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Synthesis, characterization, antibacterial and antifungal evaluation of some new platinum(II) complexes of 2-phenylbenzimidazole ligands

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A series of platinum(II) complexes of the type $[PtL_2Cl_2]$ **1**, $[PtL_2I_2]$ **2**, $[PtL_2(oxalato)]$ **3**, and $[PtL_2(malonato)]$ **4** (L= 2-phenylbenzimidazole ligands as "non-leaving groups") were synthesized and characterized by their elemental analyses, IR, ^{1}H NMR, and ESI-LC/MS. The in vitro antimicrobial activity of the synthesized compounds has been evaluated by the macrodilution method against gram-positive bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis;* gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia*; and yeast like fungi, such as *Candida glabrata* and *Candida parapsilosis*. In general, based on the data obtained in this study, synthesized complexes **1-4** might be taken into consideration as promising antifungal compounds.

Key Words: Antibacterial activity, antifungal activity, benzimidazole, carboxylato complexes, platinum(II) complexes.

Introduction

The coordination chemistry of platinum(II) has attracted a considerable attention due to its biological applications and its use in tumor treatment based on the early discovery of antitumor activity of cisplatin [*cis*-

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diamminedichloroplatinum(II)].[1,2] There are still difficulties related to its use because of its numerous side effects and toxicity.[3,4]

There is continuing interest in the development of new platinum complexes that are less toxic and have a broader spectrum of activity.[5] Variations in the nature of the amine/ammine can have a significant effect on the activity and toxicity of these complexes.[6] Several platinum complexes with N-heterocyclic ligands, such as imidazole, thiazole, benzimidazole, benzoxazole, and benzothiazole have been reported.[7–16]

Cisplatin is not very soluble in water and it can hydrolyze inside the cell, where the chloride concentration is low and the pH is neutral, to produce several species, including toxic hydroxo-bridged dimers or other oligomers.[17] A good review of the influence of structure on the activity and toxicity of platinum anticancer drugs was published.[18] The replacement of the chloro ligands by carboxylato ligands produces less toxic compounds with increased solubility in water.[19]

A second generation antitumor platinum compound, carboplatin [*cis*-diammine(1,1-cyclobutanedicarboxylatoplatinum(II)] is now commercially available in several countries. It is more soluble than cisplatin and produces an equally strong antitumor effect, but at a markedly higher dosage due to the delayed formation of the active aquated metabolites.[20] It is reported that decreased reactivity reduces the nephrotoxic and neurotoxic side effects of cisplatin. Moreover, decrease in reactivity may also lead to reduced detoxification reactions by intracellular thiols.[21] Therefore, carboxylate platinum complexes seem to be more promising than the corresponding chloro analogs. Several other amine-carboxylatoplatinum(II) compounds have also good antitumor activities like oxaliplatin [*trans*-R,R-cyclohexane-1,2-diamine oxalatoplatinum(II)] and malonatoplatin [1,2-diaminocyclohexanemalonatoplatinum(II)].[22–24]

Benzimidazole moiety is structurally related to purin bases and found in a variety of naturally occurring compounds, such as vitamin B_{12}. Substituted benzimidazole derivatives possess a range of biological activities, such as antitumor, antiparasitic, antiviral, and antimicrobial activities; this area of research has been recently reviewed.[25] In this respect, biologically important ligand benzimidazole derivatives together with transition metal complexes have been synthesized and evaluated for their antifungal and antibacterial activities.[26]

The present study is an extension of the previous studies on the synthesis and the biological activities of the platinum(II) and platinum(IV) complexes with the 2-non-or substitutedbenzimidazole ligands.[27–34] In the previous studies, it was determined that some of these complexes have in vitro cytotoxic activities on *RD*,[28] *HeLa*,[30,32–34] *HEp-2*,[34,35] and *MCF-7*[29,30,32–34] cell lines and in vitro antimicrobial,[28] mutagenic,[27,29] and genotoxic[31] activities. The effect of the some of these platinum (II)[33] and platinum (IV)[34] complexes on pBR322 plasmid DNA was also studied by gel electrophoretic mobility measurements.

In this study we report the synthesis and characterization of platinum(II) complexes with 2-phenylbenzimidazole as non-leaving ligand, and chloro, iodo, oxalate, and malonate as leaving groups as well as screening for their in vitro antimicrobial activity on gram-positive and gram-negative bacteria and yeast like fungi.

Experimental

Chemistry

Materials

All chemicals and solvents used in the synthesis were purchased from Merck and Aldrich Chemical Co.

Melting points were measured on an Electrothermal 9200 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Varian 1000 FTIR spectrometer in the range of 4000–600 cm^{-1}. Elemental analyses were performed with a LECO 932 CHNS analyzer. ^1H nuclear magnetic resonance (^1H NMR) spectra were recorded in dimethylsulfoxide-d_6 (DMSO-d_6) on a Varian Mercury 400 MHz FT NMR spectrometer. Electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS) spectra were taken on a Water Micromass ZQ connected with Waters Alliance HPLC, using ESI(+) method at Central Laboratuvary of the Faculty of Pharmacy, Ankara University (Ankara, Turkey). Thin-layer chromatography was performed on pre-coated aluminum plates Merck Silica Gel 60 F_{254}. Plates were visualized by ultraviolet light, Dragendorff's reagent or iodine vapor.

General procedure for preparation of disilver salts of malonic or oxalic acid

Disilver oxalate or disilver malonate were synthesized according to the reported methods.[23,36] Malonic (20 mmol) or oxalic acid (20 mmol) and $AgNO_3$ (40 mmol) was added to a 1 N NaOH solution (40 mL) by stirring. The mixture was stirred at room temperature in the dark overnight. The resulting white silver salts were filtered and dried thoroughly in vacuo.

Synthesis of ligand L and Pt(II) Complexes 1-4

2-Phenylenzimidazole (L)

L, used as carrier ligand in the structure of **1-4**, was prepared according to the published procedure.[37,38] Mp 293 °C, (288.5-291 °C)[39]; yield 59%. ^1H NMR (400 MHz, DMSO-d_6): δ 12.94 (broad s, 1H, N-H, exchangeable with D_2O), 8.23-8.21 (d, 2H, ArH), 7.48-7.58 (m, 5H, ArH, phenyl), 7.23-7.22 (m, 2H, ArH). IR (KBr, cm^{-1}): υ 3100-2530 (N-H) cm^{-1}.

[Dichloro-di(2-phenylbenzimidazole)platinum(II)].1.5H_2O [PtL_2Cl_2]. 1.5H_2O (1)

A solution of **L** (0.815 g, 4.20 mmol) was added into a stirred aqueous solution of K_2PtCl_4 (1.00 g, 2.41 mmol) in ethanol—water mixture (12:8 mL) by dropwise over 1 h at room temperature. The pH was adjusted to ~7 and kept constant with the addition of 0.1 M $NaHCO_3$. The reaction mixture protected from light was heated at 60 °C for 7 days. After that time the mixture was cooled to room temperature. The resulting crude precipitate was filtered off, purified by repeated washing with small portions of water, ethanol, and diethyl ether, in that order, and dried in vacuo. Yield 55%. ^1H NMR (DMSO-d_6): δ (ppm) 13.34 (s, 2H, 2x N-H, exchangeable with D_2O), 8.91-8.89 (m, 1H, ArH), 8.28-8.22 (m, 3H, ArH), 7.91-6.76 (m, 14H, ArH), IR (KBr): υ 3255-3052 (N-H, O-H) cm^{-1}, MS (ESI +) m/z: 653.33 $[M\text{-}H]^+$, calcd. 653.44, 583.47 $[M\text{-}2Cl]^+$,calcd. 583.54. Anal. Calcd. for $C_{26}H_{20}Cl_2N_4Pt$. 1.5 H_2O: C, 45.82; H, 3.40; N, 8.22. Found: C, 45.99; H, 3.35; N, 8.06.

[Diiodo-di(2-phenylbenzimidazole)platinum(II)] [PtL_2I_2] (2)

K_2PtCl_4 (1.00 g, 2.40 mmol) and KI (1.59 g, 9.60 mmol) were dissolved in water (15 mL) and stirred at 60 °C for 45 min. Then a solution of **L** (0.815 g, 4.20 mmol) in ethanol—water mixture (12:8 mL) was added dropwise over 1 h at room temperature to the resulting K_2PtI_4. The reaction mixture, protected from light, was heated at 60 °C for 2 days. The resulting yellowish precipitate was filtered off, washed several times with small portions of water, ethanol, and diethyl ether, and dried in vacuo. Yield 37%. ^{1}H NMR (DMSO-d_6): δ (ppm) 13.35 (s, 2H, 2x N-H, exchangeable with D_2O), 8.90-8.88 (m, 1H, ArH), 8.67-8.10 (m, 3H, ArH), 7.71-6.73 (m, 14H, ArH), IR (KBr): υ 3259 (N-H) cm^{-1}, MS (ESI +) m/z: 711.35 [M-I+H]$^+$, calcd. 711.46, 581.39 [M-2I-2H]$^+$,calcd. 581.53. Anal. Calcd. for $C_{26}H_{20}I_2N_4Pt$: C, 37.29; H, 2.41; N, 6.69. Found: C, 37.58; H, 2.55; N, 6.56.

[Oxalato-di(2-phenylbenzimidazole)platinum(II)] [PtL_2(oxalato)] (3)

A suspension of **1** (0.272 g, 0.40 mmol) and disilver salt of oxalic acid (0.121 g, 0.40 mmol) in water (25 mL) was stirred at 50 °C in the dark for 10 days. The resulting silver chloride was filtered through a millipore filter (0.22 μM). The filtrate was concentrated under a reduced pressure to ~10 mL in volume. The white precipitate formed was filtered off, washed with small portions of water, and dried in vacuo. Yield 13%.

^{1}H NMR (DMSO-d_6): δ (ppm) 13.23 (s, 2H, 2x N-H, exchangeable with D_2O), 8.22-8.16 (m, 3H, ArH), 7.60-6.89 (m, 15H, ArH). IR (KBr): υ 3180 (N-H), 1673 (C=O), 1419 (C-O), cm^{-1}, MS (ESI +) m/z: 671.78 [M]$^+$, calcd. 671.56. Anal. Calcd. for $C_{28}H_{20}N_4O_4Pt$: C, 50.08; H, 3.00; N, 8.34. Found: C, 50.52; H, 3.45; N, 8.74.

[Malonato-di(2-phenylbenzimidazole)platinum(II)] [PtL_2(malonato)] (4)

A procedure similar to that described for **3** was carried out using **1** and disilver salt of malonic acid (0.136 g, 0.20 mmol and 0.06 g, 0.2 mmol) at 50 °C for 11 days. Yield 22%. ^{1}H NMR (DMSO-d_6): δ (ppm) 13.20 (s, 2H, 2x N-H, exchangeable with D_2O), 8.19-8.15 (m, 3H, ArH), 7.57-6.85 (m, 15H, ArH), 6.3-5.5 (m, 2H, -CH_2-, exchangeable with D_2O). IR (KBr): υ 3160 (N-H), 1690 (C=O), 1415 (C-O), cm^{-1}, MS (ESI +) m/z: 639.18 [M- O-C=O -2H]$^+$, calcd. 639.56, 581.78 [M-malonato]$^+$, calcd. 581.53. Anal. Calcd. for $C_{29}H_{22}N_4O_4Pt$: C, 50.80; H, 3.23; N, 8.17 Found: C, 50.45; H, 3.68; N, 8.15.

Biological activity

In vitro antimicrobial activities of the synthesized compounds **L** and **1-4** were tested against gram-positive [*Staphylococcus aureus* (ATCC 25813), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (Clinical isolate)] and gram-negative [*Pseudomonas aeruginosa* (ATCC 25853), *Escherichia coli* (ATCC 25923), *Klebsiella pneumonia* (Clinical isolate)] bacteria, and fungi [*Candida glabrata* (ATCC 4322), *Candida parapsilosis* (ATCC 22019)] using the macrodilution broth method.[40,41]

Stock solutions of the compounds **L** and **1-4** were prepared in DMSO at 1000 μg/mL. Tests were carried out at pH 7.4 using the 2-fold serial dilution technique. Antibacterial and antifungal activity tests were performed in Mueller-Hinton broth (Merck) and Triptic soy broth (Merck) respectively, for microorganisms and

incubated for 24 h at 37 ± 1 °C and the final inoculum size was 10^5 CFU/mL. After incubation for bacteria and fungi for 24 h and 48 h at 37 ±1 °C, respectively, minimum inhibitory concentration (MIC) values were recorded on the lowest concentrations of the compounds that had no visible turbidity for bacteria and fungi. Ampicilline $3H_2O$ and fluconazole nitrate, purchased from Sigma Co, were used as reference in antibacterial and antifungal activity test, respectively.

Results and discussion

Chemistry

The carrier ligand of the complexes synthesized, benzimidazole **L**, was prepared according to the published procedures.[37,38] Its melting point was in accordance with the literature.[39]

The procedures of the synthesis of platinum(II) complexes **1** and **2** are shown in the Figure. Platinum(II) complex **1** was synthesized as described previously by the reaction of **L** and K_2PtCl_4 in ethanol/water solution. IR and ^{1}H NMR spectral data of the complex **1** was in accordance with the data reported previously.[28]

Reaction of K_2PtCl_4 with an excess amount of KI produced K_2PtI_4 in solution. K_2PtI_4 was then reacted with 2 equivalents of **L** to obtain platinum(II) complex of the type $[Pt(\mathbf{L})_2I_2]$ (Compound **2**).

The other platinum(II) complexes $[PtL_2(oxalato)]$ **3** and $[PtL_2(malonato)]$ **4** were prepared by reaction of the dichloroplatinum complex **1** with the disilver salt of oxalic and malonic acid, respectively, as shown in the Figure.

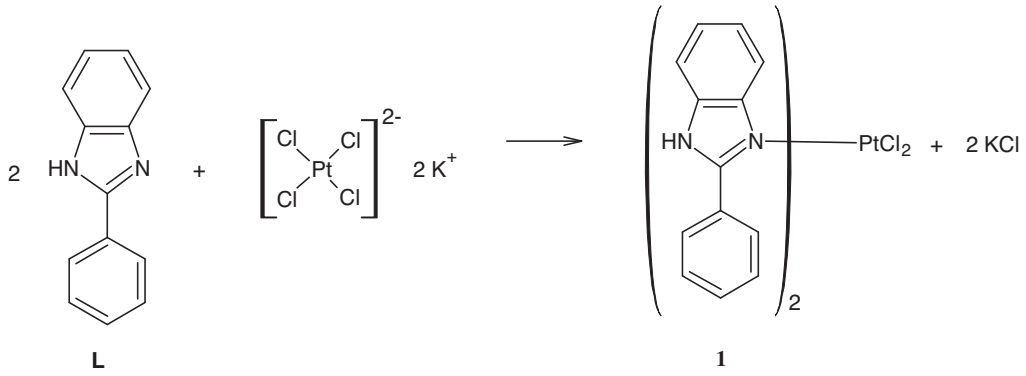
The structures of Pt(II) complexes (Compounds **1–4)** were evaluated using their spectral data, such as IR, ^{1}H NMR and ESI-LC/MS, and elemental analysis results. The elemental analysis data for each complex were in good agreement with the empirical formula proposed. In the IR spectra of all complexes (Compounds **1-4**), prominent changes were observed.[23] For complex **1** molecule of water was included as justified by the IR and elemental analysis results.

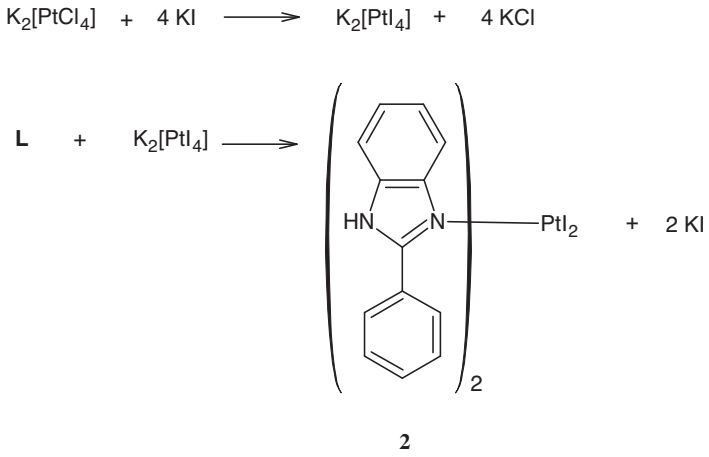
In the IR spectrum of **L**, a very broad band was seen in the region of 3100-2530 cm^{-1} due to the imidazole N-H. The complexes 1-4 exhibited υ (N—H) stretching bands centered at 3255-3160 cm^{-1} sharper than that of the free ligand due to breaking of tautomerism, indicating that the N-H group was not involved in the coordination.[42,43]

According to the kinetic transeffect,[44] the synthesis method used are expected to yield complexes with cis geometry. The υ (Pt—Cl) and υ (Pt—I) bands of the dichloro and diiodo complexes (Compounds **1** and **2**) should be observed at 320-330 and 195-183 cm^{-1} in the far-IR region of the complex's spectra.[45]

The carbonyl regions for the dicarboxylato complexes (Compounds **3** and **4**) displayed patterns characteristic of carboxylate ligands bound to the platinum metal ion.[46] The carboxylate group of the complexes (Compounds **3** and **4**) shows 2 bands due to an intense asymmetric carboxylate stretching υ (C=O) and a symmetric stretching υ (C—O), at about 1673, 1690 and 1419, 1415 cm^{-1}, respectively. Trends in the positions and separation between these bands are the most useful tool in assigning structures from IR spectra.

The insolubility of the complexes in the other organic solvents made it necessary to record ^{1}H NMR spectra in DMSO-d_6. All ^{1}H NMR measurements were recorded immediately in order to avoid the ligand exchange reaction between the platinum complexes synthesized and DMSO-d_6. The ^{1}H-NMR spectral data of





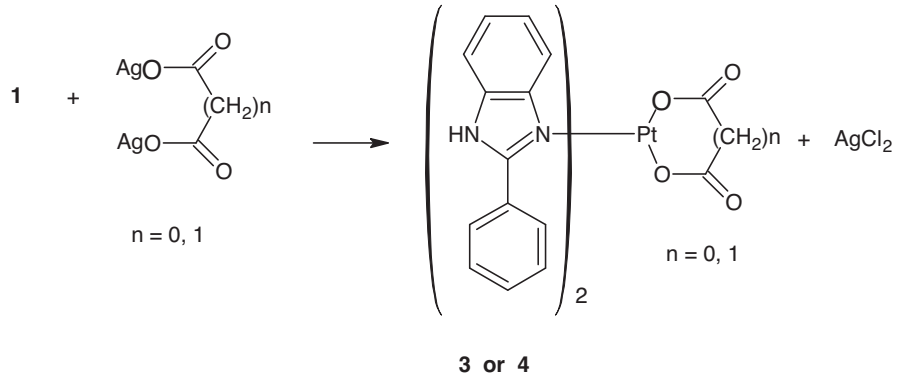


Figure. Synthesis of the platinum(II) complexes.

the ligand and the complexes are presented in the experimental section. The ^{1}H NMR spectra of all complexes were consistent with their corresponding protons as chemical shift values and the number of hydrogen. When the spectra of the complexes were compared with that of the free ligand, considerable differences were observed. The large downfield shifts in the imidazole N-H signal in the spectra of all complexes respect to their ligands are

a result of an increase in the N-H acid character after platinum binding. For the complex **4**, the malonato-CH_2-protons exchanged quite rapidly with the D atoms of the solvents DMSO-d_6. Therefore, the spectrum showed a low intensity multiplet for the –CH_2- (or -CDH- or -CD_2-), which is consistent with the literature.[17,23]

Both the retention times and the MS spectra of the peaks in samples are evidence of the purity and the excepted structures of the synthesized compounds **1-4**. Because the of 3 isotopes of Pt element, all the ESI-LC/MS spectra of the platinum complexes were found with 3 protonated ion isotopic peaks.[47]

Antimicrobial activity test

Since cisplatin is used as an effective anticancer drug, the interest in platinum group metal complexes containing N-donor ligands, which were found to be promising as antibacterial and antifungal agents, have increased.[48–53] The first report of the possible antibacterial properties of this group was that ruthenium-phenantroline complexes exerted activity against both gram-positive and gram-negative bacteria.[54] The effect of platinum species on *Escherichia coli* produced by platinum electrodes in the growth medium led to the exploitation of platinum complexes as potential anticancer drugs, the most successful of which was cisplatin.[1]

In the present study, synthesized platinum(II) complexes **1-4** were tested for their antibacterial activities against gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis,* and gram-negative *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* bacteria, and the antifungal activity against *Candida glabrata*, *Candida parapsilosis* using the macrodilution broth method. Ampicilline $3H_2O$ and Fluconazole nitrate was used as reference drugs for in vitro antibacterial and antifungal activity tests, respectively.

The minimal inhibitory concentrations (MIC) of the test compounds are presented in the Table. It was found that MIC values obtained from antibacterial and antifungal activity tests of platinum complexes are more effective than that of 2-phenylbenzimidazole ligand.

Table. In vitro antibacterial and antifungal activity of the platinum(II) complexes **1-4** and carrier ligand **L**.

Minimal Inhibitory Concentrations (μM)								
	Gram (+)			Gram (-)				
Compound	A	B	C	D	E	F	G	H
L	1.28	1.28	1.28	1.28	1.28	1.28	1.52	1.52
1	0.18	0.18	0.18	0.18	0.02	0.03	0.18	0.18
2	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
3	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
4	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Amp.	0.007	0.004	0.01	0.007	0.009	0.004	-	-
Fluc.	-	-	-	-	-	-	0.50	0.50

A*: Staphylococcus aureus* (ATCC 25813), **B**: *Bacillus subtilis* (ATCC 6633), **C***: Enterococcus faecalis* (Clinical isolate) **D**: *Pseudomonas aeruginosa* (ATCC 25853), **E**: *Escherichia coli* (ATCC 25923), **F**: *Klebsiella pneumonia* (Clinical isolate), **G**: *Candida glabrata* (ATCC 4322), **H**: *Candida parapsilosis* (ATCC 22019), Amp.: Ampicilline $3H_2O$, Fluc.: Fluconazole nitrate

The Pt(II) complexes **1** and **2** bearing chloro and iodo ligands, respectively, as leaving groups were found to show higher antibacterial activity than the other platinum(II) complexes **3**-**4** bearing oxalate and malonate ligands, respectively, as leaving groups on the gram-positive and gram-negative bacteria used.

The result of in vitro antimicrobial activity studies indicates that in general the following order of relative in vitro antifungal activity of the compounds tested could be considered: **2**>**1**>**3**> fluconazole>**4**>**L**. These results suggest that compound **1**-**4** may be worth studying further in terms of their antifungal activities.

In general, synthesized complexes **1**, **2,** which have more lipophilic leaving ligands in their structure compared to complexes **3**, **4,** were found to have higher antibacterial and antifungal activity than **3**, **4**. It might be thought that lipophilic character facilitate their crossing the bacterial cell membranes more efficiently and reach their cellular targets.[55,56] In conclusion the platinum(II) complexes tested seem to have the potential to be used as antifungal agents but further in vitro and in vivo experiments are required to verify their antimicrobial activities.

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