Electronic Supplementary Material (ESI) for MedChemComm.

Synthesis, cytotoxic and urease inhibitory activities of some novel isatin-derived *bis*-Schiff bases and their copper(II) complexes

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Material and methods

Biological assays

Cytotoxicity assay

Cell lines and cultures

Lung carcinoma (H-157), (ATCC CRL-5802) and African green monkey kidney normal cell line (Vero), (ATCC CCL-81) were kept in RPMI-1640, having heat-inactivated fetal bovine serum (10%) glutamine (2 mM), pyruvate (1 mM), 100 U/mL penicillin and 100 µg/mL streptomycin, in T-75 cm^2 sterile tissue culture flasks in a 5% CO_2 incubator at 37 °C.[1] For experiment, 96-well plates were used for growing H-157 and Vero cells by inoculating 5×10^4 cells per 100 µL per well and plates were incubated at 37 °C for 24 h in a humidified atmosphere containing 5% CO_2. Within 24 h, a uniform monolayer was formed which was used for experiments.

Cytotoxicity analysis by sulforhodamine B (SRB) assay

To perform cytotoxicity assay, with H-157 and Vero cells, a previously described method by Skehan *et al*[2] was adapted with little modifications.[3] Briefly, cells were cultured in different 96

well plates for 24 h. The compounds in different concentrations were inoculated in test wells. Furthermore, positive control vincristine was prepared in DMSO. The well containing culture media with cells having no compound or drug was taken as blank. Vero cells were treated at 100 µM test compounds to check the toxicity against normal cell lines. The plates were then incubated for 48 h. After that, cells were fixed with 50 µL of 50% ice cold trichloroacetic acid solution (TCA) and plates were incubated at 4 °C for 1 h. Subsequently, plates were washed five times with phosphate-buffered saline (PBS) and air dried. Fixed cells were further treated with 0.4% w/v sulforhodamine B dye (prepared in 1% acetic acid solution) and left at room temperature for 30 min. After that the plates were rinsed with 1% acetic acid solution and allowed to dry. In order to solubilize the dye, the dried plates were treated with 10 mM Tris base solution for 10 min at room temperature. The absorbance was measured at 490 nm, subtracting the background measurement at 630 nm.[4] All the experiments were repeated at least three times. Results reported are mean of three independent experiments (± SEM) and expressed as percent inhibitions calculated by the formula:

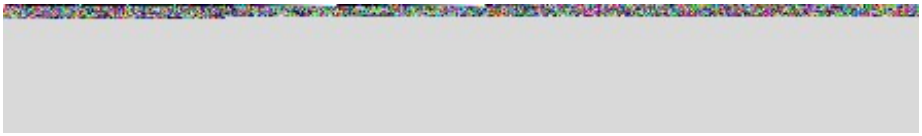
$$\text{Inhibition (\%)} = 100 - \left[\frac{\text{absorbance of the test compounds}}{\text{absorbance of the control}} \right] \times 100$$

IC_{50} values of potential inhibitors (≥50%) were determined with the help of the Graph Pad prism 5.0 Software Inc., San Diego, California, USA.[3]

Urease inhibition assay

Urease inhibition activity of the synthesized compounds was determined by indophenol method[5] with little modification.[6] Reaction mixtures comprising 40 µL of buffer (100 mmol/L urea, 0.01 mol/L K_2HPO_4, 1 mmol/ L EDTA and 0.01 mol/L $LiCl_2$, pH 8.2) and 10 µL of enzyme (5 U/mL) were incubated with 10 µL of test compounds (1 mM) and thiourea at 37 ^oC for 30 min in 96-well

plates. Urease inhibitory activity was calculated by indophenol method based on the production of ammonia. The phenol reagent (40 µL, 1 %, w/v phenol, 0.005 %, w/v sodium nitroprusside) and alkali reagent (40 µL, 0.5 %, w/v NaOH, 0.1 % active chloride NaOCl) were added to each well and after 10 min of incubation at 37 °C, the absorbance was measured at 630 nm using a microplate reader (Bio-TekELx 800™, Instruments, Inc. USA). All the experiments were performed in triplicate. Following equation was used for calculation of percentage inhibition.



Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions. The results were analyzed using PRISM 5.0 (GraphPad, San Diego, California, USA).

Docking protocols

Structure selection and preparation

Molecular docking studies were conducted to investigate putative interactions of the compounds in complex with the urease enzyme. In order to perform efficient docking studies, the crystallographic structure of Jack bean urease (PDB ID: 4H9M) was obtained from the RSCB PDB database[7] and prepared for the docking. Prior to experiments, the structures of the enzyme and the compounds were prepared as follows. The enzyme structure was protonated with the Protonate 3D[8] algorithm implemented in the molecular modeling tool MOE.[9] The structure was energy minimized using Amber99 force field including all crystallographic solvent molecules. The backbone atoms were restrained with a small force in order to avoid collapse of the binding pockets during energy minimization calculations. After minimization, the co-crystallized ligands and solvent molecules were removed. The bound ligand acetohydroxamic acid and crystallographic

water molecules were removed from the structure and hypothetical hydrogen atoms were added to the X-ray structure in standard geometries with the MOE.

Compounds preparation

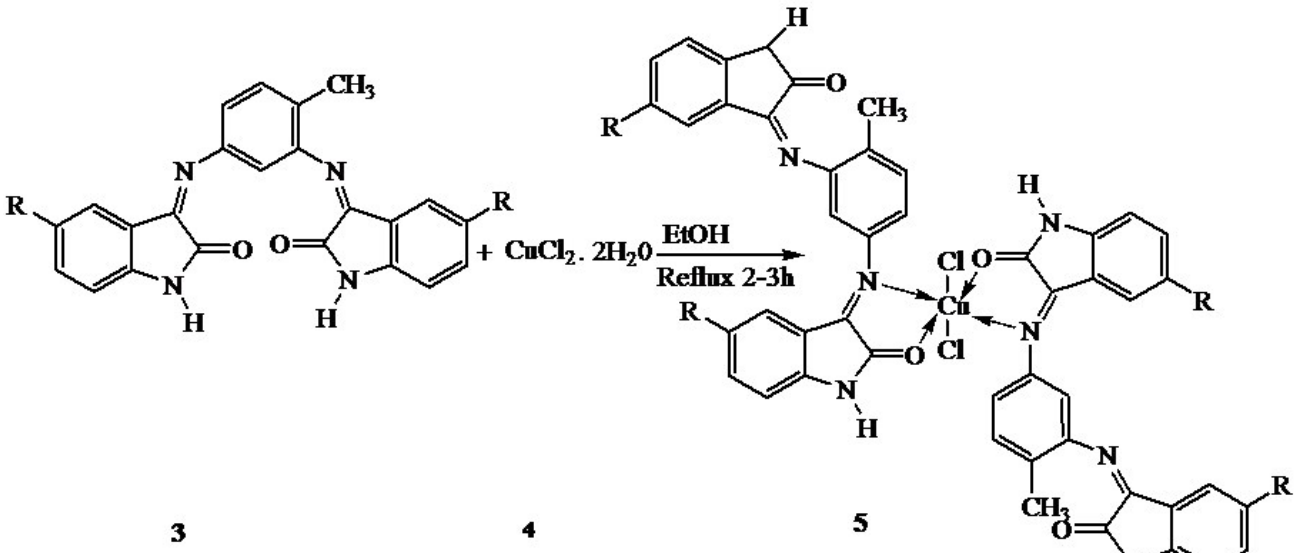
The 3D structural coordinates of the compounds were generated for all the compounds using MOE followed by assignment of protonation and ionization states in physiological pH range by using the "wash" module. Afterwards, the compounds' structures were energy minimized with the MMFF94x force field for docking studies.

Docking studies

For the docking studies, AutoDock Tools[10] was used to add atomic partial charges to the protein structure and the ligands. The point charge of the two catalytic nickel cations was manually set to +2. Gasteiger charges were calculated using AutoDock Tools.[10] The newly synthesized ligands (Schiff bases) were docked into the active sites of urease using the Lamarckian Genetic Algorithm in AutoDock 4.2 with rigid protein structure and fully flexible ligands. Possible binding modes of the Schiff bases were explored by visual inspection of the resulting docking poses. The Discovery Studio 4.0 Visualizer was used for visualizing the results.[11]

Results and Discussion

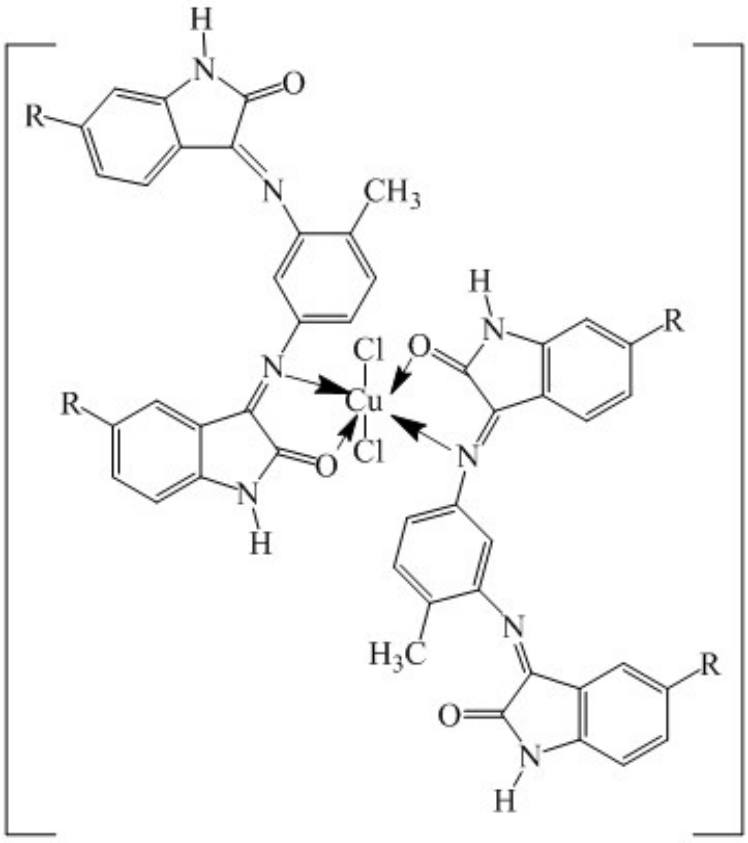
Chemistry of the copper(II) complexes 5a-g



3 **4** **5**

R:
5a = H
5b = Br
5c = F
5d = Cl
5e = CH_3
5f = SO_3H
5g = NO_2

Scheme S1. Synthesis of title Schiff base Cu(II) complexes **5a-g.**



R:

5a = H (L_1)
5b = Br (L_2)
5c = F (L_3)
5d = Cl (L_4)
5e = CH_3 (L_5)
5f = SO_3H (L_6)
5g = NO_2 (L_7)

Figure S1. The proposed structural formulae of metal complexes **5a-g.**

Raman spectra of some representative compounds

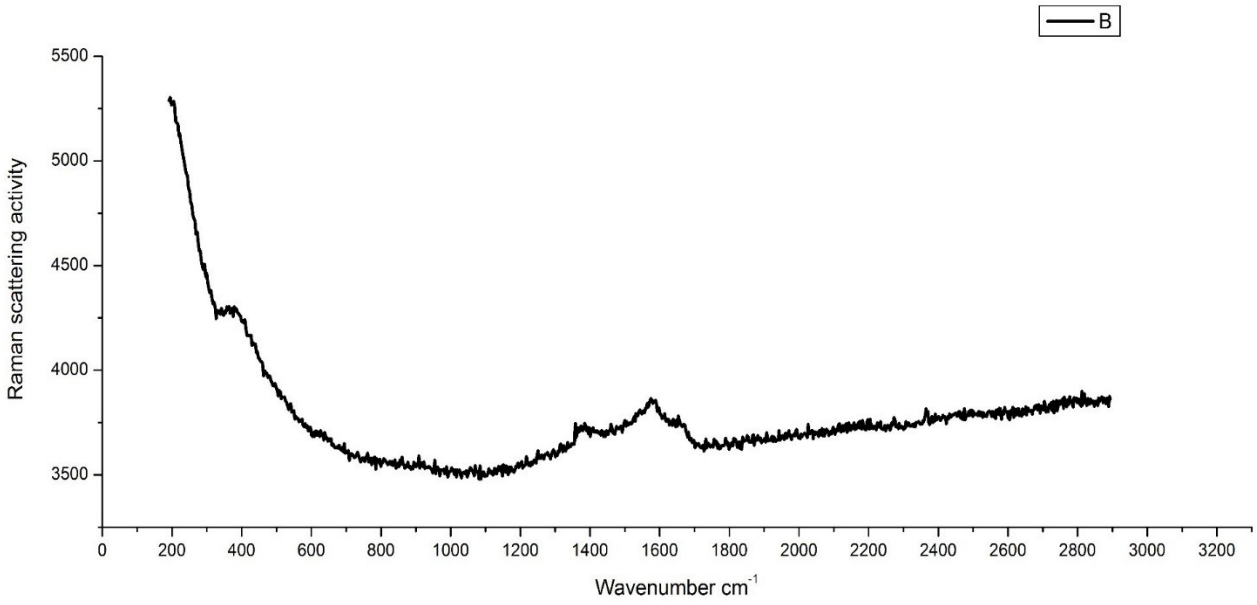


Figure S2. Raman spectrum of **5c** $[Cu(L_3)_2Cl_2]$

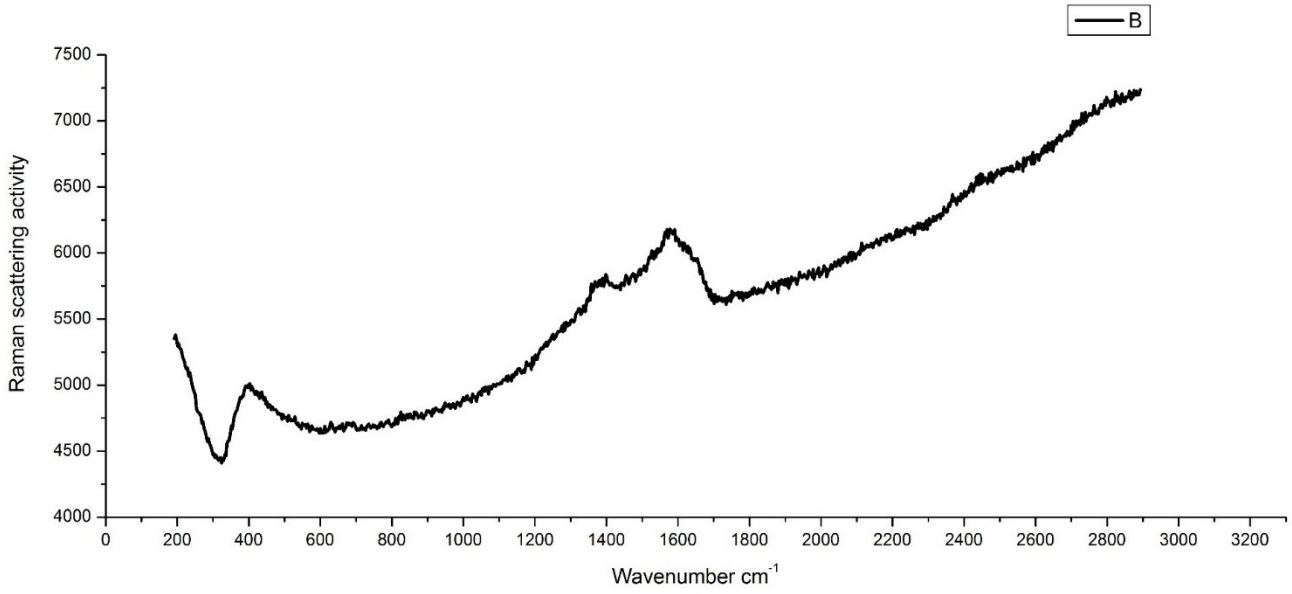


Figure S3. Raman spectrum of **5d** $[Cu(L_4)_2Cl_2]$

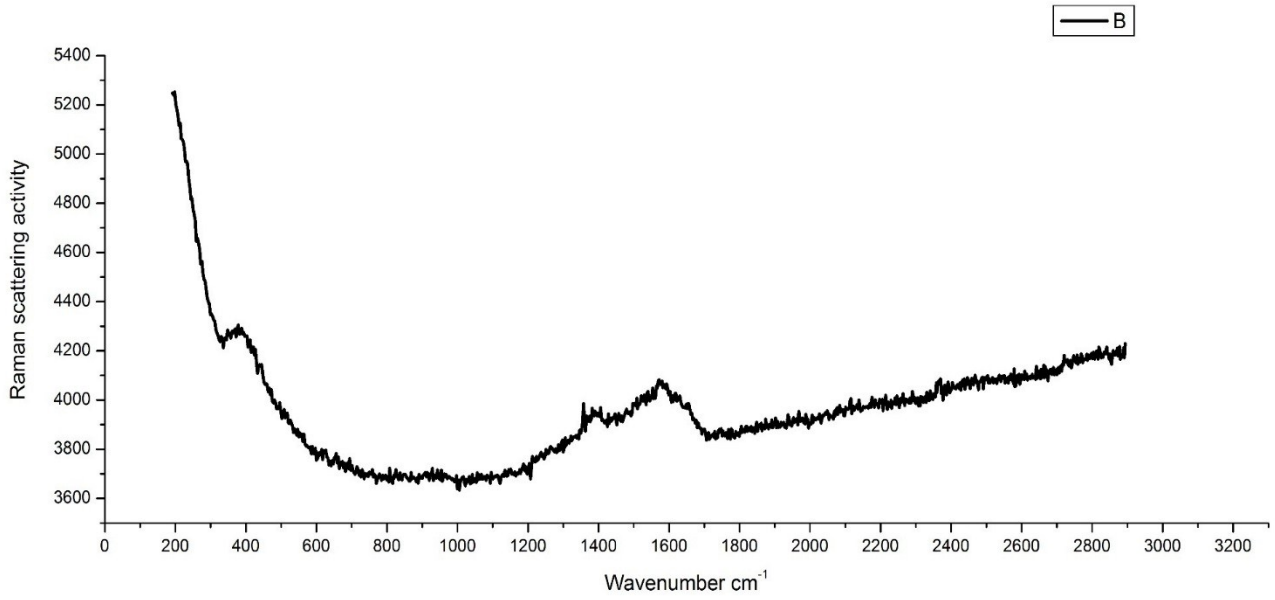


Figure S4. Raman spectrum of **5e** $[Cu(L_5)_2Cl_2]$

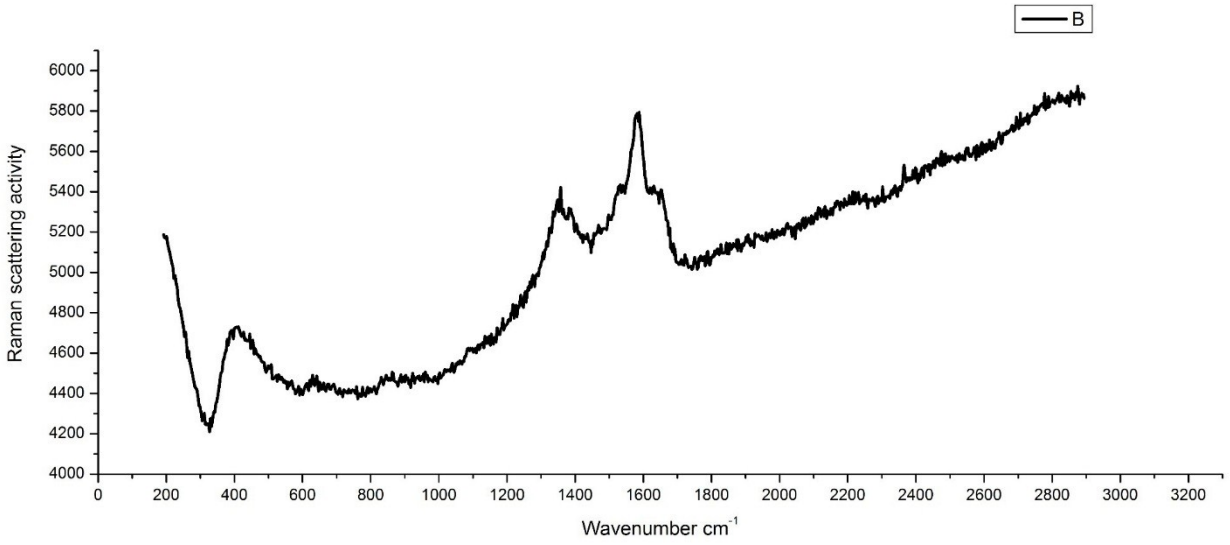
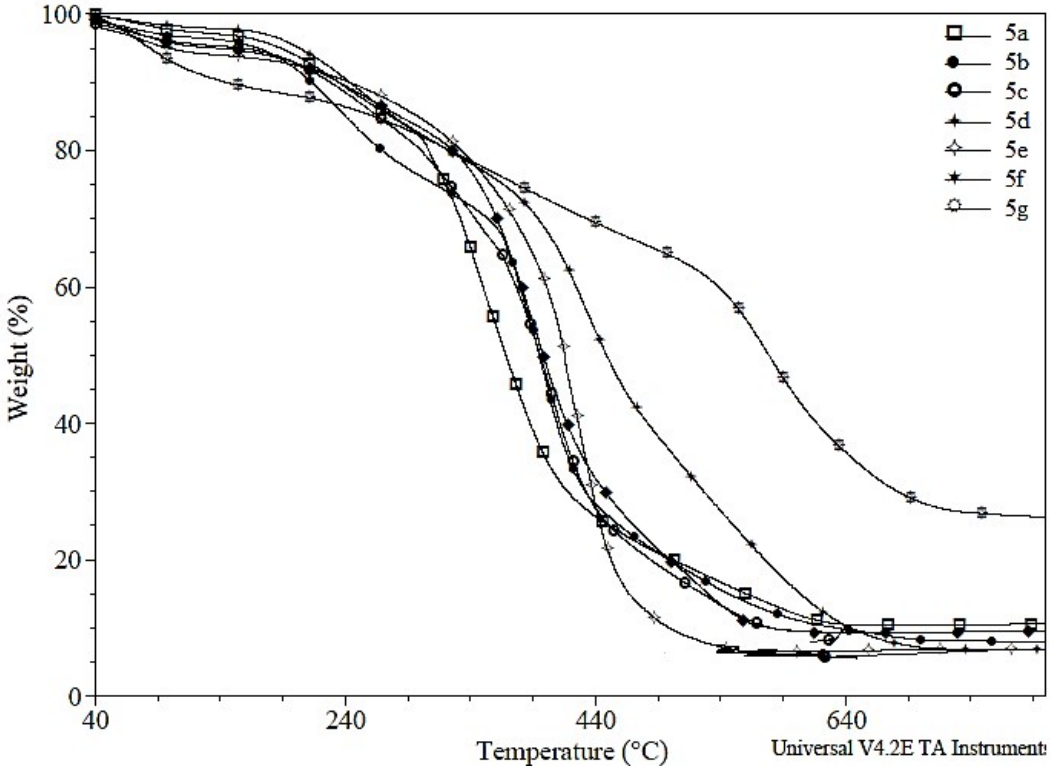


Figure S5. Raman spectrum of **5g** $[Cu(L_7)_2Cl_2]$



FigureS6. Overlaid TG curves of Cu(II) complexes **5a-g**.

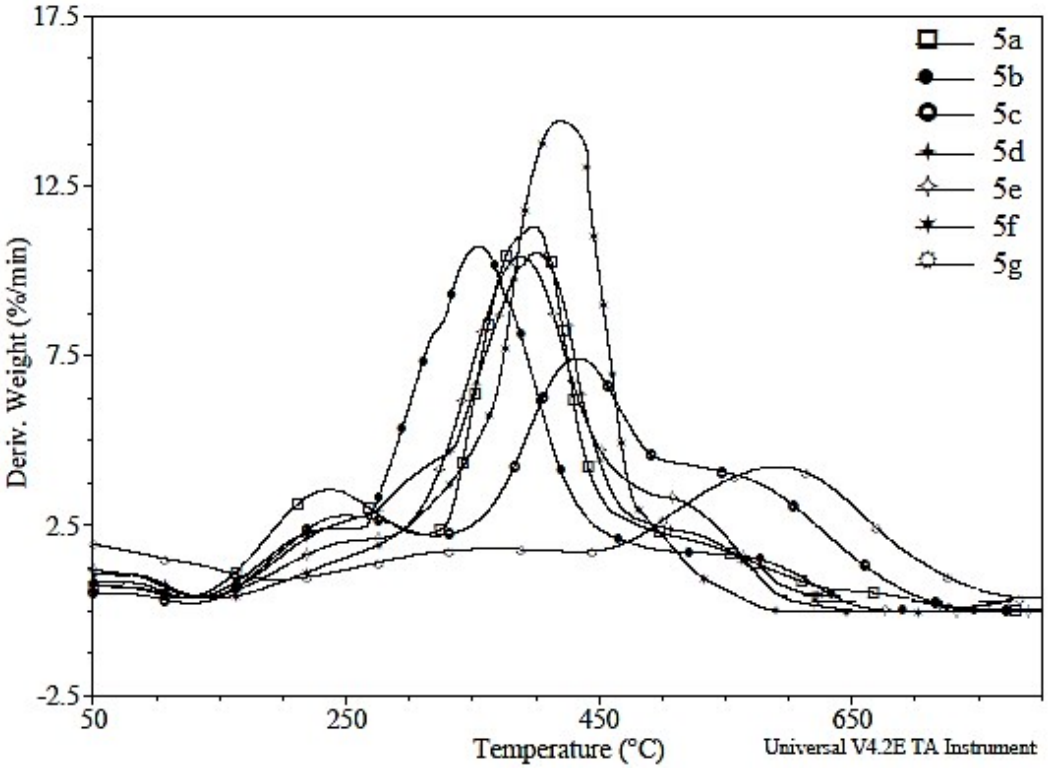


Figure S7. Overlaid DTG curves of Cu(II) complexes **5a-g**.

Table S1. Thermal degradation data of Cu(II) complexes **5a-g**

Samples	Steps	Ti (°C)	Tm (°C)	Tf (°C)	Weight loss % at Tf	Char yield (% w)
5a	I	185	234	305	18.37	8.11 at 720 °C
	II	328	395	482	51.44	
5b	I	181	235	271	10.11	10.13 at 720 °C
	II	283	358	481	63.92	
5c	I	190	257	298	13.80	6.87 at 740 °C
	II	347	436	636	72.05	
5d	I	193	247	292	12.12	5.33 at 605 °C
	II	350	402	477	60.63	
5e	I	200	248	290	8.65	9.42 at 720 °C
	II	303	392	503	63.79	
	III	513	533	557	7.26	
5f	I	307	427	495	72.20	6.19 at 650 °C
5g	I	204	350	472	21.20	20.22 at 970 °C
	II	494	579	700	38.60	
	III	784	896	954	6.10	

References

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DR:HUMAYUN PERVEZ/MAQBOOL AHMAD/MA-40_1HNMR_DMSO

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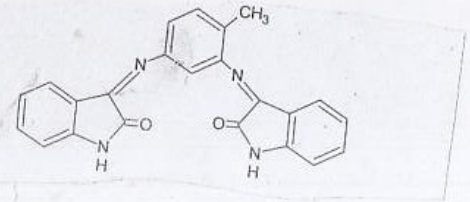

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PROCNO 1

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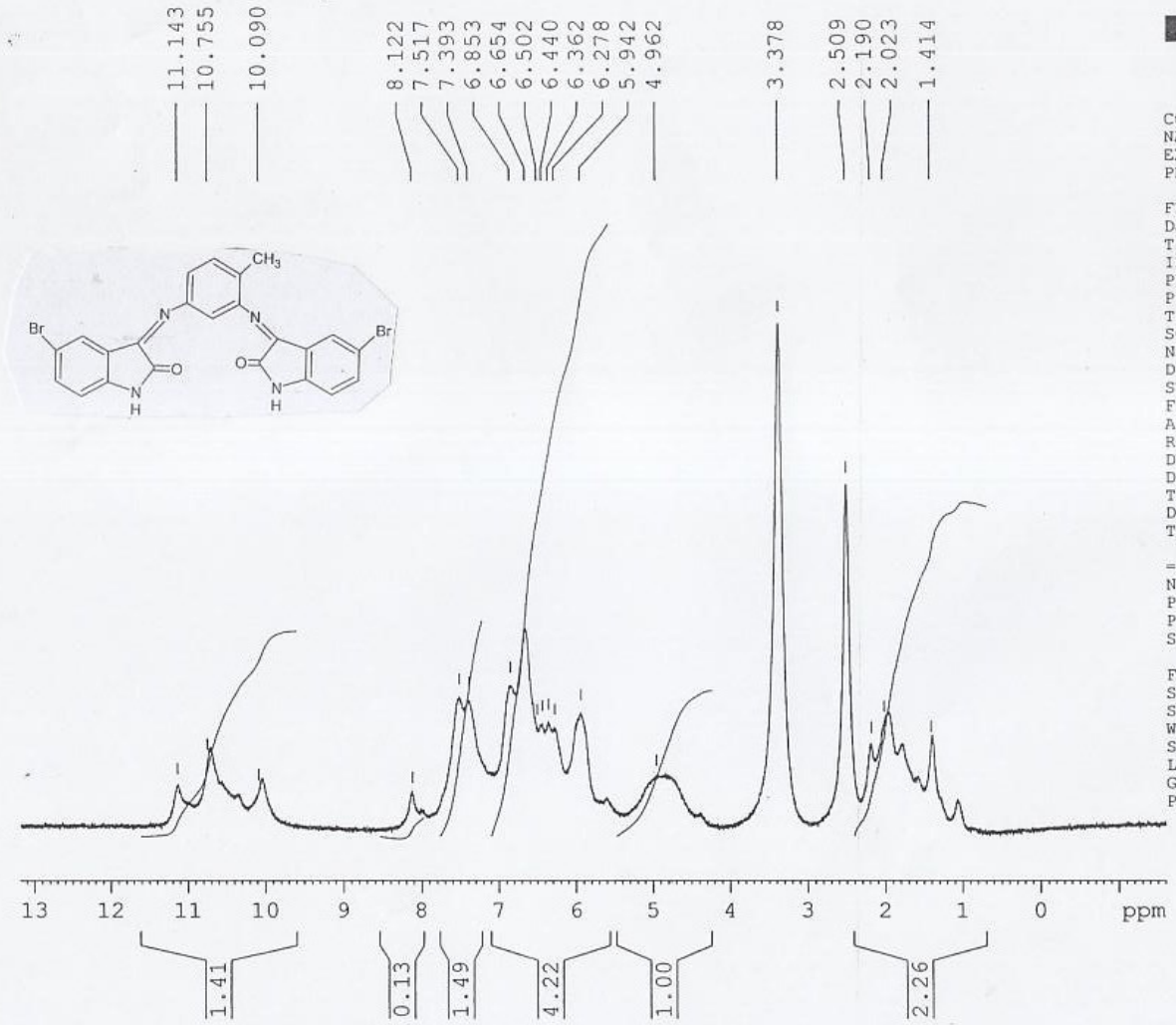
DR.HUMAYUN PERVEZ/MAQBOOL AHMAD/MA-41_1HNMR_DMSO



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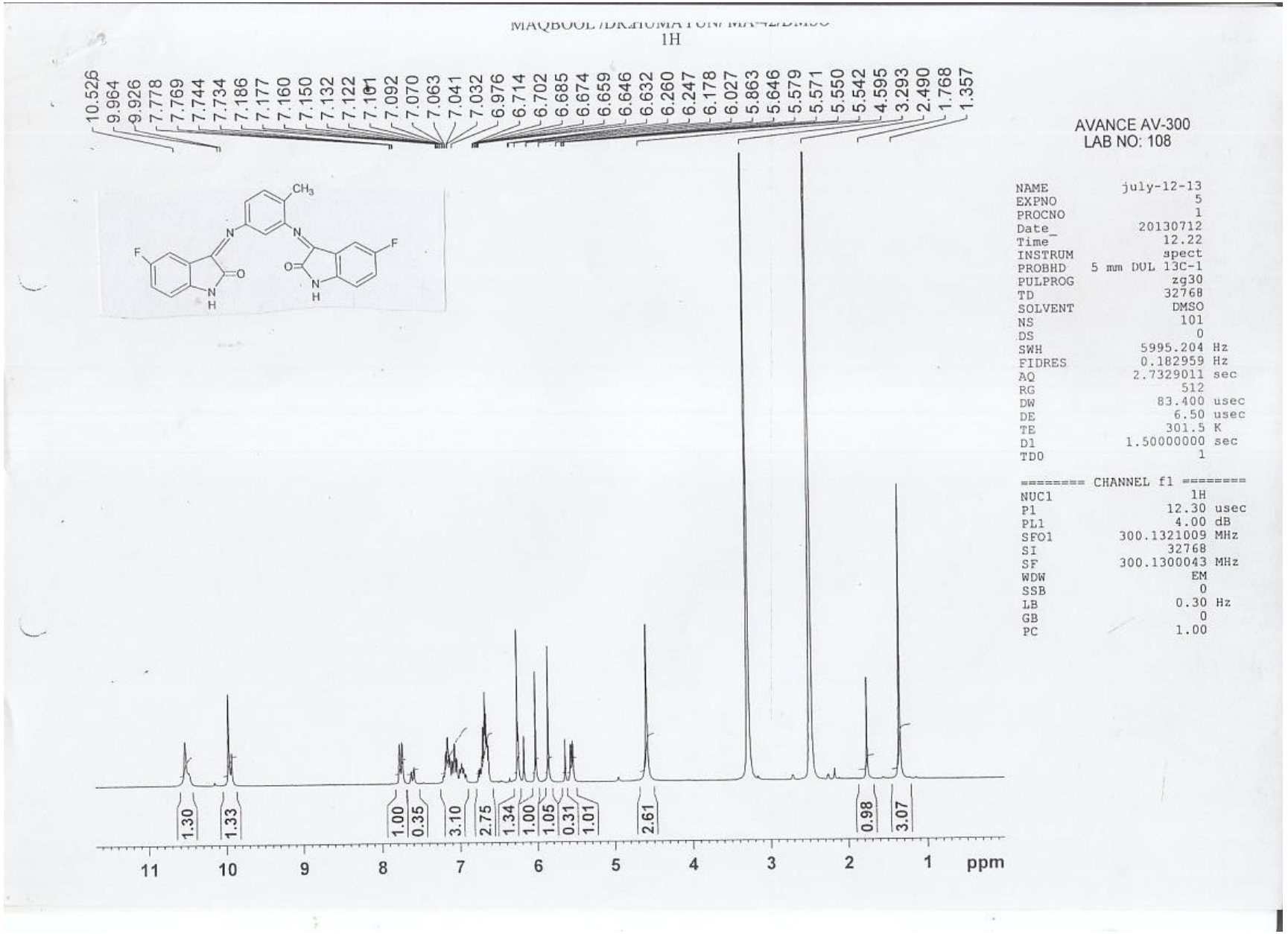

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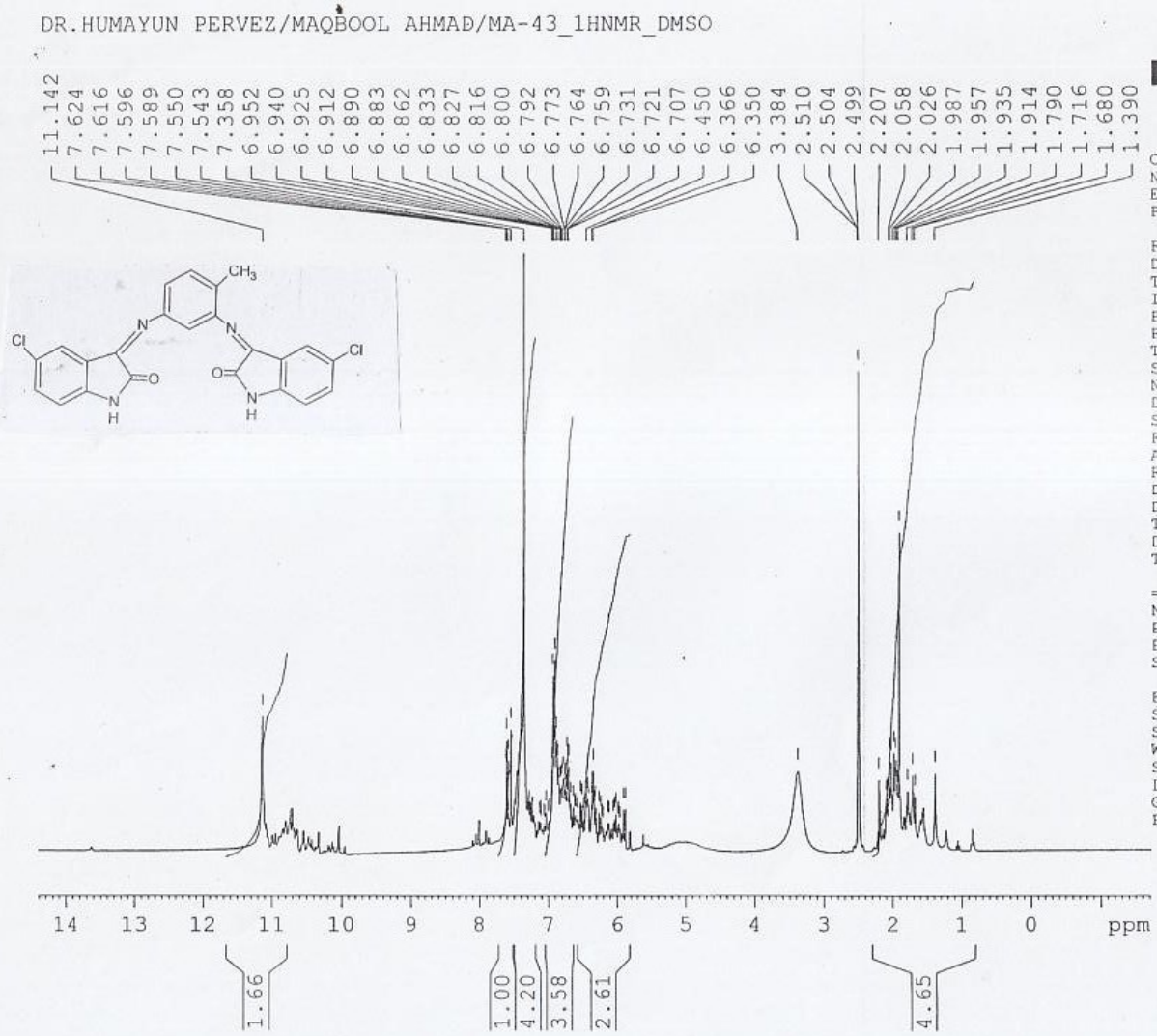

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PROCNO	1

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F2 - Processing parameters

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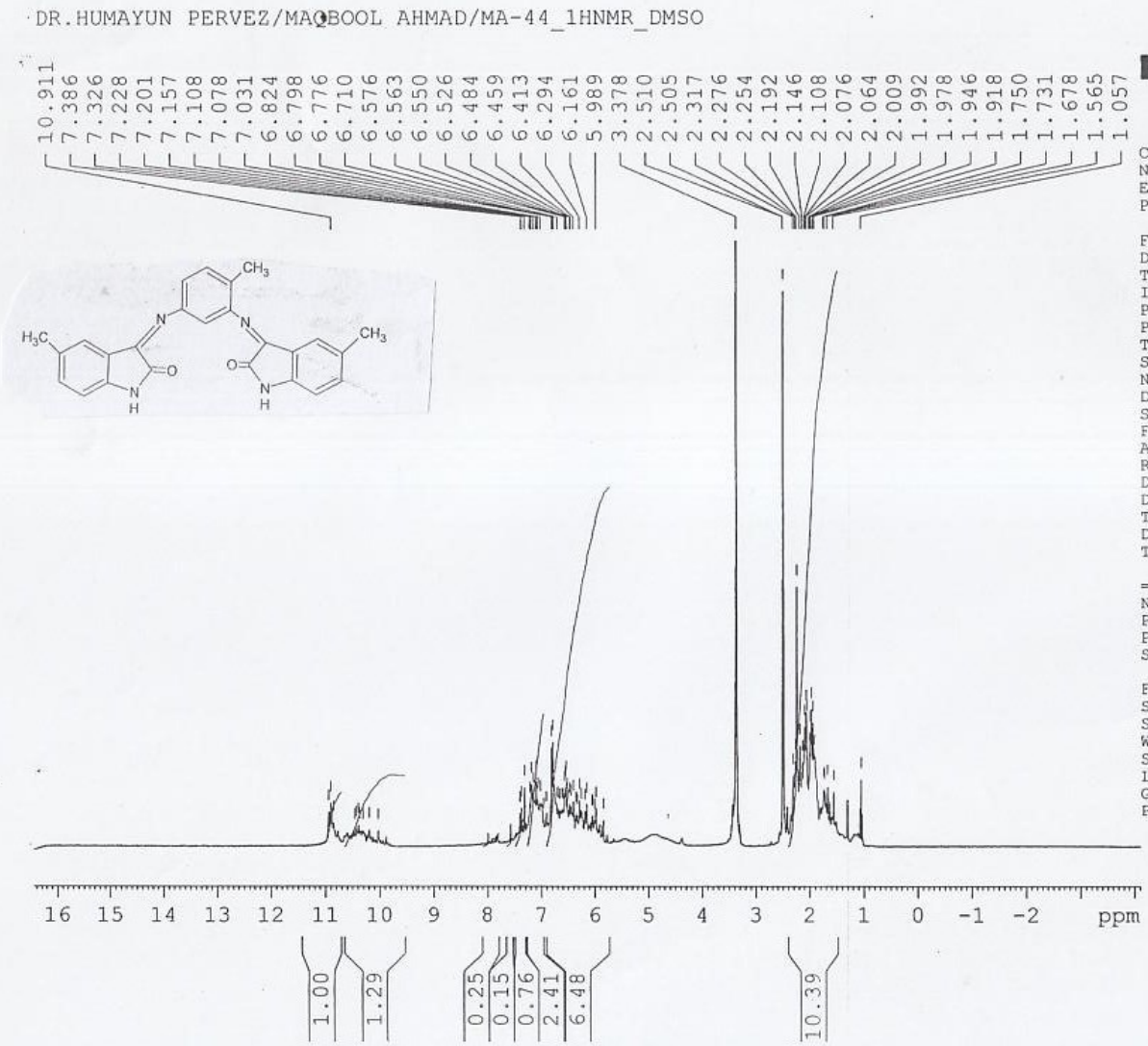

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PL1	2.00 dB
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F2 - Processing parameters

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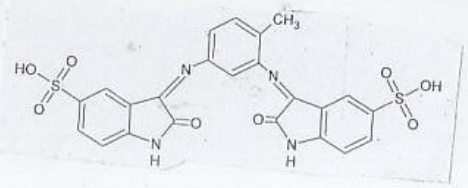

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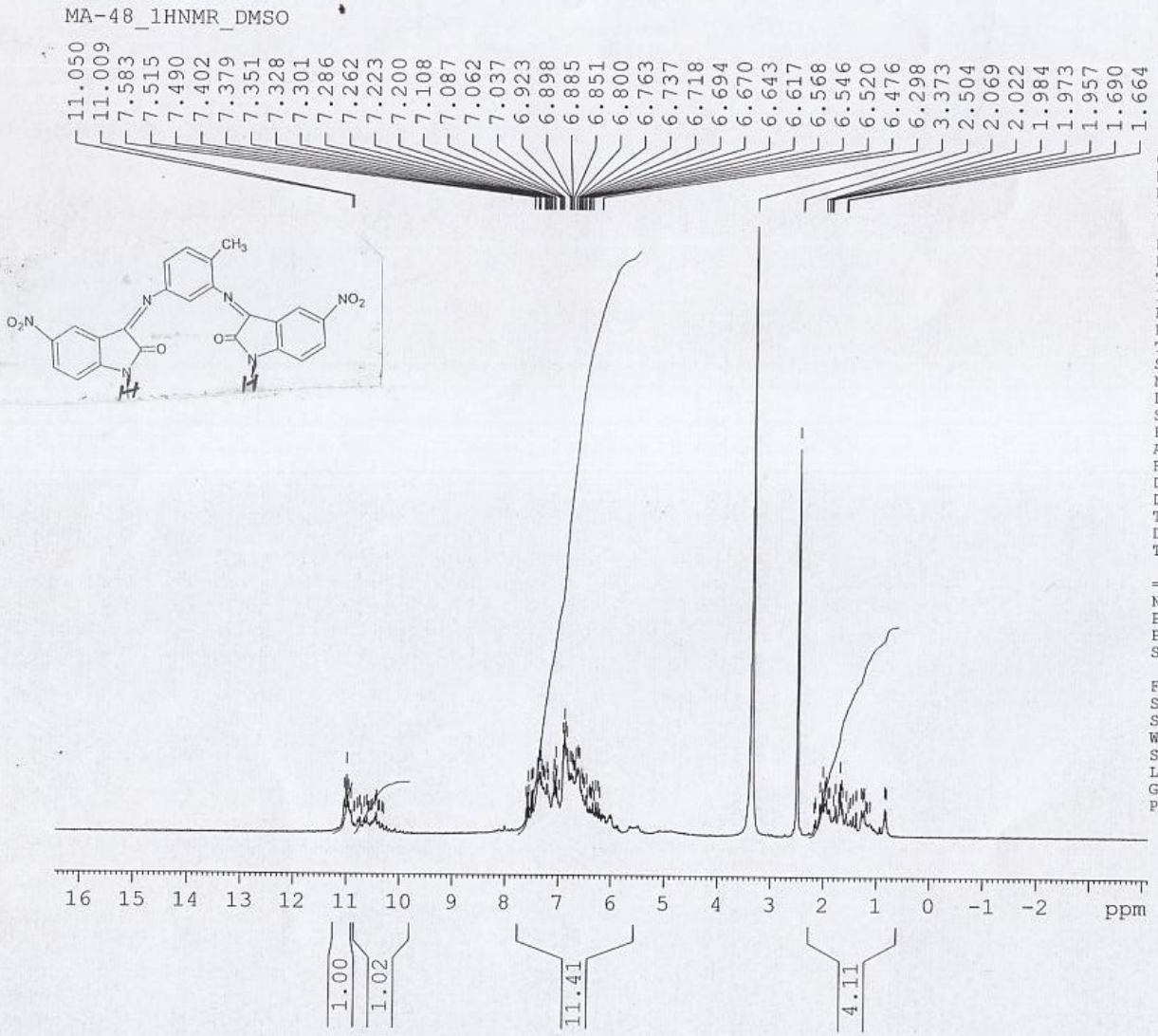

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