Supporting Information

Synthesis, characterisation, molecular docking, biomolecular interaction and cytotoxicity studies of novel Ruthenium(II)-arene-2-heteroarylbenzoxazole complexes‡

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Code	Absorption properties DMSO/DMSO-water		Emission properties DMSO/DMSO-water		Stokes shift (nm) DMSO/DMSO -water	Area DMSO/DMSO-water	Quantum yield DMSO/DMSO -water
	OD	(ε)	Excited (nm)	Emission (nm)			
5	**0.46**	**6.5 x 10^3**	**307**	--	--	1493	**0.008**
5W	0.71	1 x 10^5	312			365	0.001
5'	**0.71**	**1 x 10^4**	**299**	**440**	**141**	6272	**0.02**
5'W	0.38	5.4 x 10^3	338	413	75	1556	0.01
4	**0.46**	**6.5 x 10^3**	**281**	**451**	**170**	3300	**0.02**
4W	0.51	7.2 x 10^3	314	449	135	499	0.002
4'	**0.46**	**6.5 x 10^3**	**313**	**440**	**127**	8910	**0.05**
4'W	0.41	5.8 x 10^3	312	441	129	541	0.003
4'	**0.45**	**6.4 x 10^3**	**270**	**441**	**171**	7062	**0.04**
Ref	0.4	**5.7 x 10^3**	496	515	19	152890	0.95

Table S1. Photophysical properties of **4**, **4'**, **5** and **5'** benzoxazole based Ru(II) complexes

*W indicate DMSO: water (1:1)

Table S2. Physical characteristics of complexes

Complex	Chemical formula	Mol. wt	Solubility [mg/ml]
4	$C_{22}H_{21}BrClF_6N_2OPRu^-$	690.81	7
5	$C_{18}H_{13}BrClF_6N_2OPRu^-$	634.70	6
4'	$C_{26}H_{23}BrClF_6N_2OPRu^-$	740.87	8
5'	$C_{22}H_{15}BrClF_6N_2OPRu^-$	684.76	7

Table S3. Molar **c**onductivity study of the complexes

Complex	Concentration [M]	Molar conductivity (Λ_M) (µS/cm)			
		DMF	10% aqueous DMF	DMSO	10% aqueous DMSO
4	$5x10^{-5}$	40	45	44	55
5	$5x10^{-5}$	41	45	41	50
4'	$5x10^{-5}$	19	63	22	32
5'	$5x10^{-5}$	65	81	36	76

Table S4. Binding energy of DNA-complexes and BSA-complexes calculated from Molecular docking studies (kcal mol^{-1})

Complex	DNA Binding free energy ($\Delta G_{binding}$)$^{\alpha}$	BSA Binding free energy ($\Delta G_{binding}$)$^{\alpha}$
4	-8.2	-7.9
5	-8.7	-8.3
4'	-8.3	-7.7
5'	-8.9	-8.1

Table S5. Relative viscosity of CT-DNA interaction with Ru(II) based complex (**4)**

[Complex]/[DNA]	t1	t2	t3	Average(t)	(t-t0)	$(\eta/\eta_0)^{1/3}$
Buffer only	132.02	131.47	132.21	131.9		
Buffer+DNA	138.61	138.56	138.76	138.64	6.74	1
2µM	139.5	139.69	140.03	139.74	7.84	1.05
4µM	140.65	140.92	140.87	140.81	8.91	1.09
6µM	141.23	141.64	141.79	141.55	9.65	1.12
8µM	142.68	142.98	142.7	142.78	10.88	1.17
10µM	144.01	144.23	144.17	144.14	12.23	1.22

Table S6. Relative viscosity of CT-DNA interaction with Ru(II) based complex (**5)**

[Complex]/[DNA]	t1	t2	t3	Average(t)	(t-t0)	$(\eta/\eta_0)^{1/3}$
Buffer only	132.02	131.47	132.21	131.9		
Buffer+DNA	138.61	138.56	138.76	138.64	6.74	1
2µM	138.92	139.01	138.86	138.93	7.03	1.01
4µM	139.03	139.24	139.39	139.22	7.32	1.03
6µM	139.75	139.97	140.07	139.93	8.03	1.06
8µM	140.39	140.54	140.83	140.58	8.68	1.08
10µM	141.19	141.49	142	141.56	9.66	1.13

Table S7. Relative viscosity of CT-DNA interaction with Ru(II) based complex (**4')**

[Complex]/[DNA]	t1	t2	t3	Average(t)	(t-t0)	$(\eta/\eta_0)^{1/3}$
Buffer only	132.02	131.47	132.21	131.9		
Buffer+DNA	138.61	138.56	138.76	138.64	6.74	1
2µM	142.18	142.59	142.92	142.56	10.66	1.16
4µM	143.33	143.43	143.67	143.47	11.57	1.19
6µM	144.76	144.98	145.01	144.91	13.01	1.25
8µM	145.78	145.72	145.91	145.80	13.90	1.27
10µM	146.18	146.47	146.95	146.53	14.63	1.29

Table S8. Relative viscosity of CT-DNA interaction with Ru(II) based complex (**5')**

[Complex]/[DNA]	t1	t2	t3	Average(t)	(t-t0)	$(\eta/\eta_0)^{1/3}$
Buffer only	132.02	131.47	132.21	131.9		
Buffer+DNA	138.61	138.56	138.76	138.64	6.74	1
2µM	143.24	143.33	143.38	143.31	11.41	1.19
4µM	145.01	145.23	145.32	145.18	13.28	1.25
6µM	146.27	146.39	146.65	146.43	14.53	1.29
8µM	147.31	147.33	147.49	147.37	15.47	1.32
10µM	149.01	149.43	149.19	149.21	17.31	1.37

Table S9. Relative viscosity of CT-DNA interaction with Ru(II) based complex (**EtBr)**

[EtBr]/[DNA]	t1	t2	t3	Average(t)	(t-t0)	$(\eta/\eta0)^{1/3}$
Buffer only	132.02	131.47	132.21	131.9		
Buffer+DNA	138.61	138.56	138.76	138.64	6.74	1
2µM	144.85	145.06	144.92	144.943	13.04	1.25
4µM	146.19	146.23	146.31	146.243	14.34	1.29
6µM	147.12	147.38	147.92	147.473	15.57	1.32
8µM	148.03	148.62	148.95	148.533	16.63	1.35
10µM	149.89	150.14	150.17	150.067	18.2	1.4

Table S10. Selected bond lengths (Å) and bond angles (°) of complexes (**4**, **4'**, **5** and **5'**).

	4	**5**	**4'**	**5'**
Ru(1)–N(1)	2.0944	2.0944	2.0944	2.0944
Ru(1)–N(2)	2.1146	2.1146	2.1146	2.1146
Ru(1)–Cl(1)	2.399	2.3999	2.3999	2.3999
Ru(1)–Centroid*	2.2660	2.3678	2.1549	2.2328
N(1)–Ru(1)–N(2)	76.673	76.943	77.994	77.618
N(1)–Ru(1)–Cl(1)/Br(1)	82.593	84.711	87.334	87.083
N(2)–Ru(1)–Cl(1)/Br(1)	81.536	82.937	86.967	85.122

* N(1)= Benzoxazole nitrogen , N(2)= Pyridine nitrogen or qunioline nitrogen

Table S11. Calculated molecular electronic parameters.

Complex	**Energy (kJ/mol)**	**DM (Debay)**	**E_H (eV)**	**E_L (eV)**	**ΔE (eV)**	**χ (eV)**	**η (eV)**	**μ (eV)**	**ω (eV)**	**S (eV)**
4	-2514237.39	9.10	-8.89	-5.84	3.05	7.36	-1.53	-7.37	-17.78	-0.763
5	-2612936.52	8.33	-9.50	-6.52	2.98	8.01	-1.50	-8.10	-21.47	-0.747
4'	-2709349.02	7.75	-9.45	-6.84	2.61	8.14	-1.31	-8.14	-25.40	-0.653
5'	-2610624.73	9.15	-9.35	-6.71	2.64	8.03	-1.32	-8.03	-24.45	-0.660

Dipole moment (DM), Energy of HOMO (E_H), energy of LUMO (E_L), energy band gap (ΔE), electronegativity (χ), global hardness (η), chemical potential (μ), global electrophilicity index (ω) and global softness (S).

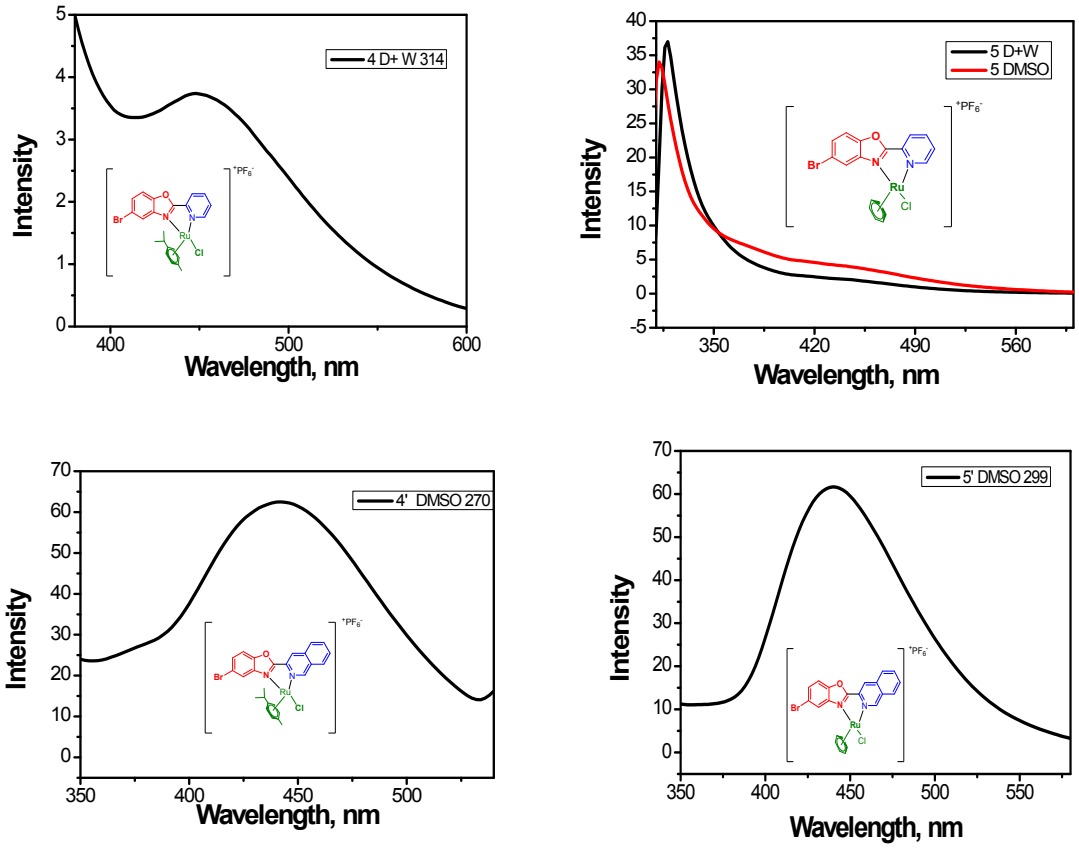
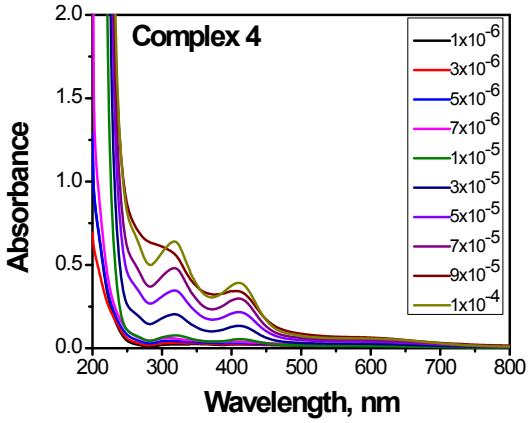
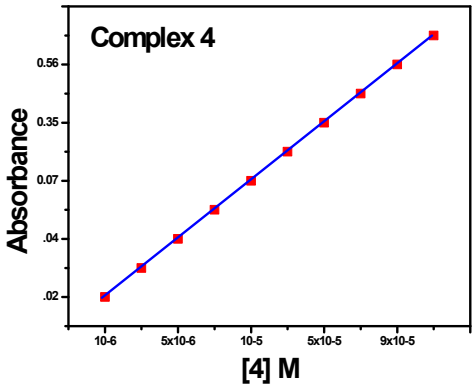
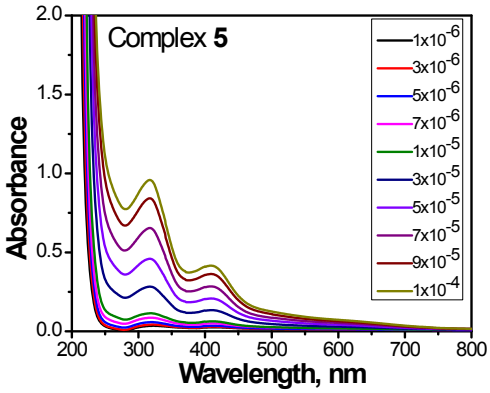
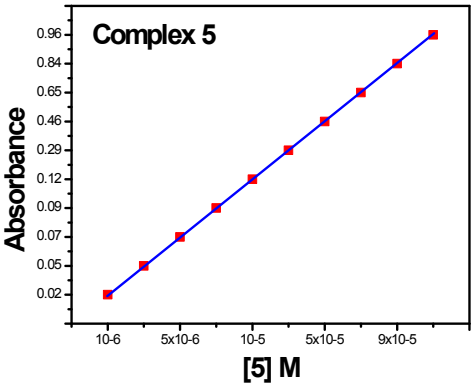


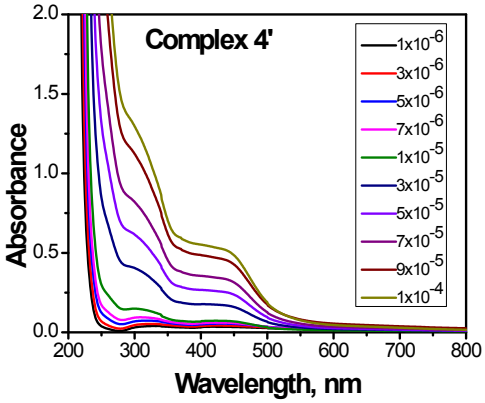
Fig. S1 Emission spectra of **4**, **5**, **4'** and **5'** in DMSO and DMSO: water media

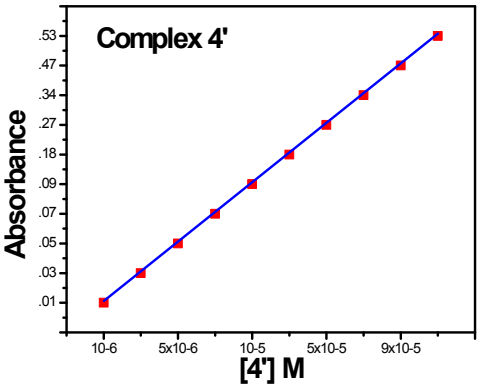

Complex 4
Absorbance
Wavelength, nm
1x10-6
3x10-6
5x10-6
7x10-6
1x10-5
3x10-5
5x10-5
7x10-5
9x10-5
1x10-4


Complex 4
Absorbance
[4] M


Complex 5
Absorbance
Wavelength, nm
1x10-6
3x10-6
5x10-6
7x10-6
1x10-5
3x10-5
5x10-5
7x10-5
9x10-5
1x10-4


Complex 5
Absorbance
[5] M


Complex 4'
Absorbance
Wavelength, nm
1x10-6
3x10-6
5x10-6
7x10-6
1x10-5
3x10-5
5x10-5
7x10-5
9x10-5
1x10-4


Complex 4'
Absorbance
[4'] M

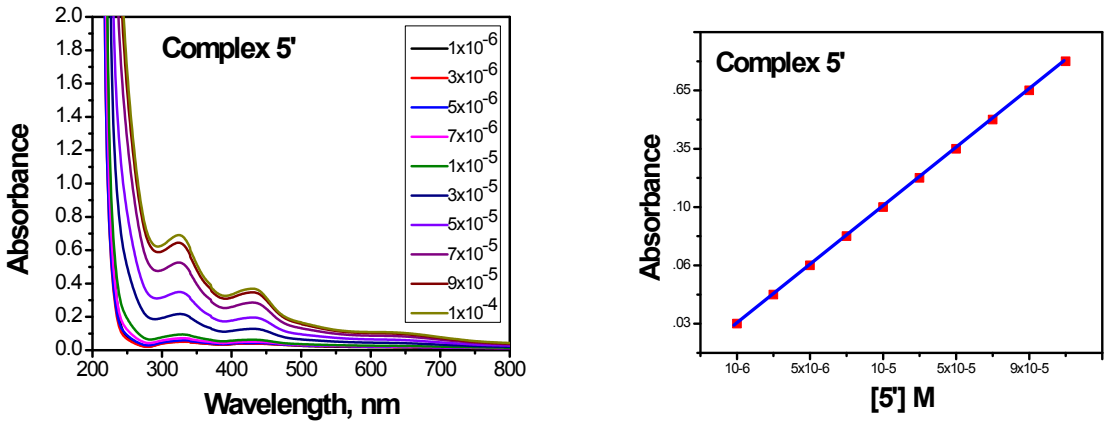
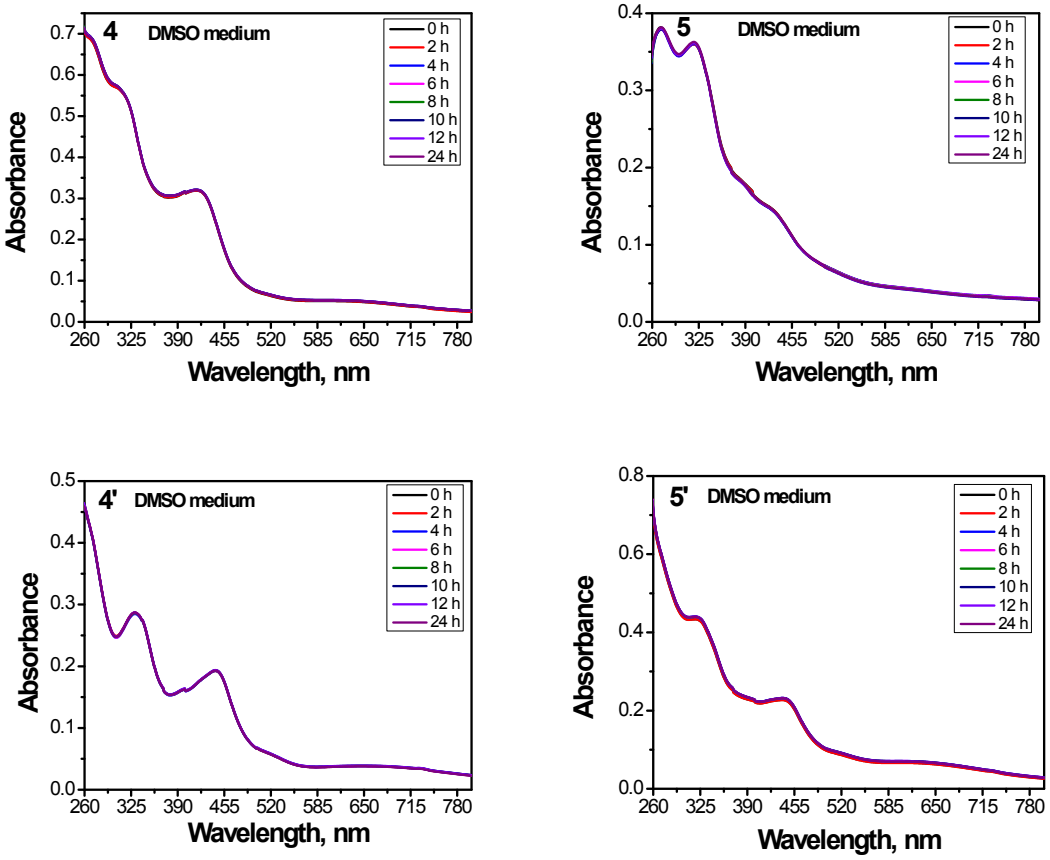
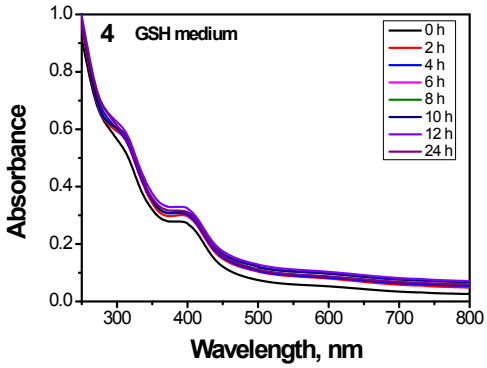


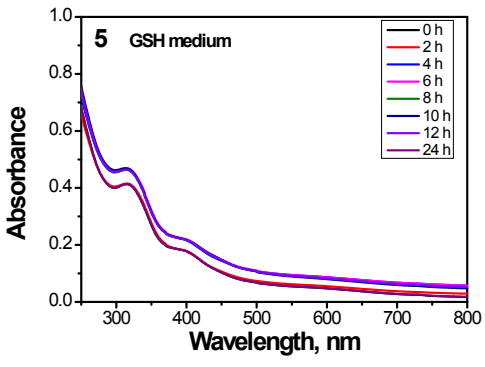
Fig. S2 Solubility study of **4**, **5**, **4'** and **5'** in DMSO: water media

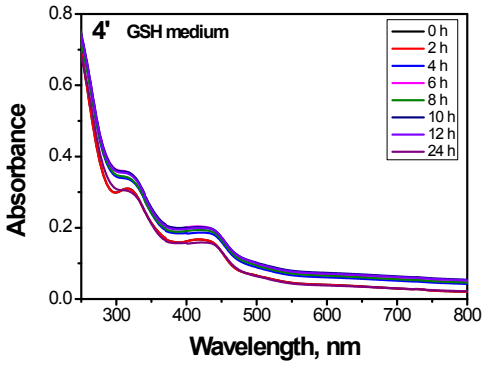
(a) Stability in DMSO medium

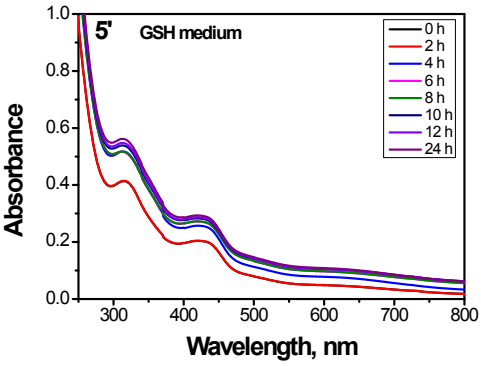


(b) Stability in GSH medium

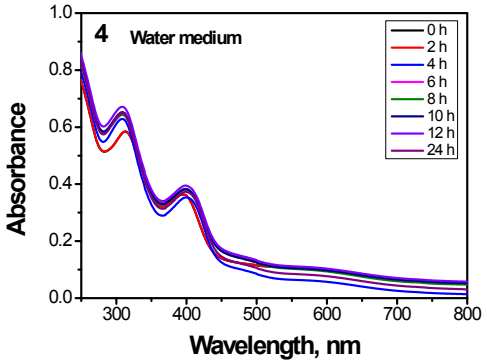


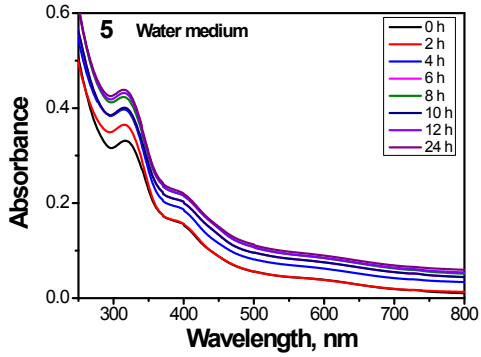


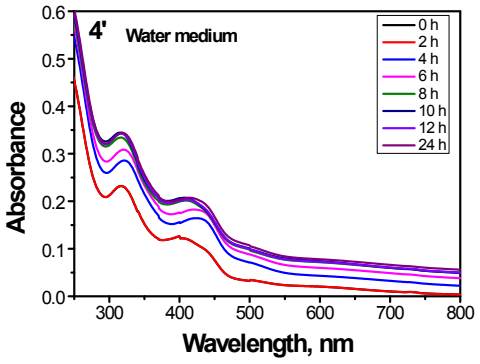




(c) Stability in Water medium







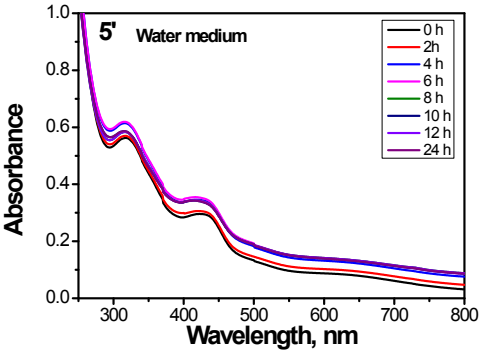


Fig. S3 Stability study of compound **4**, **5**, **4'** and **5'** complex in (a) DMSO (b) GSH (c) Water

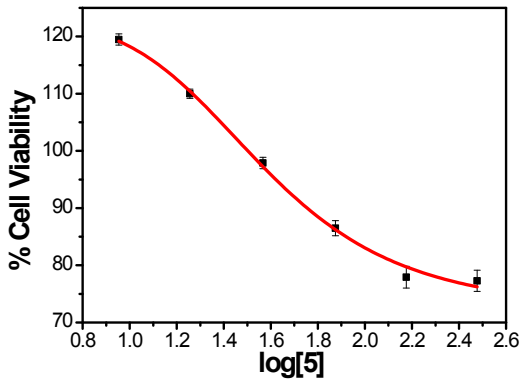
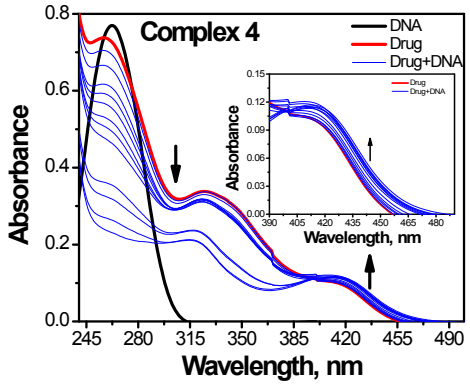
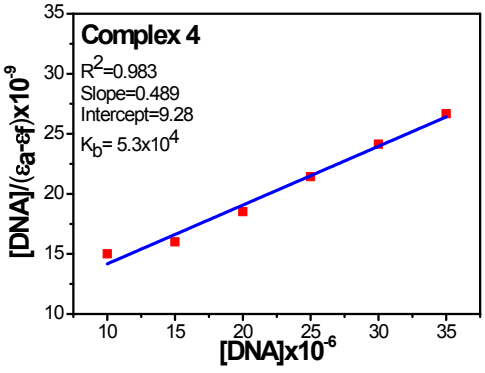
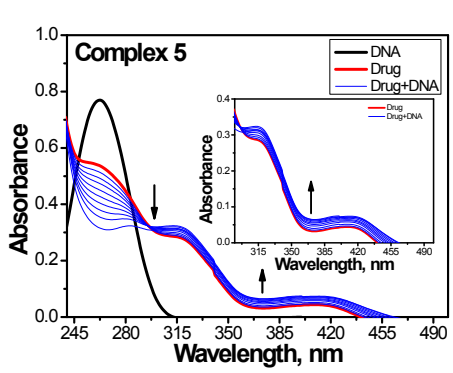
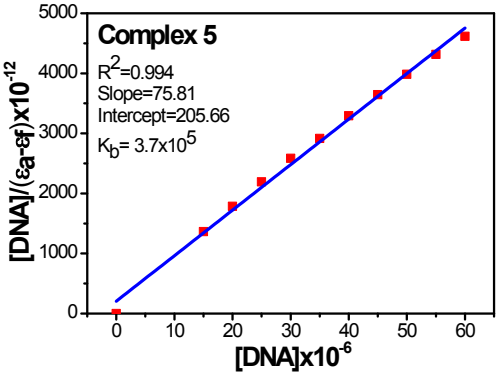


Fig. S4 Cell viability plot of complex **5** in HeLa cell line









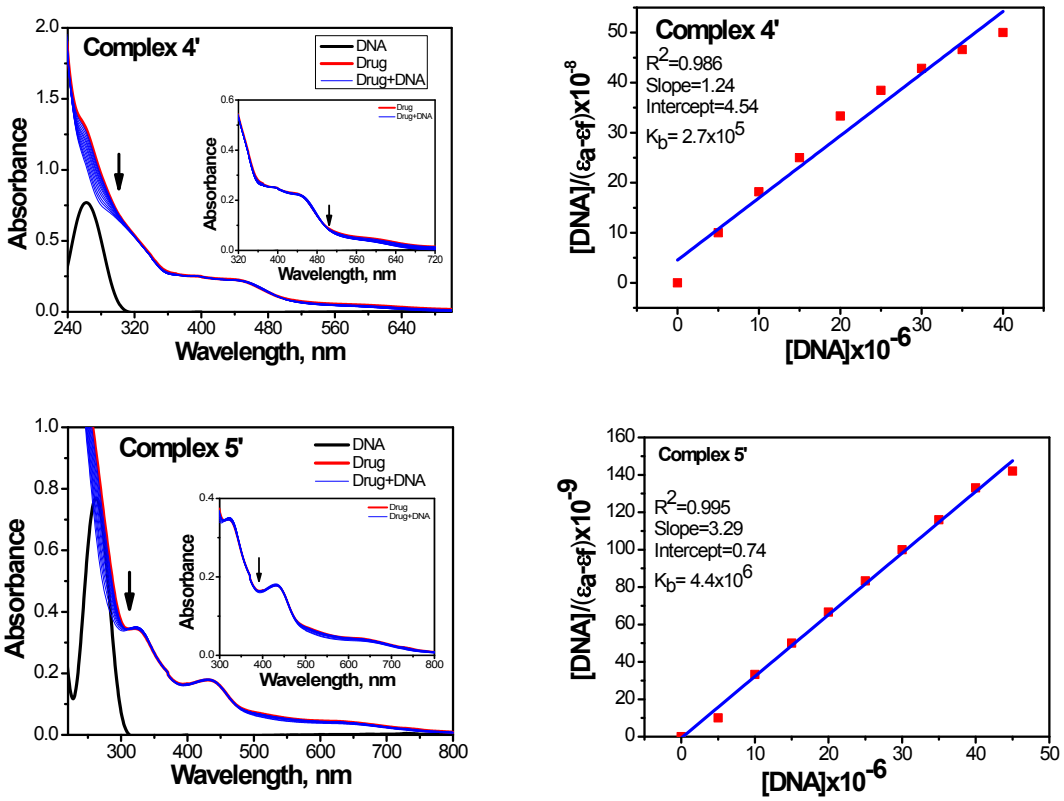
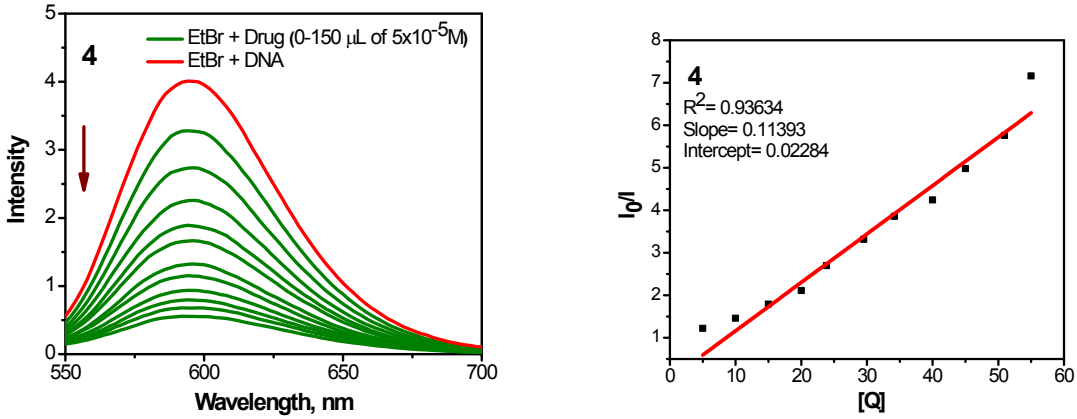


Fig. S5 (a) UV-visible titration of compounds **4**, **5**, **4'** and **5'** in 5 mM Tris-HCl/NaCl buffer with incremental addition of CT-DNA at pH 7.2. (b) [DNA]/ (ε_a-ε_f) vs. [DNA] linear plots of complexes



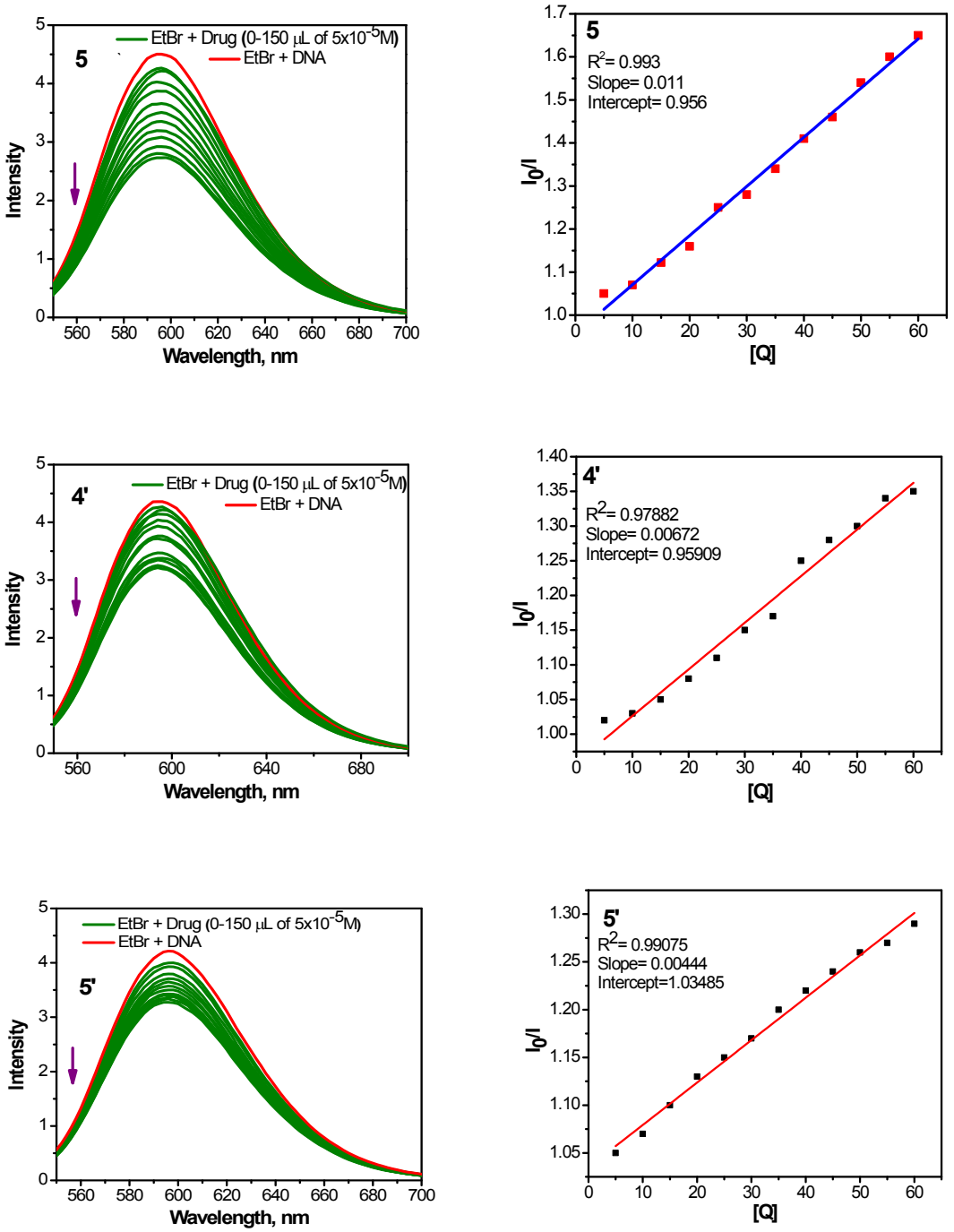
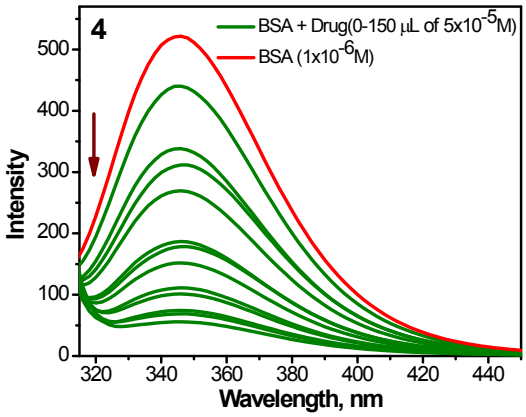
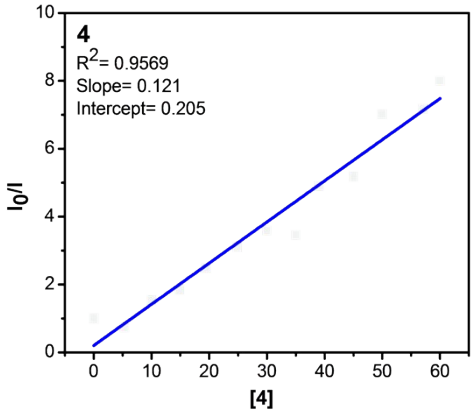
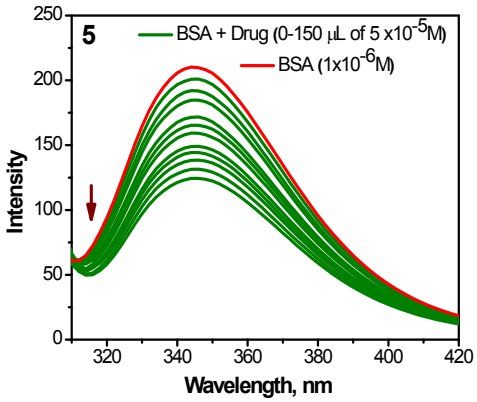
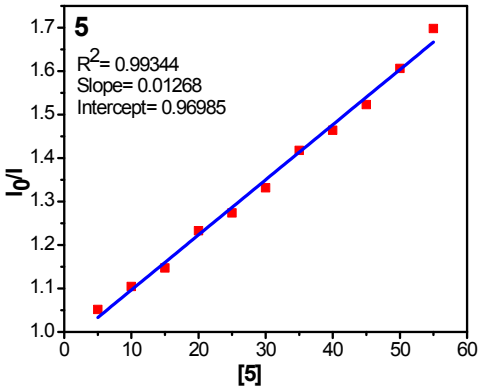


Fig. S6 Emission spectral traces of the ethidium bromide bound DNA with increasing concentration of compounds **4, 5, 4'** and **5'** in 5 mM Tris-HCl/NaCl buffer of pH 7.2. λ_{ex} = 485 nm, λ_{em} = 598 nm and 596 nm, [DNA] = 120 µM, [EthB] = 8 µM, $[\mathbf{4}]_{50}$ = 30 µM, $[\mathbf{4'}]_{50}$ = 25 µM, $[\mathbf{5}]_{50}$= 30 µM, $[\mathbf{5'}]_{50}$ = 25 µM Stern-Volmer plots of I_0/I vs. Complex.


4
BSA + Drug(0-150 µL of 5x10-5M)
BSA (1x10-6M)
Intensity
Wavelength, nm


4
R2= 0.9569
Slope= 0.121
Intercept= 0.205
I0/I
[4]


5
BSA + Drug (0-150 µL of 5 x10-5M)
BSA (1x10-6M)
Intensity
Wavelength, nm


5
R2= 0.99344
Slope= 0.01268
Intercept= 0.96985
I0/I
[5]

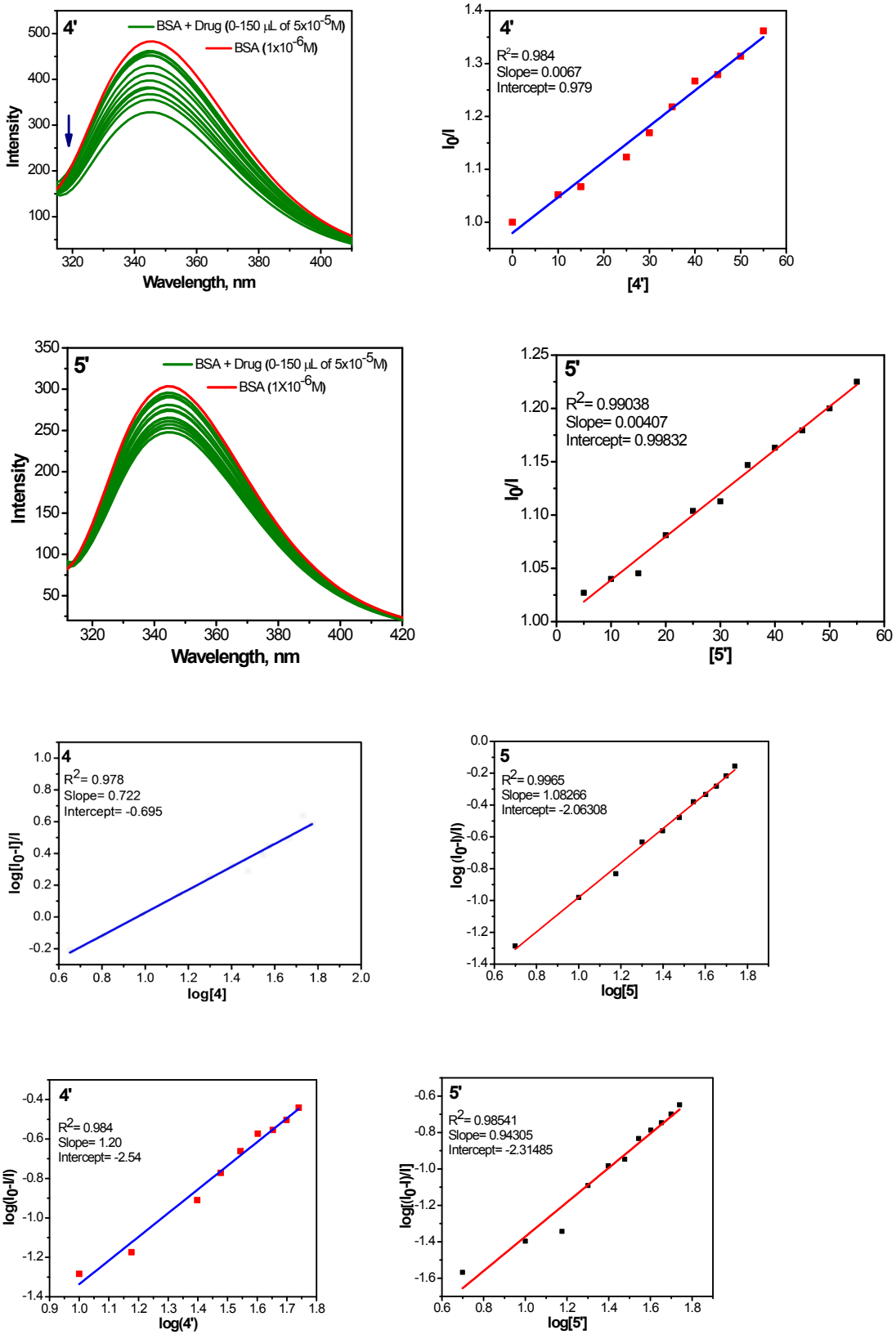


Fig. S7 (a) Fluorescence quenching of BSA on addition of compounds (a) **4, 5, 4'** and **5'** in 5 mM Tris HCl/NaCl buffer at pH 7.2 (λ_{ex} = 295; λ_{em} = 350 nm). (b) Plot of I_0/I vs. concentrations of compounds Scatchard plot of log ($[I_0-I]/I$) vs. log [compound] for BSA in the presence of complexes

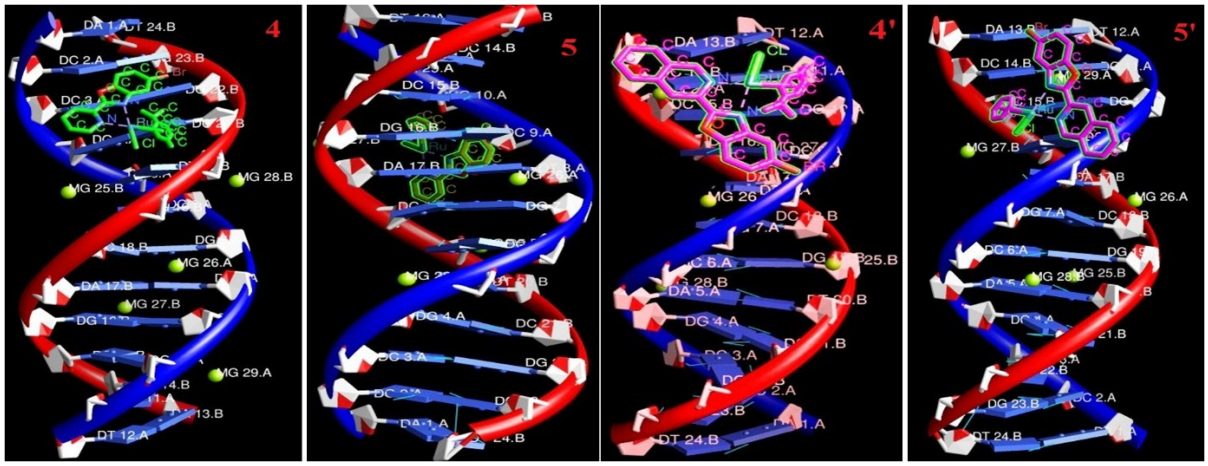


Fig. S8 Docking pose of compounds **4, 5, 4'** and **5'** within the minor groove of DNA

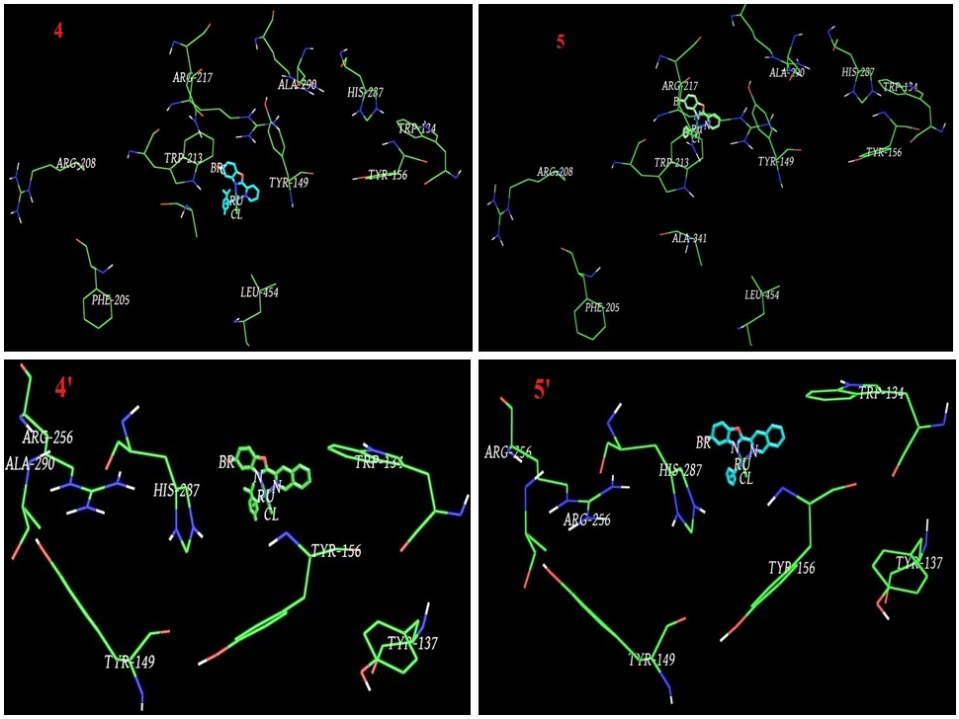


Fig. S9 Docking pose of compounds **4**, **5**, **4'** and **5'** within the active site of BSA protein

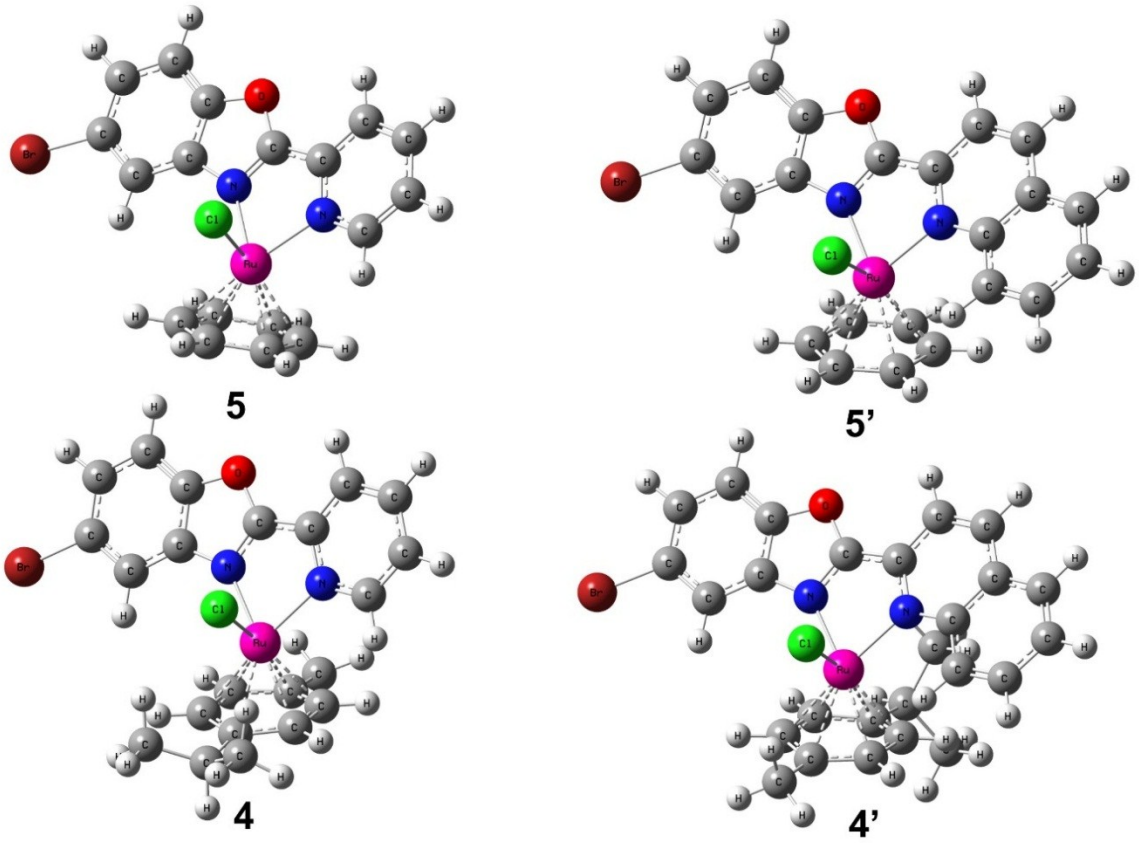


Fig. S10 Optimized structure of complexes (**4, 5, 4'** and **5'**)

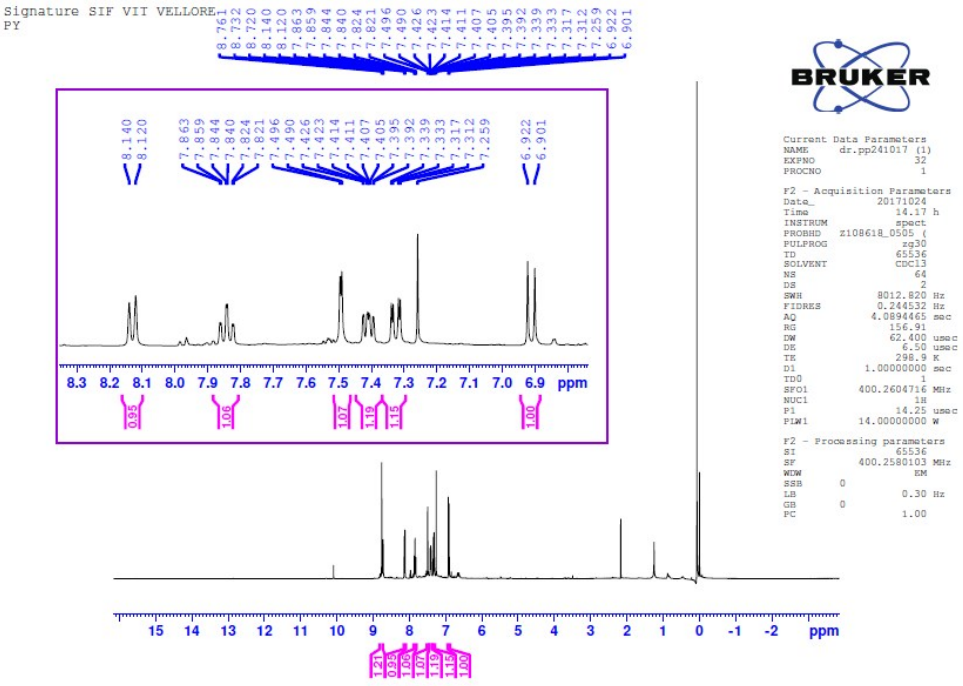


Fig. S11 ^{1}H NMR of compound **3**

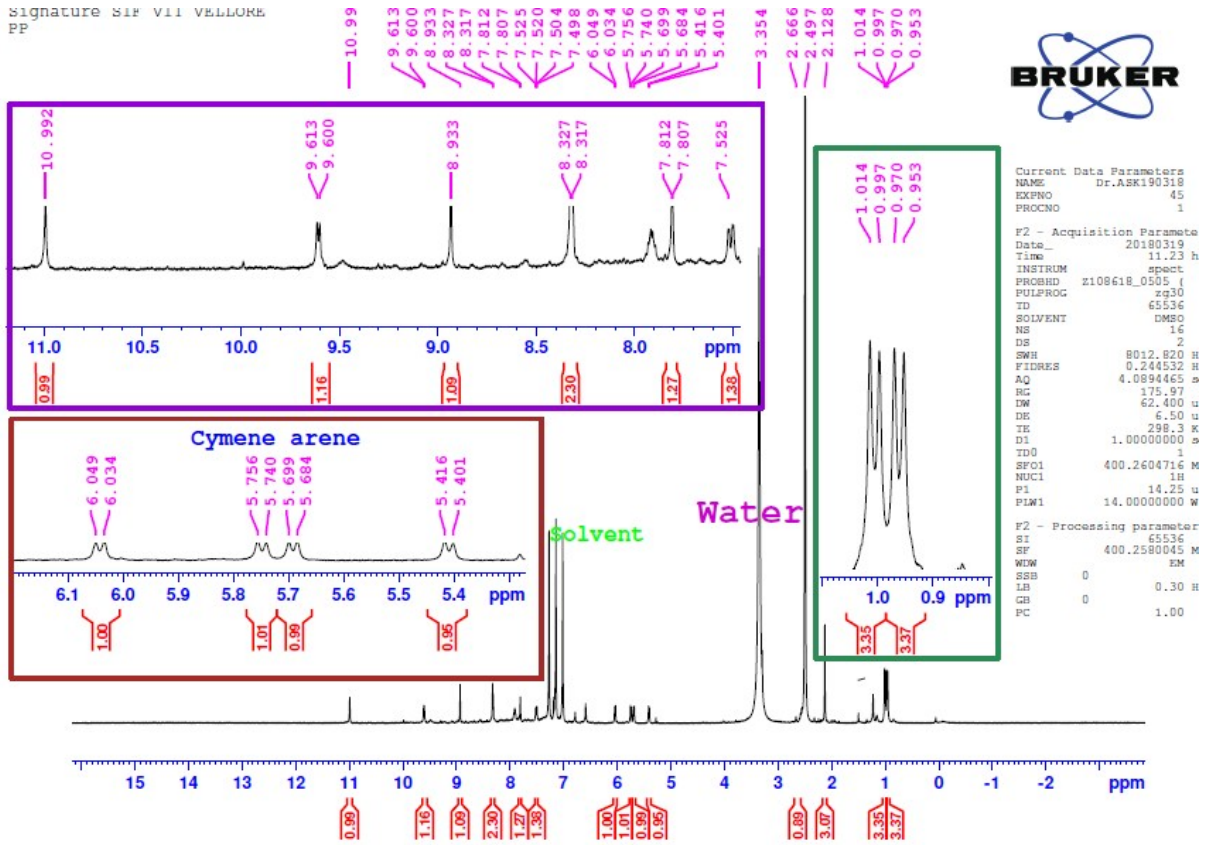


Fig. S12 ^{1}H NMR of complex **4**

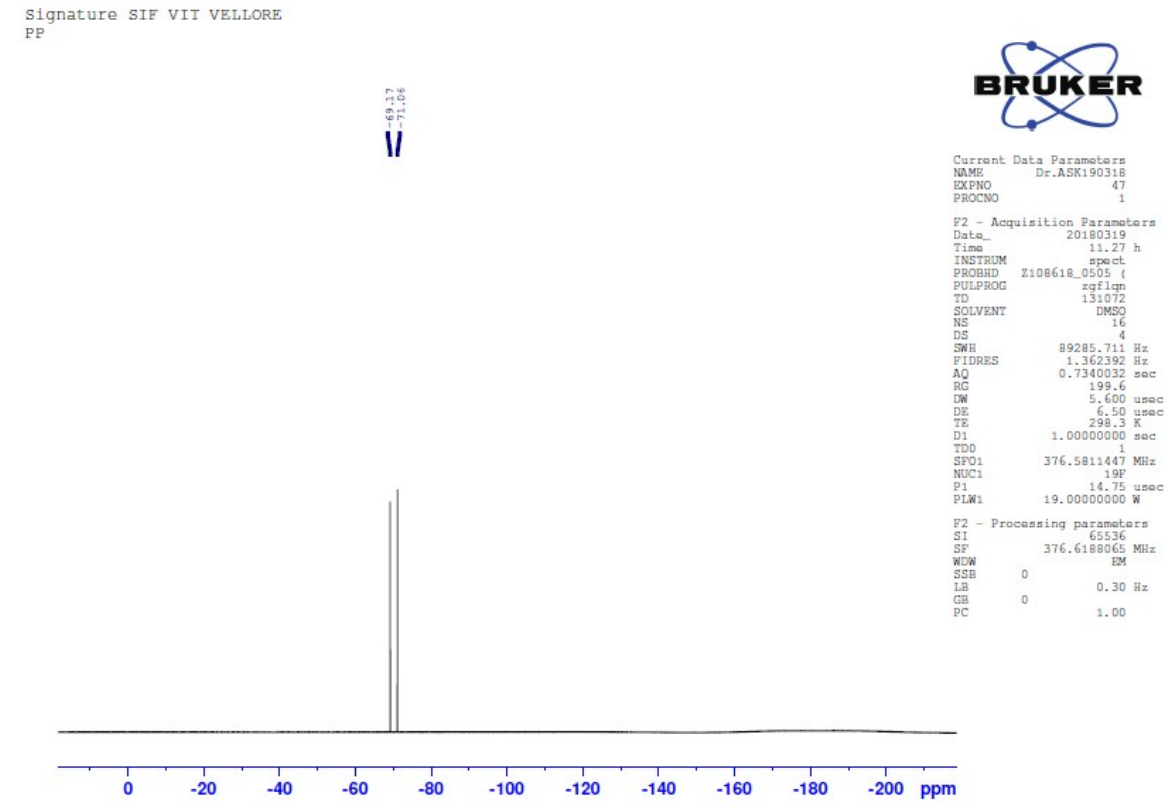


Fig. S13 ^{19}F NMR of complex **4**

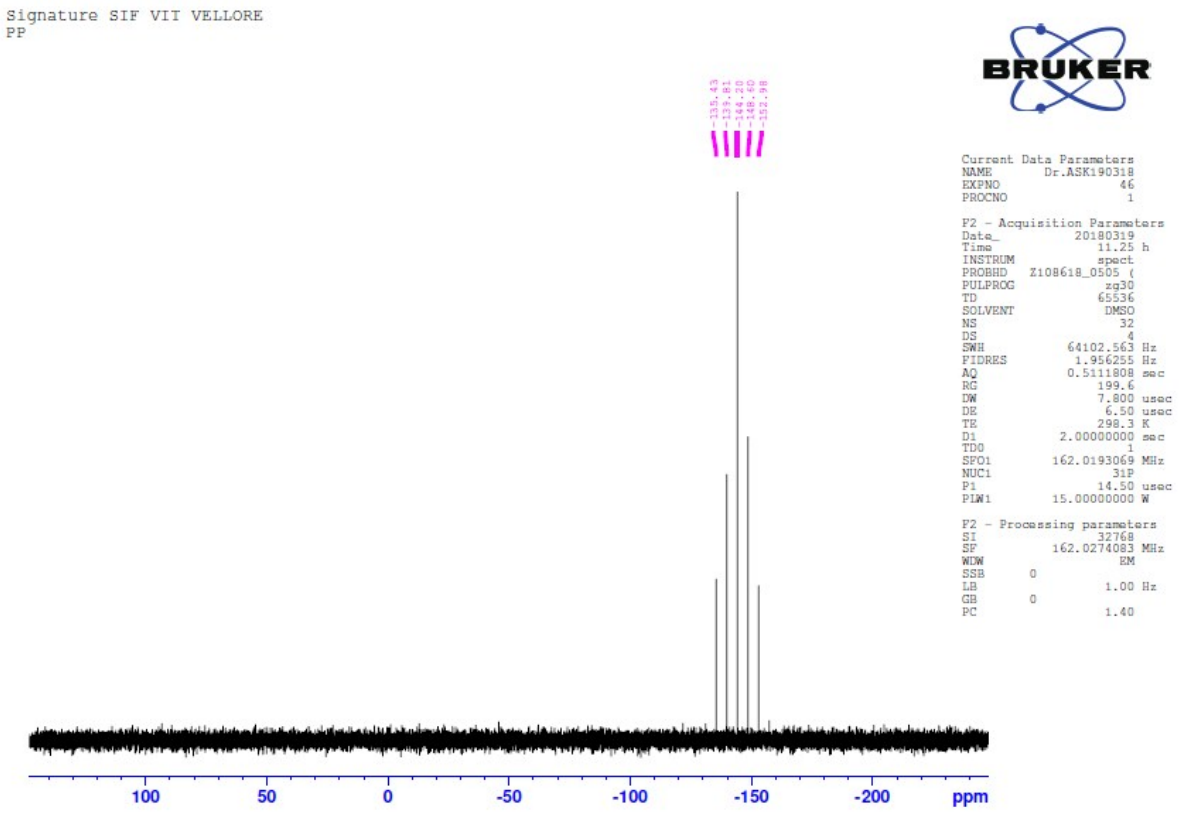


Fig. S14 ^{31}P NMR of complex **4**

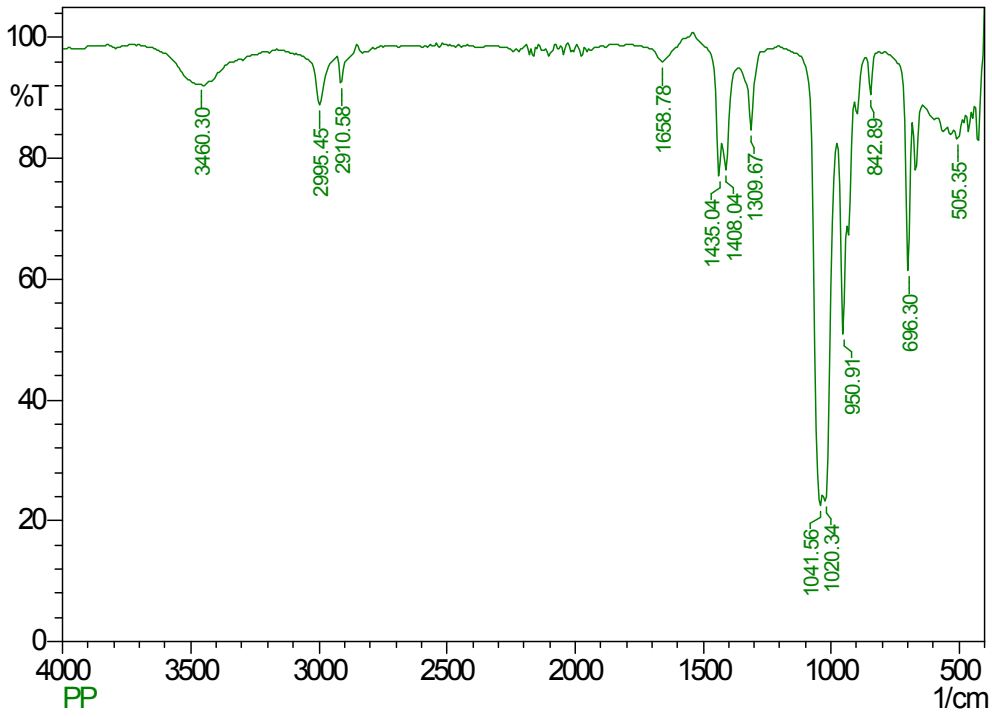


Fig. S15 FTIR spectra of complex **4**

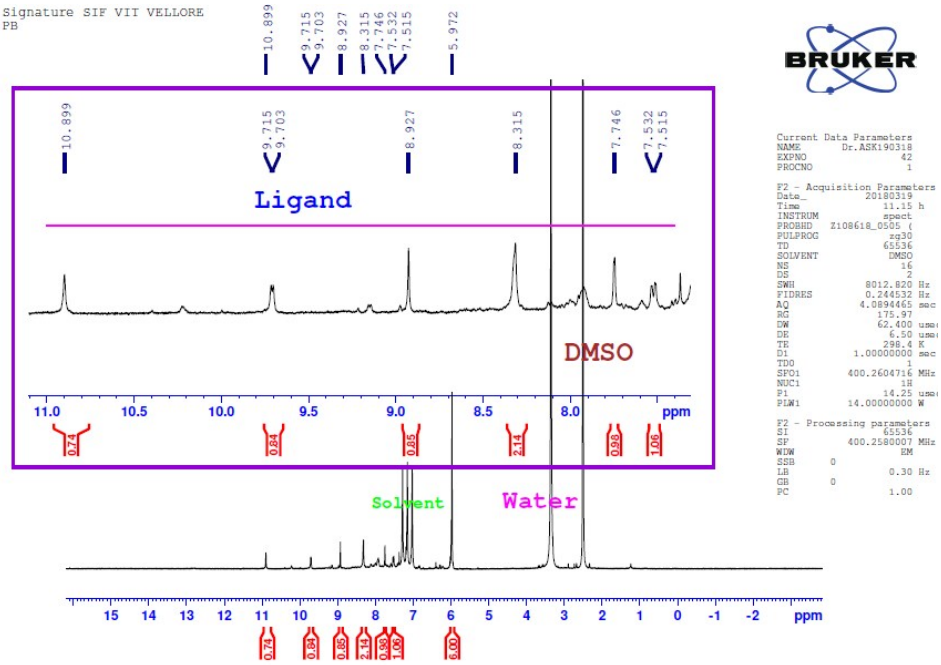


Fig. S16 ^{1}H NMR spectra of complex **5**

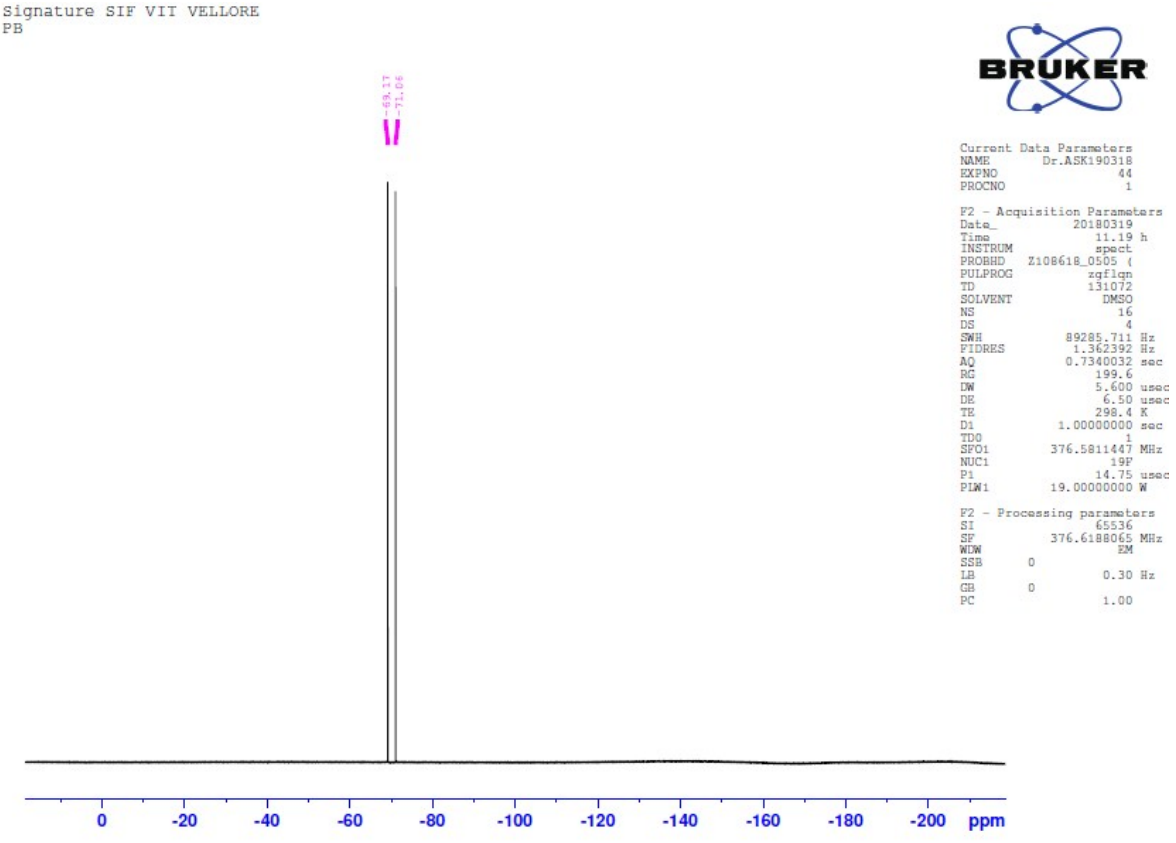


Fig. S17 ^{19}F NMR spectra of complex **5**

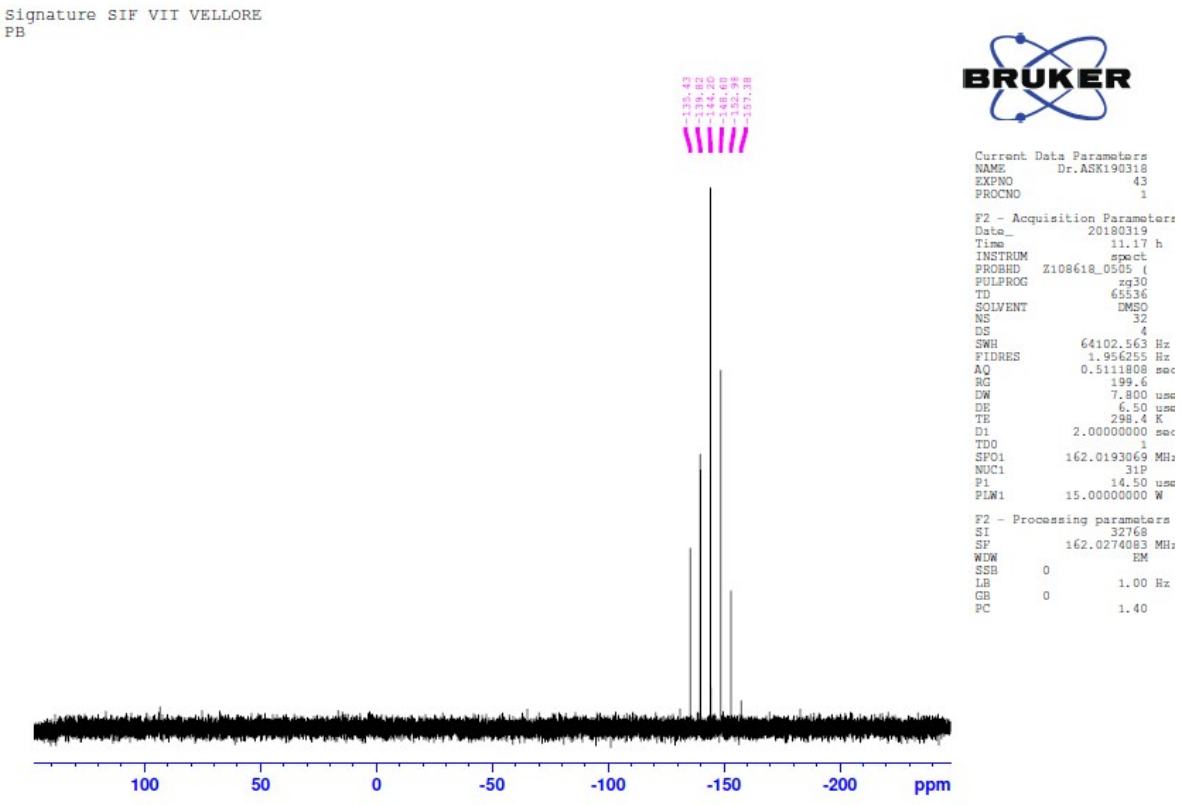


Fig. S18 ^{31}P NMR spectra of complex **5**

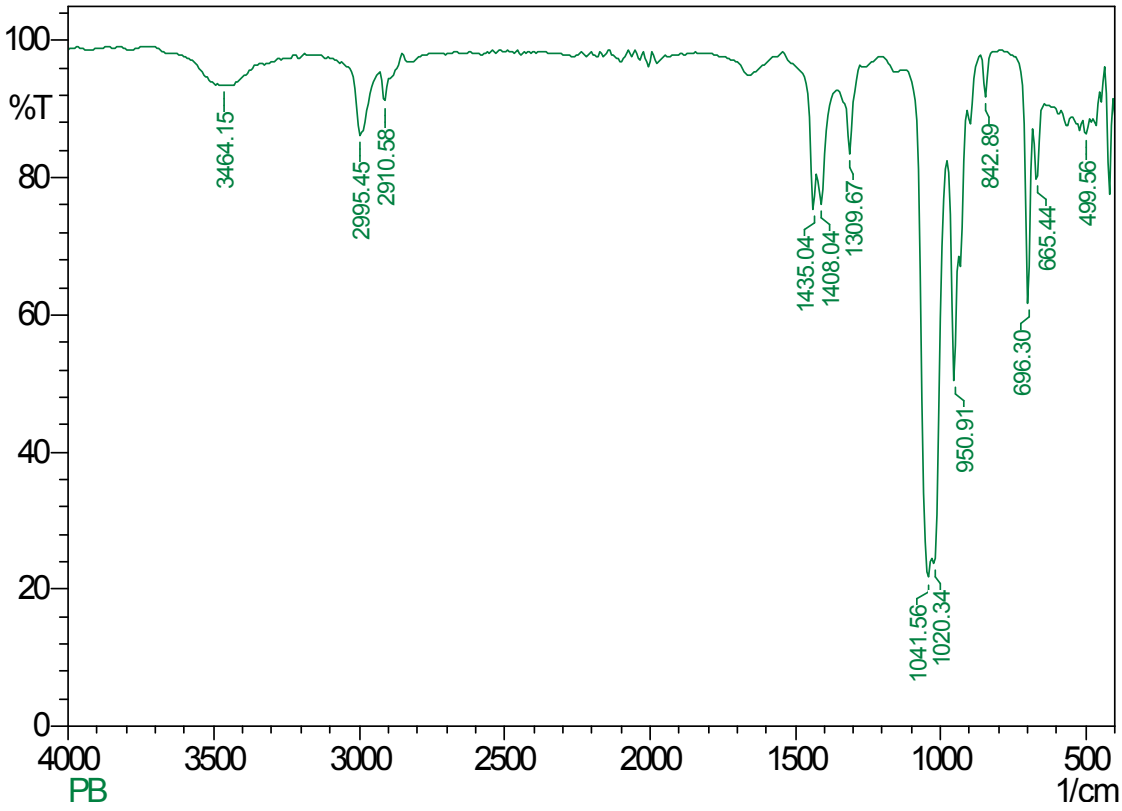


Fig. S19 FTIR spectra of complex **5**

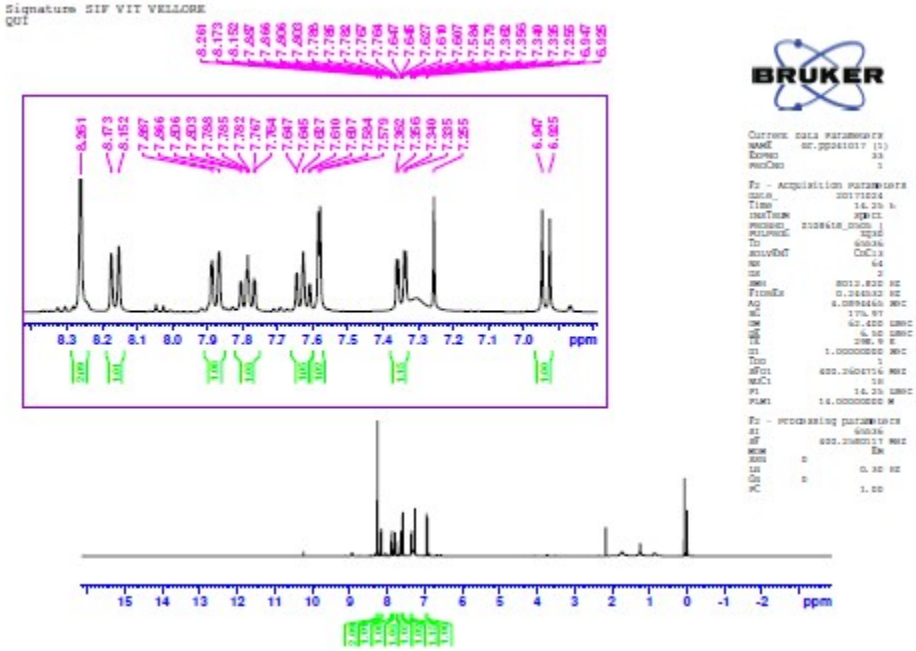


Fig. S20 ^{1}H NMR spectra of complex **3'**

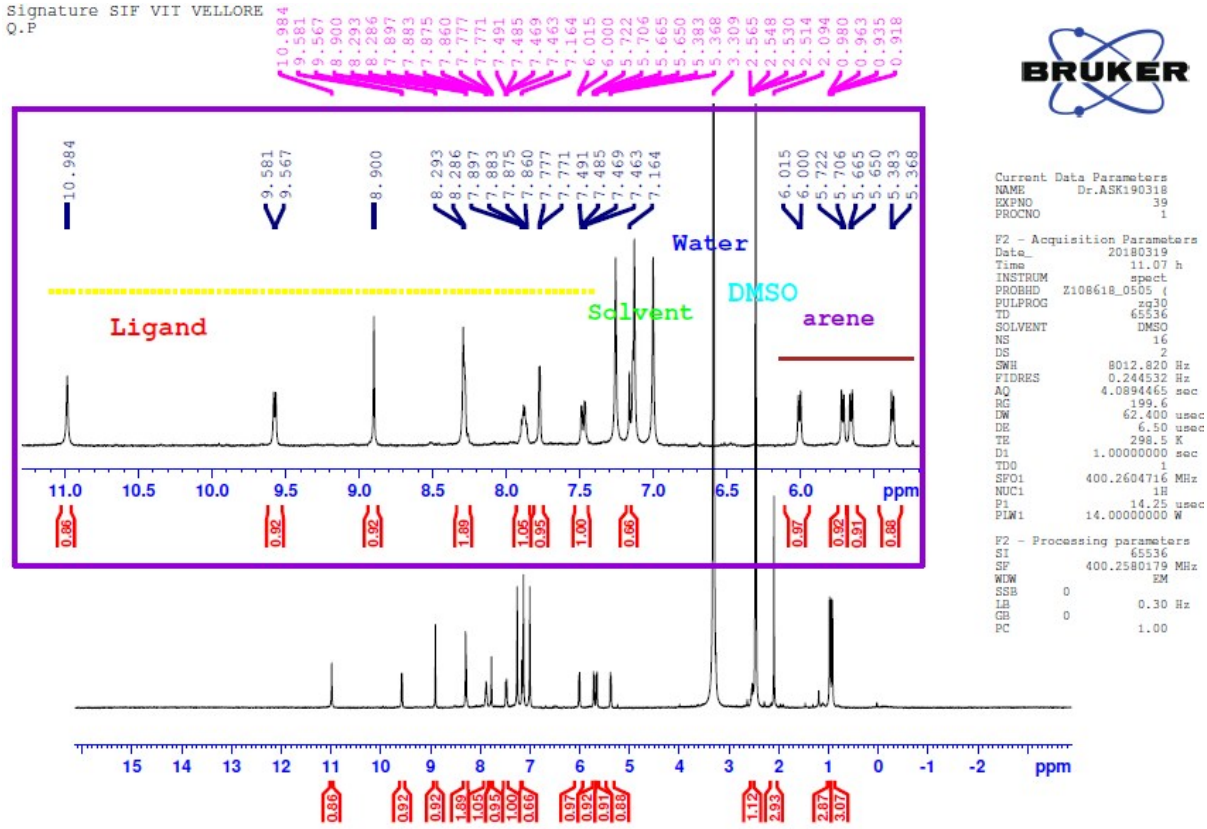


Fig. S21 ^{1}H NMR spectra of complex **4'**

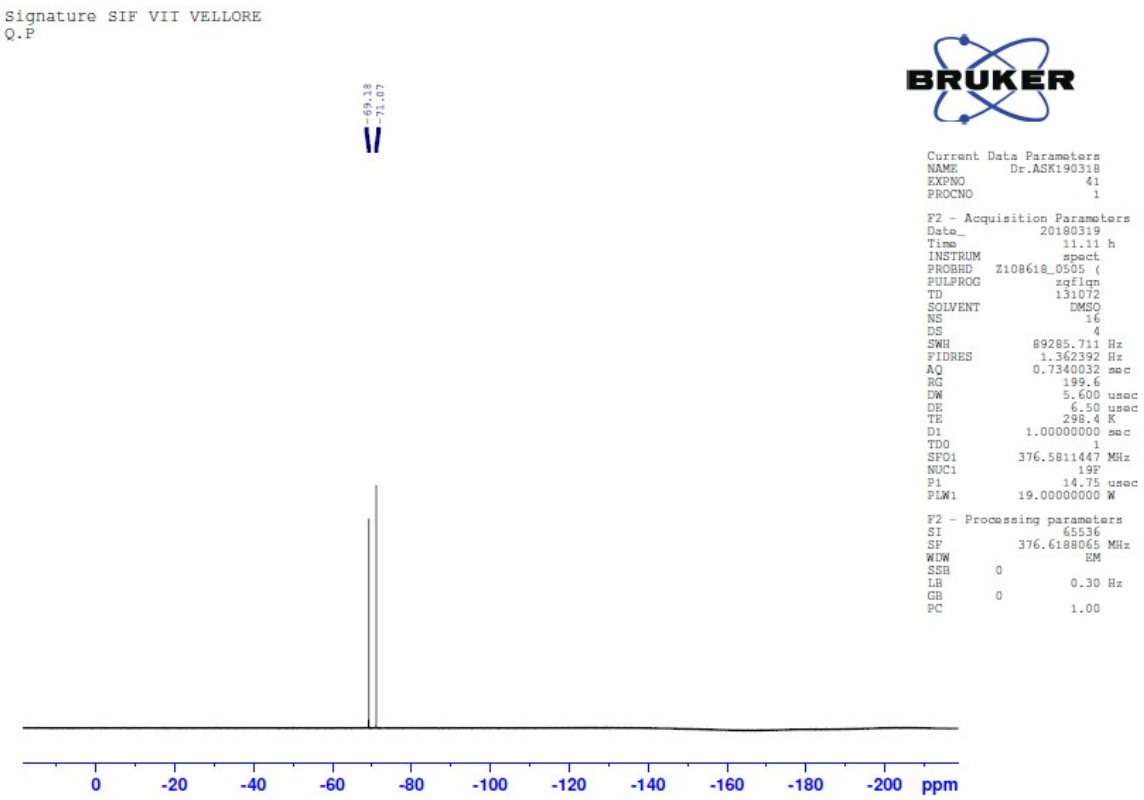


Fig. S22 ^{19}F NMR spectra of complex **4'**

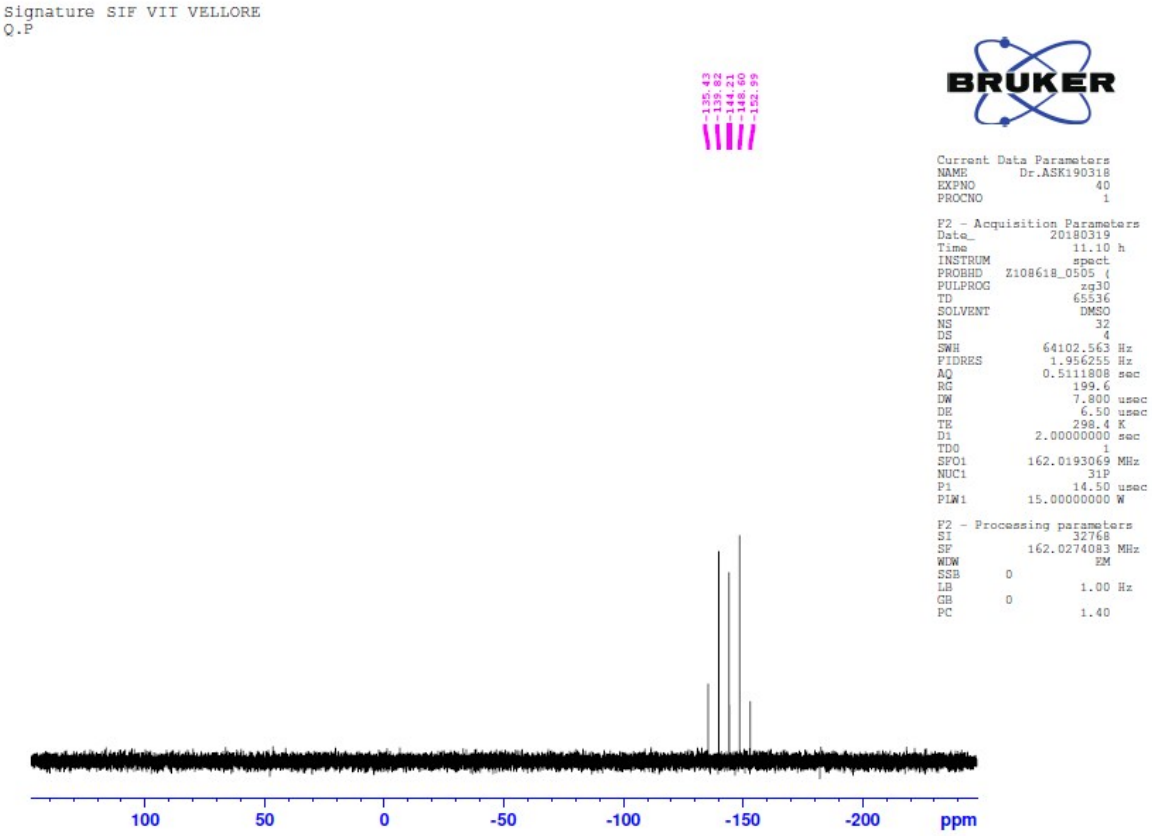


Fig. S23 ^{31}P spectra of complex **4'**

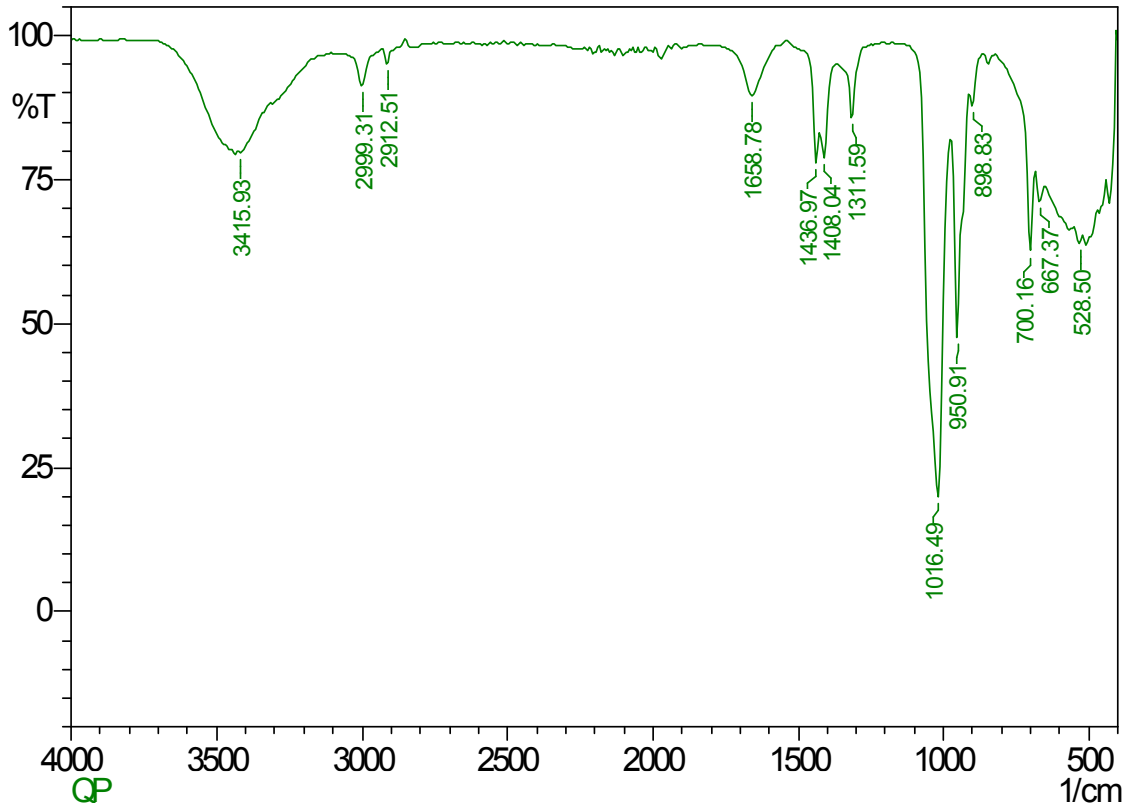


Fig. S24 FTIR spectra of complex **4'**

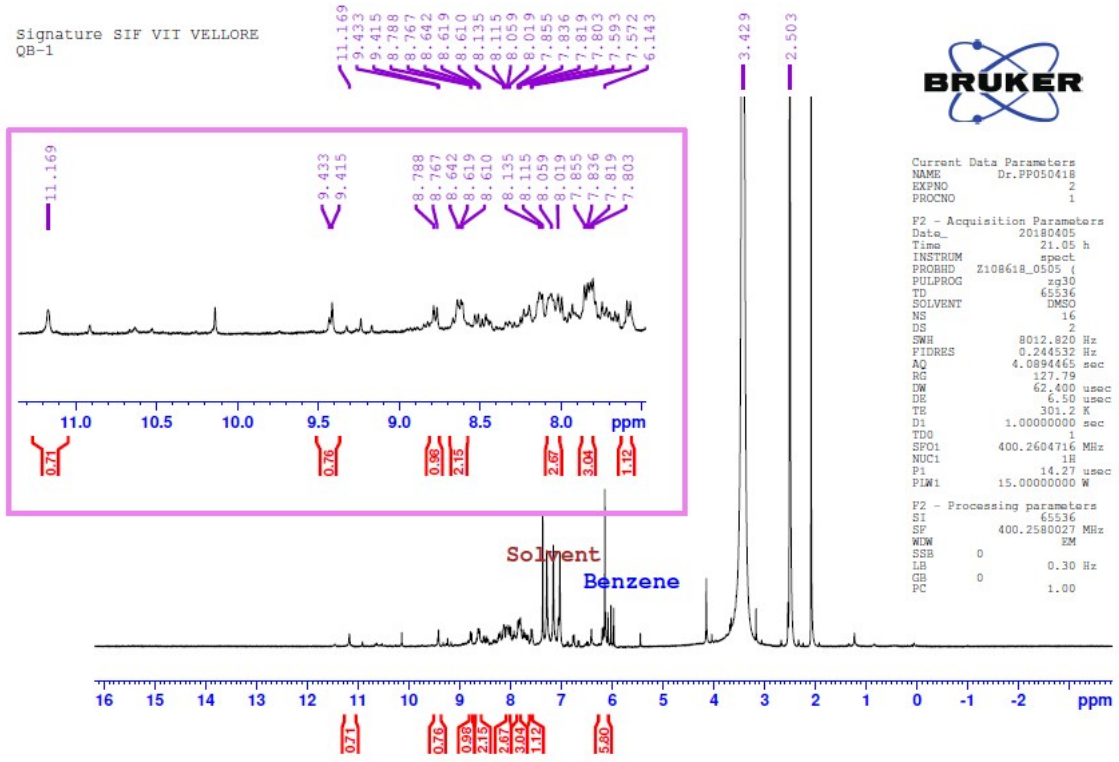


Fig. S25 ^{1}H spectra of complex **5'**

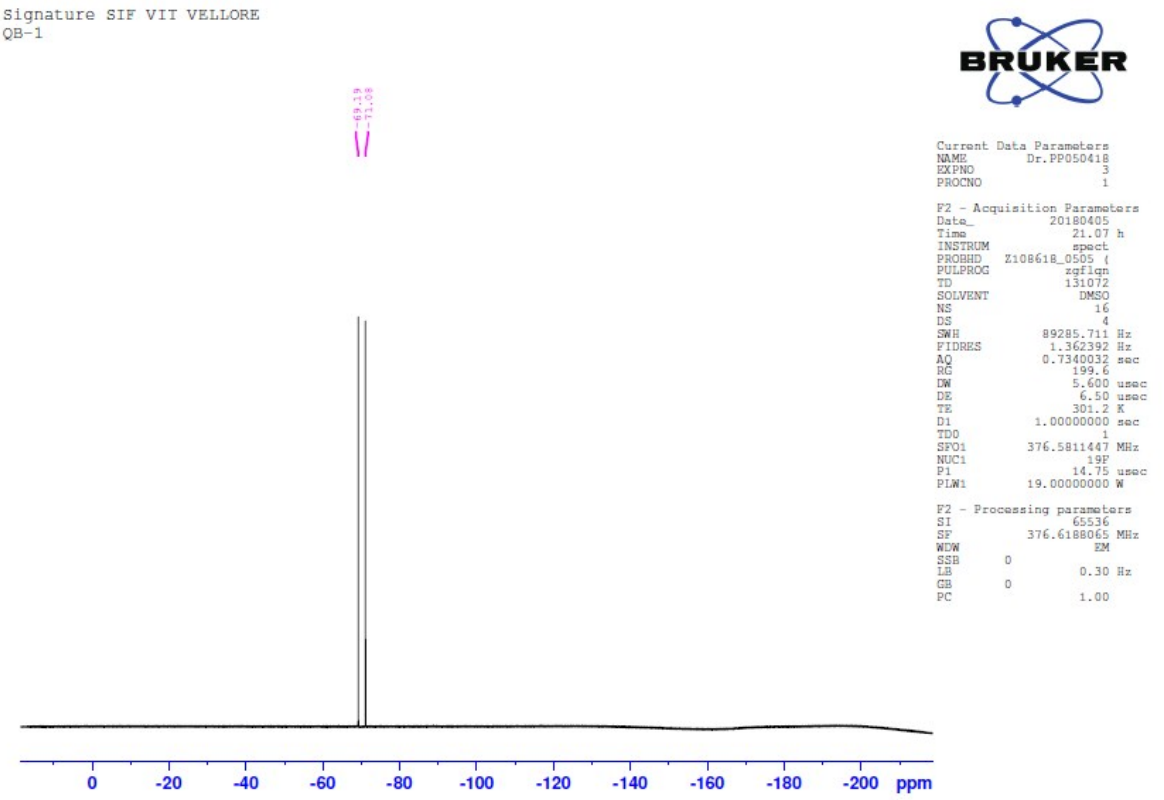


Fig. S26 ^{19}F spectra of complex **5'**

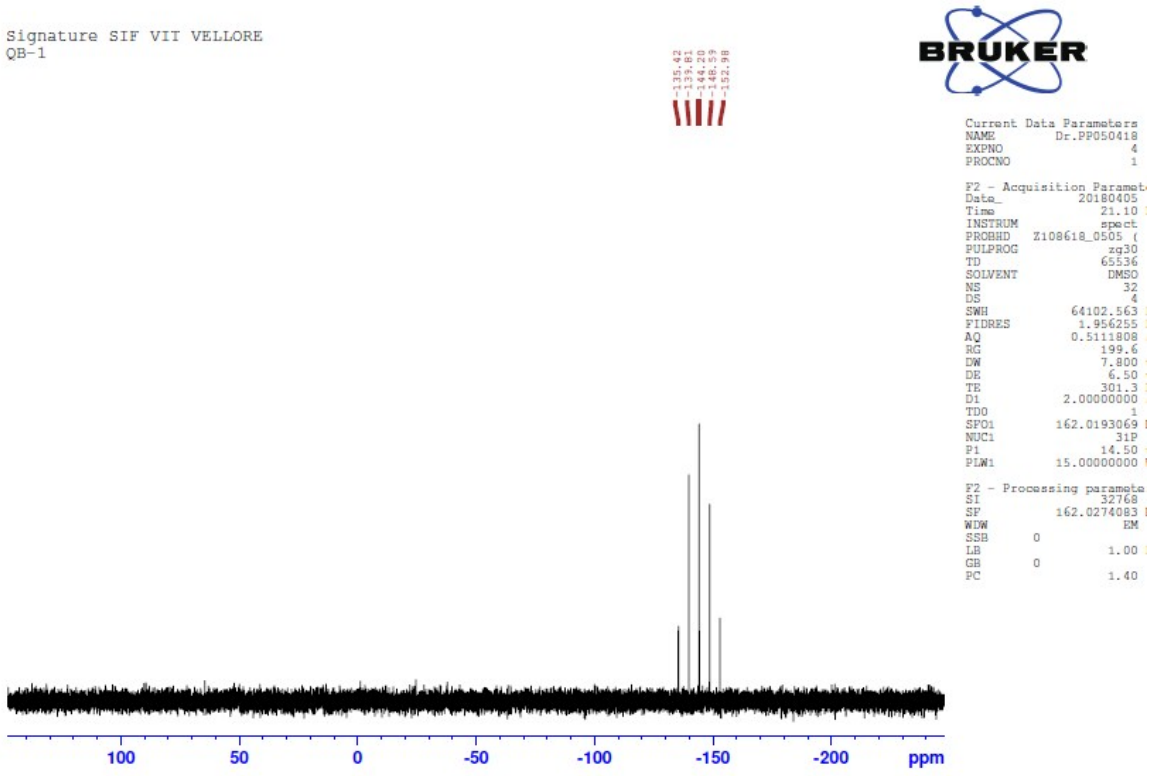


Fig. S27 ^{31}P spectra of complex **5'**

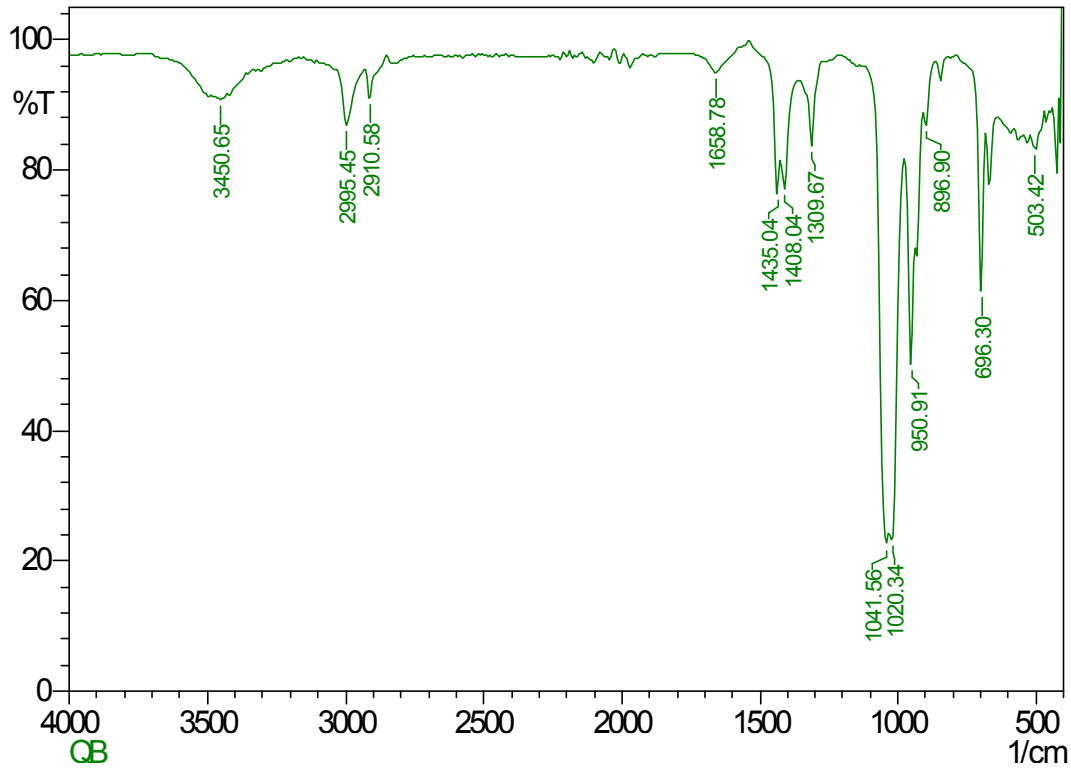


Fig. S28 FTIR spectra of complex **5'**