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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)diazenyl)-4-hydroxy-2H-chromen-2-one, (4-methylpyridin-2-yl)diaze-nyl)-4-hydroxy-2H-chromen-2-one, (6-methylpyridin-2-yl) diazenyl)-4-hydroxy-2H-chromen -2-one and(antipyrine)diazenyl -4-hydroxy-2H-chromen-2-one weresynthesized 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, 1HNMR, 13CNMR 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, andStaphylococcus aureus by Agar cup method whilefungal species usedAspergillusflavus, Penicilliumchrysogenum,Aspergillusniger and Fusariummoneliformed by the poison plate method.

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

I. INTRODUCTION

4-Hydroxycoumarin is a structurally Benz[α]pyrone derivative which contains hydroxyl group in fourth position of coumarin. The various derivatives ofcoumarin moiety found in nature havingvaried 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 agentproperty8.

In the views of above facts, we arereporting the novelarylazo4-hydroxy coumarin compounds prepared by coupling 4-Hydoxy Chromen-2-onewith diazo-heteroaryl compounds. These diazoheteroaryl 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 compoundswere characterized by IR, 1HNMR, 13CNMR and mass spectral analysis.In vitro biological screening effect of the synthesized compounds were tested against the bacterial speciesBacillus subtilis, Escherichia coli, Salmonella typhi, andStaphylococcus aureusby Agar cup method while Fungal speciesused Aspergillusflavus, Penicilliumchrysogenum,Aspergillusnigerand Fusariummoneliforme were tested by the poison plate method.

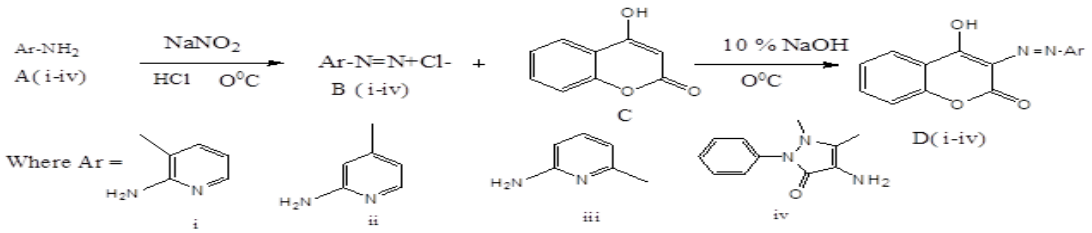
II. EXPERIMENTAL SECTION

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, EA1I08, 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 CHCl3 on a Shimadzu FTIR-8300 spectrophotometer. The 1H NMR (300 MHz) and 13C NMR (70 MHz) were run on a BrukerAvance DPX-250 spectrometer in CDCl3 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. NaNO2 (5mmol) solution wasprepared by dissolving it in minimum quantity of water and kept both the reaction mixture in ice bath for cooling. When these mixtures attain 0-50C temperature then NaNO2solutionwas added to theSubstituted 2-amino pyridinesolution drop-wise with vigorous stirring. Near 00C 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 (0-50C) for 2hours and then filtered. The crude product thus obtained was dried and recrysallized from acetic acid to give the corresponding compounds.



Reaction Scheme

Antibacterial Activity

The antibacterial activity was measured by agar cup method9.The bacterial strains used as test organism were Escherichia coliand Salmonella typhi as a gram negative bacterial strains and Bacillus subtils and Staphylococcus aureu 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 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(0.1%) was placed in separate cups as blank (negative control). 100 µl of solution of Ciprofloxacinin 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.

III. ANTIFUNGAL ACTIVITY

Procedure:

Antifungal activity was performed by Poison plate method10. A culture of Potato Dextrose Agar (PDA) medium for test of fungi wasused. 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 Aspergillusniger, Penicilliumchrysogenum, Fusariummoneliforme, Aspergillusflavuswere 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.

Table : 1Analytical data of newly synthesized azocoumarine analogues

Synthesised Azo compounds	IR (KBr, cm-1)	^{1}HNMR(CDCl3) (300 MHz)	^{13}CNMR(CDCl3) (300 MHz)	Mass Spectra

D(i) 3-(2-(3-methylpyridin-2-yl)diazenyl) -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)Strech 1020 (νC-N) Strech	7.5-7.2m,4H,Ar-H of coumarin 2.64 S,3H(–CH3) 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)diazenyl) -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)Strech 1025 (νC-N) Strech	7.4-7.2m,4H,Ar-H of coumarin 2.66 S,3H(–CH3) 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)diazenyl)-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)Strech 1034 (νC-N) Strech	7.5-7.3m,4H,Ar-H of coumarin 2.665 S,3H(–CH3) 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(–CH3) 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_3 carbon , 93 for C_4& 155 for C_5 carbon of antipyrine moiety	[M+] = 377

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)diazenyl) -4-hydroxy-2H-chromen-2-one	$C_{15}H_{11}N_3O_3$	281	62.92 (63)	3.39 (3.33)	15.72 (15.70)	185	Light Pink	80
D(ii) 3-(2-(4-methylpyridin-2-yl)diazenyl) -4-hydroxy-2H-chromen-2-one	$C_{15}H_{11}N_3O_3$	281	62.90 (62.85)	3.82 (3.54)	15.31 (15.20)	187	Brown	78
D(iii) 3-(2-(6-methylpyridin-2-yl)diazenyl)-4-hydroxy-2H-chromen-2-one	$C_{15}H_{11}N_3O_3$	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	$C_{20}H_{16}N_4O_4$	378	62.82 (63.75)	4.28 (4.25)	14.89 (14.95)	182	Red	75

IV. 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 0. 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 withIR, 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=N12while 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.

The 1H NMR spectra ofcompoundrevealed singlet for H at 15.70-15.79 assigned to phenolic OH group14. Peaks between 7.5-7.0ppm are assigned to aromatic protons of 4-hydroxy coumarin while m(8.6-7.5) indicates aromatic proton from aryl amines15 . C13NMR showed peaks between 117 to 165 ppm for 4 hydroxy coumarinmoiety while between 124 to 140 ppm for aromatic carbon of pyridine group. Assignment given to other peaks observed in 1HNMR, 13CNMR 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(Refernce)	18	24	25	25
D(i)3-(2-(3-methylpyridin-2-yl)diazenyl) -4-hydroxy-2H-chromen-2-one	20	16	20	24
D(ii)3-(2-(4-methylpyridin-2-yl)diazenyl) -4-hydroxy-2H-chromen-2-one	22	14	18	20
D(iii)3-(2-(6-methylpyridin-2-yl)diazenyl)-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.chryso-genum	A. niger	F.mone-liforme
Fluconazole (Reference)(65µg/ml)	-	-	-	-
D(i)3-(2-(3-methylpyridin-2-yl)diazenyl) -4-hydroxy-2H-chromen-2-one(64µg/ml)	+	++	+	++
D(ii)3-(2-(4-methylpyridin-2-yl)diazenyl) -4-hydroxy-2H-chromen-2-one(64µg/ml)	-	+	-	+
D(iii)3-(2-(6-methylpyridin-2-yl)diazenyl)-4-hydroxy-2H-chromen-2-one(64µg/ml)	+	+	-	+
D(iv)4-(1,3-Dimethyl-2-phenyl-3-oxo pyrazolyl)- 4-hydroxy-2H-chromen-2-one(64µg/ml)	+	++	+	++

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

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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