Preparation of new organo ruthenium(II) complexes and their nucleic acid/albumin binding efficiency and *in vitro* cytotoxicity studies

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Supporting Information

Experimental Section

Preparation of ligand AFIZ

To a methanolic solution (20 cm^3) of isonicotinic acid hydrazide (2.1922 mmol, 0.300 g), acetylferrocene (2.1922 mmol, 0.500 g) in methanol (20 cm^3) was added. The reaction mixture was refluxed for 7 h. The reddish orange precipitate formed was filtered, washed with methanol, and dried under vacuum. Yield: 72%. Mp. 156 °C. Molecular formula: $C_{18}H_{17}N_3OFe$: Molecular weight: 347.07; Anal. Calcd. C, 62.27; H, 4.94; N,12.10; Found: C, 63.34; H, 5.06; N, 12.36 %. FT-IR (cm^{-1}) in KBr: 1542 ($\nu_{C=N}$), 1648 ($\nu_{C=O}$). UV-Vis λ_{max} (nm) ε (dm^3 mol^{-1} cm^{-1}): 278 (58,555) (π-π* transition), 363 (13,043) (n-π* transition), 460 (47,809) (n-π* transition). ^{1}H NMR ($CDCl_3$, ppm): δ 4.16-4.35 (3s, 5H, unsubstituted Cp ring), δ 4.47-4.76 (4s, 4H, substituted Cp ring), δ 2.18-2.44 (3s, 3H, $-CH_3$), δ 7.75-7.78 (d, 2H, J = 13.6 Hz Pyridine protons), δ 8.77-8.81 (d, 2H, J = 18 Hz Pyridine protons), δ 8.26 (s, -NH).

Preparation of ligand BFIZ

Methanolic solution (20 cm^3) of benzoylferrocene (1.7236 mmol, 0.500 g) was added to refluxing solution of isonicotinic acid hydrazide (1.7236 mmol, 0.236 g) in methanol (20 cm^3). The reaction mixture was refluxed further for 6 h with continuous stirring. The red precipitate formed was filtered, washed with methanol, and dried under vacuum. Yield: 63%. Mp 98 °C. Molecular formula: $C_{23}H_{19}N_3OFe$: Molecular weight: 409.09; Anal. Calcd. C, 67.50; H, 4.68; N,10.27; Found: C, 67.84; H, 4.76; N, 10.42 %. FT-IR (cm^{-1}) in KBr: 1527 ($\nu_{C=N}$), 1671 ($\nu_{C=O}$). UV-Vis λ_{max} (nm) ε (dm^3 mol^{-1} cm^{-1}): 266 (56,506) (π-π* transition), 307 (50,696) (n-π* transition), 462 (60,237) (n-π* transition). ^{1}H NMR ($CDCl_3$, ppm): δ 4.18-4.21 (d, 3H, J = 15.2 Hz, unsubstituted Cp ring), δ 4.34-4.34 (d, 2H, J = 2.4 Hz, unsubstituted Cp ring), δ 4.42-4.60 (2s, 4H, substituted Cp ring), δ 7.36-7.64 (m, aromatic protons), δ 7.74-8.81 (m, Pyridine protons), δ 8.820 (s, -NH).

DNA Cleavage Studies

The cleavage of DNa was studied using agarose gel electrophoresis. Supercoiled pBR322 DNA (100 ng) in 5% DMSO and 95 % Tris buffer (5 mM, pH 7.2) with 50 mM NaCl was incubated at 37 ºC in the absence of and presence of compounds (50 µM). The DNA, compound and sufficient buffer were premixed in a vial, and the reaction was allowed to proceed for 2 h at 37 ºC befor the addition of ethylene glycol and loading onto an agarose gel. Agarose gel electrophoresis of plasmid DNA was performed at 50 V in 1 % slab gels containing 0.5 μgmL^{-1} ethidium bromide in Tris buffer. DNA was visualized by photographing the fluorescence of intercalated ethidium bromide under a UV illuminator. The cleavage efficiency was measured by determining the ability of the compounds to convert the supercoiled (SC) DNA to the nicked circular (NC) form and linear circular (LC) form. After that the gel was documented on Digital Gel Documentation system unit (Medicare, USA)

Preparation of organometallic proteomics

Synthesis of proteomics BSA-AFIZ

1 mM solution of bovine serum albumin in phosphate buffer (pH 7.2) (1 cm^3) was added to acetylferroceneisonicotinic hydrazone (**AFIZ**) in DMSO (1 mM, 1 cm^3). The mixture was kept at room temperature for nearly 10-15 days. The resulted precipitate was washed with water to eliminate traces of phosphate buffer and dried under vacuum.

Synthesis of proteomics BSA-BFIZ

1 mM solution of bovine serum albumin in phosphate buffer (pH 7.2) (1 cm^3) was added to benzoylferroceneisonicotinic hydrazone (**BFIZ**) in DMSO (1 mM, 1 cm^3). The mixture was kept at room temperature for nearly 10-15 days. The resulted precipitate was washed with water to eliminate traces of phosphate buffer and dried under vacuum.

Synthesis of proteomics BSA-PRAFIZ

1 mM solution of bovine serum albumin in phosphate buffer (pH 7.2) (1 cm^3) was added to ruthenium arene complex containing acetylferroceneisonicotinic hydrazone complex (**PRAFIZ**) in DMSO (1 mM, 1 cm^3). The mixture was kept at room temperature for nearly 10-15 days. The

resulted precipitate was washed with water to eliminate traces of phosphate buffer and dried under vacuum.

Synthesis of proteomics BSA-PRBFIZ

1 mM solution of bovine serum albumin in phosphate buffer (pH 7.2) (1 cm^3) was added to ruthenium arene complex containing benzoylferroceneisonicotinic hydrazone (**PRBFIZ**) in DMSO (1 mM, 1 cm^3). The mixture was kept at room temperature for nearly 10-15 days. The resulted precipitate was washed with water to eliminate traces of phosphate buffer and dried under vacuum.

Stability studies

The stability of the complexes in 1% aqueous DMSO was confirmed using UV-Visible spectroscopic technique. The stock solution was prepared by dissolving 1 mg of the compound in 1 ml DMSO. From this one millimolar solution was prepared by using the molecular weight of the compound.

Table S1. IR and UV-Vis spectral data of the ligands and complexes

Compound	**IR Data (cm^{-1})**			**Electronic spectra λ_{max} (nm) ε (dm^3 mol^{-1} cm^{-1})**
	$\nu_{(C=N)}$	**$\nu_{(C=O)}$**	**$\nu_{(C-O)}$**	
HL^{10}	1542	1648	-	278 (58,555), 363 (13,043), 460 (47,809)
V. 1	1511	-	1371	252 (58,354), 322 (40,149), 495 (91,138)
HL^{11}	1527	1671	-	266 (56,506), 307 (50,696), 462 (60,237)
V. 2	1521	-	1373	262 (52,140), 365 (25,034), 512 (68,298)

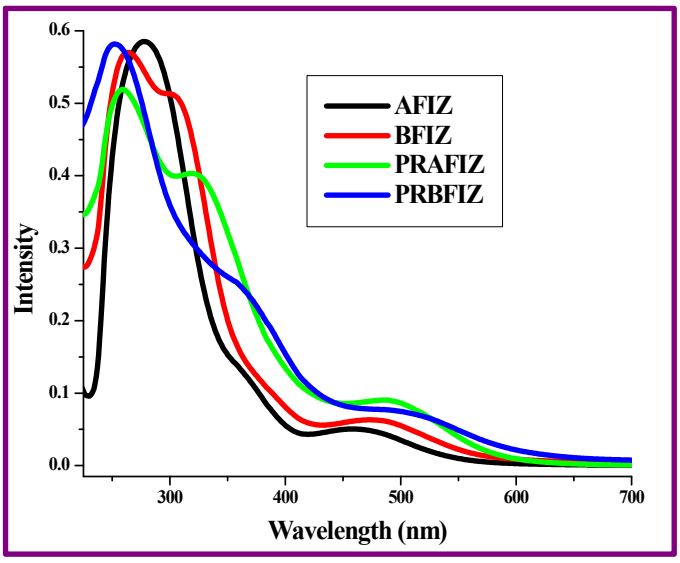


Fig. S1. Absorption spectra of the ligands and complexes

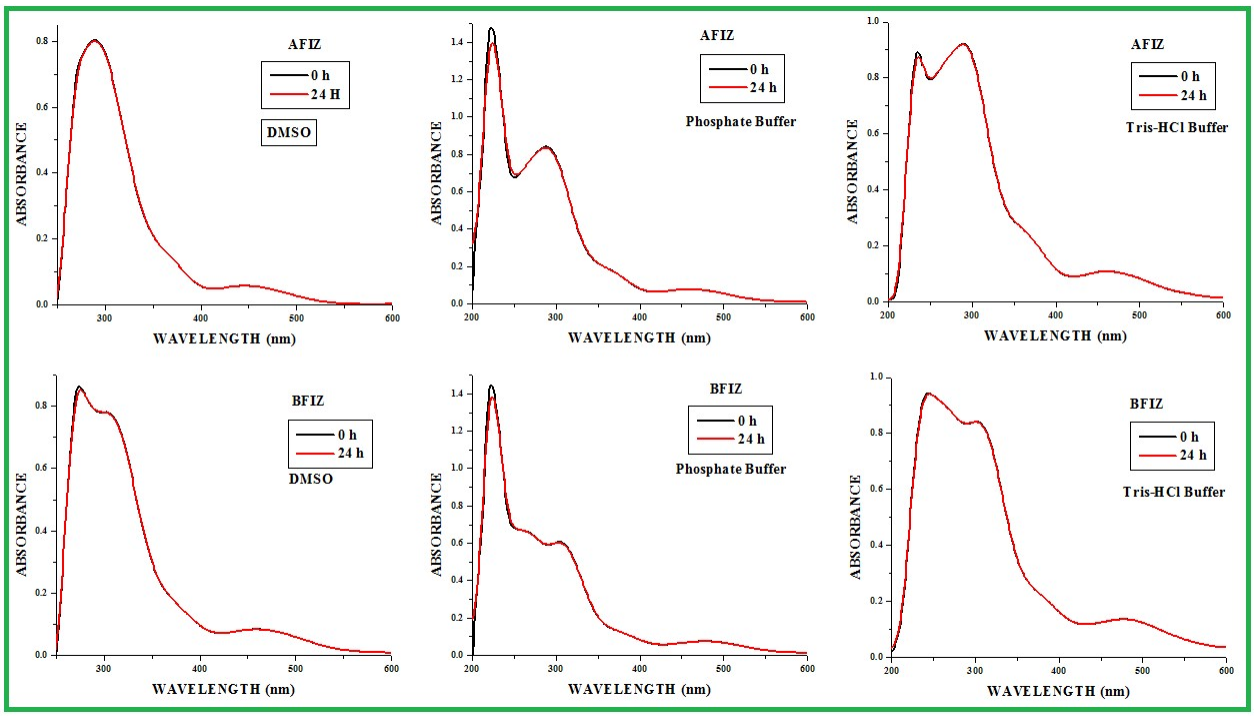


Fig. S2. UV-vis spectra of the ligands (**AFIZ** and **BFIZ**) (10 μM) in 0 h and 24 h at room temperature

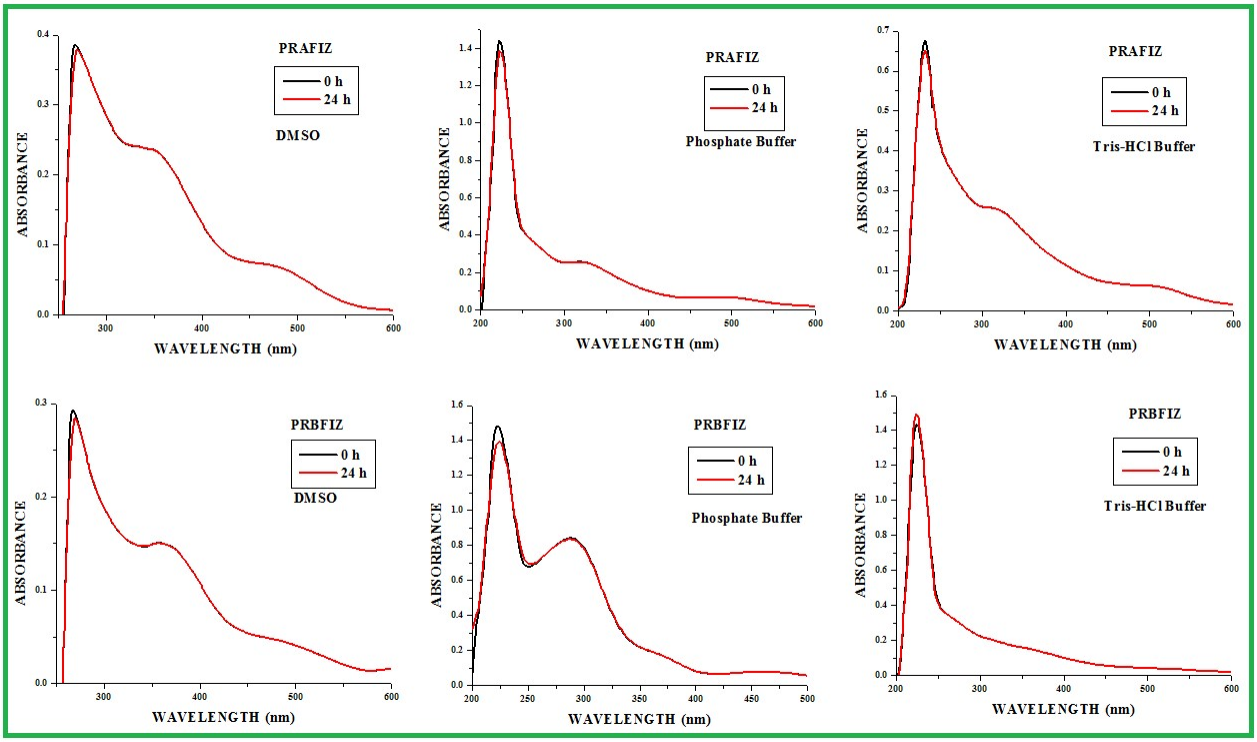
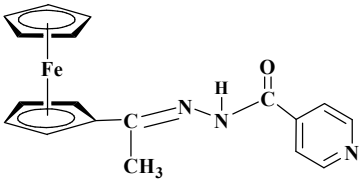
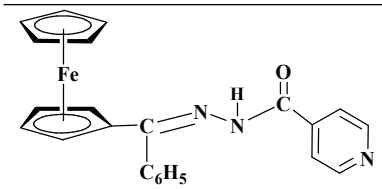
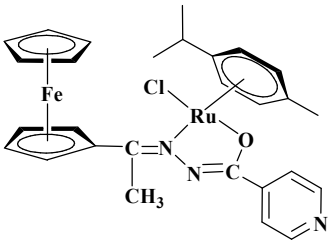
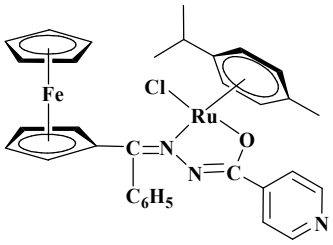


Fig. S3. UV-Vis spectra of the complexes (**PRAFIZ** and **PRBFIZ**) (10 μM) in 0 h and 24 h at room temperature

Table S2. ^{1}H NMR spectral data of the ligands and complexes

Compounds	**^{1}H NMR signals (ppm)**
	δ 4.16-4.35 (3s, 5H, unsubstituted Cp ring) δ 4.47-4.76 (4s, 4H, substituted Cp ring) δ 2.18-2.44 (3s, 3H, -CH_3) δ 7.75-7.78 (d, 2H, *J* = 13.6 Hz Pyridine protons) δ 8.77-8.81 (d, 2H, *J* = 18 Hz Pyridine protons) δ 8.26 (s, -NH)
	δ 4.18-4.21 (d, 3H, *J* = 15.2 Hz, unsubstituted Cp ring) δ 4.34-4.34 (d, 2H, *J* = 2.4 Hz, unsubstituted Cp ring) δ 4.42-4.60 (2s, 4H, substituted Cp ring) δ 7.36-7.64 (m, aromatic protons) δ 7.74-8.81 (m, Pyridine protons), δ 8.820 (s, -NH)
	δ 4.26 (s, 5H, unsubstituted Cp ring) δ 4.56-4.58 (d, 2H, *J* = 8 Hz, substituted Cp ring) δ 4.65 (s, 2H, substituted Cp ring) δ 5.20-5.25 (m, 1H, Hcymene) δ 5.30-5.31 (d, 1H, *J* = 4 Hz, Hcymene) δ 5.45-5.47 (t, *J* = 4 Hz, Hcymene) δ 5.96-5.98 (d, 1H, *J* = 8 Hz, Hcymene) δ 7.98-7.99 (d, 2H, *J* = 4 Hz, Pyridine protons)

	δ 8.60 (s, 1H, Pyridine protons) δ 8.99-9.00 (d, 1H, *J* = 4 Hz,Pyridine protons) δ 1.06-1.07 (d, 5H, 2 cymene -CH_3), δ 1.28-1.30 (d, 1H, 2 cymene -CH_3), δ 2.94 (s, 3H, cymene –CH_3) δ 4.46 (s, 1H, isopropyl -CH), δ 2.74 (s, 3H, ferrocenyl –CH_3)
	δ 4.21 (s, 5H, unsubstituted Cp ring) δ 4.44 (s, 2H, substituted Cp ring) δ 4.63 (s, 2H, substituted Cp ring) δ 5.28-5.29 (d, 1H, *J* = 8 Hz, Hcymene) δ 5.37-5.39 (d, 1H, *J* = 8 Hz, Hcymene) δ 5.62 (s, 1H, Hcymene) δ 5.67-5.68 (d, 1H, *J* = 8 Hz, Hcymene) δ 7.73-7.75 (d, 1H, *J* = 8 Hz, Pyridine protons) δ 7.99-8.01 (d, 3H, *J* = 8 Hz, Pyridine protons) δ 1.03-1.08 (dd, 6H, *J* = 8 Hz, 2 cymene -CH_3) δ 1.94 (s, 3H, cymene –CH_3) δ 3.85-3.90 (d, 1H, *J* = 20 Hz, cymene -CH) δ 7.54-7.68 (m, aromatic protons)

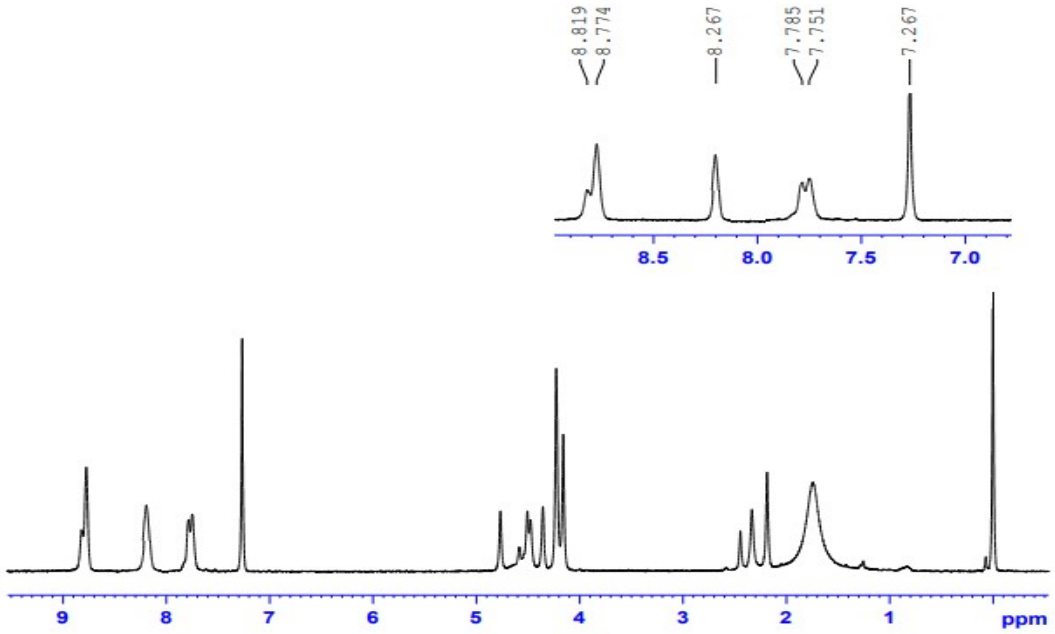


Fig. S4. [1]H NMR spectrum of **AFIZ** ligand

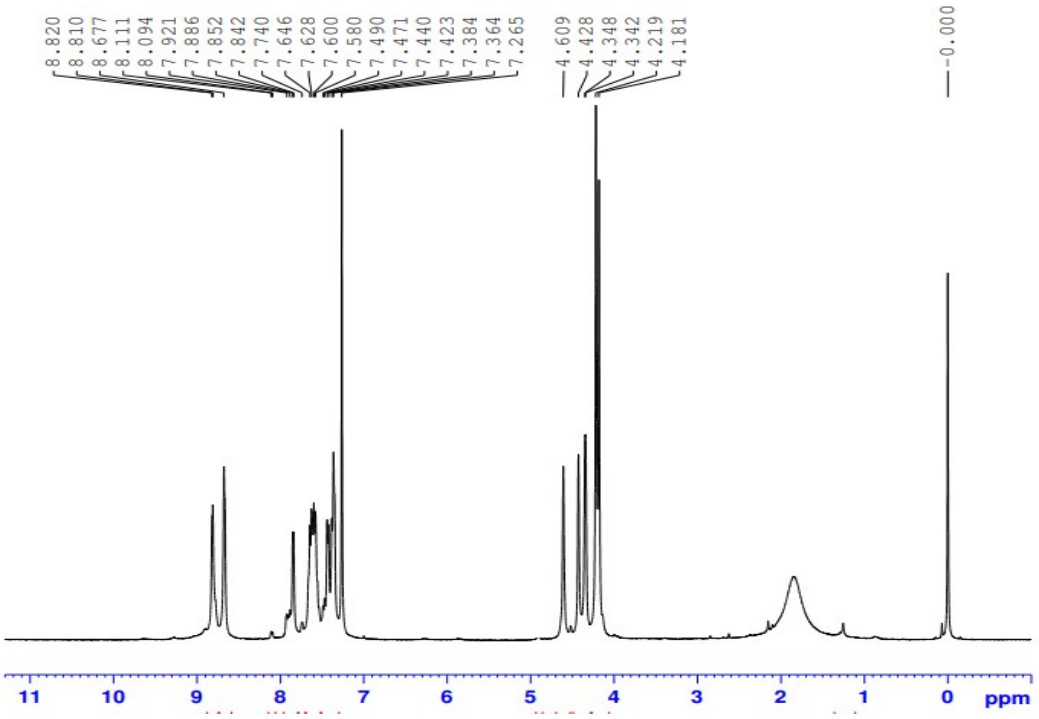


Fig. S5. ^{1}H NMR spectrum of **BFIZ** ligand

Table S3. Bond angles and bond lengths of the complex **(PRBFIZ)**

Bond Length (Aº)		**Bond Angles (˚)**	
Atom	**Length (Aº)**	**Atom**	**Angle** (˚)
Ru1-Cl1	2.4133(9)	O1-Ru1-Cl1	83.67(7)
Ru1-O1	2.059(2)	N2-Ru1-Cl1	83.78(7)
Ru1-N2	2.121(3)	N2-Ru1-O1	76.49(9)
Ru1-C11	2.205(3)	C11-Ru1-Cl1	161.70(10)
Ru1-C12	2.170(3)	C11-Ru1-O1	95.85(11)
Ru1-C13	2.207(3)	C11-Ru1-N2	113.98(12)
Ru1-C8	2.219(3)	C12-Ru1-Cl1	149.85(9)
Ru1-C9	2.187(3)	C12-Ru1-O1	125.91(11)
Ru1-C10	2.176(3)	C12-Ru1-N2	96.70(11)
Fe1-C24	2.034(3)	C12-Ru1-C11	37.69(12)

Fe1-C28	2.022(3)	C13-Ru1-Cl1	112.96(10)
Fe1-C27	2.048(4)	C13-Ru1-O1	163.34(11)
Fe1-C26	2.045(4)	C13-Ru1-N2	105.39(11)
Fe1-C25	2.037(3)	C13-Ru1-C11	68.07(13)
Fe1-C31	2.033(4)	C13-Ru1-C12	37.76(12)
Fe1-C30	2.046(4)		
Fe1-C29	2.036(4)		
Fe1-C33	2.029(4)		
Fe1-C32	2.028(4)		

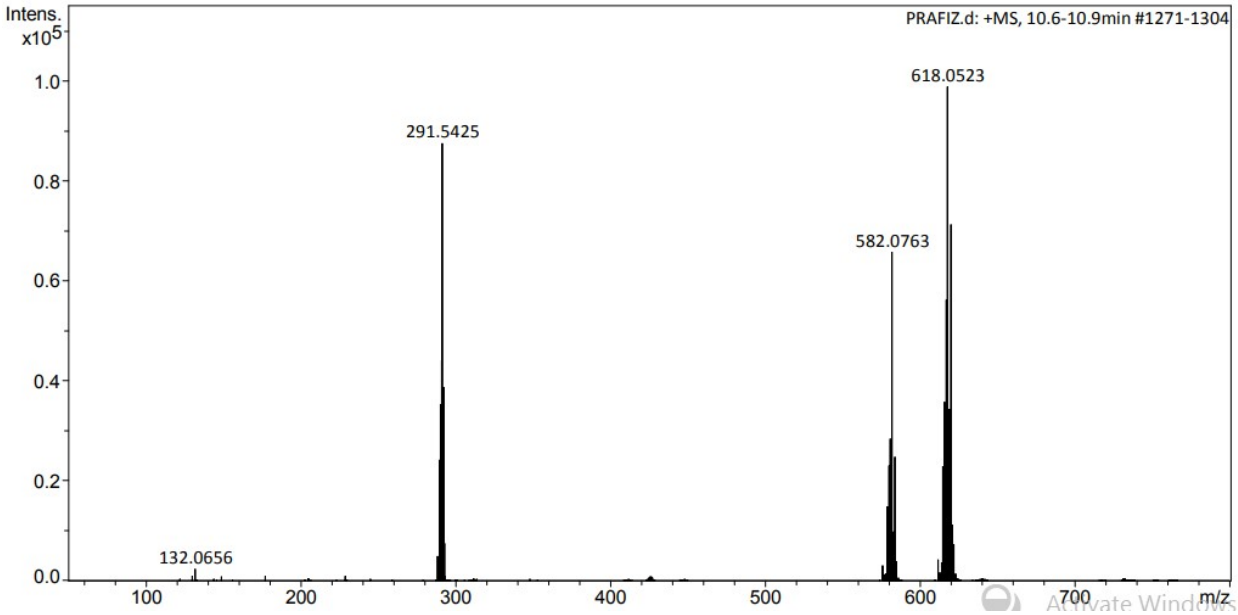


Fig. S6. Mass spectrum of **PRAFIZ** complex

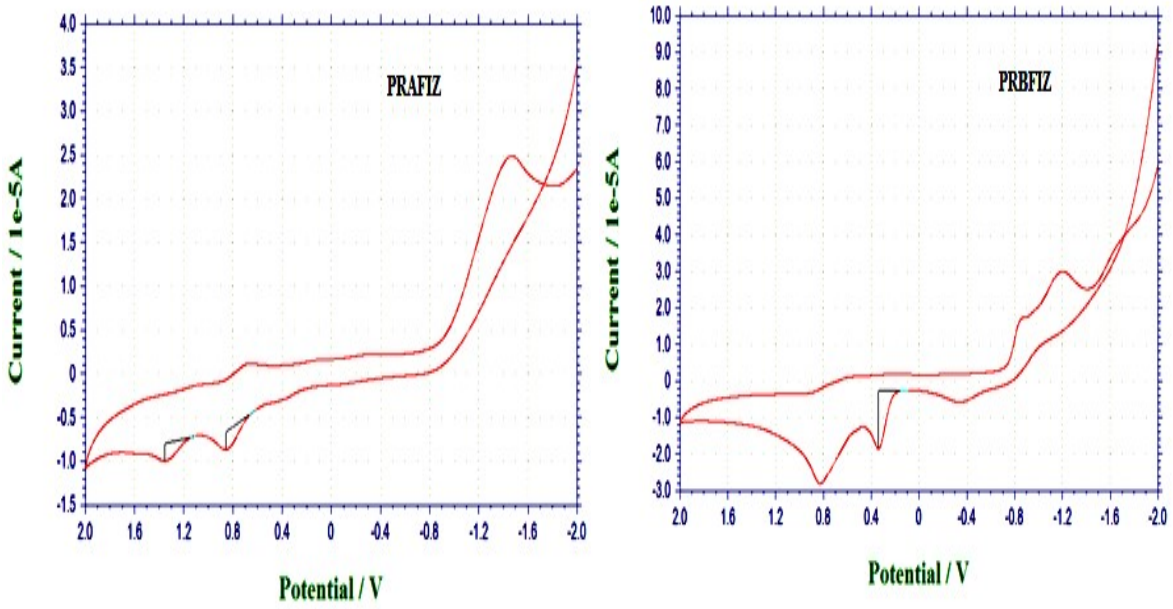


Fig. S7. Cyclic voltammogram of **PRAFIZ** and **PRBFIZ**

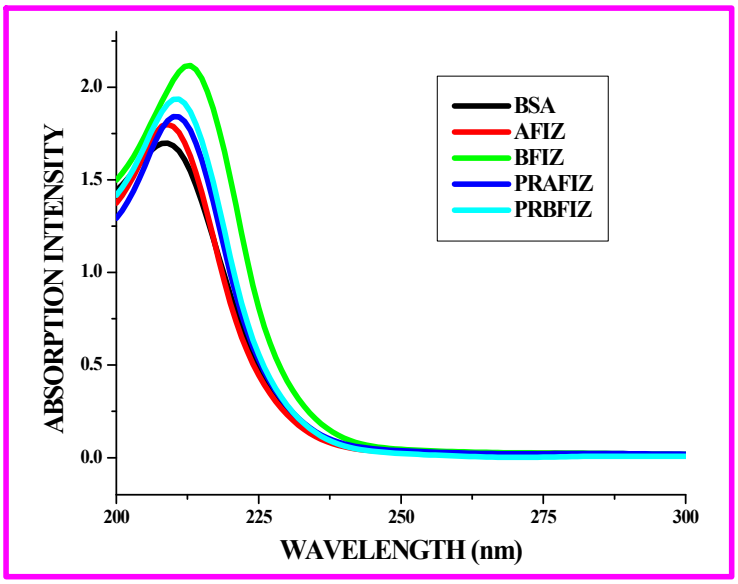


Fig. S8. UV absorption spectra of BSA (10 μM) with the ligands and complexes (10 μM)

Table S4. The IC_{50} values for the compounds with MDA-MB-231 and A549 cell lines

Complex	MDA-MB-231	A549	HEK
AFIZ	20± 1.2	26 ± 0.8	Insignificant toxicity
BFIZ	21± 1.5	27 ± 0.5	Insignificant toxicity
PRAFIZ	17± 1.4	22 ± 0.3	Insignificant toxicity
PRBFIZ	19± 0.7	24 ± 0.9	Insignificant toxicity
Cisplatin	13± 0.3	26 ± 0.8	10 ± 0.7