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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 coumarin 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 Gram +ve bacteria *Bacillus Subtilis*, Gram –ve bacteria *Serratia marcenses* and fungi *Trichoderma Sp.* The structures of all the prepared compounds are elucidated by IR, ^{1}H-NMR and Mass spectra.

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

INTRODUCTION

Coumarin derivatives one of the major classes of naturally occurring compounds, and interest in their chemistry continues unabated because of its usefulness as biologically active agents. Coumarin also represents the core skeleton of several molecules of pharmaceutical importance. Coumarin has been reported to serve as antibacterial **[Ukhov *et al.*, (2001), Abd Elhafez *et al.*, (2003), Basanagouda *et al.*, (2009) and liu *et al.*, (2008),]**, **anti-oxidant [Trapkov *et al.*, (1996) and Vukovic *et al.*, (2010)]** , anti-inflammatory **[Emmanuel *et al.*, (2001) and Hamdi *et al.*, (2007)]** **anticoagulant [Hamdi *et al.*, (2007)]** and antitumour agents **[Wang *et al.*, (2001)]** **and [Marchenko *et al.*, (2006)]** .These pharmacological properties of coumarin aroused our interest in synthesizing some coumarin derivatives with the aim of testing their microbiological activity.

EXPERIMENTAL

General :

Melting points were taken on an Electrothermal capillary melting point apparatus and are uncorrected. The microbiological screening was carried out 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.

Synthesis :

Procedure for the preparation of 7-hydroxy-4-methylcoumarin 1 is already described [11]

General procedure for the preparation of compounds 4-6

Equimolars from compound **3** and aromatic aldehydes were fused together in the presence of few drops of piperidine for about 2 hrs (120-130°C). After the reaction was completed, the mixture was cooled, treated with ethanol and poured onto ice/water. The precipitate formed was filtered out and recrystallized from appropriate solvent.

7-hydroxy-4-styryl-2H-chromen-2-one (4) recrystallized from xylene (yield 54.43%), m.p. 160°C ;^{1}H NMR (DMSO) δ (ppm): 6.895 (d, 1H-olefin, cis, J= 8.6), 6.117 - 7.598 (m, 9H, ArH), 10.491 (s, 1H, OH) ; IR (KBr) cm^{-1}: 3255, 1684, 1612 ; MS calculated for $C_{17}H_{12}O_3$: 264.08 Found: M^+ (264, 1.5%).

7-hydroxy-4-(4-methoxystyryl)-2H-chromen-2-one (5) recrystallized from toluene (yield 59.66%) m.p. 160°C ; ^{1}H NMR (DMSO) δ (ppm): 6.811 (d, 1H-olefin, cis, J=9), 3.857 (s, 3H, OCH_3), 6.105 - 7.872 (m, 8H, ArH), 9.860 (s, 1H, OH) ; IR (KBr) cm^{-1}: 3495, 1674, 1603 ; MS calculated for $C_{18}H_{14}O_4$: 294.09 Found: M^+ (294, 0.25%).

7-hydroxy-4-[2-(pyridin-3-yl)vinyl]-2H-chromen-2-one (6) recrystallized from benzene (yield 66.42%) m.p. 150°C; ^{1}H NMR (DMSO) δ (ppm): 6.813 (d, 1H-olefin, cis, J=8.7), 6.087 - 7.593 (m, 8H, ArH), 10.492 (s, 1H, OH) ; IR (KBr) cm^{-1}: 3255, 1712, 1683, 1585 ; MS calculated for $C_{16}H_{11}NO_3$: 265.07 Found: M^+ (265, 0.8%).

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 recrystallized ethanol (yield 57%), m. p. 240°C; ^{1}H NMR (DMSO) δ (ppm): 3.965 (s, 2H, $\underline{CH_2}$-NH), 4.158 (s, 2H, NH-$\underline{CH_2}$-COOH), δ=6.816 (d, 1H, olefin), 5.782 (s, 1H, NH), 6.064 - 7.818 (m, 8H, ArH), 9.089 (s, 1H, COOH) ; IR (KBr) cm^{-1}: 3415 , 3060 , 1713 and, 2931 ; MS calculated for $C_{20}H_{17}NO_5$: 351.11 Found: M^+ (351, 6.31%)

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 was completed, the mixture was poured on ice/water and the solid obtained was filtered off and recrystallized from ethanol to give (**8**) (yield

61.79%), m. p. 70°C ; ; ^{1}H NMR (DMSO) δ (ppm): 1.195-1.243 (t, 3H, CH_3-ester), 4.146-4.217 (q, 2H, CH_2-ester), 4.921 (s, 2H, O$\underline{CH_2}$CO), 6.220 - 7.706 (m, 11H, ArH) ; IR (KBr) cm^{-1}: 1720 , 1614 ; MS calculated for $C_{20}H_{17}NO_5$: 350.12 Found: M^+ (350, 25.16%)

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 was completed, the precipitate was filtered out and recrystallized from ethanol + H_2O to give (**9**) (yield 54.7%), m. p.194°C; ^{1}H NMR (DMSO) δ (ppm): 4.612 (s, 2H, O$\underline{CH_2}$CO), 6.220 - 7.711 (m, 11H, ArH), 4.346 (s, 2H, NH_2) and 9.406 (s, NH) ; IR (KBr) cm^{-1}: 3325, 3268, 1725, 1680 and 1611; MS calculated for $C_{20}H_{17}NO_5$: 336.11 Found: M^+ (336, 1%).

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 dropwise 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 recrystallized from DMF to give (**10**) (yield 68.55%), m. p. 246°C; ^{1}H NMR (DMSO-TFAA) δ (ppm): 4.636 (s, 2H, O$\underline{CH_2}$CO), 6.677 – 8.048 (m, 16H, ArH), 8.048 (s, 1H, N=$\underline{CH}$-ph), and 9.685 (s, NH) ; IR (KBr) cm^{-1}: 3342, 1686 and 1593 ; MS calculated for $C_{20}H_{17}NO_5$: 424.14 Found: M^+ (424, 0.66%).

Biological Tests :

Standard drug (Rifamycin) was used at a concentration of 1000 ppm for comparisons. The biological activity of the synthesized compounds have been evaluated by filter paper disc method [Murray *et al.,* (1995)] 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 inhibation zone.

Finally the relative inhibition was calculated according to the following formula:-

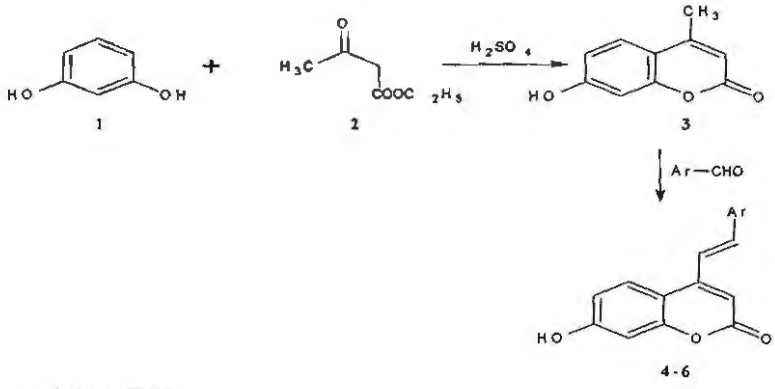
$$\text{Relative inhibition} = \frac{\text{diameter of ihibition zone around disc}}{\text{the whole diameter of growth}} \text{X100}$$

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 **[Srinivasan *et al.*, (2007)]**. The 7-hydroxy-4-methylcoumarin **3** was fused with aromatic aldehydes namely benzaldehyde, 4-methoxybenzaldehyde and pyridine-3-carboxaldehyde at (120-130°C) to give coumarin derivatives **4-6**.

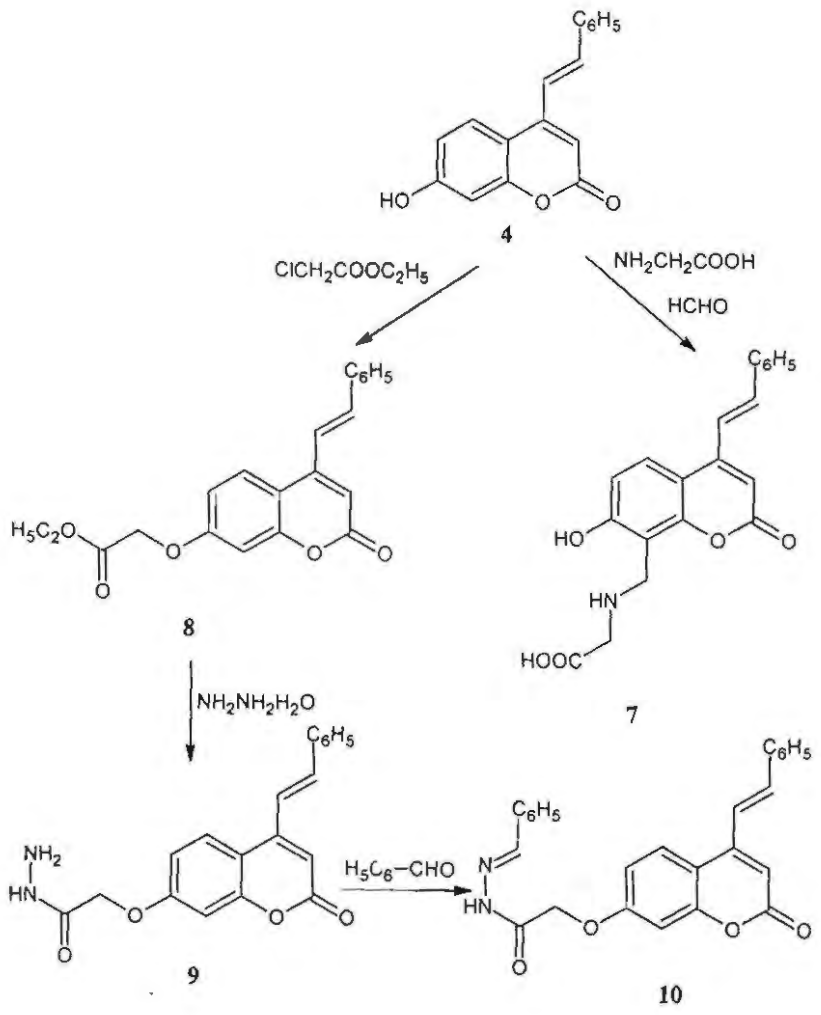
Compound **4** underwent two reactions which were Mannich reaction and alkylation as represented in Scheme 2. Mannich reaction occurred by the treatment of the solution of **4** in ethanol with a mixture of glycine in water and formalin 38-40% giving the compound **7**. Alkylation reaction occurred by 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**.

The structures of the synthesized compounds were elucidated by their ^{1}H NMR, MS and IR spectral analyses. In the IR spectra, the band due to C=N group, which is present in compounds **6** and **10** was observed at about 1585 cm^{-1} and 1593 cm^{-1}, respectively. The bands at about 3440, 3255 and 3495 were characteristic for the OH group in compounds **3** and **4-6**. About 3268 and 3342 cm^{-1} was characteristic for hydrazide and hydrazone. In ^{1}H NMR spectra, the hydroxyl proton was appeared as singlet at about δ 9.890-10.491 ppm in derivatives **3** and **4-6**. The aromatic protons were observed at about δ 6.11 to 7.818 ppm in all the synthesized compounds, the carboxylic acid proton of compound **7** appeared at about δ 9.089, NH proton was appeared at δ 9.685. In Mass spectra, molecular ion peak or M^+ was obtained from ESI-MS.



4 Ar = C_6H_5
5 Ar = $C_6H_4 - OCH_3$
6 Ar = $C_5H_4 - N$

Scheme 1



Scheme 2

Biological Activity :

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.* The results are given in Tables 1.

Table (1). Biological activity of the prepared compounds

Compound	Organism / Relative inhibition		
	Bacillus subtilis	*Serratia marcenses*	*Trichoderma Sp*
3	++ (50%)	-ve	-ve
4	++ (50%)	-ve	-ve
5	+ (25%)	+ (20%)	-ve
6	+ (25%)	+ (22%)	-ve
7	+ (12%)	+ (25%)	-ve
8	+ (10%)	-ve	-ve
9	++ (50%)	-ve	-ve
10	+ (10%)	-ve	-ve
Rifamycin	+++ (75%)	+ (25%)	-ve

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-hydroxy-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 pounds 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.*

CONCLUSION

In conclusion, the antimicrobial screening revealed that the antibacterial activity of the all synthesized compounds ranged between good and mild against the tested Gram positive bacteria. Among the synthesized compounds, only **5**, **6** and **7** showed excellent activity against the tested Gram negative bacteria. On the other hand, all the synthesized compounds showed no activity against the tested fungi.

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الملخص العربي

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" تخليق و تقييم بيولوجي لبعض مركبات الكومارين"

يبدأ تحضير مركبات الكومارين عن طريق تفاعل شهير بين رزورسينول و ايثيل اسيتو اسيتات و ينتج عن هذا التفاعل المركب الأول و هو ٧-هيدروكسي-٤-ميثيل-كومارين و بواسطة بعض التفاعلات الكيميائية المختلفة التي يمكن إجراؤها على المركب المذكور يتم الحصول على بعض المشتقات الأخرى للكومارين.

يتم تحضير محاليل للمركبات المحضرة من خلال إذابتها في ن،ن-ثنائي ميثيل فورماميد حيث تتعرض هذه المحاليل للتقييم البيولوجي من خلال اختبار نشاطها ضد نوعين من البكتريا (سالبة و موجبة الجرام) بالإضافة إلى نوع واحد من الفطريات.