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BIOLOGICAL ACTIVITY OF AZO QUINOLINE DYE AND ITS PALLADIUM(II) COMPLEX

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ABSTRACT. Azo dye derived from 8-hydroxyquinoline and 3-aminopyridine namely (E)-5-(pyridin-3-yldiazenyl)quinolin-8-ol (PQ8OL) and its palladium complex were prepared and was characterized by H-NMR, UV-Vis, and mass spectroscopies. The value of conductivity indicates that the palladium complex is non-ionic. The structure of PQ8OL dye has been solved from powder X-Ray by using Material Studio. The structure has been indicated to hydrazone form of PQ8OL dye. The PQ8OL has coordinated via nitrogen and oxygen atoms of quinoline. The UV-Vis transitions indicated that the palladium complex is square planer. The palladium complex appeared biological activity more than PQ8OL ligand. The prepared compounds appeared potential biological activity in the range of 0.6-2.0 mm against *Staphylococcus aureus* and *E. coli* bacteria. The PQ8OL ligand and its palladium complex showed potential inhibition where the inhibition was 1.0 and 6.0 mm for PQ8OL ligand and palladium complex respectively against *Staphylococcus aureus*. The inhibition for *E. coli*, by the ligand and its palladium complex was 2.0, and 7.0 mm, respectively.

KEY WORDS: Biological activity, Palladium(II) complex, Quinoline, Crystal, Pyridine

INTRODUCTION

At least azo compounds have one azo (diazene) functional group in their structure that appear chromophoric properties [1]. When the functional group of azo attach aromatic or heterocyclic aromatic substituents resulting colour stable dyes which leads to capable of absorbing electromagnetic radiation in the visible area [2]. The nitrogen atoms of azo group and carbon atoms in adjacent phenyl rings appear sp^2 hybridization which form extensive conjugated π-electron system which lead to deep their colour [3].

Some azo compounds appear interesting photochemical properties such as in high optic data storage depending on photoisomerization and in sensitizing solar cells. The 8-hydroxyquinoline fragment was observed in many biologically active molecules, like toddaquinoline and ciprofloxacin. 8-hydroxyquinoline-based dyes can be used chemosensors for metal cation detection, and can be used to detect human fingerprint [4]. Many studies, indicated that complexation potential of 8-Hq dyes have antibacterial activity [5].

Some of azo dyes having acidic groups exhibit tautomerism [6], where ability to transform between azo and hydrazo forms is called tautomerism [7]. The colour and properties of azo compounds are strongly influenced by their tautomerism process [8]. It is interested point to determine the way which form such dyes crystallize in solid state. It is possible reliable determination of the tautomeric state of azo dyes in solid state by X-ray diffraction.

The heterocyclic dyes show brighter color and higher tinctorial strength than traditional aniline-based components, besides applications in nonlinear optical materials, antitumor and antioxidant activities, optical sensing of metal ions and electronic industries [9, 10]. Heterocyclic dyes containing such as quinoline and pyridine have attracted special attention because of their excellent spectral properties and biological applications [11, 12]. Therefore, we decided to prepare

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the azo dye based on 8-hydroxyquinoline and 3-aminopyridine and its palladium complex and study of their biological activity.

EXPERIMENTAL

Material and methods

Standard operating procedures were followed in order to remove the solvents. Re-crystallization and distillation were used to purify liquid and solid aromatic amines, respectively. High purity 8-hydroxyqunoline, 3-aminopyridine, absolute ethanol, nitrite sodium and palladium(II) chloride and sodium hydroxide were purchased from Merck. IR spectra were captured by using a Shimadzu 8400 FT-IR spectrophotometer in the range of 4000-400 cm^{-1}. Using TMS as an internal standard, ^{1}H-NMR spectra were acquired using ^{1}H-NMR (500 MHz) Bruker apparatus in $CDCl_3$. A Cary UV–Vis double-beam spectrophotometer (Model 100) was used to measure the compounds' absorption spectra at 10^{-4} M. A Leco CHNS-900 analyzer was used to determine the elemental analysis. Melting temperatures were measured using an electrothermal device. XRD analysis of ligand was done on Phillips, Holland PW 1710 X-ray diffractometer system by a copper anode with nickel filter. The wavelength of radiation using by the XRD system was 1.54056 Å.

Preparation of diazotization salt for 3-aminopyridine

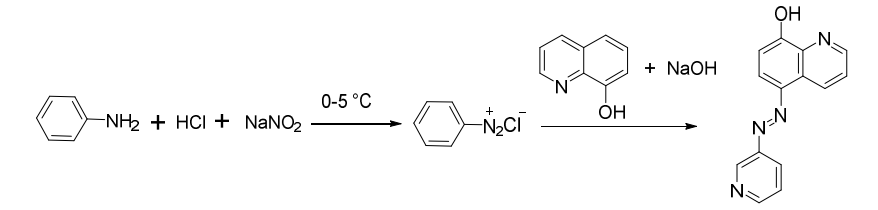
Aromatic amine of 3-aminopyridine (0.57 g, 6.0 mmol) was dissolved in a solution of hydrochloric acid (25 mL, 5 N) then, the solution was cooled to -5 °C in an ice-salt bath. Subsequently, the acidic solution of 3-aminopyridine was gradually mixed with a cold aqueous solution of nitrite sodium (10 mL, 0.4 g, 6.0 mmol), the mixture of reaction was stirred for thirty-five minutes under cooling.

Preparation of (E)-5-(pyridin-3-yldiazenyl)quinolin-8-ol (PQ8OL)

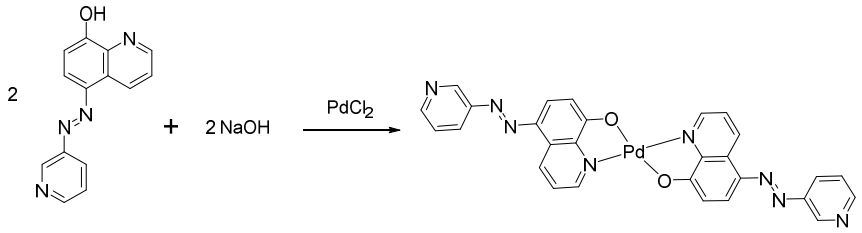
The resulting solution of diazonium salt was added gradually to the solution of dissolving (0.87 g, 6.0 mmol) of 8-hydroxyquinole in 25 mL ethanol and 10 mL, 10% of sodium hydroxide at 0-5 °C. The mixture was continuously stirred for 1 h under cooling. The precipitate underwent filtration, distilled water washing, drying, and recrystallization using absolute ethanol. The yield was 92% red powder and melting point was 220 °C. Figure 1(A) represents the preparation steps of PQ8OL ligand. The theoretical of elemental analysis for $C_{14}H_{10}N_4O$ ligand was C, 67.19; H, 4.03; N, 22.29% in perfect agreement with founding of elemental analysis, which was C, 67.01; H, 4.01; N 22.42%. Chemical shift ppm in $CDCl_3$: 9.21(H), 9.15 (H), 8.81 (H), 8.62 (H), 8.13 (H), 8.03(H), 7.57 (H), 7.38(H), 7.17 (2H) 7.26 ($CDCl_3$).

Preparation of palladium(II) complex for (PQ8OL) ligand

The metal complex of palladium(II) was prepared in a mole ratio (2:1) L: M by dissolving 0.5 g, 2.0 mmol of PQ8OL ligand in 50 mL of ethanol, then the resulting solution was added gradually to 5 mL aqueous solution having 0.18 g, 1.0 mmol of palladium chloride(II) and two drops of hydrochloric acid to complete the dissolving. The resulting solution was refluxed at 80 °C for 60 min and justified the pH at 8. The precipitate was filtered and was washed with distilled water several times, then was washed with a little of ethanol. Figure 1(B) represents the preparation steps of palladium(II) complex for PQ8OL ligand. The yield was 65% brown powder and melting point was 260 °C. The theoretical of elemental analysis for $C_{28}H_{18}N_8O_2Pd$ complex was C, 55.59; H, 3.00; N, 18.52% in perfect agreement with founding of elemental analysis, which was C, 55.46; H, 2.95.00; N, 18.57%. Chemical shift ppm in $CDCl_3$: 9.51(2H), 8.72 (2H), 8.71 (2H), 8.17 (2H), 8.01(2H), 7.54 (2H), 7.41 (2H), 7.25 (4H) 7.26 ($CDCl_3$).



A) Preparation steps of azo dye ligand derived from 3-aminopyridine and 8-hydroxyquinoline (PQ8OL)



B) Preparation of palladium (II) complex for PQ8OL

Figure 1. Preparation steps of A) PQ8OL ligand and B) palladium(II) complex for PQ8OL ligand.

Biological activity

The culture media was prepared according to the manufacturer's instructions by 38 g of (Mueller-Hinton agar) was added to 1000 mL of distilled water in a conical flask, then the mixture was heated to dissolve the powder. The medium was sterilized within a period of 15 min at 120 °C and the pressure was (15 pounds/inch). Then poured into dishes called Petri dishes 10 mL and then the temperature of the dish was lowered to room temperature to solidify. Preparation solutions of PQ8OL ligand and its palladium complex under study were 250 ppm. The bacteria were spread in the dishes on the surface of the culture media (Muller-Hinton agar) and were made three holes of 6 mm in diameter using an alcohol-sterilized cork drill with a space left between one hole and another to avoid overlapping of the damping areas between them. 0.1 mL of every compound using a fine pipette for that was added to holes, then placed in an incubator for 24 hours at 37 °C. We measured the inhibition zone of these compounds with a millimetre ruler.

RESULTS AND DISCUSSION

The azo dye of quinoline (PQ8OL) and its palladium complex have identified by different techniques including H-NMR, UV-Vis, FT-IR, mass spectroscopy, molar conductivity, and elemental analysis. The mass $(m/z)^+$ of dye was 250.2, in perfect agreement with molecular formula, and the mass of the palladium complex was 604.9 in agreement with molecular formula $C_{28}H_{18}N_8O_2Pd$. Molar conductance values of electrolytic solutions explain electrolytic behavior of metal complex solutions, which provide a concise overview of their composition and nature. Significant structural information can be reveal from molar conductance, which, determine whether a metal complexes is electrolytic or non-electrolytic base on the number of ions present in a particular solution. Molar conductivity of palladium complex was 7.0 $S.cm^2.mol^{-1}$ in DMF

solvent that means that the palladium complex is nonelectrolyte based on the molar conductivity of nonelectrolyte is less than 65 $S.cm^2.mol^{-1}$ in DMF solvent [13].

Structure analysing of PQ8OL in solid state using X-ray technique

The XRD peaks of PQ8Ol dye at 2θ showed equal to 6.6°, 13.5°, 15.1°, 24.4°, 27.3°, 31.6°, 45.3°, 56.4°, 66.1° and 75.2°. Material Studio Software was used for solving the structure of azo quinoline (PQ8OL) from its powder X-ray diffraction data. Where material studio can convert the powder X-ray data of material into crystal cell parameters and give an idea about the structure of that material. Crystal structures of PQ8OL azo dye was solved by applying Monte Carlo simulated annealing techniques to X-ray powder diffraction data and refined using the Rietveld method. The azo dye (PQ8OL) crystallizes in monoclinic symmetry (symmetry space group P2/C). Lattice parameters are: a = 6.6563 Å, b = 27.3817 Å, c = 5.5371, $\alpha = 90.0000$, $\beta = 97.6850$, $\gamma = 90.0000$. The structure of Q8POL dye is in keto form (hydazone form) as shown in Figure 2, where, the keto form (hydazone form) in the solid state is favour in the tautomeric equilibrium [6, 14]. The presence of OH, NH, or SH functional groups in para positions relative to the azo moiety presenting in dyes pay the azo-hydrazone equilibrium in azo dyes to be in hydrazone form . The hydrazone form dominates in solid state because the solid state resists the equilibrium state.

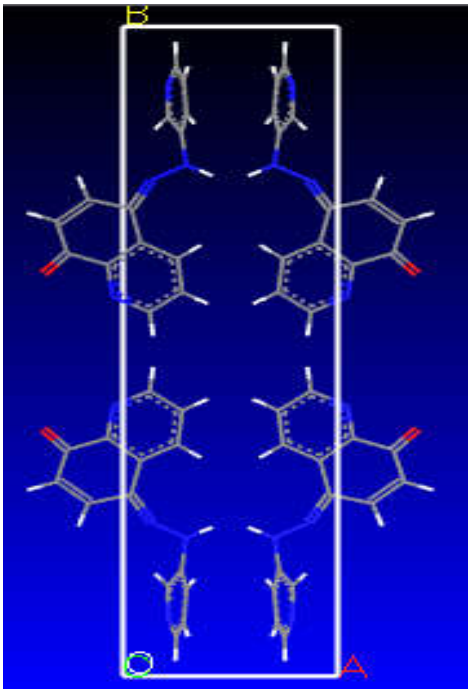


Figure 2. Structure of PQ8OL ligand from powder X-ray diffraction data by material Studio software.

^{1}H-NMR spectral of PQ8OL ligand and its palladium complex

The ^{1}H-NMR spectra of the synthesized compounds were recorded in $CDCl_3$ solvent and chemical shift expressed in ppm. The ^{1}H-NMR spectra of the synthesized compounds exhibit signals due to aromatic protons in the range of 9.51-7.01 ppm. In the ^{1}H-NMR spectrum of the PQ8OL ligand as shown in Figure 3, a singlet signal observed downfield at 9.15 ppm, integrating for one proton, which is assigned to OH.

The singlet of OH disappeared in case of palladium complex and the CH=N of quinoline was dishielding where it appeared at 9.51 ppm. These changes comparing to the free ligand indicate that PQ8OL ligand coordinated to palladium ion to form complex for palladium.

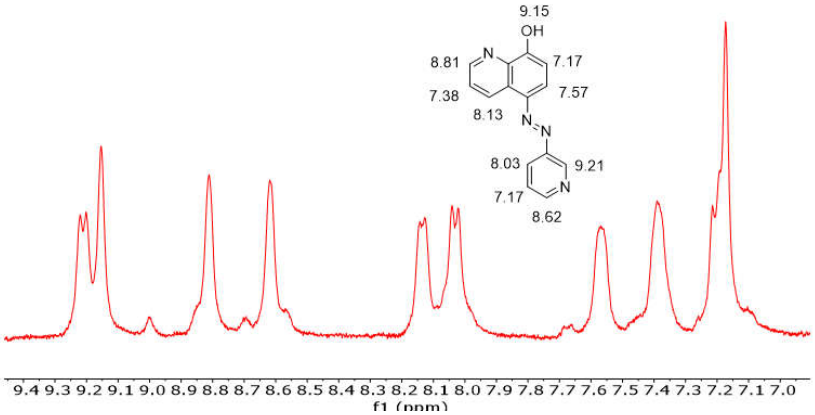


Figure 3. ^{1}H-NMR of PQ8OL ligand in $CDCl_3$ at room temperature.

IR spectra of azoquinoline and its Pd(II) complex

The IR spectrum of the free ligand (PQ8OL) as shown in Figure 4, was compared to the corresponding palladium(II) complex as shown in Figure 5. The characteristic absorptions in cm^{-1} were assigned to frequencies of OH, CH (aromatic), C=N, N=N, C=C, C-O, respectively, which listed in Table 1. The absorption at 1649 cm^{-1} corresponding to νC=N group which was found to be shifted towards lower wavenumber at 1579 in the palladium complex. Thus, it is due to ascertain the coordination of the nitrogen of the C=N group to palladium ion. The palladium complex had no broad band in the 2800–3400 cm^{-1} range, related to the stretching of intramolecular H of the OH function, which indicates that the OH group of the PQ8OL ligand is really coordinated to Pd(II).

The other characteristic bands of free ligand (PQ8OL) were not affected by metal coordination and these results suggest that the PQ8Ol ligand is bonded to the palladium(II) ion by nitrogen atom of C=N and oxygen of OH under deprotonation.

The shift in (C=N) and phenolic -OH bands by comparing the infrared spectroscopic data of palladium complex and its respective ligand are consider important indicators to form the palladium complex. Additional evidence for the coordination of the nitrogen of C=N is the presence of ν(Pd-N) band 789 cm^{-1} and ν(M-O) 445 cm^{-1} [15].

Electronic spectra of PQ8OL ligand and Pd(II) complex

In order to confirm the PQ8OL ligand binding to the metal, and the structure of palladium complex, we have analysed the spectra of the PQ8OL ligand and palladium(II) complex in the UV-Visible regions in dimethylsulphoxide as shown in Figure 6. The UV-Visible absorption spectrum of the PQ8OL ligand in 10^{-4} M at room temperature, shows two bands centred at 393 nm due to $\pi \rightarrow \pi^*$ and band at 417 nm due to $n \rightarrow \pi^*$ [16]. On the other hand, the spectrum of the Pd(II) complex shows three bands, which are 262 nm, 342 nm and 503 nm. These bands are due to intra ligand, charge transfer transition, and $^1A_{1g} \rightarrow {}^1B_{1g}$ respectively, suggesting a square planar geometry for palladium complex [17–19].

Table 1. Identification frequencies of PQ8OL ligand and its palladium complex in cm^{-1}.

Group	Ligand	Pd(II) complex
OH	3377	-
CH aromatic	3050	3047
C=N (quinoline)	1649	1579
C=N (pyridine)	1597	1597
C=C	1573, 1554	1558, 1498
N=N (azo group)	1425	1427
C-O	1332	1325
Pd-N	-	489
Pd-O	-	445

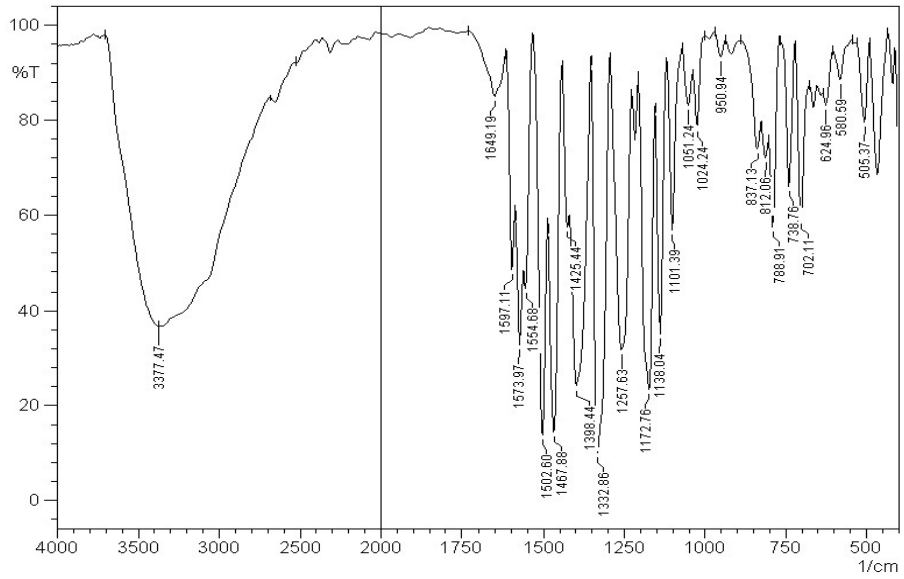


Figure 4. Infrared spectrum of PQ8OL ligand in KBr pellet.

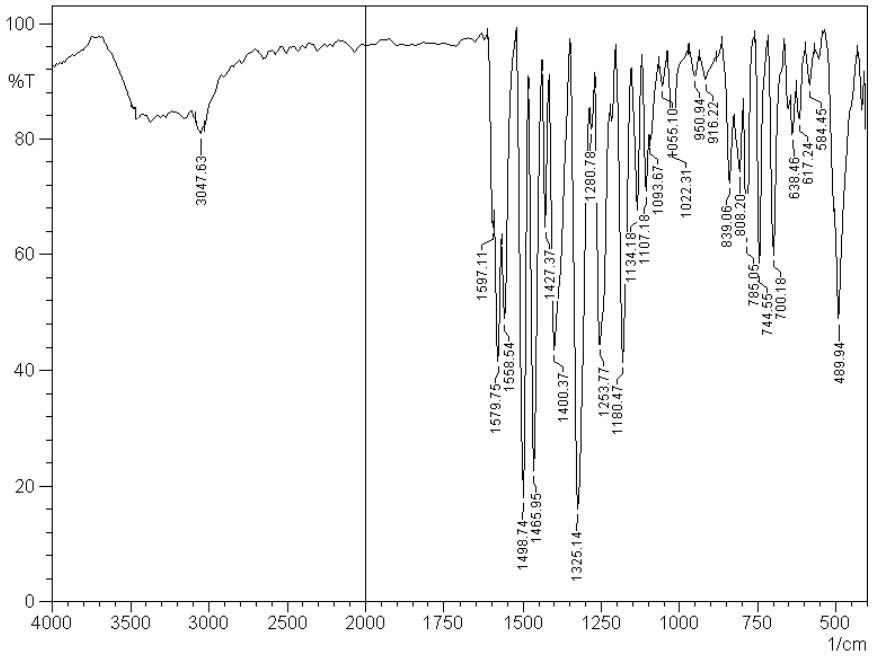


Figure 5. Infrared spectrum of Pd(II) complex for PQ8OL ligand in KBr pellet.

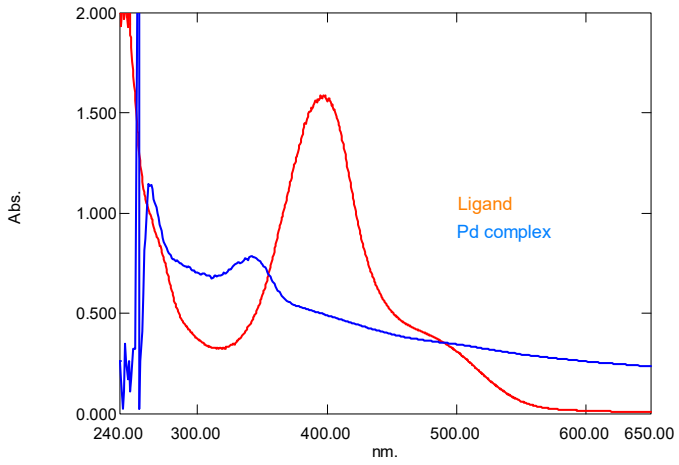


Figure 6. UV-Vis spectra of Pq8OL ligand and its palladium (II) complex in DMSO at room temperature.

Antibacterial Activities of PQ8OL ligand and its Pd(II) complex

The antibacterial activity of synthesized PQ8OL ligand and its Pd(II) complex was evaluated using different pathogens bacteria, including the Gram-positive *Staphylococcus aureus*, Gram-negative *E. coli* . Bacterial pathogens showed the different zone of inhibitions against PQ8OL ligand its Pd(II) complex as shown in Figure 7.

Considering the case of *Staphylococcus aureus*, Gram-negative *E. coli*, for synthetic compounds there were interesting zone of inhibition observed. In the case of *Staphylococcus aureus*, for synthetic compounds, the zone of inhibition was 1.0, and 6.0 mm for PQ8OL ligand and palladium complex respectively. Similarly, with *E. coli*, for synthetic compounds, the zone of inhibition was 2.0, and 7.0 mm, respectively. It is interesting to note that the PQ8OL ligand and its palladium complex showed antibacterial activity against Gram-positive and Gram-negative bacteria. This indicates the broad-spectrum ability of these compounds against the different pathogens. The palladium complex showed the highest antibacterial activity in both cases of positive and negative pathogenic bacteria when compared with the PQ8OL ligand [20–24].

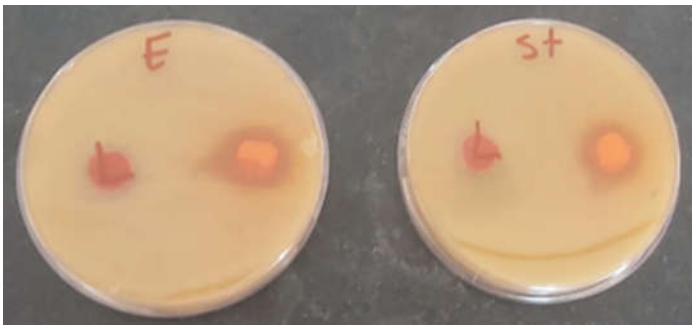


Figure 7. Biological activity of PQ8OL ligand and its palladium(II) complex against *E. coli* (left image) and *Staphylococcus* bacteria (right image).

CONCLUSION

In this work, we have prepared and characterized azo dye derived from 3-aminopyridine and 8-hydroxyquinoline and its palladium complex. The azo ligand is bidentate, which is linked by nitrogen and oxygen atoms. The structure of azo ligand gets from X-ray diffraction by Material studio software. The azo ligand is in keto form (hydrazine in solid state). The prepared compounds showed potential activity against *E.coli* and *staphylococcus* bacteria. The palladium complex is non-electrolyte and its shape is square planer with chemical formula $[Pd(PQ8OL)_2]$.

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