Biological applications of pyrazoline based half-sandwich ruthenium(III) coordination compounds

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Supplementary material 1: Synthesis and characterization of the pyrazoline ligands and Ru(III) complexes

1.1 General method for synthesis of enones (chalcones) (3a-3g)

To the solution of 2-acetyl pyridine **(1)** (10 mmol, 1.12 mL) in 10 mL of methanol, freshly prepared methanolic KOH solution (20 mmol, 1.12 g, 25 mL) was added and stirred for 15 minutes To this, appropriate aldehyde **(2a–2g)** (10 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was cooled on an ice bath and neutralized with dilute hydrochloric acid. The precipitate appeared was separated by filtration and washed three times with 60 mL distilled water to give the crude product. The obtained product was recrystallized from methanol. The purity of the products was checked on TLC by using mixture of ethyl acetate and hexane as mobile phase.

1.2 General method for synthesis of tri-substituted pyrazoline (5a-5g)

To the solution of the appropriate enones **(3a– 3g)** (6 mmol) in 10 mL of methanol, phenyl hydrazine **(4)** (6 mmol) and freshly prepared methanolic potassium *tert*-butoxide (PTB) (3 mmol, 0.34 g) solution were added and the reaction mixture was refluxed for 5–6 h. Conversion was monitored in every 60 minutes interval on precoated silica TLC plates by using mixture of ethyl acetate and hexane as mobile phase. The excess of solvent was removed under reduced pressure and the reaction mixture was cooled on an ice bath. The products precipitated out at low temperature were washed five times with 60 mL distilled water, reconstituted in minimum amount of methanol and dried under reduced pressure. The proposed reaction for the synthesis of ligands 5a-5g is shown in scheme 1.

*1.2.1 Synthesis of 2-[5-(4-fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5a)***

Prepared by above method using 3-(4-fluorophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3a)** (6 mmol, 1.36 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 82%; yellow amorphous solid. **mp:** 134 ºC; **mol. wt.:** 317.37 g/mol; **empirical formula**: $C_{20}H_{16}FN_3$, **calc. (found) (%):** C, 75.69 (75.52); H, 5.08 (5.02); N, 13.24 (13.12); **mass (m/z):** 318 $[M]^+$; **^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ/ppm:** 3.319 (1H, dd, J = 7.2 Hz, 18.0 Hz, 4-H_a), 3.994 (1H, dd, J = 12.4 Hz, 17.6 Hz, 4-H_b), 5.362 (1H, dd, J = 6.8 Hz, 12.4 Hz, 5-H), 6.830-8.598 (13H, m, 2′,3′,4′,5′,2″,3″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ/ppm:** 163.34 ($C_{4''}$), 152.06 (C_3), 149.13 ($C_{5'}$), 148.05 ($C_{1'}$), 144.17 ($C_{1'''}$), 138.04 ($C_{1''}$), 135.92 ($C_{3'}$), 128.98 ($C_{3''', 5'''}$), 127.51 ($C_{2'', 6''}$), 127.43 ($C_{4'}$), 122.73 ($C_{2'}$), 120.64 ($C_{4'''}$), 116.11 ($C_{2''', 6'''}$), 115.90 ($C_{3'', 5''}$), 63.97 (C_5), 43.17 (C_4); [Signals observed=16: Ar-C=4, Ar-CH=9, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH_2=1]; **IR (KBr, 4000–400 cm^{-1}):** 3038, ν(C–H)$_{\text{ar stretching}}$; 2888, ν(C–H)$_{\text{al stretching}}$; 1505, ν(C=N); 1327, ν(C=C); 1227, ν(C–F); 1134, ν(C–N); 1027, 879, (p–substituted aromatic ring); 778, ν(C–H)$_{\text{ar bending}}$.

*1.2.2 Synthesis of 2-[5-(3-fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5b)***

Prepared by above method using 3-(3-fluorophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3b)** (6 mmol, 1.36 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 78%; yellow amorphous solid. **mp:** 156 ºC; **mol. wt.:** 317.37 g/mol; **empirical formula**: $C_{20}H_{16}FN_3$, **calc. (found) (%):** C, 75.69 (75.54); H, 5.08 (5.01); N, 13.24 (13.15); **mas (m/z):** 318 $[M]^+$; **^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ/ppm:** 3.315 (1H, dd, J = 6.8 Hz, 17.6 Hz, 4-H_a), 3.999 (1H, dd, J = 12.8 Hz, 18.0 Hz, 4-H_b), 5.347 (1H, dd, J = 6.8 Hz, 12.4 Hz, 5-H), 6.807-8.603 (13H, m, 2′,3′,4′,5′,2″,4″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ/ppm:** 162.82 ($C_{3''}$), 151.97 (C_3), 149.13 ($C_{5'}$), 148.08 ($C_{1'}$), 144.11 ($C_{1'''}$), 140.82 ($C_{1''}$), 135.95 ($C_{3'}$), 133.30 ($C_{3''', 5'''}$), 129.31 ($C_{5''}$), 129.02 ($C_{4'}$), 127.26 ($C_{2'}$), 122.77 ($C_{6''}$), 120.65 ($C_{4'''}$), 119.82 ($C_{2''', 6'''}$), 113.59 ($C_{2''}$), 113.35 ($C_{4''}$), 64.01 (C_5), 43.09 (C_4); [Signals observed=18: Ar-C=4, Ar-CH=11, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH_2=1]; **IR (KBr, 4000–400 cm^{-1}):** 3051, ν(C–H)$_{\text{ar stretching}}$; 2925, ν(C–H)$_{\text{al stretching}}$; 1565, ν(C=N); 1332, ν(C=C); 1272, ν(C–F); 1136, ν(C–N); 871, (m–substituted aromatic ring); 785, ν(C–H)$_{\text{ar bending}}$.

*1.2.3 Synthesis of 2-[5-(4-chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5c)***

Prepared by above method using 3-(4-chlorophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3c)** (6 mmol, 1.46 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 81%; yellow

amorphous solid. **mp:** 148 ºC; **mol. wt.:** 333.82 g/mol; **empirical formula**: $C_{20}H_{16}ClN_3$, **calc. (found) (%):** C, 71.96 (71.88); H, 4.83 (4.78); N, 12.59 (12.49); **mass (m/z):** 334 $[M]^+$, 336 $[M+2]^+$; **^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ/ppm:** 3.317 (1H, dd, J = 6.8 Hz, 17.6 Hz, 4-H_a), 3.992 (1H, dd, J = 12.4 Hz, 17.6 Hz, 4-H_b), 5.362 (1H, dd, J = 6.8 Hz, 12.8 Hz, 5-H), 6.831-8.563 (13H, m, 2′,3′,4′,5′,2″,3″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ/ppm:** 160.90 (C_3), 152.04 ($C_{1'}$), 149.13 (C_5′), 148.03 ($C_{1'''}$), 144.15 ($C_{1''}$), 138.06 ($C_{4''}$), 135.95 ($C_{3'}$), 129.00 ($C_{3''', 5'''}$), 127.51 ($C_{3'', 5''}$), 127.43 ($C_{2'', 6''}$), 122.74 ($C_{4'}$), 120.64 ($C_{2'}$), 119.74 ($C_{4'''}$), 116.12 ($C_{2''', 6'''}$), 63.96 (C_5), 43.17 (C_4); [Signals observed=16: Ar-C=4, Ar-CH=9, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH_2=1]; **IR (KBr, 4000–400 cm^{-1}):** 3040, ν(C–H)$_{\text{ar stretching}}$; 2889, ν(C–H)$_{\text{al stretching}}$; 1505, ν(C=N); 1327, ν(C=C); 1128, ν(C–Cl); 1026, ν(C–N); 994, 879, (p–substituted aromatic ring); 746, ν(C–H)$_{\text{ar bending}}$.

*1.2.4 Synthesis of 2-[5-(3-chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5d)***

Prepared by above method using 3-(3-chlorophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3d)** (6 mmol, 1.46 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 85%; yellow amorphous solid. **mp:** 154 ºC; **mol. wt.:** 333.82 g/mol; **empirical formula**: $C_{20}H_{16}ClN_3$, **calc. (found) (%):** C, 71.96 (71.90); H, 4.83 (4.75); N, 12.59 (12.51); **mass (m/z):** 334 $[M]^+$, 336 $[M+2]^+$; **^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ/ppm:** 3.331 (1H, dd, J = 7.2 Hz, 18.0 Hz, 4-H_a), 4.007 (1H, dd, J = 12.8 Hz, 18.0 Hz, 4-H_b), 5.325 (1H, dd, J = 7.2 Hz, 12.8 Hz, 5-H), 6.849 -8.566 (13H, m, 2′,3′,4′,5′,2″,4″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ/ppm:** 151.92 (C_3), 149.14 (C_5′), 148.10 ($C_{1'}$), 144.51 ($C_{1'''}$), 144.12 ($C_{1''}$), 135.97 ($C_{3'}$), 135.00 ($C_{3''}$), 130.49 ($C_{5''}$), 129.06 ($C_{3''', 5'''}$), 127.86 ($C_{4''}$), 125.99 ($C_{2''}$), 124.01 ($C_{6''}$), 122.82 ($C_{4'}$), 120.70 ($C_{2'}$), 119.85 ($C_{4'''}$), 113.55 ($C_{2''', 6'''}$), 64.11 (C_5), 43.12 (C_4); [Signals observed=18: Ar-C=4, Ar-CH=11, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH_2=1]; **IR (KBr, 4000–400 cm^{-1}):** 3040, ν(C–H)$_{\text{ar stretching}}$; 2930, ν(C–H)$_{\text{al stretching}}$; 1565, ν(C=N); 1327, ν(C=C); 1141, ν(C–Cl); 1073, ν(C–N); 879, (m–substituted aromatic ring); 788, ν(C–H)$_{\text{ar bending}}$.

*1.2.5 Synthesis of 2-[5-(4-bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5e)***

Prepared by above method using 3-(4-bromophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3e)** (6 mmol, 1.73 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 80%; yellow amorphous solid. **mp:** 144 ºC; **mol. wt.:** 378.27 g/mol; **empirical formula**: $C_{20}H_{16}BrN_3$, **calc. (found) (%):** C, 63.50 (63.38); H, 4.26 (4.18); N, 11.11 (11.05); **mass (m/z):** 378 $[M]^+$, 380

[M+2]$^+$; **^{1}H NMR (400 MHz, CDCl$_3$-d$_1$) δ/ppm:** 3.311 (1H, dd, J = 6.8 Hz, 18.0 Hz, 4-H$_a$), 3.998 (1H, dd, J = 12.4 Hz, 18.0 Hz, 4-H$_b$), 5.334 (1H, dd, J = 7.2 Hz, 12.8 Hz, 5-H), 6.807-8.562 (13H, m, 2′,3′,4′,5′,2″,3″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, CDCl$_3$-d$_1$) δ/ppm:** 151.94 (C$_3$), 149.14 (C$_5$′), 148.08 (C$_{1'}$), 144.08 (C$_{1'''}$), 141.34 (C$_{1''}$), 135.96 (C$_{3'}$), 132.26 (C$_{3'',\ 5''}$), 129.03 (C$_{3''',\ 5'''}$), 127.61 (C$_{2'',\ 6''}$), 122.80 (C$_{4'}$), 121.37 (C$_{4''}$), 120.65 (C$_{2'}$), 119.83 (C$_{4'''}$), 113.57 (C$_{2''',\ 6'''}$), 64.03 (C$_5$), 43.03 (C$_4$); [Signals observed=16: Ar-C=4, Ar-CH=9, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH$_2$=1]; **IR (KBr, 4000–400 cm^{-1}):** 3014, ν(C–H)$_{\text{ar stretching}}$; 2899, ν(C–H)$_{\text{al stretching}}$; 1565, ν(C=N); 1330, ν(C=C); 1070, ν(C–Br); 1125, ν(C–N); 1002, 871, (p–substituted aromatic ring); 780, ν(C–H)$_{\text{ar bending}}$.

*1.2.6 Synthesis of 2-[5-(3-bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5f)***

Prepared by above method using 3-(3-bromophenyl)-1-(pyridin-2-yl)-prop-2-en-1-one **(3f)** (6 mmol, 1.73 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 82%; yellow amorphous solid. **mp:** 152 ºC; **mol. wt.:** 378.27 g/mol; **empirical formula**: C$_{20}$H$_{16}$BrN$_3$, **calc. (found) (%):** C, 63.50 (63.40); H, 4.26 (4.20); N, 11.11 (11.04); **mass (m/z):** 378 [M]$^+$, 380 [M+2]$^+$; **^{1}H NMR (400 MHz, CDCl$_3$-d$_1$) δ/ppm:** 3.332 (1H, dd, J = 6.8 Hz, 18.0 Hz, 4-H$_a$), 4.005 (1H, dd, J = 12.8 Hz, 18.0 Hz, 4-H$_b$), 5.310 (1H, dd, J = 7.2 Hz, 12.8 Hz, 5-H), 6.853-8.568 (13H, m, 2′,3′,4′,5′,2″,4″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, CDCl$_3$-d$_1$) δ/ppm:** 151.91 (C$_3$), 149.15 (C$_5$′), 148.12 (C$_{1'}$), 144.78 (C$_{1'''}$), 144.13 (C$_{1''}$), 135.96 (C$_{3'}$), 130.80 (C$_{2''}$), 130.77 (C$_{4''}$), 129.08 (C$_{5''}$), 128.90 (C$_{3''',\ 5'''}$), 124.47 (C$_{6''}$), 123.21 (C$_{3''}$), 122.83 (C$_{4'}$), 120.70 (C$_{2'}$), 119.87 (C$_{4'''}$), 113.56 (C$_{2''',\ 6'''}$), 64.07 (C$_5$), 43.17 (C$_4$); [Signals observed=18: Ar-C=4, Ar-CH=11, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH$_2$=1]; **IR (KBr, 4000–400 cm^{-1}):** 3040, ν(C–H)$_{\text{ar stretching}}$; 2930, ν(C–H)$_{\text{al stretching}}$; 1565, ν(C=N); 1327, ν(C=C); 1073, ν(C–Br); 1072, ν(C–N); 879, (m–substituted aromatic ring); 785, ν(C–H)$_{\text{ar bending}}$.

*1.2.7 Synthesis of 2-(1-phenyl-5-(p-tolyl)-4, 5-dihydro-1H-pyrazol-3-yl) pyridine **(5g)***

Prepared by above method using 1-(pyridin-2-yl)-3-(p-tolyl)-prop-2-en-1-one **(3g)** (6 mmol, 1.34 g) and phenyl hydrazine (6 mmol, 0.59 g) after 5–6 h reflux; **yield:** 84%; yellow amorphous solid. **mp:** 139 ºC; **mol. wt.:** 313.40 g/mol; **empirical formula**: C$_{21}$H$_{19}$N$_3$, **calc. (found) (%):** C, 80.48 (80.39); H, 6.11 (6.02); N, 13.41 (13.35); **mass (m/z):** 314 [M]$^+$; **^{1}H NMR (400 MHz, CDCl$_3$-d$_1$) δ/ppm:** 2.336 (3H, S, CH$_3$), 3.328 (1H, dd, J = 6.8 Hz, 17.6 Hz, 4-H$_a$), 3.985 (1H, dd, J = 12.8 Hz, 18.0 Hz, 4-H$_b$), 5.349 (1H, dd, J = 7.2 Hz, 12.8 Hz, 5-H), 6.814-8.561 (13H, m,

2′,3′,4′,5′,2″,3″,5″,6″,2‴,3‴,4‴,5‴,6‴-H); **^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ/ppm:** 152.23 (C_3), 149.11 (C_5′), 148.02 ($C_{1'}$), 144.35 ($C_{1'''}$), 139.34 ($C_{1''}$), 137.14 ($C_{4''}$), 135.88 ($C_{3'}$), 129.75 ($C_{3''', 5'''}$), 128.93 ($C_{3'', 5''}$), 125.84 ($C_{2'', 6''}$), 122.58 ($C_{4'}$), 120.61 ($C_{2'}$), 119.49 ($C_{4'''}$), 113.57 ($C_{2''', 6'''}$), 64.44 (C_5), 43.18 (C_4), 21.09 (-CH_3); [Signals observed=17: Ar-C=4, Ar-CH=9, pyrazole-C=1, pyrazole-CH=1, pyrazole-CH_2=1, -CH_3=1]; **IR (KBr, 4000–400 cm^{-1}):** 3024, ν(C–H)$_{\text{ar stretching}}$; 2925, ν(−CH_3); 2893, ν(C–H)$_{\text{al stretching}}$; 1505, ν(C=N); 1322, ν(C=C); 1122, ν(C–N); 1026, 871, (p–substituted aromatic ring); 748, ν(C–H)$_{\text{ar bending}}$.

1.3 General synthesis of the complexes

The half-sandwich Ru(III) metal complexes **(6a-6g)** of the general formula [(Cp*)Ru(L^n)Cl]• Cl were synthesized by the reactions of [{(Cp*)Ru(μ-Cl)Cl}$_2$] with the respective ligands **(5a-5g)** in a 1:2 molar ratio in dichloromethane/methanol.

*1.3.1 Synthesis of **[(Cp*)Ru(L^1)Cl]• Cl (6a)***

A dichloromethane/methanolic suspension of the precursor of [{(Cp*)Ru(μ-Cl)Cl}$_2$] (0.309 g, 0.5 mmol) was refluxed for 10 minutes. Then a solution of ligand **5a** (0.317 g, 1.0 mmol in 20 mL dichloromethane), was added and the reaction was stirred yielding a red-brown solution. The resulting mixture was stirred at room temperature for 16 h. Then the solution was filtered through celite in order to remove solid particles and the solvent was removed under reduced pressure. The residue was dissolved in methanol (5 mL), and the product was precipitated by addition of diethyl ether (30 mL), isolated by filtration and dried in vacuo to obtain the complex. The complex is soluble in methanol, ethanol, dichloromethane, acetonitrile, dimethylformamide and dimethyl sulphoxide but insoluble in petroleum ether and diethyl ether. The proposed reaction for the synthesis of complexes 6a-6g is shown in scheme 1. **yield:** 65%; **mp:** ≥ 300 °C; **μ_{eff}:** 2.13 BM; **mol. wt.:** 624.56 g/mol; **empirical formula:** $C_{30}H_{31}FCl_2N_3Ru$, **calc. (found) (%):** C, 57.69 (57.61); H, 5.00 (4.95); N, 6.73 (6.70); Ru, 16.18 (16.13); **IR (KBr, 4000–400 cm^{-1}):** 3061, ν(C–H)$_{\text{ar stretching}}$; 2920, ν(C–H)$_{\text{al stretching}}$; 1565, ν(C=N); 1450, ν(C=C); 1230, ν(C–F); 1159, ν(C–N); 1095, 840, (p–substituted aromatic ring); 780, ν(C–H)$_{\text{ar bending}}$, 693, ν(Ru–N); **conductance:** 44 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 360 (13680), 348 (13860), 290 (23832).

*1.3.2 Synthesis of **[(Cp*)Ru(L^2)Cl]• Cl (6b)***

It was synthesized using ligand **5b** (0.317 g, 1.0 mmol). **yield:** 68%; **mp:** ≥ 300 °C; **μ_{eff}:** 2.18 BM; **mol. wt.:** 624.56 g/mol; **empirical formula:** $C_{30}H_{31}FCl_2N_3Ru$, **calc. (found) (%):** C, 57.69

(57.63); H, 5.00 (4.97); N, 6.73 (6.68); Ru, 16.18 (16.15); **IR (KBr, 4000–400 cm^{-1}):** 3057, ν(C–H)$_{ar\ stretching}$; 2930, ν(C–H)$_{al\ stretching}$; 1568, ν(C=N); 1452, ν(C=C); 1269, ν(C–F); 1097, ν(C–N); 832, (m–substituted aromatic ring); 779, ν(C–H)$_{ar\ bending}$, 694, ν(Ru–N); **conductance:** 48 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 370 (13255), 351 (13714), 285 (23840).

1.3.3 Synthesis of ***[(Cp*)Ru(L^3)Cl]• Cl (6c)***

It was synthesized using ligand **5c** (0.334 g, 1.0 mmol). **yield:** 69%; **mp:** ≥ 300 °C; **μ$_{eff}$:** 2.23 BM; **mol. wt.:** 641.02 g/mol; **empirical formula:** $C_{30}H_{31}Cl_3N_3Ru$, **calc. (found) (%):** C, 54.21 (54.17); H, 4.87 (4.82); N, 6.56 (6.50); Ru, 15.77 (15.72); **IR (KBr, 4000–400 cm^{-1}):** 3063, ν(C–H)$_{ar\ stretching}$; 2930, ν(C–H)$_{al\ stretching}$; 1566, ν(C=N); 1454, ν(C=C); 1160, ν(C–N); 1097, ν(C–Cl); 1017, 840, (p–substituted aromatic ring); 776, ν(C–H)$_{ar\ bending}$, 694, ν(Ru–N); **conductance:** 40 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 366 (13775), 347 (14250), 289 (23856).

1.3.4 Synthesis of ***[(Cp*)Ru(L^4)Cl]• Cl (6d)***

It was synthesized using ligand **5d** (0.334 g, 1.0 mmol). **yield:** 73%; **mp:** ≥ 300 °C; **μ$_{eff}$:** 2.11 BM; **mol. wt.:** 641.02 g/mol; **empirical formula:** $C_{30}H_{31}Cl_3N_3Ru$, **calc. (found) (%):** C, 54.21 (54.15); H, 4.87 (4.84); N, 6.56 (6.52); Ru, 15.77 (15.74); **IR (KBr, 4000–400 cm^{-1}):** 3063, ν(C–H)$_{ar\ stretching}$; 2925, ν(C–H)$_{al\ stretching}$; 1563, ν(C=N); 1452, ν(C=C); 1197, ν(C–N); 1094, ν(C–Cl); 840, (m–substituted aromatic ring); 787, ν(C–H)$_{ar\ bending}$, 694, ν(Ru–N); **conductance:** 55 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 358 (12745), 345 (14365), 288 (25525).

1.3.5 Synthesis of ***[(Cp*)Ru(L^5)Cl]• Cl (6e)***

It was synthesized using ligand **5e** (0.378 g, 1.0 mmol). **yield:** 67%; **mp:** ≥ 300 °C; **μ$_{eff}$:** 2.28 BM; **mol. wt.:** 685.47 g/mol; **empirical formula:** $C_{30}H_{31}Cl_2BrN_3Ru$, **calc. (found) (%):** C, 52.57 (52.51); H, 4.56 (4.52); N, 6.13 (6.10); Ru, 14.74 (14.68); **IR (KBr, 4000–400 cm^{-1}):** 3063, ν(C–H)$_{ar\ stretching}$; 2930, ν(C–H)$_{al\ stretching}$; 1550, ν(C=N); 1452, ν(C=C); 1158, ν(C–N); 1078, ν(C–Br); 1012, 834, (p–substituted aromatic ring); 779, ν(C–H)$_{ar\ bending}$, 702, ν(Ru–N); **conductance:** 70 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 362 (12788), 350 (13956), 292 (24323).

1.3.6 Synthesis of ***[(Cp*)Ru(L^6)Cl]• Cl (6f)***

It was synthesized using ligand **5f** (0.378 g, 1.0 mmol). **yield:** 71%; **mp:** ≥ 300 °C; **μ$_{eff}$:** 2.15 BM; **mol. wt.:** 685.47 g/mol; **empirical formula:** $C_{30}H_{31}Cl_2BrN_3Ru$, **calc. (found) (%):** C, 52.57 (52.51); H, 4.56 (4.51); N, 6.13 (6.09); Ru, 14.74 (14.70); **IR (KBr, 4000–400 cm^{-1}):** 3063, ν(C–H)$_{ar\ stretching}$; 2920, ν(C–H)$_{al\ stretching}$; 1566, ν(C=N); 1452, ν(C=C); 1160, ν(C–N); 1091, ν(C–Br);

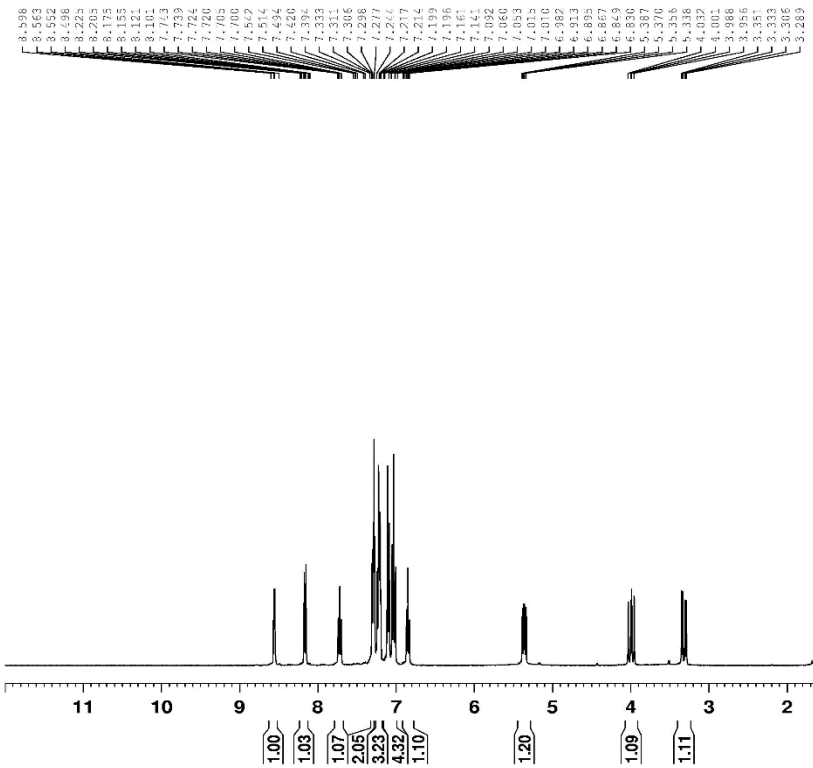
840, (m–substituted aromatic ring); 779, ν(C–H)$_{ar\ bending}$, 694, ν(Ru–N); **conductance:** 52 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 364 (13655), 347 (13860), 290 (25630).

1.3.7 Synthesis of ***[(Cp*)Ru(L^7)Cl]• Cl (6g)***

It was synthesized using ligand **5g** (0.313 g, 1.0 mmol). **yield:** 72%; **mp:** ≥ 300 °C; **μ_{eff}:** 2.25 BM; **mol. wt.:** 620.60 g/mol; **empirical formula:** $C_{31}H_{34}Cl_2N_3Ru$, **calc. (found) (%):** C, 60.00 (59.63); H, 5.52 (5.48); N, 6.77 (6.73); Ru, 16.29 (16.23); **IR (KBr, 4000–400 cm^{-1}):** 3053, ν(C–H)$_{ar\ stretching}$; 2928, ν(−CH_3); 1488, ν(C=N); 1450, ν(C=C); 1194, ν(C–N); 1093, 818, (p–substituted aromatic ring); 772, ν(C–H)$_{ar\ bending}$, 693, ν(Ru–N); **conductance:** 64 Ω^{-1} cm^2 mol^{-1}; **UV-Vis: λ (nm) (ε, L mol^{-1} cm^{-1}):** 365 (13255), 348 (14842), 292 (24940).

Supplementary material 2: ^{1}H NMR spectra of ligands (5a–5g)

1. 2-[5-(4-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5a)**

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PROCNO                      1

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SOLVENT             CDCl3
NS                     16
DS                      2
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FIDRES           0.126314 Hz
AQ                3.9584243 sec
RG                    512
DW                 60.400 usec
DE                  6.00 usec
TE                 297.0 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
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P1                  13.50 usec
PL1                 -1.00 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
SI                  32768
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WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
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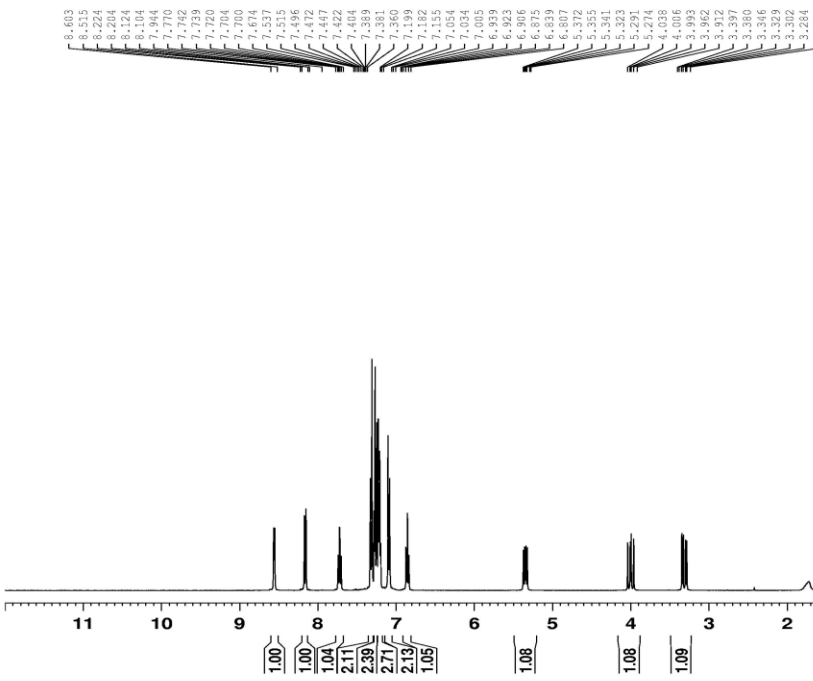
2. 2-[5-(3-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5b)**

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SOLVENT             CDCl3
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DS                      2
SWH               8278.146 Hz
FIDRES           0.126314 Hz
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DE                  6.00 usec
TE                 297.1 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
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PL1                 -1.00 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
SI                  32768
SF            400.1300000 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
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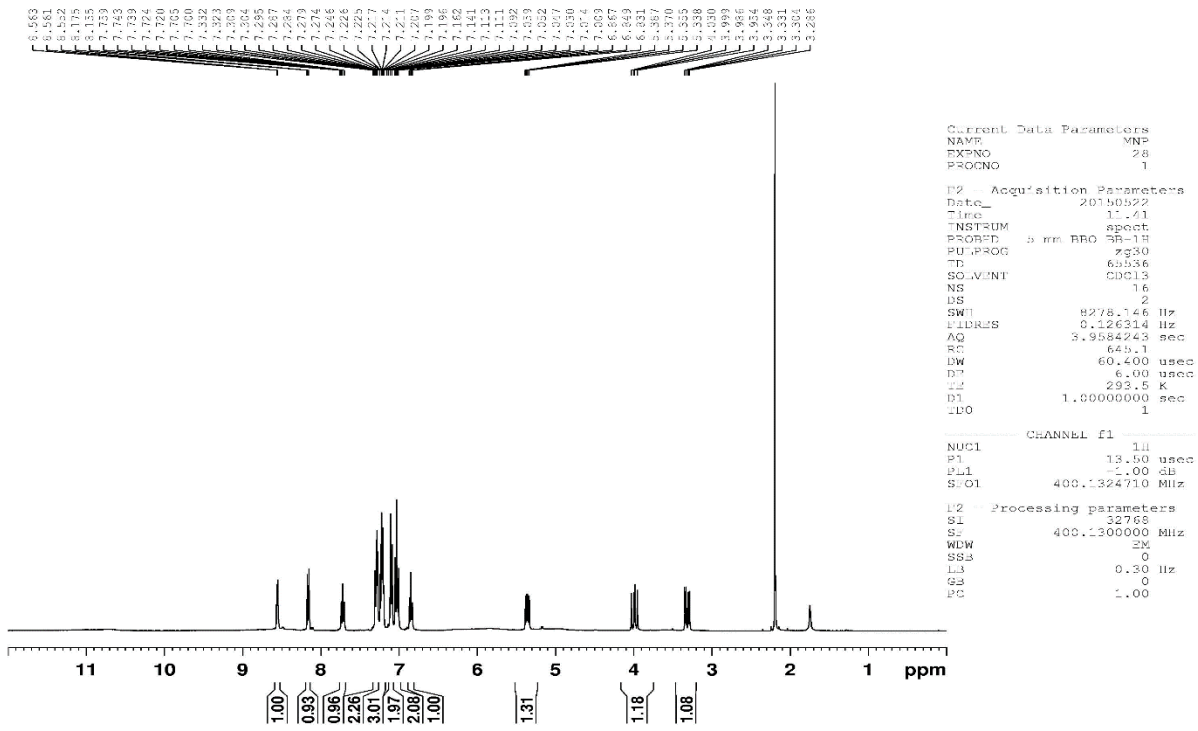
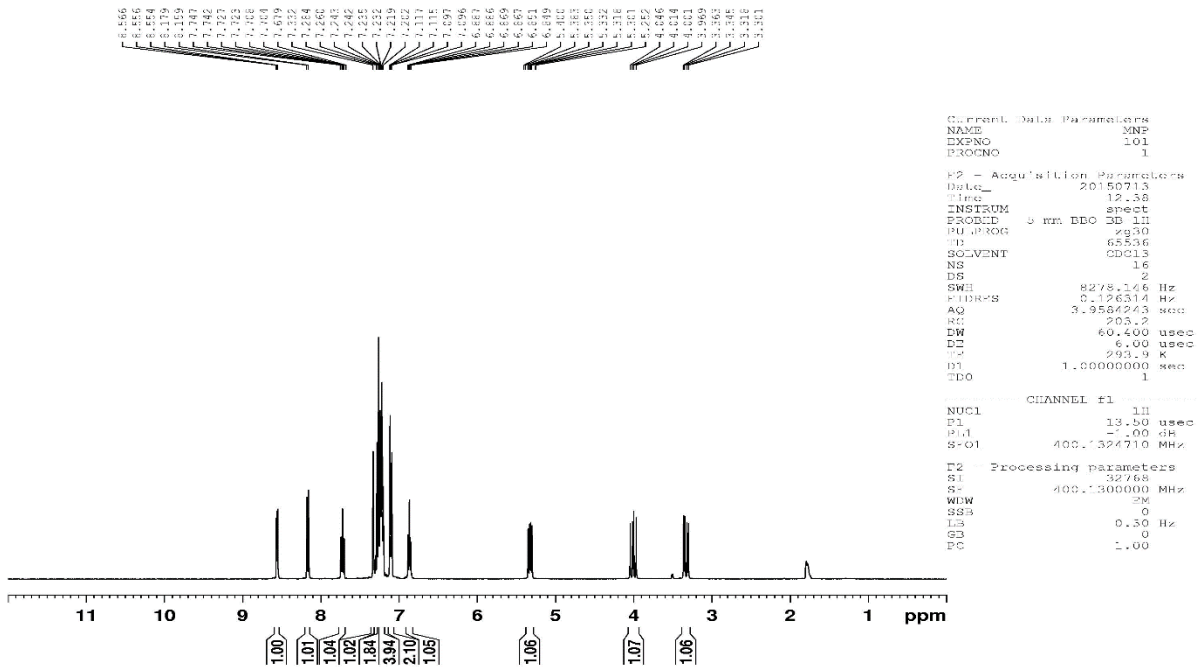
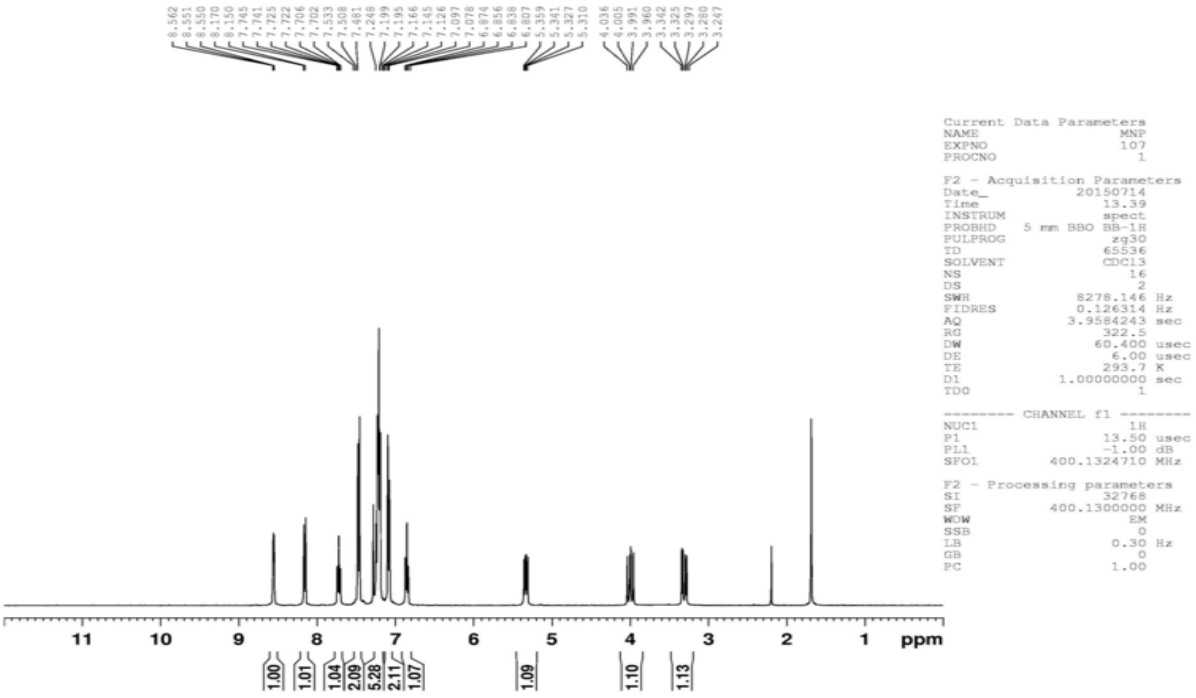
3. 2-[5-(4-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5c)**



4. 2-[5-(3-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5d)**



5. 2-[5-(4-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5e)**

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PROCNO                   1

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TD0                      1

======== CHANNEL f1 ========
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PL1                   -1.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
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SF             400.1300000 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
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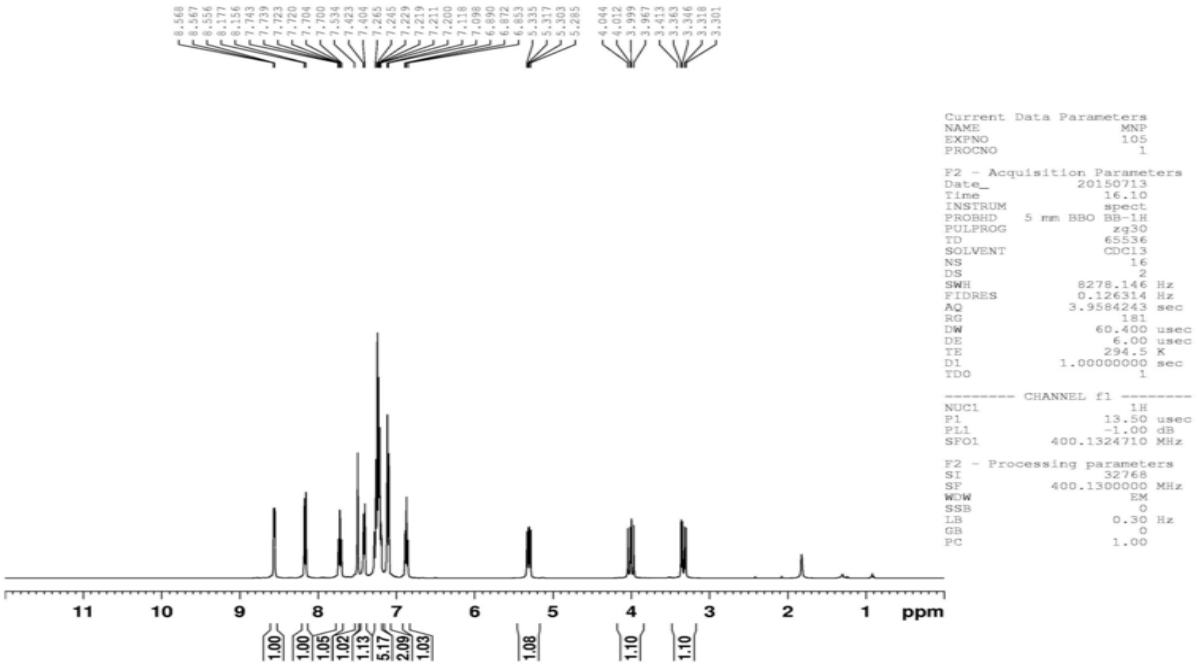
6. 2-[5-(3-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5f)**

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PROCNO                   1

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INSTRUM                spect
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TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
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FIDRES             0.126314 Hz
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RG                     181
DW                    60.400 usec
DE                     6.00 usec
TE                    294.5 K
D1                1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
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P1                    13.50 usec
PL1                   -1.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
SI                    32768
SF             400.1300000 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
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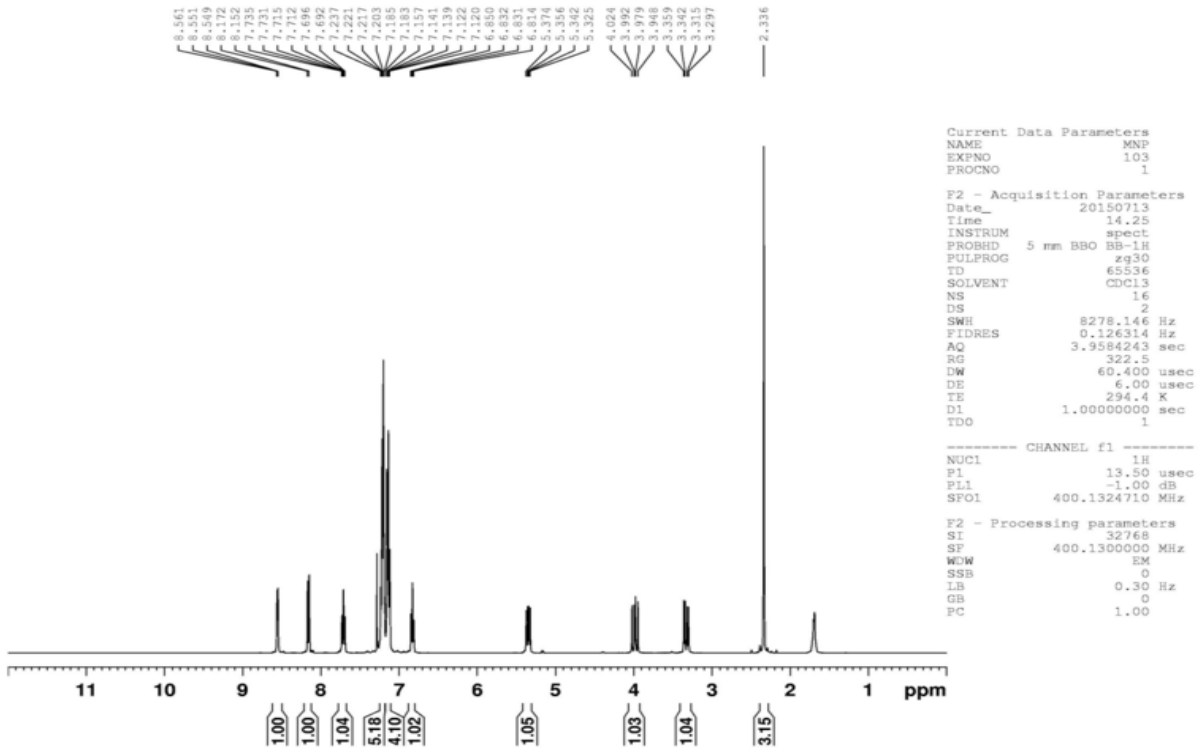
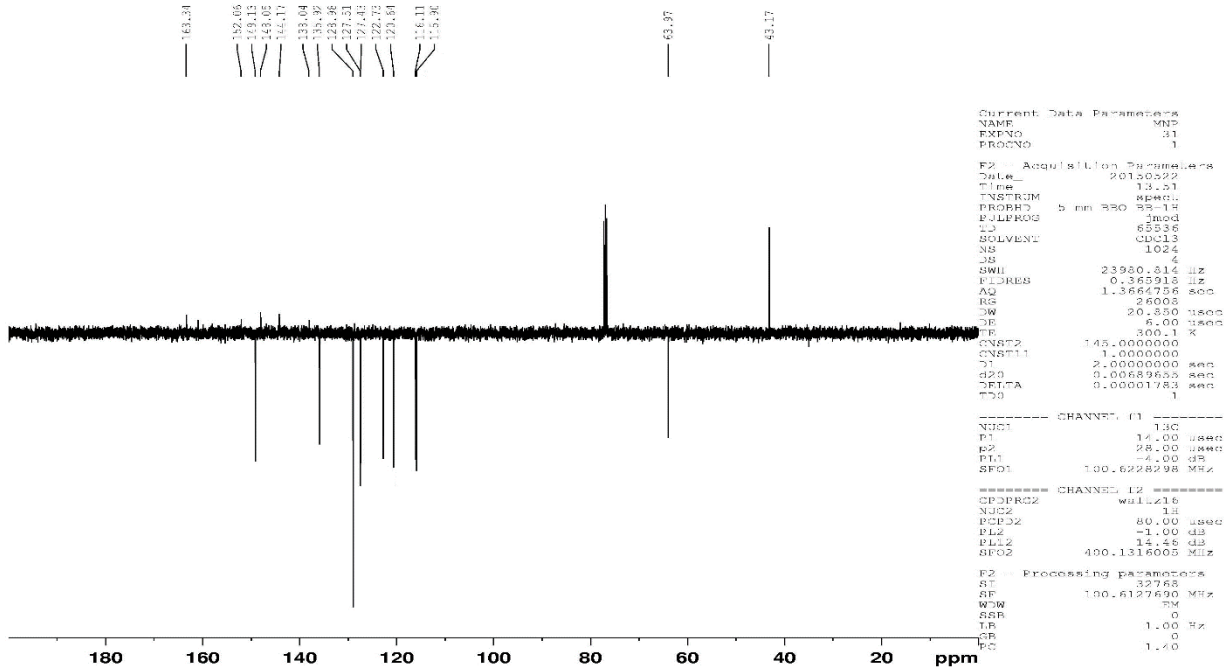
7. 2-(1-phenyl-5-(p-tolyl)-4, 5-dihydro-1H-pyrazol-3-yl) pyridine **(5g)**

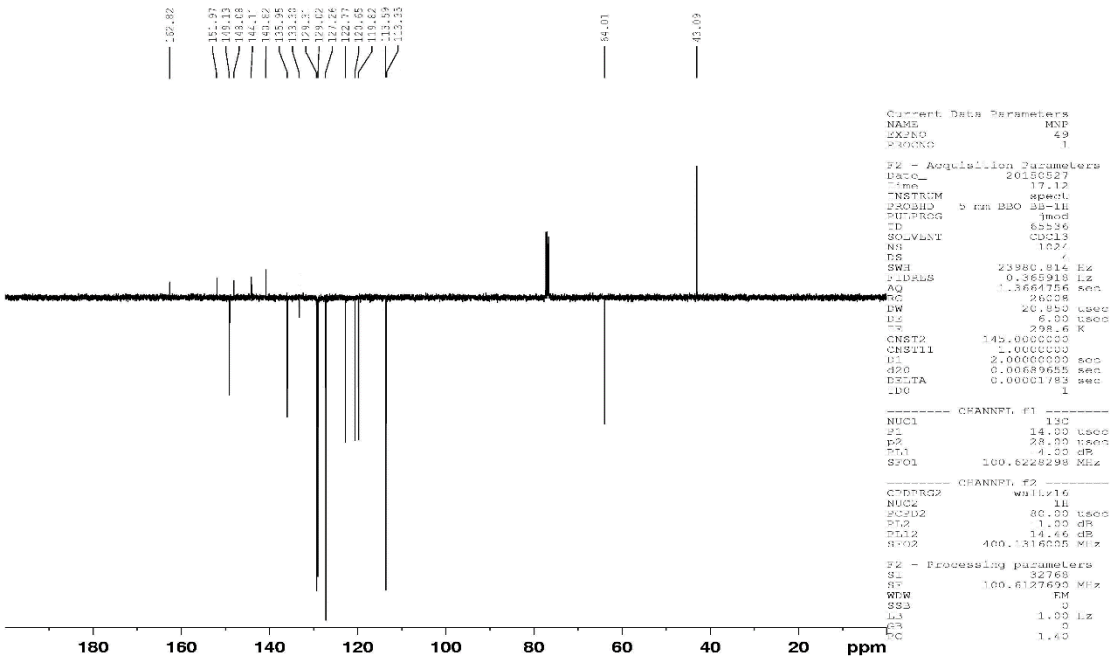


Supplementary material 3: ^{13}C NMR spectra of ligands (5a–5g)

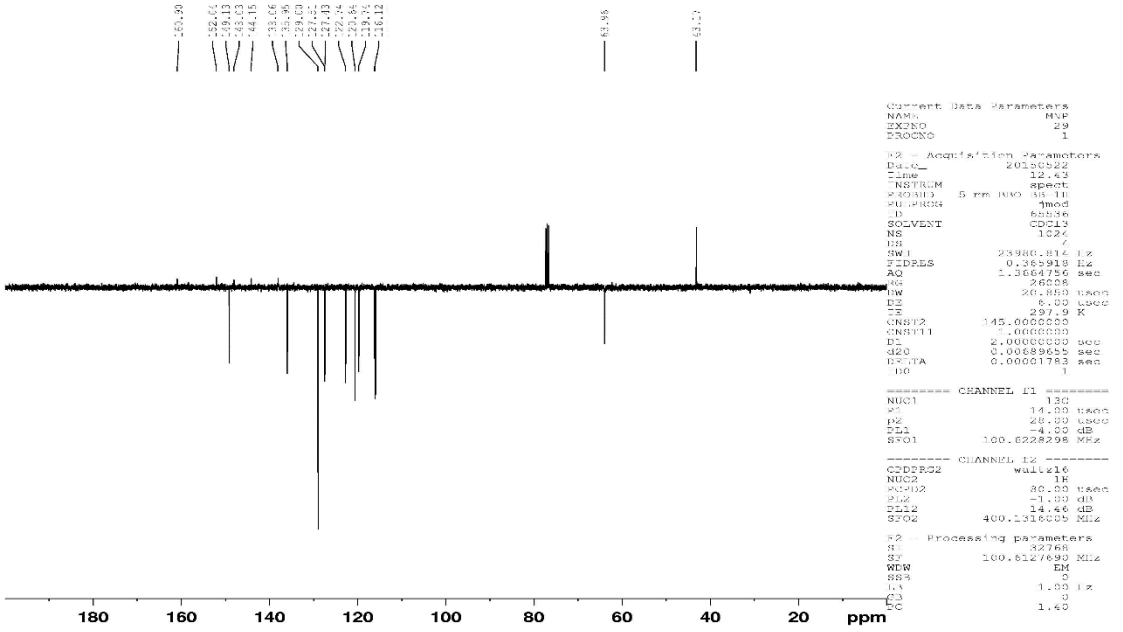
1. 2-[5-(4-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5a)**



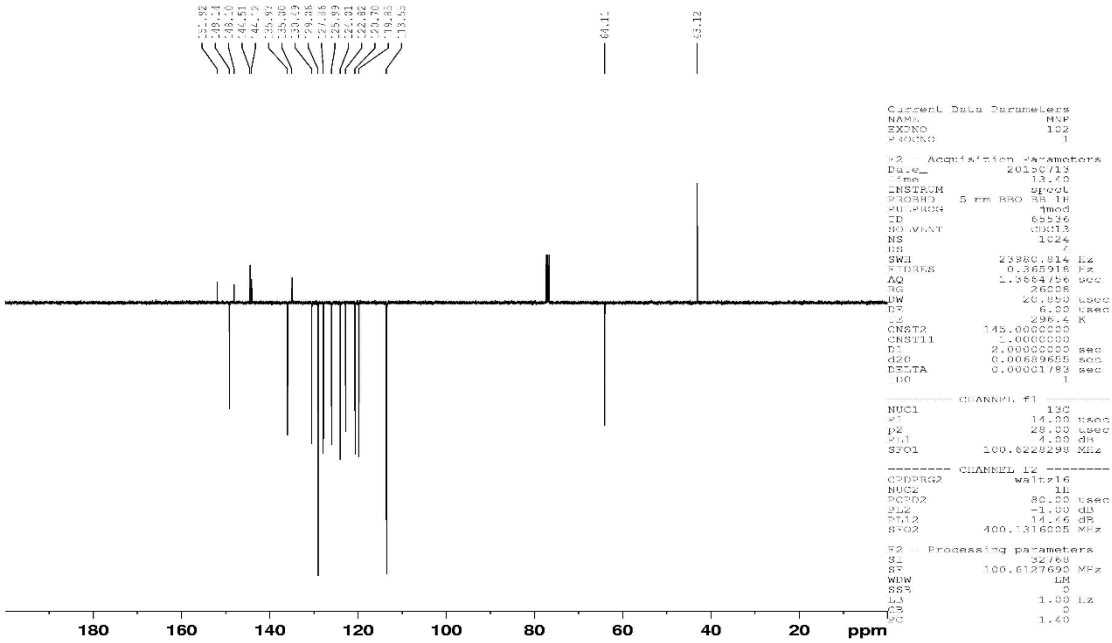
2. 2-[5-(3-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5b)**



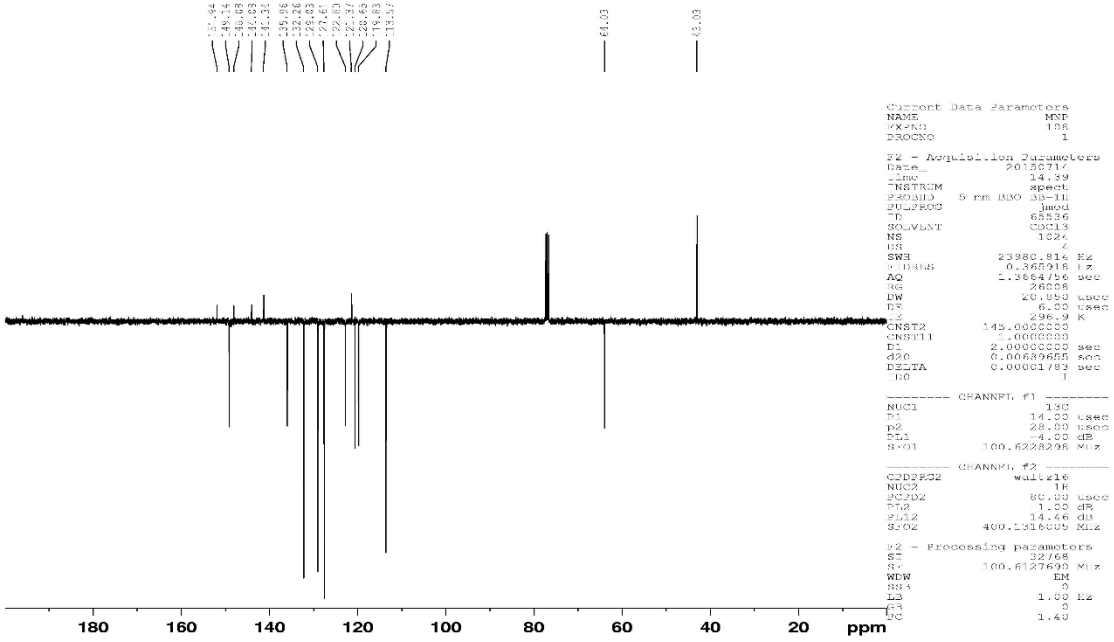
3. 2-[5-(4-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5c)**



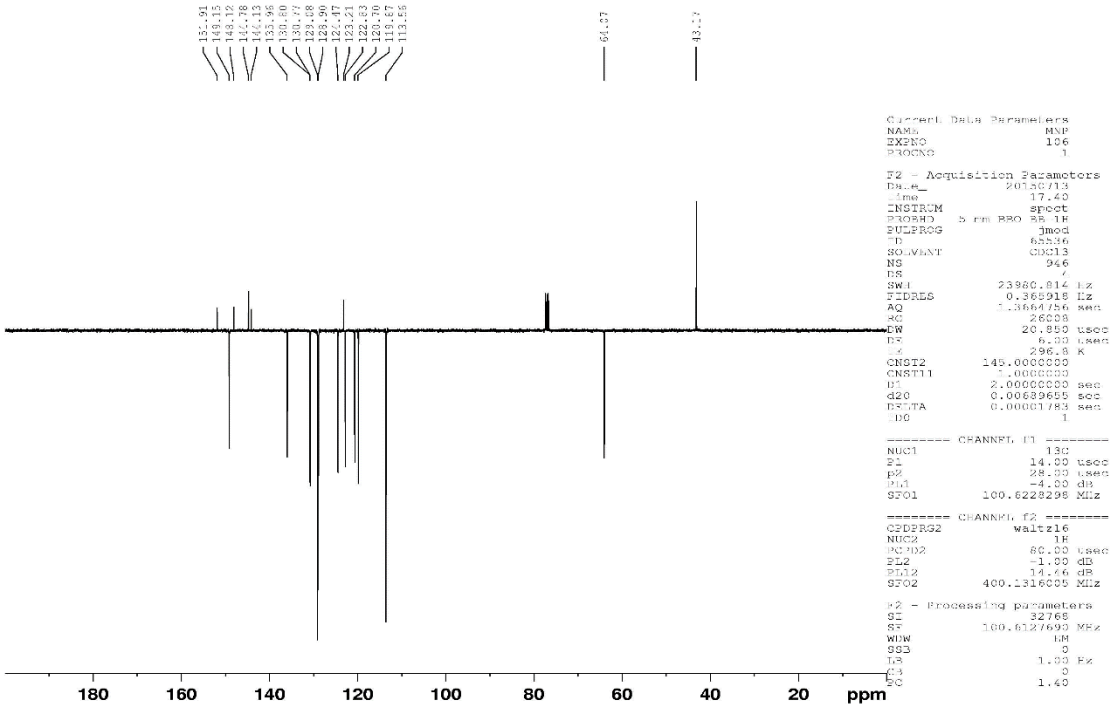
4. 2-[5-(3-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5d)**



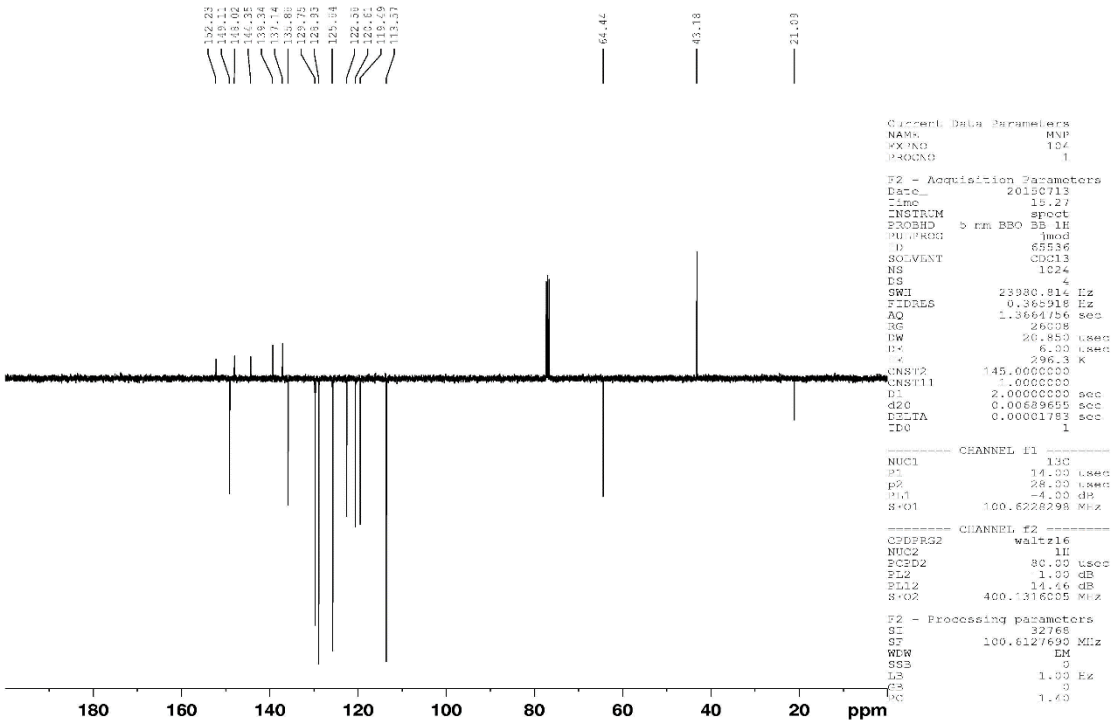
5. 2-[5-(4-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5e)**



6. 2-[5-(3-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5f)**



7. 2-(1-phenyl-5-(p-tolyl)-4, 5-dihydro-1H-pyrazol-3-yl) pyridine **(5g)**



Supplementary material 4: Characterization of Ru(III) complexes by other techniques

4.1 *Magnetic moments, electronic spectra and conductance measurements*

Magnetic moments measurement were carried out at room temperature for all the organometallic Ru(III) complexes. The $\mu_{eff.}$ values are obtained in the range of 2.11–2.28 BM, which corresponds to a single unpaired electron in low-spin $4d^5$ configuration and confirms the +3 oxidation state of ruthenium in all complexes. The $\mu_{eff.}$ values suggest a low-spin t_{2g}^5 configuration for ruthenium(III) ion in all complexes (Khan, Srinivas, Kureshy, & Khan, 1991).

UV–Visible emission spectra of the synthesized organometallic Ru(III) complexes were recorded in DMSO. The ground state of Ru(III) ion (t_{2g}^5 configuration) is $^2T_{2g}$ (Ballhausen, 1962). In most of the Ru(III) complexes, the electronic spectra show only charge transfer bands (Lever, 1984). All complexes exhibit three distinct bands, one band ranging from 361–378 nm (ε = 13250−12960 L mol^{-1} cm^{-1}), which is attributed to metal to ligand charge transfer (MLCT) transition. A high energy two bands, ranging from approximately 340–350 nm (ε = 13445−13900 L mol^{-1} cm^{-1}) and 274–292 nm (ε = 26856−23850 L mol^{-1} cm^{-1}) in the ultraviolet region are attributed to the LMCT transition (n–π*) and intra-ligand charge transfer (π–π*) transitions for the pyrazoline ligands, respectively. The pattern of the electronic spectra of all the Ru(III) complexes indicate the existence of piano-stool environment around the ruthenium(III) metal ion (Gupta *et al*., 2013; Yadav, Singh, & Pandey, 2011).

With a view to studying the electrolytic nature of the ruthenium complexes (6a-6g), their molar conductivities were measured in methanol. The molar conductance (Λ_M) values for the synthesized ruthenium complexes are found in the range of 40–70 cm^2 Ω^{-1} mol^{-1}, indicating electrolytic nature and there is one counter ion present outside the coordination sphere of all complexes. So, we conclude that all Ru(III) complexes are ionic in nature.

4.2 *LC–MS spectrum analysis*

The LC–MS spectrum and probable mass fragmentation pattern of complex (6a) are shown in below figure 1 and 2 respectively. Mass spectrum of the complex (6a) shows molecular ion peak $[M-Cl]^+$ and $[M-Cl]^{+2}$ at 589 m/z and 591 m/z, respectively due to presence of one chlorine atom. The peak at 554 m/z is due to loss of one chlorine atom. The peak at 419 m/z is due to loss of one pentamethylcyclopentadienyl ring from metal ion. The peak at 237 m/z is due to loss of neutral bidentate ligand. The peak at 317 m/z corresponds to pyrazole moiety. The peak at 135 m/z

corresponds to pentamethylcyclopentadienyl ring. The peak at 240 m/z is due to fragmentation of pyrazole ligand.

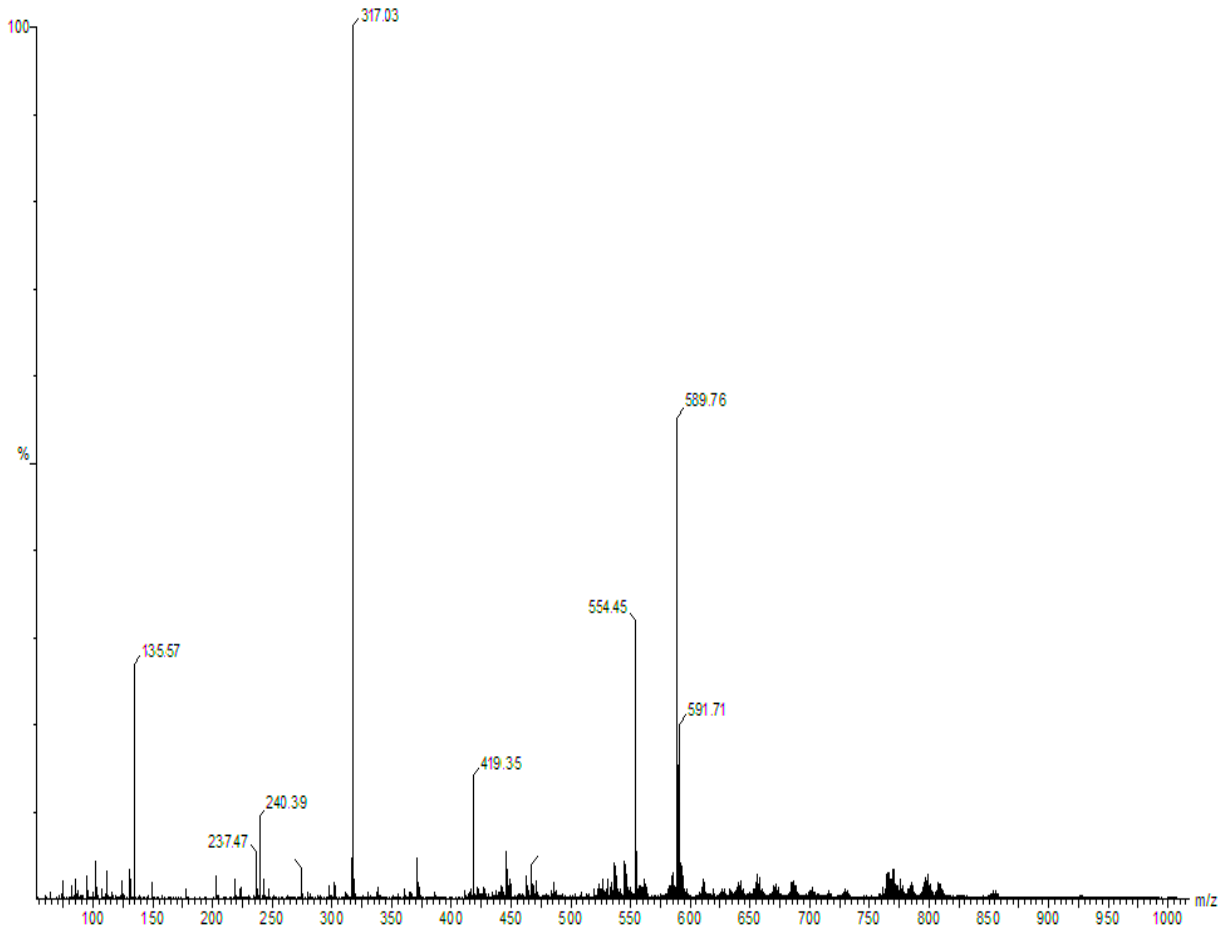


Figure 1: LC-mass spectrum of complex [(Cp*)Ru(L^1)Cl]• Cl **(6a)**

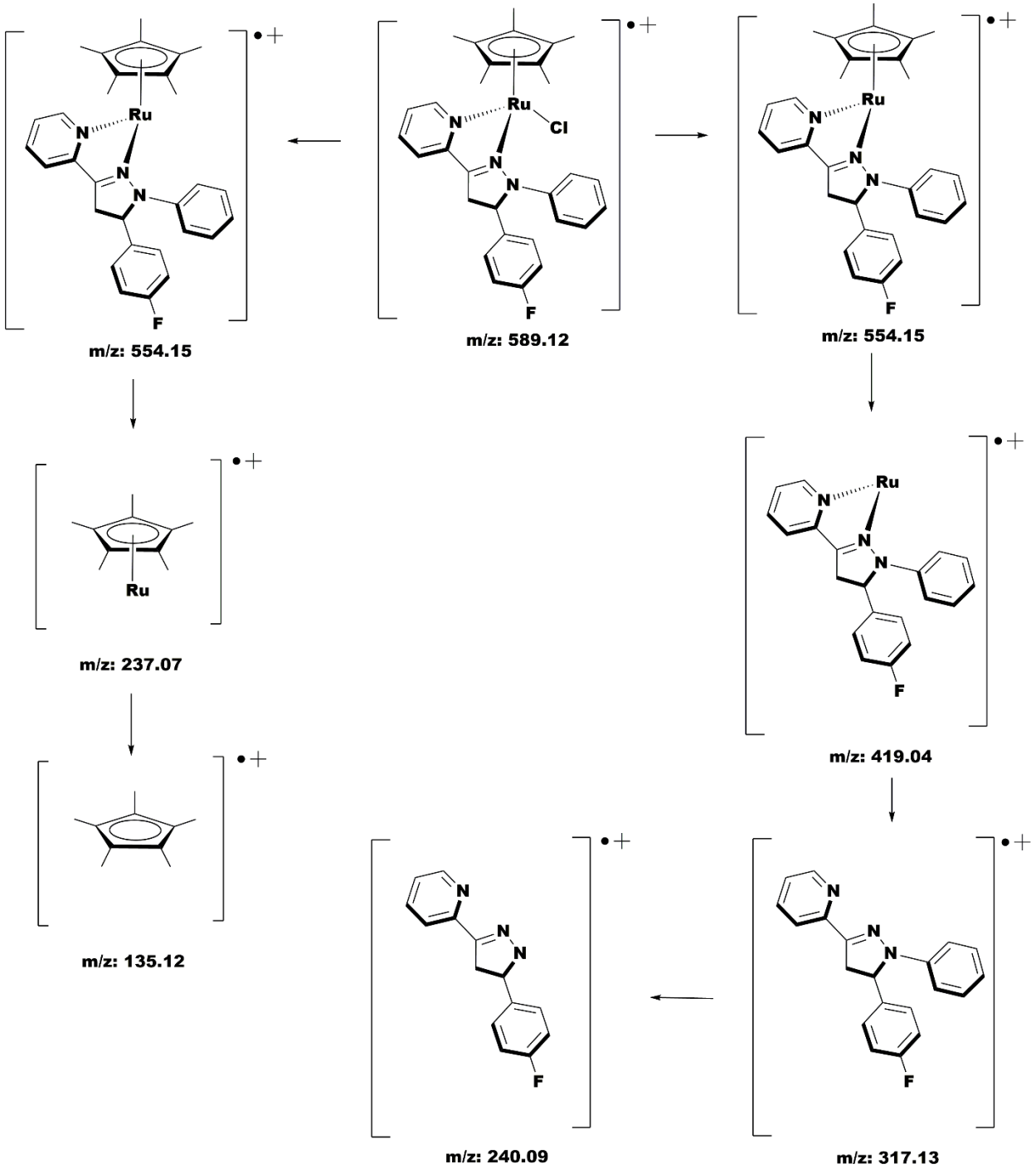


Figure 2: Mass fragmentation pattern of complex [(Cp*)Ru(L^1)Cl]• Cl **(6a)**

4.3 *Thermal analysis*

Thermo gravimetric analysis determines the weight changes of a sample, as a function of temperature. Thermo gravimetric analysis is concerned with the change in weight of a material as its temperature changes. This indicates the temperature at which the material loses weight and the loss indicates decomposition of the sample. The temperature, at which no weight loss takes place, reveals the stability of the material. Another important piece of information that can be obtained by TGA is the amount of weight lost by heating the sample to a given temperature. This information helps the chemist to determine the composition of a compound and to follow the reactions involved in its decomposition.

The characteristic thermogravimetric gram (mass loss % to temperature in °C) of complex (6a) (figure 3) shows three distinct mass losses (Mehta, Gajera, Patel, & Patel, 2015). The 1st mass loss occurs between 40–130 °C, the 2nd between 180–240 °C, and the last between 260–650 °C. Mass loss occurring during the 1st decomposition step corresponds to one molecules of chlorine atom (6 %), whereas mass loss during the 2nd step relates to decomposition of one chlorine atom which bind by one covalent bond to metal center (6 %), and the 3rd step relates to decomposition of the Cp* and neutral bidentate ligand (72 %), leaving behind metal oxide as residue.

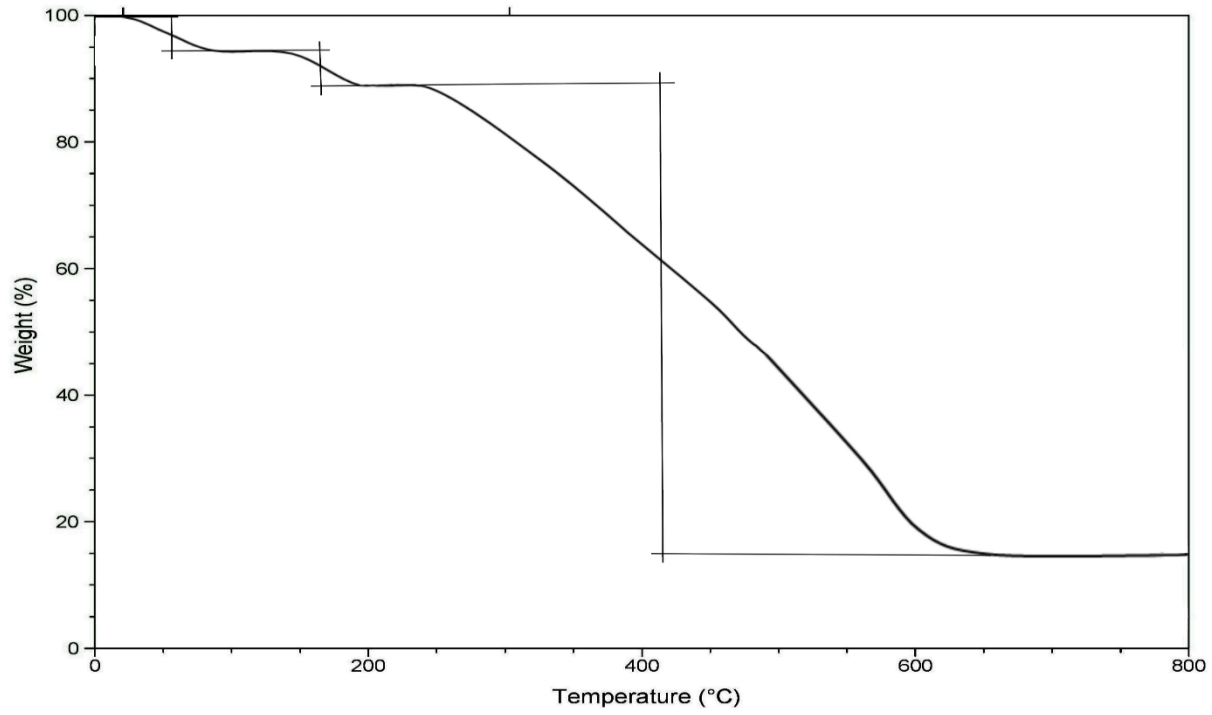


Figure 3: TGA of complex [(Cp*)Ru(L^1)Cl]• Cl **(6a)**

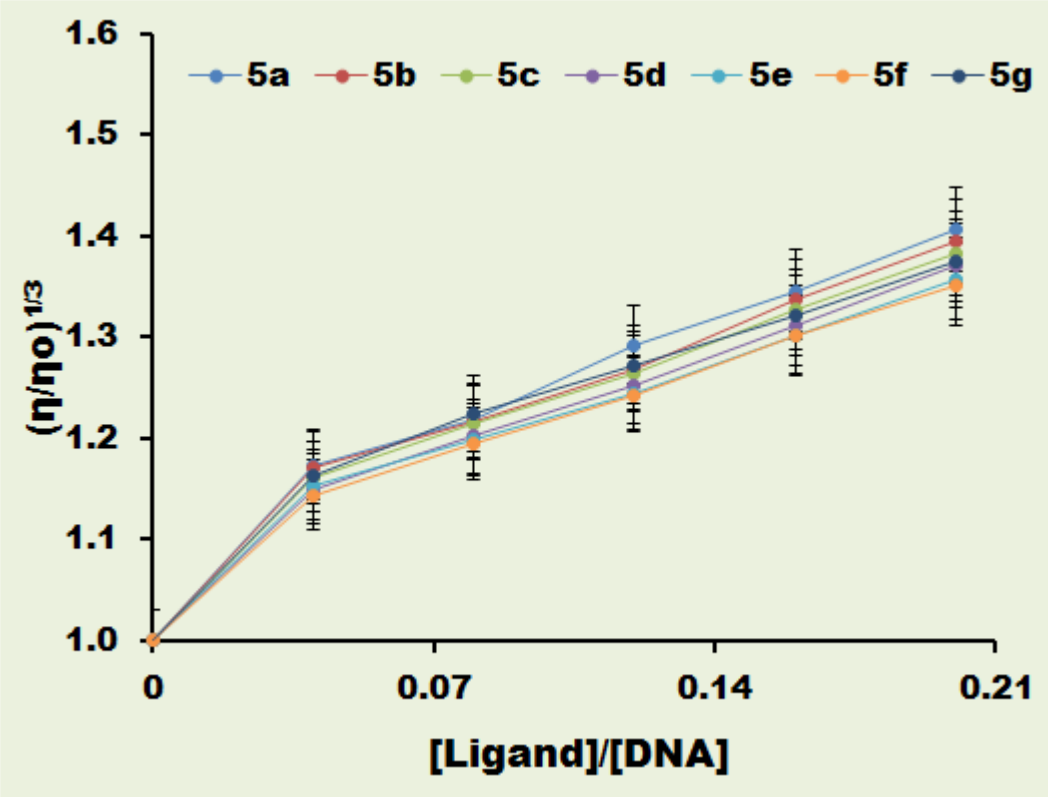
Supplementary material 5: Bacteriostatic concentration of ligands and synthesized complexes by broth dilution method in terms of MIC in µM with error uncertainty in the value ±5 %

Compounds	gram positive		gram negative		
	S. Aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
5a	242±3	233±2	260±2	232±2	240±3
5b	265±2	245±2	271±2	262±2	233±2
5c	269±2	261±2	280±2	269±2	256±2
5d	277±2	266±3	282±2	278±2	262±2
5e	302±3	285±2	294±3	290±2	275±2
5f	307±3	296±3	308±2	292±3	276±2
5g	312±3	305±2	320±3	298±2	292±3
6a	67±1	72±1	74±1	69±1	68±1
6b	69±1	73±1	78±1	76±1	73±1
6c	73±1	77±1	83±1	85±1	76±1
6d	75±1	78±1	87±1	88±1	79±1
6e	82±1	85±1	88±1	95±1	85±1
6f	84±1	84±1	90±1	93±1	89±1
6g	86±1	95±1	96±2	102±2	97±1

Supplementary material 6: Binding constant (K_b), percentage hypochromicity (%H), bathochromicity ($\Delta\lambda$), IC_{50} (antimalarial) and LC_{50} (*in vitro* cytotoxicity) values of free ligands and synthesized complexes with error uncertainty in the value ±5 %

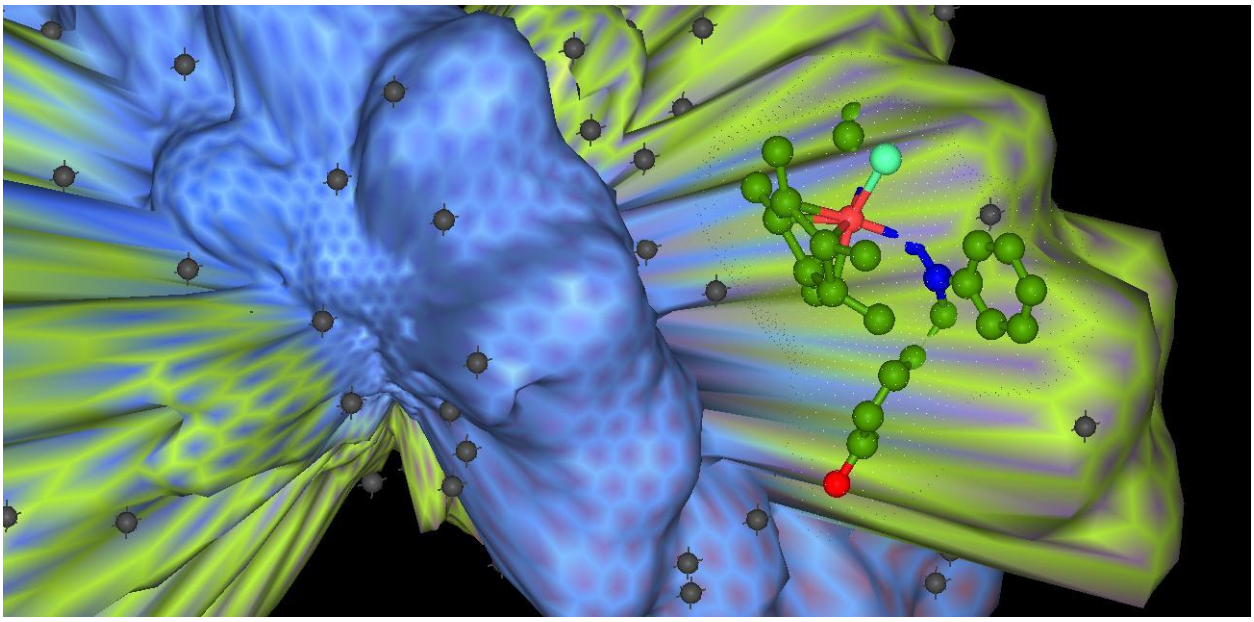
Compounds	K_b (M^{-1})	%H	$\Delta\lambda$ nm	IC_{50}(mg/L)	LC_{50}(mg/L)
5a	$0.861\pm0.03 \times 10^5$	18.18±0.53	2.6±0.09	1.68±0.04	58.479±2.11
5b	$0.751\pm0.03 \times 10^5$	17.85±0.51	3.4±0.11	1.79±0.05	65.765±2.32
5c	$0.395\pm0.01 \times 10^5$	17.52±0.67	2.8±0.11	1.83±0.05	78.704±2.74
5d	$0.357\pm0.01 \times 10^5$	17.85±0.72	2.9±0.12	1.85±0.04	77.446±2.83
5e	$0.556\pm0.01 \times 10^5$	17.37±0.64	3.0±0.10	1.90±0.05	82.985±3.12
5f	$0.447\pm0.01 \times 10^5$	17.76±0.58	3.9±0.13	2.03±0.06	80.723±3.02
5g	$0.667\pm0.02 \times 10^5$	17.91±0.53	2.7±0.09	2.15±0.05	83.368±2.95
6a	$7.28\pm0.25 \times 10^5$	22.63±0.86	3.2±0.12	0.54±0.01	5.714±0.22
6b	$5.98\pm0.19 \times 10^5$	22.38±0.53	2.25±0.08	0.55±0.01	5.741±0.21
6c	$3.01\pm0.18 \times 10^5$	21.37±0.59	2.8±0.09	0.60±0.01	7.812±0.28
6d	$1.87\pm0.19 \times 10^5$	20.49±0.69	3.2±0.11	0.63±0.02	6.850±0.27
6e	$2.57\pm0.09 \times 10^5$	18.30±0.74	3.1±0.12	0.79±0.02	7.934±0.31
6f	$2.43\pm0.11 \times 10^5$	19.11±0.76	3.1±0.13	0.88±0.02	9.238±0.30
6g	$1.04\pm0.08 \times 10^5$	18.76±0.82	3.2±0.14	0.95±0.02	11.434±0.42

Supplementary material 7: Effect on relative viscosity of HS DNA under the influence of increasing amounts of pyrazolines ligands at 27 (±0.1) °C in phosphate buffer at pH=7.2 with error uncertainty in the value ±5 %

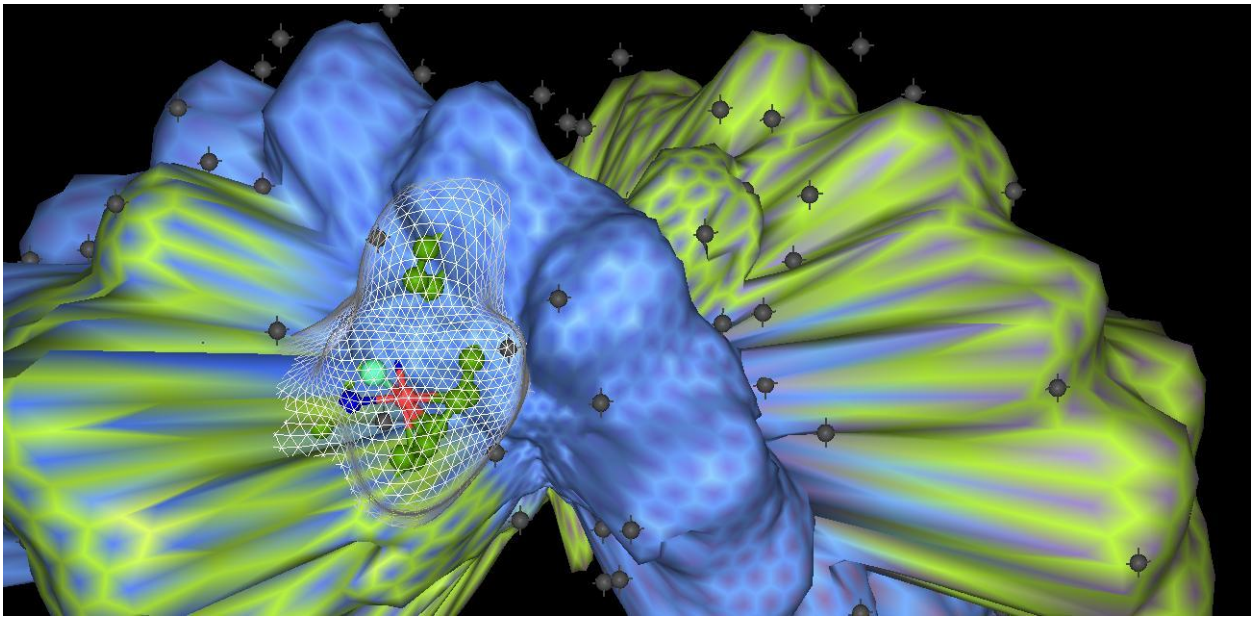


Supplementary material 8: Molecular docking of the complexes 6b–6g and pyrazolines ligands (5a-5g) (ball and stick) with the DNA duplex (VDW spheres) of sequence d(ACCGACGTCGGT)$_2$. The complex is docked in to the DNA showing intercalation between the DNA base pairs.

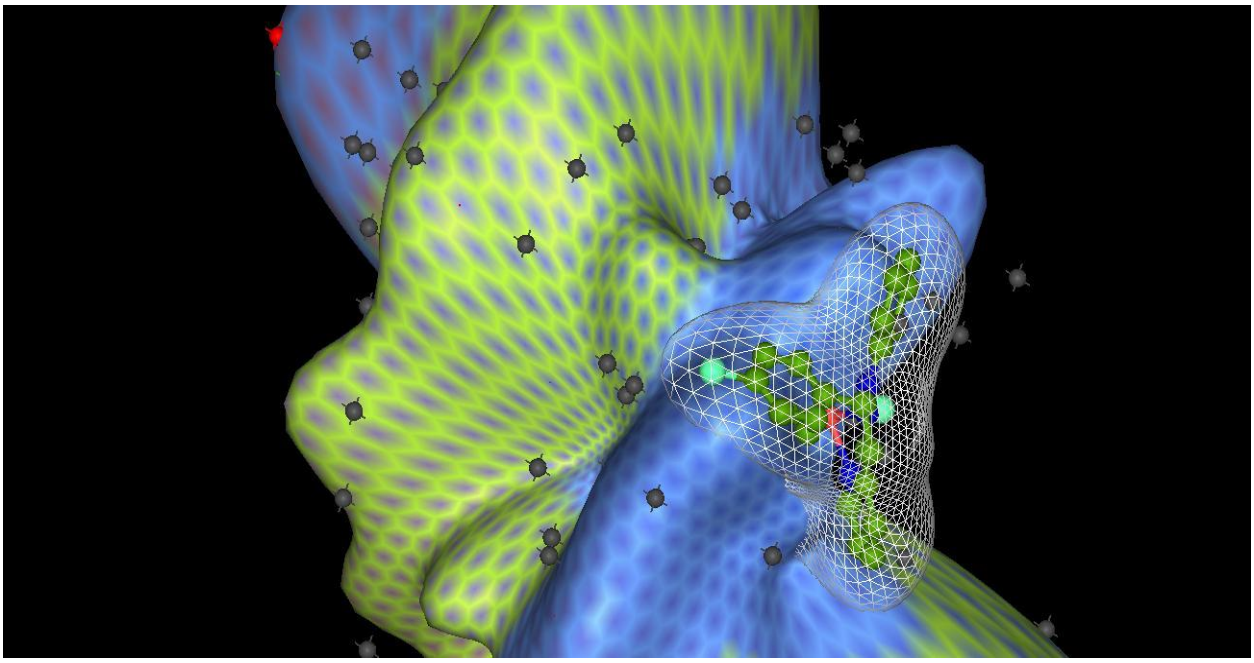
1. [(Cp*)Ru(L^1)Cl]• Cl **(6a)**



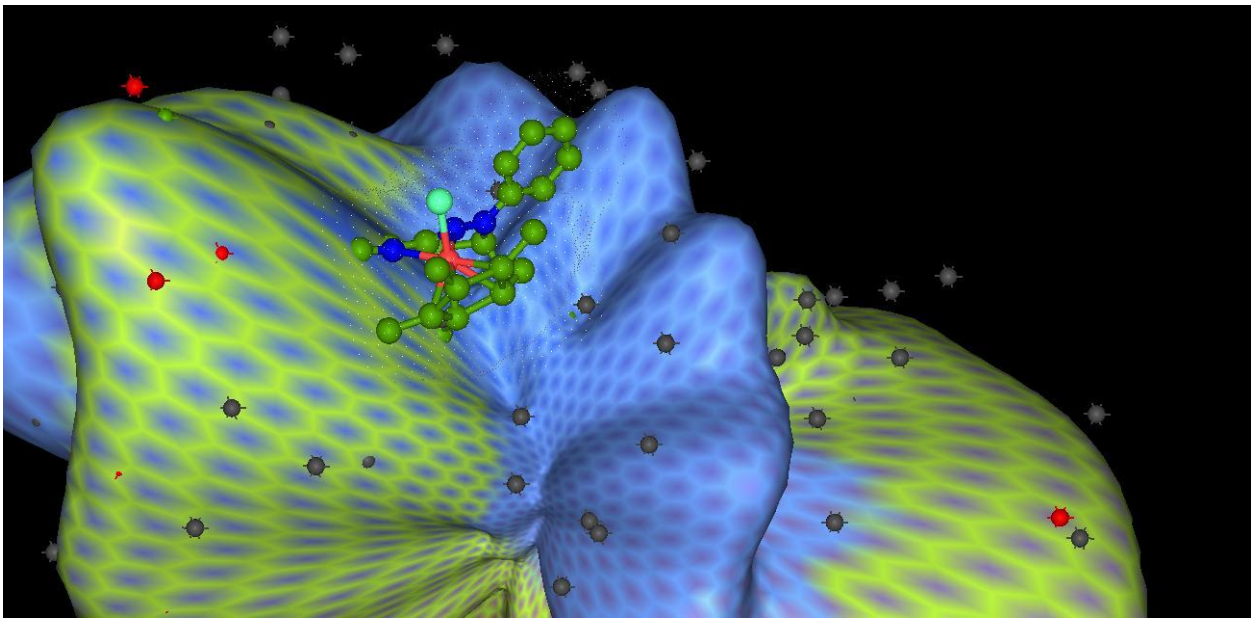
2. [(Cp*)Ru(L^2)Cl]• Cl **(6b)**



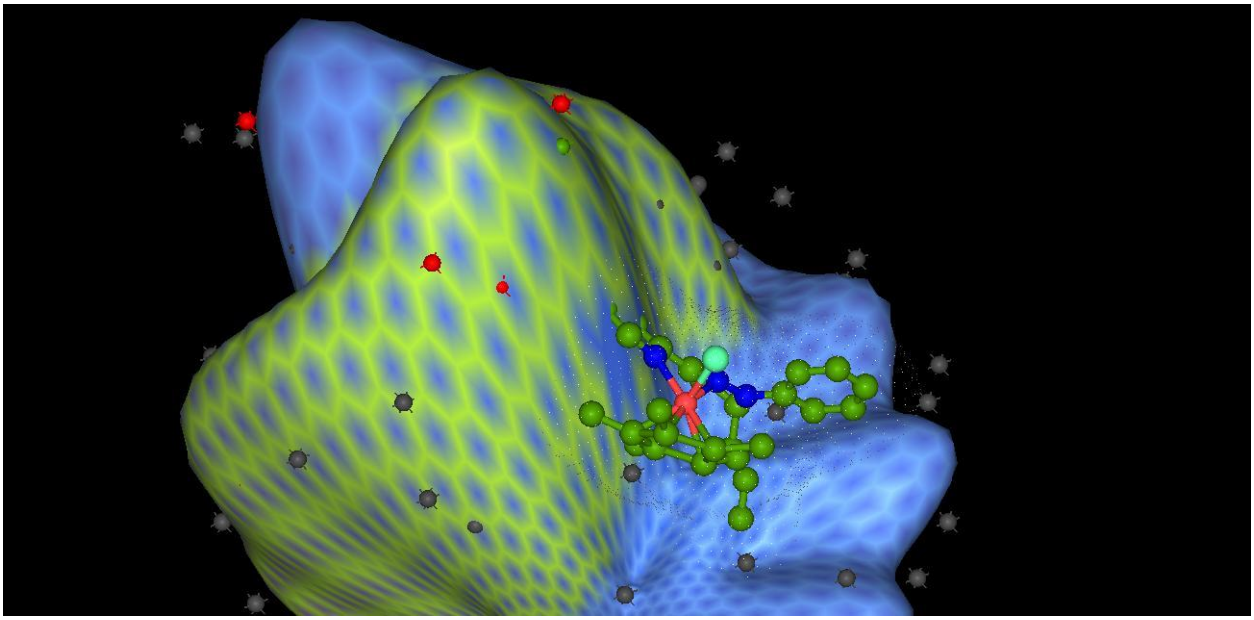
3. [(Cp*)Ru(L^3)Cl]• Cl **(6c)**



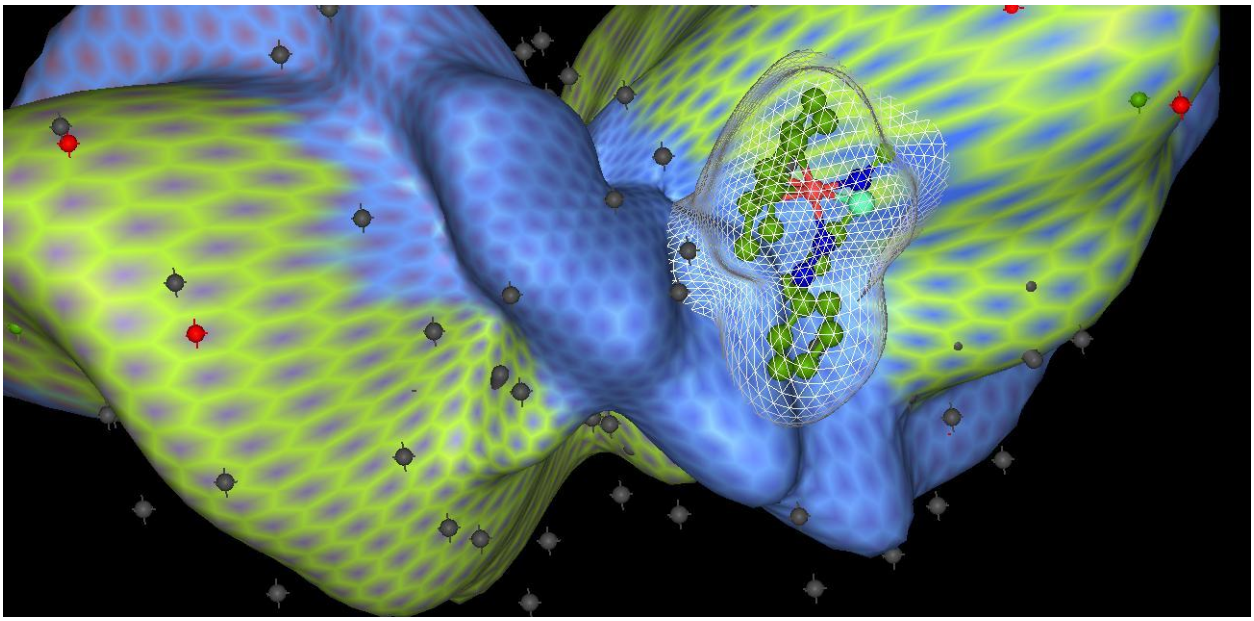
4. [(Cp*)Ru(L^4)Cl]• Cl **(6d)**



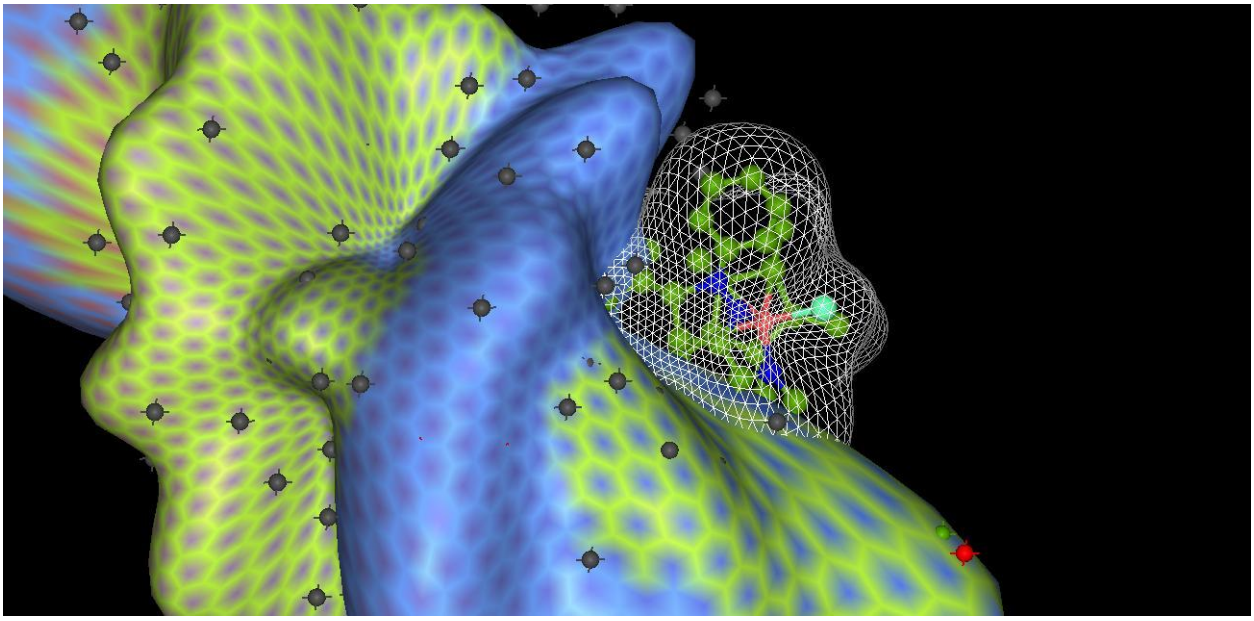
5. [(Cp*)Ru(L^5)Cl]• Cl **(6e)**



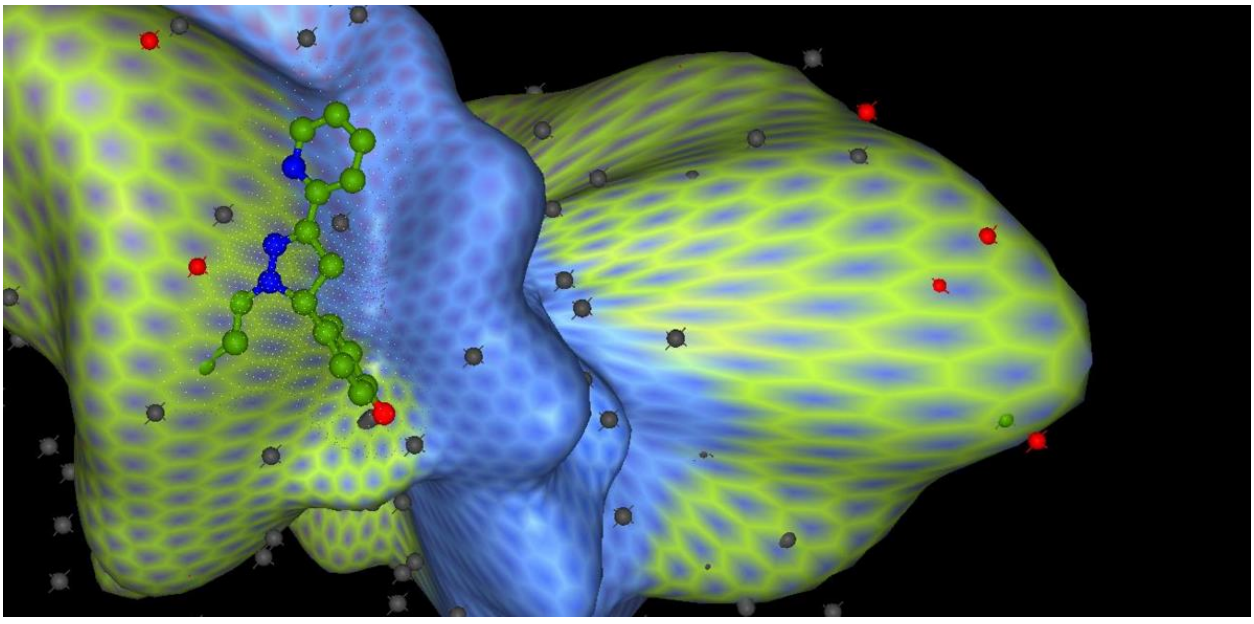
6. [(Cp*)Ru(L^6)Cl]• Cl **(6f)**



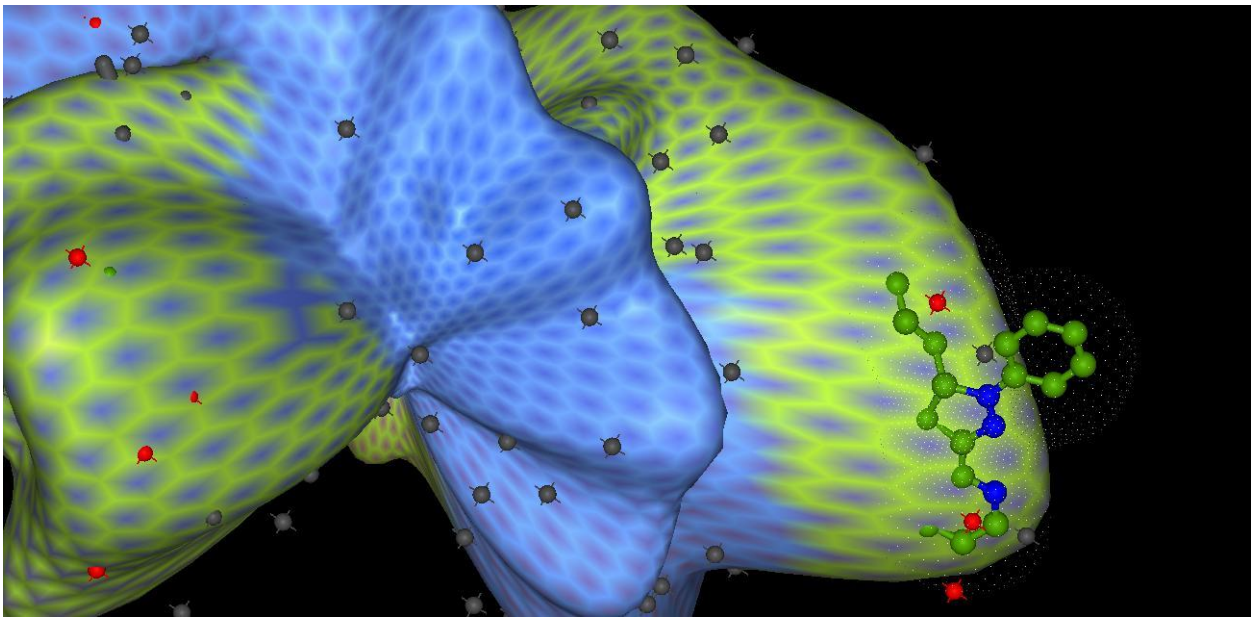
7. [(Cp*)Ru(L^7)Cl]• Cl **(6g)**



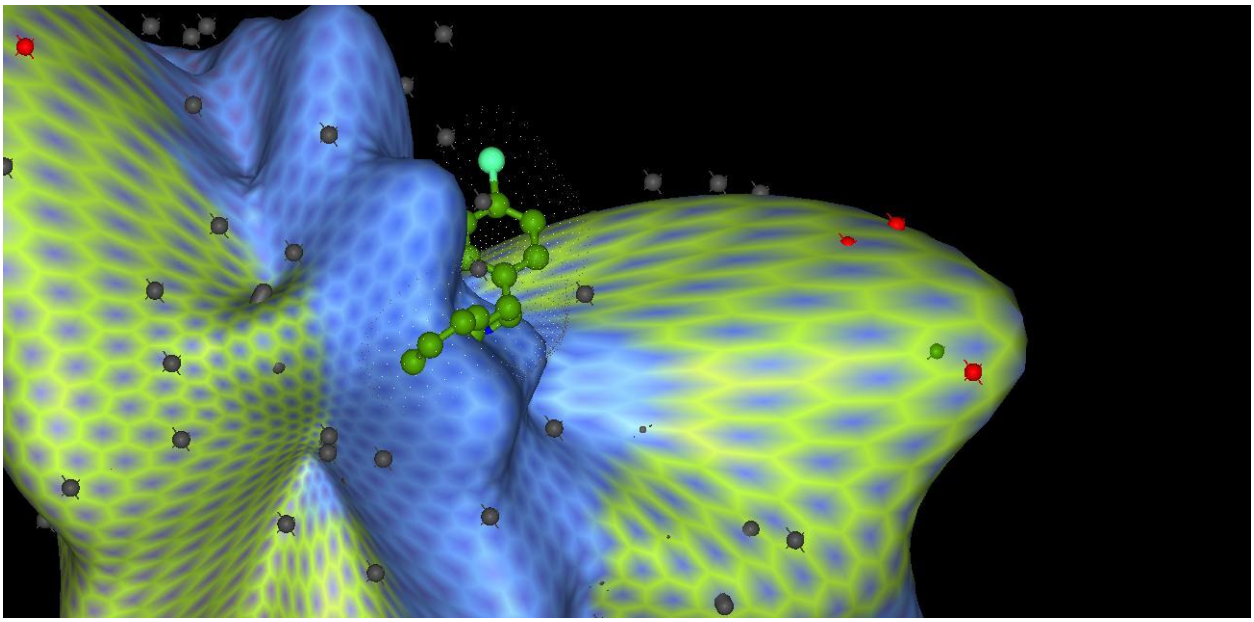
8. 2-[5-(4-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5a)**



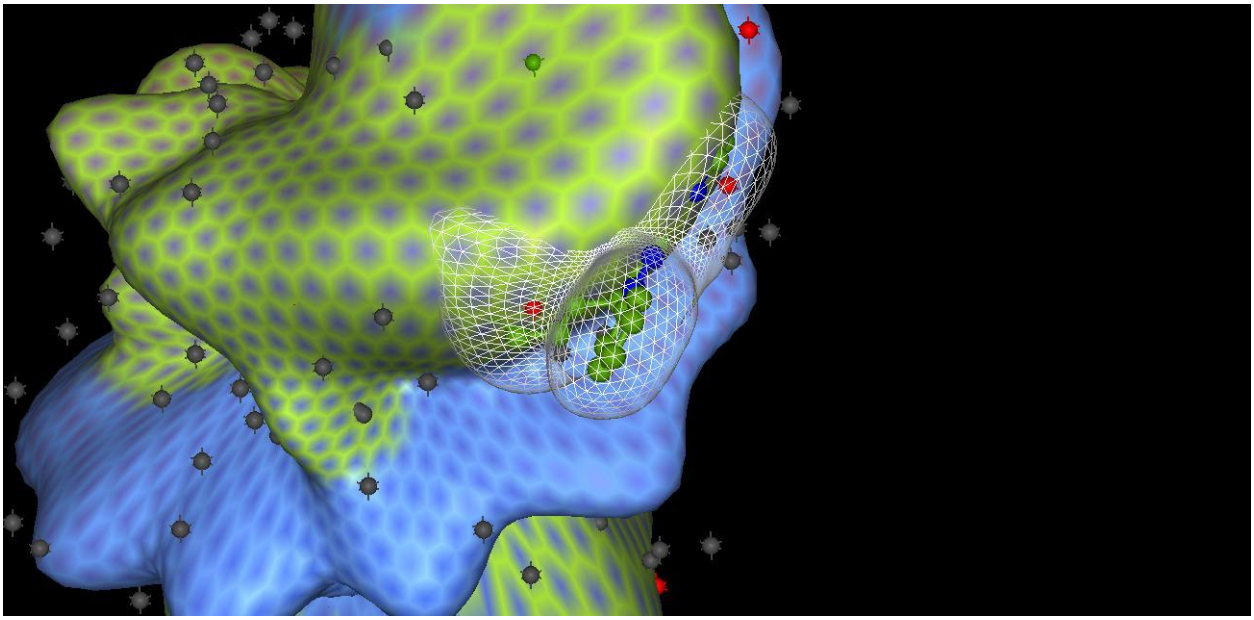
9. 2-[5-(3-Fluorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5b)**



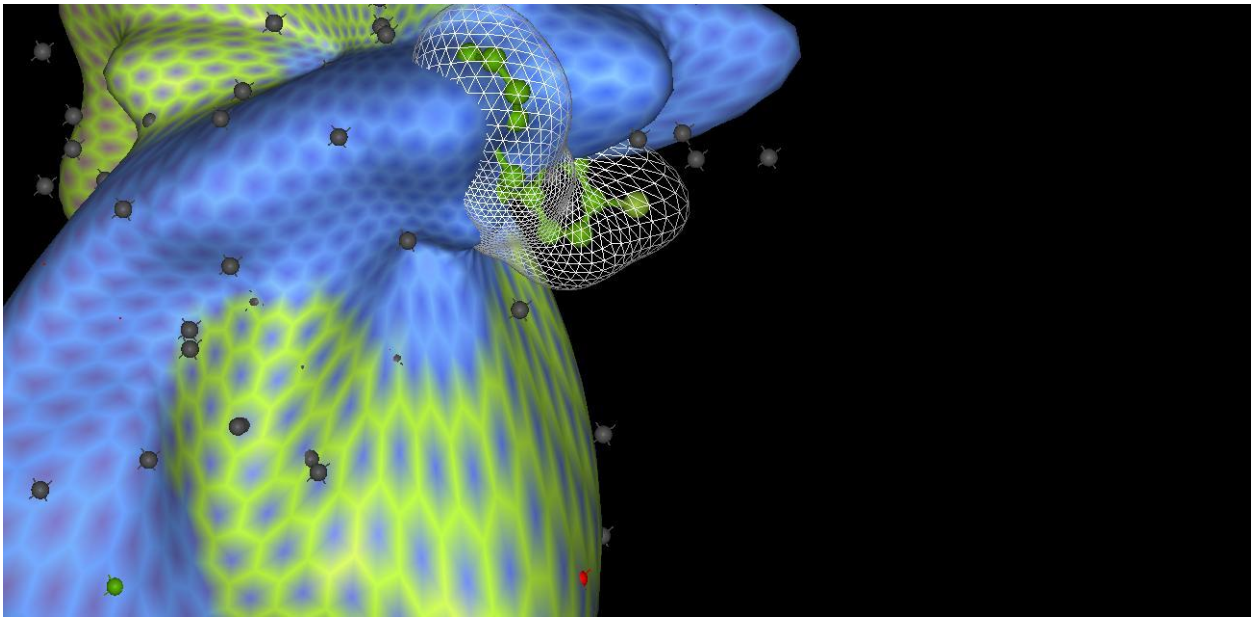
10. 2-[5-(4-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5c)**



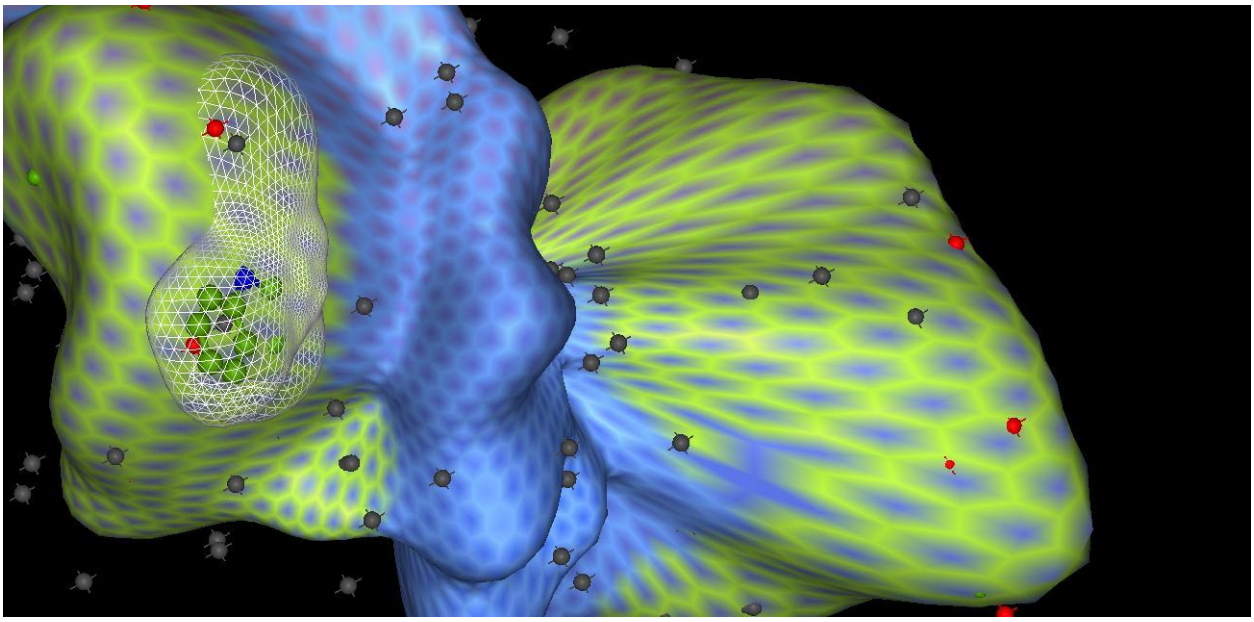
11. 2-[5-(3-Chlorophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5d)**



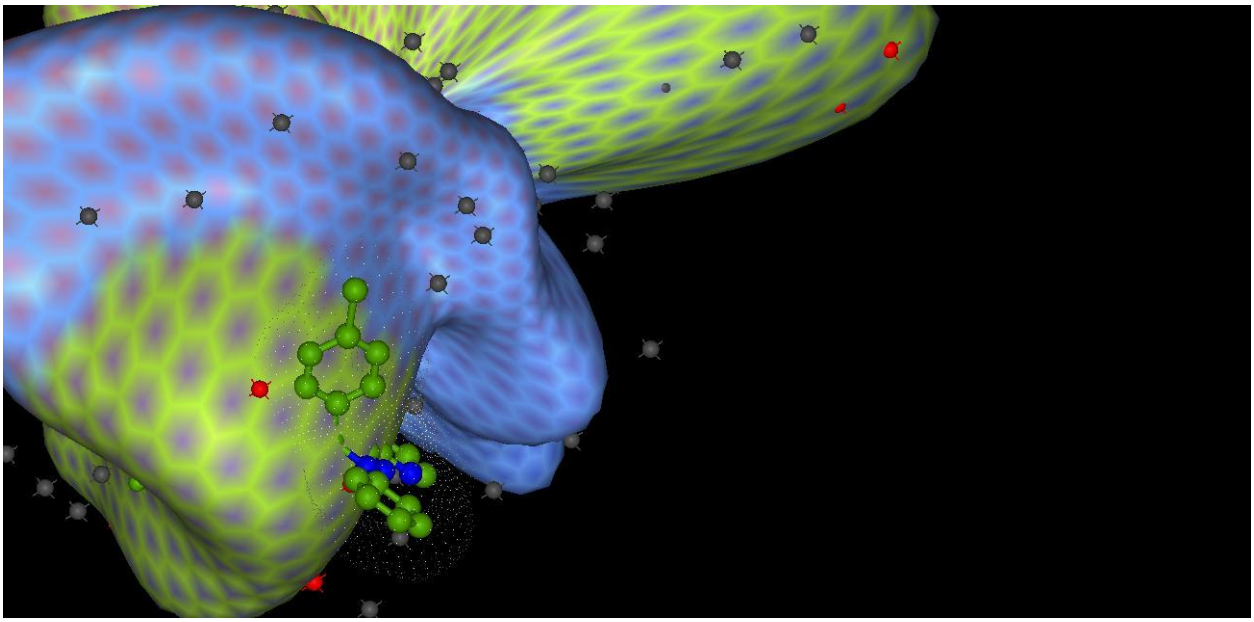
12. 2-[5-(4-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5e)**



13. 2-[5-(3-Bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5f)**



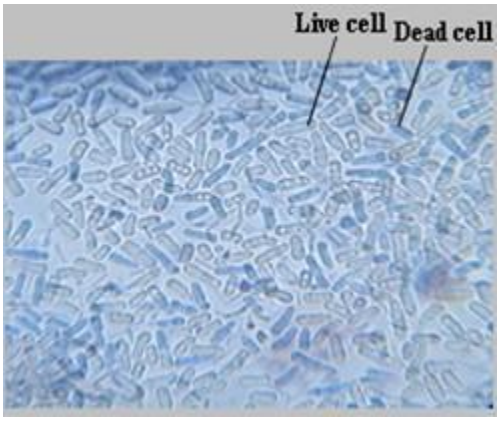
14. 2-[5-(4-Methylphenyl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl] pyridine **(5g)**



Supplementary material 9: Complex mediated DNA cleavage data by agarose gel electrophoresis with error uncertainty in the value ±5 %

Compound	Form I	Form II	Form III	% Cleavage
DNA Control	88±2	12±1	–	–
$RuCl_3 \cdot 3H_2O$	80±2	20±1	–	9.09±0.2
5a	47±1	44±1	9±1	46.59±0.8
5b	48±1	41±1	11±1	45.45±0.7
5c	50±1	35±1	15±1	43.18±0.8
5d	48±1	37±1	15±1	45.45±1.1
5e	49±1	40±1	11±1	44.31±0.7
5f	50±1	36±1	14±1	43.18±0.9
5g	49±1	36±1	15±1	44.31±1.2
6a	19±1	70±1	11±1	78.41±1.4
6b	21±1	65±1	14±1	76.13±1.3
6c	23±1	62±1	15±1	73.86±1.2
6d	24±1	63±1	13±1	72.72±1.3
6e	25±1	46±1	29±1	71.59±1.2
6f	22±1	54±1	24±1	75.00±1.2
6g	26±1	56±1	18±1	70.45±1.1

Supplementary material 10: Effect of compounds on *S. pombe* cells. Dead cells are seen dark whereas live cells are seen transparent.



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