European Journal of Medicinal Chemistry 45 (2010) 4215–4220

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Palladium(II) and platinum(II) complexes of a symmetric Schiff base derived from 2,6,diformyl-4-methylphenol with N-aminopyrimidine: Synthesis, characterization and detection of DNA interaction by voltammetry

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ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form
8 June 2010
Accepted 10 June 2010
Available online 17 June 2010

Keywords:
Pd(II) and Pt(II) complexes of
N-aminopyrimidine Schiff base
Synthesis
Characterization
DNA interaction
Voltammetry
Guanine and adenine oxidation

ABSTRACT

The two new mononuclear complexes were prepared by reacting symmetric Schiff base, containing pyrimidine rings and the metal chlorides of Pd(II) and Pt(II) in methanol. The mononuclear structure of the complexes was confirmed on the basis of elemental analyses, magnetic susceptibility, IR, UV–Vis, NMR, DTA/TGA and API-ES mass spectral data. The interaction of these metal complexes with fish sperm double-stranded DNA (dsDNA) was studied electrochemically based on the oxidation signals of guanine and adenine. Differential pulse (DP) voltammetry by using renewable pencil graphite electrode was employed to monitor the DNA interaction at the surface or in solution. The results indicate that Pd(II) and (Pt) complexes with Schiff base ligand having pyrimidine rings strongly interacted with DNA.

1. Introduction

During the last few decades there has been great interest in the chemistry of transition metals associated with nitrogen, oxygen and sulfur donor ligands, for instance, both five and six membered heterocyclic pyrazoles, pyridazines and pyrimidines [1–4]. Although extensive studies have been made on various pyridine-derived ligands [5–8], comparatively less research has centered on the transition metal chemistry with the Schiff base ligands having pyrimidine as the heterocyclic part. However, the higher π-acidity and presence of more than one hetero atom in pyrimidine play an important role in its coordination chemistry compared to that of pyridine bases and serve as better models in biological systems [9–12].

For over 40 years, the studies of metal complexes have attracted much attention due to their potential applications as anticancer, antitumor, antimutagenic and antiviral agents [13] Therefore, the researches on their interaction with DNA, that is the primary biological target of drugs, are of great importance. Development of novel clinical drugs involving metal complexes has been facilitated by the extensive knowledge of inorganic chemists about the metal coordination compounds.

The investigation of the interaction between DNA and other molecules is helpful to understand the action mechanism of some DNA-targeted drugs and toxic agents as well as the origins of some diseases like gene mutation [14–20]. Basically, a molecule may bind to DNA via covalent and/or noncovalent interactions (intercalative, electrostatic and groove binding) [21–24]. In recent years, there is a growing interest in the application of electrochemical techniques [25–30] to understand such interactions due to simplicity, cheapness, fast detection and require small amount of sample with respect to the commonly used spectroscopic methods [31–34].

Keeping the above knowledge in mind, the aim of this study is to prepare and characterize two new palladium(II) and platinum(II) complexes of Schiff base ligand derived from *N*-aminopyrimidine and 2,6-diformyl-4-methylphenol. In continuation of the study, special attention will be given to the application of electrochemical measurements based on the interaction of these metal complexes with DNA via differential pulse voltammetry using disposable pencil graphite electrode.

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doi:10.1016/j.ejmech.2010.06.016

2. Results and discussion

2.1. Chemistry

This work is an extension of recently studied symmetric Schiff base containing pyrimidine rings and its Fe(II), Cu(II), Ni(II) and Co(II) complexes in our laboratory [35]. The ligand and two complexes [Cu(II) and Co(II)] were found to show good biological activity against all tested bacteria and yeast strains. The report presented in this study includes synthesis and spectral characterization of two new palladium and platinum complexes of the formula $[PdCl_2(HL)]$ and $[PtCl_2(HL)]\cdot 4H_2O$ with the same Schiff base, and their interaction with DNA. As shown below, it was observed a square planar geometry around the both complexes, in which the ligand acts as a neutral bidentate one, coordinating through oxygen atoms of carbonyl groups.

The proposed structures of the mononuclear Schiff base complexes, $[PdCl_2(HL)]$ and $[PtCl_2(HL)]\cdot 4H_2O$, synthesized in the present study are given in Fig. 1. Both complexes were found to be very stable at room temperature in the solid state, and generally soluble in DMF and DMSO.

The corresponding data for the ligand and its complexes were presented in the Experimental section. This Schiff base had donor sites with the ONONO sequence and varied coordination abilities. This nature of the Schiff base attracted our attention and aroused our interest in elucidating the structure of metal complexes.

The metal-to-ligand ratio of bidentate Pd(II) and Pt(II) complexes was found to be 1:1 and the two additional chloride ligands were coordinated to metal ions. Molar conductance values of the Pd(II) and Pt(II) complexes were 42.8 and 31.9 Ω^{-1} cm^2 mol^{-1}, respectively. The complexes which contain coordinated chloride ions are non-conducting or show an increasing degree of conductivity with time which would be due to their replacements by DMF solvent molecules.

Comparison of the IR spectra of the free ligand with that of its Pd(II) and Pt(II) complexes, showed a approximately the same bands for stretching of $(C{=}O)_{benzoyl}$, $(OH)_{phenolic}$ and $(CH{=}N)_{azomethine}$ groups. These carbonyl stretching were found in free ligand at 1652, 1687 cm^{-1} while in the complexes at 1654 cm^{-1}. The absence of a band at 1687 cm^{-1} in the spectra of the complexes, in comparison to the free ligand, confirms the coordination through the carbonyl (C=O) group. On the other hand, in the spectra of a metal complexes, the band at 1273 cm^{-1} for the phenolic group (C–O) did not shift, suggesting that this oxygen atom of the phenolic group is not coordinated to the metal ion [35,36]. The ν(O–H)phenolic bands in the spectra of both complexes remain almost at the same position (~1270 cm^{-1}) suggesting that (C–O) phenolic group does not take part in complexation. This behavior may support bidentate coordination for Schiff base ligand through the carbonyl oxygen atoms to Pd(II) or Pt(II) ions (Fig. 1). This is also supported by the presence of ν(O–H) peak in the IR spectra and the appearance of the phenol proton peak in the ^{1}H NMR spectra of the complexes, which confirm that the ligand did not have lost phenolic proton.

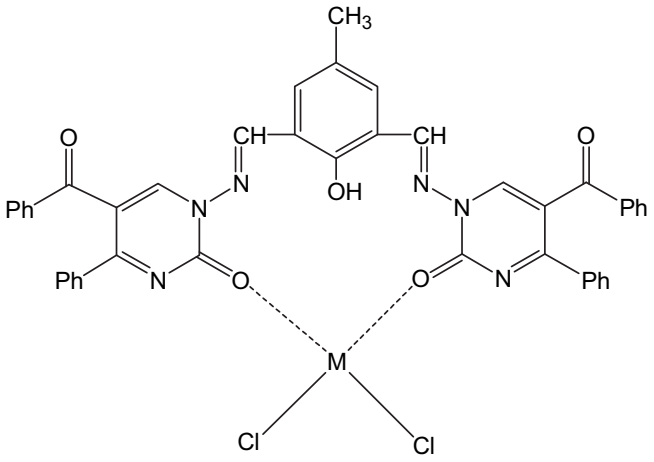

Fig. 1. Supposed structure of the Pd(II) and Pt(II) complex.

In the spectra of the complexes, the bands observed in the 445–470 cm^{-1} region might be due to ν(M–O) [35,36]. The IR spectra of the complexes were characterized by the appearance of a broad band in the region 3240–3350 cm^{-1} due to the ν(O–H) frequency of water of crystallization. This water was also identified by the elemental analyses. The Pt(II) complex had four additional water of crystallization and thermogravimetric analyses showed a percentage weight loss of 6.87 for this complex in the temperature range 65–95 °C.

DMSO-d_6 was used as a solvent to measure the ^{1}H NMR spectra of the ligand and its metal complexes. The ^{1}H NMR spectrum of the ligand showed signal at 2.35 ppm corresponding to the signals of CH_3. The sharp singlet observed at about δ 11.33 ppm due to phenolic proton of the ligand [35]. The singlet at δ 9.54 ppm and 8.85 ppm were due to azomethine and pyrimidine ring (C–H) protons in the spectrum of the ligand, respectively. The multisignals corresponding aromatic protons appeared between at δ 7.31 and 7.88 ppm. The ^{1}H NMR spectra of Pd(II) and Pt(II) complexes showed approximately the same peaks identical to those of the free ligand. The signal for the azomethine proton in the Pd(II) and Pt(II) complexes appears at 9.58 and 9.63 ppm, respectively. The aromatic protons due to phenyl rings have resonated in region δ 7.50–8.10 as a multiplet in complexes and these are shifted downfield with respect to the corresponding signal in the free ligand, indicating that the metal–oxygen bond is retained in solution.

The electronic spectra of complexes showed three d–d spin allowed transitions from the three lower lying 'd' levels to the empty $dx^2 - y^2$ orbital. The bands are attributed to $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$ and to $^1A_{1g} \rightarrow {}^1E_g$ transitions [37] (see Experimental section).

In the mass spectra of the ligand and its metal complexes, peaks were attributable to the molecular ions; *m/z*: 711.1 $[L]^+$, *m/z*: 885.0 $[PdCl_2 + LH]^+$, *m/z*: 1029.0 $[PtCl + LH + 3H_2O]^+$. The spectrum of the Pd(II) complex was shown in Fig. 2.

2.2. DNA interaction of the complexes

The redox behavior of tested compounds was investigated by DP voltammetry in 0.5 M acetate buffer pH 4.8. Voltammograms, which were monitored in blank supporting electrolyte (transfer voltammetry), obtained before/after interaction between 10 μg/mL^{-1} Pd(II) complex and 10 μg/mL^{-1} dsDNA are shown in Fig. 3 and those of Pt(II) complex and dsDNA, under similar conditions, in Fig. 4.

Pd(II) complex produced split oxidation peaks at around +0.81and +0.99 V, average two peak mean response 0.262 ± 0.012 μA ($n = 3$) with a pre-concentration step on pre-treated PG electrode at open circuit condition for 300 s at pH 4.8 as presented in Fig. 3-C. In the case of Pt(II) complex, a single oxidation peak appeared at around +0.88 V, mean response 0.167 ± 0.009 μA ($n = 3$) (Fig. 4-C).

The voltammetric measurements were also performed for free ligand and its metal complexes synthesized in reported work [35]. Ligand and its Cu(II), Ni(II) and Fe(II) complexes displayed similar DP voltammetric behavior, as shown by the Pd(II) complex while DP voltammograms of Co(II) were almost similar to those obtained for Pt(II) complex.

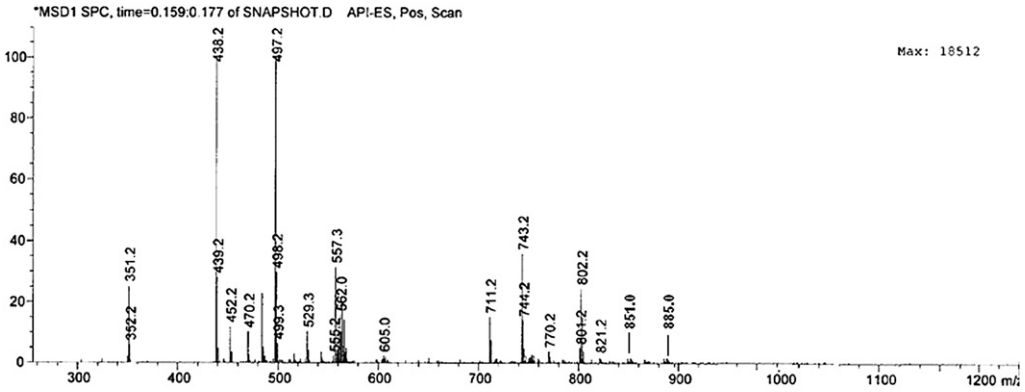

Fig. 2. API-ES spectrum of Pd(II) complex.

Under the conditions of the experiment, the redox behavior of original dsDNA exhibited one positive peak at around +1.03 V due to the oxidation of guanine residues, mean response 0.92 ± 0.054 μA ($n = 3$) (Figs. 3- and 4-B_G) and the second at around +1.29 V due to the oxidation of adenine residues, mean response 0.393 ± 0.036 μA ($n = 3$) (Figs. 3- and 4-B_A).

After interaction of complexes in solution, redox signals of guanine and adenine were decreased to ca. 75 and 88% for Pd(II) (Fig. 3-A_G and A_A) and 80 and 90% for Pt(II) (Fig. 4-A_G and A_A), respectively, comparing to the results obtained for dsDNA control solutions. On the other hand, after interaction of complexes at electrode surface corresponding peak currents were changed to ca. 50% for Pd(II) and 55% for Pt(II). The results obtained by the interaction in solution-phase were found to be much suitable for this study because of the stronger interaction (bigger bases signal changes). Thus, the assay protocol in solution-phase was chosen for remaining parts.

When the experiments were demonstrated for ligand and its other complexes in the assay conditions, the changes of all analyzed signals were negligible the compounds did not interact with DNA.

The reproducibility of DNA biosensor protocol was also evaluated based on guanine and adenine signals from three repetitive measurements for the concentration 10 μg/mL DNA and 10 μg/mL of complex. Measurements yielded reproducible results with relative standard deviations of 7.97 and 6.61% for guanine, and 12.2 and 11.59% for adenine after interaction of Pd(II) and Pt(II) complexes, respectively.

The dependence of the complex concentration (in the range 0–10 μg/mL) on guanine and adenine signals is shown in Figs. 5 and 6. In the presence of both complexes, guanine and adenine oxidation peak heights decreased gradually in the percentage of peak current ratio with concentration up to 10 μg/mL, and the signals (in special the adenine signal) almost disappeared at more concentrated solutions.

Using electrochemical DNA-based biosensors it is not possible to investigate the mode of binding of complexes to DNA. However, this method could be very useful for quick, comparative analyses of DNA interactions. Our results clearly show that the attracting ability of the Pd(II) and Pt(II) complexes to DNA is quite strong (the significantly decrease on bases oxidation signals). Moreover, guanine and adenine oxidation signal changes were almost the same for both Pd(II) and Pt(II) complexes. That is why we concluded that these complexes had the similar influence on double helix of DNA. It is well known that platinum complexes can react with DNA, by coordinating to N7 of guanine, forming the classical intra-strand and inter-strand Pt-DNA cross-links that are responsible for the cellular damage [29,38–43]. In recent years, researchers also were focused on the palladium complexes owing to their similar coordination geometry and character to that for platinum complexes [44–46].

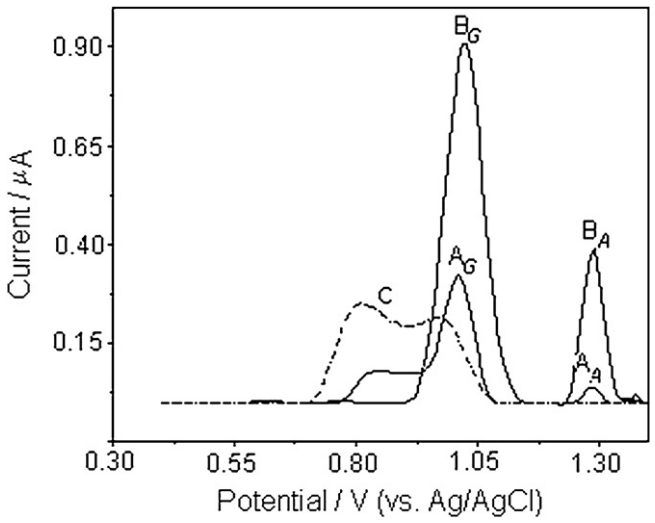

Fig. 3. DP voltammograms in supporting electrolyte pH 4.8 of (C) Pd(II) complex at bare PG electrode, and control dsDNA (B) before and (A) after interaction with Pd(II) complex in solution. *G*, guanine; *A*, adenine. See Section 4.4, for details.

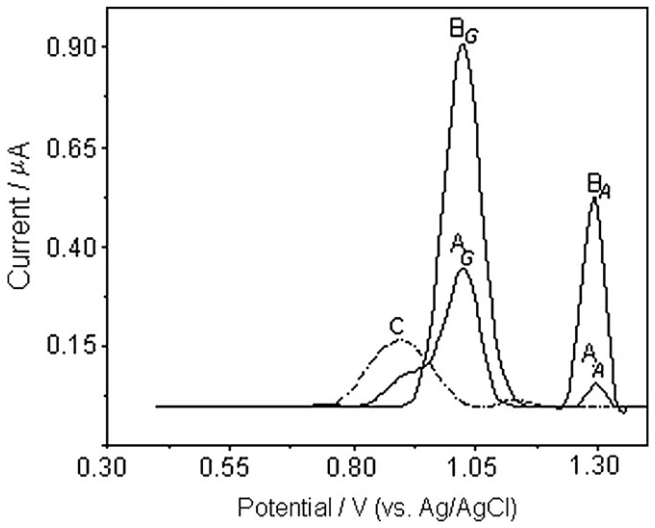

Fig. 4. DP voltammograms in supporting electrolyte pH 4.8 of (C) Pt(II) complex at bare PG electrode, and control dsDNA (B) before and (A) after interaction with Pt(II) complex in solution. *G*, guanine; *A*, adenine. See Section 4.4, for details.

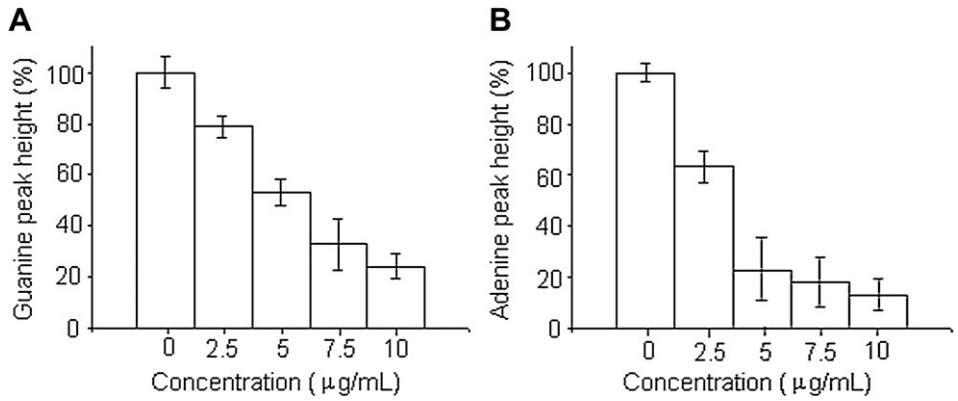

Fig. 5. The effect of Pd(II) complex upon the DP voltammetric response of guanine (A) and adenine (B) after interaction of solution-phase DNA The average values of the peak current ratio are presented in the histograms with the error bars See Section 4.4, for details.

It has been previously reported that DNA binding levels of $[PdCl_2(HL)]$ [47] and $[PtCl_2(HL)]$ [48] complexes were higher than those for $[Pd(L)_2]$ and $[Pt(L)_2]$ complexes. The former complexes, having labile chloride ligands could probably interact with DNA becoming activated through equation and reacting with nucleophilic DNA bases. Due to the planar motive contained in the $[MCl_2(HL)]$ complexes, intercalation could also be possible.

3. Conclusion

Palladium and platinum complexes have proven their effect on tumor cells and their ability to bind DNA as main antitumoral mechanism of action [49,50]. Taking into consideration the similarities between platinum and palladium, in this study two new palladium and platinum complexes with a phenol based Schiff base bidentate symmetric ligand which was recently reported, were synthesized and characterized. Compounds were tested for their DNA interaction ability with the fish sperm DNA using DP voltammetry at PG electrode. The determined DNA binding levels for platinum complex were found to be similar to those of palladium one, showing the strong interaction with DNA. However, these are preliminary observations and a more extensive study would be necessary in order to assert that the complexes act as cleavage agents.

Bearing in mind that the structures of several antitumor agents contain pyrimidine rings, results of this work can show that the approach of coordinating pyrimidine derivatives with pharmacologically interesting metals such as palladium and platinum could be a suitable strategy to develop novel therapeutic tools for the medical treatment [51].

4. Experimental

4.1. Materials

All common laboratory chemicals used in the synthesis of ligand and its metal complexes were purchased from commercial sources and used without further purification. The compounds 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one (*N*-aminopyrimidine, *N*-AP) and 2,6-diformyl-4-methylphenol (dfp) were prepared using previously published methodologies [52,53].

Complexes were tested for their DNA interaction ability using double-stranded fish sperm DNA (dsDNA) which was kindly provided by Sigma. The stock solution of the dsDNA (1000 μg/mL) was prepared in TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00), and stored at +4 °C. The solutions were then diluted to the desired concentration by mixing buffer supporting electrolyte. The stock solutions (1000 μg/mL) of ligand and its metal complexes were prepared daily in DMSO. Solutions of different concentrations of either ligand or its metal complexes were freshly prepared before each experiment by dilution of the appropriate quantity in supporting electrolyte. The supporting electrolyte solutions were 0.5 M acetate buffer pH 4.8 containing 20 mM NaCl. All aqueous solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system ($p > 18$ MΩ cm^{-1}).

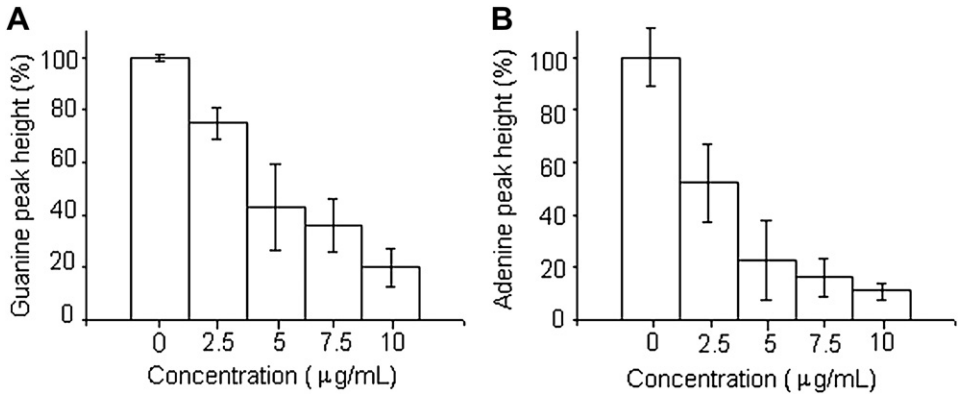

Fig. 6. The effect of Pt(II) complex upon the DP voltammetric response of guanine (A) and adenine (B) after interaction of solution-phase DNA The average values of the peak current ratio are presented in the histograms with the error bars. See Section 4.4, for details.

4.2. Instrumentation

The elemental analyses (C, H, N, S) were performed by using a Leco CHNS model 932 elemental analyzer. The IR spectra were obtained using KBr pellets (4000–400 cm^{-1}) on a Bio-Rad-Win-IR Spectrophotometer. The electronic spectra in the 200–900 nm range were recorded in DMF on a Unicam UV2-100 UV–Vis spectrophotometer. Magnetic measurements were carried out by the Gouy method using $Hg[Co(SCN)_4]$ as a calibrant. Molar conductance of the Schiff base ligand and its transition–metal complexes were determined in DMF at a room temperature by using a Jenway model 4070 conductivity meter. Thermogravimetric (TGA) measurements were obtained by a Shimadzu-50 thermal analyzer. The ^1H NMR and ^{13}C NMR spectra of the Schiff base were carried out using a Bruker 300 MHz Ultrashield TM NMR instrument. LC/MS-API-ES mass spectra were recorded with an Agilent model 1100 MSD mass spectrophotometer.

Differential pulse (DP) voltammograms were recorded with the aid of a μAutolab type III electrochemical analyzer with GPES 4.9 software package (Eco-Chemie, The Netherlands). The raw data were also treated in all DP voltammetric measurements by using Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a peak width of 0.01 V. Measurements were carried out in a home-made 5-mL glass cell using the pencil graphite (PG) electrode (active electrode area: 15.9 mm^2), with platinum wire counter electrode, and a Ag/AgCl (3 M NaCl) (Model RE-1, BAS, USA) as reference. For PG electrode, a mechanical pencil Model T 0.5 (Rotring, Germany) was used as a holder for pencil lead (Tombo, Japan), which were purchased from a local stationary. The details of its preparation have been described in our previous study [54]. Before use, glass cell was soaked into 3 M HNO_3, and rinsed several times with water and acetate buffer. The convective transport was provided with a magnetic stirring of 300 rpm.

4.3. Preparation of compounds

4.3.1. Preparation of the ligand (HL)

The Schiff base ligand (HL), [1-((*E*)-((*E*)-3-((*E*)-(5-benzoyl-2-oxo-4-phenylpyrimidine-1(2*H*)-yl imino) methyl)-2-hydroxyl-5-methylbenzilidin)amino)-5-benzoyl-4-phenylpyrimidine-2(1*H*)-on] was prepared by condensation between *N*-AP and dfp and characterized according to the literature method [35], but its synthesis is here briefly described. To a solution of 0.164 g (1 mmol) of dfp in hot ethanol (25 mL) was added 0.582 g (2 mmoL) of N-AP dissolved in 25 mL of hot ethanol and then mixed slowly with constant stirring. This mixture was refluxed on a water bath at 3 h and cooled. The precipitate was filtered, washed with hot ethanol and diethyl ether, and then dried in vacuum over P_2O_5. The product yielded yellow precipitate of HL (0.462 g, 65%); mp: 273 °C. *Anal.* Calc. for $C_{43}H_{30}N_6O_5$ (710): C, 72.67; H, 4.25; N, 11.82. Found: C, 72.20; H, 4.36; N, 11.66%. Selected IR data, (ν, cm^{-1}): 3400 (OH), 1687 $(-C{=}O)_{pyrimidine}$ 1652 (Ph–CO–), 1608 (HC=N); ^1H NMR (d_6-DMSO, ppm), δ 11.33 (s, 1H, OH), 9.54 (s, 1H, HC=N), 8.85 (s, 1H, C(6)H, 7.31–7.88 (m, 7H, Harm); 2.35 (s, 3H, CH_3); ^{13}C NMR (d_6-DMSO, ppm), δ 192.06 (OC–Ar), 179.31 (C=O, pyrimidine), 163.64 (-C6, pyrimidine ring), 157.38 (HC=N), 151.54–116.20 (C, aromatic), 20.22 (CH_3). LC-MS, *m*/*z* 711.1 [M + 1].

4.3.2. Preparation of the complexes $[PdCl_2HL]$ and $[PtCl_2HL]\cdot 4H_2O$

$[PdCl_2HL]$ was prepared according to the following procedure. First, a solution of $[PdCl_4]^{2-}$ was made by boiling $PdCl_2$ (0.5 mmol, 0.090 g) in concentrated HCl (5 mL), cooling and then diluting with distilled water (15 mL). Second, a hot solution of HL (0.5 mmol, 0.355 g) in the mixture of chloroform and acetone (100 mL; 1:1, v/v) was added to this $[PdCl_4]^{2-}$ solution. The mixture was refluxed for 30 h. The precipitated brown compound was removed by filtration, washed with diethyl ether and followed by cold methanol/water and dried in vacuum desiccators. Yield: 0.279 g (63%); mp: 300 °C. *Anal*. Calc. for $C_{43}H_{30}Cl_2N_6O_5Pd$ (886.07): C, 58.16; H, 3.40; N, 9.46. Found: C, 58.60; H, 3.71; N, 9.30%. Selected IR data (ν, cm^{-1}): 3400 (O–H)phenolic, 1654 ν(Ph–CO), 1599 (C=N). ^1H NMR (d_6-DMSO, ppm), δ 11.32 (s, 1H, OH), 9.63 (s, 1H, HC=N), 9.06 (s, 1H, C(6)H, 7.54–8.10 (m, 7H, Harm); 2.39 (s, 3H, CH_3). μ_{eff}: Dia. Λ_M (10^{-3} M, in DMF, S cm^2 mol^{-1}): 42.8. UV–Vis (in DMF, nm): 239, 254, 278, 294, 347, 460, 616. API-ES, *m*/*z*: 885 $[M]^+$ (^{106}Pd isotope).

The complex, $[PtCl_2HL]\cdot 4H_2O$, was synthesized in an identical manner as that described above using $PtCl_2$ (0.5 mmol, 0.133 g). $[PtCl_2HL]\cdot 4H_2O$ is orange-brown color compound. Yield: 0.150 g (51%); mp: 235 °C. *Anal*. Calc. for $C_{43}H_{38}Cl_2N_6O_9Pt$ (1047.17): C, 49.24; H, 3.65; N 8.01. Found: C, 48.86; H, 3.54; N, 7.67%. Selected IR data (ν, cm^{-1}): 3406 ν(O–H/H_2O), 1654 ν(–Ph–C=O), 1597, 1578 ν (C=N). ^1H NMR (d_6-DMSO, ppm), δ 12.03 (s, 1H, OH), 9.58 (s, 1H, HC=N), 8.97 (s, 1H, C(6)H, 7.51–8.27 (m, 7H, Harm); 2.33 (s, 3H, CH_3). μ_{eff}: Dia. Λ_M (10^{-3} M, in DMF, S cm^2 mol^{-1}): 31.9. UV–Vis (in DMF, nm): 229, 281, 309, 355, 389, 451. API-ES, *m*/*z*: 1029 $[M + 3H_2O]^+$ (^{194}Pt isotope).

4.4. DNA biosensor procedure

The biosensing protocol at the DNA-modified electrode consisted of the electrode pre-treatment, DNA immobilization, interaction with metal complexes, and its DP voltammetric transduction. The guanine and adenine oxidation peaks were used as the transduction signals. DP voltammograms were recorded after the transfer of the electrode into a blank supporting electrolyte. Three replicate measurements were carried out for each experiment. All data were obtained at room temperature.

Electrode pre-treatment: The PG electrode surface was pretreated by applying a potential of +1.40 V for 1 min in the blank supporting electrolyte without stirring, in order to increase the hydrophilic properties of the electrode surface through introduction of oxygenated functionalities, accomplished with an oxidative cleaning.

DNA immobilization: The dsDNA was immobilized onto the pretreated PG electrode surface by adsorptive accumulation for 5 min at +0.50 V in supporting electrolyte containing 10 μg/mL of DNA under stirred conditions. The following washing step involved dipping the electrode in a clean supporting electrolyte for 5 s (control dsDNA-modified PG electrode).

Interaction of surface-confined DNA with metal complexes: After the DNA immobilization step described above, the dsDNA-modified PG electrode was transferred to the stirred supporting electrolyte containing metal complex concentration of 10 μg/mL. The adsorption, at open circuit conditions, was allowed to proceed for 5 min. The biosensor was then rinsed for 5 s in a clean supporting electrolyte.

Interaction of solution-phase DNA with metal complexes: Metal complex in the concentration level of 10 μg/mL (unless otherwise indicated) was added to supporting electrolyte containing 10 μg/mL DNA. A PG electrode was first pre-treated as described above and subsequently immersed into the mixture solution. The accumulation of the mixture was performed for 5 min while holding the potential at +0.50 V under stirred conditions. The electrode was then washed with a clean supporting electrolyte for 5 s.

Signal transduction: The oxidation signals of guanine and adenine were measured by using DP voltammetry with the following parameters: amplitude 50 mV, step potential 8 mV, scan rate16 mV/s, between +0.45 and +1.40 V in a fresh supporting electrolyte. Successive measurements were carried out by

repeating the above assay protocol on a new PG electrode surface using a new stripping solution and cell.

References

[1] M. Viciano-Chumillas, S. Tanase, G. Aromí, J.M.M. Smits, R. de Gelder, X. Solans, E. Bouwman, J. Reedijk, Eur. J. Inorg. Chem. (2007) 2635–2640.
[2] S. Roy, T.N. Mandal, A.K. Barik, S. Pal, S. Gupta, A. Hazra, R.J. Butcher, A.D. Hunter, M. Zeller, S.K. Kar, Polyhedron 26 (2007) 2603–2611.
[3] N.A. Salih, Turk. J. Chem. 32 (2008) 229–235.
[4] S. Roy, J.A. Westmaas, F. Buda, J. Reedijk, J. Inorg. Biochem. 103 (2009) 1278–1287.
[5] M. Sönmez, İ. Berber, E. Akbaş, Eur. J. Med. Chem. 41 (2006) 101–105.
[6] J.G. Tojal, B. Donnadieu, J.P. Costes, J.L. Serra, L. Lezama, T. Rojo, Inorg. Chim. Acta 333 (2002) 132–137.
[7] P.F. Rapheal, E. Manoj, M.R.P. Kurup, E. Suresh, Polyhedron 3 (2007) 607–616.
[8] K.A. Ketcham, J.K. Swearingen, A. Castineiras, I. Garcia, E. Bermejo, D.X. West, Polyhedron 20 (2001) 3265–3273.
[9] F. Zamora, M. Kunsman, M. Sabat, B. Lippert, Inorg. Chem. 36 (1997) 1583–1587.
[10] M. Louloudi, Y. Deligiannakis, J.P. Tuchagues, B. Donnadien, N. Nadjiliadis, Inorg. Chem. 36 (1997) 6335–6342.
[11] F. Jolibois, J. Cadet, A. Grand, R. Subra, N. Raga, V. Barone, J. Am. Chem. Soc. 120 (1998) 1864–1871.
[12] A.R. Katritzky, C.W. Pees, A.J. Boulton, C. Mckillop, J. Heterocycl. Chem. 3 (1984) 57–68.
[13] B. Rosenberg, L. VanCamp, T. Krigas, Nature 222 (1969) 385–386.
[14] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65 (1993) 2317–2323.
[15] J.J. Gooding, Electroanalysis 14 (2002) 1149–1156.
[16] T.G. Drummond, M.G. Hill, J.K. Barton, Nat. Biotechnol. 21 (2003) 1192–1199.
[17] J. Wang, Anal. Chim. Acta 469 (2002) 63–71.
[18] D. Pang, H.D. Abruna, Anal. Chem. 70 (1998) 3162–3169.
[19] B. Jin, X. Ji, T. Nakamura, Electrochim. Acta 50 (2004) 1049–1055.
[20] D.E. Thurston, Brit. J. Cancer 80 (1999) 65–85.
[21] R.F. Pasternack, E.J. Gibbs, J.J. Villafranca, Biochemistry 22 (1983) 2406–2414.
[22] K.E. Erkkila, D.T. Odom, J.K. Barton, Chem. Rev. 99 (1999) 2777–2795.
[23] L.S. Ling, Z.K. He, F. Chen, Y.E. Zeng, Talanta 59 (2003) 269–275.
[24] X.Q. He, Q.Y. Lin, R.D. Hu, X.H. Lu, Spectrochim. Acta A 68 (2007) 84–190.
[25] J. Pastor, J.G. Siro, J.L. Garcia Navio, J.J. Vaquero, J. Alvarez Builla, F. Gago, B. de Pascual Teresa, M. Pastor, M.M. Rodrigo, J. Org. Chem. 62 (1997) 5476–5483.
[26] C.G. Coates, L. Jacquet, J.J. McGarvey, S.E.J. Bell, A.H.R. Alobaidi, J.M. Kelly, J. Am. Chem. Soc. 119 (1997) 7130–7136.
[27] S. Lecomte, M.H. Baron, Biospectroscopy 3 (1997) 31–45.
[28] Y. Cao, X.W. He, Spectrochim. Acta A 54 (1998) 883–892.
[29] E.J. Gao, H.X. Yin, M.C. Zhu, Y.G. Sun, X.F. Gu, Q. Wu, L.X. Ren, J. Struct. Chem. 49 (2008) 1048–1054.
[30] R.F. Pasternack, C. Bustamante, P.J. Collings, A. Giannetteo, E.J. Gibbs, J. Am. Chem. Soc. 115 (1993) 5393–5399.
[31] R.F. Pasternack, P.G. Coollings, Science 269 (1995) 935–939.
[32] C.Z. Huang, Y.F. Li, X.L. Hu, N.B. Li, Anal. Chim. Acta 395 (1999) 187–197.
[33] J.K. Barton, Comments Inorg. Chem. 3 (1985) 321–348.
[34] M.A.A.F.de C.T. Carrondo, M. Coll, J. Aymami, A.H.J. Wang, G.A. van der Marel, J.H. van Boom, A. Rich, Biochemistry 28 (1989) 7849–7859.
[35] M. Sönmez, M. Çelebi, İ Berber, Eur. J. Med. Chem. 45 (2010) 1935–1940.
[36] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds. Wiley, New York, 1986.
[37] A.B.P. Lever, Inorganic Electronic Spectroscopy, second ed.. Elsevier, Amsterdam, 1984.
[38] A.M. Oliveria Breet, H.P. Serrano, A. Macedo, D. Raimundo, M.H. Marques, M.A. La-Scalea, Electroanalysis 8 (1996) 992–995.
[39] V. Brabec, Electrochim. Acta 45 (2000) 2929–2932.
[40] A. Erdem, B. Kosmider, R. Osiecka, E. Zyner, J. Ochocki, M. Ozsoz, J. Pharm. Biomed. Anal. 38 (2005) 645–652.
[41] A.M.J. Fichtinger-Schepman, J.L. Van der Veer, J.H.J. Den Hartog, P.H.M. Lohman, J. Reedijk, Biochemistry 24 (1985) 707–713.
[42] D.Z. Yang, S.S.G.E. Van Boom, J. Reedijk, H.J. Van Boom, A.H.-J. Wang, Biochemistry 34 (1995) 12912–12920.
[43] J.-M. Malinge, M.-J. Giraud-Panis, M. Leng, J. Inorg. Biochem. 77 (1999) 23–29.
[44] G. Zhao, H. Lin, Y. Ping, H. Sun, S. Zhu, S. Xunchenge, Y. Chen, J. Inorg. Biochem. 73 (1999) 145–149.
[45] G.B. Onoa, V. Moreno, E. Freisinger, B. Lippert, J. Inorg. Biochem. 89 (2002) 237–247.
[46] A.G. Quiroga, J.M. Perez, I. Lopez-Solera, E.I. Montera, J.R. Masaquer, C. Alonso, C. Navarro-Ranninger, J. Inorg. Biochem. 69 (1998) 275–281.
[47] L. Otero, M. Vieites, L. Boiani, A. Denicola, C. Rigol, L. Opazo, C. Olea-Azar, J.D. Maya, A. Morello, R.L. Krauth-Siegel, O.E. Piro, E. Castellano, M. González, D. Gambino, H. Cerecetto, J. Med. Chem. 49 (2006) 3322–3331.
[48] V. Brabec, Prog. Nucl. Acid Res. Mol. Biol. 71 (2002) 1–68.
[49] A.S. Abu-Surrah, H.H. Al-Sa'doni, M.Y. Abdalla, Cancer Ther. 6 (2008) 1–10.
[50] A.S. Abu-Surrah, Mini. Rev. Med. Chem. 7 (2007) 203–211.
[51] E. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451–2466.
[52] Y. Akçamur, B. Altural, E. Sarıpınar, G. Kollenz, O. Kappe, K. Peters, E. Peters, H. Schering, J. Heterocylic Chem. 25 (1988) 1419–1422.
[53] R.R. Gagne, C.L. Spiro, T.J. Smith, C.A. Hamann, W.R. Thies, A.K. Shiemke, J. Am. Chem. Soc. 103 (1981) 4073–4081.
[54] Y. Yardım, E. Keskin, A. Levent, M. Özsöz, Z. Şentürk, Talanta 80 (2010) 1347–1355.