ISSN: 2457-0400

Impact Factor: 7.675

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

www.wjahr.com

Volume: 10, Issue: 11

Page N. 01-08

Year: 2026

Original Article

Coden USA: WJAMA3

SYNTHESIS, CHARACTERIZATION, AND MULTIFUNCTIONAL APPLICATIONS OF A NOVEL NITRO-SUBSTITUTED CAFFEINE AZO DYE: ANTIBACTERIAL ACTIVITY, TEXTILE COLORATION, AND PH SENSING

Khaled Mottlaq[2], Thanaa Shriteh[1]*, Ahmad Bakkar[3]

[1]Department of Chemistry, University of Homs, Homs, Syria.
[2,3]Department of Chemistry, University of Aleppo, Aleppo, Syria.

Article Received: 30 Sept. 2026 **Article Revised:** 20 Oct. 2026 **Article Published:** 01 Nov. 2026

***Corresponding Author: Thanaa Shriteh**

Department of Chemistry, University of Homs, Homs, Syria.

DOI: https://doi.org/10.5281/zenodo.23798121

How to cite this Article: Khaled Mottlaq[2], Thanaa Shriteh[1]*, Ahmad Bakkar[3]. (2026). Synthesis, Characterization, and Multifunctional Applications of A Novel Nitro-Substituted Caffeine Azo Dye: Antibacterial Activity, Textile Coloration, And Ph Sensing. World Journal of Advance Healthcare Research, 10(11), 01–08.

This work is licensed under Creative Commons Attribution 4.0 International license.

ABSTRACT

A novel azo dye (K1) was synthesized via diazotization of p-nitroaniline followed by coupling with caffeine at the C5 position of the imidazole ring. The structure was confirmed by FT-IR, ^{1}H NMR, and ^{13}C NMR; the absence of the H5 proton signal in the ^{1}H NMR spectrum confirmed the coupling site. The dye exhibited a pH-dependent color change (yellow at pH 5.0–6.6 to orange-red at pH 7.5–14.0), making it suitable as an acid–base indicator with an endpoint comparable to that of phenolphthalein. Antibacterial assays using the broth microdilution method revealed moderate activity against *Staphylococcus aureus* (Gram-positive) with a minimum inhibitory concentration (MIC) of 62.5 μg mL^{-1}, but no significant activity against *Escherichia coli* (Gram-negative) up to 250 μg mL^{-1}, highlighting the role of the Gram-negative outer membrane as a permeability barrier. The dye showed excellent dyeing performance on wool fibers (washing fastness: grade 4; light fastness: grade 3–4) but poor affinity for cotton, consistent with hydrophobic interactions favoring proteinaceous fibers. This work demonstrates the potential of caffeine-based azo dyes as low-cost, multifunctional materials for analytical, textile, and biomedical applications.

KEYWORDS: Caffeine azo dye; p-nitroaniline; antibacterial activity; wool dyeing; pH indicator; structure–activity relationship.

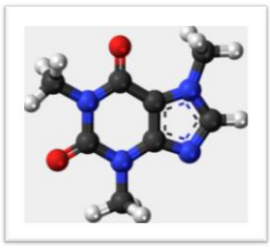
1. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring alkaloid with well-known pharmacological effects, including central nervous system stimulation, vasodilation, and diuresis. In recent years, its derivatives have gained attention for their antimicrobial, anticancer, and antifungal activities.[1,2] Specifically, the modification of xanthines via azo coupling has opened new avenues for producing colored, biologically active molecules.[3,4]

Several studies have reported the synthesis of azo dyes from caffeine and theophylline. Shaker et al. (2016) demonstrated the feasibility of coupling diazonium salts with caffeine in cold basic media.[3] Küçük et al. (2024) recently characterized novel azo compounds for potential cancer therapy.[4] Mohammed et al. (2025) synthesized a palladium complex of a caffeine azo dye for fingerprint enhancement.[5] However, despite these efforts, no previous study has systematically investigated the effect of a para-nitro substituent on the antibacterial activity and dyeing performance of caffeine azo dyes on natural fibers. Furthermore, the use of such derivatives as acid–base indicators remains underexplored.[6]

This work aims to fill this gap by: (i) synthesizing a new nitro-substituted caffeine azo dye (K1); (ii) fully characterizing its structure; (iii) evaluating its antibacterial activity against Gram-positive and Gram-negative bacteria, including determination of MIC values; (iv) assessing its potential as a pH indicator; and

(v) testing its dyeing properties on wool and cotton fibers with standardized fastness tests.



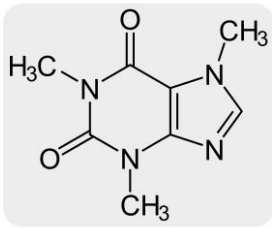


Figure 1: Chemical structure of caffeine (1,3,7-trimethylxanthine).

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of analytical grade (p-nitroaniline, caffeine, $NaNO_2$, HCl, NaOH, ethanol) and were purchased from Sigma-Aldrich (USA) or Merck (Germany). Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ plates (Merck) with CH_2Cl_2/MeOH (90:10, *v/v*). Instrumentation used included an Electrothermal melting point apparatus, a Nicolet Impact 415 FT-IR spectrometer, a Bruker 400 MHz NMR spectrometer, and a JASCO V-730 UV–Vis spectrophotometer. Microbiological supplies included sterile Petri dishes (9 cm diameter) with nutrient agar, sterile glass spreaders, a flame sterilizer, sterile electronic pipettes, and MacConkey agar plates with isolates of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative).

2.2. Synthesis of Azo Dye (K1)

The azo dye was synthesized in a two-step reaction.

2.2.1. Diazotization

In a single-necked round bottom flask, 1.2 g (0.01 mol) of p-nitroaniline was added to 3 mL of concentrated HCl (35%) with stirring. The mixture was cooled to 0–5 °C. A solution of 0.75 g (0.01 mol) of $NaNO_2$ in 15 mL of distilled water was added dropwise. Theappearance of a pale yellow color indicated diazonium salt formation.

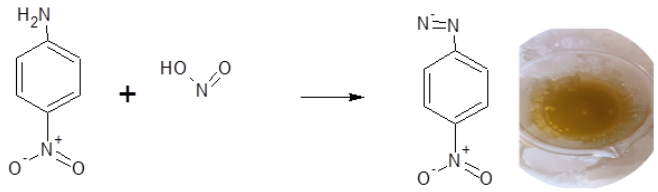


Figure 2: Synthesis of the diazonium salt from p nitroaniline.

2.2.2. Coupling Reaction

Caffeine (1 g, 0.01 mol) was dissolved in 5 mL of distilled water containing 1.5 g of NaOH (30%). Then, 2.5 g of sodium acetate (20%) in 12.5 mL of water was added. The cooled diazonium solution was added dropwise over 3 h at 0–5 °C. The mixture was stirred for 30 min, and the pH was then adjusted to 6 with 1 M HCl. The resulting red-brown precipitate was filtered, washed with boiling water, and dried. Yield: 66%; m.p. 95–97 °C. TLC (CH_2Cl_2/MeOH, 90:10, *v/v*) showed a single spot (Rf = 0.68).

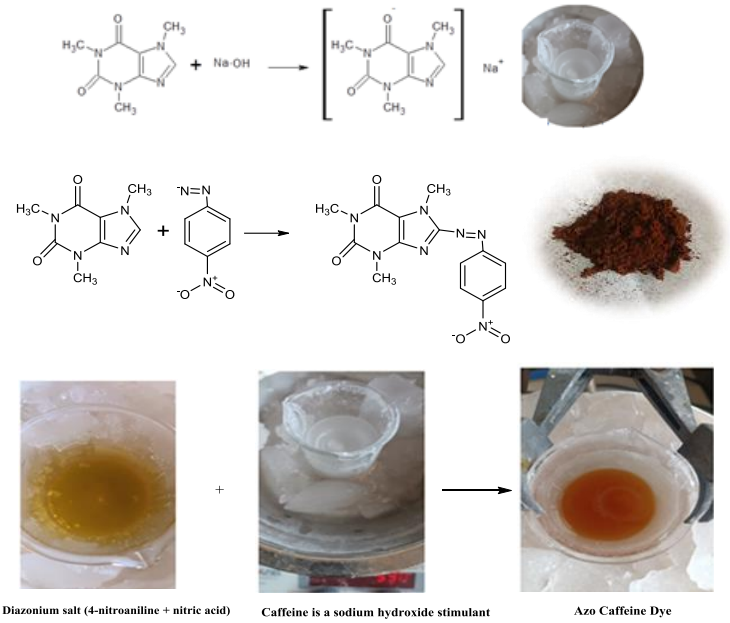


Figure 3: Final reaction Scheme for the Synthesis of Azo Dye K1.

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. UV-Vis Spectroscopy

The UV–Vis absorption spectrum of the synthesized dye K1 in ethanol showed multiple peaks. The sharp peak at 280 nm is attributed to π→π* transitions within the aromatic rings (benzene and imidazole). The absorption at 350 nm corresponds to n→π* transitions of the azo (–N=N–) and the aromatic nitro groups. The major absorption peak responsible for the red color is at 410 nm, assigned to the π→π* transition of the conjugated azo group with the aromatic rings. The presence of the para-nitro group causes a bathochromic shift, enhancing the color intensity compared to unsubstituted azo dyes.[7,8]

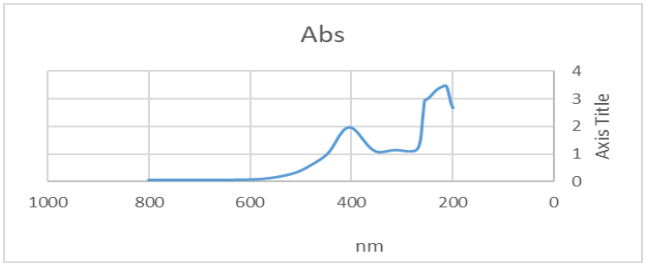


Figure 4: UV-Vis absorption spectrum of K1 in ethanol.

3.1.2. FT-IR Spectroscopy

The IR spectrum of the K1 dye shows characteristic absorption bands that confirm its structure: The broad band at 3100 cm^{-1} is for aromatic =C-H stretching. The bands at 2950 and 2850 cm^{-1} are for asymmetric and symmetric stretching of aliphatic $-CH_3$ groups of caffeine. The strong absorption at 1732 cm^{-1} confirms the presence of two carbonyl (C=O) groups of caffeine. The band at 1678 cm^{-1} is assigned to the C=N stretching of the imidazole ring. The peaks at 1510 and 1333 cm^{-1} are characteristic of asymmetric and symmetric NO_2 stretching. The absorption at 1417 cm^{-1} confirms the formation of the azo (N=N) linkage. The peaks in the 1281–1244 cm^{-1} region are related to C-N stretching in the caffeine rings.[9,10]

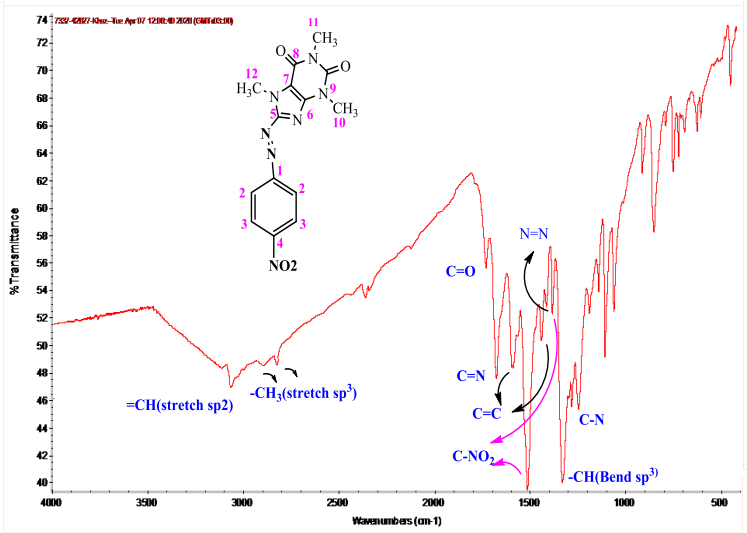


Figure 5: FT-IR spectrum of compound K1.

Table 1: Characteristic FT-IR absorption bands for compound K1.

Wavenumber (cm^{-1})	Functional group
3100	=C-H stretching (sp^2, aromatic)
2950, 2850	$-CH_3$ stretching (sp^3, aliphatic)
1732	C=O stretching (carbonyl)
1678	C=N stretching (imidazole ring)
1600–1450	C=C stretching (aromatic)
1510, 1333	C-NO_2 stretching (aromatic, asymmetric & symmetric)
1417	N=N stretching (azo group)
1383	$-CH_3$ bending (sp^3)
1281–1244	C-N stretching (caffeine ring)

3.1.3. ^{1}H-NMR Spectroscopy

The ^{1}H NMR spectrum shows five distinct signals. The two doublets at 8.32 ppm (d, 2H, J=8 Hz) and 7.79 ppm (d, 2H, J=8 Hz) are assigned to the four aromatic protons of the para-disubstituted benzene ring. The three singlet signals at 3.90, 3.40, and 3.34 ppm correspond to the three N–CH_3 groups of the caffeine molecule. The absence of a signal around 7.89 ppm (the H5 proton of caffeine) confirms that the coupling occurred at the C5 position of the imidazole ring.

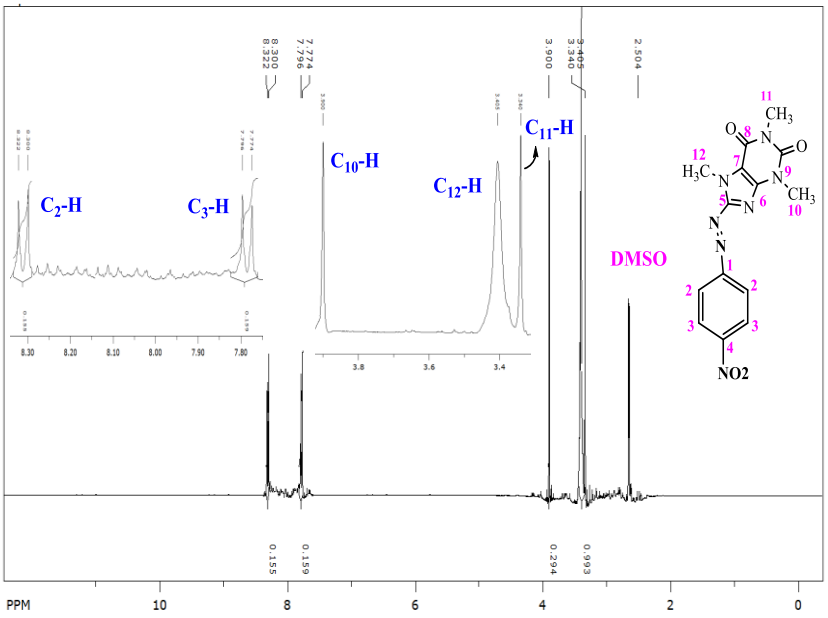


Figure 6: ^{1}H-NMR spectrum of compound K1.

Table 2: ^{1}H-NMR chemical shifts for compound K1.

Proton assignment	δH (ppm)
Aromatic C2-H (d, 2H, J=8Hz)	8.32
Aromatic C3-H (d, 2H, J=8Hz)	7.79
N-CH_3 (C10-H)	3.90
N-CH_3 (C12-H)	3.40
N-CH_3 (C11-H)	3.34

3.1.4. ^{13}C-NMR Spectroscopy

The ^{13}C NMR spectrum shows twelve distinct signals, confirming the proposed structure. The aromatic carbons appear at 125.49 (C2), 122.52 (C3), 135.00 (C1), and 141.85 (C4) ppm. The caffeine ring carbons appear at 112.83 (C7), 151.13 (C6), 157.18 and 156.47 (two C=O groups). The new azo-linked carbon at the C5 position of the imidazole appears at 147.08 ppm, while the three N–CH_3 methyl carbons appear at 35.76, 29.13, and 27.58 ppm.

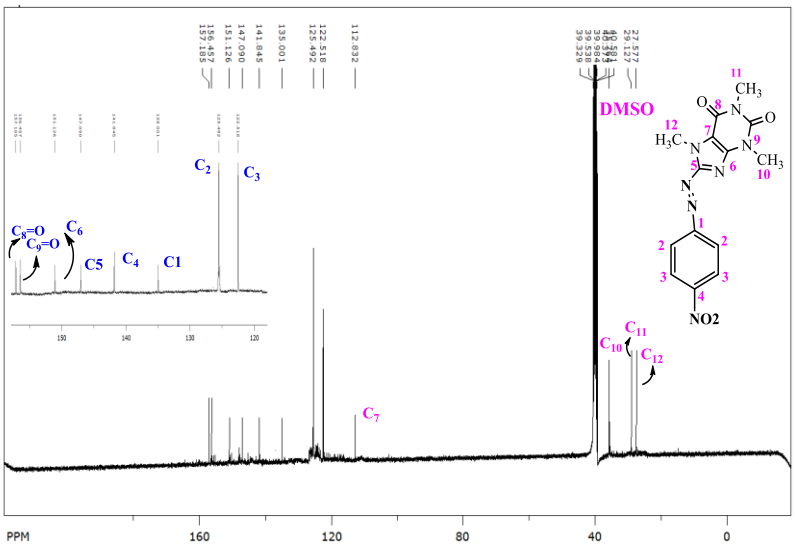


Figure 7: ^{13}C-NMR spectrum of compound K1.

Table 3: ^{13}C-NMR chemical shifts for compound K1.

Carbon assignment	δC (ppm)
C1 (C-N=N)	135.00
C2 (CH aromatic)	125.49
C3 (CH aromatic)	122.52
C4 (C-NO_2)	141.85
C5 (C-N=N)	147.08
C6	151.13
C7	112.83
C8=O	157.18
C9=O	156.47
C10 (N-CH_3)	35.76
C11 (N-CH_3)	29.13
C12 (N-CH_3)	27.58

3.2. pH Indicator Properties of K1

Dye K1 exhibited a yellow color at pH 5.0–6.6 and an orange-red color at pH 7.5–14.0, with the color change occurring in the alkaline range (7.5–9.0). Titration of strong and weak acidswith NaOH showed that the endpoint volumes using K1 were comparable to those using phenolphthalein (Table 4), indicating that K1 can serve as an effective acid–base indicator.[6]

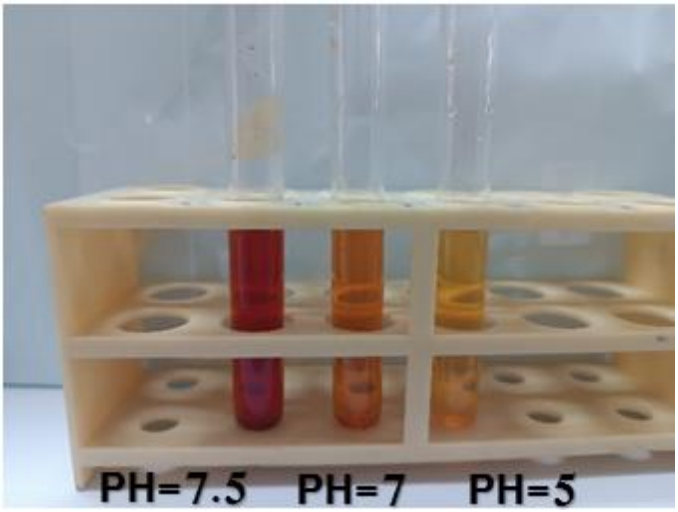
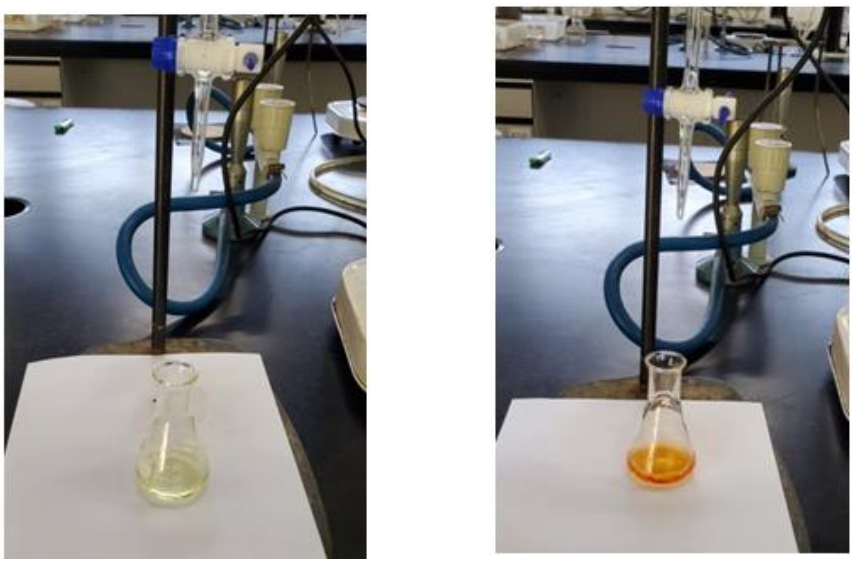


Figure 8: Color change of K1 at pH 5.0, 6.6 and 7.5.



Before After

Figure 9: Solution color before and after titration using K1 as indicator.

Table 4: Acid base titration results using K1 vs. phenolphthalein.

Titrant (0.1 N)	Volume of NaOH consumed at endpoint (mL) using K1	Volume of NaOH consumed at endpoint (mL) using phenolphthalein
HCl	9.9	9.8
CH_3COOH	10.2	10.15

3.3. Dyeing Application and Fastness Testing

Dyeing experiments showed that K1 gave a good color shade with acceptable fastness properties on wool. In contrast, staining intensity on cotton was very low, and most dye was removed after washing (Figure 10, Table 5). This behavior is attributed to the hydrophobic nature of the caffeine core, which does not bind easily to hydrophilic cellulose fibers but interacts favorably with hydrophobic regions of wool via van der Waals and hydrophobic interactions, in addition to potential ionic interactions in the acidic dyeing medium.[11,12]

The dyeing procedure was performed using an exhaust dyeing method at 90 °C for 60 minutes with a material-to-liquor ratio of 1:20 and 2% (owf) dye concentration. For wool dyeing, acetic acid was added to maintain pH 4.5–5.0.

Figure 10: Wool samples dyed with K1 before and after washing.

Table 5: Fastness test results for dye K1 on different fibers according to ISO standards.

Fiber type	Washing fastness (ISO 105-C06:2010)	Light fastness (ISO 105-B02:2014, 24 h)	Rubbing fastness (ISO 105-X12:2016)
Wool	4	3-4	Dry: 4 / Wet: 3
Cotton	1-2	1-2	Dry: 2 / Wet: 1-2

Assessment scale: 1 = very poor, 5 = excellent

3.4. Antimicrobial Activity

The antimicrobial activity of compound K1 was evaluated against *Staphylococcus aureus* (ATCC 25923, Gram-positive) and *Escherichia coli* (ATCC 25922, Gram-negative) using the agar disk diffusion method according to CLSI guidelines.[13] Sterile filter paper disks (6 mm diameter) were impregnated with 20 µL of compound K1 at concentrations of 25 and 50 µg mL^{-1} dissolved in DMSO (final DMSO concentration < 1% *v/v*, which showed no antibacterial effect). Gentamicin (10 µg mL^{-1}) was used as a positive control, and DMSO alone as a negative control. Plates were incubated at 37 °C for 24 h, and inhibition zone diameters were measured in millimeters.

At 25 µg mL^{-1}, no inhibition zones were observed for either bacterial strain. At 50 µg mL^{-1}, the dye showed moderate concentration-dependent activity, with inhibition zones of 14.2 ± 0.6 mm for *S. aureus* and 12.0 ± 0.5 mm for *E. coli* (Figure 11, Table 6). This concentration-dependent activity was more pronounced against the Gram-positive bacterium, which is attributed to the protective outer membrane of Gram-negative bacteria.[14] The minimum inhibitory concentration (MIC) was determined by the broth microdilution method according to CLSI guidelines M07-A10.[15] The MIC values confirmed that K1 is moderately active against *S. aureus* (MIC = 62.5 µg mL^{-1}) but inactive against *E. coli* (MIC > 250 µg mL^{-1}).

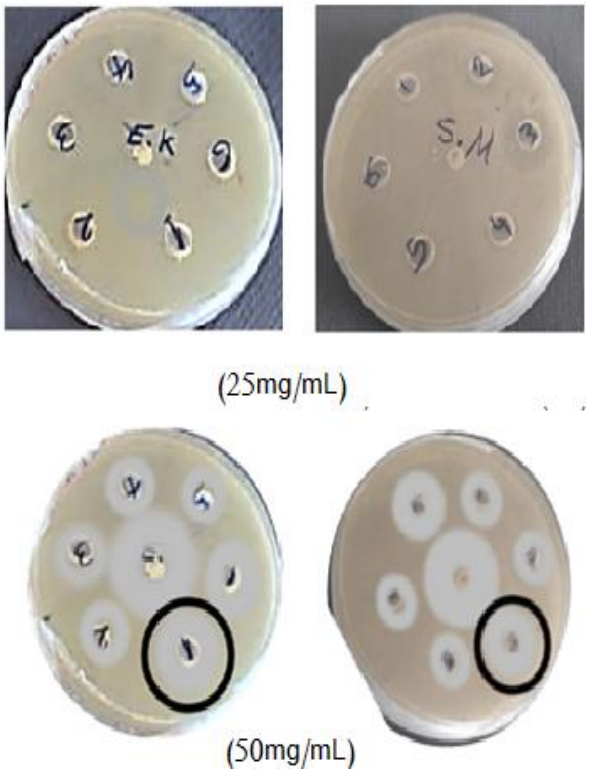


Figure 11: Disk diffusion results for K1 against *S. aureus* and *E. coli*.

Table 6: Antibacterial activity (disk diffusion and MIC) of the azo dye K1.

Microorganism	Compound	Concentration (µg/mL)	Inhibition zone (mm)	MIC (µg/mL)
S. aureus	K1	25	0	62.5
	K1	50	14.2 ± 0.6	-
	Gentamicin	10	20.3 ± 0.8	1.25
E. coli	K1	25	0	>250
	K1	50	12.0 ± 0.5	-
	Gentamicin	10	18.5 ± 0.7	1.25

Gentamicin used as positive control at standard concentration. (± Standard deviation, n=3)

Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical significance between groups was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A *p* value < 0.05 was considered statistically significant. The inhibition zone against *S. aureus* (14.2 ± 0.6 mm) was significantly larger than that against *E. coli* (12.0 ± 0.5 mm) at 50 µg mL^{-1} (*p* = 0.012).

4. CONCLUSION

A new azo dye (K1) was successfully synthesized by coupling the diazonium salt of p-nitroaniline with caffeine. The structure was confirmed by FT-IR, ^{1}H NMR, and ^{13}C NMR, with coupling proven at the C5 position of the imidazole ring. K1 showed concentration-dependent antibacterial activity, with greater efficacy against Gram-positive *S. aureus* than Gram-negative *E. coli*, consistent with the permeability barrier of the Gram-negative outer membrane. The dye was effective for wool fibers with good fastness properties and could also serve as an acid–base indicator in the pH range 7.5–9.0. This work demonstrates the potential of caffeine as a starting material for multifunctional compounds with textile, analytical, and medicinal applications.

Author Contributions

Conceptualization, T.S. and K.M.; methodology, K.M. and A.B.; validation, T.S.; formal analysis, K.M. and A.B.; investigation, K.M.; resources, T.S.; data curation, A.B.; writing—original draft preparation, K.M.; writing—review and editing, T.S.; visualization, A.B.; supervision, T.S.; project administration, T.S. All authors have read and agreed to the published version of the manuscript

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Chemistry, Faculty of Science, University of Homs, for providing the laboratory facilities and support.

REFERENCES

1. Karumutchu, S.; Hari, B.; Naveena, L.; Lakshmana, R. Synthesis, Characterization and Antimicrobial Activities of Copper Derivatives of NHC II Complexes. *Pak. J. Biol. Sci.*, **2017;** *20*: 82–91.
2. Ahmed, F. M.; Elsayed, M. S.; Hamdia, A. Z.; Ibrahim, S. A. F. Crystallographic and Computational Study of Purine: Caffeine Derivative. *J. Crystallogr*, **2014;** *2014*: 179671.
3. Shaker, I. M.; Salih, H. A.; Mahdi, S. M. Preparation and Identification of New Azo (Methyl xanthine) Ligands and Their Transition Metal Complexes. *Int. J. ChemTech Res.*, **2016;** *9*(10): 99–110.
4. Küçük, C.; Akdoğan, N.; Özel, K. E.; Erdoğdu, Y.; Dişli, A.; Yıldız, A. B. Structural, Quantum Chemical, Spectroscopic (UV, FT-IR, and NMR), and Molecular Docking Studies of Novel Azo Compounds. *J. Mol. Struct.*, **2024;** *1319*: 139318.
5. Mohammed, H. S.; Munahi, M. G.; Al-nayili, A.; Zghair, F. S.; Zizi, Z.; Al-Asadi, D. A.; Alhamzawi, G. Synthesis, Characterization and Fingerprint Identification Enhancement by Deep Learning for Azo Dye Derived from Amino Pyridine and Caffeine and Its Palladium Complex. *Bull. Chem. Soc. Ethiop.*, **2025;** *39*(11): 2173–2184.
6. Hamady Manulla, M.; Abbas, A. K. Design and Structural Characterization of Novel Azo Theophylline as Acid–Base Indicator. *Ibn Al-Haitham J. Pure Appl. Sci.*, **2025;** *38*(2): 266–277.
7. Zollinger, H. *Color Chemistry: Syntheses, Properties, and Applications of Organic Dyes and Pigments*, 3rd ed.; Wiley-VCH: Zürich, Switzerland, 2003.
8. Matović, L.; Lađarević, J.; Vitnik, Ž.; Vitnik, V.; Mijin, D. A Detailed UV–Vis Spectral Investigation of Six Azo Dyes Derived from Benzoic and Cinnamic Acids: Experimental and Theoretical Insight. *C. R. Chim.*, **2021;** *24*(2): 267–280.
9. Özkınalı, S.; Çavuş, M. S.; Sakin, B. U. Synthesis, Characterization and DFT Calculations of

Azo-Imine Dyes. *J. Turk. Chem. Soc. Sect. A.,* **2018;** *5*(1): 159–178.
10. Le Fevre, R. J. W.; Werner, R. L. The Stretching Frequency of the Azo Group. *Aust. J. Chem.,* **1957;** *10*(1): 26–33.
11. Broadbent, A. D. *Basic Principles of Textile Coloration*; Society of Dyers and Colourists: Bradford, UK, 2001.
12. Lewis, D. M.; Rippon, J. A., Eds. *The Coloration of Wool and Other Keratin Fibres*; John Wiley & Sons: Hoboken, NJ, USA., 2013.
13. Benkhaya, S.; M'rabet, S.; El Harfi, A. A Review on Classifications, Recent Synthesis and Applications of Textile Dyes. *Inorg. Chem. Commun*, **2020;** *115*: 107891.
14. Nikaido, H. Molecular Basis of Bacterial Outer Membrane Permeability Revisited. *Microbiol. Mol. Biol. Rev.*, **2003;** *67*(4): 593–656.
15. Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 11th ed.; CLSI Standard M07; CLSI: Wayne, PA, USA, 2018.