Full Paper

Selenium-Containing Heterocycles: Synthesis and Pharmacological Activities of Some New 4-Methylquinoline-2(1*H*) Selenone Derivatives

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Several selenolo[2,3-*b*]quinolines and pyrimido[4′,5′:4,5]selenolo[2,3-*b*]quinolines were prepared by annulations via reaction of NaSeH with 2-chloro-3-cyano-4-methylquinoline **1** followed by reactions with aromatic aldehydes, cycloalkanones, and acetic anhydride. Spectroscopic (IR, ^{1}H-NMR, and MS) properties of the synthesized compounds are reported. Some selected compounds **5a**, **7b**, **7c**, **8b–d**, **9a**, **11b**, and **11d** were investigated for their anti-inflammatory and analgesic activities; in addition, the most active compounds were tested for their ulcerogenicity and acute toxicity. Moreover, some of the test compounds **7c**, **9a**, **11b**, and **11d** were screened for their antibacterial and antifungal activities.

Keywords: Fused quinolines / Pharmacological Screening / Pyrimidoselenolo quinolines / Quinolines

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Introduction

Quinoline drugs, which include quinine, quinidine, chloroquine, mefloquine, and halofantrine are widely used as antimalarial agents [1, 2], also, quinoline derivatives possess a broad spectrum of biological activities such as antifungal [3], antibacterial [4], antileishmanial [5] in addition to anti-arrhythmic [6]. On the other hand, the introduction of selenium into organic compounds often permits modification of their chemical properties and biological activities [7–11]. A literature survey indicates that only few publications have mentioned the incorporation of a selenium atom in the quinoline nucleus [12–14]. Consequently, synthesis and biological screening of selenoquinoline derivatives may be considered a virgin research area. Previous work in our laboratory describes the synthesis of selenolo[2,3-*c*]pyridazine derivatives, which indicate that certain compounds bearing the selenophene and pyridazine nucleus possess significant anti-inflammatory and analgesic activities with strong fungicidal effects [15]. This work attempts to further expand the synthetic procedures of selenium- and/or sulfur-containing heterocyclic compounds [16–23]. We investigated selenophene and quinoline systems combined with a fused ring to give compact structures and screened these compounds for their inflammatory and analgesic effects. Prompted by these observations, herein we report the reaction of sodium hydrogenselenide with 2-chloro-3-cyano-4-methylquinoline **1** to give a new series of selenolo[2,3-*b*]quinoline derivatives and their pharmacological activities.

Results and discussion

Chemistry

2-Chloro-3-cyano-4-methylquinoline **1** was prepared according to a known method [24] and reacted with sodium hydrogenselenide in ethanol to give 3-cyano-4-methylquinoline-*2(1H)*selenone **2** in good yield (70%) with diquinolinyl diselenide derivative **3** as a by-product in moderate yield (22%). The two compounds **2** and **3** were isolated by fractional crystallization from ethanol.

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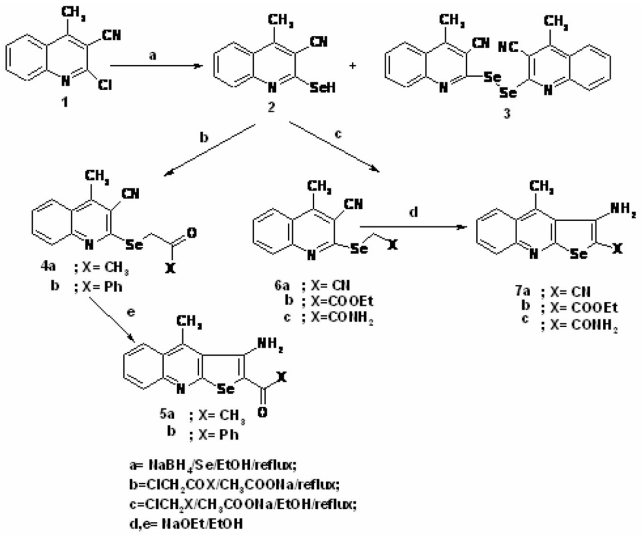
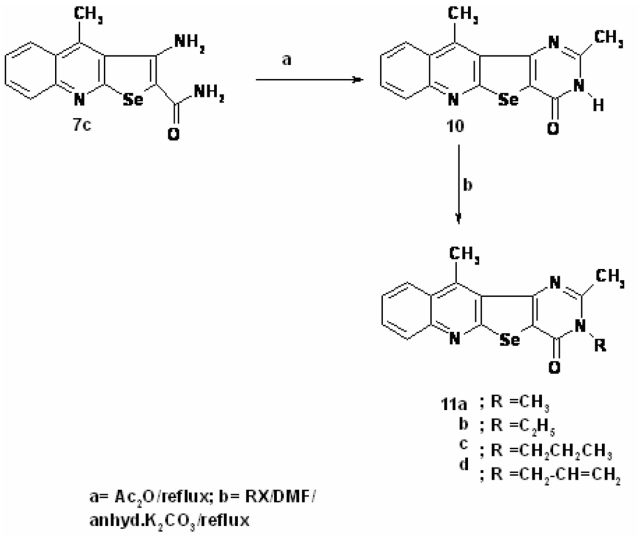
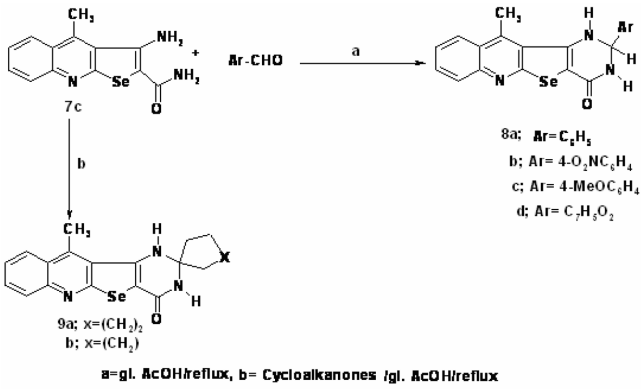


Scheme 1. Synthesis route of compounds **1**–**7**.



Scheme 3. Synthesis route of compounds **10** and **11**.



Scheme 2. Synthesis route of compounds **8** and **9**.

Upon recrystallization, compound **2** crystallized from ethanol as yellow crystals, while compound **3** crystallized from dioxane as red crystals. The structures of the obtained products were established on the basis of their mass spectra and elemental analyses. Refluxing of compound **2** with chloro acetone or phenacyl bromide in ethanol in the presence of sodium acetate as a basic catalyst, afforded 2-acetyl(benzoyl)-3-amino-4-methyl selenolo[2,3-*b*]quinoline **5a** or **5b**, respectively, in excellent yields, via the intermediates **4a** or **4b**. Furthermore, 3-cyano-4-methylquinoline-*2(1H)*selenone **2** reacted with chloro acetonitrile or ethyl chloroacetate or chloro acetamide under the same conditions and gave the corresponding selenoloacetonitrile **6a**, ethyl selenoloacetate **6b**, or selenoloacetamide **6c** quinoline derivatives. Compounds **6a–c** underwent smooth Thorpe–Ziegler cyclization, when treated with sodium ethoxide in ethanol, to give the corresponding compound derivatives **7a–c** (Scheme 1). A new series of pyrimido[4′,5′:4,5]selenolo[2,3-*b*]quinolines **8a–d** were prepared by reaction of 3-amino-4-methylselenolo[2,3-*b*]quinoline-2-carbamide **7c** with the appropriate aromatic aldehyde and/or cyclohexanone or cyclopentanone reagents, to give the corresponding spiro compounds **9a**, **b** (Scheme 2). Furthermore, compound **7c** was converted into various pyrimidine derivatives of **11a–d** through compound **10** by treatment with acetic anhydride followed by alkyl halide in the presence of anhydrous potassium carbonate in DMF (Scheme 3).

The structures of the synthesized compounds were characterized by their physical, analytical, and spectral data. The IR spectrum of compound **10** showed characteristic absorption bands at 3308 (NH) and 1660 cm^{-1} (C=O), whereas, in compounds **11a–d**, the absence of the absorption band of (NH). The results were displayed in Table 1.

Biological screening

Anti-inflammatory activity

In the present study, nine compounds, **5a, 7b, 7c, 8b–d, 9a, 11b**, and **11d** were selected and tested for their anti-inflammatory activity using the carrageenan-induced rat paw edema method in comparison to indomethacin as a reference drug [25]. The results are listed in Table 2, at a dose level of 0.028 μmol/kg. The results showed that after 3 h the inhibition effect of compounds was about 53.5–80.0% less of that of indomethacin. Compounds **7c**, **9a**, **11b**, and **11d** are the most active ones. It is worth noting that the conversion of **7b** (R = COOEt) to compound **7c** (R = $CONH_2$) resulted in an enhancement of activity. In gen-

Table 1. Physical and spectral data of compounds **2**, **3**, **4a**, **b**, **5a**,**b**, **6a–c**, **7a–c**, **8a–d**, **9a**,**b**, **10**, and **11a–d**.

Compound	Mp. (°C) Yield (%)	Mol. formula (M/wt)	IR (cm^{-1})	^{1}H-NMR (δ, ppm)
2[a]	298-300 (70)	$C_{11}H_8N_2Se$ (247.15)	2200 (CN)	DMSO-d_6 : 14.95 (s, 1H SeH, exchangeable); 7.40 – 8.10 (m, 4H Ar-H); 2.70 (s, 3H, CH_3)
3[a]	>300 (22)	$C_{22}H_{14}N_4Se_2$ (492.31)	2200 (CN), s. peak	DMSO-d_6 : 7.20 – 760 (m, 8H Ar-H); 2.70 (s, 6H 2CH_3)
4a	100 – 102 (85)	$C_{14}H_{12}N_2OSe$ (303.22)	2200 (CN),; 1685 (C=O)	$CDCl_3$: 7.40 – 7.90 (m, 4H Ar-H); 4.15 (s, 2H CH_2); 2.80 (s,3H CH_3); 2.40 (s, 3H CH_3)
4b	230 – 232 (78)	$C_{19}H_{14}N_2OSe$ (365.29)	2200 (CN), 1690 (C=O)	DMSO-d_6 : 7.40 – 8.20 (m, 9H Ar-H); 4.90 (s, 2H CH_2); 2.90 (s, 3H CH_3)
5a	218 – 220 (75)	$C_{14}H_{12}N_2OSe$ (303.22)	3320,3440 (NH_2); 1645 (C=O)	DMSO-d_6 : 7.50 – 8.10 (m, 4H Ar-H); 4.90 (s, 2H NH_2); 2.98 (s, 3H CH_3-quinoline) 2.40 (s, 3H CH_3)
5b	272 – 274 (75)	$C_{19}H_{14}N_2OSe$ (365.29)	3250, 3450 (NH_2); 1640 (C=O)	DMSO-d_6 : 7.60 – 8.10 (m, 9H Ar-H); 4.950 (s, 2H NH_2); 2.98 (s, 3H CH_3-quinoline)
6a	190 – 192 (75)	$C_{13}H_9N_3Se$ (286.19)	2200 (CN), s. peak	$CDCl_3$: 7.60 – 8.30 (m, 4H Ar-H); 4.02 (s, 2H CH_2); 2.75 (s, 3H CH_3-quinoline)
6b	142 – 144 (80)	$C_{15}H_{14}N_2O_2Se$ (333.24)	2200 (CN); 1710 (C=O ester)	$CDCl_3$: 7.70 – 8.30 (m, 4H Ar-H); 4.50 (q, 2H CH_2); 4.00 (s, 2H CH_2); 3.20 (s, 3H CH_3-quinoline); 1.60 (t, 3H CH_3)
6c	240 – 242 (85)	$C_{13}H_{11}N_3OSe$ (304.21)	3300,3430 (NH_2); 1680 (C=O); 2200 (CN)	DMSO-d_6 : 7.30 – 8.50 (m, 4H Ar-H); 7.10 (broad, 2H NH_2, exchangeable); 4.02 (s, 2H CH_2); 2.75 (s, 3H CH_3-quinoline)
7a	>300 (70)	$C_{13}H_9N_3Se$ (286.19)	3390,3200 (NH_2); 2200 (CN)	DMSO-d_6 : 7.90 – 8.00 (m, 4H Ar-H); 7.40 (s, 2H NH_2); 3.20 (s, 3H CH_3-quinoline)
7b[a]	280 – 282 (45)	$C_{15}H_{14}N_2O_2Se$ 333.24)	3400,3200 (NH_2) ; 1668 (C=O ester)	DMSO-d_6 : 7.90 – 8.00 (m, 4H Ar-H); 6.60 (s, 2H NH_2); 4.20 – 4.30 (q, 2H CH_2); 3.30 (s, 3H CH_3-quinoline); 1.28 (t, 3H CH_3)
7c[a]	275 – 277 (77)	$C_{13}H_{11}N_3OSe$ (304.21)	3500,3450, 3300, 3250 (NH_2); 1660 (C=O)	DMSO-d_6 : 8.50 (s,2H $CONH_2$); 7.90 – 8.00 (m, 4H Ar-H); 7.20 (s, 2H NH_2); 3.20 (s, 3H CH_3-quinoline)
8a	>300 (80)	$C_{20}H_{15}N_3OSe$ (392.31)	3400,3200 (2NH); 1640 (C=O)	DMSO-d_6 : 8.65 (s, 1H NH); 7.6 – 8.50 (m , 9H Ar-H); 7.30 (d, 1H CH); 5.98 (d, 1H NH); 3.20 (s, 3H CH_3-quinoline)
8b	230 – 232 (83)	$C_{20}H_{14}N_4O_3Se$ (437.31)	3420, 3200 (2NH); 1660 (C=O)	DMSO-d_6 : 8.85 (s, 1H NH); 7.90 – 8.40 (m , 8H Ar-H); 7.40 (d, 1H CH); 6.15 (d, 1H NH); 2.00 (s, 3H CH_3-quinoline)
8c	>300 (72)	$C_{21}H_{17}N_3O_2Se$ (422.34)	3400, 3200 (2NH); 1645 (C=O	DMSO-d_6 : 9.40 (s, 1H NH); 7.00 – 8.80 (m, 8H Ar-H); 6.90 (d, 1H CH); 5.8 (d, 1H NH); 4.70 (s, 3H OCH_3); 3.00 (s, 3H CH_3-quinoline)
8d	>300 (55)	$C_{21}H_{15}N_3O_3Se$ (436.32)	3400, 3200 (2NH); 1655 (C=O	DMSO-d_6 : 8.40 (s, 1H NH); 7.30 – 8.20 (m, 7H Ar-H); 6.98 (d, 1H CH); 5.9(s, 2H CH_2-aldehyde); 5.70 (d, 1H NH); 3.10 (s, 3H CH_3-quinoline)
9a[a]	>300 (76)	$C_{19}H_{19}N_3OSe$ (384.33)	3350, 3150 (2NH); 1660 (C=O	DMSO-d_6 : 8.30 (s, 1H NH); 7.30 – 8.00 (m, 4H Ar-H); 6.00 (s, 1H NH); 3.20 (s, 3H CH_3-quinoline); 1.5 – 2.4 (m, 10H-cyclohexane)
9b[a]	>300 (65)	$C_{18}H_{17}N_3OSe$ (370.31)	3300, 3100 (2NH); 1655 (C=O	DMSO-d_6 : 8.30 (s, 1H NH); 7.30 – 8.00 (m, 4H Ar-H); 6.00 (s, 1H NH); 3.20 (s, 3H CH_3-quinoline); 1.5 – 2.10 (m, 8H-cyclopentane)
10[a]	300 (52)	$C_{15}H_{11}N_3OSe$ (328.23)	3200 (NH); 1670 (C=O)	DMSO-d_6 : 8.40 (d,1H NH); 7.80 – 7.98 (m, 4H Ar-H); 2.50 (s, 3H CH_3-quinoline); 1.98 (s, 3H CH_3)
11a[a]	155 – 157 (60)	$C_{16}H_{13}N_3OSe$ (342.25)	1680 (C=O)	CDCL3: 7.20 – 7.50 (m, 4H Ar-H); 3.50 (s, 3H CH_3); 3.20 (s, 3H CH_3); 2.50 (s, 3H CH_3-)
11b[a]	170 – 172 (75)	$C_{17}H_{15}N_3OSe$ (356.28)	1680 (C=O)	CDCL3: 7.30 – 8.10 (m, 4H Ar-H); 4.00(q, 2H NCH_2); 3.20 (s, 3H CH_3-quinoline); 2.50 (s, 3H CH_3-pyrimidine); 1.3 (t, 3H CH_2CH_3)
11c[a]	165 – 167 (63)	$C_{18}H_{17}N_3OSe$ (370.31)	1675 (C=O)	DMSO-d_6 : 7.50 – 8.20 (m, 4H Ar-H); 4.20 (d, 2H NCH_2); 3.30 (s, 3H CH_3-quinoline); 2.55 (m, 2H CH_2-propane) ; 1.48 (d, 3H CH_3-pyrimidine); 1.00 (s, 3H CH_3-propane)
11d[a]	126 – 128 (70)	$C_{18}H_{15}N_3OSe$ (368.29)	1680 (C=O)	TFA : 8.2 – 8.5 (m, 4H Ar-H); 5.01 – 6.01 (m, 5H allyl-H); 4.0 (s, 3H CH_3-pyrimidine); 3.1 (s, 3H, CH_3-quinoline)

[a] For MS data of the respective compounds: see Experimental.

eral, it can be concluded that the presence of a selenium atom in cyclic structures afforded compounds, *e.g.* **7c**, **9a**, **11b**, and **11d**, with higher activity than the open structures, *e.g.* **5a**.

Analgesic activity

The most active anti-inflammatory compounds **7c**, **9a**, **11b**, and **11d** were tested for their analgesic properties relative to acetyl salicylic acid as reference drug at a dose

Table 2. Inhibitory effect of the test compounds and indomethacin upon carrageenan-induced paw edema in rats (% edema inhibition).

Compound \ Time	0.5 h	1 h	2 h	3 h	4 h	5 h
5a	3.2	9.1	20.0	22.3	30.6	30.6
7b	3.2	9.1	17.1	22.3	30.64	30.6
7c	3.2	18.1	28.6	33.4	47.3	52.8
8b	3.2	9.1	20.0	22.3	36.2	38.9
8c	3.2	12.1	22.9	25.0	36.2	41.2
8d	9.7	12.1	28.6	22.3	36.2	44.5
9a	9.7	18.1	28.6	30.6	47.3	52.8
11b	6.5	18.1	28.6	30.6	36.2	52.8
11d	3.2	9.1	28.6	30.6	41.2	44.5
Indomethacin	6.5	18.1	31.4	41.7	52.8	58.4

level of 0.028 μmol/kg, according to the reported procedures [26]. The results are given in Table 3.

The results showed that after 1 h, compounds **7c** and **9a** had about one-half the activity of the reference drug while compounds **11b** and **11d** are the most active with 100.7% and 103.9%, respectively compared to the reference drug. After 5 h, compounds **11b** and **11d** were still the most active of the tested compounds showing 114.8% and 125.7% activity, respectively compared to the reference drug. Thus, cyclization of the amide derivative **7c** into pyrimidinone derivatives **11b** and **11d** greatly improved the analgesic activity, in accordance with previously reported effects [26].

Ulcerogenicity

The following inflammatory and analgesic most active compounds **11b** and **11d** were screened for their ulcerogenicity using reported procedures [27]. The results are listed in Table 4. The tested compounds are saver concerning ulcerogenicity in animals: they showed 50% and 33% activity, respectively, for the applied doses, compared to indomethacin (100%). However, the tested compounds **11b**, **11d**, and indomethacin showed a comparable ulceration indix, see Table 4.

Table 4. Gastric ulceration effects of compounds **11b** and **11d**.

Compound	Ratio of ulceration in animals	% Ulceration	Ulceration index (M ± S.E.)
Indomethacin	6/6	100	1.08 ± 0.2
11b	3/6	50	1.05 ± 0.4
11d	2/6	33	1.04 ± 0.2

Acute toxicity (LD_{50})

The medial lethal dose (LD_{50}) of the most active compound **11d** was determined in mice according to a reported procedure [28]. The animals were injected i.p. with graded doses of the test compounds. Compound **11d** was non toxic at doses up to 160 mg/kg.

Antimicrobial effects

The most active anti-inflammatory and analgesic compounds (**7c, 9a, 11b**, and **11d**) were screened for their antibacterial and antifungal activities according to reported procedures [29] and the results are given in Table 5. The data verify that none of the synthesized compounds has a considerable antimicrobial activity, except for compound **9a**, which showed a moderate effect against *Bacillus cereus*. On the other hand, the compounds show no effect against the tested fungal species with the exception of **7c** and **9a**. A considerable antifungal effect against *Aspergillus flavus, Aspergillus niger,* and *Candida albicans* for compound **7c**, and against *Aspergillus flavus*, *Candida albicans,* **and** *Fusarium oxysporum* **for 9a.** Compound **11d** showed a moderate antifungal effect against *Candida albicans*. The minimum inhibitory concentration (MIC) of the most active compounds (**7c** and **9a**) was 50 μg mL^{-1}. The experiments also reveal that compounds **7c** and **9a** are completely inactive at 25 μg mL^{-1} against all the tested fungi and bacteria. The activities

Table 3. Analgesic activities of **7c**, **9a**, **11b**, and **11d** (on the hot plate).

Compound \ Reaction Time[a)]	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	20.1 ± 0.30	20.2 ± 0.62	20.3 ± 0.81	20.1 ± 0.61	20.1 ± 0.34	20.1 ± 0.66
Aspirin	35.1 ± 0.72	54.8 ± 0.83	46.5 ± 1.11	36.5 ± 1.02	34.2 ± 0.98	28.0[b)]± 0.72
7c	25.1 ± 0.63	27.3 ± 0.55	25.2 ± 0.47	23.4 ± 0.33	21.7 ± 0.45	20.1 ± 0.40
9a	21.9 ± 0.32	28.5 ± 0.34	30.5 ± 0.30	30.4 ± 0.42	23.6 ± 0.40	20.2 ± 0.45
11b	38.2 ± 0.65	55.2 ± 0.60	60.5 ± 0.61	40.1 ± 0.65	39.1[b)]± 0.58	32.0 ± 0.60
11d	37.2 ± 0.53	56.5 ± 0.45	58.1 ± 0.00	50.2 ± 0.60	40.3 ± 0.55	35.2[b)]± 0.63

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

Table 5. Fungal and antimicrobial activities of compounds **7c, 9a** and **11d**.

Organism	Compound	Minimum inhibitory concentration (MIC) (μg mL^{-1})							
		7c		**9a**		**11d**	Reference drug*		
		100	50	100	50	100	100	50	25
Aspergillus flavus	Fungi	8	7	7	0	0	0	0	0
Aspergillus niger		7	0	0	0	0	40	32	26
Candida albicans		9	7	12	7	7	25	18	14
Fusarium oxysporum		0	0	7	0	0	22	16	14
Bacillus cereus (+ve)	Bacteria	0	0	7	0	0	25	20	18
Escherichia coli (-ve)		0	0	0	0	0	12	10	9
P. aeruginosa (-ve)		0	0	0	0	0	29	25	20
S. marcescens (-ve)		0	0	0	0	0	24	21	19

* Reference drug: antifungal: dermatin; antibacterial: ampicillin.

of the tested compounds are considerably lower than the standard antifungal and antibacterial agents.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined using a Kofler melting point apparatus (C. Reichert, Vienna, Austria) and are uncorrected. IR (KBr) spectra were recorded on a Pye-Unicam SP3-100 instrument (Pye Unicam Ltd. Cambridge, England). ^{1}H-NMR spectra were obtained on a Varian EM 390 (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane as an internal reference. Mass spectra were recorded on a JEOL-JMS-AX 500 (JEOL, Tokyo, Japan) at Cairo National Research Center and Assiut University, Assiut, Egypt. Elemental analyses were obtained on an Elementar Vario EL 1150C analyzer (Heraeus, Germany). The purity of the compounds was checked by TLC.

*3-Cyano-4-methylquinoline-2(1H)selenone ($C_{11}H_8N_2Se$) **2***

A mixture of the corresponding chloroquinoline derivative **1** (2.02 g, 10 mmol), selenium metal (1.0 g, 12 mmol) and sodium borohydride (1.2 g, 32 mmol) was refluxed in ethanol (50 mL) for 5 h. The mixture was cooled and poured in cold HCl. The solid precipitate was filtered, dried, and recrystallized from ethanol. MS m/z (% ref. int.): 248 (18) [M$^+$], other important fragments 207 (5), 167 (25), 140 (100), 113 (20).

*2,2′-Bis(3-cyano-4-methyl)diquinolinyl diselenide ($C_{22}H_{14}N_4Se_2$) **3***

The above described reaction mixture which precipitated during refluxing now was recrystallized from dioxane. MS m/z (% ref. int.): 494 (15) [M$^+$], other important fragments 333 (55), 248 (25), 140 (100), 113 (20).

*3-Cyano-4-methyl-2-substituted selenoquinolines ($C_{14}H_{12}N_2OSe$, $C_{19}H_{14}N_2OSe$) **4a, 4b***

General procedure: A mixture of **2** (1.48 g, 6 mmol), fused sodium acetate (0.98 g, 12 mmol), and chloroacetone or phenacyl bromide (6 mmol) in 30 mL ethanol was heated under reflux for 30 min. The reaction mixture was allowed to cool and was then poured into 50 mL of ice water. The precipitate was collected by filtration and recrystallized from EtOH.

*3-Amino-4-methyl-2-acetyl or -benzoylselenolo[2,3-b]quinoline ($C_{14}H_{12}N_2OSe$, $C_{19}H_{14}N_2OSe$) **5a, 5b***

General procedure: Compounds **4a** and **4b** (6 mmol) and EtONa (0.5 g Na in 10 mL EtOH) was refluxed for 1 h, and then cooled. The precipitate was collected and recrystallized from dioxane.

*3-Cyano-4-methyl-2-substituted selenoquinolines ($C_{13}H_9N_3Se$, $C_{15}H_{14}N_2O_2Se$, $C_{13}H_{11}N_3OSe$) **6a, 6b, 6c***

General procedure: A mixture of compound **2** (0.5 g, 20 mmol), fused sodium acetate (1.4 g, 17 mmol), and chloroacetonitrile, ethyl chloroacetate or chloroacetamide (15 mmol), respectively, and 30 mL ethanol was heated under reflux for 2 h. The reaction mixture was allowed to cool and was then poured into 50 mL of ice water. The precipitate was collected by filtration and recrystallized from EtOH.

*3-Amino-4-methyl-2-cyano or -ethylcarboxylate or -carboxamide selenolo[2,3-b]quinolines ($C_{13}H_9N_3Se$, $C_{15}H_{14}N_2O_2Se$, $C_{13}H_{11}N_3OSe$) **7a, 7b, 7c***

General procedure: Compounds **6a–c** (6 mmol) and EtONa (0.5 g Na in 10 mL EtOH) were refluxed for 1 h and then cooled. The solid product was collected and recrystallized from dioxane.

7b: MS m/z 333 (50) [M$^+$ − 1] and the other important fragments are 260 (95), 182 (55), 140 (100).

7c: MS m/z 305 (100) [M$^+$]; other important fragments are 288 (80), 260 (45), 140 (55).

*2-Aryl-4-methyl-2,3-dihydropyrimido[4′,5′:4,5]-selenolo[2,3-b]quinoline-11(1H)-ones **8a-d** or 2-Spiro(cycloalkane)-4-methyl-3(H)pyrimido[4′,5′:4,5]-selenolo[2,3-b]quinoline-11(1H)-ones **9a, b***

General procedure: A mixture of **7c** (1 g, 33 mmol) and the corresponding aromatic aldehydes or cycloalkanones (33 mmol) was heated under reflux in glacial acetic acid (20 mL) for 5–7 h, the solid was collected by filtration and recrystallized from acetic acid.

9a: MS m/z 385 (53.8) [M^+], 386 (100) [$M^+ + 1$]; other important fragments are 356 (59), 342 (46), 288 (61), 236 (28), 140 (43).

9b MS m/z 372 (5.6) [$M^+ + 1$]; other important fragments are 370 (2.9), 353 (1.8), 185 (53.8), 149 (9.9), 93 (100), 78 (23.5).

*2,4-Dimethylpyrimido[4′,5′:4,5]selenolo[2,3-b]quinoline-11(1H)-one ($C_{15}H_{11}N_3OSe$) **10***

Compound **7c** (1.0 g, 33 mmol) and redistilled acetic anhydride (20 mL) were heated under reflux for 8 h, and then left to cool. The precipitate was filtered and crystallized from EtOH.

10: MS m/z 328 (52) [M^+], 329 (12) [M^+], 330 (100) [M^+] and the other important fragments are 260 (16), 179 (5), 140 (37).

*N-Alkylsubstituted-2,4-dimethylpyrimido[4′,5′:4,5]-selenolo[2,3-b]quinoline-11(1H-ones **11a–d***

General procedure: To a solution of **10** (0.5 g, 15 mmol) in DMF, anhydrous K_2CO_3 (0.5 g) and the suitable alkyl halide (15 mmol) were added. The reaction mixture was heated on a water bath for 8 – 10 h, cooled, and then diluted with ice water (20 mL). The precipitate was collected by filtration and recrystallized from EtOH.

11a: MS m/z 343 (2.4) [M^+], 342 (4.2) [$M^+ - 1$].

11b: MS m/z 357 (6.5) [M^+].

11c: MS m/z 369 (2.4) [$M^+ - 2$].

11d: MS m/z 369 (88) [$M^+ + 1$]; other important fragments are 330 (60), 300 (35), 259 (40), 140 (100).

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°C ± 2°C, 50% ± 2% relative humidity, and a 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.

Anti-inflammatory activity

The anti-inflammatory activities of compounds **5a**, **7b**, **7c**, **8b – d**, **9a**, **11b**, and **11d** were evaluated according to the method described by Winter *et al.* [25], where a pedal inflammation in rat paws was induced by subplantar injection of 0.2 mL carrageenan suspension (0.2%) into the right hind paw of the rats. Male adult albino rats (100 – 120 g) were divided into eleven groups, of five animals each. The thickness of rat paw was measured by a Veriner caliper (SMIEC, China) before and 1 h after 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 carrageenan. The control group received the vehicle (5% CMC), while the 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 intervals, after injection of the test compounds, the reference drug, and the vehicle. The results are displayed in Table 2.

Analgesic activity

The analgesic activity of **7c**, **9a**, **11b**, and **11d** was determined in mice using the hot-plate method [26] in comparison to acetyl salicylic acid. 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 containing five animals. Four test compounds and the reference drug were injected into the animals i.p. at a dose level of 10 mg kg^{-1}. A control group of animals was similarly treated with 5% CMC in normal saline. The reaction time was evaluated directly after 0.5, 1, 2, 3, 4, and 5 h intervals after injection. The results of analgesic activity of the test compounds and acetyl salicylic acid are displayed in Table 3.

Gastric ulceration

Examination of the gastrointestinal mucosa for the presence of lesions following oral administration of graded doses of the test compounds as well as the reference drug has been taken as an indication for ulcerogenic effects. Both, the frequency of ulceration (expressed as ratio of ulcerated animals) and the severity of ulceration (expressed as ulcer index) were used for comparison of the tested compound and indomethacin [27].

Three groups of six male adult albino mice each were fasted for 24 h. Compounds **11b** and **11d** and indomethacin were administered orally in doses of 10, 30, and 50 mg kg^{-1}, as suspensions in 5% CMC normal saline solution. After 6 h, the animals were killed, the stomachs were removed and gastric lesions on the mucosa were determined using a stereoscopic microscope. Ulcer was defined as one lesion that was at least 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index; results are displayed in Table 4.

Determination of acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most active and safe compound **11d** was determined in mice. Groups – each consisting of five animals – of male adult albino mice (20 – 25 g), were injected i.p. with graded doses of the test compound. The percentage of mortality was determined 72 h, after the injection. Computation of LD_{50} was processed by a graphical method [28].

Antibacterial activity

Four bacterial species representing both *Gram*-positive and *Gram*-negative strains were used to test the antibacterial activities of the target compounds **7c**, **9a**, **11b**, and **11d** *in vitro*, in comparison to ampicillin as a reference drug using the standard agar paper disc diffusion method: *Bacillus cereus* (P-70) (*Gram*-positive bacteria), *Escherichia coli* (P-69), *Pseudomonas aeruginosa* (P-72), *Serratia marcescens* (P-67) (*Gram*-negative bacteria).

Cell suspensions of bacterial stains were prepared from 48-h old cultures grown on potato dextrose agar (PDA) or Sabouraud agar (SA) media. One mL of the cell suspension was added to Petri dishes of 9 cm in diameter, and then, 15 mL of nutrient agar was poured onto the plates. Plates were shaken gently to homogenize the innoculum. Sterile 5 mm filter paper (Whatmann, UK) was saturated with 10 μg L^{-1} of the test compound, ampicillin solutions (100, 50, 25 μg mL^{-1} concentrations) as reference drug, or DMSO as negative control. Impregnated discs were then dried for 1 h and placed in the centre of each plate. The seeded plates were incubated at 35°C ± 2°C for 24 – 48 h. The radii of the inhibition zones in mm of triplicate sets were measured and the results are given in Table 5.

Antifungal activity

Compounds **7c**, **9a**, **11b**, and **11d** were screened for their antifungal activity *in vitro*, in comparison to fluconazole as a reference drug using the standard agar paper disc diffusion method against four fungi: *Aspergillus flavus* (3372), *Aspergillus niger* (3364), *Candida albicans* (421), and *Fusarium oxysporum* (208).

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 5×10^4 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 homogenize the innoculum.

Sterile 5 mm filter paper (Whatmann, UK) was saturated with 10 mg L^{-1} of the test compound, fluconazole solution (100, 50, 25 μg mL^{-1} concentrations) as reference drug or DMSO as negative control. Impregnated discs were then dried for 1 h and placed in the centre of each plate. The seeded plates were incubated at 28°C ± 2°C for 7 days. The radii of the inhibition zones in mm of triplicate sets were measured and the results had shown inhibition activity at 40, 25, and 22 mm, respectively. The results are listed in Table 5.

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