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Synthesis, Characterization and Antimicrobial Evaluation of Ru(II) and Co(III) Complexes of Phenylene-1,2-bis(iminoflavone) Derivatives

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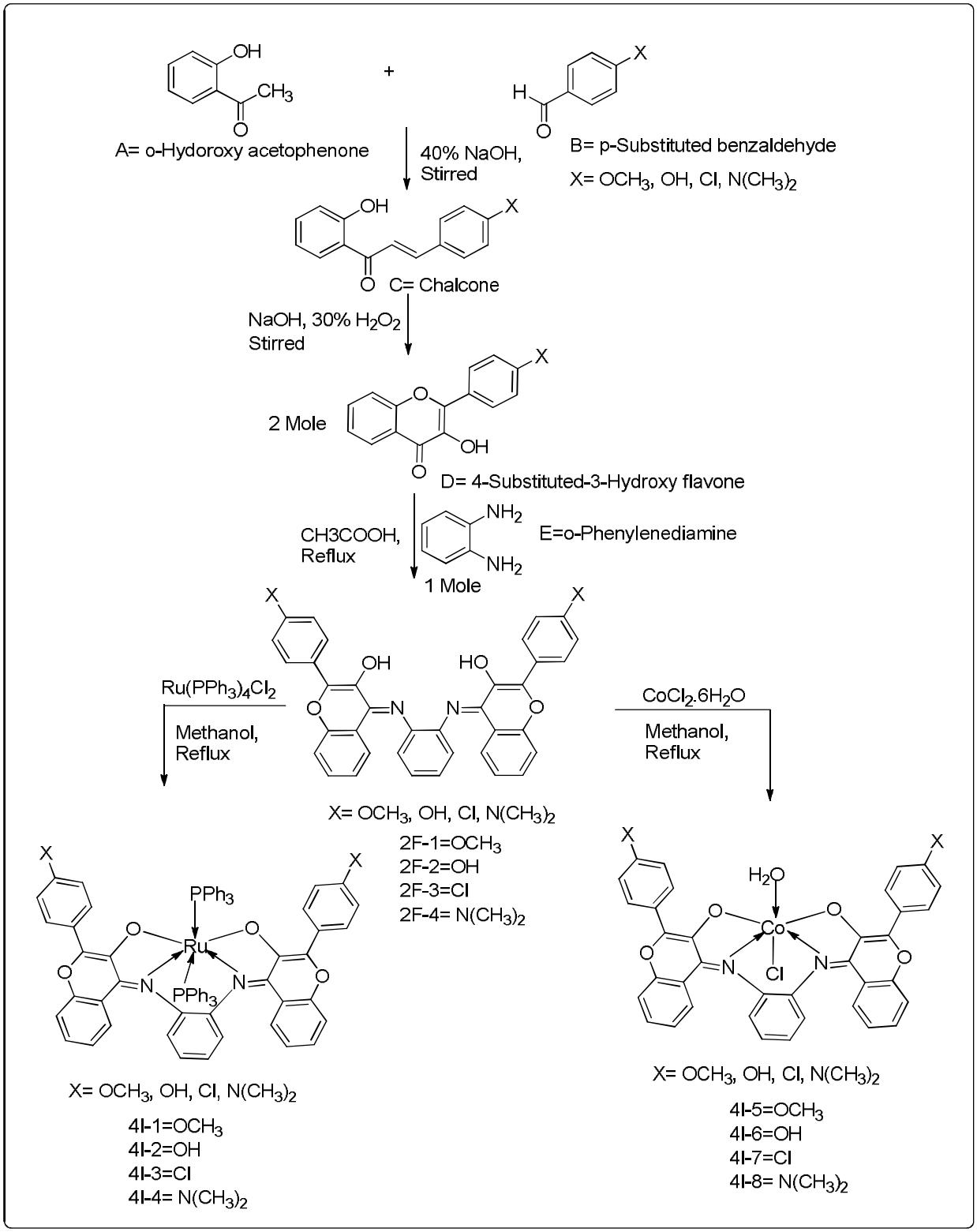
ABSTRACT: Bioactive ruthenium and cobalt scaffolds of molecular formula $[Ru(PPh_3)_2L^{1-4}]$ and $[Co(H_2O)Cl\ L^{1-4}]$ {where L^{1-4} represents the tetradentate ligands (E)-4-((2-((E)-(3-hydroxy-2-(4-substitutedphenyl)-4a,8a-dihydro-4H-chromen-4-ylidene)amino)phenyl)imino)-2-(4-substituted phenyl)-4H-chromen-3-ol (phenylenebis(iminoflavone)} have been synthesized from Ru(II) precursor $[Ru(PPh_3)_4Cl_2]$ and $CoCl_2.3H_2O$ and phenylenebis(iminoflavone). Tetradentate ligands of flavone (phenylenebis(iminoflavone)) have been derived by the condensation of 3-hydroxy-2-(4-substitutedphenyl)-4H-chromen-4-one with o-phenylenediamine. These ligands and scaffolds were characterized by elemental analysis, NMR, IR, ESI-MS, UV-Visible spectrometry, and conductometric measurement. These ligands and scaffolds were screened the in vitro toxicity for antimicrobial activity against the growth of bacterial species *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and fungal species *Candida albicans* and *Aspergillus flavus*.

Keywords: Tetradentate ligands; Ru(II) and Co(III) scaffolds; spectrometry; conductometric and magnetic moment; antimicrobial activity.

INTRODUCTION: Flavonoids, a ubiquitous heterocyclic naturally occurring compounds are found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, Propolis and honey.[1 & 2] From earlier these compounds have been used as medicine to treat human diseases as they possess wide range of biological activity such as antioxidant[3-5], antifungal[6], antibacterial[7], antiviral activity[8]. Increase in resistance power of Microbes towards antimicrobial agents has become a great problem of world as microbes have adapted to new environments more than people. Microbes are changing new properties to resist drug treatments that were once effecting at destroying them and generate new type of disease as *jeeka* virus disease.[9] Thus survival of human being against microorganism with ingenious tactics has challenged. Therefore, it is more difficult to treat the pathogenic viruses, bacteria, fungi, and protozoa with the existing drugs. In order to remove resistance against antimicrobial drugs and make them more effective other mode of action of drugs must be developed.[9] It has shown that metal complexes of flavonoids show wide range of biological activities.[10 & 11] Flavonoids itself chelates with metals such as Fe, Cu, and Zn and show enhance activity *in vivo* and provide a new route to design new metal drugs active towards various diseases related to biological role of metals.[12] Flavone complexes *cis*-dichlorobis(3-aminoflavone)platinum,[13] *cis*-dichloro bis(3-imino-2-R-O-flavanone) ruthenium(II)(R= CH_3 or CH_2CH_3),[14] cis-dichloro(3-nitrosoflavone)(3-hydroxyiminoflavan-one)ruthenium(II)[15] and dichloro(*p*cym)(6- or 7-aminoflavone) ruthenium(II), (*p*cym is η6-*p*-cymene)[16] exhibit cytotoxic activity. It has been reported that heteroatom containing flavone has antimicrobial activity. Certain flavones show antifungal activity against plant pathogen which explored against fungal pathogens causing infection in human.[17] Synthetic biflavones, amentoflavone, isocryptomerin, ginkgetin, bilobetin had shown good antifungal activity.[18-20] Natural products containing imine group play important role towards its biological activity.[21] Metal complexes containing ligands having imine group exhibit stupendous chemical and biological significance.[22] Along with ruthenium certain Co(III) complexes also showed antimicrobial activity.[21]

Here in this paper we have synthesized the phenylenebis(iminoflavone) ligands of 3-hydroxy-2-(4-

substitutedphenyl)-4H-chromen-4-one and their ruthenium(II) and cobalt(III) complexes in order to enhance the antimicrobial activity. The synthesized phenylenebis(iminoflavone) ligands and their ruthenium complexes were characterized and screened for antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* and antifungal activity against *Aspergillus flavus, Candida albicans* by using *Ciprofloxacin* and *Fluconazole* as a standard drug respectively.



Scheme 1: Synthesis of ligands and their Ru(II) and Co(III) complexes.

MATERIAL AND METHODS: All chemicals used were purchased from sigma Aldrich. The solvents used were purified by reported method.[24] Double distilled water has been used wherever necessary. $[Ru(PPh_3)_4Cl_2]$ was synthesized from $RuCl_3.H_2O$ by reported procedure.[25] The purity of the compound was monitored by TLC ($CHCl_3/CH_3OH$, 9:1), using silica gel plate (Merck). Melting points were determined with SSU melting point apparatus. Euro Vector E 3000 Elemental Analyzer was used for elemental analysis. UV visible spectra were recorded on a double beam UV -Vis near IR Labtronics LT-2900 instrument. IR spectra (KBr discs) were recorded on Agilent Cary 360 FTIR spectrometer. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on Brucker Advance 400 MHz FT NMR spectrometer. ESI mass spectrum was recorded on Waters UPLC-TQD Mass spectrometer.

Antimicrobial evaluation: The organisms were used in this study are *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* (bacteria), *Candida albicans* and *Aspergillus flavus* (fungi). All the synthesized ligands (L^1-L^4) and complexes 4I (1-8) has been screened for their *in vitro* inhibitory activity (antibacterial and antifungal activities) against strains of microorganisms for determination of minimum inhibitory concentrations (MICs) in µg/mL by micro dilution method.[26] Ciprofloxacin and Fluconazole are used as standard drugs.

Synthesis of ligands-phenylenebis(iminoflavone): The phenylenebis(iminoflavone) ligands were synthesized by reported procedure with some minor modifications.[27] To the 10 mmol solution of a 3-hydroxy-4-substituted flavone derivative (Sub.= 0.268g (-OCH_3), 0.254g (-OH), 0.364g (-Cl), 0.281g (-$N(CH_3)_2$) in methanol, o- Phenylenediamine (0.541g, 5 mmol) dissolved in methanol (20 mL) and few drops of glacial acetic acid were added. The reaction mixture was refluxed for 7 hours at 70-80℃. The resulting solution was cooled to room temperature, and then poured into ice with constant stirring. The precipitate thus obtained was filtered and washed with 10% ethanol and diethyl ether. The crude products were purified by column chromatography on silica gel. Petroleum ether/ethyl acetate (95/5%) used as eluent. The ligand was recrystallized with hot ethanol and dried in vacuo over anhydrous $CaCl_2$.

Synthesis of Ru(II) complexes (4I-1 to 4I-4): The Ru(II) complexes were prepared by reported procedure with minor modification.[28] To the 0.10 mmol (0.0994 g) solution of $[RuCl_2(PPh_3)_4]$ in 20 mL benzene, 0.1 mmol of ligand (L^1-0.610 g, L^2-0.582 g, L^3-0.619 g, L^4-0.637 g) added. The mixture was refluxed for 5h in round bottom flask and then volume was reduced up to 3 mL. The product was separated by the addition of small quantity of petroleum ether at 60℃ - 80℃. The complex was then filtered, washed and recrystallized from dichloromethane and petroleum ether mixture in 1:3 volume ratio respectively.

Synthesis of Co(III) complexes (4I-5 to 4I-8): Further, the synthesized phenylenebis(iminoflavone) ligands were interacted with the $CoCl_2.6H_2O$. The Co(III) complexes were synthesized by reported procedure with slight modification.[29] To the warm 1 mmol methanolic solution (20mL) of ligand (L^1=0.610 g, L^2=0.588 g, L^3=0.619 g, L^4=0.636 g,), 1 mmol (0.238 g) methanolic solution (15 mL) of $CoCl_2.6H_2O$ was added and refluxed for 3 hour by adding 1 mmol of sodium acetate. On cooling the reaction mixture at room temperature, the precipitate was formed, which was filtered, washed with 10% ethanol and ether and dried in vacuum over anhydrous $CaCl_2$. The complexes were recrystallized in hot ethanol. During the synthesis performed under aerobic conditions, the colour changes from violet red to dark brown which suggest the occurrence of oxidation process induced by the oxygen in air ($Co^{II} \rightarrow Co^{III}$).

(E)-4-((2-((E)-(3-hydroxy-2-(4-methoxyphenyl)-4a, 8a-dihydro-4H-chromen-4-ylidene)amino)phenyl) imino)-2-(4-methoxyphenyl)-4H-chromen-3-ol (L^1): Yield: 78% (0.281g), Colour: Brown, m.p.: 147°C, IR (KBr, νcm^{-1}): 3410(OH), 2812(CH), 1609(C=N), 1582(C=C), 1260(C-O), 1198(C-N). ^{1}H NMR(400MHz, DMSO-*d6*): δ(ppm) 3.47(6H, s), 6.79(4H, d, *J*=8.28Hz), 6.69-6.84(8H, m), 7.02-7.19(4H, m,), 7.75(4H, d, *J* = 8.68 Hz), 9.56(2H, s), ^{13}C NMR (100MHz, DMSO-*d6*, δ (ppm): 55.45, 55.45, 113.74, 113.74, 114.43, 114.43, 114.43, 114.43, 118.67, 118.67, 120.52, 120.52, 124.80, 124.80 124.24, 124.24, 128.07, 128.07, 129.27, 129.27, 131.34, 131.34, 131.71, 131.71, 131.71, 131.71, 136.22, 136.22, 154.36, 154.36, 155.67, 155.67, 155.81, 155.81, 159.58, 159.58, 160.40, 160.40, Elemental analysis (%) Calc.: C, 74.99; H, 4.64; N, 4.60; Observed: C, 75.02; H, 4.67; N, 4.59; ESI-MS: $[M + 1]^+$: 609.62 (Observed), 608.64 (Calculated). M. F.: $C_{38}H_{28}N_2O_6$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 256, (π -π*transition), 360(n-π* transition).

(E)-4-((2-((E)-(3-hydroxy-2-(4-hydroxyphenyl)-4a, 8a-dihydro-4H-chromen-4-ylidene)amino)phenyl) imino)-2-(4-hydroxyphenyl)-4H-chromen-3-ol (L^2): Yield: 62% (0.22g), Colour: Reddish brown, m.p.: 210°C, IR (KBr, νcm^{-1}): 3523(OH), 2906(CH),

1606(C=N), 1573(C=C), 1256(C-O), 1208(C-N), ^{1}H NMR (400MHz, DMSO-*d6*): δ(ppm) 6.98(4H, d, J = 8.3, Hz), 7.06(4H, d, J = 8.3, Hz), 7.28(4H, m), 7.19-7.29(8H, m), 9.11(2H, s), 9.43(2H, s), ^{13}C NMR (100MHz, DMSO-*d6*): δ (ppm): 113.74, 113.74, 115.74, 115.74, 115.74, 115.74, 118.67, 118.67, 120.50, 120.50, 124.24, 124.24, 124.80, 124.80, 128, 128, 129.27, 129.27, 131.32, 131.32, 131.41, 131.41, 131.41, 131.41, 136.24, 136.24, 154.36, 154.36, 155.65, 155.65, 155.82, 155.82, 157.83, 157.83, 159.58, 159.58, Elemental analysis (%) Calc.: C, 74.22; H, 4.50; N, 4.81; Observed: C, 74.26; H, 4.52; N, 4.79; ESI-MS: $[M + 1]^+$: 583.58(Observed), 582.60(Calculated). M. F. $C_{36}H_{26}N_2O_6$, UV-vis. λ_{max} in nm (in DMSO, 2×10^{-4}M), 271, (π -π* transitions), 410 (n-π* transition).

(E)-2-(4-chlorophenyl)-4-((2-((E)-(2-(4-chlorophenyl)-3-hydroxy-4a,8a-dihydro-4H-chromen-4-ylidene)amino)phenyl)imino)-4H-chromen-3-ol (L^3):
Yield: 68% (0.25g), Colour: Brownish black, m.p.: 121°C, IR (KBr, νcm^{-1}): 3569(OH), 2869(CH), 1618(C=N), 1564(C=C), 1286(C-O), 1180(C-N), 723(C-Cl). ^{1}H NMR (400MHz, DMSO-*d6*): δ (ppm): 7.19(4H, d, J = 8.4Hz), 7.14(4H, d, J = 8.4Hz), 7.00-7.10(8H, m), 7.29(4H, m), 9.62(2H, s), ^{13}C (100MHz, DMSO-*d6*) NMR: δ(ppm): 113.72, 113.72, 118.66, 118.66, 120.52, 120.52, 124.24, 124.24, 124.80, 124.80, 126.50, 126.50, 126.50, 126.50, 128, 128, 128.94, 128.94, 128.94, 128.94, 129.25, 129.25, 131.34, 131.34, 135.66, 135.66, 136.22, 136.22, 154.35, 154.35, 155.66, 155.66, 155.80, 155.80, 159.62, 159.62; Elemental analysis (%) Calc.: C, 69.80; H, 3.90; Cl, 11.45; N, 4.52; Observed: C, 69.82; H, 3.91; Cl, 11.44; N, 4.51; ESI-MS: $[M + 1]^+$: 620.48 (Observed), 619.49 (Calculated), M. F.: $C_{36}H_{24}Cl_2N_2O_4$, UV-vis. λ_{max} in nm (in DMSO, 2×10^{-4}M), 262(π -π* transitions), 385(n-π* transition).

(E)-2-(4-(dimethylamino)phenyl)-4-((2-((E)-(2-(4-(dimethylamino)phenyl)-3-hydroxy-4a,8a-dihydro-4H-chromen-4-ylidene)amino)phenyl)imino)-4H-chromen-3-ol (L^4):
Yield: 73% (0.27g), Colour: Brown, m.p.: 135°C, IR (KBr, νcm^{-1}): 3462(OH), 2879(CH), 1607(C=N), 1576(C=C), 1278(C-O), 1214(C-N), ^{1}H NMR (400MHz, DMSO-*d6*): δ(ppm): 2.88(12H, s), 6.93(4H, d, J = 7.5 Hz), 6.99-7.17(8H, m), 7.24(4H, m), 7.19-7.26(4H, m), 9.50(2H, s), ^{13}C NMR (100MHz, DMSO-*d6*): δ (ppm): 40.30, 40.30, 40.30, 40.30, 113.74, 113.74, 113.82, 113.82 ,113.82, 113.82, 118.66, 118.66,120.50, 120.50, 124.23, 124.23, 126.4, 126.40, 126.40, 126.40, 128, 128, 124.80, 124.80, 129.26, 129.26, 136.23, 136.23, 131.34, 131.34, 151.42, 151.42, 154.36, 154.36, 155.64, 155.64, 155.80, 155.80, 159.60, 159.60, Elemental analysis (%) Calc.: C, 75.45; H, 5.70; N, 8.80; Observed: C, 75.54; H, 5.74; N, 8.83; ESI-MS: $[M + 1]^+$: 637.74 (Observed), 636.74 (Calculated), M. F. $C_{40}H_{36}N_4O_4$ UV-vis. λ_{max} in nm (in DMSO, 2×10^{-4}M), 252(π -π* transitions), 356(n-π* transition).

Ruthenium(II) complexes of L^1 ligands: (4I-1):
Yield 71%, Colour: Brown; m. p.: 248°C, IR (KBr, νcm-1): 2833(CH), 1527(C=N), 1544(C=C), 1296(C-O), 1232(C-N), 1435(Ru-P), 1162(P-Ph), 556(Ru-O), 492(Ru -N), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS): δ (ppm): 3.47(6H, s), 7.17(PPh_3, m), 7.00-9.80(m, Ar-H), ^{31}P NMR (DMSO-*d6*, 400 MHz, 25°C, TMS): δ(ppm): 49.82(PPh_3, s), Elemental analysis (%) Calc.: C, 72.13; H, 4.58; N, 2.27; P, 5.03; Observed: C, 72.43; H, 4.66; N, 2.26; P, 5.00; Ru, 8.21; For ESI-MS: $[M + 1]^+$: 1233.24(Observed), 1232.26 (Calculated). M. F.: $C_{74}H_{56}N_2O_6P_2Ru$, UV-vis. λ_{max} (nm) (in DMSO, 2×10^{-4}M) 254(π -π* transitions), 485(n-π* transition).

Ruthenium(II) complexes of L^2 ligands: (4I-2):
Yield: 65%, Colour: Blackish brown; m. p.: 282°C, IR (KBr, νcm^{-1}): 2837(CH), 1538(C=N), 1629(C=C), 1302(C-O), 1248(C-N), 1418(Ru-P), 1178(P-Ph), 550(Ru-O), 490(Ru-N), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS): δ (ppm): 7.15(PPh_3, m), 9.11(2H, s), 6.86-8.96(Ar-H, m), ^{31}P NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 49.90(PPh_3, s), Elemental analysis (%) Calc.: C, 71.81; H, 4.35; N, 2.33; P, 5.14; Ru, 8.39, Observed: C, 71.86; H, 4.38; N, 2.31; P, 5.12; Ru, 8.40; For ESI-MS: $[M + 1]^+$ 1205.20(Observed), 1204.21(Calculated), M. F.: $C_{72}H_{52}N_2O_6P_2Ru$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 262(π -π* transitions), 505(n-π* transition).

Ruthenium(II) complexes of L^3 ligands: (4I-3):
Yield: 66%, Colour: Brown; m. p.: 228°C, IR (KBr, νcm^{-1}): 3486 (OH), 3033(CH), 1542(C=N), 1624(C=C), 1314(C-O), 1228(C-N), 1408(Ru-P), 1182(P-Ph), 548(Ru-O), 488(Ru-N), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 7.19(PPh_3, m), 7.46-9.23(m, Ar-H), ^{31}P NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 49.86(PPh_3, s), Elemental analysis (%) Calc.: C, 69.68; H, 4.06; Cl, 5.71; N, 2.26; P, 4.99; Ru, 8.14; Observed: C, 69.70; H, 4.16; Cl, 5.70; N, 2.25; P, 4.97; Ru, 8.15; For ESI-MS: $[M + 1]^+$ 1242.08(Observed), 1241.10 (Calculated). M. F.: $C_{72}H_{50}C_{l2}N_2O_4P_2Ru$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 264(π -π* transitions), 507(n-π* transition).

Ruthenium(II) complexes of L^4 ligands: (4I-4):
Yield: 68%, Colour: Brown, m. p.: 232°C, IR (KBr, νcm^{-1}): 3021(CH), 1530(C=N), 1654(C=C), 1296(C-O), 1254(C-N), 1426(Ru-P), 1170(P-Ph), 558(Ru-O), 502(Ru-N), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 2.88(6H, s), 3.96(12H, s), 7.14(PPh_3, m), 7.16-9.86(m, Ar-H), ^{31}P NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 49.02(PPh_3, s), Elemental analysis (%) Calc.: C, 72.54; H, 4.97; N, 4.45; P, 4.92; Ru, 8.03; Observed: C, 72.58; H, 4.99; N, 4.46; P, 4.90; Ru, 8.04; For ESI-MS: $[M+1]^+$: 1259.34(Observed), 1258.35(Calculated), M. F.: $C_{76}H_{62}N_4O_4P_2Ru$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M),266(π -π* transitions), 482(*n*-π* transition).

Cobalt(III) complexes of L^1 ligands: (4I-5):
Yield: 65%, Colour: Yellow brown; m. p.: 262°C, IR (KBr, νcm^{-1}): 3545(OH, broad), 798(OH), 2890(CH), 1526(C=N), 1564(C=C), 1293(C-S), 1297(C-O), 348(Co-Cl), 456(Co-N), 524(Co-O), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS): δ (ppm). 3.95(3H, s), 6.96-8.76(m, Ar-H); Elemental analysis (%) Calc.: C, 63.48; H, 3.93; Cl, 4.93; Co, 8.20; N, 3.90; Observed: C, 63.50; H, 3.97; Cl, 4.92; Co, 8.21; N, 3.89; For ESI-MS: $[M+1]^+$: 720.00(Observed), 719.02(Calculated), M. F.: $C_{38}H_{28}ClCoN_2O_7$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 265(π -π* transition), 385(*n*-π* transition), 505(*n*-π* transition).

Cobalt(III) complexes of L^2 ligands: (4I-6):
Yield: 64%, Colour: Reddish Brown; m. p. >300°C. IR (KBr, νcm^{-1}): 3547(OH), 777(OH), 2990(CH), 1526(C=N), 1614(C=C), 1281(C-S), 1307(C-O), 354(Co-Cl), 454(Co-N), 545Co-O), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 9.78(s, OH), 6.96-8.93(m, Ar-H), Elemental analysis (%) Calc.: C, 62.58; H, 3.50; Cl, 5.13; Co, 8.53; N, 4.05; Observed: C, 62.61; H, 3.55; Cl, 5.15; Co, 8.54; N, 4.04; For ESI-MS: $[M+1]^+$: 691.95(Observed), 690.97(Calculated) .M. F.: $C_{36}H_{24}ClCoN_2O_7$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 269 (π -π* transitions), 392(*n*-π* transition), 546(*n*-π* transition).

Cobalt(III) complexes of L^3 ligands: (4I-7):
Yield: 62%, Colour: Blackish brown, m. p. >300°C. IR (KBr, νcm^{-1}): 3507(OH, broad), 796(OH), 2870(CH), 1539(C=N), 1627(C=C), 1274(C-S), 1316 (C-O), 363(Co-Cl), 450(Co-N), 525(Co-O), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 6.72-8.82(m, Ar-H), Elemental analysis (%) Calc.: C, 59.40; H, 3.05; Cl, 14.61; Co, 8.10; N, 3.85; Observed: C, 59.42; H, 3.12; Cl, 14.60; Co, 8.12; N, 3.84; For ESI-MS: $[M+1]^+$: 728.85(Observed), 727.86(Calculated), M. F.: $C_{36}H_{22}Cl_3CoN_2O_5$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 277(π -π* transitions), 396(*n*-π* transition), 555(*d*-*d* transition).

Cobalt(III) complexes of L^4 ligands: (4I-8):
Yield: 68%, Colour: Yellow brown; m. p. >360°C, IR (KBr, νcm^{-1}): 3545(OH, broad), 786(OH), 2890(CH), 1529(C=N), 1564(C=C), 1302(C-S), 1297(C-O), 334(Co-Cl), 460(Co-N), 510Co-O), ^{1}H-NMR (DMSO-*d6*, 400 MHz, 25°C, TMS), δ (ppm): 3.95(3H, s), 6.96-8.76(m, Ar-H), Elemental analysis (%) Calc.: C, 64.48; H, 4.60; Cl, 4.76; Co, 7.91; N, 7.52; Observed: C, 64.51; H, 4.64; Cl, 4.75; Co, 7.92; N, 7.50; For ESI-MS: $[M+1]^+$: 746.10(Observed), 745.11(Calculated), M. F.: $C_{40}H_{34}ClCoN_4O_5$, UV-vis.: λ_{max} in nm (in DMSO, 2×10^{-4}M), 260(π -π* transition), 385(*n*-π* transition), 505(*n*-π* transition).

RESULTS AND DISCUSSION:

Spectroscopic studies:

FT-IR Spectra: The vibrational frequency of phenylenebis(iminoflavone) was compared with those of complexes in order to infer the coordination mode. The frequencies of ligands were observed at 3410-3580 cm^{-1}, 1605-1625cm^{-1}, 1250-1300cm^{-1}, and 1175-1225cm^{-1} for $\nu_{(O-H)}$ phenolic, $\nu_{(C=N)}$, $\nu_{(C-O)}$ phenolic, and $\nu_{(C-N)}$ respectively. In all the complexes, the $\nu_{C=N}$ band is shifted to lower frequency, 1565-1580 cm^{-1}, indicated that the azomethine N-atom of phenylenebis(iminoflavone) had coordinated to the metal ion.[31] Further, in all complexes, the C-O stretching vibration appears at higher frequency 1280-1320cm^{-1} inferred, coordination occurred through the phenolic O-atom. The disappearance of band due to $\nu_{(O-H)}$ in the complexes occurred during co-ordination shows deprotonation of phenolic protons. However, the new bands appeared in the metal complexes in the region 545-560 cm^{-1}, 488-504 cm^{-1}, 510-525 cm^{-1} and 450-460 cm^{-1} are attributed to $\nu_{(Ru-O)}$, $\nu_{(Ru-N)}$, $\nu_{(Co-O)}$ and $\nu_{(Co-N)}$ respectively.[32 & 33] In complexes 4I(1-4) at 1430-1440 cm-^{1}, 1165-1182 cm-^{1} and 1085-1090 cm-^{1} appeared due to $\nu_{(Ru-P)}$ and $\nu_{(P-Ph)}$, respectively.[34] On the basis of vibrational bands it is inferred that the ligands are behaving as a dibasic tetradentate ligands. The broad band in the region 3200-3500 cm^{-1} and two weaker bands in the region 750-800 cm^{-1} due to $\nu_{(O-H)}$ rocking and wagging mode of vibration respectively, indicated the presence of coordinated water molecule in complexes (4I-5 to 4I-8).[36]

^{1}HNMR Spectra: The ^{1}HNMR spectra of ligands and complexes were recorded in DMSO –*d6* and the chemical shift δ (ppm) is referenced to internal TMS. The protons of phenylenbis(iminoflavones) appeared in the region δ (ppm) δ 3.20-3.80, δ 2.2-2.9, 6.90-9.98

(broad), and δ 8.90-9.83 of methoxy (-OCH_3, L^1, 4I-1, 4I-5), N-methyl (NCH_3, L^4, 4I-4, 4I-8), aromatic (Ar-H) and phenolic (OH), protons respectively. The downfield shift of aromatic protons in the complexes from δ 6.90-9.98 to δ 6.25-8.63, shows metal ion coordinated to the ligand. Furthermore, the disappearance of the phenolic proton in the complexes shows that the de-protonation occurred during complexation of phenolic O-atom with metal.

^{31}P NMR Spectra: The ^{31}P NMR spectra of complexes (4I-1 to 4I-4) shows singlet at 25.12, 24.46, 25.04 and 24.36 ppm confirm the presence of triphenylphosphine group with magnetically equivalent phosphorous atom.

Electronic Spectra: The electronic spectral data of ligands and complexes were recorded in 2×10^{-4}M solution of DMSO. Bands of electronic spectra of Ru(II) complexes showed 2-3 bands in region 256-520nm. The complexes are diamagnetic, which showed ruthenium in (II) oxidation state and t^6_{2g} configuration in octahedral environment. The ground state is $^1A_{1g}$ arising from the t^6_{2g} configuration in an octahedral environment. The excited states corresponding to the t_{2g}^5, e_g^1 configuration are $^3T_{1g}$, $^3T_{2g}$, $^1T_{1g}$ and $^1T_{2g}$. The possible transitions are $^1A_{1g} \rightarrow {}^3T_{1g}$, $^1A_{1g} \rightarrow {}^1T_{2g}$, $^1A_{1g} \rightarrow {}^1T_{1g}$ and $^1A_{1g} \rightarrow {}^1T_{2g}$ .in order of increasing energy. The bands in the region 460-520 nm are due $^1A_{1g} \rightarrow {}^1T_{1g}$ transition[36] on the basis of low extinction coefficient and other high intensity band in the region 250-270 nm due to charge transfer transition arising from the excitation of electrons from the metal t_{2g} level to the unfilled molecular orbitals derived from the π level of legands.[37]

Electronic spectra of Co(III) complexes (4I-5 to 4I-8) exhibited three absorption bands in the region 250-280 nm, 380-400 nm and 505-560 nm due to π -π*, *n*-π* and *d-d* transitions. The absorption bands of ligands due to π -π* and *n*-π* transitions shifted at higher wavelength in complexes and a new band observed in region 505-560 nm due to *d-d* transitions. The d-d bands originate from the $^1A_1g \rightarrow {}^1T_1g$ transition for the distorted octahedral Co(III) ion in complexes.[38 & 40]

Conductometric and magnetic moment Measurement: The molar conductivity of complexes was measured in DMF (dimethylformamide) by digital TDS-Conductivity meter. All complexes of Ru(II) and Co(III) showed molar conductivity in the range 2.6-5.6 Ω^{-1} cm^2 mol^{-1} which shown all complexes are non electrolyte.[39] Also the magnetic susceptibility measurement shown all complexes are of diamagnetic (μ = 0.0 BM)

Biological Evaluation: The antimicrobial activities of the synthesized compounds have been screened in vitro, as growth inhibiting agents. The antifungal and antibacterial screening were carried out using micro dilution Method against some strains of bacteria and fungi like S. aureus, B. subtilis,(Gram positive bacteria) E. coli, S. typhi (Gram negative bacteria) and A. flavus, and C. albicans respectively. The MIC values of synthesized compounds were obtained compared with standard drug ciprofloxacin and fluconazole. The MIC values of the ligand and its complexes are given in Tables 1, 2, 3 and 4, respectively. The growth inhibitory activity of ligands and their complexes are shown in figure 1, 2, 3 and 4 respectively. The antimicrobial evaluation demonstrated that the complexes 4I-4 and 4I-8 showed good activity against different microorganism in comparison to free ligand. This gradual enhancing antimicrobial activity of the metal complexes, compared with that of Schiff bases, is conceivably owing to modification in structure due to coordination, and chelating tends to build metal complexes act as more influential antimicrobial agents, thus inhibiting the development of the microorganisms. The Overtone's principle and chelation theory illustrates the enhanced antimicrobial effect as the chelation have a tendency to make the ligand a more powerful and potent antimicrobial agent. On chelation, the polarity of the metal ion will be reduced to a better range due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. It is responsible for enhancing delocalization of π-electrons over the whole chelate ring and increases the penetration of the complexes into lipid membranes blocking of the metal binding sites in the enzymes of pathogens. In this way we can say that, metal complexes are more effective than the ligands because metal complexes may act as a vehicle for activation of ligands as a principle of cytotoxic species.

Table 1: In vitro antibacterial activity of (E)-2-(4-(substituted)phenyl)-4-((2-substitutedphenyl) imino)-4H-chromen-3-ol.

Ligands	Minimum inhibitory concentration			
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
L1	>200	>100	50	25
L2	>100	>200	>200	>200
L3	100	50	>100	50
L4	**6.5**	**75**	**7.5**	**6.5**
DMSO	00	00	00	00
Ciprofloxacin	12.5	12.5	12.5	12.5

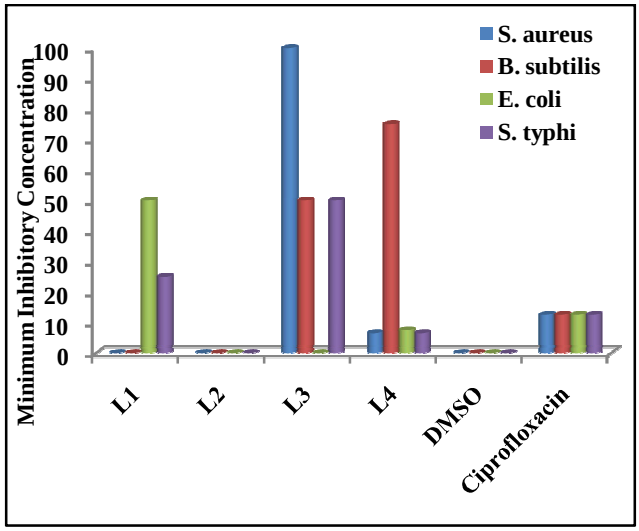


Figure 1: Bardiagram of antibacterial evaluation of ligands L1 to L4.

Table 2: In vitro antibacterial activity of Ru(II) and Co(III) complexes of (E)-4-((2-((E)-(3-hydroxy-2-(4-substitutedphenyl)-4a,8a-dihydro-4H-chromen-4-ylidene)amino)phenyl)imino)-2-(4-substitutedphenyl)-4H-chromen-3-ol.

Complexes	Minimum inhibitory concentration			
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
4I-1	>200	>100	75	50
4I-2	>100	>200	>200	>200
4I-3	100	50	>100	75
4I-4	**10.5**	**100**	**9.5**	**8.5**
4I-5	100	75	50	50
4I-6	75	>100	100	>200
4I-7	25	50	75	100
4I-8	**8.5**	**9.5**	**100**	**10.0**
DMSO	00	00	00	00
Ciprofloxacin	12.5	12.5	12.5	12.5

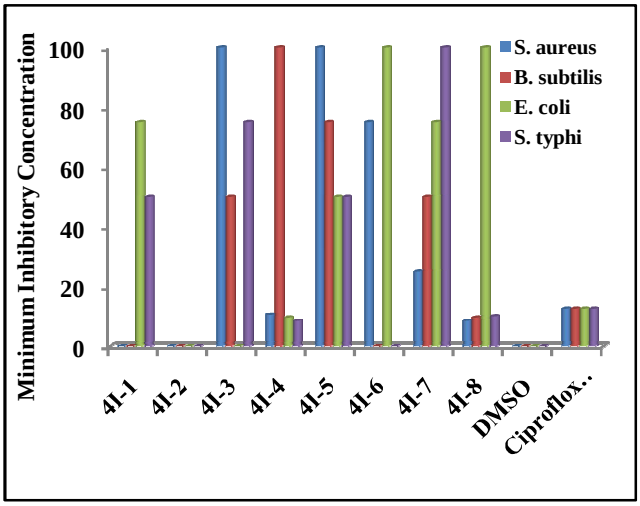


Figure 2: Bardiagram of antibacterial evaluation of Ru(II) and Co(III) complexex 41(1-8).

Table 3: In vitro antifungal activity of (E)-2-(4-(substituted)phenyl)-4-((2-substituted phenyl)imino)-4H-chromen-3-ol.

Complexes	Minimum inhibitory concentration	
	A. flavus	*C. albicans*
L1	100	>100
L2	50	25
L3	>100	75
L4	**3.75**	**75**
DMSO	00	00
Fluconazole	12.5	12.5

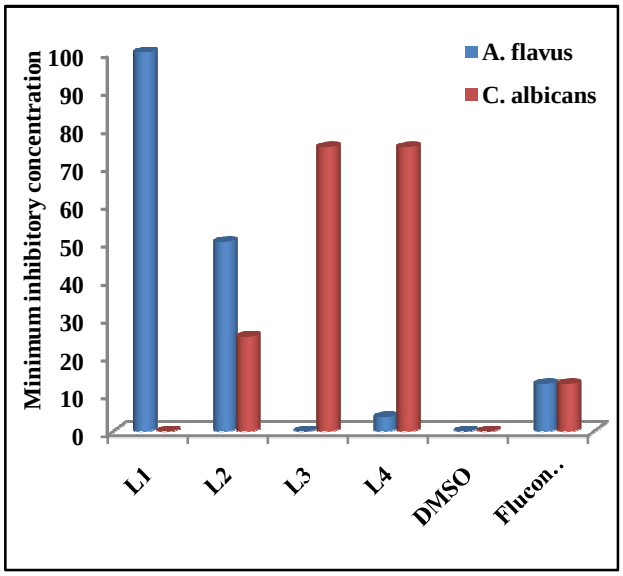


Figure 3: Bardiagram of antifungal evaluation of ligands L1 to L4.

Table 4: In vitro antifungal activity of Ru(II) and Co(III) complexes of (E)-4-((2-((E)-(3-hydroxy-2-(4-substitutedphenyl)-4a,8a-dihydro-4H-chromen-4-ylidene)amino)phenyl)imino)-2-(4-substituted phenyl)-4H-chromen-3-ol.

Complexes	Minimum inhibitory concentration	
	A. flavus	*C. albicans*
4I-1	>200	>100
4I-2	75	50
4I-3	>100	25
4I-4	**6.75**	**100**
4I-5	100	>100
4I-6	50	75
4I-7	25	>200
4I-8	**8.5**	**7.75**
DMSO	00	00
Fluconazole	12.5	12.5

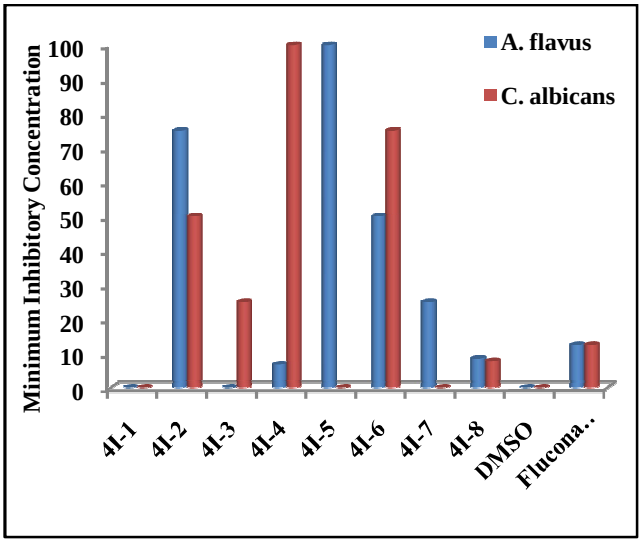


Figure 4: Bardiagram of Ru(II) and Co(III) complexes 41(1-8).

CONCLUSION: Tetradentate phenylene-bis(iminoflavone) ligands 2F(1-4) and their Ru(II) and Co (III) complexes 4I(1-8) were synthesized and characterized by using physiochemical and spectroscopic techniques and these suggest the low spin octahedral geometry of Ru(II) and Co(III) complexes. The antimicrobial activities of complexes and its ligands were evaluated against some Gram-positive and some Gram-negative bacteria as well as fungi. Among Ru(II) and Co (III) complexes **4I-1**and **4I-8** shows good antibacterial and antifungal activities. The antimicrobial activities of complexes exhibited better antimicrobial properties and showed enhanced inhibitory activities as compared to the ligands.

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