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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW COUMARIN DERIVATIVES AS ANTIMICROBIAL AGENTS

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*A series of new 7-[2-(3-alkyl/aryl-4-arylthiazol-2(3H)-ylidene)hydrazono)propoxy]-4-methyl-2H-chromen-2-ones, (**6-9a-e**), was prepared by the reaction of appropriate N-alkyl/aryl-2-[1-(4-methyl-2-oxo-2H-chromen-7yloxy)propan-2-ylidene]hydrazine carbothioamides (**4a-d**) and phenacyl bromides (**5a-e**). The purity of all new compounds was checked by TLC and elucidation of their structures was confirmed by IR, ^{1}H NMR, and mass spectrometry along with elemental microanalyses. All the target compounds were evaluated for their possible antimicrobial activity. Most of the tested compounds showed weak to moderate antibacterial activity against most of the bacterial strains used in comparison with gatifloxacin as a reference drug. The most active compounds were **6b**, **6c**, **7b**, **8b**, **8c**, and **9c** against B. cereus, E. coli and S. marcescens. Results of antifungal activity revealed that all compounds showed weak to moderate activity against S. brevicaulis, while ketoconazole as a reference drug was completely inactive. Compounds **6a**, **6b**, **6c**, **6e** and **7b** were even more active than ketoconazole against F. oxysporum.*

INTRODUCTION

Coumarins (2*H*-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in drugs and dyes[1]. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory[2]. The incorporation of other heterocyclic moiety either as substituent group or as a fused component into the parent coumarin alters the property of parent coumarin and converts it into a more useful product[3]. Coumarins are plant flavonoids widely distributed in nature. Natural coumarins are known to have antidiabetic activity[4], anabolic, antioxidant and hepato protective activities[5&6]. Substituted coumarin derivatives have been reported to have variety of biological activities including anticoagulant[7], HIV protease inhibition[8], CNS depressant[9], analgesic[10] and anti-tubercular activities[11]. The potent antibiotics like novobiocin[12], coumarmycin[13] and chlorobiocin[14] are coumarin derivatives. Recently, the interest on these compounds has been reviewed owing to their use as fluorescent markers in the biochemical determination of enzymes[15].

On the other hand, literature survey revealed that thiosemicarbazones constitute one of the most versatile classes of compounds possessing antimicrobial and anti-amoebic activities[16-20], depending on the nature of the substituents at N^1 and N^4 of thiosemicarbazone moiety. Various thiosemicarbazone derivatives have been successfully developed and documented as antimicrobial agents[16-21].

Enlightened by the aforementioned studies, the present work aims at the synthesis of some new thiazoline coumarin compounds (**6-9a-e**) derived by cyclization of coumarin thiosemicarbazones (**4a-d**) to be subjected for preliminary *in-vitro* screening of their antibacterial and antifungal activities using the agar disc diffusion method.

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MATERIALS AND METHODS

Melting points were determined on an electro thermal melting point apparatus [Stuart Scientific, model SMP3, England, UK], and were uncorrected. A pre-coated silica gel plate (kieselgel 0.25 mm, 60G F254, Merck, Germany) was used for TLC monitoring of reactions. The developing solvent system *n*-Hexane/ethylacetate (3:2 v/v) was used and the spots were detected at 254 nm wavelength using ultraviolet lamp (Spectroline, model CM-10, USA). IR spectra (KBr discs) were recorded on a shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University, Assiut. ^{1}H NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using DMSO-d_6, unless otherwise specified, as a solvent, and deuterium oxide was used for the detection of exchangeable protons. Mass spectra were recorded with JEOL JMS600 mass spectrometer (JEOL, Tokyo, Japan), Assiut University Central Lab, Assiut and at the unit of Microanalysis, Faculty of Science, Cairo University, Cairo. Elemental microanalyses were performed on a Perkin-Elmer 240 elemental analyzer (Perkin-Elmer, USA) at the unit of Microanalysis, Faculty of Science, Cairo University, Cairo. Reagents used for synthesis were purchased from Sigma-Aldrich (Gillingham – Dorest, UK) and MERCK (Schuchardt, Germany). All solvents were obtained from commercial suppliers and used without further purification.

The starting materials 7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**)[22], 4-methyl-7-(2-oxopropoxy)-*2H*-chromen-2-one (**2**)[23] 4-substituted-3-thiosemicarbazides (**3a-d**)[24], and phenacyl bromides (**5a-e**)[25]; were synthesized according to reported procedures.

Chemistry

Synthesis of *N*-alkyl/aryl-2-[1-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)propan-2-ylidene] hydrazine carbothioamides (4a-d)

To an equimolar amount of the appropriate 4-substituted-3-thiosemicarbazide (**3a-d**) (5.3 mmol) in absolute ethanol (50 mL), 4-methyl-7-(2-oxopropoxy)-*2H*-chromen-2-one (**2**) (5.3 mmol) was added. The mixture was refluxed for 4-6 hrs in the presence of 1-2 drops of glacial acetic acid. The formed precipitate of compounds *N*-alkyl/aryl-2-[1-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)propan-2-ylidene]hydrazine carbothioamides (**4a-d**) was filtered, dried and crystallized from ethanol to afford pure compounds. Yields, m.p., elemental analyses, IR, ^{1}H NMR and mass spectral data are listed in tables 1 and 2.

Synthesis of 7-[2-(3-alkyl/aryl-4-arylthiazol-2(3*H*)-ylidene)hydrazono)propoxy]-4-methyl-2*H*-chromen-2-ones (6-9a-e)

A solution of the appropriate thiosemicarbazone derivative (**4a-d**) (1.5 mmol) in absolute ethanol (30 mL) and the appropriate phenacyl bromide (**5a-e**) (1.5 mmol) was heated under reflux for 6-8 hrs in the presence of anhydrous sodium acetate (100 mg). Following up of the reaction was done by TLC. The reaction mixture was concentrated and poured onto ice cold water for complete separation of the product which was filtered, dried and crystallized from ethanol to afford pure compounds **6-9a-e**. Yields, m.p., elemental analyses, IR, ^{1}H NMR and mass spectral data are listed in tables 1 and 2.

Antimicrobial screening

a) Antibacterial activity

Organisms and culture conditions

The used bacterial cultures were obtained from Assiut University Mycological Center (AUMC), Assiut University, Assiut. The antibacterial activity of compounds **4a-d** and **6-9a-e** was determined according to the agar disc diffusion method[26].

Six bacterial species were used to test the antibacterial activity of the target compounds: *Bacillus cereus* (AUMC B70), *Staphylococcus aureus* (AUMC B71) and *Micrococcus luteus* (AUMC B68) as representatives of Gram positive strains, while the Gram negative strains were represented by *Escherichia coli* (AUMC B69), *Pseudomonas aeruginosa* (AUMC B72) and *Serratia marcescens* (AUMC B67).

Table 1: Physicochemical properties of compounds **4a-d** and **6-9a-e**.

Compd. No.	R	R^1	Yield (%)	M.p. (°C)	M.F. (M.Wt.)	Microanalysis (calculated/found)		
						C%	H%	N%
4a	C_2H_5	--	82	163-165	$C_{16}H_{19}N_3O_3S$ (333.11)	57.64 57.60	5.74 5.68	12.60 12.66
4b	$CH(CH_3)_2$	--	81	142-144	$C_{17}H_{21}N_3O_3S$ (347.13)	58.77 58.81	6.09 6.12	12.09 12.05
4c	C_6H_5	--	76	185-187	$C_{20}H_{19}N_3O_3S$ (381.11)	62.97 62.90	5.02 5.42	11.02 10.97
4d	*p*-tolyl	--	74	213-215	$C_{21}H_{21}N_3O_3S$ (395.13)	63.78 63.79	5.35 5.29	10.63 10.58
6a	C_2H_5	H	89	176-178	$C_{24}H_{23}N_3O_3S$ (433.15)	66.49 66.18	5.35 5.09	9.69 9.53
6b	C_2H_5	Br	81	205-207	$C_{24}H_{22}BrN_3O_3S$ (511.06)	56.25 55.93	4.33 4.09	8.20 8.53
6c	C_2H_5	Cl	72	201-203	$C_{24}H_{22}ClN_3O_3S$ (467.11)	61.60 61.03	4.74 4.90	8.98 8.89
6d	C_2H_5	CH_3	84	204-206	$C_{25}H_{25}N_3O_3S$ (447.16)	67.09 67.59	5.63 5.84	9.39 9.45
6e	C_2H_5	OCH_3	78	186-188	$C_{25}H_{25}N_3O_4S$ (463.16)	64.78 64.34	5.44 5.55	9.06 8.84
7a	$CH(CH_3)_2$	H	70	146-148	$C_{25}H_{25}N_3O_3S$ (447.16)	67.09 66.64	5.63 5.41	9.39 9.24
7b	$CH(CH_3)_2$	Br	80	170-172	$C_{25}H_{24}BrN_3O_3S$ (525.07)	57.04 56.87	4.60 4.33	7.98 7.69
7c	$CH(CH_3)_2$	Cl	77	192-194	$C_{25}H_{24}ClN_3O_3S$ (481.12)	62.30 62.31	5.02 5.54	8.72 8.91
7d	$CH(CH_3)_2$	CH_3	72	164-166	$C_{26}H_{27}N_3O_3S$ (461.18)	67.65 67.12	5.90 5.48	9.10 8.95
7e	$CH(CH_3)_2$	OCH_3	73	189-190	$C_{26}H_{27}N_3O_4S$ (477.17)	65.39 64.98	5.70 5.32	8.80 8.67
8a	C_6H_5	H	68	197-199	$C_{28}H_{23}N_3O_3S$ (481.15)	69.83 69.47	4.81 5.14	8.73 9.12
8b	C_6H_5	Br	63	204-206	$C_{28}H_{22}BrN_3O_3S$ (559.06)	60.00 59.48	3.96 3.52	7.50 7.02
8c	C_6H_5	Cl	70	199-201	$C_{28}H_{22}ClN_3O_3S$ (515.11)	65.17 65.15	4.30 4.38	8.14 7.89
8d	C_6H_5	CH_3	89	210-212	$C_{29}H_{25}N_3O_3S$ (495.16)	70.28 70.24	5.08 5.17	8.48 8.26
8e	C_6H_5	OCH_3	75	192-194	$C_{29}H_{25}N_3O_4S$ (511.16)	68.08 67.85	4.93 5.30	8.21 7.86
9a	*p*-tolyl	H	74	136-138	$C_{29}H_{25}N_3O_3S$ (495.16)	70.28 70.61	5.08 5.04	8.48 8.44
9b	*p*-tolyl	Br	88	202-205	$C_{29}H_{24}BrN_3O_3S$ (573.07)	60.63 60.24	4.21 4.17	7.31 7.67
9c	*p*-tolyl	Cl	81	212-214	$C_{29}H_{24}ClN_3O_3S$ (529.12)	65.71 65.43	4.56 4.25	7.93 7.48
9d	*p*-tolyl	CH_3	70	196-198	$C_{30}H_{27}N_3O_3S$ (509.18)	70.70 70.46	5.34 5.03	8.25 8.15
9e	*p*-tolyl	OCH_3	64	193-195	$C_{30}H_{27}N_3O_4S$ (525.17)	68.55 68.41	5.18 4.99	7.99 7.87

Table 2: Spectral characterization of compounds **4a-d** and **6-9a-e**.

Compd. No.	IR (KBr) (ύ, cm^{-1})	^{1}H NMR (DMSO-d_6, δ ppm)	MS (*m/z*)
4a	3390, 3350 (NH), 1622 (C=N), 1579, (Ar-C=C)	0.9-1.3 (t, 3H, $CH_2C\underline{H}_3$), 2-2.6 (m, 8H, $CH_3C\underline{H}_2$, $NCCH_3$, and coumarin-CH_3), 5 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.8-7.3 (m, 2H, Ar-H), 7.4-7.6 (d, 1H, Ar-H), 9.2 (s, 1H, NH, D_2O exchangeable), 10.4 (s, 1H, NH, D_2O exchangeable).	334.12 (M^++1)
4b	3381, 3350 (NH), 1625 (C=N), 1580, (Ar-C=C)	1.1-1.3 (d, 6H, $CH(C\underline{H}_3)_2$), 2.2 (s, 3H, $NCC\underline{H}_3$), 2.5 (s, 3H, coumarin-CH_3), 3.1-3.3 (m, 1H, $C\underline{H}(CH_3)_2$), 4.8 (s, 2H, OCH_2), 6.4 (s, 1H, C-H_3 of coumarin), 6.8-7.2 (m, 2H, Ar-H), 7.4-7.6 (d, 1H, Ar-H), 8.9 (s, 1H, NH, D_2O exchangeable), 10.8 (s, 1H, NH, D_2O exchangeable).	348.13 (M^++1)
4c	3385, 3351 (NH), 1622 (C=N), 1589, (Ar-C=C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.4 (s, 3H, coumarin-CH_3), 4.9 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.9-7.8 (m, 8H, Ar-H), 9.6 (s, 1H, NH, D_2O exchangeable), 10.3 (s, 1H, NH, D_2O exchangeable)	382.12 (M^++1)
4d	3390, 3348 (NH), 1620 (C=N), 1598, (Ar-C=C)	2.1 (s, 3H, Ar-CH_3), 2.3-2.5 (m, 6H, $NCC\underline{H}_3$, and coumarin-CH_3), 4.8 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.9-7.7 (m, 7H, Ar-H), 9.6 (s, 1H, NH, D_2O exchangeable), 10.5 (s, 1H, NH, D_2O exchangeable).	396.13 (M^++1)
6a	1562 (C=N), 1528, 1491 (C=C), 1248, 1171 (C-S-C)	1.1-1.3 (t, 3H, $CH_2C\underline{H}_3$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.7 (s, 3H, coumarin-CH_3), 3.9-4.4 (q, 2H, $C\underline{H}_2CH_3$), 5.1 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.95-7.8 (m, 8H, Ar-H).	434.15 (M^++1)
6b	1572 (C=N), 1525, 1491 (C=C), 1248, 1171 (C-S-C)	1.2-1.35 (t, 3H, $CH_2C\underline{H}_3$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.8 (s, 3H, coumarin-CH_3), 3.8-4.2 (q, 2H, $C\underline{H}_2CH_3$), 5.1 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.7 (s, 1H, CH of thiazoline), 6.9-7.8 (m, 7H, Ar-H).	512.06 (M^++1)
6c	1562 (C=N), 1518, 1471 (C=C), 1248, 1176 (C-S-C)	1.1-1.4 (t, 3H, $CH_2C\underline{H}_3$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.8 (s, 3H, coumarin-CH_3), 3.7-4.1 (q, 2H, $C\underline{H}_2CH_3$), 5.1 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.9-7.8 (m, 7H, Ar-H).	468.11 (M^++1)
6d	1573 (C=N), 1518, 1480 (C=C), 1248, 1178 (C-S-C)	1.1-1.4 (t, 3H, $CH_2C\underline{H}_3$), 2.4 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 6H, Ar-CH_3 and coumarin-CH_3), 3.7-4.1 (q, 2H, $C\underline{H}_2CH_3$), 5.1 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.8-7.8 (m, 7H, Ar-H).	448.17 (M^++1)
6e	1562 (C=N), 1518, 1491 (C=C), 1248, 1171 (C-S-C)	1.1-1.4 (t, 3H, $CH_2C\underline{H}_3$), 2.4 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 3.7-4.1 (q, 2H, $C\underline{H}_2CH_3$), 4.3 (s, 3H, OCH_3), 5.0 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.8-7.8 (m, 7H, Ar-H).	464.16 (M^++1)
7a	1634 (C=N), 1576, 1529 (C=C), 1260, 1187 (C-S-C)	1.2-1.5 (d, 6H, $CH(C\underline{H}_3)_2$), 2.2 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 2.9-3.2 (m, 1H, $C\underline{H}(CH_3)_2$), 5.0 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.5 (s, 1H, CH of thiazoline), 6.9-7.8 (m, 8H, Ar-H).	448.17 (M^++1)
7b	1625 (C=N), 1567, 1520 (C=C), 1260, 1187 (C-S-C)	1.3-1.5 (d, 6H, $CH(C\underline{H}_3)_2$), 2.4 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 2.9-3.3 (m, 1H, $C\underline{H}(CH_3)_2$), 5.1 (s, 2H, OCH_2), 6.4 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.8-7.8 (m, 7H, Ar-H).	526.08 (M^++1)
7c	1630 (C=N), 1556, 1531 (C=C), 1260, 1185 (C-S-C)	1.2-1.5 (d, 6H, $CH(C\underline{H}_3)_2$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 2.9-3.2 (m, 1H, $C\underline{H}(CH_3)_2$), 5.0 (s, 2H, OCH_2), 6.4 (s, 1H, C-H_3 of coumarin), 6.5 (s, 1H, CH of thiazoline), 6.9-7.8 (m, 7H, Ar-H).	482.13 (M^++1)

Table 2: Continued.

Compd. No.	IR (KBr) (ύ, cm^{-1})	^{1}H NMR (DMSO-d_6, δ ppm)	MS (*m/z*)
7d	1634 (C=N), 1576, 1525 (C=C), 1260, 1187 (C-S-C)	1.1-1.5 (d, 6H, $CH(C\underline{H}_3)_2$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 6H, Ar-CH_3 and coumarin-CH_3), 2.8-3.1 (m, 1H, $C\underline{H}(CH_3)_2$), 5.0 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.8-7.8 (m, 7H, Ar-H).	462.18 (M^++1)
7e	1630 (C=N), 1576, 1525 (C=C), 1260, 1187 (C-S-C)	1.2-1.5 (d, 6H, $CH(C\underline{H}_3)_2$), 2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 2.9-3.2 (m, 1H, $C\underline{H}(CH_3)_2$), 4.1 (s, 3H, OCH_3), 5.0 (s, 2H, OCH_2), 6.4 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 6.8-7.8 (m, 7H, Ar-H).	478.18 (M^++1)
8a	1578 (C=N), 1515, 1453 (C=C), 1250, 1181 (C-S-C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.5 (s, 3H, coumarin-CH_3), 4.9 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 13H, Ar-H).	482.17 (M^++1)
8b	1578 (C=N), 1521, 1460 (C=C), 1250, 1180 (C-S-C)	2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 5.1 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.2-7.9 (m, 12H, Ar-H).	560.08 (M^++1)
8c	1578 (C=N), 1520, 1460 (C=C), 1250, 1178 (C-S-C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 3H, coumarin-CH_3), 5.0 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.5 (s, 1H, CH of thiazoline), 7.1-7.8 (m, 12H, Ar-H).	516.16 (M^++1)
8d	1578 (C=N), 1522, 1453 (C=C), 1250, 1176 (C-S-C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.5 (s, 6H, Ar-CH_3 and coumarin-CH_3), 5.0 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.5 (s, 1H, CH of thiazoline), 7.1-7.8 (m, 12H, Ar-H).	496.18 (M^++1)
8e	1578 (C=N), 1515, 1457 (C=C), 1250, 1178 (C-S-C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.5 (s, 3H, coumarin-CH_3), 4.2 (s, 3H, OCH_3), 4.9 (s, 2H, OCH_2), 6.2 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 12H, Ar-H).	512.17 (M^++1)
9a	1578 (C=N), 1517, 1463 (C=C), 1250, 1179 (C-S-C)	2.2 (s, 3H, $NCC\underline{H}_3$), 2.4 (s, 6H, Ar-CH_3 and coumarin-CH_3), 4.9 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 12H, Ar-H).	496.18 (M^++1)
9b	1558 (C=N), 1515, 1453 (C=C), 1250, 1178 (C-S-C)	2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 6H, Ar-CH_3 and coumarin-CH_3), 5.0 (s, 2H, OCH_2), 6.4 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.1-7.9 (m, 11H, Ar-H).	574.11 (M^++1)
9c	1587 (C=N), 1515, 1457 (C=C), 1250, 1178 (C-S-C)	2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 6H, Ar-CH_3 and coumarin-CH_3), 4.9 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 11H, Ar-H).	530.16 (M^++1)
9d	1572 (C=N), 1512, 1459 (C=C), 1250, 1180 (C-S-C)	2.3 (s, 3H, $NCC\underline{H}_3$), 2.5 (s, 9H, 2Ar-CH_3 and coumarin-CH_3), 5.0 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 11H, Ar-H).	510.18 (M^++1)
9e	1581 (C=N), 1520, 1450 (C=C), 1250, 1181 (C-S-C)	2.3 (s, 3H, $NCC\underline{H}_3$), 2.6 (s, 6H, Ar-CH_3 and coumarin-CH_3), 4.1 (s, 3H, OCH_3), 4.9 (s, 2H, OCH_2), 6.3 (s, 1H, C-H_3 of coumarin), 6.6 (s, 1H, CH of thiazoline), 7.0-7.8 (m, 11H, Ar-H).	526.18 (M^++1)

Materials and method

Cell suspension of bacterial strains was prepared from 48 hrs old cultures grown on nutrient agar (NA) in sterilized water[26]. One mL suspension was added to Petri dishes of 9 cm in diameter and then 15 mL of NA was poured into the plates. Plates were shaken gently to homogenize the inocula.

Sterile 5-mm filter paper disc (Whatman) was saturated with 10 µL solutions of the test compounds or gatifloxacin as a reference drug (53 µmol-mL^{-1} in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as a negative control. The discs were then dried for 1 hr and placed in the center of each plate. The seeded plates were incubated at 35±2°C for 24-48 hrs. The radii of inhibition zones (in mm) were measured in triplicate and the results are given in table 3.

b) Antifungal activity

Organisms and culture conditions

The used Sabouraud Agar (SA) media were prepared in Assiut University Mycological Center (AUMC), Assiut University, Assiut. The antifungal activity of compounds **4a-d** and **6-9a-e** was determined according to the agar disc diffusion method[26].

Seven pathogenic (*Trichophyton rubrum* (Castellani) Sabouraud AUMC 1145 and *Candida albicans* (Robin) Berkhout AUMC 421), phytopathogenic (*Fusarium oxysporum* Schlechtendal AUMC 208) and food deteriorating fungal species (*Aspergillus flavus* Link AUMC 3372, *Aspergillus niger* Van Tieghem AUMC 3364, *Geotrichum candidum* Link AUMC 228 and *Scopulariopsis brevicaulis* (Saccardo) Bainier AUMC 363) were used in the present study.

Materials and method

Spore suspension in sterile distilled water was prepared from 2-5 days old culture of the test fungi growing on Sabouraud agar (SA) medium[26]. The final spore concentration was nearly 5×10^4 spores-mL^{-1}. About 15 mL of growth medium was introduced on sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. Antifungal activity of the test compounds **4a-d** and **6-9a-e** was performed by the standard agar disc diffusion method as follows.

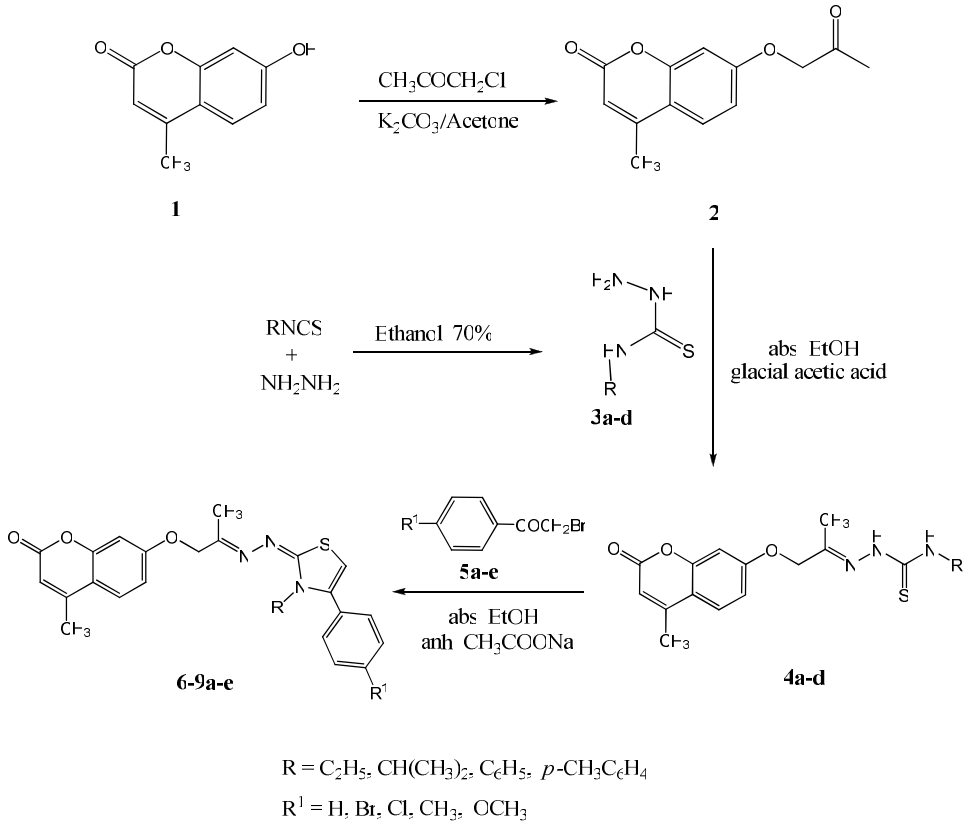
Sterile 5-mm filter paper disc (Whatman) was saturated with 10 µL solutions of the test compound or ketoconazole (40 µmol-mL^{-1} in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as a negative control. The disks were then dried for 1 hr and placed in the center of each plate. The seeded plates were incubated at 28±2°C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and results are presented in table 4.

RESULTS AND DISCUSSION

Chemistry

The starting material 4-methyl-7-(2-oxopropoxy)-*2H*-chromen-2-one (**2**) was prepared according to a reported procedure through reaction of 7-hydroxy-4-methyl-*2H*-chromen-2-one (**1**) with chloroacetone in the presence of anhydrous potassium carbonate using acetone as a solvent[25]. Structure of compound **2** was confirmed by comparison of its physical and spectral data with the reported ones[25]. ^{1}H NMR spectrum of compound **2** showed two singlets at δ 2 and 2.10 ppm (two methyl groups), singlet at δ 4.9 ppm (OCH_2), singlet at δ 6.3 ppm (C-H_3 of coumarin ring) besides multiplet (2H) at 6.8-7.3 ppm corresponding to C-H_6 and C-H_8 of coumarin, in addition to a doublet (1H) at 7.4-7.6 ppm corresponding to C-H_5 coumarin.

The key intermediates, *N*-alkyl/aryl-2-[1-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)propan-2-ylidene]hydrazine carbothioamides (**4a-d**) were prepared by condensation of thiosemicarbazides (**3a-d**) derived from an aryl or alkyl isothiocyanates and hydrazine[19], with compound **2** (Scheme 1). Structures of compounds **4a-d** were confirmed from IR, ^{1}H NMR spectra as well as elemental analyses (Tables 1 and 2). IR spectrum showed two bands at 3390 and 3350 cm^{-1} (two NH) and a band at 1625 cm^{-1} corresponding to C=N. ^{1}H NMR spectrum revealed the presence of two broad singlet (two NH groups) exchangeable with D_2O, singlet at δ 2.3 ppm (C_4-CH_3), singlet at δ 5 ppm (OCH_2), singlet (C-H_3 of coumarin), in addition to characteristic patterns of coumarin aromatic protons.



R = C_2H_5, $CH(CH_3)_2$, C_6H_5, *p*-$CH_3C_6H_4$

R^1 = H, Br, Cl, CH_3, OCH_3

Scheme 1: Synthetic route of compounds **4a-d** and **6-9a-e**.

Table 3: Percentage inhibition zones of compounds **4a-c**, **6a-c, 6e, 7a-e**, **8a-c**, **9c** and **gatifloxacin**.

Compd. No.	% Inhibition zones			
	Gram-Positive	Gram-Negative		
	B. cereus	*E. coli*	*P. aeruginosa*	*S. marcescens*
4a	24.2	27.8	--	34.1
4b	23.4	32.4	--	32.8
4c	22.2	28.7	--	--
6a	26.5	--	--	--
6b	26.5	33.3	--	88.2
6c	52.9	--	--	64.7
6e	35.3	60.0	--	29.4
7a	23.5	46.7	--	--
7b	29.4	53.3	--	44.1
7c	20.6	--	--	--
7d	--	40.0	50.0	--
7e	--	--	--	20.6
8a	29.4	--	--	58.8
8b	38.2	--	--	67.6
8c	41.2	--	--	44.1
9c	29.4	--	41.7	--
Gatifloxacin	100	100	100	100

-- No inhibition.

Table 4: Percentage inhibition zones of compounds **4a-c**, **6a-c**, **6e**, **7b**, **8a-c** and **ketoconazole**.

Compd. No.	% Inhibition zones					
	C. albicans	*F. oxysporum*	*A. flavus*	*A. niger*	*G. candidum*	*S. brevicaulis*
4a	25.8	56.8	--	--	37.2	--
4b	--	48.9	--	--	25.9	--
4c	--	50.4	--	--	--	15.2
6a	--	125.0	58.8	75.0	--	--
6b	--	150.0	29.41	62.5	31.2	--
6c	--	162.5	58.8	75.0	46.9	10*
6e	25.0	162.5	58.8	83.3	37.5	--
7b	--	125.0	--	50.0	--	9*
8a	--	--	29.4	--	--	20*
8b	--	--	38.2	--	--	23*
8c	--	--	41.2	--	--	15*
Ketoconazole	100	100	100	100	100	--

* In mm -- No inhibition.

In the present investigation, 7-[2-(3-alkyl/aryl-4-arylthiazol-2(3*H*)-ylidene)hydrazono)propoxy]-4-methyl-2*H*-chromen-2-ones (**6-9a-e**) were prepared by refluxing thiosemicarbazone derivatives (**4a-d**) with equimolar amount of phenacyl bromides (**5a-e**) in the presence of anhydrous sodium acetate in absolute ethanol for 6-8 hrs (Scheme 1).

The IR spectra of compounds **6-9a-e** are characterized by some general features such as lack of the characteristic bands due to NH and NCS functions and exhibited a band attributed to C=N stretching vibration at 1634-1562 cm^{-1}. Moreover, all compounds showed the characteristic bands at 1577-1500 and 1523-1478 cm^{-1} attributed to C=C function as well as bands at 1255-1248 and 1187-1171 cm^{-1} due to C-S-C[27].

^{1}H NMR spectra of 7-[2-(3-ethyl-4-arylthiazol-2(3*H*)-ylidene)hydrazono)propoxy]-4-methyl-2*H*-chromen-2-ones (**6a-e**) are characterized by some general features such as the presence of a triplet signal at δ 1.1-1.3 ppm and quartet at 3.7-4.4 ppm corresponding to C_2H_5 moiety, a singlet signal at δ 5-5.1 ppm of OCH_2 group, a singlet signal at δ 6.3 ppm corresponding to the coumarin H_3, a singlet signal at δ 6.6-6.7 ppm corresponding to the thiazoline proton, in addition to disappearance of the signals corresponding to the NH groups.

Mass spectrum of compound **6c** revealed a molecular ion peak M^+ at *m/z* 467.1 (100%) corresponding to the molecular weight of this compound which is also the base peak. Also, the spectrum showed a peak at M^++2 (*m/z* 469.1, 36%) due to $CH^{37}ClNOS$.

^{1}H NMR spectra of 7-[2-(3-isopropyl-4-arylthiazol-2(3*H*)-ylidene)hydrazono)propoxy]-4-methyl-2*H*-chromen-2-ones (**7a-e**) are characterized by some general features such as the presence of a doublet signal at δ 1.1-1.5 ppm and multiplet at 2.8-3.3 ppm corresponding to $CH(CH_3)_2$ moiety, a singlet signal at δ 5-5.1 ppm of OCH_2 group, a singlet signal at δ 6.3 ppm corresponding to the coumarin H_3, a singlet signal at δ 6.6-6.7 ppm corresponding to the thiazoline proton, in addition to disappearance of the signals corresponding to NH groups.

Mass spectrum of compound **7a** (M.Wt. 447.16) revealed a molecular ion peak M^+ at *m/z* 447.1 (100%) corresponding to the molecular weight of this compound which is also the base peak.

^{1}H NMR spectra of compounds **8a-e** and **9a-e** are characterized by some general features such as the presence of a singlet signal at δ 4.9-5.0 ppm of OCH_2 group, a singlet signal at δ 6.2-6.4 ppm corresponding to the coumarin H_3, a singlet signal at δ 6.5-6.6 ppm corresponding to the thiazoline proton, besides the characteristic pattern of aromatic protons. Additionally the signals corresponding to the NH groups have disappeared.

Mass spectrum of compound **9c** revealed the molecular ion peak M^+ at *m/z* 529.1 corresponding to the base peak (100%). Also,

the spectrum showed a peak M^++2 at *m/z* 531.1 (38.7%) due to ^{37}Cl.

Antimicrobial activity and SAR

Antibacterial activity

Results of the antibacterial activity (Table 3) indicated that *S. aureus* and *M. luteus* were completely resistant to the tested compounds, while *B. cereus*, *E. coli* and *S. marcescens* were the most sensitive organisms to the tested compounds. Compounds **4d** (R= *p*-tolyl), **6d** (R= C_2H_5 and R^1= CH_3), **8d** (R= C_6H_5 and R^1= CH_3), **8e** (R= C_6H_5 and R^1= OCH_3), **9a** (R= *p*-tolyl and R^1= H), **9b** (R= *p*-tolyl and R^1= Br), **9d** (R= *p*-tolyl and R^1= CH_3) and **9e** (R= *p*-tolyl and R^1= OCH_3) were completely inactive against all the tested organisms. Also the test compounds were inactive against *P. aeroginosa* except compounds **7d** and **9c** which are moderately active. On the other hand, the majority of the tested compounds appeared to be weakly active against *B. cereus*. Nevertheless some of them, **6c**, **6e**, **8b** and **8c** showed moderate activity, and, compounds **6e**, **7a**, **7b**, and **7d** exhibited moderate to good activity against *E. coli* correlated to the standard drug gatifloxacin. Moreover, some of the test compounds showed good activity against *S. marcescens* and compound **6b** exhibited excellent activity.

SAR

1- Generally, it was observed that compounds containing thiazoline nucleus (**6-9a-e**) seems to be more effective than their precursor's hydrazine carbothioamides (**4a-d**).
2- The most active compounds seem to be compounds containing Cl or Br as an R^1 substituent.
3- It was also noticed that the introduction of a *p*-tolyl group in compounds **9a-e** resulted in a decrease of their antibacterial activity. Also, the ethyl substituent at R seems to be crucial for the antibacterial activity against *S. marcescens*.

Antifungal activity

Results of antifungal activity (Table 4) revealed that all the tested compounds were inactive against *T. rubrum*. They were also inactive against *C. albicans*, except for compounds **4a** and **6e** since they showed 25% activity compared to ketoconazole. Several compounds showed weak to moderate activity against *S. brevicaulis*, while the reference drug was completely inactive. Compounds **6a**, **6b**, **6c**, **6e** and **7b** were even more active than ketoconazole against *F. oxysporum*. The most active compounds were **6b**, **6c**, **7b**, **8b** and **8c** comprising Cl or Br as an R^1 substituent.

SAR

1- Again, it was observed that compounds containing thiazoline nucleus (**6-9a-e**) seems to be more effective than their precursor's hydrazine carbothioamides (**4a-d**).
2- The most active compounds seem to be compounds containing electron withdrawing group (Cl or Br) as an R^1 substituent.
3- It was also noticed that the introduction of a bulky group (*p*-tolyl) in compounds **9a-e** resulted in a complete loss of antifungal activity while small group (ethyl substituent) at R seems to be crucial for the antifungal activity against *F. oxysporum, A. flavus* and *A. niger*.

Conclusions

A number of 7-[2-(3-alkyl/aryl-4-arylthiazol-2(3*H*)-ylidene)hydrazono)propoxy]-4-methyl-2*H*-chromen-2-ones (**6-9a-e**) were prepared and tested for their antimicrobial activity. The antibacterial data indicated that most of the test compounds showed moderate to good activity against *B. cereus*, *E. coli* and *S. marcescens* and some of them showed antibacterial activity against and *P. aeroginosa*. On the other hand, they showed no activity against *S. aureus* and *M. luteus*. Results of antifungal activity revealed that all the tested compounds were inactive against *T. rubrum* and *C. albicans*, except for compounds **4a** and **6e**. Several compounds showed weak to moderate activity against *S. brevicaulis*, while the reference drug was completely inactive. Compounds **6a**, **6b**, **6c**, **6e** and **7b** were even more active than ketoconazole against *F. oxysporum*. Generally, compounds containing thiazoline nucleus (**6-9a-e**) seems to be more effective both as antibacterial and antifungal than their precursor's hydrazine carbothioamides (**4a-d**). *N*-ethyl series (**6a-e**) was the most active both as antibacterial and antifungal agents among the series. It was also noticed that the halo substituted derivatives were the most active ones both as antibacterial and antifungal agents.

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نشرة العلوم الصيدلية
جامعة أسيوط

تشييد وتقييم الفاعلية البيولوجية لبعض مشتقات الكيومارين الجديدة كمضادات للميكروبات

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قسم الكيمياء العضوية الصيدلية ، كلية الصيدلة ، جامعة أسيوط ، أسيوط ٧١٥٢٦ ، مصر

تم في هذا البحث تشييد مجموعة جديدة من المركبات وهي (٣-ألكيل/ أريل – ٤- ثيازول – ٢(٣يد)-وايلدين)هيدرازونو)بروبكسى)-٤-ميثيل-٢يد- كرومين- ٢- ا ون **(6-9a-e)** وذلك من خلال تفاعل المركبات **(4a-d)** مع فيناثيل البروماید **(5a-e)**. ولقد اختبرت درجة نقاوة جميع المركبات الجديدة بواسطة كروماتوجرافيا الطبقة الرقيقة ، كما تم التحقيق من التراكيب البنائية لها اعتماداً على نتائج التحاليل الطيفية المختلفة (الأشعة دون الحمراء، الرنين المغناطيسي للهيدروجين ومطياف الكتلة) بالإضافة إلى التحليل الكمي الدقيق لعناصر تلك المركبات.

وقد تم اختبار الفاعلية البيولوجية لجميع المركبات المستهدفة كمضادات للبكتريا والفطريات مقارنة بعقاري الجاتيفلوكساسين وكيتوكينازول على التوالي في الأنبوب.

وقد أظهرت المركبات التي تم اختبارها فاعلية بيولوجية متوسطة التأثير مقارنة بالعقار المستخدم ضد البكتريا بينما أظهرت بعض المركبات فاعلية تفوق العقار المرجعي ضد بعض الفطريات.