Synthesis and Biological Evaluation of Some Coumarin Derivatives

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Abstract

Starting from 7-hydroxy-4-methylcoumarin which was prepared via pechmann condensation, some comarin derivatives were synthesized depending on several reactions which are condensation with aldehydes, Mannich reaction, alkylation and reaction with hydrazine hydrate. The prepared compounds were evaluated for their microbiological activity against Grame +ve bacteria *Bacillus Subtilis*, Gram –ve bacteria *Serratia marcenses* and fungi *Trichoderma Sp.*

Keywords: 7-hydroxy-4-methylcoumarin, 7-hydroxy-4-styryl-2H-chromen-2-one, antimicrobial activity

Introduction

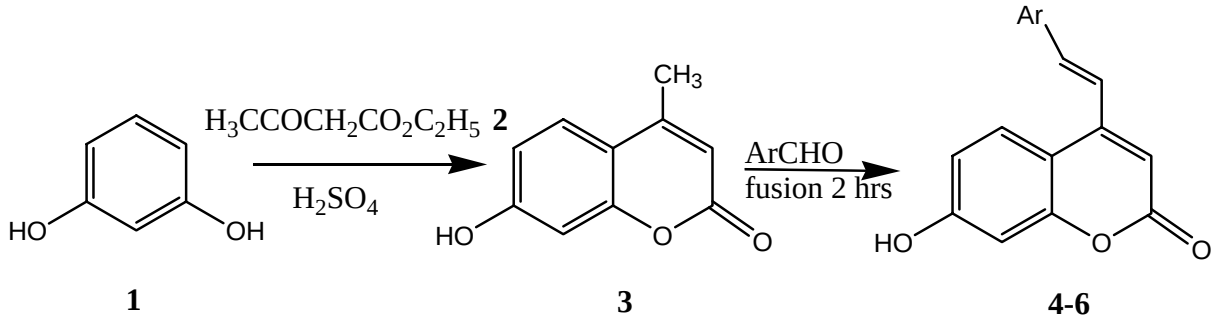
Coumarin constitutes one of the major classes of naturally occurring compounds, and interest in its chemistry continues unabated because of its usefulness as biologically active agents. It also represents the core structure of several molecules of pharmaceutical importance. Coumarin has been reported to serve as antibacterial[1-4], anti-oxidant[5,6], anti-inflammatory[7,8], anticoagulant[8] and antitumour[9,10] agents. These pharmacological properties of coumarin aroused our interest in synthesizing some coumarin derivatives with the aim of testing their microbiological activity.

Results and Discussion

In order to prepare a variety of coumarin derivatives, the 7-hydroxy-4-methylcoumarin **3** was prepared as the starting compound. As depicted in Scheme 1, 7-hydroxy-4-methylcoumarin **3** was synthesized via Pechmann reaction by the condensation between

resorcinol **1** and ethyl acetoacetate **2** in the presence of concentrated sulfuric acid [11]. The 7-hydroxy-4-methylcoumarin **3** was fused with aromatic aldehydes namely benzaldehyde, anisaldehyde and nicotinaldehyde at (120-130°C) to get coumarin derivatives **4**-**6**.

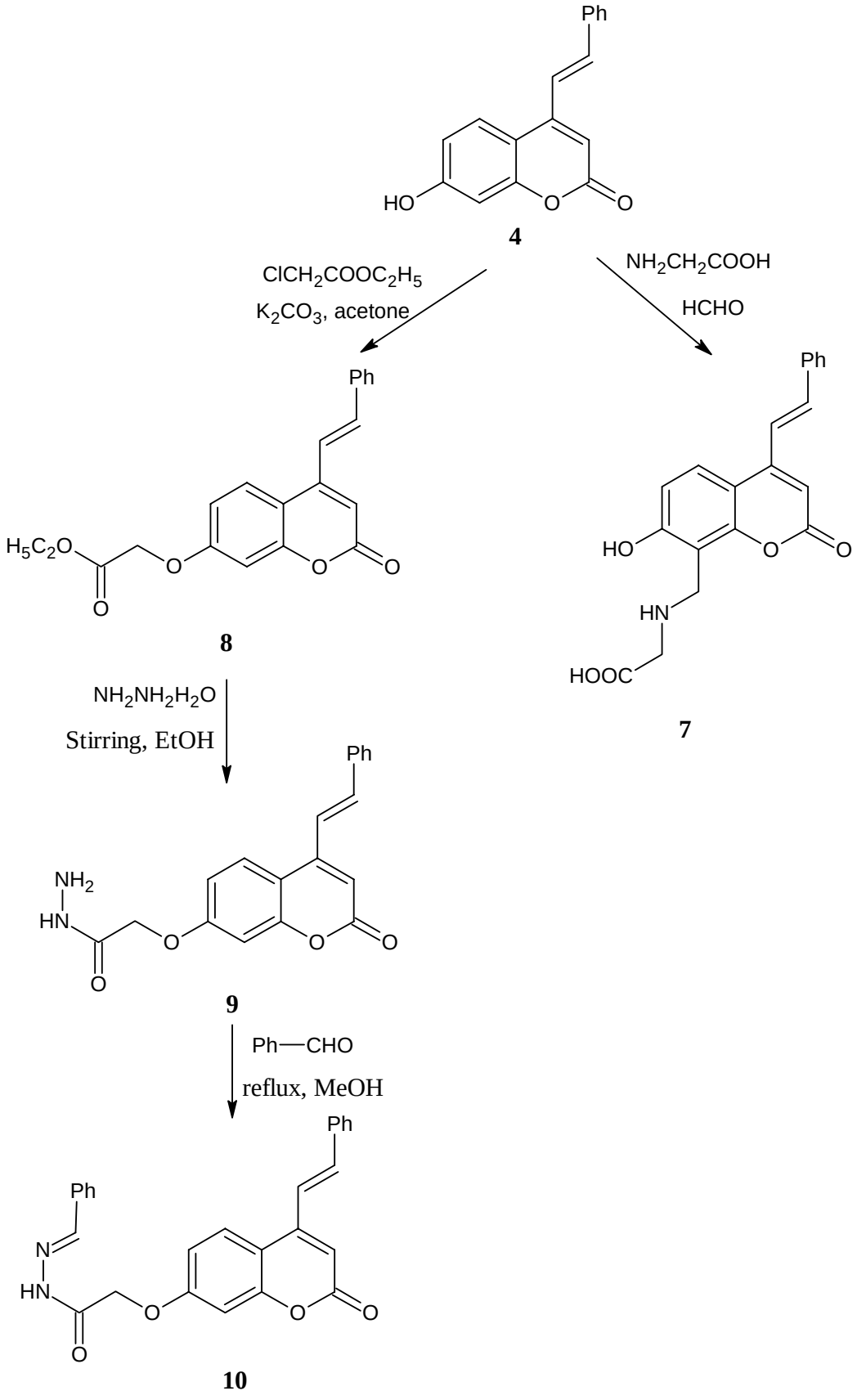
Compound **4** underwent two reactions which were Mannich reaction and alkylation as represented in Scheme 2. Mannich reaction occurred by the treatment of the solution of **4** in ethanol with a mixture of glycine in water and formalin 38-40% giving the compound **7**. Alkylation reaction occurred by treatment of **4** with ethyl chloroacetate in acetone in the presence of K_2CO_3 giving compound **8**. Stirring of **8** with hydrazine hydrate over night at room temperature gave the corresponding hydrazide **9**. Condensation of **9** with benzaldehyde by reflux in methanol gave the corresponding hydrazone **10**.



1 **3** **4-6**

4 Ar = C_6H_5
5 Ar = $C_6H_4OCH_3$
6 Ar = C_5H_4-N

Scheme 1


Ph
HO
O
O
4
ClCH2COOC2H5
K2CO3, acetone
NH2CH2COOH
HCHO
Ph
H5C2O
O
O
O
O
8
Ph
HO
O
O
HN
HOOC
7
NH2NH2H2O
Stirring, EtOH
Ph
NH2
HN
O
O
O
O
9
Ph—CHO
reflux, MeOH
Ph
Ph
N
HN
O
O
O
O
10

Scheme 2

The structural assignment of all synthesized compounds is based on ^{1}H NMR, IR and mass analyses.

The ^{1}H NMR spectrum of the coumarin derivative **5** exhibits signals at δ 2.349, 3.857 and 9.860 ppm corresponding to; CH (olefin), OMe, OH group respectively, Figure 1.

The ^{1}H NMR spectrum of the coumarin derivative **7** showed characteristic signals at δ 3.965, 4.158, 5.782 and 9.089 ppm corresponding to; two CH_2, NH group and COOH group respectively, Figure 1.

The ^{1}H NMR The ^{1}H NMR spectrum of the coumarin derivative **8** showed characteristic signals at δ 1.195, 4.146 and 4.921 ppm corresponding to; ester and CH_2 respectively, Figure 1.

The ^{1}H NMR The ^{1}H NMR spectrum of the coumarin derivative **10** showed characteristic signals at δ 4.636 , 5.946 and 9.685 corresponding to; CH_2, CH and NH group respectively, Figure 1.

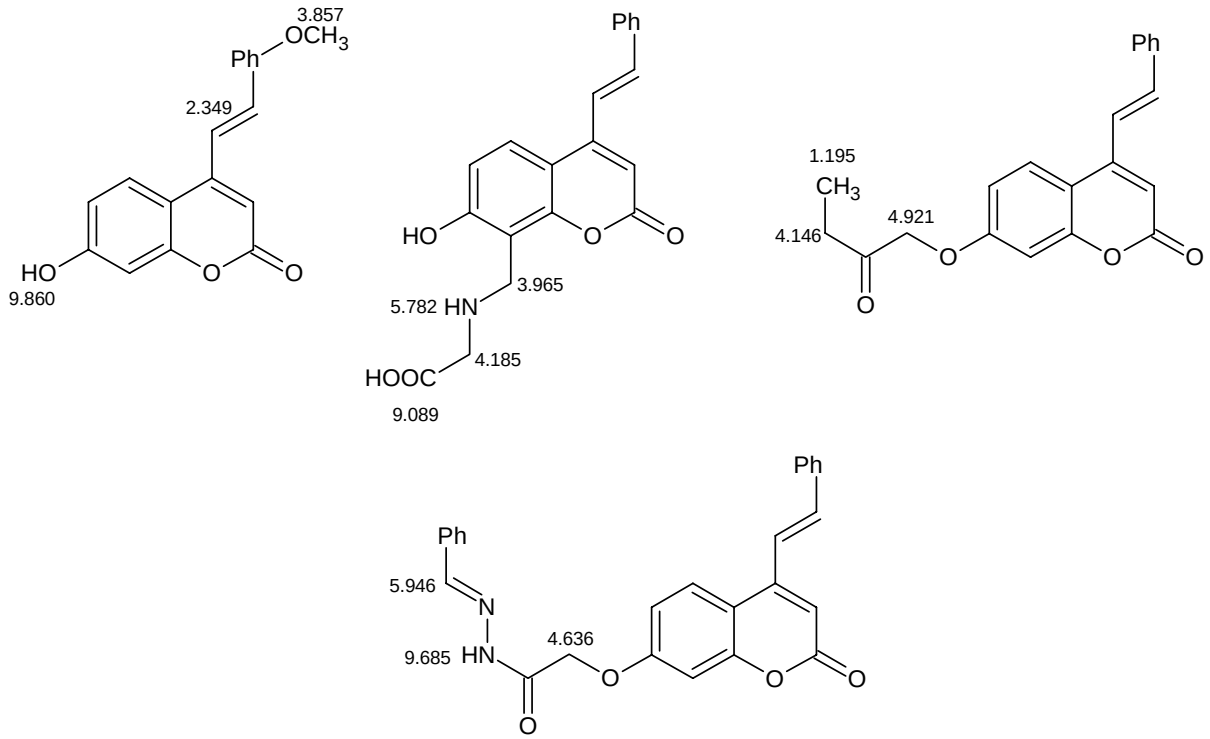


Figure 1. Selected 1H NMR of compunds 5, 7, 8 and 10

Antimicrobial studies

All the compounds prepared were screened for their activity against Gram-positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Serratia marcenses*, as well as fungi *Trichoderma Sp*. Standard drug (Rifamycin) was used at a concentration of 1000 ppm for comparisons. The biological activity of these compounds have been evaluated by filter paper disc method [12] after dissolving them in *N*,*N*dimethylformamide to obtain a 0.5mg/mL solution (500 ppm). The inhibition zones of microbial growth surrounding the filter paper disc (5 mm) were measured in millimeters at the end of an incubation period of 3 days at 28°C. *N*,*N*-dimethylformamide alone showed no inhibition zone. Antimicrobial activity was evaluated by measuring the inhibition zone formed around the discs.

It is apparent from the data listed in Table 1 that some of the synthesized compounds showed antibacterial activity comparable to that of the Rifamycin reference drug used. However, concerning the activity against Gram-positive bacteria (*Bacillus subtilis*), the 7-htdroxy-4-methyl coumarin **3**, 7-hydroxy-4-styryl-2H-chromen-2-one (**4**) and 2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetohydrazide (**9**) showed good activity, compounds 7-hydroxy-4-(4-methoxystyryl)-2H-chromen-2-one (**5**) and 7-hydroxy-4-[2-(pyridin-3-yl)vinyl]-2H-chromen-2-one (**6**) exhibit moderate activity, whereas compounds 2-[(7-hydroxy-2-oxo-4-styryl-2H-chromen-8-yl)methylamino]acetic acid (**7**), ethyl 2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetate (**8**) and N'-benzylidene-2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetohydrazide (**10**) showed mild activity . On the other hand, the Gram – negative bacteria (*Serratia marcenses*) showed high responses to three of the prepared products. 7-hydroxy-4-(4-methoxystyryl)-2H-chromen-2-one (**5**), 7-hydroxy-4-[2-(pyridin-3-yl)vinyl]-2H-chromen-2-one (**6**) and 2-[(7-hydroxy-2-oxo-4-styryl-2H-chromen-8-yl)methylamino]acetic acid (**7**) showed the excellent activity comparable to that of the Rifamycin reference drug used.

Concerning the data of antifungal activity, all the prepared compounds showed no activity against *Trichoderma Sp.*

Table 1. Biological activity of the prepared compounds

Compound	Organism / Relative inhibition		
	Bacillus subtilis	*Serratia marcenses*	*Trichoderma Sp*
3	++	-	-
4	++	-	-
5	+	+	-
6	+	+	-
7	+	+	-
8	+	-	-
9	++	-	-
10	+	-	-
Rifamycin	+++	+	-

Experimental

General

Melting points were taken on an Electrothermal capillary melting point apparatus and are uncorrected. The microanalyses were done at Faculty of Agriculture, Suez Canal University. Infrared spectra were recorded on a Perkin Elmer 1650 FT-IR instrument, using KBr disks. ^{1}H-NMR Spectra were recorded on Varian-400 MHz NMR Spectrometer. Mass spectra were recorded on Shimadzu GCMS-QP1000 EX spectrometer at 70 eV.

General procedure for the preparation of compounds 4-6

Equimolars from compound **3** and aromatic aldehydes are fused together in the presence of catalytic amount of piperidine for about 2 hrs (120-130°C). After the reaction has finished, the mixture was cooled, treated with ethanol and poured onto ice/water. The formed precipitate was filtered out and recrystalized from appropriate solvent as shown Table 2.

Table 2. Physical properties of compounds (4-6)

Compound	M. P.	Yield	Solvent of recrystalization
(**4**)	160°C	54.43%	xylene
(**5**)	167°C	59.66%	toluene
(**6**)	150°C	66.42	benzene

Procedure for the preparation of 2-[(7-hydroxy-2-oxo-4-styryl-2H-chromen-8-yl)methylamino]acetic acid (7)

A warm solution of compound (**4**) (1.32 g, 0.005 mole) in ethanol was treated with a solution of glycine (0.38 g, 0.005 mole) in water and formalin (0.45 ml). The reaction mixture was held at 80-90°C for 6 hrs. The resulting precipitate was filtered out and recrystalized ethanol (yield 57%), m. p. 240°C.

Procedure for the preparation of ethyl 2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetate (8)

A mixture of compound (**4**) (3.7 g, 0.014 mol), anhydrous potassium carbonate (1.93g, 0.014 mol) and ethyl chloroacetate (1.7 ml, 0.014 mol) in dry acetone was refluxed for 24 h. After the reaction has finished, the mixture was poured on ice/water. The solid obtained was filtered off and recrystallized from ethanol to give (**8**) (yield 61.79%), m. p. 70°C.

Procedure for the preparation of 2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetohydrazide (9)

Compound (**8**) (1.73 g, 0.0049 mole), was stirred with hydrazine hydrate (0.2 ml, 0.004) in ethanol overnight. After the reaction has finished, the precipitate was filtered out and recrystalized from ethanol + H_2O to give (**9**) (yield 54.7%), m. p.194°C.

Procedure for the preparation of N'-benzylidene-2-(2-oxo-4-styryl-2H-chromen-7-yloxy)acetohydrazide (10)

A solution of benzaldehyde (0.31 ml, 0.0029 mole) in methanol was added drop wise to a well-stirred solution of compound (**9**) (0.98 g, 0.0029 mole) in boiling methanol, the reaction mixture was refluxed for about 2 hrs. After the reaction has finished, the precipitate was filtered out and recrystalized from DMF to give (**10**) (yield 68.55%), m. p. 246°C.

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