Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 127 (2014) 261–267

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

DNA interaction and cytotoxic activities of square planar platinum(II) complexes with N, S-donor ligands

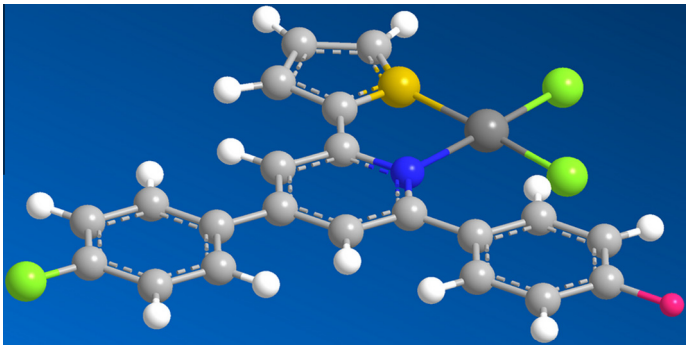
Mohan N. Patel *, Chintan R. Patel, Hardik N. Joshi, Khyati P. Thakor

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India

HIGHLIGHTS

- Synthesis of N, S-donor ligands and their Pt(II) complexes.
- Characterization by elemental analysis, IR, UV–visible, ^{1}H NMR and mass spectrometry.
- All Pt(II) complexes have been tested for their cytotoxic activity.
- The interaction of DNA with complexes has been performed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form 30 January 2014
Accepted 11 February 2014
Available online 27 February 2014

Keywords:
Pt(II) complexes
N, S-donor ligand
DNA binding
DNA cleavage
Unwinding
Cytotoxicity

ABSTRACT

The platinum(II) complexes with N, S-donor ligands have been synthesized and characterized by physicochemical methods viz. elemental, electronic, FT-IR, ^{1}H NMR and LC–MS spectra. The binding mode and potency of the complexes with HS DNA (Herring Sperm) have been examined by absorption titration and viscosity measurement studies. The results revealed that complexes bind to HS DNA via covalent mode with the intrinsic binding constant (K_b) in the range 1.37–7.76 × 10^5 M^{-1}. Decrease in the relative viscosity of HS DNA also supports the covalent mode of binding. The DNA cleavage activity of synthesized complexes has been carried out by gel electrophoresis experiment using supercoiled form of pUC19 DNA; showing the unwinding of the negatively charged supercoiled DNA. Brine shrimp (*Artemia Cysts*) lethality bioassay technique has been applied for the determination of toxic property of synthesized complexes in terms of μM.

Introduction

In the view of excellent anticancer efficiency of *cisplatin*, but at the same toxicity associated with its therapeutic applications, thousands of new platinum compounds have been synthesized and investigated. Several of them have been tested in clinical trials, and up to date only few have been approved for clinical use, e.g. carboplatin, tetraplatin, or oxaliplatin [1]. The goal behind the design of new platinum compounds is the synthesis of compounds that remain active against resistant cell lines, with a wider spectrum of antitumor activity and with a lower toxicity than *cisplatin*. It is generally accepted that the efficiency of *cisplatin* is based on its strong binding to DNA nucleobases resulting in a locally unwound and kinked helix [2,3].

In order to reduce the toxicity and to modulate activity of the known anticancer drug, *cisplatin*, a new strategy is the design of novel metal complexes containing N, S-donor ligands [4,5]. The interactions of heavy metals such as platinum, gold with N, S-donor atoms have been recognised for their anti-carcinogenic

* Corresponding author. Tel.: +91 2692 226856x218.
E-mail address: jeenen@gmail.com (M.N. Patel).

http://dx.doi.org/10.1016/j.saa.2014.02.053
1386-1425/

properties with the potential to develop metal-based drug [6,7]. This interest has probably initiated from detoxicant properties of sulfur-containing ligands against heavy metal intoxication [8]. The chemistry of N, S containing heterocyclic ligands with platinum group metals has been of considerable interest both from structural point of view [9,10] and also for their antitumor activity [11,12]. High tendency of platinum to bind sulphur and isolation of thiolate complexes (e.g., [Pt(methionine)$_2$] [13]) from the urine of *cisplatin* treated patients have been a driving force to explore platinum complexes with sulphur ligands so as to overcome the side effects of *cisplatin* and have improved toxicity and/or their applicability to a wide range of tumours [14].

In continuation of our previous work [15], we describe the synthesis and characterization of some N, S-donor ligand and their platinum(II) complexes. The mode and extent of interaction of complexes have been determined by viscosity measurements and absorption titration using HS DNA. Gel electrophoresis technique has been used to determine the unwinding angle of pUC19 DNA. The experimental studies provide information regarding nuclease behavior of synthesized metal complexes. Cytotoxic activity of the complexes has been carried out by Brine shrimp (*Artemia Cysts*) lethality bioassay technique to measure the LC_{50} (μM) value of the compounds.

Experimental

Materials and instrumental details

All solvents, chemicals and reagents used were of analytical reagent grade and used as such; double distilled water was used throughout. Potassium tetrachloroplatinate was purchased from Chemport India Pvt Ltd Mumbai. 4-Fluorobenzaldehyde, benzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-methyl benzaldehyde and 4-methoxybenzaldehyde were purchased from SpectrochemPvt. Ltd., Mumbai (India). Agarose, ethidium bromide, TAE (Tris-Acetyl-EDTA), bromophenol blue and xylene cyanol FF were purchased from Himedia, India. Herring Sperm DNA was purchased from Sigma Chemical Co. (India). Culture of pUC19 bacteria (MTCC 47) was purchased from Institute of Microbial Technology (Chandigarh, India).

Elemental analyses (C, H, N and S) of the synthesized complexes were performed with a model 240 Perkin Elmer elemental analyzer, Massachusetts (USA). The electronic spectra were recorded on a UV-160A UV–Vis. spectrophotometer, Shimadzu, Kyoto (Japan). Infrared spectra were recorded on a FT-IR ABB Bomen MB-3000 (Canada) spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The thermogram of complexes was recorded on a Mettler Toledo TGA/DSC 1 Thermogravimetric analyzer. The LC–MS spectra were recorded using Thermo mass spectrophotometer (USA). ^{1}H and ^{13}C NMR were recorded on a BrukerAvance (400 MHz). Photo quantization of the gel after electrophoresis was done using AlphaDigiDoc™ RT. Version V.4.0.0 PC-Image software, California (USA).

Synthesis of ligands

All ligands were prepared by modified kronke pyridine synthesis [16]. A halo pyridinium salt of 2 acetyl thiophene and ammonium acetate was mixed with different chalcones in methanol. The mixture was refluxed for 6–7 h on sand bath. The product was obtained by keeping solution in ice bath and purified by crystallization in n-hexane Ligands were characterized by elemental analysis, ^{1}H and ^{13}C NMR spectra. (General synthesis of ligands is kept in Supplementary material 1).

2-(4-Chlorophenyl)-4-(4-fluorophenyl)-6-(thiophen-2-yl)pyridine (L^1)

Anal. Calc. for $C_{21}H_{13}ClFNS$: Calc. (Found): C, 68.94 (68.80); H, 3.58 (3.50); N, 3.83 (3.70); S, 8.76 (8.65). Yield: 65%, mp: 138–140 °C, mol. wt. 365.85;^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.14–8.11 (complex, 2H, $H_{3,5}$), 7.74–7.69 (complex, 5H, $H_{5',2''',3''',5''',6'''}$), 7.51–7.46 (complex, 3H, $H_{3',3'',5''}$), 7.26–7.22 (complex, 2H, $H_{2'',6''}$), 7.19–7.16 (complex, 1H, $H_{4'}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 164.75 ($C_{4'''}$), 156.16 (C_6), 152.93(C_2), 149.32 ($C_{2'}$), 145.09 ($C_{1'''}$), 137.34 ($C_{1''}$), 134.76 ($C_{3''',5'''}$), 134.73 ($C_{2''',6'''}$), 128.94 ($C_{5'}$), 128.91 ($C_{3'}$), 128.86 ($C_{4'}$), 128.30 ($C_{5''}$), 128.03 ($C_{3''}$), 127.93 ($C_{6''}$), 124.85 ($C_{2''}$), 116.07 (C_3), 115.32 (C_5). IR (KBr, 4000–400 cm^{-1}): 3066, 3056 ν(C—H)$_{ar}$; 1545, 1491 ν(C=C); 1397 ν(C=N); 1179 ν(C=S); 1229 ν(C—F); 1092 ν(C—Cl); 1381, 1362 (pyridine skeleton band); 1112, 828 (*p*-substituted aromatic ring); 800 δ(C—H).

2-(4-Chlorophenyl)-4-phenyl-6-(thiophen-2-yl)pyridine(L^2)

Anal. Calc. for $C_{21}H_{14}ClNS$: Calc. (Found): C, 72.51 (71.95); H, 4.06 (4.02); N, 4.03 (4.01); S, 9.22 (9.14). Yield: 55%, mp: 118–120 °C, mol. wt. 347.86;^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.16–8.14 (complex, 2H, $H_{3,5}$), 7.81–7.74 (complex, 5H, $H_{2''',3''',4''',5''',6'''}$), 7.58–7.46 (complex, 6H, $H_{3',5',2'',3'',5'',6''}$), 7.18 (t, 1H, $H_{4'}$, J = 5.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 156.08 (C_2), 152.87 (C_6), 150.40(C_4), 145.24 ($C_{2'}$), 138.67 ($C_{1'''}$), 137.48 ($C_{1''}$), 135.29 ($C_{5'}$), 129.16 ($C_{4''}$), 128.89 ($C_{3''',4''',5'''}$), 128.32 ($C_{3'',5''}$), 128.01 ($C_{2'',6''}$), 127.83 ($C_{4'}$), 127.13 ($C_{2''',6'''}$), 124.78 ($C_{3'}$), 116.55 (C_5), 115.56 (C_3). IR (KBr, 4000–400 cm^{-1}): 3066 ν(C—H)$_{ar}$; 1544, 1490 ν(C=C); 1397 ν(C=N); 1162 ν(C=S); 1099 ν(C—Cl); 1381, 1362 (pyridine skeleton band); 1162, 827 (*p*-substituted aromatic ring); 740 δ(C—H).

2,4-Bis(4-chlorophenyl)-6-(thiophen-2-yl)pyridine (L^3)

Anal. Calc. for $C_{21}H_{13}Cl_2NS$: Calc. (Found): C, 65.97 (65.88); H, 3.43 (3.35); N, 3.66 (3.50); S, 8.39 (8.30). Yield: 60%, mp: 139–141 °C, mol. wt. 382.31;^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.13 (d, 2H, $H_{3,5}$, J = 8.4 Hz), 7.75 (d, 3H, $H_{5',3''',5'''}$, J = 7.2 Hz), 7.68 (d, 2H, $H_{2''',6'''}$, J = 8.4 Hz), 7.54–7.46 (complex, 5H, $H_{3',2'',3'',5'',6''}$), 7.18 (dd, 1H, $H_{4'}$, J = 8.8 and 1.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 156.28 (C_2), 153.03 (C_6), 151.26(C_4), 149.13 ($C_{2'}$), 145.02 ($C_{1'''}$), 137.54 ($C_{1''}$), 137.09 ($C_{4'''}$), 135.44 ($C_{4''}$), 129.38 ($C_{3'',5''}$), 128.94 ($C_{3''',5'''}$), 128.40 ($C_{2'',6''}$), 128.29 ($C_{2''',6'''}$), 128.06 ($C_{5'}$), 127.98 ($C_{4'}$), 124.89 ($C_{3'}$), 116.21 (C_5), 115.26 (C_3). IR (KBr, 4000–400 cm^{-1}): 3061 ν(C—H)$_{ar}$; 1546, 1492 ν(C=C); 1397 ν(C=N); 1176 ν(C=S); 1202 ν(C—Cl); 1383, 1304 (pyridine skeleton band); 1123, 830 (*p*-substituted aromatic ring); 778 δ(C—H).

4-(4-Bromophenyl)-2-(4-chlorophenyl)-6-(thiophen-2-yl)pyridine (L^4)

Anal. Calc. for $C_{21}H_{13}ClBrNS$: Calc. (Found): C, 59.10 (59.90); H, 3.07 (3.08); N, 3.28 (3.30); S, 7.51 (7.45). Yield: 62%, mp: 122–124 °C, mol. wt. 426.76;^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.12 (d, 2H, $H_{3,5}$, J = 8.0 Hz), 7.73 (t, 3H, $H_{5',3''',5'''}$, J = 4.8 Hz), 7.68 (d, 2H, $H_{2''',6'''}$, J = 8.4 Hz), 7.60 (d, 2H, $H_{2'',6''}$, J = 8.4 Hz), 7.48 (dd, 3H, $H_{3',3'',5''}$, J = 18.4 and 4.8 Hz), 7.18 (t, 1H, $H_{4'}$, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 156.29 (C_2), 153.92 (C_6), 153.07 (C_4), 149.19 ($C_{2'}$), 145.00 ($C_{1'''}$), 137.54 ($C_{1''}$), 137.26 ($C_{4'''}$), 135.46 ($C_{4''}$), 132.34 ($C_{3'',5''}$), 128.93 ($C_{3''',5'''}$), 128.68 ($C_{2'',6''}$), 128.29 ($C_{2''',6'''}$), 128.04 ($C_{5'}$), 128.00 ($C_{4'}$), 124.92 ($C_{3'}$), 116.12 (C_5), 115.17 (C_3). IR (KBr, 4000–400 cm^{-1}): 3052 ν(C—H)$_{ar}$; 1545, 1485 ν(C=C); 1390 ν(C=N); 1170 ν(C=S); 1095 ν(C—Cl); 1012 ν(C—Br); 1380, 1360 (pyridine skeleton band); 1150, 815 (*p*-substituted aromatic ring); 750 δ(C—H).

2-(4-Chlorophenyl)-6-(thiophen-2-yl)-4-p-tolylpyridine (L^5)

Anal. Calc. for $C_{22}H_{16}ClNS$: Calc. (Found): C, 73.02 (73.10); H, 4.46 (4.40); N, 3.87 (3.80); S, 8.86 (8.75). Yield: 64%, mp: 120–122 °C, mol. wt. 361.89; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.12 (d, 2H, $H_{3,5}$, J = 8.4 Hz), 7.79 (t, 3H, $H_{5',3''',5'''}$, J = 5.2 Hz), 7.64 (d, 2H, $H_{2''',6'''}$, J = 7.6 Hz), 7.48 (dd, 3H, $H_{3',2'',6''}$, J = 19.2 Hzand 6 Hz), 7.36 (d, 2H, $H_{3'',5''}$, J = 8 Hz), 7.18 (dd, 1H, $H_{4'}$, J = 8.4 Hz and 0.8 Hz), 2.47 (s, 3H, —CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 155.90 (C_2), 152.59 (C_6), 150.53 (C_4), 144.70 ($C_{2'}$), 139.51 ($C_{1'''}$), 135.47 ($C_{1''}$), 135.39 ($C_{4'''}$), 129.91 ($C_{4''}$), 128.88 ($C_{3'',5''}$), 128.67 ($C_{3''',5'''}$), 128.46 ($C_{2'',6''}$), 128.10 ($C_{2''',6'''}$), 127.95 ($C_{5'}$), 126.97 ($C_{4'}$), 125.25 ($C_{3'}$), 116.52 (C_5), 115.52 (C_3), 21.29 (—CH_3). IR (KBr, 4000–400 cm^{-1}): 3050 ν(C—H)$_{ar}$; 2918 ν(C—H)$_{al}$; 1543, 1492 ν(C=C); 1397 ν(C=N); 1231 ν(C=S); 1091 ν(C—Cl); 1381, 1360 (pyridine skeleton band); 1145, 826 (*p*-substituted aromatic ring); 800 δ(C—H).

2-(4-Chlorophenyl)-4-(4-methoxyphenyl)-6-(thiophen-2-yl)pyridine (L^6)

Anal. Calc. for $C_{21}H_{16}ClNOS$: Calc. (Found): C, 69.92 (69.60); H, 4.27 (4.15); N, 3.71 (3.65); S, 8.49 (8.35). Yield: 53%, mp: 114–116 °C, mol. wt. 377.89; ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm:8.13 (d, 2H, $H_{3,5}$, J = 8.8 Hz), 7.77–7.69 (complex, 5H, $H_{3',2''',3''',5''',6'''}$), 7.47 (dd, 3H, $H_{5',3'',5''}$, J = 23.6 Hz and 10.0 Hz), 7.17 (dd, 1H, $H_{4'}$, J = 8.8 Hz and 0.8 Hz), 7.07 (d, 2H, $H_{2'',6''}$, J = 8.8 Hz), 3.91 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 160.64 ($C_{4'''}$), 156.00 (C_2), 152.78 (C_6), 149.81 (C_4), 145.37 ($C_{2'}$), 137.60 ($C_{1''}$), 135.19 ($C_{1'''}$), 130.85 ($C_{3'',5''}$), 128.86 ($C_{3''',5'''}$), 128.30 ($C_{2'',6''}$), 128.29 ($C_{4''}$), 127.97 ($C_{2''',6'''}$), 127.71 ($C_{5'}$), 124.67 ($C_{4'}$), 116.03 ($C_{3'}$), 115.01 (C_5), 114.58 (C_3), 55.45 (—OCH_3). IR (KBr, 4000–400 cm^{-1}): 3040 ν(C—H)$_{ar}$; 2836 ν(C—H)$_{al}$; 1543, 1492 ν(C=C); 1401 ν(C=N); 1235 ν(C=S); 1086 ν(C—Cl); 1261 ν(C—O—C)$_{sy}$; 1030 ν(C—O—C)$_{asy}$; 1382, 1362 (pyridine skeleton band); 1181, 825 (*p*-substituted aromatic ring); 796 δ(C—H).

Synthesis of complexes

Synthesis of [$PtCl_2(L^1)$] (1)

Complex 1was synthesized by heating 1:1 ratio of L^1(2-(4-chlorophenyl)-4-(4-fluorophenyl)-6-(thiophen-2-yl)pyridine) (0.450 mmol, 0.164 g) and K_2PtCl_4 (0.450 mmol, 0.186 g) in water–methanol system (50 mL) at 80 °C. A drop of hydrochloric acid (free acid is to avoid displacement of Cl^- by OH^-) added until the solution became colorless (0.5–5 h) Reaction mixture was allowed to cool at room temperature. The obtained product was washed with hot water and dried under vacuum. Similarly rest of all the complexes were prepared. (General synthesis of complexes is kept in Supplementary material 2).

Yield: 75%, mp: 148–150 °C, mol. wt. 631.84, Anal. for $C_{21}H_{13}Cl_3FNPtS$, Calc. (found) (%), C 39.92 (39.23), H 2.07 (2.12), N 2.22 (2.09), S 5.07 (5.16). UV–Vis: λ_{max} (nm) (ε dm^3 mol^{-1} cm^{-1}): 333 (10,287), 269 (25,841), LC MS: m/z = 629.59. FT-IR (KBr, 4000–400 cm^{-1}): 3063 ν(C—H)$_{ar}$; 1643, ν(C=C); 1489 ν(C=N); 1381, 1273 (pyridine skeleton band); 1227 ν(C—F); 1157 ν(C=S); 1095 ν(C—Cl); 1101, 825 (*p*-substituted aromatic ring); 764 δ(C—H), 555 ν(Pt—N)$_{sym}$, 475 ν(Pt—N)$_{asym}$, 450 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.37 (d, 2H, $H_{3,5}$, J = 8.4 Hz), 8.31 (d, 1H, $H_{5'}$, J = 8.4 Hz), 8.24–8.06 (complex, 4H, $H_{3'',5'',2''',6'''}$), 7.69 (d, 1H, $H_{3'}$, J = 5.2 Hz), 7.61 (d, 1H, $H_{2'',6''}$, J = 8.8 Hz), 7.41 (t, 2H, $H_{3''',5'''}$, J = 8.8 Hz), 7.23 (t, 1H, $H_{4'}$, J = 4.8 Hz).

Synthesis of [$PtCl_2(L^2)$] (2)

It was prepared using 2-(4-chlorophenyl)-4-phenyl-6-(thiophen-2-yl)pyridine (L^2) (0.450 mmol, 0.156 g) and K_2PtCl_4 (0.450 mmol, 0.186 g). Yield: 70%, mp: 128–130 °C, mol. wt. 613.84, Anal. for $C_{21}H_{14}Cl_3NPtS$, Calc. (found) (%), C 41.09 (40.95), H 2.30 (2.22), N 2.28 (2.39), S 5.22 (5.15). UV–Vis: λ_{max}(nm) (ε dm^3 mol^{-1} cm^{-1}): 334 (41,253), 271 (15,260), LC MS: m/z = 611.95. IR (KBr, 4000–400 cm^{-1}): 3063 ν(C—H)$_{ar}$; 1643 ν(C=C); 1489 ν(C=N); 1381, 1281 (pyridine skeleton band), 1142 ν(C=S); 1088 ν(C—Cl);; 1018, 825 (*p*-substituted aromatic ring); 764 δ(C—H), 540 ν(Pt—N)$_{sym}$, 470 ν(Pt—N)$_{asym}$, 445 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.32 (d, 2H, $H_{3,5}$, J = 8.8 Hz), 8.18 (d, 1H, $H_{3'}$, J = 21.6 Hz), 8.07 (d, 1H, $H_{5'}$, J = 3.2 Hz), 8.04 (d, 3H, $H_{2'',3'',5''}$, J = 7.2 Hz), 7.70 (d, 1H, $H_{6''}$, J = 4.8 Hz), 7.62–7.53 (complex, 5H, $H_{2''',3''',4''',5''',6'''}$), 7.23 (t, 1H, $H_{4'}$, J = 4.8 Hz).

Synthesis of [$PtCl_2(L^3)$] (3)

It was prepared using 2,4-bis(4-chlorophenyl)-6-(thiophen-2-yl)pyridine (L^3) (0.450 mmol, 0.172 g) and K_2PtCl_4 (0.450 mmol, 0.186 g). Yield: 80%, mp: 172–174 °C, mol. wt. 648.28, Anal. for $C_{21}H_{13}Cl_4NPtS$, Calc. (found) (%), C 38.91 (38.45), H 2.02 (2.12), N 2.16 (2.10), S 4.94 (4.85). UV–Vis: λ_{max} (nm) (ε dm^3 mol^{-1} cm^{-1}): 319 (20,689), 271 (17,032), LC MS: m/z = 647.19. IR (KBr, 4000–400 cm^{-1}): 3047 ν(C—H)$_{ar}$; 1643 ν(C=C); 1481 ν(C=N); 1373, 1273 (pyridine skeleton band); 1134 ν(C=S); 1080 ν(C—Cl); 1031, 825 (*p*-substituted aromatic ring); 750 δ(C—H), 548 ν(Pt—N)$_{sym}$, 480 ν(Pt—N)$_{asym}$, 455 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.19 (d, 3H, $H_{3,5,3'}$, J = 8.4 Hz), 7.99 (d, 1H, $H_{4'}$, J = 15.6 Hz), 7.88 (d, 3H, $H_{5',2'',6''}$, J = 8.4 Hz), 7.75–7.64 (complex, 6H, $H_{3'',5'',2''',3''',5''',6'''}$).

Synthesis of [$PtCl_2(L^4)$] (4)

It was prepared using 4-(4-bromophenyl)-2-(4-chlorophenyl)-6-(thiophen-2-yl)pyridine (L^4) (0.450 mmol, 0.192 g) and K_2PtCl_4 (0.450 mmol, 0.186 g). Yield: 75%, mp: 173–175 °C, mol. wt. 692.74, Anal. for $C_{21}H_{13}$ $BrCl_3NPtS$, Calc. (found) (%), C 36.41 (35.95), H 1.89 (1.78), N 2.02 (2.08), S 4.63 (4.65). UV–Vis: λ_{max} (nm) (ε dm^3 mol^{-1} cm^{-1}): 319 (19,691), 270 (17,032), LC MS: m/z = 689.87. IR (KBr, 4000–400 cm^{-1}): 3155 ν(C—H)$_{ar}$; 1643 ν(C=C); 1481 ν(C=N); 1389, 1272 (pyridine skeleton band); 1120 ν(C=S); 1072 ν(C—Cl); 1003 ν(C—Br); 910, 810 (*p*-substituted aromatic ring); 663 δ(C—H), 540 ν(Pt—N)$_{sym}$, 485 ν(Pt—N)$_{asym}$, 465 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.19 (d, 3H, $H_{3,5,3'}$, J = 8.4 Hz), 7.99 (d, 1H, $H_{4'}$, J = 15.6 Hz), 7.88 (d, 3H, $H_{5',2'',6''}$, J = 8.4 Hz), 7.76–7.64 (complex, 6H, $H_{3'',5'',2''',3''',5''',6'''}$).

Synthesis of [$PtCl_2(L^5)$] (5)

It was prepared using 2-(4-chlorophenyl)-6-(thiophen-2-yl)-4-p-tolylpyridine (L^5) (0.450 mmol, 0.162 g) and K_2PtCl_4 (0.450 mmol, 0.186 g). Yield: 65%, mp: 121–123 °C, mol. wt. 625.87, Anal. for $C_{22}H_{16}$ Cl_3NPtS, Calc. (found) (%), C 42.08 (41.45), H 2.57 (2.65), N 2.23 (2.15), S 5.11 (5.05). UV–Vis: λ_{max} (nm) (ε dm^3 mol^{-1} cm^{-1}): 331 (12,644), 275 (31,350), LC MS: m/z = 625.97. IR (KBr, 4000–400 cm^{-1}): 3055 ν(C—H)$_{ar}$; 2916 ν(C—H)$_{al}$; 1643 ν(C=C); 1481 ν(C=N); 1381, 1273 (pyridine skeleton band); 1142 ν(C=S); 1088 ν(C—Cl); 1011, 810 (*p*-substituted aromatic ring); 756 δ(C—H), 540 ν(Pt—N)$_{sym}$, 485 ν(Pt—N)$_{asym}$, 465 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.32 (d, 2H, $H_{3,5}$, J = 8.0 Hz), 8.17 (t, 2H, $H_{4',5'}$, J = 11.6 Hz), 8.07 (d, 1H, $H_{3'}$, J = 2.4 Hz), 7.96 (d, 2H, $H_{2'',6''}$, J = 7.6 Hz), 7.69–7.60 (complex, 3H, $H_{3'',5'',6'''}$), 7.39 (d, 2H, $H_{3''',5'''}$, J = 7.6 Hz), 7.23 (d, 1H, $H_{2'''}$, J = 4.0 Hz), 2.41 (s, 3H,—CH_3).

Synthesis of [$PtCl_2(L^6)$] (6)

It was prepared using 2-(4-chlorophenyl)-4-(4-methoxyphenyl)-6-(thiophen-2-yl)pyridine (L^6) (0.450 mmol, 0.170 g) and K_2PtCl_4 (0.450 mmol, 0.186 g). Yield: 68%, mp: 103–105 °C, mol. wt. 643.87, Anal. for $C_{22}H_{16}$ Cl_3NOPtS, Calc. (found) (%), C 41.04 (41.15), H 2.50 (2.59), N 2.18 (2.05), S 4.98 (4.90). UV–Vis: λ_{max} (nm) (ε dm^3 mol^{-1} cm^{-1}): 293 (50,550), LC MS: m/z = 641.96. IR

(KBr, 4000–400 cm^{-1}): 3001 ν(C—H)$_{ar}$; 2908 ν(C—H)$_{al}$; 1643 ν(C=C); 1435 ν(C=N); 1250 ν(C—O—C)$_{sy}$; 1373, 1160 (pyridine skeleton band); 1142 ν(C=S); 1088 ν(C—Cl); 1011 ν(C—O—C)$_{asy}$; 1180, 879 (*p*-substituted aromatic ring); 818 δ(C—H), 540 ν(Pt—N)$_{sym}$, 480 ν(Pt—N)$_{asym}$, 460 ν(Pt—S). ^{1}H NMR (DMSO-d6, 400 MHz) δ/ppm:8.31 (d, 2H, $H_{3,5}$, J = 8.8 Hz), 8.17–8.12 (complex, 6H, $H_{2'',3'',5'',6'',2''',6'''}$), 8.02 (d, 1H, $H_{4',5'}$, J = 8.8 Hz), 7.61 (d, 1H, $H_{3'}$, J = 8.8 Hz), 7.12 (d, 2H, $H_{3''',5'''}$, J = 8.8 Hz), 3.86 (s, 3H, —OCH_3).

Evaluation of binding constant by absorption titration using HS DNA

The experiment was performed using HS DNA (ε = 12858 $dm^3\ mol^{-1}\ cm^{-1}$) in phosphate buffer solution (pH 7.2). The stock solutions of the complexes were prepared by dissolving the complexes in DMSO. The absorption titration was carried out by keeping the concentration of complex constant (20 μM) and varying the concentration of DNA and incubated for 10 min at room temperature. The change in absorbance was recorded after each addition of DNA aliquot. The K_b value was determined from the ratio of the slope to intercept [17].

DNA binding study by viscosity measurement

Cannon–Ubbelohde viscometer maintained at a constant temperature of (37.0 ± 0.1 °C) in a thermostatic jacket was used to measure the relative viscosity of DNA solutions in the presence of platinum(II) complexes. Digital stopwatch with least count of 0.01 s. was used for flow time measurement with accuracy of ±0.1 s. The [Complex]/[DNA] ratio was maintained in the range of 0–0.2.The flow time of each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta_0)^{1/3}$ versus [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone [18]. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta \propto t$–t_0 [19].

Unwinding of supercoiled pUC19 DNA

Unwinding of supercoiled pUC19 DNA was measured by agarose gel electrophoresis mobility shift assay [20]. The unwinding angle ϕ, induced per Pt-DNA adduct was calculated by following equation,$\phi = -18\sigma/r_b$(c)where, σ is the superhelical density and r_b is the bound drug-to-nucleotide ratio, r_b(c) is the ratio at which the supercoiled and nicked forms comigrate i.e. the ratio at which the complete transformation of the supercoiled to relaxed form of the plasmid is attained. Plasmid were incubated with platinum complex for 48 h, precipitated by ethanol and redissolved in TAE buffer (0.04 M Tris-acetate + 1 mM EDTA, pH 7.0). An aliquot of the precipitated sample was subjected to electrophoresis on 1% agarose gel at room temperature in TAE buffer at constant voltage of 50 V. The gel was stained with aqueous EB, followed by photo capturing on UV–transilluminator. The superhelical density (r) of the plasmid was determined from r_b(c) values obtained for *cisplatin* and its known unwinding angle (ϕ = 13° for the site-specific 1,2-intrastrand cross-link) [21].

Cytotoxic activity

Brine shrimp (*Artemia Cysts*) lethality bioassay technique was applied for the determination of general toxic property of complexes. The *in vitro* lethality test has been carried out using brine shrimp eggs i.e. *Artemia cysts*. Brine shrimp eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighter side. A stock solution of the test complex was prepared in DMSO. From this stock solution, solutions were transferred to the vials to make final concentration 2, 4, 6, 8, 10, 12 μM, etc. (three for each dilutions were used for each test sample and LC_{50} is the mean of three values) and three vial was kept as control having of DMSO only. After two days, when the of nauplii were ready, 1 mL of seawater and 10 of nauplii were added to each vial and the volume was adjusted with seawater to 2.5 mL per vial [22]. After 24 h each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. Data were analyzed by simple logit method to determine the LC_{50} values, in which log of concentration of samples were plotted against percent of mortality of nauplii [23].

Results and discussion

Thermogravimetric analysis and magnetic moments

Thermal analyses of the Pt(II) complexes were carried out in order to ascertain the nature of associated water molecules and the compositional difference of the complexes. No weight loss occurs up to 320 °C, indicates the absences of coordinated as well as lattice water molecules. The weight loss occurs from 320 to 550 °C corresponds to loss of ligands and leaving behind the metal residue.

The magnetic moment value of the synthesized complexes was found to be zero at room temperature, suggesting d^8-system with low-spin configuration i.e. dsp^2 hybridization, pointing toward square planar Pt(II) complexes.

Electronic absorption analyses

For low-spin square-planar d^8-metal complexes, three d–d spin allowed transitions are expected corresponding to the transitions from three lower lying d-levels to the empty $\boldsymbol{d}_{x^2-y^2}$ orbital. These transitions are designated as $\boldsymbol{d}_{z^2}(\boldsymbol{a}_{1g}) \rightarrow \boldsymbol{d}_{x^2-y^2}(\boldsymbol{b}_{1g})$, $\boldsymbol{d}_{xz,yz}(\boldsymbol{e}_g) \rightarrow \boldsymbol{d}_{x^2-y^2}(\boldsymbol{b}_{1g})$, $\boldsymbol{d}_{xy}(\boldsymbol{b}_{2g}) \rightarrow \boldsymbol{d}_{x^2-y^2}(\boldsymbol{b}_{1g})$ and observed at ~30,000 cm^{-1}, ~26,000 cm^{-1} and ~23,000 cm^{-1}, respectively [24,25]. In the synthesized Pt(II) complexes, only one d–d band ~330 nm (~30,303 cm^{-1}) is observed, which is assigned to $\boldsymbol{d}_{z^2}(\boldsymbol{a}_{1g}) \rightarrow \boldsymbol{d}_{x^2-y^2}(\boldsymbol{b}_{1g})$ transition, where as one charge transfer transition band is observed ~270 nm (~37,037 cm^{-1}) [26], The reason for only one charge transfer transition band is the masking of high intensity charge transfer bands. All these bands points toward the low spin complex of d^8-system with square planar geometry.

The IR spectroscopic measurements of the ligands show bands at ca. 1550 cm^{-1} and ca. 1400 cm^{-1} corresponding to C=C and C=N ring stretching, whereas the bands at 750 cm^{-1} and 3060 cm^{-1} are due to aromatic C—H out-of-plane bending and stretching respectively. The band for C—H out-of-plane ring deformation appears at ca. 763 cm^{-1} and C—Cl stretching appears at 1080 cm^{-1}. Major changes occur in the bands of C=C and C=N ring stretching as they are shifted from 1550, 1400 to 1643 and 1490 cm^{-1} respectively. This is further supported by the binding of metal atom to nitrogen and sulphur atom of ligand skeleton, by a sharp band at ca. 550 and ca. 470 cm^{-1}, characteristic bands of symmetric and asymmetric stretching of the Pt—N bond [27] and stretching frequency of Pt—S bond at ca. 450. The stretching mode of Pt—Cl is expected in the region below 400 cm^{-1} i.e. ca. 320 cm^{-1} [28].

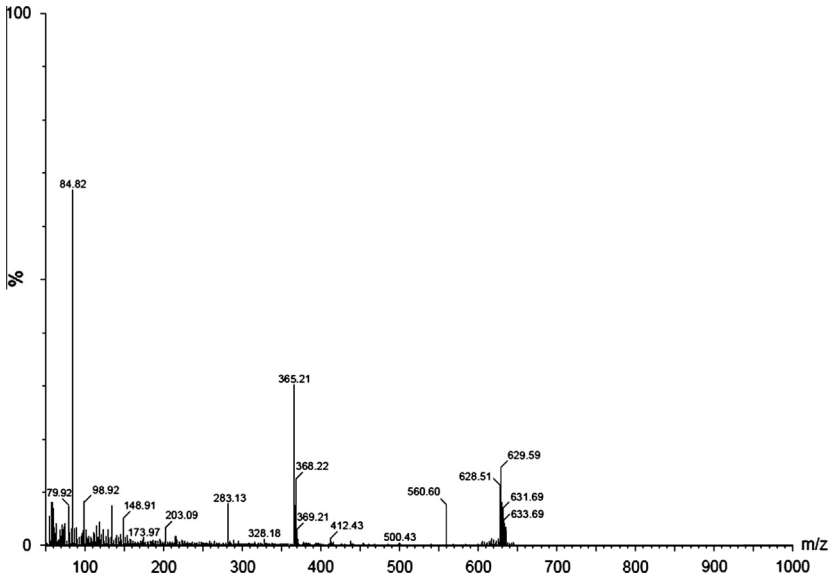


Fig. 1. LC–MS spectrum of complex-1.

Fig. 1 represents mass spectrum of complex $[PtCl_2(L^1)]$ (1). Mass spectrum of complex 1 shows molecular ion peak $[M^+]$ at 629.59 *m/z*, [M + 2] at 631.69 *m/z* and [M + 4] at 633.69 *m/z*. Peak at 560.60 *m/z* is due to ligand attached with platinum metal ion i.e. due to loss of two chlorine atoms from the complex. The peak at 365.21 *m/z* is due to ligand i.e. loss of platinum metal, which is attached to ligand. The other peaks at 283.13, 203.09, 173.97, 98.92, 84.82 and 79.92 *m/z* are due to the fragmentation of ligand.

The ^{1}H NMR spectra of all the complexes show downfield for protons. Protons $H_{3,5}$ in ligands are in the most downfield region, while for the complexes these protons show more downfield shift in the spectra. The coordination of ligand results in shifting of all ^{1}H NMR peaks in the downfield region. These suggest that the coordination occur through N and S atom, which is also supported by the IR data.

Absorption spectral study

Absorption titration is employed universally to determine the binding mode and binding strength of the complexes with DNA [17]. Presence of red shift indicates coordination of complex with DNA through N7 position of guanine. The extent of hyperchromism also reveals the nature of binding affinity [29]. The absorption spectra of Pt(II) complex with increasing concentration of HS DNA is shown in Fig. 2. As the concentration of DNA increases, hyperchromism is observed in the charge transfer band of each complex along with the red shift of about 2 nm which suggest covalent binding of metal complexes with DNA. The K_b values were obtained for Pt(II) complexes are in the range of 1.37×10^5–7.76×10^5 (Table 1). These values are much lower than that of the classical intercalator

Table 1
Binding constant (K_b) values of synthesized complexes.

Complex	K_b (M^{-1})
$[PtCl_2(L^1)]$	7.76×10^5
$[PtCl_2(L^2)]$	2.99×10^5
$[PtCl_2(L^3)]$	6.94×10^5
$[PtCl_2(L^4)]$	5.96×10^5
$[PtCl_2(L^5)]$	2.38×10^5
$[PtCl_2(L^6)]$	1.37×10^5

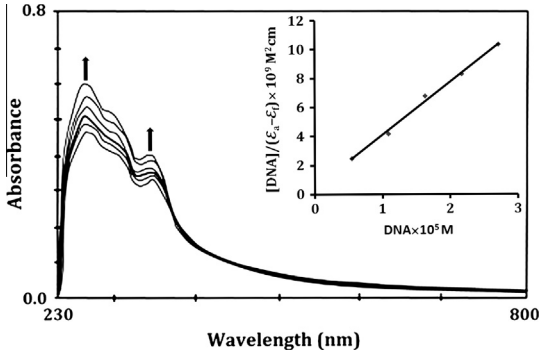


Fig. 2. Absorption spectral traces of complex 1 with increasing amount of Herring Sperm DNA in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2). [complex] = 50 μM, [DNA] = 0–60 μM with incubation period of 15 min at room temperature. Arrow shows the absorbance change upon increasing DNA concentrations. Inset: Plots of [DNA]/(ε_a–ε_f) versus [DNA] for the titration of DNA with platinum(II) complexes.

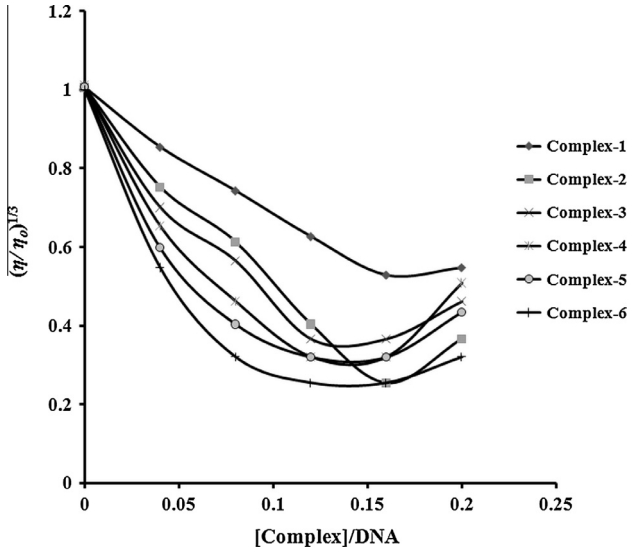


Fig. 3. Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 37 ± 0.1 °C in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2).

Table 2
Data for determining the unwinding angle of pUC19 DNA under influence of Complexes.

Complex	Complex-to-nucleotide ratio[a] r_b (C)	Unwinding angle ϕ
1	0.071	14.02°
2	0.092	10.76°
3	0.075	13.20°
4	0.081	12.38°
5	0.098	10.10°
6	0.108	9.17°

[a] Complex-to-nucleotide ratio at which supercoiled and nicked forms comigrates.

(ethidium bromide) but these values are higher than intercalative [$PtCl_2$(4,7-Me_2phen)] complex ($6.35 \pm 0.2 \times 10^4\ M^{-1}$) [30]. While the K_b values of the synthesized Pt(II) complexes are similar to the value observed for [Pt(dmphen)(CO_3)]·H_2O (dmphen = 2,9-dimethyl-1,10-phenanthroline) ($1.8 \times 10^5\ M^{-1}$) [31], [Pt(bpy)(pip)](NO_3)$_2$ and [Pt(bpy)(hpip)](NO_3)$_2$· $2H_2O$ [bpy = 2,2′-bypyridine; pip = 2-phenylimidazo[4,5-f]1,10-phenanthroline; hpip = 2-(2-hydroxyphenyl) imidazo [4,5-f]1,10-phenanthroline)] [32].

Viscosity measurement

Viscosity measurement study was carried out to confirm the binding mode of the complexes. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta \propto t - t_0$ [19,18]. The viscosity of a DNA solution was measured in the presence of compounds is regarded as the least ambiguous and most critical test of a DNA binding model [33], and affords a stronger argument for DNA binding mode [34]. A classical intercalation mode results in the lengthening of the DNA helix, leading to an increase in the DNA viscosity. In contrast, partial intercalators as well as covalent binders could bend DNA helix, reduce its effective length and thereby its viscosity [35]. During the relative specific viscosity measurement with increase in ratio of [complex]/[DNA] from 0.02 to 0.2, decrease the relative viscosity of DNA solution (Fig. 3) resulting from bending or kinking of the DNA helix, which attributes to covalent binding of complexes with DNA bases [36].

Unwinding of pUC19 DNA

Unwinding of supercoiled pUC19 DNA was measured by agarose gel electrophoresis mobility shift assay [20]. The unwinding induced in negatively supercoiled pUC19 plasmid determined by monitoring the degree of supercoiling (Supplementary material 3). The degree of supercoiling decrease upon binding of unwinding agents, which causes a decrease in the rate of migration through agarose gel, which makes it possible to observe and to quantify the mean value of unwinding. Due to binding of unwinding agents linear form of DNA is not observed and supercoiled form of DNA is completely converted to open circular form. Under the present experimental conditions, r was calculated to be −0.055 on the basis of the data of *cisplatin* for which the r_b(c) was determined

Table 3
Data for determining the LC_{50} values of synthesized complexes.

Compound	Conc. (μM)	Log conc.	No. of nauplii taken	No. of nauplii dead	% of mortality	LC_{50} (μM)
1	2	0.301	10	6	40	
	3	0.477	10	5	50	
	4	0.602	10	4	60	3.02
	5	0.699	10	3	70	
	6	0.778	10	2	80	
	8	0.903	10	1	90	
2	2	0.301	10	9	10	
	5	0.699	10	8	20	
	6	0.778	10	7	30	9.77
	8	0.903	10	6	40	
	10	1.000	10	5	50	
	12	1.079	10	4	60	
3	2	0.301	10	7	30	
	4	0.602	10	6	40	
	5	0.699	10	5	50	5.37
	8	0.903	10	4	60	
	10	1.000	10	3	70	
	12	1.079	10	2	80	
4	2	0.301	10	8	20	
	4	0.602	10	7	30	
	6	0.778	10	6	40	7.08
	7	0.845	10	5	50	
	10	1.000	10	4	60	
	12	1.079	10	3	70	
5	2	0.301	10	9	10	
	6	0.778	10	8	20	
	12	1.079	10	7	30	21.38
	18	1.255	10	6	40	
	20	1.301	10	5	50	
	30	1.477	10	4	60	
	38	1.580	10	3	70	
6	2	0.301	10	10	00	
	4	0.602	10	9	10	
	6	0.778	10	8	20	28.18
	10	1.000	10	7	30	
	20	1.301	10	6	40	
	30	1.477	10	5	50	
	40	1.602	10	4	60	
	50	1.699	10	3	70	

in this study and $\phi = 13°$ was assumed. Using this approach, the DNA unwinding angles were determined (09.17–14.02°) (Table 2). The highest unwinding angle observed for complex 1 (14.02°), while the lowest unwinding angle observed for complexes 6 (09.17°). These are similar to those found for platinum complexes *cis*-[$PtCl_2(Me_2N(CH_2)_3PPh_2$-*P*)$_2$] ($\phi = 14° \pm 1°$) [37], *trans*-diamminedichloroplatinum ($\phi = 9°$) [20], *cis*-diamminedichloroplatinum ($\phi = 13°$) [20], *trans*-[$PtCl_2(NH_3)$(quin)] ($\phi = 15°$) and *trans*-[$PtCl_2(NH_3)_2$] ($\phi = 10°$) [38].

Cytotoxicity

A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in complex is the brine shrimp lethality bioassay [22,23]. All the synthesized compounds screened for their cytotoxicity using the protocol of Meyer et al. [22]. LC_{50} values of test complexes observed in the range of 03.02–28.18 μM after 24 h. (Table 3). In brine shrimp lethality bioassay, which is comparable to that of *cis-platin* complexes reported using cell line (MCF-7, HL-60, EJ, BGC823 and HCT-8 cells) [35,39].

Conclusions

From analytical and spectral investigations, we can conclude that square planar nature of all Pt(II) complexes derived from N, S-donor ligands. The electronic absorption data are in good accordance with viscosity titration curves, which suggest that the complexes bind to HS DNA via covalent binding or partial intercalation mode. Among all the complexes, complex 1 binds more strongly. The measurement of unwinding angles of the DNA upon binding with Pt(II) complexes using gel electrophoresis analysis shows similarity with many reported *cisplatin* analogues complexes. The cytotoxic activity of the synthesized complexes shows that the complexes have high potency against brine shrimps. It also concluded from the results that as the electron withdrawing ability of substituent (F, Cl and Br) on the intercalative ligand increases, the DNA interaction ability and cytotoxicity of the complexes increases and vice-versa for the electron releasing substituent (CH_3, OCH_3).

Acknowledgments

Authors thank Head, Department of Chemistry, Sardar Patel University, India for making it convenient to work in laboratory and U.G.C. for providing financial support under the scheme "UGC Research Fellowship in Science for Meritorious Students".

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.02.053.

References

[1] E. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451–2466.
[2] P.M. Takahara, C.A. Frederick, S.J. Lippard, J. Am. Chem. Soc. 118 (1996) 12309–12321.
[3] P.M. Takahara, A.C. Rosenzweig, C.A. Frederick, S.J. Lippard, Nature 377 (1995) 649–652.
[4] C. Makedonas, C.A. Mitsopoulou, Inorg. Chim. Acta 360 (2007) 3997–4009.
[5] C. Makedonas, C.A. Mitsopoulou, F.J. Lahoz, A.I. Balana, Inorg. Chem. 42 (2003) 8853–8865.
[6] K.J. Barnham, M.I. Djuran, P. del Socorro Murdoch, P.J. Sadler, J. Chem. Soc., Chem. Commun. (1994) 721–722.
[7] H.S.A. Sigel, Metal Ions in Biological Systems, vol. 32, Marcel Dekker, New York, 1996, p. 641.
[8] C. Makedonas, C.A. Mitsopoulou, Euro. J. Inorg. Chem. 3 (2006) 590–598.
[9] E.S. Raper, Coord. Chem. Rev. 165 (1997) 475–567.
[10] V.K. Jain, L. Jain, Coord. Chem. Rev. 249 (2005) 3075–3197.
[11] K. Matsumoto, K. Sakai, in: A.G. Sykes (Ed.), Adv. Inorg. Chem. 49 (1999) 375–427.
[12] E. Zangrando, F. Pichierri, L. Randaccio, B. Lippert, Coord. Chem. Rev. 156 (1996) 275–332.
[13] W.W. Alden, A.J. Repta, Chem. Biol. Interact. 48 (1984) 121–124.
[14] J. Reedijk, Chem. Rev. 99 (1999) 2499–2510.
[15] M. Patel, D.S. Gandhi, P.A. Parmar, B.S. Bhatt, A.P. Patidar, Z. Anorg, Allg. Chem. 638 (5) (2012) 838–843.
[16] F. KrÖHnke, Synthesis 1 (1976) 1–24.
[17] A. Wolfe, G.H. Shimer, T. Meehan, Biochemistry 26 (1987) 6392–6396.
[18] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Biochemistry 32 (1993) 2573–2584.
[19] S. Basili, A. Bergen, F. Dall'Acqua, A. Faccio, A. Granzhan, H. Ihmels, S. Moro, G. Viola, Biochemistry 46 (2007) 12721–12736.
[20] M.V. Keck, S.J. Lippard, J. Am. Chem. Soc. 114 (1992) 3386–3390.
[21] S.F. Bellon, J.H. Coleman, S.J. Lippard, Biochemistry 30 (1991) 8026–8035.
[22] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, Planta Med. 45 (1982) 31–34.
[23] M.R. Islam, S.M.R. Islam, A.S.M. Noman, J.A. Khanam, S.M.M. Ali, S. Alam, M.W. Lee, Mycobiology 35 (2007) 25–29.
[24] W.R. Mason, H.B. Gray, J. Am. Chem. Soc. 90 (1968) 5721–5729.
[25] D.N. Sathyanarayana, Electronic Absorption Spectroscopy and Related Techniques, Universities Press, Hyderabad, India, 2001, p. 191.
[26] V.X. Jin, J.D. Ranford, Inorg. Chim. Acta 304 (2000) 38–44.
[27] J. Stawińska, M. Cieślak-Golonka, Z. Staszak, R. Gancarz, Transition Metal Chem. 26 (2001) 153–159.
[28] B. Miller, S. Wild, H. Zorbas, W. Beck, Inorg. Chim. Acta 290 (1999) 237–246.
[29] C.S. Allardyce, P.J. Dyson, D.J. Ellis, S.L. Heath, Chem. Commun. (2001) 1396–1397.
[30] J. Kasparkova, T. Suchankova, A. Halamikova, L. Zerzankova, O. Vrana, N. Margiotta, G. Natile, V. Brabec, Biochem. Pharmacol. 79 (2010) 552–564.
[31] E.-J. Gao, L. Wang, M.-C. Zhu, L. Liu, W.-Z. Zhang, Eur. J. Med. Chem. 45 (2010) 311–316.
[32] B. Coban, U. Yildiz, A. Sengul, J. Biol. Inorg. Chem. 18 (2013) 461–471.
[33] D.S. Pilch, M.J. Waring, J.-S. Sun, M. Rougée, C.-H. Nguyen, E. Bisagni, T. Garestier, C. Hélène, J. Mol. Biol. 232 (1993) 926–946.
[34] B. Nordén, F. Tjerneld, Biopolymers 21 (1982) 1713–1734.
[35] F. Gumus, G.k.e. Eren, L. Acık, A. Celebi, F. Ozturk, S.u.k. Yılmaz, R.a.I.ı. Sagkan, S. Gur, A. Ozkul, A. Elmalı, Y.ı. Elerman, J. Med. Chem. 52 (2009) 1345–1357.
[36] M. Chauhan, K. Banerjee, F. Arjmand, Inorg. Chem. 46 (2007) 3072–3082.
[37] K. Neplechová, J. Kašpárková, O. Vrána, O. Nováková, A. Habtemariam, B. Watchman, P.J. Sadler, V. Brabec, Mol. Pharmacol. 56 (1999) 20–30.
[38] U. Bierbach, Y. Qu, T.W. Hambley, J. Peroutka, H.L. Nguyen, M. Doedee, N. Farrell, Inorg. Chem. 38 (1999) 3535–3542.
[39] J. Zhang, Y. Gong, X. Zheng, M. Yang, J. Cui, Euro. J. Med. Chem. 43 (2008) 441–447.