2CH3); EIMS m/z: 766 [M+, (17.54)]; Anal. Calcd. for C30H30N4O6S2Se2 (765.99): C, 47.12; H, 3.95; N, 7.33. Found: C, 47.22; H, 3.86; N, 8.40.

Diethyl 5,5'-((5,5'-diselanediylbis(4-phenylthiazole-5,2-diyl)) bis(azanediyl))bis(5-oxopentanoate)(13)

Orange powdery crystals; yield 93%; mp 271oC; Rf = 0.8 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1725(C=O), 1677(C=O), 1665(C=C), 1220(C-O), 1150(C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.80 – 7.76 (m, 2H, Ar-H), 7.63 – 7.59 (m, 2H, Ar-H), 7.41-7.38 (m, 4H, Ar-H), 7.01-6.68 (m, 2H, Ar-H), 4.1 (q, J= 7.19 Hz, 4H, 2CH2), 2.20- 2.18 (m, 8H, 4CH2), 2.01-1.96 (m, 4H, 2CH2), 1.20 (t, J= 7.20 Hz, 6H, 2CH3); EIMS m/z: 779 (M+-CH3, (10.92)]; Anal. Calcd. for C32H34N4O6S2Se2 (794.03): C, 48.49; H, 4.32; N, 7.07%. Found: C, 48.38; H, 4.26; N, 7.12.

(2Z,2'Z)-Diethyl 4,4'-((diselanediylbis(2-methyl-4,1-phenylene))bis(azanediyl))bis(4-oxobut-2-enoate) (21)

Brown powdery crystals; yield 60%; mp 170oC; Rf = 0.25 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1728(C=O), 1652(C=O), 1620(C=C), 1161(C-O), 1024(C-N); 1H NMR (300 MHz, DMSO-d6) δ 8.26-8.20 (m, 2H, Ar-H), 7.81 (d, J = 10.6 Hz, 4H, Ar-H), 6.65 (dd, J= 18.2 Hz, 4H, 2CH=CH),4.29 (q, J= 7.16 Hz, 4H, 2CH2), 2.50 (s, 6H, 2CH3), 1.92 (t, J= 7.20 Hz, 6H, 2CH3); EIMS m/z (%) [M+, (2.48)]; Anal. Calcd. for C26H28N2O6Se2 (624.03): C, 50.17; H, 4.53; N, 4.50. Found: C, 50.22; H, 4.61; N, 4.62.

Diethyl 4,4'-((diselanediylbis(2-methyl-4,1-phenylene)) bis(azanediyl))bis(4-oxobutanoate) (22)

Yellow powdery crystals; yield 80%; mp 158oC; Rf = 6.5 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1724(C=O), 1652(C=O), 1637(C=C), 1121(C-O), 1096(C-N); 1H NMR (300 MHz, DMSO-d6) δ 8.1 (s , 2H, 2NH), 7.76 – 7.49 (m, 5H, Ar-H), 7.21 – 7.10 (m, 5H, Ar-H), 4.10 (q, J = 7.18 Hz, 4H, 2CH2), 2.52-2.49 (m, 8H, 4CH2), 2.22 (s, 6H, 2CH3), 1.23 (t, J= 7.18 Hz, 6H, 2CH3); EIMS m/z (%) 628 [M+, (9.05)]; Anal. Calcd. for C26H32N2O6Se2 (628.06): C, 49.85; H, 5.15; N, 4.47. Found: C, 50.03; H, 5.00; N, 4.52.

Diethyl 5,5'-((diselanediylbis(2-methyl-4,1-phenylene)) bis(azanediyl))bis(5-oxopentanoate) (23)

Yellow powdery crystals; yield 75%; mp 165oC; Rf = 6.5 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 1728(C=O), 1652(C=O), 1419(C=C), 1078(C-N),1024(C-O); EIMS m/z: 658 (M++2, 8.92), 657 (M++1,4.46), 656 (M+, 18.70), 655 (5.81), 654 (5.59), 626 (0.59), 602 (0.59), 115 (100.0, base peak), 113 (1.31), 110 (1.17), 100 (2.57), 95 (3.38); Anal. Calcd. for C28H36N2O6Se2 (656.09): C, 51.38; H, 5.54; N, 4.28. Found: C, 51.29; H, 5.61; N, 4.21.

General procedure for the preparation of cyclic imides 14, 15, 16, 24, 25 and 26

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 oC. The reaction was cooled and quenched with ice water and the separated solid was recrystallized from ethanol.

1,1'-(5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis(1H-pyrrole-2,5-dione) (14)

Yellow powdery crystals; yield 75%; mp 265oC; Rf = 5.3 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3020(C-H), 1821(C=O), 1422(C=C), 1140(C-N); 1H NMR (300 MHz, DMSO-d6) δ 8.02-7.93 (m, 5H, Ar-H), 7.58- 7.48 (m, 5H, Ar-H), 6.76 (dd, J= 7.5 Hz, 4H, 2CH=CH); EIMS m/z (%) 670 [M+, (0.26)]; Anal. Calcd. for C26H14N4O4S2Se2 (669.88): C, 46.72; H, 2.11; N, 8.38. Found: C, 46.67; H, 2.22; N, 8.39.

1,1'-(5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis (pyrrolidine-2,5-dione) (15)

Orange powdery crystals; yield 70%; mp 292oC; Rf = 6 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3020(C-H), 1821(C=O), 1653(C=C),1095(C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.82 – 7.75 (m, 2H, Ar-H), 7.70 – 7.52 (m, 2H, Ar-H), 7.32-7.28 (m, 4H, Ar-H), 7.03-6.69 (m, 2H, Ar-H), 2.50 (s, 8H, 4CH2); EIMS m/z (%) 674 [M+, (10.48)]; Anal. Calcd. for C26H18N4O4S2Se2 (673.91): C, 46.44; H, 2.70; N, 8.33. Found: C, 46.36; H, 2.62; N, 8.47.

1,1'-(5,5'-Diselanediylbis(4-phenylthiazole-5,2-diyl))bis (piperidine-2,6-dione) (16)

Orange powdery crystals; yield 75%; mp < 300oC; Rf = 5.7 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3109(C-H), 1744(C=O), 1713(C=O), 1088(C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.96 – 7.90 (m, 2H, Ar-H), 7.66 – 7.62 (m, 2H, Ar-H), 7.44-7.40 (m, 4H, Ar-H), 7.13-6.69 (m, 2H, Ar-H), 2.20- 2.18 (m, 8H, 4CH2), 2.93-1.85 (m, 4H, 2CH2); EIMS m/z (%) [702 (16.55)]; Anal. Calcd. for C28H22N4O4S2Se2 (701.94): C, 48.01; H, 3.17; N, 8.00. Found: C, 48.09; H, 3.12; N, 8.07.

1,1'-(Diselanediylbis(2-methyl-4,1-phenylene))bis(1H-pyrrole-2,5-dione) (24)

Yellow powdery crystals; yield 70%; mp 255oC; Rf = 4.8 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3063(C-H), 1746(C=O), 1422(C=C), 1174(C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.81-7.79 (m, 3H, Ar-H), 7.28- 7.20 (m, 3H, Ar-H), 6.86 (dd, J= 7.46 Hz, 4H, 2CH=CH), 2.23 (s, 6H, 2CH3); EIMS m/z (%) 532 [M++1, (11.03)]; Anal. Calcd. for C22H16N2O4Se2 (531.94): C, 49.83; H, 3.04; N, 5.28. Found: C, 49.79; H, 3.14; N, 5.15.

1,1'-(Diselanediylbis(2-methyl-4,1-phenylene))bis (pyrrolidine-2,5-dione) (25)

Yellow powdery crystals; yield 70%; mp 155oC; Rf = 4.8 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3063(C-H), 1746(C=O), 1422(C=C), 1174(C-N); 1H NMR (300 MHz, DMSO-d6) δ 8.38 – 8.30 (m, 2H, Ar-H), 6.80-6.77 (m, 4H, Ar-H), 2.81 (s, 8H, 4CH2), 2.37 (s, 6H, 2CH3); 13C NMR (75 MHz, DMSO) δ 176.62, 138.20, 136.87, 133.60, 129.59, 128.89, 125.30, 120.74, 28.60, 17.06; EIMS m/z (%) 520 [M+-O, (0.03)]; Anal. Calcd. for C22H20N2O4Se2 (535.98): C, 49.45; H, 3.77; N, 5.24. Found: C, 49.38; H, 3.79; N, 5.30.

1,1'-(Diselanediylbis(2-methyl-4,1-phenylene))bis (piperidine-2,6-dione) (26)

Yellow powdery crystals; yield 80%; mp 158oC; Rf = 4.4 [pet. ether (60-80)/ethyl acetate (4:1)]; IR (KBr): υmax. cm-1: 3063(C-H), 1796 (C=O), 1718(C=O), 1054(C-N); 1H NMR (300 MHz, DMSO-d6) δ 7.90 – 7.72 (m, 3H, Ar-H), 7.64-7.59 (m, 3H, Ar-H), 2.35 (s, 6H, 2CH3), 2.25- 2.19 (m, 8H, 4CH2), 2.10-2.08 (m, 4H, 2CH2); EIMS m/z: 564 [M+, 0.14)]; Anal. Calcd. for C24H24N2O4Se2 (564.01): C, 51.26; H, 4.30; N, 4.98. Found: C, 51.33; H, 4.21; N, 5.11.

BIOLOGICAL STUDIES

Cytotoxicity assay

The mammary gland breast cell line (MCF-7) and human fibroblast cell line (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 and Fetal Bovine serum was purchased from GIBCO, UK. The cells 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 oC in a humidified atmosphere containing about 15% (v/v) CO2 in air.

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) was used to measure the metabolic activity of cells which are capable of reducing it by dehydrogenases to a violet colored formazan product. Briefly, 120 μL aliquots of a cell suspension (50,000 cells mL-1) in 96-well microplates were incubated at 37 °C and 10% CO2 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 oC and 10% CO2, 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 color was measured at 590 nm using an ELISA plate reader. All investigations were carried out in two parallel experiments. The IC50 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 individually 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 1x108 CFU/mL of pathological tested bacteria and 1x104 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.

Acknowledgements

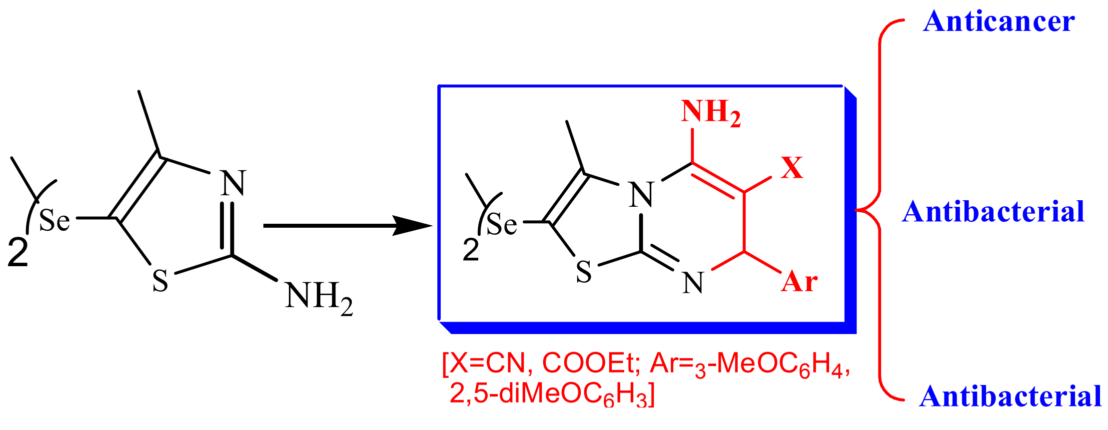
The authors thank the Egyptian Ministry of Higher Education and Mansoura University for financial support. We would like also to thank Mr Ahmed Abbas for carrying out the biological testing.

Conflict of Interest

The authors have declared no conflict of interest.

SHORT SUMMARY

A novel series of symmetrical thiazolediselenides were synthesized and their cytotoxic properties were evaluated on human breast adenocarcinom and compared with their cytotoxicity in normal fibroblast cells (WI-38) employing standard MTT assay. Of the tested compounds, 4b, 5, 7, 18 and 23 exhibited therapeutic indices (TI) up to eleven fold and were higher than that of 5-fu suggesting their effectiveness as anti-cancer agents.



REFERENCES

1. J. Ferlay, H. R. Shin, F. Bray, D. Forman, C. Mathers, D. M. Parkin, Int. J. Cancer 2010, 127, 2893-2917.
2. R. Schmadeka, B. E. Harmon, M. Singh, Am. J. Clin. Pathol. 2014, 141, 462-477.
3. J. H. Goldie, Cancer Metastasis Rev. 2001, 20, 63-68.
4. Z. Mihaly, A. M. Szasz, B. Gyorffy, Biomark Med. 2014, 8, 77-79.
5. R. J. Shamberger, J. Natl. Cancer Inst. 1970, 44, 931-936.
6. C. Ip, Biol. Trace Elem. Res. 1983, 5, 317-330.
7. P. D. Whanger, Br. J. Nutr. 2004, 91, 11-28.
8. C. W. Nogueira, J. B. Rocha, Arch. Toxicol. 2011, 85, 1313-1359.
9. R. Naithani, Mini Rev. Med. Chem. 2008, 8, 657-668.
10. M. Doering, L. A. Ba, N. Lilienthal, C. Nicco, C. Scherer, M. Abbas, A. A. Zada, R. Coriat, T. Burkholz, L. Wessjohann, M. Diederich, F. Batteux, M. Herling, C. Jacob, J. Med. Chem. 2010, 53, 6954-6963.
11. E. E. Martinez, A. K. Darke, C. M. Tangen, P. J. Goodman, J. H. Fowke, E. A. Klein, S. A. Abdulkadir, Cancer. Prev. Res. (Phila) 2014, 7, 950-957.
12. I. Thompson Jr, A. Kristal, E. A. Platz, Am. Soc. Clin. Oncol. Educ. Book 2014, e76-80.
13. B. F. Abdel-Wahab, S. Shaaban, Synthesis 2014, 46, 1709-1716.
14. S. Shabaan, L. A. Ba, M. Abbas, T. Burkholz, A. Denkert, A. Gohr, L. A. Wessjohann, F. Sasse, W. Weber, C. Jacob, Chem. Commun. (Camb) 2009, (31), 4702-4704.
15. S. Mecklenburg, S. Shaaban, L. A. Ba, T. Burkholz, T. Schneider, B. Diesel, A. K. Kiemer, A. Röseler, K. Becker, J. Reichrath, A. Stark, W. Tilgen, M. Abbas, L. A. Wessjohann, F. Sasse, C. Jacob, Organic and Biomolecular Chemistry 2009, 7, 4753-4762.
16. S. Shaaban, R. Diestel, B. Hinkelmann, Y. Muthukumar, R. P. Verma, F. Sasse, C. Jacob, Eur. J. Med. Chem. 2012, 58, 192-205.
17. S. Shaaban, M. A. Arafat, W. S. Hamama, ARKIVOC 2014, i, 470-505.
18. S. Shaaban, A. Negm, E. E. Ibrahim, A. A. Elrazak, Oncology Reviews 2014, 8, 25-35.
19. M. A. Metwally, S. Shaaban, B. F. Abdel-Wahab, G. A. El-Hiti, Current Organic Chemistry 2009, 13, 1475-1496.
20. W. S. Hamama, M. A. Ismail, S. Shaaban, H. H. Zoorob, J. Heterocycl. Chem. 2008, 45, 939-956.
21. S. Shaaban, F. Sasse, T. Burkholz, C. Jacob, Bioorg. Med. Chem. 2014, 22, 3610-3619.
22. S. Shaaban, M. A. Arafat, H. E. Gaffer, W. S. Hamama, Der Pharma Chemica 2014, 6, 186-193.
23. M. A. Gouda, Y. E. Sherif, M. S. Elsherbini, Phosphorus, Sulfur, and Silicon and the Related Elements 2014, 189, 1633-1643.
24. S. Brauch, M. Henze, B. Osswald, K. Naumann, L. A. Wessjohann, S. S. van Berkel, B. Westermann, Org. Biomol. Chem. 2012, 10, 958-965.
25. [25] G. Smith, U. D. Wermuth, Acta Crystallogr. C 2012, 68, o253-6.
26. V. A. Kachanov, Y. O. Slabko, V. O. Baranova, V. E. Shilova, A. V. Kaminskii, Tetrahedron letters 2004, 45, 4461-4463.
27. D. Plano, Y. Baquedano, D. Moreno-Mateos, M. Font, A. Jiménez-Ruiz, J. A. Palop, C. Sanmartín, Eur. J. Med. Chem. 2011, 46, 3315-3323.