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Synthesis of Coumarin Derivatives with Cytotoxic, Antibacterial and Antifungal Activity

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Dedicated to Prof. Dr. H. C. W. Voelter on the Occasion of His 65th Birthday

The synthesis and selective biological screening of 7-hydroxy-4-methyl-2*H*-chromen-2-one (2), 7-hydroxy-4,5-dimethyl-2*H*-chromen-2-one (15) and some of their derivatives were carried out. Compound 13 was found to be most potent cytotoxic agent with LD_{50} = 126.69 μg/ml. In antibacterial assay the compounds showed a broad spectrum of activities. Compound 11 exhibited a very high degree of plant growth inhibition at three levels of concentration. Compound 4 showed very promising antifungal activity against *Candida albicans*. Compounds 12 and 13 demonstrated excellent antioxidant activity.

Keywords: Coumarin derivatives; Antibacterial; Antifungal; Cytotoxic agent

INTRODUCTION

A diversity of biological effects is associated with the coumarins and their derivatives, some of which are reported to exhibit a wide spectrum of pharmacological activities.[1,2] Osthol, alloimperatorin, isopimpinellin possess antimicrobial properties. Among a number of furocoumarins, *viz.* xanthotoxin, imperatorin, psoralen, angelicin, the most potent therapeutic agent is psoralen, which participates in the restoration of melanin-depleted skin, *i.e.* in the treatment of vitiligo and psoriasis.[3] The oestrogenic activities of caumentans[4] and the insecticidal properties of rotenoids[5] are well established. Phytoalexin activities are associated with the 3-aryl-4-hydroxy-2*H*-1-benzopyran-2-ones.[6] In view of the structural similarities of 3-aryl-4-hydroxy-2H-1-benzopyran-2-ones to warfarin and dicoumarol, their anticoagulant activities were investigated[7] and several compounds exhibited even stronger antivitamin K properties than dicoumarol. Coumadin[8] is the most widely prescribed anti-thrombotic in North America.[9] The major metabolites of *S*-warfarin are *S*-6-hydroxy and *S*-7-hydroxywarfarin, which are derived by the 2C9P450 isoenzyme.[10] The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic has stimulated discovery of therapeutic agents to arrest the replication[11] of the causative virus, human immunodeficiency virus (HIV). Recently, 3-substituted-4-hydroxycoumarin, phenoprocaumen [3-(*d*-ethyl-benzyl)-4-hydroxycoumarin] and analogous compounds have been identified as active nonpeptidic HIV protease inhibitors.[12] Recent reports[13–17] on new synthetic routes to this class of compounds and their different important and interesting biological and pharmacological activities initiated us to synthesize and explore their selected biological activities. We have synthesized very simple derivatives of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**2**) and 7-hydroxy-4,5-dimethyl-2*H*-chromen-2-one (**15**) in search of their biological and pharmacological activities and found very encouraging results.[18,19] This work has been extended here.

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MATERIALS AND METHODS

General Procedure for the Preparation of Compounds 3–10, 16 and 17

To a solution of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**2**) or 7-hydroxy-4,5-dimethyl-2*H*-chromen-2-one (**15**) (1.0 mmol) in THF (10 ml) was added solid potassium carbonate (0.5 mmol) and the resultant mixture was stirred at room temperature for 15 min. To this heterogenous mixture appropriate halides (1.1 mmol) were added (see Scheme 1). The mixture was refluxed until completion of the reaction (TLC analysis) and the solid was filtered off. The filtrate was evaporated *in vacuo* and the resultant solid residue was crystallized from an appropriate solvent to afford the pure derivatives **3–10**, **16** and **17**, respectively.[18,19]

4,5-Dimethyl-2-oxo-2H-chromen-7-yl 4-methylbenzenesulfonate (16)

Yield (89%); colorless solid; mp 178–180°C (chloroform/*n*-hexane); R_f 0.36 (1:1, ethyl acetate/*n*-hexane); UV λ_{max} (MeOH) 281 (log ε 4.46) nm; IR ν_{max} (KBr) 1715 (C=O), 1605 (C=C), 1443 (S=O), 1147 (C–O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (2H, d, $J = 8.4$ Hz, H-2′/H-6′), 7.37 (2H, d, $J = 8.4$ Hz, H-3′/H-5′), 7.04 (1H, d, $J = 1.9$ Hz, H-6), 6.73 (1H, d, $J = 1.9$ Hz, H-8), 6.14 (1H, s, H-3), 2.49 (3H, s, CH_3-4), 2.47 (3H, s, CH_3-4′), 2.32 (3H, s, CH_3-5); EIMS (rel. int. %): *m/z* 344 [M^+] (35), 280 (5), 190 (10), 155 (70), 91 (100), 76 (10), 65 (18), 51 (10); Anal. Calcd. for $C_{18}H_{16}O_5S$ (344.38): C, 62.78; H, 4.68. Found: C, 62.82; H, 4.62%.

Ethyl 2-[(4,5-dimethyl-2-oxo-2H-chromene-7-yl)oxy]acetate (17)

Yield (86%); colorless crystalline solid; mp 249–250°C (methanol/chloroform); R_f 0.60 (1:1, ethyl acetate/*n*-hexane); UV λ_{max} (MeOH) 317 (log ε 4.20) nm; IR ν_{max} (KBr) 1730 (C=O), 1710 (C=O), 1605 (C=C), 1170 (C–O) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.48 (1H, d, $J = 1.4$ Hz, H-6), 6.70 (1H, d, $J = 1.4$ Hz, H-8), 6.17 (1H, s, H-3), 4.52 (2H, s, CH_2-1′), 4.28 (2H, q, $J = 7.2$ Hz, CH_3-2′), 2.49 (3H, s, CH_3-4), 2.32 (3H, s, CH_3-5), 1.32 (3H, t, $J = 7.2$ Hz, CH_3-3′); EIMS (rel. int. %): *m/z* 276 [M^+] (5), 262 (100), 234 (75), 219 (10), 189 (22), 175 (25), 161 (100), 147 (10), 133 (15), 115 (25), 77 (51), 51 (22); Anal. Calcd. for $C_{15}H_{16}O_5$ (276.28): C, 65.21; H, 5.84. Found: C, 65.27; H, 5.78%.

Biological Protocols

DPPH Free Radical Scavenging Activity

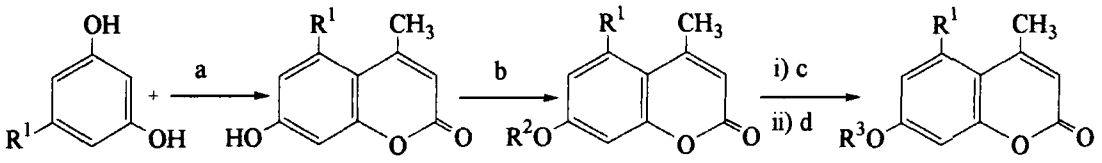
The reaction mixture for this activity contained 5 μL of test compounds in DMSO and 95 μL of DPPH (Sigma-Aldrich (Milan, Italy)) in ethanol.[20] Different concentrations of test compounds were used in the reaction mixture, while the concentration of DPPH was kept as 300 μM. The reaction mixtures were put into 96 well microtitre plates (*Molecular Devices, USA*) and incubated at 37°C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity of the compounds was determined in comparison with a DMSO treated control group. IC_{50} Values represent the concentration of sample, which is required to scavenge 50% DPPH free radicals. 3-*t*-Butyl-4-hydroxyanisole (BHA) and propyl gallate were used as positive controls.

Determination of Cytotoxicity (In Vitro)

Brine shrimp (*Artemia salina* Leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial seawater, which was prepared by dissolving commercial salt mixture (Instant ocean, Aquarium system, Inc., Mentor, Ohio, USA) in double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the smaller compartment was open to ordinary light. After two days a pipette collected nauplii from the lighted side. A sample was prepared by dissolving 20 mg of the test compound in 2 ml of methanol. From this stock solution 500, 50 and 5 μg/ml were transferred to 9 vials, three for each dilution, and one vial was kept as control containing 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 ml of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 ml per vial. After 24 h the number of survivors were counted.[21,22] Data were analysed by a Finney Computer programme to determine the LD_{50}.[23]

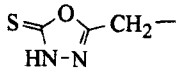
Antibacterial Bioassay (In Vitro)

The synthesized compounds were screened for antibacterial activity against six Gram-negative bacterial strains *i.e. Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae* and four Gram-positive bacterial strains *i.e. Bacillus cereus, Corynebacterium diphtheriae, Staphylococcus aureus* and *Streptococcus pyogenes* using the agar well diffusion method.[24–29] Two to eight hours old bacterial inoculums containing approximately 10^4–10^6 colony forming units (CFU)/ml were used. Wells were dug in the media with the help of a sterile metal borer with centres at least 24 mm. A recommended concentration (100 μl) of the test sample (1 mg/ml in DMSO) was introduced into



1, 14 **2, 15** **3- 10, 16-17** **11-13**

a = Methyl acetoacetate, H_2SO_4, 10°C to r.t.; **b** = R^2X, THF, K_2CO_3; **c** = NH_2NH_2, reflux;
d = Phenyl isothiocyanate, EtOH, reflux, 72 h or CS_2, r.t., KOH, reflux, 24 h.

Compound	R^1	R^2	R^3	X	Yield %
1[a]	H	-	-	-	-
2	H	-	-	-	81
3	H	$H_3CO-\overset{O}{\overset{\|}{C}}-CH_2-$	-	Br	80
4	H	$H_3C-C_6H_4-\overset{O}{\overset{\|}{S}}(=O)-$	-	Cl	85
5	H	$H_3C-\overset{O}{\overset{\|}{S}}(=O)-$	-	Cl	90
6	H	$H_3C-\overset{O}{\overset{\|}{C}}-$	-	$H_3C-\overset{O}{\overset{\|}{C}}-O-$	92
7	H	$(H_3CO)_3C_6H_2-\overset{O}{\overset{\|}{C}}-$	-	Cl	86
8	H	$C_6H_5-\overset{O}{\overset{\|}{C}}-$	-	Cl	88
9	H	H_3C-CH_2-	-	I	84
10	H	$H_3C-(CH_2)_3-CH_2-$	-	I	86
11	CH_3	-	$H_2NHN-\overset{O}{\overset{\|}{C}}-CH_2-$	-	85
12	CH_3	-	$PhHN-\overset{S}{\overset{\|}{C}}-HNHN-\overset{O}{\overset{\|}{C}}-CH_2-$	-	87
13	CH_3	-		-	85
14[a]	CH_3	-	-	-	-
15	CH_3	-	-	-	87
16	CH_3	$H_3C-C_6H_4-\overset{O}{\overset{\|}{S}}(=O)-$	-	Cl	89
17	CH_3	$H_3CO-\overset{O}{\overset{\|}{C}}-CH_2-$	-	Br	86

[a]Starting material

SCHEME 1 Structures, synthesis and yields for the coumarins.

respective wells. Other wells supplemented with DMSO and reference antibacterial drugs *i.e.* imipenum serving as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive and negative controls.

Lemna Welv. Phytotoxic Bioassay

This test was performed according to the modified protocol of McLaughlin *et al.*[21,22] The test compounds were incorporated into sterilized E-medium at different concentrations *i.e.* 5, 50 and 500 μg/ml in methanol. Sterilized conical flasks were inoculated with compounds of the desired concentration prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 ml of sterilized E-medium and ten *Lemna aecquinoctialis* Welv. each containing a Roselle of three fronds. Other flasks were supplemented with methanol serving as negative control and reference inhibitor *i.e.* paraquat serving as positive control. Treatments were replicated three times and the flasks incubated at 30°C in a Fisons Fi-Totran 600 H growth cabinet for seven days, 9000 lux light intensity, 56 ± 10 rh (relative humidity), and a 12 h day length. Growth of *Lemna acquinoctialis*, in flasks containing the compounds, was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to the negative control.

Antifungal Activity (In Vitro)

Antifungal activities of all compounds were studied against fifteen fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspension and transferred to petri plates. Discs soaked in 20 ml (10 μg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for seven days. The results were recorded as zones of inhibition in mm.[30]

RESULTS AND DISCUSSION

Chemistry

Resorcinol (**1**) or 5-methyl resorcinol (**14**) was treated with methyl acetoacetate in the presence of sulphuric acid at 10°C to give 7-hydroxy-4-methyl-2*H*-chromen-2-one (**2**) or 7-hydroxy-4,5-dimethyl-2*H*-chromen-2-one (**15**) respectively. Reaction of compound **2** with ethyl bromoacetate in the presence of potassium carbonate using THF as a solvent afforded ethyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetate (**3**) and in a similar manner *p*-toulenesulfonyl chloride gave (4-methyl-2-oxo-2*H*-chromen-7-yl)-4-methylbenzenesulfonate (**4**). Likewise, the compounds 4-methyl-2-oxo-2*H*-chromen-7-yl-methanesulfonate (**5**), 4-methyl-2-oxo-2*H*-chromen-7-yl acetate (**6**), 4-methyl-2-oxo-2*H*-chromen-7-yl 3,4,5-trimethoxybenzoate (**7**) and 4-methyl-2-oxo-2*H*-chromen-7-yl benzoate (**8**) were synthesized by treatment of compound **2**, using THF as a solvent, with methanesulfonyl chloride, acetic anhydride, trimethoxybenzoyl chloride and benzoyl chloride respectively in the presence of potassium carbonate at room temperature for 24 h. The syntheses of 7-ethoxy-4-methyl-2*H*-chromen-2-one (**9**) and 4-methyl-7-(pentyloxy)-2*H*-chromen-2-one (**10**) were accomplished by treatment of compound **2** with ethyl iodide and iodopentane respectively, in THF in the presence of potassium carbonate for 10 days, (Scheme 1).[18,19]

Ethyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-acetate (**3**) was treated with hydrazine hydrate in ethanol at room temperature for 15 min to provide 2-[(4,5-dimethyl-2-oxo-2*H*-chromen-7-yl)oxy] acetohydrazide (**11**), which when reacted with phenyl isothiocyanate in ethanol on refluxing for 72 h yielded 2-{3-[4,5-dimethyl-2-oxo-2*H*-chromen-7-yl)oxy]-2-oxoethyl}-*N*-phenyl-1-hydrazinecarbothioamide (**12**). Similarly, when compound **11** was treated with carbon disulfide at room temperature in ethanol and then with 2*N* potassium hydroxide with refluxing for 24 h it afforded 4,5-dimethyl-7-[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy]-2*H*-chromen-2-one (**13**) (Scheme 1).[18,19]

Compounds 4,5-dimethyl-2-oxo-2*H*-chromen-7-yl 4-methylbenzenesulfonate (**16**) and ethyl 2-[(4,5-dimethyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**17**) were prepared by treating compound **15** in THF with *p*-toluenesulfonyl chloride or ethyl bromoacetate respectively in the presence of potassium carbonate, (Scheme 1).

Antioxidant Activity

Some of the synthesized compounds were screened for their free radical scavenging activities according to literature protocol[20] and the results are depicted in Table I. Compound **13** demonstrated the highest activity (94.01%) compared to standard propyl gallate (91.00%). Compound **12** also displayed 93.29% activity, which is higher than the standard. The hydrazide **11** also exhibited significant scavenging activity but less than propyl gallate; however, its precursor **3** was completely inactive (data not shown). The benzoate ester **8** showed an some activity. The structure–activity relationship

TABLE I Antioxidant bioassay results for the coumarins

Compound	Free radical scavenging (%)*
2	–
3	–
4	–
5	–
6	–
7	–
8	36.8
9	–
10	–
11	72.7
12	93.3
13	94.0
15	–
16	–
17	–
Propyl gallate	91.0
BHA	95

(–) = Not tested. * Scavenging at 1 mM concentrations against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (2 determinations).

(SAR) demonstrated that the thiocarbonyl group could be responsible for this activity in addition to other electronic factors. In the case of compound **13** the thiocarbonyl group appeared in the 1,3,4-oxadiazole-2(3*H*)-thione ring, whereas in compound **12**, it occurred as an open chain hydrazinecarbothioamide residue. This difference in activities indicated that the thiocarbonyl in the ring form is in some way a better radical scavenger than an open chain hydrazinecarbothioamide. Further extensive work on this type of compound is required in order to get a more potent antioxidant.

Brine-shrimp Lethality Bioassay

The compounds were examined for cytotoxicity in the brine-shrimp lethality bioassay using the protocol of Meyer *et al.*[21–23] From the results, it is evident that only two compounds (**9** and **13**) displayed cytotoxicity towards *Artemia salina*, while other compounds were inactive in this assay. Compound **13** having an 1,3,4-oxadiazol-2-(3*H*)-thione ring showed maximum activity (LD_{50} = 126.69 μg/ml). Compound **9**, an ethoxycoumarin showed (LD_{50} = 157.27 μg/ml) less activity than compound **13**. The structure–activity relationship studies (SAR) revealed that cytotoxicity increases when the alkyl group of the ether moiety in the coumarin is an ethyl group, however, if it is a pentyl group a dramatic decline in activity was observed as demonstrated by compound **10**. In the case of compound **13** the higher level of cytotoxicity shown than for compound **9** may be due to the presence of the 1,3,4-oxadiazole-2(3*H*)-thione ring in addition to an ether residue. These results showed that an ether linkage with a small alkyl chain, in addition to the 1,3,4-oxadiazole-2(3*H*)-thione ring, might fulfil the electronic requirements for the cytotoxic effect.

Antibacterial Activity

All fourteen compounds were tested against six Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae*) and four *Gram*-positive (*Bacillus cereus, Corynebacterium diphtheriae, Staphylococcus aureus, Streptococcus pyogenes*) bacterial strains according to literature protocol.[24–29] The results were compared with those of the standard drug imipenum (Table II). Compounds **2**, **3**, **4**, **8** and **11** exhibited significant activity against *K. pneumoniae* and moderate activity against *S. aureus*. Compound **5**, **6** and **7** were significantly active against *S. dysenteriae* and moderate active against *E. coli, K. pneumoniae, P. mirabilis, S. typhi, B. cerous* and *S. pyogenes*, whereas compound **12** showed moderate activity against all Gram-negative bacteria but was completely inactive against all Gram-positive tested microbes. Compounds **9** and **10** have significant to moderate antibacterial activity against all tested microbes except *S. pyogenes*. Compound **13** showed significant to moderate activities against all tested bacterial strains except *S. aureus* and *S. pyogenes*. Compound **15**, **16** and **17** exhibited significant

TABLE II Antibacterial bioassay results for the coumarins (1 mg/mL in DMSO)

	Compound (zones of inhibition in mm)*															
Bacteria	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**15**	**16**	**17**	SD*
Gram-negative																
Escherichia coli	00	00	00	12	13	13	00	14	14	00	12	17	14	14	14	30
Klebsiella pneumoniae	17	15	19	12	13	13	18	11	15	15	12	16	00	00	00	30
Proteus mirabilis	00	00	00	13	13	12	00	13	18	00	13	19	13	13	12	33
Pseudomonas aeruginosa	00	00	00	00	00	00	00	14	17	00	13	18	14	16	15	25
Salmonella typhi	00	00	00	13	12	13	00	12	15	00	12	12	00	00	00	14
Shigella dysenteriae	00	00	00	14	14	15	00	12	12	00	11	13	00	00	00	33
Gram-positive																
Bacillus cereus	00	00	00	13	12	13	00	13	15	00	00	14	14	14	15	31
Corynebacterium diphtheriae	00	00	00	00	00	00	00	13	16	00	00	14	11	12	11	25
Staphylococcus aureus	13	12	13	00	00	00	13	10	13	11	00	00	00	00	00	43
Streptococcus pyogenes	00	00	00	11	12	13	00	00	00	00	00	00	00	00	00	35

* Significant 14–20 mm, Moderate 7–13 mm, Weak <7 mm. Standard Drug (imipenum).

activity against *E. coli*, *P. aeruginosa* and *B. cerous* and moderate activity against *P. mirabilis* and *C. diphtheriae*.

The structure–activity relationship studies (SAR) suggested that compound **13** with an additional 1,3,4-oxadiazole-2(3*H*)-thione at the C-7 position of the coumarin moiety was found to be active against a wide range of *gram positive* and *gram negative bacteria*. However, compound **10** having a pentyloxy group at the same position showed an equipotent effect against the same organisms as compound **13**. A slight difference was noted in the behaviour of compounds **9** and **10** having an ethyl and pentyl group respectively, at the same position (C-7), **10** being slightly more active. These results suggest that the coumarins **2** and **15** have some antibacterial effects, but if an ether linkage is present at the 7-position, their activity dramatically increases. Another group which may increase the activity of coumarines **2** and **15** is the 1,3,4-oxadiazole-2(3*H*)-thione moiety in addition to the ether moiety.

Phytotoxic Bioassay

The synthesized compounds **2–13** and **15–17** were screened for their phytotoxicity using a literature protocol.[21,22] These experiments were done at three different concentrations *i.e.* 500, 50 and 5 μg/ml. The results of the phytotoxic bioassay are shown in Table III. Compound **11** was found to be the most active in the series with 100% plant growth inhibition at all three levels of concentrations. Compound **3** exhibited promising phytotoxicity with 100% plant growth inhibition at 500 and 50 μg/ml concentration levels. Compound **2** showed weak activity at all three concentrations whereas compound **4** demonstrated significant activity at 500 μg/ml concentration and relatively weak activities at lower concentrations. Compound **13** showed promising phytotoxic activities with 100% plant growth inhibition at 500 μg/ml level, no activity at 50 μg/ml level and very weak activity at 5 μg/ml concentration level. Compounds **8** and **9** exhibited promising activity with 100% plant growth inhibition at 500 μg/ml, however, a considerable decrease in activity occurred at 50 and 5 μg/ml concentration levels. The acyl hydrazide derivative **11** having an ether linkage with a coumarin moiety showed the highest degree of plant growth inhibition, which could be due to the presence of the hydrazide in addition to an ether linkage. Comparing the results for compound **3**, having an ester linkage, and compound **11**, containing a hydrazide moiety, it may be concluded that the hydrazide moiety is probably responsible for plant growth inhibition.

TABLE III *Lemna acquinoctialis Welv.* phytotoxic bioassay results for the coumarins

Compound	Growth Inhibition (%)		
	500 μg/ml	50 μg/ml	5 μg/ml
2	54	51	48
3	100	100	29
4	86	51	59
5	−40	−8.33	0
6	0	−16.6	−23.08
7	−60	0	0
8	100	21	19
9	100	16.67	23.08
10	−30	0	0
11	100	100	100
12	0	16.69	23.08
13	100	0	23.08
15	0	0	−15.39
16	−20	8.33	7.7
17	−50	0	15.39

Standard Drug: Paraquat 0.902 μg/ml. The negative figures mean that the growth is enhanced, not inhibited.

Antifungal Bioassay

The antifungal screening of the compounds was carried out against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberate* fungal strains according to literature protocol.[30] The results illustrated in Table IV indicate that out of fifteen compounds, eight are completely inactive against

TABLE IV Antifungal bioassay results for the coumarins (200 μg/mL in DMSO)

Organism	Compound (% Inhibition)															
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**15**	**16**	**17**	SD*
Trichophyton longifusus	27	32	84	00	00	00	00	65	00	00	00	65	00	00	00	A
Candida albicans	00	00	100	00	00	00	00	00	00	00	00	00	00	00	00	B
Aspergillus flavus	09	00	00	00	00	00	37	00	00	60	00	00	00	00	00	C
Microsporum canis	00	00	00	00	00	00	90	87	00	00	00	55	00	00	00	D
Fusarium solani	00	00	00	00	00	00	80	00	00	00	00	00	00	00	00	E
Candida glaberata	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	F

*Standard Drugs MIC μg/mL: A = Miconazole (70 μg/mL); B = Miconazole (110.8 μg/mL); C = Amphotericin B (20 μg/mL);D = Miconazole (98.4 μg/mL); E = Miconazole (73.25 μg/mL); F = Miconazole (110.8 μg/mL).

either of the tested fungal strains, however, the remaining seven compounds showed varying degrees of activity against some fungal strains. The results were compared with the standard drugs miconazole and amphotericin B. Compounds **4**, **9** and **13** exhibited significant activities against *T. longifusus*, while compounds **2** and **3** showed weak activity. Compounds **8**, **9** and **13** showed prominent activity against *M. canis*, while compound **11** was found to be active against *A. flavus* only. Compound **8** was also found to be significantly active against *F. solani*. Surprisingly compound **4** was found to be the only highly active compound amongst this series against *C. albican*, which indicated that it may be considered as a lead structure for drug design against candidiasis. Compound **4** was also significantly active against *T. longifusus*. The structure of compound **8** suggested that its activity against *M. canis* and *F. solani* might be due to the presence of a reduced electron withdrawing effect of the ester group. However, compound **9** showed significant activity against *T. longifusus* and *M. canis*, which might be due to the presence of the ether moiety. Compound **13** also inhibited the growth of *T. longifusus* and *M. canis*, which could be due to the presence of the 1,3,4-oxadiazole-2(3*H*)-thione moiety.

CONCLUSION

This study provides the basis for the development of 7-hydroxy-4-methyl-2*H*-chromen-2-one and 7-hydroxy-4,5-dimethyl-2*H*-chromen-2-one derivatives as cytotoxic, phytotoxic, antibacterial, antifungal, and antioxidant agents.

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References

[1] Murray, R.D.H., Mendez, J. and Brown, S.A. (1982) *The Natural Coumarins: Occurrence, Chemistry and Bio-chemistry* (John Wiley & Sons Ltd, New York), p 21.
[2] Murray, R.D.H. (1991) *Prog. Chem. Org. Nat. Prod.* **58**, 83.
[3] Musajo, L. and Rodighiero, G. (1962) *Experientia* **18**, 153–200.
[4] Montgomery, G.W., Martin, G.B., Le Bars, J. and Pelletier, J. (1985) *J. Reprod. Fertil.* **73**, 457–463.
[5] Fukami, H. and Nakajima, M. (1971) In: Jacobson, M.D.G., ed., *Naturally Occurring Insecticides* (Dekker, New York), p 71.
[6] Ravise, A. and Kirkiacharian, B.S. (1976) *Phytopathol. Z.* **85**, 74.
[7] Queval, P., Falconet, B., Susini-Garnier, M., Krikorian-Manoukian, A., Courmarcel, D. and Buu-Hoi, N.P. (1972) *Chim. Ther.* **7**, 300.
[8] Coumadin® (warfarin sodium) is a trade mark of the DuPont Merck Pharmaceutical Company.
[9] Raskob, G.E., Comp, P.C., Pineo, G.F. and Hull, R.D. (1994) In: Green, D., ed., *Anticoagulants: Physiologic, Pathologic and Pharmacologic Applications* (CRC Press, Boca Raton (FL)), p 213.
[10] Trager, W.F. (1990) In: Miklos, S., ed., *Problems and Wonders of Chiral Molecules* (Akad. Kiado, Budapest, Hungary).
[11] De Clercq, E. (1995) *J. Med. Chem.* **38**, 2491.
[12] Thaisrivongs, S., Romero, D.L., Tommasi, R.A., Janakiraman, M.N., Strohbach, J.W., Turner, S.R., Biles, C., Morge, R.R., Johnson, P.D., Aristoff, P.A., Tomich, P.K., Lynn, J.C., Horng, M.M., Chong, K.T., Hinshaw, R.R., Howe, W.J., Finzel, B.C. and Watennpaugh, K.D. (1996) *J. Med. Chem.* **39**, 4630.
[13] Mitra, J. and Mitra, A.K. (1992) *Ind. J. Chem. Sect. B* **31**, 693.
[14] Bandyopadhyay, C., Sur, K.R., Patra, R. and Sen, A. (2000) *Tetrahedron* **56**, 3583.
[15] Mitra, J. and Mitra, A.K. (1996) *Ind. J. Chem. Sect. B* **35B**, 588.
[16] Mohanty, N., Rath, P.C. and Rout, M.K. (1967) *J. Ind. Chem. Soc.* **44**, 1001.
[17] Takechi, H., Oda, Y., Nishizono, N., Oda, K. and Machida, M. (2000) *Chem. Pharm. Bull.* **48**, 1702.
[18] Khan, K.M., Saify, Z.S., Hayat, S., Khan, M.Z., Noor, F., Makhmoor, T., Choudhary, M.I., Atta-ur-Rahman, Zia-Ullah and Perveen, S. (2002) *J. Chem. Soc. Pak.* **24**, 226.
[19] Khan, K.M., Saify, Z.S., Begum, S., Khan, M.Z., Hayat, S., Noor, F., Choudhary, M.I., Perveen, S. and Zia-Ullah (2003) *Nat. Prod. Rep.* **17**, 115.
[20] Fujita, Y., Uehara, I., Morimoto, Y., Nakashima, M. and Hatano, T. (1998) *Yakugaku Zasshi* **108**, 129.
[21] McLaughlin, J.L., Chang, C.-J. and Smith, D.L. (1991) In: Atta-ur-Rahman, ed., *Studies in Natural Products Chemistry* (Elsevier Science Publishers B.V., The Netherlands) Vol. **9**, "Bench-Top" Bioassays for the Discovery of Bioactive Natural Products: an update, Structure and Chemistry (part-B), p 383.
[22] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. (1982) *Planta Medica* **45**, 31.
[23] Finney, D.J. (1971) *Probit Analysis*, 3rd Edn. (Cambridge University Press, Cambridge).
[24] Atta-ur-Rahman, Choudhary, M.I. and Thomsen, W.J. (2001) *Bioassay Techniques for Drug Development* (Harwood Academic Publishers, The Netherlands), p 16.
[25] Khan, K.M., Saify, Z.S., Zeeshan, Khan, A., Ahmed, M., Saeed, M., Abdel-Jalil, R.J., Grubler, G. and Voelter, W. (1999) *Naturforsch, 54b*, 1210.
[26] Khan, K.M., Saify, Z.S., Zeeshan, Khan, A., Ahmed, M., Saeed, M., Schick, M., Kohlbau, H.J. and Voelter, W. (2000) *Arzneim-Forsch/Drug Res.* **50**, 915.
[27] Saify, Z.S., Khan, K.M., Haider, S.M., Zeeshan, Shah, S.T.A., Saeed, M., Shekhani, M.S. and Voelter, W. (1999) *Z. Naturforsch* **54b**, 1327.
[28] Zaidi, J.H., Naeem, F., Iqbal, R., Choudhary, M.I., Khan, K.M., Shah, S.T.A., Hayat, S. and Voelter, W. (2001) *Z. Naturforsch* **56b**, 689.
[29] Khan, K.M., Rahat, S., Choudhary, M.I., Atta-ur-Rahman, Ghani, U., Perveen, S., Khatoon, S., Dar, A. and Malik, A. (2002) *Helv. Chim. Acta* **85**, 559.
[30] Atta-ur-Rahman, Choudhary, M.I. and Thomsen, W.J. (2001) *Bioassay Techniques for Drug Development* (Harwood Academic Publishers, The Netherlands), p 22.