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Synthesis, characterization and Antimicrobial activity of some new Schiff's bases derived from 3-acetyl-4-hydroxy-2*H*-chromen-2-one and primary aromatic amines

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ABSTRACT

4-hydroxy-3-(1-(arylimino)ethyl)chromen-2-one were synthesized by condensation of primary aromatic amines with 3-acetyl-4-hydroxy-2H-chromen-2-one. These were characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. In vitro biological screening effects of the investigated compounds were tested against the bacterial species Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Bacillus subtilis by Agar cup method. Fungal species Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme and Aspergillus flavus by the poison plate method.

Key word: 3-acetyl-4-hydroxychromen-2-one, aromatic amines, Schiff base, Spectra, Antibacterial, Antifungal.

INTRODUCTION

The substituted coumarin moiety is a common fused heterocyclic nucleus found in many natural products of medicinal importance. Several of these exhibit exceptional biological and pharmacological activities such as antibacterial, antiviral, anti-HIV, anticoagulant and cytotoxic properties [1-9]. Further, coumarin derivatives have been used as food additives, perfumes, cosmetics, dyes and herbicides [10,11]. In addition, schiff's bases perform important role in biological systems, where the >C=N- linkage is an essential structural requirement for biological activity.[12] Many Schiff bases exhibited remarkable antibacterial,[13,14] antifungal,[15,16] anticancer,[17] diuretic activities [18] and can also be regarded as mimetic systems for enzyme models.[19] Some substituted Schiff bases, such as *N*4-arylideneaminotriazole derivatives, exhibited anti-HIV activity.[20]

Literature survey reveals that work has been carried out on of Schiff bases derived from chromen-2-one having substitutions at benzene nucleus.[21] No attempt has been made on the Schiff bases derived from 3-acetyl-4-hydroxychromen-2-one and primary aromatic amine. In the views of above facts, we herein report the preparation of new 3-acetyl-4-hydroxy-2H-chromen-2-one from 4-hydroxy-2H-chromen-2-one by earlier reported method [22] with some modification. The later was condensed with aromatic amines such as aniline, 4-toluidine, 4-chloroaniline, 4-bromoaniline, 4-iodoaniline, 4-methoxyaniline, 4-ethoxyaniline to synthesize 4-hydroxy-3-(1-(arylimino)ethyl)chroman-2-one. These were characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis.

EXPERIMENTAL SECTION

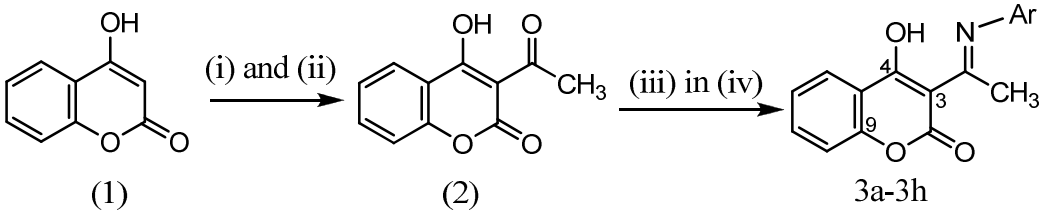
All the chemical and solvents used were of A.R. grade. All chemicals used were of E-Merck and S.D. fine Ltd. Melting points were determined in an open capillary tube and are uncorrected. The purity of the compound has been checked by TLC. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (70 MHz) were run on a BrukerAvance 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. The in vitro biological screenings of the investigated compounds were tested against the bacterial species by agar cup method and fungal species by the poison plate method.

Procedure for the synthesis of 3-acetyl-4-hydroxychromen-2-one: To a solution of 4-hydroxy-chromen-2-one (3g, 18.6 mmoles) in acetic acid (16 ml) phosphrousoxychloride (5.6 ml) was added. The mixture was heated at reflux for 30 minutes. After cooling, the precipitate was collected and recrystallized from ethanol. 3-acetyl-4-hydroxy-2H-chromen-2-one (2) is collected as white needles. Yield of 2.7 g (90%), mp 134-36^{0}C.

Characterization of 3-acetyl-4-hydroxy-2H-chromen-2-one(2)
Colour: White; Yield: 90%; m.p. 134-136^{0}C ; **IR (KBr,cm^{-1}):** 3600-2600 (broad phenolic ν_{OH}), 1722 ($\nu_{C=O}$) of lactone,1690 ($\nu_{C=O}$) of 3-acetyl, 1547 and 1489 aromatic ($\nu_{C=C}$), 1333 (ν_{C-O}) phenolic-OH); **^{1}HNMR:**δ2.78 (S, 3H, acetyl –CH_3), 7.28-8.06 (Ar-H of coumarin moiety), 16.75 (S,1H, O–H); **^{13}CNMR:**δ9.58 (acetyl-CH_3 carbon), 101.62 for C^3, 136-115.56 for aromatic carbons, 155.02 for C^9, 159.85.4 for lactone carbon, 178.83 for C^4, and 205.83 for acetyl carbonyl carbon. Mass Spectra: [M^+]=204.89

General procedure for the synthesis of 4-hydroxy-3-(1-(arylimino)ethyl)chroman-2-ones: The Schiff bases(3a-3h, Fig.1) were prepared by adding 3-acetyl-4-hydroxy-chromen-2-one(0.01 mole) and the corresponding aromatic amine (0.01 mole) in ethanol (50 ml) and refluxing the mixture for 4 hrs. After cooling, the product was crystallized from ethanol. The purity of the ligands was checked by m.p. and TLC. These are characterized by IR, ^{1}HNMR , ^{13}CNMR and mass spectral studies.



(i) AcOH, (ii) $POCl_3$, (iii) Ar-NH_2 and (iv) EtOH

Ar = phenyl (a), toluyl (b), 2,4-dimethylphenyl (c), 4-chlorophenyl (d), 4-bromophenyl (e), 4-iodophenyl (f), 4-methoxyphenyl (g) and 4-ethoxyphenyl (h)

Fig. 1 : 4-hydroxy-3-(1-(arylimino)ethyl)chroman-2-ones

Characterization of synthesized Schiff's Bases (3a-h)
Analytical data of *4-hydroxy-3-(1-(phenylimino)ethyl)-2H-chromen-2-one(3a)*
Colour: yellowish green; Yield: 90%; m.p. 163-165^{0}C ; **IR (KBr,cm^{-1}):**3600-2600 (broad phenolic ν_{OH}), 1710 ($\nu_{C=O}$) of lactone,1628 ($\nu_{C=N}$) of imine, 1564 and 1483 aromatic ($\nu_{C=C}$), 1335 (ν_{C-O}) phenolic-OH);**^{1}HNMR:**δ2.66 (S, 3H, imine –CH_3), 7.5-7 (m, 5H, Ph–H), 8.06 and 7.5-7.2 (Ar-H of coumarin moiety), 15.75 (S,1H, O–H); **^{13}CNMR:**δ20.39 (imine-CH_3 carbon), 98.12 for C^3, 138-116 for aromatic carbons, 154 for C^9, 162.4 for lactone carbon, 175.9 for C^4, and 181.7 for imine carbon. Mass Spectra: [M^+]=279.08

Analytical data of *4-hydroxy-3-(1-(p-tolylimino)ethyl)-2H-chromen-2-one(3b)*
Colour: yellowish green; Yield: 78%; m.p. 178-180^{0}C ; **IR (KBr,cm^{-1}):**3590-2500 (broad phenolic ν_{OH}), 1709 ($\nu_{C=O}$) of lactone ,1630 ($\nu_{C=N}$) of imine, 1567, 1483 aromatic ($\nu_{C=C}$), 1336 (ν_{C-O})phenolic-OH; **^{1}HNMR:**δ2.65 (S, 3H, imine –CH_3), 2.38 (S, 3H, for p-CH_3) 7.08 and 7.5 dd, 4H, (-C_6H_4-, *p*-substituted),8.04 and 7.5-7.2 (Ar-H of coumarin moiety), 15.74 (S,1H, O–H); **^{13}CNMR:**δ20.38 (imine-CH_3 carbon), 21.04 (*p*-CH_3), 98.07 for C^3, 138-116 for aromatic carbons, 154 for C^9, 162.38 for lactone carbon, 175.99 for C^4 and 181.78 for imine carbon. Mass Spectra: [M^+]=294.35

Analytical data of *4-hydroxy-3-(1-(2,4-dimethylphenylimino)ethyl)-2H-chromen-2-one(3c)*
Colour: pale yellow; Yield: 76%; m.p. 188-190^{0}C ; **IR (KBr,cm^{-1}):**3600-2580 (broad phenolic ν_{OH}), 1711 ($\nu_{C=O}$) of lactone ,1632 ($\nu_{C=N}$) of imine, 1570, 1488 aromatic ($\nu_{C=C}$), 1338 (ν_{C-O})phenolic-OH; **^{1}HNMR:** δ2.64 (S, 3H, imine –CH_3), 2.42 (S, 6H, for -2,4-CH_3) 7.08 and 7.06 (m, 3H, Ph–H), 8.04 and 7.4-7.1 (Ar-H of coumarin moiety), 15.54 (S,1H, O–H); **^{13}CNMR:**δ20.38 (imine-CH_3 carbon), 18.6 and 21.04 (2,4-CH_3), 99.16 for C^3, 138-116 for aromatic carbons, 152 for C^9, 162.56 for lactone carbon, 176.02 for C^4 and 181.78 for imine carbon. Mass Spectra: [M^+]=307.04

Analytical data of *4-hydroxy-3-(1-(p-clorophenylimino)ethyl)-2H-chromen-2-one(3d)*
Colour: yellowish Green; Yield: 82%; m.p. 168-170^{0}C ; **IR (KBr,cm^{-1}):**3600-2600(broad phenolic ν_{OH}),1720 ($\nu_{C=O}$) of lactone ,1626 ($\nu_{C=N}$) of imine,1565 and 1484 aromatic ($\nu_{C=C}$), 1336 (ν_{C-O}) phenolic-OH; **^{1}HNMR:** δ2.68 (S, 3H, imine –CH_3), 7.19 and 7.47dd, 4H, (-C_6H_4-, *p*-substituted), 8.04 and 7.5-7.2 (Ar-H of coumarin moiety), 15.92 (S,1H, O–H); **^{13}CNMR:**δ20.43 (imine-CH_3 carbon), 98.07 for C^3, 133.9-116.7 for aromatic carbons, 154.1 for C^9, 162.12 for lactone carbon, 176.13 for C^4 and 182.11 for imine carbon. Mass Spectra: [M$^+$]=307.04; [M$^+$]=314.23 and 316.19 (in isotopic ratio of Chlorine)

Analytical data of *4-hydroxy-3-(1-(p-bromophenylimino)ethyl)-2H-chromen-2-one(3e)*
Colour: Green; Yield: 78%; m.p. 180-182^{0}C ; **IR (KBr,cm^{-1}):** 3600-2600 (broad phenolic ν_{OH}),1718 ($\nu_{C=O}$) of lactone ,1627 ($\nu_{C=N}$) of imine, 1563 and 1481 aromatic ($\nu_{C=C}$), 1337 (ν_{C-O}) phenolic-OH; **^{1}HNMR:**δ2.72 (S, 3H, imine –CH_3), 7.22 and 7.51, dd, 4H, (-C_6H_4-, *p*-substituted) , 8.14 and 7.4-7.1 (Ar-H of coumarin moiety),15.63 (S,1H, O–H); **^{13}CNMR:** δ20.58 (imine-CH_3 carbon), 98.26 for C^3, 134.4-115.8 for aromatic carbons, 153.8 for C^9, 163.16 for lactone carbon, 178.42 for C^4 and 182.06for imine carbon. Mass Spectra: [M$^+$]=358.49 and 360.27 (in isotopic ratio of Bromine)

Analytical data of *4-hydroxy-3-(1-(p-iodophenylimino)ethyl)-2H-chromen-2-one(3f)*
Colour: Green; Yield: 72%; m.p. 198-201^{0}C ; **IR (KBr,cm^{-1}):** 3600-2600 (broad phenolic ν_{OH}),1716 ($\nu_{C=O}$) of lactone ,1629 ($\nu_{C=N}$) of imine, 1560 and 1483 aromatic ($\nu_{C=C}$), 1333 (ν_{C-O}) phenolic-OH; **^{1}HNMR:**δ2.64 (S, 3H, imine –CH_3), 7.09 and 7.26, dd, 4H, (-C_6H_4-, *p*-substituted) , 8.10 and 7.5-7.2 (Ar-H of coumarin moiety), 15.71 (S,1H, O–H); **^{13}CNMR:**δ20.36 (imine-CH_3 carbon), 98.02 for C^3, 132.6-115.9 for aromatic carbons, 153.08 for C^9, 162.02 for lactone carbon, 176.16 for C^4 and 181.91for imine carbon. Mass Spectra: [M$^+$]=404.87

Analytical data of *4-hydroxy-3-(1-(p-methoxyphenylimino)ethyl)-2H-chromen-2-one(3g)*
Colour: Light Green; Yield: 82%; m.p. 213-215^{0}C ; IR (KBr,cm^{-1}) : 3600-2600 (broad phenolic ν_{OH}),1710 ($\nu_{C=O}$) of lactone,1632 ($\nu_{C=N}$) of imine, 1563 and 1483 aromatic ($\nu_{C=C}$), 1338 (ν_{C-O}) phenolic-OH; **^{1}HNMR:**δ2.66 (S, 3H, imne –CH_3), 3.84 (S, 3H, of p-OCH_3), 6.97 and 7.14dd, 4H, (-C_6H_4-, *p*-substituted) 8.05 and 7.5-7.2 (Ar-H of coumarin moiety), 15.68 (S,1H, O–H); **^{13}CNMR:**δ20.34 (imine-CH_3 carbon), 55.60(*p*-OCH_3 carbon), 98.04 for C^3, 133.9-116.7 for aromatic carbons, 154 for C^9, 159.7 for lactone carbon, 176.07 for C^4 and 181.59 for imine carbon. Mass Spectra:[M$^+$]=309.98

Analytical data of *4-hydroxy-3-(1-(p-ethoxyphenylimino)ethyl)-2H-chromen-2-one(3h)*
Colour: Light Green; Yield: 70%; m.p. 237-240^{0}C ; IR (KBr,cm^{-1}) : 3600-2600 (broad phenolic ν_{OH}),1708 ($\nu_{C=O}$) of lactone,1630 ($\nu_{C=N}$) of imine, 1564 and 1480 aromatic ($\nu_{C=C}$), 1333 (ν_{C-O}) phenolic-OH; **^{1}HNMR:**δ 2.72(S, 3H, imne –CH_3), 4.04 and 1.07 (q for2H and t for 3H of p-OCH_2CH_3) 7.02 and 7.2 dd, 4H, (-C_6H_4-, *p*-substituted) 8.2 and 7.4-7.1(Ar-H of coumarin moiety), 15.88 (S,1H, O–H); **^{13}CNMR:**δ16.06 (ethoxy methyl), 20.02 (imine-CH_3 carbon), 55.80 (*p*-OCH_2- carbon), 98.26 for C^3, 134.7-116.3for aromatic carbons, 156.2 for C^9, 162.4 for lactone carbon, 176.82 for C^4 and 180.94for imine carbon. Mass Spectra: [M$^+$]=323.06

Antibacterial Activity
Procedure:
The antibacterial activity was measured by agar cup method.[23] Nutrient agar (Himedia) was prepared and sterilized at 15 Psi for 15 minutes in the autoclave. It was allowed to cool below 45°C and seeded with turbid suspension of test bacteria separately, prepared from 24 hours old slant cultures. 3% inoculate were used every time. The bacterial cultures selected were, two gram negative cultures viz. *Escherichia coli*, *Salmonella typhi* and two Gram positive cultures viz. *Staphylococcus aureu*, *Bacillus subtilis*. This seeded preparation was then poured separately in sterile petri plate under aseptic condition and allowed it to solidify.

Cups of 10mm diameter were made in the agar plate with sterile cork borer. 100 µl of compound solution prepared in ethanol (0.1%) was added in the cups under aseptic condition with the help of micropipette. 100µl of ethanol was placed in separate cups as blank (negative control). 100 µl of solution of penicillin in ethanol (0.1%) was also placed on the seeded nutrient agar surface as standard reference antibiotic (positive control).

The plates were kept in refrigerator for 15 minutes to allow diffusion of the compound from agar cup into the medium. Then the plates were shifted to incubator at 37°C and incubated for 24 hours.

After incubation plates were observed for the zone of inhibition of bacterial growth around the agar cup. Results were recorded by measuring the zone of inhibition in millimeter (mm) 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	24	18	21	14
(3a)	16	-	13	7
(3b)	11	-	9	8
(3c)	10	-	7	7
(3d)	20	7	14	11
(3e)	19	7	13	11
(3f)	21	8	15	13
(3g)	18	-	11	8
(3h)	17	-	12	7

Antifungal Activity

Procedure:

Antifungal activity was performed by Poison plate method.[23] The medium used was Potato Dextrose Agar (Himedia). The medium was prepared and sterilized at 10 Psi in autoclave for 15 minutes. Then the compound to be tested is added to the sterile medium in aseptic condition so as to get final concentration as 1%. A plate with ethanol was prepared as blank (negative control) similarly a plate with 1% Gresiofulvin was prepared as standard reference plate (positive control).

*Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme, Aspergillus flavus*were selected as test fungal cultures. They were allowed to grow on slant for 48 hours so as to get profuse sporulation. 5ml of 1:100 aqueous solution of Tween 80 was added to the slant and spores were scraped with the help of nichrome wire loop to form suspension.

The fungal suspension was inoculated on the plates prepared using compound with the help of nicrome wire loop. The plates were incubated at room temperature for 48 hours.

After incubation plates were observed for the growth of inoculated fungi. Results were recorded (**Table-2**) as moderate growth of fungi (++), reduced growth of fungi (+) and no growth of inoculated fungi (-) antifungal activity.

Table-3 Anti fungal activity

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

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

RESULTS AND DISCUSSION

All the reactions were carried out under conventional methods. 3-acetyl-4-hydroxy-2H-chromen-2-one(**2**)was the intermediate that required for preparing Schiff bases 4-hydroxy-3-(1-(arylimino)ethyl)chromen-2-ones (**3a-3h**). 3-acetyl-4-hydroxy-2H-chromen-2-one (**2**) was prepared from 4-hydroxy coumarin (**1**) by action of $POCl_3$in acetic acid. The reactions were carried out in a protective hood. The intermediate product (**2**) formed was recrystallized in ethanol and purity was tested by TLC. 4-hydroxy-3-(1-(arylimino)ethyl)chroman-2-ones (**3a-3h**) were obtained by refluxing in ethanol for 4 hrs. Increase in the time of refluxing did not improve the yield of product.

Assignment of significant peaks observed in IR, ^{1}HNMR, ^{13}CNMR spectra of the compounds **3a-3h** is clarified in the analytical data. The IRspectra of compound **3a-3h** showed high intensity band observed at 1632-1626 cm^{-1} is assigned to ν(C=N) vibration suggesting the formation of Schiff base. Broad weak band around 3590-2580 cm^{-1} is assigned to H bonded –OH in the Schiff bases. The band at 1567-1480 cm^{-1} is assigned to the combination of ν(C=C) of the aromatic ring. A high intensity band in the region 1338-1333 cm^{-1} is assigned to phenolic ν(C-O) vibration and 1720-1708 cm^{-1} for lactone carbonyl.[24]

Each one of the ^{1}H NMR spectraof**3a-3h** revealed singlet for 3H between 2.64-2.72 ppm assigned to imino methyl group. Peaks between 8.2-7.0ppm are assigned to aromatic protons. All ^{1}HNMR spectra of compounds **3b-3h** showed double doublet confirming para substitution at aryl moiety bonded to imino nitrogen. A broad singlet at 15.63-15.92 ppm confirms the presence of 4-hydroxyl group. Compound **3g** revealed a peak at 3.84 ppm assigned to -OCH_3. Methylene proton of –OC_2H_5 revealed a peak at 4.04 ppm. ^{13}CNMR showed peaks between 159.7-163.16 ppm for lactone carbon, between 182.11-180.94 ppm 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-3h.** The Schiff's bases 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 imines have shown lesser activity against *E. coli, S. aureus and B. subtilis* compared with penicillin taken as standard. The activity of compounds **3d-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 **3d-3f**have reduced the growth of these organisms. Therefore it may be concluded from results that antibacterial activity may be due to the presence of halogen in the molecule.

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