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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NOVEL 4-HYDROXY COUMARIN DERIVATIVES BEARING AZO MOIETY

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ABSTRACT

The objective of this study was to investigate *in vitro* antimicrobial activity of ten 4-hydroxycoumarin derivatives bearing azo group at C-3 position. Some new derivatives of coumarin dyes 3(a-j) were prepared by the coupling of 4-hydroxycoumarin with derivatives diazonium salts, which were obtained by diazotization of aniline derivatives in presence of sodium nitrite and concentrated HCl. All the synthesized derivative compounds were characterized by elementary analysis, IR, ^{1}H NMR and further screened individually to their preliminary *in-vitro* antimicrobial activity against four human pathogenic bacteria viz., *E.coli, S.aureas, S. typhi* and *Pseudomonas* species. Antibacterial activity of each compound was compared with standard Ciprofloxacin. *In-vitro* antibacterial activity results revealed that all the synthesized compounds inhibited the growth of *E.coli* and *S. aureus* as compared to other bacterial pathogens except 3f; but all the products were ineffective against *P. aeruginosa.* The compounds like 3b, 3c and 3h have excellent activity against all bacterial species.

Keywords: 4-Hydroxy coumarin, Derivative of aniline, diazotization, Antibacterial activity.

INTRODUCTION

Azo dyes are the largest and the most popular group of dyes showing the full palette of colours. Azo dyes compounds are containing –N=N– group as a characteristic chromophore, and mainly obtained in diazotization and coupling reaction. As per literature survey, it was found that azo dyes have been most widely used in different application fields, such as dying textile fibres, biomedical studies and advanced in organic synthesis as well as shows variety of interesting biological activities including antibacterial and pesticide activities[1-5]. Synthesis of azo dyes involves diazotization of a primary aromatic amine followed by coupling with one or more nucleophiles. Amino, hydroxy and active methylene groups are commonly used coupling compounts[6]. In pharmaceutical, azo linkage was used to protect drug from undesirable reaction such as prontosil was found to protect against streptococcal infections in mice and inactive on bacterial cultures. 4-Hydroxycoumarin is a structurally Benz[α]pyrone derivative which contains hydroxyl group in fourth position of coumarin. The chemistry and pharmacological action of 4-hydroxy coumarin have great interest to medicinal chemistry because of its derivative possess various biological activity such as anticoagulant, antibacterial, antifungal, anticancer, antiinflammatory, antiviral, antioxidant, and analgesic[7-13]. The azo dye sulphonamides antibacterial drugs were the first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infection in humans. The above observations prompted us to synthesize the title compounds with the presumption that introduction of Azo group in 4-hydroxy coumarin would produce new compounds with significant antibacterial activity.

EXPERIMENTAL

The chemicals used in the present studies were of synthetic grade, Merck company Ltd. The products were characterized by IR and ^{1}H NMR. The melting points were determined by open capillary method and found to be uncorrected. The IR spectra were recorded on Perkin-Elmer spectrum FTIR instrument in the form of KBr pellet. The ^{1}H NMR spectra were measured in $CDCl_3$ solutions on a Brucker 400 MHz

spectrometer using TMS as an internal reference (δ ppm). The purity of compounds was checked by TLC. Elemental (C, H, N) analysis indicated that the calculated and found values were within the acceptable limits (±0.4%).

General synthesis procedure of 4-hydroxy-3-(substituted phenyldiazenyl)-2H-chromen-2-one compounds (3a-j) [14]

Ten different individual substituted aromatic amines were mixed with 2.5 mL conc. HCl and 2.5 mL (4 N) cold solution of $NaNO_2$ was added with the frequent stirring. The temperature of the reaction was maintained up to 0-5^{0}C. Diazonium salt solution prepared above and was added drop wise to the alkaline solution of 4-hydroxy coumarin. The reaction mixture stirred for 10-20 minutes maintaining the temperature 5-10^{0}C. The colored products obtained were filtered, washed with water and finally the products were dried. The entire product individually recrystallized from 50% ethanol.

Table -1: Characterization of synthesized Azo compounds by IR & ^{1}HNMR Spectral data

Compd.	Aryl substituent	IR(cm-1)	^{1}HNMR(δ)	Recrystallized by
3a	Phenyl	3000(Ar-H), 1615 (C=C of pyrone), 1555(N=N), 1720(C=O str, α pyrone), 730(C-H out plane, mono disub).	7.42- 7.88(m,9H,Ar-H), 17.07(s, 1H, enolic OH).	50% ethanol
3b	4-methoxyphenyl	1616 (C=C of pyrone), 1557(N=N), 1720(C=O, str, α pyrone) 1220, (C-O str of Ar-OCH_3), 825(C-H out plane, Para disub phenyl).	6.99-7.90(m, 8H, Ar-), 3.83(d, 3H, -OCH_3), 17.07(s, 1H, enolic OH).	50% ethanol
3c	4-chlorophenyl	1615 (C=C of pyrone), 1555(N=N), 1717(C=O, str, α pyrone), 772(Ar C- Cl str) 835(C-H def Para disub phenyl).	7.27-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).	50% ethanol
3d	4-bromophenyl	1620 (C=C of pyrone), 1558(N=N), 1715(C=O str, α pyrone), 695(Ar C-Br str) 835(C-H out plane para disub phenyl).	7.27-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).	50% ethanol
3e	4-hydroxy phenyl	1625 (C=C of pyrone), 1558(N=N), 3610(phenolic-OH). 1715(C=O, str, α pyrone).	6.82-7.84(m, 8H, Ar-H), 17.07(s, 1H, enolic OH), 9.86(s, 1H.phenolic-OH).	50% ethanol
3f	2-carboxyphenyl	1620 (C=C of pyrone), 1560(N=N), 1720(C=O, str, α pyrone) 3400(Ar C=C COOH), 1400(C-O str).	7.42-8.21(m,8H,Ar-H), 12.82(m, 1H, carboxylic-OH).	Ethanol+DMF
3g	4-nitrophenyll	2890(Ar-H), 1558(N=N), 1720(C=O, str, α pyrone), 1520, 1350(NO_2str).	7.18-8.10(m, 8H, Ar-H), 17.07(s, 1H, enolic OH).	50% ethanol
3h	4-sulphamidophenyl	2900(Ar-H), 1555(N=N), 1720(C=O, str,α pyrone), 1320(sym str-SO_2), 1149 (asym str-SO_2,) 3200, 3260(N-H str, SO_2NH_2), 902(S-N str).	7.42-7.86(m,8H,Ar-H), 17.07(s, 1H, enolic OH), 7.39(s, 2H,-NH_2).	Ethanol+DMF

3i	4-bromo3-methylphenyl	1618 (C=C of pyrone), 1555(N=N), 1720(C=O, str, α pyrone), 850(C-H out plane trisubs of phenyl).	7.08-7.86(m,7H,Ar-H), 17.07(s, 1H, enolic OH), 2.36(s, 3H, CH_3).	50% ethanol
3j	Napthyl	1620(C=C of pyrone), 1555(N=N), 1720(C=O, str, α pyrone).	7.44-8.0(m,11H,Ar-H), 17.07(s, 1H, enolic OH).	50% ethanol

Antimicrobial activity

Ten (3a-j) newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Salmonella typhi* (recultured) bacterial strains by using zone of inhibition method[15]. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Ciprofloxacin as standard. All the newly synthesized compounds were dissolved in Dimethylsulphoxide (DMSO) to prepare chemicals stock solution of 100 µg/ml. Zone of inhibition was determined for (3a-j) and the results are expressed in Table-3.

Table-2: Physical data of synthesized compounds (3a-j)

Compd.	Aryl	Molecular Formula	Mol. Wt.	Analysis % C,H,N found (calculated)			Physical state	Yield (%)
				Carbon	Hydrogen	Nitrogen		
3a	Phenyl-	$C_{15}H_{10}N_2O_3$	266	67.67 (67.62)	3.79 (3.75)	10.52 (10.50)	Cream white	75
3b	4-methoxyphenyl	$C_{16}H_{12}N_2O_4$	296	64.86 (64.85)	4.08 (4.07)	9.46 (9.45)	White	65
3c	4-chlorophenyl	$C_{15}H_9ClN_2O_3$	300	59.91 (59.85)	3.01 (2.98)	9.32 (9.30)	White	80
3d	4-bromophenyl	$C_{15}H_9BrN_2O_3$	345	52.20 (52.18)	2.63 (2.60)	8.12 (8.10)	Light brown	75
3e	4-hydroxy phenyl	$C_{15}H_{10}N_2O_4$	282	63.83 (63.82)	3.57 (3.55)	9.92 (9.90)	White	85
3f	2-carboxyphenyl	$C_{16}H_{10}N_2O_5$	310	63.94 (63.90)	3.25 (3.22)	9.03 (9.0)	Yellow	45
3g	4-nitrophenyl	$C_{15}H_9N_3O_5$	311	57.88 (57.85)	2.91 (2.90)	13.50 (13.48)	Light green	85
3h	4-sulphamidophenyl	$C_{15}H_9N_3O_5$	345	52.17 (52.15)	3.21 (3.20)	12.17 (12.15)	Pale yellow	80
3i	4-bromo3-methylphenyl	$C_{16}H_{11}BrN_2O_3$	359	53.5 (53.49)	3.09 (3.08)	7.80 (7.75)	White	60
3j	Naphthyl	$C_{19}H_{17}N_2O_3$	316	72.15 (72.13)	3.82 (3.80)	8.86 (8.83)	White	85

RESULTS AND DISCUSSION

A series of 4-hydroxy-3-(substituted phenyldiazenyl)-2H-chromen-2-one(3a-i) were synthesized by coupling of diazonium salt of aniline derivatives 2(a-j) with 4-hydroxycoumarin in presence of sodium hydroxide (Scheme-1). The coupling mechanism of organic reaction is summarized in the scheme. The crude products re-crystallized from 50% ethanol while some compounds from mixture of ethanol and DMF. All prepared compounds were physically characterized by IR (cm^{-1}) and ^{1}HNMR (chemical shift δ) as mentioned in Table-1 and 2. 4-hydroxy coumarin nucleus is containing active hydrogen group at C_3

position which on attacked by strong N_2^+electrophiles to produce 3-azosubstitutedcoumarin. The following peaks are confirmed the structure of 3-azosubsituted of 4-hydroxy coumarin; the peaks at 1570-1560cm^{-1}, 1620-1615cm^{-1},1720-1715cm^{-1} in FTIR are the groups of –N=N-, -C=C-aromatic str and C=O str respectively. The compound 3h shows IR peak at 1360-1306 cm^{-1}, 1154-1149 cm^{-1} for N-H (str) and N-H (def) $NH_2$of SO_2NH_2 respectively. The NH proton of the SO_2NH_2 group observed NMR at δ7.00-7.39 ppm as singlet. The ^{1}HNMR spectra showed board singlet at δ 17.07, which suggests the presence of enolic OH in all the prepared compounds. The enolic –OH group of all the compounds were chemically detected by the treatment with $FeCl_3$ solution, which gives characteristic colour.

Table-3: Antimicrobial properties of the synthesized Expressed as zone of Inhibition

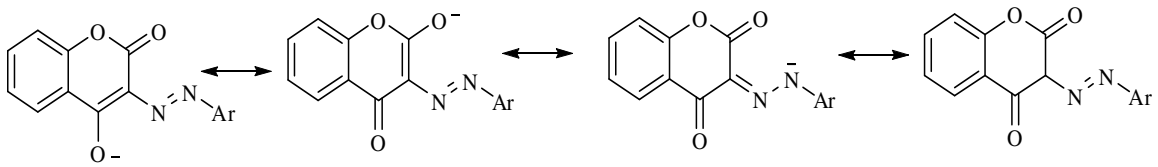
Compounds (conc.100 µg/ml)	Micro-organism & Zone of Inhibition(mm)			
	E.coli	*S. aureus*	*S. typhi*	*P. aeruginosa*
3a	14	18	----	8
3b	19	23	20	----
3c	18	23	18	9
3d	15	18	14	-----
3e	15	12	12	-----
3f	----	-----	13	---
3g	16	22	22	----
3h	18	20	18	---
3i	12	17	09	8
3j	15	18	-----	----
DMSO	----	------	-----	-----
Ciprofloxacin (conc.100 µg/ml)	20	25	25	25

----:- No Antimicrobial growth

Antibacterial activity

Antibacterial activity results of ten 3(a-j) derivatives of azo coumarin dyes are shown in Table-3. The results of these studies revealed that all the synthesized compounds exhibited tremendous inhibition of *E.coli* and *S. aureus* when compared to other bacterial strains except 3f. The compounds 3a, 3d and 3e were found to moderate to weak activities against all bacterial stains but all the products were ineffective against *P. aeruginosa.* Due to the presence of Cl, -OCH_3, -NO_2, SO_2NH_2 as structural substitution at para position of phenyl ring, the compounds like 3b, 3c, 3g & 3h have an excellent activity against all bacterial strains. But the products, like 3a, 3d, and 3e were found to moderate to weak activities against all bacterial pathogens.

Resonance structural Hybrids of coupled azocoumarin

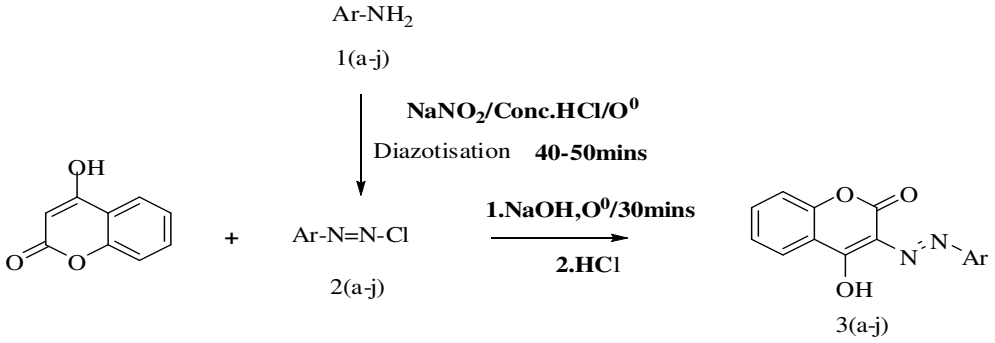


Structure-1

CONCLUSION

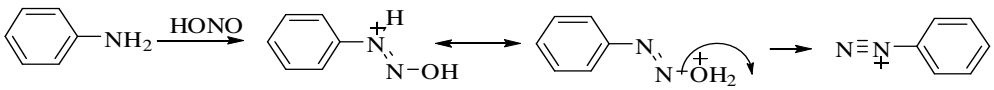
The present research work involves the synthesis of novel 3-substituted azocoumarin derivative to explore their antimicrobial activity. All the compounds were recrystallized and structurally interpreted by IR and

[1]HNMR. Compounds 3b, 3c, 3g and 3h were exhibited excellent antibacterial activity against *E.coli* and *S. aureus*, whereas the synthesized compounds (3a, 3i) were found to be moderate to weak activity. Hence, it is concluded that there is a scope for further study in developing some lead compounds for the treatment of bacterial and fungal diseases.

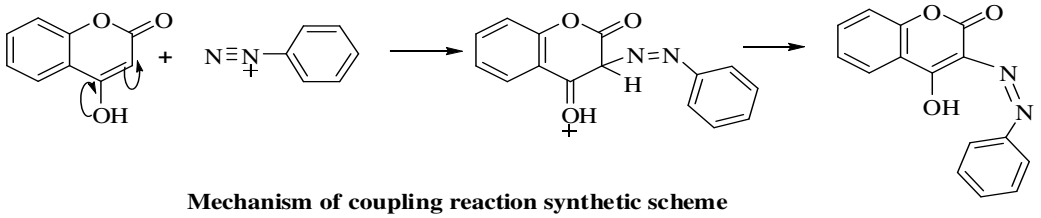


Ar;phenyl,4-methoxy phenyl ,4-chloro phenyl, 4-bromophenyl, 4-hydroxy phenyl, 2-carboxy phenyl, 4-nitro phenyl, 4-sulphamido phenyl ,3-bromo 4-methyl phenyl, napthyl.

Formation of diazonium salt, generation of electrophile species



Coupling Reaction of diazonium electrophile with 4-hydroxy coumarin



Mechanism of coupling reaction synthetic scheme

Scheme-1

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