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Synthesis, Docking Studies And Biological Evaluation Of Thiophene-Oxadiazole Hybrid Derivatives.

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Abstract

The novel thiophene-oxadiazole hybrid derivatives were schemed and substituted, and then they were checked for docking scores against the Human topoisomerase I enzyme. From that, the 10 best docked compounds are selected and synthesized, and spectral data of the synthesized compounds was obtained from IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. Then the ten compounds are subjected to *in-vitro* cytotoxicity studies by the MTT assay method with the MCF-7 cell line. Among the tested compounds, derivative **T11** substituted with a salicylic aldehyde moiety shows a significant IC_{50} value (45.68 nM) in the tested cell lines and possesses cytotoxic activity.

Keywords: Human topoisomerase I enzyme, MCF-7 cell line, salicylic aldehyde moiety, cytotoxic activity.

INTRODUCTION:

According to WHO, Cancer is a large group of diseases that can start in almost any organ or tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body and / or spread to other organs. The latter process is called metastasizing and is a major cause of death from cancer. Other names of cancer are neoplasm and malignant tumor**[1]** . As per GLOBOCAN 2020, the number of new cancer cases diagnosed in 2020 was 19.3 million, and almost 10.0 million died due to cancer. GLOBOCAN predicts that the number of cancer cases will increase to 28.4 million in 2040. Worldwide, Female breast cancer is the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%),and 6.9% mortality rate . Breast cancer is the second major cause of the cancer deaths across the world**[2]** . The molecular hybridization (MH) is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybridarchitectures that maintain pre-selected characteristics of the original templates**[3]**.

Oxadiazole core:
oxadiazole is a heterocyclic aromatic chemical compound of azole family with molecular formula $C_2H_2N_2O$.

There are 4 isomers of oxadiazole namely,
1. 1,2,3 oxadiazole
2. 1,2,4 oxadiazole
3. 1,2,5 oxadiazole
4. 1,3,4 oxadiazole

1,3,4-oxadiazole is a 5membered aromatic ring containing one oxygen & 2 Nitrogen atoms seen in many synthetic molecules **[4]**. Structural feature of 1,3,4-oxadiazole ring with pyridine type of Nitrogen atom is effective for its derivatives in binding with different enzymes.1,3,4-Oxadiazole with therapeutic potency are used to treat different ailments & entire medicinal chemistry such as anticancer **[5]**, anti-fungal **[6]**, anti-bacterial **[7]**, anti-tubercular [8], anti-inflammatory **[9]**, anti- neuropathic **[10]**, anti-hypertensive **[11]**, anti-viral **[12]**, anti-histaminic **[13]**, anti-parasitic **[14]**, anti-obesity & other medicinal activities.

Thiophene core:
Thiophene belongs to a class of heterocyclic compounds containing a five membered ring made up of one sulphur as heteroatom with the molecular formula C_4H_4S. Thiophene structure can be found in certain natural products and is also incorporated in several pharmacologically active compounds **[15]**. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications.
The thiophene nucleus has been recognized as an important entity in the synthesis of heterocyclic compounds with promising pharmacological characteristics. Thiophene moiety and their derivatives are known as antidiabetic, antihypertensive, antimicrobial, analgesic and anti-inflammatory, cholesterol inhibitors, antiviral, and antitumor agents **[16 – 20].** In light of afore-mentioned facts and in continuance of our research on the improvement of thiophene derivatives, we hereby report the synthesis and biological screening of thiophene derivatives.

MATERIALS AND METHODS:

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 .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 centimetres the band positions are noted. Nuclear magnetic spectra (^{1}H NMR) were obtained from Bruker DRX-300 (500 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.

Chemistry
Synthesis of 5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (figure 1)
Semicarbazide (50 mmol), thiophene-2-carboxylic acid (50mmol) and sulphuric acid (13 mL) were mixed, and the mixture was heated for 35 to 45 minutes in a fuming cupboard at 65 to 75 °C. After heating, cool the mixture to 27°C in an ice bath before adding approximately 100 mL of distilled water. Following the installation of a reflux condenser, the mixture was heated for around 22–24 hours. The completion of the reaction was monitored by TLC and the mobile phase of n-hexane: ethyl acetate (5:5). The liquid was cooled and basified following the addition of 50% NaOH and continuous stirring. After washing the filtered sediments in distilled water, the product was air dried. Ethanol was utilized to recrystallize.

General procedure for synthesis of title compounds (figure 2)
Refluxing a mixture of 5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (0.01 mol) and the corresponding aromatic or heteroaromatic aldehyde (0.01 mol) in 100% ethyl alcohol (10–12 mL) with NaOH (pellet) as catalyst. The mobile phase for TLC was N-hexane: ethyl acetate (5:5), and the mixture was decanted into a

Petri plate. Once the solvent had air dried, the result was scraped out. This product was washed with diluted HCl in order to carefully remove the surplus base by neutralization.

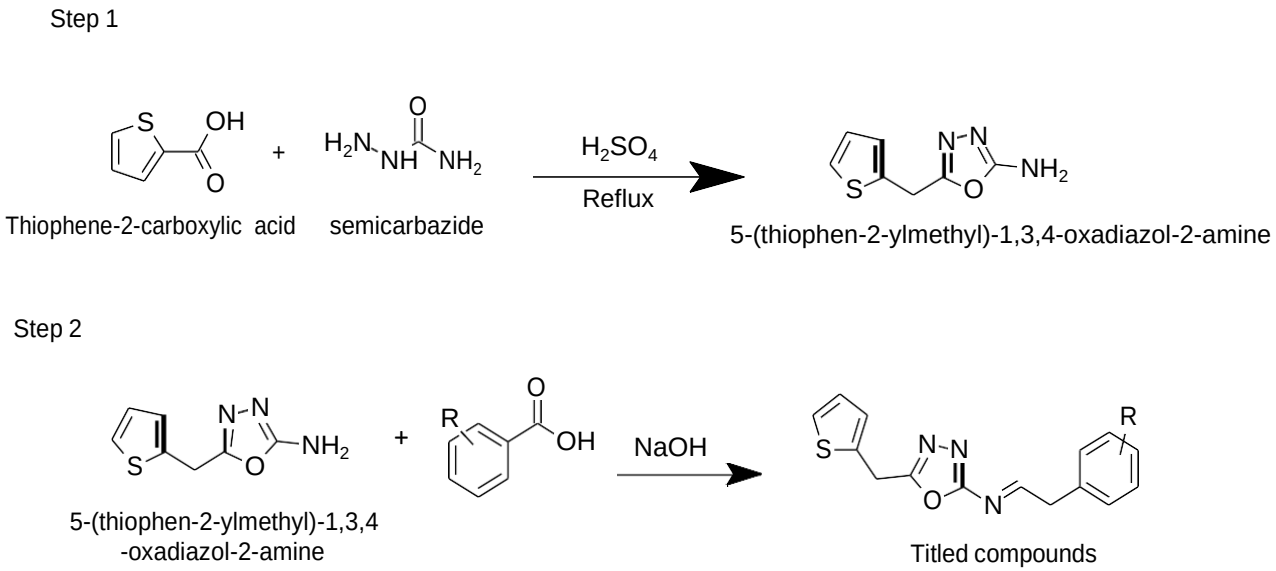

Figure 1. Synthesis of 5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine

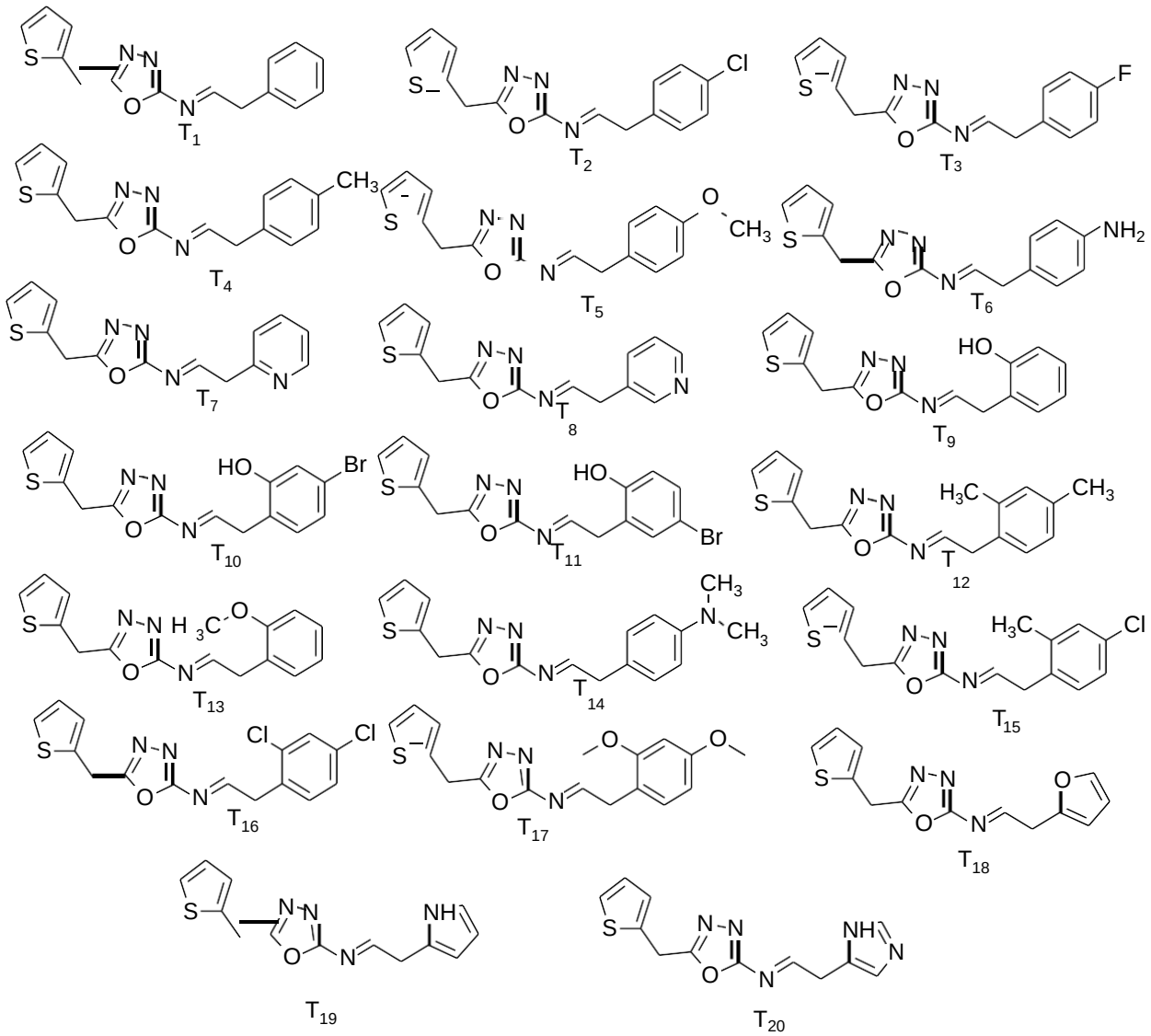

Figure 2. Newly designed compounds

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

Devices and materials

The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some offline programming's like protein data bank (PDB: 1A35) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through PyRx docking software.

Preparation of protein

By utilizing the online program protein data bank (PDB, we take the Human topoisomerase I (PDB ID: 1A35) was obtained from PDB website. From the protein 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 online tool in google. From this, we found the active amino acid present in the protein.

Preparation of Ligands

By utilizing the Marvin sketch tool, the designed molecules are sketched 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.

Molecular Docking

PyRx virtual screening tool is used . Because, it showed higher docking accuracy than other stages of the docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%) in the market coordinates in PDB format. Non- polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Molecular docking was performed using Molecular docking engine of PyRx software. The binding site was defined as a spherical region which encompasses all protein atoms within 15.0 A^{o} of bound crystallographic ligand atom. Default settings were used for all the calculations. Docking was performed using a grid resolution.

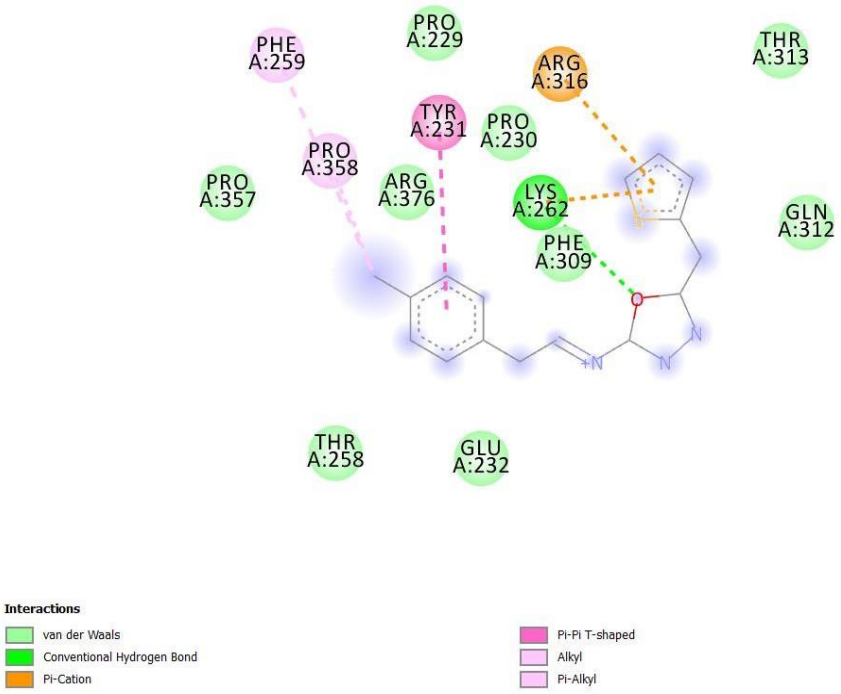

Figure 3. 2D docking interaction of compound T2 against Human topoisomerase I enzyme

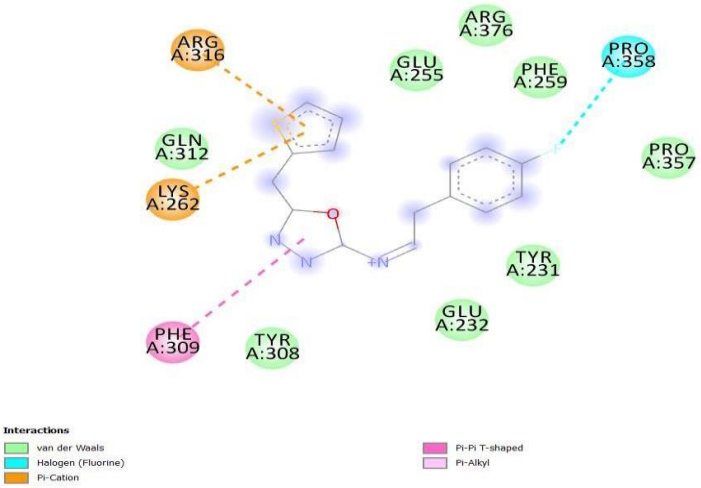

Figure 4. 2D docking interaction of compound T3 against Human topoisomerase I enzyme'

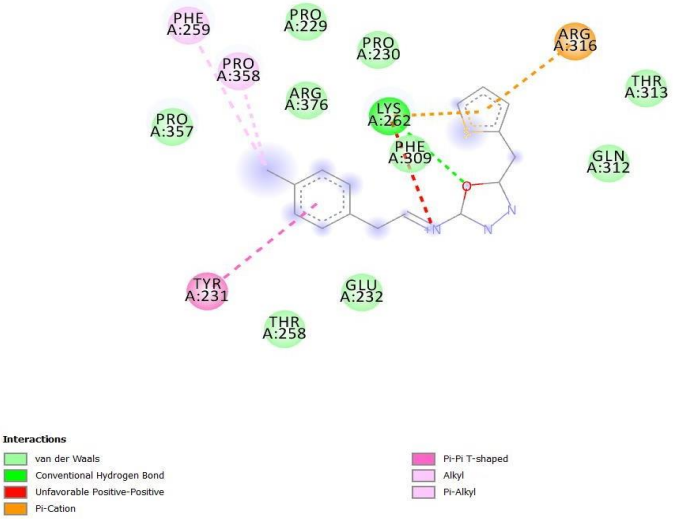

Figure 5. 2D docking interaction of compound T4 against Human topoisomerase I enzyme

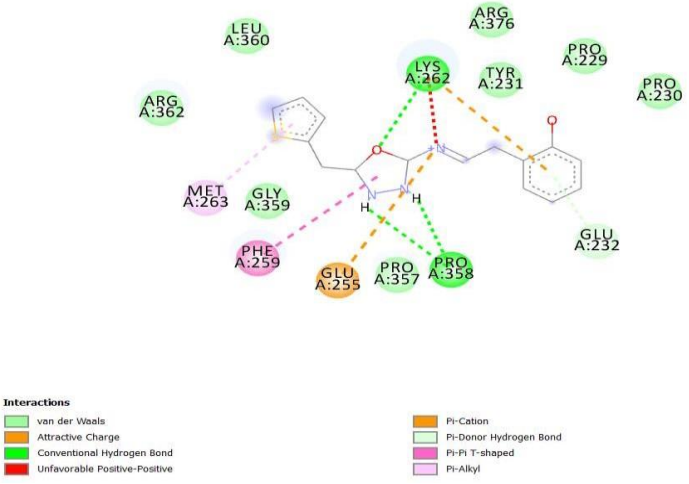

Figure 6. 2D docking interaction of compound T9 against Human topoisomerase I enzyme

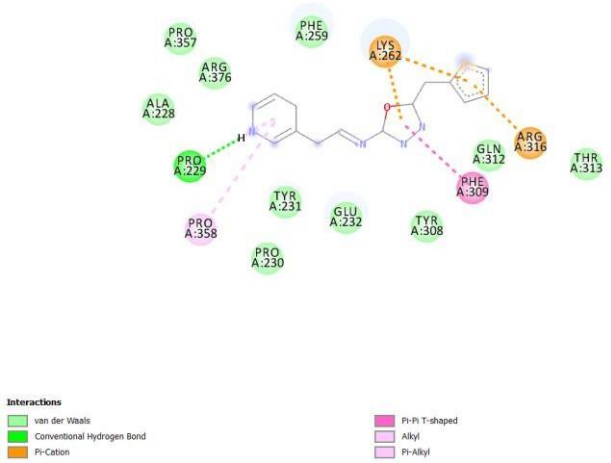

Figure 7. 2D docking interaction of compound T10 against Human topoisomerase I enzyme

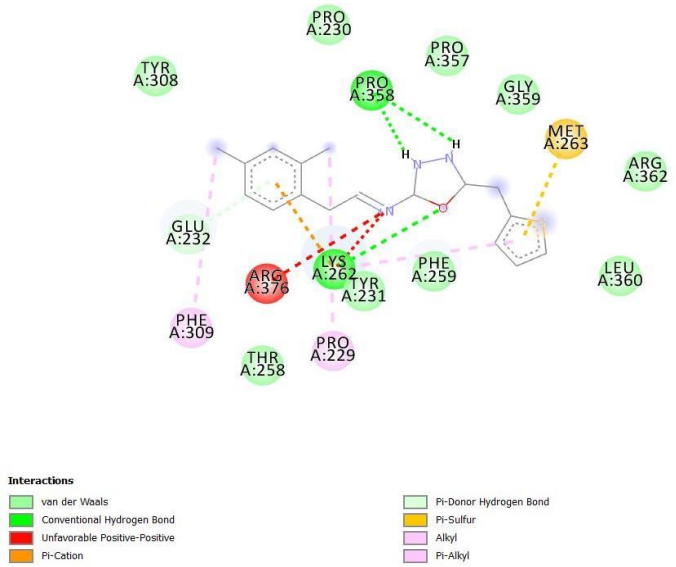

Figure 8. 2D docking interaction of compound T11 against Human topoisomerase I enzyme

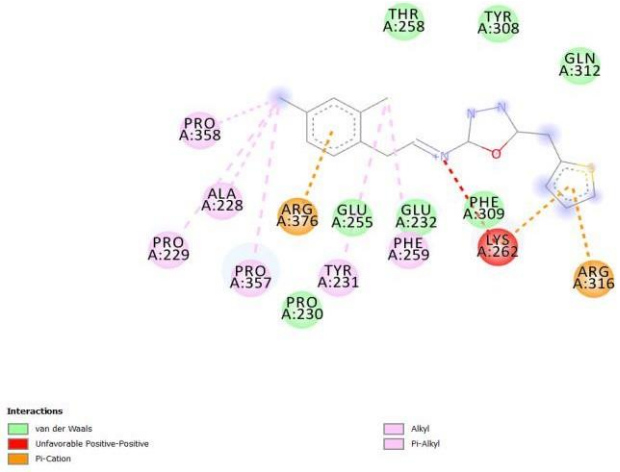

Figure 9. 2D docking interaction of compound T12 against Human topoisomerase I enzyme

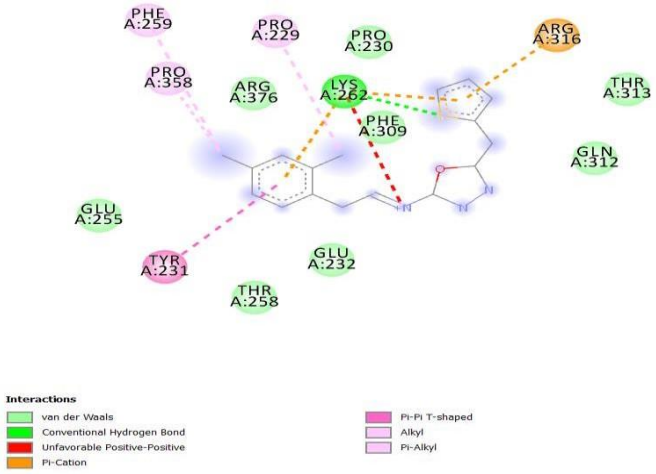

Figure 10. 2D docking interaction of compound T15 against Human topoisomerase I enzyme

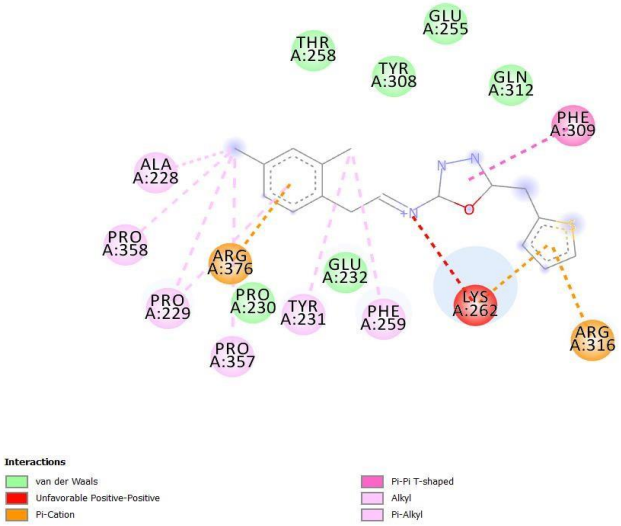

Figure 11. 2D docking interaction of compound T16 against Human topoisomerase I enzyme

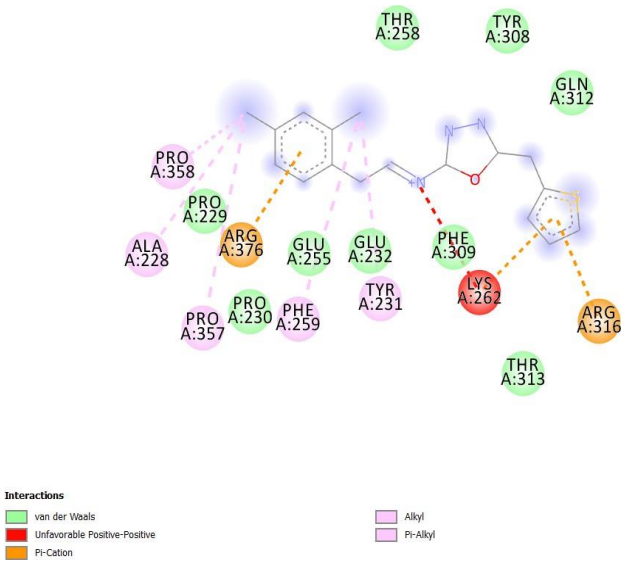

Figure 12. 2D docking interaction of compound T17 against Human topoisomerase I enzyme

Table 1. Binding energy of studied compounds.

Ligand	Binding Affinity
T1	-7.4
T2	**-8.6**
T3	**-8.6**
T4	**-8.7**
T5	-7.5
T6	-7.5
T7	-7.3
T8	-7.2
T9	**-8.6**
T10	**-8.9**
T11	**-9**
T12	**-8.9**
T13	-7.5
T14	-7.4
T15	**-8.8**
T16	**-8.3**
T17	**-8.9**
T18	-8.3
T19	-8.1
T20	-6.9

Results of Molecular docking

From the results (table 1) it clearly shows that, all the compounds have promising interaction with targeted enzyme topoisomerase I. The interaction is mainly due to the presence of lipophilic factor of aromatic heterocyclic ring. From the docking results, compound T11 (9 kcal/mol) shows highest binding affinity toward Human topoisomerase I enzyme compared to standard drug doxorubicin. This compound produced two conventional hydrogen bonds between carbonyl oxygen and nitrogen of oxadiazole moiety with residues of Pro 358 and Lys 262 respectively. The following amino acids such as Pro 230, Try 231, Glu 232, Phe 259, Tyr 308, Pro 357, Gly 359, Leu 360, Arg 362, are interact with ligand through hydrophobic bond. These interactions due to the aromatic character of ligands. The remaining of 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 Human Topoisomerase I enzyme and also play important role in the inhibition of the ligand-binding domain of topoisomerase I 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 T2, T3, T4, T9, T10, T11, T12, T15, T16, and T17 are selected for the conventional synthesis and it was further evaluated for the cytotoxicity studies against the MCF-7 cells.

Spectral Data of synthesized compounds:

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: (**figure 13 to 52**).

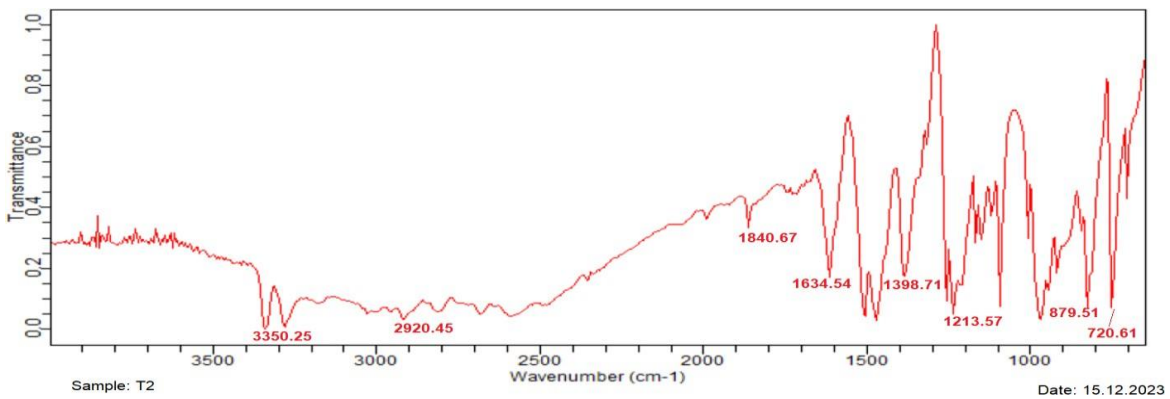

Figure 13: IR Spectra for compound T2

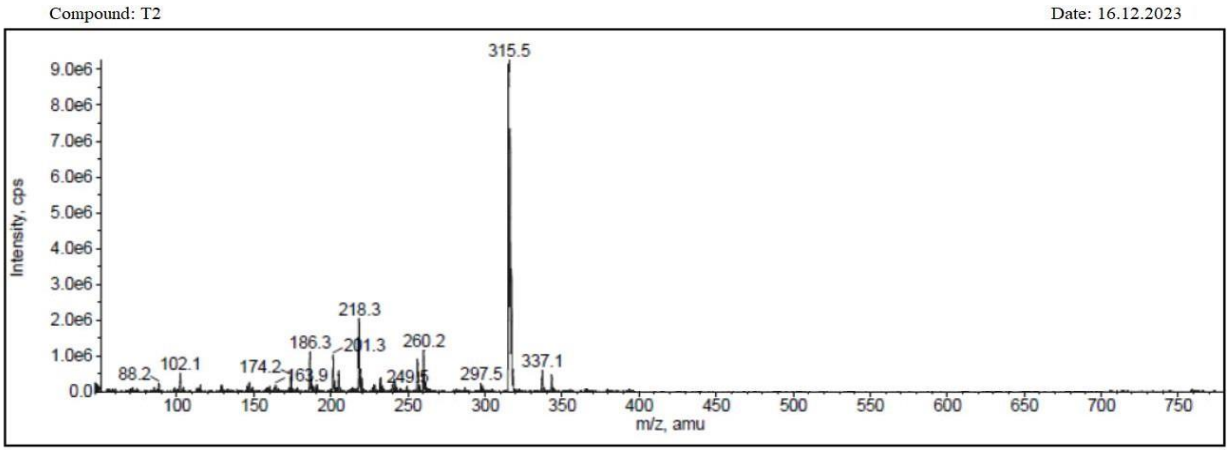

Figure 14: Mass Spectra for compound T2

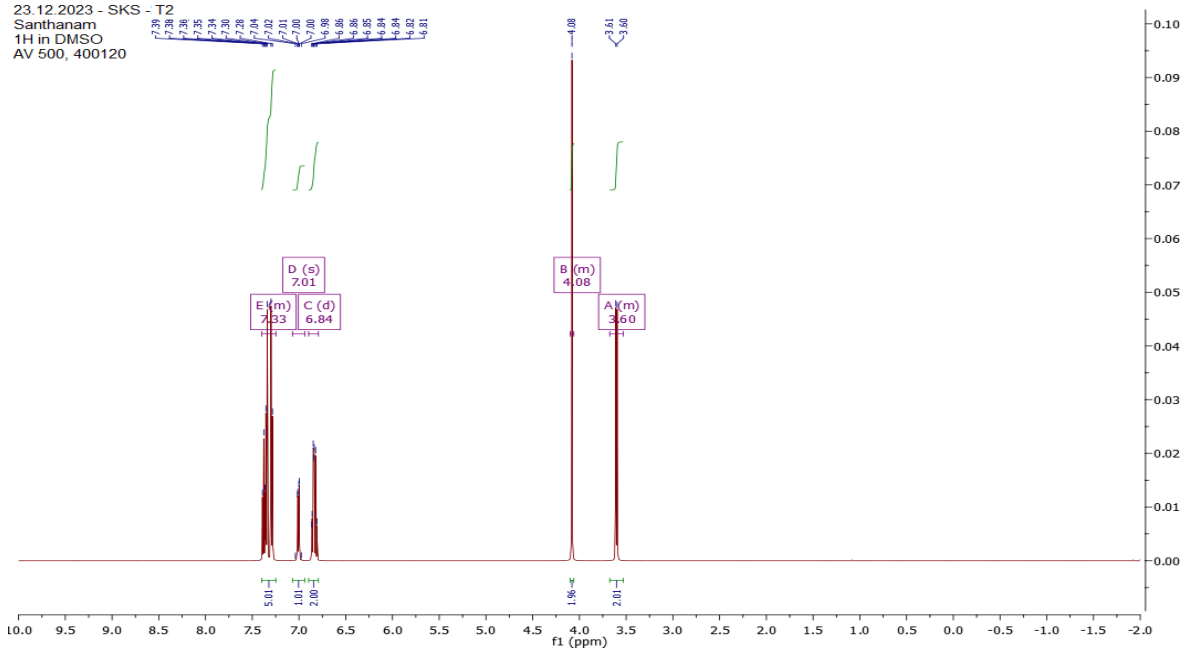

Figure 15: ^{1}H NMR Spectra for compound T2

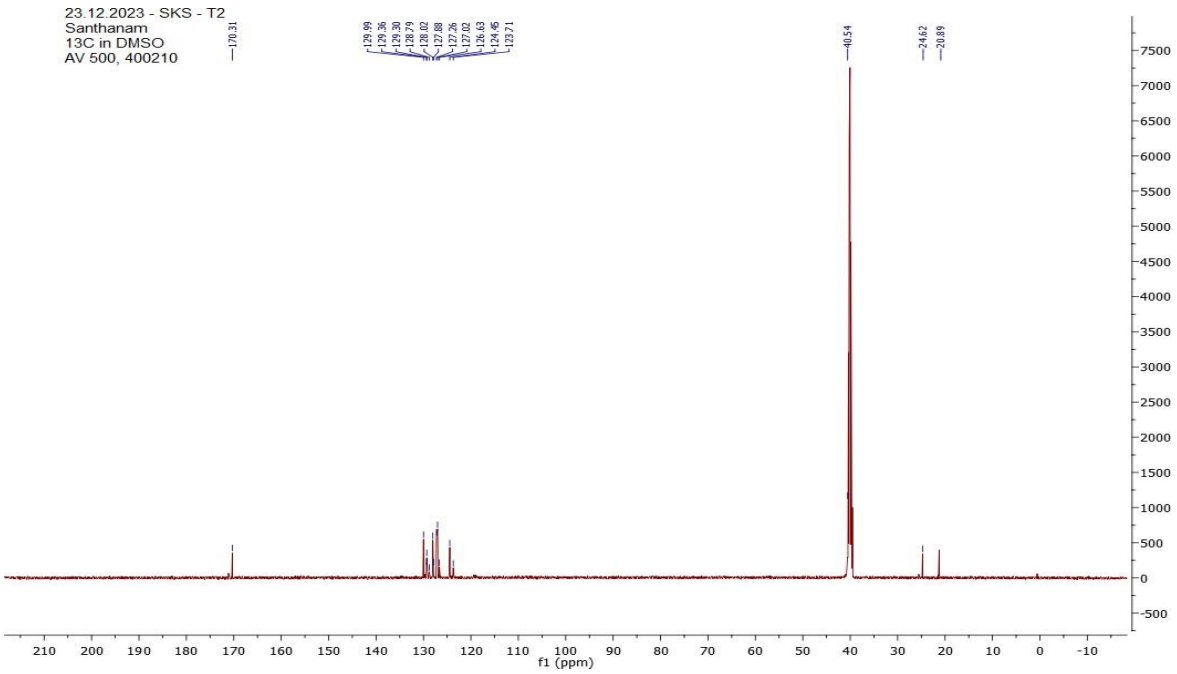

Figure 16: ^{13}C NMR Spectra for compound T2

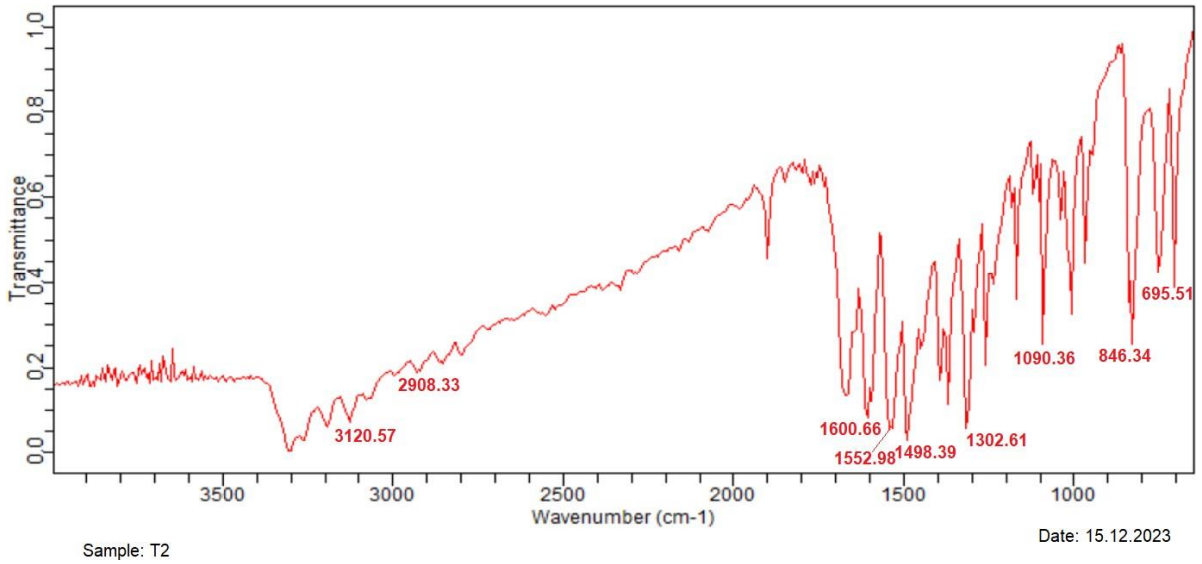

Figure 17: IR Spectra for compound T3

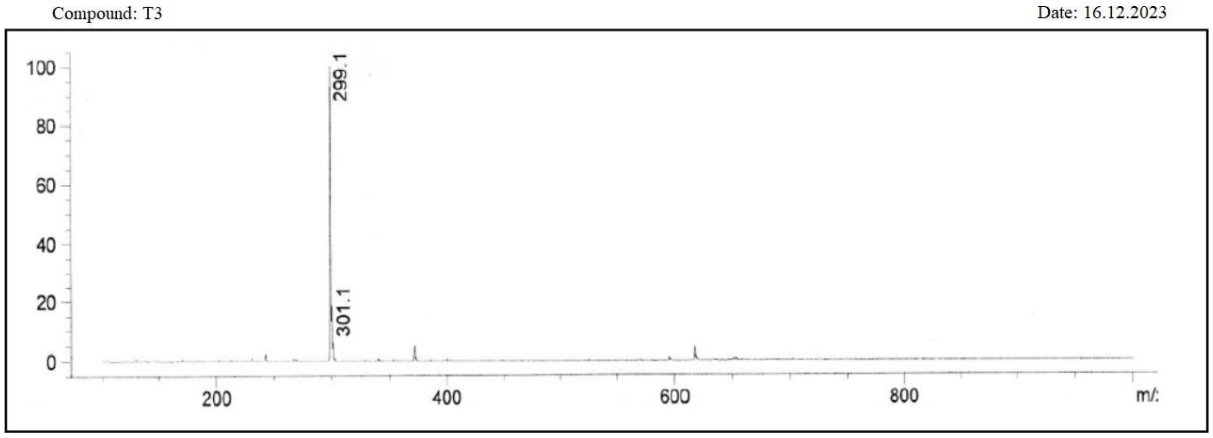

Figure 18: Mass Spectra for compound T3

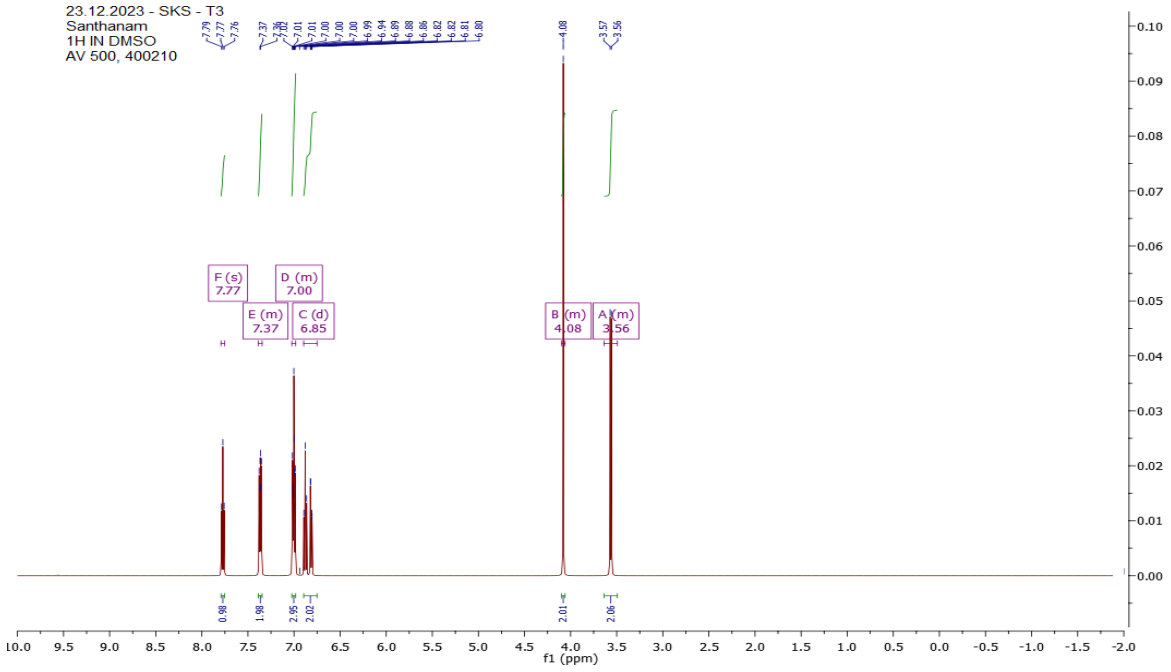

Figure 19: ^{1}H NMR Spectra for compound T3

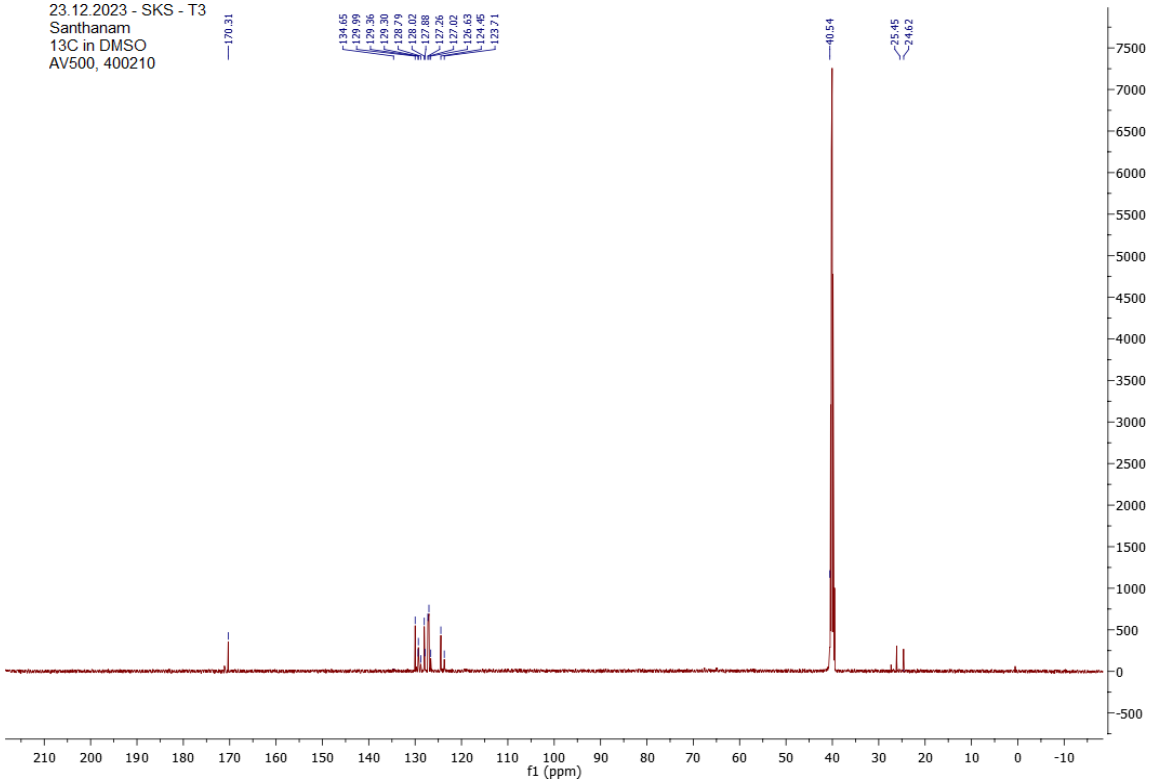

Figure 20: ^{13}C NMR Spectra for compound T3

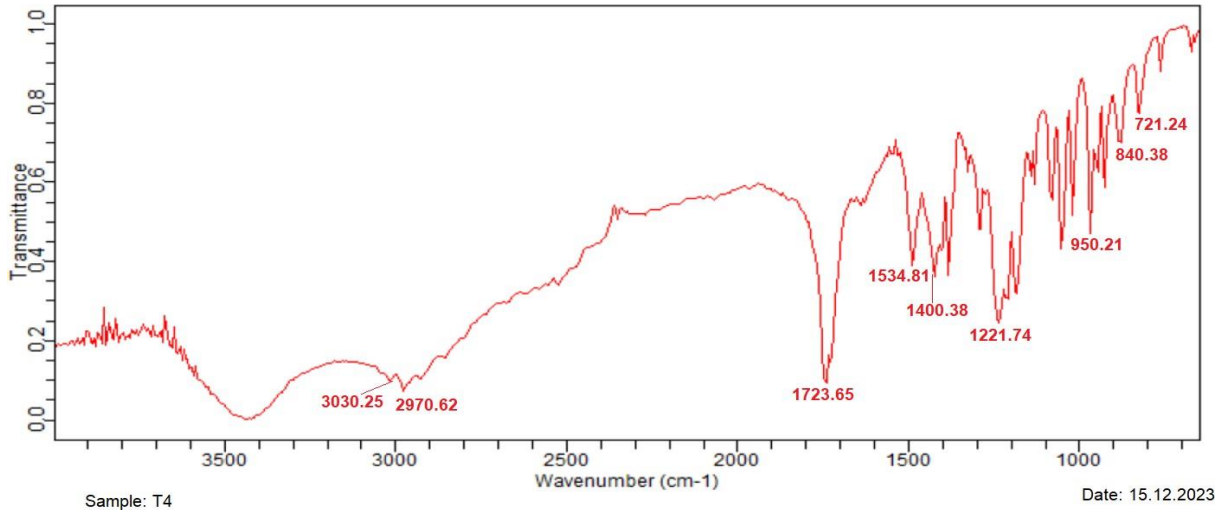

Figure 21: IR Spectra for compound T4

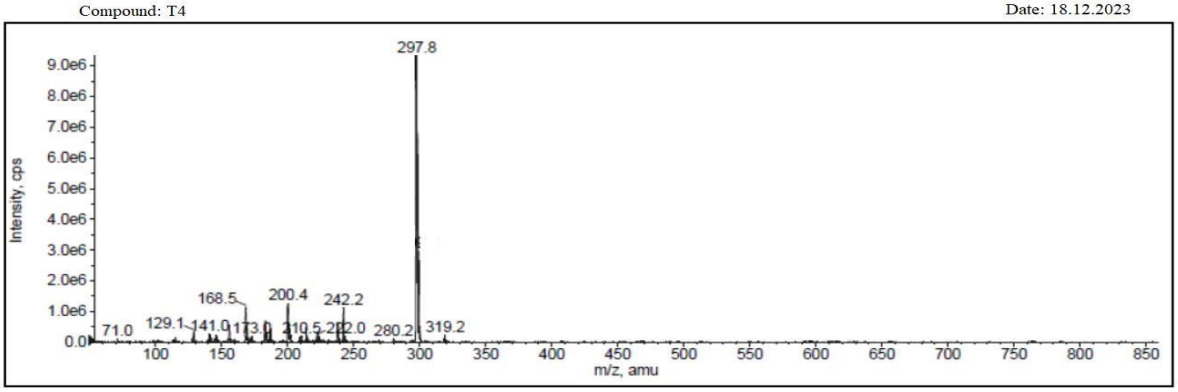

Figure 22: Mass Spectra for compound T4

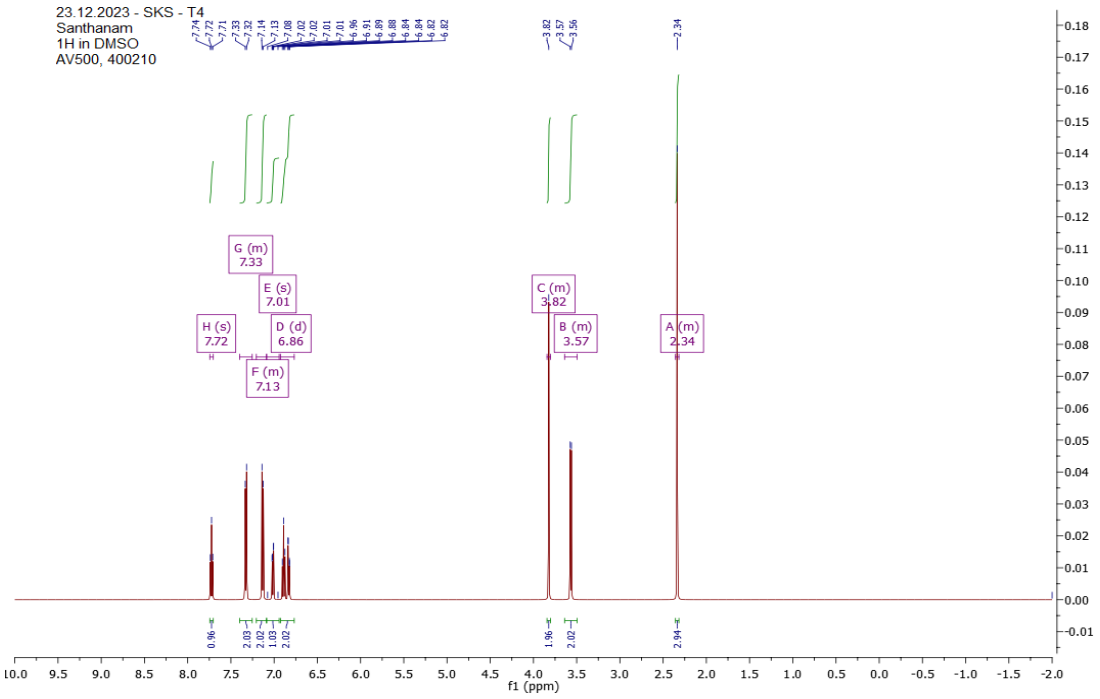

Figure 23: ^{1}H Spectra for compound T4

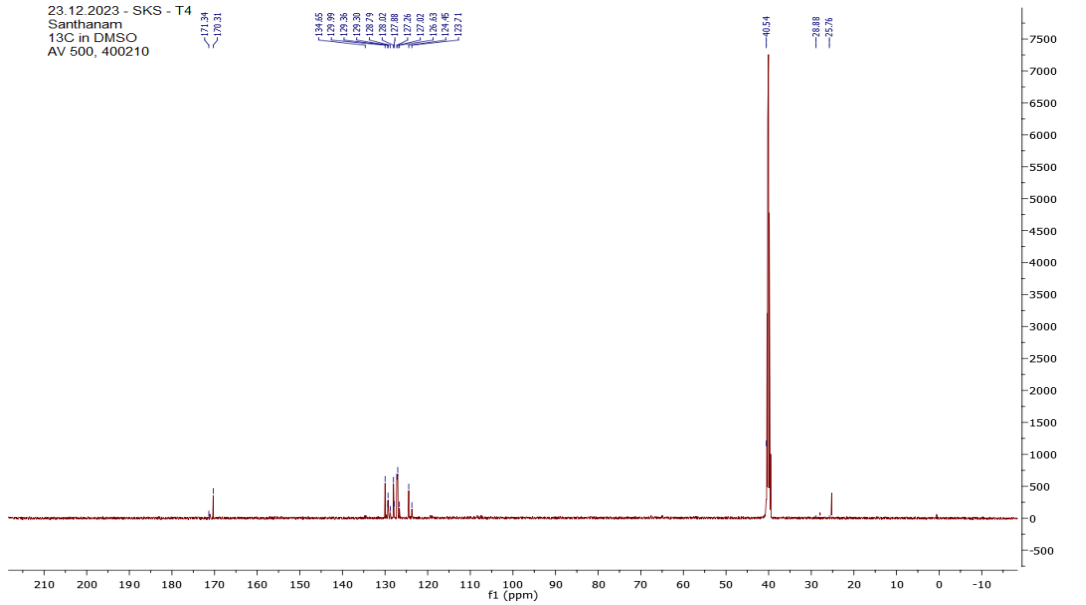

Figure 24: ^{13}C NMR Spectra for compound T4

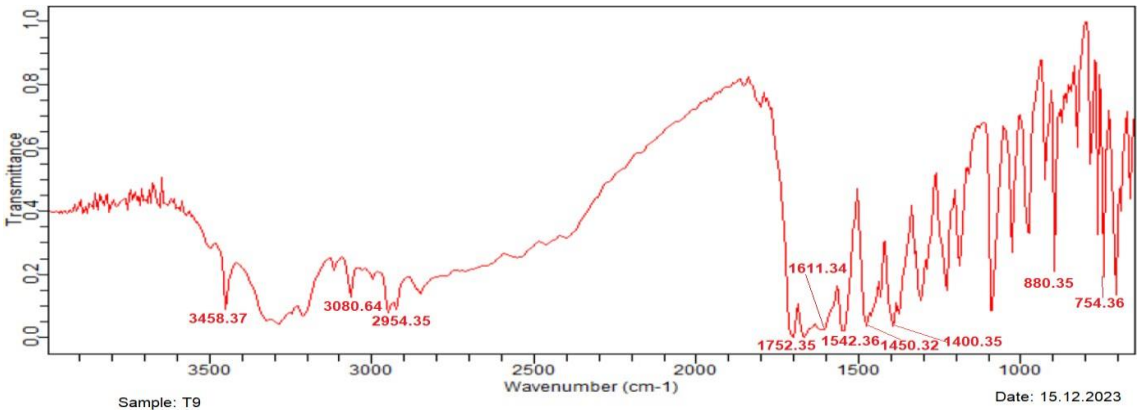

Figure 25: IR Spectra for compound T9

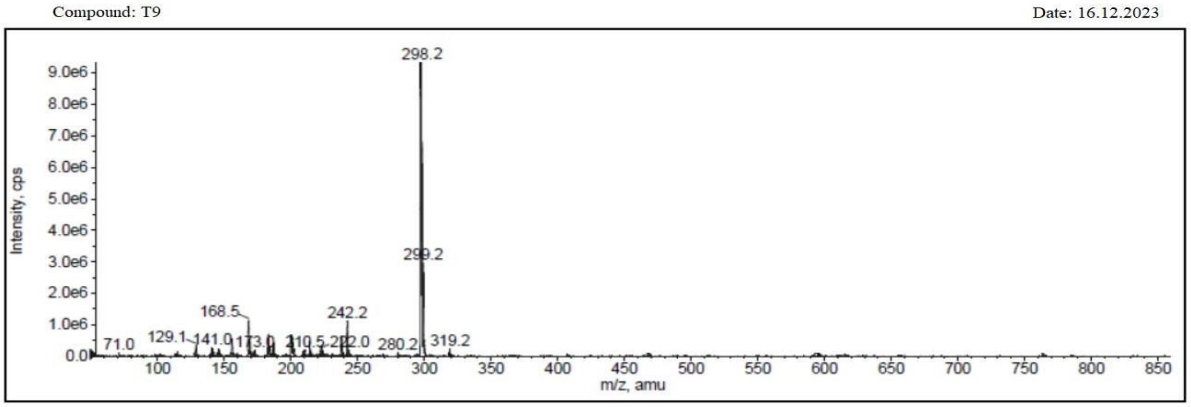

Figure 26: Mass Spectra for compound T9

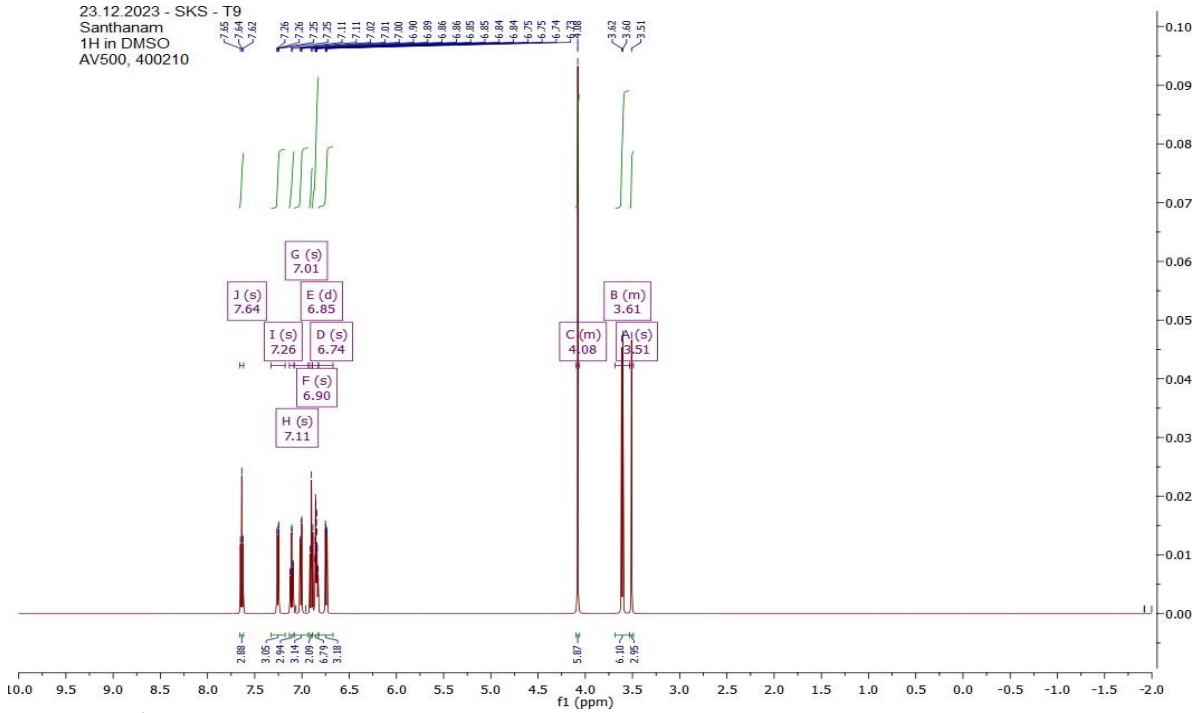

Figure 27: ^{1}H NMR Spectra for compound T9

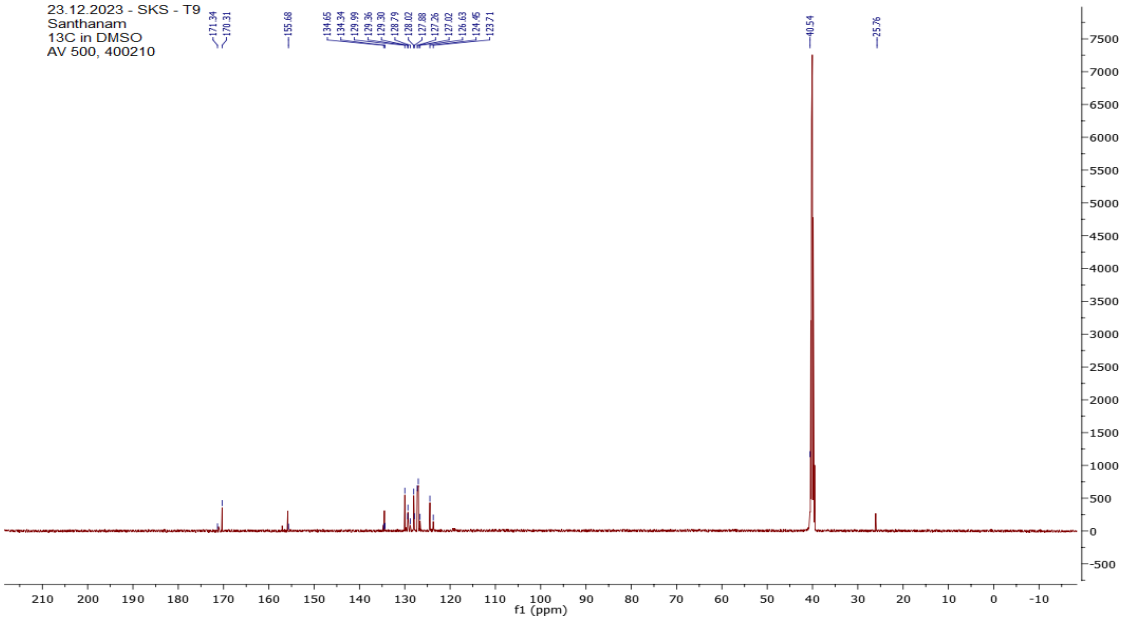

Figure 28: ^{13}C NMR Spectra for compound T9

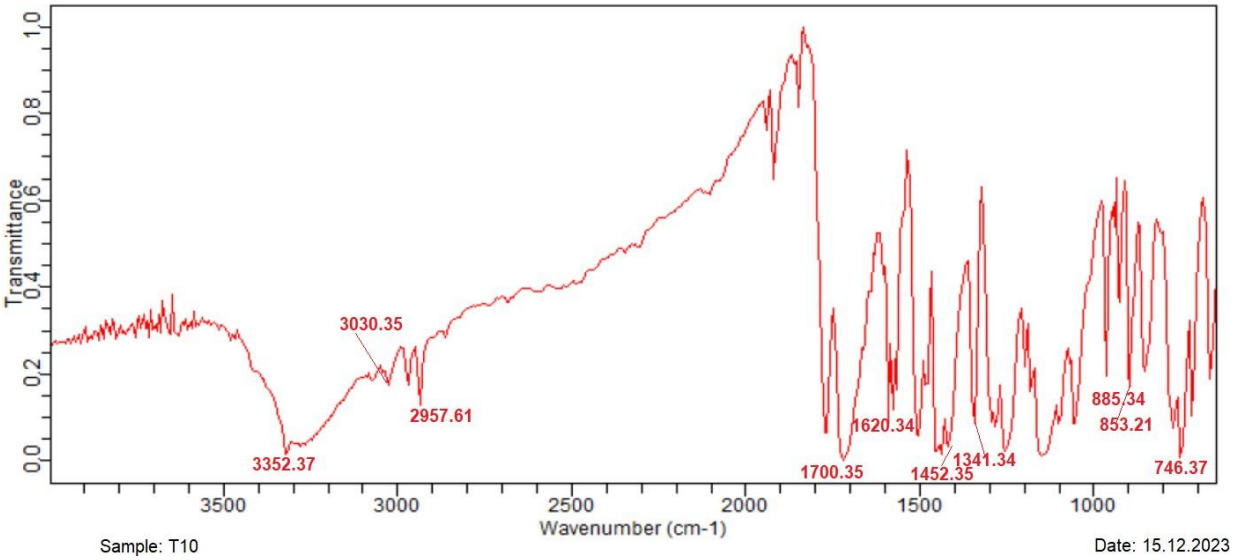

Figure 29: IR Spectra for compound T10

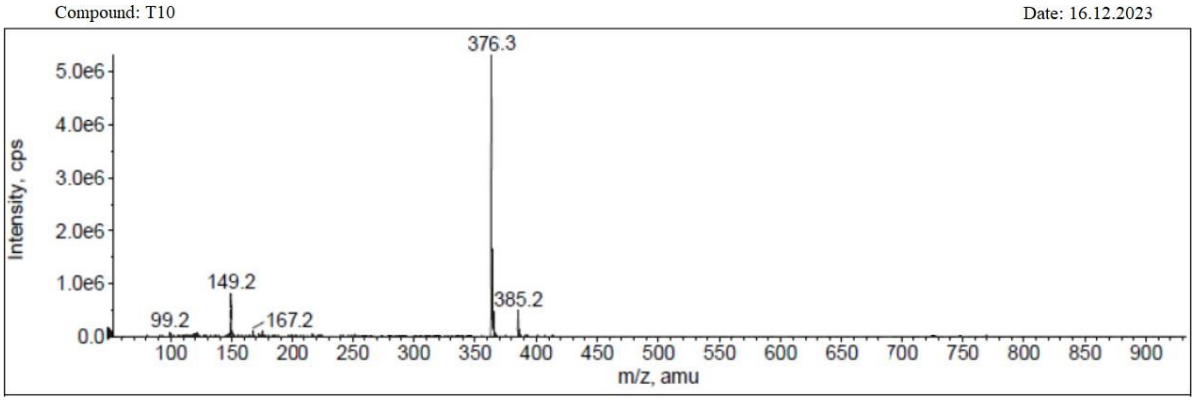

Figure 30: Mass Spectra for compound T10

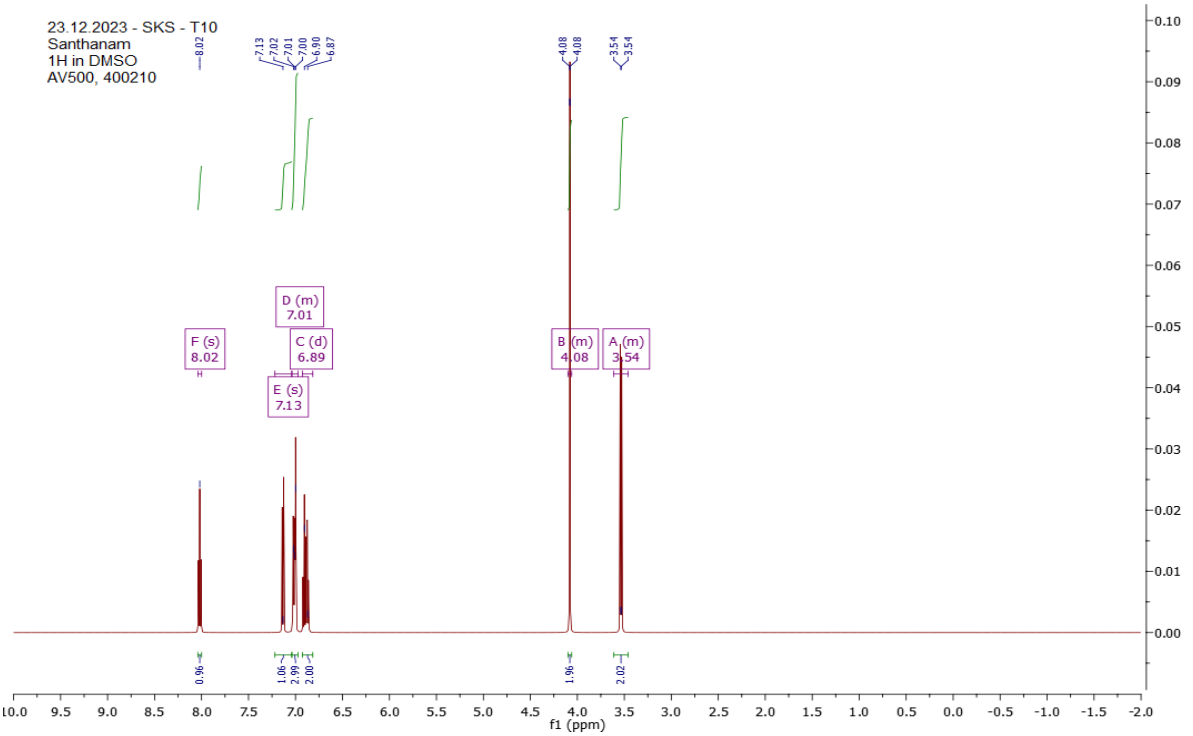

Figure 31: ^{1}H NMR Spectra for compound T10

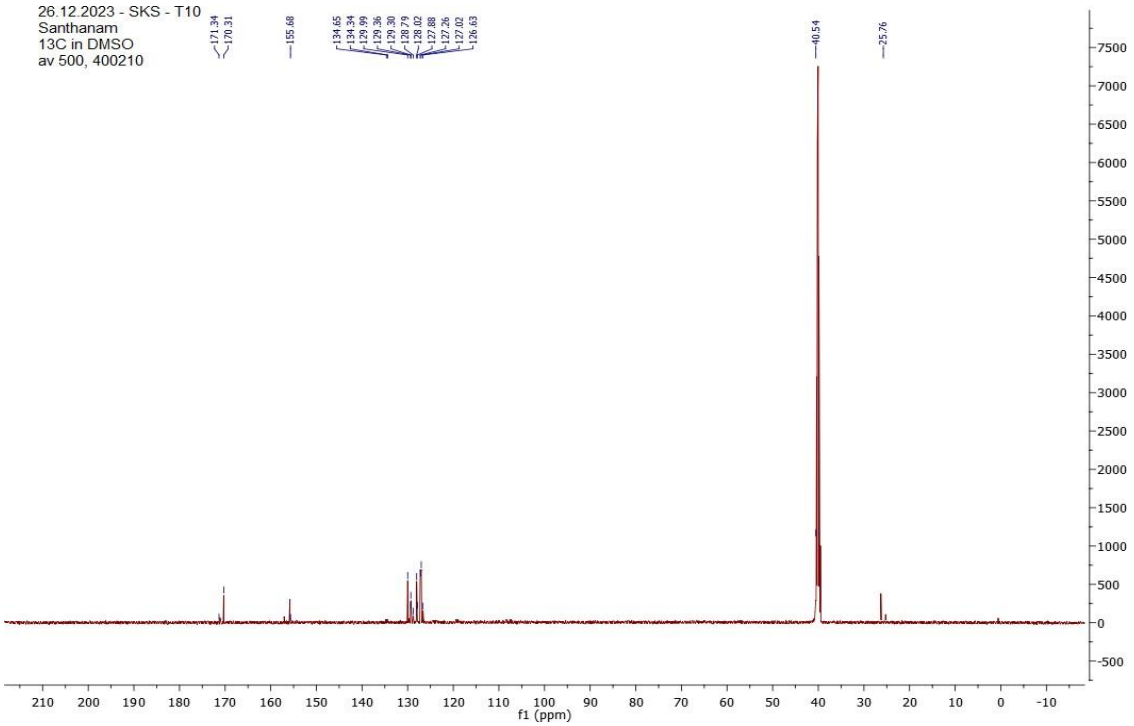

Figure 32: ^{13}C NMR Spectra for compound T10

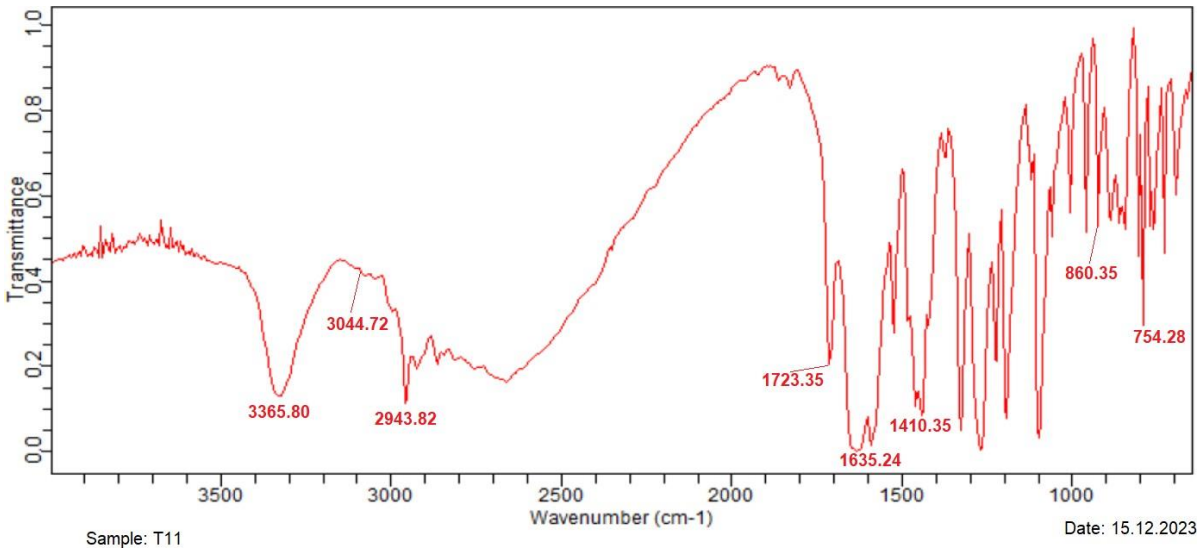

Figure 33: IR Spectra for compound T11

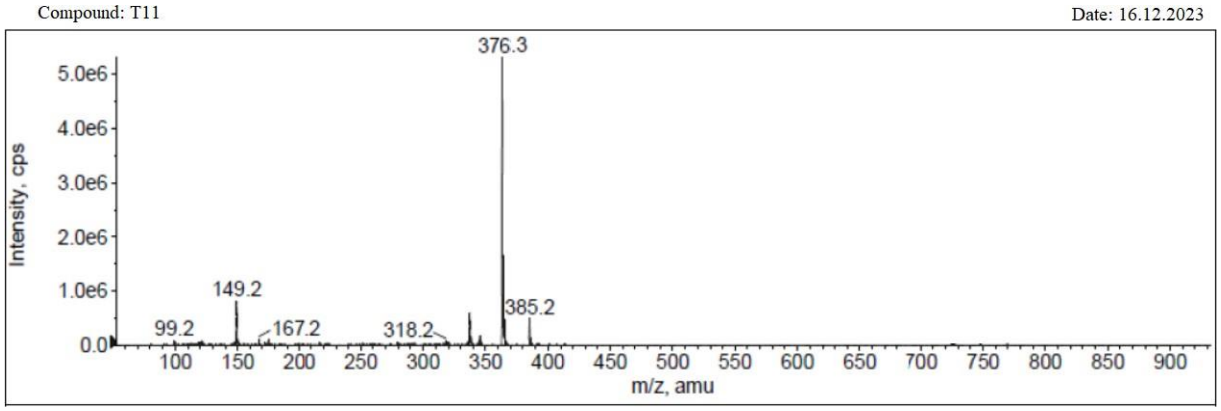

Figure 34: Mass Spectra for compound T11

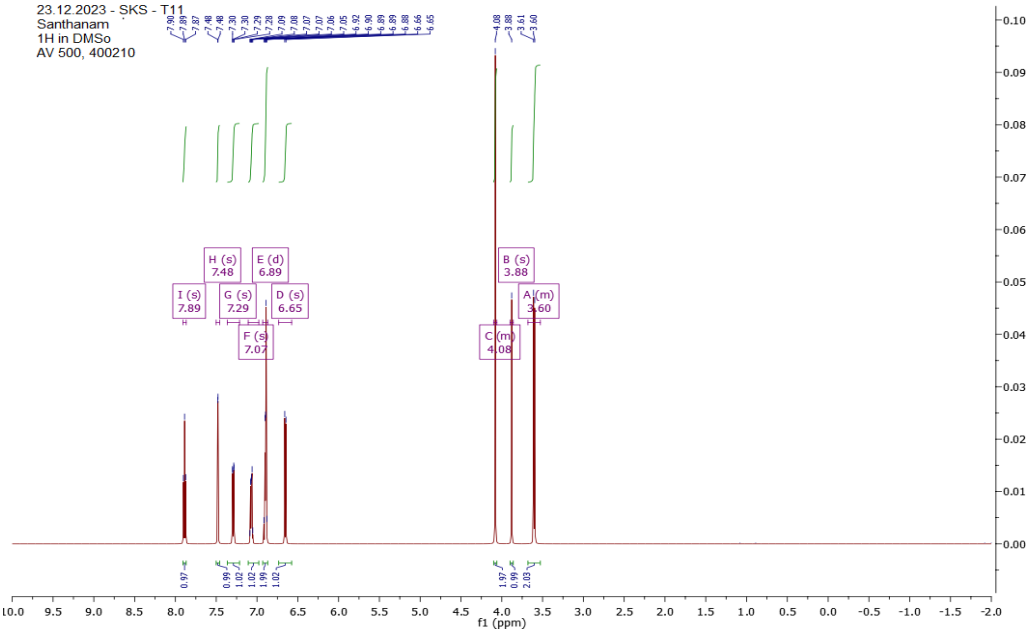

Figure 35: ^{1}H NMR Spectra for compound T11

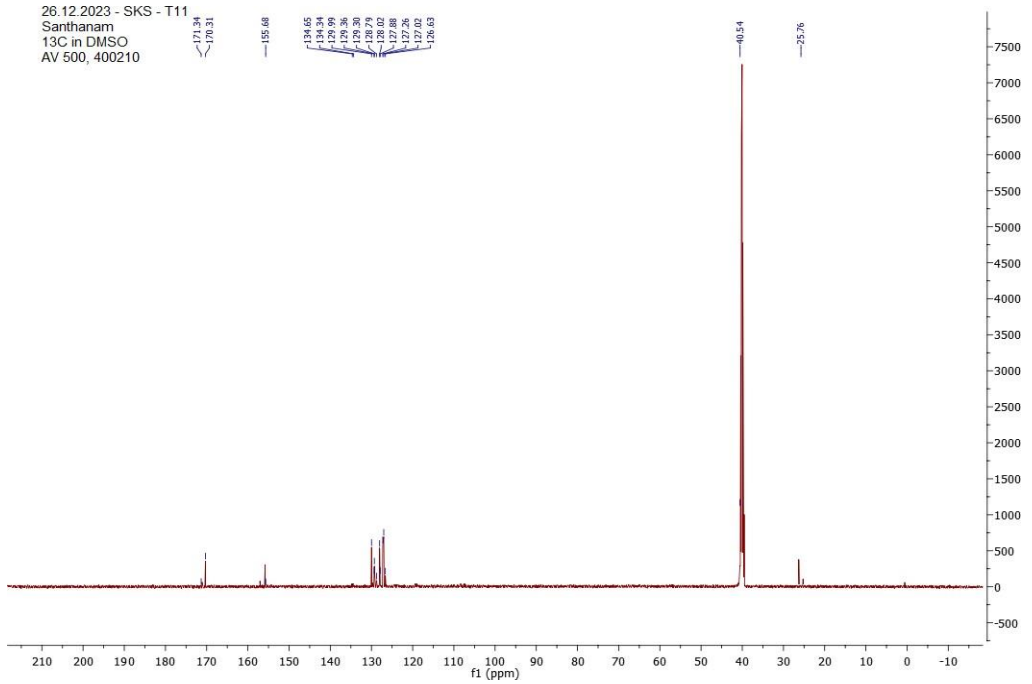

Figure 36: ^{13}C NMR Spectra for compound T11

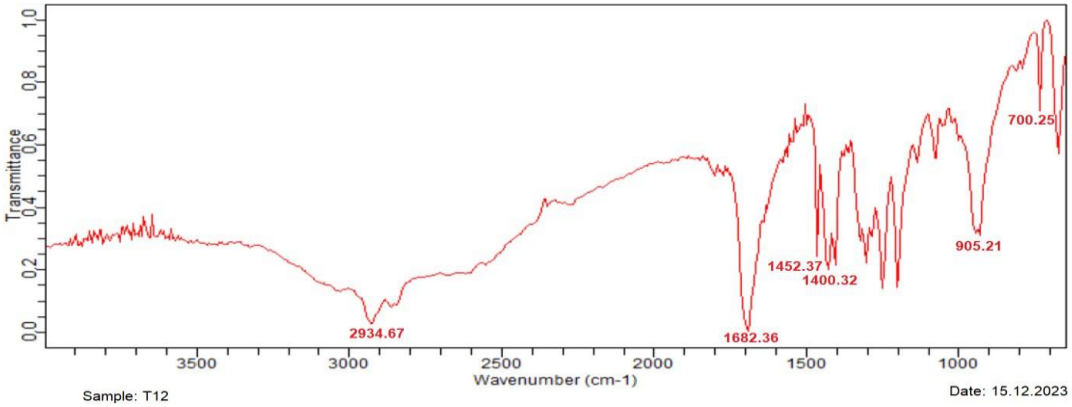

Figure 37: IR Spectra for compound T12

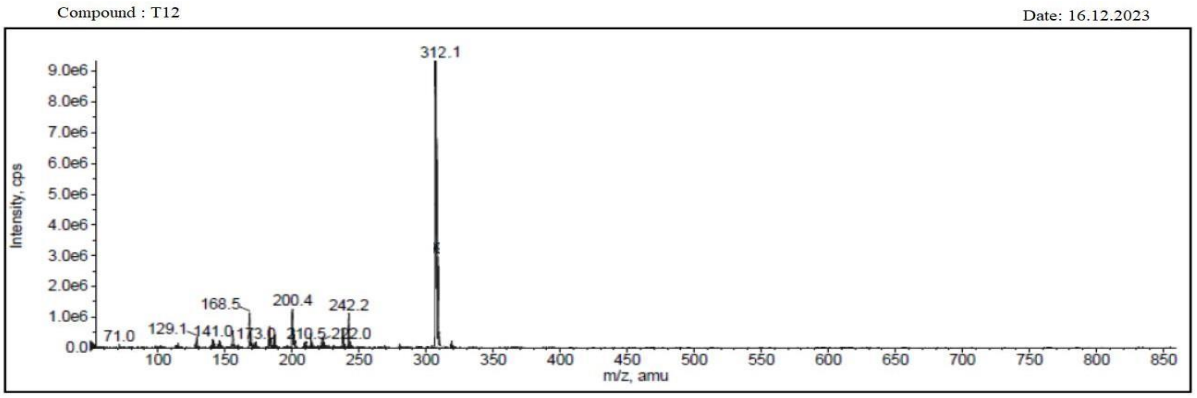

Figure 38: Mass Spectra for compound T12

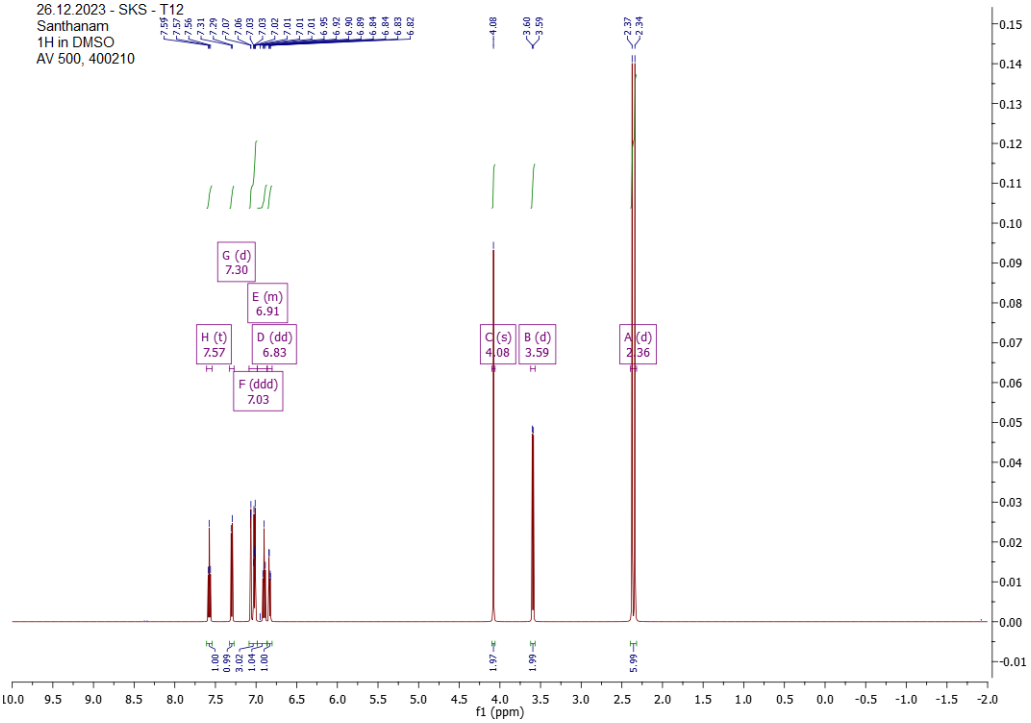

Figure 39: ^{1}H NMR Spectra for compound T12

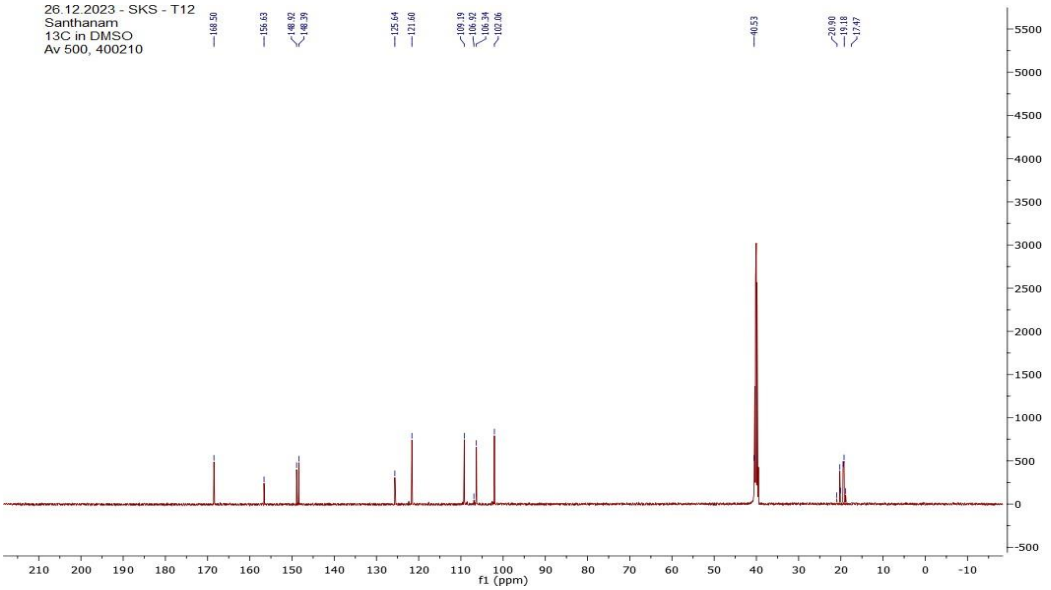

Figure 40: ^{13}C NMR Spectra for compound T12

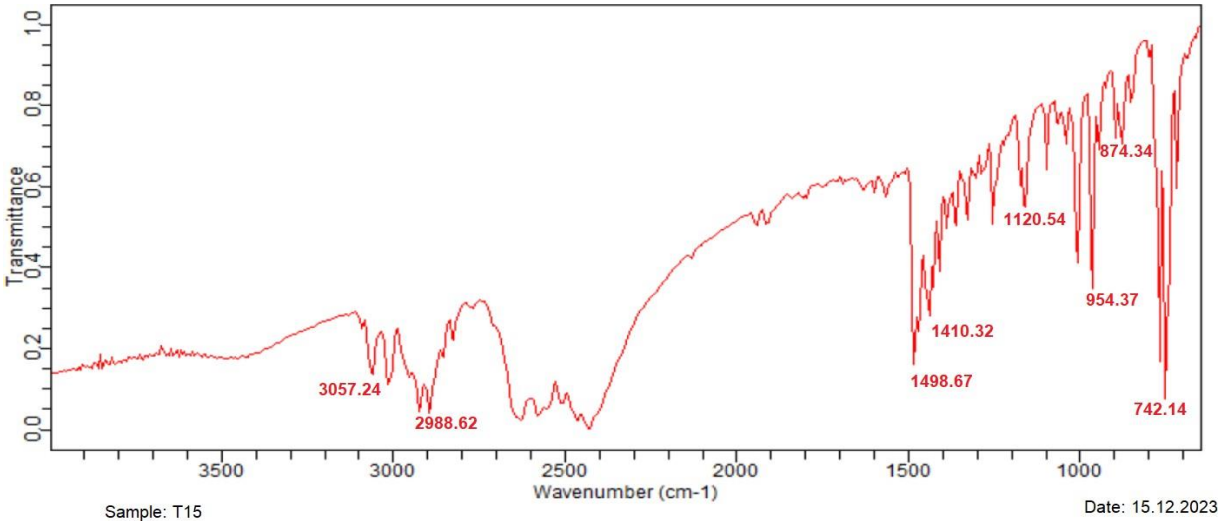

Figure 41: IR Spectra for compound T15

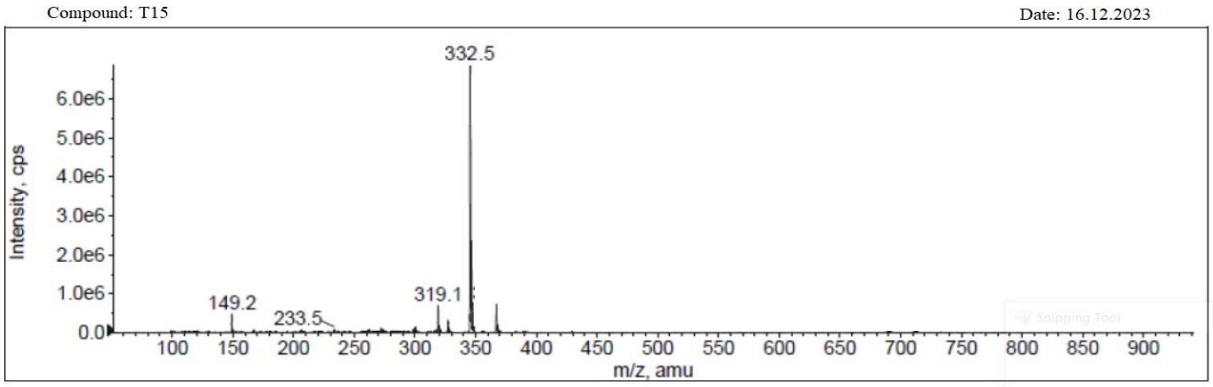

Figure 42: Mass Spectra for compound T15

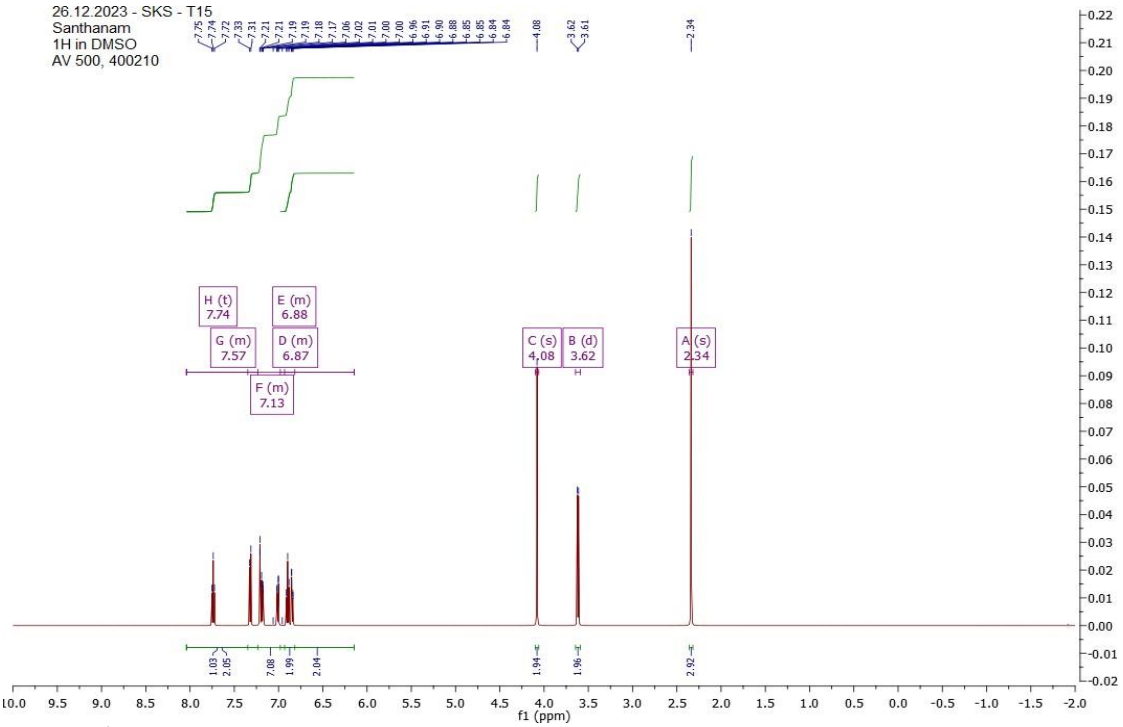

Figure 43: ^{1}H NMR Spectra for compound T15

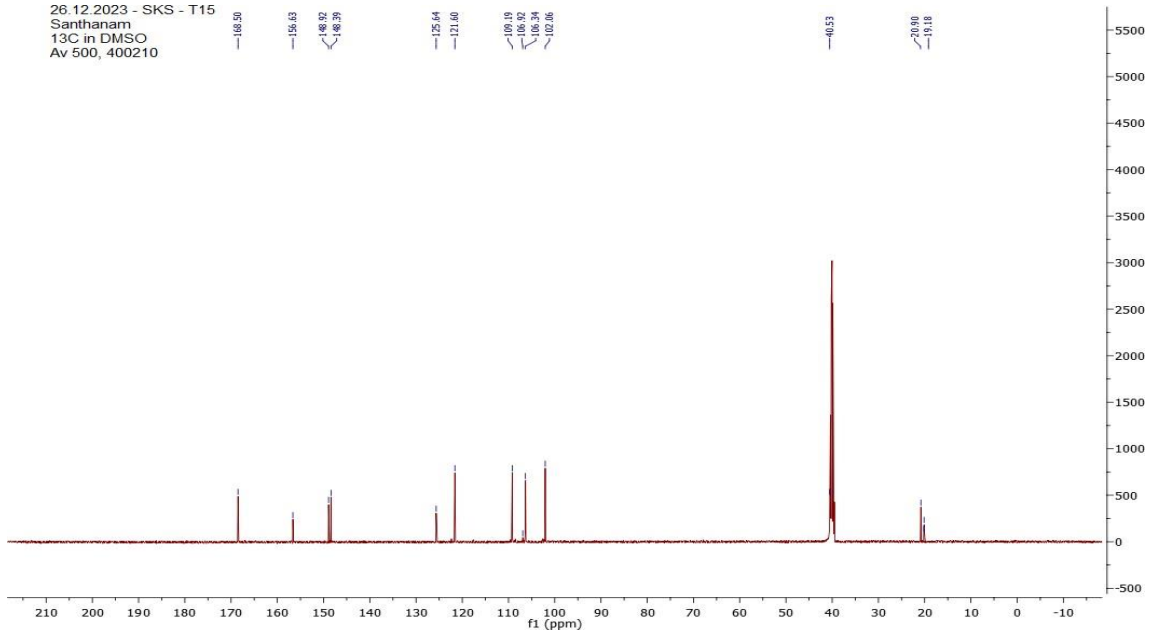

Figure 44: ^{13}C NMR Spectra for compound T15

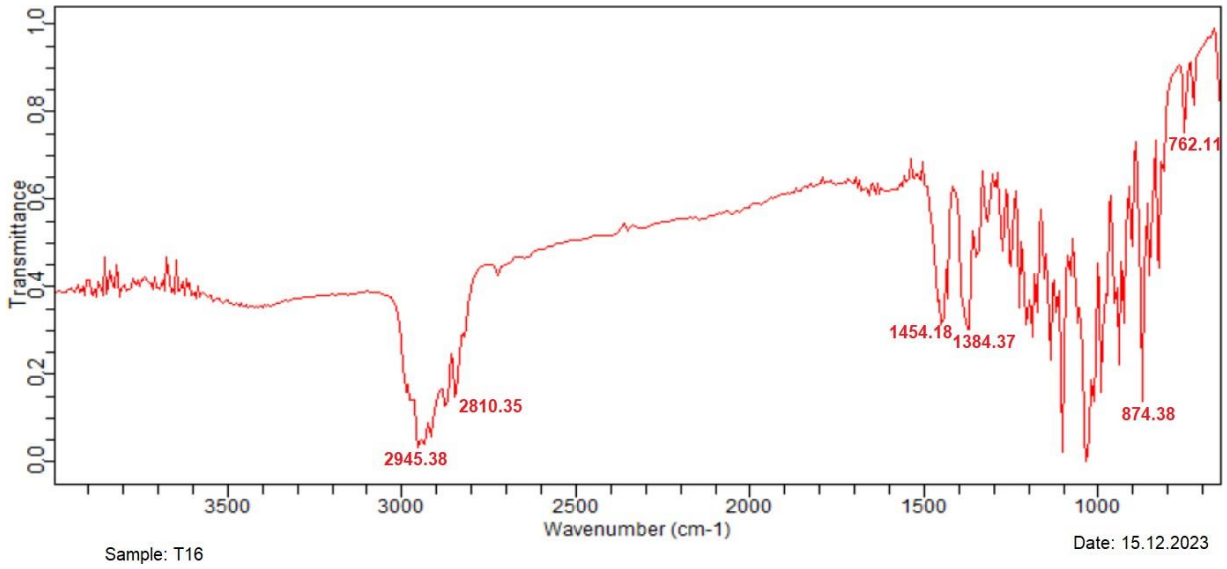

Figure 45: IR Spectra for compound T16

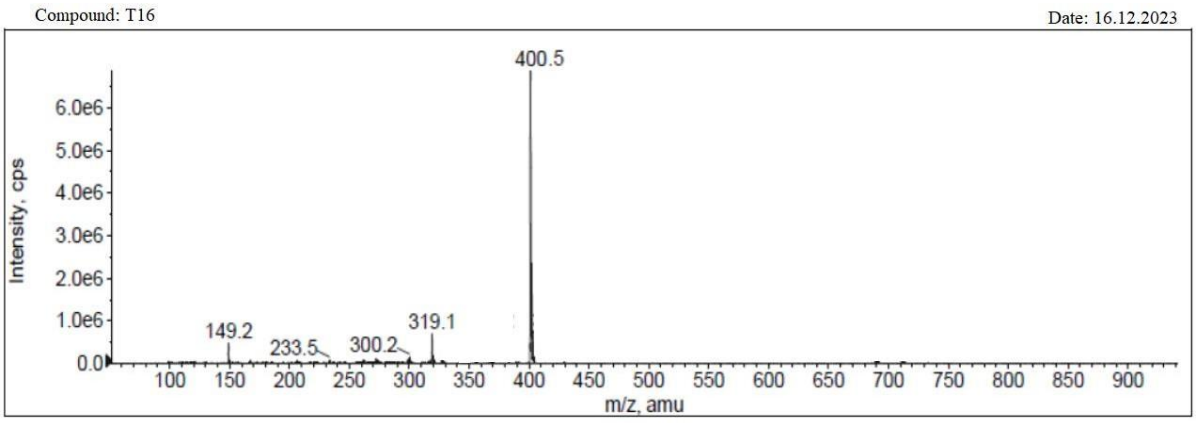

Figure 46: Mass Spectra for compound T16

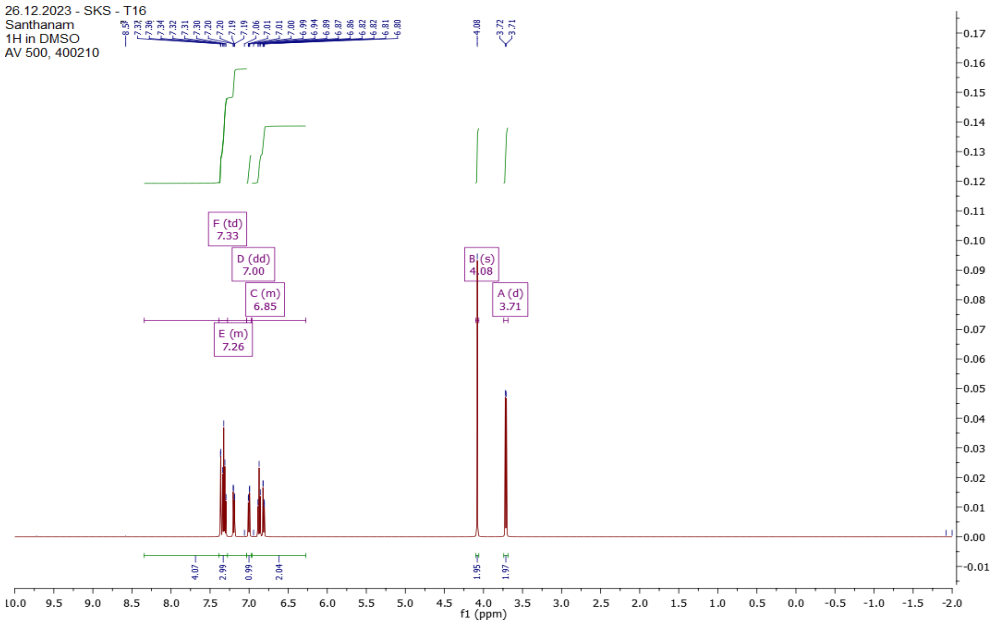

Figure 47: ^{1}H NMR Spectra for compound T16

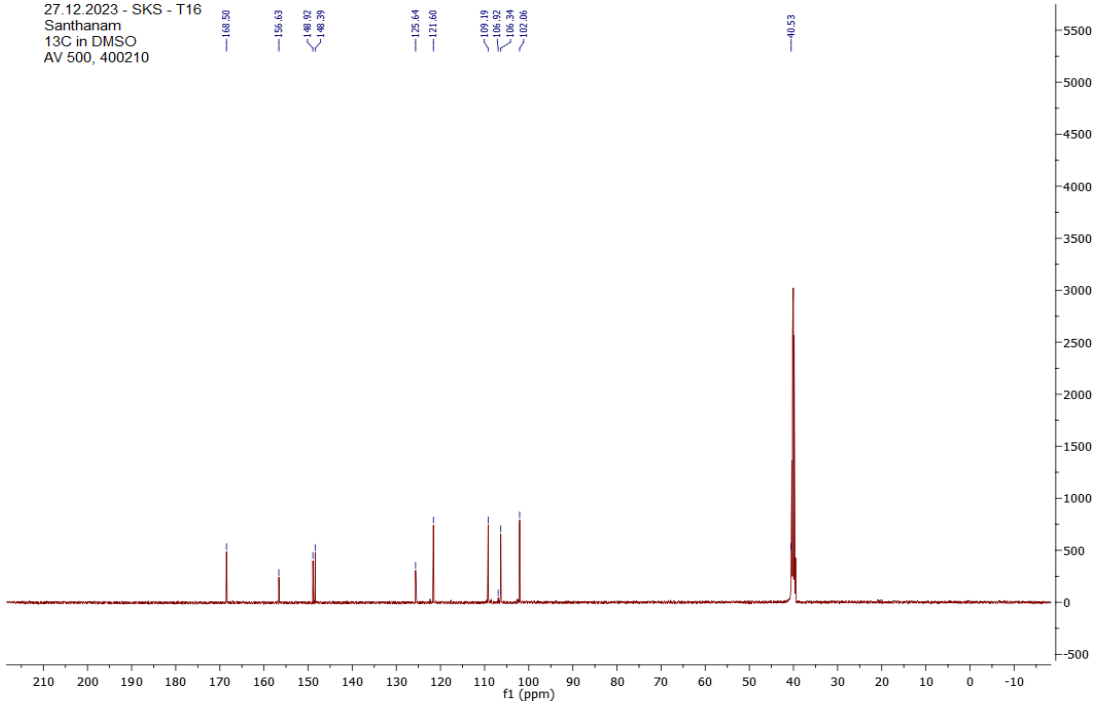

Figure 48: ^{13}C NMR Spectra for compound T16

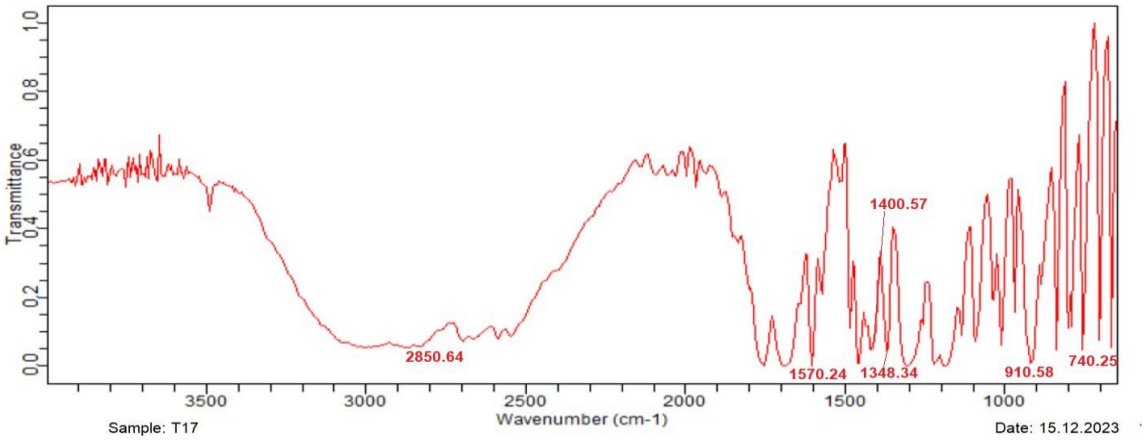

Figure 49: IR Spectra for compound T17

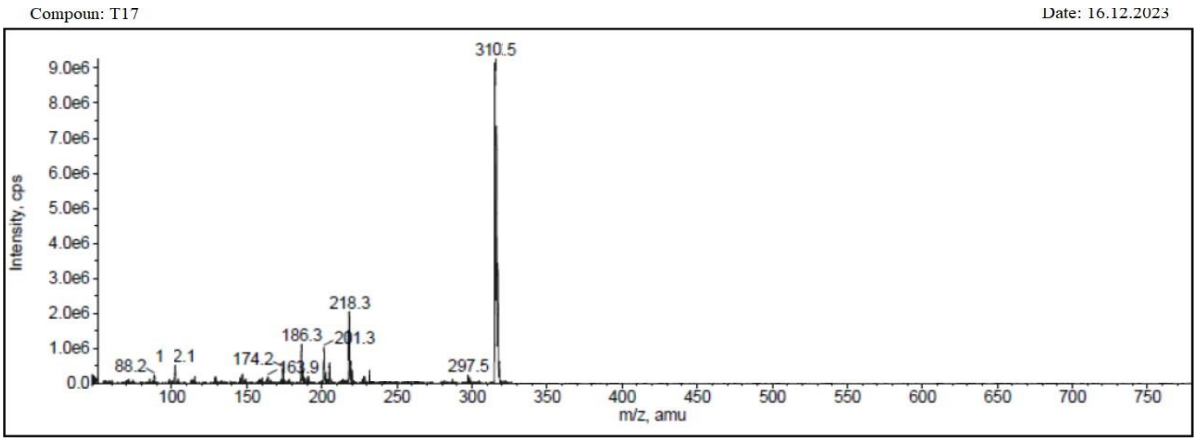

Figure 50: Mass Spectra for compound T17

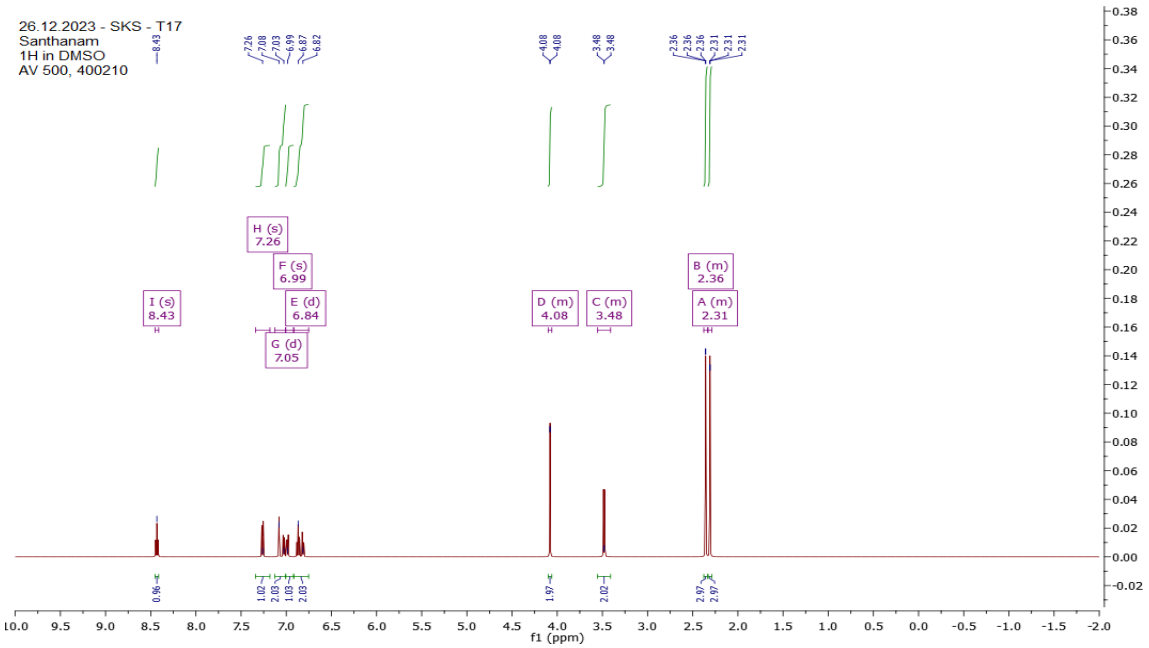

Figure 51: ^{1}H NMR Spectra for compound T17

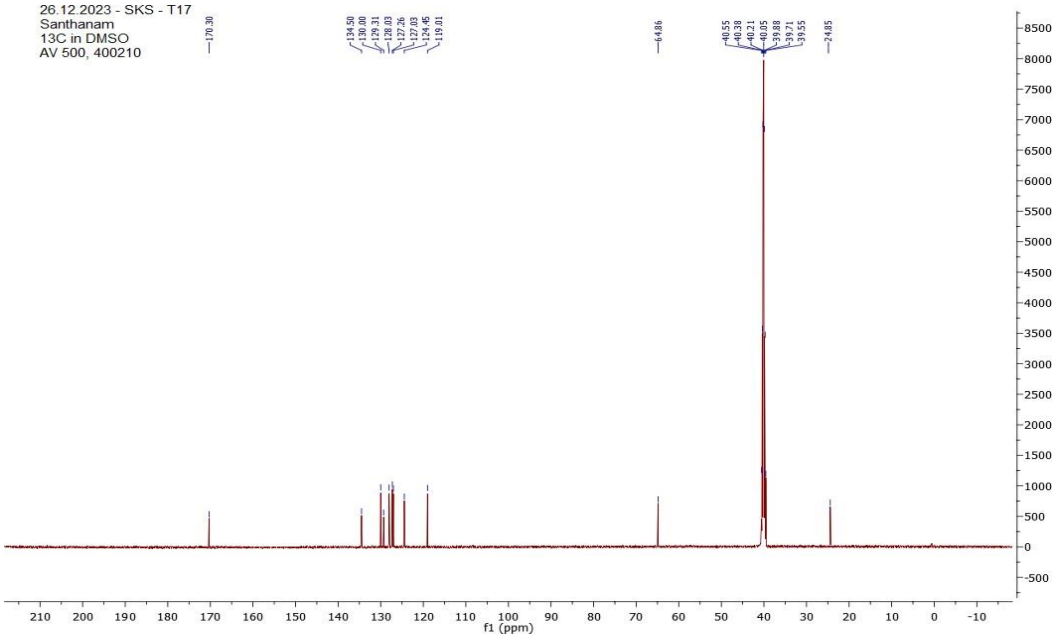

Figure 52: ^{13}C NMR Spectra for compound T17

Characterization of synthesized compounds

1.(E)-N-(2-(4-chlorophenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T2)

$C_{15}H_{12}ClN_3OS$; White solid; MP: 120 – 123^{O}C; Rf: 0.74; IR (KBr) cm^{-1}: 3350 (CH str alkene); 2920 (CH str alkane); 1840 (C=O amide); 1634 (C=O ketone) 1398 (CN bending); 879 (Aromatic ring); 720 (C-Cl str);^{1}H NMR (500 MHz, DMSO) δ 7.40 – 7.25 (m, 5H), 7.01 (s, 1H), 6.84 (d, *J* = 13.8 Hz, 2H), 4.10 – 4.06 (m, 2H), 3.68 – 3.53 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 170.31, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 40.54, 24.62, 20.89.; Mass: Actual: 317m/z; Found: 315 (M-2) m/z

2. (E)-N-(2-(4-fluorophenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T3)

$C_{15}H_{12}FN_3OS$; White solid; MP: 117 – 119^{O}C; Rf: 0.71; IR (KBr) cm^{-1}: 3120 (CH str alkene); 2908 (CH str alkane); 1600 (C=O amide); 1552 (C=O ketone) 1498 (CN bending); 846 (Aromatic ring); 695 (C-Cl str); ; ^{1}H NMR (500 MHz, DMSO) δ 7.77 (s, 1H), 7.39 – 7.35 (m, 2H), 7.02 – 6.98 (m, 3H), 6.85 (d, *J* = 31.9 Hz, 2H), 4.10 – 4.06 (m, 2H), 3.64 – 3.49 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 170.31, 134.65, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 40.54, 25.45, 24.62.; Mass: Actual: 301 m/z; Found: 301 m/z.

3.(E)-5-(thiophen-2-ylmethyl)-N-(2-(p-tolyl)ethylidene)-1,3,4-oxadiazol-2-amine (T4)

$C_{16}H_{15}N_3OS$; White solid; MP: 129 – 131^{O}C; Rf: 0.73; IR (KBr) cm^{-1}: 3030 (CH str alkene); 2970 (CH str alkane); 1723 (C=O amide); 1534 (C=O ketone) 1400 (CN bending); 950 (Aromatic ring);^{1}H NMR (500 MHz, DMSO) δ 7.72 (s, 1H), 7.40 – 7.25 (m, 2H), 7.21 – 7.09 (m, 2H), 7.01 (s, 1H), 6.86 (d, *J* = 29.2 Hz, 2H), 3.84 – 3.80 (m, 2H), 3.64 – 3.50 (m, 2H), 2.36 – 2.32 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 171.34, 170.31, 134.65, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 40.54, 28.88, 25.76; Mass: Actual: 297; Found 297 m/z:

4. (E)-2-(2-((5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-yl)imino)ethyl)phenol (T9)

$C_{15}H_{13}N_3O_2S$; White solid; MP: 104 – 107^{O}C; Rf: 0.79; IR (KBr) cm^{-1}: 3458 (OH str); 3080 (CH str alkene); 2954 (CH str alkane); 1752 (C=O amide); 1611 (C=O ketone) 1400 (CN bending); 850 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.64 (s, 3H), 7.26 (s, 3H), 7.11 (s, 3H), 7.01 (s, 3H), 6.90 (s, 2H), 6.85 (d, *J* = 1.3 Hz, 7H), 6.74 (s, 3H), 4.10 – 4.06 (m, 6H), 3.68 – 3.53 (m, 6H), 3.51 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 171.34, 170.31, 155.68, 134.65, 134.34, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 40.54, 25.76; Mass: Actual: 297 m/z; Found 298 (M+1) m/z.

5.(E)-5-bromo-2-(2-((5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-yl)imino)ethyl)phenol (T10)

$C_{15}H_{12}BrN_3O_2S$; White solid; MP: 123 – 126^{O}C; Rf: 0.81; IR (KBr) cm^{-1}: 3352 (OH str); 3030 (CH str alkene); 2957 (CH str alkane); 1700 (C=O amide); 1620 (C=O ketone) 1452 (CN bending); 853 (Aromatic ring); 746 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 8.02 (s, 1H), 7.13 (s, 1H), 7.04 – 6.97 (m, 3H), 6.89 (d, *J* = 17.2 Hz, 2H), 4.10 – 4.06 (m, 2H), 3.61 – 3.46 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 171.34, 170.31, 155.68, 134.65, 134.34, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 40.54, 25.76; Mass: Actual: 376 m/z; Found: 376 m/z

6.(E)-4-bromo-2-(2-((5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-yl)imino)ethyl)phenol (T11)

$C_{15}H_{12}BrN_3O_2S$; White solid; MP: 120 – 122^{O}C; Rf: 0.81; IR (KBr) cm^{-1}: 3365 (OH str); 3044 (CH str alkene); 2943 (CH str alkane); 1723 (C=O amide); 1635 (C=O ketone) 1410 (CN bending); 860 (Aromatic ring); 754 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 7.89 (s, 1H), 7.48 (s, 1H), 7.29 (s, 1H), 7.07 (s, 1H), 6.89 (d, *J* = 3.9 Hz, 2H), 6.65 (s, 1H), 4.10 – 4.06 (m, 2H), 3.88 (s, 1H), 3.68 – 3.53 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 171.34, 170.31, 155.68, 134.65, 134.34, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 40.54, 25.76; Mass: Actual: 376 m/z; Found: 376 m/z.

7.(E)-N-(2-(2,4-dimethylphenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T12)

$C_{17}H_{17}N_3OS$; White solid; MP: 116 – 119^{O}C; Rf: 0.83; IR (KBr) cm^{-1}: 2934 (CH str alkane); 1682 (C=O ketone) 1452 (CN bending); 905 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.57 (t, *J* = 7.0 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.03 (ddd, *J* = 10.7, 7.5, 1.3 Hz, 3H), 6.99 – 6.87 (m, 1H), 6.83 (dd, *J* = 7.5, 1.6 Hz, 1H), 4.08 (s, 2H), 3.59 (d, *J* = 7.0 Hz, 2H), 2.36 (d, *J* = 17.2 Hz, 6H); ^{13}C NMR (126 MHz, DMSO) δ 168.50, 156.63, 148.92, 148.39, 125.64, 121.60, 109.19, 106.92, 106.34, 102.06, 40.53, 20.90, 19.18, 17.47. Mass: Actual: 311 m/z; Found: 312 (M+1) m/z.

8.(E)-N-(2-(4-chloro-2-methylphenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T15)

$C_{16}H_{14}ClN_3OS$; White solid; MP: 104 – 107^{O}C; Rf: 0.83; IR (KBr) cm^{-1}: 3057 (CH str alkene); 2988 (CH str alkane); 1498 (C=O ketone); 1410 (CN bending); 874 (Aromatic ring); 742 (C-Cl str); ; ^{1}H NMR (500 MHz, DMSO) δ 7.74 (t, *J* = 7.1 Hz, 1H), 8.04 – 7.23 (m, 2H), 8.04 – 6.14 (m, 7H), 6.98 – 6.14 (m, 2H), 6.93 – 6.82 (m, 2H), 4.08 (s, 2H), 3.62 (d, *J* = 7.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 168.50, 156.63, 148.92, 148.39, 125.64, 121.60, 109.19, 106.92, 106.34, 102.06, 40.53, 20.90, 19.18, 17.47. Mass: Actual: 331m/z; Found: 332 (M+1) m/z

9.(E)-N-(2-(2,4-dichlorophenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T16)
$C_{15}H_{11}Cl_2N_3OS$; White solid; MP: 122 – 124^{O}C; Rf: 0.83; IR (KBr) cm^{-1}: 2995 (CH str alkene); 2810 (CH str alkane); 1454 (C=O ketone); 1384 (CN bending); 874 (Aromatic ring); 762 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 7.33 (td, *J* = 14.1, 4.3 Hz, 3H), 8.34 – 7.03 (m, 4H), 7.00 (dd, *J* = 7.3, 1.6 Hz, 1H), 6.96 – 6 .28 (m, 2H), 4.08 (s, 2H), 3.71 (d, *J* = 7.0 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 168.50, 156.63, 148.92, 148.39, 125.64, 121.60, 109.19, 106.92, 106.34, 102.06, 40.53; Mass: Actual: 399 m/z; Found: 400 (M+1) m/z
10.(E)-N-(2-(2,4-dimethoxyphenyl)ethylidene)-5-(thiophen-2-ylmethyl)-1,3,4-oxadiazol-2-amine (T17)
$C_{17}H_{17}N_3O_3S$; White solid; MP: 102 – 104^{O}C; Rf: 0.83; IR (KBr) cm^{-1}: 2850 (CH str alkane); 1700 (C=O amide); 1570 (C=O ketone); 1400 (CN bending); 910 (Aromatic ring); 740 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 8.43 (s, 1H), 7.26 (s, 1H), 7.05 (d, *J* = 27.4 Hz, 2H), 6.99 (s, 1H), 6.84 (d, *J* = 26.2 Hz, 2H), 4.10 – 4.06 (m, 2H), 3.55 – 3.41 (m, 2H), 2.38 – 2.34 (m, 3H), 2.33 – 2.29 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 108.40, 64.86, 40.55, 40.38, 40.21, 40.05, 39.88, 39.71, 39.55, 24.85. Mass: Actual: 343 m/z; Found: 343 m/z

***In-silico* ADMET prediction**

The *in-silico* ADME properties of the designed ligands were carried out through SWISS ADME software. The molecular weight of the designed compounds ranged between 297 and 378. The estimated number of hydrogen bond donors is zero to one. The estimated number of hydrogen bond acceptors range of 4 - 6. The predicted octanol/water partition co-efficient was in the range of 2 to 2.8 and the number of likely metabolic reactions was between 1–3. The number of violations of Lipinski's rule of five was 0. All the compounds have high human oral absorption and all the compounds does not have BBB penetration. From almost all the properties of the compounds are within the recommended values. The details of the *in-silico* ADMET properties for the compounds are shown in **Table 2**.

Table 2. *In-silico* ADMET properties of synthesized compounds

Code	MW	H-bond acceptors	H-bond donors	TPSA	iLOGP	Lipinski violations	GI absorption	BBB permeant
T2	317	4	0	79.52	3.44	0	High	NO
T3	301	5	0	79.52	3.30	0	High	NO
T4	297	4	0	79.52	3.40	0	High	NO
T9	299	5	1	99.75	2.83	0	High	NO
T10	378	5	1	99.75	3.15	0	High	NO
T11	378	5	1	99.15	3.05	0	High	NO
T12	311	4	0	79.52	3.54	0	High	NO
T15	331	4	0	79.52	3.52	0	High	NO
T16	352	4	0	79.52	3.60	0	High	NO
T17	346	6	0	97.98	3.56	0	High	NO

***In vitro* anti-cancer evaluation**

Cell culture and conditions

MCF-7 (TNBC) cell lines (ATCC) were maintained in DMEM, supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). The cells were grown at 37°C with 5% CO_2.

MTT assay

DMEM media with 10% FBS was used to adjust the cell culture to 1.0105 cells/mL. 100 µL of distilled cell suspension (about 10,000 cells/well) was added to each well of a 96 well flat bottom micro titre plate. After the cell population was determined to be sufficient after 24 hours, the cells were centrifuged, and the pellets were suspended in 100 µL of various test sample concentrations made in maintenance media. The plates were incubated for 48 hours at 37°C in a 5% CO_2 environment, with observations recorded every 24 hours. MTT (2 mg/mL) in MEM-PR (MEM without phenol red) was added after 48 hours. The plates were incubated at 37°C for 2 hours (5% CO_2 atmosphere). The 100 µL of DMSO was added and the plates were shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage of cell viability was calculated using the formula.

$$\% \text{ Cell viability} = \frac{\text{Mean OD of individual sample}}{\text{Mean OD of control}} \times 100$$

Table 3. Results for *in vitro* MTT assay of cell viability of the compounds

Compounds	**IC_{50}[nanomolar (nM)]values**
T2	68.48
T3	54.57
T4	78.34
T9	99.29
T10	56.37
T11	45.68
T12	50.37
T15	61.52
T16	105.34
T17	112.40
Doxorubicin	23.54

1.1. *In-vitro* anticancer activity

Results of anticancer activity of the compounds were expressed as IC_{50} values which were determined by plotting the percentage cell viability versus concentration of sample on a logarithmic graph and reading off the control. The experiments were performed in triplicates, and then, the final IC_{50} values were calculated by taking average of triplicate experimental results. The results of *in-vitro* anti-cancer activity expressed in IC_{50} (nM) are expressed in **table 2** and were compared to Doxorubicin. The ten compounds are subjected to *in-vitro* cytotoxicity study by MTT assay method with MCF-7. Among the tested compounds, derivative **T11** substituted with salicylic aldehyde moiety shows a significant IC_{50} value (45.68 nM) in the tested cell lines. The derivatives **T11** also possesses significant docking score **9** kcal/mol Remaining all other tested compounds shows good to moderate cytotoxic activity on tested cell line. The **Table 3** shows the results for *in vitro* MTT assay of cell viability of the compounds.

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 Topoisomerase I protein (PDB ID: 1A35) in molecular docking studies. The calculated docking energies indicated that its interaction with Human Topoisomerase I enzyme is favourable, but only to a limited extent. The ADME properties of the compounds are also assessed by SWISS ADME online tool and all the compounds are within the limit. All the synthesized compounds were screened for their *in vitro* viability test against MCF-7 cancer cell line. Compound **T11** emerged to be the most active compounds against in tested cell line. The study thus serves as an attempt to progress toward the discovery of novel lead molecule for cancer treatment. 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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CONFLICT OF INTEREST:

The author has no conflict of interest.

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