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Research Article

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Synthesis, characterization and antimicrobial study of some new schiff's bases derived from 3-acetyl-4-hydroxy-*2H*-chromen-2-one

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ABSTRACT

2-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazinecarboxamide, 2-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene) hydrazinecarbothioamide,4-hydroxy-3-(1-(2-hydroxyethylimino)ethyl)-2H-chromen-2-one and 4-hydroxy-3-(1-(2-mercaptoethylimino)ethyl)-2H-chromen-2-one were synthesized by condensation of semicarbazide, thiosemicarbazide, 2-aminoethanol and 2-aminothioethanolamine with 3-acetyl-4-hydroxy-2H-chromen-2-one. These Schiff bases were characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. In vitro biological screening effects of the synthesized 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 were tested by the poison plate method.

Keywords: 3-acetyl-4-hydroxychromen-2-one, semicarbazide, thiosemicarbazide, 2-aminoethanol and 2-aminothio ethanolamine, Schiff's base, Biological activity.

INTRODUCTION

Syntheses of Schiff bases have been the subject of investigation for last few decays. They have significant biological activity due to >C=N- structure. These Schiff bases exhibited variety of biological action like antibacterial,[1,2] antifungal,[3,4] anticancer,[5] and diuretic activities.[6]Some substituted Schiff bases, such as *N*4-arylideneaminotriazole derivatives, exhibited anti-HIV activity.[7]

Coumarin plays an vital role in synthetic organic chemistry and natural products. The substituted coumarins exhibit unique biological and pharmacological activities as antibacterial, antiviral, anti-HIV, anticoagulant and cytotoxic properties [8-13]. In addition, coumarin compounds are used as food additives, perfumes, cosmetics, dyes and herbicides [14-15].

Literature survey reveals that work has been carried out on of Schiff bases derived from chromen-2-one having substitutions at benzene nucleus.[16]We have reported synthesis of 4-hydroxy-3-(1-(arylimino)ethyl)chroman-2-one.[17] No attempt has been made on the Schiff bases derived from 3-acetyl-4-hydroxychroman-2-one and primary aliphatic amine. In the views of above facts, we herein report the preparation of new imine by condensationof 3-acetyl-4-hydroxy-2H-chromen-2-one with semicarbazide, thiosemicarbazide, 2-aminoethanol and 2-aminothio ethanolamine. These are characterized by elemental, IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis.

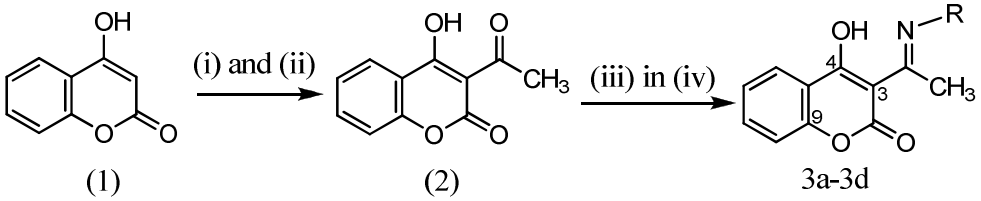
EXPERIMENTAL SECTION

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. The C, H, N analysis of ligands were carried out by microcombustion method using CHNSO, EA1I08, Elemental analyzer model-CARLO-ERBA Instruments, Italy, at micro analysis division, National

Chemical Laboratory, Pune and Elemental Analyzer "PERKINELMER" model No. 2400 at School of Chemical Sciences, North Maharashtra University, Jalgaon. The samples weighing between 1-10 mg were used for the analysis. The molecular stoichiometry of each compound was established on the basis of elemental analysis.IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8300 spectrophotometer. The ^1H 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. 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.

General procedure for the synthesis of Schiff's Bases:

3-acetyl-4-hydroxy-2H-chromen-2-one (2) is prepared as per previous reported method.[17]The imines (3a-3d, Fig.1) were prepared by adding 3-acetyl-4-hydroxy-chromen-2-one(0.01 mole) and semicarbazide, thiosemicarbazide, 2-aminoethanol and 2-aminothioethanolamine(0.01 mole each) 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) R-NH_2 and (iv) EtOH

R= -$NHCONH_2$(a), -$NHCSNH_2$(b), -CH_2CH_2OH (c) and -CH_2CH_2SH (d)

Fig. 1

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

Data of 2-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)hydrazinecarboxamide*(3a)*

Colour: yellow; Yield: 88%; m.p. 230^{0}C ; **IR (KBr,cm^{-1}):** 3500-2600 (3464,3293,3154) (broad phenolic $\nu_{OH}\nu_{NH2}$), 1708 ($\nu_{C=O}$) of lactone, 1666 ($\nu_{C=O}$) of semicarbazide, 1606 ($\nu_{C=N}$) of imine, 1538 and 1490 aromatic ($\nu_{C=C}$), 1366 (ν_{C-O}) phenolic-OH); **^{1}HNMR:**δ2.64 (S, 3H, imine –CH_3),7.9 and 7.5-7.2 (Ar-H of coumarin moiety), 14.5 (S,1H, O–H), 6.90(S,2H, NH_2), 5.30(S,1H, NH);**^{13}CNMR:**δ20.3 (imine-CH_3 carbon), 99.10 for C^3, 138-116 for aromatic carbons, 155.4 for C^9, 163.2 for lactone carbon, 175.6 for C^4, and 181.4 for imine carbon, 200.00 for C of carbonyl carboxide. Mass Spectra: [M^+]=261

Data of2-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)hydrazinecarbothioamide*(3b)*

Colour: yellow; Yield: 85%; m.p. 196^{0}C ; **IR (KBr,cm^{-1}):** 3500-2600 (3460,3287,3225) (broad phenolic $\nu_{OH}\nu_{NH2}$), 1711 ($\nu_{C=O}$) of lactone,1608 ($\nu_{C=N}$) of imine, 1542 and 1500 aromatic ($\nu_{C=C}$), 1352 (ν_{C-O}) phenolic-OH),1290 ($\nu_{C=S}$) of thiosemicarbazide; **^{1}HNMR:**δ2.6 (S, 3H, imine –CH_3),7.8 and 7.5-7.2 (Ar-H of coumarin moiety), 14.5 (S,1H, O–H), 6.90(S,2H, NH_2), 5.30(S,1H, NH);**^{13}CNMR:**δ20 (imine-CH_3 carbon), 98.0 for C^3, 140-120 for aromatic carbons, 156.2 for C^9, 160.2 for lactone carbon, 175.6 for C^4, and 182 for imine carbon, 176.0 for C=S of thiocarboxide. Mass Spectra: [M^+]=277

Data of4-hydroxy-3-(1-(2-hydroxyethylimino)ethyl)-2H-chromen-2-one *(3c)*

Colour: White; Yield: 90%; m.p. 196^{0}C ; **IR (KBr,cm^{-1}):** 3500-2600 (broad phenolic ν_{OH}), 1710 ($\nu_{C=O}$) of lactone,1612 ($\nu_{C=N}$) of imine, 1540 and 1510 aromatic ($\nu_{C=C}$), 1348 (ν_{C-O}) phenolic-OH; **^{1}HNMR:** δ2.62 (S, 3H, imine –CH_3),7.9 and 7.5-7.2 (Ar-H of coumarin moiety), 14.5 (S,1H, O–H), 4.10(S,1H, OH),2.31(t, 2H, CH_2 [a]), 2.50(t, 2H, CH_2 [b]);**^{13}CNMR:**δ20 (imine-CH_3 carbon), 96.0 for C^3, 140-120 for aromatic carbons, 156.4 for C^9, 162.0 for lactone carbon, 175 for C^4, and 182 for imine carbon, 45.5 for C of CH_2 [a],57.5 for C of CH_2 [b]. Mass Spectra: [M^+]=247

Data of4-hydroxy-3-(1-(2-mercaptoethylimino)ethyl)-2H-chromen-2-one*(3d)*

Colour: Dirty white; Yield: 88%; m.p. 210^{0}C ; **IR (KBr,cm^{-1}):** 3500-2600 (broad phenolic ν_{OH}), 1706 ($\nu_{C=O}$) of lactone,1610 ($\nu_{C=N}$) of imine, 1540 and 1510 aromatic ($\nu_{C=C}$), 1348 (ν_{C-O}) phenolic-OH, 850 due to >C-S; **^{1}HNMR:**δ2.64 (S, 3H, imine –CH_3),7.8 and 7.5-7.2 (Ar-H of coumarin moiety), 14.5 (S,1H, O–H), 3.6 (S,1H, OH), 2.3(t, 2H, CH_2 [a]), 2.46(t, 2H, CH_2 [b]);**^{13}CNMR:**δ20 (imine-CH_3 carbon), 98.0 for C^3, 150-120 for aromatic

carbons, 156 C^9, 162.0 for lactone carbon, 176 for C^4, and 180 for imine carbon, 46 for C of CH_2 [a],58 for C of CH_2 [b]. Mass Spectra: $[M^+]$=263

ANTIBACTERIAL ACTIVITY

Procedure:

The antibacterial activity was measured by agar cup method.[18] 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	**26**	**20**	**23**	**15**
(3a)	**12**	**-**	**9**	**8**
(3b)	**17**	**-**	**15**	**8**
(3c)	**13**	**5**	**10**	**9**
(3d)	**21**	**7**	**16**	**12**

ANTIFUNGAL ACTIVITY

Procedure:

Antifungal activity was performed by Poison plate method.[18] 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)	-	-	+	-

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**) as per earlier reported method. The reactions were carried out in a protective hood. The intermediate product (**2**) formed was recrystallized in ethanol and purity was tested by TLC. The imines (**3a-3d**) were obtained by refluxing in ethanol with respective amines 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-3d**isclarified in the analytical data. The IR spectra of compound **3a-3d** showed high intensity band observed at 1608-1612 cm^{-1} is assigned to ν(C=N) vibration suggesting the formation of Schiff base. Broad weak band around 3600 cm^{-1} is assigned to –OH in the Schiff bases. The band at 1542-1490 cm^{-1} is assigned to the combination of ν(C=C) of the aromatic ring. A high intensity band in the region 1348-1366cm^{-1} is assigned to phenolic ν(C-O) vibration and 1706-1711 cm^{-1} for lactone carbonyl. A band at 1290cm^{-1}observed for '3b' is assigned to C=S.[19]Beside that >N-N<, >N-H and C-H stretch vibration observed around 1000cm^{-1}, 1670cm^{-1},2930cm^{-1} in respective imines.

Each one of the ^{1}H NMR spectraof**3a-3d**revealed singlet for 3H between 2.64-2.6 ppm assigned to imino methyl group. Peaks between 7.9-7.2 ppm are assigned to aromatic protons. A broad singlet around 14.5 ppm confirms the presence of 4-hydroxyl group. Compound 3c and **3d**revealed a peak at 2.3 ppm assigned to $–CH_2$(a), 2.5 ppm assigned to $–CH_2$(b) and SH revealed a peak at 3.60 ppm.

^{13}CNMR showed peaks between 160.2-163.2 ppm for lactone carbon, between 180-182 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-3d.**

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**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**have reduced the growth of these organisms.

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