Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2021, 14(1), 306-313
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2021, 14(1), 306-313
DOI: 10.18185/erzifbed.875774
Research Article

Synthesis and Cytotoxic Activity Evaluation of New Metal Complexes

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Geliş / Received: 07/02/2021, Kabul / Accepted: 16/03/2021

Abstract

Salicylaldehyde and 1-amino-5-(4-methylbenzoyl)-4-*p*-tolylpyrimidin-2(1*H*)-one were used for preparing a Schiff base, which is 1-(2-hydroxybenzylideneamino)-5-(4-methylbenzoyl)-4-*p*-tolylpyrimidin-2(1*H*)-one (**S1**). Three new complexes (Ag(I), Pd(II), and Pt(II)) were synthesized from this chelate ligand (**S1**). The Ag(I) complex was screened against the human colon cancer cell line, and the silver complex was found to have more cytotoxic activity than a standard drug used.

Keywords: Cytotoxic activity; Cancer; Metal complexes; Schiff base.

Yeni Metal Komplekslerinin Sentezi ve Sitotoksik Aktivite Değerlendirmesi

Öz

Salisilaldehit ve 1-amino-5-(4-metilbenzoil)-4-*p*-tolilpirimidin-2(1*H*)-on bir Schiff bazı olan 1-(2-hidroksibenzilidenamino)-5-(4-metilbenzoil)-4-*p*-tolilpirimidin-2(1*H*)-on (S1) 'u hazırlamak için kullanıldı. Üç yeni kompleks (Ag(I), Pd(II) ve Pt(II)) bu şelat ligantdan (S1) sentezlendi. Ag(I) kompleksi insan kolon kanser hücre hattına karşı tarandı ve gümüş kompleksinin kullanılan standart ilaçtan daha fazla sitotoksik aktiviteye sahip olduğu bulundu.

Anahtar Kelimeler: Sitotoksik aktivite; Kanser; Metal kompleksleri; Schiff bazı.

1. Introduction

Schiff bases and their metal complexes are significant compounds in terms of chemistry. They have broad ranges of biological activity applications. For example, some metal complexes including zinc, cadmium, cobalt, palladium, silver or mercury have antimicrobial (Dorosti et al., 2019; İlkimen et al., 2016; Mallela et al., 2018; Sarı et al., 2016), antifungal (Biswas et al., 2014; Ghazal et al., 2019; Yousef et al., 2016), antioxidant (Fetoh et al., 2018; Yousef et al., 2016), and anticancer (Akkoç, 2019a; Ali et al., 2019; Aslan et al., 2020; Bahron et al., 2019; Yaghobi et al., 2019) activities. Furthermore, these type compounds have also many different properties such as catalytic activity, porosity, magnetism and conductivity (Pettinari et al., 2012; Correa-

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Ayala et al., 2019; Gangu et al., 2019; Madhupriya et al., 2014; Akkoç et al., 2014). As an example to catalytic activity studies, compounds based Schiff bases are used as catalyst in oxidation reactions, which are one of the significant reactions in organic chemistry. Gupta and Sutar studied the catalytic activity properties of Co(II), Fe(II), Ni(II), and Pd(II) Schiff base complexes. The authors tested catalytic effects of complexes in the following reaction types; allylic alkylations, hydrosilation, decomposition of hydrogen peroxide, isomerization (Gupta et al., 2008). Karvembu et al. synthesized ruthenium(II) type, [Ru(CO)(B), (L)] (B = complexes of ASPH, pyridine, piperidine or morpholine) tetradentate Schiff bases and tested their catalytic activity (Karvembu et al., 2003).

Three new metal complexes were synthesized in the present study. The structures of the related complexes were characterized by 1H NMR, 13C NMR, IR, LC-MS. The biological activities of the compounds S1 and Ag(I) were screened towards human colon colorectal adenocarcinoma (DLD-1) (ATCC® CCL-221TM) cell line, which was purchased from ATCC (USA), using MTT assay method. The results showed that the silver complex had higher cytotoxic activity with an IC50 value of 5.98 μM than the ligand (S1) and standard drug used.

2. Material and Method

2.1. General considerations

The silver chloride (AgCl), potassium tetrachloropalladate(II) (K_2PdCl_4) and potassium tetrachloroplatinate(II) (K_2PtCl_4) salts were used for the synthesis of the complexes. Ethyl alcohol and diethyl ether were used as solvents. The solutions of complexes were prepared in chloroform for conductivity studies. A Mattson 1000 IR spectrometer, an Agilent 1100 (LC-MS), a Bruker 400 MHz Spectrometer, a Perkin Elmer Diamond TG/DTA thermal analyzer and a Siemens WPA C35 conductometer were used.

2.2. General procedure for the synthesis

Compound **S1** was prepared according to our previous study protocol (Aslan et al., 2018). 10 mL of ethyl alcohol was added to over 2.10^{-4} mmoles of **S1**. The reaction was conducted at 60 ºC. After that, K_2PdCl_4 and K_2PtCl_4 were prepared in the chloroform-ethyl alcohol mixture. The AgCl solution was prepared in a 1:1 mixture of pure water-ethyl alcohol. The solutions of the prepared salts were added drop wise to the ligand solution. The reaction mixture was refluxed for 2h. After this process, the temperature was dropped, and the reaction was conducted at 25 ºC for 24h. After completion of the synthesis procedures, the solvents in the reaction medium were aspirated. The complexes obtained were washed several times with warm ethyl alcohol and dried in desiccators.

2.2.1. Bis[(1-(2-hydroxybenzylideneamino)-5-(4-methylbenzoyl)-4-p-tolylpyrimidin-2(1H)-one] chloroargentat(I)

Color: white; yield: 51%; m.p.: 174 °C. IR (cm^{-1}) ν(CH)aro: 3045, ν(C-N): 1640, ν(C-OH): 1264, ν(Ag-N): 602. Conductivity (Λ_0, ohm^{-1} cm^2 mol^{-1}): 124. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.62 (s, 2H, -OH), 9.37 (s, 2H, -CH), 8.63 (s, 2H, -$CH_{pyr.ring}$), 7.82-6.93 (m, 24H, ArH), 2.50, 2.49, 2.33 and 2.27 (s, 12H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 191.71, 170.65, 164.24, 159.05, 151.68, 148.64, 144.58, 141.26, 134.97, 134.72, 134.51, 130.44, 129.75,

129.34, 129.16, 128.94, 120.09, 118.35, 117.20, 116.05, 21.63 and 21.36. LC/MS (m/z): Ag(I) [M^+]-5H: 990.2, found: 985; [M^+]-Cl: 952, found: 955.

2.2.2. Bis[(1-(2-hydroxybenzylideneamino)-5-(4-methylbenzoyl)-4-p-tolylpyrimidin-2(1H)-one] palladium(II) chloride

Color: dark yellow; yield: 52%; m.p.: 277 °C. IR (cm^{-1}) ν(CH): 2917, ν(C-N): 1658, ν(C-OH): 1270, ν(Pd-N): 590. Conductivity (Λ_0, ohm^{-1} cm^2 mol^{-1}): 268. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.62 (s, 1H, -OH), 9.39-6.38 (m, 29H), 2.50, 2.31 and 2.26 (s, 12H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 191.77, 149.07, 144.38, 140.72, 134.80, 134.69, 130.24, 129.81, 129.67, 129.21, 129.11, 115.44, 21.59 and 21.30. LC/MS (m/z): [M+]: 1022.42; [M^+]-Cl: 986.92, found 986.70.

2.2.3. [(1-(2-hydroxybenzylideneamino)-5-(4-methylbenzoyl)-4-p-tolylpyrimidin-2(1H)-one] dichloro platinum(II)

Color: grey; yield: 41%; m.p: 204 °C. IR (cm^{-1}) ν(CH)aro: 3065, ν(CH): 2922, ν(C-N): 1663, ν(C-OH): 1260, ν(Pt-N): 588. Conductivity (Λ_0, ohm^{-1} cm^2 mol^{-1}): 250. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.68 (s, 1H, -OH), 9.54 (s, 1H, -OH), 9.37 (s, 1H, -CH), 8.68 (s, 1H, -CH), 8.63 (s, 1H, -$CH_{pyr.ring}$), 8.39 (s, 1H, -$CH_{pyr.ring}$), 7.66-7.09 (m, 24H, Ar-H), 2.50, 2.32 and 2.23 (s, 12H, -CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 191.76, 169.32, 153.59, 149.05, 144.37, 140.71, 134.81, 134.70, 130.23, 129.67, 129.21, 129.10, 115.45, 21.59 and 21.30. LC/MS (m/z): [M^+]: 688.55, found: 688; [M^+]-2Cl: 618.55, found 618.

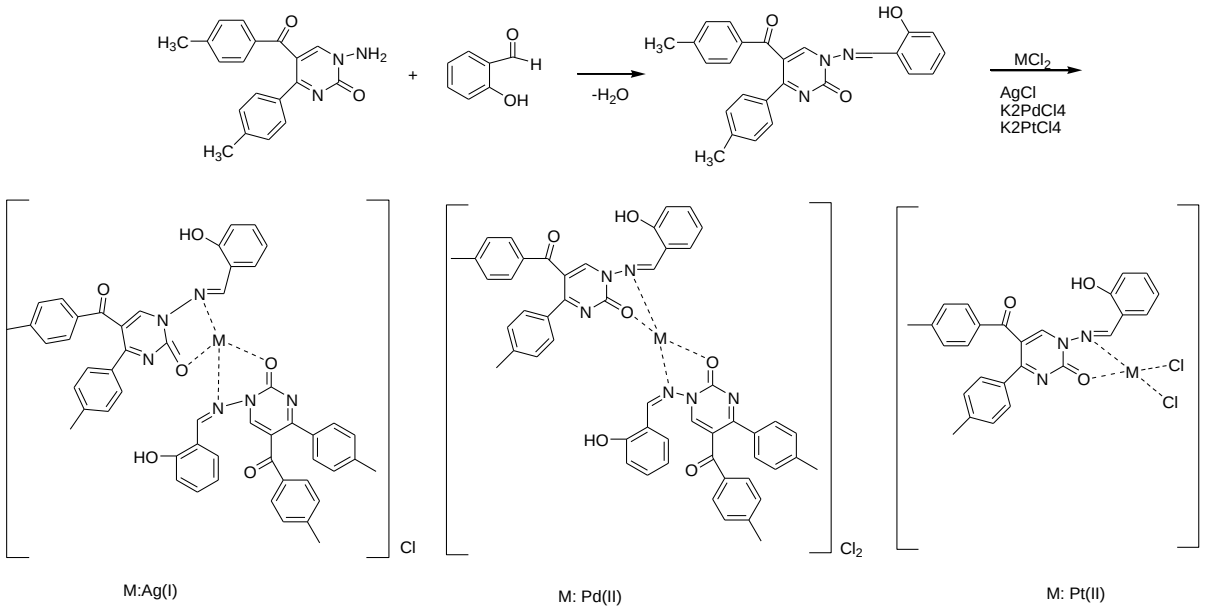
2.3. Cytotoxic activity studies

The cytotoxic activity procedure was performed according to the method in the literature (Akkoç, 2019b, 2021). The DLD-1 cells were cultured in Dulbecco's modified eagle's medium-high glucose (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% glutaMAX, and 1% penicillin-streptomycin. The cells were seeded into sterile 96-well plates at a density of 5 x 10^3 cells/well. The cells were treated with the ligand **S1** and silver complex at six different concentrations (5, 10, 20, 50, 100 and 200 μM). Following this, the 96-well plates were incubated for 48 h. After the incubation completed, the medium in each well was carefully aspirated. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) stock solution (5 mg/mL, 50 μL) was added and the plates were incubated for further 3h. Then, 200 μL dimethyl sulfoxide (DMSO) was added into each well, and the absorbance values were measured at 560 nm.

3. Results and Discussion

3.1. Characterization of complexes

According to IR and 1H NMR data, the ligand acts as a neutral bidentate ligand, coordinating through its nitrogen and oxygen atoms. The structure of complexes can be seen in Scheme 1.



Scheme 1. The open structures of complexes.

In the IR spectrum, the intensity ν(C=N) stretching vibrations for ligand was observed at 1650 cm^{-1}. It was shifting to the lower wave number for the Ag(I), Pd(II), Pt(II) complexes, and observed at 1601.64, 1602.53, 1602.44 cm^{-1}, respectively. This result indicates that the imine nitrogen is bound to the metal. The second bond with the metal was thought to be with the ligands -C=O group in the pyrimidine ring.

Conductometric measurements (S/M) were taken in 10^{-3} M of complexes at 25 ºC. According to these results, the Ag(I) complex gave two ions. Pd(II) and Pt(II) complexes gave three and one ions, respectively. Molar susceptibility (B.M.) studies were carried out at 20 °C. The molar susceptibility of the Ag(I), Pd(II), and Pt(II) complexes were obtained as 0 B.M. The Ag(I) complex is tetrahedral, the Pd (II) and Pt (II) complexes are square planar.

3.2. Cytotoxic activity evaluation

The cytotoxic activity studies of compounds **S1**, Ag(I) for evaluating the relative IC_{50} values expressed in μM concentration were screened against the DLD-1 cell line. However, the biological activity studies of Pd(II) and Pt(II) complexes could not be performed because they were not completely dissolved in 0.5% DMSO. Cisplatin was used as a standard drug. The results are given in Table 1.

Table 1. IC_{50} results for compounds against a human cell line.

Compounds	IC_{50} (μM)
	DLD-1
S1	126.30
Ag(I)	5.98
Cisplatin	57.58

The compounds screened in the colon cancer cell line showed antiproliferative activity for 48h at determined concentrations between 200 and 5 μM.

Starting matter (**S1**) inhibited DLD-1 cell growth, and IC_{50} value was found to be 126.30 µM. Silver complex had more toxic effects than **S1** and cisplatin. Ag(I) complex displayed cytotoxic effect at very low micro molar concentration with a considerable IC_{50} value of 5.98 µM. For this reason, the silver complex is promising, and it can be developed for further studies in colon cancer as well as other cancer types.

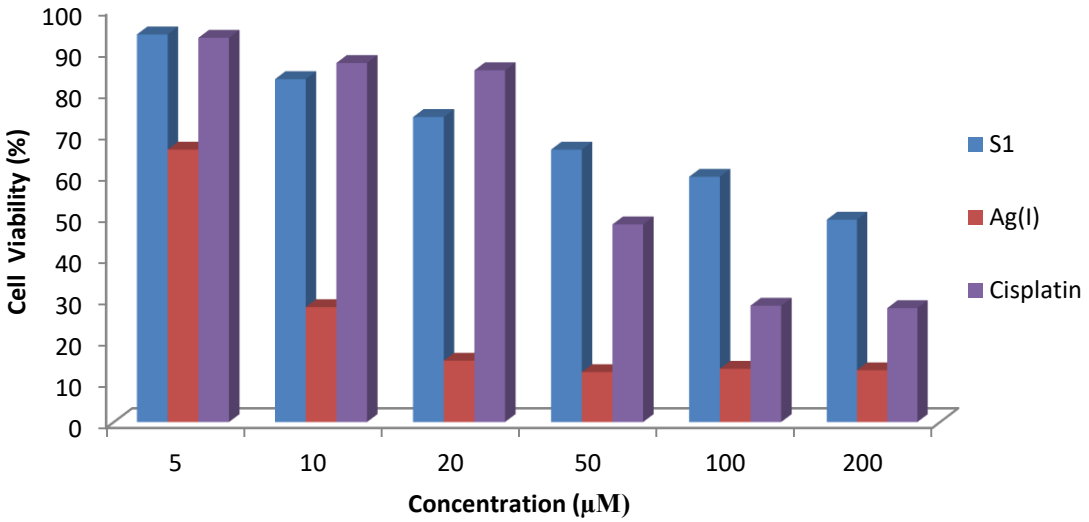


Figure 1. Cell viability ratio of DLD-1 cells after applying with drug candidates.

The results of the cytotoxic activity studies revealed that the cell viability of DLD-1 is dose-dependent. The above figure shows that the Ag(I) complex exhibited excellent growth inhibitory potential on DLD-1 cells, even a concentration of 10 µM. While cell viability of DLD-1 was 65% at 5 µM of Ag(I), this rate decreased to 27% at 10 µM of the same compound. At 20 µM concentration of Ag(I), it dropped to 14%. A sharp change in cell viability ratio was not observed with the concentration change of **S1**.

4. Conclusion

In the present investigation, three new metal complexes Ag(I), Pd(II) and Pt(II) were synthesized and characterized. The antiproliferative effect of the ligand and silver complex was tested on human colon cancer cell line. The silver complex was found to have antiproliferative effect at low micro molar concentration towards DLD-1 cells, and the IC_{50} value for it was calculated as 5.98 µM. Furthermore, Ag(I) complex demonstrated higher cytotoxic activity compared to the standard drug cisplatin.

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgment

The authors are grateful to Erciyes University's Research Foundation (FBA-2013-4659) for financial support.

Authors' contribution

G. Aslan designed the study, conducted all experiments of metal complexes, and data analysis.

S. Akkoç conducted biological activity studies, and wrote the paper.

Z. Kökbudak synthesized and analyzed the starting material (ligand).

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