



Synthesis of Biological Active N'-(1-(4-Hydroxycumarinyl)Ethylidene) Benzohydrazides

SS Chandole¹ and SG Shirodkar^{2*}

¹Shri Guru Buddhiswami Mahavidyalaya, Purna, Maharashtra, India

²Department of Chemistry and Research Centre, N.S.B. College, Nanded, Maharashtra, India

ABSTRACT

2-substituted-N'-(1-(4-hydroxycumarinyl) ethylidene)benzohydrazide were synthesized by condensation of 2-substituted benzohydrazide with 3-acetyl-4-hydroxycumarine. The structure of each was established by IR, ¹HNMR, ¹³CNMR and mass spectral studies. Antibacterial studies of these compounds were tested in vitro against *E. coli*, *S. typhi*, *S. aureus*, and *B. subtilis* by agar cup method. Antifungal studies were tested against *A. niger*, *A. flavus*, *P. notatum* and *F. moneliforme* by poison plate method.

Keywords: Benzohydrazide; 3-acetyl-4-hydroxycumarine; Imines; Antibacterial; Antifungal in vitro studies

INTRODUCTION

Present paper deals with synthesis of few novel imines which are combination of two biological active moieties such as hydrazide and Coumarins. Hydrazides are important pharmacological active molecule and show diverse activity like CNS acting [1], anti-HIV [2], anti-tuberculosis agent [3]. Hydrazides are used as important intermediates in synthesis of various heterocyclics viz. 1,2,4-triazoles, 1,3,4-thiadiazoles, 1,3,4-oxadiazoles, 1,2,4,5-tetrazines possessing different pharmacological properties. Coumarine structural unit is a usually observed in many of those natural products having medicinal values such as antibacterial, antiviral, anti-HIV, anticoagulant and cytotoxic properties [4-9]. Coumarins are also been utilized in production of various cosmetics, perfume, dyes, and pesticides. Imines are important compounds because of their wide reactive ability, which has led to their extensive use in laboratory and industrial applications [10,11]. They have ability to interact with DNA, RNA, Lipids and Protein, there by inhibiting or accelerating several biological processes. [12-14].

Relevant survey shows that scope for synthesis of potential imines as 2-substituted-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidene)benzohydrazide can be carried out by condensation of 2-substituted benzohydrazides with 3-acetyl-4-hydroxychromen-2-one. The structure of each was established by spectral analysis.

EXPERIMENTAL SECTION

AR grade chemicals and solvents of E-Merck and S.D. fine Ltd were used in the present study. Melting points were measured using capillary tube. By TLC, purity of compounds was proved. IR spectra were recorded and evaluated using Bruker Alpha FTIR (ATR) spectrophotometer. The ¹H NMR (300 MHz) and ¹³C NMR (70 MHz) spectral values in δ scale were recorded on Bruker Avance DPX-250 spectrometer in CDCl₃ using TMS as an internal standard. Mass spectra were determined on a Shimadzu GC MS-QP 1000 EX mass spectrometer. *In vitro* microbial investigations of the synthesized hydrazides were tested against the bacterial and fungal species by agar cup method and by the poison plate method respectively.

General Procedure for Synthesis of 3-Acetyl-4-Hydroxycumarine

3-acetyl-4-hydroxycumarine (d) was synthesized by conventional method [15]. 4-hydroxycumarine (3 g, 18.6 mmoles), 16 ml of acetic acid and 5.6 ml phosphoryl chloride (5.6 ml) mixture was heated at refluxing temperature for 30-40 minutes. On cooling, the precipitate the precipitate formed was recrystallized from ethanol. Yield = 2.7 g (90%), mp 134-36°C.

General Procedure for Synthesis of 2-Substituted Benzohydrazide(3a-f)

Various methods are known for synthesis of hydrazides. These can be prepared by conventional as well as by new methods [16]. Survey reveals a highly efficient solvent free synthesis of hydrazides using grinding technique [17]. In present investigation the 2-substituted benzohydrazide were synthesized in following two steps by reported conventional method [18]. The mixture of aryl acid (I) (0.25 moles), absolute ethanol (0.25 moles) and 0.5 gm of concentrated Sulphuric acid was refluxed for 3 hours. Excess of alcohol was distilled off on water bath. The contents were cooled. The aryl ester (II) formed was separated by CCl₄ using separating funnel. Excess of free acid was removed by NaHCO₃, washed with water and dried over anhydrous MgSO₄. In second step mixture of aryl ester (2a-f) (0.01 mole) and hydrazine hydrate (0.01) and ethyl alcohol was refluxed about 5 hours. After the completion of the reaction the ethanol was distilled off to give the desired benzohydrazides(3a-f), which were recrystallized by ethanol and dried over anhydrous magnesium sulphate.

General Procedure for the Synthesis of 2-Substituted-N'-(1-(4-Hydroxy-2-oxo-2H-Chromen-3-yl) Ethylidene)Benzohydrazide

The imines 4a-4f, (Figure 1) were prepared by adding 3-acetyl-4-hydroxycumarine (d) (0.01 mole) and the corresponding 2-substituted benzohydrazide (III) (0.01 mole) in ethanol (50 ml) and refluxing the mixture for 4 hrs. After cooling, the product was recrystallized from ethanol. The purity of the imines was checked by m.p. and TLC. These were characterized by spectral studies.

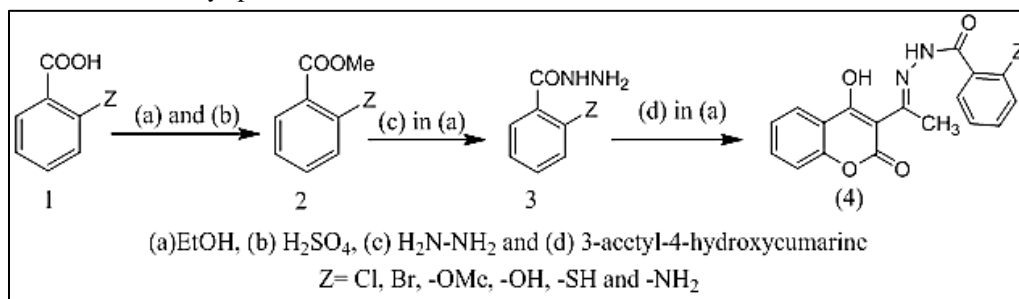


Figure 1: Synthesis of 2-substituted-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidene) benzohydrazide

Analytical data of Synthesized Imines (5a-5f)

2-Chloro-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidene)benzohydrazide (5a):

Colour: Yellowish green; Yield: 85%; m.p. 175°C; IR (cm⁻¹): 3610-2640 (3474,3273) (broad phenolic νOH and νNH), 1708(νC=O) of lactone, 1674(νC=O) of arylhydrazides, 1615 (νC=N) of imine, 1565 and 1500 aromatic (νC=C), 1340 (νC-O) phenolic-OH), 770-735 (ortho substitution).

¹HNMR(δ) : 2.32(s, 3H, imine -CH₃), 8.1-7.0(m, Ar-H), 15.9 (s, 1H, O-H), 5.13(s,1H, NH); ¹³CNMR(δ) : 20.2 (imine-CH₃carbon), 85 for C³, 140-125 for aromatic carbons, 157 for C⁹, 163.1 for lactone carbon, 177.5 for C⁴, and 163.5 for imine carbon, 168.5 for carbonyl carbon of aryl hydrazides, Mass Spectra: (M⁺)=357, CHN % for C₁₈H₁₃ClN₂O₄; Analytical: C 60.38, H 3.6, N 7.42; Calculated: C, 60.63; H, 3.69; N, 7.82.

2-Bromo-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidene)benzohydrazide (5b):

Colour: Yellowish brown; Yield: 80%; m.p. 193°C; IR (cm⁻¹): 3600-2745 (3500, 3300) (broad phenolic νOH and νNH), 1705 (νC=O) of lactone, 1696 (νC=O) of arylhydrazides, 1620 (νC=N) of imine, 1575 and 1510 aromatic (νC=C), 1342(νC-O) phenolic-OH), 780-740 (ortho substitution).

¹HNMR(δ) : 2.61 (s, 3H, imine -CH₃), 8.2-7.3(m, Ar-H), 16.3 (s, 1H, O-H), 6.35 (s,1H, NH); ¹³CNMR(δ) : 20.1 (imine-CH₃carbon), 94 for C³, 145-125 for aromatic carbons, 157.1 for C⁹, 163.2 for lactone carbon, 175.2 for C⁴, and 162.4 for imine carbon, 168.1 for carbonyl carbon of arylhydrazides, Mass Spectra: (M⁺)=402, CHN % for C₁₈H₁₃BrN₂O₄; Analytical: C 60.38, H 3.6, N 7.42; Calculated: C, 60.52; H, 3.70; N, 7.87.

2-Methoxy-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidenebenzohydrazide (5c):

Colour: Greenish yellow; Yield: 84%; m.p. 210°C; IR (cm⁻¹): 3600-2670 (3500, 3350) (broad phenolic νOH and νNH), 1711 (νC=O) of lactone, 1688 (νC=O) of arylhydrazides, 1601 (νC=N) of imine, 1564 and 1507 aromatic (νC=C), 1340 (νC-O) phenolic-OH), 765-745 (ortho substitution).

¹HNMR(δ) : 2.60 (S, 3H, imine -CH₃), 4.1(S, 3H, -OCH₃), 8.3-7.1 (m, Ar-H), , 16.3 (S, 1H, O-H), 6.43(S,1H, NH); ¹³CNMR(δ) : 20.1 (imine-CH₃ carbon), 93.5 for C³, 130-125 for aromatic carbons, 153.1 for C⁹, 160.2 for lactone carbon, 168.2 for C⁴, 161for imine carbon and 162 for carbonyl carbon of arylhydrazides, Mass Spectra: (M⁺)=353, CHN % for C₁₉H₁₆N₂O₅; Analytical: C 53.30, H 3.60, N 6.40; Calculated: C, 53.77; H, 3.23; N, 6.91.

2-Hydroxy-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidenebenzohydrazide (5d):

Colour: Yellowish white ; Yield: 81%; m.p. 219°C; IR (cm⁻¹): 3600-2700 (3500,3330) (broad phenolic νOH and νNH), 1707 (νC=O) of lactone, 1695 (νC=O) of arylhydrazides, 1605 (νC=N) of imine, 1569 and 1515 aromatic (νC=C), 1343(νC-O) phenolic-OH), 790-750(ortho substitution).

¹HNMR(δ): 2.67 (S, 3H, imine -CH₃), 5.10 (S, 1H, -OH), 8.1-7.2 (m, Ar-H), 16.0 (S, 1H, O-H), 6.40(S,1H, NH); ¹³CNMR(δ): 20.3 (imine-CH₃ carbon), 939 for C³, 132-125 for aromatic carbons, 155.1 for C⁹, 162.2 for lactone carbon, 169.2 for C⁴, 161for imine carbon and 161for carbonyl carbon of arylhydrazides ,Mass Spectra: (M⁺)=339, CHN % for C₁₈H₁₄N₂O₅; Analytical: C 53.30, H 3.60, N 6.40; Calculated: C, 53.77; H, 3.23; N, 6.91.

2-Mercapto-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidenebenzohydrazide (5e):

Colour: Yellow; Yield: 85%; m.p. 227°C; IR (cm⁻¹): 3600-2710 (3500, 3350) (broad phenolic νOH and νNH), 1701 (νC=O) of lactone, 1690 (νC=O) of arylhydrazides, 1610 (νC=N) of imine, 1563.and 1520 aromatic (νC=C), 1343(νC-O) phenolic-OH), 790-750(ortho substitution).

¹HNMR(δ) : 2.64 (S, 3H, imine -CH₃), 5.15(S, 1H, -SH), , 8.3-7.4 (m, Ar-H), 16.3 (S, 1H, O-H), 6.42(S,1H, NH); ¹³CNMR(δ) : 20.5 (imine-CH₃ carbon), 941 for C³, 132-127 for aromatic carbons, 155.5 for C⁹, 162.4 for lactone carbon, 165.2 for C⁴, 164 for imine carbon and 162 for carbonyl carbon of arylhydrazides, Mass Spectra: (M⁺)=355, CHN % for C₁₈H₁₄SN₂O₄; Analytical: C 53.30, H 3.60, N 6.40; Calculated: C, 53.77; H, 3.23; N, 6.91.

2-Amino-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidenebenzohydrazide (5f):

Colour: Yellow; Yield: 82%; m.p. 231°C; IR (cm⁻¹): 3600-2720(3500, 3340) (broad phenolic νOH and νNH), 1707(νC=O) of lactone, 1687 (νC=O) of arylhydrazides, 1613 (νC=N) of imine, 1560.and 1523 aromatic (νC=C), 1345(νC-O) phenolic-OH), 780-760 (ortho substitution).

¹HNMR(δ) : 2.67 (S, 3H, imine -CH₃), 5.11(S, 2H, -NH), 8.3-7.1 (m, Ar-H), 16.3 (S, 1H, O-H), 6.40 (S,1H, NH); ¹³CNMR(δ) : 20.3 (imine-CH₃ carbon), 945 for C³, 132-129 for aromatic carbons, 151.5 for C⁹, 161.4 for lactone carbon, 163.2 for C⁴, 169 for imine carbon and 161 for carbonyl carbon of arylhydrazides, Mass Spectra: (M⁺)=338, CHN % for C₁₈H₁₅N₃O₄; Analytical: C 53.30, H 3.60, N 6.40; Calculated : C, 53.77; H, 3.23; N, 6.91.

Anti-bacterial Activity

Procedure adopted for antibacterial studies was by poison plate method as reported [19,20]. Two gram negative cultures viz. *E. coli*, *S. typhi* and two Gram positive cultures viz. *S. aureus*, *B. subtilis* were selected for antibacterial screening. The zones of inhibition (in mm) were recorded using zone reader (Table 1).

Table 1: Anti-bacterial activity

| Compound | Zone of Inhibition (diameter in mm) | | | |
|------------|-------------------------------------|-----------------|-----------------|--------------------|
| | <i>E. coli</i> | <i>S. typhi</i> | <i>S.aureus</i> | <i>B. subtilis</i> |
| Penicillin | 25 | 21 | 24 | 17 |
| 4a | 17 | 5 | 7 | 11 |
| 4b | 19 | 4 | 12 | 13 |
| 4c | 15 | 6 | 9 | 8 |
| 4d | 20 | - | 18 | 14 |
| 4e | 16 | 5 | 7 | 10 |
| 4f | 22 | 8 | 21 | 12 |

Anti-fungal Activity

Poison plate method was used for measurement of antifungal activity as explained in previous reported studies [21,22]. Standard cultures of *A. niger*, *A. flavus*, *P.notatum* and *F. moneliforme* were selected for anti-fungal

activity. Results were recorded as adequate growth of inoculated fungi (++) , decreased growth of inoculated fungi (+) and zero growth of inoculated fungi (-) as antifungal activity (Table 2).

Table 2: Anti-fungal activity

| Compound | Growth of Fungi | | | |
|--------------|-----------------|------------------|----------------------|----------------------|
| | <i>A. niger</i> | <i>A. flavus</i> | <i>P.chrysogenum</i> | <i>F.moneliforme</i> |
| Gresiofulvin | - | - | - | - |
| 4a | + | + | + | - |
| 4b | ++ | ++ | + | + |
| 4c | - | + | + | - |
| 4d | + | + | + | + |
| 4e | - | + | - | + |
| 4f | + | + | + | ++ |

Adequate (++) , Decreased (+) and Zero growth (-) of inoculated fungi

RESULTS AND DISCUSSION

Aryl hydrazides(3a-f) and 3-acetyl-4-hydroxycumarine (d) were prepared by known procedure. The Purity of imines was tested by m.p. and TLC. The benzohydrazides (4a-4f) were obtained by adding 3-acetyl-4-hydroxycumarine and aryl hydrazides (3a-f) and heating at 120-130°C for 4 hrs. Principal significant peaks viewed in IR, ¹HNMR, ¹³CNMR spectra of 4a-4f are assigned and presented in analytical data. The IR spectral studies of 4a-4f revealed high intensity bands observed in the range 1601-1620 cm⁻¹ and are given to ν(C=N) vibration, confirming the formation of hydrazone. Broad weak bands around 3600-3300 cm⁻¹ and around 3300-2670 cm⁻¹ are ascribed to -OH and >NH respectively. The bands in the region 1570-1500 cm⁻¹ are assigned to aromatic ν(C=C). High intensity bands in the region 1340-1345 cm⁻¹ are ascribed to νC-O (phenolic) vibration. 1701-1711 cm⁻¹ and around 1601-1620 cm⁻¹ for νC=O of lactone and hydrazide. The bands in the region 790-754 cm⁻¹ specify ortho di-substitution at benzene ring system.

¹H NMR spectra of 4a-4f exhibited singlet (3H) at 2.5 ppm given to imino -CH₃ group. Peaks in the range 7-8.5 ppm are given to aromatic protons. A singlet at around 16.4 ppm is assigned for hydrogen of 4-hydroxyl group. Singlet for 1H around 6.5 ppm is assigned for -NH group. 5c revealed a peak at delta 4.1 assigned to -OCH₃ while compound 5d, 5e and 5f shows singlet for -OH, SH and -NH₂ at delta value 5.10, 5.15 and 5.11 respectively. ¹³CNMR showed peaks around δ160 to 166 are attributed for imine and carbonyl carbon. Molecular ion peaks in mass spectra, NMR (H and ¹³C) spectral data confirms the structures of benzohydrazides 4a-4f. 2-substituted-N'-(1-(4-hydroxycumarinyl) ethylidene)benzohydrazide prepared were screened for anti-microbial activity on specific strains of bacteria and fungi. Results are exhibited in Tables 1 and 2.

CONCLUSION

All have shown lesser activity against *E. coli*, *S. aureus* and *B. subtilis* compared with penicillin taken as standard. Compound 4f has shown higher activity against *S. typhi* and fungi. Antifungal property observed against *Aspergillus* species was encouraging in comparison with *P. chrysogenum* and *F. moneliforme*. However, compounds 4f have decreased the growth of *Aspergillus* species and *P. chrysogenum*.

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