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18. Synthesis of Biological Active N'-(1-(4-hydroxycumarinyl)ethylidene)benzohydrazides

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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 wasestablished by IR, ^{1}HNMR, ^{13}CNMR and mass spectral studies. Antibacterial studies of these compounds were tested in vitro against *E. coli, S.typhi, Staph.aureus*, and *B. subtilis* by agar cup method. Antifungal studieswere tested against *A.niger, A.flavus, P.notatum and F.moneliforme* by poison plate method.

Key word: Benzohydrazide,3-acetyl-4-hydroxycumarine, imines, antibacterial and 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]. Coumarinsare 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]

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

Experimental Section: A.R. grade chemicals and solvents of E-Merck and S.D. fine Ltd were used in the present study.Melting points were measuredusing capillary tube.By TLC, purity of compoundswas proved.IR spectra were recorded and evaluated using Bruker Alpha FTIR (ATR) spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (70 MHz) spectral values in δscale wererecorded onBrukerAvance DPX-250 spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were determined on a Shimadzu GC MS-QP 1000 EX mass spectrometer. In vitro microbialinvestigations 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 (3g, 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^{0}C.

General procedure for synthesis of2-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) (0.25moles),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 CCl_4 using separating funnel. Excess of free acid was removed by $NaHCO_3$, washed with water and dried over anhydrous $MgSO_4$.

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.

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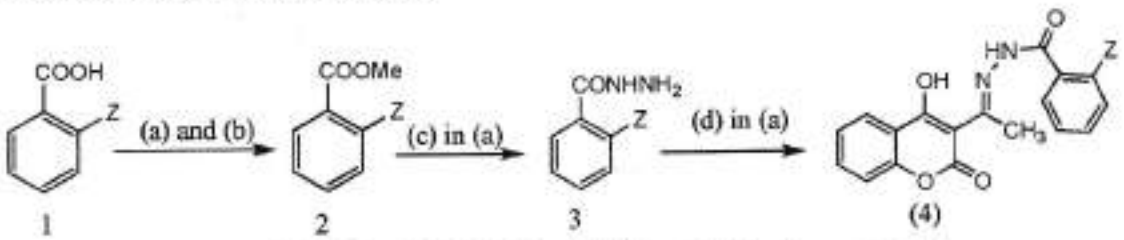
General procedure for the synthesis of 2-substituted-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide: The imines 4a-4f, (Fig.1) were prepared by adding 3-acetyl-4-hydroxycumarine (d) (0.01 mole) and the corresponding 2-substituted benzohydrazide (III) (0.01 mole) in ethanol (50 ml) and 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.



(a)EtOH, (b) H_2SO_4, (c) H_2N-NH_2 and (d) 3-acetyl-4-hydroxycumarine

Z= Cl, Br, -OMe, -OH, -SH and -NH_2

Fig. 1 : (IV) 2-substituted-*N'*-(1-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide

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

2-Chloro-N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl) ethylidenebenzohydrazide (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) **^{1}HNMR(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); **^{13}CNMR(δ) :** 20.2 (imine-CH_3carbon), 85 for C^3, 140-125 for aromatic carbons, 157 for C^9, 163.1 for lactone carbon, 177.5 for C^4, and 163.5 for imine carbon, 168.5 for carbonyl carbon of aryl hydrazides, **Mass Spectra:** $[M^+]$=357, CHN % for $C_{18}H_{13}ClN_2O_4$; 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) ethylidenebenzohydrazide (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)

^{1}HNMR(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); **^{13}CNMR(δ) :** 20.1 (imine-CH_3carbon), 94 for C^3, 145-125 for aromatic carbons,

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157.1 for C^9, 163.2 for lactone carbon, 175.2 for C^4, and 162.4 for imine carbon, 168.1 for carbonyl carbon of arylhydrazides, **Mass Spectra**: $[M^+]$=402, CHN % for $C_{18}H_{13}BrN_2O_4$; 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)

^{1}HNMR(δ) : 2.60 (S, 3H, imine $-CH_3$), 4.1(S, 3H, $-OCH_3$), 8.3-7.1 (m, Ar-H), , 16.3 (S, 1H, O–H), 6.43(S,1H, NH); **^{13}CNMR(δ) :** 20.1 (imine-CH_3 carbon), 93.5 for C^3, 130-125 for aromatic carbons, 153.1 for C^9, 160.2 for lactone carbon, 168.2 for C^4, 161for imine carbon and 162 for carbonyl carbon of arylhydrazides, **Mass Spectra**: $[M^+]$=353, CHN % for $C_{19}H_{16}N_2O_5$; 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)**^{1}HNMR(δ) :** 2.67 (S, 3H, imine $-CH_3$), 5.10 (S, 1H, -OH), 8.1-7.2 (m, Ar-H), 16.0 (S, 1H, O-H), 6.40(S,1H, NH); **^{13}CNMR(δ) :** 20.3 (imine-CH_3 carbon), 939 for C^3, 132-125 for aromatic carbons, 155.1 for C^9, 162.2 for lactone carbon, 169.2 for C^4, 161for imine carbon and 161for carbonyl carbon of arylhydrazides , **Mass Spectra**: $[M^+]$=339, CHN % for $C_{18}H_{14}N_2O_5$; 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(orthosubtitution)

^{1}HNMR(δ) : 2.64 (S, 3H, imine $-CH_3$), 5.15(S, 1H, -SH),), 8.3-7.4 (m, Ar-H), 16.3 (S, 1H, O–H), 6.42(S,1H, NH); **^{13}CNMR(δ) :** 20.5 (imine-CH_3 carbon), 941 for C^3, 132-127 for

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aromatic carbons, 155.5 for C^9, 162.4 for lactone carbon, 165.2 for C^4, 164 for imine carbon and 162 for carbonyl carbon of arylhydrazides , **MassSpectra**: $[M^+]$=355, CHN % for $C_{18}H_{14}SN_2O_4$; 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 (orthosubtitution)

^{1}HNMR(δ) : 2.67 (S, 3H, imine $-CH_3$), 5.11(S, 2H, -NH), 8.3-7.1 (m, Ar-H), 16.3 (S, 1H, O–H), 6.40 (S,1H, NH); **^{13}CNMR(δ) :** 20.3 (imine-CH_3 carbon), 945 for C^3, 132-129 for aromatic carbons, 151.5 for C^9, 161.4 for lactone carbon, 163.2 for C^4, 169 for imine carbon and 161 for carbonyl carbon of arylhydrazides , **Mass Spectra: $[M^+]$**=338, CHN % for $C_{18}H_{15}N_3O_4$; 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. *Staph.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)			
	E. coli	*S. typhi*	*S.aureus*	*B. subtilis*
Penicillin	**25**	**21**	**24**	**17**
4a	**17**	**5**	**7**	**11**
4b	**19**	**4**	**12**	**13**
4c	**15**	**6**	**9**	**08**
4d	**20**	**-**	**18**	**14**
4e	**16**	**5**	**07**	**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] Standard cultures of *A.niger, A. flavus, P.notatum and F.moneliforme*were selected foranti-fungal activity. Results were recorded as adequate growth of inoculated fungi (++), decreasedgrowth of inoculated fungi (+) and zero growth of inoculated fungi (-) as antifungal activity. **(Table-2)**

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Table-2 Anti-fungal activity

Compound	Growth of Fungi			
	A. niger	*A. flavus*	*P.chrysogenum*	*F.moneliforme*
Gresiofulvin	-	-	-	-
4a	+	+	+	-
4b	++	++	+	+
4c	-	+	+	-
4d	+	+	+	+
4e	-	+	-	+
4f	+	+	+	++

Adequate (++), Decreased (+) and Zero growth (-) of inoculatedfungi

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, ^{1}HNMR, ^{13}CNMR 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} specifyortho di-substitution at benzene ring system.

^{1}H NMR spectra of **4a-4f** exhibited singlet (3H)at 2.5 ppm given to imino $-CH_3$ 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 $-OCH_3$ while compound 5d,5e and 5f shows singlet for –OH,SH and $-NH_2$ at delta value 5.10, 5.15 and 5.11 respectively . ^{13}CNMR showed peaks around δ160 to 166 areattributed for imine and carbonyl carbon.

Molecular ion peaks in mass spectra,NMR (H and ^{13}C) spectral dataconfirms the structures of benzohydrazides**4a-4f**.

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2-substituted-N'-(1-(4-hydroxycumarinyl)ethylidene)benzohydrazide prepared were screened for anti-microbial activity onspecific strains of bacteria and fungi. Results are exhibited in **Table-1** and **Table-2**. 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 decreasedthe growth of *Aspergillus* species and*P. chrysogenum*.

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