Heteroleptic C, N- Donor Pd(II) Complexes: Synthesis, Characterization, DNA/BSA Binding Interactions And Biological Studies

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Abstract

A series of trisubstituted pyrazole-based ligands (L$_1$-L$_6$) and corresponding palladium(II) complexes were synthesized and characterized by conductivity measurement, $^1$H NMR, $^{13}$C NMR, Fourier Transform infrared (FT-IR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS). Synthesized compounds were screened for various biological activities. UV-Vis spectroscopy, viscosity measurement, fluorescence spectroscopy and molecular docking studies were used to determine the binding mode between HS-DNA and complexes, which suggest intercalation mode of binding. The protein binding study of complexes was evaluated by UV-visible spectroscopy. Antibacterial study of the complexes was screened against two Gram (+ve) and three Gram (-ve) bacteria and results show that all complexes are more effective against microorganisms than their respective ligands. The cytotoxicity of the synthesized compounds was tested against brine shrimp and MCF-7 Cells. The LC$_{50}$ values of the ligand and complexes were found in the range of 9.24-4.12 µg/mL and 5.68-7.94 µg/mL, respectively.

Introduction

All life born on the earth creates something in this world and ends with some reason. In some cases, whenever we analyze the cause of life end at the global level, that analysis states that the cause of life end is cardiovascular disorders followed by cancer[1]. Cancer is a pensive health problem in society. The WHO study says that 9.6 million lives ended in 2018 with stress of cancer and total death expected in 2040 will double due to various types of cancer[2]. Anticancer drug available in the market has limitations like high toxicity, missing target specialty, acquired resistance and heavy dosage, so to work out the efficient anticancer drug, target-specific and having a minimum side effect is a pivotal in medicinal chemistry[3]. At the global level, many researchers are committed to working out metal coordination compounds that can bind with DNA (by covalent mode or non-covalent mode) to produce an equipotential activity of cisplatin[4–9].

Organometallic Pd(II) coordination compounds show unusual activity in tumor curing agents compared to first-generation anti-tumor drugs, which show drug resistance, nephrotoxicity, and other problems[10]. Due to the structural similarity of Pd(II) and Pt(II), coordination compounds are ideal as metallodrugs. The ligand, which is incorporated with metal ions, plays a vital role in bio-activity[11–14]. Predominantly various bio-activities of the ligands will spank results when it combines with the cation in metal complexes[15].

Due to diverse bio-activities, pyrazole moiety with different derivatives is on the list of studies of many researchers in medicinal chemistry[16–20]. Many researchers reported that coordination compound with pyrazole moiety with the different metal and various substituted pyrazole shows a long bio-activity list [21–24].

Experimental
2.1 Materials and methods

All the chemicals and solvents were of the reagent grade and used without further purification. Sodium tetrachloropalladate(II) was purchased from S.D. Fine Chem limited(SDFCL). 5-Bromothiophene-2-carbaldehyde and substituted acetophenone were purchased from Sigma Aldrich. Herring sperm (HS)-DNA was purchased from Sigma Aldrich Chemical Co (India). Nutrient broth (NB), agarose, ethidium bromide (EtBr), tris-acetyl EDTA(TAE) and bromophenol blue were purchased from Himedia (India). All chemicals and reagents used for DNA binding were purchased from SRL (Sisco Research Limited India), MCF-7 was purchased from NCCS, Pune, INDIA. The solvents used for spectral measurements were of HPLC grade.

2.2 Physical measurements

$^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded on a Brucker Avance (400 MHz) spectrometer using deuterated dimethyl sulphoxide (DMSO-$d_6$) solvent with chemical shift ($\delta$ in ppm) reference to internal TMS. IR Spectra were recorded on an FT-IR ABB Bomen MB-3000 spectrophotometer. Melting points were determined on thermocal 10 melting point apparatus (Analab Scientific PVT.LTD, India) in open capillaries. Electronic spectra were recorded with UV-160A UV-Vis spectrophotometer, Shimadzu, Kyoto (Japan). Conductance measurements were carried out using conductivity meter model number space E-660A. C, H, N- elemental analysis was performed on model Euro vector EA 3000(for ligands) and Perkin-Elmer 240(for complexes) elemental analyzer. Electron spray ionization (ESI) mass spectra were obtained using thermo scientific mass spectrometer (USA).

2.3 Preparation of thiophene-pyrazole based C, N- donor ligands ($L^1$-$L^6$)

Substituted thiophene-based enones ($\alpha$, $\beta$-unsaturated ketones) were synthesized by 5-bromothiophene-2-carboxyldehyde and substituted acetophenone with the help of base (sodium hydroxide). The ketones were used for further reaction to getting ligands ($L^1$–$L^6$). Potassium tertiary butoxide added in an alcoholic solution of substituted thiophene-based enones and substituted phenylhydrazine hydrochloride were added and refluxed at 60 °C for 6–7 h in methanol. It gave thiophene-pyrazole-based C, N donor ligands ($L^1$–$L^6$). The reaction scheme for the preparation of thiophene-pyrazole-based C, N- donor ligands is depicted in scheme 1.

2.3.1 3-(5-(5-Bromothiophen-2-yl)-1-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) pyridine ($L^1$)

This ligand ($L^1$) was prepared through the addition of $\alpha$, $\beta$-unsaturated enone (3a) (1.5 mmol) and phenyl hydrazine (123 mg, 1.479 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: $C_{18}H_{13}BrClN_3S$ Yield: 79.82% Melting point: 289–292°C Molecular weight: 418.74 gm/mol calc.(%): C, 51.63; H, 3.13; N, 10.04; S, 7.66; Found. (%): C, 51.56; H, 3.24; N, 10.39; S, 7.52; m/z(%): 417 (100) [M]+, 419
[M \ddagger]^+; ^1H NMR (400 MHz, DMSO-\textit{d}_6) \delta/\text{ppm}: 8.489 (1H, dt, H 4″), 7.284 (1H, S, H 2″), 7.217 (1H, dt, H 6″), 7.133 (1H, dt, H 5″), 6.893 (4H, d, J = 4.0 Hz, H 2″, 3″, 5″, 6″), 6.774 (2H, d, J = 3.6 Hz, H 3.4), 5.553 (1H, dd, J 1 = 6.4 Hz, J 2 = 12.4 Hz, H3′), 3.917 (1H, dd, J 1 = 12 Hz, J 2 = 18.0 Hz, H 4′a), 3.447 (1H, dd, J 1 = 6.4 Hz, J 2 = 18.0 Hz, H 4′b).

13C NMR (100 MHz, DMSO-\textit{d}_6) \delta/\text{ppm}: 152.08 (C 5′, -Cquat), 143.63 (C1″′, -Cquat), 135.11 (C 5, -Cquat), 134.78 (C 4″′, -Cquat), 124.94 (C 1″, -Cquat), 119.04 (C 2, -Cquat), 109.10 (C 3″′, 5″′, -CH), 42.85 (C 4′, -CH₂), 153.13 (C 4″, -CH), 143.07 (C 2″, -CH), 142.94 (C 6″, -CH), 139.81 (C 3, -CH), 129.81 (C 3″′, 5″′, -CH), 115.34 (C 5″, -CH), 110.13 (C 2″′, 6″′, -CH) [Total signal observed = 15: signal of Cquat and –CH₂ = 8, signal of –CH and –CH₃ = 7].

IR Spectra (KBr, 4000–400 cm⁻¹): 3035 \nu (=C-H) ar. Stretching, 1604 \nu (C=O) ar. Stretching, 1504 \nu (C=N) ar. Stretching, 1213 \nu (C-N) ar. Stretching, 956 \nu (C-Br) stretching, 817 \nu (C-Cl) stretching, 756 \nu (C-Br) bending, 678 \nu (C-H) bending.

2.3.2 5-(5-Bromothiophen-2-yl)-1-(4-chlorophenyl)-3-(3-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole (L₂)

This ligand (L₂) was prepared through the addition of \(\alpha, \beta\)-unsaturated enone (3b) (1.5 mmol) and 4-Cl phenyl hydrazine (123 mg, 1.479 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: \(\text{C}_{20}\text{H}_{13}\text{BrClF}_3\text{N}_2\text{S}\) Yield: 76.32% Melting point: 278–280°C Molecular weight: 485.75 gm/mol. ^1H NMR (400 MHz, DMSO-\textit{d}_6) \delta/\text{ppm}: 7.755 (2H, dd, J₁ = 4.4 Hz, J₂ = 8.0 Hz, H3″′,5″′), 7.328 (2H, dd, J₁ = 1.6 Hz, J₂ = 6.8 Hz, H2″′,6″′), 7.263 (1H, S, H2″), 7.043 (3H, dt, H4″,5″,6″), 6.899 (1H, d, J = 3.6 Hz, H3), 6.779 (1H, d, J = 3.6 Hz, H4), 5.422 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H3′), 3.796 (1H, dd, J₁ = 11.6 Hz, J₂ = 16.8 Hz, H4′a), 3.280 (1H, dd, J₁ = 6.4 Hz, J₂ = 16.8 Hz, H4′b) 13C NMR (100 MHz, DMSO-\textit{d}_6) \delta/\text{ppm}: 156.16 (C5′, -Cquat), 152.08 (C1″′, -Cquat), 146.85 (C3″′, -Cquat), 142.04 (C5, -Cquat), 140.15 (C1″, -Cquat), 128.76 (C4″′, -Cquat), 112.08 (-CF₃, -Cquat), 108.55 (C3″, -Cquat), 107.47 (C2, -Cquat), 43.76 (C4′, -CH₂), 155.58 (C4″, -CH), 147.84 (C3″′, 5″′, -CH), 131.83 (C5″, -CH), 129.82 (C6″, -CH), 126.26 (C2″, -CH), 126.16 (C3″, -CH), 124.70 (C4″, -CH), 115.45 (C2″′, 6″′, -CH) [Total signal observed = 18: signal of Cquat and –CH₂ = 10, signal of –CH and –CH₃ = 8].

IR Spectra (KBr, 4000–400 cm⁻¹): 3252 \nu (-NH) stretching, 2934 \nu (-CH) stretching, Alkane, 2819 \nu (= CH) stretching, 2332 \nu (-C-N) stretching, 1576, 1468 \nu (C = N), 1222 \nu (C = C) stretching, 1067, 805 \nu (para substitution), 620, 558 \nu (C-Cl).

2.3.3 5-(5-Bromothiophen-2-yl)-1-(4-chlorophenyl)-3-(4-(methylthio)phenyl)-4,5-dihydro-1H-pyrazole (L³)

This ligand (L³) was prepared through the addition of \(\alpha, \beta\)-unsaturated enone (3C) (1.5 mmol) and 4-CH₃ phenyl hydrazine (1.5 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: \(\text{C}_{20}\text{H}_{16}\text{BrClN}_2\text{S}_2\) Yield: 79.15% Melting point: 255–270°C Molecular weight: 463.84 gm/mol calc. (%): C, 51.79; H, 3.48; N, 6.04; S, 13.82 Found. (%): C, 51.46; H, 3.53; N, 5.97; S, 13.61; m/z(%): 462 (100) [M]+, 464 [M + 2]+; ^1H NMR (400 MHz, DMSO-\textit{d}_6) \delta/\text{ppm}: 7.640 (2H, d, J = 8.4 Hz, H2″,6″), 7.328 (2H, d, J = 8.8 Hz, H3″,5″), 7.254 (1H, dd, J = 8.4 Hz, H2″,6″), 7.036 (2H, d, J = 9.2 Hz, H3″,5″), 6.899 (1H, d, J = 4.0 Hz, H3), 6.779 (1H, d, J = 3.6 Hz, H4), 5.422 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H3′), 3.796 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H3′), 3.280 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H4′a), 3.280 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H4′b).
17.2 Hz, H4'a), 3.268 (1H, dd, J1 = 6.4 Hz, J2 = 16.8 Hz, H4'b), 2.536 (3H, S, -SCH3), 13C NMR (100 MHz, DMSO-\(d_6\)) δ/ppm:156.13 (C5', -Cquat), 155.07 (C1''', -Cquat), 149.26 (C4'', -Cquat), 145.90 (C1'', -Cquat), 144.63 (C5, -Cquat), 132.36 (C4'', -Cquat), 129.46 (C2, -Cquat), 115.03 (C3', -Cquat), 43.69 (C4', -CH2), 150.48 (C3''',5''', -CH), 140.44 (C3, -CH), 128.88 (C5'', -CH), 127.35 (C3'',5'', -CH), 126.57 (C2'',6'', -CH), 126.24 (C2''',6''', -CH), 15.46 (-SCH3, -CH3) [Total signal observed = 16 : signal of Cquat and –CH2 = 9, signal of -CH and –CH3 = 7] IR Spectra (KBr, 4000–400 cm\(^{-1}\)): 3080 \(\nu\) (=C-H) ar. Stretching, 1604 \(\nu\) (C= C) ar. Stretching, 1481 \(\nu\) (C= N) ar. Stretching, 1319 \(\nu\) (C-N) ar. Stretching, 856 \(\nu\) (C-Br) stretching, 794 \(\nu\) (C-Cl) stretching, 725 \(\nu\) (=C-H) bending, 663 \(\nu\) (C-H) bending.

2.3.4 3-(1-(4-bromophenyl)-5-(5-bromothiophen-2-yl)-4,5-dihydro-1H-pyrazol-3yl) pyridine (L\(^4\))

This ligand (L\(^4\)) was prepared through the addition of \(\alpha,\beta\)-unsaturated enone (3d) (1.5 mmol) and 4-NO\(_2\) phenyl hydrazine (1.5 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: C\(_{18}\)H\(_{13}\)Br\(_2\)N\(_3\)S Yield: 80.76% Melting point: 250–254°C Molecular weight: 463.19 gm/mol calc. (%): C, 46.68; H, 2.83; N, 9.07; S, 6.92 Found. (%): C, 46.74; H, 2.81; N, 8.99; S, 6.80 m/z(%):462 (100) [M\(^+\)], 464 [M + 2]+; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) δ/ppm: 7.640 (2H, d, J = 8.4 Hz, H2''',6'''), 7.335 (2H, dt, H4'',5''), 7.264 (1H, S, H2''), 7.043 (1H, dt, H6''), 6.899 (2H, d, J = 3.6 Hz, H3''',5'''), 6.779 (2H, d, J = 4.0 Hz, H3,4), 5.418 (1H, dd, J1 = 8.4 Hz, J2 = 12.0 Hz, H3'), 3.795 (1H, dd, J1 = 11.2 Hz, J2 = 17.2 Hz, H4'a), 3.267 (1H, dd, J1 = 6.4 Hz, J2 = 12.0 Hz, H4'b) 13C NMR (100 MHz, DMSO-\(d_6\)) δ/ppm: 153.28 (C5', -Cquat), 147.73 (C1''', -Cquat), 146.83 (C5, -Cquat), 143.71 (C1'', -Cquat), 140.25 (C4''', -Cquat), 128.69 (C2, -Cquat), 111.93 (C3', -Cquat), 110.33 (C2''',6''', -CH), 3.267 (1H, dd, J1 = 6.4 Hz, J2 = 12.0 Hz, H4'a), 3.267 (1H, dd, J1 = 6.4 Hz, J2 = 12.0 Hz, H4'b) 13C NMR (100 MHz, DMSO-\(d_6\)) δ/ppm: 152.45 (C5', -Cquat), 148.58 (C1''', -Cquat), 145.15 (C1'', -Cquat), 144.26 (C3'', -Cquat), 137.63 (C5, -Cquat), 118.44 (-CF\(_3\), -Cquat), 110.51 (C4', -Cquat), 107.12 (C3', -Cquat), 36.34 (C4', -CH2) 151.94 (C6*, -CH), 138.10

2.3.5 1-(4-Bromophenyl)-5-(5-bromothiophen-2-yl)-3-(3-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole (L\(^5\))

This ligand (L\(^5\)) was prepared through the addition of \(\alpha,\beta\)-unsaturated enone (3e) (1.5 mmol) and 4-bromo phenyl hydrazine (1.5 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: C\(_{20}\)H\(_{13}\)Br\(_2\)F\(_3\)N\(_2\)S Yield: 81.48% Melting point: 254–259°C Molecular weight: 530.20 gm/mol; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) δ/ppm: 7.783 (1H, S, H2''), 7.699 (2H, d, J = 8.0 Hz, H3''',5'''), 7.367 (2H, d, J = 8.8 Hz, H2'',6'''), 7.316 (2H, d, J = 8.4 Hz, H4'',6''), 7.064 (1H, t, J = 4.4 Hz, H5'''), 7.004 (2H, d, J = 3.6 Hz, H3,4), 5.851 (1H, dd, J1 = 4.8 Hz, J2 = 11.2 Hz, H3'), 3.840 (1H, dd, J1 = 11.6 Hz, J2 = 17.2 Hz, H4'a), 3.260 (1H, dd, J1 = 6.4 Hz, J2 = 12.0 Hz, H4'b) 13C NMR (100 MHz, DMSO-\(d_6\)) δ/ppm: 152.45 (C5', -Cquat), 148.58 (C1''', -Cquat), 145.15 (C1'', -Cquat), 144.26 (C3'', -Cquat), 137.63 (C5, -Cquat), 118.44 (-CF\(_3\), -Cquat), 110.51 (C4', -Cquat), 107.12 (C3', -Cquat), 36.34 (C4', -CH2) 151.94 (C6*, -CH), 138.10
(C3′′′,5′′′, -CH), 131.52 (C5′′′, -CH), 130.07 (C4′′, -CH), 126.41 (C3′′, -CH), 125.64 (C4′, -CH), 115.45 (C2′′′,6′′′, -CH) [Total signal observed = 17: signal of Cquat and –CH2 = 10, signal of -CH and –CH3 = 7]. IR Spectra (KBr, 4000–400 cm⁻¹): 3016 ν= (C=H) ar. Stretching, 1614 ν(C = C) ar. Stretching, 1462 ν(C = N) ar. Stretching, 1226 ν(C=N) ar. Stretching, 856 ν(C-Br) stretching, 786 ν=(C=H) bending, 671 ν(CH) bending.

2.3.6 1-(4-bromophenyl)-5-(5-bromothiophen-2-yl)-3-(4-(methylthio)phenyl)-4,5-dihydro- 1H-pyrazole (L₆)

This ligand (L₆) was prepared through the addition of α,β-unsaturated enone (3f) (1.5 mmol) and 4-OCH₃ phenyl hydrazine (1.5 mmol), after 5–6 h reflux; Color: yellowish crystalline solid; Empirical formula: C₂₀H₁₆Br₂N₂S₂ Yield: 80.79% Melting point: 246–250°C Molecular weight: 508.29 gm/mol Calc. (%): C, 47.26; H, 3.17; N, 5.55; S, 12.61 Found. (%): C, 47.18; H, 3.23; N, 5.37; S, 12.55 m/z(%): 507 (100) [M]+, 509 [M+2]+; ¹H NMR (400 MHz, DMSO-d₆) δ/ppm: 7.640 (2H, d, J = 8.4 Hz, H₃′′′,5′′′), 7.328 (2H, dd, J₁ = 2.0 Hz, J₂ = 6.8 Hz, H₂″″,6″″), 7.251 (1H, d, J = 8.0 Hz, H₃), 7.036 (2H, dd, J₁ = 2.0 Hz, J₂ = 6.8 Hz, H₃″″,5″″), 6.899 (2H, d, J = 3.6 Hz, H₂″″,6″″), 6.781 (1H, d, J = 3.6 Hz, H₄), 5.470 (1H, dd, J₁ = 5.2 Hz, J₂ = 8.8 Hz, H₃′), 3.799 (1H, dd, J₁ = 12.0 Hz, J₂ = 16.8 Hz, H₄′a), 3.268 (1H, dd, J₁ = 6.8 Hz, J₂ = 17.2 Hz, H₄′b), 2.537 (3H, S, -SCH₃) ¹³C NMR (100 MHz, DMSO-d₆) δ/ppm: 151.85 (C5′′, -Cquat), 145.58 (C1′′′, -Cquat), 144.15 (C1′′, -Cquat), 138.88 (C4′, -Cquat), 124.70 (C2′, -Cquat), 117.38 (C5, -Cquat), 113.12 (C4′′, -Cquat), 112.94 (C3′, -Cquat), 43.68 (C4′, -CH₂) 151.30 (C3′, -CH), 142.31 (C4′, -CH), 131.77 (C3′′′,5′′′, -CH), 129.77 (C2′′,6′′, -CH), 126.59 (C3′′,5′′, -CH), 126.26 (C2′′′,6′′′, -CH), 15.46 (SCH₃, -CH₃) [Total signal observed = 16: signal of Cquat and –CH₂ = 9, signal of -CH and –CH₃ = 7]. IR Spectra (KBr, 4000–400 cm⁻¹): 3096 ν= (C=H) ar. Stretching, 1650 ν(C = C) ar. Stretching, 1566 ν(C = N) ar. Stretching, 1319 ν(C=N) ar. Stretching, 910 ν(C-Br) stretching, 794 ν=(C=H) bending, 678 ν(CH) bending.

2.4 General method for synthesis of Palladium(II) complexes (I-VI)

The thiophene-pyrazole-based ligands(1.0 mmol) (L₁ – L₆) dissolved in a small amount of toluene. Ligands solution was added dropwise in 1.0 mmol sodium tetrachloropalladate(II) solution and refluxed in the presence of toluene for 12 h at 150°C. After 12 h, a brownish colour product was obtained. Synthesized compounds washed with methanol. The reaction scheme for the preparation of organometallic compounds (I - VI) is depicted in scheme 1.

2.4.1 Characterization of heteroleptic palladium(II) complex Na[Pd(L₁)Cl₂] (I)

It was synthesized using ligand (L₁) (1.0 mmol). Color: brown crystalline solid; Empirical formula: C₁₈H₁₂BrClN₃NaPdS Yield: 59.78% Melting point: > 300°C Molecular weight: 618.04 gm/mol Calc. (%): C, 34.98; H, 1.96; N, 3.72; Pd, 17.22; S, 5.19 Found. (%): C, 35.03; H, 1.53; N, 3.79;Pd, 17.33; S, 5.24
m/z(%): 350.4 (100) [M+], 352.4 [M + 2] Conductance: 14.72 ohm-1cm2mol⁻¹; ¹H NMR (400 MHz, DMSO-
\(d_6\)) \(\delta/\text{ppm}: 7.907 (2H, d, J = 4.0 Hz, H3,4), 7.626 (1H, d, J = 7.2 Hz, H4''), 7.534 (2H, dd, J1 = 8.4 Hz, J2 = 15.2 Hz, H3'',5''), 7.360 (1H, t, J = 8.8 Hz, H5''), 7.055 (2H, dd, J1 = 6.8 Hz, J2 = 8.8 Hz, H2'',6''), 6.907 (1H, d, J = 3.6 Hz, H6''), 5.511 (1H, dd, J1 = 6.4 Hz, J2 = 12.8 Hz, H3''), 3.847 (1H, dd, J1 = 12.0 Hz, J2 = 16.8 Hz, H4'a), 3.323 (1H, dd, J1 = 6.0 Hz, J2 = 16.8 Hz, H4'b). ¹H NMR (400 MHz, DMSO-
\(d_6\)). ¹³C NMR (100 MHz, DMSO-
\(d_6\)) \(\delta/\text{ppm}: 154.97 (C2'', -Cquat), 147.08 (C1''', -Cquat), 143.28 (C5', -Cquat), 132.22 (C5, -Cquat), 130.87 (C4'', -Cquat), 126.43 (C1'', -Cquat), 115.67 (C2, -Cquat), 110.99 (C3', -Cquat), 43.05 (C4', -CH₂) 154.04 (C4'', -CH), 149.80 (C6'', -CH), 130.04 (C3''',5'''', -CH), 128.73 (C3, -CH), 127.70 (C4, -CH), 125.40 (C5'', -CH), 123.93 (C2'',6'', -CH) [Total signal observed = 16: signal of Cquat and –CH₂ = 9, signal of -CH and –CH₃ = 7]. IR Spectra (KBr, 4000–400 cm⁻¹): 2977 \(\nu (=\text{C-H})\text{ ar. Stretching}, 1596 \(\nu =\text{C} =\text{C}\text{ ar. Stretching}, 1502 \(\nu =\text{C} =\text{N}\text{ ar. Stretching}, 1211 \(\nu =\text{C-N}\text{ ar. Stretching}, 965 \(\nu =\text{C-Br}\text{ stretching}, 825 \(\nu =\text{C-Cl}\text{ stretching}, 748 \(\nu (=\text{C-H})\text{ bending}, 694 \(\nu (=\text{C-H})\text{ bending}, 555 \(\nu =\text{Pd-N}\).

2.4.2 Characterization of heteroleptic palladium(II) complex Na[Pd(L²)Cl₂] (II)

It was synthesized using ligand (L²) (1.0 mmol). Empirical formula: C₂₀H₁₂BrCl₃F₃N₂NaPdS Yield: 57.24%; Melting point: > 300°C Molecular weight: 685.05 gm/mol Calc. (%): C, 35.07; H, 1.77; N, 4.09; Pd, 15.53; S, 4.68 Found. (%): C, 35.19; H, 1.63; N, 4.14; Pd, 15.79; S, 4.75 Conductance: 16.57 ohm⁻¹cm²mol⁻¹; ¹H NMR (400 MHz, DMSO-
\(d_6\)) \(\delta/\text{ppm}: 8.499 (2H, dt, H4'',6'''), 7.379 (1H, t, H5''), 7.087 (2H, d, J₁ = 2.4 Hz, J₂ = 7.2 Hz, H3''',5'''), 6.778 (2H, d, J₁ = 3.6 Hz, H3,4), 5.557 (1H, dd, J₁ = 6.4 Hz, J₂ = 12.0 Hz, H4'a), 3.923 (1H, dd, J₁ = 12.4 Hz, J₂ = 18.0 Hz, H4'b) ¹³C NMR (100 MHz, DMSO-
\(d_6\)) \(\delta/\text{ppm}: 159.14 (C1''', -Cquat), 158.54 (C5', -Cquat), 154.46 (C1'', -Cquat), 146.86 (C5, -Cquat), 129.82 (C3'', -Cquat), 124.94 (C4'', -Cquat), 115.75 (-CF₃, -Cquat), 113.23 (C3', -Cquat), 42.86 (C4', -CH₂) 153.64 (C6'', -CH), 143.64 (C4'', -CH), 143.11 (C3, -CH), 142.97 (C4, -CH), 132.32 (C3''',5'''', -CH), 132.09 (C5', -CH), 131.98 (C2'',6'', -CH), 114.82 (C2', -CH) [Total signal observed = 18: signal of Cquat and –CH₂ = 10, signal of -CH and –CH₃ = 8]. IR Spectra (KBr, 4000–400 cm⁻¹): 2923 \(\nu (=\text{C-H})\text{ ar. Stretching}, 1627 \(\nu (\text{C} = \text{C})\text{ ar. Stretching}, 1496 \(\nu (\text{C} = \text{N})\text{ ar. Stretching}, 1257 \(\nu (\text{C-N})\text{ ar. Stretching), 864 \(\nu (\text{C-Br})\text{ stretching}, 756 \(\nu (=\text{C-H})\text{ bending}, 632 \(\nu (\text{C-H})\text{ bending}, 524 \(\nu (\text{Pd-N}).

4.4.3 Characterization of heteroleptic palladium(II) complex Na[Pd(L²)Cl₂] (III)

It was synthesized using ligand (L³) (1.0 mmol). Color: brown crystalline solid; Empirical formula: C₂₀H₁₅BrCl₃N₂NaPdS₂ Yield: 53.74% Melting point: > 300°C Molecular weight: 663.14 gm/mol Calc. (%): C, 36.22; H, 2.28; N, 4.22; Pd, 16.05; S, 9.67 Found. (%): C, 36.16; H, 2.20; N, 4.15; Pd, 15.88; S, 9.77 Conductance: 15.96 ohm⁻¹cm²mol⁻¹; ¹H NMR (400 MHz, DMSO-
\(d_6\)) \(\delta/\text{ppm}: 7.948 (1H, S, H3''), 7.906 (1H, d, J = 7.2 Hz, H3''), 7.624 (1H, d, J = 7.6 Hz, H6''), 7.554 (1H, d, J = 8.4 Hz, H3), 7.361 (2H, d, J = 2.0 Hz, H3'',5''), 7.062 (2H, d, J = 9.2 Hz, H2'',6''), 6.907 (1H, d, J1 = 4.0 Hz, H4), 5.510 (1H, dd, J1 = 6.4 Hz, J2 = 12.0 Hz, H3''), 3.847 (1H, dd, J1 = 12.4 Hz, J2 = 17.2 Hz, H4'a), 3.322 (1H, dd, J1 = 6.4 Hz, J2 = 17.2 Hz,
H4'b), 2.281 (3H, S, -SCH3) 13C NMR (100 MHz, DMSO-d6) δ/ppm: 154.62 (C1'', -Cquat), 147.74 (C5', -Cquat), 144.49 (C4'', -Cquat), 140.26 (C5', -Cquat), 126.26 (C2'', -Cquat), 126.16 (C4'', -Cquat), 124.70 (C1'', -Cquat), 112.08 (C3', -Cquat), 111.93 (C2, -Cquat), 43.68 (C4', -CH2) 153.13 (C3'',5'', -CH), 143.35 (C6'', -CH), 131.77 (C3, -CH), 129.77 (C4, -CH), 115.45 (C3'', -CH), 108.10 (C5'', -CH), 107.04 (C2'',6'', -CH), 15.47 (-SCH3, -CH3) [Total signal observed = 18: signal of Cquat and –CH2 = 10, signal of -CH and –CH3 = 8]. IR Spectra (KBr, 4000–400 cm−1): 3047 υ (=C-H) Ar. Stretching, 1589 υ (C=O) Ar. Stretching, 1488 υ (C=N) Ar. Stretching, 1380 υ (C-N) Ar. Stretching, 817 υ (C=O), 748 υ (=C-H) Bending, 667 υ (C-H) Bending, 570 υ (Pd-N).

2.4.4 Characterization of heteroleptic palladium(II) complex Na[Pd(L^{4})Cl_{2}] (IV)

It was synthesized using ligand (L^{4}) (1.0 mmol). Color: brown crystalline solid; Empirical formula: C_{18}H_{12}Br_{2}Cl_{2}N_{3}NaPdS Yield: 57.48% Melting point: > 300°C Molecular weight: 662.49 gm/mol Calc. (%): C, 32.63; H, 1.83; N, 6.34; Pd, 16.06; S, 4.84 Found. (%): C, 32.14; H, 1.82; N, 6.54; Pd, 16.22; S, 4.71; Conductance: 18.04 ohm^{-1}cm^{2}mol^{-1}; 1H NMR (400 MHz, DMSO-d6) δ/ppm: 7.939 (1H, t, J = 8.8 Hz, H5''), 7.610 (1H, d, J = 6.8 Hz, H4''), 7.556 (1H, d, J = 8.8 Hz, H6''), 7.354 (2H, dd, J1 = 8.0 Hz, J2 = 14.0 Hz, H3'',5'''), 7.043 (2H, dd, J1 = 11.2 Hz, J2 = 15.6 Hz, H2'',6'''), 6.902 (1H, d, J = 8.0 Hz, H4), 6.871 (1H, d, J = 8.0 Hz, H3), 5.511 (1H, dd, J1 = 6.4 Hz, J2 = 11.6 Hz, H3'), 3.857 (1H, dd, J1 = 6.8 Hz, J2 = 16.0 Hz, H4'a), 3.325 (1H, dd, J1 = 6.4 Hz, J2 = 17.6 Hz, H4'b) 13C NMR (100 MHz, DMSO-d6) δ/ppm: 158.41 (C1'', -Cquat), 156.46 (C2'', -Cquat), 149.78 (C5', -Cquat), 138.86 (C5', -Cquat), 132.71 (C1'', -Cquat), 128.04 (C4'', -Cquat), 122.58 (C2, -Cquat), 114.49 (C3', -Cquat), 33.12 (C4', -CH2) 150.73 (C4'', -CH), 146.46 (C6'', -CH), 141.66 (C3'',5'', -CH), 133.94 (C3, -CH), 124.77 (C4, -CH), 117.04 (C5'', -CH), 108.93 (C2'',6'', -CH) [Total signal observed = 16: signal of Cquat and –CH2 = 9, signal of -CH and –CH3 = 7]. IR Spectra (KBr, 4000–400 cm−1): 3047 υ (=C-H) Ar. Stretching, 1596 υ (C=C) Ar. Stretching, 1488 υ (C=N) Ar. Stretching, 1380 υ (C=N) Ar. Stretching, 817 υ (C=O), 748 υ (=C-H) Bending, 667 υ (C-H) Bending, 570 υ (Pd-N).

2.4.5 Characterization of heteroleptic palladium(II) complex Na[Pd(L^{5})Cl_{2}] (V)

It was synthesized using ligand (L^{5}) (1.0 mmol). Color: brown crystalline solid; Empirical formula: C_{20}H_{12}Br_{2}Cl_{2}F_{3}N_{2}NaPdS Yield: 60.14% Melting point: > 300°C Molecular weight: 729.50 gm/mol Calc. (%): Pd, 14.59; Found. (%): Pd, 14.35; Conductance: 20.18 ohm^{-1}cm^{2}mol^{-1} 1H NMR (400 MHz, DMSO-d6) δ/ppm: 7.793 (1H, t, J = 12.8 Hz, H5''), 7.640 (1H, d, J = 8.4 Hz, H4''), 7.441 (1H, d, J = 2.4 Hz, H6''), 7.354 (1H, d, J = 8.4 Hz, H4), 6.902 (1H, d, J = 8.0 Hz, H4), 6.871 (1H, d, J = 8.0 Hz, H3), 5.511 (1H, dd, J1 = 6.4 Hz, J2 = 11.6 Hz, H3'), 3.799 (1H, dd, J1 = 6.8 Hz, J2 = 16.0 Hz, H4'a), 3.269 (1H, dd, J1 = 6.4 Hz, J2 = 16.8 Hz, H4'b) 13C NMR (100 MHz, DMSO-d6) δ/ppm: 155.45 (C1'', -Cquat), 150.73 (C5', -Cquat), 144.82 (C3'', -Cquat), 133.51 (C5, -Cquat), 129.91 (C1'', -Cquat), 127.56 (C2'', -Cquat), 119.49 (C4'', -Cquat), 115.30 (C2, -Cquat), 112.95 (C3', -Cquat), 104.20 (-CF_{3}, -Cquat), 31.13 (C4', -Cquat).
CH₂) 136.63 (C6", -CH), 130.69 (C3",5", -CH), 130.60 (C5", -CH), 126.51 (C3, -CH), 121.80 (C4, -CH), 116.62 (C4", -CH), 116.41 (C2",6", -CH) [Total signal observed = 18: signal of Cquat and CH₂ = 11, signal of -CH and –CH₃ = 7]. IR Spectra (KBr, 4000–400 cm⁻¹): 3109 ν(= C-H) ar. Stretching, 1609 ν(C = C) ar. Stretching, 1535 ν(C = N) ar. Stretching, 1257 ν(C-N) ar. Stretching, 906 ν(C-Br) stretching, 810 ν(= C-H) bending, 624 ν(C-H) bending, 563 ν(Pd-N).

2.4.6 Characterization of heteroleptic palladium(II) complex Na[Pd(L⁶)Cl₂] (VI)

It was synthesized using ligand (L⁶) (1.0 mmol). Color: brown crystalline solid; Empirical formula: C₂₀H₁₅Br₂Cl₂NaPdS₂ Yield: 51.49% Melting point: > 300°C Molecular weight: 707.59 gm/mol Calc. (%): C, 33.95; H, 2.14; N, 3.96; Pd, 15.04; S, 9.06; Found. (%): C, 33.82; H, 2.03; N, 4.05; Pd, 15.21; S, 9.16; Conductance: 18.76 ohm⁻¹cm²mol⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ/ppm: 7.640 (2H, dd, J₁ = 2.0 Hz, J₂ = 6.8 Hz, H3"'), 7.328 (2H, d, J = 9.2 Hz, H3",4"'), 7.265 (1H, S, H6"'), 7.037 (2H, dd, J₁ = 2.0 Hz, J₂ = 6.8 Hz, H2"',6"'), 6.898 (1H, d, J = 3.6 Hz, H4), 6.778 (1H, d, J = 3.6 Hz, H3), 5.421 (1H, dd, J₁ = 6.4 Hz, J₂ = 11.6 Hz, H3'), 3.795 (1H, dd, J₁ = 11.6 Hz, J₂ = 16.8 Hz, H4'a), 3.268 (1H, dd, J₁ = 6.4 Hz, J₂ = 16.8 Hz, H4'b), 2.536 (3H, S, -SCH₃) ¹³C NMR (100 MHz, DMSO-d₆) δ/ppm: 153.21 (C1"', -Cquat), 149.22 (C5", -Cquat), 146.35 (C4", -Cquat), 132.62 (C5, -Cquat), 129.59 (C2", -Cquat), 127.69 (C1", -Cquat), 123.20 (C4", -Cquat), 123.12 (C2, -Cquat), 111.10 (C3", -Cquat), 39.34 (C4", -CH₂) 149.09 (C3",5", -CH), 134.98 (C3",5", -CH), 129.00 (C6", -CH), 126.26 (C3, -CH), 115.17 (C4, -CH), 109.34 (C2",6", -CH), 17.24 (-SCH₃, -CH₃) [Total signal observed = 17: signal of Cquat and -CH₂ = 10, signal of -CH and –CH₃ = 7]. IR Spectra (KBr, 4000–400 cm⁻¹): 3039 ν(= C-H) ar. Stretching, 1674 ν(C = C) ar. Stretching, 1596 ν(C = N) ar. Stretching, 1404 ν(C-N) ar. Stretching, 918 ν(C-Br) stretching, 825 ν(= C-H) bending, 686 ν(C-H) bending, 547 ν(Pd-N).

2.5 Biological screening of compound

2.5.1 MIC by broth dilution method

All synthesized compounds were tested for MIC using the broth dilution method. The method used to evaluate MIC value is according to the reported process[25]. Serially two-fold dilution of the test compound added to three Gram(−ve) microorganisms, namely Pseudomonas aeruginosa (MTCC P-09), Escherichia Coli (MTCC 433), Serratia marcescens (MTCC 7103) and two Gram(+ve) bacteria, namely Bacillus subtilis Staphylococcus aureus (MTCC 3160), (MTCC 7193).

2.5.2 Evaluation of in vitro cytotoxicity

The cytotoxicity of complexes was studied on brine shrimp, Artemia cysts, according to the method reported by Meyer et al. [25].

2.5.3 Cell proliferation assay
Tested compounds were diluted in DMSO with a regular interval in RPMI cell tissue medium in quadruplicate. Cells were sustained in RPMI-1640 implement completed with 10% fetal bovine serum (FBS) without antibiotics. Cells were cultured in monolayer and sustained with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at 37°C with CO₂ (5%) for 1 day. At the end of the treatment period, medium was withdrawn from cells and added 0.5 mg/mL MTT, prepared from PBS and incubated in 5% CO₂ for four h. After four h, the MTT implement was discarded from the cell and washed utilizing PBS (300 µL). The formed precipitates were soluble in 100 µL DMSO and yellow dye turned into purple formazan, which color intensity was observed at 570 nm.

2.5.4 DNA interaction studies

2.5.4.1 Absorption spectra

A DNA binding assay is a powerful technique for investigating the biological activity of a new compound and studying its ability to interfere with DNA replication and transcription. Among the different methods, we selected spectroscopic titration experiments to determine the binding mode. The investigation was carried out according to previously published literature[26].

2.5.4.2 Viscosity measurement

Further clarification of the interaction between the complexes and DNA was carried out by viscosity measurements. Measurement of viscosity is a simple, complementary and excellent method to know the binding mode of metal complexes to DNA. The detailed experimental process was as reported in the literature[27].

2.5.4.3 Fluorescence quenching analysis

Emission spectral study of EB–DNA complex used to recognize the binding of test complexes to DNA regardless of their binding modes and only judge their capacity impact the EB intensities in EB–DNA complex. Metal complexes quenching constants to HS-DNA were diagnosed by fluorescence titration study using EB, which contrives highly fluorescent EB-DNA compound with emission property at 610 nm. Fluorescence experiments were performed by addition of synthesized compounds to PB of EB-DNA. In this study, EB concentration was fixed at 33.3 µm and 10 µm of HS-DNA added in PB solution. The solution was incubated at R.T for 10 min. After the addition of the above solution, emission spectra were recorded at 500–800 nm in Fluoromax-4, HORIBA spectrofluorometer with 1 cm path length quartz cell. Competitive spectral study of EB bound HS-DNA with or without quencher (complex) have been noted for [EB] = 33.3 µm and [HS-DNA] = 10 µm against insertion of increasing quantity of compounds (10–100 µm).

2.5.4.4 Molecular docking study

Molecular docking is a vital tool in computational drug design[28]. Docking study showed the binding affinity and some hydrogen bonds. It is interesting to note that the binding affinities have negative values. Molecular docking is an approach used in drug design that functions by placing a small molecule into a
receptor site and studying the fit orientation bind mainly via non-covalent mode. The molecules were allowed to run in chembio3D software to refine the molecule according to the most stable conformational arrangement. The molecules were formatted as PDB files and were allowed to interact with B-DNA in HEX 8.0 software on CORE i3 processor in windows 8.1 operating system.

2.5.5 Protein binding studies

2.5.5.1 Absorption titration study

According to previously reported literature, the absorption study of the complexes with bovine serum albumin is performed [29].

2.5.5.2 Fluorescence quenching study

Fluorescence quenching of metal complex and protein is performed according to previously published literature[4, 5].

Result And Discussion

3.1 \(^1\)H NMR and \(^{13}\)C NMR spectra

The shifting of the peak in \(^1\)H NMR spectra suggests the involvement of pyridine ring nitrogen as a coordinating atom. In the case of ligands (L\(^1\)-L\(^6\)) and Pd(II) coordination complexes (I-VI), aromatic protons are observed in the range of 8.49–6.77 δ ppm and 8.50–6.78 δ ppm, respectively. The H\(_{4'a}\) and H\(_{4'b}\) protons in ligands (L\(^1\)-L\(^6\)) and complexes (I-VI) are observed in the range of 3.79–3.91, 3.77–3.92 δ ppm and 3.26–3.44, 3.29–3.46 δ ppm, respectively.

In the case of ligands (L\(^1\)-L\(^6\)) and organometallic palladium(II) complexes (I-VI), a signal of C\(_{4'}\) are observed at 36.3–43.7 ppm and 31.3–43.5 ppm, respectively. In ligands, L\(^2\) and L\(^5\) and complexes II and V, the signal of −CF\(_3\) carbon is observed at 112.0, 118.4 ppm and 115.7, 104.2 ppm, respectively. In ligands, L\(^3\) and L\(^6\) and complexes III and VI, the signal of −SCH\(_3\) carbon is observed at 15.5, 15.4 ppm, and 15.4, 17.2 ppm, respectively. The \(^1\)H and \(^{13}\)C NMR spectra are shown in supplementary material 1.

3.2 FT-IR spectroscopy

IR frequency of heteroleptic palladium(II) complexes (I-VI) are slightly shifted than respective ligands (L\(^1\)-L\(^6\)). In ligands (L\(^1\)-L\(^6\)) and complexes (I-VI), frequency of ν(C = C)\(_{\text{ar. stretching}}\) are observed at around 1523–1620 cm\(^{-1}\) and 1512–1604 cm\(^{-1}\), respectively. Frequency of ν(C = O)\(_{\text{stretching}}\) in complexes (I-VI) are observed at about 1735–1897 cm\(^{-1}\). In ligands (L\(^1\)-L\(^6\)) and complexes (I-VI), frequency of ν(C = N)\(_{\text{ar. stretching}}\) are observed at around 1523–1620 cm\(^{-1}\) and 1488–1596 cm\(^{-1}\), respectively.
3.3 Electronic spectra, magnetic behaviour and conductance measurement

An electronic spectral study using a UV-visible spectrometer confirmed the geometry of palladium(II) compounds. The electronic spectra of organometallic Pd(II) complexes (I-VI) show bands in the range of 258–269 nm, 317–333 nm and 366–390 nm due to MLCT transition, CT transition and d-d transition, respectively.

The magnetic moments of Pd(II) compounds are zero B.M., which is similar to the theoretical value for square planar complexes with dsp² hybridization, low-spin d⁸ system and diamagnetic. The conductance of complex in DMSO (10⁻³ M) was measured using E-660A conductometer to examine the electrolytic response of metal complex. Molar conductance of complexes (I-VI) is observed in the range of 14–21 Ω⁻¹ cm² mol⁻¹, suggesting a negative charge of complex and one sodium ion presence in the outer sphere.

3.4 Biological screening of synthesized compounds

3.4.1 In vitro antibacterial activity

The ligands L², L⁴, and L⁶ exhibit significant activity than L¹, L³, and L⁵ against five organisms. Complexes I, III and V exhibit significant activity than complexes II, IV and VI against five organisms. The coordination compounds (45–105 µm) show significant activity than ligands (255–375 µM), and are comparable with reported palladium (II) complexes[27]. Graphical representation of MIC value of ligand(L¹-L⁶) and Pd(II) complexes(I-VI) is shown in Fig. 1

The higher antimicrobial activity (lower MIC value) of the metal complexes (MIC = 45–105 µM) compared to the metal salt (MIC > 1000 µM) and respective ligand is due to nuclearity of the metal center in the complexes, chelate effects, nature of the ligands, and ion neutralizing the complexes[25].

3.4.2 In vivo brine shrimp lethality bioassay

The BSLB is a valuable method for assurance of lethal nature of synthesized compounds and demonstrates the pharmacological exercises of the compounds. It is widely used in the bioassay. Figure 2 represents the graph of LC₅₀ value of ligands (L¹-L⁶) and Pd(II) complexes (I-VI), respectively. Order of the LC₅₀ value: I > V > III > II > IV > I > L³ > L¹ > L² > L⁵ > L⁴ > L⁶, Complexes I and V show potent cytotoxicity activity than complexes II, III, IV, and VI. In ligands, L¹ and L³ exhibit potent cytotoxicity activity than L², L⁴ L⁵ and L⁶. LC₅₀ value of heterocyclic agents and Pd(II) complexes are observed range in range of 10.56–16.63 µg/mL, 5.70–7.58 µg/mL, respectively which is comparable to reported Pd(II) complexes (LC₅₀ = 5.68–7.94 µg/mL)[29]. The complexes exhibit good cytotoxicity activity than heterocyclic compounds.

3.4.3 Cell proliferation
Complexes (I, II and III) exhibit higher activity than remaining complexes over MCF-7 cell line. The IC\textsubscript{50} value of Pd(II) complexes IC\textsubscript{50} value for MCF-7 are found in sequence of I (65 µg/mL) > III (68 µg/mL) > II (80 µg/mL) > V (86 µg/mL) > VI (89 µg/mL) > IV (98 µg/mL). Complexes I, II, and III exhibit higher activity than other complexes over MCF-7 cell line due to presence of Cl-group at the para position of benzene ring. Graph of % cell proliferation vs. [complex] and IC\textsubscript{50} value graph represent in Fig. 4.

### 3.4.4 DNA binding studies

#### 3.4.4.1 Electronic absorption titration study

The C, N donor pyrazole based ligands (L\textsubscript{1}-L\textsubscript{6}) and palladium(II) complexes (I-VI) exhibit hypochromism shift and bathochromic shift, which show non-covalent interaction (i.e. intercalation) types of binding with K\textsubscript{b} value in range of 0.22 × 10\textsuperscript{5} – 2.90 × 10\textsuperscript{5} M\textsuperscript{-1}. Binding affinity order of ligands (L\textsubscript{1}-L\textsubscript{6}) and palladium complexes (I-VI) are: IV > I > II > III > V > VI > L\textsubscript{4} > L\textsubscript{1} > L\textsubscript{5} > L\textsubscript{3} > L\textsubscript{2} > L\textsubscript{6}. The K\textsubscript{b} value of complexes (I-VI) are comparable with [Pd(sac)(terpy)](sac).4H\textsubscript{2}O (1.0 × 10\textsuperscript{5} M\textsuperscript{-1}) and [Pdcl(terpy)](sac).2H\textsubscript{2}O (2.0 × 10\textsuperscript{5} M\textsuperscript{-1}) complexes[30].

Equation [(A\textsubscript{free} - A\textsubscript{bound})/A\textsubscript{free} × 100] and ΔG = -RTlnK were used to calculated % hypochromicity and Gibb’s free energy, respectively. The % hypochromicity and ΔG are established in the range 5.0 – 43.5 and -24.5 to -31.0 kJ mol\textsuperscript{-1}. The K\textsubscript{b} value graph of ligand1(L\textsubscript{1}) and respective complex is as shown in Fig. 4.

#### 3.4.4.2 Viscosity measurements

The hydrodynamic length of DNA generally increases upon partial intercalation. While it does not lengthen upon groove binding. Viscosity measurements can sensitively detect the lengthening of a DNA helix induced by the binding of intercalators. And thus provide evidence of intercalation for small DNA-binding molecules[31]. The data of the viscosity measurement are represented as the plot of the relative viscosity, i.e. (η)\textsuperscript{1/3} vs. [complex]/[DNA].

Viscosity order of compounds in decreasing form: VI > II > V > IV > III > I > L\textsubscript{3} > L\textsubscript{1} > L\textsubscript{4} > L\textsubscript{2} > L\textsubscript{5} > L\textsubscript{5}. The figure shows increased viscosity via HS-DNA with a higher concentration of compounds, which indicates the intercalation binding. The viscosity result of complexes (I-VI) is comparable with reported Pd(II) complexes[32]. A representative graph of the viscosity measurement of ligands and respective complexes is shown in Fig. 5.

#### 3.4.4.3 Fluorescence quenching analysis

Fluorescence spectroscopy has beneficial applications as a tool to study DNA–complex interactions due to its ability to examine the effects of environmental conditions on various DNA interactions. It is considered a non-destructive and sensitive technique for the characterization of DNA–complex
interactions. A competitive binding study of each complex with EB was carried out to determine whether it can displace EB from its HS DNA–EB complex. So, a comparison of the intrinsic emission spectra of the free EB–DNA complex and the metal complex’s presence can help to evaluate the mode of DNA interaction with the complexes. Here we have used the more concentrated solution of the EB than the tested compound, giving less gap between two sequential readings and an accurate binding constant.

In emission titration of Pd(II) complexes, emission intensity decrease on insertion of quencher to EB bound HS-DNA was recorded at 610 nm, which preferred intercalative binding. Value of \( K_{sv} \), binding constant (\( K_a \)), and binding sites (n) are observed in the range of \( 2.1–8.2 \times 10^3 \text{ M}^{-1} \), \( 0.20–6.1 \times 10^4 \text{ M}^{-1} \) and \( 0.904–1.298 \), respectively, which is comparable with reported palladium complexes[33, 34]. The order of binding constant \( K_a \) of pyrazole-based palladium complexes (I - VI) are: I < V < VI < II < IV < III.

**3.4.4.4 Molecular docking study**

The adequate energy of interaction in which molecule be fit with DNA was counted as its docking energy. Studies of protein-ligand interaction and structural information by docking stimulation from complexes clarify the mechanism of molecular recognition. Docking software hex 8.0 with PDB (1BNA) having sequence (5'-d(CGCGAATTCGCG)-3')\textsubscript{2} was used to find out binding energy, binding modes and binding affinity between proteins/DNA and ligands. The docking structure of synthesized ligands (L\textsubscript{1}-L\textsubscript{6}), Pd(II) complexes (I-VI) are represented in Fig. 7. Docking energy of complexes I, II, III, IV, V and VI are \(-281.84, -281.71, -281.84, -268.44, -303.48, -284.52 \text{ kJ/mol}\) while, their respective ligands are \(-248.93, -300.25, -272.67, -249.70, -277.46, -266.52 \text{ kJ/mol}\). Docked structure of complex I is given in Fig. 7. Docked images of all the compounds are given in Supplementary Material.

**3.5.5 Protein binding studies**

**3.5.5.1 Absorption titration study**

In absorption titration spectra, solution of BSA during titration over the addition of an increasing amount of ligands (L\textsubscript{1} – L\textsubscript{6}) and Pd(II) complexes (I-VI) at around 250–550 nm. Increase the band's intensity without any change of position, which can observe an increasing amount of complexes with BSA solution. The \( K_b \) value of ligands (L\textsubscript{1} – L\textsubscript{6}) and Pd(II) complexes (I-VI) are observed in the range of \( 0.41–1.18 \times 10^4 \) and \( 0.99–1.63 \times 10^4 \), respectively, which is comparative with reported palladium complexes[35].

representative graph of the ligand1(L\textsubscript{1}) and complex-I is shown in Fig. 8

**3.5.5.2 Fluorescence quenching study**

In emission intensity of Pd(II) complexes, emission intensity decrease with a blue shift in maximum emission wavelength (\( \lambda_{em} \)) of BSA is observed with an increase in quencher concentration at 345 nm, indicating the associative interaction between the BSA and quenchers. The intensity of fluorescence
regularly decreases with a concentration of complexes increase (Fig. 10) and results suggest that complex quenches intrinsic fluorescence of BSA. Value of $K_{sv}$, binding constant ($K_a$), and binding sites (n) are in range of $1.07–2.80 \times 10^3 \text{ M}^{-1}$, $0.10–0.58 \times 10^5 \text{ M}^{-1}$, and $0.92–1.12$, respectively which is comparable with reported literature[36, 37]. The order of binding constant $K_a$ of pyrazole-based palladium(II) complexes: I < VI < II < V < IV < III.

Conclusion

In this work, thiophene-pyrazole-based C, N- donor ligands ($L^1$-$L^6$) and their complexes with Pd(II) were synthesized and characterized by various spectral and analytical techniques. The $^1$H NMR and $^{13}$C NMR spectra signals are slightly shifted in complexes (I-VI) than corresponding ligands. Similarly, IR frequency changed upon complexation. The molar conductance value of complexes (I-VI) observed in range $14–21 \text{ cm}^2 \text{ mol}^{-1} \text{ Ω}^{-1}$ suggests a negative charge of complex and one sodium ion presence in the outer sphere. The geometry of Pd(II) coordination complexes are four coordinated with dsp$^2$ hybridization, low spin-d$^8$ system and diamagnetic. The data suggest that all complexes exhibit square planar geometry. The UV-visible, fluorescence titration viscosity measurement and molecular docking studies of Pd(II) complexes reveal that the interaction of synthesized complexes with HS-DNA is specifically through non-covalent interaction (i.e., intercalation) types of binding with DNA base pairs. The binding ability and binding energy of complex-DNA are higher compared to the corresponding ligand. All Pd(II) complexes exhibit good antibacterial activity with low MIC values against Gram(+ ve) and Gram(-ve) bacteria, which indicates that the complexes show good activity as compared to ligands. The platinum(II) and palladium(II) complexes exhibit higher potency in cellular level cytotoxicity and brine shrimp lethality bioassay than the pyrazole-based ligands. Pd(II) complexes show significant cytotoxicity against MCF-7 (human adenocarcinoma cell line) cell line. And comparable with clinically used metallodrugs such as cisplatin, carboplatin and oxaliplatin.

Declarations

Conflict of interest

The author declares no conflict of interest.

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**Figures**

![Graph Image](image-url)
Figure 1

MIC of ligand (L1-L6) and Pd(II) complexes (I-VI)

![Graph showing MIC values for ligands and complexes.]

Figure 2

LC50 value of ligand and metal complexes

![Graph showing LC50 values for ligands and complexes.]

Figure 3

% cell proliferation of Pd(II) complexes (I-IV)

![Graph showing percentage cell proliferation for Pd(II) complexes.]

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Figure 4

DNA binding of ligand 1 (L1) and Pd(II) complex I

Figure 5

Viscosity of ligands and Pd(II) complexes
Figure 6
Fluorescence spectra of Pd(II) complex

Figure 7
Molecular docking of ligand1(L1) and Pd(II) complex I
Figure 8

BSA absorption titration curve of complex-I
Figure 9

Fluorescence emission spectra of BSA quenching of complex-I

Supplementary Files

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- Scheme1.png
- SupplementaryMaterial.docx