RESEARCH ARTICLE

Synthesis and Characterization of New Coumarin Derivatives as Possible Antimicrobial Agents

Shams S. Abdulraheem[1*], Mohammed K. Hadi[2]

[1]*The National Center for Drug Control and Research, Department of Technical Matters, Ministry of Health, Baghdad, Iraq*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

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ABSTRACT

Umbelliferon, the natural crenelated coumarin distributed in the plants of a piaceae family, has shown various biological activities. The study aims to synthesize new coumarin derivatives, offer thorough chemical characterization, and evaluate their antimicrobial capabilities, given the potential interest of heterocyclic compounds. Two new series of 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide (III) has been synthesize: Schiff bases (N-acyl hydrazones) IV(a-c) and (VII), N-alkyl hydrazide derivatives of (III): V (a-c). Salicylaldehyde react with ethyl acetoacetate in the presence of piperidin at room temperature to produce compound 3-acetyl-2H-chromen-2-one (VI). 7-hydroxy-4- methycoumarin (I) react with ethylbromoacetate in the presence of K_2CO_3 and dry acetone to produce ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetate (II), which then reacted with hydrazine hydrate in the presence of ethanol to produce 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide (III). These newly formed Coumarin derivatives were tested for antibacterial activity against a variety of microorganisms; two species of (G+ve) bacteria (*Staphylococcus aureus, Streptococcus pneuomoniae*) and two species of (G-ve) bacteria (*Pseudomonas aeruginosa, Escherichia coli*) and antifungal activity against *Candida albicans*. In the present study, the new coumarin derivatives are synthesized and characterized by attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) and 1H-Nuclear magnetic resonance (1HNMR) spectra. The preliminary study of the antimicrobial activity of final compounds showed that compound VII has a significant antibacterial and antifungal activity followed by compounds IVb and IVc. In contrast, compounds IVa, Va, Vb and Vc showed slight to moderate antibacterial activity and antifungal activity, except compound IVa showed high antifungal activity.

Keywords: 4-methylumbelliferone, 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide, 3-acetylcoumarin, Antibacterial activity, Antifungal activity, Ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetate.

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INTRODUCTION

This work aims to synthesize new coumarin derivatives, offer thorough chemical characterization, and evaluate their antimicrobial capabilities, taking into account the potential interest of heterocyclic compounds. Heterocyclic compounds have paid tremendously to society in the form of many drugs for the treatment of various diseases and have occupied an eminent place in medicinal chemistry due to their varied biological activities.[1] The combination of heterocycles offers a new opportunity to create novel multicyclic compounds having improved biological activity.[2] These natural chemicals have gotten much interest from the scientific community in recent years because of their vast variety of biological actions (such as the ability to interact with biological systems). Heterocycles' role in the biological system is very important. Biochemical processes of components of living organisms like RNA, DNA etc., are based on heterocycles.[3] Privileged structures have been frequently used as a template for discovering and investigating very significant compounds. Coumarins are a basic scaffold found in a wide variety of plants and some fungi and bacteria.[4] Coumarins (chromen-2-ones) are a benzopyrone class that is extensively found in nature. They are a lactone type with a benzene ring fused to α-pyrone ring Figure 1.[5] Due to their wide range of applications, coumarin derivatives are one of the most important groups of heterocycles in synthetic and medicinal chemistry. They have a wide range

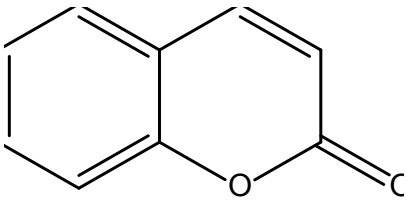


Figure 1: Structure of coumarin

**Author for Correspondence: shams.rahim1202@copharm.uobaghdad.edu.iq*

of biological activity and numerous pharmacological aspects and biochemical and therapeutic applications, depending on the substitution pattern.[6] The coumarin nucleus has proven to be simple to synthesize and decorate, allowing researchers to create new coumarin-based molecules and test their efficacy in the treatment of a variety of ailments. Coumarin derivatives are widely used in the perfumery, cosmetics, food additives, optical brighteners[7] and dye lasers.[8] They also have many bioactivities from the following groups: anticoagulant,[9] analgesic and hypothermic,[10] antioxidant,[11] antibacterial,[12] antifungal,[13] pesticides,[14] anticancer,[15] dermal photosensitization,[16] molluscicidal and anthelmintic,[17] anti-inflammatory.[18]

The main representatives of the class are its hydroxy derivatives 7-hydroxy-4-methylcoumarin (4-methylumbelliferone, hymecromone), as shown in Figure 2. It has been intensively researched for various biological roles, including 4-methylumbelliferone was utilized as fluorescent probes to detect Hg2+ in neat aqueous solutions,[19] and hypochlorite in tap water and cancer cells.[20] 4-Methylumbelliferone derivatives have a variety of biological characteristics, including anticonvulsant,[21] antifungal,[22] herbicidal activity,[23] antidepressant,[24] anaphylaxis,[25] anticancer,[26,27] antioxidant,[28] antibacterial,[29] antipsychotic,[30] anti-inflammatory[31] properties. Although some coumarins are hazardous, 4-methylumbelliferone is a harmless chemical employed as an active ingredient in several FDA-approved medications.[32]

The purpose of this review is to provide a critical overview of Heterocycles, 4-methylumbelliferone; coumarins; biological activity and Schiff bases. We have reported the synthesis of N-acyl hydrazones and N-alkyl hydrazide derivatives of acid hydrazide, as well as their structural characterization, based on the preceding observations and extensive results on coumarin analogues. The biological activities were also done to see if the inhibitory effect on the organisms was there.

EXPERIMENTAL SECTION

Chemistry

All chemicals were purchased from commercial sources and were not purified before using them. The ascending thin layer chromatography (TLC) was run using silica gel S.GF 254 (type 60) pre-coated aluminum sheets, Merck (Germany). TLC was used for monitoring the reaction progress and checking out the purity of the final products. The spots were detected by visualization of TLC sheets with UV 254 nm lamp light. Melting points were measured by using Electro-thermal capillary apparatus (Stuart SMP30) and are uncorrected. The infrared spectra were recorded using Shimadzu Specac GS 10800-R IRAffnity-1Spectrometer (Shimadzu, Japan) at the University of Baghdad-College of Pharmacy, the measurement unit (ύ, cm^{-1}). ^{1}HNMR spectra were achieved on Varian model ultra-shield (500) MHz spectrophotometer with tetramethylsilane (TMS) as an internal standard, Dimethyl sulfoxide (DMSO)-d6 used as a solvent for samples, chemical shift values expressed as (δ=ppm) and coupling constant in (j= Hz). ^{1}HNMR was run at Tehran University, Islamic Republic of Iran. The antimicrobial study of the synthesized final products was done at the University of Al-mustansiriyah, College of Science Department of Biology and in a private laboratory called Chemistry Analysis Center (CAC), Baghdad, Iraq.

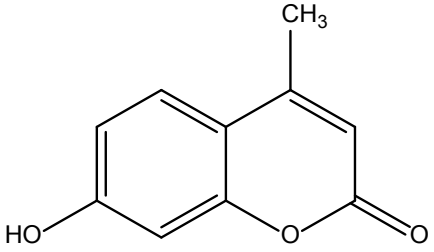


Figure 2: Structure of 4-methylumbelliferone

Synthesis of ether, ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetate (compound II)[33]

Mixture of 7-hydroxy-4- methycoumarin (I) (1,76g, 0.01mol), ethyl bromoacetate (2.5 g, 0.015 mol) and potassium carbonate (2.07 g, 0.015 mol) in 50 mL dry acetone for around 14–16 hours, it was refluxed. The solvent was extracted at decreased pressure after the mixture was filtered. The solid that resulted was then washed with much water. Crystallization of the crude product from ethanol was used to purify it to give white crystals, yield: 91.2%, melting point: 98-100°C, Rf value: 0.71 (mobile phase: ethyl acetate 1: hexane 1), ATR-FTIR: (3062 cm^{-1} C-H aromatic Str.), (2981, 2954 and 2870 cm^{-1} asymmetrical and symmetrical C-H aliph. Str.), (1739 cm^{-1} C=O Str. ester), (1712 cm^{-1} C=O Str. δ Lactone [coumarin]), (1608 cm^{-1}, 1508 cm^{-1} C=C aro. Str.), (1188 cm^{-1} C-O Str. ester), (1211 cm^{-1} asymmetrical, 1060.8 cm^{-1} symmetrical Ar-O-C str.), (1442 asymmetrical, 1396 symmetrical cm^{-1} C-H bending), (840 cm^{-1} C-H out of plane bending), (709 cm^{-1} C=C out of plane bending). ^{1}H-NMR (500 MHz, DMSO- d6, δ=ppm); (1.22-1.25): (t, 3H, CH_3), 2.40: (s, 3H, CH_3), (4.17-4.22): (q, 2H, CH_2), 4.94: (s, 2H, CH_2), 6.23: (s, 1H, Ar-H), (6.99-7.70): (d,d,s,3H,Ar-H),

Calculated for $C_{14}H_{14}O_5$, exact Mass: 262.08, molecular Weight: 262.26**.**

Synthesis of acid hydrazide, 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide (compound III):[34,35]

A mixture of compound II (2.6 g, 0.01 mol) in 25 mL of absolute ethanol was refluxed with 100% hydrazine hydrate (1.0012 gram, 0.02 mol) for about four hours. The resulting solid was washed with an excess of cold ethanol. After concentrating the reaction mixture, the crude product was purified by crystallization from ethanol to give white crystalline powder, yield: 90.2%, m.p.: 202–204 °C, Rf value: 0.51 (mobile phase; chloroform 9: methanol 1), ATR-FTIR: (3332 asymmetrical, 3263 symmetrical cm^{-1} N-H str. In primary amide), (3082 cm^{-1} and 3062 cm^{-1} C-H aro. Str.), (2981 cm^{-1} and 2958 cm^{-1} asymmetrical, 2908 cm^{-1} and 2858 cm^{-1} symmetrical C-H aliphatic Str.), (1720 cm^{-1} C=O Str. δ Lactone [coumarin]), (1674 cm^{-1} C=O Str. of amide), (1604 cm^{-1}, 1508 cm^{-1} C=C aro. Stretching), (1527 cm^{-1} N-H bending), (1438 asymmetrical, 1384 symmetrical cm^{-1} C-H bending), (1265 cm^{-1} C-N str.), (1203 cm^{-1} asymmetrical, 1053.1 cm^{-1} symmetrical Ar-O-C str.), (813 cm^{-1} N-H out of plane bending), (840 cm^{-1} C-H out

of plane bending), (675 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, DMSO- d6, δ=ppm); 2.39: (s, 3H, CH3), 4.34: (s, 2H, NH2), 4.63: (s, 2H, CH2), 6.21: (s, 1H, Ar-H), (6.96-7.69): (d,d,s, 3H, Ar-H), 9.43: (s, 1H, NH), Calculated for C12H12N2O4, exact Mass: 248.08, molecular Weight: 248.24.

Synthesis of Schiff base derivatives of 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide [N-acyl Hydrazones] (compounds IV a-c):[36]

A mixture of compound III (0.002 mol) in 60 mL ethanol was refluxed with appropriate aromatic aldehydes (0.002 mol) in the presence of a catalytic amount of glacial acetic acid (3–5 drops) for 16 hours. A solid mass separated and recrystallized from ethanol after cooling to room temperature, yielding the necessary products (IVa-c).

The appearance, yield, m.p., Rf value, the 1H-NMR and ATR-FTIR spectra of Compounds IVa, IVb, IVc are listed below:

N'-(3,4-dimethylbenzylidene)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide (compound IVa)

White powder, m.p.: 223–224 °C, yield: 97.8%, Rf value: 0.7 (mobile phase: ethyl acetate 3: hexane 1), ATR-FTIR: (3186 cm^{-1} N-H stretching of secondary amine), (3097 cm^{-1} C-H aro. Stretching), (2974 cm^{-1}, 2920 cm^{-1} C-H asymmetrical str.), (2866 cm^{-1} and 2854 cm^{-1} C-H symmetrical str.), (1712 cm^{-1} C=O str. δ Lacton [coumarin]), (1681 cm^{-1} C=O stretching of amide), (1612 cm^{-1} C=N- stretching of Schiff base), (1508 cm^{-1}, 1500 cm^{-1} C=C aro. Str.), (1562 cm^{-1} N-H bending), (1435 cm^{-1} asymmetrical, 1392 cm^{-1} symmetrical C-H bending), (1269 cm^{-1} C-N str.), (1199 asymmetrical, 1056 symmetrical Ar-O-C str.), (833 cm^{-1} C-H out of plane bending), (698 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, DMSO- d6, δ=part per million[ppm]); 6.23: (s,1H, Ar-H), 2.41: (s, 3H, methyl), 4.81: (s, 2H, CH_2), 11.58: (s, 1H, NH), 8.26: (s, 1H, CH), 2.26: (2s, 6H, $2CH_3$), (6.98-7.97): (m,6H, Ar-H), Calculated for $C_{21}H_{20}N_2O_4$, exact Mass: 364.14, molecular Weight: 364.40.

N'-(4-hydroxy-3,5-dimethoxybenzylidene)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide (compound IVb)

Off-white powder, m.p.: 213–214 °C, yield: 92.4%, Rf value: 0.65 (mobile phase: methanol 1: chloroform 9), ATR-FTIR: (3441. cm^{-1} O-H str.), (3186 cm^{-1} N-H str. of secondary amine), (3082 cm^{-1} C-H aro. Stretching), (2981 cm^{-1} and 2935 cm^{-1} asymmetrical C-H stretching), (2831 cm^{-1} symmetrical C-H stretching), (1735 cm^{-1} C=O str. δ Lacton [coumarin]), (1678 cm^{-1} C=O str. of amide), (1624 cm^{-1} C=N stretching of Schiff base), (1558 cm^{-1} and 1504 cm^{-1} C=C aro. Str.), (1593 cm^{-1} N-H bending), (1423 cm^{-1} asymmetrical, 1396 cm^{-1} symmetrical C-H bending), (1315 cm^{-1} C-N str.), (1153 cm^{-1} asymmetrical, 1080 cm^{-1} symmetrical Ar-O-C str.), (732 cm^{-1} C-H out of plane bending), (709 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, Dimethylsulfoxide[DMSO-d6], δ=part per million[ppm]); 2.41: (s, 3H, methyl), 3.82: (s, 6H, $2CH_3$), 4.80: (s, 2H, CH2), 6.22: (s, 1H, Ar-H), (6.96-7.70): (m, 5H, Ar-H), 8.21: (s, 1H, CH), 8.87: (s, 1H, OH), 11.49: (s, 1H, NH).

Calculated for C21H20N2O7, exact Mass: 412.13, molecular Weight: 412.40.

2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(thiophen-3-ylmethylene) acetohydrazide (compound IVc):

White powder, m.p.: 253–255 °C, yield: 96.8%, Rf value: 0.61 (mobile phase: ethyl acetate 3: hexane 1), ATR-FTIR: (3109 cm^{-1} N-H stretching of secondary amine), (3051 cm^{-1} C-H aro. Stretching), (2970 cm^{-1} and 2931 cm^{-1} asymmetrical C-H aliphatic stretching), (2916 cm^{-1} symmetrical C-H aliphatic str.), (1708 cm^{-1} C=O str. δ Lacton [coumarin]), (1681 cm^{-1} C=O str. of amide), (1612 cm^{-1} C=N str. of Schiff base), (1543 cm^{-1} and 1512 cm^{-1} C=C aro. Str.), (1562 cm^{-1} N-H bending), (1419 cm^{-1} asymmetrical and 1392 symmetrical cm^{-1} C-H bending), (1269 cm^{-1} C-N str.), (1161 cm^{-1} asymmetrical, 1083 cm^{-1} symmetrical Ar-O-C stretching), (624 cm^{-1} C-S str.), (759 cm^{-1} C-H out of plane bending), (694 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, Dimethylsulfoxide[DMSO-d6], δ=part per million [ppm]); 2.41: (s, 3H, methyl), 4.80: (s, 2H, CH_2), 6.22: (s, 1H, Aromatic-H), (6.99-7.75): (m, 6H, Aromatic-H), 7.94: (s, 1H, CH), 9.91: (s, 1H, NH).

Calculated for C17H14N2O4S, exact Mass: 342.07, molecular Weight: 342.37

Synthesis of N- alkyl derivatives of 2-[(4-methyl-2-oxo-2H-chromen-7-yl)oxy] acetohydrazide [N- alkyl hydrazide], (compounds V a-c)[37]

A mixture of compound III (0.7 g, 0.002 mol) and other required Halides (0.002 mol) in dry dimethylformamide [DMF] (5–10 mL) and trimethylamine [TEA] (0.285 g, 0.002 mol) was heated for 24 hours at 70°C. TLC was used to ensure that the reaction was completed. For 10 minutes, the reaction mixture was placed onto crushed ice (100 mL) and stirred. Suction filtration was used to collect the precipitate, washed with cold water before being purified by crystallization from ethanol to get the desired products (Va-c).

The appearance, yield, m.p., Rf value, the ^{1}H-NMR and ATR-FTIR spectra of Compounds Va, Vb, Vc are listed below:

N'-benzyl-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide (comp. Va):

White crystalline powder, m.p.: 186–188°C, yield: 94%, Rf value: 0.666 (mobile phase: ethyl acetate 7: toluene 3cc: hexane 1), ATR-FTIR: (3186 cm^{-1} N-H stretching of secondary amine), (3086 cm^{-1}, 3070 cm^{-1} C-H aro. Str.), (2981 cm^{-1} asymmetrical and 2927 cm^{-1} symmetrical C-H aliphatic str.), (1716 cm^{-1} C=O str. δ Lacton [coumarin]), (1689 cm^{-1} C=O stretching of amide), (1612 cm^{-1}, 1508 cm^{-1} C=C aro. Stretching), (1558 cm^{-1} N-H bending), (1435 cm^{-1} asymmetrical, 1392 cm^{-1} symmetrical C-H bending), (1261 cm^{-1} C-N str.), (1138 cm^{-1} asymmetrical, 1080 cm^{-1} symmetrical Ar-O-C str.), (844 cm^{-1} C-H out of plane bending), (698 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, DMSO- d6, δ=ppm); 2.36: (s, 3H, CH_3), 3.79: (s, 2H, CH_2), 4.42: (s, 2H, CH_2), 5.31: (s, 1H, NH), 6.18: (s, 1H, Aromatic-H), 8.80: (s, 1H, NH), (7.02-7.75): (m, 8H, Aromatic-H).

Calculated for C19H18N2O4, exact Mass: 338.13, molecular Weight: 338.36

2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-phenylaceto-hydrazide (comp. Vb):

Orange to brown crystalline powder, m.p.: 234–236°C, yield: 92%, Rf value: 0.634 (mobile phase: ethyl acetate 7: toluene 3cc: hexane 1), ATR-FTIR: (3186 cm^{-1} N-H stretching of secondary amine), (3074 cm^{-1} C-H aro. Str.), (2970 cm^{-1} asymmetrical and 2927 cm^{-1} symmetrical C-H aliph. str.), (1708 cm^{-1} C=O str. δ Lacton [coumarin]), (1685 cm^{-1} C=O stretching of amide), (1616 cm^{-1}, 1512 cm^{-1} C=C aro. Str.), (1562 cm^{-1} N-H bending), (1415 cm^{-1} asymmetrical, 1392 symmetrical cm^{-1} C-H bending), (1273 cm^{-1} C-N stretching), (1161 cm^{-1} asymmetrical, 1083 cm^{-1} symmetrical Aromatic-O-C stretching), (837 cm^{-1} C-H out of plane bending), (686 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, dimethylsulfoxide[DMSO-d6], δ=part per million[ppm]); 2.40: (s, 3H, methyl), 4.63: (s, 2H, CH2), 6.23: (s, 1H, Aromatic-H), (6.84-7.71): (m, 8H, Aromatic-H), 9.48: (s, 1H, NH), 10.09: (s, 1H, NH).

Calculated for $C_{18}H_{16}N_2O_4$, exact Mass: 324.11, molecular Weight: 324.34

2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(4-nitrobenzyl) acetohydrazide (compound Vc):

Yellow crystalline powder, m.p.: 254–256 °C, yield: 92%, Rf value: 0.6 (mobile phase: ethyl acetate 5: toluene 3cc: hexane 1), ATR-FTIR: (3113 cm^{-1} N-H stretching of secondary amine), (3082 cm^{-1} C-H aro. Str.), (2966 cm^{-1} and 2920 cm^{-1} asymmetrical and 2854 cm^{-1} symmetrical C-H aliphatic str.), (1732 cm^{-1} C=O str. δ Lacton [coumarin]), (1685 cm^{-1} C=O stretching of amide), (1616 cm^{-1}, 1510 cm^{-1} C=C aro. Str.), (1562 cm^{-1} N-H bending), (1431 cm^{-1} asymmetrical, 1388 symmetrical cm^{-1} C-H bending), (1261 cm^{-1} C-N stretching), (1157 cm^{-1} asymmetrical, 1080 cm^{-1} symmetrical Aromatic-O-C stretching), (829 cm^{-1} C-H out of plane bending), (690 cm^{-1} C=C out of plane bending), (1523 cm^{-1} and 1342 cm^{-1} NO2 str.), ^{1}H-NMR (500 MHz, dimethylsulfoxide[DMSO-d6], δ=part per million[ppm]); 2.40: (s, 3H, methyl), 3.91: (s, 2H, CH2), 4.57: (s, 2H, CH2), 5.31: (s, 1H, NH), 6.21: (s, 1H, Ar-H), (7.00-8.32): (m, 7H, Ar-H), 8.85: (s, 1H, NH).

Calculated for $C_{20}H_{18}N_2O_6$, exact Mass: 382.12, molecular Weight: 382.37

Synthesis of 3-acetyl-2H-chromen-2-one (compound VI)[38,39]

A mixture of salicylaldehyde (7.02 g, 0.05 mole) and ethyl acetoacetate (7.446 g, 0.05 mole) and a few drops of piperidine were mixed for 5–30 minutes at room temperature without any solvent and with continuous stirring. The product was isolated by filtration. The solid separated washed with cold ethanol then cold distilled water. The final compound was then recrystallized by ethanol to give needle shape yellow crystal, m.p.: 119–121 °C, yield: 78.3%, Rf value: 0.84 (mobile phase: ethanol 3: ethyl acetate 3: toluene 4: hexane 6), ATR-FTIR: (3047 cm^{-1} C-H aro. Str.), (2985 cm^{-1} asymmetrical, 2931 cm^{-1} symmetrical C-H aliphatic str.), (1720 cm^{-1} C=O str. δ lacton [coumarin]), (1674 cm^{-1} C=O str. acetyl str.), (1604 cm^{-1} and 1554 cm^{-1} C=C aro. Str.), (1454 cm^{-1} asymmetrical, 1354 cm^{-1} symmetrical C-H bending), (1199 cm^{-1} C-O str.), (756 cm^{-1} C-H out of plane bending), (636 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, DMSO- d6, δ=part per million[ppm]); 2.59: (s, 3H, CH3), 8.63: (s, 1H, Ar-H), (7.39-7.94):(m, 4H, Ar-H).

Calculated for $C_{11}H_8O_3$, exact Mass: 188.05, molecular Weight: 188.18

Synthesis of 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(1-(2-oxo-2H-chromen-3-yl)ethylidene)acetohydrazide (compound VII)[36]

A mixture of compound III (0.002 mol) in 60 mL ethanol refluxed with equimolar amount of 3-acetylcoumarin (0.002 mol) in the presence of a catalytic amount of glacial acetic acid for about 20 hours. A solid mass isolated and recrystallized from ethanol after cooling to room temperature to give pale yellow crystalline powder, m.p.: 249–251°C, yield: 60.17%, Rf value: 0.31 (mobile phase: ethyl acetate 3: hexane 1), ATR-FTIR: (3282 cm^{-1} N-H stretching of secondary amine), (3093 cm^{-1} and 3043 cm^{-1} C-H aro. Stretching), (2970 cm^{-1}, 2939 cm^{-1} asymmetrical C-H aliphatic str.), (2916 cm^{-1} symmetrical C-H aliphatic str.), (1724 cm^{-1} C=O str. δ Lacton [coumarin]), (1681 cm^{-1} C=O str. of amide), (1612 cm^{-1} C=N str. of Schiff base), (1539 cm^{-1} C=C aro. Str.), (1558 cm^{-1} N-H bending), (1489 cm^{-1} asymmetrical and 1365 symmetrical cm^{-1} C-H bending), (1300 cm^{-1} C-N str.), (1153 cm^{-1} asymmetrical, 1080 cm^{-1} symmetrical Ar-O-C str.), (748 cm^{-1} C-H out of plane bending), (671 cm^{-1} C=C out of plane bending), ^{1}H-NMR (500 MHz, DMSO- d6, δ=ppm); 6.25: (s, 1H, Ar-H), 2.41: (s, 3H, CH_3), 2.09: (s, 3H, CH3), 4.85: (s, 2H, CH_2), 10.31: (s, 1H, NH), (6.99-7.97): (m, 8H, Aromatic-H).

Calculated for $C_{23}H_{18}N_2O_6$, exact Mass: 418.12, molecular Weight: 418.41.

Antimicrobial Evaluation

In vitro Antimicrobial Evaluation:[40]

The antimicrobial activities of the synthesized derivatives (IVa, IVb, IVc, Va, Vb, Vc, VII) were measured by well diffusion technique, using two species of (G+ve) and two species of (G-ve) bacteria, in comparison to [amoxicillin, Ciprofloxacin and Neomycin] as standard antibacterial agents, and against one species of fungi, in comparison to [Fluconazole and Miconazole] as standard "antifungal agents". DMSO was used as a solvent and as a control. The filter paper disc-diffusion method was used for preliminary screening of the tested compounds. At a concentration of 1-mg/mL, the compounds were evaluated. The inhibition zone was measured in millimeters and compared to a reference standard. The compounds evaluated have a high level of effectiveness against Gram positive and Gram negative bacteria except compounds (Va, Vb and Vc) displayed slight to moderate activity. The results of antimicrobial screening studies are reported in Table 1.

Table 1: *In-vitro* antimicrobial activity of the synthesized compounds at 1 mg/mL

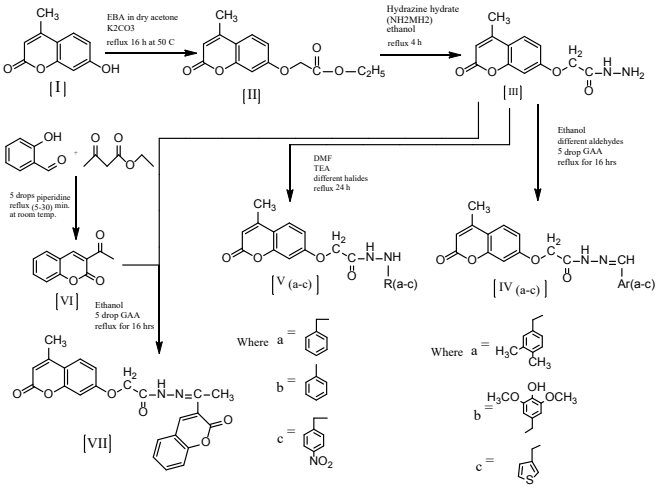
Comp. Name		*S. aureus*	*S. pneuomoniae*	*P. aeruginosa*	*E. coli*	*C. albicans*
		Zone of inhibition(ZI) in (mm)				
IVa		19	11	12	25	22
IVb		22	12	20	30	25
IVc		25	15	22	33	23
Va		12	-	11	11	9
Vb		14	6	10	15	11
Vc		11	-	9	11	9
VII		25	23	20	29	22
Amoxicillin		25	29	-	22	-
Ciprofloxacin		35	30	38	38	-
Neomycin		-	-	15	6	-
Fluconazole		-	-	-	-	22
Miconazole		-	-	-	-	20
DMSO	Control and solvent	-	-	-	-	-

(-) = No activity, (zone of inhibition between 5–10 mm) = slightly active, (zone of inhibition between 10-15 mm) = moderately active, (zone of inhibition more than 15 mm) = highly active

RESULTS AND DISCUSSION

Chemistry

The pathway for the synthesis of the targeted compounds is depicted in scheme 1. beginning from 4-methyl umbelliferone, compound (II); ether, synthesized by the reflux of ethyl bromoacetate, 4-methyl umbelliferone, anhydrous K_2CO_3 in the presence of dry acetone. The ATR-FTIR spectra of comp. (II) exhibit absorption band at 1739 cm^{-1} due to C = O stretching (ester carbonyl) and absorption band at 1188 cm^{-1} due to stretching vibration of (C-O) ester. The ^{1}H-NMR spectrum showed a triplet at δ (1.22–1.25) ppm due to methyl protons (3H of CH3), a quartet at δ (4.17–4.22) part per million due to methylene protons (2H of CH2) and a (s) at δ4.94 part per million due to (2H of CH2), Comp. (II) react with hydrazine hydrate (NH2NH2) to give acid hydrazide (III) in a good yield. The ATR-FTIR spectrum for Comp. III (acid hydrazide) exhibit absorption bands at 3332, 3263 cm^{-1} N-H (asymmetric and symmetric) str. of primary amine, 1674 cm^{-1} due to (C=O) stretching vibration of amide, 1527 cm^{-1} due to (N-H) bending vibration and an absorption band at 1265 cm^{-1} due to (C-N) stretching vibration. ^{1}H-NMR spectra exhibited a (s) at δ4.63 part per million due to (2H of CH2), a (s) at δ9.43 part per million due to (1H of NH) and a (s) at δ4.34 part per million due to (2H of NH2). Compounds IV (a-c) were synthesized by the reflux of 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide (III) with different aryl/hetero aromatic aldehydes. Also compound (III) was reacted with compound (VI) at the same condition to produce compound (VII). The ATR-FTIR spectrum show absorption bands at (1678–1681) cm^{-1} due to C=O str. of amide, (1612–1624) cm^{-1} due to C=N str. of Schiff base, (3109–3186) cm^{-1} due to a single weak band of



Scheme 1: General synthetic pathway of target compounds

secondary amine and bands at (1269-1315) cm^{-1} due to C-N str. also, N-acyl hydrazones (NAH) formation were confirmed by the disappearance of the absorption band of primary amine at (3332-3263) cm^{-1}. ^{1}H-NMR spectrum exhibited a singlet at δ (7.94-8.26) due to one proton of CH (-N=CH-). Compounds V (a-c) synthesize by the reaction of compound (III) with different halides in the presence of dry DMF, the ATR-FTIR spectrum show absorption bands at (1685-1689) cm^{-1} due to C=O str. of amide, (3113-3186) cm^{-1} due to N-H str. of a secondary amine and (1261-1273) cm^{-1} due to C-N stretching. also, the N-alkyl hydrazides formation was confirmed by the disappearance of absorption band of primary amine at (3332-3263) cm^{-1}. ^{1}H-NMR spectrum exhibited a singlet at δ (5.31-9.48) due to one proton of NH (-CONH-NH-).

Antimicrobial Activity

Table 2 shows the antibacterial and antifungal activity of the synthesized compounds against the selected microorganism at a concentration of 1-mg/mL.

Most of the synthesized compounds were screened for their antibacterial activity against two gram positive (G+ve) bacteria; S. aureus and S. pneumonia, two gram negative (G-ve) bacteria; *P. aeruginosa* and *E. coli* one fungi species; *C. albicans*. A [MIC] of 1000 ug/mL was used for all derivatives in DMSO.

From the recorded data in table (3.2), all the derivatives showed high activity toward (G+ve) and (G-ve) bacteria in comparison with Amoxicillin except compounds (Va, Vb, and Vc) showed moderate activity in comparison with Amoxicillin, compounds (IVb and IVc) showed comparable activity against *E.coli* in comparison with ciprofloxacin. All the tested compounds showed high activity against *C. albicans* compared to Fluconazole and Miconazole except compound Vb showed moderate activity, and compounds Va and Ve showed slight activity against *C. albicans* in comparison with Fluconazole and Miconazole.

CONCLUSIONS

New 7-hydroxy-4-methylcoumarin derivatives were effectively synthesized using conventional methods, and their antimicrobial activities were investigated., Characterization and identification of the synthesized compounds were achieved by determining physical properties, ATR-FTIR spectroscopy and ^{1}HNMR. The preliminary study of the antimicrobial activity of final compounds showed that compound VII has a significant antibacterial and antifungal activity followed by compounds IVb and IVc. In contrast, compounds IVa, Va, Vb and Vc showed slight to moderate antibacterial activity and antifungal activity except compound IVa showed high antifungal activity.

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