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Research Article

Synthesis, characterization and antibacterial activity of ruthenium complex bearing 3,3′-dicarboxy-2,2′-bipyridine ligand

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Abstract

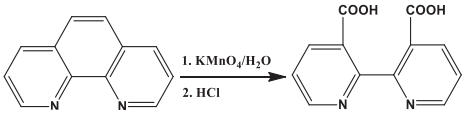
The development of bacteria has been one of the most significant advances in medical science. The need for the development of novel antibacterial is clear, but the development of new antibacterial classes is more important. In this study, the new ruthenium complex bearing the NN chelating ligand 3,3'-dicarboxy-2,2'-bipyridine (3,3'-dcbpy) and the dimethylsulphoxide (DMSO) ligand, [Ru(3,3'-dcbpy) $(DMSO)_2Cl_2$], was synthesized and characterized using FT-IR, ^{1}H- and ^{13}C-NMR spectroscopy, elemental analysis, and UV-Vis spectrometry. In addition, the Ru-complex was examined for its activity as an antibacterial against gram-positive and gramnegative strains. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of the compound were assessed using sterile 96-well plates, in accordance with the Clinical and Laboratory Standards Institute. The results show that the compound shows a MIC value of 35 μg mL^{-1} against *Staphylococcus aureus* and 65 μg mL^{-1} against *Escherichia coli* with the bacteria's mode of action. In conclusion, Ru(3,3'-dcbpy) $(DMSO)_2Cl_2$ complex can be candidate as antibacterial agent against both gram-positive and gram-negative bacteria.

Keywords

Ruthenium, Antibacterial, 3,3'-Dicarboxy-2,2'-bipyridine, *Staphylococcus aureus*, *Escherichia coli*

Introduction

The 3,3'-dicarboxy-2,2'-bipyridine ligand, (3,3'-dcbpy), is derived from the simple oxidation of 1,10-phenanthroline with potassium permanganate, $KMnO_4$, to obtain 3,3'-dcbpy in its pure form (Equation 1) (Kanungo et al. 2003).



(1)

3,3'-dcbpy is both a σ-donor and a π-acceptor ligand. The lone pair of electrons on each nitrogen atom can form a σ-bond with the central metal atom, while the aromatic system can take part in π-back-bonding (Rauchfuss et al. 1977). This mode of bonding helps stabilize the metal center, especially for transition metal ions in low oxidation states such as ruthenium in a +2 oxidation state (Qin and Peng 2012). In addition, 3,3'-dcbpy, with its two N-donor atoms, is able to form a chelate ring with metal centers. This ability to

coordinate has led to the formation of complexes with many metal ions that constitute the basis of many important industrial catalytic processes (Reynal and Palomares 2011). The transition metal complexes of ligands containing nitrogen donor atoms is of considerable interest due to their carcinostatic (Deghadi et al. 2022), antitumor (Salman 2021), antiviral (Laxman Sangle 2022), antifungal (Dhabale et al. 2022), and antibacterial activity (Mohammad et al. 2021), in addition to the industrial applications of complexes derived from them (Graur et al. 2022). Because the complexes contain nitrogen donor atoms, they are effective and stereospecific catalysts for oxidation, reduction, hydrolysis and biological activity (Bonaccorso et al. 2020; Egza et al. 2020; Borase et al. 2021; Dayan et al. 2021; Ghanghas et al. 2021). Although there are numerous reports on the chemistry of ruthenium(II) complexes with nitrogen donor sites, only a few reports on Ru(II) complexes containing 3,3'dcbpy ligand are available. (Machado et al. 2021; Ezugwu et al. 2022; Fudo et al. 2022; Zhou et al. 2022).

Therefore, the aim of this study, the ruthenium (II) mixed ligands complex bearing 3,3'-dcbpy and dimethylsulphoxide, DMSO, [Ru(3,3'-dcbpy)$(DMSO)_2Cl_2$], was synthesized. It was characterized by FTIR, ^{1}H- and ^{13}C-NMR, and UV-Vis spectroscopies and the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* of the complex was examined.

Materials and methods

Materials and physical measurements

3,3'-dicarboxy-2,2'-bipyridine (Kanungo et al. 2003) and [$RuCl_2(DMSO)_4$] (Evans et al. 1973) were prepared according to published methods. 1,4-Dioxane (Merck) was purified and dried following literature procedures (Armarego W L F 2017). Ethanol and methanol were redistilled in a nitrogen atmosphere. All manipulations were carried out in the N_2-atmosphere. The solvent distilled water was used to dissolve the tested samples.

The infrared spectra were recorded on Nicolet Impact-400 FT-IR spectrometer, as KBr discs. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE III-500 MHz spectrometer. Melting points were determined with Philip-Harris melting point apparatus. UV-Visible spectra were carried out for 1.0×10^{-5} M solutions in CH_2Cl_2 at 25 °C using Cary 100 Bio UV-Vis spectrophotometer.

Bacteria strains

Staphylococcus aureus (ATCC 29213) and *Escherichia coli* (ATCC 25922) were obtained from American Type Culture Collection and used in this study.

Preparation of dichloro-3,3′-dicarboxy-2,2′-bipyridyl-bis(dimethylsulphoxide)ruthenium(II) [$RuCl_2$(3,3′-dcbpy)$(DMSO)_2$]

To a suspension of [$RuCl_2(DMSO)_4$] (0.242 g, 0.500 mmol) in dry ethanol (20 mL), a suspension of 3,3'-dcbpy compound (0.121 g, 0.500 mmol) in dry ethanol (20 mL) was added. The reaction mixture was heated to reflux under a nitrogen flow, for 2 hrs. During which time, the solution changed color to brown-red. The reaction was allowed to cool to room temperature, and then it was filtered. The solvents were removed to achieve dryness. The residual solid was dissolved in a minimum amount of dry methanol and filtered. Diethyl ether (20 mL) was added to obtain a brown solid. The resulting product was filtered, washed with diethyl ether (2×10 mL) and dried in vacuo at 60 °C for 4 hrs. Yield 89.5%, m.p. 195–200 °C.

Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) for [$RuCl_2$(3,3′-dcbpy)$(DMSO)_2$]

The MIC and MBC of the compound were assessed using sterile 96-well plates, in accordance with the Clinical and Laboratory Standards Institute (Andrews J M 2001). The bacteria were grown on Muller Hinton Broth (MHB), and then diluted to 10^6 $CFUmL^{-1}$ in the same medium. Several dilutions of the compound with final concentrations ranging from 0.5 to 100 M were prepared. An aliquot of 50 µL of each solution was poured in the wells of the 96-well plates, to which 50 µL of diluted bacterial suspension were added. Each concentration test was repeated in three consecutive wells. The plates were then incubated for 18 h at 37 °C. The bacterial growth was quantified using an ELISA-OD plate reader at 570 nm. A column in the plate was used as a positive control, where the wells containing 50 µL MHB were inoculated with 50 µL bacterial suspension without antimicrobial agents. Another column was used for the negative control, where 100 µL of MHB was added alone. MBCs were determined by taking 10 µL from clear wells and cloudy positive control wells, which were seeded on sterile agar medium and incubated for 24 h at 37 °C. The concentration that causes 0.1% of the cells to be live was considered the MBC value.

Results

Dichloro-3,3′-dicarboxy-2,2′-bipyridyl-bis(dimethylsulphoxide) ruthenium(II) [Ru(3,3′dcbpy)$(DMSO)_2Cl_2$]

The new ruthenium complex was prepared by the direct reaction of $RuCl_2(DMSO)_4$ with 3,3'dcbpy in dry etha-

nol, where 3,3'-dcbpy is 3,3'-dicarboxy-2,2'-bipyridine. Mixing of $RuCl_2(DMSO)_4$ with one equivalent of 3,3'-dcbpy ligand gives $Ru(3,3'\text{-dcbpy})(DMSO)_2Cl_2$ (Equation 2). The formulation of the complex was confirmed by micro elemental analysis. This complex was characterized by FT-IR and UV-Vis.

$$[RuCl_2(DMSO)_4] + 3,3'\text{-dcbpy} \xrightarrow{\text{Dry EtOH/ Reflux}} [RuCl_2(3,3'\text{-dcbpy})(DMSO)_2] \quad (2)$$

The brown-colored complex, $Ru(3,3'\text{-dcbpy})(DMSO)_2Cl_2$, has good solubility in H_2O, CH_3OH, CH_3CH_2OH, acetone, tetrahydrofuran, and dimethylsulphoxide and insoluble in CH_2Cl_2, $CHCl_3$, pet. ether, and diethyl ether.

^{1}H- and ^{13}C-NMR Spectral and elemental analysis

Figs 1, 2 shown ^{1}H NMR and ^{13}C NMR, ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.75 (d, 1H), 9.57 (d, 1H), 8.47 (d, 1H), 8.32 (d, 1H), 7.65 (dd, 1H), 7.57 (dd, 1H) (bipyridyl protons), 3.55 (s, 3H), 3.28 (s, 3H) (S-bonded DMSO), 2.62 (s, 3H) (O-bonded DMSO). ^{13}C NMR (500 MHz, $CDCl_3$): δ 155.6, 137.7, 125.8, 125.3, 125.2, 124.9 (bipyridyl carbons), 47.5, 44.9, 43.8 (S-bonded DMSO), 39.5 (O-bonded DMSO). Anal. Calc. for $C_{16}H_{20}N_2S_2O_6Cl_2Ru$ (%): C, 33.57; H, 3.52; N, 4.89; S, 11.20. Found (%):C, 33.78; H, 3.99; N, 4.63; S, 10.61.

Table 1. Infrared spectral data for (N-P), $[RuCl_2(DMSO)_4]$ and $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$ complexes.

Mode	Compounds		
	(3,3'-dcbpy)	$[RuCl_2(DMSO)_4]$	$[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$
νO-H	3392	–	3422
νC-H (Aromatic)	3073	–	3076
νC-H (Aliphatic)	–	3002, 2920	2919, 2599
νC-C (Aromatic)	1578. 1433	–	1573, 1419
νC=O	1717	–	1719
νS=O (S-bonded)	–	1100, 1021	1088, 1016
νS=O (O-bonded)	–	927	–

FT-IR Spectral analysis

The characteristic bands in the spectra of the ligand, the Ru-DMSO precursor and the newly synthesized Ru-complex are shown in Table 1 (Fig. 3).

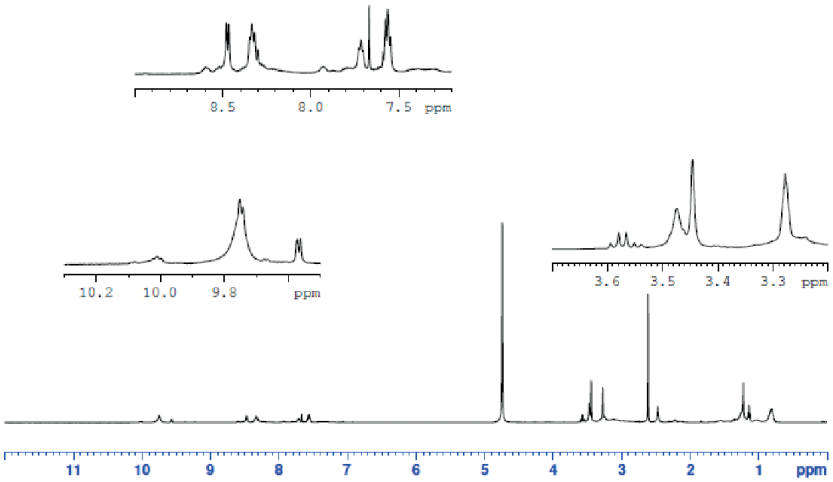


Figure 1. ^{1}H-NMR ($CDCl_3$) spectrum of $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$.

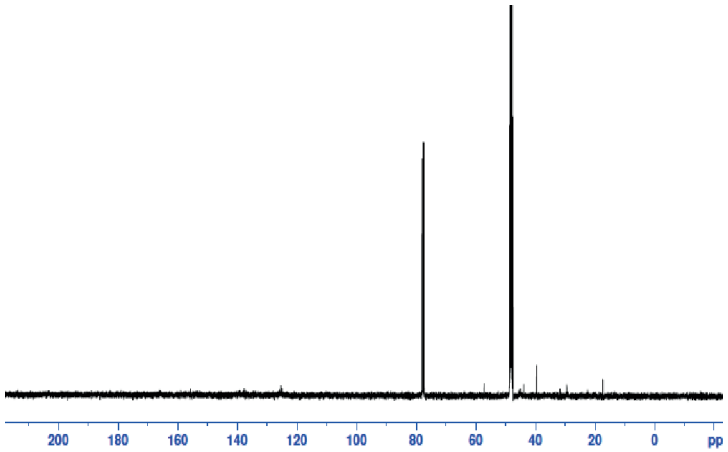


Figure 2. ^{13}C-NMR ($CDCl_3$) spectrum of $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$.

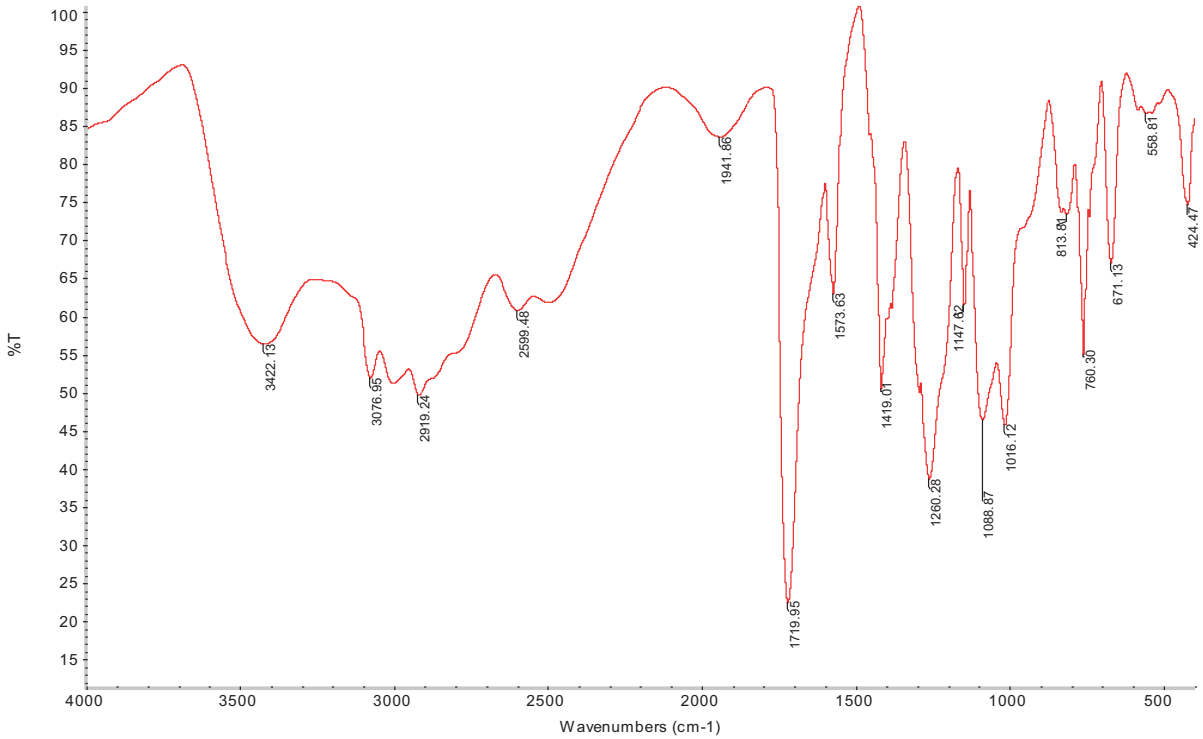


Figure 3. FTIR spectrum of [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$].

UV-Vis spectroscopy

The UV-visible spectrum of Ru-complex, [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$], was measured in MeOH solution. The complex exhibits three absorption bands at 381, 302 and 204 nm, shown in Fig. 4.

Antibacterial activity

In this study, Ru-complex was tested against both strains of gram positive bacteria (*Staphylococcus aureus* (ATCC 29213)) and gram negative strain (*Escherichia coli* (ATCC 25922)). The MIC and MBC value of this compound is shown in Table 2.

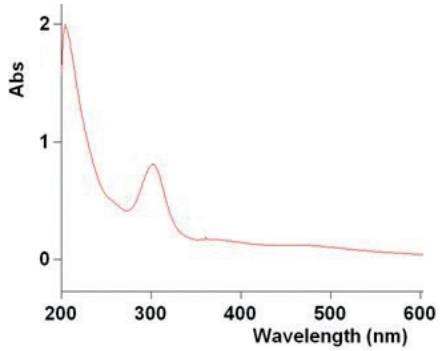


Figure 4. UV-Visible spectrum of [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$].

Table 2. The results of the MIC and MBC value of the compound.

Bacteria stains	MIC (μg mL^{-1})	MBC (μg mL^{-1})
Staphylococcus aureus	35	85
Escherichia coli	65	93

Discussion

The 3,3'-dcbpy ligand shows bands for ν_{C-H} (aromatic) at 3073 cm^{-1}, C-C ring stretching vibrations at 1578 and 1433 cm^{-1}, The stretching vibration of the carboxylate group appears as a strong band at 1719 cm^{-1} and a broad band at 3422 cm^{-1} was assigned for ν_{O-H} of the carboxylate group. The complex, [$RuCl_2(DMSO)_4$] shows two types of $\nu_{S=O}$ for S-bonded DMSO (1100, 1021 cm^{-1}) and O-bonded DMSO (927 cm^{-1}). The Ru-complex, [Ru(3,3'-dcbpy)$(DMSO)_2Cl_2$] shows in addition to the ν_{C-H} (aromatic and aliphatic) bands due to aromatic ring vibrations at 3079, 2919, 2599 cm^{-1}, ν_{C-C} (aromatic) at 1573 and 1419 cm^{-1}, ν_{O-H} at 3422 cm^{-1} and $\nu_{C=O}$ at 1719 cm^{-1} and $\nu_{S=O}$ for S-bonded DMSO at 1088, 1016 cm^{-1}, shown Fig. 2. The complex, [Ru(3,3'-dcbpy)$(DMSO)_2Cl_2$] does not show any peaks due to $\nu_{S=O}$ for O-bonded DMSO.

Ruthenium complex gave as absorption very broad band in the visible region at 381 nm ($\varepsilon = 1.70 \times 10^4$ cm^{-1}M^{-1}) was assigned to the metal-to-ligand charge-transfer (MLCT). The band at 302 nm ($\varepsilon = 8.07 \times 10^4$ cm^{-1}M^{-1}) in the ultraviolet region was assigned to metal-centered (MC) and the last band at 204 nm ($\varepsilon = 1.98 \times 10^5$ cm^{-1}M^{-1}) in the UV-region was assigned to the ligand-tometal charge-transfer (LMCT) transition (Kalyanasundaram and Gratzel 1998).

The results show that the compound shows good activity against the tested bacteria strains with a bacteriostatic mode of action. The activity of the ruthenium complex may be due to the easier dissociation constant of L2 after chelation. This will help decrease the pH of the medium. The bacteria have limitations on their acidity tolerance. So, the growth of bacteria doesn't prefer this acidic medium. Drastic variations in cytoplasmic pH can harm bacteria by disrupting the

plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Most prokaryotes die if the internal pH drops much below 5.0 to 5.5 (Nandanwar and Kim 2019). The difference in the activity of the compound between the gram-positive and gram-negative can be explained by the structural differences in their cell wall composition. Gram-negative bacteria contain a thin peptidoglycan layer that is surrounded by an outer membrane composed of a lipopolysaccharides layer, while Gram-positive bacteria possess a thick peptidoglycan lipid but lack an outer layer of lipopolysaccharides (Mai-Prochnow et al. 2016).

Conclusion

In this study, a new ruthenium complex, [Ru(3,3'-dcbpy)$(DMSO)_2Cl_2$], has been prepared and characterized by FTIR, ^{1}H- and ^{13}C-NMR, and UV-Vis spectroscopies, and the antibacterial activity of the complex is examined. The complex has good antibacterial activity against gram-positive and gram-negative strains. The UV-Vis spectra revealed significant absorptions in the UV region when compared to the visible region.

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References

Andrews JM (2001) Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy 48(suppl_1): 5–16. https://doi.org/10.1093/jac/48.suppl_1.5

Armarego WLF (2017) Purification of Laboratory Chemicals (8th edn.). Butterworth-Heinemann, Canberra, 95–634. https://doi.org/10.1016/B978-0-12-805457-4.50003-3

Bonaccorso C, Marzo T, La Mendola D (2020) Biological applications of thiocarbohydrazones and their metal complexes: A Perspective review. Pharmaceuticals 13(1): 4–23. https://doi.org/10.3390/ph13010004

Borase JN, Mahale RG, Rajput SS (2021) Design, synthesis and biological evaluation of heterocyclic methyl substituted pyridine Schiff base transition metal complexes. SN Applied Science 3: 1–13. https://doi.org/10.1007/s42452-021-04144-z

Dayan S, Tercan M, Özdemir FA, Aykutoğlu G, Özdemir N, Şerbetçi Z, Dinçer M, Dayan O (2021) Catalytic and biological activities of homoleptic palladium(II) complexes bearing the 2-aminobenzothiazole moiety. Polyhedron 199: e115106. https://doi.org/10.1016/j.poly.2021.115106

Deghadi RG, Elsharkawy AE, Ashmawy AM, Mohamed GG (2022) Can one novel series of transition metal complexes of oxy-dianiline schiff base afford advances in both biological inorganic chemistry and materials science? Comments on Inorganic Chemistry 42(1): e146. https://doi.org/10.1080/02603594.2021.1962310

Dhabale RH, Shah S, Tiwari N, Patani P (2022) Review of Semicarbazone, Thiosemicarbazone, and their transition metal complexes, and their biological activities. Journal of Pharmaceutical Negative Results 13(5): 2416–2424. https://doi.org/10.47750/pnr.2022.13.S05.377

Egza TF, Mitiku AA, Lemma TS, Eeranna BC (2020) A review on pharmacological activity of some selected transition metal complexes with heterocyclic ligands. World Journal of Pharmaceutical Research 9(12): 100–127. https://doi.org/10.20959/wjpr202012-18836

Evans IP, Spencer A, Wilkinson G (1973) Dichlorotetrakis-(dimethylsulphoxide)ruthenium(II) and its use as a source material for some new ruthenium(II) complexes. Journal of the Chemical Society, Dalton Transactions: Inorganic Chemistry (2): 204–209. https://doi.org/10.1039/dt9730000204

Ezugwu CI, Liu S, Li C, Zhuiykov S, Roy S, Verpoort F (2022) Engineering metal-organic frameworks for efficient photocatalytic conversion of CO_2 into solar fuels. Coordination Chemistry Reviews 450: e214245. https://doi.org/10.1016/j.ccr.2021.214245

Fudo Z, Ajibade PA, Odularu AT (2022) Synthesis, characterization, and electrochemical activities of ruthenium(II) bipyridyl-dithiocarbamate complexes. International Journal of Photoenergy 6875515: 1–10. https://doi.org/10.1155/2022/6875515

Ghanghas P, Choudhary A, Kumar D, Poonia K (2021) Coordination metal complexes with Schiff bases: Useful pharmacophores with comprehensive biological applications. Inorganic Chemistry Communications 130: e108710. https://doi.org/10.1016/j.inoche.2021.108710

Graur V, Chumakov Y, Garbuz O, Hureau C, Tsapkov V, Gulea A (2022) Synthesis, structure, and biologic activity of some copper, nickel, cobalt, and zinc complexes with 2-formylpyridine N^4-allylthiosemicarbazone. Bioinorganic Chemistry and Applications 2022: e2705332. https://doi.org/10.1155/2022/2705332

Kalyanasundaram K, Gratzel M (1998) Applications of functionalized transition metal complexes in photonic and optoelectronic devices. Coordination Chemistry Reviews 177(1): 347–414. https://doi.org/10.1016/S0010-8545(98)00189-1

Kanungo BK, Baral M, Bhattacharya S, Sahoo Y (2003) Synthesis of new macrocycles with 2,2`-bipyridyl and polyamine functions. Synthetic Communications 33(18): 3159–3164. https://doi.org/10.1081/SCC-120023436

Laxman Sangle S (2022) Introduction to schiff base. Schiff Base in Organic, Inorganic and Physical Chemistry: 1–13. https://doi.org/10.5772/intechopen.108289

Machado JF, Correia JDG, Morais TS (2021) Emerging molecular receptors for the specific-target delivery of ruthenium and gold complexes into cancer cells. Molecules 26(11): e31533182. https://doi.org/10.3390/molecules26113153

Mai-Prochnow A, Clauson M, Hong J, Murphy AB (2016) Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. Scientific Reports 6(1): 1–11. https://doi.org/10.1038/srep38610

Mohammad N, Uddin E, Uddin S, Babar IH, Hossain S, Bitu NA, Khan MN, Asraf A, Hossen F, Kudrat-E-Zahan M (2021) Exploring Schiff base chemistry - An overview. International Journal of Chemistry

and Pharmaceutical Sciences 9: 18–22. http://www.pharmaresearch-library.com/ijcps

Nandanwar SK, Kim HJ (2019) Anticancer and antibacterial activity of transition metal complexes. Chemistry Select 4(5): 1706–1721. https://doi.org/10.1002/slct.201803073

Qin Y, Peng Q (2012) Ruthenium sensitizers and their applications in dye-sensitized solar cells. International Journal of Photoenergy 291579: 1–21. https://doi.org/10.1155/2012/291579

Rauchfuss TB, Clements JL, Agnew SF, Roundhill DM (1997) Synthesis and reactions of and catalytic homogeneous hydrogenation by chlorocarbonyl(o-(diphenylphosphino)-N,N-dimethylaniline)iridium(I) and Chlorocarbonyl(o-(diphenylphosphino)-N,N-dimethylbenzylamine)iridium(I). Inorganic Chemistry 16(4): 775–778. https://doi.org/10.1021/ic50170a010

Reynal A, Palomares E (2011) Ruthenium Polypyridyl sensitizers in dye solar cells based on mesoporous TiO_2. European Journal of Inorganic Chemistry 2011(29): 4509–4526. https://doi.org/10.1002/ejic.201100516

Salman WA (2021) Synthesis, characterization, and biological activity for a new ligand 2,5bis[(butan2-ylidene)hydrazinyl]-1,3,4-thiadiazole with some transition metal complexes. International Journal of Drug Delivery Technology 11: 711–715. https://doi.org/10.25258/ijddt.11.3.10

Zhou J, Liu L, Li Y, Wang L, Xie Z (2022) Multivariate strategy preparation of nanoscale rudoped metal–organic frameworks with boosted photoactivity for bioimaging and reactive oxygen species generation. Inorganic Chemistry 61(11): 4647–4654. https://doi.org/10.1021/acs.inorgchem.1c03649