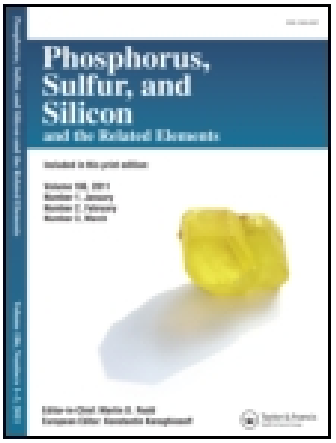

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

One Pot Synthesis of Some New Heteroylselenoglycolic Acids and Their Biological Activity

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Accepted author version posted online: 09 Aug 2012.Published online: 16 Aug 2013.

To cite this article: Shams H. Abdel-Hafez , Laila M. Break & Amani M. Alsaadi (2013) One Pot Synthesis of Some New Heteroylselenoglycolic Acids and Their Biological Activity, Phosphorus, Sulfur, and Silicon and the Related Elements, 188:9, 1281-1295, DOI: 10.1080/10426507.2012.717138

To link to this article: http://dx.doi.org/10.1080/10426507.2012.717138

Phosphorus, Sulfur, and Silicon, 188:1281–1295, 2013

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426507.2012.717138

ONE POT SYNTHESIS OF SOME NEW HETEROYLSELENOGLYCOLIC ACIDS AND THEIR BIOLOGICAL ACTIVITY

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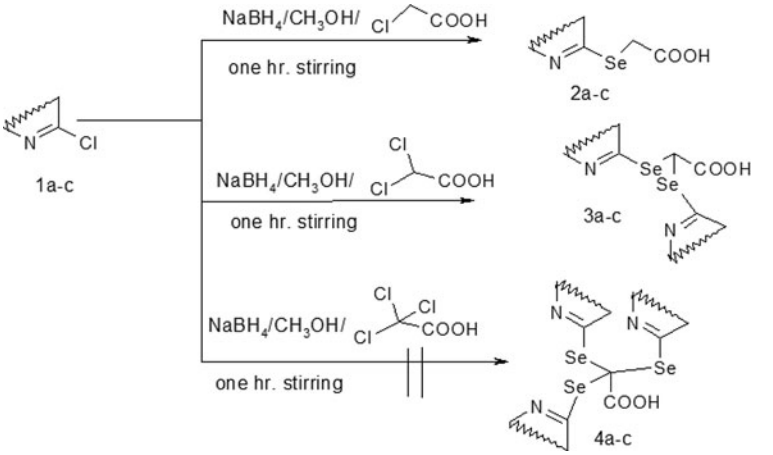
GRAPHICAL ABSTRACT



Abstract *A convenient one pot three-stage synthesis was used for obtaining new heteroylselenoglycolic and di-heteroylselenoglycolic acids by nucleophilic substitution reaction of the starting compounds pyridineselenol, pyridazineselenol, and quinolineselenol with α-chloro- or α,α-dichloroacetic acids for 1-h stirring. The newly synthesized compounds were screened biologically for anti-microbial and anti-oxidant activities. The structure of all new compounds was confirmed by ^{1}H NMR, ^{13}C NMR, Mass, and IR spectroscopy and elemental analyses.*

Keywords Selenoglycolic acids; pyridineselenol; pyridazineselenol; quinolineselenol; anti-oxidant activity; anti-microbial activity

Received 29 May 2012; accepted 9 July 2012.

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INTRODUCTION

Organoselenium compounds have continued to attract the attention of investigators because of a series of their unique properties. There are data showing that organoselenium compounds are capable of sensitizing processes in living organism.[1] Therefore, despite high toxicity of many selenium compounds,[2–4] antitumor,[5–7] anticancer,[8,9] as well as biologically active substances exhibiting antiviral[10–13] and antimicrobial activities[14–17] have been produced on the basis of organic derivatives of selenium. A literature survey indicates that only few publications have mentioned the incorporation of a selenium atom into glycolic acid. Consequently, synthesis and biological screening of selenoglycolic acid may be considered a virgin research area. Athayde-Filho et al.[18] described a convenient one-pot three-stage synthesis for obtaining three new aroylselenoglycolic acids. In the first stage, powdered gray selenium reacts with $NaBH_4$ in aqueous solution to form NaHSe. This reacts with selected aroyl chlorides to form sodium aroylselenides. In the third stage, these react with α-chloroacetic acid to form the desired aroylselenoglycolic acids. Stimulated by our recent work on the synthesis of selenium containing sulfa drugs,[19] and the synthesis of selenium containing amino acid analogues,[20] we decided to expand our interest to introduce selenium into heterocyclic molecules followed by α-chloro- or α,α-dichloroacetic acids, hoping to obtain heteroylselenoglycolic and di-heteroylselenoglycolic acid systems of anticipated biological and pharmaceutical effects.

RESULTS AND DISCUSSION

Chemistry

The present work was planned to synthesize some new heteroylselenoglycolic acids and to test their biological activity as anti-bacterial, anti-fungal, and anti-oxidant agents. Our approach to synthesize target compounds started from 2-chloro-4,6-dimethylpyridine-3-carbonitrile**,** 3-chloro-5,6-diphenylpyridazine-4-carbonitrile, and 2-chloro-4,6-dimethylquinoline **(1a–c)** respectively, which were prepared as described previously.[19, 20]

Our work plan consists of the following three steps:

(1) Synthesis of some new heteroylselenoglycolic acids (one pot three-stage synthesis of **2a–c**):

A convenient one pot three-stage synthesis was used for obtaining new heteroylselenoglycolic acids such as (3-cyanopyridin-2-yl)selenoglycolic acid (**2a**), (4-cyanopyridazin-3-yl)selenoglycolic acid (**2b**), and (quinolin-2-yl)selenoglycolic acid (**2c**) by nucleophilic substitution reaction of compounds **1a–c** with α-chloroacetic acid in the presence of sodium borohydride as a basic catalyst by 1-h stirring (see Scheme 1).

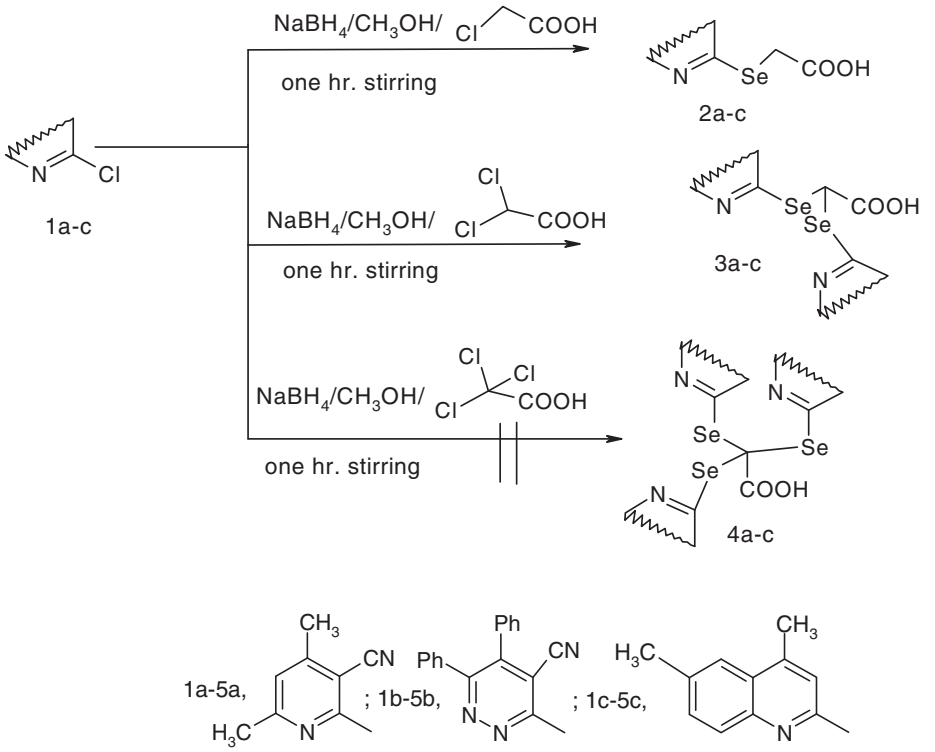
The first stage:

Reaction of sodium borohydride with powdered gray selenium in methanol or water as a solvent to produce sodiumhydrogen selenide.

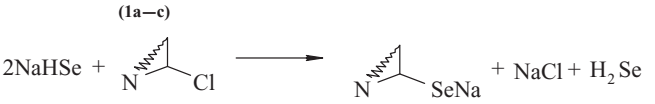
$$4NaBH_4 + 2Se + 7H_2O \rightarrow 2NaHSe + Na_2B_4O_7 + 14H_2$$

The second stage:

Reaction of NaHSe with heteroyl chlorides for 1 h with stirring to form sodiumheteroyl selenide.



Scheme 1 Synthesis of compounds **2a–c** and **3a–c.**



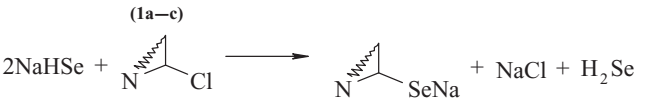
The third stage:

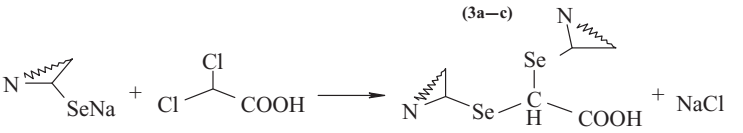
Production of heteroylselenoglycolic acids by the reaction of sodiumheteroyl selenide with α-chloroacetic acid for 1 h.

Trials to prepare compounds **4a–c** with trichloroacetic acid failed and this may be due to steric hindrance effects.

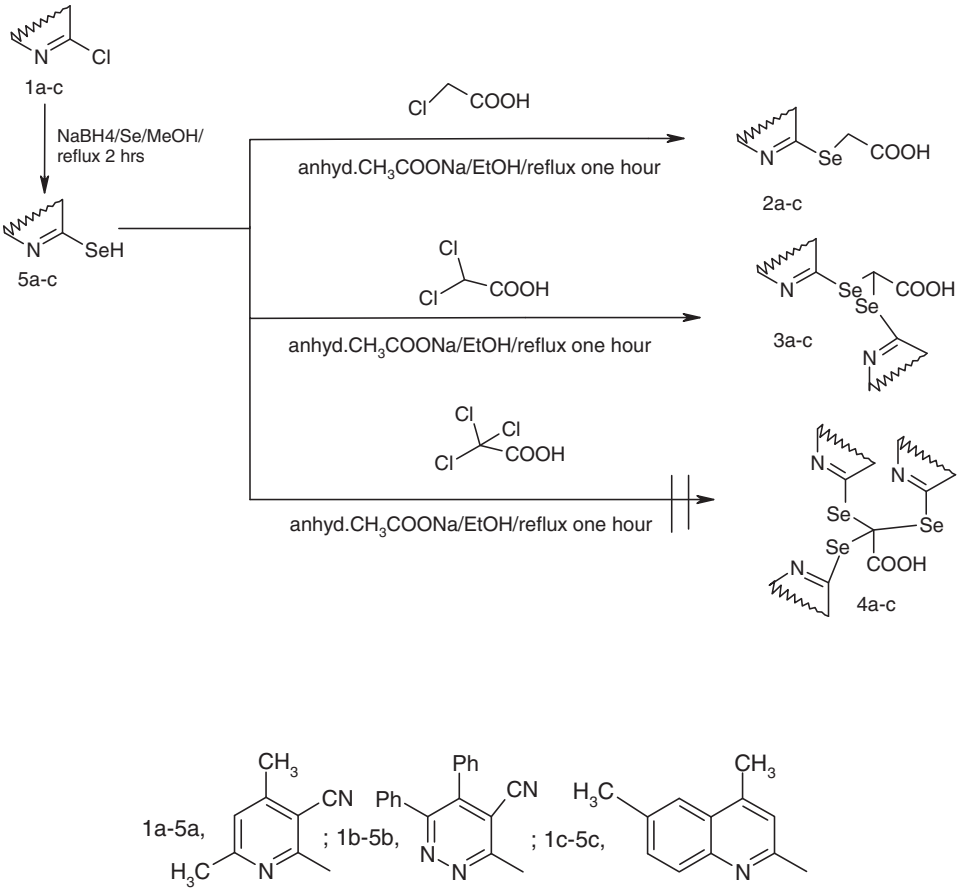
The synthesis of new di-heteroylselenoglycolic acids (one pot three-stage synthesis of **3a–c**) also consists of the following three reaction steps:

$$4NaBH_4 + 2Se + 7H_2O \rightarrow 2NaHSe + Na_2B_4O_7 + 14H_2$$





Unequivocal synthesis of 2a–c and 3a–c through the intermediate compounds 5a–c. To confirm the structure of the new compounds **2a–c** and **3a–c**, we first separated compounds **5a–c** and then added α,α-dichloroacetic acids in the presence of anhydrous sodium acetate to yield the identical products **2a–c** and **3a–c** (see Scheme 2). In the same manner, the reaction consists of three steps as mentioned above. Trials to prepare compounds **4a–c** with trichloroacetic acid via the intermediate compounds **5a–c** failed also. All the products gave satisfactory mass spectra and elemental analyses. The characteristic band of the IR spectra is the C=O stretch of the carboxyl group at 1730, 1740, and 1720 for **2a**, **2b,** and **2c** respectively. In the ^{1}H NMR spectra of **2a**, **2b**, and **2c**, the hydrogen atoms of the $SeCH_2$ group have chemical shifts at $\delta = 3.32$, 3.36, and 3.40 ppm respectively. The carboxy hydrogen atoms exhibit chemical shifts around 14.15 ppm for **2b** and 11.53 ppm for **2c**, while the signal of the COOH group disappeared for **2a** as a result of the hydrogen



Scheme 2 Synthesis of compounds **2a–c** and **3a–c** through the intermediates **5a–c.**

bond formation. The ^{13}C NMR signals appeared at δ 20.58–24.53 $(CH_3)_2$, 29.70 (CH_2), 108 (C—CN), 114 (CN), 122 (H—C), 151–152 (C—CH_3), 155 (C—Se), 162 (COOH) for **2a**, at δ 56 (CH_2), 112 (C—CN), 121 (CN), 127.78–128.94 (Ph) 2, 130 (Ph—C—C), 136 (Ph—C—N), 161 (C—Se), 172 (COOH) for **2b,** and at δ 18.6–18.7 $(CH_3)_2$, 21.8 (CH_2), 120 (CH_3—C), 122 (Ph—C), 126–147 (Ph), 149 (C—Se), 153 (COOH) for **2c**. The characteristic IR band is the C = O stretching of the carboxyl group at 1730, 1742, and 1725 respectively. In the ^1H NMR spectra of **3a**, **3b**, and **3c**, the hydrogen atoms (1H, SeCH) have chemical shifts at δ 3.34, 4.53, and 3.42 respectively. The hydrogen atoms of the acid group exhibit chemical shifts around 14.13 ppm for **3b** and disappeared in both **3a** and **3c** as a result of hydrogen bond formation. The ^{13}C NMR spectra were characterized by peaks average at δ 20.58–24.53 $(CH_3)_2$, especially for **3a** and **3a**, 29.7 (CH_2), 108 (C-CN), 114 (CN), 122 (H-C), 151–152 (C-CH_3), 155 (C-Se), and 162 (COOH) for **3a–c**.

Biological Activity

Anti-microbial activity. The antimicrobial screening of the synthesized compounds was undertaken using the agar well diffusion assay.[21] Table 1 lists the screening

Table 1 Antibacterial activity of **2a–c** and **3a–c** (inhibition zone in mm)

Bacterial species Sample No.	**2a** monopyrd	**3a** dipyrd	**2b** monopyrdz	**3b** dipyrdz	**2c** monoquino	**3c** diquino	12*
Staphylococcus aureus AUMC No. B-54	18	0	0	0	12	0	27
Bacillus cereus AUMC No. B-52	23	0	0	0	16	0	32
Escherichia coli AUMC No. B-53	16 p.i.	0	0	0	0	0	30
Pseudomonas aeruginosa AUMC No. B-73	0	0	0	0	0	0	24
Serratia marcescens AUMC No. B-55	0	0	0	0	0	0	41
Fungal species Sample No.	**2a**	**3a**	**2b**	**3b**	**2c**	**3c**	**12****
Candida albicans AUMC No. 418	28	0	9	0	14	9	30
Geotrichum candidum AUMC No. 226	18	8	0	0	10	0	24
Trichophyton rubrum AUMC No. 1804	34	17	14	0	16	14	35
Fusarium oxysporum AUMC No. 5119	32	10	12	0	16	12	22
Scopulariopsis brevicaulis AUMC No. 729	11	0	18	17	12	0	26
Aspergillus flavus AUMC No. 3214	30	14	18	0	14	13	27

Notes: The amount added in each pore is 50 μL.
12* = Choramphenicol as antibacterial standard.
12** = Clotrimazole as antifungal standard.
p.i. = Partial inhibition.
AUMC = Assiut University Mycological Center.

Table 2 Anti-bacterial activity (minimum inhibition concentration, MIC)

	2a											**2c**								
Organism Sample No.	50	25	12.5	6.25	3.125	1.56	0.78	0.4	0.2	0.1	0.05	50	25	12.5	6.25	3.125	1.26	0.78	0.4	0.2
Staphylococcus aureus AUMC No. B-54	18	10	10	9	9	8	8	0	—	—	—	12	12	10	10	8	0	—	—	—
Bacillus cereus AUMC No. B-52	23	23	18	17	14	12	12	10	10	10	0	16	16	14	13	12	0	—	—	-<

	12*								
Organism Sample No.	20	10	5	2.5	1.25	0.6	0.3	0.15	0.08
Staphylococcus aureus AUMC No. B-52	27	17	17	15	13	12	10	10	0
Bacillus cereus AUMC No. B-52	32	32	32	30	28	25	18	16	0

	2a											**2c**								
Organism Sample No.	50	25	12.5	6.25	3.125	1.56	0.78	0.4	0.2	0.1	0.05	50	25	12.5	6.25	3.125	1.26	0.78	0.4	0.2
Candida albicans AUMC No. 418	28	26	24	20	16	14	13	11	0	—	—	14	14	12	10	0	—	—	—	—
Geotrichum candidum AUMC No. 226	18	18	18	14	12	12	12	12	0	—	—	10	10	10	10	0	—	—	—	—
Trichophyton rubrum AUMC No. 1804	34	26	18	15	12	10	8	8	0	—	—	26	26	16	14	14	12	**10**	**0**	—
Fusarium oxysporum AUMC No. 5119	32	26	22	16	12	12	0	—	—	—	—	16	16	13	12	8	0	—	—	—
Scopulariopsis brevicaulis AUMC No. 729	11	11	10	10	0	—	—	—	—	—	—	12	10	0	—	—	—	—	—	—
Aspergillus flavus AUMC No. 3214	30	16	14	14	14	13	13	10	0	—	—	14	8	0	—	—	—	—	—	—

	12**						
Organism Sample No.	20	10	5	2.5	1.25	0.6	0.3
Candida albicans AUMC No. 418	30	30	30	26	26	26	26
Geotrichum candidum AUMC No. 226	24	22	22	22	22	22	22
Trichophyton rubrum AUMC No. 1804	35	34	34	34	34	34	34
Fusarium oxysporum AUMC No. 5119	22	22	22	22	18	18	18
Scopulariopsis brevicaulis AUMC No. 729	26	24	23	23	20	20	20
Aspergillus flavus AUMC No. 3214	27	27	27	25	25	25	25

Note: 12* = Choramphenicol as antibacterial standard.

results of the compounds tested against Gram-negative and Gram-positive bacteria. Also, Table 1 (continued) lists the screening results of the compounds tested against fungi. Five bacterial test organisms such as *Staphylococcus aureus* (B-54), *Bacillus cereus* (B-52), *Escherichia coli* (B-53), *Serratia marcescens* (B-55), and *Pseudomonas aeruginosa* (B-73), and six fungi test organisms such as *Candida albicans* (418), *Geotri_chum candidum* (226), *Trichophyton rubrum* (1804), *Fusarium oxysporum* (5119), *Scopulariopsis brevicaulis* (729), and *Aspergillus flavus* (3214) were obtained from the Mycological Center of Assiut University. Chloramphenicol was used as an antibacterial standard, while clotrimazole was used as an antifungal standard (Table 2). DMSO was used as a blank, which exhibited no activity against any of the used organisms. The obtained data revealed that most of the selected compounds **2a–c** and **3a–c** showed no activity against all organisms of bacteria used except for **2a** and **2c**, which showed remarkably good activity potential against *Staphylococcus aureus* and *Bacillus cereus* with inhibition zone of 18 and 23 mm for **2a** at a concentration of 20 mg/mL, and inhibition zone of 12 and 16 mm for **2c** at a concentration of 20 mg/mL. The minimum inhibition concentration (MIC) values were found to be 8 (0.78) and 10 (0.1) mg/mL for **2a**, and 8 (3.125) and 12 (3.125) for **2c** respectively (Table 3). All compounds **2a–c** and **3a–c** showed strong fungicidal effect against all species of fungi, especially the two compounds **2a** and **2c**. The MIC values of these compounds were 11, 12, 12, 10, 8, and 10 mg/mL (0.4%) for **2a** and 10, 10, 8, 8, and 10 mg/mL (6.25%) for **2c**. The strong fungicidal and bactericidal effects of these two compounds are presumably due to the presence of either cyano- or selenoglycolic acid groups in **2a** and the selenoglycolic acid in **2c**. In addition, the effect of the pyridine and quinoline nuclei may play a role. These compounds seem to be good bactericidal and fungicidal candidates against all species of bacteria and fungi.

Generally, it was noticed that the activities of the tested compounds are less pronounced than those of standard antifungal and antibacterial agents used. Also, organoselenium compounds that contain bis-(pyridine, pyridazine, and quinoline) selenylglycolic acid moieties do not exhibit antimicrobial activity against the tested organisms. It seems

Table 3 Antibacterial activity of chemical compounds tested. Inhibition zone (mm) and MIC (mg/mL) are given in parentheses

Sample	*Staphylococcus aureus* AUMC No. B-54	*Bacillus cereus* AUMC No. B-52				
2a	8 (0.78)	10 (0.1)				
2c	8 (3.125)	12 (3.125)				
12* (CHL)	10 (0.15)	16 (0.15)				
Sample	*Candida albicans* AUMC No. 418	*Geotrichum candidum* AUMC No. 226	*Fusarium oxysporum* AUMC No. 5119	*Aspergillus flavus* AUMC No. 3214	*Trichophyton rubrum* AUMC No. 1804	*Scopulariopsis brevicaulis* AUMC No. 729
2a	11 (0.4)	12 (0.4)	12 (1.56)	10 (0.4)	8 (0.4)	10 (6.25)
2c	10 (6.25)	10 (6.25)	8 (3.125)	8 (25)	10 (0.78)	10 (25)
12** (CLO)	26 (0.3)	22 (0.3)	18 (0.3)	25 (0.3)	34 (0.3)	20 (0.3)

12** = Clotrimazole (CLO) was used as standard.

obvious that quinolineselenyl and pyridineselenylglycolic acids demonstrated the best results in biological screening.

Screening of In-vitro Antioxidant Activities of Mono- and Diselenoglycolic Acid Derivatives of 2a–c and 3a–c

Screening of in-vitro antioxidant activities of mono- and diselenoglycolic acid derivatives of pyridine, pyridazine, and quinoline nuclei was investigated. The antioxidant activities, studied by three methods, viz., Diphenylpicrylhydrazyl (DPPH) assay, β-carotene/linoleic acid bleaching assay, and ferric reducing power assay, revealed significant antioxidant activity compared with synthetic antioxidant activity.

Pharmacological Results and Discussion

The potential antioxidant activity of mono- and diselenoglycolic acid derivatives and the parent selenols of pyridine, pyridazines, and quinoline were evaluated on the basis of three methods: the scavenging activity of the stable free radical DPPH (EC_{50} value); inhibition of the coupled oxidation of linoleic acid and beta-carotene and ferric reducing antioxidant power. The obtained result was given EC_{50} values, which is defined as the half maximum effective concentration. The data are presented in Table 4 and Figure 1. Generally, each compound with a lower value of EC_{50} has the higher free radical scavenging activity. The resulting data showed that the parent pyridineselenol and the mono- and diselenoglycolic acid derivatives have a good free radical scavenging activity, while quinolidineselenol and its mono- and diseleno derivatives have moderate free radical scavenging activity compared with other tested compounds. Regarding EC_{50} values of pyridineselenol and mono- or diseleno derivatives, the DPPH values were 121.01 ± 9.81, 102.39 ± 7.34, and 95.259 ± 4.60 μg/mL respectively. For EC_{50} β-carotene, values of 24.51 ± 2.07, 21.13 ± 1.54, and 17.31 ± 1.93 μg/mL were found, and for EC_{50} ferric, reducing values of 46.21 ± 3.11, 41.12 ± 2.32, and 35.17 ± 1.77 μg/mL were found. The DPPH values

Table 4 EC50 values (μg/mL) of mono- and diselenoglycolic acids and their parent pyridine-2-selenol, pyridazine-2-selenol, and quinolineselenol

Sample	DPPH assay EC_{50}	β-carotene assay EC_{50}	Ferric reducing assay EC_{50}
BHT*	37.14 ± 3.21^a	2.54 ± 0.87	nt
α-Tocopherol*	9.64 ± 1.87^a	3.91 ± 0.73	nt
EDTA*	nt	nt	7.32 ± 1.05
Pyridine-2-selenol	121.01 ± 9.81^b	24.51 ± 2.07	46.21 ± 3.11
Pyridine-monoselenoglycolic acid	102.39 ± 7.34^b	21.13 ± 1.54	41.12 ± 2.32
Pyridine-diselenoglycolic acid	95.259 ± 4.60^b	17.31 ± 1.93	35.17 ± 1.77
Pyridazineselenol	256.24 ± 6.87^b	56.23 ± 2.43	45.40 ± 2.34
Pyridazine-monoselenoglycolic acid	235.76 ± 4.67^b	47.12 ± 2.93	39.22 ± 2.11
Pyridazine-diselenoglycolic acid	226.31 ± 3.14^b	42.23 ± 1.98	35.09 ± 3.08
Quinolidineselenol	145.76 ± 6.78^b	41.12 ± 1.93	67.20 ± 4.64
Quinoline-monoselenoglycolic acid	140.22 ± 5.91^b	39.47 ± 1.31	51.31 ± 4.12
Quinoline-diselenoglycolic acid	138.32 ± 3.72^b	33.45 ± 2.11	46.32 ± 2.08 >

*Standards; nt = not tested; [a, b]significant compared with standards.

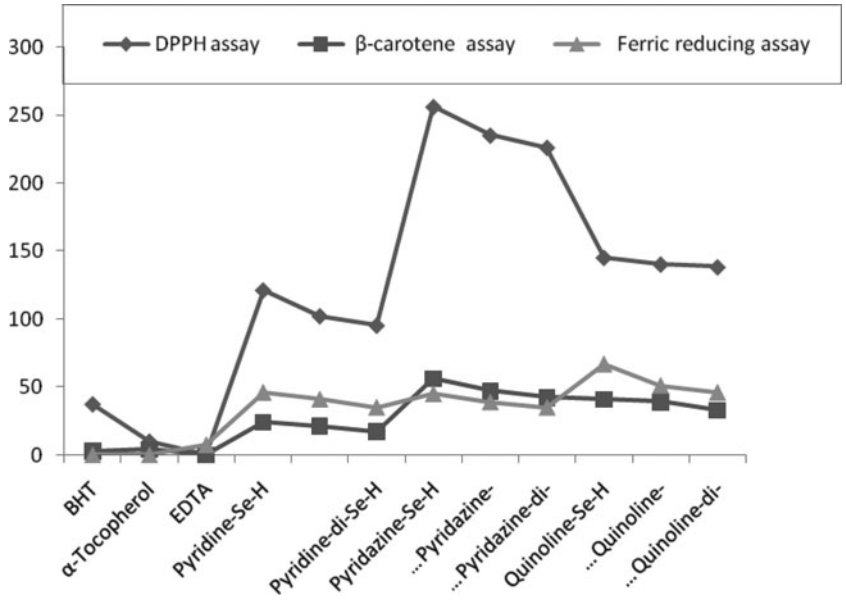


Figure 1 EC_{50} values of tested compounds with different antioxidants assays.

for quinolinemono- and -di- derivatives were 145.76 ± 6.78, 140.22 ± 5.91, and 138.32 ± 3.72 μg/mL.

Generally, pyridineselenol and the mono- and diselenoglycolic acid derivatives **5a**, **2a**, and **3a** respectively, have better free radical scavenging activity than quinolineselenol and the mono- and diselenoglycolic acid derivatives **5c**, **2c**, and **3c**, respectively, which have a moderate antioxidant activity. The pyridazineselenol and the mono- and diselenoglycolic acid **5b**, **2b** and **3b**, respectively have the lowest antioxidant activity in this class of compounds as compared with other derivatives. The order of decreasing antioxidant activities was diselenoglycolic acid of pyridine/quinoline > monoselenopyridine/quinoline > their parent pyridine/quinolineselenols. The data in Fig. 1 show that the efficiency of the tested compounds as a DPPH free radical scavenger, more effective than the two other ways β-carotene linoleic acid system and ferric reducing assay, where the seleno compound reacts with the hydrated free radical (OH·H) to form stable compounds before they destroy biological macromolecules.[22,23] Lipid peroxidation is an oxidative deterioration process of polyunsaturated fatty acids which is induced by free radicals. In the β-carotene linoleic acid system assay, effective compounds block the chain reaction of lipid peroxidation mainly by scavenging the generated intermediate lipid peroxyl radicals.[24] In the reducing power assay, the presence of antioxidants in the tested samples would cause reducing ferricyanide complex in ferrous form, turning the yellow color of test solution into various shades of green and blue colors, which depends on the reducing power of antioxidant samples.[25]

EXPERIMENTAL

Chemistry

Melting points were determined by using the Kofler melting point apparatus, and were uncorrected. IR (KBr, cm^{-1}) spectra were recorded on a Pye-Unicam SP3–100 instrument.

^{1}H NMR spectra were obtained on a Varian (400 MHz) EM 390 USA instrument at King Abdel-Aziz University by using TMS as internal reference.[13] C NMR spectra were recorded on a JNM-LA spectrometer (100 MHz) at King Abdel-Aziz University, Saudi Arabia. Elemental analyses were obtained on an Elementar Vario EL 1150C analyzer. Purity of the compounds was checked by thin layer chromatography (TLC) using silica gel plates. Mass spectra were recorded on a JEOL-JMS-AX 500 at Cairo National Research Center, Cairo, Egypt and JEOL-JMS 600 at Assiut University, Assiut, Egypt. Purity of the compounds was checked by TLC.

General procedure (1).

*One pot three-stage synthesis of monoheteroylselenoglycolic acids **2a–c**.* An aqueous solution of $NaBH_4$ (26.5 mmol) was added with magnetic stirring at room temperature to powder gray selenium (12.7 mmol) suspended in distilled water or methanol. Within a few minutes, a considerable amount of H_2 was liberated and the selenium was consumed in approximately 5 min. The almost colorless solution of the resultant NaHSe was used without further treatment. Heteroyl chloride (12.6 mmol) was added and magnetically stirred for 1 h. A dark red solution was formed, and shortly thereafter α-chloroacetic acid (12.6 mmol) was added in small portions and magnetically stirred for another 1 h. A black gray solid precipitated, which was poured on crushed ice, then filtered off, washed with distilled water, dried at room temperature, and recrystallized from chloroform.

(3-Cyano-4,6-dimethylpyridin-2-yl)selenoglycolic acid **(2a)**

M.p. 96–97 °C ($CHCl_3$), orange crystals, 69% yield. IR: 3410 (OH), 3050 (CH_{ar}), 2200 (CN), 1730 (CO). ^{1}H NMR (DMSO-d_6): δ 7.43 (s, 1H, $CH_{pyridine}$), 3.32 (s, 2H, $SeCH_2$), 2.48 (s, 3H, CH_3), 2.47 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.58–24.53 $(CH_3)_2$, 29.70 (CH_2), 108(C—CN), 114 (CN), 122 (H—C), 151–152(C—CH_3), 155 (C—Se), 162 (COOH). MS: *m*/*z* (%) 269 $[M+1]^+$. Calcd. for $C_{10}H_{10}O_2N_2Se$: C, 44.62; H, 3.74; N, 10.41. Found C, 44.35; H, 3.70; N, 10.33.

(4-Cyano-5,6-diphenylpyridazin-3-yl)selenoglycolic acid **(2b)**

M.p. 110–112 °C ($CHCl_3$), brown crystals, 55% yield. IR: 3405 (OH), 2200 (CN), 1740 (CO). ^{1}H NMR (DMSO-d_6): δ 14.15 (s, 1H, COOH), 7.43–7.29 (m, 10H, ArH), 3.36 (s, 2H, $SeCH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 56 (CH_2), 112(C—CN), 121 (CN), 127.78–128.94 (Ph) 2, 130 (Ph—C—C), 136 (Ph—C—N), 161 (C—Se), 172 (COOH). MS: *m*/*z* (%) 394 $[M]^+$. Calcd. for $C_{19}H_{13}O_2N_3Se$ C, 57.88; H, 3.32; N, 10.66. Found C, 57.65; H, 3.15; N, 10.23.

(4,6-Dimethylquinolin-2-yl)selenoglycolic acid **(2c)**

M.p. 115–117 °C ($CHCl_3$), beige crystals, 50% yield. IR: 3400 (OH), 1720 (CO). ^{1}H NMR (DMSO-d_6): δ 11.53 (s, 1H, COOH), 7.82–7.38 (m, 4H, $CH_{quinoline}$), 3.40 (s, 2H, $SeCH_2$), 2.62 (s, 3H, CH_3), 2.51 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.6–18.7 $(CH_3)_2$, 21.8 (CH_2), 120 (CH_3—C—Se), 122 (Ph—C—C—Se), 126–147 (Ph), 149 (C—Se), 153 (COOH). MS: *m*/*z* (%) 294 $[M+2]^+$. Calcd. for $C_{13}H_{13}O_2NSe$: C, 53.07; H, 4.45; N, 4.76. Found C, 52.88; H, 4.19; N, 4.21.

General procedure (2).

*One pot three-stages synthesis of di-heteroylselenylglycolic acids (**3a–c**).* An aqueous solution of $NaBH_4$ (26.5 mmol) was added with magnetic stirring at room temperature to powdered gray selenium (12.7 mmol) suspended in distilled water or methanol. Within a few minutes, a considerable amount of H_2 was liberated, and the selenium was consumed in approximately 5 min. The almost colorless solution of resultant NaHSe was

used without further treatment. The heteroyl chloride (12.6 mmol) was added and magnetically stirred for 1 h. Dark red solution was formed, and shortly thereafter dichloroacetic acid (12.6 mmol) was added in small portions and magnetically stirred for 1h. Within a few minutes, a black gray solid precipitated, which was poured on crushed ice, then filtered off, washed with distilled water, dried at room temperature, and recrystallized from chloroform.

Bis-](3-cyano-4,6-dimethylpyridin-2-yl)selenyl]ethanoic acid **(3a)**

M.p 105–107 °C, deep orange crystals, 35% yield. IR: 3410 (OH), 2200 (CN), 1730 (CO). ^{1}H NMR (DMSO-d_6,): δ 7.42 (s, 2H, $CH_{pyridine}$), 3.34 (s, 1H, SeCH), 2.49–2.46 (s, 12H, CH_3), COOH disappeared as a result of H-bond. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.28–24.81[$(CH_3)_2)_2$], 29.70 (CH-COOH), 108–109 (C-CN)$_2$, 114–115 $(CN)_2$, 122–123 (H-C)$_2$, 151–152 (C-CH_3)$_2$, 154–155 (C-Se)$_2$, 162 (COOH). MS: *m*/*z* (%) 475 $[M+1]^+$. Calcd. for $C_{18}H_{16}O_2N_4Se_2$: C, 45.20; H, 3.37; N, 11.71. Found C, 45.00; H, 3.14; N, 11.46.

Bis-[(4-cyano-5,6-diphenylpyridazin-3-yl)selenyl]ethanoic acid **(3b)**

M.p. 127–129 °C, deep brown crystals, 20% yield. IR: 3400 (OH), 2200 (CN), 1742 (CO). ^{1}H NMR (DMSO-d_6): δ 14.13 (s, 1H, COOH), 7.54–7.21 (m, 20H, ArH), 3.42 (s, 1H, SeCH). MS: *m*/*z* (%) 728 $[M-28]^+$. Calcd. for $C_{36}H_{22}O_2N_6Se_2$: C, 59.35; H, 3.04; N, 11.54. Found C, 59.30; H, 2.99; N, 11.26.

Bis-[(4,6-dimethylquinolin-2-yl)selenyl]ethanoic acid **(3c)**

M.p. 150–152 °C, yellow crystals, 25% yield. IR: 3410 (OH), 2200 (CN), 1740 (CO). ^{1}H NMR (DMSO-d_6): δ 7.84–7.39 (m, 8H, $CH_{quinoline}$); 3.35 (s, 1H, SeCH), 2.62–2.49 (s, 12H, CH_3), COOH disappeared as a result of H-bond. MS: *m*/*z* (%) 528 $[M]^+$. Calcd. for $C_{24}H_{22}O_2N_2Se_2$: C, 54.56; H, 4.20; N, 5.30. Found C, 54.33; H, 4.10; N, 5.1.

General procedure (3).

*Unequivocal synthesis of compounds **2a–c** and **3a–c** through the intermediate compounds **5a–c**.* Compounds **5a–c** were prepared as previously described.[19,20] All new compounds **2a–c** and **3a–c**, prepared through procedure (3), were spectroscopically identical with the compounds obtained via procedures (1) and (2). It is noticeable that the yields obtained via procedures (1) and (2) are much better compared with those prepared from procedure (3).

Biological Assay

Antibacterial activity. Five bacterial species, representing both Gram-positive and Gram-negative strains, were used to test the antibacterial activities of the target compounds **2a–c** and **3a–c** in vitro in comparison to chloramphenicol as a reference drug using the standard agar paper disc diffusion method[21] against *S. aureus* (B-54), *B. cereus* (B-52), *E. coli* (B-53), *S. marcescens* (B-55), and *P. aeruginosa* (B-73). Cell suspensions of bacterial strains were prepared from 48-h old cultures grown on potato dextrose agar (PDA) or Sabouraud agar (SA) media. Cell suspension, 1 mL, was added to Petri dishes of 9 cm in diameter, and then 15 mL of nutrient agar was poured into plates. Plates were shaken gently to homogenize the innoculum. Sterile 5-mm filter paper (Whatmann, UK) was saturated with 10 mg·mL^{-1} of the test compound, chloramphenicol solutions (200, 100, 50, 25, 15.5, 6 mg·mL^{-1} concentration) as a reference drug, or DMSO as negative control. Then impregnated discs were dried for 1 h, and placed in the center of each plate. The seeded plates were incubated at 35 ± 2 °C for 24–48 h. The radii of inhibition zones, in millimeter of triplicate sets, were measured. The results are given in Tables 1–3.

Antifungal activity. Compounds **2a–c** and **3a–c** were screened for their antifungal activity in vitro, in comparison to clotrimazole as a reference drug using the standard agar

paper disc diffusion method[21] against six fungi: *C. albicans* (418), *G. candidum* (226), *T. rubrum* (1804), *F. oxysporum* (5119), *S. brevicaulis* (729), and *A. flavus* (3214). A spore suspension in sterile distilled water was prepared from 2–3 days old culture of the fungi growing on potato dextrose agar (PDA) or Sabouraud agar (SA) media. The final spore concentration was 56104 spores mL^{-1}. About 15 mL of the growth medium was placed into sterile Petri dishes of 9 cm in diameter and incubated with 1 mL of the spore suspension. Plates were shaken gently to 28 ± 2 °C for 7 days. The radii of the inhibition zones in millimeter of triplicate sets were measured. The results are shown in Tables 1–3.

Screening of Anti-oxidant Activity

Chemicals. DPPH, methanol, *n*-hexane, 2-deoxy-2-ribose, β-carotene, and linoleic acid; BHT; α-tocopherol and EDTA were procured from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Tween 40 and dimethyl sulfoxide (DMSO) were from Merck (Darmstadt, Germany). All chemical reagents used were of analytical grade.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The potential antioxidant activity of mono- and diseleno derivatives of pyridineselenol, pyridazineselenol, and quinolineselenol were measured in terms of hydrogen-donation or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as reagent.[26] 50 μL of each compound (100 μg/mL) was added to 2 mL of 60-μM buffered methanol solution of DPPH (pH = 5.5). Absorbance measurements were read at 517 nm after 20 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of buffered methanol and DPPH solution acted as control, and BHT was used as standard antioxidant. All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to the following formula:

$$\%\text{Inhibition} = [(A_B - A_A)/A_B] \times 100,$$

where A_B is the absorption of the blank sample ($t = 0$ min) and A_A is the absorption of the tested compound or standard substance solution ($t = 30$ min).

β-Carotene/linoleic acid bleaching assay. The antioxidant activity was determined by measuring the inhibition of conjugated dienehydroperoxides arising from linoleic acid oxidation and method running as described by Miraliakbari and Shahidi.[27] A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 mL $CHCl_3$, 25 μL of linoleic acid, and 200-mg Tween 40. The solvent was evaporated under vacuum, and 100 mL of aerated distilled water was added to the residue. Each tested compound dissolved in DMSO, and 350 μL of solution was added to 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50 °C for 2 h, together with two tubes, one containing the antioxidant α-Tocopherol as standard, and the other containing the same volume of DMSO instead of compounds' solutions. The test tube with BHT maintained its yellow color during the incubation period. The absorbencies were measured at 470 nm on a spectrometer. Antioxidant activities (inhibition percentage, I%) of the samples were evaluated in terms of the bleaching of β-carotene using the following equation:

$$\text{Inhibition}\% = [\text{A}(\beta - \text{carotene after 2-h assay})/\text{A}(\text{initial } \beta\text{-carotene})] \times 100,$$

where A(β-carotene after 2-h assay) is the absorbance of β-carotene after 2-h assay remaining in the samples, and A(initial β-carotene) is the absorbance of β-carotene at the

beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicates.

Ferric Reducing Antioxidant Power Assay

The reductive potential of the mono- and diseleno derivatives of pyridineselenol, pyridazineselenols, and quinolineselenol and the standard (EDTA) was determined according to the method of Oyaizu.[28] Each tested compound or standard was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$: 2.5 mL, 1%). Then the mixture was incubated at 50 °C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increase of reducing power.

Statistical Analysis

Each of the measurements described was carried out in three replicate experiments, and the results were recorded as mean ± standard deviation. The significant difference was calculated at $p \leq 0.05$.

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