Synthesis, Characterization and Biological Activity Study of Some New Palladium (II) Complexes Containing Amine or Azomethine Groups

Adil A. Al-Fregi*, Ahmed L. Al- Fadhly, Bushra K. Al-Salami

Department of Chemistry, College of Science, University of Basrah

*Corresponding author Tel: +964 7707377383, e-mail address: dr.adilalfregi@yahoo.com

Abstract

This study concern the preparative methods of two types of palladium (II) complexes. The first type concerned two newly palladium (II) complexes derived from bidentate amine ligands while the second type concerned six newly palladium(II) complexes derived from bidentate Schiff base ligands. All the synthesized complexes have been characterized by elemental analysis CHN, conductivity measurements, and u.v.- visible, FT-IR and ^{1}H-NMR spectral data. *In vitro* all the prepared complexes have also been tested for their growth inhibitory activity against gram negative bacteria *Escherichia coli* [ATCC 25922] and gram positive *Staphylococcus aureus*[ATCC 25923]as well as determined the minimum inhibitory concentration (MIC). Furthermore, complexes- human DNA interactions were studied.

Keywords: palladium(II) complexes, amines ligands, Schiff base, antibacterial activity complexes- human DNA interactions,

Introduction

Cancer may be defined as a disease or a group of diseases, in which the cells divided and multiply without control, have the capacity to metastasize in the body, destroy healthy tissue and endanger life. It is one of the major causes of death in many countries of the world. Cancer is one of the top three killers worldwide disease, second only to heart disease and diabetes. A bout 20 million cancer cases are expected to occur in the next two decades, which renders the quest for new and improved antineoplastic agents.(1) While many drug molecules are "organic" in nature, other elements in the periodic table, particularly platinum metal, offer a much more diverse chemistry and have important therapeutic applications.(2)

Cis-diamminedichloroplatinum(II) ,Cisplatin, is one of the most potent and effective antitumor agents was introduced to chemotherapeutic treatment in 1978,(3) but it lacks selectivity for tumor tissue and many tumors are growing resistance to this platinum complex.(4) To solve this problem modified versions of cisplatin, leading to second and third generation platinum-based drugs have been synthesized over the past 30 years and have got their less toxic effect to the host tissue.(5)

In recent years a great deal of effort has been devoted to developing transition metal antitumor agents which have better therapeutic properties than prototype drug cisplatin.(6, 7)Although the bulk of the work to date has involved investigations of platinum(II) complexes as potential antitumor agents, some investigations involving transition metals such as palladium(II) have been done.(8, 9) The significant similarity between the coordination chemistry of compounds has advocated studies of Pd(II) complexes as anticancer drugs. A key factor that might explain why platinum is most useful comes from the ligand- exchange kinetics. The hydrolysis in oalladium complexes is too rapid: 10^5 times faster than for their corresponding platinum analogues. The lability of palladium complexes for dissociate readily in solution leading to very reactive species that are unable to reach their pharmacological targets.(10)

One of the main challenges in the rational design of metal-containing chemotherapy agents is to enhance their cytostatic activity while simultaneously reducing toxicity.(11,12) For the most part, palladium complexes have shown little or no antitumor activity. This has been attributed to the higher lability and lack of stability of palladium(II) complexes compared to platinum(II). Compared to cisplatin, the corresponding cispalladium, *cis*-$[PdCl_2(NH_3)_2]$does not show antitumoral activity. It is well known that it undergoes an active trans-conformation and that the two compounds hydrolyze very fast assuming that they interact in vivo with a lot of molecules particularly proteins preventing them to reach the DNA, their pharmacological target.(17) Thus, whereas platinum compounds such as cisplatin maintain their structural integrity *in vivo* long

enough to reach their cellular targets, analogous palladium compounds undergo hydrolysis and / or various substitution reactions more quickly to be effective as antitumor agents. Therefore, if an antitumor palladium drug is to be developed , it must somehow be stabilized so that it can reach the cancerous cells intact. The considerably higher activity of palladium complexes implies that if an antitumor palladium drug is to be developed, it must somehow be stabilized by a chelate or strongly coordinated, bulky monodentate nitrogen ligand and a suitable leaving groups.[9, 14] Due to the steric effect that results from the bulk on the donor atoms, these ligands could minimize any possible cis-trans isomerism.[15] In fact, carefully designed platinum and palladium complexes structurally different from cisplatin and its second-generation analogues are prone to display an altered spectrum of clinical activity and toxicity, due to differences in cellular biochemical pharmacology.[16] Therefore, the parameters ruling their cytotoxicity may not follow the patterns applied to cisplatin-like agents. In an effort to solve this problem we have adopted the approach proposed by Gill. This approach involves the use of chelating ligands to stabilize the palladium complex. [17]

On the other hand, amines are suitable chelating ligands for transition metal ions such as palladium, yielding stable and reasonably water soluble coordination compounds. Linear aliphatic amines, in particular, are recognized to have a high conformational freedom and may be designed to display suitable flexibility and polidenticity features, which constitutes an advantage for an efficient interaction with metal ions and biological receptors.[18]

In fact, the high conformational freedom and the dual hydrophilic-lipophilic character of the amine or Schiff bases ligands, comprising both cationic amine and imine groups and variable length hydrophobic alkyl linkers, allow these metal complexes to interact with DNA through a nonconventional way, both covalently (through direct binding of the metal center to the purine bases) and non-covalently (via hydrophobic and hydrogen-bonding close contacts).

Studies of palladium complexes with biologically active carriers yielding promising results in the field of anticancer chemistry. Many reports have been demonstrated that some complexes which contain Schiff base or ferrocenyl derivatives are highly active against several diseases, including cancer.[19-23]

Recently, we reported the synthesis, characterization and biological activity study of two type of bidentate ligands and their complexes with platinum(II) ion .[24] The first type of ligand includes bidentate amines while the other includes bidentate Schiff bases based on ferrocene moiety.[24] Herein, We have chosen the same bidentate ligands in our previously study to synthesis, characterization and antitumor activity study of these ligands with palladium ion. Antibacterial activity of all the prepared palladium (II) complexes beside their interaction with human DNA were investigated.

Experimental

N-(3-phenylpropyl)ethane-1,2-diamine, N,N'-bis(3-phenylpropyl) ethane-1,2-diamine, N-2-furan methyledinebenzene-1,2-diamine , N,N'-bis(2-furanmethyledine)benzene-1,2-diamine, N-ferrocen methyledinebenzene-1,2-diamine , N,N'-bis(ferrocenmethyledine)benzene-1,2-diamine, N-1-ferrocen ethylidenebenzene-1,2-diamin, N,N'-bis(ferrocenethylidene)benzene-1,2-diamine were prepared in our previously paper.[24] Dichlorobis(benzonitrile)palladium(II) was prepared by the literature method. **[25]**

Elemental analyses were performed by University of AL al-Bayt, Al-Mafraq, Jordan using a Euro vector EA 3000A Elemental Analysis (Italy). Infrared spectra were recorded for KBr pellets on a FT-IR spectrophotometer Shimadzu model 8400S in range 4000-400 cm^{-1} at Department of Chemistry, College of Education for Pure Science, University of Basrah. UV-Vis spectra for the synthesized complexess were recorded at Department of Chemistry, College of Science, University of Basrah by using Scan 80D (England) at range 200-800 nm using chloroform as a solvents and 1cm^3 pathway quartz cells. ^{1}H- NMR spectra were recorded at Al al-Bayt University, Jordan by using a Bruker 300 MHz (Germany). Chemical shift of all ^{1}H- spectra were recorded in δ(ppm) unit downfield from the internal reference tetramethyl silane (TMS), using DMSO-d_6 solvent. Conductivity measurements were measured in $1x10^{-3}$ M solutions of dimethyl sulfoxide solvent at room temperature using a Konduktoskop model 365B using standard conductivity cell with constant equal to 0.81 cm^{-1}.

Synthesis of complexes:

Synthesis of dichloro[N-(3-phenylpropyl)ethane-1,2-diamine]palladium(II) (1)

To dibenzonitriledichloropalladium (II),$PdCl_2(PhCN)_2$, (0.383 g; 1 mmol) in absolute ethanol (15 mL), *N*-(3-phenylpropyl)ethane-1,2-diamine (0.355 g ,1 mmol) dissolved in absolute ethanol (15 mL) was slowly added. After stirring for 48 h at room temperature, the product was isolated by filtration. The solid product was washed with water, ethanol and dried *in vacuo* to give a pale yellow solid product of complex **1** in 62 % yield, m.p. 75-77 ^{0}C(dec.); IR data cm^{-1}: 3346 , 3066. 2929, 2861, 1494, 1454, 1239, 752, 686, 666.

Synthesis of dichloro[N,N'-bis(3-phenylpropyl)ethane-1,2-diamine]palladium(II) (2)

To a stirring solution of dibenzonitriledichloropalladium (II), PdCl2$(PhCN)_2$, (0.383 g, 1 mmol) in absolute ethanol (15 mL), a solution of N,N'-bis(3-phenylpropyl)ethane-1,2-diamine (0.489 g, 1 mmol) in 25mL of absolute ethanol. After stirring for 48h at room temperature , a brown precipitate had formed. The crude solid was filtered, washed with water, then ethanol, and was dried *in vacuo* to afford complex **2** as a orange solid in 71% yield, m.p. 90-92^{o}C (dec.). IR data cm^{-1}:3390, 3022, 2924, 2854, 1496, 1452, 1266, 750, 698, 617

Synthesis of dichloro[N-(2-furanmethyledine)benzene-1,2-diamine]palladium(II) (3)

To a freshly prepared absolute ethanol (15 mL) solution of $PdCl_2(PhCN)_2$(0.383 g, 1 mmol) was added N-(2-furfuralydine)benzene-1,2-diamine(0.377 g, 1 mmol). The mixture was stirred at room temperature for 48h . a dark brown participate was formed and was collected by filtration. The solid was then recrystallized from chloroform to afford complex **3** as a dark brown solid in 60 % . m.p 129-131(dec.). IR data cm $^{-1}$: 3359, 3068, 2926, 2852, 1656, 1500, 1454, 1234, 746, 673, 602.

Synthesis of dichloro[N,N'-bis(2-furanmethyledine)benzene-1,2-diamine]palladium (II) (4)

To a absolute ethanol solution of $PdCl_2(PhCN)_2$(0.383 g, 1 mmol) was N,N'-bis(2-furfuralydine)benzene-1,2-diamine (0.455 g, 1 mmol). The mixture was stirred at room temperature for 48h . a brown participate was formed and was collected by filtration. The solid was then recrystallized from chloroform to afford complex **4** as a brown solid in 69 % yield, . m.p 168-179^{0}C (dec.). IR data cm^{-1}: 3022, 2926, 2862, 1610, 1510, 1462, 1226, 748, 666, 602.

Synthesis of dichloro[N-(ferrocenmethyledine)benzene-1,2-diamine]palladium(II) (5)

A filtered solution of N-(ferrocenylidene)benzene-1,2-diamine(0.524 g, 1 mmol) in absolute ethanol (20 mL)was added to a solution of $PdCl_2(PhCN)_2$ (0.383 g, 1 mmol) in absolute ethanol (15 mL) with continuous stirring. After 48h stirring at room temperature, a brown solid was formed. The solid product was purified by extraction by diethyl ether (5×4 ml)and dried *in vacuo*. Complex **5** was obtained as orangein 67 % yield , m.p. 115-117^{0}C(dec.). IR data cm^{-1}:3350, 3030, 2930, 2853, 1656, 1566, 1462, 1274, 746, 666, 613.

Synthesis of dichloro[N,N'-bis(ferrocenmethyledine)benzene-1,2-diamine]palladium(II) (6)

A filtered solution of N,N'-bis(ferrocenmethyledine)benzene-1,2-diamine (0.749 g, 1 mmol) in absolute ethanol (20 mL)was added to a solution of $PdCl_2(PhCN)_2$ (0.383 g, 1 mmol) in absolute ethanol (15 mL) with continuous stirring. After 48h stirring at room temperature, a orange solid was formed. The solid product was purified by extraction by diethyl ether (5×4 ml)and dried *in vacuo*. Complex **6** was obtained as orange solid in 72 % yield, m.p. 117-119^{0}C(dec.). IR data cm^{-1}: 3066, 2924, 2852, 1653, 1493, 1466, 1266, 772, 746, 666.

Synthesis of dichloro[N-(1-ferrocenylethylidine)benzene-1,2-diamine]palladium(II) (7)

A mixture of N-(1-ferrocenylethylidine)benzene-1,2-diamine (0.537 g, 1 mmol) and $PdCl_2(PhCN)_2$ (0.383 g, 1 mmol) in 30 mL of absolute ethanol was stirred at room temperature for 48h. a brown precipitate was obtained which was then collected by filtration. The solid product was purified by extraction by diethyl

ether (10 × 4 ml)and dried *in vacuo*. Complex **7** was obtained as a reddish brown solid in 64 % yield , m.p. 129-131(dec.). IR data cm^{-1}: 3333,3066, 2933, 2856, 1653, 1513, 1466, 1226, 759, 746, 666.

Synthesis of dichloro[N,N'-bis(1-ferrocenylethylidine)benzene-1,2-diamine]palladium(II) (8)

A mixture of N,N'-bis(1-ferrocenylethylidine)benzene-1,2-diamine (0.775 g, 1 mmol) and $PdCl_2(PhCN)_2$ () in 30 mL of absolute ethanol was stirred for 48h at room temperature. A brown solid was obtained which was then collected by filtration. The solid product was purified by extraction by diethyl ether (10× 4 ml) and dried *in vacuo*. Complex **8** was obtained as a brown solid in 60% yield, m.p. 91-93(dec.). IR data cm^{-1}: 3092, 2933, 2853, 1653, 1458, 1375, 1280, 693, 666, 626.

Preparation of stock solution of palladium (II) complexes

For all biological experiments, stock solution of compounds 1-8 [1000 µg/ ml] were prepared as follows:

For Pd(II) complexes, dimethyl sulfoxide DMSO was used as a solvent to prepare their stock solutions (the final concentration of the solvent should be 1 %).

Antimicrobial activity

In vitro antibacterial screening is generally performed by the agar diffusion method for testing antibacterial activity of the prepared palladium(II) complexes. This method includes agar well diffusion assay and disc assay. In this test, the antimicrobial compound is applied to an agar plate on a paper disc or in a well. The compound diffuses into agar resulting in a concentration gradient that is inversely proportional to the distance from the disc or well. The diameter of the inhibition zone around the disc or well is a measure of the degree of degree of inhibition. The resulting of the test are generally qualitative.[(26)]

For antimicrobial activity, compound stock solutions[1000µg/ ml] from which, serial dilutions of work concentrations were diluted sufficiently for this assay with sterilized water to avoid solvent interferences. The solvent was used as a negative control in each separated assay.

The primary screening of antibacterial activity of the palladium(II) complexes compared with cisplatin, were determined at concentration of [250 µg/ disc] against two kinds of bacterial species Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative (*Eschericha coli* ATCC25922) bacteria as described by by the disc diffusion method.[(26)]

Watman no.4 filter paper was used for the preparation of diffusion disc (7 mm in diameter), and the discs were saturated with Pd- complexes and other standard complexes at a concentration of [250 µg/ ml]. the medium used in this respect was nutrient agar, the plates were inoculated with tested bacteria, and the complexes-impregnated disc was a specially placed on the agar surface. The plates were incubated for 24 hrs, zone diameter were measured, and the results were compared with cisplatin.

The minimum inhibitory (MIC)

The minimum inhibitory concentration of the complexes was determined against *Staphylococcus aureus* and *Eschericha coli* by serial dilution technique which comprises the serial dilution of the antimicrobial agent at a concentration ranging from [1-15 µg/ ml] inoculated with the organism as it is described by the previously literature. [(27)] The medium used in this respect was nutrient broth.

Preparation of disc

The ligand/ complex (30 µg) in DMSO (0.01 cm^3) was mounted on a paper disc (prepared from blotting paper (5 mm diameter) with the help of a micropipette. The discs were left at room temperature till dryness and then on the microorganism-grown agar plates.

Preparation of agar plates

Minimal agar used for growth of specific microbial species. The preparation of agar plates for against two types of bacteria: *Gram-positive Staphylococcus aureus* and Gram-negative *Eschericha coli* utilized nutrient agar (2.30 g: obtained from Panreac Quimica SA, Spain) suspended in freshly distilled water (100 mL).

This was allowed to soak for 15 min. and then boiled on a water bath until the agar was completely dissolved. The mixture was autoclaved for 20 min. at 121 ^{0} C and then poured into previously washed and sterilized Petri dishes and stored at 30^0 C for inoculation

.**Procedure of inoculation**

Inoculation was done with the help of a platinum wire loop, which was heated to red-hot in a flame, cooled and then used for the application of the microbial stains.

Application of the discs

Sterilized forceps were used for th application of paper discs to the already included agar plates. The discs were then incubated at 37 ^{0}C for 24 h. the diameter of the zone of inhibition was measured around the disc.

***In vitro* antimicrobial activity :**

1-2 mL of nutrient broth was inoculated with the test organisms and incubated at 37 ^{0}C for 24 hr. Sterile nutrient agar plates were also prepared and holes of 5 mm diameter were cut. The test organisms after 24 hr of incubation were spread onto separate agar plates. The compounds which dissolved in DMSO were poured into labeled holes. The plates of each bacterial strain was prepared. The plates were incubated aerobically at 37 ^{0}C for 24 hr. The antimicrobial activity was determined by measuring the diameter of the zone (mm) showing complete inhibiting with respect to control (DMSO).

DNA concentration

DNA concentration per nucleotides was determined by electronic absorption spectroscopy at λ_{max} =260 nm in human blood, each 1 absorption unit (AU)= 50 µg DNA/ mL at 258 nm

Study of Palladium(II) complexes- human DNA interaction

The binding of the prepared Pd (II)-complexes **1-8** with DNA were studied by following of the change of their absorbance (concentrations) with the time by U.V-Visible spectroscopy technique as following:

1- absorbance of DMSO solutions (80 µM) of complexes 1-8 were measured at 200-800 nm.

2- absorbance of mixture (0.1 ml of human DNA and 3ml of 80 µM of the prepared complexes 1-8, respectively) were measured at 200- 800 nm range with different times(i.e. at time= 0, 1h, 2h, and 24h).[28]

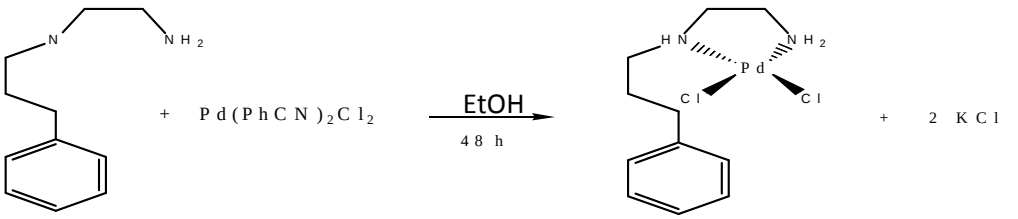
Extraction of Human DNA

Human DNA was extracted from human blood by Sambrook method [29]as following:

To special test tube containing 1.0 ml of human blood, 0.6 ml of RBC buffer was added. Then, the result mixture was centrifuged at 5000 cycle/ min for 15 min several times and then white participate was obtained. To the solid participate , a mixture of (0.6 ml of sodium dodecyl sulfate and 0.03 ml of proteinase K and 0.03 ml of NLB) was added and then was occupied on water bath at 65 ^{0}C for 2h. After incubated time was finished, 0.1 ml of 5 M NaCl solution was added and re- incubated again in water bath at 65 ^{0}C for 10 min. After that, 0.75 ml of chloroform: isopropyl alcohol (24:1) was added for each sample and was centrifuged at 12000 cycle/ min for 8 min. Three layers were obtained, the upper layer was selected and then 0.55 ml of cold absolute ethanol was added and was kept deep freezer at -20 ^{0}C

Results and Discussion

Eight complexes of palladium(II) were synthesized by reacting of dibenzonitrile dichloropalladium(II) with N-(3-phenylpropyl)ethane-1,2-diamine , N,N'-bis(3-phenylpropyl) ethane-1,2-diamine, N-2-furan methyledinebenzene-1,2-diamine , N,N'-bis(2-furanmethyledine)benzene-1,2-diamine, N-ferrocen methyledinebenzene-1,2-diamine , N,N'-bis(ferrocenmethyledine)benzene-1,2-diamine,N-1-ferrocene thylidenebenzene-1,2-diamin, N,N'-bis (ferrocenethylidene)benzene-1,2-diamine respectively in absolute at room temperature for 48 h, *Scheme* 1.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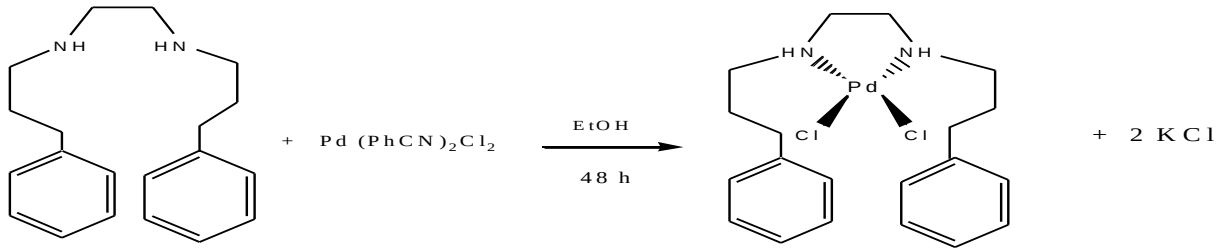
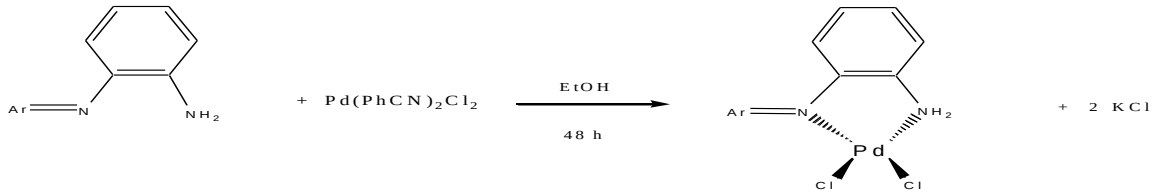
+ Pd(PhCN)2Cl2
EtOH
48 h
HN
NH2
Pd
Cl
Cl
+ 2 KCl

(1)


NH
HN
+ Pd (PhCN)2Cl2
EtOH
48 h
HN
NH
Pd
Cl
Cl
+ 2 KCl

(2)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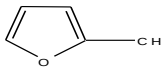
N
NH2
+ Pd(PhCN)2Cl2
EtOH
48 h
Ar
N
NH2
Pd
Cl
Cl
+ 2 KCl

Ar =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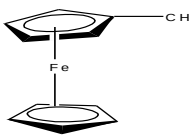
CH

(3)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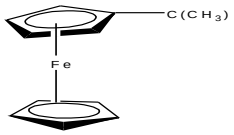
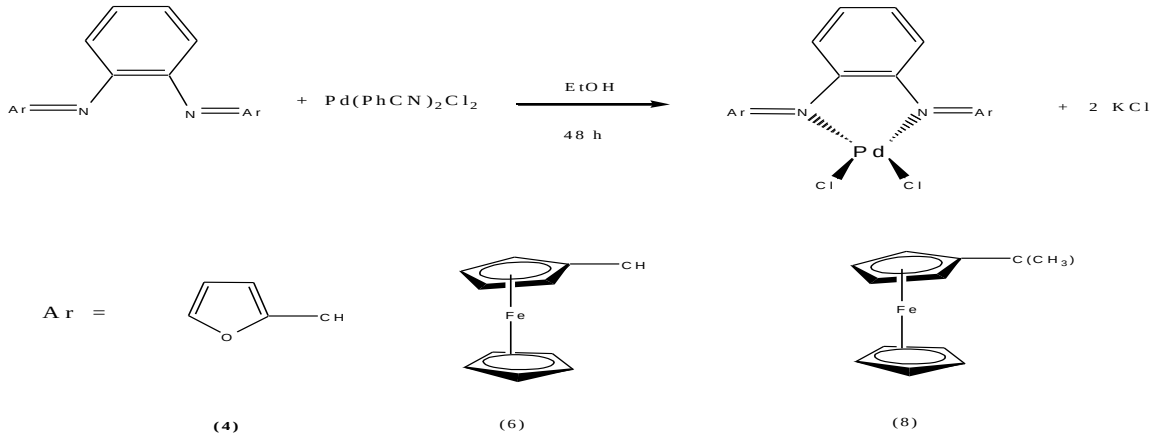
Fe

(5)


C(CH3)
Fe

(7)



Scheme 1: preparative methods of complexes **1-8**

In general, all the prepared Pd(II)-complexes **1-8** are pale yellow to orange solids with moderate melting points(dec.) ranged 75-168 ^{0}C , and they are soluble in common organic solvents such as carbon tetrachloride, chloroform, dichloromethane, dimethylformamide and dimethylsulfoxide which indicate that these complexes are neutral.

The carbon, hydrogen, and nitrogen analyses for Pd(II)-complexes **1-8** agreed well with the calculated values and are presented in *Table* 1. Elemental analysis of these complexes showed that all the palladium(II) ion to the ligand ratio in the dichloro complexes is 1:1. Due to geometrical reasons and the nature of ligands which are bidentate, we believe that all the synthesized complexes adopted to *cis*- form.

The molar conductivities were measured for complexes **1-8** in 1×10^{-3} M solutions of DMSO solvents at room temperature. The molar conductance of complexes **1-8** were found at range 5.00- 8.50 $ohm^{-1}.cm^{2}.mol^{-1}$. This indicate that these complexes behave as non-electrolytes which are in agree well with previous literatures.[30]

Table 1: molar conductivity and elemental analysis for the prepared complexes **1-8**

complex No.	molar conductivity $ohm^{-1}.cm^{2}.mol^{-1}$	elemental analysis %; theoretical/ (practical)		
		C	H	N
1	6.50	37.17 (37.15)	5.19 (5.10)	7.91 (7.88)
2	5.10	50.79 (50.70)	6.00 (5.96)	5.99 (5.91)
3	5.40	36.34 (36.34)	2.79 (2.77)	7.77 (7.71)
4	5.00	43.53	2.78	6.49

		(43.52)	(2.74)	(6.34)
5	7.45	42.41 (42.41)	3.39 (3.35)	5.89 (5.82)
6	6.30	49.79 (49.64)	3.68 (3.57)	4.33 (4.13)
7	8.50	43.79 (43.63)	2.65 (3.66)	4.90 (5.65)
8	8.40	51.06 (51.07)	4.19 (4.00)	4.00 (3.97)

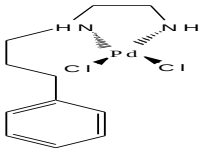
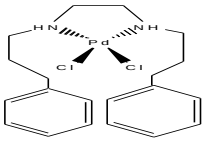
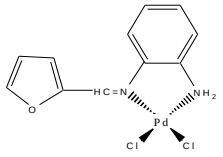
The IR spectra of all new synthesized complexes **1-8** display common feature in certain region and characteristic bands in the fingerprint and other regions. The IR spectra of complexes **1**, **2**, **3**, **5**, **7** show a broad band at 3333 - 3359 cm^{-1} range can be attributed to N-H stretching.[31, 32] Shift was observed toward low frequency for amino groups than the free ligands [24] at range 8 - 58 cm^{-1} due to coordinate the palladium(II) ion with the amino groups. The IR spectra of complexes **3-8** show a strong band at 1610-1656 cm^{-1} attributed to CH=N stretching. [31, 32] This band shifted to higher frequency at range 9 -45 cm^{-1} compared with the corresponding ligands can be considered to coordinate azomethine groups in these ligands with palladium ion.[31, 32] These shift can be attributed to the π – back donation between empty π^* of CH=N and d-orbitals of palladium(II). Two strong bands appeared in the range1458 - 1566 cm^{-1} and 1375 - 1466 cm^{-1} can be attributed to asymmetrical and symmetrical stretching of aromatic (C=C) respectively. **[31,32]** The IR spectra of compounds **1-8** show a weak band in the range 3022 - 3092 cm^{-1} due to aromatic C-H stretching. **[32, 33]** Two weak bands were appeared at 2924 – 2933 cm^{-1} and 2853 - 2862 cm^{-1} due to asymmetrical and symmetrical stretching of aliphatic C-H bands respectively. [32, 33] Furthermore, three variable bands between 695-772 cm^{-1} range can be assigned to aromatic C-H bending while the band at 1226-1280 cm^{-1} due to aliphatic C-H bending. **[31, 32]** Two well defined bands were appeared at range 628- 620 cm^{-1} can be assigned to ν(Pd-N) which are confirm that palladium ion coordinate to the corresponding ligand through nitrogen atoms of amine and imine groups and in cis geometry.[34, 35]

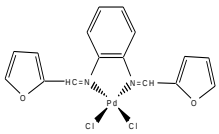
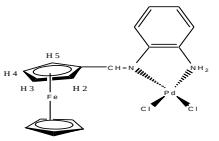
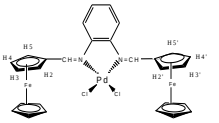
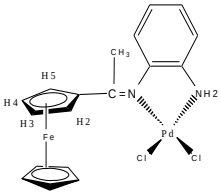
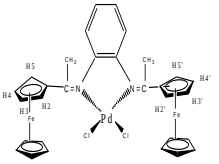
The UV-visible spectra of all Pd(II)-complexes **1-8** were recorded in 1×10^{-4} M solution of DMSO solvent at range 200- 800 nm using quartz cells. The UV-Vis. spectra for complexes **1** and **2** showed one absorption region at 300 nm with molar extinction ranged (ε = 1050 - 1820 $M^{-1}.cm^{-1}$) which may be attributed to π-π^* transition of phenyl group.[36, 37] The UV-Vis. spectra for Pd(II)-complexes **3-8** showed three electronic transitions, the first band appeared at range 300 - 312 nm with molar extinction ranged 2280- 6100 $M^{-1}.cm^{-1}$ can be attributed to π-π^* transition of phenylene group.[36,37] The second band was observed at range 322-390 nm with molar extinction 2670-4011 $M^{-1}.cm^{-1}$ which is due to π-π^*transition of the aromatic rings (*i.e* furan ring for complexes **3** and **4**; ferrocenyl groups for complexes **5-8**).[36-39] The third band was observed between 450 and 460 (ε= 2409-4041 $M^{-1}.cm^{-1}$) which is attributed to π-π^* of the azomethine(CH=N) groups.[36-38] The azomethine band were observed to be red shifted at range 14-30 nm when comparison complexes **3-8** with the corresponding ligands which can be attributed to coordinate this groups with palladium(II) ion. No d-d transitions were observed for all complexes **1-8**, this may be due to their overlap with π-π^* transition of the phenylene group which lead to masked it.

The ^{1}H NMR spectra of all the synthesized complexes **1- 8** were taken in DMSO-d_6 solvent and are summarized in *Table* 2 . In general, ^{1}H NMR spectra of the recorded complexes show the expected signals in proper intensity ratio. The ^{1}H NMR spectra of Pd(II)-complexes **1** and **2** display singlet signal in the range

between 5.80 and 5.95 ppm due to protons of N-H.[40] These protons shifted toward higher chemical shift (downfield) compared to their free ligands[24] at range 1.05 - 1.34 ppm , which are confirm the coordination between palladium (II) ion with corresponding ligands through nitrogen atoms of amine groups. The protons due to phenyl group were found in their expected regions as multiple signals at 7.10 -7.40 ppm.[40, 41] Also, ^{1}H NMR spectra of complexes **1** and **2** show several signals at range 1.14 - 3.41 ppm due to different methylene groups CH_2 as shown in *Table* 2. On the other hand, the ^{1}H NMR spectra of complexes **3-6** showed a singlet signal in the range between 8.45 and 8.66 ppm which can be attributed to the imines protons CH=N.[40,42] These signals also shifted toward higher chemical shift (downfield) than their free ligands signals at the range 0.10- 0.44 ppm which can be indicated as an evidence to coordinate palladium (II) ion with the corresponding ligand via nitrogen atom of azomethine groups.^{1}H NMR spectra of complexes **3**,**5**,**7** showed a singlet signal at 8.81, 9.09, 9.54 ppm, respectively attributed to protons of terminal amine group NH_2.[40] Multiple signals were observed in ^{1}H NMR spectra of complexes **3-8** at the range between 6.20 and8.02 ppm attributed to aromatic protons.[40-42] On the other hand, the complexes which contain ferrocenyl moiety (*i.e.* copmplexes **5- 8**) showed all the expected proton signals which are in well agreement with the previously literatures.[40,43] These spectra showed a singlet signal in the range 4.16-4.40 ppm attributed to protons of unsubstituted cyclopentadienyl ring because all these protons are magnetically equivalent. The substituted cyclopentadienyl ring appeared two singlet singles at the ranges 4.28-4.71 due to (H2 and H5) ; while the second signal at the range 4.35 - 4.92 ppm due to H3 and H4. These protons shifted downfield because increasing the resonance due to the coordination between the corresponding ligands and the palladium ion. Furthermore, ^{1}H NMR spectra of complexes **7** and **8** showed a singlet signal at 2.50 and 2.98 ppm, respectively attributed to methyl group with only a minor shift for this signal could be observed compared with the corresponding free ligands.[24]

Table 2: ^{1}H NMR data of complexes **1-8**

Comp. No.	complex structure	chemical shift (ppm); TMS= 0 ppm
1		1.14 (t , 2H , $\underline{CH_2}$Ph); 1.80 (qu , 2H , $CH_2\underline{CH_2}CH_2$); 2.60(t , 2H , $CH_2CH_2\underline{CH_2}NH$); 3.79 (t , 2H , $\underline{CH_2}CH_2NH_2$) 4.46 (t , 2H , $CH_2CH_2NH_2$); 5.80 (s , H ,NH); 5.90 (s , 2H , NH_2); 7.19-7.35 (m , 5H , Ar-H)
2		1.23(t , 4H , 2 $\underline{CH_2}$Ph); 1.79 (qu , 4H , 2 $CH_2\underline{CH_2}CH_2$); 2.60 (t , 4H , 2 $CH_2CH_2\underline{CH_2}NH$); 3.01 (t , 4H , 2 $\underline{CH_2}NH$); 5.95 (s , 2H , 2NH); 7.10-7.4 (m , 10H , Ar-H)
3		6.38-7.78 (m , 7H , Ar-H); 8.45 (s , 1H , CH=N); 8.81 (s , 2H , NH_2)

4		6.38-8.02 (m , 10H , Ar-H); 8.66 (s , 2H , 2CH=N)
5		4.20 (s , 5H , C_5H_5); 4.28 (d , 2H , J = H_Z , H_2 and H_5); 4.35 (d , 2H , , H_3 and H_4) 6.15-7.96 (m , 4H , Ar-H); 8.45 (s , 1H , CH=N); 9.09 (s , 2H , NH_2)
6		4.16 (s , 10H , 2 C_5H_5); 4.35 (d , 4H , $2H_2$& $2H_5$); 4.85 (d , 4H , $2H_3$& $2H_4$) 6.20-7.91 (m , 4H , Ar-H); 8.60 (S , 2H , 2 CH=N)
7		2.50 (s , 3H , CH_3) ; 4.40 (s , 5H , C_5H_5) ; 4.71 (s , 2H , $H_2$and H_5); 4.90 (s , 2H , H_3 and H_4); 6.55-7.68 (m , 4H , Ar-H) ; 9.54 (s , 2H , NH_2)
8		2.98 (s , 6H , $2CH_3$); 4.40 (s , 10H , 2 C_5H_5); 4.71 (s , 4H , $2H_2$&$2H_5$); 4.92 (s , 4H , $2H_3$&$2H_4$) 6.60-7.49 (m , 4H , Ar-H)

In vitro antibacterial screening is generally performed by the disc diffusion method against two types of bacteria: Gram positive *Staphylococcus aureus* ATCC 25923 and Gram negative *Escherichia coli* ATCC 25922 bacteria for the primary selection of the complexes as a therapeutic agents and compared with commercial drug, cisplatin.

The results of antibacterial activity of Pd(II) complexes in the concentration of [250 µg/ml] and minimum inhibitory concentration MIC (define as the lowest concentration of the compound in a medium without visible growth of the test organisms) in concentration ranging from [1 -250 µg/ ml] are shown in *Table* 3.

The results of this study indicate that all Pd(II) –complexes have variable antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli* bacteria. In comparison with the ligands[24], the Pd(II) complexes were found to be more antimicrobial activity. In general, the results of screening show that most of Pd(II)- complexes which contain imino groups (*i.e.* complexes **3**-**8**) are more antibacterial active than those which contain amine groups(*i.e.* complexes **1** and **2**) . On the other hand, the antibacterial activity of Pd(II)- complexes which contain ferrocenyl moiety, and has a better activity against both *Staphylococcus aureus and Escherichia coli bacteria ,* in which complex **5** showed a maximum activity against both tested bacterial strains, whereas complex **4** which contain furan moiety has worst against both tested bacterial strains. From the present investigation we can conclude that antibacterial activity of Pd(II) complexes is ordered as follows: **5** > **7** > **6** > **8** > **3** > **4** >**1** > **2**

Table 3: *In vitro* antibacterial activity and minimum inhibitory concentration (MIC) values of the Pd(II) complexes and cisplatin against *Staphylococcus aureus and Escherichia coli bacteria*

Staphylococcus aureus									
	Concentration (µg/ml)								
complex	250	200	150	100	50	25	10	1	MIC
1	28	24	17	13	7	NI	NI	NI*	50
2	27	23	15	12	5	NI	NI	NI	50
3	34	30	21	15	11	7	NI	NI	25
4	32	30	19	14	9	5	NI	NI	25
5	50	43	34	28	20	13	9	7	1
6	38	28	20	17	13	10	5	NI	10
7	48	40	34	28	19	11	6	NI	10
8	47	36	28	20	11	8	NI	NI	25
cisplatin	25	19	14	11	8	NI	NI	NI	50
Escherichia coli									
	Concentration (µg/ml)								
complex	250	200	150	100	50	25	10	1	MIC
1	12	8	NI	NI	NI	NI	NI	NI	200
2	8	5	NI	NI	NI	NI	NI	NI	200
3	17	14	11	8	NI	NI	NI	NI	100
4	14	12	10	8	NI	NI	NI	NI	100
5	40	32	26	18	13	9	6	NI	10
6	21	17	14	10	7	NI	NI	NI	50
7	35	26	17	12	8	6	NI	NI	25
8	18	15	13	9	6	NI	NI	NI	50
cisplatin	28	21	18	13	9	7	NI	NI	25

*NI= No Inhibition

It is known that, the complexes containing Schiff base ligands tend to act as more powerful and potent bactericidal agents to kill the microorganism. The explanation of that in the complex, the metal has partially positive charge coordinate with the donor atom of ligand as well as there is π-electron delocalize over the whole chelate ring.[48, 49] This, in turn, increase the lipophilic character of the metal chelate and favors its permeation through the lipoid layers of the microorganism membranes. Apart from this, other factors, such as solubility conductivity and dipole moment may also be the possible reasons for increasing this activity.

In order to determine the binding which can occur between the prepared complexes and human DNA, we studied the change of concentration of the prepared complexes 1-8 (2 ml of 80 µM) when added human DNA (2 ml of 80 µg) in different times (0, 1h, 2h, 24h, 1 week) by UV.-Visible spectroscopy. The data confirm the binding between the synthesized complexes and human DNA by decreasing the absorbance of bands which adopt to complexes 1-8. when the time increase, the drop in absorbance be greater. These data encourage to use these complexes as drug alternatives.

Conclusions

In summary, we have prepared a eight of bidentate amine and Schiff base palladium(II) complexes. The resulting complexes assume a neutral square planar configuration in *cis*-form. We are examine the biological activity for antibacterial activity of all the prepared complexes 1-8 beside their interaction with human DNA. The antibacterial activity of compounds **1-8** against two types of bacterial : the first negative towards Gram stain (*i.e. Escherichia coli*) and against positive towards Gram stain (*i.e. Staphylococcus aureus*) were tested. These data proved that all Palladium(II) complexes show a significant antibacterial activity for growth inhibition of *Staphylococcus aureus* and *Escherichia coli* bacteria. Antibacterial activity of all Pd(II) complexes show slightly active against G(-ve)bacteria and has a better activity against G(+ ve) bacteria which complex **5** showed a maximum activity against both tested bacteria strains 40 and 50 mm ,respectively. On the other hand, DNA interaction study of complexes 1-8 with human DNA showed that all these complexes are binding with DNA which is enhances the probability of using these complexes as drug alternatives.

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