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Research Article

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Characterization and antimicrobial study of some new N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)arylhydrazide synthesized from 3-acetyl-4-hydroxy-2H-chromen-2-one

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ABSTRACT

We report synthesis of N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)arylhydrazide by condensing 3-acetyl-4-hydroxy-2H-chromen-2-one with arylhydrazides. The structures of synthesized compounds were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and Mass Spectral Studies. The present reaction and was found to be effective in terms of product yield. Some of the compounds synthesized showed significant cytotoxic activity when tested in vitro.

Keywords: 3-acetyl-4-hydroxychromen-2-one, arylhydrazides, hydrazone, antimicrobial activity.

INTRODUCTION

A single molecule prepared by combination of two molecules with individual biological activity possess dual activity.[1]Substituted2H-chromen-2-one exhibit unique biological and pharmacological activities as antifungal[2], anti-tumer[3], anti-HIV[4],CNS stimulants[5], antibacterials,[6]anti-inflammatory[7] and anti-coagulants[8] properties. Moreover, hydroxycoumarins, showed antioxidant properties by preventing free radical injury by scavenging reactive oxygen species [9] Hydrazones showed anti-tubercular[10], anticonvulsant[11] andanti-malerial activity[12]. This class of compounds shows an extensive range of pharmacological properties especially antitumor and anti HIV activities. [13-14]. Hydrazones and substituted hydrazones on the other hand, because of their distinctive structural features and presence of azomethine group, continue to attract the attention of the medical researchers [15-16].

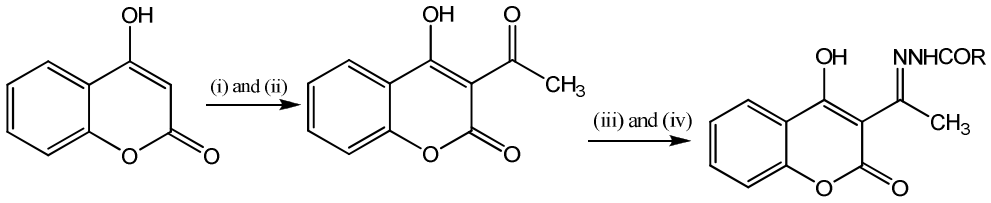
Considering the above remarkable facts and pharmaceutical and industrial applications prompted us to synthesize, characterize and assess antibacterial and antifungal activities of hybrid molecules of 3-acetyl-4-hydroxy-2H-chromen-2-one with aryl hydrazides

EXPERIMENTAL SECTION

All the chemical and solvents were of AR grade purchased from S.D. fine chemical Ltd. The purity of the compounds was confirmed by TLC and melting point. Melting points were determined in an open capillary tube and are uncorrected. The C, H, N analysis of compounds were carried out by microcombustion method using elemental Analyzer "PERKINELMER" model No. 2400 at School of Chemical Sciences, North Maharashtra University, Jalgaon. The molecular stoichiometry of each compound was established on the basis of elemental analysis.IR spectra were recorded in Bruker'salpha ATR-FTIR spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (70 MHz) were run on a Bruker Avance DPX-250 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Chemical shift values are given in δ scale. Mass spectra were recorded on Finnigan Mat LCQ Mass Spectrometer using methanol as mobile phase. In vitro biological screenings of the synthesized compounds were tested against the bacterial species by agar cup method and fungal species by the poison plate method.

General procedure for the synthesis of Schiff's Bases:

Aryl hydrazides are synthesized by treating aryl ester with hydrazine hydrate as per reported procedure.[17] 3-acetyl-4-hydroxy-2H-chromen-2-one is prepared as per previous reported method.[18]N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)arylhydrazide(3a-3f, Fig.1) were prepared by adding 3-acetyl-4-hydroxy-chromen-2-one(0.01 mole) and Aryl hydrazides(0.01 mole each) in ethanol (50 ml) and refluxing the mixture for 4 hrs. After cooling, the products were crystallized from ethanol. The purity of each was checked by m.p. and TLC. These were characterized by IR, ^{1}HNMR,^{13}CNMR and mass spectral studies.



(i) AcOH, (ii) $POCl_3$, (iii) R-$CONHNH_2$ and (iv) EtOH

R= (a)C_6H_5-, (b)4-$CH_3C_6H_4$-, (c) 4-ClC_6H_4-, (d) 4-BrC_6H_4-, (e) 4-$CH_3OC_6H_4$-and (f)4-$NO_2C_6H_4$-

Fig. 1

CHARACTERIZATION OF SYNTHESIZED SCHIFF'S BASES (3a-3f)

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

Colour: Yellow; Yield: 87%; m.p. 176^{o}C ; **IR (cm^{-1}):** 3600-2650 (3460,3280) (broad phenolic $\nu_{OH\ and}$ ν_{NH}), 1708 ($\nu_{C=O}$) of lactone, 1678 ($\nu_{C=O}$) of arylhydrazides, 1610 ($\nu_{C=N}$) of imine, 1545 and 1490 aromatic ($\nu_{C=C}$), 1360 (ν_{C-O}) phenolic-OH),**^{1}HNMR:**δ2.62(S, 3H, imine –CH_3),7.2-7.3(Ar-H), 15.5 (S,1H,O–H), 5.30(S,1H, NH);**^{13}CNMR:** δ20.3 (imine-CH_3 carbon), 90.10 for C^3, 138-116 for aromatic carbons, 155.4 for C^9, 163.2 for lactone carbon, 165.6 for C^4, and 163.4 for imine carbon, 163.2 for carbonyl carbon of arylhydrazides,Mass Spectra: [M^+]=323,CHN % for $C_{18}H_{14}N_2O_4$; Analytical: C 66.72, H 4.32, N 8.60; Calculated: C 67.07, H 4.38, N 8.69.

(2) N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)-4-methylbenzohydrazide(3b)

Colour: Yellow; Yield: 82%; m.p. 187^{o}C; **IR (cm^{-1}):** 3600-2650 (3450,3270) (broad phenolic ν_{OHand} ν_{NH}), 1700 ($\nu_{C=O}$) of lactone, 1670 ($\nu_{C=O}$) of arylhydrazides, 1620 ($\nu_{C=N}$) of imine, 1558 and 1498 aromatic ($\nu_{C=C}$), 1350 (ν_{C-O}) phenolic-OH), **^{1}HNMR:** δ2.6(S, 3H, imine –CH_3), δ2.38(S, 3H, –CH_3), 8.1-7.4(Ar-H) δ8.1(dd 2H) and δ7.4(dd 2H) assigned for para substituted aryl hydrazide moiety, 15.5 (S, 1H, O–H), 5.30(S,1H, NH); **^{13}CNMR:** δ20.0 (imine-CH_3 carbon), 92.20 for C^3, 130-120 for aromatic carbons, 157.6 for C^9, 163.2 for lactone carbon, 165.8 for C^4, and 162.4 for imine carbon, 162.4 for carbonyl carbon of arylhydrazides, Mass Spectra: [M^+]=337, CHN % for $C_{19}H_{16}N_2O_4$; Analytical: C 67.78, H 4.72, N 8.52; Calculated:C, 67.85; H, 4.79; N, 8.33.

(3) N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)-4-chlorobenzohydrazide(3c)

Colour: greenish Yellow; Yield: 88 %; m.p. 170^{o}C; **IR (cm^{-1}):** 3600-2650 (3474,3273) (broad phenolic $\nu_{OH\ and}$ ν_{NH}), 1705 ($\nu_{C=O}$) of lactone, 1676 ($\nu_{C=O}$) of arylhydrazides, 1614 ($\nu_{C=N}$) of imine, 1568 and 1500 aromatic ($\nu_{C=C}$), 1346 (ν_{C-O}) phenolic-OH), **^{1}HNMR:** δ2.64(S, 3H, imine –CH_3), 8.0-7.4(Ar-H),δ8.0(dd 2H) and δ7.64(dd 2H) assigned for para substituted aryl hydrazide moiety, 16.4 (S, 1H, O–H), 6.10(S,1H, NH); **^{13}CNMR:** δ20.0 (imine-CH_3 carbon), 84 for C^3, 140-120 for aromatic carbons, 157.4 for C^9, 162.8 for lactone carbon, 177.2 for C^4, and 163.1 for imine carbon, 168.8 for carbonyl carbon of arylhydrazides, 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, 60.60; H, 3.67; N, 7.85.

(4) N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)-4-bromobenzohydrazide (3d)

Colour: dark Yellow; Yield: 75 %; m.p. 190^{o}C; **IR (cm^{-1}):** 3600-2700 (3500,3290) (broad phenolic $\nu_{OH\ and}$ ν_{NH}), 1710 ($\nu_{C=O}$) of lactone, 1694 ($\nu_{C=O}$) of arylhydrazides, 1616 ($\nu_{C=N}$) of imine, 1572 and 1508 aromatic ($\nu_{C=C}$), 1352 (ν_{C-O}) phenolic-OH), **^{1}HNMR:** δ2.63(S, 3H, imine –CH_3), 8.7-7.3 (Ar-H), δ7.66(dd 2H) and δ7.44(dd 2H) assigned for para substituted aryl hydrazide moiety, 16.5 (S, 1H, O–H), 6.30(S,1H, NH); **^{13}CNMR:** δ20.0 (imine-CH_3 carbon), 94 for C^3, 140-120 for aromatic carbons, 156.8 for C^9, 163.5 for lactone carbon, 175.8 for C^4, and 162.8 for imine carbon, 168.4 for carbonyl carbon of arylhydrazides, 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, 60.60; H, 3.67; N, 7.85.

(5) N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)-4-methoxybenzohydrazide**(3e)**

Colour: greenish yellow; Yield: 80 %; m.p. 208^{o}C; **IR (cm^{-1}):** 3600-2660 (3500,3300) (broad phenolic $\nu_{OH\ and}$ ν_{NH}), 1708 ($\nu_{C=O}$) of lactone, 1686 ($\nu_{C=O}$) of arylhydrazides, 1608 ($\nu_{C=N}$) of imine, 1566 and 1504 aromatic ($\nu_{C=C}$), 1347 (ν_{C-O}) phenolic-OH), **^{1}HNMR:** δ2.62(S, 3H, imine –CH_3), δ4.1(S, 3H, –OCH_3), 8.1-7.2 (Ar-H), δ8.1(dd 2H) and δ7.2(dd 2H) assigned for para substituted aryl hydrazide moiety, 16.5 (S, 1H, O–H), 6.40(S,1H, NH); **^{13}CNMR:**

δ20.0 (imine-CH_3 carbon), 93.8 for C^3, 130-120 for aromatic carbons, 153.3 for C^9, 160.2 for lactone carbon, 168.4 for C^4, and 163 for imine carbon, 164 for carbonyl carbon of arylhydrazides, Mass Spectra: $[M^+]$=402, CHN % for $C_{18}H_{13}BrN_2O_4$; Analytical: C 53.3, H 3.6, N 6.4; Calculated:C, 53.89; H, 3.27; N, 6.98.

(6) N'-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene))-4-nitrobenzohydrazide**(3f)**
Colour: brown; Yield: 74 %; m.p. 246^{o}C; **IR (cm^{-1}):** 3600-2500 (3500,3280) (broad phenolic $\nu_{OH\ and}\ \nu_{NH}$), 1710 ($\nu_{C=O}$) of lactone, 1680 ($\nu_{C=O}$) of arylhydrazides, 1612 ($\nu_{C=N}$) of imine, 1560 and 1500 aromatic ($\nu_{C=C}$), 1352 (ν_{C-O}) phenolic-OH), **^{1}HNMR:** δ2.6(S, 3H, imine –CH_3), 8.8-7.4 (Ar-H), δ8.8(dd 2H) and δ8.1(dd 2H) assigned for para substituted aryl hydrazide moiety, 16.5 (S, 1H, O–H), 6.40(S,1H, NH); **^{13}CNMR:** δ20.0 (imine-CH_3 carbon), 88.8 for C^3, 130-110 for aromatic carbons, 152.3 for C^9, 159.4 for lactone carbon, 166.2 for C^4, and 163.3 for imine carbon, 164.2 for carbonyl carbon of arylhydrazides, Mass Spectra: $[M^+]$=368, CHN % for $C_{18}H_{13}BrN_2O_4$; Analytical: C 58.3, H 3.3, N 10.9; Calculated:C, 58.86; H, 3.57; N, 11.44.

ANTIBACTERIAL ACTIVITY

Procedure:
The antibacterial activity was evaluated as per the earlier reported procedure [19, 20] selecting two gram negative cultures viz. *Escherichia coli, Salmonella typhi* and two Gram positive cultures viz. *Staphylococcus aureus,Bacillus subtilis*. The zone of inhibition in millimeter (mm) using zone reader were recorded (**Table-1**).

Table-1 Anti Bacterial activity

Compound	Zone of Inhibition (diameter in mm)			
	E. coli	***S. typhi***	***S.aureus***	***B. subtilis***
Penicillin	**26**	**20**	**23**	**15**
(3a)	**18**	**6**	**8**	**8**
(3b)	**16**	**4**	**14**	**10**
(3c)	**22**	**7**	**18**	**12**
(3d)	**20**	**5**	**16**	**10**
(3e)	**14**	**-**	**14**	**8**
(3f)	**23**	**12**	**20**	**12**

ANTIFUNGAL ACTIVITY

Procedure: Poison plate method was adopted for evaluation of antifungal activity as described in earlier reported work. [20,21] *Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme, Aspergillus flavus* were selected as test fungal cultures.

Results were recorded (**Table-2**) as moderate growth of fungi (++), reduced growth of fungi (+) and no growth of inoculated fungi (-) antifungal activity.

Table-2 Anti-fungal activity

Compound	Growth of Fungi			
	A. niger	***P.chrysogenum***	***F.moneliforme***	***A. flavus***
Gresiofulvin	-	-	-	-
(3a)	+	+	+	+
(3b)	+	++	+	++
(3c)	-	+	-	+
(3d)	-	+	+	-
(3e)	+	+	+	+
(3f)	-	-	-	-

Moderate growth (++), Reduced growth (+) and No growth (-) of fungi

RESULTS AND DISCUSSION

All the reactions were carried out by conventional methods. Aryl hydrazide were prepared as per reported procedure.[17] 3-acetyl-4-hydroxy-2H-chromen-2-one was prepared as reported earlier.[18] Purity of intermediates were tested by m.p. and TLC. The hydrozones (**3a-3f**) were obtained by adding 3-acetyl-4-hydroxy-2H-chromen-2-one(**2**) and aryl hydrazides and refluxing for 4 hrs at 120-130 ^{o}C.

Important peaks observed in IR, ^{1}HNMR, ^{13}CNMR spectra of the compounds **3a-3f** are assigned with clarification in the analytical data. The IR spectra of compound **3a-3f**showed high intensity band observed at 1608-1612 cm^{-1} is assigned to ν(C=N) vibration suggesting the formation of hydrazone. Broad weak band around 3500-3450 cm^{-1} and

around 3300-3270 cm^{-1} are assigned for -OH and >NH respectively. The band at 1568-1490 cm^{-1} is assigned to ν(C=C) of the aromatic ring. A high intensity band in the region 1360-1346cm^{-1} is assigned to phenolic ν(C-O) vibration. 1710-1700 $cm^{-1}$and 1694 -1678 $cm^{-1}$for lactone and hydrazide carbonyl stretching vibration.

^{1}H NMR spectraof**3a-3f** showed singlet for 3H around 2.6 ppm assigned to methyl group bonded to imino carbon. Compound **3e** revealed a peak at δ4.1 assigned to $–OCH_3$. Peaks between 8.8-7ppm are assigned to aromatic protons. A broad singlet around 16.5 ppm confirms the presence of 4-hydroxyl group. Double doublet in aromatic region in ^{1}H NMR spectra of**3b-3f** confirms Para substitution in aryl hydrazide moiety.

^{13}CNMR showed peaks around163 ppm ascribed for imine carbon. Assignment given to other peaks observed in ^{1}HNMR, ^{13}CNMR spectra and also molecular ion peaks in mass spectra justifies the structures of compounds **3a-3f.** The hydrazones synthesized were evaluated for anti-bacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown 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. The activity of compounds **3f** was higher in comparison and has also shown activity against *S. typhi*and fungi.

Antifungal activity observed against *Aspergillus* species was encouraging in comparison with *Penicillium chrysogenum* and *Fusarium moneliforme.* However, compounds **3f** have reduced the growth of these organisms.

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REFERENCES

[1] FW Muregi; AIshih.*Drug Dev. Res.* **2010**, 71, 20-32.
[2] C Montagner;SM De Souza; C Groposoa; F DelleMonache; EF Smânia; A Smânia Jr.,**2008**63 (1-2), 21–28.
[3] RG Harvey;C Cortez;TP Ananthanarayan;S Schmolka,*J Org Chem*, **1988**, 53, 3936–3943.
[4] I Kostova; SRaleva; P Genova; RArgirova, *Bioinorg. Chem. Appl.* **2006**, 68274.
[5] RS Moffet,*J. Med. Chem.* **1964**, 7, 446-449.
[6] MA Al-Haiza;MS Mostafa;MY El-Kady,*Molecules*,**2003**, 8, 275–286.
[7] KC Fylaktakidou; DJHadjipavlou-Litina;KE Litinas;DN Nicolaides,*Curr. Pharm Des*,**2004**, 10, 3813–3833.
[8] JC Jung;YJ Jung; OS Park,*Synth. Commun.* **2001**, 31, 1195-1200.
[9] M Payá;B Halliwell;JR Hoult.*BiochemPharmacol*. **1992**, 44(2), 205-14.
[10] SG Kucukguzel; S Rollas; IKucukguzel; MKiraz,*Eur. J. Med.Chem*., **1999**, 34, 1093-1100.
[11] HnDogan; A Duran; SGRollas; GSener;Y Armutak;M Keyer-Uysal, *Med. Sci. Res.*,**1998**, 26, 755- 758.
[12] S Gemma; GKukreja; CFattorusso; M Persico; M Romano; MAltarelli;L Savini; G Campiani; EFattorusso;N Basilico,*Bioorg. Med. Chem. Lett.*,**2006**, 16, 5384-5388.
[13] N Terzioglu;AGursoy,*Eur. J. Med. Chem.*,**2003**, 38, 781-786.
[14] L Savini; LChiasserini; V Travagli; CPellerano; E Novellino; S Cosentino; MB Pisano,*Eur. J. Med. Chem.*,**2004**, 39, 113-122.
[15] JLM Tributino; CD Duarte; RS Corrêa; AC Doriguetto; J Ellena; NC Romeiro; NG Castro; ALP Miranda; EJ Barreiro; CAM Fraga,*Bioorg. Med. Chem.* **2009**, *17*, 1125.
[16] LW Zheng;LL Wu;BX Zhao; WL Dong; JY Miao. *Bioorg. Med. Chem.* **2009**, *17*, 1957.
[17] BS Furniss BS;V Rogers; PWG Smith;AR Tatchell,*Vogel's textbook of practical organic chemistry*, 5th Edn. (Longman Scientific and Technical, Essex England), **1989**, pp 1269-70.
[18] MV Girgaonkar; SG Shirodkar, *J. Chem. Pharm. Res.*, **2012**,4(1):260-264
[19] RJ Cruickshank; PDuguid; RR Swain,*Medical Microbiology*,**1998,** Vol. 1, Churchill Livingstone.
[20] VV Kodgire; SS Chandole; SG Shirodkar,*J. Chem. Pharm. Res.*, **2015**, 7(4):199-203
[21] R Tada; N Chavda; MK Shah,*J. Chem. Pharm. Res.*, **2011,** 3(2), 290-297.