



AN EFFICIENT SYNTHESIS OF NOVEL BIOACTIVE NEW COUMARIN SCHIFF'S BASE.

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Abstract:

Due to the potential antibacterial activity of Schiff base and coumarin moieties, hybrid compounds containing both structures have been synthesized in good yield. A new efficient method was developed to synthesize Schiff base incorporating coumarin nuclei in excellent yields starting from 7-hydroxy-4-methyl coumarin, Chloroethyl acetate. The method was also successfully applied to the synthesis of coumarin derivatives containing Schiff base. The five novel coumarin bearing different functionalities have been synthesized and confirmed on the basis of their spectral data (1H-, 13C-NMR, UV, IR and mass). They were examined for the first time for antimicrobial activity.

Keywords:

Coumarin, Schiff base, antifungal, Efficient

Introduction:

Coumarins and their derivatives have been found to exhibit different biological and pharmacological activities [1]. The 4-methylcoumarins have been found to possess anti-inflammatory [2], cholestatic [3], analgesic [4], antispermatogenic [5] and diuretic [6] properties. Apart from the medicinal applications coumarins are also used as sweetener, fixative of perfumes, enhancer of natural oils such as lavender, a food additive in combination with vanillin, a flavour/odour stabilizer in tobacco, an odour masker in paints and rubber. Owing to the widespread applications, synthetic and biological activity evaluation of coumarins and their derivatives has been a subject of intense investigations. The emergence of multidrug-resistant gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) has made treatment of infectious diseases difficult and over the last decades, became a serious medical problem. As pathogenic bacteria continuously evolve resistance to





currently used antibacterial agents, so the discovery of novel and potent antibacterial agents is the best way to overcome bacterial resistance and develop effective therapies [7]. The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. It involves an effort to combine the separate pharmacophoric groups of similar activity into one compound, thus making structural changes in the biological activity. Here, we reported the synthesis of new coumarin derivatives containing Schiff base moieties. It is achieved when 7-hydroxy-4-methylcoumarin condensed with Chloroethylacetate to yield 2, which on refluxed with hydrazine hydrate gave 3. The intermediate moiety 3 formed Schiff base 4 on condensation with aromatic aldehydes. Because of the enormous biological importance of coumarin derivatives, the target was to synthesize some coumarin based Schiff's base in a goal to increase their biological relevance so that these compounds could be used for potential antifungal/antibacterial/antiviral activity.

Material and Method:

1. GENERAL: - All the chemicals and solvents were obtained from E Merck, India (AR grade) and were used without further purification. Melting points were taken in an open capillary tube. IR spectra were recorded on a Shimadzu Dr- 8031 instrument. ¹H NMR spectra of the synthesized compounds were recorded on a Bruker- Avance (300 MHz), Varian-Gemini (200 MHz) spectrophotometer using CDCl₃ solvent and TMS as the internal standard. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source.

2. GENERAL SYNTHESIS 2.1. Synthesis of 7-OH-4-Methylcoumarin: - 7-Hydroxy-4- methylcoumarin (1) was prepared in good yield (Scheme.1.) using a Pechmann procedure by condensation of ethylacetoacetate with an equimolar amount of resorcinol in presence of conc [8]. H₂SO₄. The product was isolated with 95% yield. The uncorrected mp was 179- 181 °C and this closely matched the literature value of 180- 182 °C. 2.2. Synthesis of 2-(4-methyl-2-oxo-2H-





chromen-7-yloxy) acetic acid: - A mixture of 7-HO-4- methylcoumarin (1) (0.01 mole), ethyl chloroacetate (0.01 mole) and anhydrous K₂CO₃ (0.01 mole) in dry acetone was refluxed on a water bath for 8-9 h. The mixture was then filtered and solvent was removed under reduced pressure. The resulting solid was crystallized from ethanol to afford product (2).

2.3. Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide:-A mixture of compound (2) (0.01 mole) and hydrazine hydrate (0.02 mole) in ethanol was refluxed on a water bath for 9 h. After cooling, the solid that separated was washed with water, dried and recrystallized from ethanol. Needle shaped crystals of (3) were obtained.

2.4. Synthesis of new coumarin derivatives (4a-ae):- A mixture of compound 3 (0.01 mole) in ethanol (30 mL), benzaldehyde a (0.01 mole) and 1 ml. of glacial acetic acid was refluxed on water bath for 8h. After cooling, the solvent was removed under reduced pressure and the separated solid was crystallized from methanol to yield 4a.

2.5. In Vitro Antimicrobial Screening: For biological screening, the agar cup plate method using Hi-Media agar medium was employed to study the antibacterial activity of 4a-4e against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (50 mg) was dissolved in DMF (50 mL, 1000 mg/mL), which was used as sample solution. Sample size for all the compounds was fixed at 0.1 mL. Using a sterilized cork borer, cups were scooped out of Agar. Medium contained in a Petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cups and the Petri dishes were subsequently incubated at 37°C for 48 h. Ampicillin and Streptomycin were used as reference drugs and DMF as a negative control. Zones of inhibition produced by each compound were measured in millimeters, and the results are listed in Table 1

Table 1	Antibacterial	antifungal	bioactivity of compounds 4a-4e
Compd.	MW	g/mol	Gram + Gram - Fungal B. subtitis
S. aureus	S. typhyl	E. coli	A. niger
4a	336	++	+++
4b	352	+++	+++





+++ +++ ++ +++ ++ 4c 370 ++ ++ +++ +++ +++ 4d 366 ++ +++ ++ ++ ++ 4e 371
++ +++ ++ ++ ++ AMP - +++ ++ ++ +++ ++ STREP - ++ +++ +++ ++ +++ STREP:
Streptomycin; AMP: Ampicillin; - =. Inactive (inhibition zone <5 mm); + =
slightly active (inhibition zone 5-10 mm); ++ = moderately active (inhibition
zone 10-15 mm); +++ = highly active (inhibition zone >15 mm. Scheme. 1. 2.6.
Spectroscopic characterization of 4a-4e 1. (E)-N'-benzylidene-2-(4-methyl-2-
oxo-2H-chromen-7-yloxy) acetohydrazide (4a) :- M.P.: 139; % yield: 75; IR (KBr,
cm-1): 1090 (C-O str.), 1754 (lactone, C=O str.), 1660 (C=N str.), 2958 (-CH₃
str.), 3012 (Ar-H str.), 3421 (-NH str. Sec. amine).¹HNMR (400 Mz, DMSOd₆,
ppm): 2.4 (s, 3H, CH₃), 6.95-7.83 (m, 8H, Ar-H), 6.22 (s, 1H, vinylic -CH),
11.20 (s, 1H, N-H, Sec. amine), 8.53 (s, 1H, benzydenium). ¹³C NMR (DMSO-
d₆, ppm): 125.0 (C1-coumarin), 155.4 (C2-coumarin), 115.6 (C3-coumarin),
123.4 (C4-coumarin), 115.7 (C5-coumarin), 155.8 (C6- coumarin), 160.0 (C7-
coumarin), 112.5 (C8-coumarin), 152.6 (C9-coumarin), 20.4 (C10-coumarin),
144.9 (CH=N, imine), 173 (amide) , 133.7-128.8 (Phenyl ring). MS
(C₁₉H₁₆N₂O₄): m/z 336 (Mp, 100%). Elemental analysis: calcd. (found): C,
67.7867 (67.7521), H, 4.7569 (4.7552), N, 8.3246 (8.3224). 2. (E)-N'-(4-
hydroxybenzylidene)-2-(4-methyl-2-oxo-2H-chromen-7-
yloxy)acetohydrazide(4b) M.P.: 145; % yield: 75; IR (KBr, cm-1): 1090 (C-O str.),
1720 (lactone, C=O str.), 1650 (C=N str.), 2958 (-CH₃ str.), 3120 (Ar-H str.),
3421 (-NH str. Sec. amine) 3415 (-OH str.).¹HNMR (400 Mz, DMSOd₆, ppm):
2.4 (s, 3H, CH₃), 10.1 (s, 1H, -OH) 6.95-7.83 (m, 8H, Ar-H), 6.12 (s, 1H, vinylic
-CH), 11.36 (s, 1H, N-H, Sec. amine), 8.50 (s, 1H, benzydenium). ¹³C NMR
(DMSO-d₆, ppm): 124 (C1-coumarin), 153.4 (C2-coumarin), 113.6 (C3-
coumarin), 122 (C4-coumarin), 114 (C5-coumarin), 156 (C6- coumarin), 160
(C7-coumarin), 112.5 (C8-coumarin), 152.6 (C9-coumarin), 20.1 (C10-
coumarin), 144.9 (CH=N, imine), 173 (amide) , 131-127 (Phenyl ring). MS
(C₁₉H₁₆N₂O₅): m/z 352 (Mp, 100%). Elemental analysis: calcd. (found): C,
64.7727 (64.7600), H, 4.5454 (4.5120), N, 7.9545 (7.9445). 3. (E)-N'-(4-
chlorobenzylidene)-2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetohydrazide(4c)





:- M.P.: 152; % yield: 75; IR (KBr, cm⁻¹): 1090 (C-O str.), 1715 (lactone, C=O str.), 1658 (C=N str.), 2958 (-CH₃ str.), 3251 (Ar-H str.), 3421 (-NH str. Sec. amine). ¹H NMR (400 Mz, DMSO-d₆, ppm): 2.4 (s, 3H, CH₃), 6.95-7.83 (m, 7H, Ar-H), 6.31 (s, 1H, vinylic -CH), 10.69 (s, 1H, N-H, Sec. amine), 8.34 (s, 1H, benzydenium). ¹³C NMR (DMSO-d₆, ppm): 125.3 (C1-coumarin), 155.5 (C2-coumarin), 116.2 (C3-coumarin), 123.4 (C4-coumarin), 115.7 (C5-coumarin), 155 (C6-coumarin), 161.0 (C7-coumarin), 112.5 (C8-coumarin), 152.6 (C9-coumarin), 21 (C10-coumarin), 144.9 (CH=N, imine), 171 (amide), 133.7-128.8 (Phenyl ring). MS (C₁₉H₁₅ClN₂O₄): m/z 370 (Mp, 100%). Elemental analysis: calcd. (found): C, 61.6216 (61.6120), H, 4.0540 (4.0041), N, 7.5675 (7.5420).

4. (E)-N'-(4-methoxybenzylidene)-2-(4-methyl-2-oxo-2H-chromen-7-

oxy)acetohydrazide(4d) :- M.P.: 134; % yield: 75; IR (KBr, cm⁻¹): 1090 (C-O str.), 1725 (lactone, C=O str.), 1696 (C=N str.), 2958 (-CH₃ str.), 3099 (Ar-H str.), 3421 (-NH str. Sec. amine). ¹H NMR (400 Mz, DMSO-d₆, ppm): 2.4 (s, 3H, CH₃), 3.6 (s, 3H, OCH₃), 6.95-7.83 (m, 7H, Ar-H), 6.10 (s, 1H, vinylic -CH), 11.82 (s, 1H, N-H, Sec. amine), 8.4 (s, 1H, benzydenium). ¹³C NMR (DMSO-d₆, ppm): 124.0 (C1-coumarin), 155.9 (C2-coumarin), 115.2 (C3-coumarin), 123 (C4-coumarin), 115.2 (C5-coumarin), 155.7 (C6-coumarin), 161.2 (C7-coumarin), 112.1 (C8-coumarin), 152.2 (C9-coumarin), 20.9 (C10-coumarin), 144 (CH=N, imine), 174 (amide), 130-128 (Phenyl ring). MS (C₂₀H₁₈N₂O₅): m/z 366 (Mp, 100%). Elemental analysis: calcd. (found): C, 65.5737 (65.1240), H, 4.9180 (4.8254), N, 7.6502 (7.4510).

5. (E)-N'-(4-

(dimethylamino)benzylidene)-2-(4-methyl-2-oxo-2H-chromen-7-
oxy)acetohydrazide(4e) :- M.P.: 167; % yield: 75; IR (KBr, cm⁻¹): 1090 (C-O str.), 1730 (lactone, C=O str.), 1637 (C=N str.), 2958 (-CH₃ str.), 3042 (Ar-H str.), 3421 (-NH str. Sec. amine). ¹H NMR (400 Mz, DMSO-d₆, ppm): 2.4 (s, 3H, CH₃), 2.9 (s, 6H, N(CH₃)₂), 6.95-7.83 (m, 7H, Ar-H), 6.30 (s, 1H, vinylic -CH), 11.12 (s, 1H, N-H, Sec. amine), 8.3 (s, 1H, benzydenium). ¹³C NMR (DMSO-d₆, ppm): 125.0 (C1-coumarin), 155.2 (C2-coumarin), 115 (C3-coumarin), 123.6 (C4-coumarin), 115.1 (C5-coumarin), 155.3 (C6-coumarin), 160.0 (C7-





coumarin), 112.5 (C8-coumarin), 153.6 (C9-coumarin), 22.4 (C10-coumarin), 144 (CH=N, imine), 171 (amide), 133.7-128.8 (Phenyl ring). MS (C₂₁H₂₁N₃O₄): m/z 371 (Mp, 100%). Elemental analysis: calcd. (found): C, 67.9245 (67.8921), H, 5.6603 (5.4769), N, 11.3207 (11.2905).

Result and Discussion:

1.SYNTHESIS: - The coumarin was synthesized by the well known Pechmann condensation in quantitative yield. The intermediates 2 and 3 were synthesized by usual reaction with some modification in reaction conditions. Series of five new Schiff bases with coumarin structure 4a-4e have been prepared by simple methodology reported for first time Scheme-1.

2.SPECTRAL STUDY: -Structure of the synthesized compound was established on the basis of IR, ¹H NMR, ¹³C NMR and Mass spectral data. The general spectral characters of 4a-4e showed absorption bands ranging from 1696-1637 cm⁻¹ for imine (-C=N) formation and 1754-1715 cm⁻¹ for (C=O) of coumarin. The ¹H-NMR spectra were recorded in DMSO-d₆ at room temperature using TMS as internal standard. In particular, it must be pointed out that ¹H NMR of compounds 4a-4e showed presence of a singlet between δ 8.3-8.5 ppm indicated the formation of imine (-CH=N-) by simple condensation process. Further characteristic peaks at δ 2.4 ppm indicated the presence of 4-CH₃ group of coumarin ring in their structure. All synthesized compounds have showed prominent peak around δ 11 ppm confirmed secondary amine structural unit. The compounds 4a-4e showed prominent singlet around δ 6.22 ppm for 3rd proton of coumarin and multiplet at δ 6.95-7.83 ppm for aromatic protons. The presence of lactone carbonyl group is in agreement with their IR value. The IR peak in between 3300- 3100 corresponds to aromatic hydrogen stretching. All other peaks in the spectra are in well agreement with the contents of functionalities in the synthesized coumarin Schiff base derivatives. The other signals and peaks of ¹H-NMR and IR are in complete agreement with the assigned structures. ¹³C NMR spectral data for the title





compounds most characteristic peak around δ 144.9 and 173 ppm indicated the formation Schiff base linkage and amide moiety in molecule. Electron impact mass spectra showed an accurate molecular ion peaks which are in agreement with molecular mass of synthesized compounds. All the compounds have given the satisfactory elemental analysis. The synthesized compounds were evaluated for in vitro antibacterial and antifungal activity against various gram-positive, gram-negative bacteria and fungal strains using agar cup plate method. The results are shown in table-1 suggest that compounds 4a, 4b and 4c are highly active against bacterial strain as well as fungi. Compounds 4d and 4e are moderately active against bacterial strain but highly active against fungi

Conclusion:

In this paper we report the synthesis and antimicrobial activity of novel series of Schiff base. The derivatives were prepared by condensation between 7-hydroxy-4-methylcoumarin derivatives with various substituted aromatic aldehydes to obtain schiff bases compounds (4a–4e). The preliminary in vitro antimicrobial activity of these novel series of derivative has evidenced that derivatives are more active than that of schiff bases. Further studies are required for the detailed mechanism of action of these derivatives. Some of Compounds were found to be equally potent as compared to Ampicillin and Streptomycin.

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