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GREEN SYNTHESIS, DOCKING STUDIES AND ANTI DIABETIC ACTIVITY OF NOVEL AMINO FUSED TRIZOLE SCAFFOLD.

Sumathi K[1]*, Subhashini D[2], Senthil Kumar N[3]

[1],[2,3]Department of pharmaceutical chemistry, JKKMRF-Annai J.K.K Sampoorani ammal college of pharmacy, Namakkal, Affliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai , Tamil Nadu - 638183. E mail id : subhashini27091999@gmail.com Phone number :+91 6379883753[2] Email id : senthilkumarjkkm@gmail.com Phone number :+91 9842024640[3] Email id : kabhisumi@gmail.com Phone number :+91 9080189507.[1]*

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***Corresponding Address**: Sumathi K*

[*]Department of pharmaceutical chemistry, JKKMRF-Annai J.K.K Sampoorani ammal college of pharmacy, Namakkal, Affliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai , Tamil Nadu -638183. Email id :kabhisumi@gmail.com Phone number :+91 9080189507.

Abstract

In the present work, the green synthesis of novel amino fused triazole scaffold hybird heterocyclic derivatives have been carried out and substituted,and they were checked for docking scores against the DPP IV topoisomerase II enzyme.From that, ten best docked compounds are selected and synthesized and characterization of spectral data of the synthesized comopunds was obtained from IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy,then the ten compounds are subjected to *in-vitro* antidiabetic study by α-amylase enzyme inhibition assay method. Among the tested compounds, derivative **T9** (92.33838%) substituted with furan moiety shows a significant activity against α-amylase enzyme at different concentrations possesses anti-diabetic activity.

Keywords: DPP IV topoisomerase II enzyme, α-amylase enzyme inhibition, furan moiety, anti-diabetic activity.

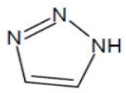
INTRODUCTION:

Diabetes Mellitus is chronic metabolic disorder, which there are high blood sugar level over a prolonged period. The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination. It is a fastgrowing global problem with huge social, health and economic consequences. It is estimated that in 2010 there were globally 285 million people (approximately 6.4% of the adult population) suffering from this disease. This number is estimated to increase to 430 million in the absence of better control or cure.An ageing population and obesity are two main reasons for the increase. Furthermore it has been shown that almost 50% of the putative diabetics are not diagnosed until 10 years after onset of the disease, hence the real prevalence of global diabetes must be astronomically high [1-4]. Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE with type 1 associated with youth and type 2 with being overweight. Effective treatment was not developed until the early part of the 20th

century, when Canadians Frederic Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922. This was followed by the development of the long-acting insulin NPH in the 1940s [5-7].

TRIAZOLE:

Triazoles are an important class of heterocyclic compounds containing three nitrogen atoms in a five-membered ring having molecular formula $C_2H_3N_3$.These exist in two isomeric forms depending upon the position of the nitrogen atom in the heterocyclic ring: 1,2,3-triazoles or 1,2,4-triazoles. [8,9].



1, 2, 3-triazole

1, 2, 4-triazole

Out of the two isomeric forms of triazoles, 1,2,4- triazole derivatives are synthesized in the form of fused ring systems or as heterocyclic or aromatic substitutions [10]. In medicinal chemistry, five-member heterocyclic nitrogen-containing compounds such as triazole are of great importance due to their wide range of biological applications such as anticonvulsant [11,12] antimicrobial [13], antiviral [14], antitubercular [15], antidiabetic [16], anti-inflammatory [17], anti-proliferative [18], antioxidant [19], anti-urease [20], and antimalarial activities [21].

1,2,4 TRIAZOLE:

Being polar in nature, the triazole nucleus can increase the solubility of the ligands and contribute better pharmacokinetic and pharmacodynamic properties [22]. Over the last decades, increased research has been devoted to 1,2,4 triazole drugs [23]. Among them, 3-amino-1,2,4-triazoles derivatives have attracted special attention as they demonstrated a broad spectrum of bioactivities, including potential applications against thrombotic disorders [24], fibrotic [25], auto-immune diseases, central nervous system disorders [26], obesity, diabetes, Alzheimer's disease [27], microbial infections [28 - 30] cancer [31].

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 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 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 2-{3-amino-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid(figure 1)

The parent amino acid coupled triazole derivatives were obtained using a green, beign multicomponent reaction of cinnamaldehyde with glycine and thiosemicabazide in ethanol/ H_2O:(1:7) using lemon juice as a natural green acidic catalyst at 100^{O}C for 2 to 3 hours. The completion of the reaction was monitored by TLC and the mobile phase of n-hexane: ethyl acetate (5:5).

General procedure for synthesis of title compounds(figure 2)

Refluxing a mixture of 2-{3-amino-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (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.

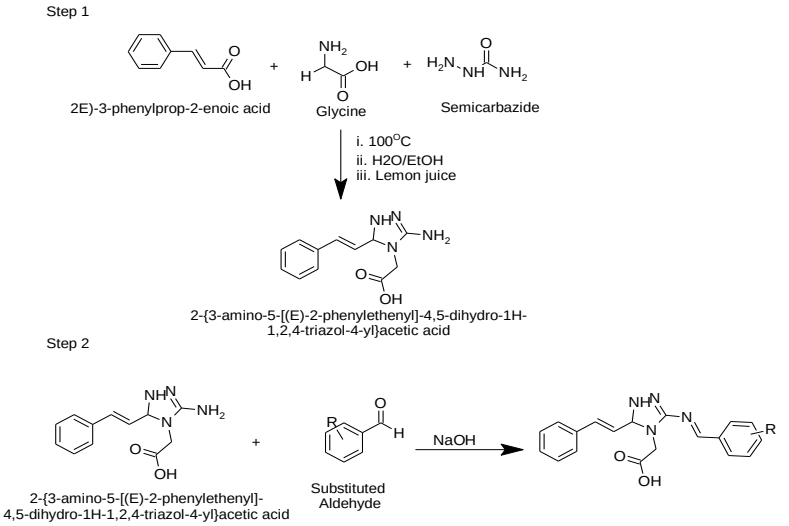


Figure 1. General scheme for the synthesis

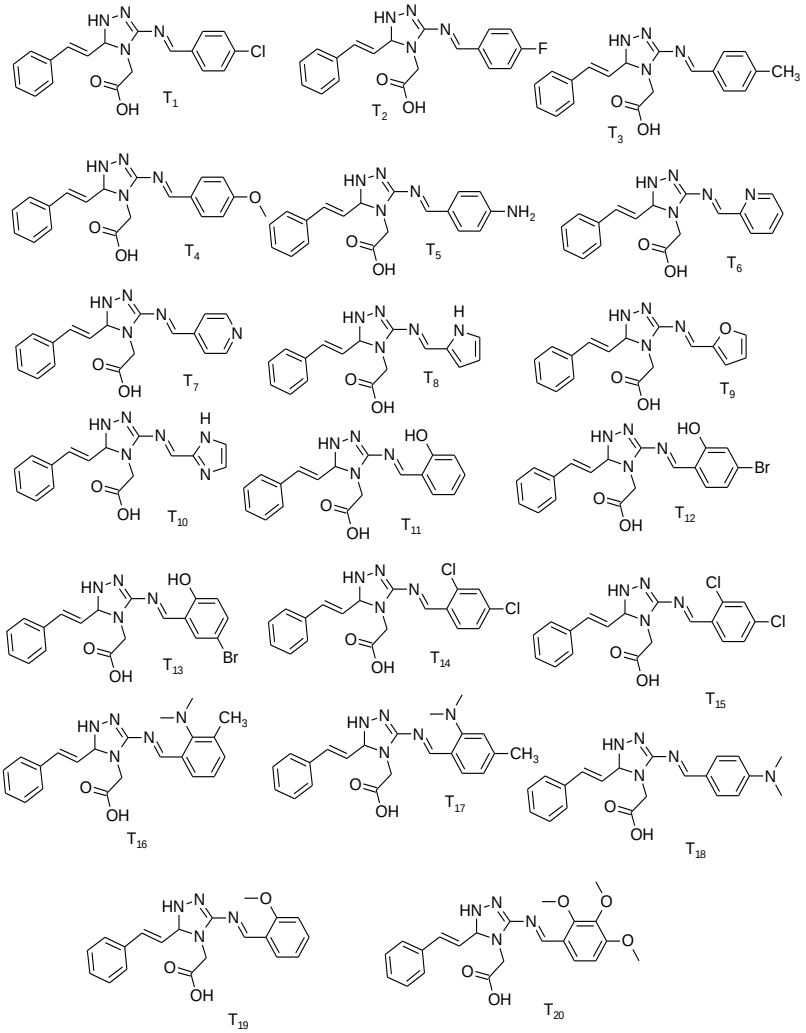


Figure 2: Newly designed compounds

***In silico* molecular docking studies:**

Devices and materials

The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some of the online programming's like protein data bank 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, we take the DPP-IV (PDB ID: 6EOR) 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 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.

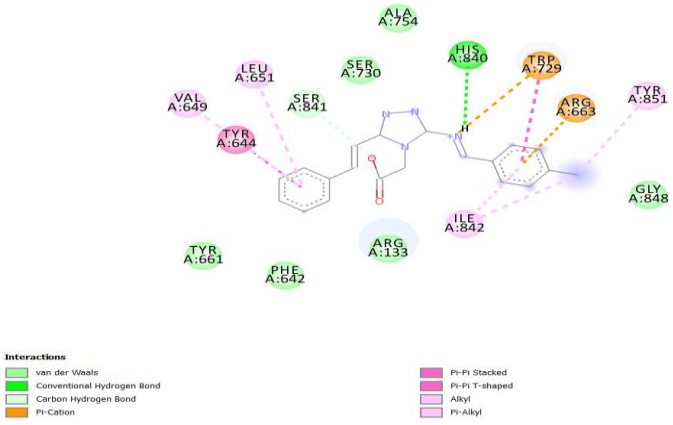


Figure 3. 2D docking interaction of compound T1 against DPP-IV enzyme

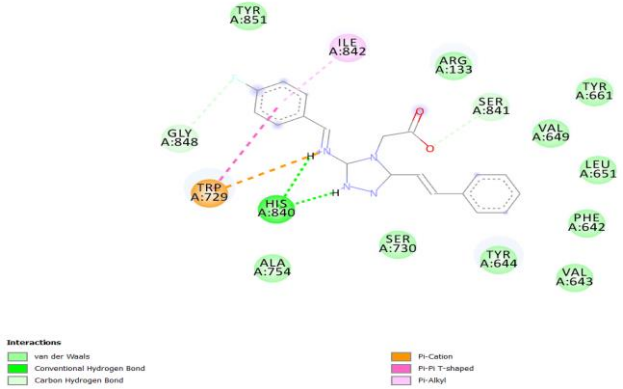


Figure 4. 2D docking interaction of compound T2 against topoisomerase DPP-IV enzyme

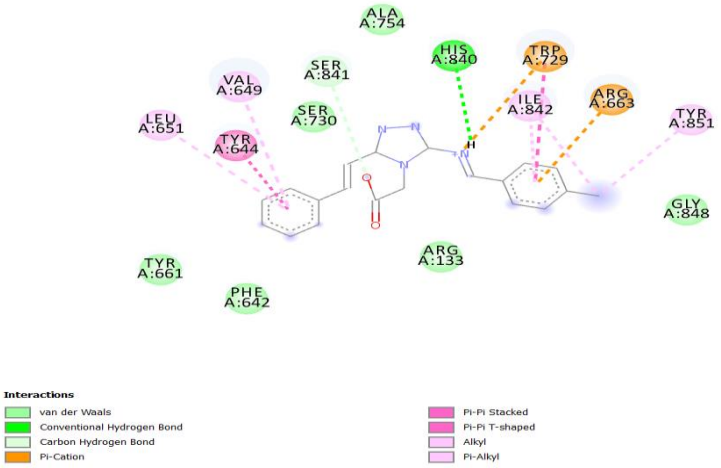


Figure 5. 2D docking interaction of compound T3 against topoisomerase DPP-IV enzyme

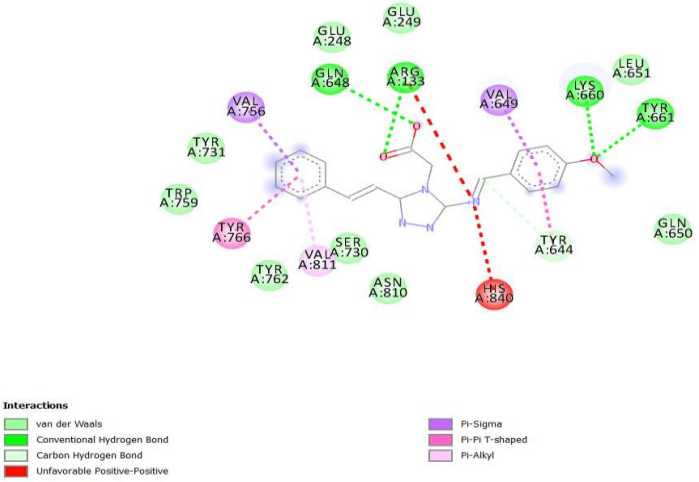


Figure 6. 2D docking interaction of compound T4 against topoisomerase DPP-IV enzyme

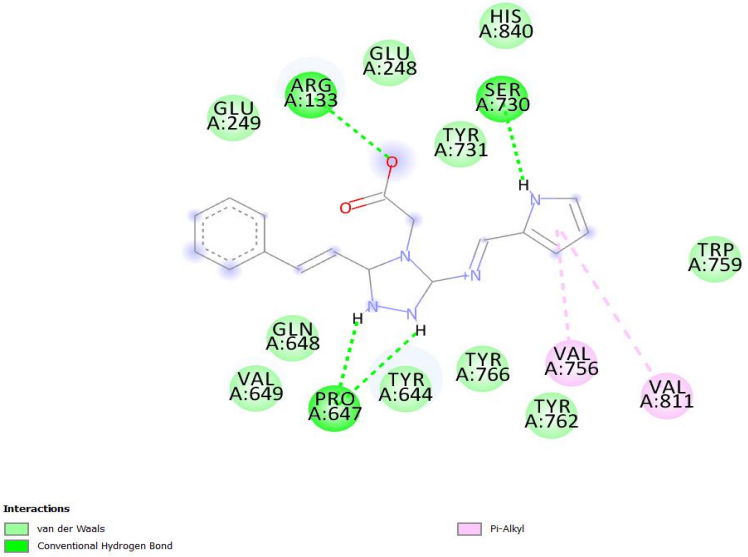


Figure 7. 2D docking interaction of compound T8 against DPP-IV enzyme

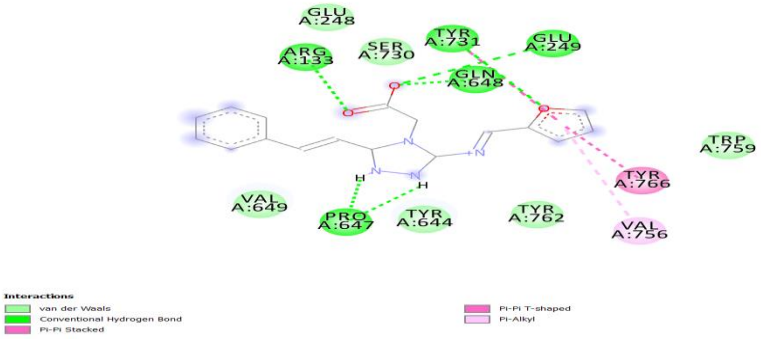


Figure 8. 2D docking interaction of compound T9 against DPP-IV enzyme

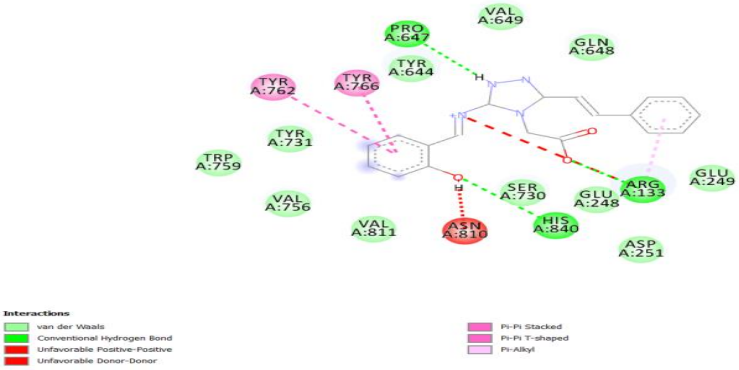


Figure 9. 2D docking interaction of compound T11 against DPP-IV enzyme

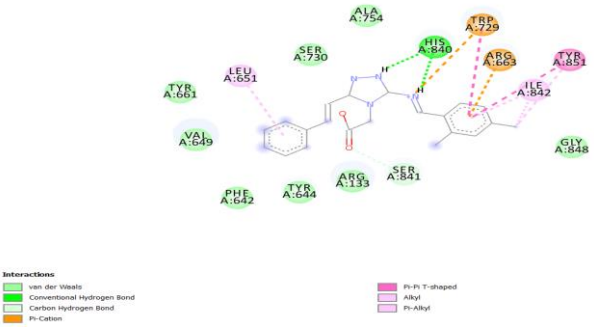


Figure 10. 2D docking interaction of compound T14 against DPP-IV enzyme

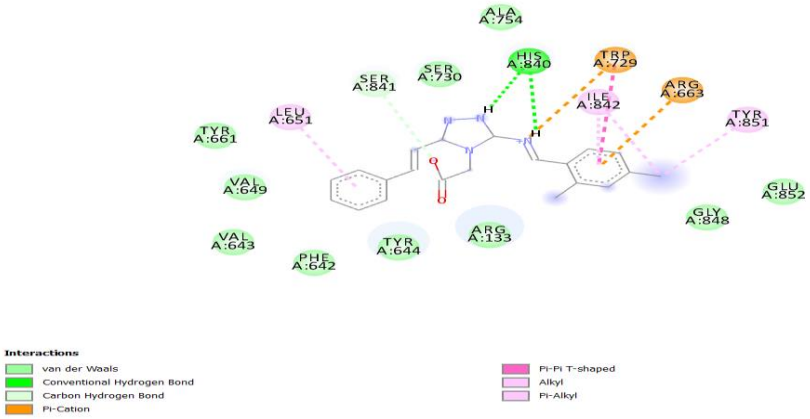


Figure 11. 2D docking interaction of compound T15 against DPP-IV enzyme

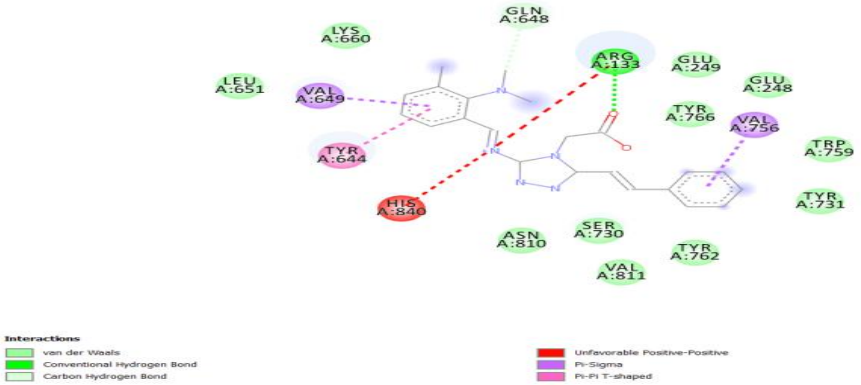


Figure 12. 2D docking interaction of compound T16 against DPP-IV enzyme.

Table 1. Binding energy of studied compounds.

Ligand	Binding Affinity
T1	**-8.5**
T2	**-9**
T3	**-8.5**
T4	**-8.6**
T5	-8.1
T6	-8.4
T7	-8.1
T8	**-8.5**
T9	**-9.7**
T10	-8.3
T11	**-8.6**
T12	-8
T13	-8.3
T14	**-8.6**
T15	**-8.7**
T16	**-9**
T17	-8.5
T18	-8.4
T19	-8.3
T20	-8.1
Gliclazid	-11.2

RESULT OF MOLECULAR DOCKING:

From the results it shows that, all the compounds have promising interaction with targeted enzyme topoisomerase II. The interaction is mainly due to the presence of lipophilic factor of aromatic heterocyclic ring. From the docking results, compound T9 (9.7 kcal/mol) shows highest binding affinity toward topoisomerase II enzyme compared to standard drug gliclazide. This compound produced five conventional hydrogen bonds between carbonyl oxygen, acid oxygen from hydroxyl group and nitrogen of triazole moiety with residues of Arg 133, Glu 249, Pro 647, Gln 648 and Try 731 respectively. 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 Topoisomerase II enzyme and also play important role in the inhibition of the ligand-binding domain of topoisomerase 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 T1, T2, T3, T4, T8, T9, T11, T14, T15, T16, and T17 are selected for the conventional synthesis and it was further evaluated for the in vitro anti-diabetic activity using corresponding cell line.

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 from (**Figure 13 to 52).**

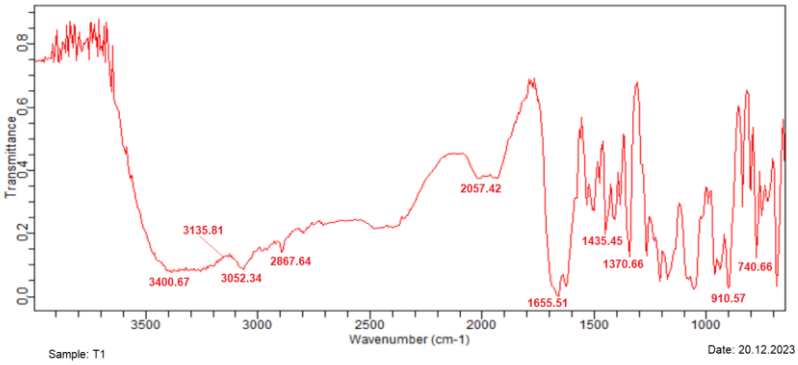


Figure 13: IR Spectra for compound T1

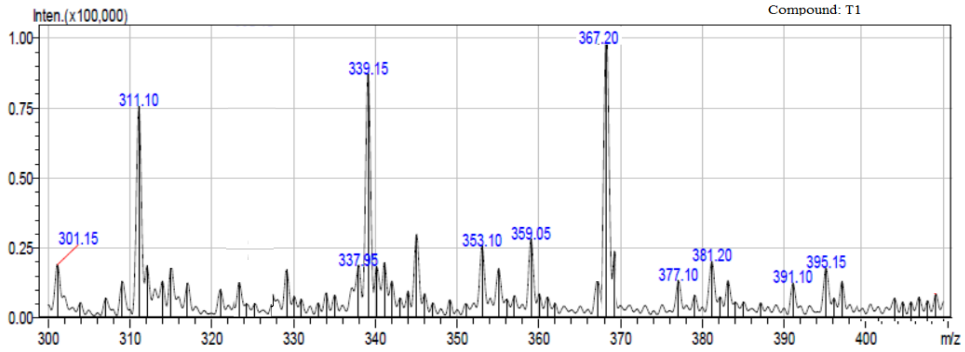


Figure 14: Mass Spectra for compound T1

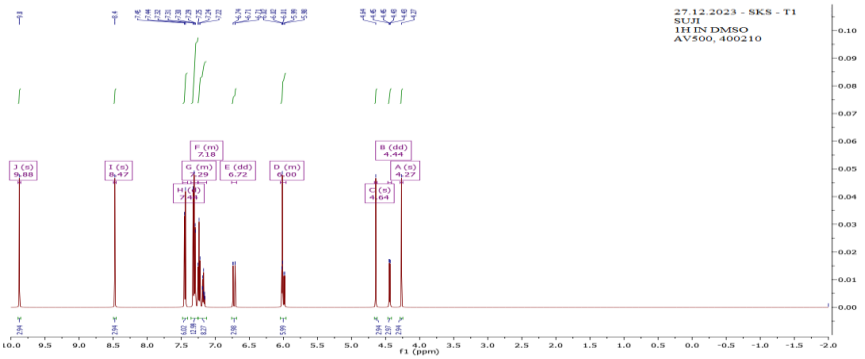


Figure 15: [1]H NMR Spectra for compound T1

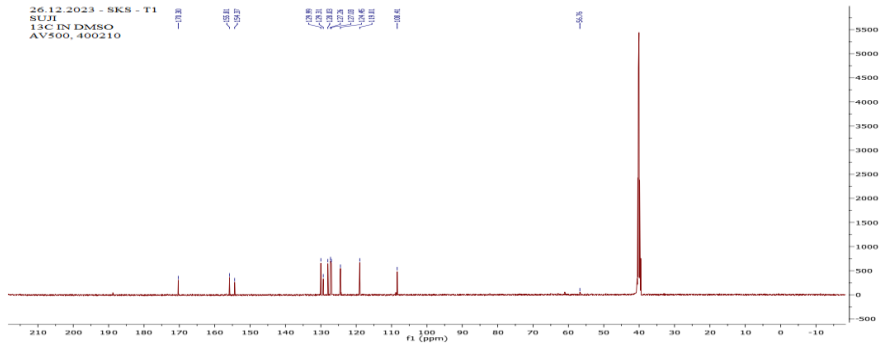


Figure 16: [13]C NMR Spectra for compound T1

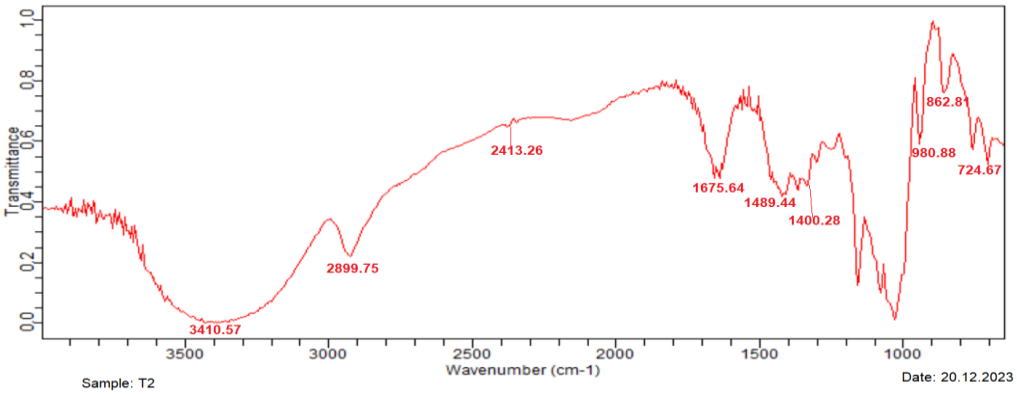


Figure 17: IR Spectra for compound T2

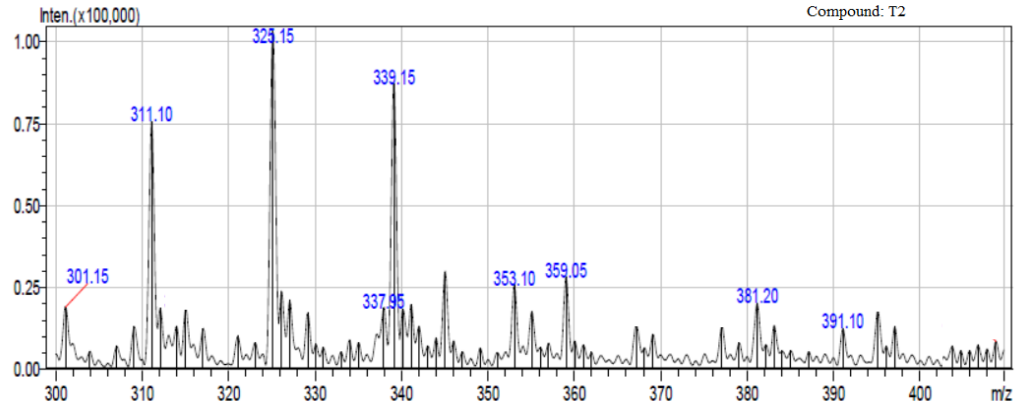


Figure 18: Mass Spectra for compound T2

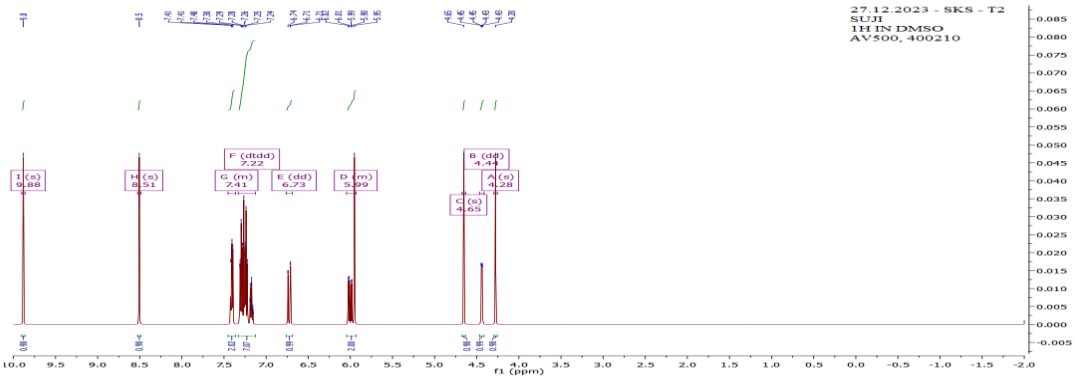


Figure 19: ^{1}H NMR Spectra for compound T2

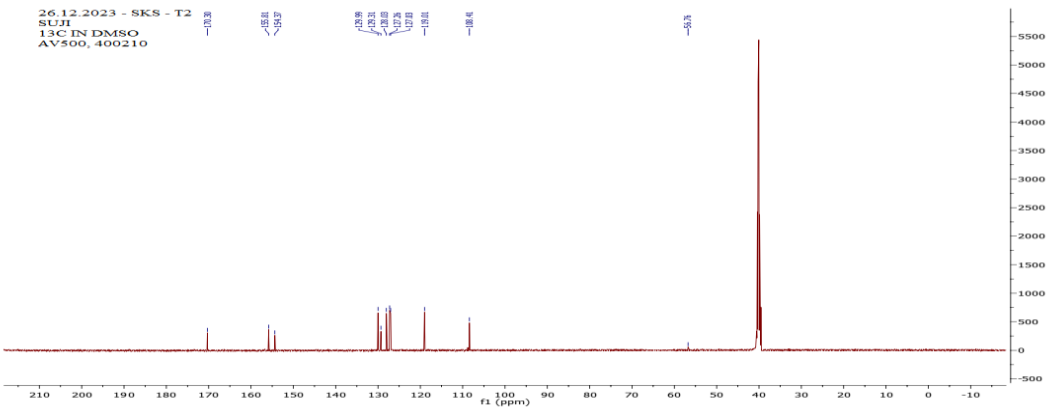


Figure 20: ^{13}C NMR Spectra for compound T2

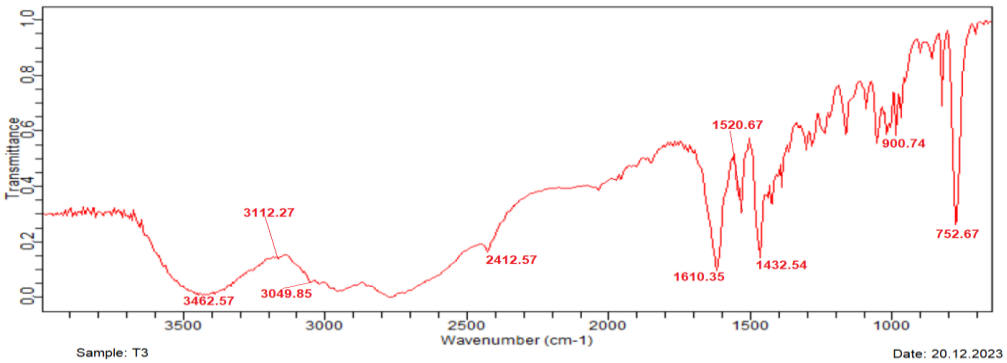


Figure 21: IR Spectra for compound T3

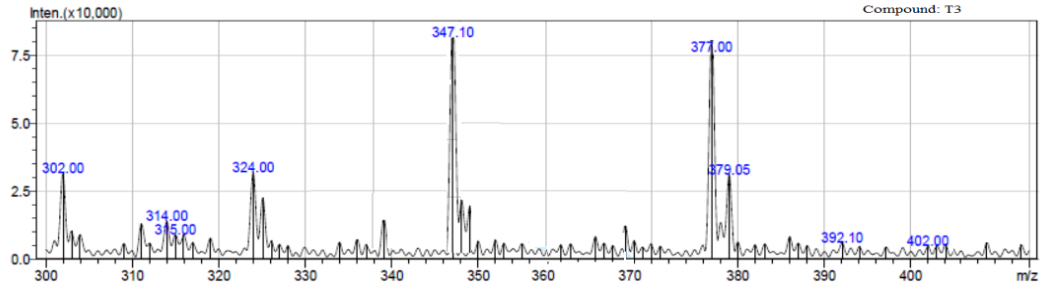


Figure 22: Mass Spectra for compound T3

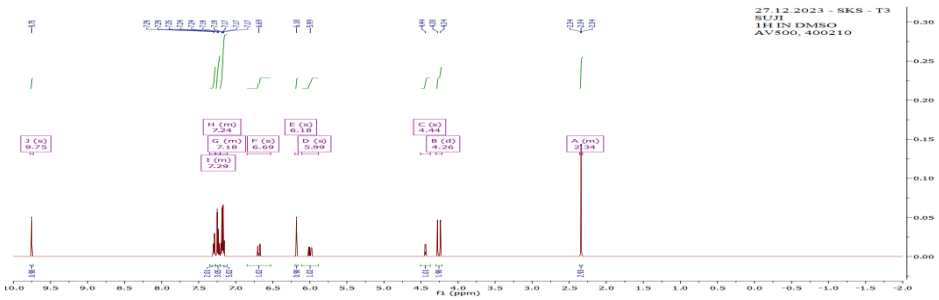


Figure 23: ^{1}H Spectra for compound T3

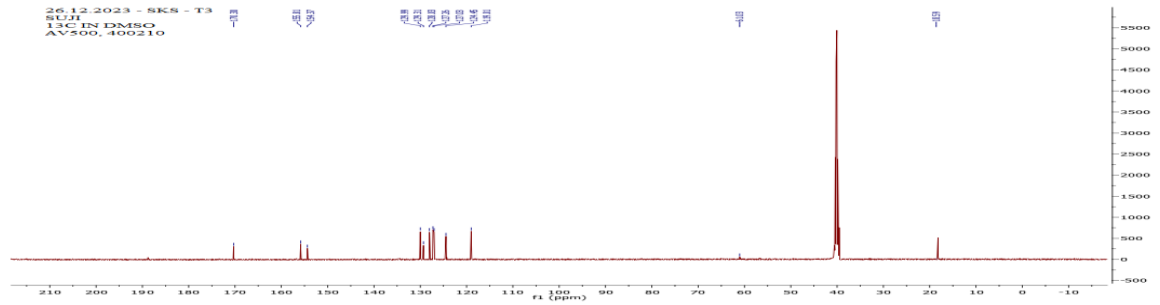


Figure 24: ^{13}C NMR Spectra for compound T3

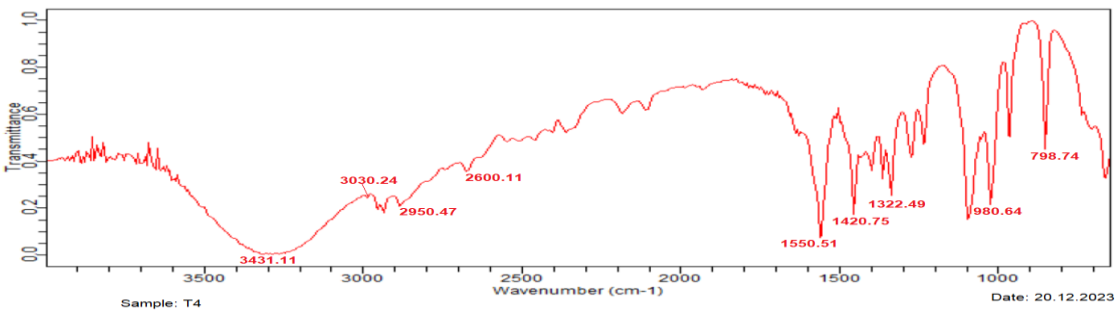


Figure 25: IR Spectra for compound T4

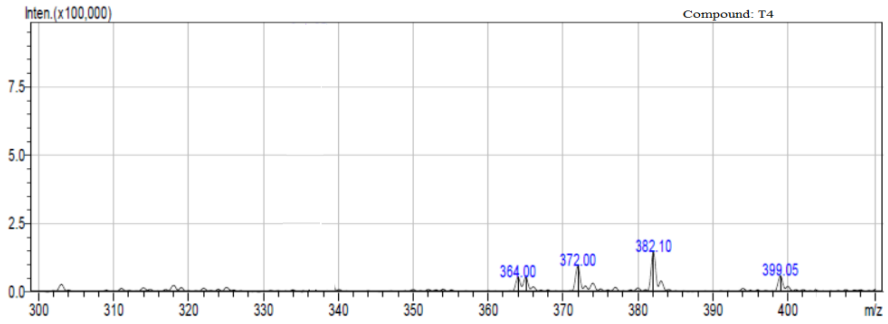


Figure 26: Mass Spectra for compound T4

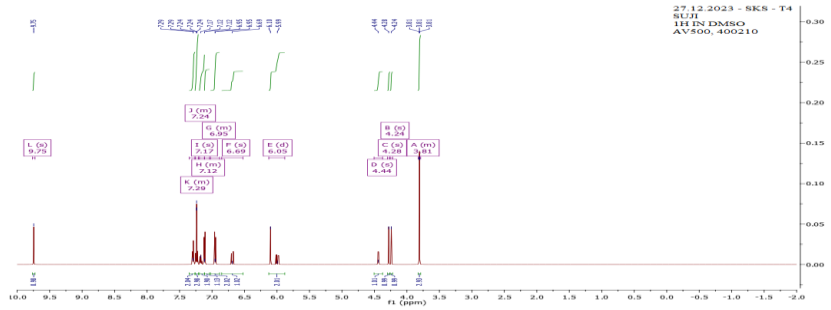


Figure 27: ^{1}H NMR Spectra for compound T4

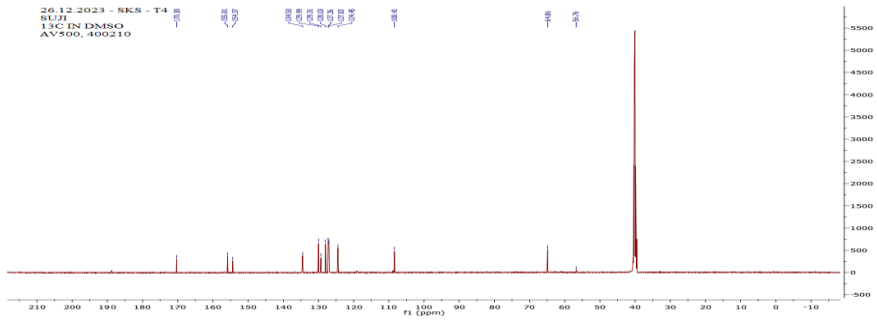


Figure 28: ^{13}C NMR Spectra for compound T4

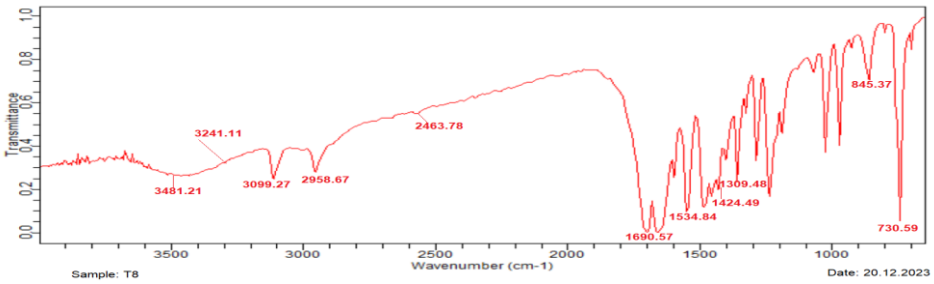


Figure 29: IR Spectra for compound T8

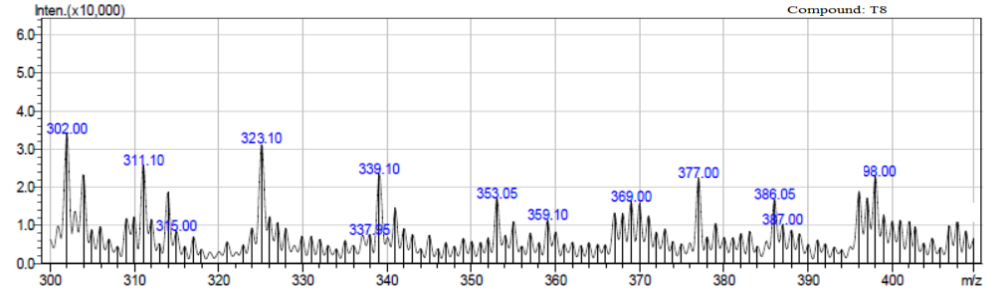


Figure 30: Mass Spectra for compound T8

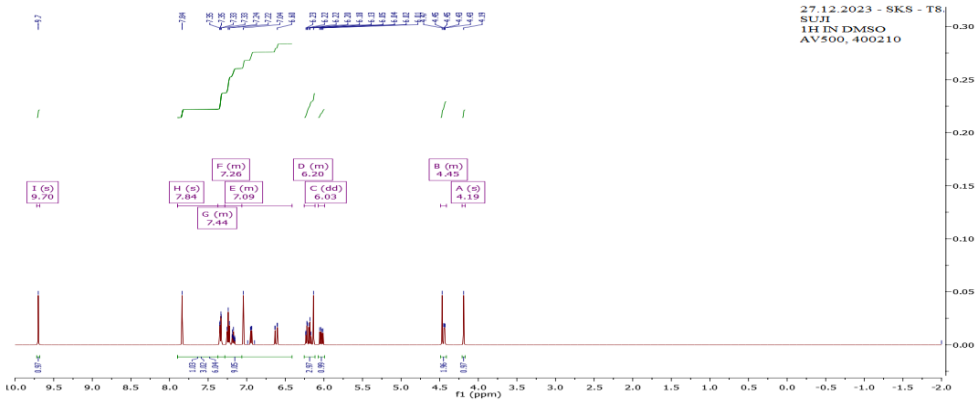


Figure 31: [1]H NMR Spectra for compound T8

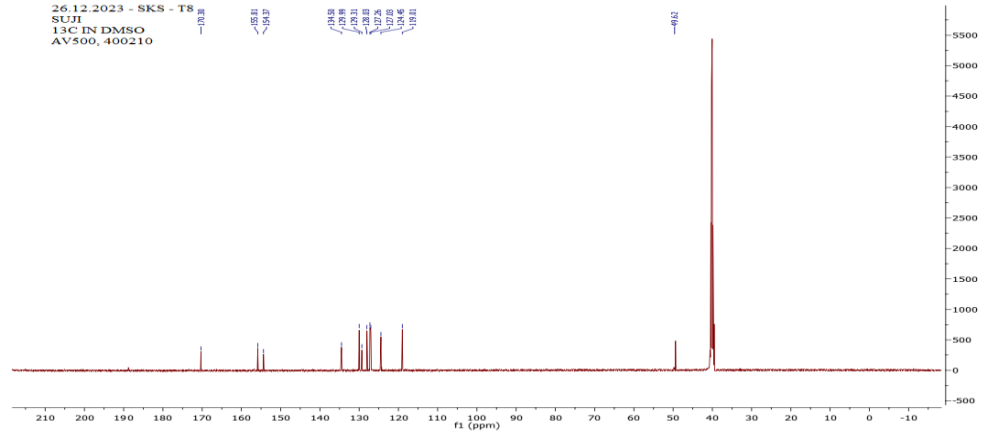


Figure 32: [13]C NMR Spectra for compound T8

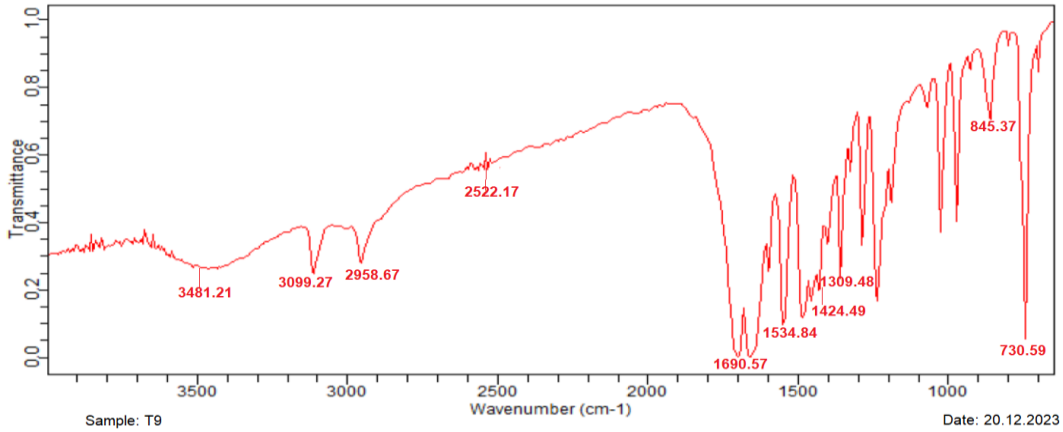


Figure 33: IR Spectra for compound T9

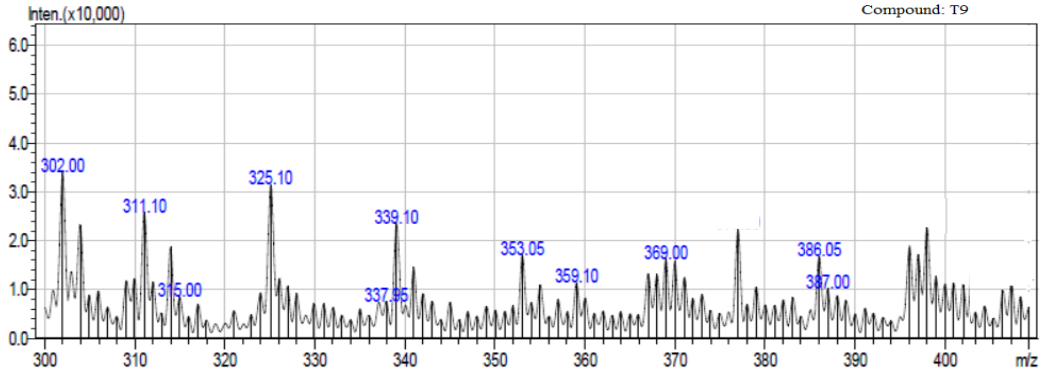


Figure 34: Mass Spectra for compound T9

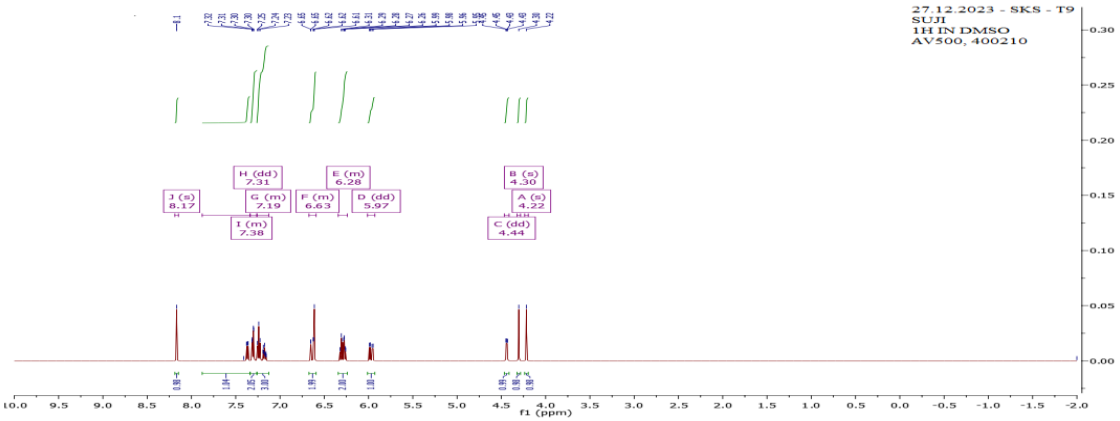


Figure 35: [1]H NMR Spectra for compound T9

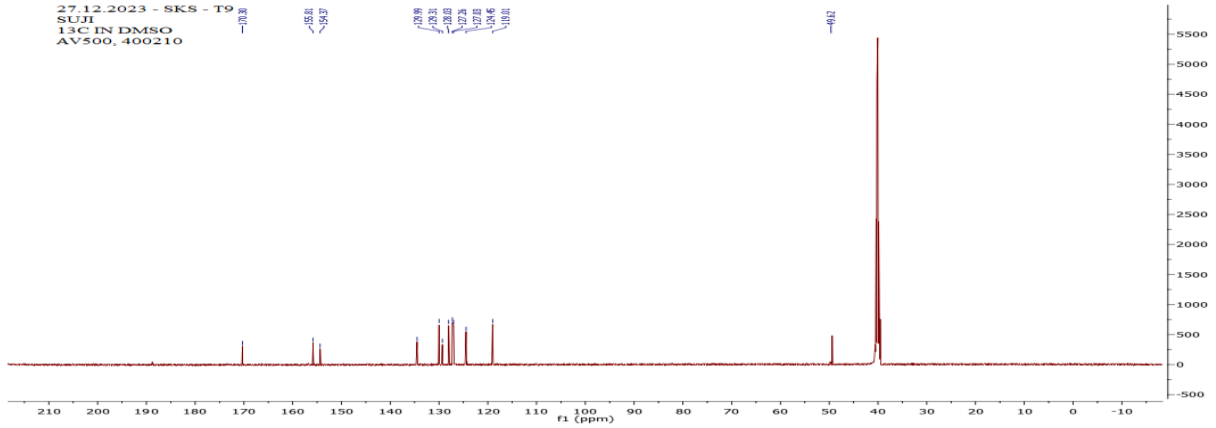


Figure 36: [13]C NMR Spectra for compound T9

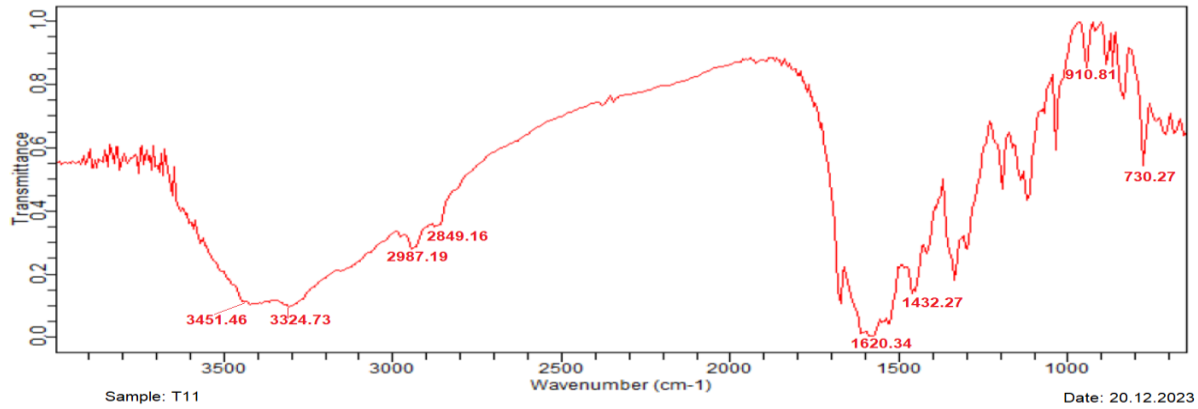


Figure 37: IR Spectra for compound T11

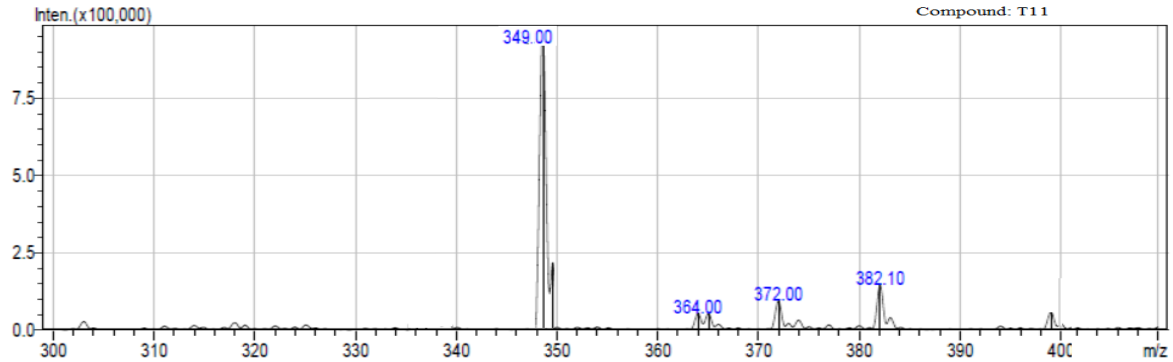


Figure 38: Mass Spectra for compound T11

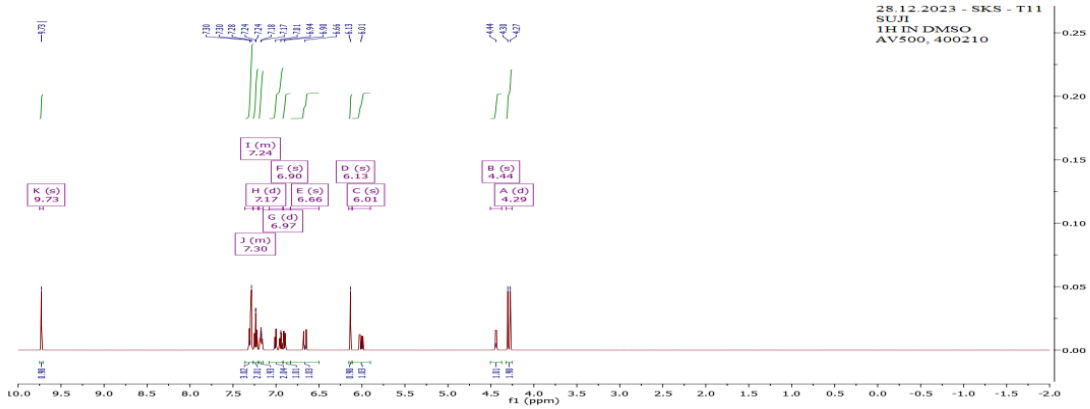


Figure 39: [1]H NMR Spectra for compound T11

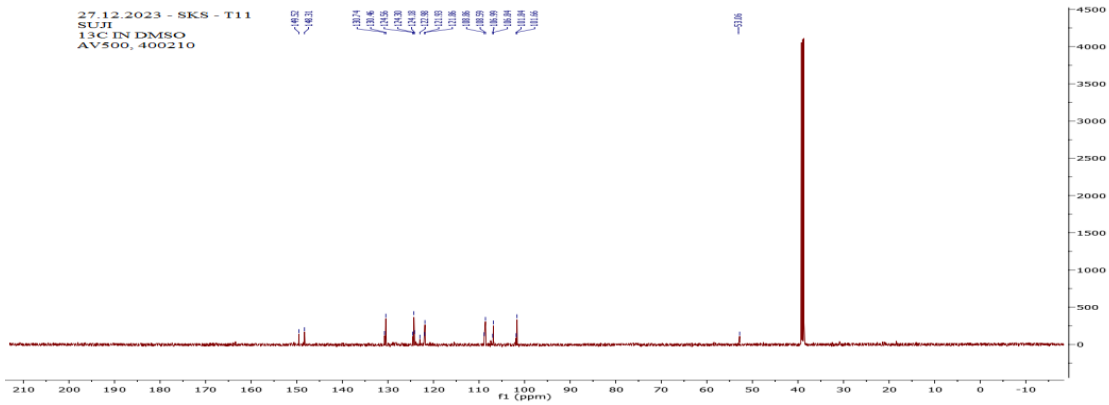


Figure 40: [13]C NMR Spectra for compound T11

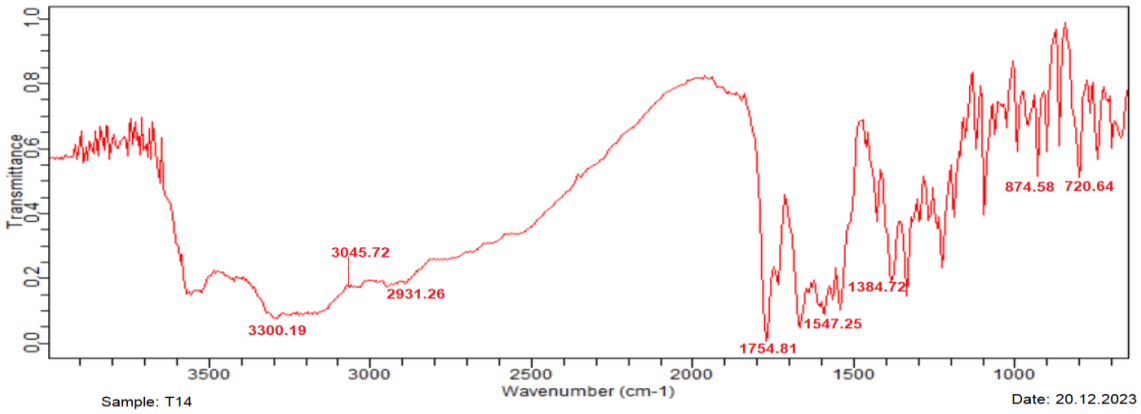


Figure 41: IR Spectra for compound T14

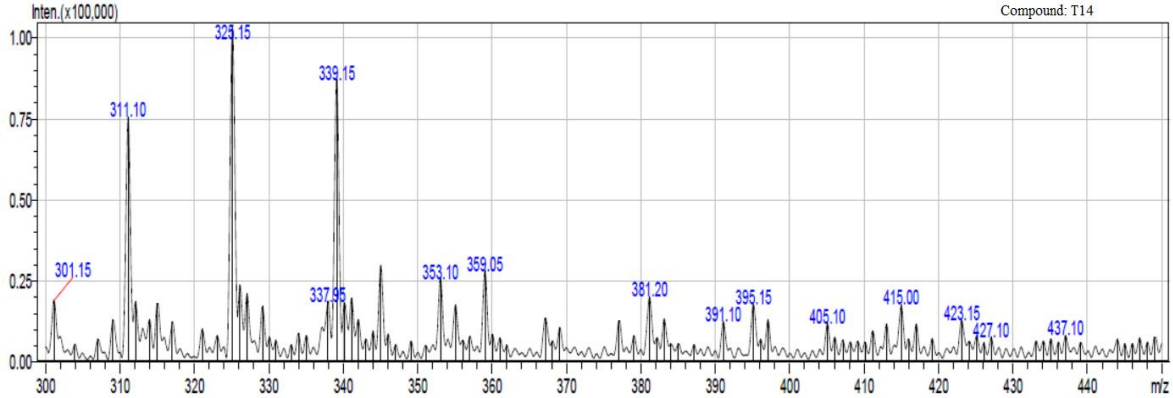


Figure 42: Mass Spectra for compound T14

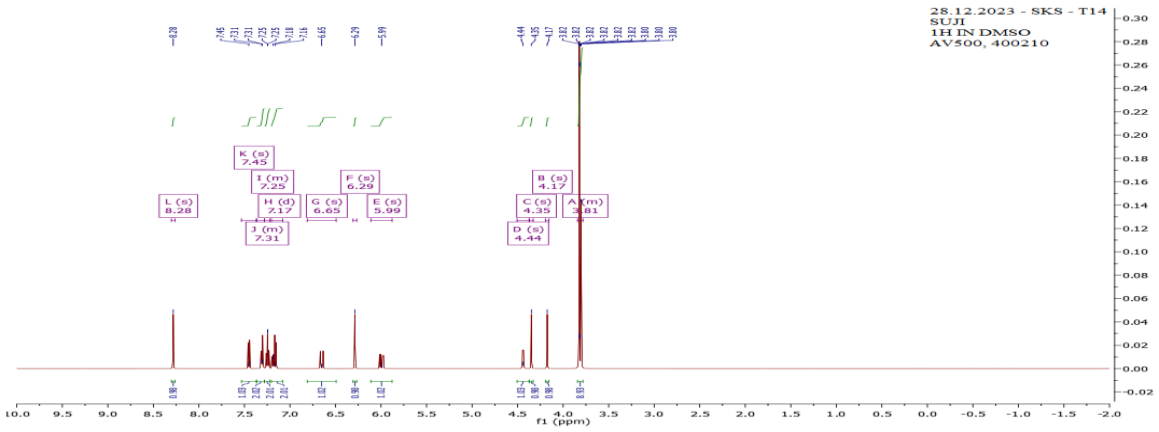


Figure 43: [1]H NMR Spectra for compound T14

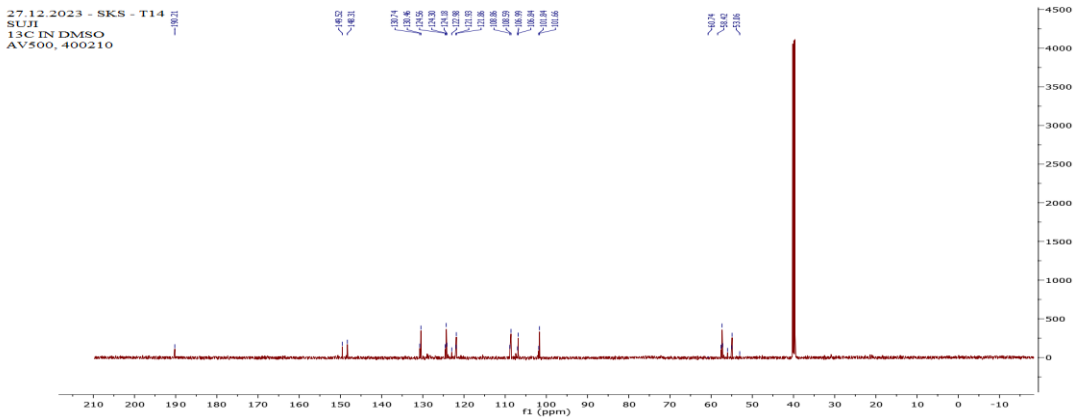


Figure 44: [13]C NMR Spectra for compound T14

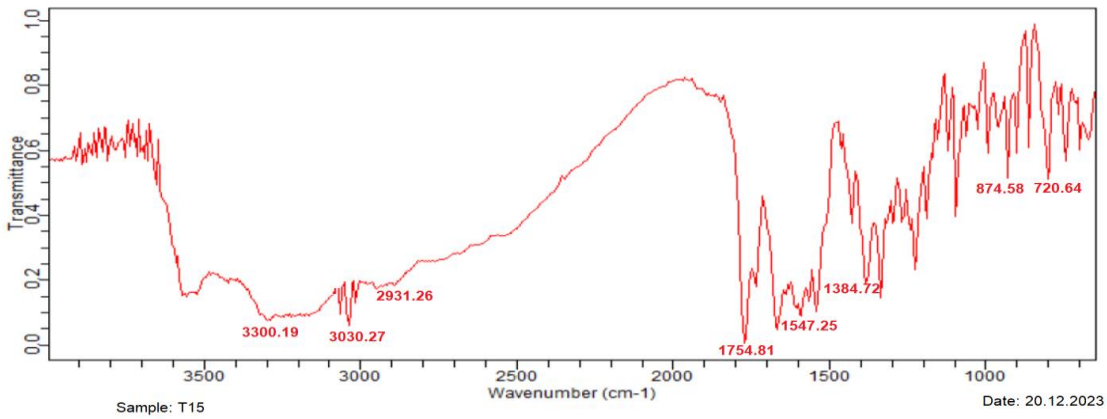


Figure 45: IR Spectra for compound T15

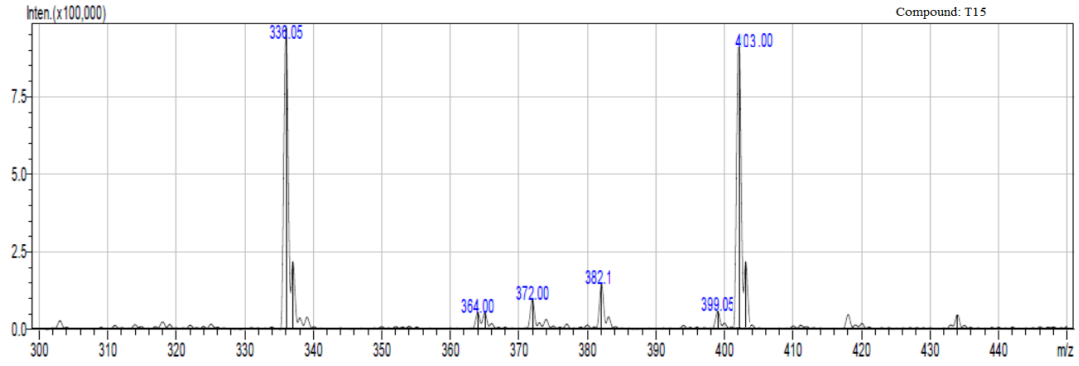


Figure 46: Mass Spectra for compound T15

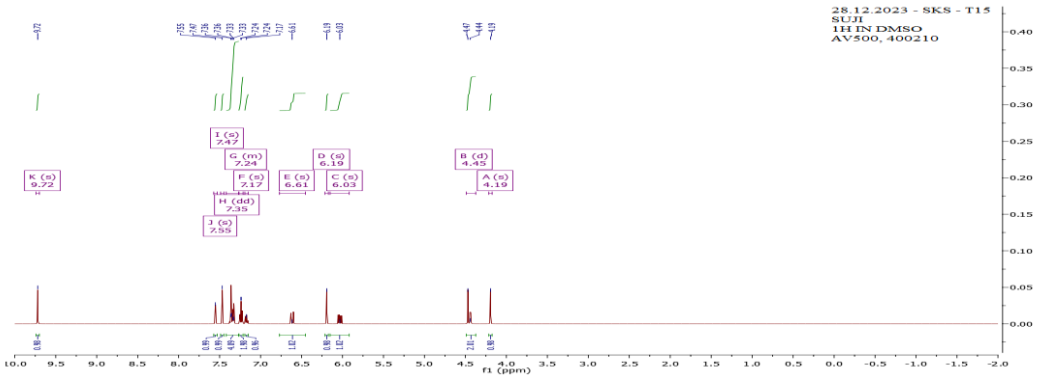


Figure 47: [1]H NMR Spectra for compound T15

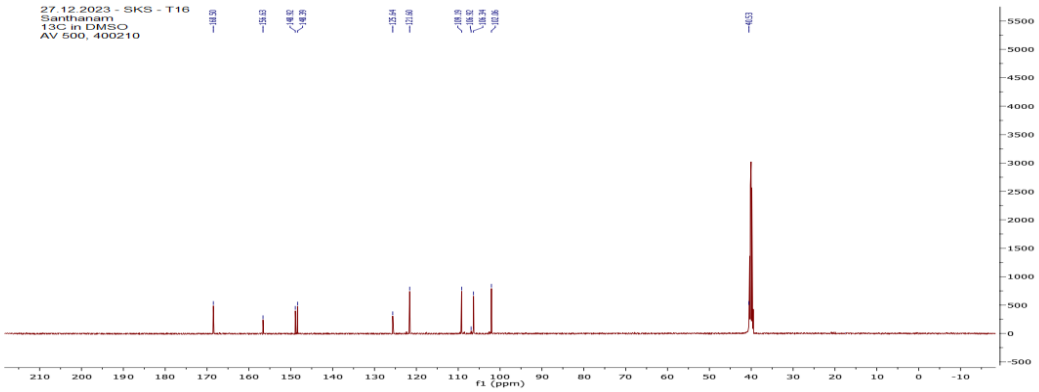


Figure 48: [13]C NMR Spectra for compound T15

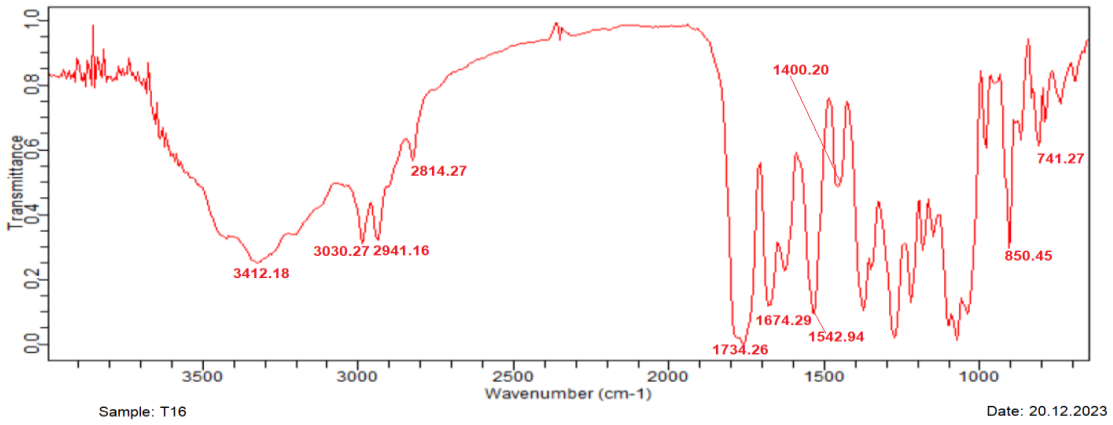


Figure 49: IR Spectra for compound T16

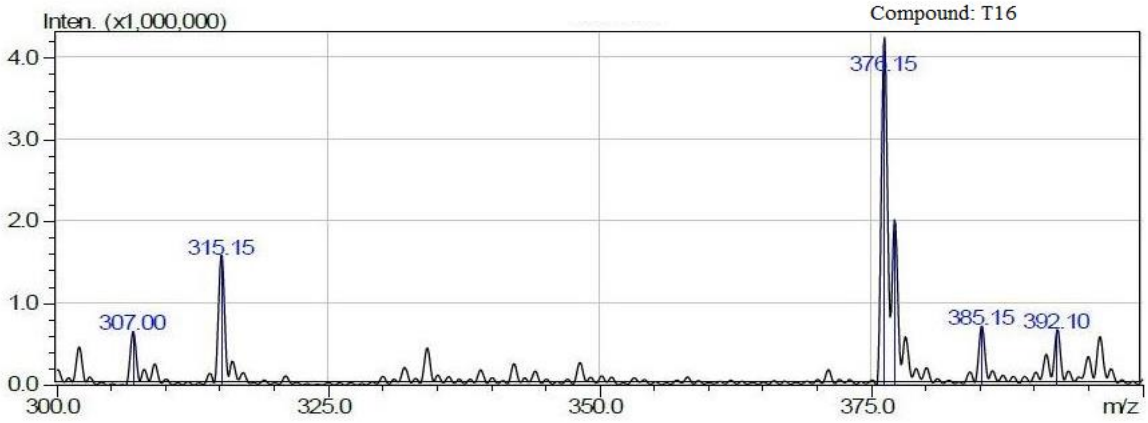


Figure 50: Mass Spectra for compound T16

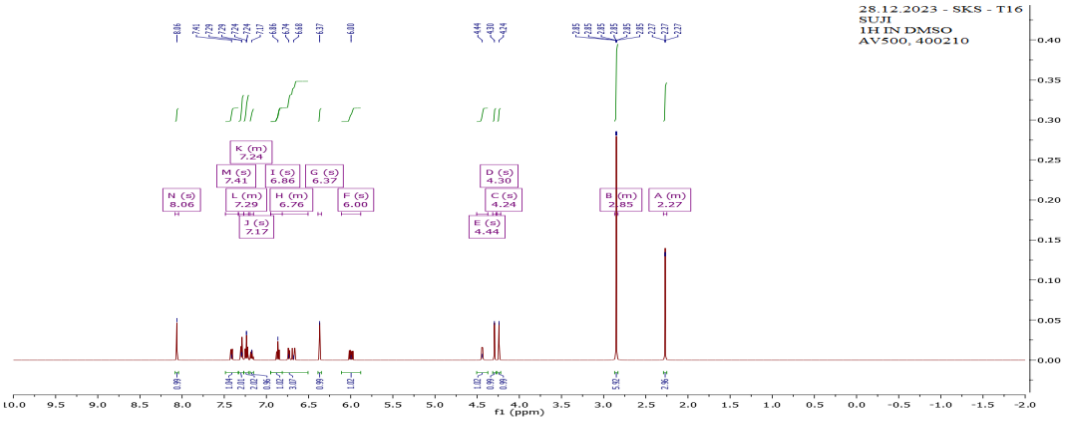


Figure 51: [1]H NMR Spectra for compound T16

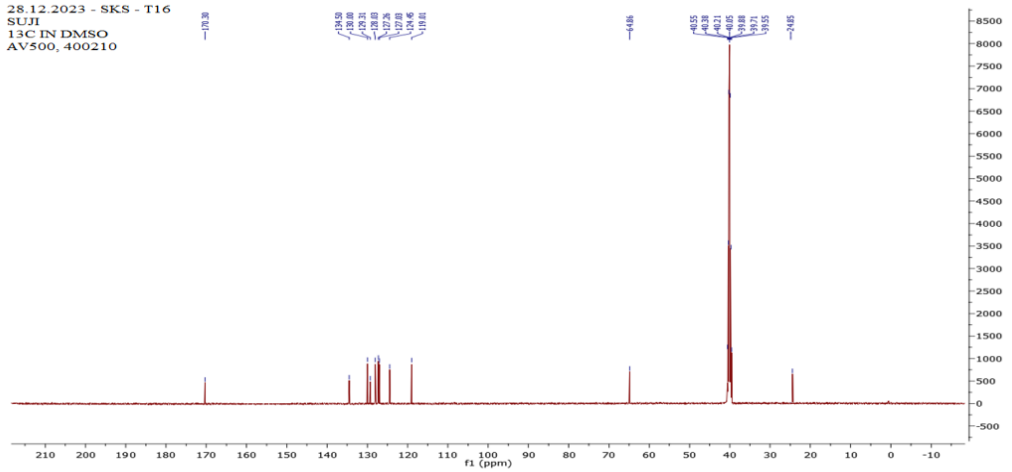


Figure 52: [13]C NMR Spectra for compound T16

Characterization of synthesized compounds

2-{3-[(E)-[(4-chlorophenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T1)

$C_{19}H_{17}ClN_4O_2$; White colour solid; MP: 110 – 113^{O}C; yield: 81%; Rf value: 0.71; IR (KBr) cm^{-1}: 3400 (OH str acid); 3135 (NH str amine); 3052 (CH str alkene); 1655 (C=O str carbonyl carbon); 1435 (CN bending); 910 (Aromatic ring); 740 (C-Cl str); ^{1}H NMR (500 MHz, DMSO) δ 9.88 (s, 3H), 8.47 (s, 3H), 7.44 (d, J = 7.5 Hz, 6H), 7.36 – 7.25 (m, 13H), 7.25 – 7.13 (m, 8H), 6.72 (dd, J = 15.1, 0.8 Hz, 3H), 6.04 – 5.96 (m, 6H), 4.64 (s, 3H), 4.44 (dd, J = 6.2, 0.6 Hz, 3H), 4.27 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 108.41, 56.76. Mass: actual: 368 m/z; Found: 367 (M-1) m/z.

2-{3-[(E)-[(4-fluorophenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T2)

$C_{19}H_{17}FN_4O_2$; White colour solid; MP: 115 – 118^{O}C; yield: 79%; Rf value: 0.69; IR (KBr) cm^{-1}: IR (KBr) cm^{-1}: 3410 (OH str acid); 2899 (CH str alkene); 1675 (C=O str carbonyl carbon); 1489 (CN bending); 862 (Aromatic ring); 724 (C-Cl str); ^{1}H NMR (500 MHz, DMSO) δ 9.88 (s, 1H), 8.51 (s, 1H), 7.45 – 7.36 (m, 2H), 7.22 (dd, J = 8.7, 6.4, 5.2, 1.4 Hz, 7H), 6.73 (dd, J = 15.1, 0.8 Hz, 1H), 6.04 – 5.93 (m, 2H), 4.65 (s, 1H), 4.44 (dd, J = 6.2, 0.6 Hz, 1H), 4.28 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 129.99, 129.31, 128.03, 127.26, 127.03, 119.01, 108.41, 56.76. Mass: actual: 352 m/z; Found : 353 (M+1) m/z.

2-{3-[(E)-[(4-methylphenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T3)

$C_{20}H_{20}N_4O_2$; White colour solid; MP: 112 – 114^{O}C; yield: 81%; Rf value: 0.72; IR (KBr) cm^{-1}: 3462 (OH str acid); 3112 (NH str amine); 3049 (CH str alkene); 2912 (CH_3); 1610 (C=O str carbonyl carbon); 1432 (CN bending); 900 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.75 (s, 1H), 7.35 – 7.27 (m, 2H), 7.27 – 7.21

(m, 3H), 7.21 – 7.12 (m, 5H), 6.69 (s, 1H), 6.18 (s, 1H), 5.99 (s, 1H), 4.44 (s, 1H), 4.26 (d, J = 19.7 Hz, 2H), 2.36 – 2.32 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 61.03, 18.59. Mass: actual: 348 m/z; Found: 347 (M-1) m/z.

2-{3-[(E)-[(4-methoxyphenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T4)

$C_{20}H_{20}N_4O_3$; off White colour solid; MP: 116 – 118^{O}C; yield: 69%; Rf value: 0.74; IR (KBr) cm^{-1}: 3431 (OH str acid); 3030 (CH str alkene); 2950 (CH_3); 1550 (C=O str carbonyl carbon); 1420 (CN bending); 980 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.75 (s, 1H), 7.35 – 7.26 (m, 2H), 7.26 – 7.21 (m, 3H), 7.17 (s, 2H), 7.12 – 7.04 (m, 1H), 7.02 – 6.88 (m, 2H), 6.69 (s, 1H), 6.05 (d, *J* = 52.9 Hz, 2H), 4.44 (s, 1H), 4.28 (s, 1H), 4.24 (s, 1H), 3.83 – 3.79 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 134.50, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 108.41, 64.86, 56.76.. Mass: actual: 364 m/z; Found : 364 m/z.

2-{5-[(E)-2-phenylethenyl]-3-[(E)-(1H-pyrrol-2-ylmethylidene)amino]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T8)

$C_{17}H_{17}N_5O_2$; White colour solid; MP: 103 - 106^{O}C; yield: 72%; Rf value: 0.82; IR (KBr) cm^{-1}: 3481 (OH str acid); 3241 (NH str); 3099 (CH str alkene); 2958 (CH_3); 1534 (C=O str carbonyl carbon); 1424 (CN bending); 848 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.70 (s, 1H), 7.84 (s, 1H), 7.90 – 7.28 (m, 3H), 7.90 – 7.06 (m, 6H), 7.90 – 6.41 (m, 9H), 6.26 – 6.11 (m, 3H), 6.03 (dd, *J* = 15.0, 6.2 Hz, 1H), 4.49 – 4.41 (m, 2H), 4.19 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 134.50, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 49.62; Mass: actual: 323 m/z; Found : 323 m/z.

2-{3-[(E)-(furan-2-ylmethylidene)amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T9)

$C_{17}H_{16}N_4O_3$; White colour solid; MP: 121 - 123^{O}C; yield: 73%; Rf value: 0.89; IR (KBr) cm^{-1}: 3481 (OH str acid); 3099 (CH str alkene); 1534 (C=O str carbonyl carbon); 1429 (CN bending); 845 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.17 (s, 1H), 7.88 – 7.34 (m, 1H), 7.31 (dd, *J* = 7.5, 1.5 Hz, 2H), 7.26 – 7.13 (m, 3H), 6.67 – 6.59 (m, 2H), 6.34 – 6.24 (m, 2H), 5.97 (dd, *J* = 15.1, 6.1 Hz, 1H), 4.44 (dd, *J* = 6.2, 0.6 Hz, 1H), 4.30 (s, 1H), 4.22 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 170.30, 155.81, 154.37, 129.99, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 49.62.; Mass: actual: 324 m/z; Found : 325 (M-1) m/z.

2-{3-[(E)-[(2-hydroxyphenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T11)

$C_{19}H_{18}N_4O_3$; White colour solid; MP: 119 - 122^{O}C; yield: 71%; Rf value: 0.88; IR (KBr) cm^{-1}: 3451 (OH str acid); 3324 (NH str); 2987 (CH str alkene); 1620 (C=O str carbonyl carbon); 1432 (CN bending); 910 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.73 (s, 1H), 7.36 – 7.27 (m, 3H), 7.27 – 7.21 (m, 2H), 7.17 (d, *J* = 2.2 Hz, 2H), 6.97 (d, *J* = 31.3 Hz, 2H), 6.90 (s, 1H), 6.66 (s, 1H), 6.13 (s, 1H), 6.01 (s, 1H), 4.44 (s, 1H), 4.29 (d, *J* = 14.5 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 149.52, 148.31, 130.74, 130.46, 124.56, 124.30, 124.18, 122.98, 121.93, 121.86, 108.86, 108.59, 106.99, 106.84, 101.84, 101.66, 53.06; Mass: actual: 350 m/z; Found : 349 (M-1) m/z.

2-{5-[(E)-2-phenylethenyl]-3-[(E)-[(2,3,4-trimethoxyphenyl)methylidene]amino]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T14)

$C_{22}H_{24}N_4O_5$; White colour solid; MP: 117 - 119^{O}C; yield: 76%; Rf value: 0.84; IR (KBr) cm^{-1}: 3300 (OH str acid); 3048 (NH str); 2950 (CH str alkene); 2849 (CH_3); 1620 (C=O str carbonyl carbon); 1432 (CN bending); 910 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.73 (s, 1H), 7.36 – 7.27 (m, 3H), 7.27 – 7.21 (m, 2H), 7.17 (d, *J* = 2.2 Hz, 2H), 6.97 (d, *J* = 31.3 Hz, 2H), 6.90 (s, 1H), 6.66 (s, 1H), 6.13 (s, 1H), 6.01 (s, 1H), 4.44 (s, 1H), 4.29 (d, *J* = 14.5 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 190.21, 149.52, 148.31, 130.74, 130.46, 124.56, 124.30, 124.18, 122.98, 121.93, 121.86, 108.86, 108.59, 106.99, 106.84, 101.84, 101.66, 60.74, 58.42, 53.06; Mass: actual: 424 m/z; Found : 423 (M-1) m/z.

2-{3-[(E)-[(2,4-dichlorophenyl)methylidene]amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl}acetic acid (T15)

$C_{19}H_{16}Cl_2N_4O_2$; White colour solid; MP: 125 - 127^{O}C; yield: 74%; Rf value: 0.71; IR (KBr) cm^{-1}: 3300 (OH str acid); 3030 (CH str alkene); 1547 (C=O str carbonyl carbon); 1384 (CN bending); 874 (Aromatic ring): 720 (C-Cl str); ^{1}H NMR (500 MHz, DMSO) δ ^{1}H NMR (500 MHz, DMSO) δ 9.72 (s, 1H), 7.55 (s, 1H), 7.47

(s, 1H), 7.35 (dd, *J* = 13.3, 2.3 Hz, 4H), 7.27 – 7.21 (m, 2H), 7.17 (s, 1H), 6.61 (s, 1H), 6.19 (s, 1H), 6.03 (s, 1H), 4.45 (d, *J* = 14.8 Hz, 2H), 4.19 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 190.21, 149.52, 148.31, 130.74, 130.46, 124.56, 124.30, 124.18, 122.98, 121.93, 121.86, 108.86, 108.59, 106.99, 106.84, 101.84, 101.66, 60.74, 58.42, 53.06; Mass: actual: 403 m/z; Found : 403 m/z.

2-[(5R)-3-[(E)-{[2-(dimethylamino)-3-methylphenyl]methylidene}amino]-5-[(E)-2-phenylethenyl]-4,5-dihydro-1H-1,2,4-triazol-4-yl]acetic acid (T16)

C22H25N5O2; White colour solid; MP: 121 - 123^{O}C; yield: 76%; Rf value: 0.72; IR (KBr) cm^{-1}: 3412 (OH str acid); 3030 (CH str alkene); 2941 (CH_3); 1542 (C=O str carbonyl carbon); 1400 (CN bending); 850 (Aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.06 (s, 1H), 7.41 (s, 1H), 7.33 – 7.27 (m, 2H), 7.27 – 7.21 (m, 2H), 7.17 (s, 1H), 6.86 (s, 1H), 6.95 – 6.51 (m, 3H), 6.37 (s, 1H), 6.00 (s, 1H), 4.44 (s, 1H), 4.30 (s, 1H), 4.24 (s, 1H), 2.87 – 2.83 (m, 6H), 2.29 – 2.25 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 190.21, 149.52, 148.31, 130.74, 130.46, 124.56, 124.30, 124.18, 122.98, 121.93, 121.86, 108.86, 108.59, 106.99, 106.84, 101.84, 101.66, 60.74, 58.42, 53.06; Mass: actual: 391 m/z; Found : 392 (M-1) m/z.

IN VITRO ANTIDIABETIC STUDY:

Inhibition of α-amylase enzyme

Starch solution (0.1%) was prepared by dissolving 0.1 g of starch in 100 mL of sodium acetate buffer (pH = 4.8, 16 mM). An enzyme solution was prepared by dissolving 27.5 mg of α-amylase in 100 mL of deionized H_2O. A colorimetric reagent was prepared by dissolving 1 g of 3,5-dinitro salicylic acid in deionized H_2O (20 mL) and 0.16 g sodium hydroxide (in 10 mL deionized H2O) and 4 g of sodium potassium tartrate was added gradually to the mixture. The mixture was mixed well and the volume was made up to 100mL using deionized H_2O. Both control (100 µL) and the sulfonylurea derivatives (100 µL) were separately mixed with the starch solution (100 µL) and left for 30 minutes to react with the α-amylase solution (under alkaline conditions at 25^{O}C). The action was recorded after 5 minutes. The liberated maltose was measured quantitatively by the reduction of 3,5-dinitro salicylic acid to 3-amino-5-nitrosalicylic acid. This reaction was measured at 540 nm.

Lipinski's rule of five parameters

The rule describes molecular properties important for drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion ("ADME"). However, the rules do not predict whether a compound is pharmacologically active or not. The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity as well as drug-like properties as described by Lipinski's rule.

Toxicity studies

TOPKAT (Toxicity prediction by computer assisted technology) is constitutive methods in modern drug discovery to predict the pharmacokinetics and toxicity. These studies predict ADMET properties of the designed derivative and also help in structural refinements to improve ADME and remove toxicities. ADMET properties are important for the selection and development of drug candidates. The ADMET properties were estimated using by Discovery Studio 4.1. The results predicted for properties of human intestinal absorption (HIA) after oral administration, aqueous solubility, blood-brain penetration (blood brain barrier, BBB) after oral administration, CYP2D6 enzyme inhibition using 2D chemical structure, potential organ toxicity for a wide range of structurally diverse compounds and whether a compound is likely to be highly bound (>= 90% bound) to carrier proteins in the blood for screened structures. The toxicity predicted on male, female mouse and rat which calculated Carcinogenity, Weight of Evidence, Ames test for mutagenicity, Developmental Toxicity Potential, Rat Oral Dose, Mouse Carcinogenic Potency, Rat Carcinogenic Potency, Rat maximum tolerated dose, Rat inhalation, LOAEL (Lowest observed adverse effect level), Fat head minnow, Dalphnia, Biodegradability, Skin Irritancy, Skin sensitization and Ocular skin irritancy.

RESULTS OF IN VITRO ANTI-DIABETIC STUDY:

Inhibition of α-amylase enzyme:

Results of α-amylase enzyme inhibition activity of the compounds were expressed in percentage values which were determined by suitable formulae. The experiments were performed in triplicates, and then, the final values were calculated by taking average of triplicate experimental results. The results of *in-vitro* antidiabetic activity expressed in % are expressed in **table 2** and were compared to Gliclazide. The ten compounds are subjected

to *in-vitro* antidiabetic study by α-amylase enzyme inhibition assay method. Among the tested compounds, derivative **T9** (92.33838%) substituted with furan moiety shows a significant activity against α-amylase enzyme at different concentrations. The derivatives **T11** also possesses significant docking score **9.7** kcal/mol Remaining all other tested compounds shows good to moderate cytotoxic activity on tested cell line. The **Table 2** shows the results for *in vitro* anti-diabetic activity of synthesized compounds.

Table 2. Results for *in vitro* anti-diabetic activity of synthesized compounds

Compound	% inhibition
T1	39.61486
T2	79.57359
T3	91.65062
T4	87.15268
T8	89.02338
T9	92.33838
T11	27.52407
T14	63.92022
T15	29.348
T16	69.13343
Gliclazide	98.24467

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/) 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
T1	2.18	77.29	12	4	2	0	6
T2	2.22	77.29	12	5	2	0	6
T3	2.14	77.29	12	4	2	0	6
T4	2.28	86.52	12	5	2	0	6
T8	1.74	93.08	12	4	3	0	6
T9	1.56	90.43	12	5	2	0	6
T11	1.90	97.52	12	5	3	0	6
T14	2.60	104.98	12	7	2	0	6
T15	2.66	77.29	12	4	2	0	6
T16	2.28	80.53	12	4	2	0	6

Druglikeness Properties of Designed triazole 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 5. Based on the result of druglikness properties of designed benzimidazole derivatives shows 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
T1	-0.16	-0.32	-0.11	-0.22	-0.65	-0.07
T2	-0.16	-0.32	-0.12	-0.23	-0.65	-0.10

T3	-0.13	-0.25	-0.16	-0.18	-0.62	-0.07
T4	-0.13	-0.32	-0.08	-0.18	-0.65	-0.07
T8	-0.07	-0.27	-0.05	-0.22	-0.62	-0.06
T9	-0.28	-0.42	-0.16	-0.36	-0.76	-0.16
T11	-0.20	-0.39	-0.16	-0.23	-0.68	-0.12
T14	-0.19	-0.37	-0.12	-0.20	-0.67	-0.10
T15	-0.13	-0.25	-0.16	-0.18	-0.62	-0.07
T16	-0.20	-0.37	-0.14	-0.23	-0.67	-0.12

Toxicity studies:

The toxicity (TOPKAT) studies suggests that all the studied compounds are non-carcinogen to Mouse NTP Model (Female). All the compounds are also non-carcinogen in Mouse FDA male/female and Rat FDA male/female models. The Weight of Evidence (WoE) of most of the compounds are non-carcinogen. The compound T9 showed the lowest TD_{50} value with 5.00 mg/kg/day in mouse followed by compound T1 (7.51 mg/kg/day). The compound T5 showed higher LC50 value with 0.39 g/kg.

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 DPP-IV protein (PDB ID: 6OER) in molecular docking studies. The calculated docking energies indicated that its interaction with DPP-IV 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* anti-diabetic activity. Compounds **T9** emerged to be the most active compounds against in tested enzyme. The study thus serves as an attempt to progress toward the discovery of novel lead molecule for diabetic 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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