Synthesis And Bio-Evaluation Of Some 4-Hydroxycoumarin Derivatives

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Abstract

Coumarins are naturally occurring oxygen heterocyclic compounds having multifarious medicinal properties, hence used as lead compounds for designing new potent analogs. The modification of 4-hydroxycoumarin by different reaction steps was done to yield target compounds. The structures of the synthesized compounds were characterized by spectroscopic methods, Infrared Spectra FT-IR and ^1H Nuclear Magnetic Resonance Spectra and elemental analysis techniques, melting point and thin layer chromatography. The newly synthesized derivatives contain different functional groups, and as such they could exhibit microbiological activity. Therefore, we tested the microbiological activity of these derivatives on various species of bacteria and fungi. The tested compounds have shown different activity in terms of growth inhibition of microorganisms. Newly synthesized derivatives exhibit antibacterial activities.

1- Introduction

Coumarin derivatives from natural and synthetic sources are useful in a number of fields and are commonly used in medicine, often in the form of glycosides. Plants from the families of Rutaceae, Rubiaceae, Asteraceae, Apiaceae, Oleaceae, Fabaceae, Solanaceae and Hippocastanaceae, as well as bacteria of *Aspergillus* and *Streptomyces* strains are the most inspiring and the richest source of leading structures containing the coumarin system. Numerous efforts, including the isolation and purification of naturally occurring coumarins from a variety of plants as well as laboratory synthesis of coumarin derivatives with novel structures and properties, have been focusing on the research and development of coumarins as potential drugs [1–4]. Treatment approaches for bacterial infections remain a challenging therapeutic problem. Despite many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic resistant bacterial strains in recent decades has increased the need for new classes of antibacterial agents [5]. Nowadays 4-hydroxycoumarin and its derivatives are widely used anticoagulant rodenticides as well as antithrombotic agents [6]. The 4-hydroxycoumarin anticoagulants are antagonists of vitamin K and their target is vitamin K 2,3-epoxide reductase in the liver microsomes. Finally, they are also useful key intermediates for many industrial products such as dyes [7] and liquid crystals [8]. The chemical synthesis, structural modification, and a wide variety of biological activities of 4-hydroxycoumarins have been reported in many papers [9–11]. The goal of this research is to summarize recent synthetic of 4-hydroxycoumarin derivatives and their biological activities.

2- Experimental Details

Infrared (IR) spectra were recorded at room temperature from 4000-400 cm^{-1} with KBr pellets, using Avatar 330 equipped with DTGS detector. The ^1H NMR was measured on a Bruker AMX-400 instrument at room temperature; the ^1H NMR was measured in $\text{DMSO}-d_6$ using TMS as internal reference. Melting points were determined in open capillaries and are uncorrected. All reagents were purchased from Aldrich.

2.1 General procedure with Spectral data:

A. Procedure for the preparation of methyl 2-(2-oxo-2H-chromen-4-yloxy)acetate (**2**) : a suspension of 4-hydroxy methylcoumarin (1.086 g, 6.17 mmol) in acetone (30ml) was refluxed with methyl bromoacetate (1.528 g, 9.15 mmol) and potassium carbonate K_2CO_3 (4.69 g, 33.91 mmol) for 12 hr. After cooling, the mixture was evaporated to remove the moisture. Then the residue was separated into $CHCl_3$ (50 ml) and water (50 ml), the crude product was purified by crystallization from ethanol [12].

B. Procedure for the preparation of 2-(2-oxo-2H-chromen-4-yloxy)acetohydrazide (**3**), 2-(2-(2-oxo-2H-chromen-4-yloxy)acetyl)hydrazinecarbothioamide (**4**): Conventional method: A solution of the compound (**2**) (2.48 g, 10 mmol) in 25 ml of ethanol was refluxed with hydrazine hydrate (7.5 g, 15 mmol) for 4 hr. to yield compound (**3**), with thiosemicarbazide to yield compound (**4**), The residue were dried and then desiccated to afford a crystalline powders. [12].

C. Procedure for the preparation of (N',N'E,N',N"E)-N',N"-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(2-(2-oxo-2H-chromen-4-yloxy)acetohydrazide) (**5**), (N',N'E,N',N"E)-N',N"-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(2-(2-(2-oxo-2H-chromen-4 yloxy)acetyl)hydrazinecarbothioamide) (**6**): a mixture of compound(**4**) (0.01 mol) in chloroform methanol (1:1v/v) mixture (30 mL), terephthalaldehyde (0.005mol) and 1 mL of glacial acetic acid was refluxed on a water bath for 3 h. The mixture was allowed to cool and then the separated solid was filtered, washed with excess of methanol [13].

2.2. Antibacterial Activities.

Antibacterial activities of the synthesized coumarins were examined utilizing diffusion technique [14] on G+ bacteria species named (*Staphylococcus aureus* and *Streptococcus pyogenes*) and G- bacteria species named (*Pseudomonas aeruginosa* and *E. coli*) and antifungal activities was examined utilizing diffusion technique on *C. albicans*. A nutrient agar was utilized to estimate the antimicrobial activity. Results were demonstrated as inhibition zones and shown in mm. Stock solutions at a concentration of 1mg/mL for synthesized target compounds were prepared by dissolving the target compounds in dimethyl sulfoxide, and the inhibition zone was measured (in millimeters) at 37°C after an 18 h incubation period. The concentrations of the prepared solutions were as follows: 0.5, 0.25, 0.125, 0.0625, and 0.03125mg/mL. To clarify any effect of DMSO on biological screening, a separate study was performed using dimethyl sulfoxide (99.5% DMSO) as a control, and the result showed that there was no activity against any of the selected bacteria. For comparison, a standard antibiotic drug, gentamycin, was used as a standard for antimicrobial activity.

3. Results And Discussion

3.1. Chemistry

The reactions for the synthesis of the new compounds, namely, methyl 2-(2-oxo-2H-chromen-4-yloxy)acetate(**2**), 2-(2-oxo-2H-chromen-4-yloxy)acetohydrazide (**3**), 2-(2-(2-oxo-2H-chromen-4-yloxy)acetyl)hydrazinecarbothioamide(**4**), (N',N"E,N',N"E)-N',N"-(1,4-phenylenebis(methan-1-yl-1-

ylidene))bis(2-(2-oxo-2H-chromen-4-yl)oxy)acetohydrazide) (**5**), (N,N'E,N,N'E)-N,N'-(1,4-phenylenebis(methan-1-yl-1-ylidene))bis(2-(2-(2-oxo-2H-chromen-4-yl)oxy)acetyl)hydrazinecarbothioamide) (**6**), were successfully synthesized and completed under conventional methods and the reaction sequence for the synthesis of the compounds is shown in scheme 1, starting from 4-hydroxycoumarin. Many researchers synthesized coumarin derivatives from 4-hydroxycoumarin [15–24].

3.2. Antibacterial Activity

The antibacterial activities data demonstrate that the newly synthesized compounds (**1–6**) exhibit antibacterial action and that (**1–6**) they display additional restraint effects beyond the parent 4-hydroxycoumarin. Delocalization of pi-electrons above the entire synthesized molecule area electrons belonging to certain molecules is not attached to a particular atom or bond in that molecule. These electrons are said to be “delocalized” because they do not have a specific location (are not localized). Electrons become delocalized in order to stabilize a structure [25]. This raises the lipophilic capacity of the synthesized compounds (**1–6**), increases its penetration through the lipid-layer membrane of the bacteria. The lipophilic character of the synthesized compounds (**1–6**) was increased and seems to be responsible for the enhanced potent antimicrobial activity. The mechanism of the synthesized compounds (**1–6**) for killing bacteria may involve deactivation of different cellular enzymes, playing a vital role in diverse metabolic pathways of selected bacteria types. We suggest that the final action of the toxicant impairs the proteins in the cell, resulting in denaturation of natural cellular processes. Other factors that may also raise the activity are as follows: conductivity, solubility, and bonds lengths. As a result of the antibacterial screening study of (**1–6**), as shown in Table 2. First, all compounds exhibited antibacterial activity toward all selected types of tested organisms. Second, compounds **3, 4, 6** have more activity for the tested bacteria compared with compounds **1, 2** and **3** combined. Third, compounds **3, 4, 6** have (NH-NH₂) group and compounds **4, 6** have (C = S) group which via the inductive effect; this may be the main mechanism by which compound **3, 4, 6** acts against bacteria.

Table 1
Physical properties and spectral data of the prepared compounds

NO. COMP.	M.P (°C)	Yield (%)	Molecular formula (M.wt)	Spectral data	
				IR (cm ⁻¹)	¹ H- NMR (δ, ppm)
2	83– 85 lit. 84– 85	80 (lit. 85)	C ₁₂ H ₁₀ O ₅ 234.20	2960(C-H,aliphatic) 3083.4(C-H,aromatic) 1760.3(C = O,ester) 1723.1 (C = O, lactone) 1624.5 (C = C, alkene) 1567.2(C = C, aromatic)	3.6 (s, 3H, CH ₃), 4.79 (s, 2H, CH ₂), 5.58 (s, 1H, -C = C-H) 7.3111–7.896 (m, 4H, aromatic ring)
3	200– 202 lit. 198– 200	55 (lit.60)	C ₁₁ H ₁₀ N ₂ O ₄ 234.06	3300.1(NH ₂), 3233.3, 3210 (N-H) 2959.0(C-H,, liphatic) 3083.9(C-H, aromatic),1721.4(C = O,lactone),1624.2 (C = O, amide)	4.45 (s, 2H, CH ₂), 4.75 (s, 2H, NH ₂), 5.43 (s, 1H, -C = C- H) 7.41–7.78 (m, 4H, aromatic ring), 8.21 (s, 1H, NH)
4	210– 213	50	C ₁₂ H ₁₁ N ₃ O ₄ S 293.30	3310.15(NH ₂),3230.1, 3215 (N-H) ,2950 (C-H, aliphatic) ,3080(C-H, aromatic) ,1720 (C = O,lactone) 1625 (C = O, amide) 455(C = S)	2.0 (s,1H,NH, amine),8.0(s,1H, NH sec. amide), 8.49(s, 2H,NH ₂ amine), 7.41–7.78 (m, 4H, aromatic ring), 4.67 (s, 2H, CH ₂), 5.39 (s, 1H, -C = C-H)
5	255– 258	40	C ₃₀ H ₂₂ N ₄ O ₈ 566.52	3235, 3220 (N-H), 3085(C-H, aromatic), 1710(C = O,lactone) 1620 (C = O, amide)	8.1(s,1H, NH amide), 7.42– 7.65 (m, 4H, aromatic ring), 4.79 (s, 2H, CH ₂), 5.35 (s, 1H, -C = C-H), 8.35(s,1H,CH- N), 8.0(s,4H,aromatic)
6	268– 270	45	C ₃₂ H ₂₄ N ₆ O ₈ S ₂ 684.70	3233, 3225 (N-H), 3089(C-H, aromatic), 1705(C = O,lactone), 1621 (C = O, amide), 465(C = S)	2.1 (s,1H,NH amine), 8.3(s,1H, NH amide), 7.40– 7.67 (m, 4H, aromatic ring), 4.49 (s, 2H, CH ₂), 5.22 (s, 1H, -C = C-H), 8.33 (s,1H,CH- N), 8.1(s,4H,aromatic)

Table 2
Antimicrobial activity of the prepared compounds, expressed as the inhibition zone (mm)

Microorganism	<i>S. aureus</i>	<i>Staphylococcus epidermidits</i>	<i>Streptococcus.sp</i>	<i>Klebsiella sp.</i>	<i>E. coli</i>	<i>C. albicans</i>
Compound (1 mg/ml)	INHIBITION ZONES (mm)					
1	19	19	-	12	13	13
2	-	-	-	10	11	-
3	14	-	-	11	-	12
4	30	32	-	31	30	30
5	13	14	-	-	11	9
6	11	-	-	12	14	-
Tetracycline	19	11	12	12	21	—

Conclusion

A series of coumarin derivatives was designed, synthesized and evaluated for its antimicrobial activities. The biological screening results indicated that the compounds **1,4,5**, and **6**, the best antimicrobial activity comparable to the standard drugs. Hence these compounds may be taken as lead compound for further development of novel antimicrobial.

Declarations

Compliance with Ethical Standards

By submitting the manuscript, the authors recognized that the material presented in this paper has not been published before, nor has it been submitted for publication to another journal. The corresponding author attests that this study has been approved by all the co-authors concerned.

Conflict of Interest: The authors declare that they have no conflict of interest

References

1. A. Abate, V. Dimartino, P. Spina, P.L. Costa, C. Lombardo, A. Santini, M. Del Piano, P. Alimonti, Hymecromone in the treatment of motor disorders of the bile ducts: a multicenter, double-blind, placebo-controlled clinical study. *Drugs Exp. Clin. Res.* **27**, 223–231 (2001)
2. M. Božič-Mijovski, Advances in monitoring anticoagulant therapy. *Adv. Clin. Chem.* **90**, 197–213 (2019)

3. C.L. Dávila-Fajardo, X. Díaz-Villamarín, A. Antúnez-Rodríguez, A.E. Fernández-Gómez, P. García-Navas, L.J. Martínez-González, J.A. Dávila-Fajardo, J.C. Barrera, Pharmacogenetics in the treatment of cardiovascular diseases and its current progress regarding implementation in the clinical routine. *Genes (Basel)* **10**, 261–286 (2019)
4. D. Opherk, G. Schuler, W. Waas, R. Dietz, W. Kubler, 1990. Intravenous carbochromen: a potent and effective drug for estimation of coronary dilatory capacity. *11*, 342–347. DOI: 10.1093/oxfordjournals.eurheartj.a059708
5. I. Chopra, C. Schofield, M. Everett et al., Treatment of healthcare-associated infections caused by Gram-negative bacteria: a consensus statement. *Lancet. Infect. Dis* **8**(2), 133–139 (2008) “,”,
6. M.M. Samama, A century of progress. From biology to antithrombotic agents. *J. Mal. Vasc.* <background-color:#66FF66;bvertical-align:super;>2001</background-color:#66FF66;bvertical-align:super;>, *26*, 165–168
7. M. Bulut, C. Erk, Improved synthesis of some hydroxycoumarins. *Dyes Pigments* <background-color:#66FF66;bvertical-align:super;>1996</background-color:#66FF66;bvertical-align:super;>, *30*, 99–104
8. H. Kang, K.S. Kwon, D. Kang, J.C. Lee, Enhanced, perpendicular liquid-crystal alignment on rubbed films of a coumarin-containing polystyrene. *Macromol. Chem. Phys.* <background-color:#66FF66;bvertical-align:super;>2007</background-color:#66FF66;bvertical-align:super;>, *208*, 1853–1861
9. N. Au, A.E. Rettie, Pharmacogenomics of 4-hydroxycoumarin anticoagulants. *Drug Metab. Rev.* <background-color:#66FF66;bvertical-align:super;>2008</background-color:#66FF66;bvertical-align:super;>, *40*, 355–375
10. N. Hamdi, M. Saoud, A. Romerosa, 4-Hydroxy Coumarine: A versatile reagent for the synthesis of heterocyclic and vanillin ether coumarins with biological activities. *Top. Heterocycl. Chem.* <background-color:#66FF66;bvertical-align:super;>2007</background-color:#66FF66;bvertical-align:super;>, *11*, 283–301
11. N. Hamdi, M. Saoud, A. Romerosa, R.B. Hassen, Synthesis, spectroscopic and antibacterial investigations of new hydroxy ethers and heterocyclic coumarin derivatives. *J. Heterocycl. Chem.* <background-color:#66FF66;bvertical-align:super;>2008</background-color:#66FF66;bvertical-align:super;>, *45*, 1835–1841
12. K. Yasameen, A. Al-Majedy, H. Amir, A. Kadhum, Ahmed, Al-Amiery, Abu Bakar Mohamad ; Synthesis and Characterization of Some New 4-Hydroxycoumarin Derivatives, *Molecules* 2014,19, 11791–11799
13. V.S.V. SATYANARAYANA, MADHUMITA Rakshitand, A. SIVAKUMAR: Microwave-Assisted Synthesis of 2,4,5-Triphenyl-1H-imidazole Containing Schiff Base Derivatives with Potential Antioxidant and Anticancer Activities. *Asian J. Chem.* **23**(3), 1212–1218, (2011)
14. R. Ahmad, A.M. Ali, D.A. Israf, N.H. Ismail, K. Shaari, N.H. Lajis, “Antioxidant, radical-scavenging, anti-inflammatory, cytotoxic and antibacterial activities of methanolic extracts of some Hedyotis species,”

15. Y.K. Al-Majedy, A.A. Al-Amiery, A.A.H. Kadhum, A.B. Mohamad, "Antioxidant activities of 4-methylumbelliferone derivatives," *PLoS ONE*, vol. 11, no. 5, Article ID e0156625, 2016
16. Y.Al-Majedy,A.Amir Kadhum, H. Ibraheem, A. Al-Amiery, Ahmed Abdel Moneim, Abu Bakar Mohamad," A Systematic Review on Pharmacological Activities of 4-Methylumbelliferon". *Sys Rev. Pharm.* **9**(1), 49–54 (2018)
17. A.A. Al-Amiery, Y.K. Al-Majedy, A.A.H. Kadhum, A.B. Mohamad, Novel macromolecules derived from coumarin: Synthesis and antioxidant activity. *Sci Rep.* 5, 2015
18. A.A. Al-Amiery, Y.K.A. -Majedy, A.A.H. Kadhum, A.B. Mohamad, New coumarin derivative as an eco-friendly inhibitor of corrosion of mild steel in acid medium. *Molecules.* **20**(1), 366–383 (2015)
19. Y.K. Al-Majedy, A.A.H. Kadhum, A.A. Al-Amiery, A.B. Mohamad, Synthesis and characterization of some new 4-hydroxycoumarin derivatives. *Molecules.* **19**(8), 11791 9 (2014)
20. DunyaL. AL-Duhaidahawi¹, YasameenK. Al-Majedy, HibaH. Ibraheem, AbdulAmirH. Kadhum, AhmedA. Al-Amiery, " Macro Coumarins as Novel Antioxidants", *Orient. J. Chem.*, Vol. 34(5), 2018
21. A.S. Al-Ayed, N. Hamdi, A new and efficient method for the synthesis of novel 3-acetyl coumarins oxadiazoles derivatives with expected biological activity. *Molecules* **19**(1), 911–924 (2014) “,”,
22. N. Hamdi, V. Passarelli, A. Romerosa, Synthesis, spectroscopy and electrochemistry of new 4-(4-acetyl-5- substituted- 4, 5-dihydro-1, 3,4-oxodiazol-2-yl)methoxy)-2H-chromen- 2-ones as a novel class of potential antibacterial and antioxidantderivatives. *C. R. Chim.* **14**(6), 548–555 (2011) “,”,
23. Z. Chen, Q. Zhu, W. Su, A novel sulfonic acid functionalized ionic liquid catalyzed multicomponent synthesis of 10,11- dihydrochromeno[4,3-b]chromene-6,8(7H,9H)-dione derivatives in water. *Tetrahedron Lett.* **52**(20), 2601–2604 (2011) “,”,
24. H.Y. Guo, Y. Yu, One-pot synthesis of 7-aryl-11,12-dihydrobenzo[h]pyrimido-[4,5-b]quinoline-8,10(7H,9H)-diones via three-component reaction in ionic liquid. *Chin. Chem. Lett.* **21**(12), 1435–1438 (2010) “,”,
25. V.M. Krishnamurthy, G.K. Kaufman, A.R. Urbach et al., Carbonic anhydrase as a model for biophysical and physicalorganic studies of proteins and protein–ligand binding. *Chem. Rev.* **108**(3), 946–1051 (2008) “,”,
26. H. Hiba Ibraheem,New derivative of 1,2-Dihydropyridine-3-Carbonitrile as Corrosion Inhibitor; *Mater. Sci. Eng.* 579 012052 (2019)
27. Y.K. Al-Majedy, H.H. .Ibraheem, M.S. Falih, and A A. Al-Amiery, New coumain derivatives as corrosion inhibitor. *Mater. Sci. Eng.* **579**, 012051 (2019)
28. A.A.H.,Kadhum AA.Al-Amiery, Y.K. Al-Majedy, ,H.I. Ibraheem, A.A. Al-Temimi, R.I. Al-Bayati, The legend of 4-aminocoumarin: Use of the Delépine reaction for synthesis of 4-iminocoumarin. *Res. Chem. Intermed* **39**(3), 1385–1391 (2013)

Scheme

Scheme 1 is available in the Supplemental Files section

Supplementary Files

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