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# SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME COUMARINE-SCHIFFS BASE DERIVATIVE

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## ABSTRACT

8-Hydroxycoumarine reacts with ethyl chloroacetate in the presence of anhydrous $K_2CO_3$ to yield ethyl [(2-oxo-2*H*-chromen-4-yl)oxy]acetate (1). The ethyl ester reacts with hydrazine hydrate and forms 2-[(2-oxo-2*H*-chromen-4-yl)oxy]acetohydrazide (2). The hydrazide upon reaction with various substituted acetophenone yields substituted 2-[(2-oxo-2*H*-chromen-4-yl)oxy]-*N*'-[(1*E*)-1-Phenylethylidene]acetohydrazide (3a-e). The newly synthesized compounds were assigned on the basis IR, MASS and 1H NMR spectral data. All the compounds have been screened for their antibacterial and antifungal activity by the cup-plate method. Some of the compounds showed good activity against all the organisms.

**KEYWORDS:** Coumarine, Schiff bases, Hydrazones, Antibacterial, and Antifungal activity**.**

## 1.0 INTRODUCTION

Heterocyclic chemistry is one of the largest areas of research in organic chemistry and it is growing rapidly. Of all published organic chemistry literature, papers on heterocyclic synthesis accounted for around 60 % in 1998,[1] but nowadays the fraction is much larger considering that novel heterocyclic compounds are published in different fields such as biochemistry, pharmaceuticals, materials and others. A similar trend is seen for coumarin, a heterocyclic system with a very large number of different derivatives. Coumarin is a compound with varied biological activities and in 1954 it was classified as a carcinogenic substance.[2] Main representatives of the class are its hydroxy derivatives 4-hydroxycoumarin (**1**) and 7-hydroxycoumarin (*umbeliferone*), also biologically active and very important for synthesis of other coumarin derivatives. Until now, an enormous number of compounds with coumarin systems in their structure have been synthesized. Those derivatives have shown a remarkably broad spectrum of pharmacological and physiological activities and they are used as anticoagulant,[3-5] antibacterial,[6,7] antiviral,[8,9] antitumor,[10-13] bactericidal,[14] fungicidal,[15] and anti-inflammatory agents.[16] Also, in recent times there are references to derivatives with anti-HIV activity.[17-20]

## 2.0 MATERIAL AND METHODS

All raw materials used in the synthesis have been obtained from M/s Fluka AG (Bachs-Switzerland) and M/s Sigma Aldrich chemicals and Co. Inc. (Milwoukee, WI, USA). Melting points were recorded on a Thermonik Melting point apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. IR spectra were recorded on an IR-Affinity, Shimadzu using DRS system. $^{1}$H-NMR spectra have been recorded on a JEOL AL-400 FT-NMR spectrometer (400 MHz- JEOL Ltd. Tokyo, Japan), using TMS as internal standard in solvent DMSO. Elemental analysis has been carried out on a C, H, N Elemental Analyzer (Thermo-Finnigan Flash, EA 1112, Italy). Mass data have been recorded on Agilent GC-MS.

### 2.1 EXPERIMENTAL

#### 2.1.1 Preparation of ethyl [(2-oxo-2*H*-chromen-4-yl)oxy]acetate (2)

4-Hydroxy coumarin (0.01 mol), ethyl chloro acetate (0.01 mol) and anhydrous K2CO3 was dissolved in dry acetone was refluxed on water bath for 24 hrs. The resulting reaction mixture was cooled and filtered. The acetone was removed from the filtrate by distillation the remaining filtrate was poured into well stirred, ice-cold water. The organic layer was

extracted with diethyl ether. The ether was then removed by evaporation on a water bath and the remaining liquid vacuum distilled to afford pure product.

Yield 74%; white colour solid; mp; 95°C

$^1$H NMR (400 MHz, DMSO-δ6) δ (ppm) 3.2(s, 2H, $CH_2$), 3.5 (q, 2H, $CH_2$), 3.8 (t, 3H, $CH_3$) 7.11-8.28 (m, 5H, Ar-H)

Anal. calcd for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87;

Found: C, 62.92; H, 4.89;

IR (KBr) $cm^{-1}$: 1753.29(C=O), 1116.78(-O-), 752.24(Di substituted Ar-H)

MS (m/z): 249[$M^+$] ($C_{13}H_{12}O_5^+$).

**2.1.2 Preparation of 2-[(2-oxo-2*H*-chromen-4-yl)oxy]acetohydrazide (3)**

Crystallized product of compound **(2)** (ethyl [(2-oxo-2*H*-chromen-4-yl)oxy]acetate) was dissolved in alcohol reflux the reaction mixture. To this solution Hydrazine hydrate was added and refluxes the reaction mixture for 5 hours. Reaction was monitoring by TLC technique. From the resulting mixture, the excess of ethanol was removed by distillation. On cooling, white, needle-like crystals of the required products separated. The product was dried and recrystallized by ethanol.

Yield 72%; colourless solid; mp; 168°C

$^1$H NMR (400 MHz, DMSO-δ6) δ (ppm) 5.4 (s, 2H, $NH_2$), 6.1(s, 1H, NH), 3.2(s, 2H, $CH_2$), 7.12-8.48 (m, 5H, Ar-H)

Anal. calcd for $C_{11}H_{10}N_2O_4$: C,56.41; H,4.30; N,11.96;

Found: C, 56.67; H, 4.46; N, 11.99

IR (KBr) $cm^{-1}$: 3325.28(-NH), 1058.92(-O-), 1554.63(-C-O), 1647.21(-NH2), 1215.15(-CN)

MS (m/z): 235 [$M^+$] ($C_{11}H_{10}N_2O_4^+$).

**2.1.3.1 Preparation of 2-[(2-oxo-2H-chromen-4-yl)oxy]-N'-[(1Z)-1-phenylethylidene] acetohydrazide (4a)**

A mixture of crystallized product compound **(3)** (2-[(2-oxo-2*H*-chromen-4-yl)oxy]aceto hydrazide) (0.01 mol) was dissolved in ethanol (20mL) by warming, and then substituted acetophenone (0.01 mol) in ethanol and 1 ml. of glacial acetic acid was added and refluxes the reaction mixture on water bath for 6 hrs. The reaction mixture is cooled and poured into 100mL of ice cold water and the precipitated compound is filtered and recrystallized from alcohol to yield product.

Yield 68%; white colour solid; mp; 176°C

$^1$H NMR (400 MHz- DMSO-$d_6$) δ (ppm) 3.8(s,2H, $CH_2$), 4.7(s, 3H, $CH_3$), 5.6(s,1H, NH), 7.2-8.2(m, 10H, Ar-H)

Anal. Calc. for $C_{19}H_{16}N_2O_4$: C,67.85; H,4.79; N,8.33;

found: C, 67.90; H, 4.80; N, 8.55;

IR (KBr) $cm^{-1}$: 1034(-O-), 1620(CONH), 1566(C=N)

MS (m/z): 337 [$M^+$] ($C_{19}H_{16}N_2O_4^+$).

**2.1.3.2 Preparation of N'-[(1Z)-1-(4-hydroxyphenyl)ethylidene]-2-[(2-oxo-2H-chromen-4-yl)oxy]acetohydrazide (4b)**

Yield 74%; yellow colour solid; mp; 182°C

$^1$H NMR (400 MHz- DMSO-$d_6$) δ (ppm) 3.4(s,2H, $CH_2$), 4.3(s, 3H, $CH_3$), 5.1(s,1H, NH), 8.2(s,1H, OH), 7.2-8.2(m, 9H, Ar-H)

Anal. Calc. for $C_{19}H_{16}N_2O_5$: C,64.77; H,4.58, N,7.95;

found: C, 64.97; H, 4.66; N, 7.98;

IR (KBr) $cm^{-1}$: 1034(-O-), 1620(CONH), 1536(C=N), 1246(OH)

MS (m/z): 353 [$M^+$] ($C_{19}H_{16}N_2O_5^+$).

**2.1.3.3 Preparation of N'-[(1Z)-1-(4-methylphenyl)ethylidene]-2-[(2-oxo-2H-chromen-4-yl)oxy]acetohydrazide (4c)**

Yield 62%; yellow colour solid; mp; 168°C

$^1$H NMR (400 MHz- DMSO-$d_6$) δ (ppm) 3.2(s,2H, $CH_2$), 4.5(s, 3H, $CH_3$), 5.4(s,1H, NH), 2.3(s, 3H, $CH_3$), 7.2-8.2(m, 9H, Ar-H)

Anal. Calc. for $C_{20}H_{18}N_2O_4$: C,68.56; H,5.18; N,8.00;

found: C, 68.60; H, 5.35; N, 8.10;

IR (KBr) $cm^{-1}$: 1180(-O-), 1682(CONH), 1472($CH_3$), 1682(C=N)

MS (m/z): 351 [$M^+$] ($C_{20}H_{18}N_2O_4^+$).

**2.1.3.4 Preparation of *N*'-[(1*Z*)-1-(4-methoxyphenyl)ethylidene]-2-[(2-oxo-2*H*-chromen-4-yl)oxy]acetohydrazide (4d)**

Yield 43%; yellow colour solid; mp; 155°C

$^1$H NMR (400 MHz- DMSO-$d_6$) δ (ppm) 3.9(s,2H, $CH_2$), 4.8(s, 3H, $CH_3$), 5.7(s,1H, NH), 2.5(s, 3H, $CH_3$), 7.2-8.2(m, 9H, Ar-H)

Anal. Calc. for $C_{20}H_{18}N_2O_5$: C,65.57; H,4.95; N,7.65;

found: C, 65.69; H, 4.99; N, 7.70;

IR (KBr) $cm^{-1}$: 1142(-O-), 1626(CONH), 1626(C=N), 2814($OCH_3$)

MS (m/z): 367 [$M^+$] ($C_{20}H_{18}N_2O_5^+$).

**2.1.3.5 Preparation of N'-[(1Z)-1-(4-chlorophenyl)ethylidene]-2-[(2-oxo-2H-chromen-4-yl)oxy]acetohydrazide (4e)**

Yield 78%; white colour solid; mp; 212°C

$^1H$ NMR (400 MHz- DMSO-$d_6$) δ (ppm) 3.4(s,2H, CH2), 4.4(s, 3H, CH3), 5.6(s,1H, NH), 7.4-8.2(m, 9H, Ar-H)

Anal. Calc. for $C_{19}H_{15}ClN_2O_4$: C,61.55; H,4.08; N,7.56;

found: C, 61.67; H, 4.16; N, 7.70;

IR (KBr) $cm^{-1}$: 1102(-O-), 1620(CONH), 1566(C=N), 755(Cl)

MS (m/z): 371 [$M^+$] ($C_{19}H_{15}ClN_2O_4$ ).

**2.2 Antimicrobial Activity[21]**

The antimicrobial activity of all these compounds was screened by using cup-plate agar diffusion methods in DMF, using standard Ampicillin 10 µg/ml against gram positive and gram negative bacteria such as *E. coli, S. aureus, and C. albicans.* While all compounds were also screened for their antifungal activities by using standard Ketonazole 10 µg/ml against *C. albicans* in Tables no 2.

**Table 1**: - **Physical and analytical data of compound 3a-e.**

| Sr No. | Comp. | R | mp ($^0C$) | Colour | Yield |
|---|---|---|---|---|---|
| 1 | 4a | H | 176 | White | 68% |
| 2 | 4b | 4-OH | 182 | Pale Yellow | 74% |
| 3 | 4c | 4-$CH_3$ | 168 | Yellow | 62% |
| 4 | 4d | 4-$OCH_3$ | 155 | Yellow | 43% |
| 5 | 4e | 4-Cl | 212 | White | 78% |

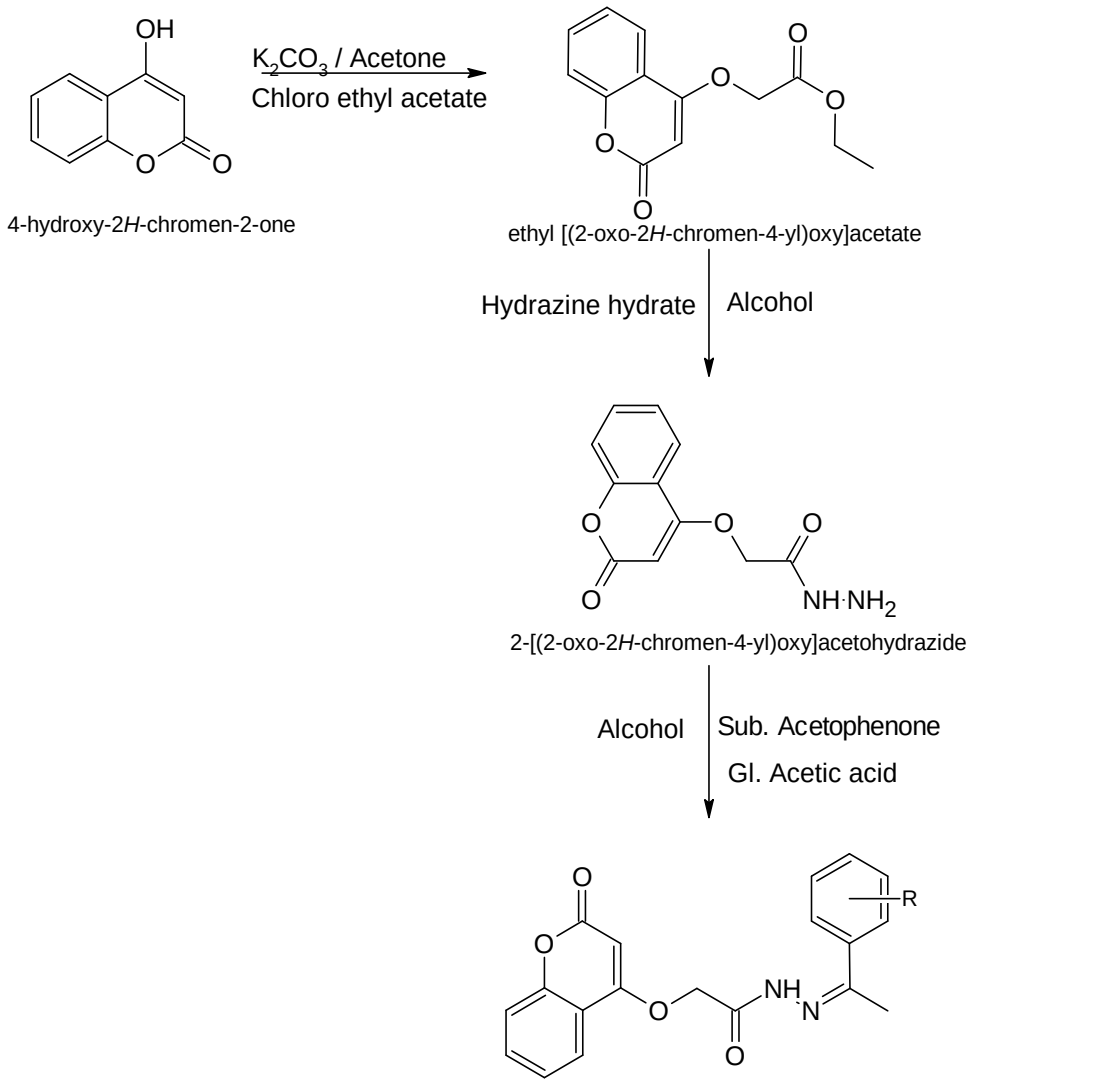
**Table 2**: - **Anti-microbial activity of Synthesized compound**.

| Sr. No. | Comp. | Substituent's R | Anti-microbial Activity (µg/ml) | | |
|---|---|---|---|---|---|
| | | | Anti-Bacterial Strain | | Anti-Fungal Strain |
| | | | *E. coli* | *S. aureus* | *C. albicans* |
| 1 | 5a | OH | -ve | -ve | -ve |
| 2 | 5b | diOH | -ve | -ve | -ve |
| 3 | 5c | Cl | -ve | -ve | -ve |
| 4 | 5d | di$OCH_3$ | -ve | -ve | -ve |
| 5 | 5e | F | -ve | -ve | -ve |
| 6 | Ampicillin | - | +ve (24 mm) | +ve (24 mm) | - |

| | | | | | |
|---|---|---|---|---|---|
| 7 | Kitonazole | - | - | - | +ve (24 mm) |

- Ampicillin (MIC-10 µg/ml) used as standard against *E. coli & S. aureus.*
- Kitonazole (MIC- 10 µg/ml) as standard against *C. albicans* and *A. niger*.

**Table No 3: - SCHEMATIC REPRESENTATION**




## 3.0 RESULTS AND DISCUSSION

Compounds (4a-e) were prepared according to reported procedures. Target compounds 2-[(2-oxo-2H-chromen-4-yl)oxy]-N'-[(1Z)-1-phenylethylidene]acetohydrazide derivatives (4a-e) were prepared by fusing compounds (3) and substituted acetophenone. Structures of compounds were supported by IR, $^1$H-NMR and MS in addition to elemental method of

analyses. IR spectra of compounds (4a-e) were characterized by the characteristic bands due to –C=N at 1590-1690 $cm^{-1}$. $^{1}$H-NMR spectra of compounds (4a-e), $CH_2$ and $CH_3$ of thiazolidinone ring showing signal between 2-3 and 4-5 ppm. NH which is present in between coumarine and acetophenone ring characterized signal between 5-6 ppm. MS of compound (4a-e) revealed the molecular ion peaks $M^+$ corresponding to the molecular weight for compounds 4a, 4b, 4c, 4d, 4e.

## 4.0 CONCLUSION

A number of schiff base derivatives were prepared through 3 steps reaction. This protocol involves the formation of 2-[(2-oxo-2H-chromen-4-yl)oxy]-N'-[(1Z)-1-phenylethylidene] acetohydrazide which done by the treatment hydrazino derivative with acetophenone. The structures of target compounds (4a-e) were elucidated depending upon different spectral data as well as the elemental methods of analyses. In addition, MS were carried out. The entire series of test compounds (4a-e) showed weak to moderate antibacterial and antifungal activity in comparison to ampicillin and ketonazole as a standard drug.

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## 7.0 CONFLITCT OF INTEREST

"The author(s) declare(s) that there is no conflict of interest regarding publication of this article"

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