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Original article

Selenium containing heterocycles: Synthesis, anti-inflammatory, analgesic and anti-microbial activities of some new 4-cyanopyridazine-3(2*H*)selenone derivatives

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Abstract

New series of selenolo[2,3-*c*]pyridazine and pyrimido[4′,5′:4,5]selenolo[2,3-*c*] pyridazine derivatives were prepared from new 4-cyano-5,6-diphenylpyridazine-3(2*H*)selenone (**2**). Elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data confirmed the structure of the newly synthesized compounds. Some selected compounds **2**, **4a**,**b**, **6a**–**c**, and **11a** were investigated for their anti-inflammatory activity. Compounds **4b** and **6a**–**c** which showed activity comparable to the standard drug Indomethacin were screened for their analgesic activity. In addition, the most active compounds were tested for their acute toxicity. Moreover, some of the tested compounds **4b**, **6a**, **6b** and **6c** were screened for their antibacterial and antifungal activities.

Keywords: Pyridazines; Fused pyridazines; Pyrimidoselenolo pyridazines; Anti-inflammatory; Analgesic; Anti-microbial activities

1. Introduction

Heterocyclic annulated pyridazines continue to attract considerable attention, which mainly arises from the large variety of interesting pharmacological activities, herbicides, insecticides and fungicides [1,2]. On the other hand, the current interest in selenium containing heterocycles is a result of their chemical properties and biological activities [3–7]. In addition numerous recent publications deals with the pharmaceutical potential of selenium compound [8–13] and therefore, new efficient syntheses are an attractive goal of chemical research [14–17]. In connection with these facts and in continuation of our efforts on the preparation of a new heterocyclic systems containing selenium and/or sulfur atoms [18–24], we have investigated reactions to discover new useful compounds for the treatment of inflammatory diseases, by replacing the chlorine atom in pyridazine moiety with additional selenium atom containing heterocycles which expected to possess an interesting profile of anti-inflammatory activity with significant analgesic effect. The heterocycles reported here are selenolo[2,3-*c*] pyridazine, pyrimido[4′,5′:4,5]selenolo[2,3-*c*]pyridazine derivatives.

2. Chemistry

The starting compound 4-cyano-5,6-diphenylpyridazine-3(2)selenone (**2**) was successfully prepared by the reaction of 3-chloro-4-cyano-5,6-diphenyl pyridazine (**1**) with sodium hydrogenselenide in ethanol in excellent yield (90%) with new dipyridazinyl diselenide derivative (**3**) as a byproduct in 5% yield. It was noticed that the natural oxidation of a selenol **2** into diselenide **3** can be accelerated by air and heating [23]. The two compounds **2** and **3** were isolated by fractional crystallization from ethanol. Upon recrystallization, compound **2** crystallized from ethanol as yellow crystals, while compound **3** crystallized from dioxan as orange crystals. The reaction of **2** with chloroacetone or phenacyl bromide in refluxing ethanol in the presence of sodium acetate as a basic catalyst afforded

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2-acetyl(benzoyl)-3-amino-4,5-diphenylselenolo[2,3-*c*]pyridazine (**4a** and **4b**), respectively, in excellent yields. In contrast, the reaction of **2** with chloro acetonitrile or ethyl chloroacetate or chloro acetamide under the same above conditions gave the corresponding selenoloacetonitrile (**5a**) or ethyl selenoloacetate (**5b**) or selenoloacetamide (**5c**) pyridazines. Treatment of **5a–c** with sodium ethoxide in refluxing ethanol underwent Thorpe–Ziegler cyclization to give the corresponding (**6a–c**) derivatives (Scheme 1).

5-Amino-3,4-diphenylselenolo[2,3-*c*]pyridazine-6-carboxamide (**6c**) was used as key intermediate in the synthesis of some new heterocyclic systems thus, the reactivity of amino group of compound **6c** was tested via its condensation with some carbonyl reagents. Reactions of **6c** with appropriate aromatic aldehydes by refluxing in acetic acid didn't give the expected Schiff's bases (**7a–c**) or (**8a–c**) instead, the tetrahydropyrimidinone derivatives (**9a–c**) were formed. Under similar conditions, compound **6c** was condensed with cyclopentanone or cyclohexanone to afford the corresponding spiro compounds (**11a**,**b**) instead of the expected Schiff's bases (**10a**,**b**) (Scheme 2).

Finally cyclocondensation of **6c** with triethylortho formate by heating in acetic anhydride led to formation of 6-methyl-3,4-diphenylpyrimido[4′,5′:4,5] selenolo[2,3-*c*]pyridazin-8(7*H*)-one (**12**). By the same manner, reaction of **6c** with acetic anhydride in absence of triethyl orthoformate gave **12** which was allowed to react with ethyl iodide in *N*,*N*-dimethylformamide, the corresponding *N*-ethylated product (**13**) was obtained (Scheme 3).

The structures of the synthesized compounds were confirmed by their physical, analytical and spectral data. Results were displayed in Table 1.

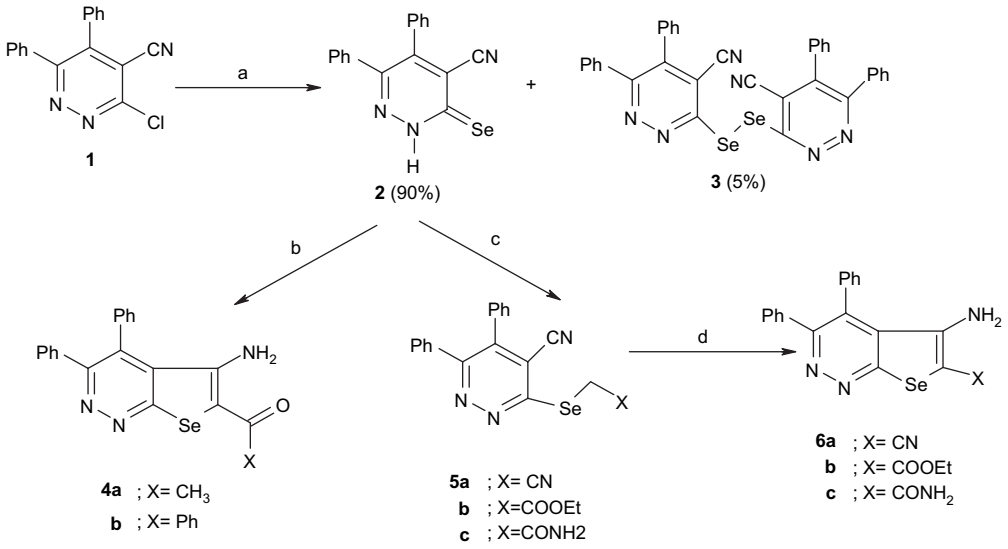
3. Pharmacological results and discussion

3.1. Anti-inflammatory activity

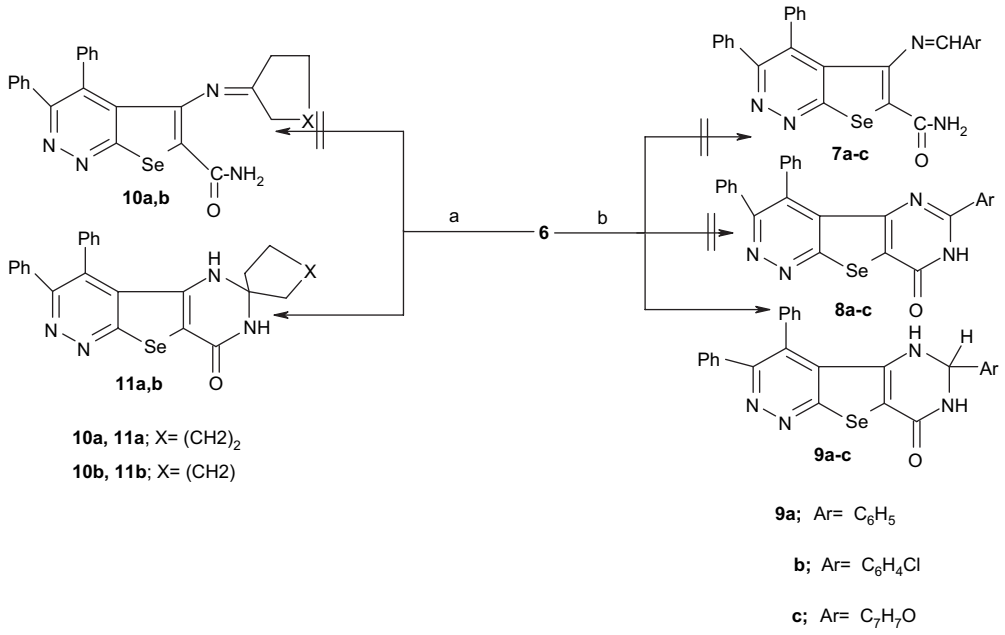
Anti-inflammatory activity of the synthesized compounds **2**, **4a**,**b**, **6a–c** and **11a** was evaluated by carrageenan induced paw edema method of Winter et al. [25]. The data are taken at 0.5, 1, 2, 3, 4 and 5 h interval. The compounds were tested at doses of 10 mg kg^{-1}. The anti-inflammatory activity of selenopyridazine derivatives is in the range from 8.9 to 50.00 (Table 2), whereas after 3 h interval the inhibition effect of compounds **2**, **4a**,**b**, **6a–c** and **11a** showed (20.5–44.4) reduction in edema of that of standard drug Indomethacin (44.1). The resulted data at 3 h interval showed that compounds **6b** and **6c** are the most active anti-inflammatory (35.2 and 44.4) respectively. On the other hand, after 5 h interval, the inhibition effect of compounds **6a–c** less about 44.4–50.00 of that Indomethacin (57.5). Compound **6c** showed anti-inflammatory activity 87% of that Indomethacin. The relation between the structure and the data which mentioned before can be discussed as follows: when 4-cyanopyridazine-3(2*H*) derivative (**2**) was cyclized into 2-acetyl-3-selenopyridazine (**4a**), the reactivity of anti-inflammatory was found to be the same as in compound **2** (33.5), while replacing benzoyl group instead of acetyl group in position 2, the reactivity of anti-inflammatory of compound (**4b**), increased to 38.8. Replacing acetyl or benzoyl groups in position 2 by cyano (**6a**) or ester (**6b**), the reactivity of anti-inflammatory was increased to 44.4. Compound **6c** having amide group in position 2 showed the greatest activity (50.0) as compared to Indomethacin (57.5). As final conclusion the presence of amide group in position 2 showed maximum anti-inflammatory activity than cyano or ester groups.

3.2. Analgesic activity

The most active anti-inflammatory compounds **4b** and **6a–c** were tested for further exploration of their analgesic properties relative to acetyl salicylic acid as reference drug at a dose level of 0.028 μmol kg^{-1} using the hot plate protocol as described in Section 4 [26]. The data are presented in Table 3. The result showed that after 3 h interval analgesic activity ranging from 36.5 to 98.4 of that reference drug (45.6). From the resulted data at 5 h interval showed that analgesic



Scheme 1. a = $NaBH_4$/Se/EtOH/reflux; b = $ClCH_2COX/CH_3COONa$/reflux; c = $ClCH_2X/CH_3COONa$/EtOH/reflux; d = NaOEt/EtOH.



Scheme 2. a = Cycloalkanones/AcOH; b = ArCHO/AcOH.

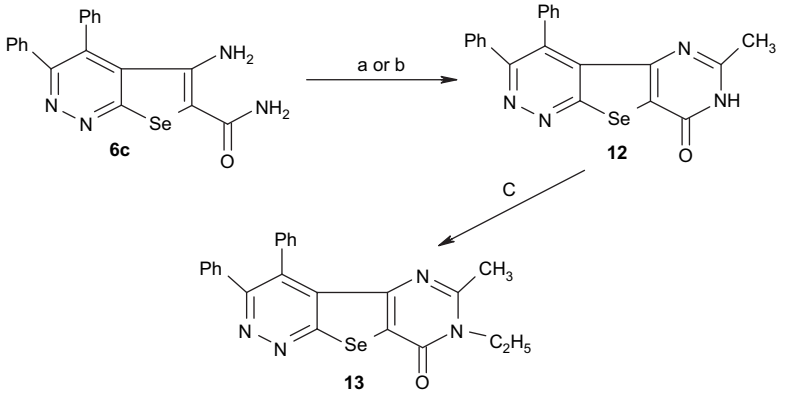
activity ranging from 24.2 to 83.4 of that of standard drug (30.0). The relation between the structure and the reactivity is as follows: when 4-cyanopyridazine-3(2*H*) derivative (**2**) was cyclized into 2-benzoyl-3-amino-selenolopyridazine (**4b**), the reactivity was found to be decreased (24.2). When benzoyl group in position 2 replaced by ester (**6b**), the reactivity was increased to 33.4. Also, when benzoyl group replaced cyano group in position 2, the analgesic activity was sharply increased than those of benzoyl or ester groups to 65.3 as compared with the standard drug. The presence of amide group in position 2 in compound **6c** showed the greatest analgesic effect (83.4) as compared to the standard drug (30.0) and less analgesic activity than amide group **4b**, **6a** and **6b**. Moreover, the activities of the tested compounds are very much higher than those of standard agents used.

3.3. Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most active compound **6c** was determined (i.p.) in mice according to reported procedures [27]. The animals got injection (i.p.) of a certain grade. The results showed that the (LD_{50}) of tested compound **6c** was non-toxic at doses up to 250 mg kg^{-1}.

3.4. Anti-microbial effects

The results obtained from the anti-microbial screening of some newly synthesized compounds (**4b** and **6a–c**) against representatives of bacteria and fungi are listed in Table 4 and according to reported method [28]. The used organisms are; *Bacillus cereus* (as Gram-positive bacteria), *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens* (as Gram-negative bacteria) and four fungal species including *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Candida albicans*. Data showed that none of the synthesized compounds has a considerable antimicrobial activity against the tested organisms except for **6a** and **6c** which showed strong fungicidal effect while **6b** exhibited a moderate fungicidal effect against *C. albicans*. The minimum inhibition concentration (MIC) of these compounds was 0.5 μg ml^{-1} (50%). The strong fungicidal effect of these



Scheme 3. a = Triethylorthoformate/Ac2O/8 h; b = Ac2O/8 h; c = C_2H_5I/K_2CO_3.

Table 1
Physical and spectral data of compounds (**2**, **3**, **4a**,**b**, **5a–c**, **6a–c**, **9a–c**, **11a**,**b**, **12** and **13**)

Compd. no.	Mp °C (yield %)	Mol. formula (M/wt)	IR (cm^{-1})	^1H NMR (δ, ppm)
2[a,b]	218–220 (90)	$C_{17}H_{11}N_3Se$ (336.27)	3190 (NH), 2200 (CN)	DMSO-d_6: 7.9 (s, 1H, NH); 7.15–7.50 (m, 10H, Ar–H)
3	>300 (5)	$C_{34}H_{20}N_6Se_2$ (670.52)	2200 (CN)	DMSO-d_6: 7.20–760 (m, 20H, Ar–H)
4a	210–212 (86)	$C_{20}H_{15}N_3OSe$ (392.34)	3320, 3440 (NH_2); 1645 (C=O)	DMSO-d_6: 7.3–7.8 (m, 10H, Ar–H); 6.4 (s, 2H, NH_2); 2.3 (s, 3H, CH_3)
4b	215–217 (60)	$C_{25}H_{17}N_3OSe$ (454.41)	3250, 3450 (NH_2); 1640 (C=O)	DMSO-d_6: 7.3–7.7 (m, 15H, Ar–H); 6.8 (s, 2H, NH_2)
5a	114–116 (63)	$C_{19}H_{12}N_4Se$ (375.31)	2200 (CN)	$CDCl_3$: 7.2–7.6 (m, 10H, Ar–H); 4.10 (s, 2H, CH_2)
5b	82–84 (72)	$C_{21}H_{17}N_3O_2Se$ (422.37)	2200 (CN); 1720 (C=O ester)	$CDCl_3$: 7.2–7.7 (m, 10H, Ar–H); 4.13 (s, 2H, CH_2); 3.95 (q, 2H, CH_2); 1.38 (t, 3H, CH_3)
5c	135–137 (74)	$C_{19}H_{14}N_4OSe$ (393.33)	3400, 3250 (NH_2); 2200 (CN); 1624 (C=O)	$CDCl_3$: 7.3–7.6 (m, 10H, Ar–H); 6.13 (s, 2H, NH_2); 4.01 (s, 2H, CH_2)
6a[a]	270, decomposed (80)	$C_{19}H_{12}N_4Se$ (375.31)	3390, 3200 (NH_2); 2200 (CN)	DMSO-d_6: 7.2–7.40 (m, 10H, Ar–H); 5.80 (s, 2H, NH_2)
6b[a]	180–182 (75)	$C_{21}H_{17}N_3O_2Se$ (422.37)	3400, 3200 (NH_2); 1667 (C=O ester)	$CDCl_3$: 7.3–7.8 (m, 10H, Ar–H); 4.89 (s, 2H, NH_2); 3.95 (q, 2H, CH_2); 1.23 (t, 3H, CH_3)
6c[a,b]	280–282 (85)	$C_{19}H_{14}N_4OSe$ (393.33)	3500, 3450, 3300, 3250 (2 NH_2); 1650 (C=O)	DMSO-d_6: 7.22–7.45 (m, 12H, Ar–H + NH_2); 5.87(s, 2H, NH_2)
9a	>300 (82)	$C_{26}H_{18}N_4OSe$ (481.44)	3400, 3200 (2NH); 1640 (C=O)	DMSO-d_6: 7.2–7.50 (m, 15H, Ar–H); 8.6 (s, 1H, CONH); 5.8 (s, 1H, CH–tetrahydropyrimidine); 4.8 (s, 1H, NH)
9b[b]	>300 (92)	$C_{26}H_{17}N_4OClSe$ (515.88)	3390, 3180 (2NH); 1640 (C=O)	DMSO-d_6: 7.1–7.40 (m, 14H, Ar–H); 8.5 (s, 1H, CONH); 5.9 (s,1H, CH–tetrahydropyrimidine); 4.95 (s, 1H, NH)
9c	>300 (80)	$C_{27}H_{20}N_4O_2Se$ (511.47)	3390, 3200 (2NH), 1640 (C=O)	DMSO-d_6: 7.1–7.40 (m, 14H, Ar–H); 8.7 (s, 1H, CONH); 5.5 (s, 1H, CH–tetrahydropyrimidine); 4.6 (s, 1H, NH); 3.7 (s, 3H, OCH_3)
11a[a,b]	>300 (83)	$C_{25}H_{22}N_4OSe$ (473.47)	3400, 3200 (2NH); 1650 (C=O)	DMSO-d_6: 7.99 (s, 1H, CONH); 7.26–7.45 (m, 10H, Ar–H); 4.12 (s, 1H, NH); 1.24–3.55 (m, 10H, $5CH_2$–cyclohexylidene)
11b	>300 (80)	$C_{24}H_{20}N_4OSe$ (459.44)	3400, 3200 (2NH); 1650 (C=O)	DMSO-d_6: 8.00 (s, 1H, CONH); 7.2–7.5 (m, 10H, Ar–H); 4.5 (s, 1H NH); 1.4–2.3 (m, 8H, $4CH_2$–cyclopentylidene)
12	>300 (72)	$C_{21}H_{14}N_4OSe$ (417.35)	3200 (NH); 1670 (C=O)	DMSO-d_6: 8.01 (s, 1H, NH); 7.3–7.5 (m, 10H, Ar–H); 1.98 (s, 3H, CH_3)
13	155–157 (75)	$C_{23}H_{18}N_4OSe$ (445.41)	1680 (C=O)	DMSO-d_6: 7.3–7.6 (m, 10H, Ar–H); 2.01 (s, 3H, CH_3); 1.19–1.31 (t, 3H, CH_3); 3.95–4.01 (q, 2H, CH_2)

[a] MS (see Section 4).
[b] ^{13}C NMR of compounds **2**, **6c**, **9b** and **11a** (see Section 4).

compounds is presumably due to presence of either cyano or amide group. These compounds seem to be good fungicidal candidates against dermatophytic fungi. Generally, it was noticed that the activities of the tested compounds are much less than those of standard antifungal and antibacterial agents used.

4. Experimental protocols

4.1. Chemistry

Meting points were determined using a Kofler melting point apparatus and were uncorrected. IR (KBr) spectra were recorded on a Pye-Unicam SP3-100 instrument. ^1H NMR spectra were obtained on a Varian EM 390 USA at Assiut University using tetramethylsilane as an internal reference. ^{13}C NMR spectra were recorded on a Mercury-300BB NMR300 at Cairo University. Mass spectra were recorded on a JEOL-JMS-AX 500 at Cairo National Research Center and JEOL-JMS 600 at Assiut University, Assiut, Egypt. Elemental analyses were obtained on an Elementer Vario EL 1150C analyser. Purity of the compounds was checked by TLC.

All physical and spectral data in Table 1.

*4.1.1. 4-Cyano-5,6-diphenylpyridazine-3(2H)selenone (**2**, $C_{17}H_{11}N_3Se$)*

A mixture of the corresponding chloropyridazine **1** (2.91 g, 10 mmoles), selenium metal (1 g, 12 mmol) and sodium

Table 2
Anti-inflammatory activity of **2**, **4a**,**b**, **6a**, **c** and **11a** on carrageenan induced paw edema in rats (% inhibition ± S.E.M[a])

Time	Indomethacin	**2**	**4a**	**4b**	**6a**	**6b**	**6c**	**11a**
0.5	11.7 ± 0.15	8.8 ± 0.03	8.8 ± 0.03	9.0 ± 0.04	8.8 ± 0.12	8.8 ± 0.03	8.7 ± 0.03	8.7 ± 0.34
1	20.5 ± 0.02	17.2 ± 0.01	17.2 ± 0.01	9.0 ± 0.04	9.9 ± 0.08	17.2 ± 0.01	14.7 ± 0.03	9.9 ± 0.08
2	32.3 ± 0.01	20.5 ± 0.11	20.5 ± 0.11	26.4 ± 0.10	29.4 ± 0.06	23.5 ± 0.06	26.4 ± 0.10	23.5 ± 0.06
3	44.1 ± 0.09	23.5 ± 0.06	20.5 ± 0.11	29.4 ± 0.06	29.4 ± 0.06	35.2 ± 0.14	44.4 ± 0.09	23.5 ± 0.06
4	52.7 ± 0.04	30.5 ± 0.04	28.5 ± 0.01	35.5 ± 0.03	36.1 ± 0.04	41.6 ± 0.04	47.2 ± 0.04	30.5 ± 0.04
5	57.5 ± 0.29	30.5 ± 0.04	33.5 ± 0.08	38.8 ± 0.08	44.4 ± 0.00	44.4 ± 0.00	50.0 ± 0.08	38.8 ± 0.08

[a] S.E.M. = standard error mean and all showed at least significant difference at $p < 0.05$ in comparison with control group.

Table 3
Analgesic activity of **4b**, **6a–c** on hot plate[a]

Compd. no.	Reaction time					
	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	17.7 ± 0.55	17.0 ± 0.61	18.0 ± 1.11	18.7 ± 0.71	19.1 ± 0.80	18.5 ± 0.66
Aspirin	36.2 ± 0.68	58.9 ± 0.72	50.5 ± 0.67	45.6 ± 0.56	39.2 ± 0.65	30.01 ± 0.60
6a	67.9 ± 1.01	77.9 ± 0.96	75.5 ± 1.00	79.8 ± 0.78	75.5 ± 0.80	65.3 ± 0.75
6b	21.6[b] ± 0.78	42.8 ± 0.36	39.8 ± 0.45	41.3 ± 0.48	39.6 ± 0.60	33.4 ± 0.30
6c	90.3 ± 0.65	97.8 ± 0.60	99.4 ± 0.68	98.4[b] ± 0.50	90.5 ± 0.49	83.4 ± 0.51
4b	25.2 ± 0.32	27.1 ± 0.49	30.6 ± 0.53	36.5 ± 0.62	31.9 ± 0.36	24.2 ± 0.43

[a] Each value represents the mean ± S.E. and all showed at least significant difference at $p < 0.05$ in comparison with control group.
[b] Not significant.

borohydride (1.2 g, 32 mmol) were refluxed in 50 ml ethanol for 5 h. The mixture was cooled and poured in ice/HCl. The solid thus separated out was filtered, dried and recrystallized from ethanol. ^{13}C NMR (DMSO-d_6, 75 MHz) δ 158.90 (C=N of pyridazine), 155.98, 144.84, 142.08, 134.86, 134.08, 129.99, 129.37, 128.92, 128.30, 127.88 (aryl), 118.02 (CN), 114.78 (C–CN); mass spectrum of compound **2** exhibited molecular ion peak at *m/z*, (%) 336 (M^+, 45%) and the other important fragments were observed at 337 ($M^+ + 1$, 87%), 338 ($M^+ + 2$, 19%), 265 [60], 178 [100], 140 [50], 77 [80].

4.1.2. 3,3′-Bis(4-cyano-5,6-diphenyl)dipyridazinyldiselenide (**3**, $C_{34}H_{20}N_6Se_2$)

3,3′-Bis(4-cyano-5,6-diphenyl)dipyridazinyldiselenide precipitated during reflux of the above reaction mixture and recrystallized from dioxan as orange crystals.

4.1.3. 5-Amino-3,4-diphenyl-6-substitutedselenolo[2,3-c]pyridazines (**4a**, $C_{20}H_{15}N_3OSe$; **4b**, $C_{25}H_{17}N_3OSe$)

A mixture of compound **2** (1.96 g, 6 mmoles), fused sodium acetate (0.98 g, 12 mmol) and chloroacetone or phenacyl bromide (6 mmol) in 30 ml ethanol was heated under reflux for 2 h, the reaction mixture allowed to cool and poured into 50 ml ice water. The solid product was collected by filtration and recrystallized from ethanol.

4.1.4. 4-Cyano-5,6-diphenyl-3-substitutedselenolopyridazines (**5a**, $C_{19}H_{12}N_4Se$; **5b**, $C_{21}H_{17}N_3O_2Se$; **5c**, $C_{19}H_{14}N_4OSe$)

A mixture of compound **2** (0.5 g, 15 mmol), fused sodium acetate (1.4 g, 17 mmol) and chloroacetonitrile or ethyl chloroacetate or chloroacetamide (15 mmol) in 30 ml ethanol was heated under reflux for 2 h, the reaction mixture allowed to cool and poured into 50 ml ice water. The solid product was collected by filtration and recrystallized from ethanol.

4.1.5. 5-Amino-3,4-diphenyl-6-substitutedselenolo[2,3-c]pyridazines (**6a**, $C_{19}H_{12}N_4Se$; **6b**, $C_{21}H_{17}N_3O_2Se$; **6c**, $C_{19}H_{14}N_4OSe$)

Compounds **5a–c** (13 mmol) and sodium ethanolate (0.5 g Na in 10 ml EtOH) was refluxed for 1 h, and then allowed to cool. The solid product was collected and recrystallized: compounds **6a** and **6b** from ethanol while **6c** recrystallized from dioxan. ^{13}C NMR (DMSO-d_6, 75 MHz) for compound **6c** δ 167.68 (C=O), 162.79 (C=N of pyridazine), 156.12, 146.41, 143.54, 136.76, 135.11, 132.58, 129.89, 129.46, 129.04, 128.31, 127.86, 127.51 (aryl), 103.10 (C=C–selenophene ring); mass spectra of compounds **6a** exhibited molecular ion peak at *m/z*, (%) 375 (M^+, 61%) and the other important fragments were observed at 374 ($M^+ - 1$, 35%), 348 [30], 268 [10], 240 [17], 77 [4], for **6b** at 422 (M^+, 100%), and the other important fragments were observed at 423 ($M^+ + 1$, 25%), 376 [85], 348 [77], 240 [42], 77 [55]

Table 4
Fungal and anti-microbial activity of compounds **4b**, **6a–c**

Organism		Sample no.																
		6a (2%)			**6b** (1%)		**6c** (2%)			**4b** (1%)		Reference[a]						
		Minimum inhibitory concentration (μg ml^{-1})																
		1%	0.5%	0.25%	0.5%	0.25%	1%	0.5%	0.25%	0.5%	0.25%	1%	0.5%	0.25%	0.125%	0.1%	0.05%	0.25%
A. flavus	Fungi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. niger		0	0	0	0	0	0	0	0	0	0	40	32	26	18	12	8	0
C. albicans		17	9	0	9	0	14	9	0	0	0	25	18	14	10	0	0	0
F. oxysporum		0	0	0	0	0	0	0	0	0	0	22	16	14	8	0	0	0
B. cereus (+ve)	Bacteria	0	0	0	0	0	0	0	0	0	0	25	20	18	16	9	8	0
E. coli (−ve)		0	0	0	0	0	0	0	0	0	0	12	10	9	8	0	0	0
P. aeruginosa (−ve)		0	0	0	0	0	0	0	0	0	0	29	25	20	18	10	8	0
S. marcescens (−ve)		0	0	0	0	0	0	0	0	0	0	24	21	19	18	16	10	0

[a] Reference: (antifungal = Dermatin), (antibacterial = Ampicillin).

and for **6c** at 393 (M^+, 62%), 394 ($M^+ + 1$, 100%), 348 [30], 202 [24], 77 [28].

4.1.6. Reaction of **6c** with aromatic aldehydes or cycloalkanones; formation of 6-substitutedaryl-3, 4-diphenylpyrimido[4′,5′:4,5]selenolo[2,3-c] pyridazine-8(7H)-one (**9a–c**)or 6-spiro(cycloalkane)-3, 4-diphenylpyrimido [4′,5′:4,5]selenolo[2,3-c]pyridazine-8(7H)-one (**11a,b**)

A mixture of **6c** (1 g, 25 mmol) and the corresponding aromatic aldehydes or cycloalkanones (25 mmol) was heated under reflux in gl. acetic acid (20 ml) for 5–7 h. The solid was collected by filtration and recrystallized from acetic acid. ^{13}C NMR (DMSO-d_6, 75 MHz) for compounds **9b** δ (164.2) (C=O), 161.54 (C=N of pyridazine), 156.02, 143.84, 143.54, 139.54, 136.53, 135.44, 132.80, 132.49, 129.91, 129.52, 129.05, 128.15, 127.99, 127.95 (aryl), 113.82 and 65.14 (CH–pyrimidine) and **11a** δ (161.32) (C=O), 155.86 (C=N of pyridazine), 142.65, 136.43, 132.82, 129.92, 129.41, 129.10, 128.47, 128.05, 127.63 (aryl), 69.63, 66.29 (CH_2–spiro), 35.47, 23.63, 20.82 ($3CH_2$–cyclohexane); mass spectrum of compound **11a** exhibited molecular ion peak at 473 (M^+, 11%), 474 ($M^+ + 1$, 45%), 475 ($M^+ + 2$, 14%) and the other important fragments were observed at, 431 [100], 202 [15], 77 [11].

4.1.7. 6-Methyl-3,4-diphenylpyrimido[4′,5′:4,5]selenolo [2,3-c]pyridazine-8(7H)-one (**12**, $C_{21}H_{14}N_4OSe$)

Compound **6c** (0.78 g, 20 mmol) and triethylortho formate (2 ml) in redistilled acetic anhydride 20 ml was heated under reflux for 8 h, and then left to cool. The precipitate was filtered and crystallized from dioxan. The same product (**12**) was obtained upon treatment with acetic anhydride only.

4.1.8. Reaction of **12** with ethyl iodide; formation of N-ethylated product (**13**, $C_{23}H_{18}N_4OSe$)

To a solution of **12** (0.5 g, 12 mmol) in DMF, anhydrous K_2CO_3 (0.5 g) and ethyl iodide (0.15 ml, 12 mmol) were added. The resulting mixture was heated on a water bath for 5 h and then left to cool and diluted with 20 ml ice water. The precipitate was collected by filtration and recrystallized from ethanol.

4.2. Biological screening

The biological screening was carried out at the Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt. Animals were obtained from the animal house of the Faculty of Medicine. The experiments were performed with albino rats of Wister strain of either sex, weighing 100–120 g. The animals were maintained at 25 ± 2 °C and 50 ± 2% relative humidity, 12 h light/dark cycle. Food and water were freely available up to the time of experiments. The test compounds were dissolved in 1% carboxyl methyl cellulose (CMC) solution.

4.2.1. Anti-inflammatory activity

The anti-inflammatory activity of seven representatives of the synthesized compounds (**2**, **4a**,**b**, **6a–c** and **11a**) was evaluated according to the method described by Winter et al. [25], where a pedal inflammation in rat paws induced by sub-plantar injection of 0.2 ml carrageenan (0.2%) suspension into the right hind of the rats. Male adult albino rats (100–120 g) were divided into six groups, each of five animals. The thickness of rat paw was measured by a Veriner caliper (SMIEC, China) before and after 1 h of carrageenan injection to detect the inflammation induced by carrageenan. Test compounds at doses of 10 mg kg^{-1} were injected i.p. to nine groups of rats 1 h after injection of carageenan. Control group received the vehicle (5% gum acacia), while reference group received Indomethacin at 10 mg kg^{-1}. The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1, 2, 3, 4 and 5 h, after injection of the test compounds, the reference drug, and the vehicle. The percent anti-inflammatory activity was calculated according to the formula given below.

% Anti-inflammatory activity $= (V_c - V_t/V_c) \times 100$; where V_t represents the mean increase in paw volume in rats treated with the test compounds and V_c represents the mean increase in paw volume in control group of rats. Data are expressed as mean ± S.E.M. The results are listed in Table 2.

4.2.2. Analgesic activity

The analgesic activity of compounds (**4a** and **6a–c**) was determined in mice using the hot plate method [26] in comparison to Indomethacin. In this method, the time taken by the mouse to lick its feet or to jump within a Plexiglas cylinder placed on a hot plate surface (55 °C) was determined. This reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity. Male adult albino mice (20–25 g) were divided into six groups, each of five animals. Nine test compounds and the reference drug were injected i.p. at a dose level of 10 mg kg^{-1} into mice. Control group of animals was similarly treated with 5% gum acacia. The reaction time was evaluated directly after 0.5, 1, 2, 3, 4 and 5 h of injection. % Analgesic activity $= (n - n'/n) \times 100$; where n' represents the mean number of writhes of the test compounds and n represents the mean number of writhes of control group of rats. Data are expressed as mean ± S.E.M. The results are listed in Table 3.

4.2.3. Determination of acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most active compound **6c** was determined in mice. A group of male adult albino mice of five animals (25–30 g) was injected (i.p.) at a certain grade. The percentage of mortality was determined 72 h after injection. Computation of LD_{50} was processed by a graphical method [27].

4.2.4. Antibacterial and antifungal activities

The antibacterial activity of some newly synthesized compounds (**4a** and **6a–c**) was determined in vitro by using disc diffusion method [28] against variety of pathogenic micro organisms; *B. cereus* (P-70) (Gram-positive bacteria), *E. coli* (P-69), *P. aeruginosa* (P-72) and *S. marcescens* (P-67) (Gram-negative bacteria) at 100, 50, 25 μg ml^{-1} concentrations,

respectively, in the nutrient agar media by measuring the zone of inhibition in mm. The solutions required concentrations (100, 50, 25 μg ml^{-1}) in DMF as a solvent using Ampicillin as a reference drug and the results are listed in Table 4. The antifungal screening of the compounds (**4a** and **6a–c**) was carried out in vitro by paper disc method against four fungi *A. flavus* (3372), *A. niger* (3364), *C. albicans* (421) and *F. oxysporum* (208) by using Dermatin in 100 μg ml^{-1} as a reference and the results are listed in Table 4.

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