Synthesis, Characterization, Antimicrobial, DNA Cleavage and Fluorescent Activity of Metal ion(II) Coordinate with 2H-Chromene Azo novel ligand

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ABSTRACT
A new ligand 2H-chromene containing azo group 2-(4-nitrophenyl)-N-(4-(phenyldiazenyl)-2H-chromen-4-amine (AH), were synthesized from the condensation reaction (1:2) of 2'-hydroxychalcone and p-aminoazobenzene. Co(II), Cu(II) and Ni(II) complexes of the new ligand have been synthesized and characterized using C.H.N. analysis, 1H NMR spectra, FT.IR, UV/Visible, magnetic susceptible, conductance measuring, and fluorescence spectral spectroscopy; 13CNMR spectroscopy of the ligand was also studied. Spectroscopic results revealed the 2H-Chromene Azo (AH) ligand behaves as monodentate chelating via the nitrogen atom of amine group at position 4 having 1:1 [M:L] ratio; suggested that the cobalt and nickel complexes have the tetrahedral structure and a distorted tetrahedral geometry for the copper complex, indicating their non-electrolyte nature. The new ligand shows a fluorescence emission comparing with this fluorescence quenching was noticed in its metal complexes. The antibacterial potency of the free ligand and its chelates with metal ion(II) were screened against E.coli, K.pneumoniae, Staph.aureus and B.Subtilis; The DNA cleavage activity of the free ligand and its 2H-chromene azo metal (II) complexes was performed by the gel electrophoresis process, which given that these compounds are effective upon DNA cleavage.

INTRODUCTION
Heterocyclic compounds represent a large family of organic compounds (Katritzky et al., 2010; Brimble et al., 2008), they are known for widespread applications in various fields because of their biological importance, medicinal chemistry, and chemical biology. The 2H-chromene (2H-1-benzopyran) moiety is one of the most important heterocycles detected in many biologically active natural products and in synthetic compounds (Kolokythas et al., 2007; P et al., 2009; Schweizer and Meeder, 1977) 2H-chromenes were reported to display significant antioxidant (Kwak et al., 2006), antiviral (Martinez-Grau and Marco, 1997), antimalarial (Li et al., 2018) anti-inflammatory (Gebhardt et al., 2007) and antifungal properties (Chanu et al., 2017). Furthermore, several metal complexes of chromene derivatives possess antimicrobial (Soliman et al., 2016) DNA cleavage activities (Prabhakara et al., 2015) and photoluminescence properties (Philip et al., 2019). Azo compounds and their complexes are highly important due to their applications as textile dyes, Zhigang et al. (2009) indicators (Abbas et al., 2018), medicinal importance (Philip et al., 2019) and are also inhibition of DNA, RNA and protein cre-
in the present study, we display the synthesis and characterization of Co(II), Cu(II), and Ni(II) complexes with new azo-linked chromene ligand (AH). The newly ligand and its complexes were examined for their antibacterial potency against some types of gram-positive and negative bacteria; DNA cleavage activity, and also fluorescence properties.

**MATERIALS AND METHODS**

All chemicals were from BDH, Merck, and Fluka products. $^1$H and $^{13}$C-NMR spectra were performed using Burker 500.13 MHz. Elemental analyses were carried through a Perkin CHN Elmer analyzer. The metal content was determined by varian-AAS6200 flame atomic absorption spectrophotometer. Galenkamp M.P. apparatus were used. Molar conductance of the prepared complexes with $10^{-3}$M in EtOH were measurement using SIEMENS conductivity meter. Magnetic susceptibility measurements were obtained using the MSB-MK-1 model. The UV-Vis spectra were obtained with SHIMADZU-1800 spectrophotometer using a quartz cell (200-1100 nm). The IR spectra were measured using SHIMADZU FTIR-8400S spectrophotometer using (KBr & CsI discs). The fluorescence measurements of the prepared compounds were recorded on a SHIMADZU Fluorescence instrument.

**Synthesis of The Starting Material**

**Synthesis of chalcone compound (B) [1-(2-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one].**

A mixture of p-nitrobenzaldehyde (10 mmol), 2'-hydroxyacetophenone (10 mmol) were dissolved in (25 ml) methanol, Potassium hydroxide (30 mmol) was added with stirring at room temperature for 10 h to obtain a blood red mixture, then infused into 1N HCl (20 ml), and concentrated HCl was added until the solution was acidic. The precipitate was filtered and washed with the hot solvent; the prepared compounds were recorded on a SHIMADZU Fluorescence instrument.

**Synthesis of 2H-Chromene Azo Ligand (AH) 2-(4-nitrophenyl)-N-(4-(phenyldiazenyl)) -2H-Chromen-4-Amine.**

A mixture of the prepared chalcone compound (1.67 mmol, 0.44 g) and p-amino azobenzene (3.34 mmol, 1.49 g) was refluxed in absolute ethanol (50 ml) with concentrated hydrochloric acid for 30 h. The precipitate was filtered and washed with the hot solvent; Scheme 1. $\text{C}_{27}$H$_{25}$N$_4$O$_4$ (448 g mol$^{-1}$), Dark purple solid yield: 85 %, m.p.: 228-230 $^\circ$C, FTIR (KBr, cm$^{-1}$): 3485 (N-H), 3041 (aromatic C-H), 1658 (aromatic C=C), 1452 (N=N), $[^1]$H-NMR (500 MHz, DMSO-d$_6$, $\delta$ ppm) $\delta$ 9.23 (s, NH), 7.91 (d, $J=5$Hz, 1H), 7.85 (d, $J=10$Hz, 1H), 7.54 (dt, $J=15$, 7.5 Hz, 1H), 7.33 (d, $J=10$Hz,1H), 3.44($q$, $J=6.6$Hz, 1H), 2.07 & 1.05 (s, $t$, $J=7.5$Hz, CH, CH); $^{13}$C-NMR (125 MHz,C$_6$D$_6$, $\delta$ ppm) $\delta$ 159.8 (C-O), 151.9 (C-N), 148.9 (C-N), 148.2 (C-N), 131.4 (C), 130.0 (C), 129.9 (C), 129.7 (C), 125.1 (C), 122.7 (C), 122.5 (C), 96.5 (C), 76.9 (C), 58.6 (C).

**Synthesis of 2H-Chromene Azo Metal Complexes**

To a stirred solution of 2-(4-nitrophenyl)-N-(4-(phenyldiazenyl))-2H-chromen-4-amine (AH) (0.3 mmol, 0.134 g) in (20 ml) ethanol, (0.6 mmol) of metal chloride (M= CoCl$_2$, CuCl$_2$; NiCl$_2$ 6H$_2$O were added at room temp. The solution mixture was reflux for two hours, then allowed to cool and left overnight at room temperature. Then filtered and washed with EtOH, ethyl ether, and dried at the desiccator on CaCl$_2$ .

For Co(AH)(H$_2$O)$_2$Cl: pink powder; yield: 69 %, FTIR (CsI, cm$^{-1}$): 3419, 771 (H$_2$O coor.), 3047 (aromatic C-H), 545 (Co-O), 360 (Co-N), 316 (Co-Cl). For Cu(AH)(H$_2$O)$_2$Cl: olive green powder; yield: 74 %, FTIR (CsI, cm$^{-1}$): 3438, 840 (H$_2$O coor.), 3058 (aromatic C-H), 553 (Cu-O), 345 (Cu-N), 283 (Cu-Cl). For Ni(AH)(H$_2$O)$_2$Cl: Reddish pink powder; yield: 71 %, FTIR (CsI, cm$^{-1}$): 3315, 845 (H$_2$O coor.), 3047 (aromatic C-H), 441 (Ni-O), 356 (Ni-N), 310 (Ni-Cl).

**Biological Studies**

**Antibacterial Studies**

The biological activities (in vitro) of the prepared ligand (AH) and its chelates were performed by disc diffusion method (Bauer et al., 1966) against two gram-positive (Bacillus subtilis, Staphylococcus aureus) and two gram-negative (Escherichia coli, Klebsiella pneumonia) using Muller Hinton agar for growing cell and the activity was measured in terms of zone of inhibition. DMSO has been applied as a negative control with ciprofloxacin (1 mg.ml$^{-1}$) as the standard drug.

**DNA Cleavage Studies**

The newly prepared ligand (AH) and its metal (II) complexes were analyzed as DNA cleaving agents using the bacterial previous studied DNA as targets by the agarose gel electrophoresis method (Sam-
RESULTS AND DISCUSSION

The analytical results of all compounds are presented in Table 1. The elemental analysis, metal determination, and the chloride content (by Mohr’s method) of the prepared complexes revealed that all complexes with 1:1 stoichiometry and the calculated values are in a good acceptance with the found values.

Biological Studies

Antibacterial Studies

The antimicrobial activity of the (AH) ligand and its metal (II) complexes were investigated against two Gram-positive (Bacillus subtilis, Staphylococcus aureus) and two Gram-negative (Escherichia coli, Klebsiella pneumoniae). The inhibition zones were determined and compared with ciprofloxacin (standard drug), as shown in Figure 1. The results indicate that the ligand (AH) and Co(II) complex has no effectiveness versus Staphylococcus aureus, but, the Cu(II) complex has more inhibition than the Ni(II) complex against this bacteria. The promoter activity of these metal complexes over the ligand can be elucidated on the principle of chelation theory (Sharma and Chandra, 2011) (Chohan et al., 2006). In chelating, the positive charge of the metal ion is partial participate with the donator group present in the ligand and probable π-electron delocalization upon chelated mode (El-Wahab et al., 1989). Test samples (1 mg.ml\(^{-1}\)) were dissolved in DMSO.

Figure 1: Graphical depicting of antibacterial activity of ligand (AH) and its chelates metal (II) against different bacterial strains in concentration (1 mg.ml\(^{-1}\)).
Table 1: Physical properties and analytical data for AH(ligand) and its metal complexes.

| Compound          | Color               | Melt. point °C | M.Wt g.mol⁻¹ | Elemental Analysis | Metal Found % (Calc.) | Chloride Found % (Calc.) |
|-------------------|---------------------|----------------|--------------|-------------------|-----------------------|--------------------------|
| AH ligand         | Dark purple         | 228-230        | 448.15       | C% 72.31 H% 4.53 N% 12.56 | ___ ___ ___           | ___ ___ ___             |
| Co(II) complex    | Pink                | 190 dec.       | 577.4        | C% 56.14 H% 4.05 N% 9.71 | 10.45 6.10            | ___ ___             |
| Cu(II) complex    | Olive green         | 180            | 582.15       | C% 55.73 H% 4.07 N% 9.63 | 10.79 6.01            | ___ ___             |
| Ni(II) complex    | Reddish pink        | 180 dec.       | 577.25       | C% 56.15 H% 4.1 N% 9.72 | 10.32 6.17            | ___ ___             |

Table 2: Molar conductance and magnetic moment values of the prepared metal complexes

| Compound       | Molar Cond. S.cm².mol⁻¹ | μeff. (B.M) |
|----------------|-------------------------|-------------|
| Co(II) complex | 12.3                    | 4.65        |
| Cu(II) complex | 10.4                    | 1.89        |
| Ni(II) complex | 13.2                    | 3.31        |

Figure 2: C, control DNA of S. aureus; S. aureus DNA remedied by: Lane 1, AH(ligand); Lane 2, Co(II) complex; Lane 3, Ni(II) complex; Lane 4, Cu(II) complex.

Figures 3: Emission spectra of ligand (AH) and its chelates in Ethanol at λex = 380 nm.

DNA cleavage studies

The DNA cleavage activity of the new ligand AH and its complexes have been studied against DNA of *Staphylococcus aureus*, from the results of DNA cleavage experiment as shown in Figure 2, it was noticed that the DNA control (untreated DNA) does not exhibit any cleaving and there was a difference in the migration of ligand alone and its chelates. This damage of DNA in AH ligand, Co(II), Cu(II) and Ni(II) complex has no activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus subtilis* bacteria, whereas Co(II) complex has just action against *Bacillus subtilis*, the prepared compounds showed variable effectiveness against different organisms which refers either on the impermeability into microbes cells or on variance in ribosome of microbial cells (İspir, 2009).
Ni(II) complexes could be attributed to the cleavage of DNA. It was observed that the lane Cu(II) complex showed complete DNA cleavage of *Staphylococcus aureus*, but the free ligand, Co(II), and Ni(II) complexes showed partial cleavage of DNA. These data refer to the significant property of coordination to metal ion (Karekal *et al.*, 2013) (Mahalakshmi and Rajavel, 2014)

Many previous studies infer that the compound was to split the DNA; it can prohibit the evolution of the pathogenic organism via the genome cleaving (Yousef *et al.*, 2013)

**Fluorescence studies**

The fluorescence properties of newly synthesized 2H-chromene azo ligand and its complexes were measured in (10<sup>-5</sup> M) Ethanol and DMSO solutions. The fluorescent spectra of the free ligand and its complexes when excited at 387 nm in Ethanol, showed a maxima band at 425 nm, as in Figure 3. The low intensity of the band corresponding to complexes is due to a decrease in electron density on the ligand. The low band intensity that belongs to complexes caused the decreasing electron density of the ligand (Yamgar *et al.*, 2009)

The free ligand exhibits an emission maximum at 569 nm with excited at 530 nm in DMSO, and the Cu(II) complex noted an emission band at 565 nm as in Figure 4: but, at the same excited band (530 nm), the Co(II) and Ni(II) complexes possess no fluorescence. The chelation of the metal with ligand performing the posing of the nonfluorescent complex; consequently, the fluorescent intensity is decreased and promotes quenching (Philip *et al.*, 2019)

**NMR Studies**

The <sup>13</sup>C-NMR spectrum of the 2'-hydroxy chalcone (B) shows a significant signal at 203.6 ppm corresponding to carbonyl group that was absent in the spectrum of the 2H-chromene azo ligand.

The <sup>1</sup>H-NMR spectrum of the 2'-hydroxy chalcone (B), signal at 11.84 ppm was referred to the phenolic (–OH) proton, while in the spectrum of 2H-chromene azo ligand (AH), the signal at 9.23 ppm (s, 1H, NH) is due to NH proton (El-Deeb *et al.*, 2008), the disappearance of this signal in all spectra of metal complexes indicate its involvement in complexation with metal ion through nitrogen atom via deprotonation.

**Infrared spectral studies**

The IR spectrum of the prepared starting material 2'-hydroxy chalcone shows the characteristic band at 3548 cm<sup>-1</sup> indicated that the presence of hydroxyl group and 1639 cm<sup>-1</sup> remarked the α, β unsaturated carbonyl (>C=O) (Sumathi *et al.*, 2011)

In IR spectrum of 2H-chromene azo ligand (AH) exhibited significant features, the peak at 3485 cm<sup>-1</sup> was assigned to the secondary (NH) vibration of the ligand which was absent in all-metal complexes, due to deprotonation of (NH) group on binding with metal ions, while the band at 3045 cm<sup>-1</sup> was characteristic for the existing of aromatic (-C-H) stretching.

No shift group was observed in the stretching vibration of the azo group (N=N) of the ligand. This supports the non-involved of an azo group in the metal coordination (Tunçel and Serin, 2006)

The appearance of lower frequencies due to bands of metal bonds with nitrogen, oxygen, and chloride in all metal complexes and show other bands about the region (3438-3315 cm<sup>-1</sup>) and at the range (845-771 cm<sup>-1</sup>) ascribed to coordinated water (Aazam *et al.*, 2012)
Electronic Spectra, Magnetic Susceptibility, and Molar Conductivity Measurements

The electronic spectra of ligand and all prepared complexes were recorded in DMSO at r.t.; in the spectra of free ligand showed two absorption bands. The first at 255 nm attributed to $\pi-\pi^*$ transition of the benzene ring (Khannomhaddi and Darvishpour, 2009) (Khedr et al., 2005). The second band noticed at 326 nm can be attributed to $n-\pi^*$ transition. An azo of free ligand (403 nm) did not note a significant shift in all the complexes, it was considered as evidence that no participation of the azo group in complex formation. The Co(II) complex showed two bands at 677 and 615 nm, which can be assigned to the d-d transitions $[^4A_2(F)\rightarrow ^4T_1(F)]$ & $[^4A_2(F)\rightarrow ^4T_1(P)]$. These bands, with the magnetic moment of the complex ($\mu = 4.65$ B.M.), usually correspond to a tetrahedral environment around Co(II) ion (Lechat et al., 1993) (Knittl et al., 2018). The electronic spectrum of Cu(II) complex exhibited band at 989 nm and other at 891 nm that might be assigned to $^2B_2\rightarrow^2E$ and $^2B_2\rightarrow^2B_1(^2A_1)$ transitions, indicating the Cu(II) complex has distorted tetrahedral geometry (Lever, 1984) (Pahontu et al., 2015) with the magnetic moment ($\mu = 1.89$ B.M.). In the Ni(II) complex, the electronic spectrum showed bands at 646 nm and 785 nm; these bands may be attributed to $^2A_2(F)\rightarrow^2T_2(F)$ and $^2A_2(F)\rightarrow^2T_1(F)$ transitions, respectively. It suggests a tetrahedral geometry of the Ni(II) complex (Lever et al., 1984), and its the magnetic susceptibility equals 3.31 B.M., which sited at the typical field for a tetrahedron geometry.

Figure 5 showed the proposed structure of all metal complexes. Molar conductance of the complexes was recorded in ethanol ($10^{-3}$ M). The lower value of molar conductance for all prepared complexes indicates their non-electrolytic character (Geary, 1971), as shown in Table 2.

CONCLUSION

In this study, the 2H-Chromene azo ligand and its chelates with Cobalt(II), Copper(II) and Nickel(II) ions have been synthesized and characterized using various analytical and spectral data. The results demonstrate that the ligand is monodentate; all metal chelates exhibited non-electrolyte and tetrahedral geometry. Antibacterial activities of the free ligand and its metal complexes were investigated against gram (+) and Gram (-) bacteria strains and revealed that Cu(II) and Ni(II) complexes were effective comparables with free ligand against S. aureus bacteria. Cu(II) complex activity upon the DNA cleavage studies showed more effective against the DNA isolated from S. aureus bacteria than the free ligand and other its metal chelates.

REFERENCES

Aazam, E. S., Husseiny, A. F. E., Al-Amri, H. M. 2012. Synthesis and photoluminescent properties of a Schiff-base ligand and its mononuclear Zn(II), Cd(II), Cu(II), Ni(II), and Pd(II) metal complexes. Arabian Journal of Chemistry, 5(1):45–53. Ni(II), and Pd(II) metal complexes.

Abbas, N., S.A., T., G. S., A. S., G., H., P.A., C., R. S., M. A. 2018. Chromium (III) complexes of azo dye ligands: Synthesis, characterization, DNA binding, and application studies. Inorganic and Nano-Metal Chemistry. 48(1):57–66.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology, 45(4_ts):493–496.

Brimble, M. A., Gibson, J. S., Sperry, J. 2008. Pyrans and their Benzo Derivatives: Synthesis. In Comprehensive Heterocyclic Chemistry III, pages 419–699.

Chanu, I. H., Devi, L. R., Khumanthem, N., Singh, N. I., Kumar, D., Singh, O. M. 2017. Synthesis of functionalized benzo[1]H-chromenes and evaluation of their antimicrobial activities. Russian Journal of Bioorganic Chemistry, 43(2):177–185.

Chohan, Z. H., Arif, M., Akhtar, M. A., Supuran, C. T. 2006. Metal-Based Antibacterial and Antifungal Agents: Synthesis, Characterization, and In Vitro Biological Evaluation of Co(II), Cu(II), Ni(II), and Zn(II) Complexes with Amino acid derived Compounds. Bioinorganic Chemistry and Applications, pages 1–13. Cu(II), Ni(II), and Zn(II) Complexes with Amino acid-derived Compounds.

El-Wahab, Z. H. A., Mashaly, M. M., Salman, A. A., El-Shetary, B. A., Faheim, A. A. 2004. Co(II), Ce(III) and UO2(VI) bis-salicylatothiosemicarbazide complexes. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 60(12):2861–2873.

Geary, W. J. 1971. The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. Coordination Chemistry Reviews, 7(1):81–122.

Gebhardt, P., Dornberger, K., Gollmick, F. A., Gräfe, U., Härtl, A., Görts, H., Hertweck, C. 2007. Quercinol, an anti-inflammatory chromone from the wood-rotting fungus Daedalea quercina (Oak Mazegill). Bioorganic & Medicinal Chemistry Letters, 17(9):2558–2560.

I El-Deeb, I., Ryu, J., Lee, S. 2008. Synthesis of New N-Arylpyrimidin-2-amine Derivatives Using a Pal-
Ispir, E. 2009. The synthesis, characterization, electrochemical character, catalytic and antimicrobial activity of a novel, azo-containing Schiff bases, and their metal complexes. *Dyes and Pigments*, 82(1):13–19.

Karekal, M. R., Biradar, V., Mathada, M. 2013. Synthesis, Characterization, Antimicrobial, DNA Cleavage, and Antioxidant Studies of Some Metal Complexes Derived from Schiff Base Containing Indole and Quinoline Moieties. *Bioinorganic Chemistry and Applications*, pages 1–16.

Katritzky, A. R., Ramsden, C. A., Joule, J. A., Zhdankin, V. 2010. Handbook of Heterocyclic Chemistry. volume 3, pages 815–822, Oxford. Elsevier.

Khanmohammadi, H., Darvishpour, M. 2009. New azo ligands containing azomethine groups in the pyridazine-based chain: Synthesis and characterization. *Dyes and Pigments*, 81(3):167–173.

Khedr, A., Gaber, M., Issa, R., Erten, H. 2005. Synthesis and spectral studies of 5-[3-(1,2,4-triazolyl-azo)-2,4-dihydroxybenzaldehyde (TA) and its Schiff bases with 1,3-diamino propane (TAAP) and 1,6-diamino hexane (TAAH). Their analytical application for micro spectrophotometric determination of cobalt. *Dyes and Pigments*, 67(2):117–126.

Knittl, E. T., Abou-Hussein, A. A., Linert, W. 2018. Syntheses, characterization, and biological activity of novel mono- and binuclear transition metal complexes with a hydrazone Schiff base derived from a coumarin derivative and oxalyldihydrazine. *Monatshefte Für Chemie - Chemical Monthly*, 149(2):431–443.

Kolokythas, G., Kostakis, I. K., Pouli, N., Marakos, P., Kousidou, O. C., Tzanakakis, G. N., Karamanos, N. K. 2007. Design and synthesis of new pyranoxanthenones bearing a nitro group or an amino-substituted side chain on the pyran ring. Evaluation of their growth inhibitory activity in breast cancer cells. *European Journal of Medicinal Chemistry*, 42(3):307–319.

Kwak, J. H., Kang, H. E., Jung, J. K., Kim, H., Cho, J., Lee, H. 2006. Synthesis of 7-hydroxy-4-Oxo-4H-chromene- and 7-hydroxycroman-2-carboxylic acidN-alkyl amides and their antioxidant activities. *Archives of Pharmacal Research*, 29(9):728–734.

Lechat, S., Khan, M. A., Bouet, G., Vierling, F. 1993. Spectrophotometric study of cobalt(II) chloride complexes in ethanol and propane-2-ol. *Inorganica Chimica Acta*, 211(1):33–36.

Lever, B. P. 1984. Inorganic Electronic Spectroscopy. New York. 2nd Edition.

Li, M., Zhao, X., Yang, W., Zhong, F., Yuan, L., Ren, Q. 2018. Asymmetric synthesis and biological evaluation of 3-nitro-2H-chromenes as potential antibacterial agents. *Tetrahedron Letters*, (39):3511–3515.

Mahalakshmi, N., Rajavel, R. 2014. Synthesis, spectroscopic, DNA cleavage, and antibacterial activity of binuclear schiff base complexes. *Arabian Journal of Chemistry*, 7(4):509–517.

Martínez-Grau, A., Marco, J. 1997. Friedländer reaction on 2-amino-3-cyano-4H-pyrans: Synthesis of derivatives of 4H-pyran [2,3-b] quinoline, new tacrine analogues. *Bioorganic & Medicinal Chemistry Letters*, 7(24):3165–3170.

Moorthy, J. N., Venkatakrishnan, P., Sengupta, S., Baidya, M. 2006. Facile Synthesis, Fluorescence, and Photochromism of Novel Helical Pyrones and Chromenes. *Organic Letters*, 8(21):4891–4894.

P, E. G., IM, L., P, E. G., E, W.-V. 2009. The Chemistry of Heterocyclic Compounds: Chromenes, Chromanones, and Chromones. volume 31, pages 1–1196, New York.

Pahontu, E., Ilieş, D. C., Shova, S., Paraschivescu, C., Badea, M., Gulea, A., Roşu, T. 2015. Synthesis, Characterization, Crystal Structure and Antimicrobial Activity of Copper(II) Complexes with the Schiff Base Derived from 2-Hydroxy-4-Methoxybenzaldehyde. *Molecules*, 20(4):5771–5792.

Park, C., Lim, J. S., Lee, Y., Lee, B., Kim, S. W., Lee, J., Kim, S. 2007. Optimization and morphology for decolorization of reactive black 5 by Funalia trogii. *Enzyme and Microbial Technology*, 40(7):1758–1764.

Philip, S., Thomas, P. S., Mohanan, K. 2019. Synthesis, characterization, fluorescence imaging, and cytotoxicity studies of a uracil-based azo derivative and its metal complexes. *Journal of the Chinese Chemical Society*, 66(1):21–30.

Prabhakara, C. T., Patil, S. A., Kulkarni, A. D., Naik, V. H., Manjunatha, M., Kinnal, S. M., Badami, P. S. 2015. Synthesis, spectral, thermal, fluorescence, antimicrobial, anthelmintic, and DNA cleavage studies of mononuclear metal chelates of bisdentate 2H-chromene-2-one Schiff base. *Journal of Photochemistry and Photobiology B: Biology*, 148:322–332.

Sambrook, J., Fritsch, E. F., Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Second Edition.
istry of Heterocyclic Compounds, pages 1–10.

Sharma, A. K., Chandra, S. 2011. Complexation of nitrogen and sulphur donor Schiff’s base ligand to Cr(III) and Ni(II) metal ions: Synthesis, spectroscopic, and antipathogenic studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 78(1):337–342.

Soliman, M. H., Mohamed, G. G., Elgemeie, G. H. 2016. Novel synthesis of 2-imino-2H-chromene-3-carboximide metal complexes. *Journal of Thermal Analysis and Calorimetry*, 123(1):583–594.

Sumathi, S., Tharmaraj, P., Sheela, C. D., Ebenezer, R. 2011. Synthesis, spectral, bioactivity, and NLO properties of chalcone metal complexes. *Journal of Coordination Chemistry*, 64(10):1707–1717.

Tunçel, M., Serin, S. 2006. Synthesis and characterization of new azo-linked Schiff bases and their cobalt(II), copper(II) and nickel(II) complexes. *Transition Metal Chemistry*, 31(6):805–812.

Yamgar, B. A., Sawant, V. A., Sawant, S. K., Chavan, S. S. 2009. Copper(II) complexes of thiazolylazo dye with triphenylphosphine and N3 or NCS – as coligands: synthesis, spectral characterization, electrochemistry, and luminescence properties. *Journal of Coordination Chemistry*, 62(14):2367–2374.

Yousef, T. A., El-Reash, A., El-Gammal, G. M., Bedier, O. A., . . ., R. A. 2013. Synthesis, characterization, optical band gap, in vitro antimicrobial activity, and DNA cleavage studies of some metal complexes of pyridyl thiosemicarbazone. *Journal of Molecular Structure*, 1035:307–317.

Zhigang, Y., Chunxia, Z., Defeng, Z., Freeman, H., Peitong, C., Jie, H. 2009. Monoazo dyes based on 5,10-dihydrophenophosphazine, Part 2: Azo acid dyes. *Dyes and Pigments*, 81(2):137–143.