Electronic Supplementary Material (ESI) for RSC Advances.

Synthesis of 1, 3, 5-trisubstituted pyrazoline derivatives and its applications

Jugal V. Mehta,[a] Sanjay B. Gajera,[a] Parth Thakor,[b] Vasudev R. Thakkar[b] and Mohan N. Patel*[a]

[a]Department of Chemistry, Sardar Patel University,

Vallabh Vidyanagar–388 120, Gujarat, India.

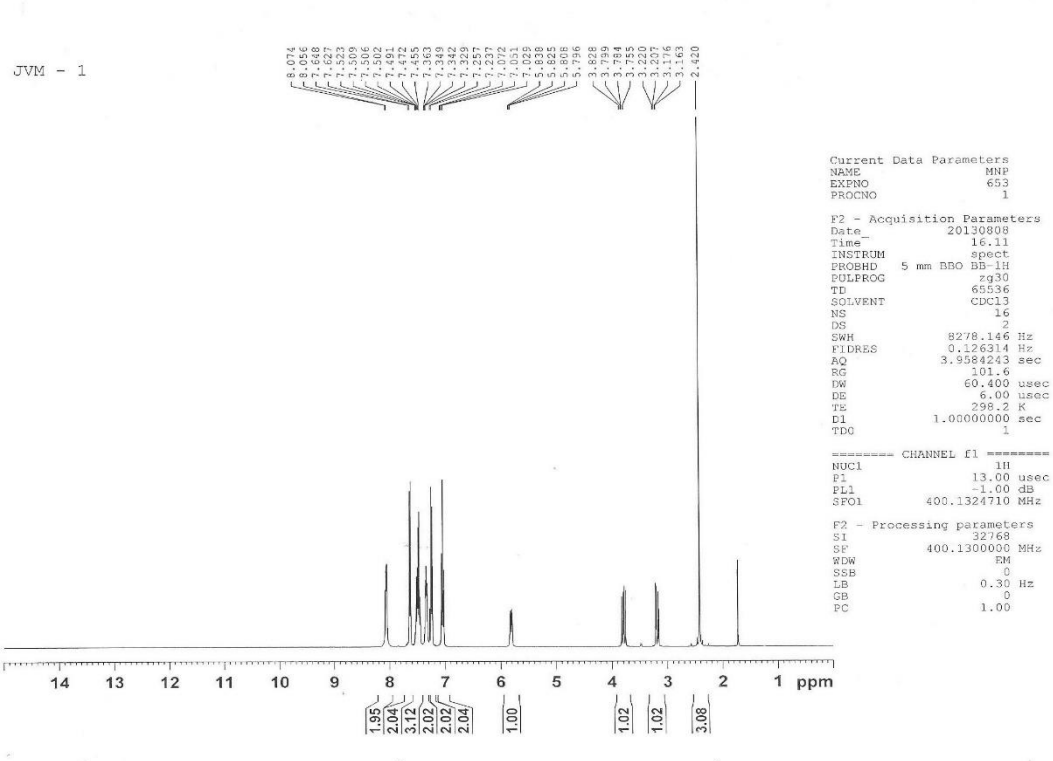
Corresponding author. Tel.: +91 2692 226856 E-mail: jeenen@gmail.com

[b]B. R. Doshi School of Bioscience, Sardar Patel University,

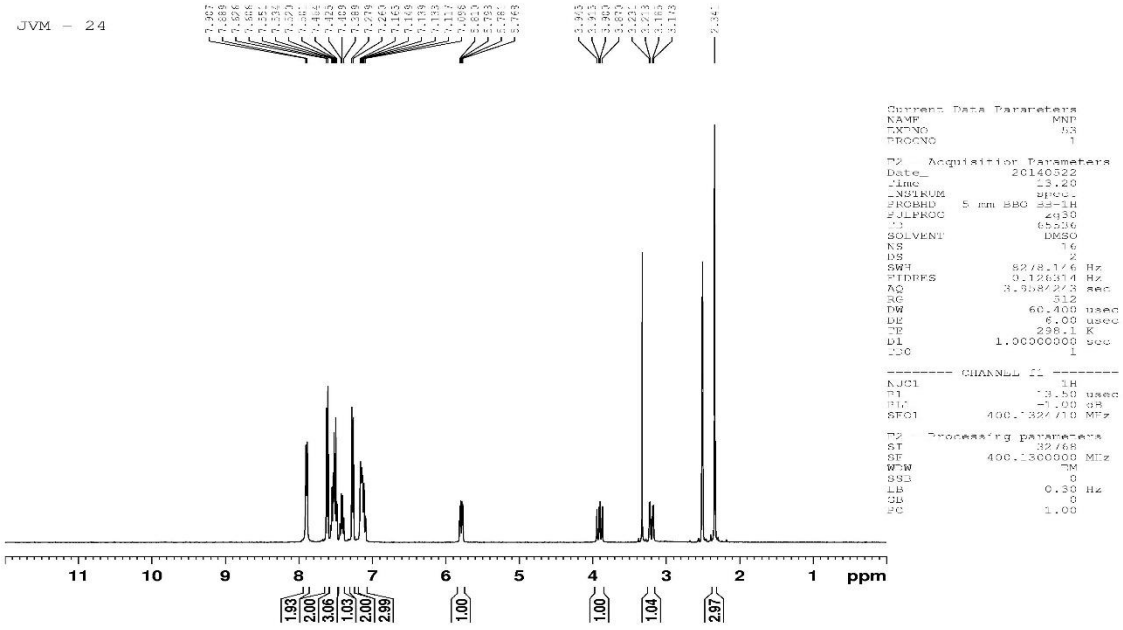
Vallabh Vidyanagar-388 120, Gujarat, India

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

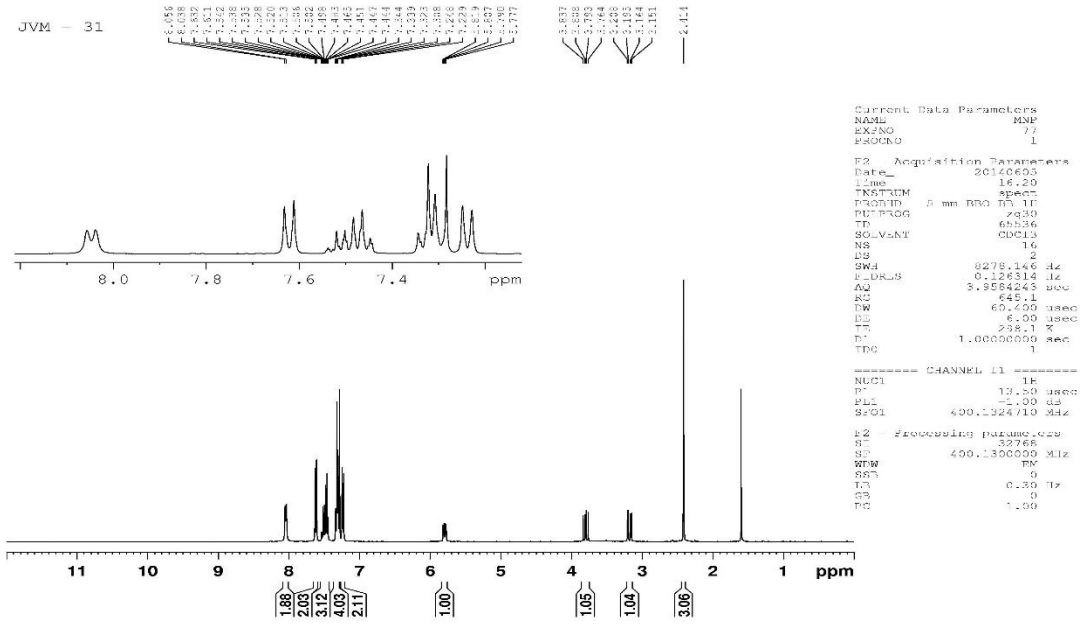
1. [5-(4-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5a)**



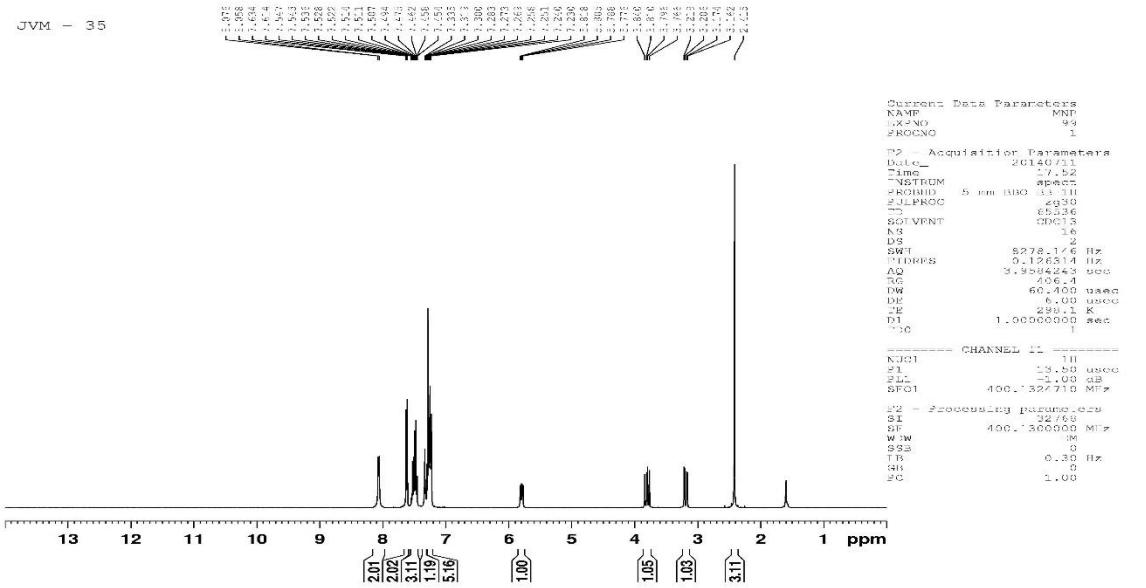
2. [5-(3-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5b)**



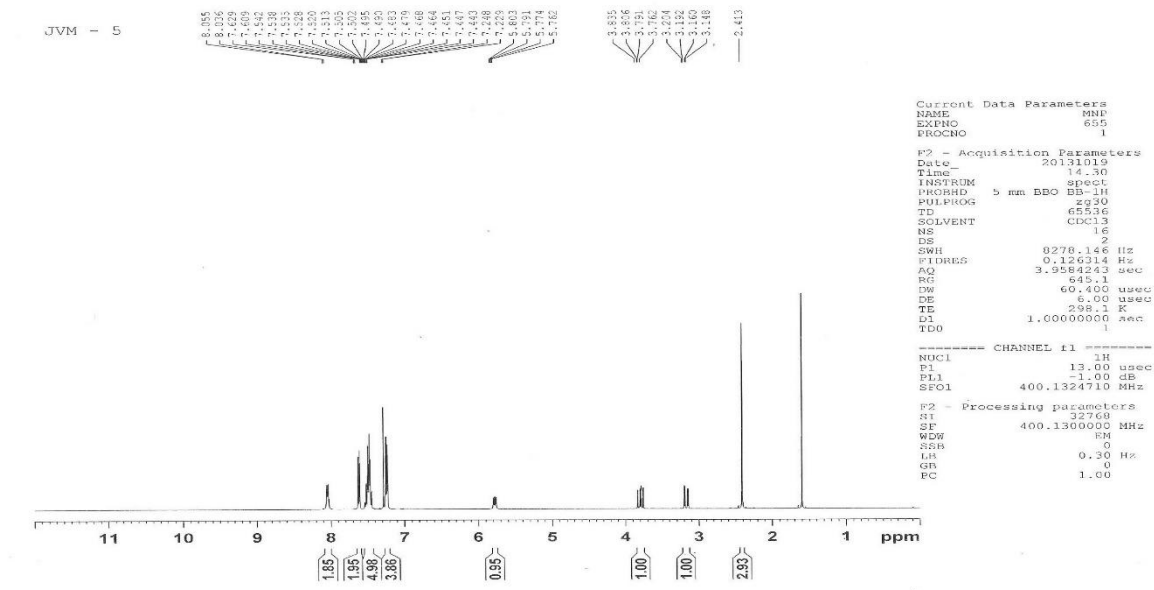
3. [5-(4-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5c)**



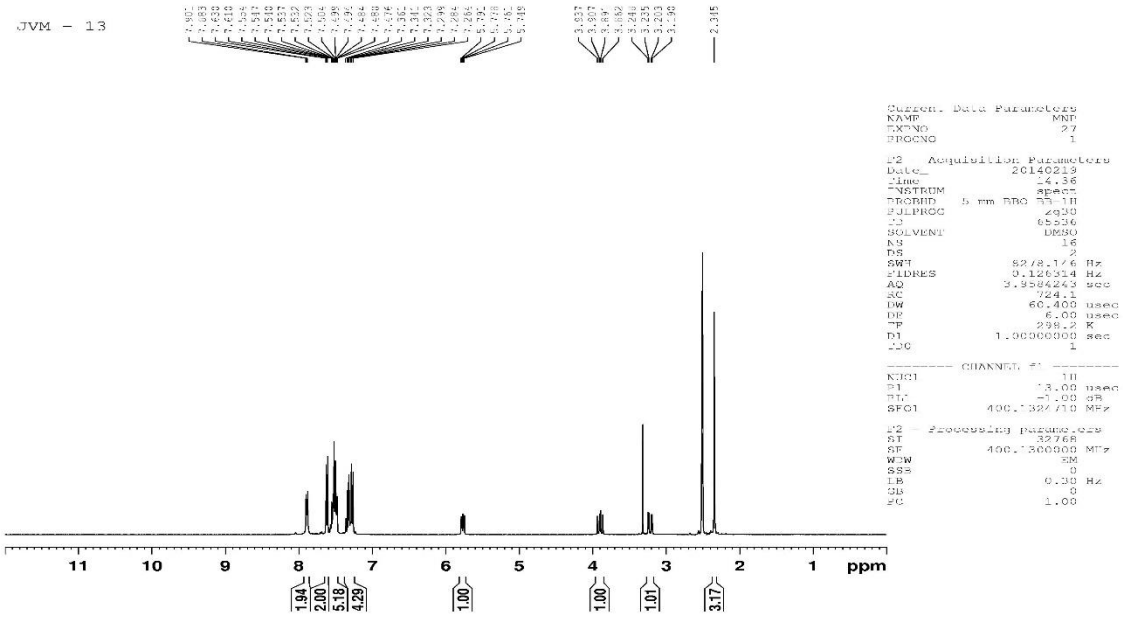
4. [5-(3-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5d)**



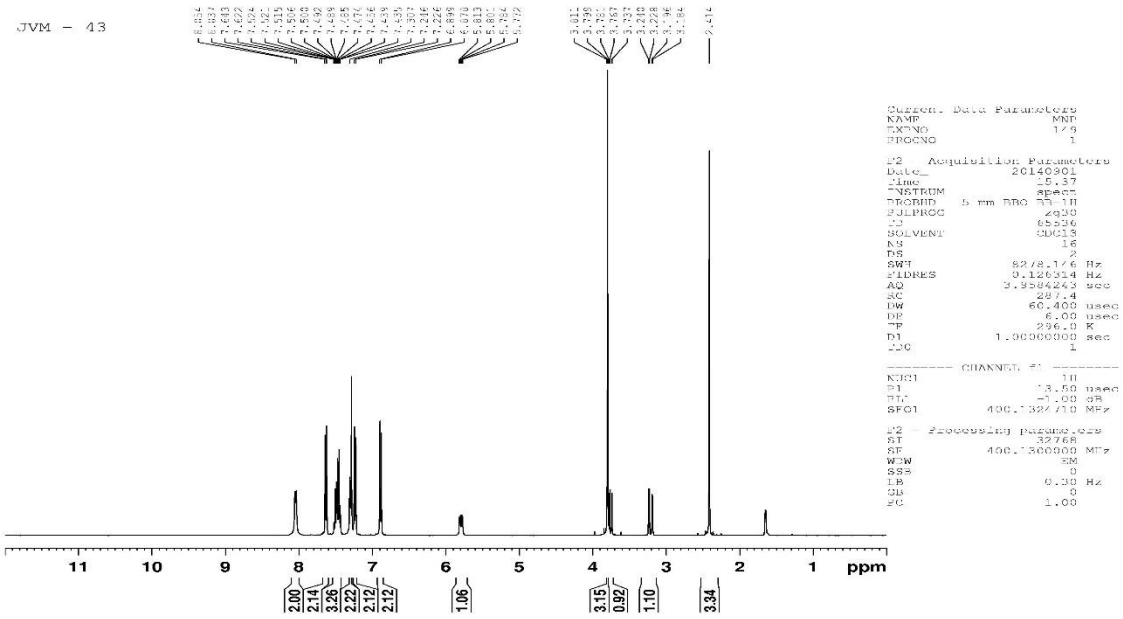
5. [5-(4-Bromophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5e)**



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

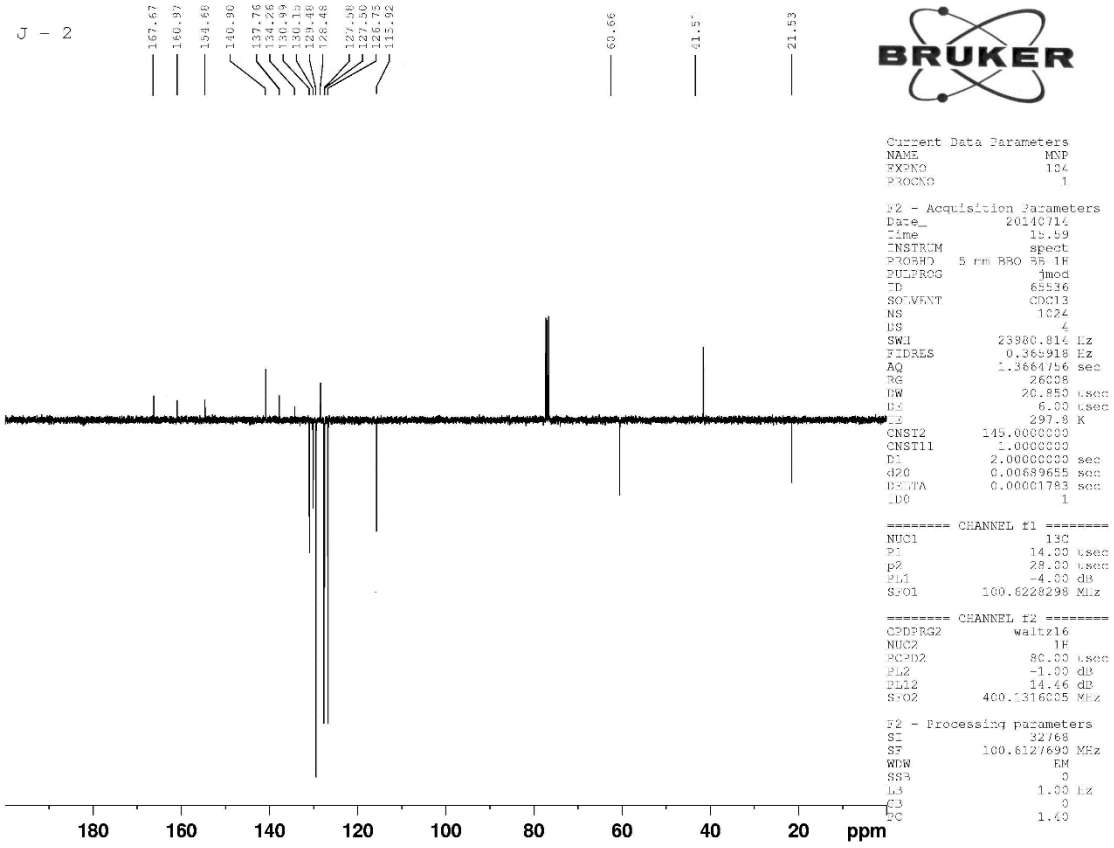


7. [5-(4-Methoxyphenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5g)**

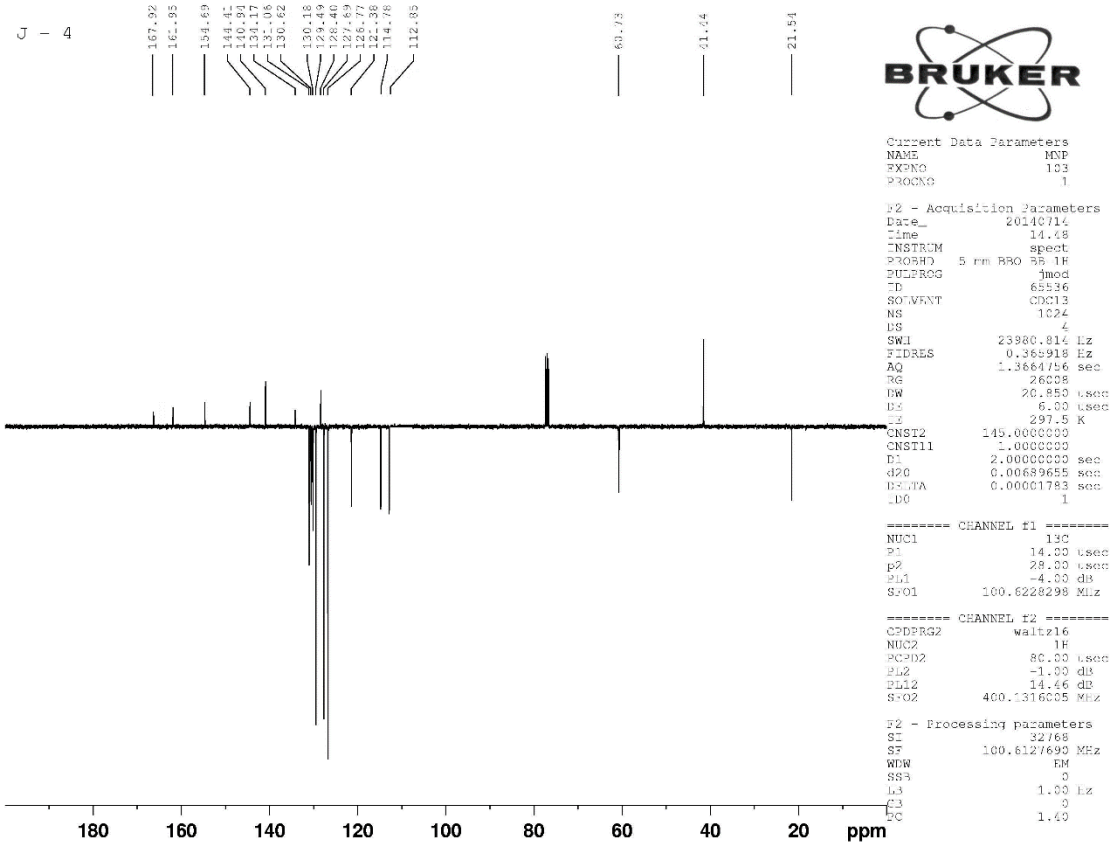


Supplementary material 2: ^{13}C NMR spectra of ligands (5a–5g)

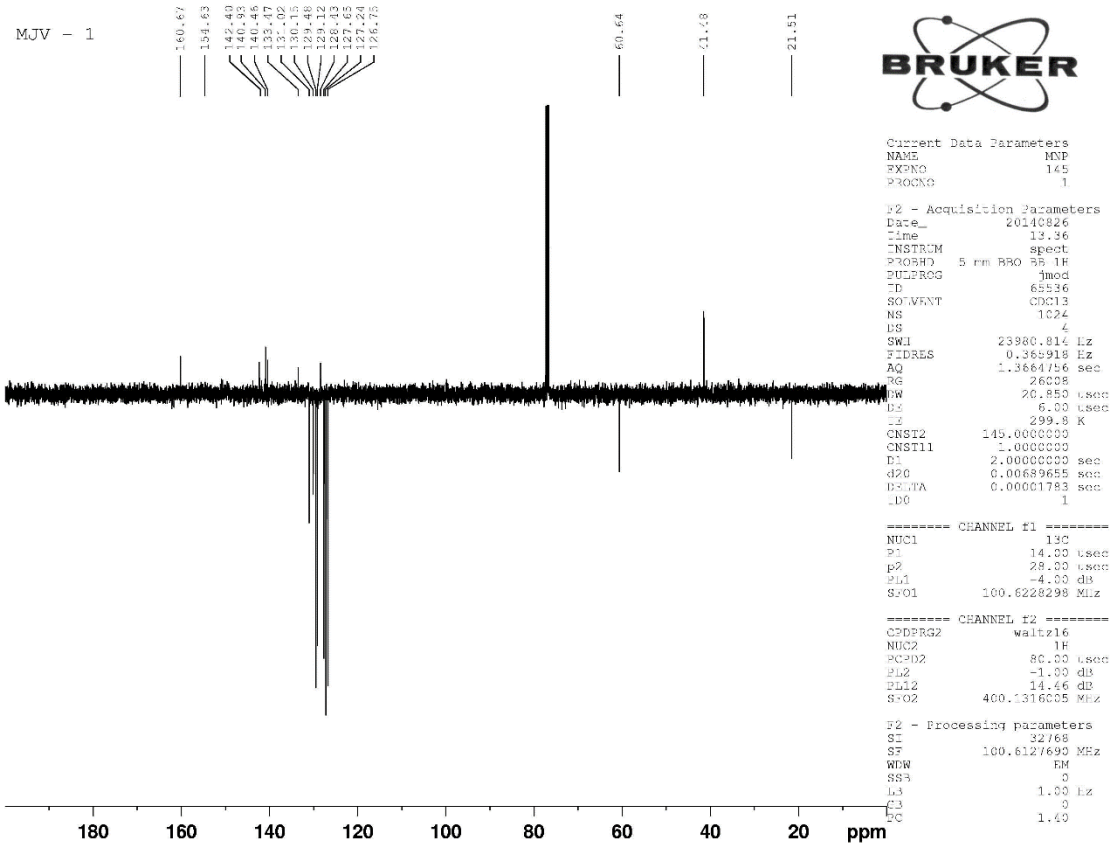
1. [5-(4-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5a)**



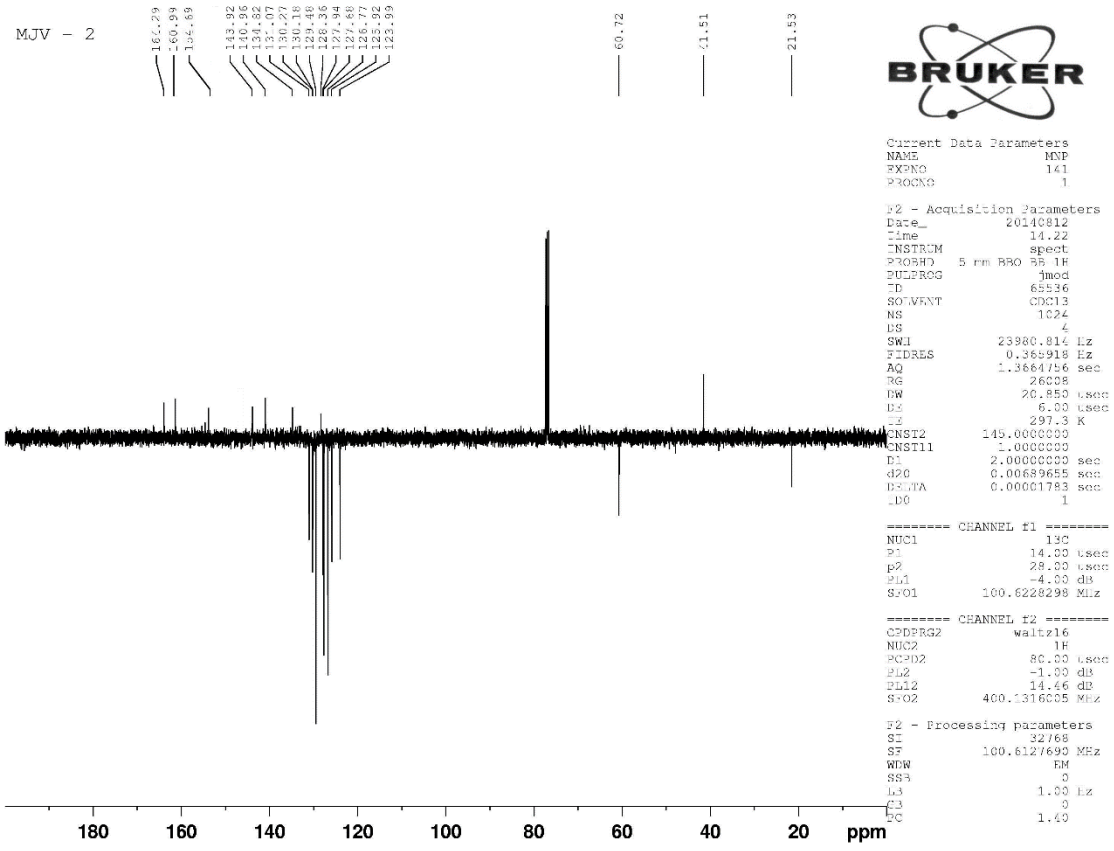
2. [5-(3-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5b)**



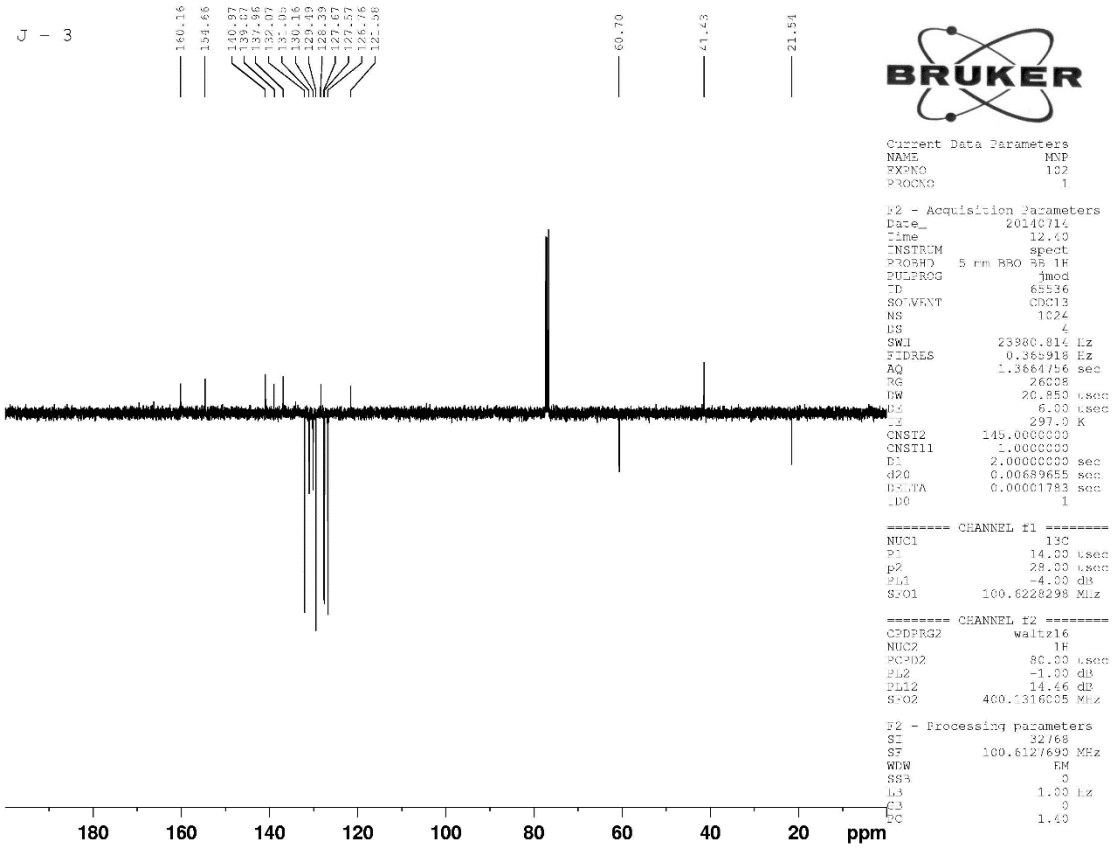
3. [5-(4-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5c)**



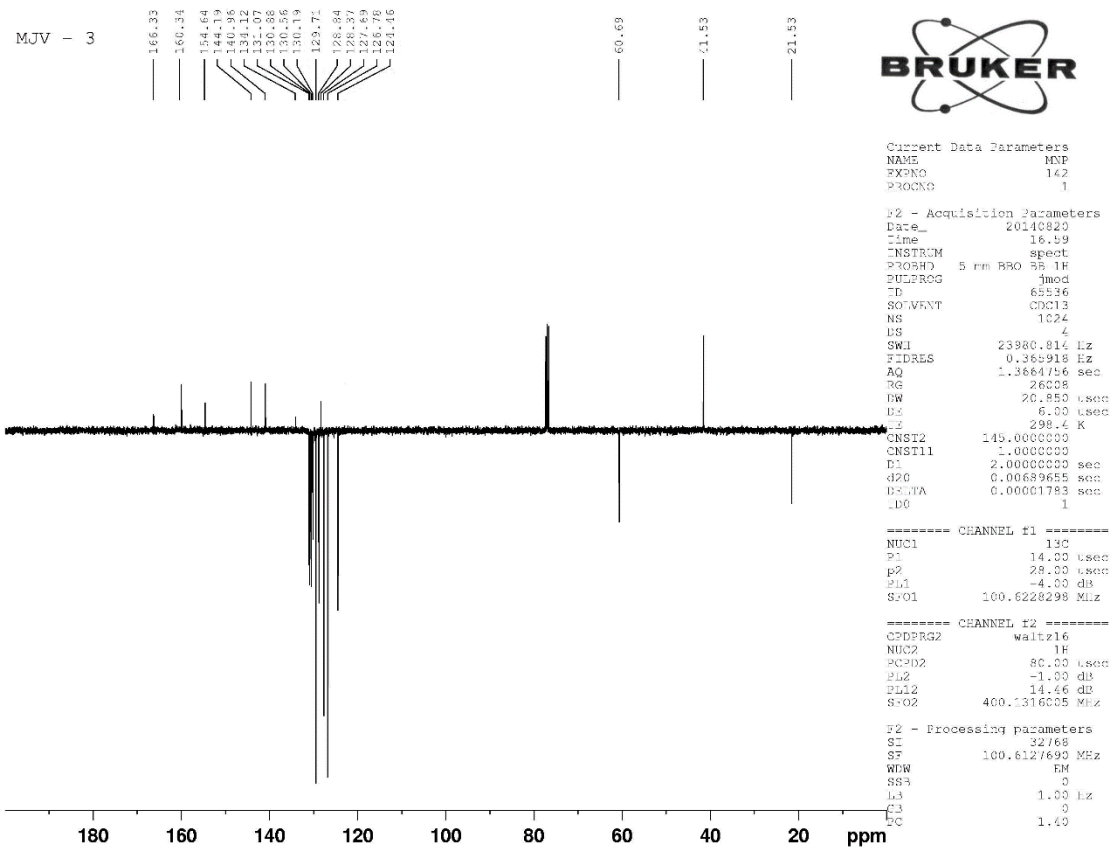
4. [5-(3-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5d)**



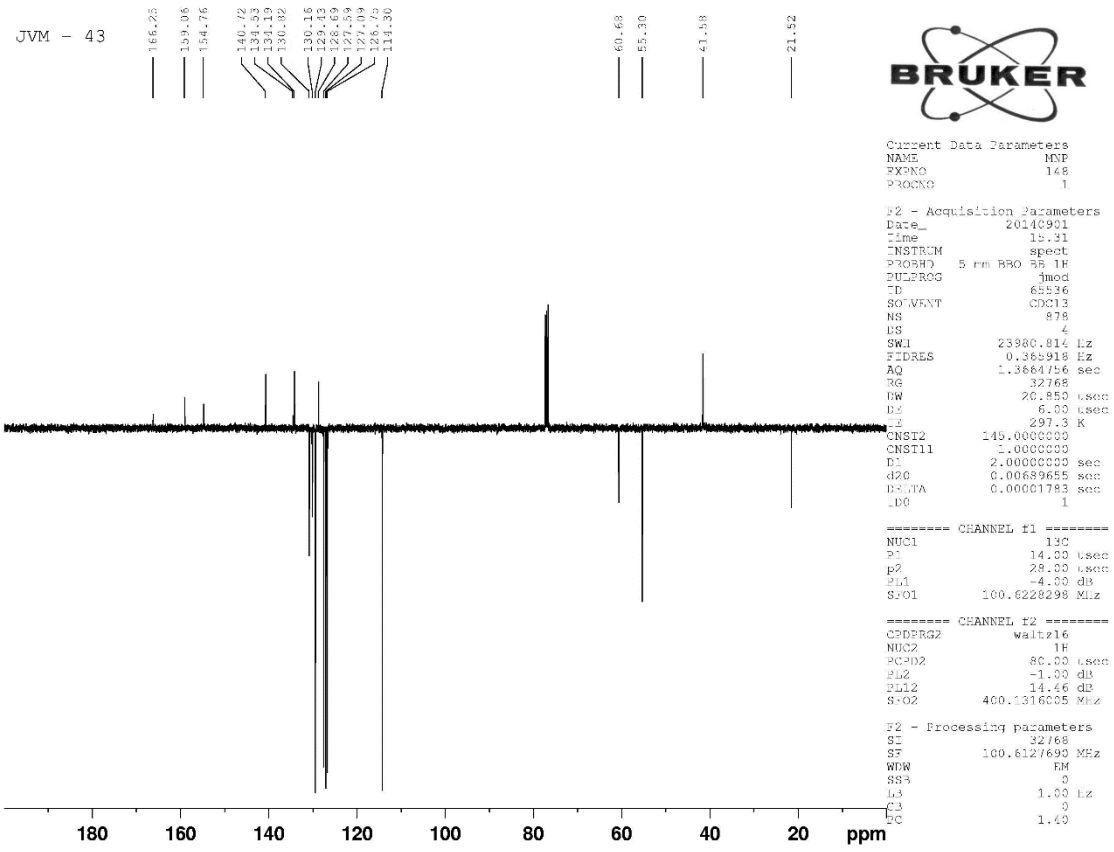
5. [5-(4-Bromophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5e)**



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

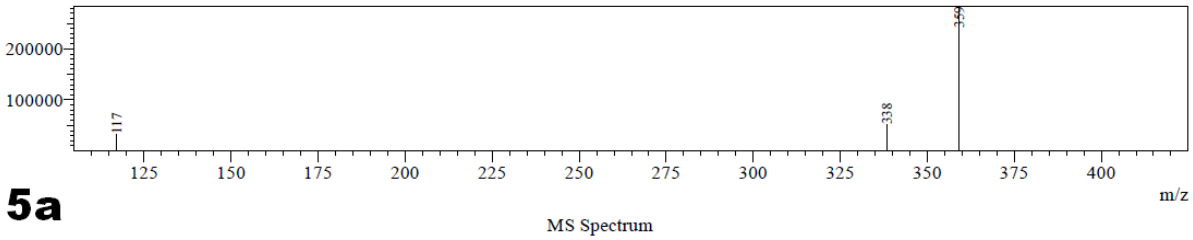


7. [5-(4-Methoxyphenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5g)**



Supplementary material 3: Mass spectra of ligands (5a–5g)

1. [5-(4-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5a)**

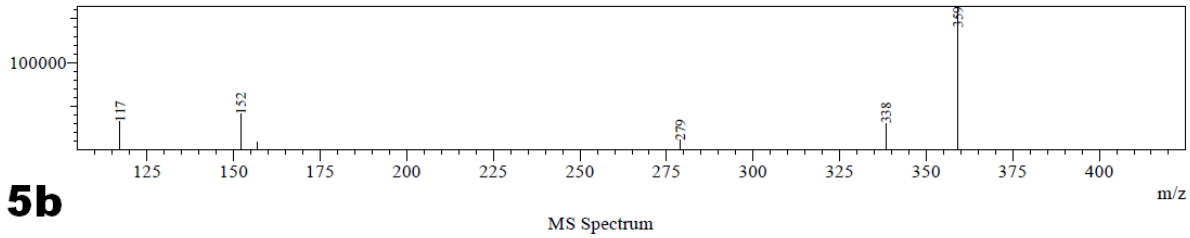


5a

MS Spectrum

Line#:1 R.Time:----(Scan#:----)
MassPeaks:3
Spectrum Mode:Averaged 2.917-3.567(176-215) Base Peak:359(283100) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

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

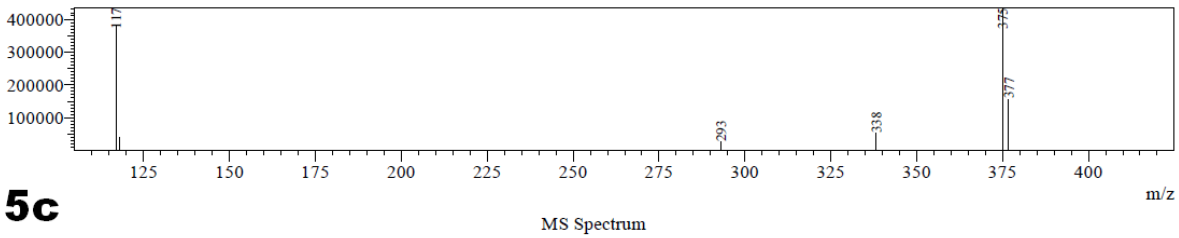


5b

MS Spectrum

Line#:1 R.Time:----(Scan#:----)
MassPeaks: 5
Spectrum Mode:Averaged 0.633-3.300(39-199) Base Peak:359(164140) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

3. [5-(4-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5c)**

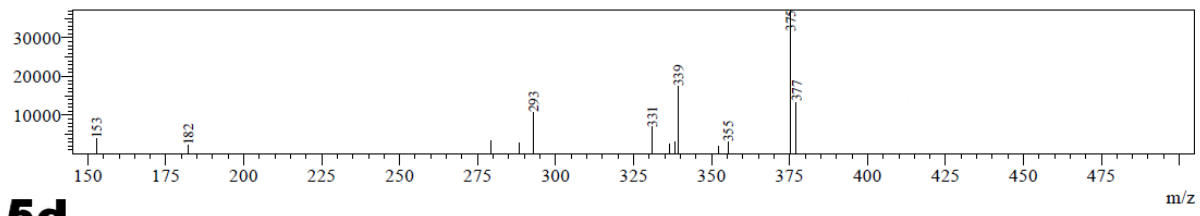


5c

MS Spectrum

Line#:1 R.Time:3.250(Scan#:196)
MassPeaks:6
Spectrum Mode:Single 3.250(196) Base Peak:375(434436) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

4. [5-(3-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5d)**

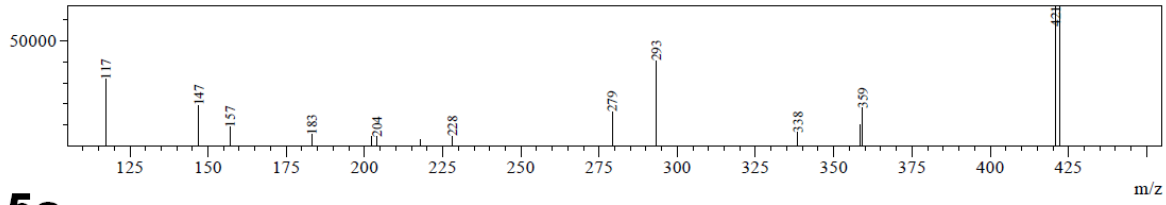


5d

MS Spectrum

Line#:1 R.Time:2.817(Scan#:170)
MassPeaks:13
Spectrum Mode:Single 2.817(170) Base Peak:375(37137) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

5. [5-(4-Bromophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5e)**

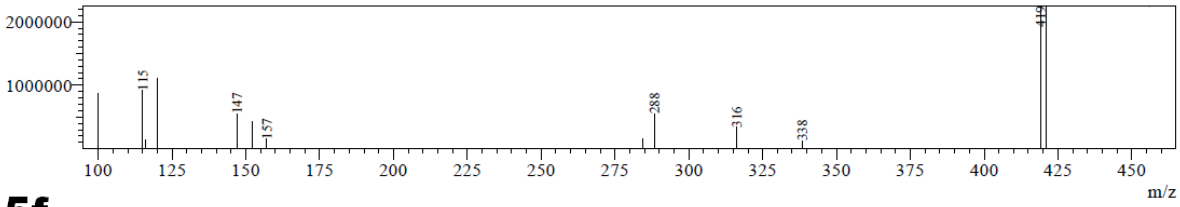


5e

MS Spectrum

Line#:1 R.Time:3.033(Scan#:183)
MassPeaks:15
Spectrum Mode:Single 3.033(183) Base Peak:421(66517) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

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

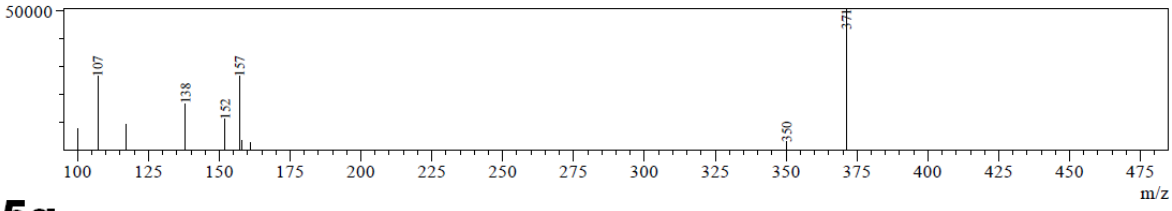


5f

MS Spectrum

Line#:1 R.Time:2.850(Scan#:172)
MassPeaks:13
Spectrum Mode:Single 2.850(172) Base Peak:419(2247667) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

7. [5-(4-Methoxyphenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5g)**



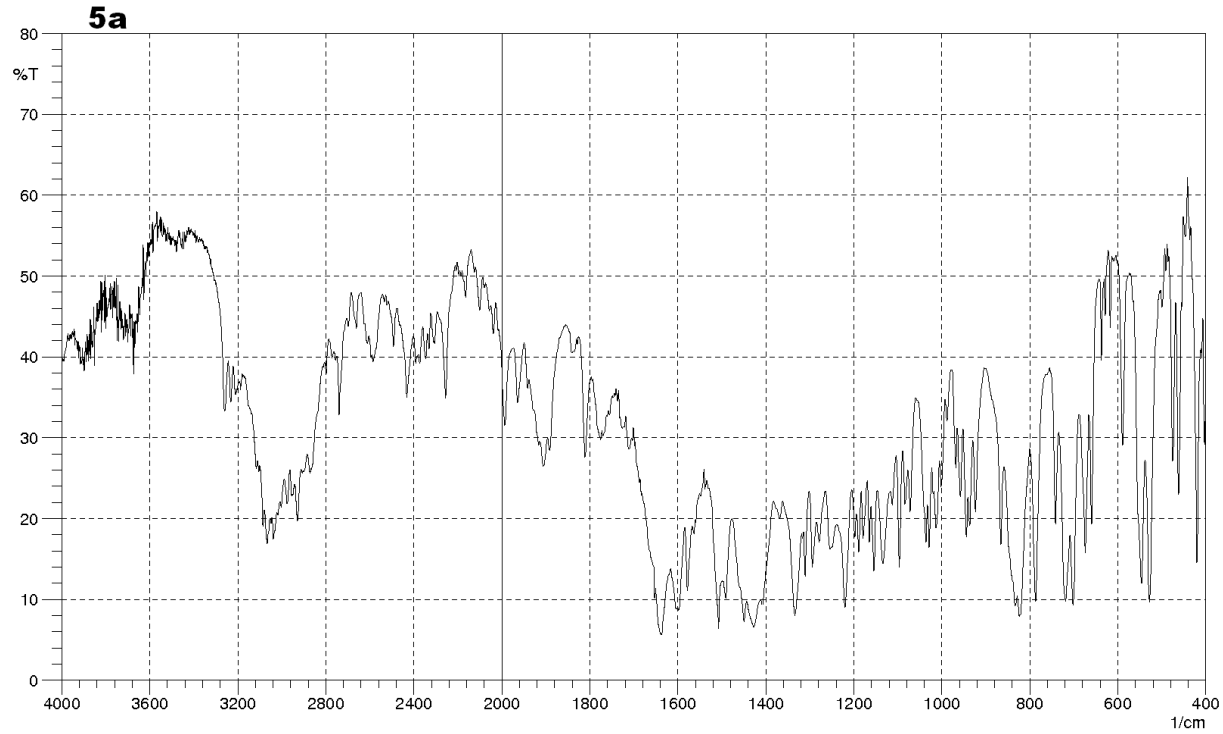
5g

MS Spectrum

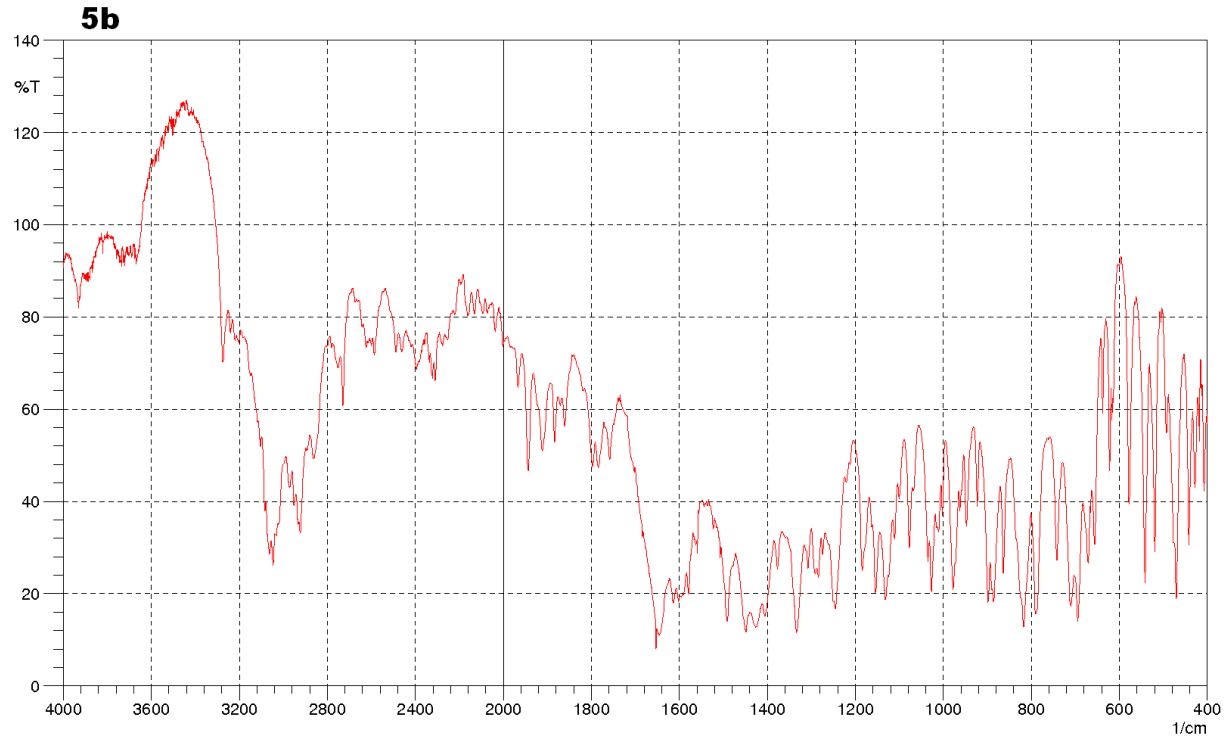
Line#:1 R.Time:----(Scan#:----)
MassPeaks:10
Spectrum Mode:Averaged 2.267-3.750(137-226) Base Peak:371(50947) Relative Intensity:100.00
BG Mode:None Segment 1 - Event 1

Supplementary material 4: FT-IR spectra of ligands (5a–5g)

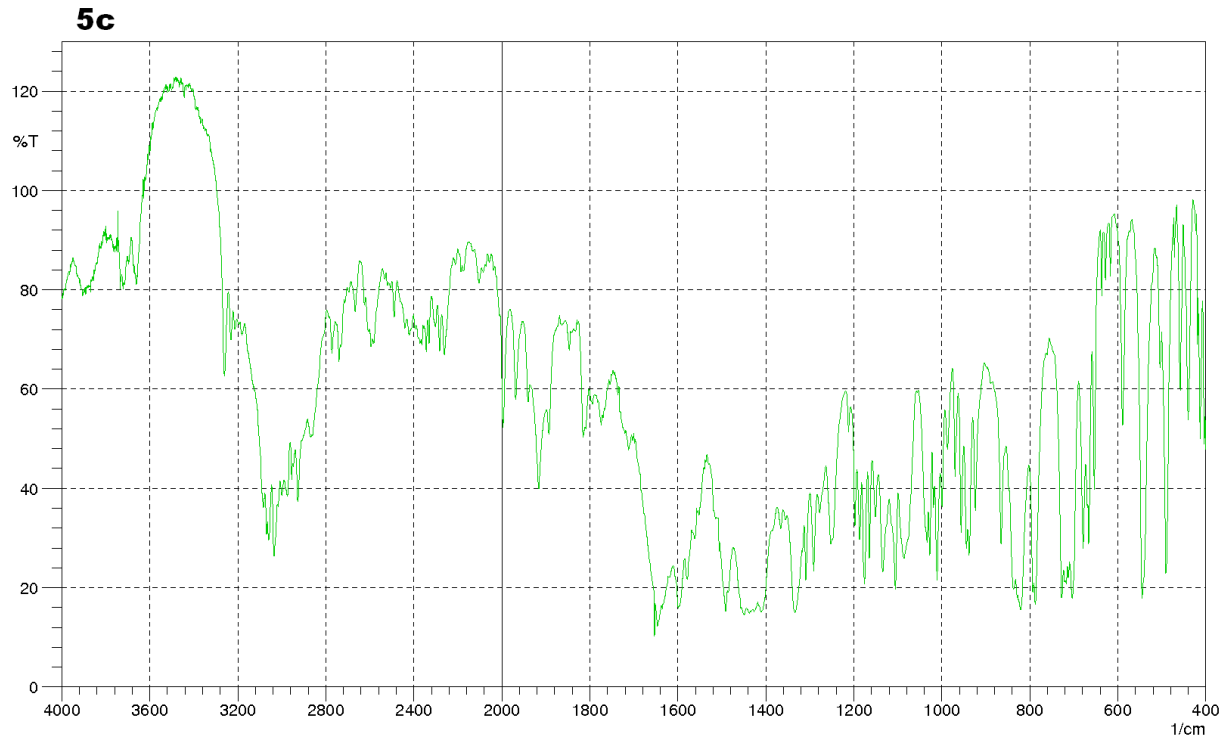
1. [5-(4-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5a)**



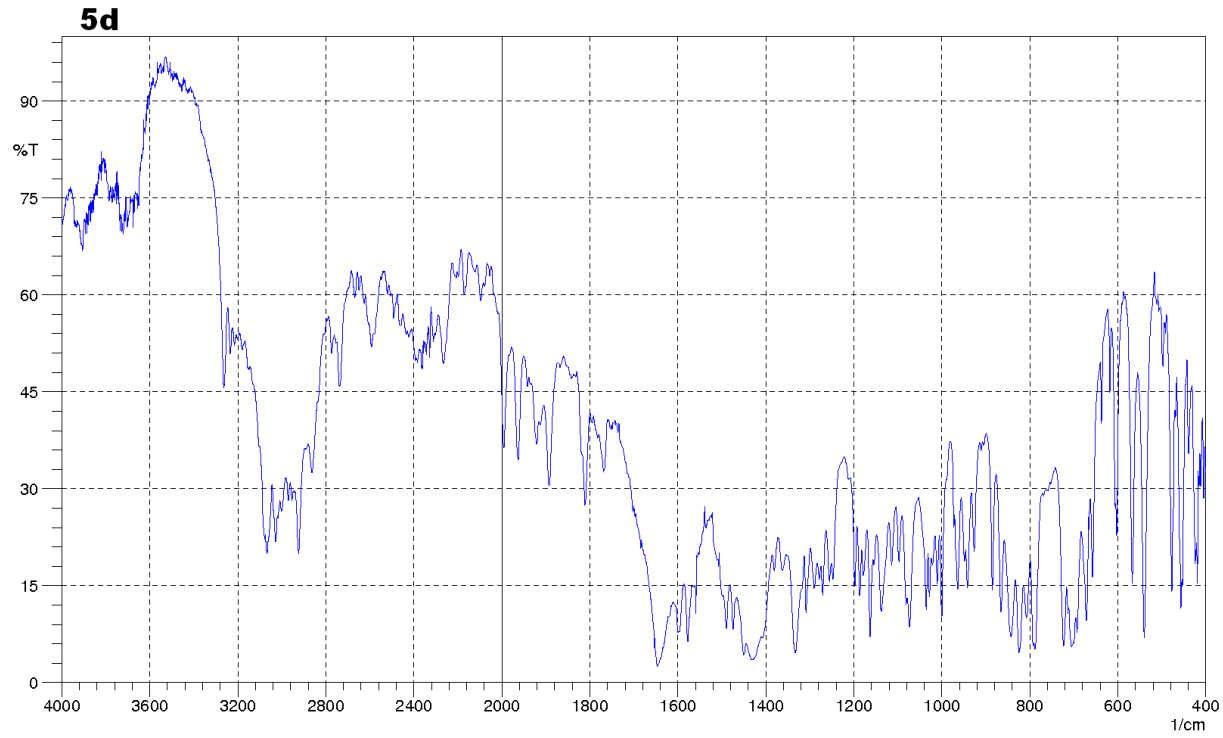
2. [5-(3-Fluorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5b)**



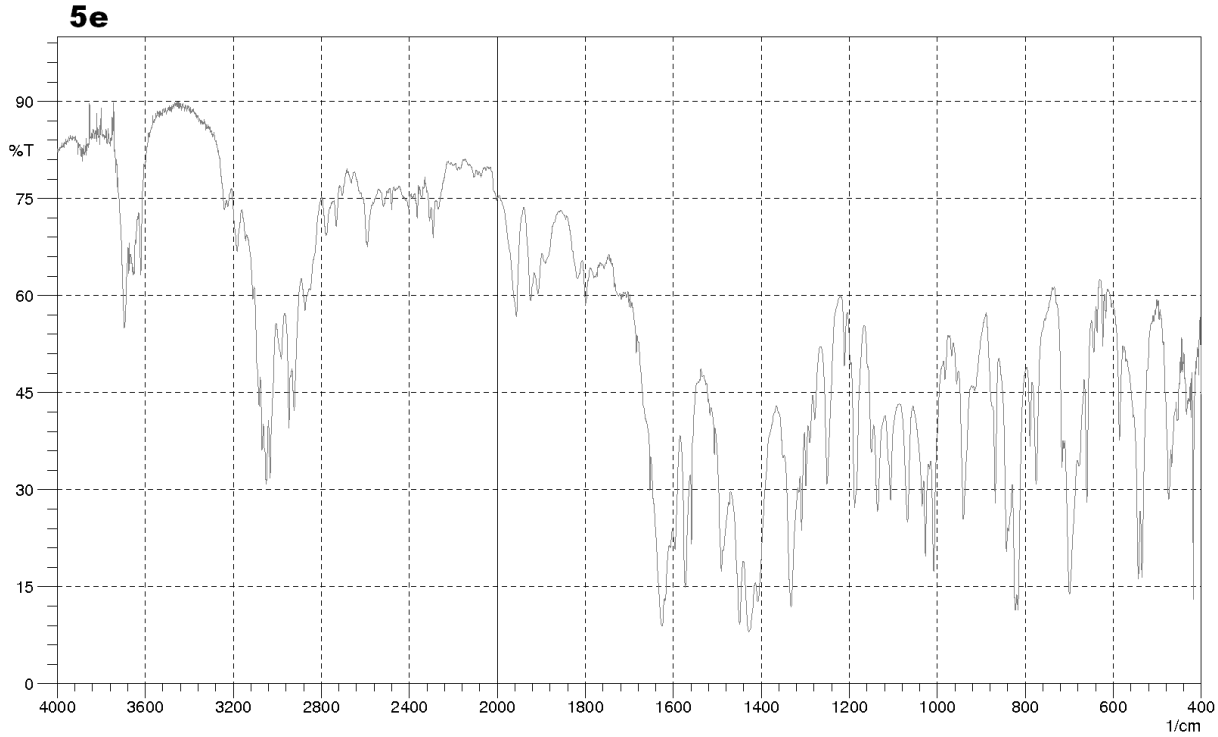
3. [5-(4-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5c)**



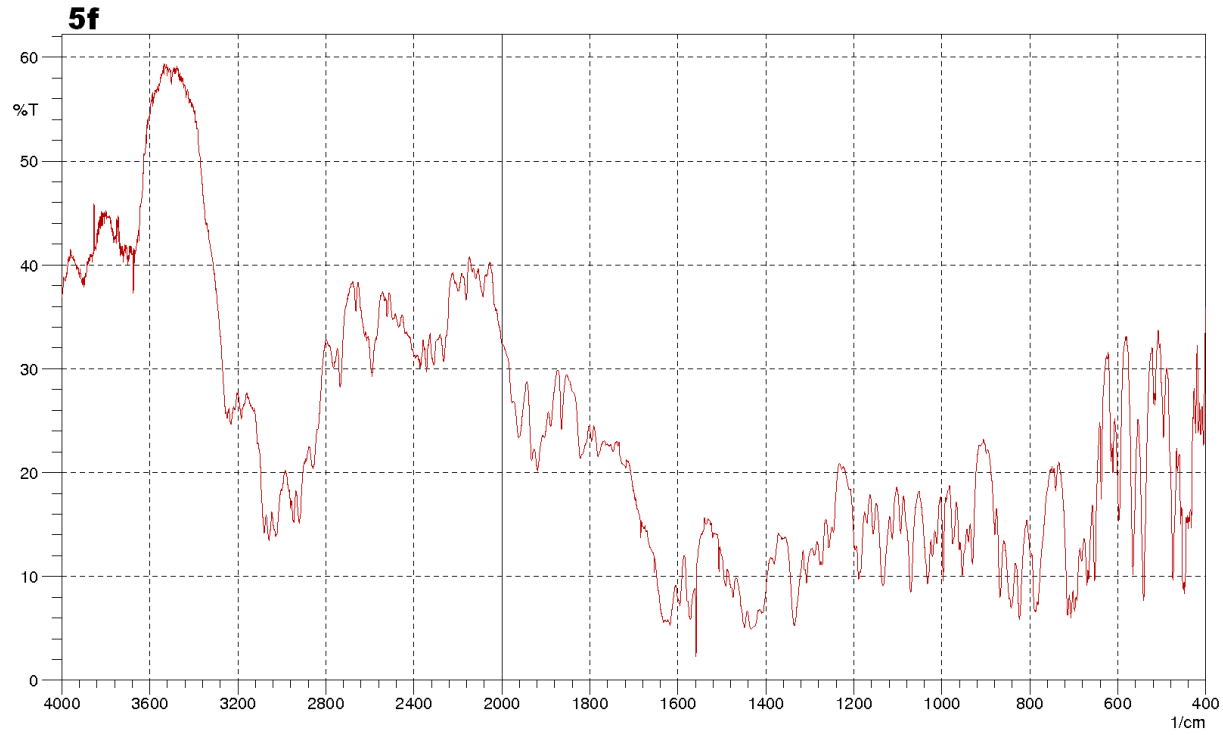
4. [5-(3-Chlorophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5d)**



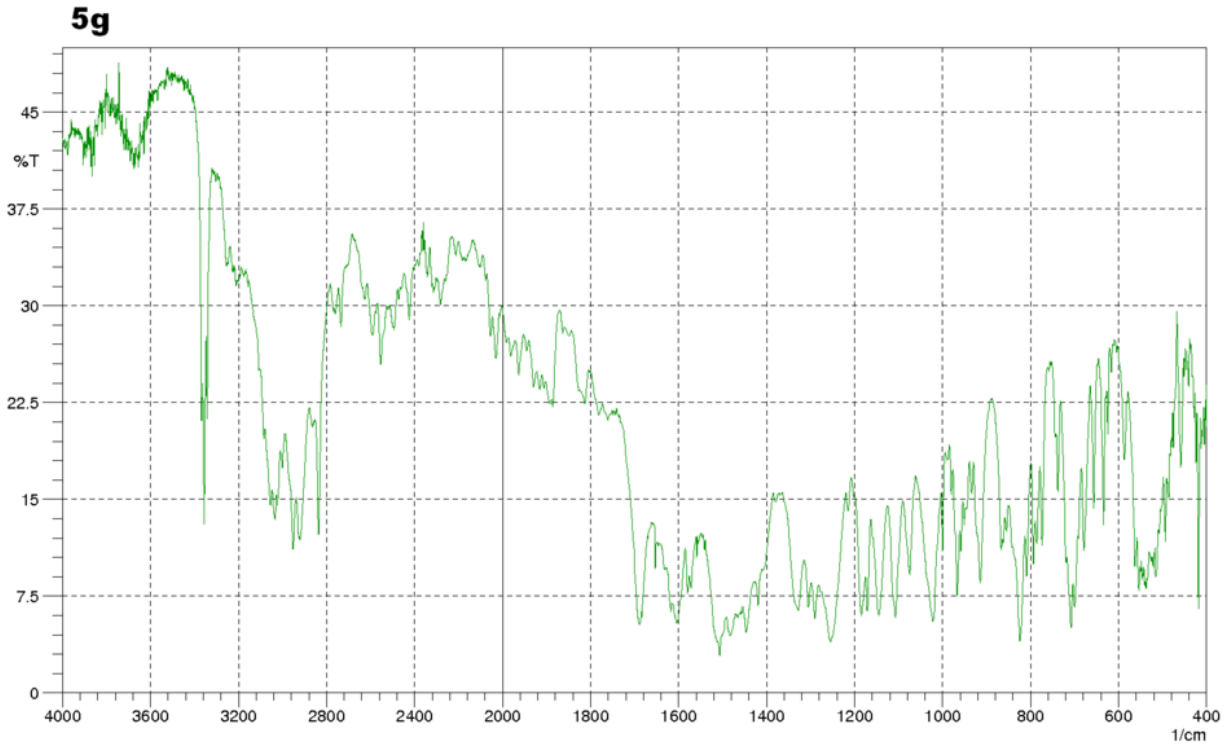
5. [5-(4-Bromophenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5e)**



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

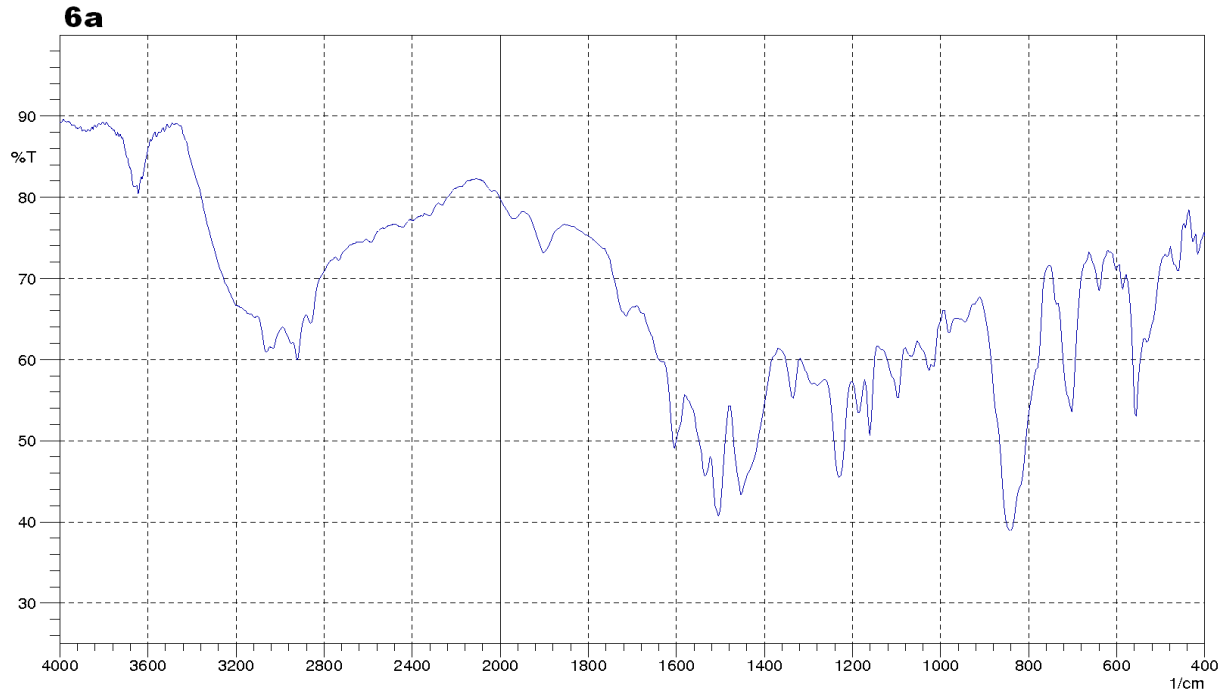


7. [5-(4-Methoxyphenyl)-3-(p-tolyl)-4, 5-dihydro-1H-pyrazol-1-yl]-phenyl methanone **(5g)**

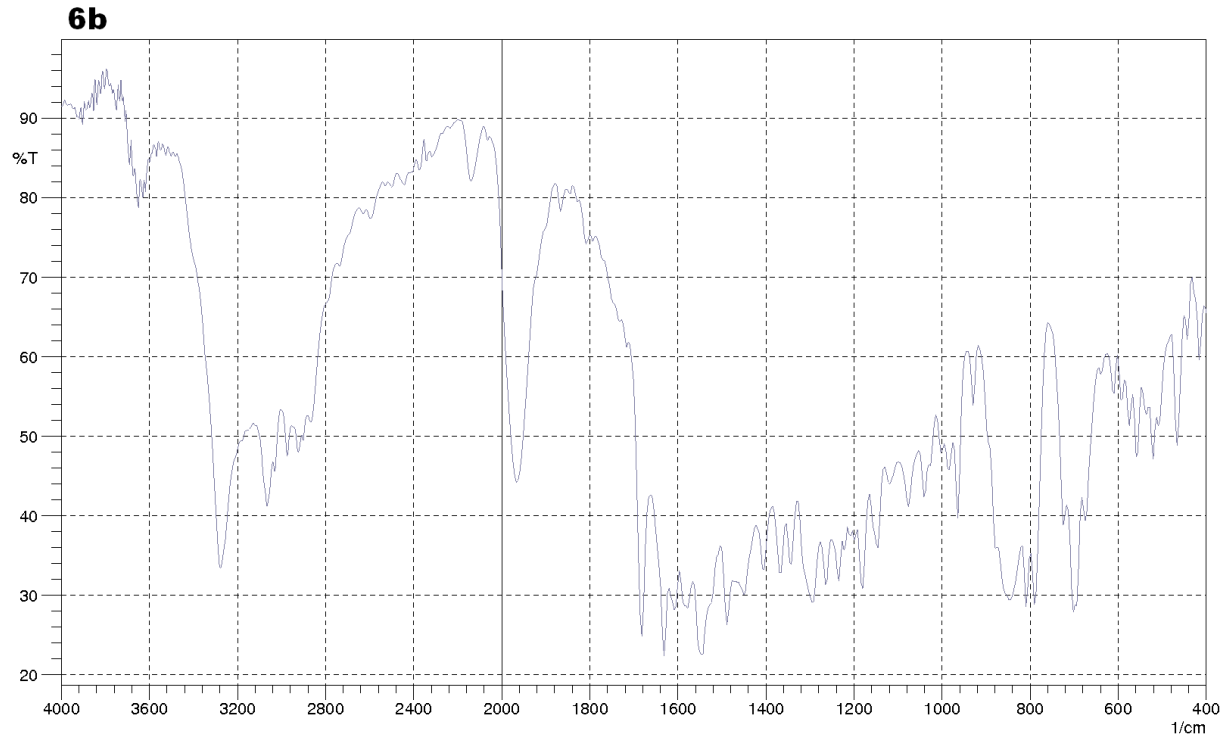


Supplementary material 5: FT-IR spectra of complexes (6a–6g)

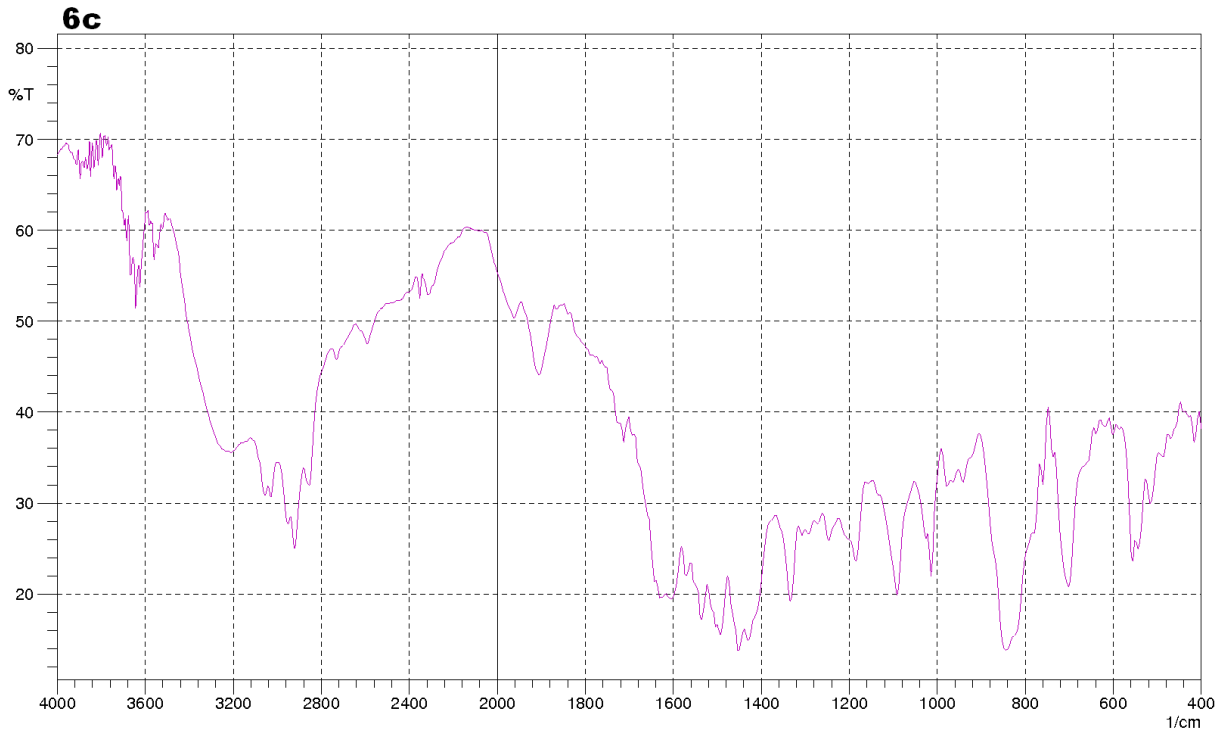
1. Complex 6a



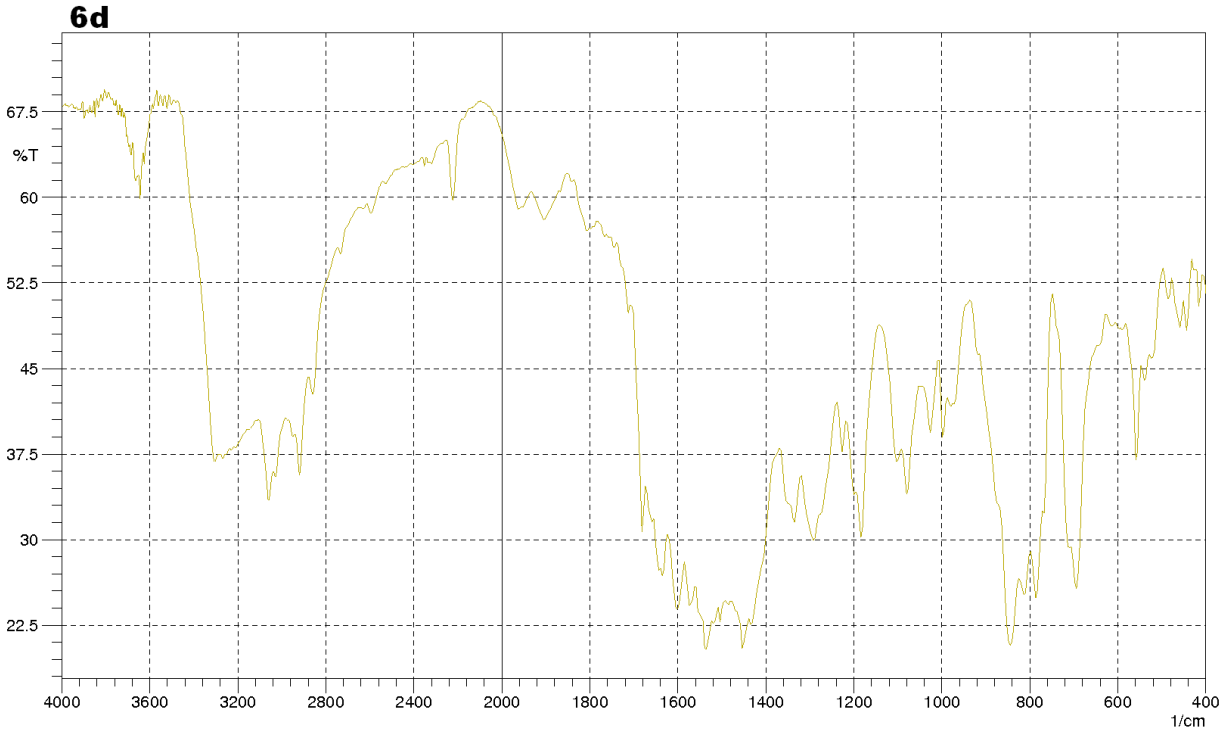
2. Complex 6b



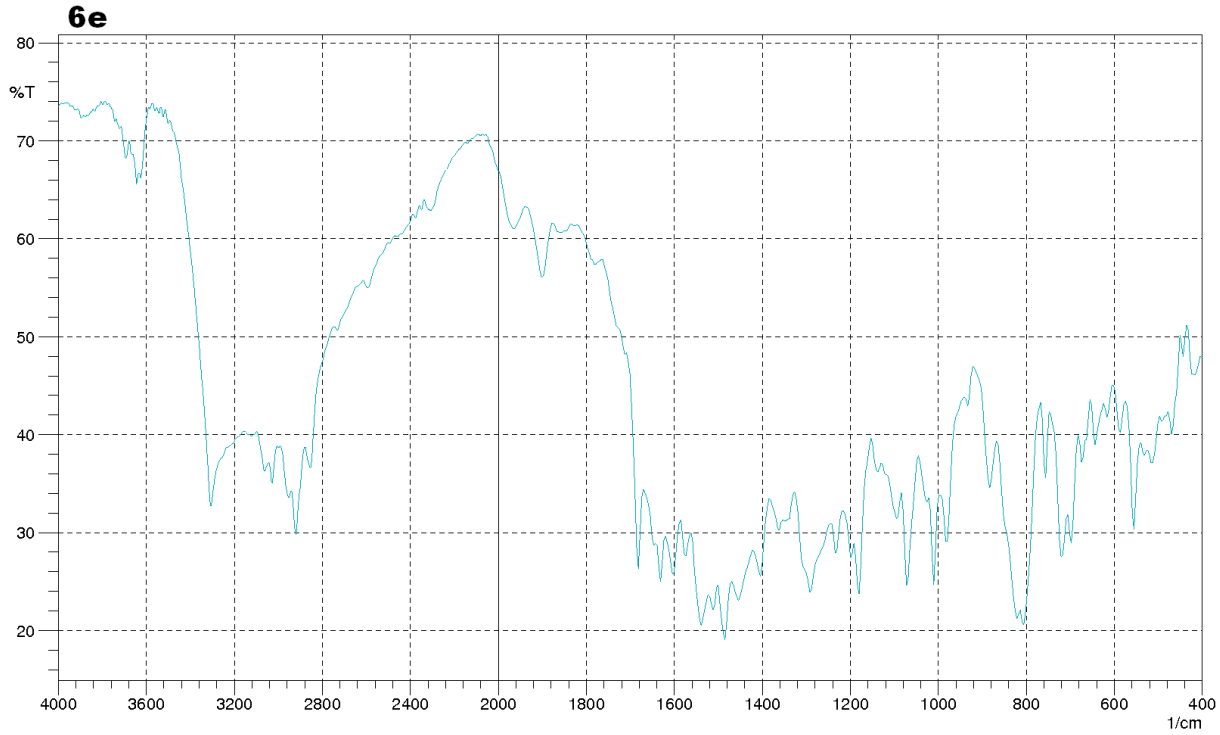
3. Complex 6c



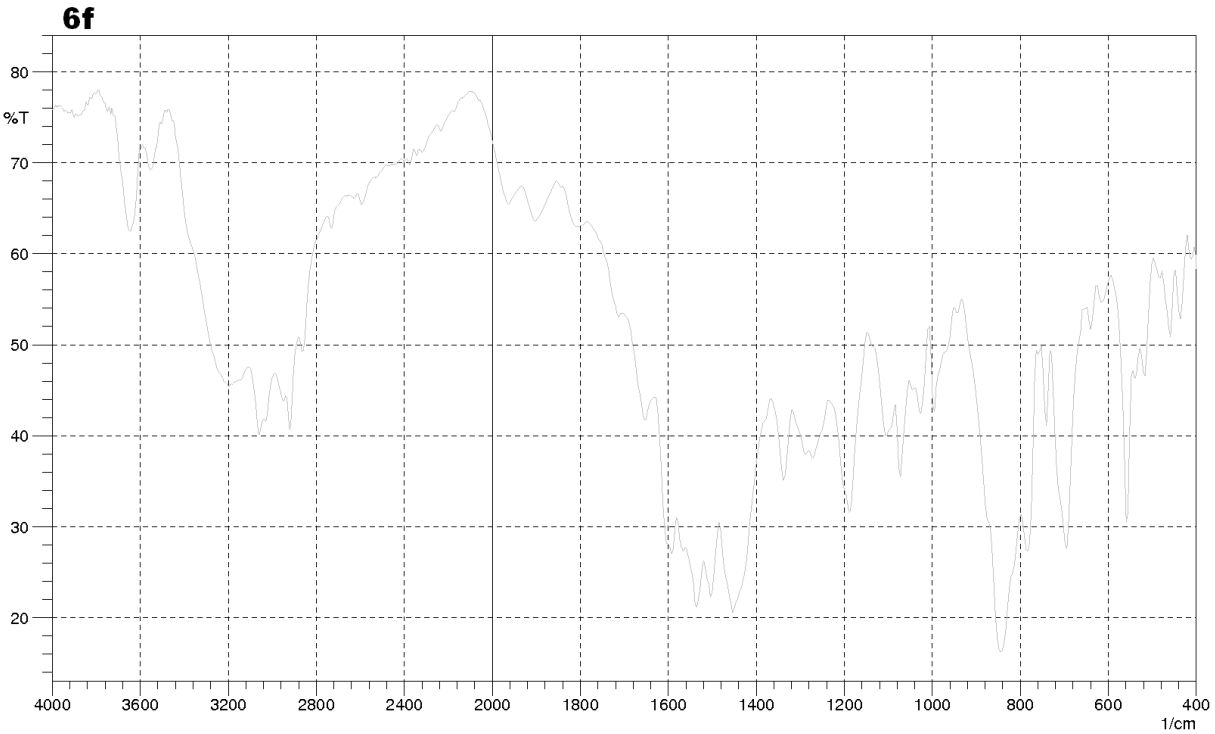
4. Complex 6d



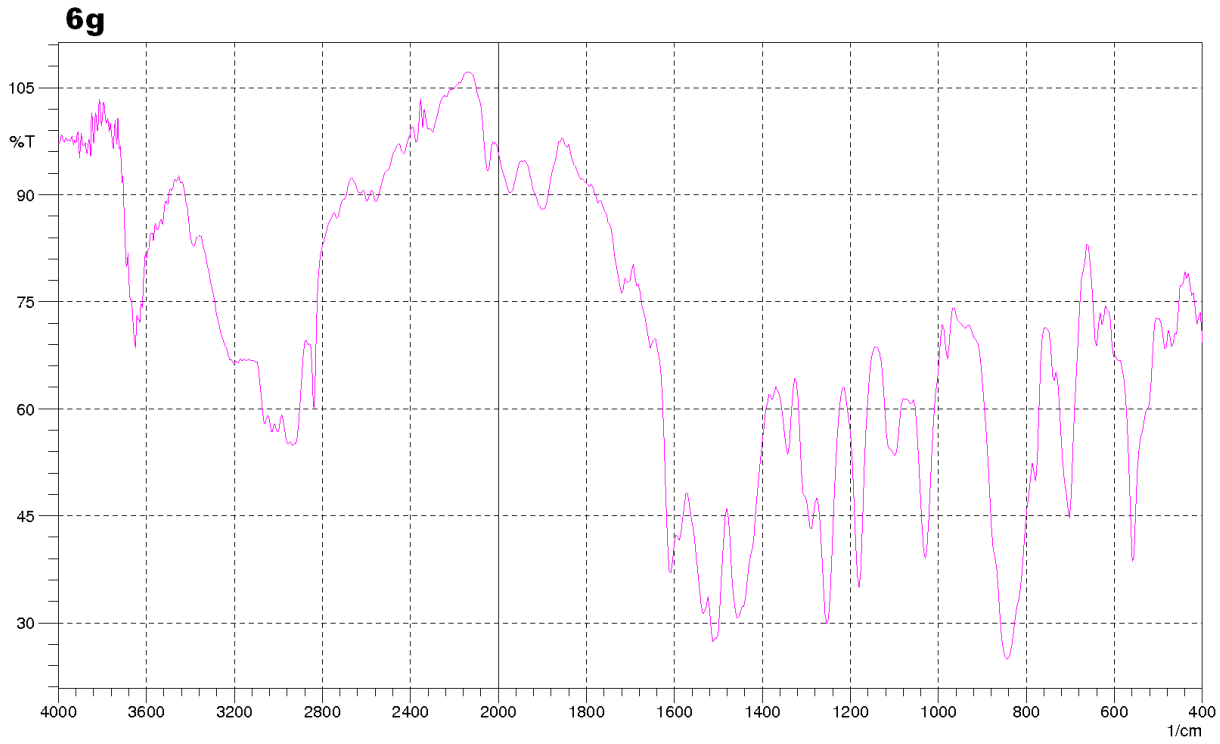
5. Complex 6e



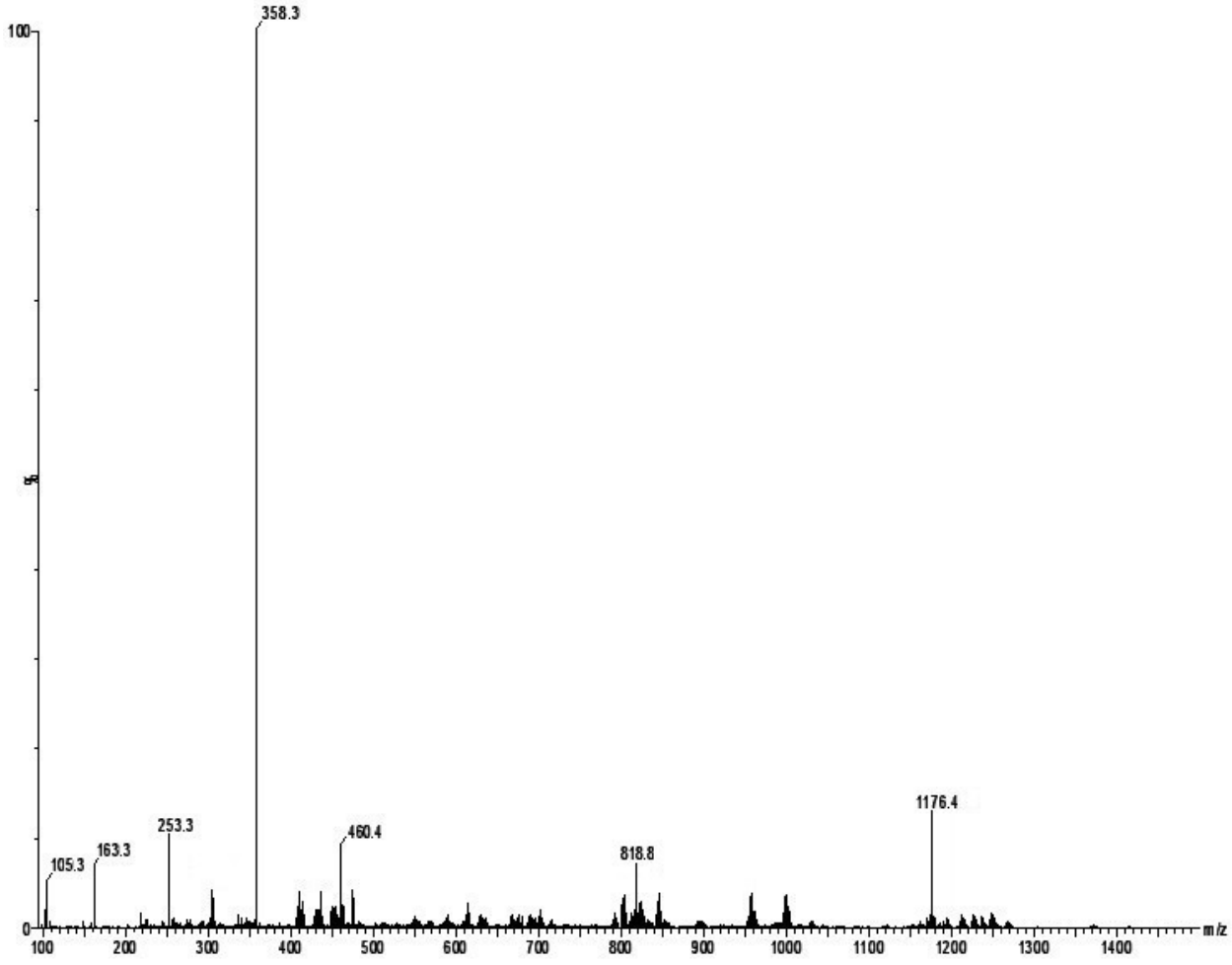
6. Complex 6f



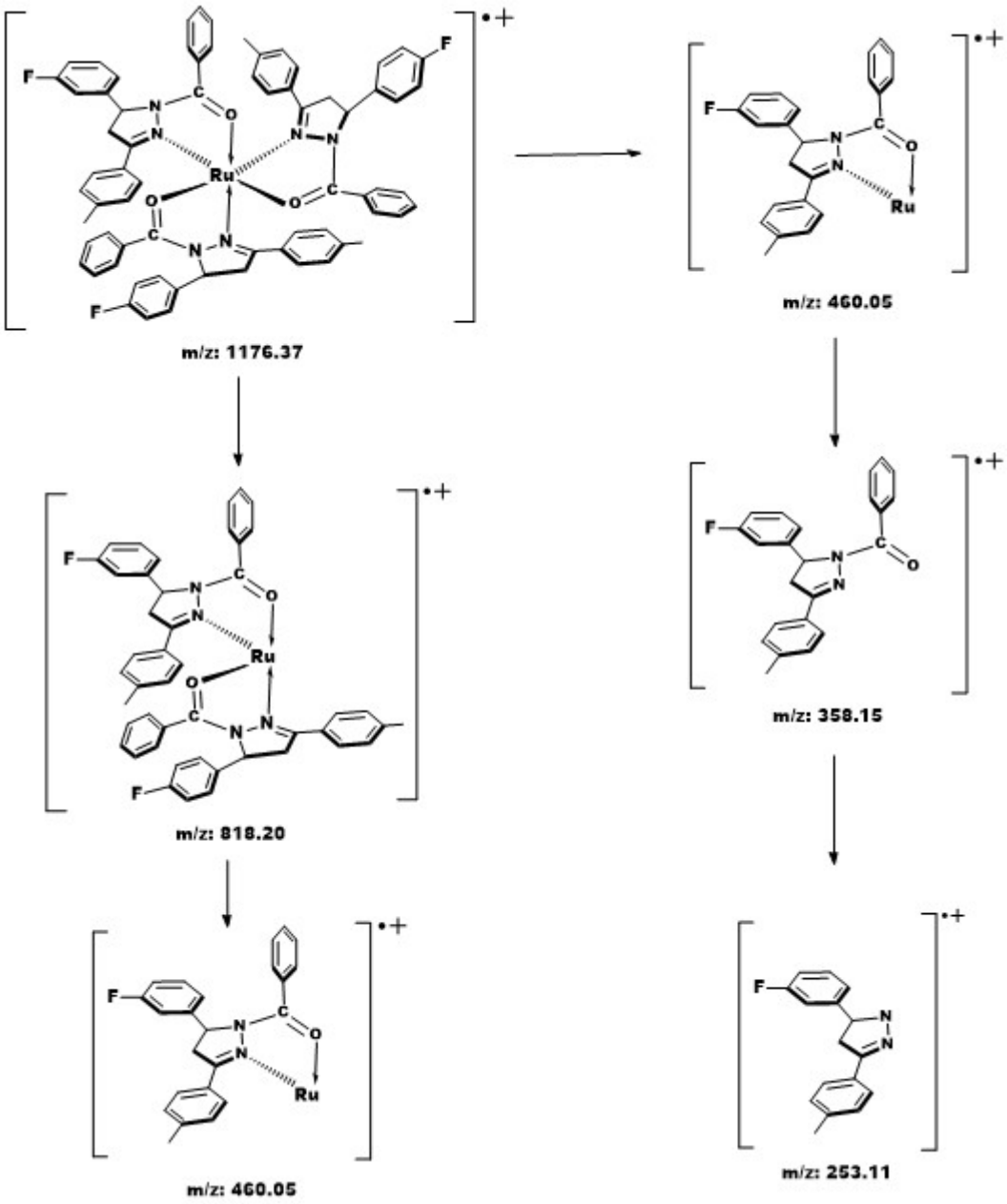
7. Complex 6g


6g
105
%T
90
75
60
45
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm

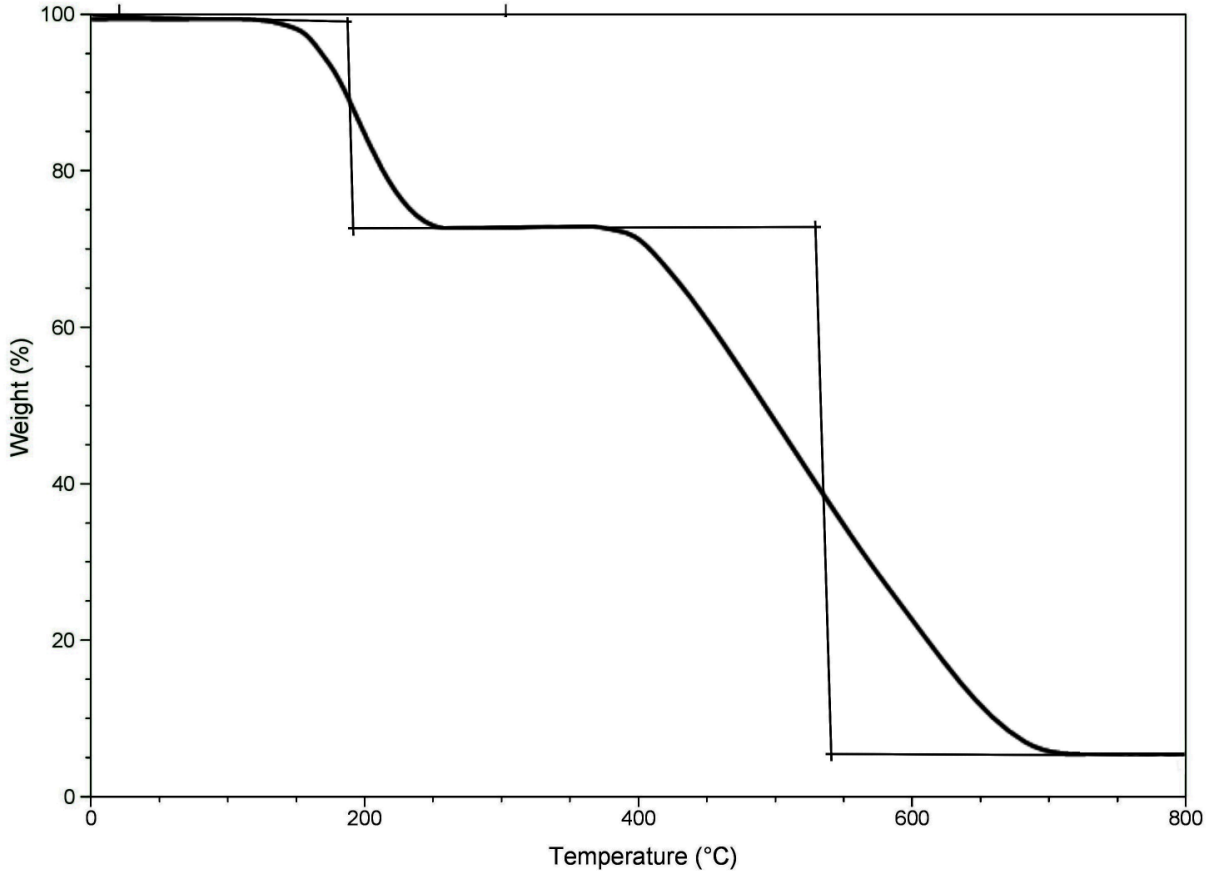
Supplementary material 6: LC-mass spectrum of complex 6a



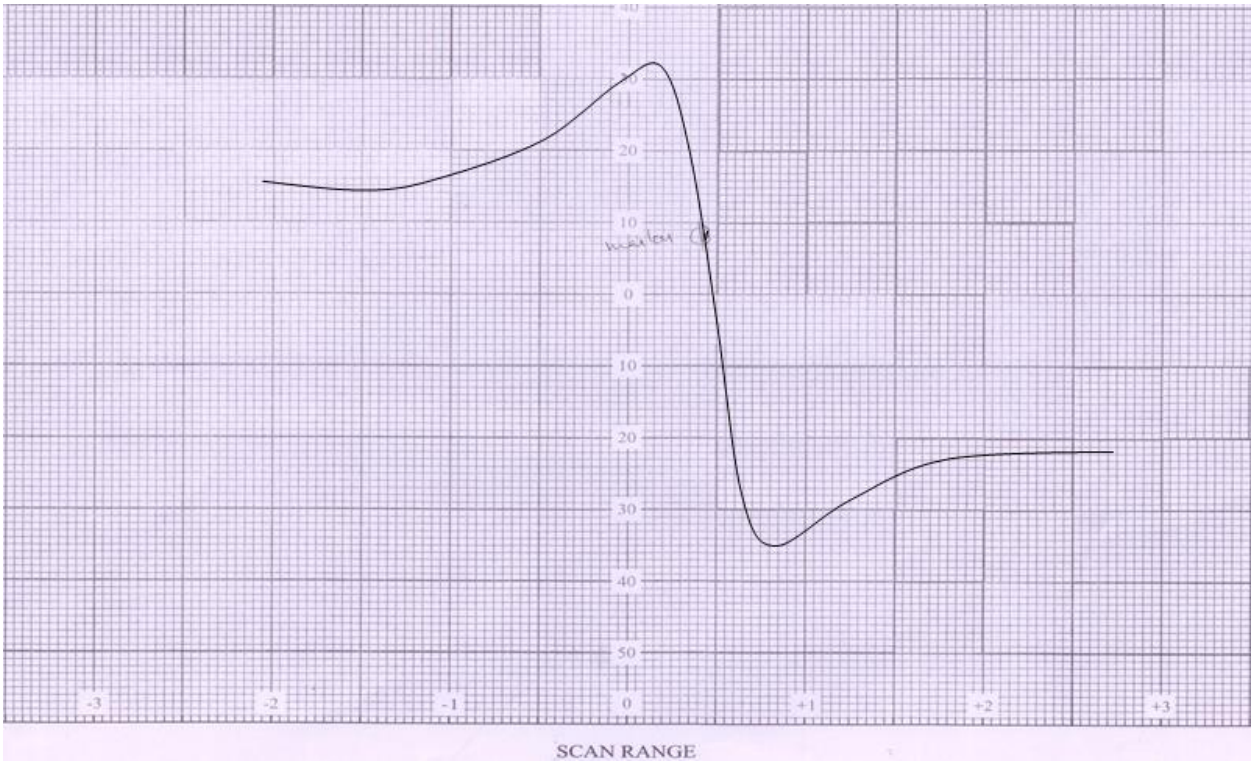
Supplementary material 7: Mass fragmentation pattern of complex 6a



Supplementary material 8: Thermogravimetric analysis (TGA) of complex 6a



Supplementary material 9: An electron paramagnetic resonance (EPR) spectrum of complex 6a



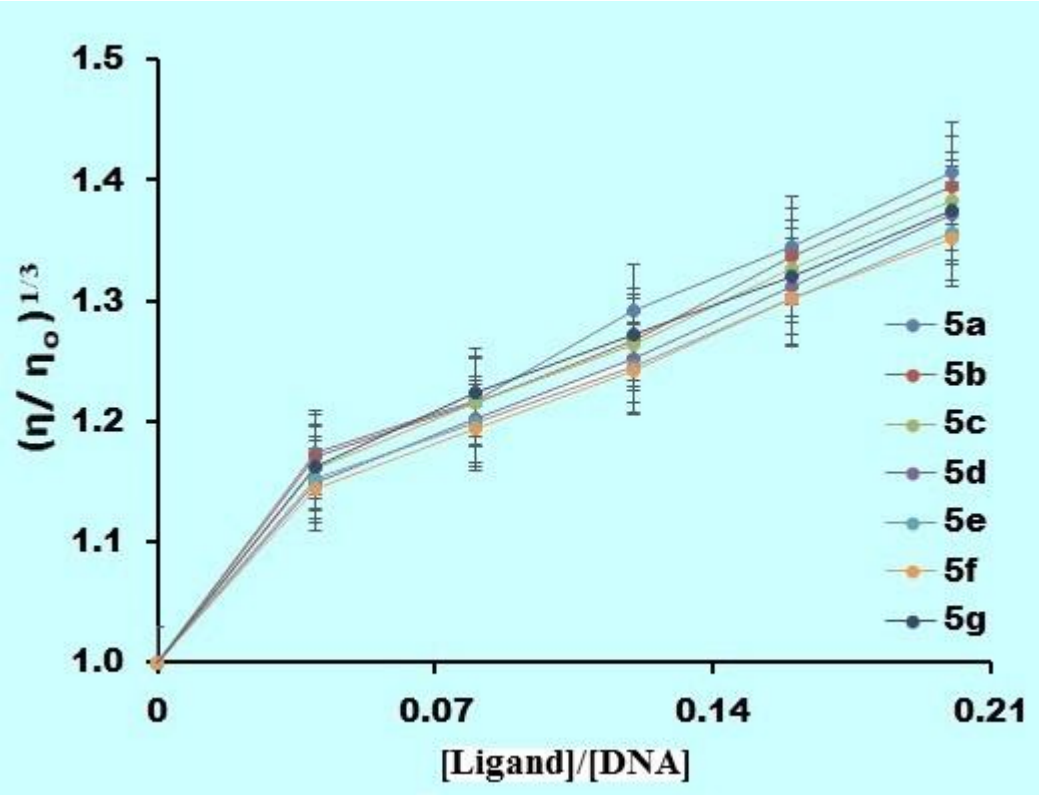
Supplementary material 10: Bacteriostatic concentration of free ligands and synthesized complexes by broth dilution method in terms of MIC in µM with error uncertainty in the value ±5 %

Compounds	Gram(+ve)		Gram(-ve)		
	S. Aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
5a	260±3	242±3	281±2	272±2	210±2
5b	271±3	250±3	285±3	278±3	214±3
5c	287±2	273±2	295±3	310±3	240±2
5d	302±4	285±2	307±4	298±3	245±2
5e	310±2	297±2	315±4	311±3	257±2
5f	305±2	315±3	328±2	327±3	267±3
5g	292±3	295±2	305±3	307±3	247±2
6a	82±1	80±2	86±1	91±1	56±1
6b	87±1	83±1	87±1	94±1	60±1
6c	92±2	88±1	91±1	104±1	72±1
6d	95±1	94±1	97±1	102±1	75±1
6e	98±1	105±1	106±2	107±2	81±1
6f	101±1	102±2	105±1	112±2	83±1
6g	93±1	97±1	119±1	109±1	73±1

Supplementary material 11: 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 %

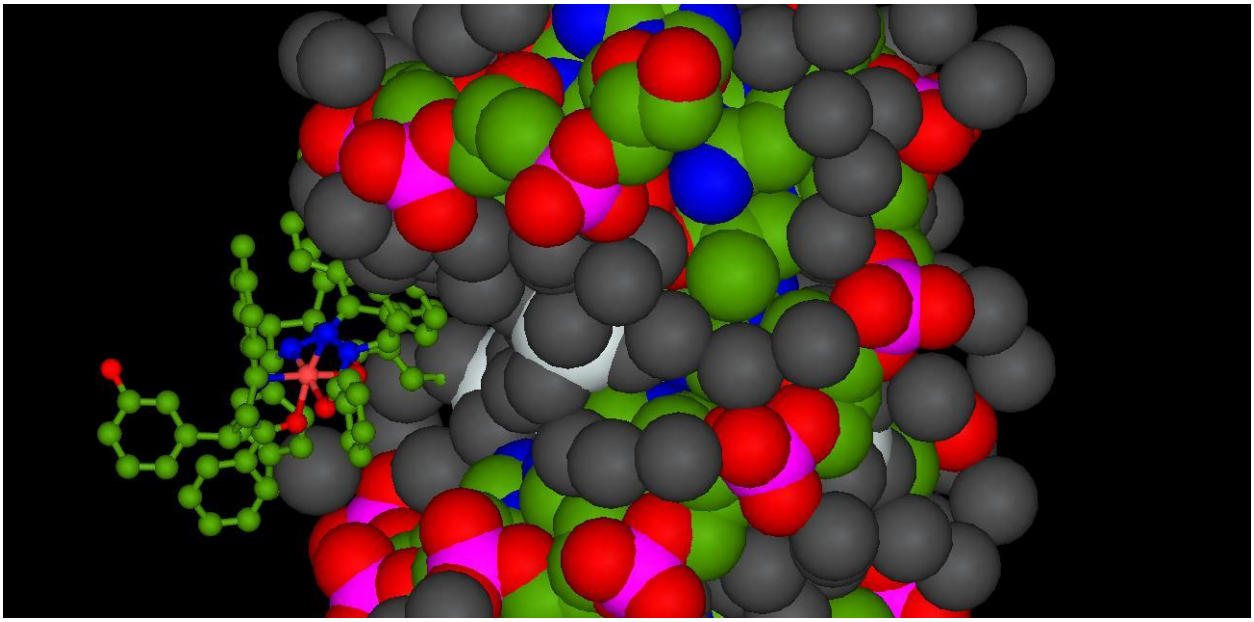
Compounds	K_b (M^{-1})	%H	$\Delta\lambda$ nm	IC_{50}(µg/ml)	LC_{50}(µg/ml)
5a	$0.505\pm0.01 \times 10^5$	19.67±0.58	3.0±0.3	1.10±0.05	10.658±0.24
5b	$0.446\pm0.02 \times 10^5$	17.83±0.54	3.0±0.2	1.14±0.05	11.803±0.28
5c	$0.441\pm0.01 \times 10^5$	18.41±0.87	3.0±0.2	1.15±0.06	15.739±0.35
5d	$0.419\pm0.01 \times 10^5$	18.35±0.77	3.0±0.2	1.20±0.05	16.143±0.46
5e	$0.417\pm0.01 \times 10^5$	17.94±0.81	3.0±0.2	1.23±0.06	15.703±0.48
5f	$0.346\pm0.01 \times 10^5$	18.40±0.62	3.0±0.3	1.22±0.07	16.143±0.54
5g	$0.344\pm0.01 \times 10^5$	18.51±0.60	3.0±0.1	1.34±0.06	17.579±0.66
6a	$6.07\pm0.25 \times 10^5$	22.10±0.56	3.0±0.2	0.54±0.01	5.296±0.21
6b	$5.50\pm0.19 \times 10^5$	18.52±0.64	3.0±0.3	0.56±0.01	5.631±0.23
6c	$4.32\pm0.18 \times 10^5$	18.47±0.98	2.5±0.3	0.69±0.02	7.538±0.31
6d	$5.19\pm0.19 \times 10^5$	19.47±0.80	3.0±0.3	0.75±0.02	7.645±0.32
6e	$2.42\pm0.09 \times 10^5$	20.16±0.85	3.0±0.3	0.78±0.03	7.670±0.34
6f	$3.80\pm0.11 \times 10^5$	19.21±0.79	3.0±0.1	0.80±0.03	7.897±0.33
6g	$3.11\pm0.08 \times 10^5$	21.44±0.66	2.5±0.1	0.92±0.04	7.925±0.35

Supplementary material 12: Effect on relative viscosity of HS DNA under the influence of increasing amounts of ligands at 27 (±0.1) °C in phosphate buffer at pH=7.2 with error uncertainty in the value ±5 %

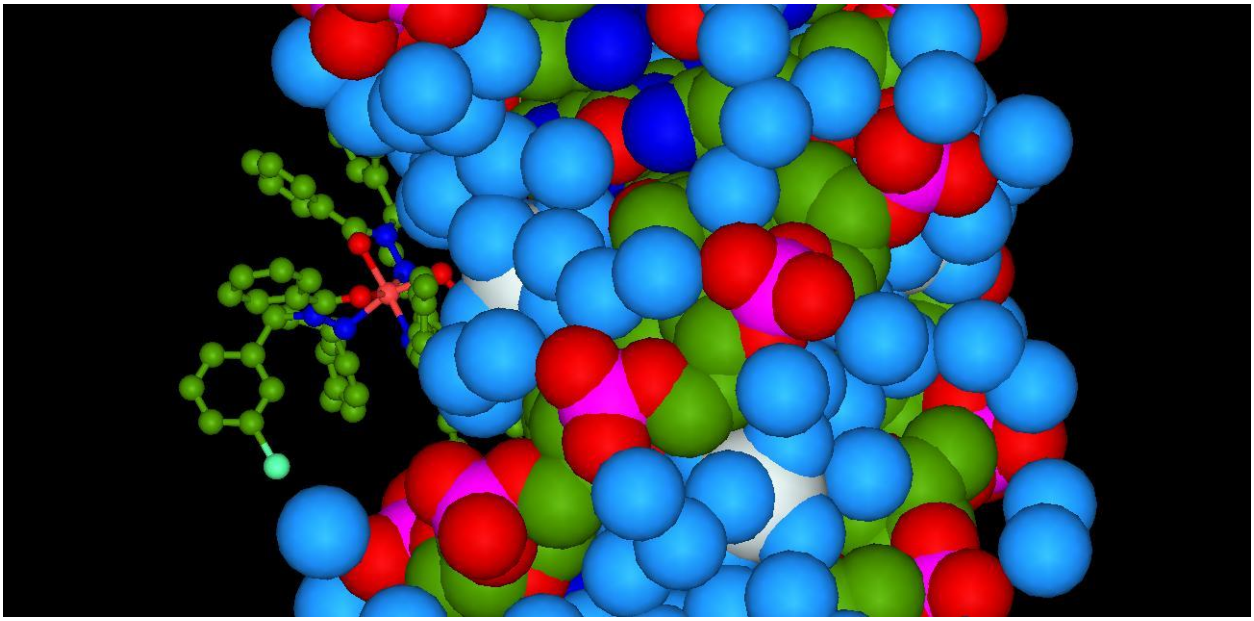


Supplementary material 13: Molecular docking of the complexes 6b–6g (ball and stick) with the DNA duplex (VDW spheres) of sequence d(ACCGACGTCGGT)$_2$. The complex is docked in to the DNA showing intercalation between the DNA base pairs.

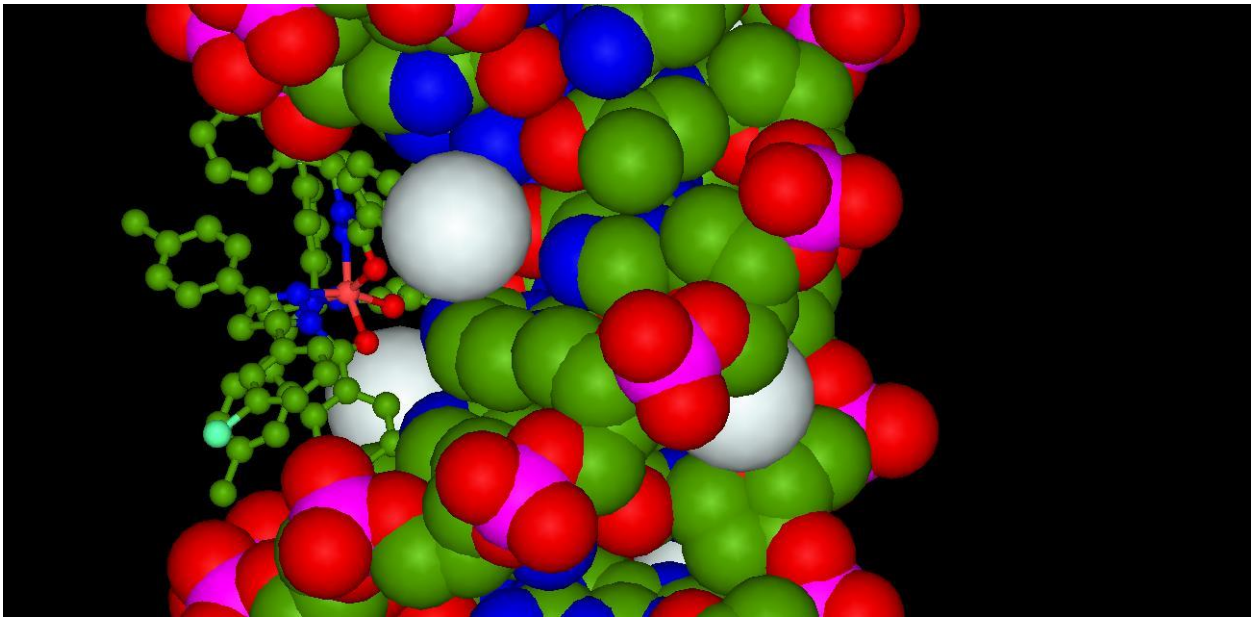
1. $[Ru^{III}(5b)_3](PF_6)_3$ **(6b)**



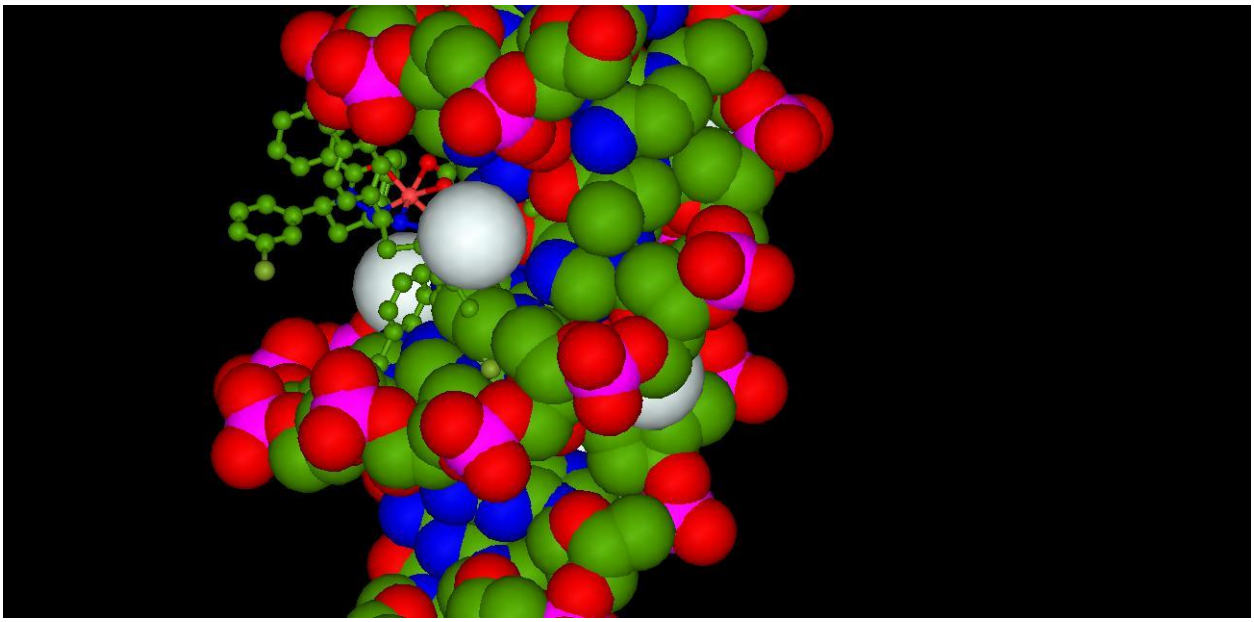
2. $[Ru^{III}(5c)_3](PF_6)_3$ **(6c)**



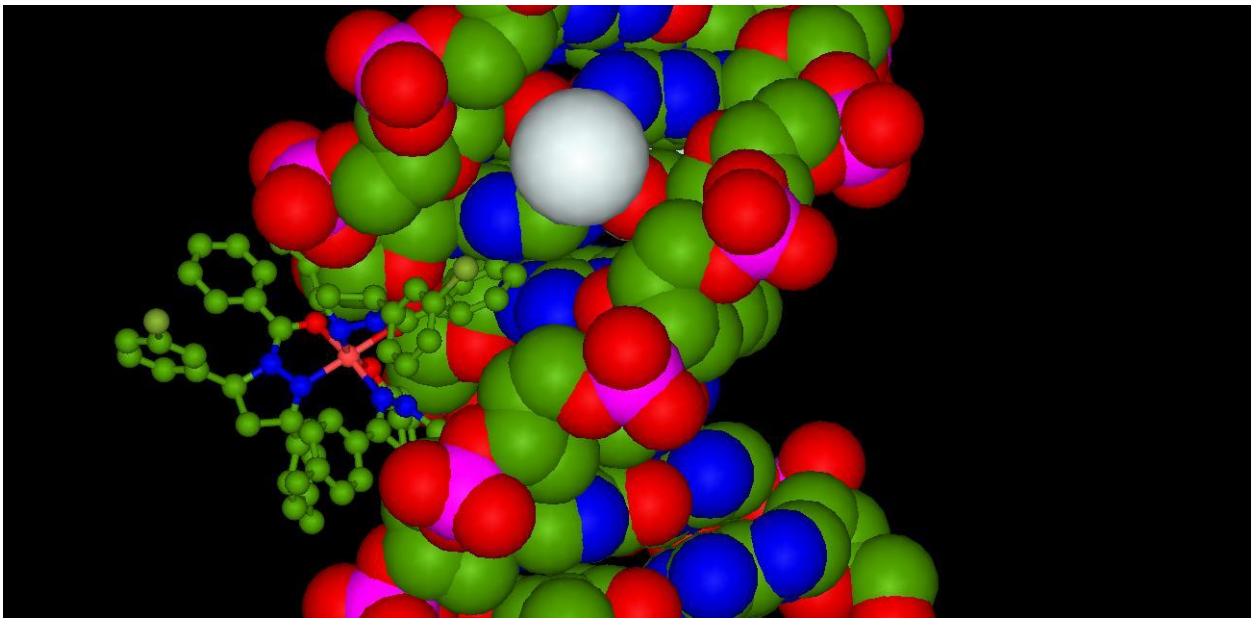
3. $[Ru^{III}(5d)_3](PF_6)_3$ **(6d)**



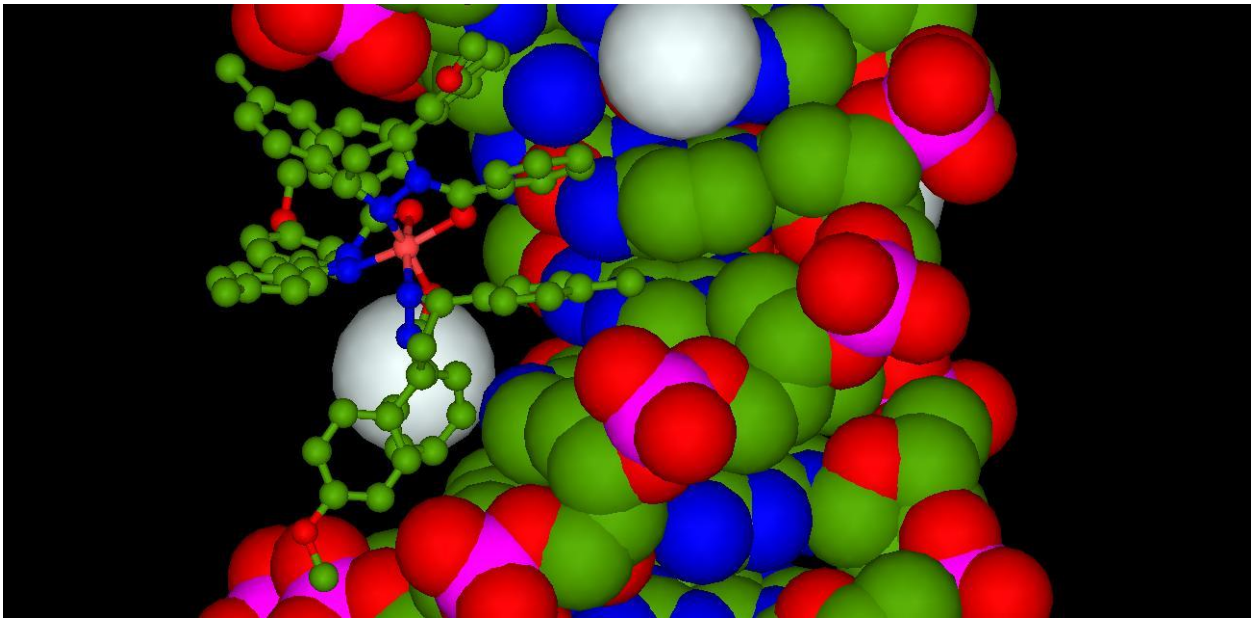
4. $[Ru^{III}(5e)_3](PF_6)_3$ **(6e)**



5. $[Ru^{III}(5f)_3](PF_6)_3$ **(6f)**



6. $[Ru^{III}(5g)_3](PF_6)_3$ **(6g)**



Supplementary material 14: Complex mediated DNA cleavage data by agarose gel electrophoresis with error uncertainty in the value ±5 %

Lane No.	Compound	Form I	Form II	Form III	% Cleavage
1	DNA Control	89±2	11±1	–	–
2	$RuCl_3 \cdot 3H_2O$	82±2	18±1	–	07.86±0.2
3	6a	25±1	51±1	24±1	71.91±1.5
4	6b	28±1	40±1	32±1	68.54±1.4
5	6c	29±1	44±1	27±1	67.42±1.4
6	6d	30±1	45±1	25±1	66.29±1.2
7	6e	32±1	48±1	20±1	64.04±1.3
8	6f	35±1	45±1	20±1	60.67±1.2
9	6g	34±1	47±1	19±1	61.80±.4
10	5a	61±2	21±1	18±1	31.46±0.7
11	5b	62±2	21±1	17±1	30.34±0.5
12	5c	60±2	20±1	20±1	32.58±0.6
13	5d	49±1	24±1	27±1	44.94±0.8
14	5e	59±1	18±1	23±1	33.71±0.5
15	5f	52±1	23±1	25±1	41.57±0.8
16	5g	57±	16±1	27±1	35.96±0.7

Supplementary material 15: Effect of compounds on viability of *S. Pombe* cells at different concentrations with error uncertainty in the value ±5 %

Concentration(µg/mL)	**2**	**4**	**6**	**8**	**10**
Compounds			**% Viability**		
5a	70	68	61	60	58
5b	72	68	63	61	59
5c	76	67	65	62	60
5d	78	68	65	63	61
5e	80	77	72	68	64
5f	83	78	70	70	65
5g	82	75	70	68	60
6a	60	54	50	46	42
6b	65	61	57	55	46
6c	66	62	60	57	52
6d	67	62	59	54	53
6e	70	65	62	58	55
6f	72	67	63	60	57
6g	68	62	60	55	52
DMSO			96		
Untreated cells			97		