



Novel Synthesis, Characterization and Study of Biological Activity of 3-Arylazo-4-Hydroxy 2-H-Chromen-2-One Moiety

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ABSTRACT

(3-methylpyridin-2-yl)diazonyl-4-hydroxy-2H-chromen-2-one, (4-methylpyridin-2-yl)diazonyl-4-hydroxy-2H-chromen-2-one, (6-methylpyridin-2-yl)diazonyl-4-hydroxy-2H-chromen-2-one and (antipyrine)diazonyl-4-hydroxy-2H-chromen-2-one were synthesized by using coupling of 2-amino 3-methyl pyridine, 2-amino 4-methyl pyridine, 2-amino 6-methyl pyridine, 4-amino antipyrine with 4-hydroxy-2H-chromen-2-one. These azo compounds were characterized by IR, ¹H NMR, ¹³C NMR and mass spectral analysis. In vitro biological screening effects of the synthesized compounds were tested for their antibacterial and antifungal activity. For antibacterial activity the bacterial species used were *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* by Agar cup method while fungal species used *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium moniliforme* by the poison plate method.

Keyword: 4-hydroxychromen-2-one, amino pyridine, amino antipyrine, biological activity.

1. INTRODUCTION

4-Hydroxycoumarin is a structurally Benz[α]pyrone derivative which contains hydroxyl group in fourth position of coumarin. The various derivatives of coumarin moiety found in nature having varied biological importance¹. Coumarin moiety contains a fused heterocyclic nucleus which shows variety of medicinal application. Several of these exhibit exceptional biological and pharmacological activities such as anti-inflammatory activity², antioxidant³, anti-HIV⁴, anticoagulant⁵ and cytotoxic properties⁶. Its applications not only restricted to medicine but also found in food additives, perfumes, cosmetics, dyes and herbicides⁷. Like coumarin, azo dye functional group containing compound have importance because of its anti-microbial, and food coloring agent property⁸.

In the views of above facts, we are reporting the novel arylazo-4-hydroxy coumarin compounds prepared by coupling 4-Hydroxy Chromen-2-one with diazo-heteroaryl compounds. These diazo-heteroaryl compounds were prepared by diazotization with 2-amino 3-methyl pyridine, 2-amino 4-methyl pyridine, 2-amino 6-

methyl pyridine and amino antipyrine. These synthesized compounds were characterized by IR, ^1H NMR, ^{13}C NMR and mass spectral analysis. In vitro biological screening effect of the synthesized compounds were tested against the bacterial species *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* by Agar cup method while Fungal species used *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium moniliforme* were tested by the poison plate method.

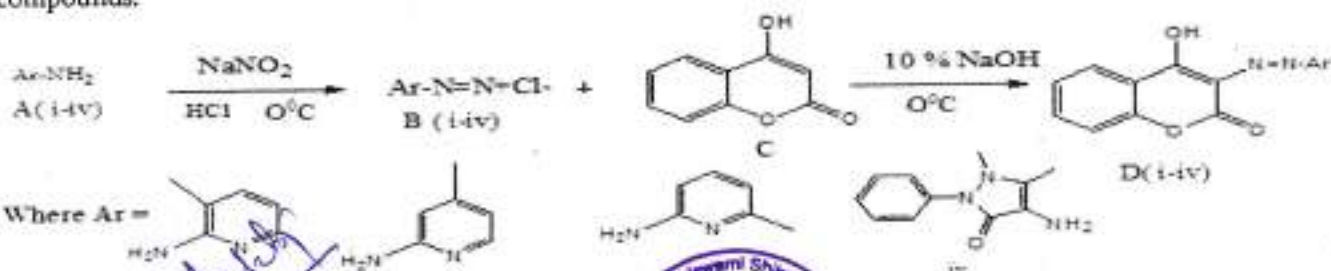
II. EXPERIMENTAL SECTION

The solvents and the reagents used in present study were of analytical grade and obtained from 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 synthesized compounds were carried out by micro combustion method using CHNSO, EA1108, Elemental analyzer model-CARLO-ERBA Instruments, at micro analysis division, National Chemical Laboratory, Pune. 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.

Procedure:

Substituted 2-amino pyridine (5mmol) were dissolved in 8ml water and 5ml conc. HCl, mixture is heated until amine hydrochloride is completely dissolved. NaNO_2 (5mmol) solution was prepared by dissolving it in minimum quantity of water and kept both the reaction mixture in ice bath for cooling. When these mixtures attain 0-50°C temperature then NaNO_2 solution was added to the substituted 2-amino pyridine solution drop-wise with vigorous stirring. Near 00°C temperature was maintained throughout the reaction. After the complete addition reaction mixture was kept in ice bath for 15 minutes with occasional stirring.

The diazotized reaction mixture was then poured in ice cooled solution of 4-hydroxy coumarin (5mmol) in 25 ml of 10% sodium hydroxide solution. This mixture was allowed to stand (0-50°C) for 2 hours and then filtered. The crude product thus obtained was dried and recrystallized from acetic acid to give the corresponding compounds.



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Reaction Scheme



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III ANTIBACTERIAL ACTIVITY

The antibacterial activity was measured by agar cup method⁹. The bacterial strains used as test organism were *Escherichia coli* and *Salmonella typhi* as gram negative bacterial strains and *Bacillus subtilis* and *Staphylococcus aureus* as gram positive bacterial strains. Nutrient agar (Himedia) was prepared and sterilized and kept for 15 minutes in the autoclave. All bacteria were cultured aerobically at 37°C in LB agar and LB broth medium. Before experimental use, cultures from agar medium were sub cultivated in liquid media, incubated for 12 h (37°C). The media plate were seeded with this both culture. Cups of 10mm diameter were made in the agar plate with sterile cork borer. 100 μ l of compound solution prepared in ethanol (0.1%) was added in the cups under aseptic condition with the help of micropipette. 100 μ l of ethanol (0.1%) was placed in separate cups as blank (negative control). 100 μ l of solution of Ciprofloxacin in ethanol (0.1%) was also placed on the seeded nutrient agar surface as standard reference antibiotic (positive control). The plates were allowed for diffusion of the compound from agar cup into the medium. Then the plates were incubated for 24 hours. Record the zone of inhibition of bacterial growth around the agar cup in millimeter (mm) using zone reader.

IV ANTIFUNGAL ACTIVITY

Procedure:

Antifungal activity was performed by Poison plate method¹⁰. A culture of Potato Dextrose Agar (PDA) medium for test of fungi was used. The compound to be tested is added to the sterile medium in aseptic condition. A plate with ethanol was prepared as blank (negative control) similarly a plate with 1% Fluconazole was prepared as standard reference plate (positive control). For testing the fungal activity *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Aspergillus flavus* were selected. 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 plates were incubated at room temperature for 48 hours. After incubation plates were observed for the growth of inoculated fungi. Results were recorded.


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Table : 1 Analytical data of newly synthesized azocoumarine analogues

Synthesised Azo compounds	IR (KBr, cm ⁻¹)	¹ HNMR(CDCl ₃) (300 MHz)	¹³ CNMR(CDCl ₃) (300 MHz)	Mass Spectra
D(i) 3-(2-(3-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	3425(νO-H) stretch 3018 (νAr-H) stretch 1710(νC=O) of lactone 1610 (νC=C stretch coumarin 1557 (νN=N) Stretch 1020 (νC-N) Stretch	7.5-7.2m, 4H, Ar-H of coumarin 2.64 S, 3H(-CH ₃) 15.70 S, 1H, (-O-H); 8.3-7.5m, 3H (Ph-H) of pyridine	164.9 for C ₃ carbon, 158 for C ₂ carbon & 117 to 154 for other carbon of coumarine moiety. 24.8 for CH ₃ and 119 to 158 for carbon of pyridine moiety	[M ⁺] = 280.06
D(ii) 3-(2-(4-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	3420(νO-H) stretch 3025 (νAr-H) stretch 1715(νC=O) of lactone 1620 (νC=C stretch coumarin) 1552 (νN=N) Stretch 1025 (νC-N) Stretch	7.4-7.2m, 4H, Ar-H of coumarin 2.66 S, 3H(-CH ₃) 15.75 S, 1H, (-O-H); 8.3-7.5m, 3H (Ph-H) of pyridine	164.9 for C ₃ carbon, 160 for C ₂ & 117 to 154 for other carbon of coumarine moiety. 15.3 for CH ₃ and 121 to 151 for carbon of pyridine moiety	[M ⁺] = 280
D(iii) 3-(2-(6-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	3428(νO-H) stretch 3022 (νAr-H) stretch 1722(νC=O) of lactone 1618 (νC=C stretch coumarin) 1555 (νN=N) Stretch 1034 (νC-N) Stretch	7.5-7.3m, 4H, Ar-H of coumarin 2.665 S, 3H(-CH ₃) 15.71 S, 1H, (-O-H); 8.3-7.5m, 3H (Ph-H) of pyridine	164.9 for C ₃ carbon, 159 for C ₂ & 117 to 154 for other carbon of coumarine moiety. 24.8 for CH ₃ and 124 to 149 for carbon of pyridine moiety	[M ⁺] = 280
D(iv) 4-(1,3-Dimethyl-2-phenyl-3-oxo pyrazolyl)-4-hydroxy-2H-chromen-2-one	3445(νO-H) stretch 3015 (νAr-H) stretch 1720(νC=O) of lactone 1620 (νC=C) stretch of coumarin 1548 (νN=N) Stretch 1018 (νC-N) Stretch Pyrazolone Stretch 1660 (νC=O) stretch	7.5-7.4m, 4H, Ar-H of Coumarine 2.63 S, 3H(-CH ₃) 15.79 S, 1H, (-O-H); 8.6-7.8m, 3H (Ph-H) of antipyrine	164.9 for C ₃ carbon, 164 for C ₂ & 117 to 154 for other carbon of coumarine moiety. 162.2 for C ₄ carbon, 93 for C ₅ & 155 for C ₆ carbon of antipyrine moiety	[M ⁺] = 377

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Table : 2 Physical data of newly synthesized azo compounds

Synthesised Azo compounds	Molecular Formula	Mol. Wt	Elemental analysis Found (Calculated)			Melting point	Colour	Yield (%)
			C	H	N			
D(i) 3-(2-(3-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	C ₁₅ H ₁₁ N ₃ O ₃	281	62.92 (63)	3.39 (3.33)	15.72 (15.70)	185	Light Pink	80
D(ii) 3-(2-(4-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	C ₁₅ H ₁₁ N ₃ O ₃	281	62.90 (62.85)	3.82 (3.54)	15.31 (15.20)	187	Brown	78
D(iii) 3-(2-(6-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	C ₁₅ H ₁₁ N ₃ O ₃	281	62.30 (62.10)	3.72 (3.52)	15.53 (15.30)	185	Yellow	70
D(iv) 4-(1,3-Dimethyl-2-phenyl-3-oxo pyrazolyl)-4-hydroxy-2H-chromen-2-one	C ₂₀ H ₁₆ N ₄ O ₄	378	62.82 (63.75)	4.28 (4.25)	14.89 (14.95)	182	Red	75

V RESULT AND DISCUSSION

The scheme of reaction approaching to the target aryl azo compounds is outlined above. In present investigation we report newly synthesized four aryl azo compounds. They were prepared by coupling 4-hydroxy-2H-chromen-2-one with diazotized aryl amines. The products formed were recrystallized in ethanol and purity was tested by TLC. Different aryl amines were firstly undergoing diazotization by the action of sodium nitrate at 0-5 °C. This diazotised mixture produces N₂⁺ as strong electrophile which triggers the coupling reaction with 4-hydroxy coumarine11. The synthesized compounds were summarized in table.

The Characterization of the synthesized compounds were done with IR, ¹HNMR, ¹³CNMR techniques. The significant peaks observed in the spectra are summarized in the table-1.

The IR spectra of compound showed high intensity band observed at 1548-1557 cm⁻¹ is assigned to ν(N=N) vibration suggesting the presence of N=N12 while Broad weak band around 3420-3445 cm⁻¹ is assigned to H bonded -OH in the compound. 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 1018-1034 cm⁻¹ is assigned to ν(C-N) vibration and 1722-1710 cm⁻¹ for lactone carbonyl13.


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The ^1H NMR spectra of compound revealed singlet for H at 15.70-15.79 assigned to phenolic OH group¹⁴. Peaks between 7.5-7.0 ppm are assigned to aromatic protons of 4-hydroxy coumarin while m(8.6-7.5) indicates aromatic proton from aryl amines¹⁵. ^{13}C NMR showed peaks between 117 to 165 ppm for 4-hydroxy coumarin moiety while between 124 to 140 ppm for aromatic carbon of pyridine group. Assignment given to other peaks observed in ^1H NMR, ^{13}C NMR spectra and also molecular ion peaks in mass spectra justifies the structures of compounds.

Table: 3 Anti-Bacterial Activity

Synthesised Azo compounds	Zone of Inhibition (diameter in mm)			
	B. subtilis	E. coli	S. typhi	S. aureus
Ciprofloxacin(Reference)	18	24	25	25
D(i)3-(2-(3-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	20	16	20	24
D(ii)3-(2-(4-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	22	14	18	20
D(iii)3-(2-(6-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one	23	15	19	21
D(iv)4-(1,3-Dimethyl-2-phenyl-3-oxo pyrazolyl)-4-hydroxy-2H-chromen-2-one	24	18	21	28

Table: 4 Anti-fungal Activity

Synthesised Azo compounds	Growth of Fungi			
	A. flavus	P. chrysogenum	A. niger	F. moniliforme
Fluconazole (Reference)(65 µg/ml)	-	-	-	-
D(i)3-(2-(3-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one(64 µg/ml)	+	++	+	++
D(ii)3-(2-(4-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one(64 µg/ml)	-	+	-	+
D(iii)3-(2-(6-methylpyridin-2-yl)diazanyl)-4-hydroxy-2H-chromen-2-one(64 µg/ml)	+	+	-	+
D(iv)4-(1,3-Dimethyl-2-phenyl-3-oxo pyrazolyl)-4-hydroxy-2H-chromen-2-one(64 µg/ml)	+	++	+	++

Moderate growth (++)₁ Reduced growth (+) and No growth (-) of fungi

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The aryl azo compounds synthesized were evaluated for anti-bacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown in Table-3 and Table-4. All azo compounds show good antimicrobial activity against *B.subtilis* compared to Ciprofloxacin as control. While D (iv) showed antimicrobial against *S.typhi* and *S.aureus* alongwith *B.subtilis*. All compounds showed encouraging antifungal activity against *Aspergillus* species as compared to *P.crysogenum* and *F.moniliforme* with D(ii) and D(iii) showing highly effective as compared to Fluconazole as control. The growth of the later was also reduced by these compounds.

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