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Synthesis, Characterization, and Biological Studies of Novel 4-Hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one Based Azo Dyes

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Abstract—Objective: Due to wide range of biological activities exhibited by pyran derivatives such as antibacterial, antioxidant, anticancer and other activities, we have synthesized and investigated the biological activity of some pyran derivatives. **Methods:** A series of 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one based azo dyes (**IIIa–IIIf**) have been synthesized by a conventional diazo coupling reaction. The antioxidant activity was determined via DPPH assay and Antimicrobial activity through disc diffusion test. **Results and Discussion:** Compound (**IIIb**) has shown excellent radical scavenging activity with an IC_{50} value of 20.22 ± 7.36 μg/mL compared to the standard. Furthermore, compounds (**IIIc**) and (**IIId**) showed a good antibacterial and antifungal activity against *Escherichia coli* and *Aspergillus niger* compared to other prepared azo compounds. **Conclusions:** We propose that in future, synthesized compounds may act as a promising antimicrobial drugs.

Keywords: antimicrobial activity, azo dyes, antioxidant activity, 4-hydroxy pyranone

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INTRODUCTION

Azo dyes are the most widely used and oldest class of industrially synthesised organic dyes because of their numerous uses in a variety of industries, including the dyeing of textiles, leather, paper, food, and cosmetic products [1], as well as biomedical research and applications [2, 3]. In high-tech fields like laser [4], photodynamic therapy [5], and dye-sensitized solar cells [6], they are also employed. Azo compounds link two aromatic systems with at least one conjugated azo chromophore (–N=N–) [7–9]. Azole dye synthesis typically takes two steps. Azo dyes are usually synthesised in two steps. The first step is called diazotization, which is the conversion of an aromatic amine to a diazo compound. The second step is called diazo coupling, which is the reaction of the titled diazo compound with a nucleophilic component to produce the corresponding azo dye [10–12].

In both naturally occurring and biologically active compounds, the chromoene moiety is frequently seen as a crucial structural component [13–15]. It has been observed in certain natural alkaloids [17] and is commonly carried out in flavonoids [16, 17]. A unique class of medicinal scaffolds, 2-amino-4*H*-chromenes is particularly useful among the various types of chromene systems; it is used to treat a variety of conditions, including viral hepatitis [18], Alzheimer's disease [19], cardiovascular disorders, epilepsy, inflammatory bowel syndrome [20], hypertension, and atherosclerosis [21]. A growing body of research is being done on 2-amino-4*H*-chromene derivatives with a nitrile group because they are being used to treat inflammatory human diseases like cancer [22], arthritis [23], leukaemia [24], and cancer therapy [25].

These encouraging reports led to the selection of 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one as

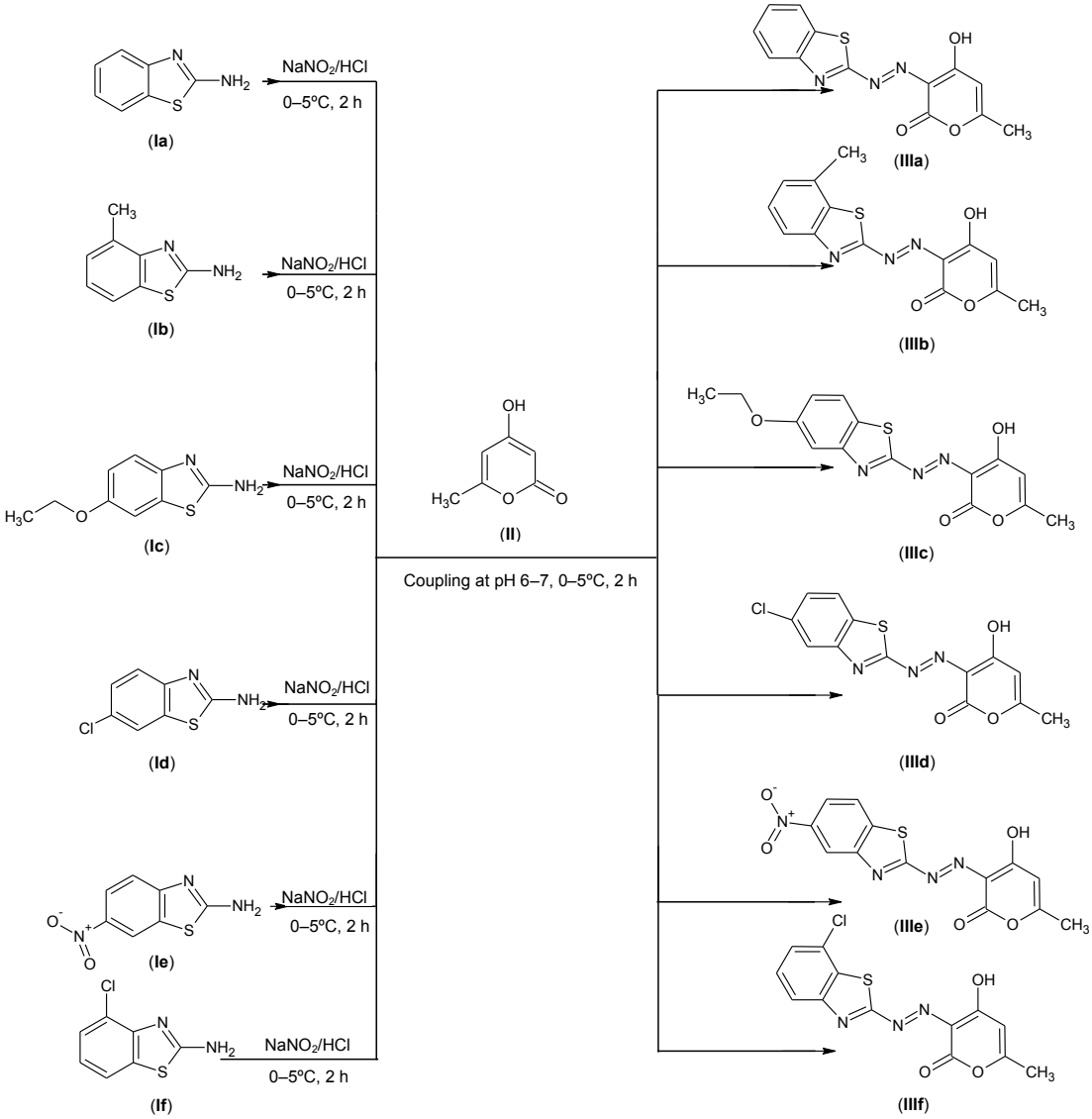
the coupling component and some diazotized aromatic amines as the diazo component for the reaction. All the synthesised compounds' structures were verified by ^{1}H NMR, ^{13}C NMR, IR, and LC-MS.

RESULTS AND DISCUSSION

Chemistry

The diazotization route was used to create the six novel pyran-based azo dyes (**IIIa–IIIf**), which were then characterised using a variety of analytical methods. Scheme 1 depicts the synthetic process, and the supporting information shows the spectra.

Table 1 lists the antioxidant activity of synthetic azo dyes (**IIIa–IIIf**) against DPPH radicals. Based on the findings, compound (**IIIb**) exhibited remarkable radical scavenging ability, with an IC_{50} value of 20.22 ± 7.36 μg/mL in comparison to the reference. Comparing the compounds (**IIIb**), (**IIIe**), and (**IIIf**) to standard, their IC_{50} values were 108.62 ± 1.0, 130.36 ± 31.69, and 103.36 ± 22.10 μg/mL, respectively, indicating good scavenging activity. Compounds (**IIIa**) and (**IIId**) that



Scheme 1. Synthetic route for pyran-thiazole based azo dyes (antioxidant activity: DPPH radical scavenging activity of the synthesized azo dyes (**IIIa–IIIf**)).

Table 1. The results of the DPPH radical scavenging activity of compounds (**IIIa–IIIf**)

Compound	IC_{50} (μg/mL)
(**IIIa**)	470.50 ± 41.63
(**IIIb**)	108.62 ± 1.0
(**IIIc**)	20.22 ± 7.36
(**IIId**)	314.36 ± 28.96
(**IIIe**)	130.36 ± 31.69
(**IIIf**)	103.36 ± 22.10
Standard (ascorbic acid)	20.37 ± 1.4

Table 2. Antimicrobial activity data of synthesized colourants (**IIIa–IIIf**)

No.	Compound	Zone of inhibition in mm			
		antibacterial		antifungal	
		E. coli	*S. aureus*	*A. niger*	*P. chrysogenum*
1	(**IIIa**)	8.0	6.0	6.0	5.0
2	(**IIIb**)	9.0	7.0	6.0	4.0
3	(**IIIc**)	16	14	17	16
4	(**IIId**)	15	14	16	15
5	(**IIIe**)	5.0	4.0	6.0	5.0
7	(**IIIf**)	4.0	3.0	7.0	6.0
8	Amoxicillin	26.0	26.0	–	–
9	Fluconazole	–	–	32	30

remained showed reduced activity, with IC_{50} values of 470.50 ± 41.63 and 314.36 ± 28.96μg/mL, in that order.

Antimicrobial Activity

A disc diffusion method was used to determine the antibacterial activity of all compounds (**IIIa–IIIf**) against bacterial strains. The results are displayed in Table 2. Comparing compound (**IIIc**) and (**IIId**) to amoxicillin, the antibacterial activity results revealed that they had better activity against *Escherichia coli*. In comparison to amoxicillin, compounds (**IIIb**) and (**IIIf**) showed moderate activity against *Escherichia coli*. Less activity was shown by the remaining compounds (**IIIa**) and (**IIIe**).

EXPERIMENTAL

Materials and methods. The reagents and chemicals were bought from Sigma Aldrich. Using KBr pellets, the FTIR-Alpha T Brucker instrument was used to obtain the infrared spectra. Using TMS as the internal reference, ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on a Brucker Avance spectrometer at 400 and 100 MHz, respectively. Water's SYNAPT G2

QTOF instrument was used to obtain the mass spectra (HRMS).

General procedure for the synthesis of 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one based azo dyes (IIIa–IIIf). The cooled solution of heterocyclic amines (**Ia–If**) in 8 mL of HCl was mixed dropwise with the ice-cooled solution of $NaNO_2$ in 5 mL of water. During 2 h, the solution was agitated at 0 to 5°C. The ice-cold 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one KOH solution (10%) was mixed with the diazonium mixture dropwise. At the same temperature, the mixture was stirred for an additional two hours. A 10% bicarbonate solution was used to bring the pH down to 6–7. Following the formation of colour, the product was filtered, cleaned with distilled water, and then dried and recrystallized using ethanol diluted with HCl. The corresponding azo dyes (**IIIa–IIIf**) yielded the product.

3-[(*E*)-1,3-Benzothiazol-2-yldiazenyl]-4-hydroxy-6-methyl-2*H*-pyran-2-one (IIIa). FT-IR (KBr) ν_{max}, cm^{-1}: 1715 (C=O), 1590 (C=N), 1448 (N=N), 730 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6), ppm 3.01 (m, 3H, $–CH_3$), 7.50 (d, J = 8 Hz, 1H, ArH), 7.76 (t, J = 4 Hz, 2H, ArH), 7.92 (t, J = 8 Hz, 2H, ArH), 9.84 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.98, 27.25, 110.30, 109.25, 112.03, 116.07, 123.30, 130.38, 137.20, 139.17, 154.10, 163.50; HRMS: *m/z* 287.2106 $[M]^+$; Calculated, %: C, 54.35; H, 3.16; N, 14.63; Found, %: C, 54.25; H, 3.01; N, 14.52.

4-Hydroxy-6-methyl-3-[(*E*)-(7-methyl-1,3-benzothiazol-2-yl)diazenyl]-2*H*-pyran-2-one (IIIb). FT-IR (KBr) ν_{max}, cm^{-1}: 1710 (C=O), 1585 (C=N), 1440 (N=N), 725 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 3.09 (m, 6H, $–CH_3$), 7.52 (d, J = 8 Hz, 1H, ArH), 7.78 (t, J = 4 Hz, 2H, ArH), 7.96 (t, J = 8 Hz, 1H, ArH), 9.88 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.90, 27.15, 110.15, 109.20, 112.25, 116.15, 123.26, 130.23, 137.15, 139.15, 154.16, 163.60; HRMS: *m/z* 301.3301 $[M]^+$; Calculated, %: C, 55.80; H, 3.68; N, 13.95; Found, %: C, 55.71; H, 3.56; N, 13.84.

3-[(*E*)-(5-Ethoxy-1,3-benzothiazol-2-yl)diazenyl]-4-hydroxy-6-methyl-2*H*-pyran-2-one (IIIc). FT-IR (KBr) ν_{max}, cm^{-1}: 1720 (C=O), 1580 (C=N), 1442 (N=N), 721 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 2.10 (m, 2H, $–CH_2$), 3.09 (m, 3H, $–CH_3$), 3.30 (m, 3H, $–CH_3$), 7.56 (d, J = 8 Hz, 1H, ArH), 7.70 (t, J = 6 Hz, 2H, ArH), 7.92 (t, J = 8 Hz, 1H, ArH), 9.82 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.78, 27.24, 110.20, 109.27, 112.15, 116.24, 123.56, 131.23, 134.15, 137.15, 154.20, 163.24; HRMS: *m/z* 331.3478 $[M]^+$; Calculated, %: C, 54.37; H, 3.95; N, 12.68; Found, %: C, 54.25; H, 3.84; N, 12.60.

3-[(*E*)-(5-Chloro-1,3-benzothiazol-2-yl)diazenyl]-4-hydroxy-6-methyl-2*H*-pyran-2-one (IIId). FT-IR (KBr) ν_{max}, cm^{-1}: 1725 (C=O), 1580 (C=N), 1438 (N=N), 740 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 3.03 (m, 3H, $–CH_3$), 7.48 (d, J = 8 Hz, 1H, ArH), 7.79 (t, J = 4 Hz, 2H, ArH), 7.90 (t, J = 8 Hz, 1H, ArH), 9.80 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.90, 27.15, 110.20, 109.15, 112.23, 116.47, 123.33, 130.45, 137.78, 139.85, 154.21, 163.41; HRMS: *m/z* 321.6548 $[M]^+$; Calculated, %: C, 48.53; H, 2.51; N, 11.02; Found, %: C, 48.49; H, 2.45; N, 10.96.

4-Hydroxy-6-methyl-3-[(*E*)-(5-nitro-1,3-benzothiazol-2-yl)diazenyl]-2*H*-pyran-2-one (IIIe). FT-IR (KBr) ν_{max}, cm^{-1}: 1712 (C=O), 1582 (C=N), 1444 (N=N), 724 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 3.06 (m, 6H, $–CH_3$), 7.47 (d, J = 9 Hz, 1H, ArH), 7.73 (t, J = 6 Hz, 2H, ArH), 7.91 (t, J = 8 Hz, 1H, ArH), 9.89 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.50, 27.21, 110.54, 109.78, 112.23, 116.88, 123.74, 130.86, 137.21, 139.74, 154.23, 163.74; HRMS: *m/z* 332.4125 $[M]^+$; Calculated, %: C, 46.99; H, 2.43; N, 16.86; Found, %: C, 46.84; H, 2.35; N, 16.74.

3-[(*E*)-(7-Chloro-1,3-benzothiazol-2-yl)diazenyl]-4-hydroxy-6-methyl-2*H*-pyran-2-one (IIIf). FT-IR (KBr) ν_{max}, cm^{-1}: 1725 (C=O), 1580 (C=N), 1438 (N=N), 740 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6), ppm: 3.03 (m, 3H, $–CH_3$), 7.48 (d, J = 8 Hz, 1H, ArH), 7.79 (t, J = 5 Hz, 2H, ArH), 7.90 (t, J = 8 Hz, 1H, ArH), 9.80 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6), ppm: 24.90, 27.15, 110.20, 109.15, 112.23, 116.47, 123.33, 130.45, 137.78, 139.85, 154.21, 163.41; HRMS: *m/z* 321.6548 $[M]^+$; Calculated, %: C, 48.53; H, 2.51; N, 11.02; Found, %: C, 48.49; H, 2.45; N, 10.96.

Antioxidant activity. DPPH radical scavenging activity of the synthesized azo dyes (IIIa–IIIf). The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity of the synthesized azo dyes (**IIIa–IIIf**) was investigated. The methodology was followed, albeit slightly modified, from the published report [26]. The compounds were added to test tubes in different concentrations (15.62–1000 μg/mL), and methanol was used to bring the final volume of each test tube to 4 mL. After adding DPPH in methanol (0.004%, 3 mL) to the mixtures, they were incubated for approximately 24 h at 37°C and then for 30 min in the dark. To measure the absorbance at 517 nm, a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) was utilized. A blank consisting of 95% methanol was taken, and ascorbic acid in the same volume was used as a control. The test was run three times, and the results were reported as an IC_{50}.

Antimicrobial activity. Using the disc diffusion method, the antimicrobial activity of synthetic compounds (**IIIa–IIIf**) was investigated [27]. 1 mL of lag phase bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*, was added to the tubes as an inoculant. *Penicillin chrysogenum* and *Aspergillus niger* were two examples of the fungal strains used. Potato dextrose agar medium was used for fungi, and sterile nutrient agar medium was used for the growth of bacteria. Various quantities (10–100 mg) of synthesized conjugates (**IIIa–IIIf**) were added to the correspondingly labelled discs and combined with DMSO. The bacterial plate was then incubated for 24 h at 37°C and for 48 h for fungi at 27°C. For every compound, the zone of inhibition was measured.

CONCLUSIONS

The goal of this work was to create new azo dyes based on 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one. The spectroscopic techniques of ^{1}H NMR, LC-MS, and FT-IR were used to confirm the structures of azo dyes. Additionally, the produced derivatives underwent screening for the ability of synthetic azo dyes to scavenge DPPH radicals was used to assess their antioxidant potential. Upon DPPH scavenging activity, all synthesized compounds demonstrated good efficacy, with IC_{50} values ranging from 20.22 ± 7.36 to 470.50 ± 41.63 μg/mL. Compounds (**IIIc**) and (**IIId**) demonstrated strong antibacterial and antifungal activity against *Escherichia coli* and *Aspergillus niger*, according to the results of the antimicrobial activity tests. The remaining compounds' antifungal and antibacterial activity was lower.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflicts of interest was declared by the authors.

AUTHOR CONTRIBUTION

The author KYC–methodology, investigation, writing–original draft; the author MB–writing and analysis; the author NBP–investigation, supervision, and editing.

All authors participated in the discussions.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1068162024040320

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