

Anderson Type $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6]\cdot 8\text{H}_2\text{O}$: Novel and Green Catalyst for Quinoxaline Synthesis and Biomedical Applications

Pallavi Kadam^a, Sandip Sabale^b, Vashistha Gurame^c and Amit Supale^{a*}

^aDepartment of Chemistry, Bharati Vidyapeeth's, Dr. Patangrao Kadam Mahavidyalaya, Sangli, Maharashtra, 416416, India.

^bDepartment of Chemistry, Jaysingpur College, Jaysingpur, Maharashtra, 416101, India.

^cDepartment of Chemistry, Shri. Shivaji College, Barshi, Maharashtra, 413411, India.

*Corresponding Author: amit.supale@bharativedyapeeth.edu

Results and discussion:

The synthesized Al-POM was characterized by FT-IR, XRD, SEM, EDS, BET and TGA analysis. The obtained results are in good agreement with previously reported literature, confirming the successful formation of Anderson-type POM. The detailed characterization data are provided in the Supporting Information (Figure A to F).

At first, the Al-POM was synthesised as per the previously reported literature. Several spectroscopic techniques were used to characterise and validate the Al-POM. In Figure A, the Mo-O-Mo bond vibration is responsible for the peak at 645 cm^{-1} in FTIR measurement, while the Mo-Ot bond's distinctive peak is located at 938 cm^{-1} . The maxima of water molecules are located at 1636 and 3497 cm^{-1} . The bond between Al-O atoms is the primary distinctive peak that seen at 451 cm^{-1} [49]. The diffraction peaks in the X-ray diffraction (XRD) pattern are observed at 16.76° , 19.16° , 30.40° , 31.73° , 41.80° , 48.36° , 57.87° , and 61.39° with crystallographic planes (1 1 1), (2 0 0), (3 1 0), (3 1 1), (4 1 1), (4 2 2), (4 4 1), and (6 1 0), respectively (Figure B). It is **perceived** that these diffraction peaks demonstrate a primitive cubic structure.

Figure A - FT-IR spectra of $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6]\cdot 8\text{H}_2\text{O}$

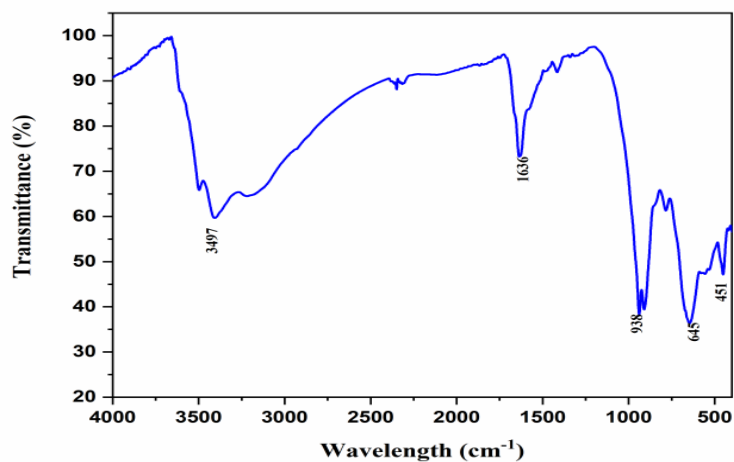
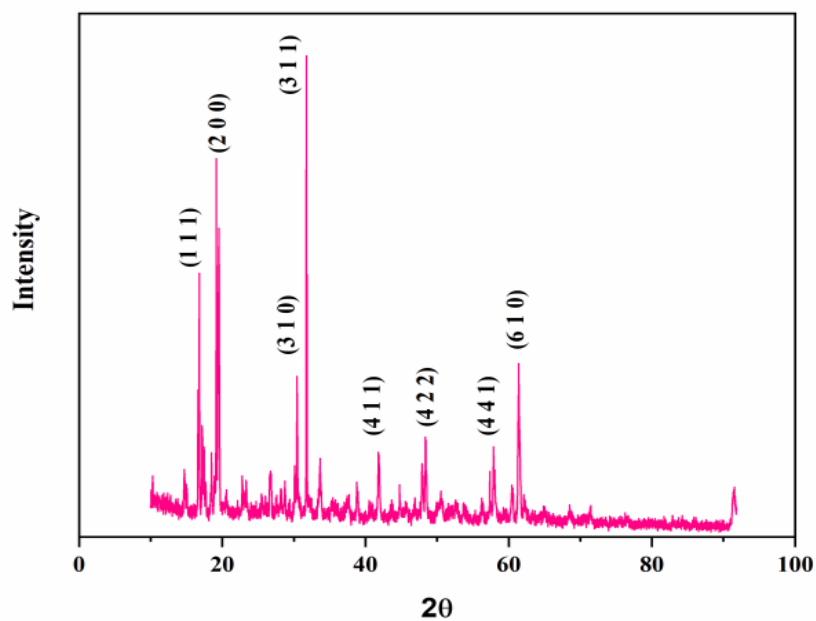


Figure B - XRD spectra of $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6]\cdot 8\text{H}_2\text{O}$



The EDX spectrum, as seen in Figure C, reveals that the main components are molybdenum (Mo), sodium (Na), aluminium (Al) and oxygen (O), with no discernible contaminants. The appearance of the catalyst had been investigated through SEM analysis. Figure D depicts a SEM micrograph of the Al-POM, which had an elliptical shape and a size of 63 to 85 nm.

Figure C - EDX analysis of Anderson-type $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6]\cdot 8\text{H}_2\text{O}$ polyoxometalate

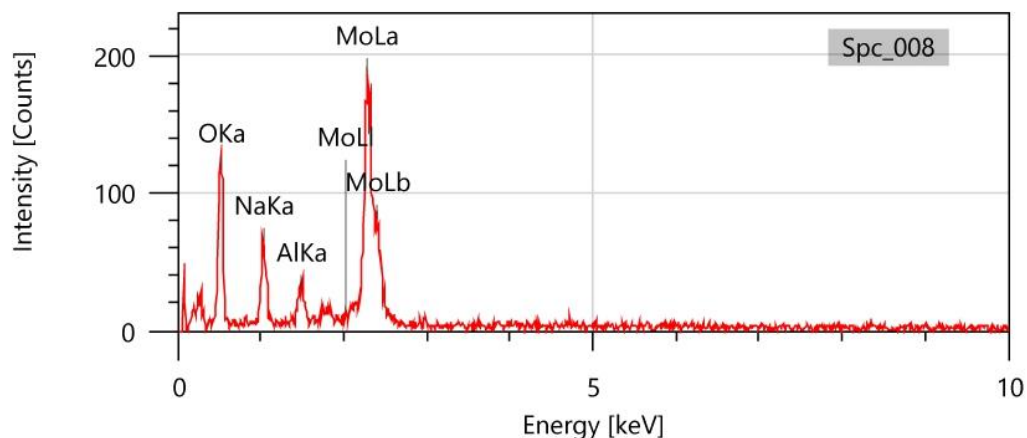
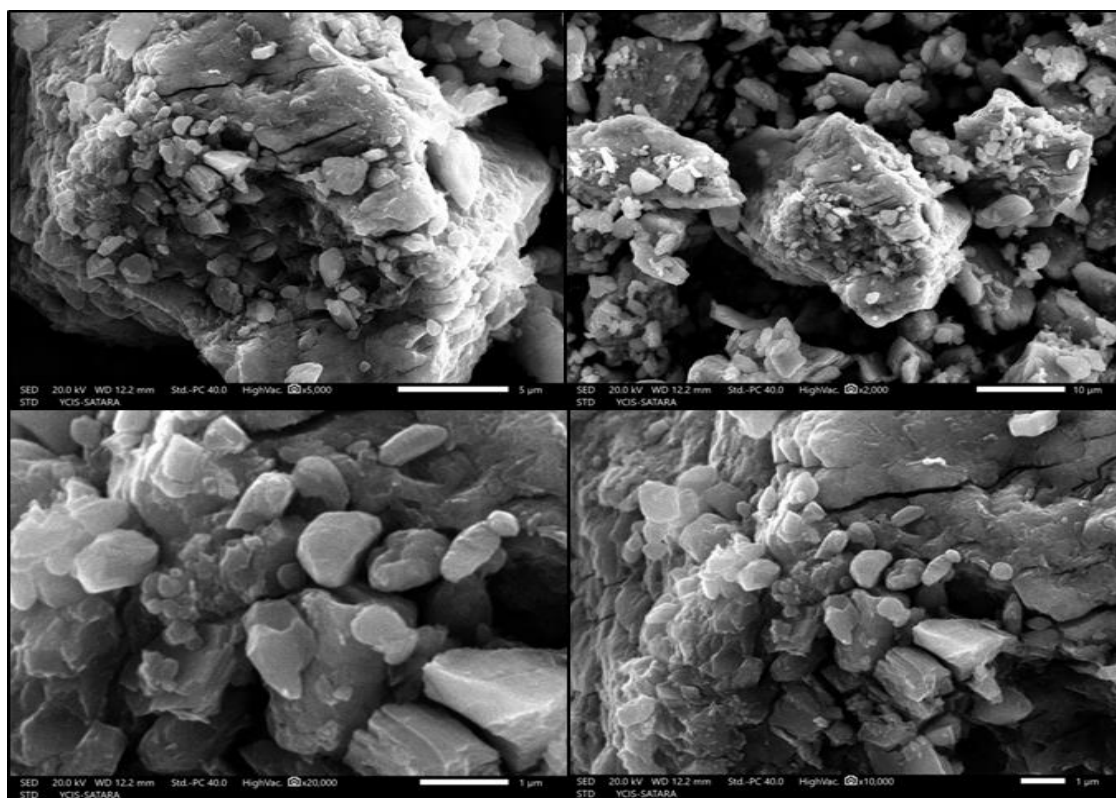


Figure D - SEM images of Anderson-type $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6]\cdot 8\text{H}_2\text{O}$ polyoxometalate



The TGA and DTA curves for the catalyst are shown in Figure E. At temperatures lower than 110 °C, removal of water molecules results in a small initial mass decrease. Around 288 °C, the hydroxyl molecules are responsible for the second loss. The second weight loss occurred with the breakdown of the Anderson-type anion. The TGA curve confirms the total weight

loss of 15.81%. The BET surface area of the prepared Al-POM catalyst was obtained by nitrogen adsorption and desorption measurement (Figure F). The surface area, pore volume and pore size of the Al-POM catalyst were $1.608 \text{ m}^2\text{g}^{-1}$, $0.005966 \text{ cm}^3\text{g}^{-1}$, 14.841 nm .

Figure E - TGA-DTA analysis of Anderson-type $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6] \cdot 8\text{H}_2\text{O}$ catalyst

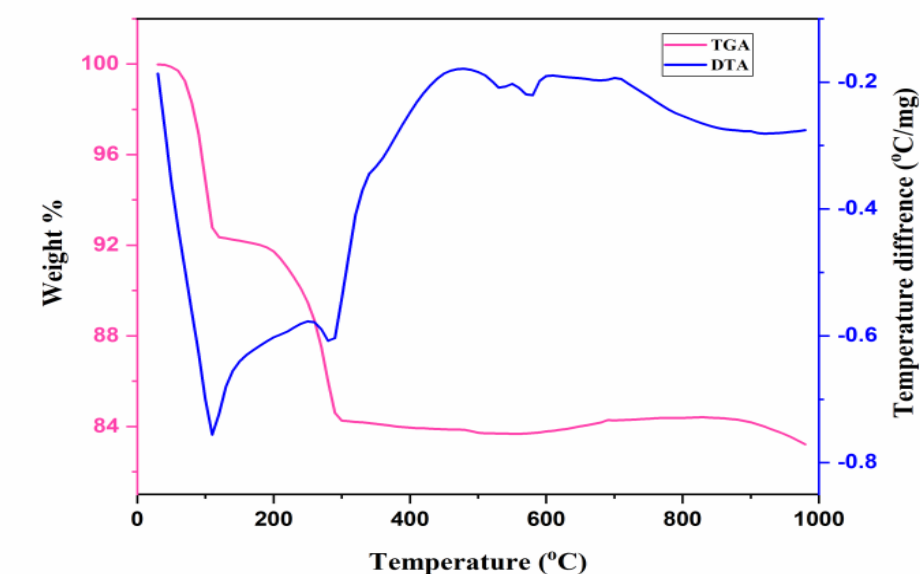
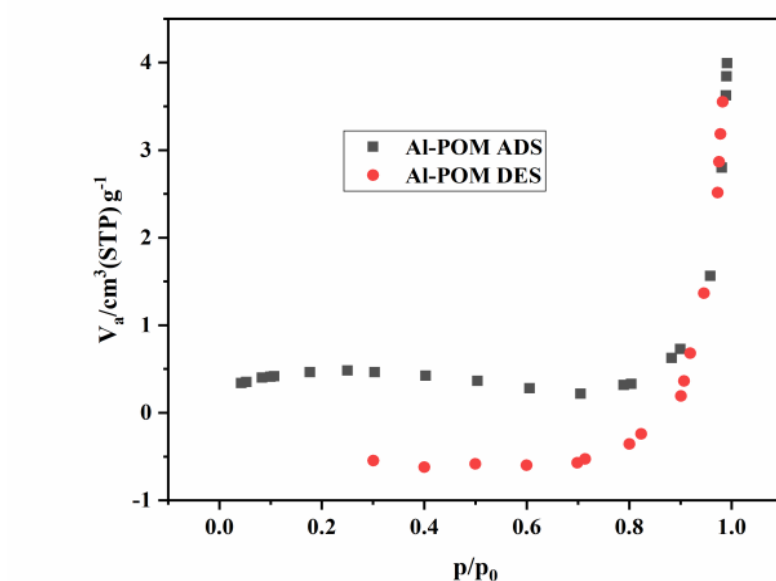


Figure F - N_2 ADS-DES isotherm analysis of Anderson-type $\text{Na}_3[\text{AlMo}_6\text{O}_{24}\text{H}_6] \cdot 8\text{H}_2\text{O}$ catalyst



To confirm the heterogeneous nature of the catalyst, the filtrate (Run 4) was characterised by ICP-OES for Al metal. This analysis revealed that the concentration of Al was below detection limits. The elemental analysis confirms no leaching of the catalyst Al-POM.

Table - ICP-OES analysis for Al

Solution label	Al (308.215 nm)	Al (396.152 nm)
Blank	-0.08 u (ppm)	-0.01 u (ppm)
Sample Al	-0.11 u (ppm)	-0.04 u (ppm)
Blank	-0.08 u (ppm)	-0.01 u (ppm)

Anti HIV Assay:

1. Materials and Methods

1.1 Chemical required:

Enzyme Pepsin (Himedia), Hemoglobin (Himedia), Valacyclovir, Silver nanoparticle of valacyclovir, Sodium Acetate tri hydrates (Qualigens), Sodium Chloride (Himedia), Tri Chloro Acetic acid (TCA) (Sdfine). Acetate buffer was prepared by 50 Mm Na Acetate tri hydrate and 0.1 M NaCl with pH 3.5.

1.2 Instruments required

Spectrophotometer - Karry 100, Rami for 8000 rpm, Micropipette 5-40 µl, 40-200 µl, 200-1000 µl, Eppendorff (1.75ml), electronic balance.

1.3 Enzyme Pepsin activity inhibition Assay: - Pepsin has a quite close resemblance in proteolytic activity with HIV-1 protease one of key enzyme of HIV-1 life cycle as both of them belong to same Aspartate enzyme family. This enzyme was used as a substitute of HIV-1 protease to check out anti HIV activity of valacyclovir and silver nanoparticle of valacyclovir in this experiment.

1.4 Procedure Enzyme inhibition assay

Protease has a quite resemblance in proteolytic activity with HIV-protease one key enzyme of HIV-1 life cycle as both of them belongs to same aspartate enzyme family¹⁸. This enzyme was used as a substitute of HIV-1 protease to check out anti- HIV activity of silver nanoparticle of valacyclovir in the present investigation. To measure the inhibition of the protease enzyme (Sigma Aldrich) used with their corresponding substrate (Sigma Aldrich).

For this assay, 50 µg pepsin, 800 µg hemoglobin and different crude plant extracts were taken in 500 µl of reaction mixture. The mixture was allowed to incubate at 37°C, after 20 min 700 µl of 5% TCA was added to stop the reaction. It was then centrifuged at 14000 g for 5 min and the supernatant was collected. Optical Density (OD) was recorded spectrophotometrically at 280 nm. Separate blanks were used or both positive and negative controls as well as for sample. For negative control, enzyme and substrate were taken and followed the above procedure and for positive control protease was taken as a well-known inhibitor of HIV-protease, lopinavir was taken. Each sample was taken in triplicate, so this assay gives reproducible results. Percentage of inhibition was calculated by using a formula. Inhibition (%) = [(OD of negative control - OD of sample) / OD of negative control] × 100.

Several experiments were performed to get optimum activity of enzyme:

Parameter	Optimum range
pH	2- 4
Incubation period	30 min.
Reaction volume	1000 μ L
Incubation temperature	37 °C
Centrifugation	14000 rpm.

Result:

Table no. 1 **Anti HIV property of Extracts**

Sr. no.	Compound	Concentration	Reading	% inhibition
1	Control		0.315	
2	Pepstatin (std)	100 μ g/m	0.078	75.23
3	Al-POM	100 μ g/m	0.158	49.84

Anti-cancer Assay:

Procedure:

1. MCF-7 (**Human Mammary gland Breast Adenocarcinoma Cell line**) was procured from National center for cell sciences (NCCS), Pune maintained in **MEM Medium** supplemented with 10% fetalbovineserum.
2. Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5% CO₂.
3. Cells were seeded at a concentration(70 μ l) 10⁴ cells/wellin 100 μ l culture medium and 100 μ l.
 - i. Samples (10-100 μ g/ml) into microplatesrespectively (tissue culture grade, and 96 wells).
4. Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.
5. Cell cultures were incubated for 24 h at 37°C and 5% CO₂ in CO₂ incubator (**Thermo scientificBB150**)
6. After incubation, the medium was completely removed and Added 20 μ l of MTT reagent (5mg/min PBS)
7. After addition of MTT, cells incubated for 4hrs at 37°C in CO₂ incubator.
 - i. Observed the wells for formazan crystal formation under microscope. The Yellowish MTT was reduced to dark colored formazan by viable cells only.
8. After removing the medium completely. Added 200 μ l of DMSO (kept for 10 min) and incubate at 370°C (wrapped with aluminum foil).
 - i. Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplate reader (**Benesphera E21**) at a wavelength of 570 nm.

Effects of Sample against MCF-7 (Human Mammary gland Breast Adenocarcinoma Cell line) by MTT assay

Sr. no.	Concentration (μ g/ml)	Absorbance (O D)				Cell viability (%)	IC50 (μ g/ml)
		1	2	3	Average		
1.	Control	2.107	2.114	2.11	2.110		
2.	Al-POM						144.573
	10	1.821	1.825	1.821	1.822	86.36	
	40	1.522	1.524	1.526	1.524	72.22	
	100	1.32	1.321	1.314	1.318	62.48	

Spectral data:**2,3-diphenylquinoxaline (3a):**

IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3026, 2932, 1679, 1669, 1538, 1473, 1344; ^1H NMR (400 MHz, CDCl_3) (δ ppm): 8.2 (dd, $J=6.4$, 6.4, 2H), 7.7 (dd, $J=6.4$, 6.4, 2H), 7.5 (m, 4H), 7.3 (m, 6H); ^{13}C NMR (400 MHz, CDCl_3) (δ ppm): 153.45, 141.15, 138.93, 130.05, 129.85, 129.15, 128.86, 128.29

6-chloro-2,3-diphenylquinoxaline (3b):

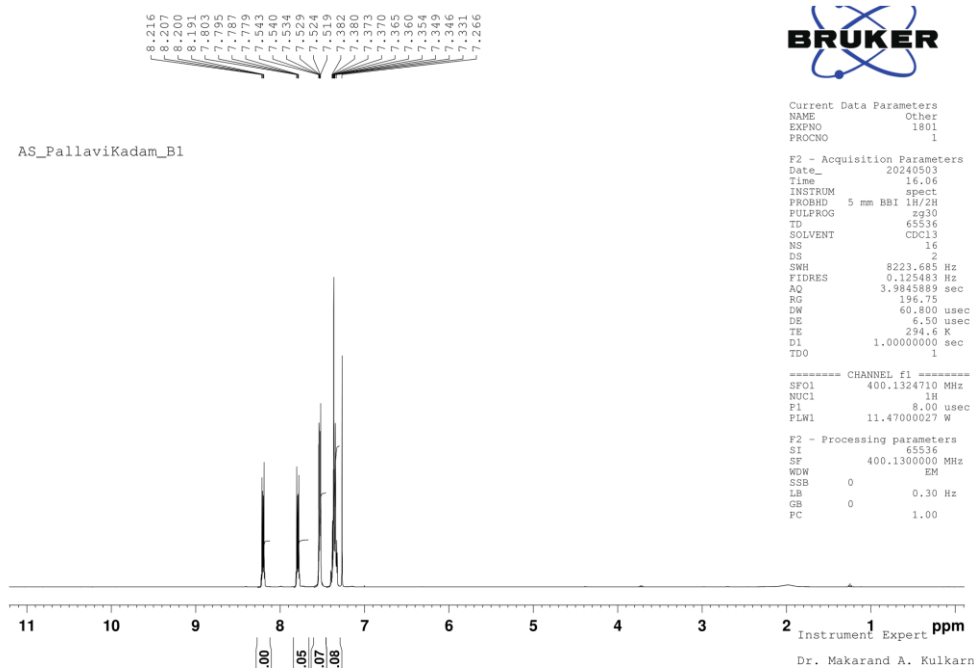
IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3899, 3738, 1712, 1648, 1539, 1421, 1068, 535; ^1H NMR (400 MHz, CDCl_3) (δ ppm): 8.1 (s, 1H), 8.2-8.1 (d, $J=8.4$ Hz, 1H), 7.73-7.70 (dd, $J=9.2$ Hz, $J=8.8$ Hz, 1H), 7.52-7.50 (m, 4H), 7.39-7.32 (m, 6H) ^{13}C NMR (400 MHz, CDCl_3) (δ ppm): 154.30, 153.63, 141.48, 139.72, 138.71, 135.66, 130.98, 129.83, 129.79, 129.04, 128.34, 128.08

6-nitro-2,3-diphenylquinoxaline (3c):

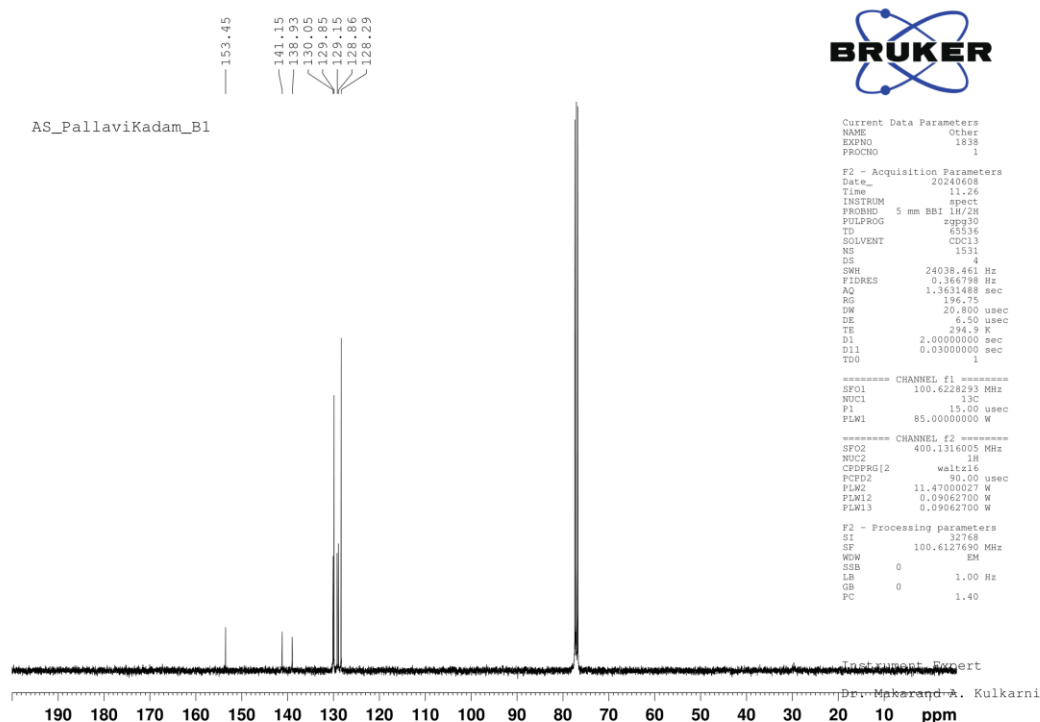
IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3436, 3091, 3061, 1646, 1519, 1400, 1342, 1054, 996, 734, 593; ^1H NMR (400 MHz, CDCl_3) (δ ppm): 9.07 (d, $J=2.4$ Hz, 1H), 8.55-8.52 (dd, $J=9.2$ Hz, $J=9.2$ Hz, 1H), 8.31-8.29 (d, $J=9.2$ Hz, 1H), 7.58-7.55 (m, 4H), 7.43-7.35 (m, 6H) ^{13}C NMR (400 MHz, CDCl_3) (δ ppm): 123.32, 125.64, 128.48, 129.82, 130.76, 138.00, 138.07, 139.97, 143.59, 147.86, 155.70, 156.33

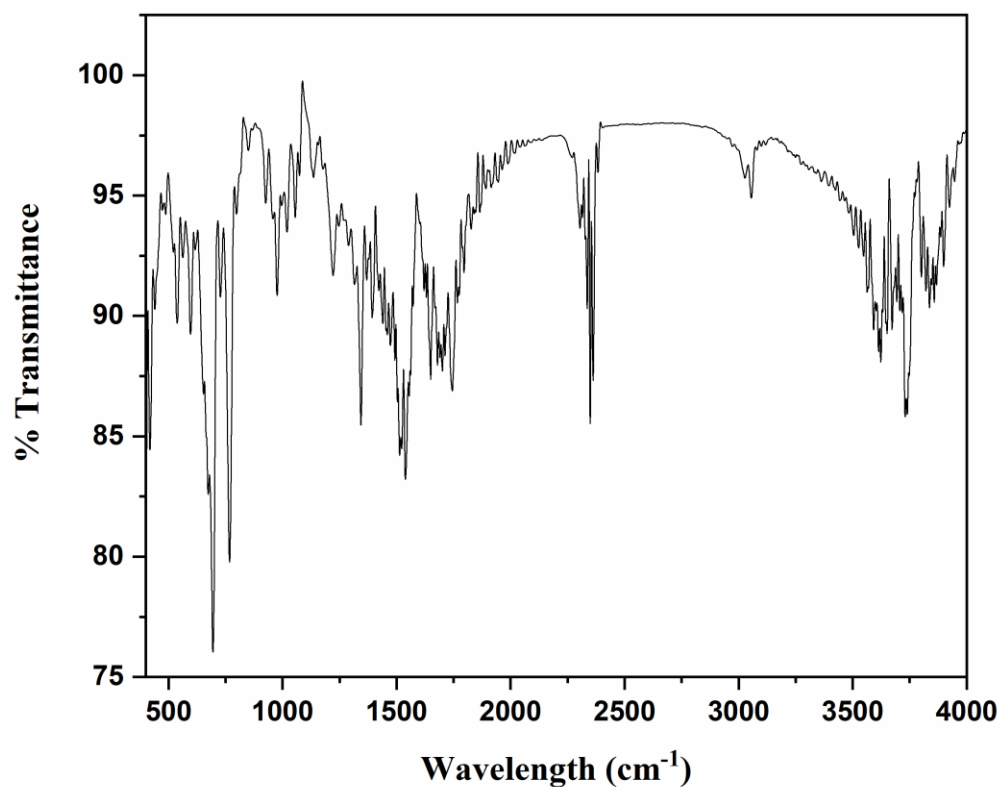
5,6-diphenyl-2,3-dihydropyrazine (3d): IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3028, 2943, 2831, 1649, 1543, 1317, 1263, 765; ^1H NMR (400 MHz, CDCl_3) (δ ppm): 7.366-7.387 (m, 2H), 7.269-7.316 (m, 1H), 7.209-7.248 (m, 2H), 3.684 (s, 2H); ^{13}C NMR (400 MHz, CDCl_3) (δ ppm): 160.28, 137.70, 129.63, 128.11, 127.87, 45.77

¹H NMR of 2,3-diphenylquinoxaline:

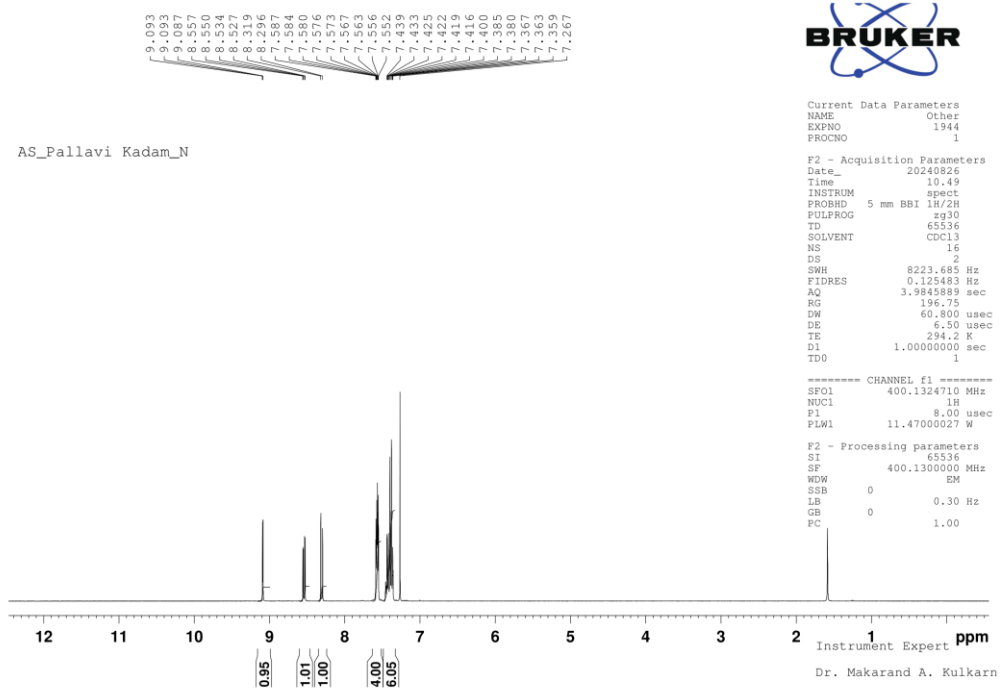


¹³C NMR of 2,3-diphenylquinoxaline:

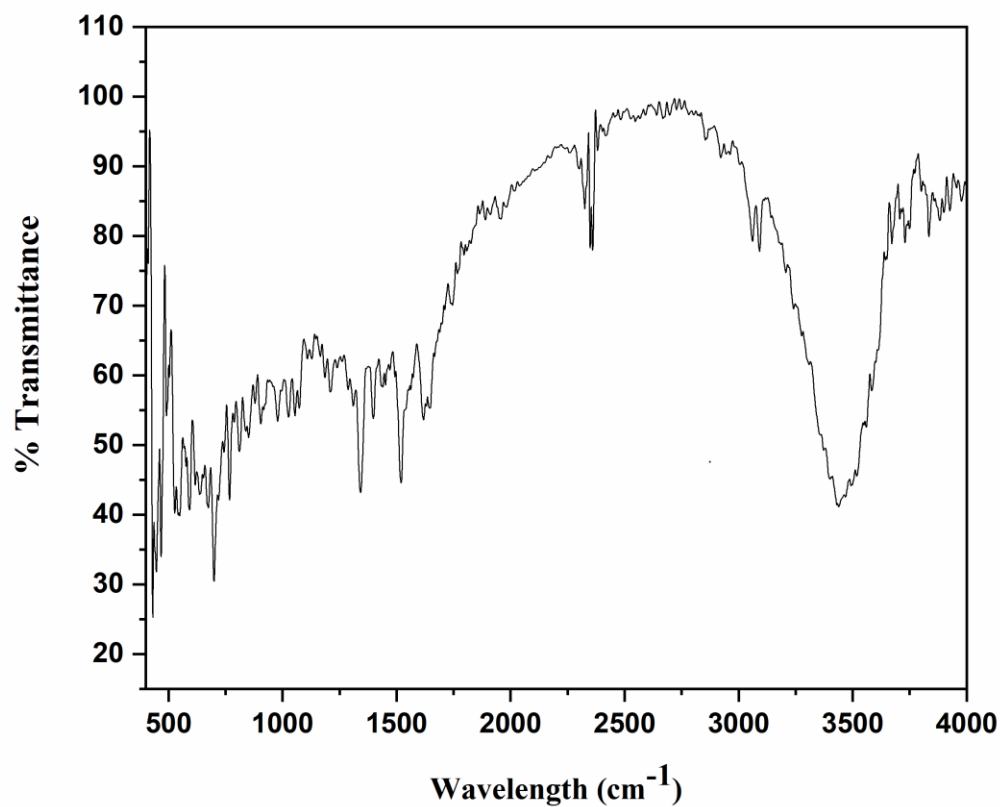
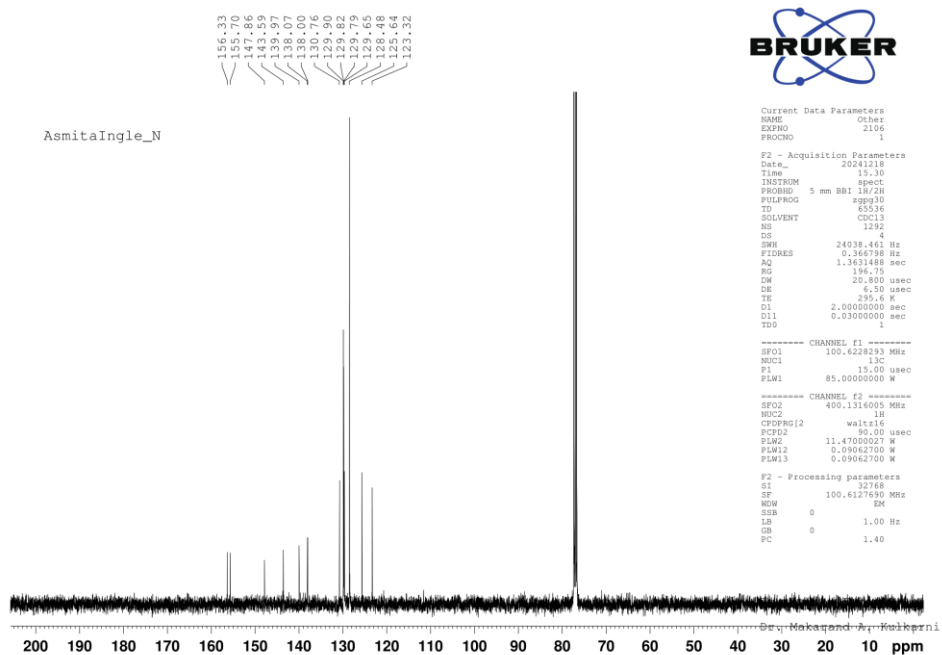




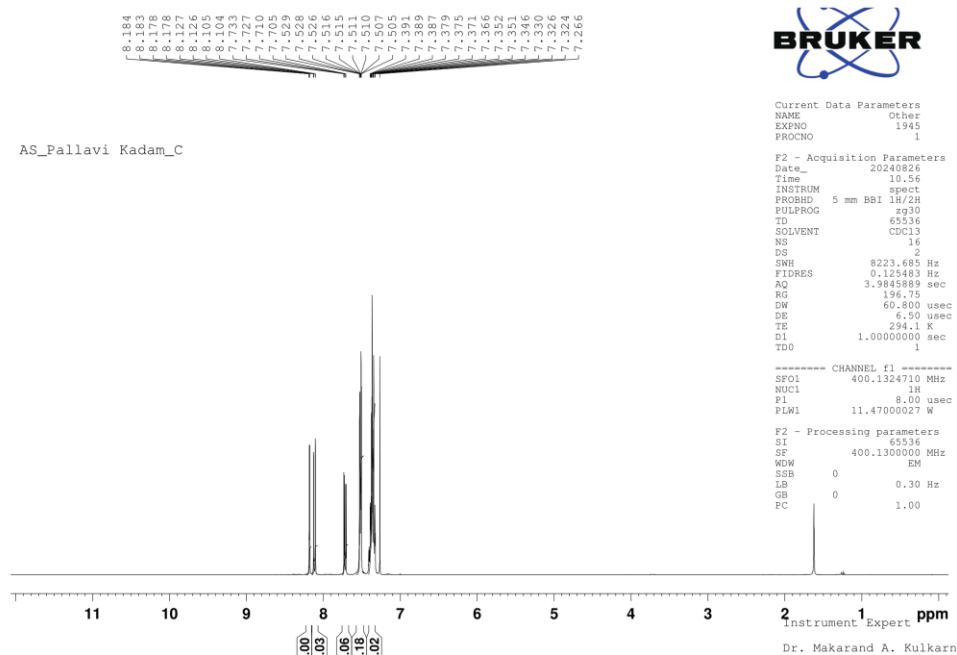
¹H NMR 6-nitro-2,3-diphenylquinoxaline:



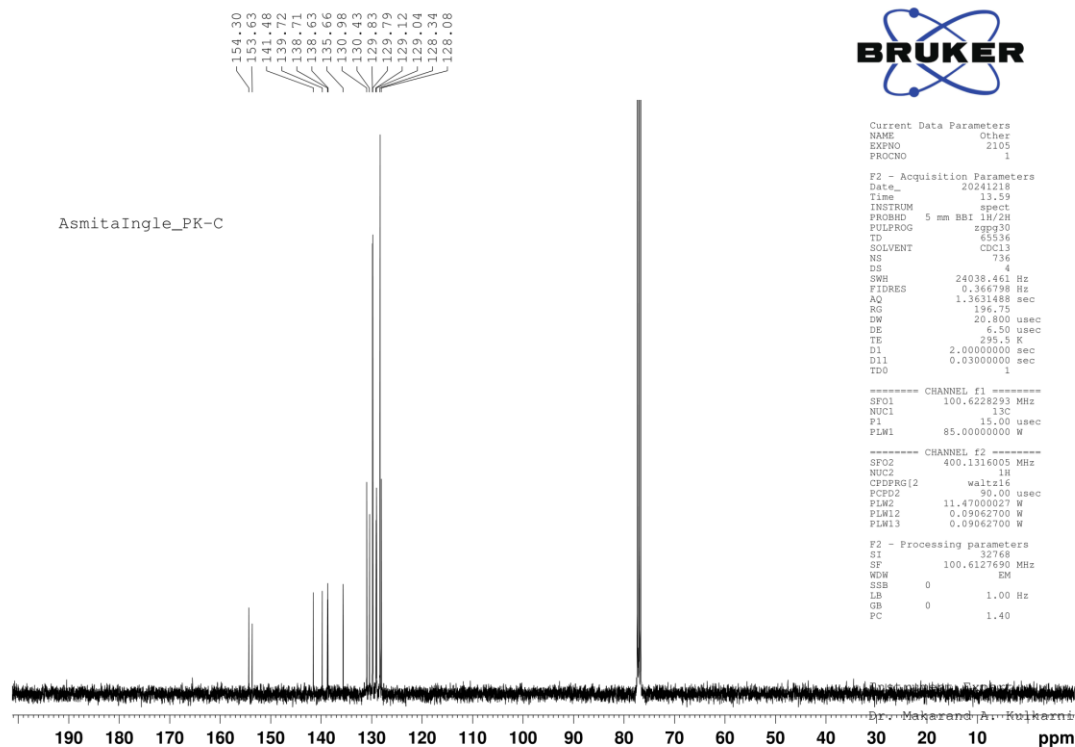
¹H NMR 6-nitro-2,3-diphenylquinoxaline:

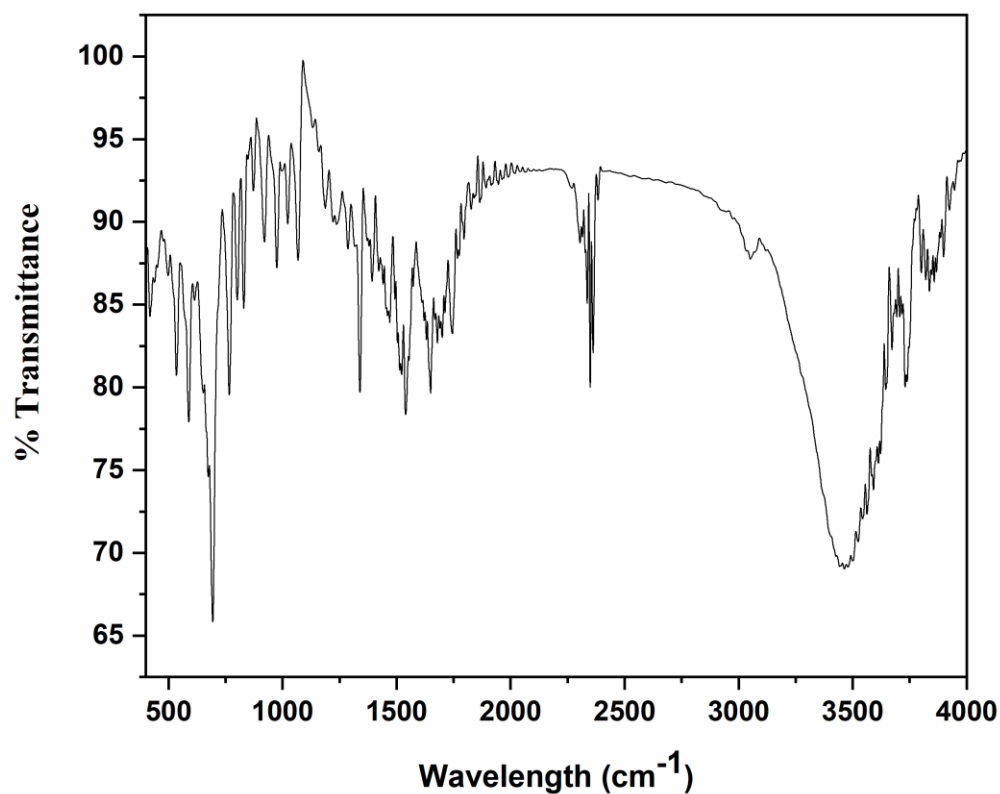


¹H NMR of 6-chloro-2,3-diphenylquinoxaline:

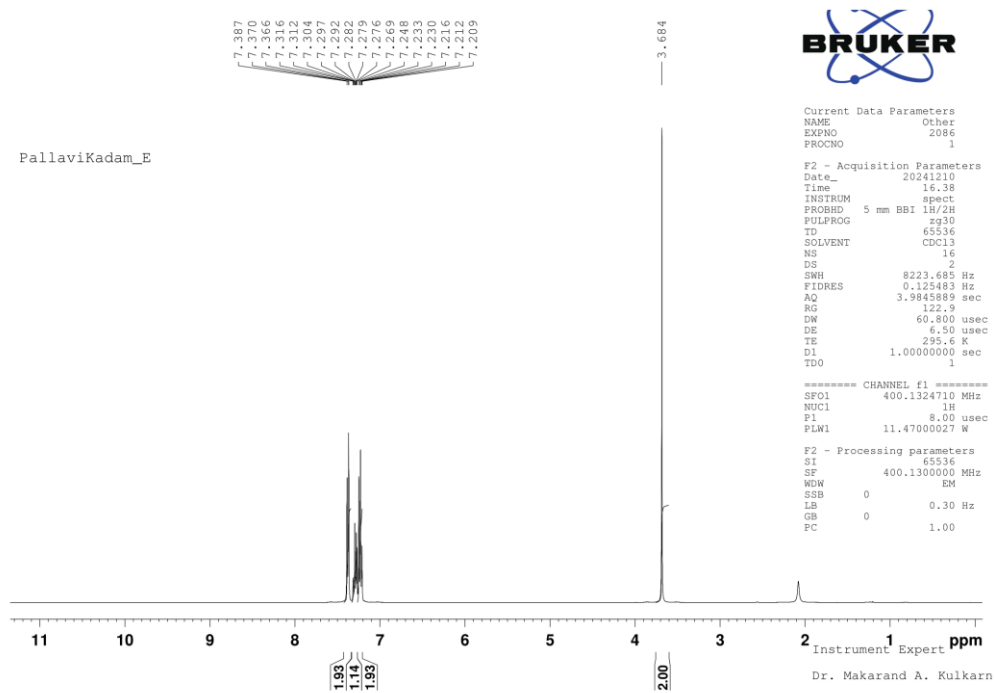


¹³C NMR of 6-chloro-2,3-diphenylquinoxaline:





¹H NMR of 5,6-diphenyl-2,3-dihydropyrazine



¹³ C NMR of 5,6-diphenyl-2,3-dihydropyrazine

