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3-(2, 6-Dichlorophenyl)-4-hydroxy-6-nitrocoumarin: Synthesis, Characterization, and Antibacterial Properties

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Abstract

Herein, 4-hydroxycoumarin was first treated with sodium nitrate in the presence of concentrated sulfuric acid to afford 6-nitro-4-hydroxycoumarin in a reasonable yield. 6-Nitro-4-hydroxycoumarin was then reacted with the diazonium salt derived from 2, 6-dichloroaniline, and the corresponding azo dye was prepared and purified. This compound was characterized using Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopic techniques. The UV-vis spectroscopic behavior of the dye was then analyzed in six organic solvents with different polarities: ethanol, dimethyl sulfoxide, dimethyl formamide, chloroform, acetic acid, and acetonitrile. Fourier transform Infrared (FT-IR), Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy confirmed the presence of two distinct azo-enol and hydrazone-keto isomers of the proposed tautomeric forms, both in the solid state and in solution. The UV-vis absorption spectra of the dyes remained largely unaffected by solvent changes, likely due to intramolecular hydrogen bonding within their molecular structures. The antibacterial activities of the azo-nitro product dissolved in DMSO were evaluated using the well diffusion method against *Staphylococcus aureus* ATCC 25923 bacterial strains, and the results were compared with a standard specimen.

Key words:6-nNitro-4-hydroxycomarin, Spectroscopy, Nitration, Azo dye, Antibacterial activities

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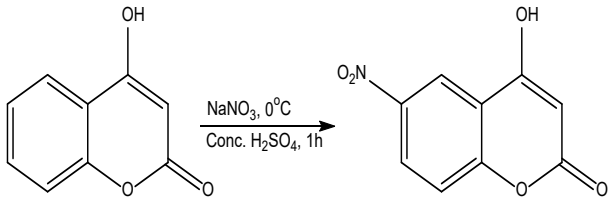
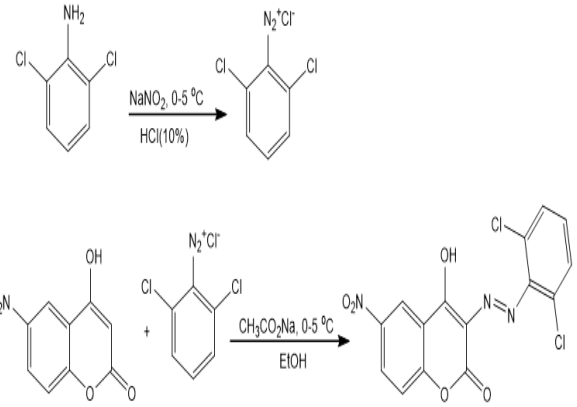
Introduction

4-hydroxycoumarin derivatives are highly valued in various fields due to their unique molecular structures(Jung and Park, 2009; Luchini et al., 2008; Chohan et. al., 2006; Abdou et al., 2019; Bouhaoui et al., 2021). They are known for their pharmacological properties and are used in the synthesis of dyes and pigments (Knierzinger and Wolfbeis, 1980; Dondon and Fery-Forgues, 2001; Bečić et al., 2011; Al-Majedy et al., 2015; Sßenkuytu et al., 2017; Yasameen et al., 2021). The reaction between 4-hydroxycoumarin and phenyldiazonium salt was first reported by Yoder et al., leading to the formation of E and Z geometric isomers of the phenylhydrazone derivative(Yoder et al., 1972; Rahmani et al., 2022). Research on aryl-azo hydroxycoumarin dyes, including their structural characterization and tautomeric behaviors, has continued over the years (Giri and Mishra, 1984; Noroozi Pesyan and Rashidnejad, 2016; Jyotirmaya and Kumar, 2017; Nagaraja et al., 2020; Yordanov et al., 2021; Manjunatha et al., 2021). Karci and Yildirim synthesized 4-hydroxycoumarin-based heterocyclic disazo dyes using substituted amino-thiophenes and other heterocyclic diazotizing agents (Karci, 2005; Karci and Ertan, 2005; Karci and Karci, 2012; Yildirim et al., 2016). These dyes have been found suitable for traditional and heat transfer printing on polyester and nylon 6 fabrics, as well as exhibiting antibacterial activity against various strains (Gaffer et al., 2016; Sudhir Kumar et al., 2013; Sahoo et al., 2015; Jyotirmaya and Kumar, 2017). Additionally, 4-hydroxycoumarin-based azo compounds have been used as ligands to form complexes with transition metals (Shoair, 2007; Jabbar et al. 2021; Abbas Bayader et al. 2022). Recent research by Brahmachari and colleagues explored aryl azo derivatives of 4-hydroxycoumarin, highlighting their preference for the hydrazone tautomeric structure (Brahmachari et al., 2021). Inspired by previous research on 4-hydroxycoumarin derivatives, we synthesized 6-nitro-4-hydroxycoumarin following a modified procedure (Huang et al., 2007) (Scheme 1). The molecule was reacted with 2, 6-dichlorobenzendiazonium hydrogen chloride to produce the related azo derivative in a reasonable yield (Scheme 2).The antimicrobial activity of the dye was examined using the well diffusion method.



Scheme1. Synthesis of 6-nitro-4-hydroxycoumarine



Scheme 2. Synthetic pathway to 3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin.

Materials and methods

Chemicals and solvents were purchased from Aldrich-Sigma and Merck chemical companies. The FT-IR spectra were recorded using a Perkin Elmer FT-IR Spectrophotometer with pressed KBr discs. NMR spectra were obtained with a Brucker Avance spectrometer in DMSO-d6 using TMS as an internal standard. Melting points were measured with a Barnstead Electrothermal 9100 melting point apparatus in open capillary tubes, and the values were not corrected.

Synthesis of 4-hydroxy-6-nitro-coumarin

In a solution of sulfuric acid (20 mL) cooled with ice, potassium nitrate (0.623 g, 6.17 mmol) was dissolved, followed by the addition of 4-hydroxycoumarin (1.0 g, 6.17 mmol). The reaction mixture was stirred at 0°C for 1 hour, then poured into ice-cold water to precipitate the product. The precipitate was collected by filtration, and the crude product was recrystallized from a 6:4

mixture of ethyl acetate and cyclohexane, resulting in a white solid with a 64% yield. The melting point was found to be 251–253°C (literature value: 253–254°C, Huang et al., 2007).1H NMR (DMSO-d6, 300 MHz) 13.16 (1H, br. OH), δ 8.48 (1H, d, J=1.6 Hz), 8.40 (dd, J= 5.5, 1.6Hz), 7.56 (1H, d, J= 5.5 Hz), 5.66 (s, 1H); 13C NMR (75 MHz, DMSO-d6) 165.13, 161.40, 157.72, 143.78, 127.93, 119.72, 118.71, 117.00, 92.58.

Synthesis of 3-(2,6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin

3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin was synthesized following literature reports (Moradi Rufchahi and Ghanadzadeh Gilani, 2012; Yahyazadeh et al. 2024). A solution of 2, 6-dichloroaniline (3.0 mmol) was prepared in 10% HCl (v: v, 9.0 mL) and stirred for 15 minutes at room temperature. The mixture was then cooled to 0-5˚C. Sodium nitrite (0.25 g, 3.6 mmol) dissolved in water (3.0 mL), was added dropwise to the solution over 20 minutes. The mixture was left stirred at 0-5˚C for 1 hour. The diazonium solution was slowly added over 30 minutes to a stirred mixture of sodium acetate (2.0 g) and 6-nitro-4-aminocoumarin (0.62 g, 3.0 mmol) in ethanol (20 mL) at 0-5˚C. After complete addition, the mixture was stirred for 2 hours at 0-5˚C and an additional 4 hours at room temperature. The yellow precipitates formed were collected by filtration, washed with water, and recrystallized from absolute ethanol to obtain pure crystals of the desired dyes. The characterization details of the synthesized compound can be found as follows:

3-(2,6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin:

: brick red powder, yield 80%; m.p. 211-213°C; IR (KBr): ν (cm-1); 3446 (r, OH), 3056 (=C-H), 1756 (C=O, ester), 1624 (C=N, hydrazone), 1518, 1454 and 1385 (Ar-C=C),1254 and 1209 (C-O), (C-Cl); 1H NMR (300 MHz, DMSO-d6); δ ppm 15.18 (br., NH, Hydrazone), 13.53(br., OH), 8.68 (1H, d, J=3.8Hz), 8.57(1H, dd, J= 2.8, 9.0, Hz), 7.72-7.68 (2H, m, overlapped), 7.64 (1H, d, J=9.0Hz), 7.50(1H, t, J=9.0).

3. Results and discussion

3.1. Synthesis and characterization

6-Nitro-4-hydroxycoumarin was synthesized by reacting 4-hydroxycoumarin with potassium nitrate in concentrated sulfuric acid at 0°C for 1 hour. The 1H NMR spectrum confirmed the structure, showing characteristic signals for the coumarin core and nitro-coumarin moiety. The 13C NMR spectrum in DMSO-d6 supported the proposed structure with nine distinct signals.

The 3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin was prepared according to methods reported in the literatures [Karci, 2005; Karci and Ertan, 2005; Karci and Karci, 2012; Yildirim et al., 2016]. The 1H NMR spectra of this compound is presented in Figure1. The spectra exhibit two distinct broad signals in the ranges of 15.18 ppm and 13.53 ppm, which are assigned to the hydrazone (=N–NH) and enolic hydroxyl (C=C-OH) protons, respectively [Nagaraja et al., 2020; Yildirim et al., 2016; Yordanov et al., 2021]. These results indicate that the dyes exist as an equilibrium mixture of tautomeric forms, specifically the azo-enol and hydrazone-keto forms, in the solution state (Scheme 3).

3.2. Evaluation of Antibacterial Activity

The antibacterial properties of the three synthesized dye was evaluated using the well diffusion method on Mueller-Hinton agar (MHA). The compounds were prepared in DMSO, and *Staphylococcus aureus* (ATCC 25923) *Escherichia coli* (ATCC 25922) served as reference strains for the assay. After 24 hours of incubation, the inhibition zones were measured in millimeters (mm) and are presented in Table 2. The bacterial strains were adjusted to a turbidity of 0.5 McFarland standard (equivalent to 1.5×108 CFU/ml) and cultured on MHA under aseptic conditions. Wells with a diameter of 6 mm were loaded with 80 µl of dye solution at concentrations of 1 mg/ml and 2 mg/ml, followed by incubation at 37°C for 24 hours. Ciprofloxacin was the positive control, while DMSO was the negative control. After incubation, the diameters of the inhibition zones were measured in millimeters.

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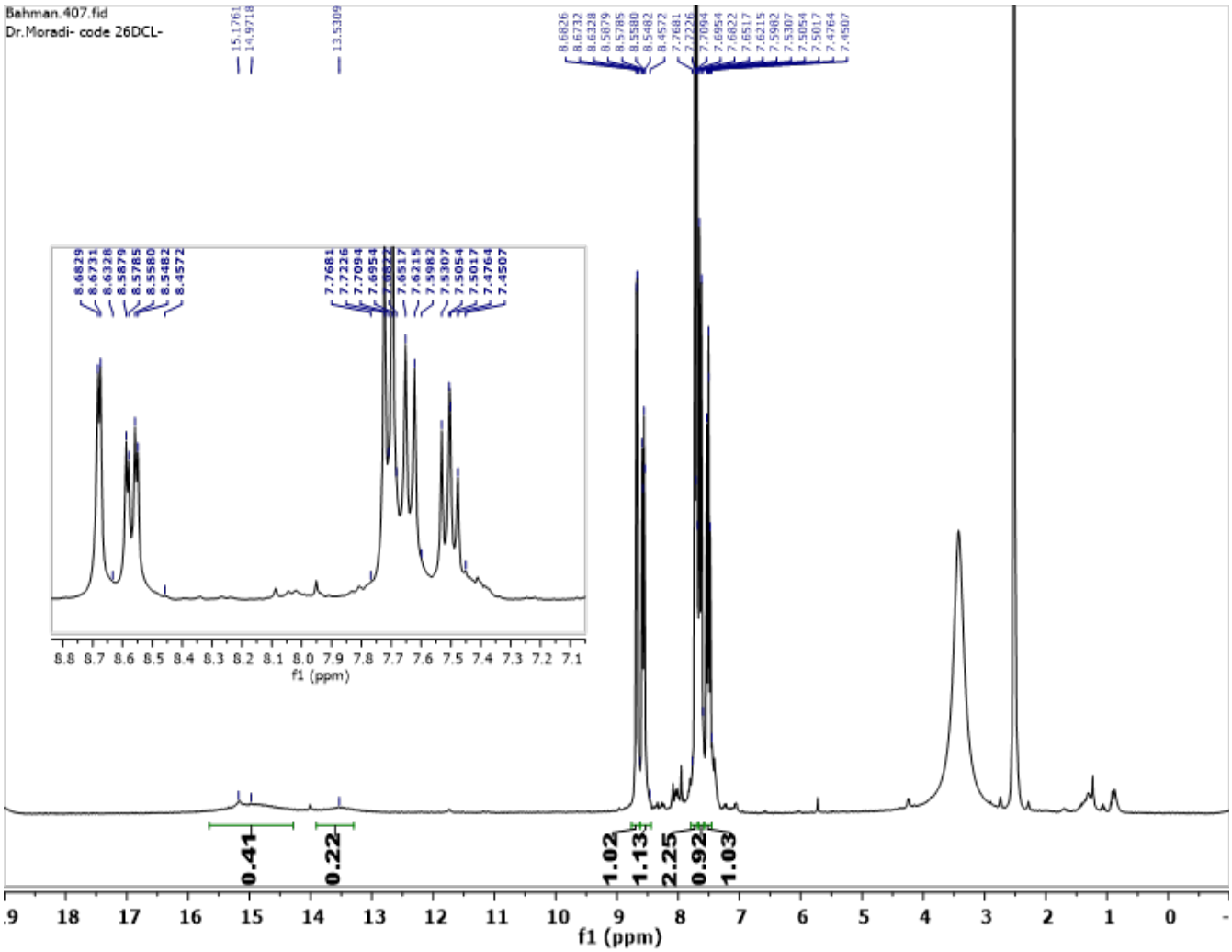
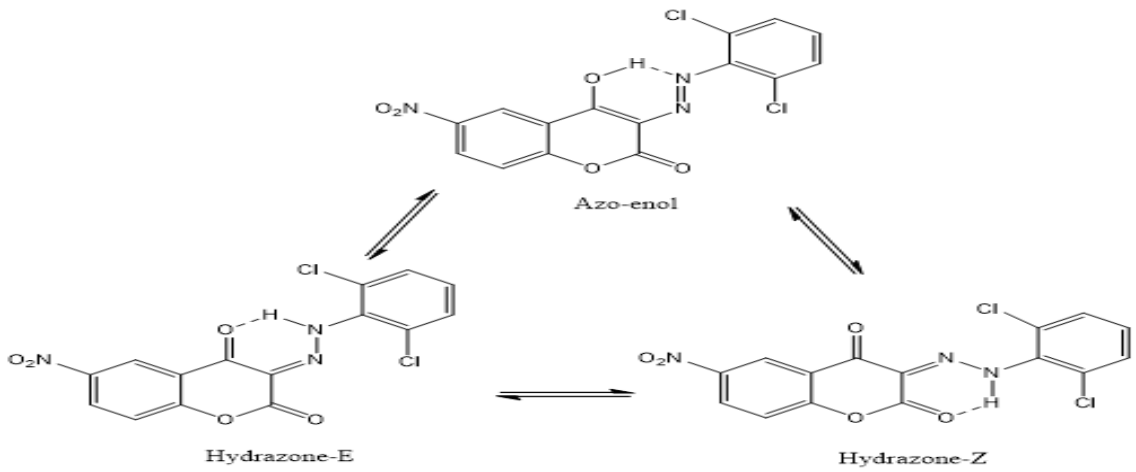


Figure 1. [1]H NMR spectrum of 3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin in DMSO-d6 at ambient temperature.



Scheme 3. Three proposed isomeric forms, including intramolecular hydrogen bonding, suggested for compound 3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin.

Based on the activity index, the well diffusion assay indicates that this compound, which contains chlorine substituents at 2 and 5 positions at the phenyl ring, exhibits moderate antimicrobial activity against the tested microorganisms. This was demonstrated by forming inhibition zones (Table 3 and Figure 2). Specifically, this compound showed inhibition zones ranging from 7 to 8 mm against the Gram-negative bacterium Escherichia coli and 8 to 15 mm against

Table 2. Inhibition zone (mm) of the synthesized compound

	Gram-negative bacteria *Escherichia coli*		Gram-positive bacteria *Staphylococcus aureus*	
	0.1 mg/ml	0.2 mg/ml	0.1 mg/ml	0. 2/ml mg
Compound	7	12	8	15
Standard	34	40	30	37

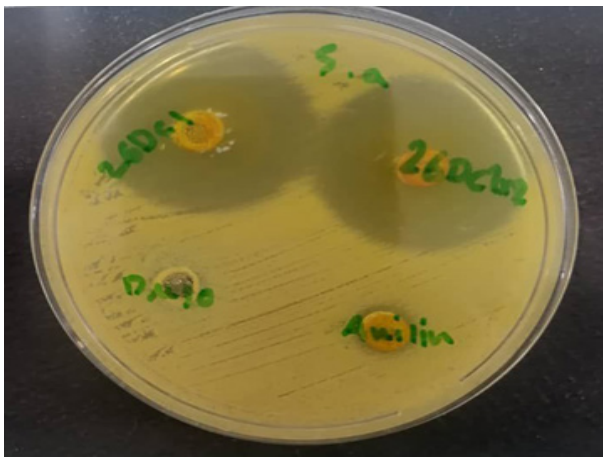


Figure 2. Zone of inhibition for the compound (80 µl, 0.002 g/ml) against *Staphylococcus aureus* by well diffusion meth-

4. Conclusion

Conclusively, in the presented research work, 6-nitro-4-hydroxycoumarin was synthesized and used as an enol type coupling component for the synthesis and characterization of 3-(2, 6-dichlorophenylazo)-4-hydroxy-6-nitrocoumarin. The compound with chlorine and nitro groups exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Further research on similar chlorine-containing compounds could provide insights into their mechanisms and potential applications in fighting bacterial infections.

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