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Synthesis And Biological Activity Of New Indole Based Derivatives As Potent Anti-Inflammatory Agent

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Abstract

Indole, a versatile outstanding heterocyclic compound, engaged in numerous pharmacological properties due to their multiple biochemical processes. The remarkable indole moiety resembles with numerous protein structures. The fascinating molecular framework of indole makes its suitable for drug development. Indole derivatives mimic the peptides structure and bind reversibly to several enzymes, which contribute enormous opportunities to develop novel drugs with distinct mechanism of action Indole, a versatile outstanding heterocyclic compound, engaged in numerous pharmacological properties due to their multiple biochemical processes. The remarkable indole moiety resembles with numerous protein structures. The fascinating molecular framework of indole makes its suitable for drug development. Indole derivatives mimic the peptides structure and bind reversibly to several enzymes, which contribute enormous opportunities to develop novel drugs with distinct mechanism of actionIndole, a versatile outstanding heterocyclic compound, engaged in numerous pharmacological properties due to their multiple biochemical processes. The remarkable indole moiety resembles with numerous protein structures. The fascinating molecular framework of indole makes its suitable for drug development. Indole derivatives mimic the peptides structure and bind reversibly to several enzymes, which contribute enormous opportunities to develop novel drugs with distinct mechanism of actionIndole, a versatile outstanding heterocyclic compound, engaged in numerous pharmacological properties due to their multiple biochemical processes. The remarkable indole moiety resembles with numerous protein structures. The fascinating molecular framework of indole makes its suitable for drug development. Indole derivatives mimic the peptides structure and bind reversibly to several enzymes, which contribute enormous opportunities to develop novel drugs with distinct mechanism of action In the present work, synthesis and biological activity of new indole based derivatives as potent anti-inflammatory agent. The derivatives were schemed and substituted, they were checked for docking scores against the Cyclooxygenase II enzyme. From that 10 best docked compounds are

selected and synthesized spectral data of the synthesized compounds was obtained from IR , ^{1}HNMR, ^{13}CNMR, and mass spectroscopy, among the tested compounds. From the docking results, compound A7 and A10 (9.2 Kcal/mol) shows the highest binding affinity against Cyclooxygenase II enzyme possesses anti-inflammatory activity.

Keywords: Cyclooxygenase II enzyme, Anti-inflammatory activity indole derivatives.

INTRODUCTION:

INDOLES:

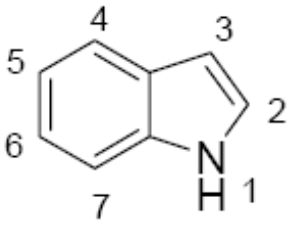


Figure 1:

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six membered benzene ring fused to a five membered nitrogen containing ring. Indole is widely distributed in the natural environment and can be produced by a variety of bacteria. As an intercellular signal molecule, indole regulates various aspects of bacterial physiology, including spore formation, plasmid stability, resistance to drugs, biofilm formation, and virulence. The amino acid tryptophan is an indole derivative and the precursor of the neurotransmitter serotonin [1, 2].

INFLAMMATION:

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.1,2 Symptoms of inflammation include pain, swelling, red coloration to the area, and sometimes loss of movement or function. The potent mediators of inflammation are derivatives of arachidonic acid a 20- carbon unsaturated fatty acid produced from membrane phospholipids. [3,4].

ANTI-INFLAMMATORY:

This prevents the enzymes responsible for the inflammatory response. This anti-inflammatory range also acts by inhibiting the activation of T-lymphocytes, which are abundant in the inflamed tissues, and release the lymphokines, which play an important role in mediating inflammation [5 – 6].

MATERIALS AND METHODS:

Reagents and Instrumentation:

- Oven dried glass wares were used to perform all the reactions. Procured reagents were of analytical grade and solvents of laboratory grade and purified as necessary according to techniques mentioned in Vogel's Textbook of Practical Organic Chemistry.
- In an open glass capillary tubes using Veego VMP-1 apparatus, melting points have been determined in ^{0}C and are uncorrected.
- Ascending TLC on precoated silica-gel plates (MERCK 6 F254) visualized under UV light was utilized to routinely monitor the progress and purity of the synthesized compounds. Solvents used during TLC are n-hexane, ethyl acetate, methanol, petroleum ether, chloroform and dichloromethane.
- The Infrared Spectra was plotted by Perkin-Elmer Fourier Transform-Infrared Spectrometer and in reciprocal centimeters the band positions are noted.
- Nuclear magnetic spectra (^{1}H NMR) were obtained from Bruker DRX-300 (300 MHz FT-NMR) spectrophotometer using DMSO as solvent with TMS as the internal standard ^{13}C NMR have been recorded

utilizing Bruker with Dimethyl sulphoxide as solvent. Shimadzu LC-MS was employed to record Mass Spectra.

***In-silico* molecular docking studies**

Devices and materials:

In the molecular scenario in the modern drug design, the docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some of the online programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through PyRx.

Preparation of protein:

By utilizing the online program protein data bank (PDB), we take the cyclooxygenase II (PDB: 5W58) with a resolution of 1.90A^{o} was obtained. From the protein (5W58) we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot.

Identification of active sites:

Identification of active amino acid present in the protein is detected by using Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in google. From this, I found the active amino acid present in the protein .

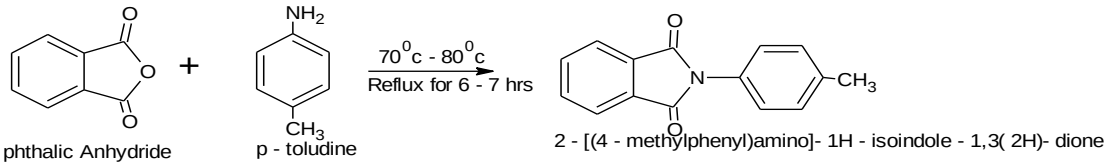
Preparation of Ligands:

By utilizing the Marvin sketch tool, the molecules are designed in two and three-dimensional structures. After designed molecule, the structure was optimized in 3D optimization in Marvin sketch and saved as a pdb format.

Chemistry:

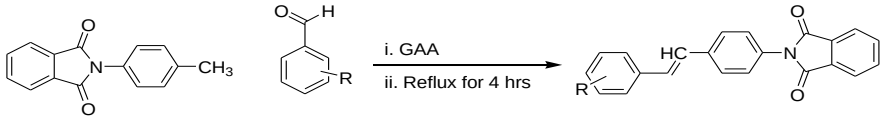
Step 1: synthesis of 2-[(4-methylphenyl)amino]-1H-isoindole-1,3(2H)-dione

- Add 0.1M phthalic anhydride and 0.01M p-toludine in a round bottom flask containing 5ml of glacial acetic acid.
- Reflux the reaction mixture at a temperature 70-80^{o}c for 6-7hours.
- The completion of the reaction is monitored by TLCchloroform : methanol (9 : 1).
- After completion of the reaction, the precipitate is cooled, recrystallized with ethanol and dried.
- The recrystallized dried product is 2-[(4-methylphenyl)amino]-1*H*-isoindole-1,3(2H)-dione, the yield is calculated.



Step 2: Synthesis of 2-{4-[(E)-2-substitiuted-ethenyl}phenyl}-1H-isoindole-1,3(2H)-dione

- The 2-[(4-methylphenyl)amino]-1*H*-isoindole-1,3(2H)-dione and equimolar concentrations of various substituted aldehyde is added to the round bottom flask and add 5ml of glacial acetic acid .
- The reaction mixture was refluxed at a temperature 70-80^{o}c for 3-4 hours.
- The completion of the reaction is monitored by TLC chloroform:methanol (9 : 1).
- After completion of the reaction, the precipitate is cooled, recrystallized with ethanol and dried.



Assessment of in vitro anti-inflammatory activity:

Inhibition of albumin denaturation:

The anti-inflammatory activity of all synthesized compounds was studied according to the protocol of Mizushima et al. and Sakat et al. [8,9] with some modifications. Inhibition of albumin denaturation was done according to the protocol. The reaction mixture consists of an equal volume of test compounds of different concentrations (100–500 µg/ml) and 1% aqueous solution of bovine albumin (Fraction V). The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min. The absorbance was measured after cooling the samples at room temperature. The turbidity formed was measured at 660 nm using ultraviolet (UV)-visible spectrophotometer (Model: Shimadzu UV-1800). The percentage inhibition of protein denaturation was calculated as follows:

$$\%\,\text{inhibition} = \frac{\text{Abs control -Abs sample}}{\text{Abs control}} \times 100$$

RESULT AND DISCUSSION:

Molecular docking:

The molecular docking studies for the designed compounds **(figure 2)** were carried out through PyRx molecular docking software to determine the free energy binding towards targeted enzymes. The docking pose for the ligand enzyme interaction was visualized with discovery studio. The binding free energy for all the ligands was tabulated in table. From the results it clearly shows that, all the compounds have promising interaction with targeted enzyme cyclooxygenase II. The interaction is mainly due to the presence of lipophilic factor of aromatic heterocyclic ring. From the docking results, compound A7 and A10 (9.2 kcal/mol) shows highest binding affinity toward cyclooxygenase II enzyme compared to standard drug indomethacin. The compound A7 shows 3 hydrogen bond interactions with amino acid such as Lys 493, Thr 501, and Asp 533. The following amino acids such as His 367, Gly 490, Asn 491, Thr 498, Lys 532 and Ser 534 are interact with ligand through hydrophobic bond. These interactions due to the aromatic character of ligands. The remaining the entire studied compound shows good to moderate binding affinities to the selected enzymes. These amino acids have been repeatedly implicated during ligand interaction with the cyclooxygenase II enzyme and also play important role in the inhibition of the ligand-binding domain of cyclooxygenase II inhibitors. These non-covalent interactions, van der Waals, columbic interaction, π–π interaction, and hydrogen interaction, are shown in **Figure 3 to 12**. The table 1 shows the binding energy of studied compounds. Based on the docking score the following derivatives like A2, A4, A5, A6, A7, A8, A9, A10, A12, A14, and A15 are selected for the conventional synthesis and it was further evaluated for the in-vitro anti-inflammatory activity.

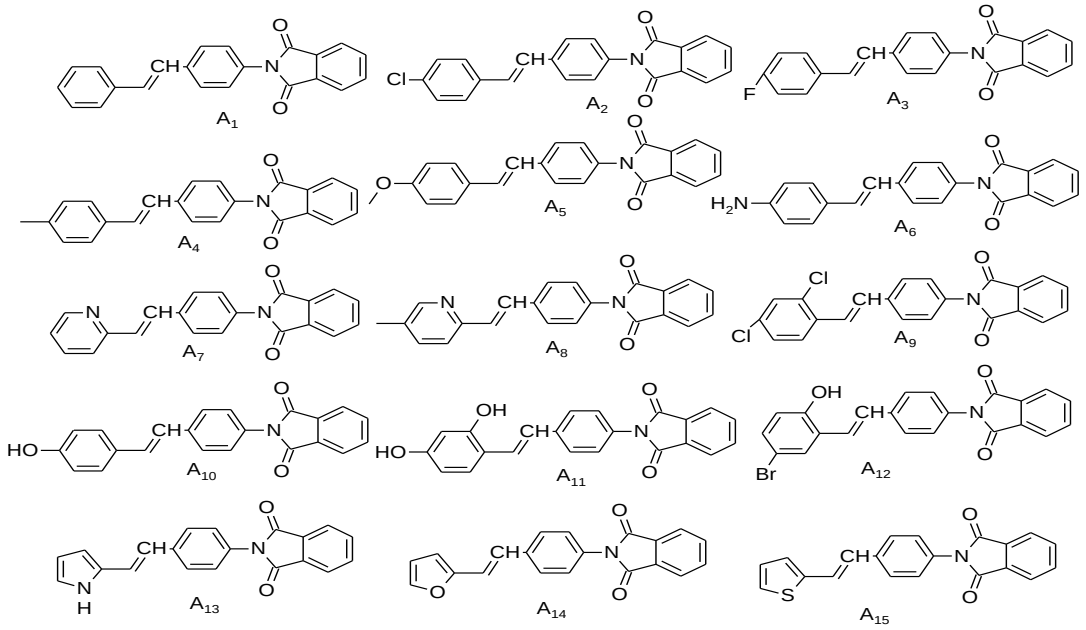


Figure 2. Newly designed compounds

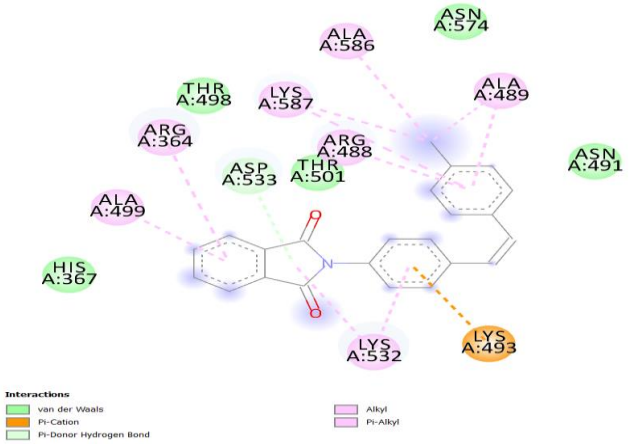


Figure 3. 2D docking interaction of compound A2 against cyclooxygenase II enzyme

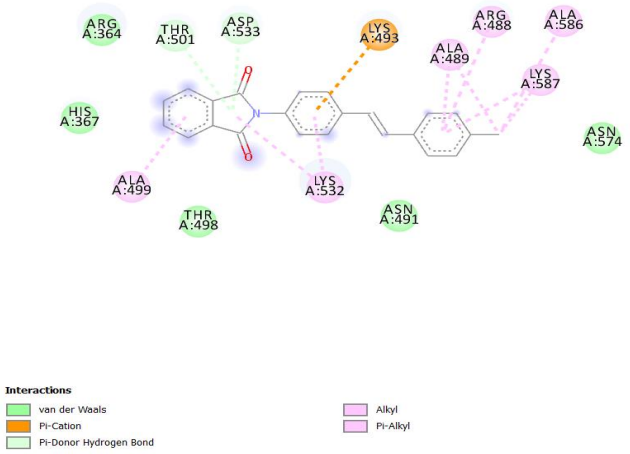


Figure 4. 2D docking interaction of compound A4 against cyclooxygenase II enzyme

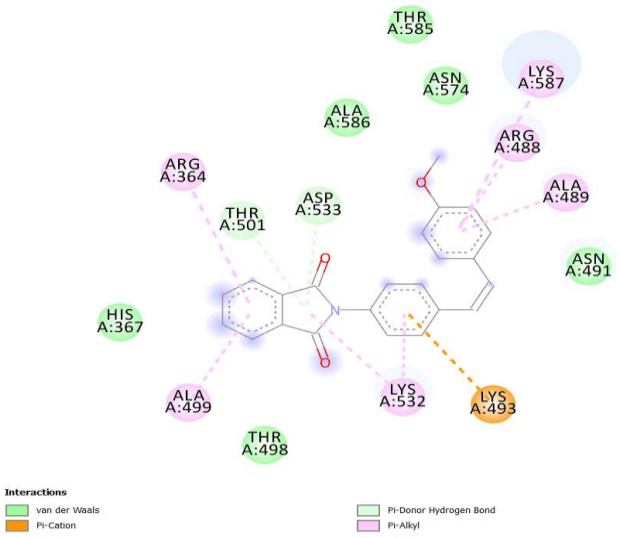


Figure 5. 2D docking interaction of compound A5 against cyclooxygenase II enzyme

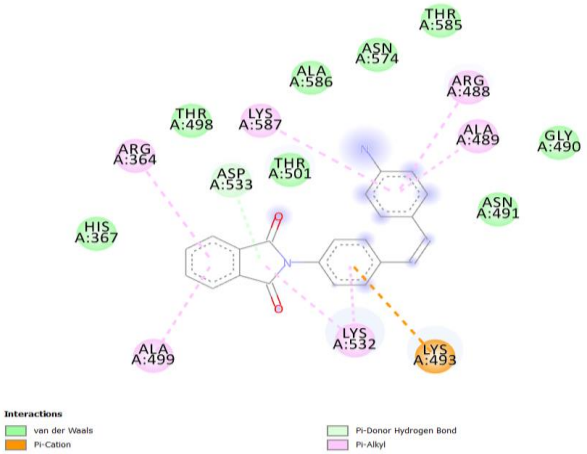


Figure 6. 2D docking interaction of compound A6 against cyclooxygenase II enzyme

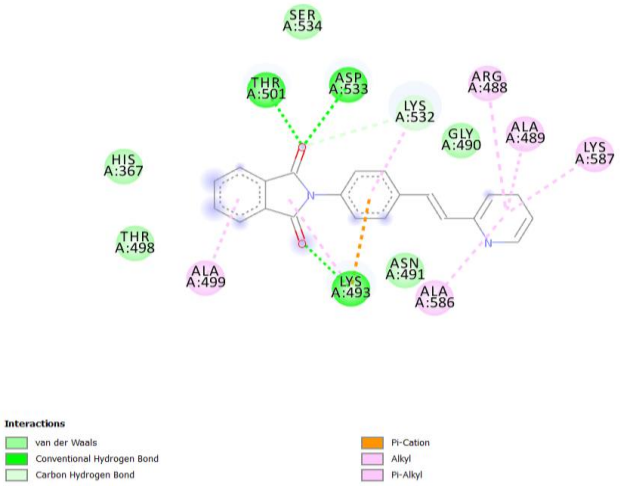


Figure 7. 2D docking interaction of compound A7 against cyclooxygenase II enzyme

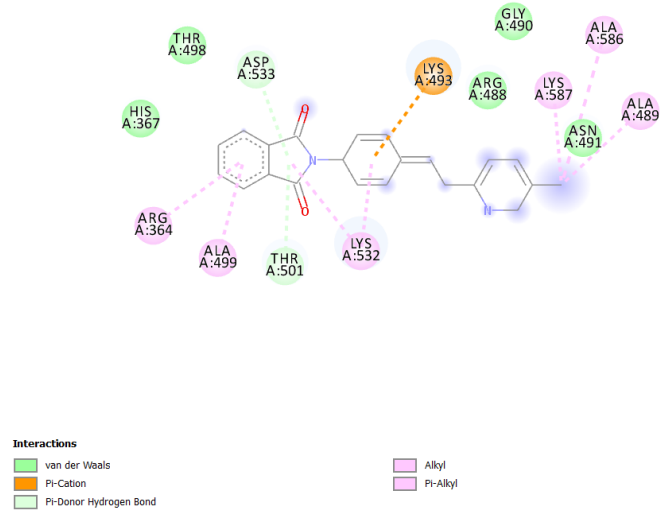


Figure 8. 2D docking interaction of compound A8 against cyclooxygenase II enzyme

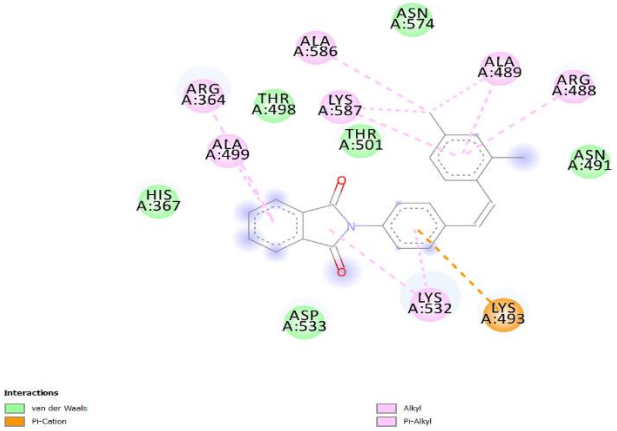


Figure 9. 2D docking interaction of compound A9 against cyclooxygenase II enzyme

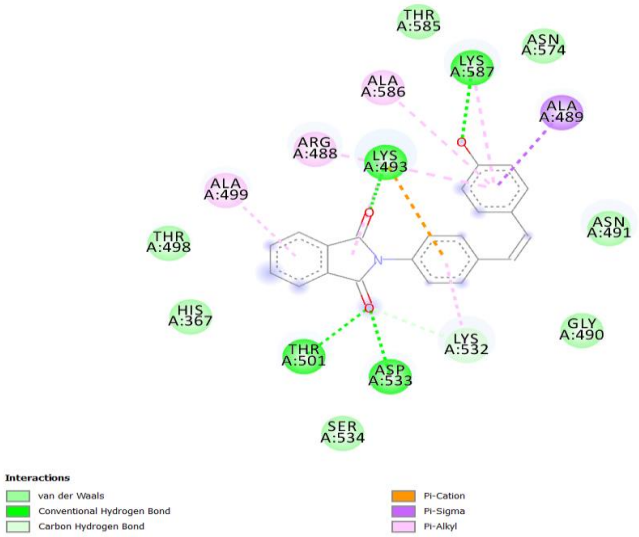


Figure 10. 2D docking interaction of compound A10 against cyclooxygenase II enzyme

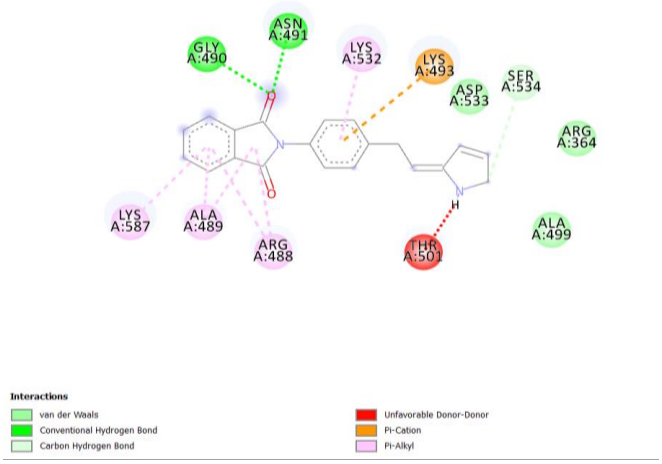


Figure 11. 2D docking interaction of compound A13 against cyclooxygenase II enzyme

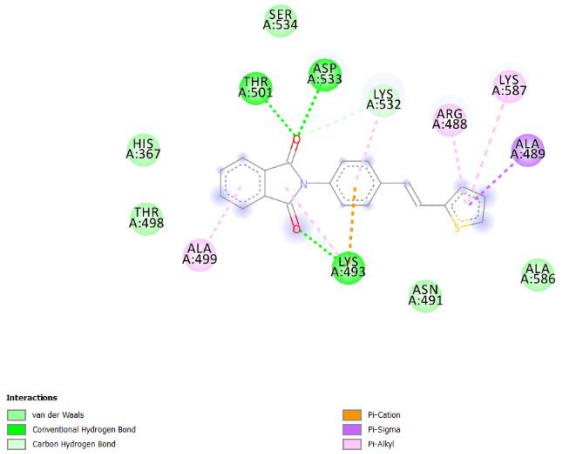


Figure 12. 2D docking interaction of compound A15 against cyclooxygenase II enzyme

Table 1. Binding energy of studied compounds.

Ligand	Binding Affinity
A1	-7
A2	**-8.9**
A3	-7.2
A4	**-9**
A5	**-8.9**
A6	**-8.6**
A7	**-9.2**
A8	**-8.9**
A9	-7.9
A10	**-9.2**
A11	-7.8
A12	**-9**
A13	**-8.6**
A14	-7.7
A15	**-8.5**
Indomethacin	**-10.4**

Chemistry:

The final derivatives of chalcone based indole derivatives were achieved by two step process. In first step, Phthalic anhydride and p-toludine and 5ml of glacial acetic acid was reflux at a 70-80^{o}C for 7 hours. The completion of the reaction is monitored by TLC by chloroform: methanol (9:1) as mobile phase. After completion of the reaction, the precipitate of 2-[(4-methylphenyl)amino]-1*H*-isoindole-1,3(2H)-dione (A) is cooled, recrystallized with ethanol and dried. Further the compound A is reacted with equimolar concentrations of various substituted aldehyde under reflux at a temperature 70-80^{o}C for 3-4 hours. The completion of the reaction is monitored by TLC chloroform: methanol (9:1). The structure of synthesized compounds was elucidated by various spectral analyses. From the spectral analysis, it evident that all the compounds showed a corresponding signals in all the spectral data. The spectral data for all the compounds are given below:

Spectral Data of synthesized compounds:

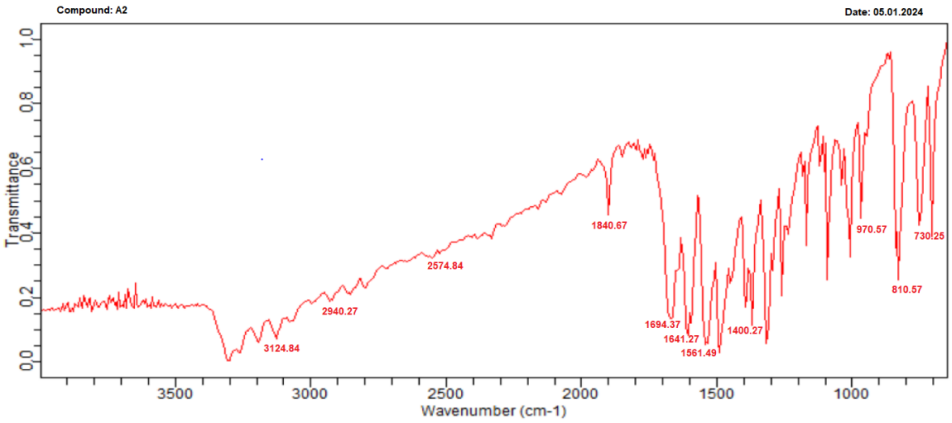


Figure 13: IR Spectra for compound A2

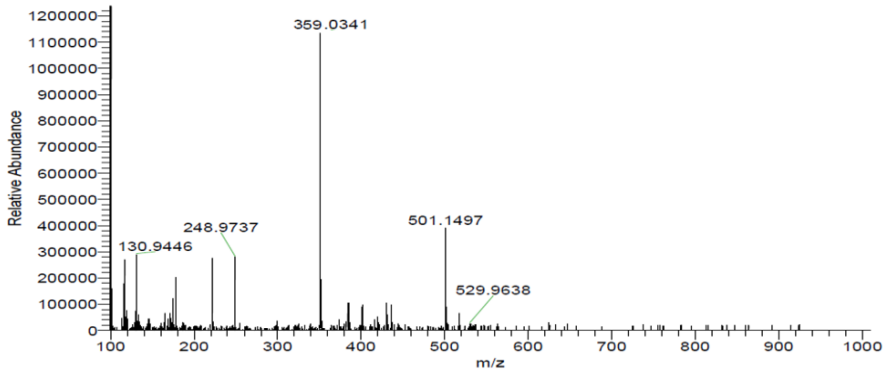


Figure 14: Mass Spectra for compound A2

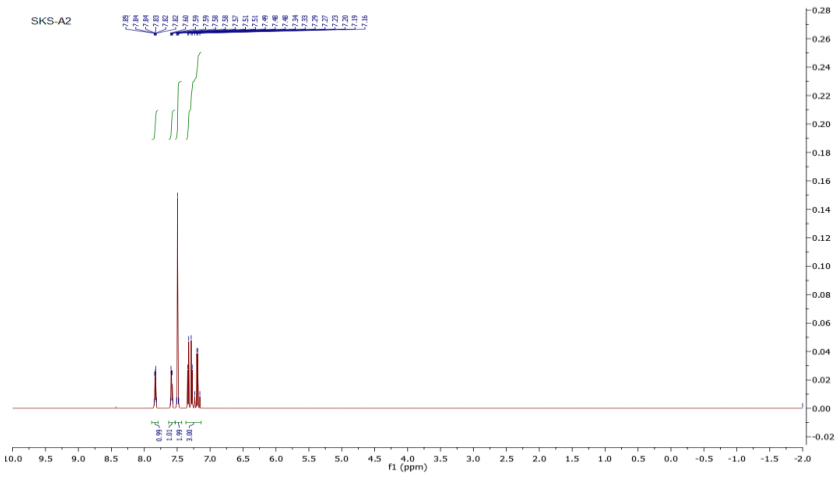


Figure 15: ^{1}H NMR Spectra for compound A2

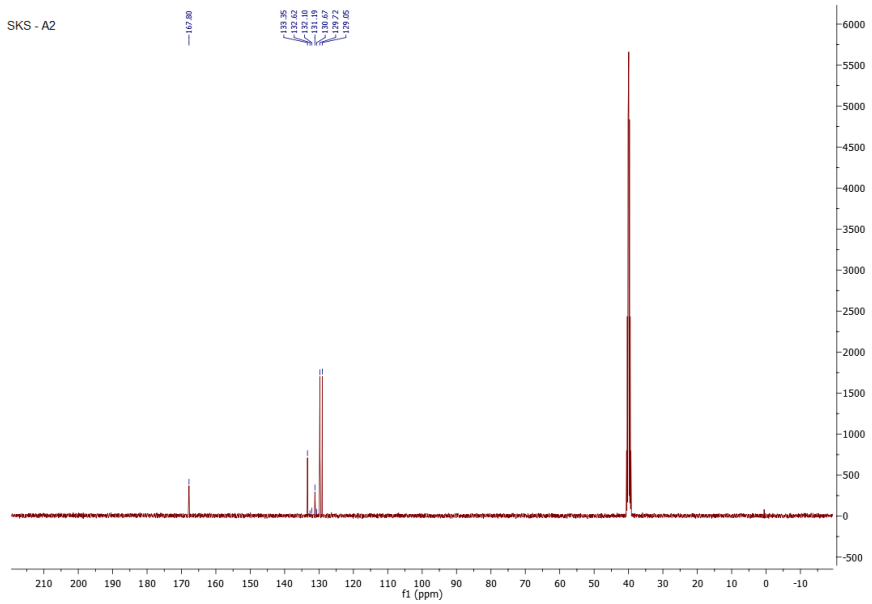


Figure 16: ^{13}C NMR Spectra for compound A2

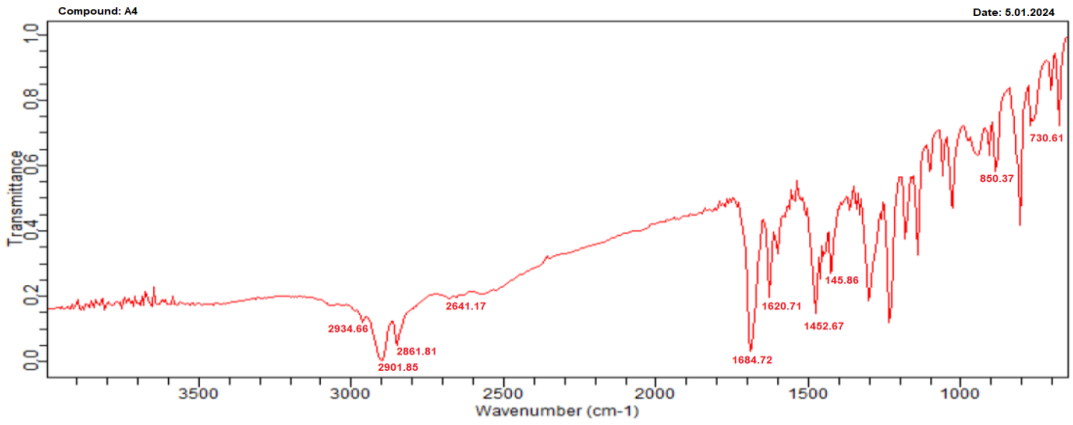


Figure 17: IR Spectra for compound A4

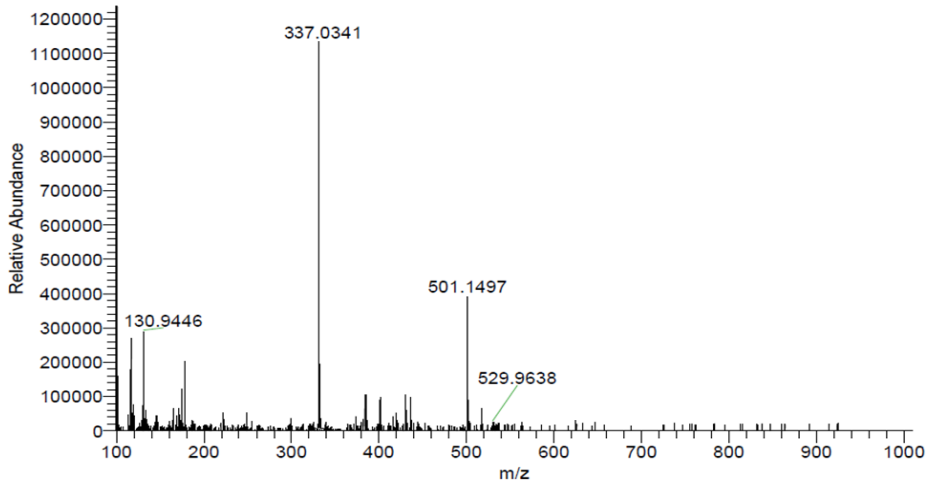


Figure 18: Mass Spectra for compound A4

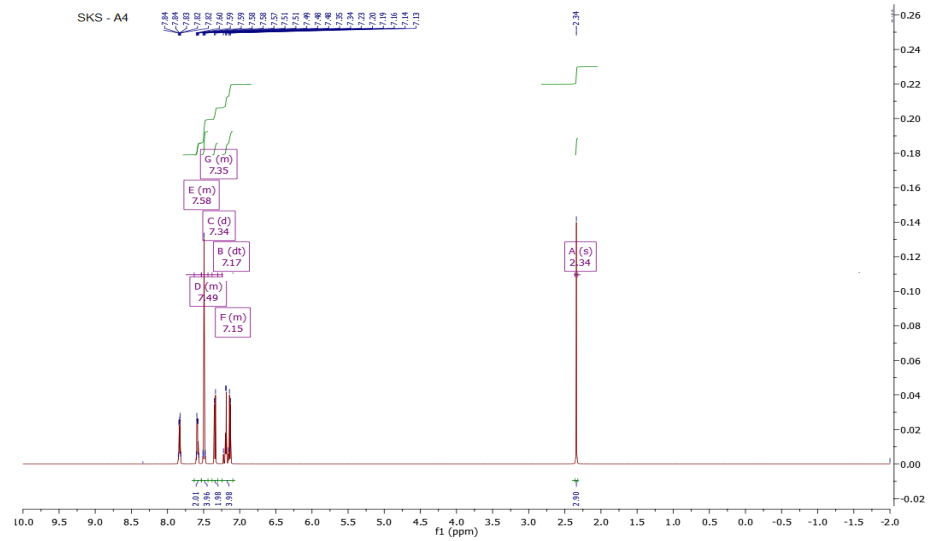


Figure 19: ^{1}H NMR Spectra for compound A4

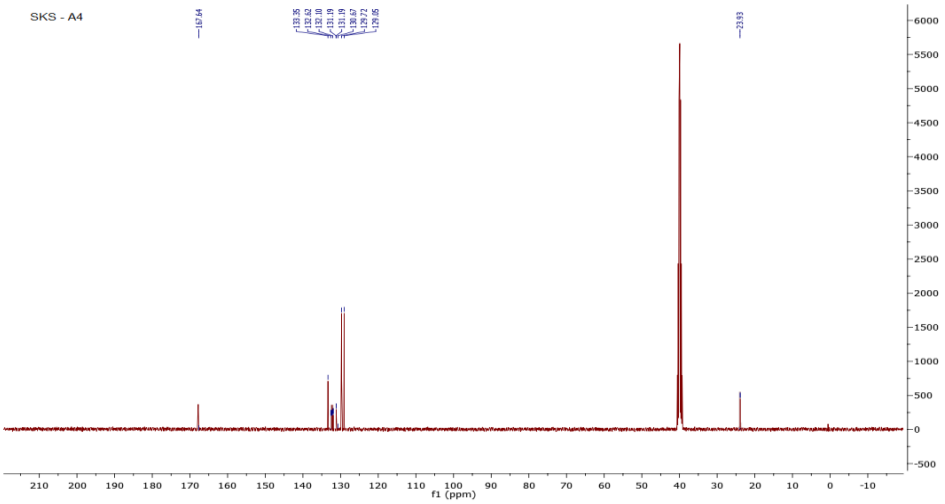


Figure 20: ^{13}C NMR Spectra for compound A4

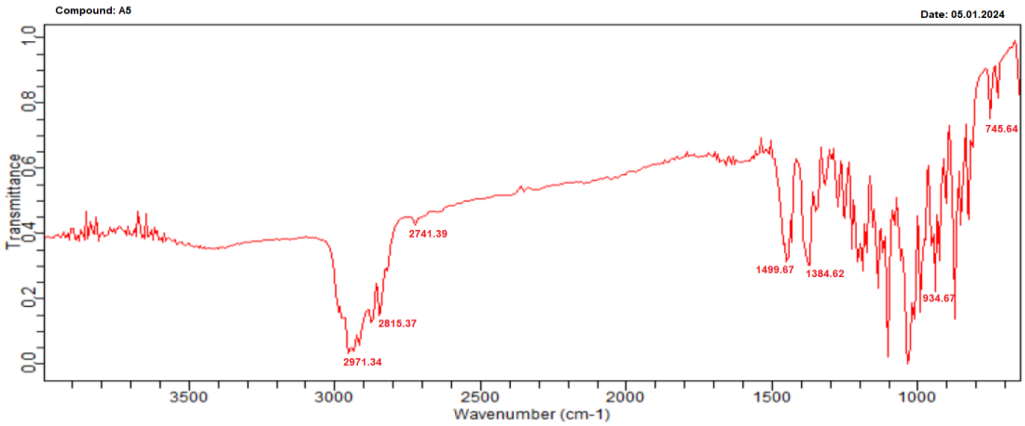


Figure 21: IR Spectra for compound A5

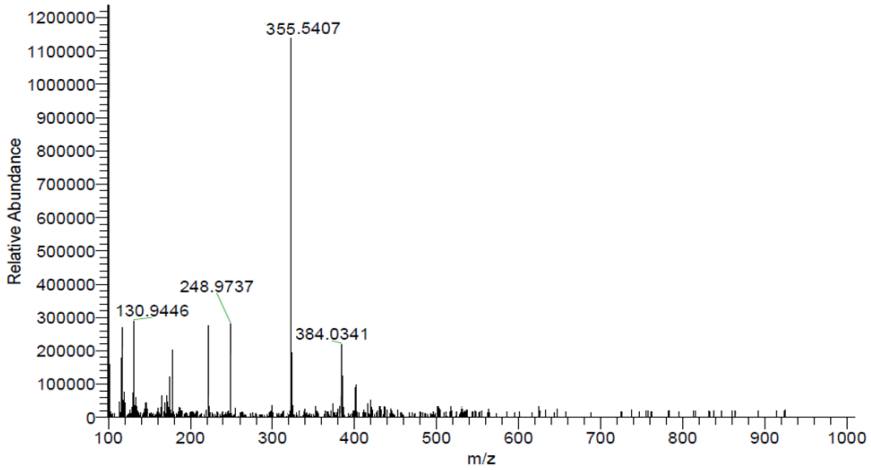


Figure 22: Mass Spectra for compound A5

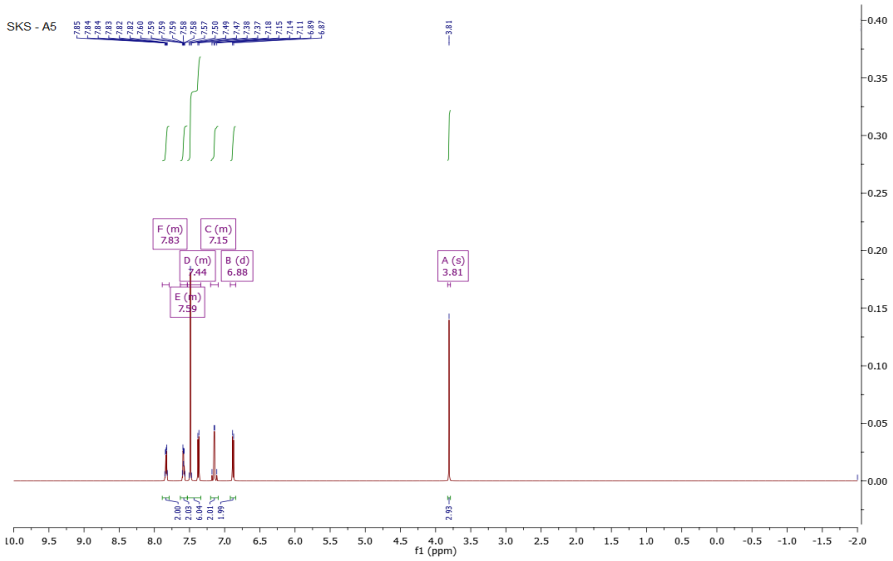


Figure 23: ^{1}H NMR Spectra for compound A5

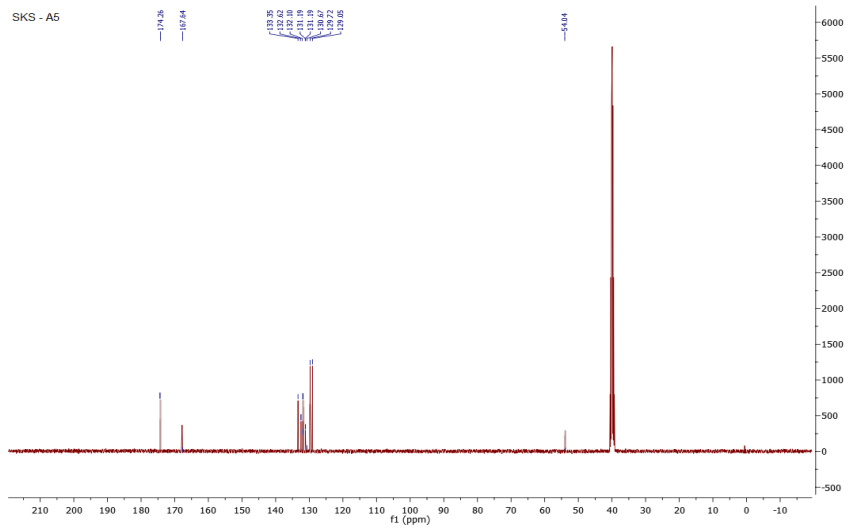


Figure 24: ^{13}C NMR Spectra for compound A5

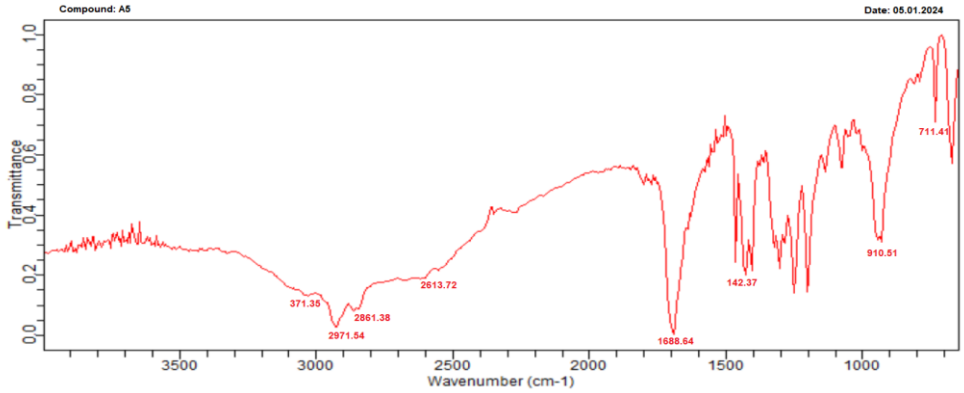


Figure 25: IR Spectra for compound A6

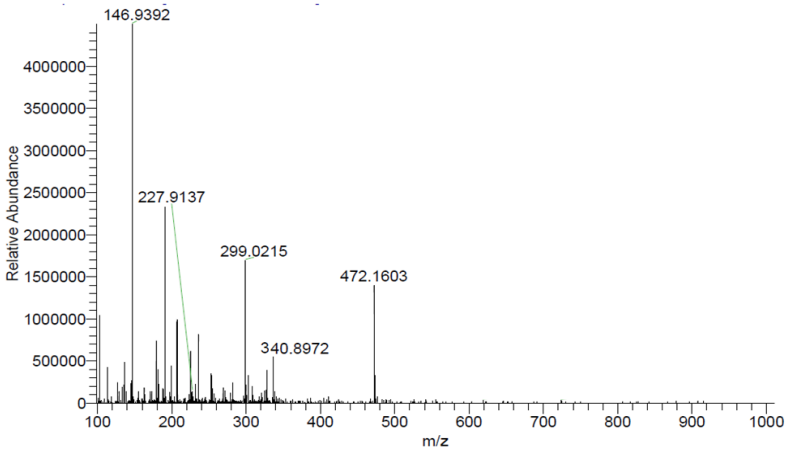


Figure 26: Mass Spectra for compound A6

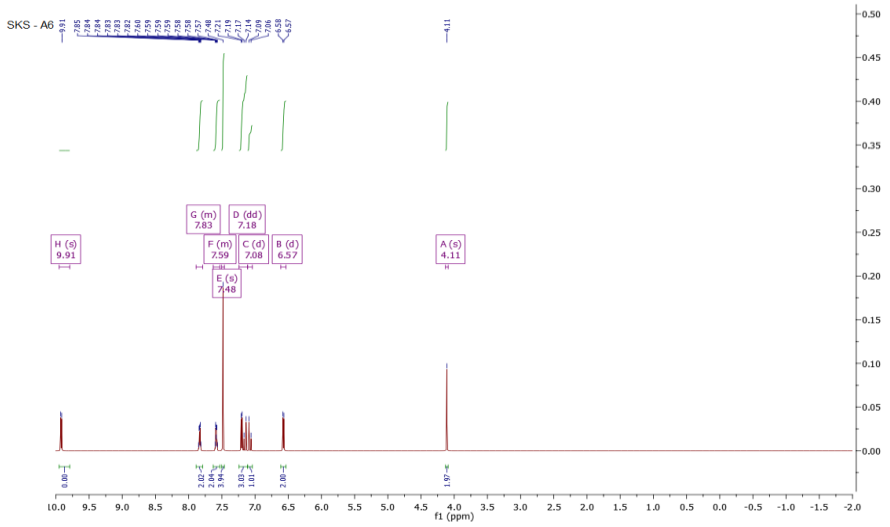


Figure 27: ^{1}H NMR Spectra for compound A6

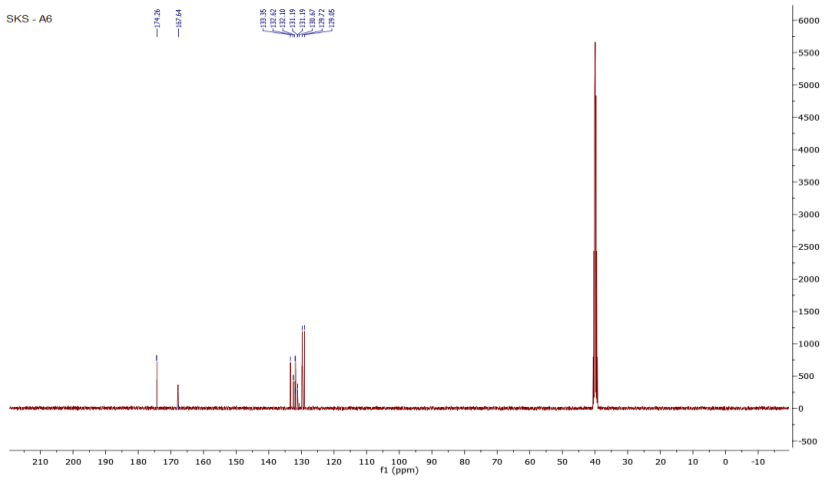


Figure 28: ^{13}C NMR Spectra for compound A6

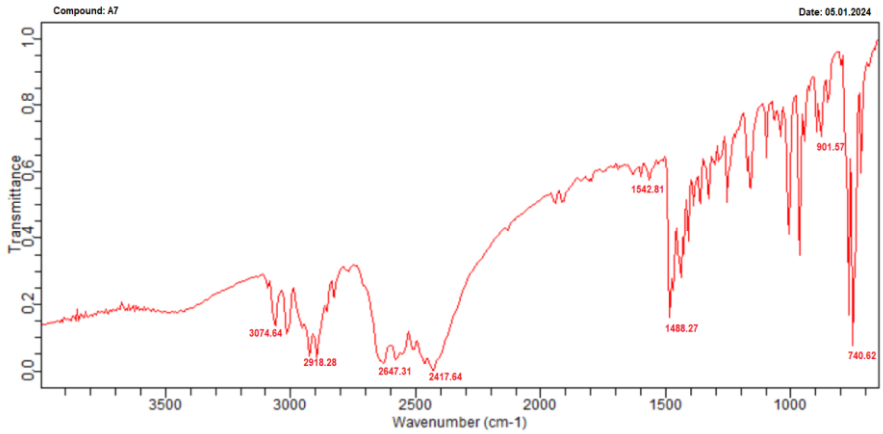


Figure 29: IR Spectra for compound A7

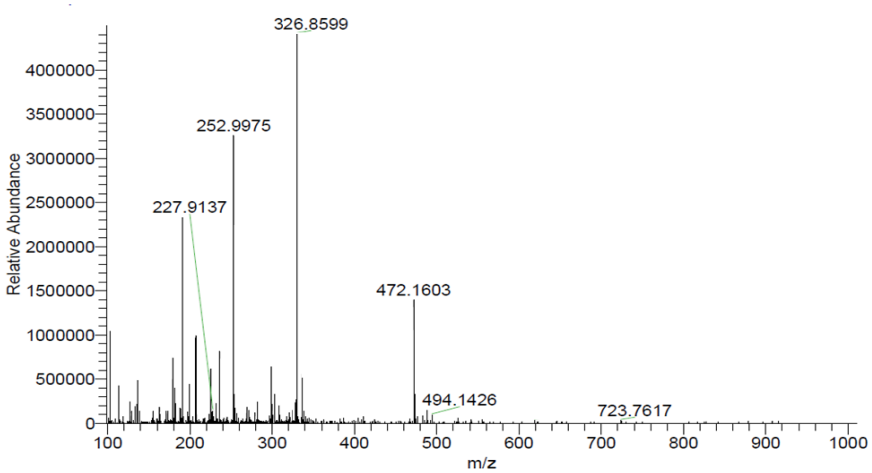


Figure 30: Mass Spectra for compound A7

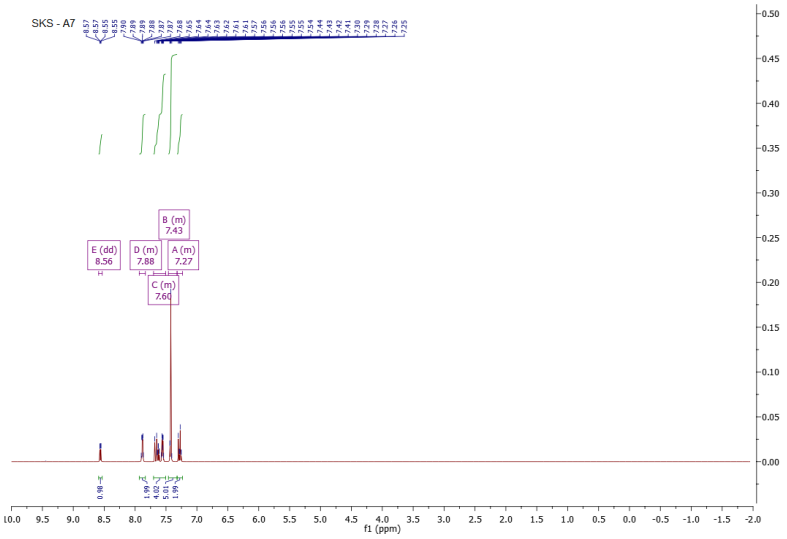


Figure 31: ^{1}H NMR Spectra for compound A7

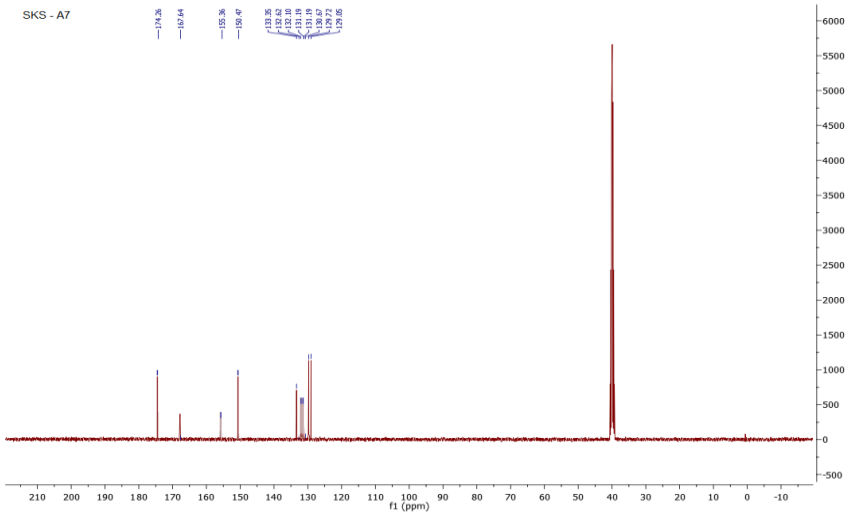


Figure 32: ^{13}C NMR Spectra for compound A7

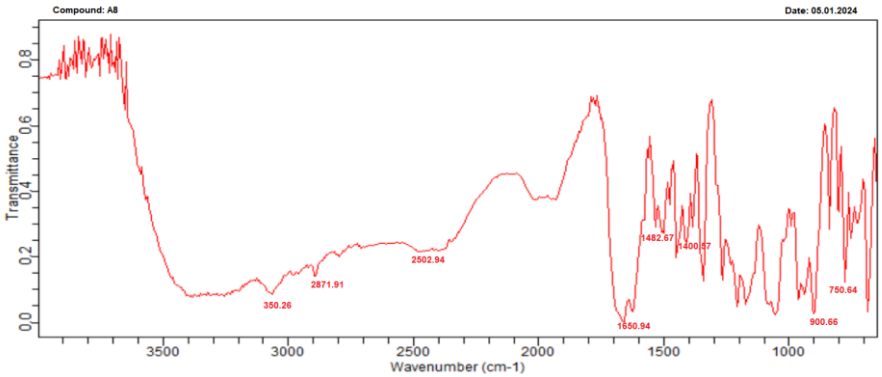


Figure 33: IR Spectra for compound A8

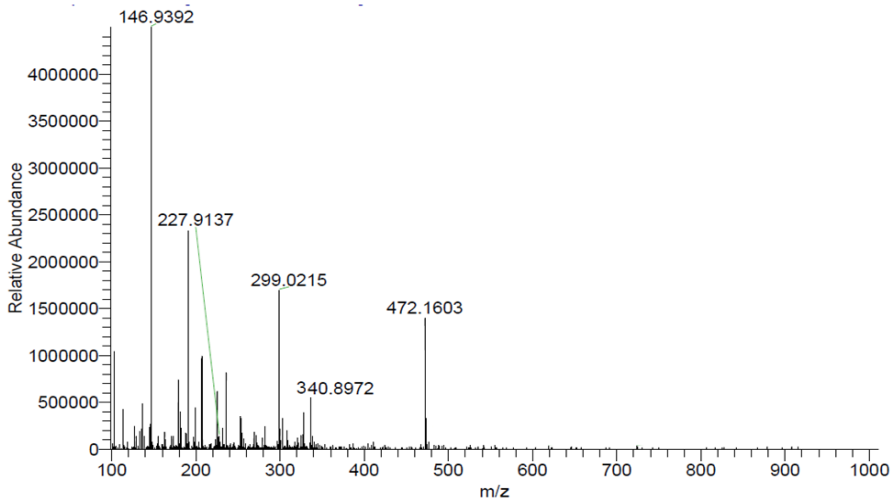


Figure 34: Mass Spectra for compound A8

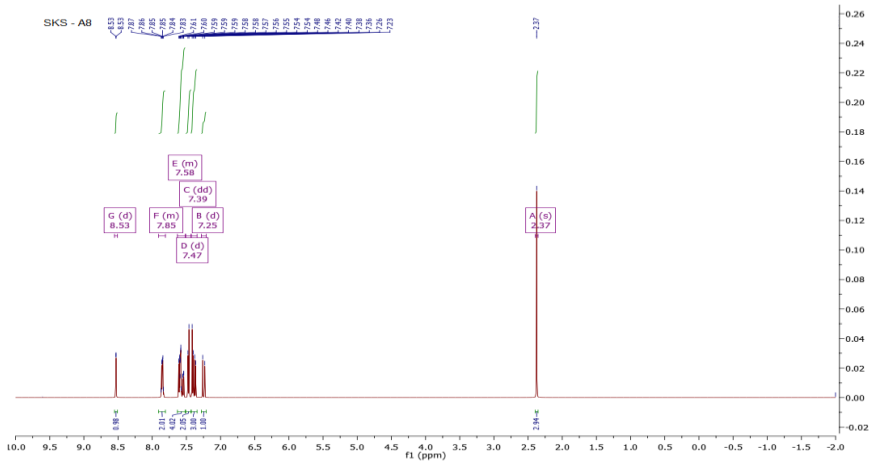


Figure 35: ^{1}H NMR Spectra for compound A8

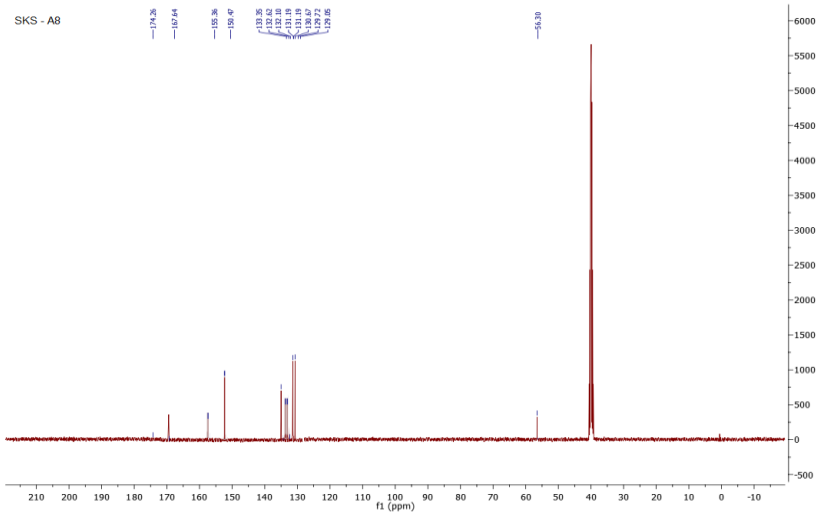


Figure 36: ^{13}C NMR Spectra for compound A8

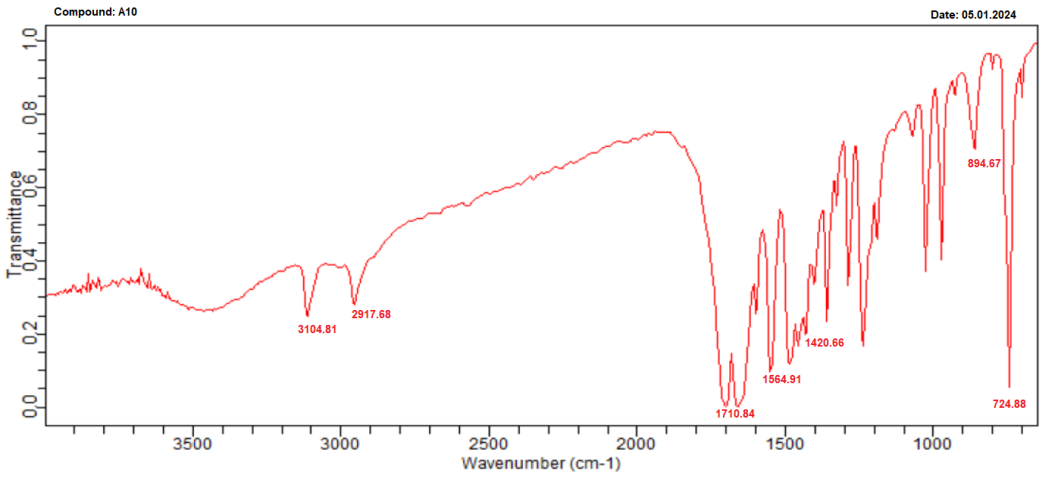


Figure 37: IR Spectra for compound A10

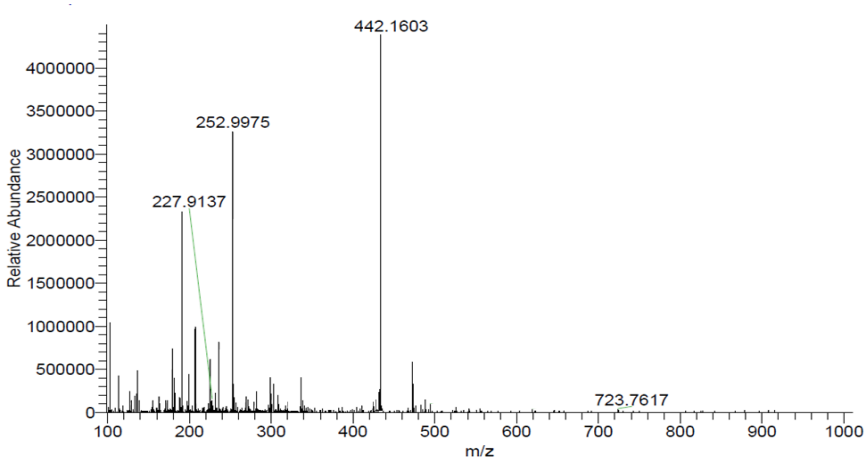


Figure 38: Mass Spectra for compound A10

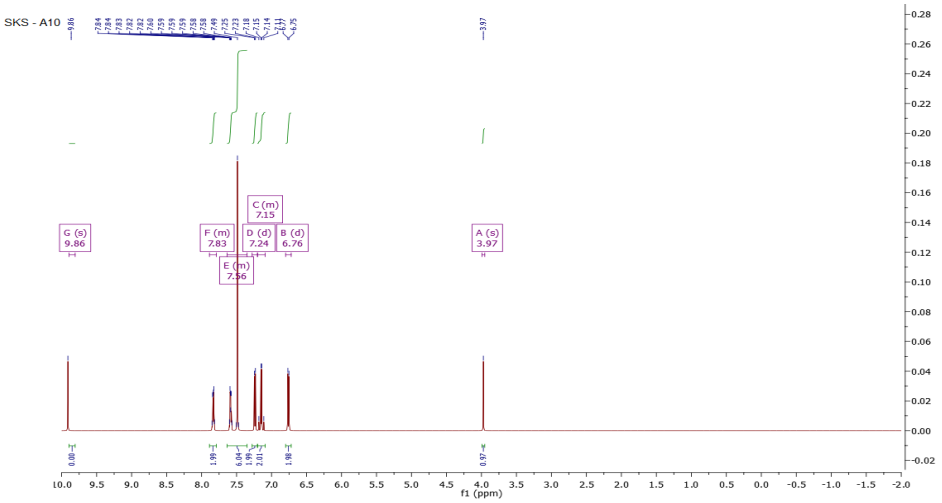


Figure 39: ^{1}H NMR Spectra for compound A10

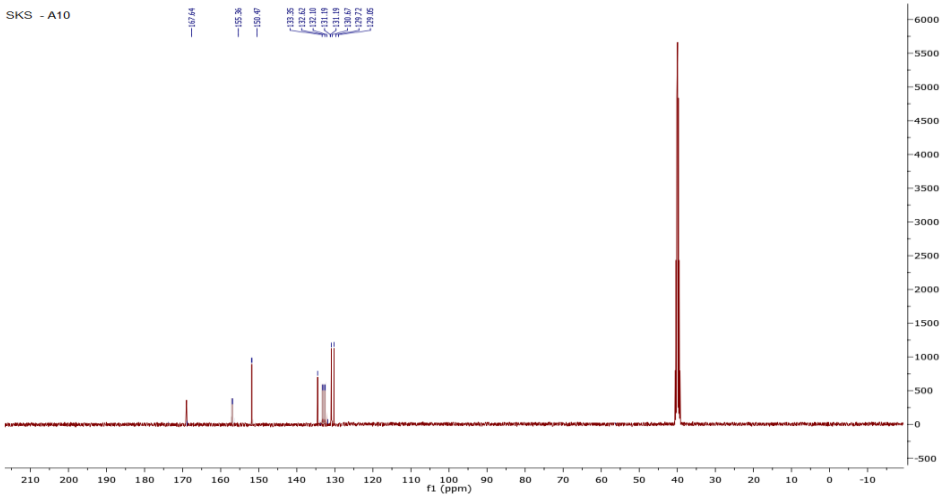


Figure 40: ^{13}C NMR Spectra for compound A10

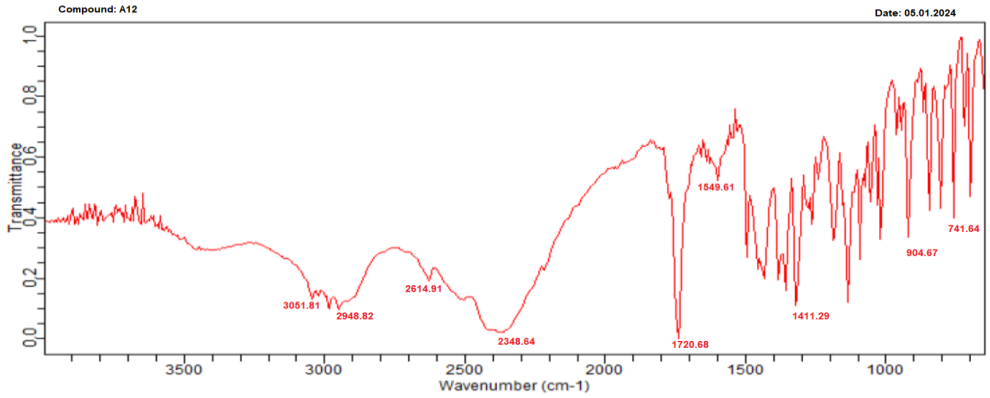


Figure 41: IR Spectra for compound A12

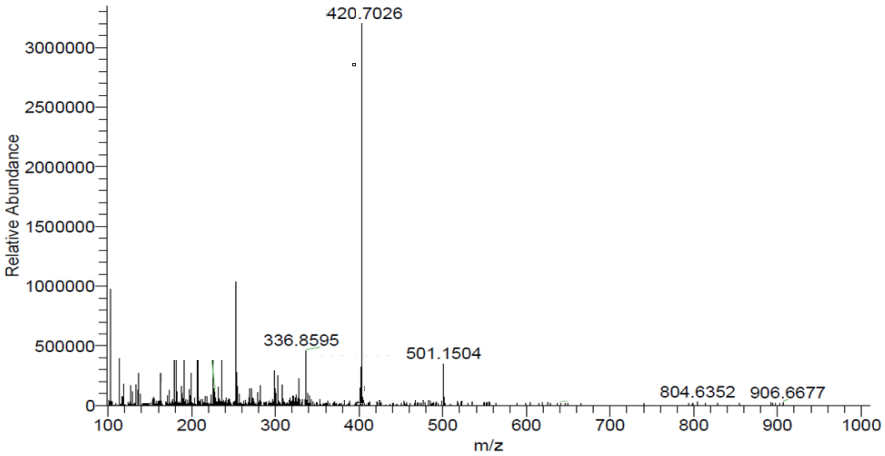


Figure 42: Mass Spectra for compound A12

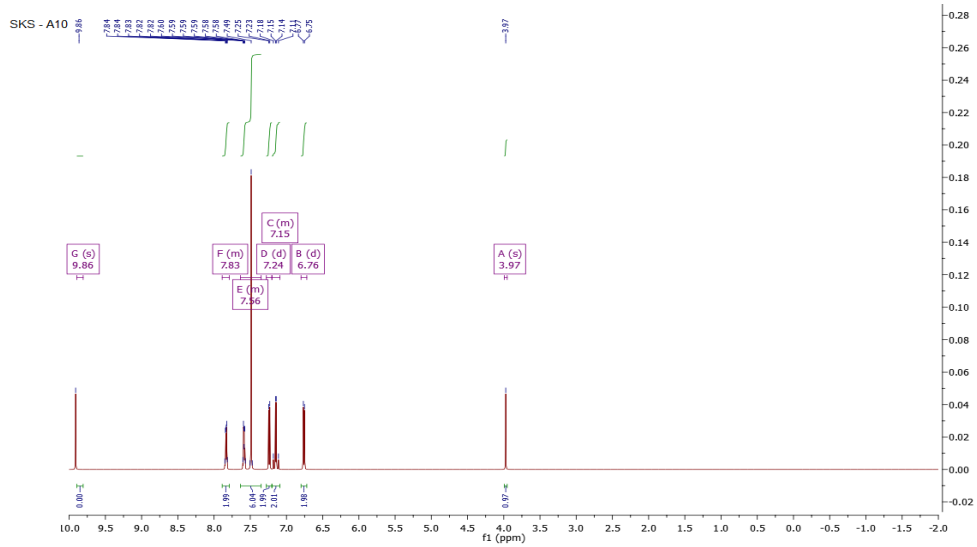


Figure 43: ^{1}H NMR Spectra for compound A12

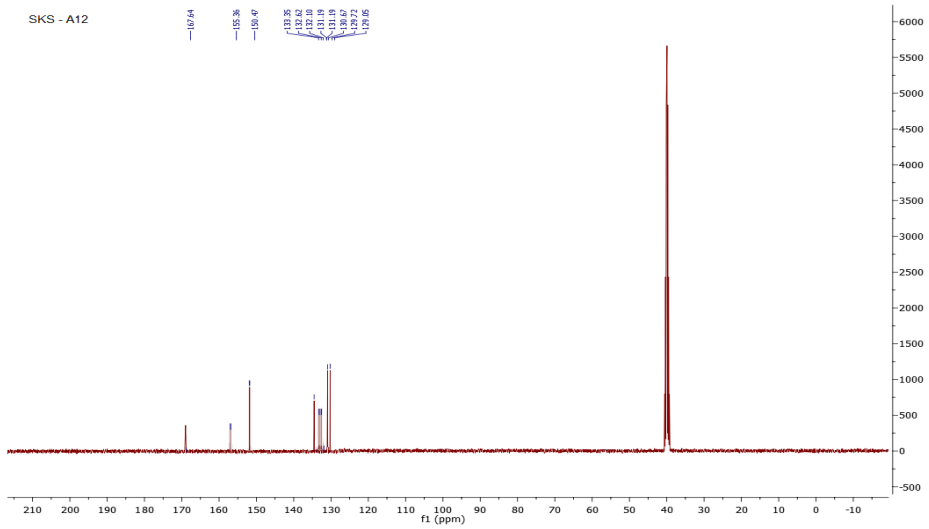


Figure 44: ^{13}C NMR Spectra for compound A12

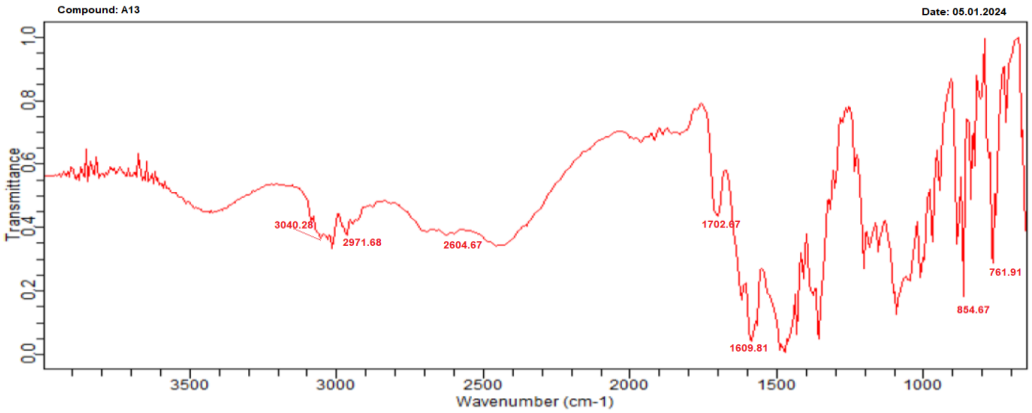


Figure 45: IR Spectra for compound A13

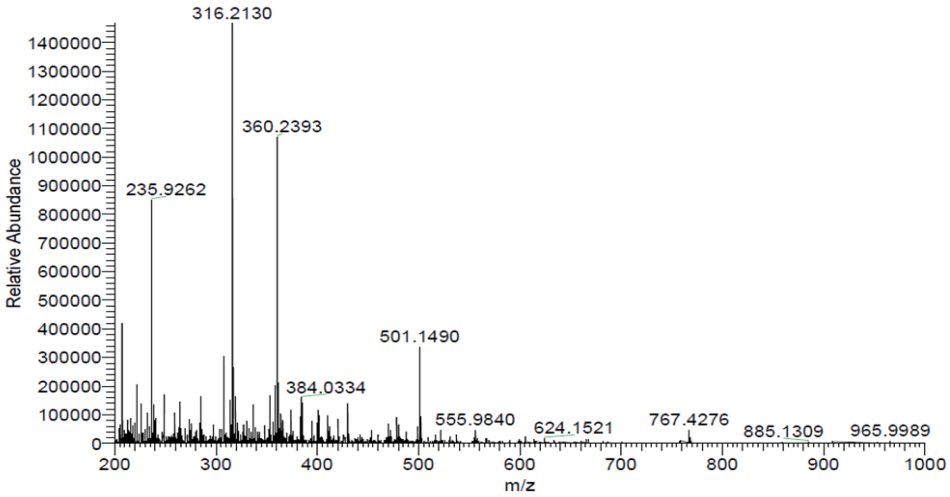


Figure 46: Mass Spectra for compound A13

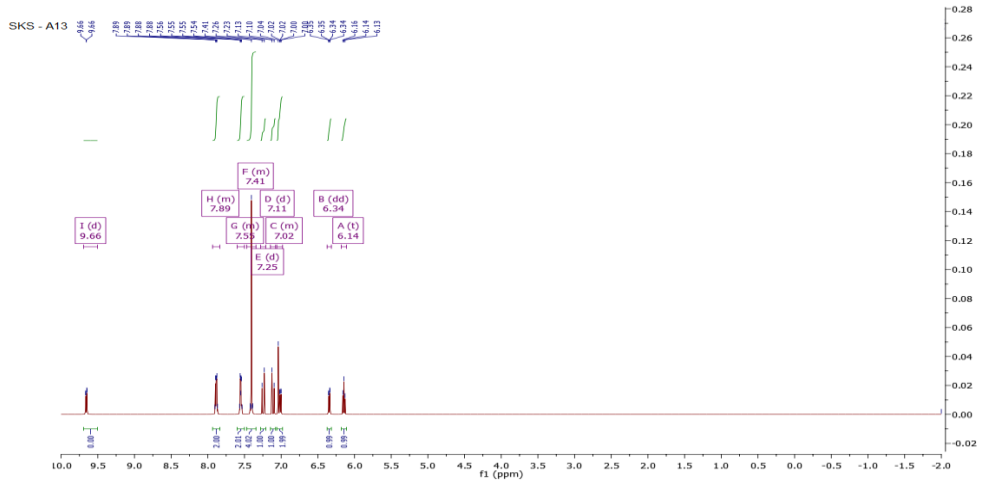


Figure 47: ^{1}H NMR Spectra for compound A13

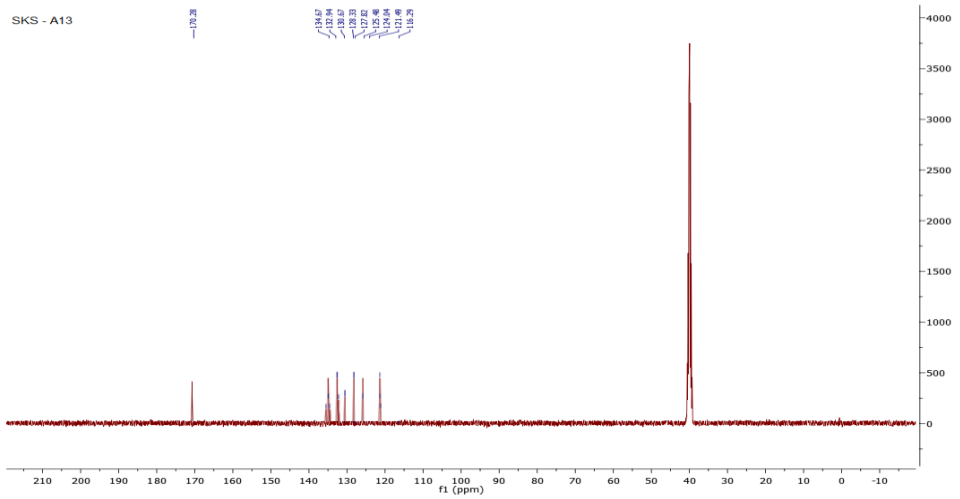


Figure 48: ^{13}C NMR Spectra for compound A13

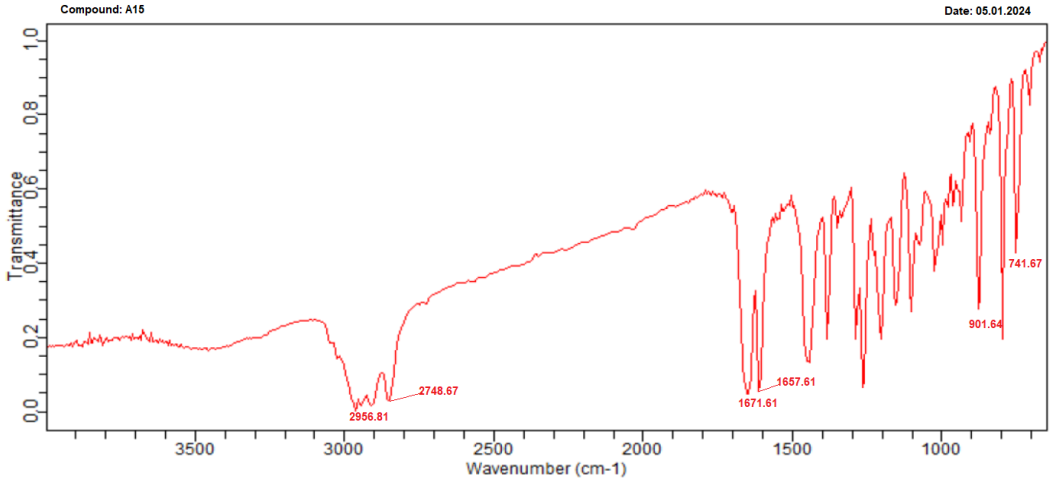


Figure 49: IR Spectra for compound A15

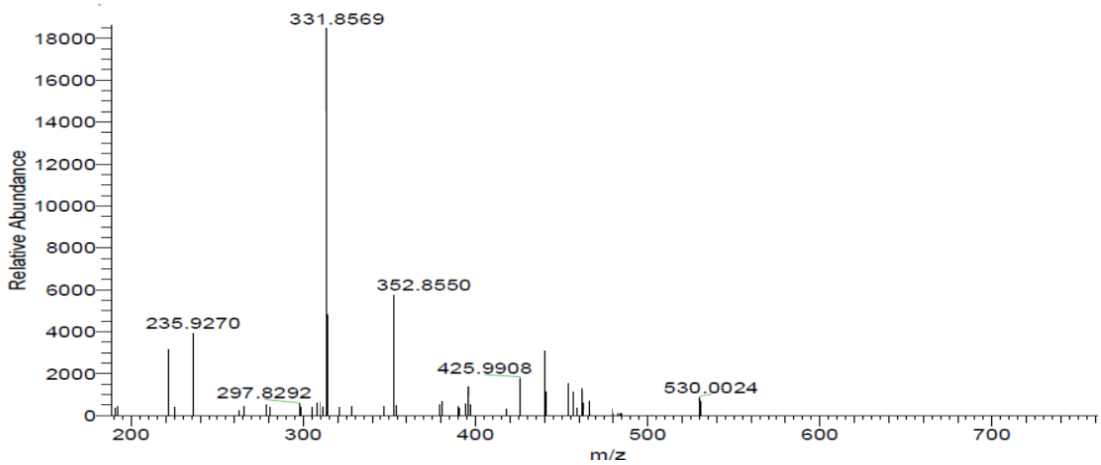


Figure 50: Mass Spectra for compound A15

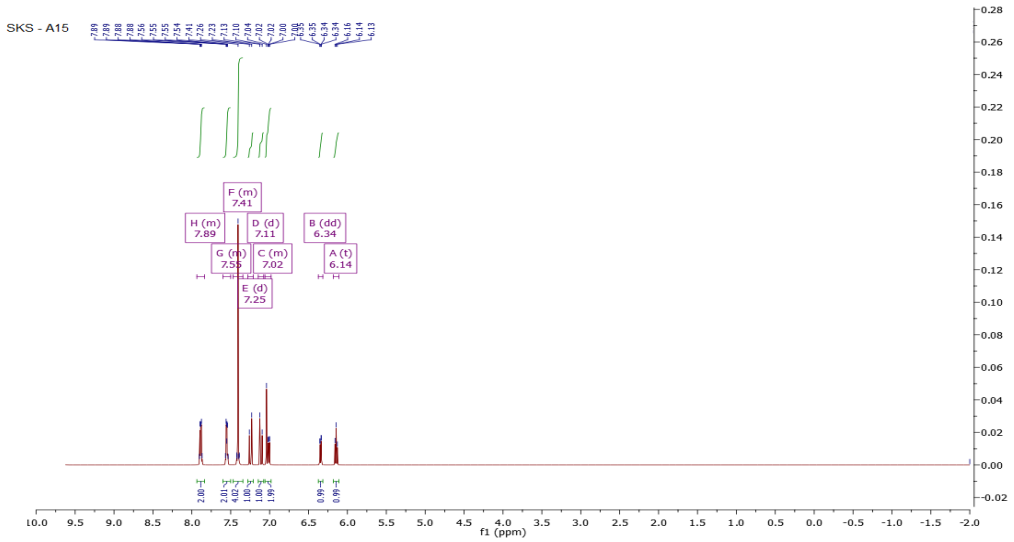


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Figure 51: ^{1}H NMR Spectra for compound A15

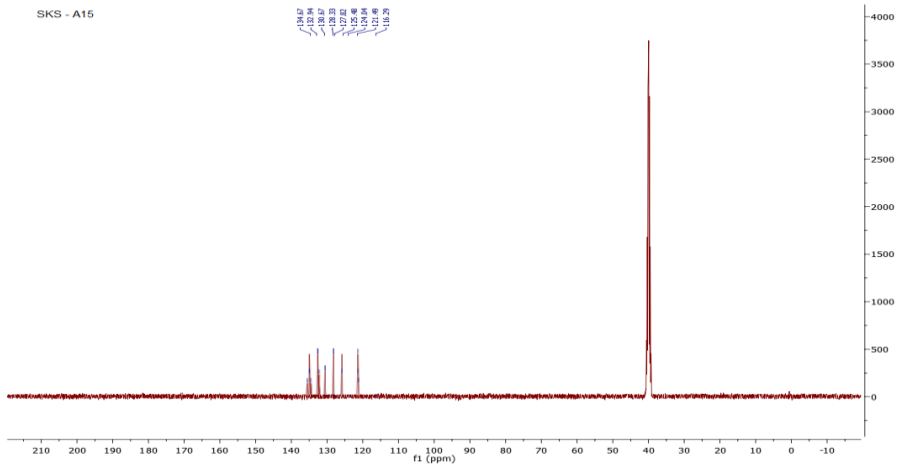

Figure 52: ^{13}C NMR Spectra for compound A15

Characterization of synthesized compounds:

a. (E)-2-(4-(4-chlorostyryl)phenyl)isoindoline-1,3-dione (A2)

$C_{22}H_{14}ClNO_2$; Brown colour solid; MP: 102 – 105^{O}C; Rf: 0.57; IR (KBr) cm^{-1}: 3124 (CH str alkene), 2574 (CH str aromatic), 1694 (C=O str ketone), 970 (aromatic ring), 720 (C-Cl str); ^{1}H NMR (500 MHz, DMSO) δ 7.89 – 7.79 (m, 1H), 7.63 – 7.53 (m, 1H), 7.53 – 7.43 (m, 2H), 7.25 (ddd, *J* = 38.5, 25.0, 11.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 167.80, 133.35, 132.62, 132.10, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 359 m/z; found: 359 m/z.

b. (E)-2-(4-(4-methylstyryl)phenyl)isoindoline-1,3-dione (A4)

$C_{23}H_{17}NO_2$; Brown colour solid; MP: 110 – 113^{O}C; Rf: 0.57; IR (KBr) cm^{-1}: 2934 (CH str alkene), 2901 (CH str CH3), 2641 (CH str aromatic), 1684 (C=O str ketone), 850 (aromatic ring), 730 (C-Cl str); ^{1}H NMR (500 MHz, DMSO) δ 7.63 – 7.53 (m, 2H), 7.53 – 7.44 (m, 4H), 17.24 – -1.57 (m, 17H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.17 (dt, *J* = 14.3, 11.3 Hz, 4H), 7.79 – -1.57 (m, 15H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 23.93; Mass: Actual: 339 m/z; found: 338 (M-1) m/z.

c. (E)-2-(4-(4-methoxystyryl)phenyl)isoindoline-1,3-dione (A5)

$C_{23}H_{17}NO_3$; Brown colour solid; MP: 106 – 109^{O}C; Rf: 0.61; IR (KBr) cm^{-1}: 2971 (CH str alkene), 2815 (CH str CH_3), 2741 (CH str aromatic), 1499 (C=O str ketone), 934 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.89 – 7.79 (m, 2H), 7.63 – 7.53 (m, 2H), 7.53 – 7.34 (m, 6H), 7.20 – 7.09 (m, 2H), 6.88 (d, *J* = 7.4 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 54.04. Mass: Actual: 355 m/z; found: 355 (m/z).

d. (E)-2-(4-(4-aminostyryl)phenyl)isoindoline-1,3-dione (A6)

$C_{22}H_{16}N_2O_2$; Yellow colour solid; MP: 116 – 118^{O}C; Rf: 0.63; IR (KBr) cm^{-1}: 3171 (NH str amine), 2971 (CH str alkene), 2866 (CH str aromatic), 1688 (C=O str ketone), 910 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.91 (s, 1H), 7.89 – 7.79 (m, 2H), 7.63 – 7.53 (m, 2H), 7.48 (s, 3H), 7.18 (dd, *J* = 25.6, 11.3 Hz, 3H), 7.08 (d, *J* = 15.2 Hz, 1H), 6.57 (d, *J* = 7.5 Hz, 2H), 4.11 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 340 m/z; found: 340 m/z.

e. (E)-2-(4-(2-(pyridin-2-yl)vinyl)phenyl)isoindoline-1,3-dione (A7)

$C_{21}H_{14}N_2O_2$; Red colour solid; MP: 113 – 116^{O}C; Rf: 0.51; IR (KBr) cm^{-1}: (CH str alkene), (CH str aromatic), (C=O str ketone), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.56 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.93 – 7.83 (m, 2H), 7.70 – 7.51 (m, 4H), 7.46 – 7.32 (m, 5H), 7.32 – 7.23 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 326 m/z; found: 326 m/z.

f. (E)-2-(4-(2-(5-methylpyridin-2-yl)vinyl)phenyl)isoindoline-1,3-dione (A8)

$C_{22}H_{16}N_2O_2$; Red colour solid; MP: 121 – 123^{O}C; Rf: 0.59; IR (KBr) cm^{-1}: 3074 (CH str alkene), 2918 (CH str CH_3), 2417 (CH str aromatic), 1542 (C=O str ketone), 901 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.53 (d, *J* = 1.4 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.63 – 7.52 (m, 4H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.39 (dd, *J* = 18.3, 7.5

Hz, 3H), 7.25 (d, *J* = 15.2 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 56.30; Mass: Actual: 340 m/z; found: 340.

g. (E)-2-(4-(4-hydroxystyryl)phenyl)isoindoline-1,3-dione (A10)

$C_{22}H_{15}NO_3$; Orange colour solid; MP: 118 – 121^{O}C; Rf: 0.62; IR (KBr) cm^{-1}: 3050 (OH str), 2871 (CH str alkene), 2502 (CH str aromatic), 1650 (C=O str ketone), 900 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 7.89 – 7.79 (m, 1H), 7.63 – 7.35 (m, 2H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.20 – 7.09 (m, 3H), 6.76 (d, *J* = 7.5 Hz, 1H), 3.97 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 341 m/z; found: 342 (M+1).

h. (E)-2-(4-(5-bromo-2-hydroxystyryl)phenyl)isoindoline-1,3-dione (A12)

$C_{22}H_{14}BrNO_3$; Orange colour solid; MP: 114 – 117^{O}C; Rf: 0.64; IR (KBr) cm^{-1}: 3051 (OH str), 2948 (CH str alkene), 2348 (CH str aromatic), 1720 (C=O str ketone), 904 (aromatic ring), 741 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 9.86 (s, 1H), 7.89 – 7.79 (m, 1H), 7.63 – 7.35 (m, 2H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.20 – 7.09 (m, 3H), 6.76 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 56.30; Mass: Actual: 419 m/z; found: 420 (M+1) m/z.

i.(E)-2-(4-(2-(1H-pyrrol-2-yl)vinyl)phenyl)isoindoline-1,3-dione (A13)

$C_{20}H_{14}N_2O_2$; Brown colour solid; MP: 112 – 115^{O}C; Rf: 0.65; IR (KBr) cm^{-1}:3040 (NH str amine), 2971 (CH str alkene), 2604 (CH str aromatic), 1702 (C=O str ketone), 854 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.66 (d, *J* = 0.6 Hz, 1), 7.93 – 7.84 (m, 2H), 7.60 – 7.50 (m, 2H), 7.47 – 7.34 (m, 1H), 7.25 (d, *J* = 15.0 Hz, 2H), 7.11 (d, *J* = 15.0 Hz, 2H), 7.06 – 6.98 (m, 1H), 6.34 (dd, *J* = 7.5, 1.4 Hz, 2H), 6.14 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 170.28, 134.67, 132.94, 130.67, 128.33, 127.82, 125.48, 124.04, 121.49, 116.29; Mass: Actual: 314 m/z; found: .

j. (E)-2-(4-(2-(thiophen-2-yl)vinyl)phenyl)isoindoline-1,3-dione (A15)

$C_{20}H_{13}NO_2S$; Brown colour solid; MP: 122 – 125^{O}C; Rf: 0.51; IR (KBr) cm^{-1}: 2956 (CH str alkene), 2748 (CH str aromatic), 1671 (C=O str ketone), 901 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.66 (d, *J* = 0.6 Hz, 1), 7.93 – 7.84 (m, 2H), 7.60 – 7.50 (m, 2H), 7.47 – 7.34 (m, 1H), 7.25 (d, *J* = 15.0 Hz, 2H), 7.11 (d, *J* = 15.0 Hz, 2H), 7.06 – 6.98 (m, 1H), 6.34 (dd, *J* = 7.5, 1.4 Hz, 2H), 6.14 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 134.67, 132.94, 130.67, 128.33, 127.82, 125.48, 124.04, 121.49, 116.29; Mass: Actual: 331 m/z; found: 331 m/z.

Anti-inflammatory:

There are many methods to estimate the anti-inflammatory action of drugs. At present, a study has been done with different concentrations of four synthesized compounds for anti-inflammatory activity. The anti-inflammatory study was compared with a standard drug paracetamol. Results of in-vitro anti-inflammatory activity of the compounds were expressed as percentage values which were determined by plotting the absorption versus concentration of sample on a logarithmic graph and reading off the control. The experiments were performed in triplicates, and then, the final values were calculated by taking average of triplicate experimental results. The study was also carried out with the standard drug indomethacin. All the tested compounds displayed significant inhibition at a concentration range of 100–500 µg/mL. Among the tested compounds, the compound A10 which is substituted with hydroxyl benzene and the compound A7 substituted with pyridine moiety displayed considerable anti-inflammatory activity compared to standard drug indomethacin. Primarily the inhibition of protein (albumin) denaturation was studied and was found the maximum in ethanolic solution of A10 with 98.55% at concentration of 500 µg/mL, and followed by the ethanolic solution of A7 with 97.55% at concentration of 500 µg/mL. Results are represented in **Table 2**.

Table 2. Result for *invitro* anti-inflammatory activity

Percentage of albumin denaturation (%)											
Concentration (µg/mL)	**A2**	**A4**	**A5**	A6	**A7**	**A8**	**A10**	**A12**	**A13**	**A15**	**Standard**
100	31.05	25.56	36.88	15.25	35.82	31.05	45.56	25.25	16.35	15.37	51.84
200	39.04	31.05	52.21	22.89	41.73	39.04	51.05	42.89	24.61	27.61	63.33
300	65.56	48.42	64.13	28.28	68.48	65.56	68.42	58.28	31.58	31.67	76.76
400	72.36	57.68	70.33	33.25	77.57	72.36	79.68	63.25	47.67	51.67	85.89
500	79.02	67.55	82.94	47.80	97.55	79.02	98.55	87.80	59.34	74.64	99.37

Calculation of molecular properties:
The molecular properties were calculated on the basis of simple molecular descriptors used by 'Lipinski's rule of 5'. The five properties consist of Molecular weight, Hydrogen bond donor; hydrogen bond acceptors, log P, and Total polar surface area (TPSA) which was calculated using the online cheminformatics tool molinspiration (http://www.molinspiration.com/) 18 and the results were shown in **Table 3**.

Table 3. Molecular descriptor properties of designed compounds

Compound Code	Log P	TPSA	N Atoms	n ON	N OHNH	N Violation	N rotb	Volume
A2	6.04	39.08	26	3	0	1	3	307.02
A3	5.52	39.08	26	3	0	1	3	299.12
A4	5.81	39.08	26	3	0	1	3	310.75
A5	5.14	48.31	27	4	0	1	4	319.73
A6	4.43	65.10	26	4	2	1	3	305.14
A7	4.01	51.97	25	4	0	0	3	290.03
A8	4.46	51.67	26	4	0	0	3	306.59
A10	4.88	59.30	26	4	1	0	3	302.00
A12	5.90	59.30	27	4	1	1	3	320.09
A13	4.33	54.87	24	4	1	0	3	279.19
A15	5.08	39.08	24	3	0	1	3	284.09

Druglikeness Properties of Designed Derivatives:
The Molinspiration virtual screening is fast (100,000 molecules may be screened in about 30 minutes) and therefore allows processing of very large molecular libraries. Validation tests performed on various target classes (including kinase inhibitors, various GPCR targets, different enzymes, etc.,) show 10 to 20- fold increases in hit rate in comparison with a standard / random selection of molecules for screening. The data's for drug likeness properties were depicted in table 4. Based on the result of druglikness properties of designed derivatives show no violations in their pharmacological action.

Table 4: Drug likeness properties of designed compounds

Compound Code	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
A2	-0.07	-0.10	-0.14	-0.02	-0.18	-0.09
A3	-0.06	-0.11	-0.09	0.02	-0.17	-0.07
A4	-0.11	-0.17	-0.16	-0.03	-0.20	-0.12
A5	-0.11	-0.17	-0.15	-0.02	-0.19	-0.10
A6	-0.03	-0.05	-0.02	-0.06	-0.07	0.02
A7	0.08	0.02	0.07	0.02	-0.06	0.06
A8	0.04	-0.05	0.01	-0.01	-0.13	0.00
A10	-0.03	-0.06	-0.08	0.13	-0.13	-0.01
A12	-0.17	-0.19	-0.17	-0.04	-0.28	-0.11
A13	0.04	-0.04	0.04	-0.00	-0.25	0.06
A15	-0.17	-0.35	-0.15	-0.10	-0.33	-0.14

SUMMARY AND CONCLUSION:

The physicochemical and spectroscopic data confirmed the structural integrity of the newly synthesized compounds. The investigated molecules displayed a similar manner to protein binding to the active site of cyclooxygenase in molecular docking studies. The calculated docking energies indicated that its interaction with cyclooxygenase is favourable, but only to a limited extent. The molecular discreptor value was studied by molinsipration software and all the parameters were within a limit. All the synthesized compounds were screened for their *in vitro* anti-inflammatory activity activity. Compounds A10 and A7 are emerged to be the most active compounds against in tested enzyme and these compounds also posses a significant docking score for the studied enzyme. The study thus serves as an attempt to progress toward the discovery of novel lead molecule for the treatment of inflammation. In future the additional derivatives may be prepared and further

extended in-depth investigations into *in-vivo* activity would be implemented to establish a SAR (Structural activity relationship) for rational study.

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Contribution:

1. **SUMATHI K**[*] - Contributed for the conceptual work in schemes of research work.
2. **ANITHA M**[1] - Contributed for the Liberatory works in research and literature works.
3. **SENTHIL KUMAR N**[1] - Contributed for the Literature works and a moral support.