



Design, Synthesis and Characterization of Thiophene Substituted Chalcones for Possible Biological Evaluation

Mejo Joseph^{1*} and S. Alaxander²

¹Nehru College of Pharmacy, Thiruvilvamala, Thrissur Dist. Kerala, 680588, India.

²Vinayaka Mission College of Pharmacy, Vinayaka Mission Research Foundation (Deemed to be University), Salem, Tamil Nadu, 636308, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Development of new antimicrobial agents is a better solution to rectify drug resistance problems in society. In this circumstances new functionalized sulphur bearing heterocyclic moiety were designed, synthesized and evaluated for their *in vitro* antibacterial activity. The present work encompasses the designing novel series of thiophene substituted analogous linked to para amino acetophenone and different aldehydes were successfully synthesized and biological activity was predicted using various computational software's such as Chemsketch, Molinspiration, and admetSAR. Among the synthesized thiophene substituted chalcones T-IV-I and thiophene T-IV-B displayed significant activity against *Streptococcus aureus*. Compounds T-IV-J, T-IV-H and T-IV-C bearing sulphur moiety possess better activity against *Staphylococcus aureus*. Moreover T-IV-C and T-IV-J exhibits good antibacterial activity against *E. coli* and *Pseudomonas aeruginosa*. In general, most of the synthesized compounds exhibited remarkable antibacterial activity due to the presence of sulphur atom in the heterocyclic moieties as well as its lipophilic characters. Molecular docking studies indicated that the synthesized compounds are potent inhibitor of microsomal enzyme Glutathione-S-transferases (PDB ID: 1GNW) also find the different interacting residues,

*Corresponding author: E-mail: mejojoseph000@gmail.com;

bond distance and nature of bonding between the target and the ligand molecules. The results provide important information for the future design of more effective antibacterial agents.

Keywords: Thiophene; antibacterial agents; chalcone; antimicrobial; docking.

1. INTRODUCTION

In sulphur containing heterocyclic, thiophene substituted chalcone derivatives are at the focus as these candidates have structural similarities with active compounds to develop new potent lead molecules in drug design. Thiophene scaffold is one of the privileged structures in drug discovery as this core exhibits various biological activities allowing them to act as antimicrobial, antioxidant, antitubercular, antifungal [1] actions. Further, numerous thiophene -based compounds as clinical drugs have been extensively used to treat various types of diseases with high therapeutic potency, which has led to their extensive developments. Due to the wide range of biological activities of substituted thiophenes, their structure activity relationships (SAR) have generated interest among medicinal chemists, and this has culminated in the discovery of several lead molecules against numerous diseases [2]. The present review is endeavouring to highlight the progress in the various pharmacological activities of thiophene substituted chalcone derivatives [3]. Also biological studies that highlight the chemical groups responsible for evoking the pharmacological activities of synthesized derivatives are studied and compared. Design of *In-silico* filters to eliminate compounds with undesirable properties poor activity or poor Absorption, Distribution, Metabolism, Excretion and Toxicity, (ADMET)) and select the most promising candidate. Fast expansion in this area has been made possible by advances in software and hardware computational power and sophistication. Identification of molecular targets and an increasing database of publicly available target protein structures like the protein data bank www.pdb.org. CADD is being utilized to identify this active drug candidates, select lead compounds (most likely candidates for further evaluation), and optimize leads compounds i.e. transform biologically active compounds into suitable drugs by improving their physicochemical, pharmaceutical, ADMET/PK (pharmacokinetic) properties. Virtual screening is used to discover new drug candidates from different chemical scaffolds by searching commercial, public, or private 3-dimensional chemical structure databases [4].

In-silico technique is reducing the number of molecules synthesized and helping researchers in the process of drug development. Tools and models available are used to estimate the ADMET properties, and structure-based molecular docking, helps in predicting the possible interactions with the target under study. Major information whether the compound under study can work as a drug at an early stage of development is provided by *in-silico* physico chemical properties such as saturation, size, lipophilicity, solubility, polarity, and flexibility [5].

The purpose of the current study was to perform simulated screening of molecules through molecular docking strategy and identify possible lead molecules which could serve as a template for designing new proposed molecules with improved binding affinities, and better molecular interactions with the receptor. Additionally *in-silico* ADME and drug likeness properties of the designed compounds were also evaluated for oral bioavailability and safety of the compound. Discovery studio 2020 prediction was performed on selected compounds to assess the probability of antimicrobial activity.

2. DOCKING METHODOLOGY

2.1 Protein Target

3D Structures of protein were procured from PDB. The protein structures were cleaned (water molecules and other hetero atoms removed), prepared and minimized before docking.

2.2 Docking

Docking module LibDock using Discovery Studio 2020 was used to study interaction between the Protein and ligand molecules. The binding site of the protein defined and the docking performed. The LibDock scores, nature of bonding and bond length of the docked ligands were estimated.

2.3 Docking with Discovery Studio

In Discovery Studio there are some pre docking steps to perform docking procedures. Open the files of both protein and ligand to dock in DS. For that click on, File open choose the file again open.

2.4 Protein Preparation

Prepare the protein structure before docking because, In general PDB structures contain water molecules, all water molecules are removed except the important ones in protein preparation. Hydrogen atoms will be missing in PDB structure; many docking programs need the protein to have explicit hydrogen. Hydrogen can be added unambiguously except in the case of acid/ basic side chains through protein preparation. The PDB structure can be incorrect in some protein side chains. The crystallographic structure gives electron density, not molecular structure. Click on, Macromolecule then prepare protein then automatic preparation followed by prepare protein give input protein (select the saved protein structure) apply run. Then save the resultant prepared structure in a new file.

2.5 Ligand Preparation

Preparation of ligand is also done because of some reasons; a reasonable 3D structure is needed as starting point. Protonation state and tautomeric form of a particular ligand could influence its hydrogen bonding ability. Small molecule Prepare then alter-ligands then prepare ligand add input ligand (select the saved ligand structure) finally run. The resultant prepared structures of ligands are saved in new file

2.6 Define Binding Site

After the protein and ligand preparation, next step is to define binding site for docking. Receptor ligand interaction then defines & Edit binding site [In Define site, there are 3 options: From Receptor Cavities, From PDB Site Records, and From Current Selection]. Selected the residues (eg: ILE225, ASN226, ILE227, LEU228, SER229, GLU230, PRO231, PRO232, LYS233, ARG234, and LYS235.) Then click on file select from current selection part.

2.7 Docking

Click on Receptor ligand interaction open dock Ligands click LibDock which was used for docking, because a target needs to dock with multiple ligands. After docking each and every poses of dock result are analysed in detail. Then the result was screened based on presence of Hydrogen-bond interaction and Libdock score, and are listed out. The listed ligand poses are screened based on the presence of H-bond interaction at GLU230 residue and the molecular

properties of these ligands are calculated in DS by ADMET descriptors and toxicity prediction. The binding energy of the ligands was also calculated.

3. MATERIALS AND METHODS

3.1 *In silico* Molecular Studies

In silico molecular modeling studies were carried out for different derivatives using different softwares like ACD Lab Chems sketch, Molinspiration, admet SAR and Biovia Discovery Studio 2020. Analysis of Lipinski Rule of Five was carried out for the proposed analogues using Molinspiration software. *In silico* molecular modeling using ACD Lab Chems sketch software was carried out as ACD Lab Chem sketch is a chemically intelligent drawing interface that allows drawing almost all chemical structure including organics, organo metallics, polymers and Markush structures. Use it to produce professional looking structures and diagrams for reports and publications. Determination of drug likeness and Lipinski rule of five using Molinspiration software [6] indicated in Table 1.

Determination of drug likeness is an important aspect of the drug design. These properties mainly electronic distribution, hydrophobicity, molecular size, hydrogen bonding characteristic, flexibility and presence of various pharmacophoric features influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity metabolic stability, toxicity and many others. Drug likeness score is calculated by Mol inspiration software [7] mentioned in Table 2.

The Lipinski Rule of five provides a measure for determining the oral bioavailability of a compound specified in Table 3.

3.2 Data's Computed from Software

Antimicrobial activity was predicted using Discovery Studio 2020 software

3.3 Determination of ADMET Profile Using AdmetSAR

In total, 22 highly predictive qualitative classification models were implemented in admetSAR software. These models includes human intestinal absorption, blood-brain barrier penetration, Caco-2 permeability, P-glycoprotein

substrate and inhibitor, CYP450 substrate and inhibitor (CYP1A2, 2C9, 2D6, 2C19, and 3A4), hERG inhibitors, AMES mutagenicity, carcinogens, fathead minnow toxicity, honey bee toxicity, and tetrahymenapyriformis toxicity. In

addition, all classification models were given a probability output instead of simple binary output. In scientific community of ADMET prediction, quantitative predictions are more useful. The report was summarized in Table 5

Table 1. Details of Lipinski's Rule of Five

Sl.No	Compounds	LogP	MW	HBD	HBA	Lipinski's rule alert index
1	T-IV-A	5.26	367.86	1	3	1
2	T-IV-B	5.05	347.44	1	3	1
3	T-IV-C	4.12	349.14	2	4	0
4	T-IV-D	5.41	412.17	1	3	1
5	T-IV-E	4.70	376.48	1	4	0
6	T-IV-F	4.60	333.14	1	3	0
7	T-IV-G	5.05	367.86	1	3	1
8	T-IV-H	4.33	378.41	1	6	0
9	T-IV-I	4.5	378.41	1	6	0
10	T-IV-J	3.37	323.37	1	4	0

Table 2. Drug likeness analysis of novel analogues

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitors	Enzyme inhibitors
T-IV-A	0.24	0.41	0.24	0.44	0.28	0.21
T-IV-B	0.28	0.47	0.27	0.45	0.30	0.24
T-IV-C	0.20	0.36	0.18	0.28	0.23	0.13
T-IV-D	0.34	0.48	0.27	0.53	0.35	0.25
T-IV-E	0.21	0.39	0.19	0.37	0.24	0.18
T-IV-F	0.25	0.42	0.23	0.43	0.26	0.19
T-IV-G	0.26	0.43	0.35	0.42	0.30	0.26
T-IV-H	0.36	0.43	0.40	0.50	0.44	0.13
T-IV-I	0.36	0.43	0.35	0.48	0.35	0.28
T-IV-J	0.18	0.44	0.30	0.52	0.36	0.17

Table 3. Molecular descriptors of synthesized derivatives

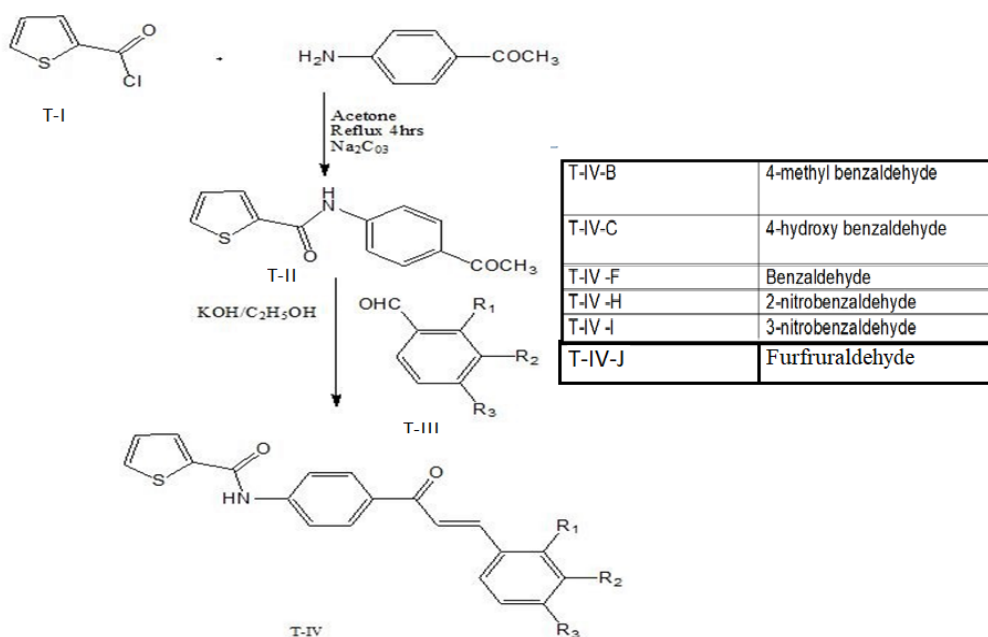
Compound code	MR,cm ³	MV,cm ³	Parachor, cm ³	Surface tension, dynes/cm	Polarizability,cm ³
T-IV-A	104.96±0.3	270.1±3.0	749.6±4.0	59.3±3.0	41.61±0.510-24
T-IV-B	104.89±0.3	274.4±3.0	751.3±4.0	56.1±3.0	41.58±0.510-24
T-IV-C	101.95±0.3	256.6±3.0	728.7±4.0	65.0±3.0	40.41±0.510-24
T-IV-D	107.75±0.3	274.3±3.0	764.2±4.0	60.2±3.0	42.71±0.510-24
T-IV-E	114.38±0.3	296.1±3.0	815.7±4.0	57.5±3.0	45.34±0.510-24
T-IV-F	100.06±0.3	258.1±3.0	713.7±4.0	58.4±3.0	39.67±0.510-24
T-IV-G	104.96±0.3	270.1±3.0	749.6±4.0	59.3±3.0	41.61±0.510-24
T-IV-H	106.61±0.3	270.0±3.0	769.2±4.0	65.8±3.0	42.26±0.510-24
T-IV-I	106.61±0.3	270.0±3.0	796.2±4.0	65.8±3.0	42.26±0.510-24
T-IV-J	92.36±0.3	240.9±3.0	667.7±4.0	58.9±3.0	36.61±0.510-24

Table 4. Data's computed from Discovery Studio 2020 Software

Sl.no Ligands	Targets 1GNW LibDock Score	Substitution of different aldehyde group at T-II
T-IV-A	No Interaction	4-chlorobenzaldehyde
T-IV-B	67.4646	4-methyl benzaldehyde
T-IV-C	99.4459	4-hydroxy benzaldehyde
T-IV-D	No Interaction	4-bromo benzaldehyde
T-IV-E	No Interaction	4-dimethylamino benzaldehyde
T-IV-F	92.2231	Benzaldehyde
T-IV-G	No Interaction	2-chlorobenzaldehyde
T-IV-H	82.7068	2-nitrobenzaldehyde
T-IV-I	108.335	3-nitrobenzaldehyde
T-IV-J	99.4675	Furfuraldehyde

Table 5. ADMET prediction of the ligands

PubChem ID	Solubility	BBB	CYP2D6	Hepatotoxic	Absorption	PBB	A Log P	PSA	Ames Mutagenicity
T-IV-A	0	0	false	true	0	true	0.000	00.00	Non-mutagen
T-IV-B	2	1	false	true	0	true	4.698	47.411	Non-mutagen
T-IV-C	2	2	false	true	0	false	3.97	68.227	Non-mutagen
T-IV-D	2	1	false	true	0	false	4.983	47.411	Non-mutagen
T-IV-E	0	0	false	true	0	false	0/00	00.000	Non-mutagen
T-IV-F	2	1	false	true	0	true	4.212	47.411	Non-mutagen
T-IV-G	0	0	false	true	0	false	0.000	00.000	Non-mutagen
T-IV-H	2	2	false	true	0	true	4.107	90.234	Non-mutagen
T-IV-I	2	2	false	true	0	true	4.107	90.234	Non-mutagen
T-IV-J	2	2	false	true	0	true	3.316	59.966	Non-mutagen



Scheme 1. Synthetic scheme for biologically potent thiophene substituted chalcones according to the docking score

4. EXPERIMENTAL

A mixture of ketone like 4-(acetyl phenyl) thiophene 2-carboxamide and different ortho, meta, para substituted benzaldehyde ((0.01m) and 40% aqueous potassium hydroxide is added to 30 ml of ethanol and was stirred at room temperature for about 2-6 hrs. The resulting products was keep overnight in refrigerator. The solid separated out was filtered, washed with water and recrystallized from ethanol yields pure crystalline products. After drying in an oven at about 70°C yield was 68%. Melting point was found to be 132°C. TLC was checked by n-hexane and chloroform (9:1) ratio as an eluent. All compounds were prepared by same method. Chemical characterization in synthesized derivatives summarized in Table 6.

4.1 N-(4-(3-p-Tolylacryloyl)Phenyl)Thiophene-2-Carboxamide(T-IV-B)

Yield was 74%. IR vales.(cm⁻¹) 3340(N-H Stretch),2880(CH₃ Stretching), 1721(C=O Stretching Ketone),1655(C=O Stretching Amide), 1588(C=C,Stretching,Aromatic),1376(CH₃Stretching),1023(=C-Cl,Stretch),807(CH=CH Stretch),695 (C-S-C, Stretching).

¹H-NMR-δppm:9.15(s,1H,CONH),8.37-7.18 (d,11H,ArH),7.877.85(d,2H,CH=CH),2.03(s,3H,C H₃).¹³C-NMRδppm:139.4ArC,130.3ArC(CH),131.9 ArC(CH)129.0ArC(CH)(C=O)Thiophene,143.7,13 3.5ArC(NC=O)Benzene,132.2,137.6ArC(C=C)Be nzene,122.1,131.4ArC(NC=O)Benzene,128.5,12 8.9ArC(C=C)Benzene,161.8(NAmide),189.7ArC(C=C),145.1,121.3ArC(C=O)Ethylene,121.3ArC (CH₃). M⁺(m/z): 348.44

4.2 N-(4-(3-(4-Hydroxyphenyl)Acryloyl) Phenyl)Thiophene-2Carboxamide (T-IV-C)

Yield was 67%. IR vales. (cm⁻¹) 3487(O-H Stretch), 3357(N-H Stretch), 1711 (C=O Stretch, Ketone),1657(C=O, Stretch Amide), 1587(C=C, Stretch, Aromatic),1042(=C-Cl Stretch),671(C-S-C, Stretch),642(CH=H, Stretch).

¹H-NMR-δppm:9.15(s,1H,CONH),8.33-6.59 (m,11H,Ar-H),7.61,7.60(d,2H,CH=CH),5.35 (s,1H,OH).¹³C-NMR:δppm:139.4,130.3,131.9, 129.0-ArC(C=O)Thiophene,157.7,127.8,115.8, 130.6-ArC(C=C)Benzene,143.7,133.5,122.1, 131.4ArC(NC=O)Benzene,161.8(N-Amide),

189.7-ArC(C=C),145.1,121.3ArC(C=O) Ethylene. M⁺(m/z): 350.41

4.3 N-(4-Cinnamoylphenyl)Thiophene-2-Carboxamide (T-IV-F)

Yield was 65%. IR vales.(cm⁻¹) 3336(N-H Stretching), 1716(C=O Stretching Ketone), 1657 (C=O Stretching Amide).1551(C=C Stretching Aromatic),1022(=C-Cl Stretch), 724(C-H Bending),692(C-S-C, Stretching),657(CH=CH Stretching).

¹H-NMR-δppm:9.18(s,1H,CONH),8.49-7.21 (m,13H,ArH),7.21-6.36(d,2H,CH=CH).¹³C-NMR- δppm:139.4,130.3,131.9,129.0ArC(C=O)Thiophe ,143.7,133.5, 122.1,131.4ArC(N-C=O)Benzene, 161.8(N-Amide),189.7ArC(C=C),145.1,121.3 ArC (C=O)Ethylene,135.2,127.9,128.5,ArC(C=C)Ben- zene. M⁺(m/z):334.14

4.4 N-(4-(3-(2-Nitrophenyl)Acryloyl) Phenyl)Thiophene-2-Carboxamide (T-IV-H)

Yield was 63%. IR vales.(cm⁻¹)3372(N-H Stretch),1723(C=O Stretch Ketone),1652 (C=O, Stretch,Amide),1526(C=C, Stretching, Aromatic), 1365-1391(NO₂,Stretch),1172(=C-ClStretch)931, (CH=CH, Stretching),789(C-S-C, Stretching).

¹H-NMR-δppm: 9.15(s,1H, CONH), 8.30-7.27 (m,11H,ArH),7.87-7.85(d,2H,CH=CH).¹³C-NMR- δppm:139.4,130.3,131.9,129.0ArC(C=O) Thiophene,147.7,127.3,123.8,128.8,134.7ArC(N (=O)=O,(C=C)Benzene,143.7,133.5,122.1,131.4 ArC(C=O)Benzene,161.8(N-Amide),189.7-ArC (C=C)145.1,121.3ArC(C=O)Ethylene,135.2, 127.9,128.6ArC(C=C)Benzene.M⁺(m/z):379.41

4.5 N-(4-(3-(3-Nitrophenyl)Acryloyl) Phenyl)Thiophene-2-Carboxamide (T-IV-I)

Yield was 69%. IR vales.(cm⁻¹) 3380(N-H Stretching),721(C=O Stretching Ketone), 1678 (C=O Stretching Amide),1604(C=C Stretch), 1380(NO₂ Stretching),1015(=C-Cl, Stretching), 975(CH=CH Stretching). 794(C-S-C, Stretching).

¹H-NMR-δppm:9.15(s,1H,CONH),8.31-7.23(m, 11H, ArH),8.17-7.84(d, 2H, CH=CH).¹³C-NMR-δppm:139.4,130.3,131.9.0ArC(C=O) Thiophene,147.7,127.3,123.8,128.8,134.7-ArC (N(=O)=O,(C=C)Benzene,143.7,133.5,122.1 ArC (N-C=O),(C=O)Benzene,161.8(N-Amide)145.1, 121.3ArC(C=O)Ethylene. M⁺(m/z): 379.41

4.6 N-(4-(3-(Furan-3-yl)Acryloyl)Phenyl) Thiophene-2-Carboxamide(T-IV-J)

Yield, (70%).IR-vales.(cm⁻¹)3310(N-H, Stretch), 1713(C=O, Stretch, Ketone), 1679 (C=O Stretch Amide),1548(C=C Stretch Aromatic), 1170(C-O-C Stretching, Aromatic),1015(=C-Cl, Stretching), 826 (CH=CH Stretch), 628(C-S-C, Stretching).

¹H-NMR-δppm:9.15-(s,1H,CONH),8.31-7.12 (m,11H,ArH),7.59-7.13(d,2H,CH=CH).¹³C-NMR-δppm:139.4,ArC,130.3(CH),131.9(CH),129.0(CH),ArC(C=O)Thiophene,139.0,143.0,124.4,108.2-ArC(C=C)Furan,143.7,133.5,122.1,131.4,122.1,128.5-ArC(N-C=O)Benzene,161.8(NAmide),189.7 (C=C),145.1,127.3ArC(C=O)Ethylene.M⁺(m/z): 324.37

5. RESULTS

5.1 Docking Study of Antibacterial Activity

5.1.1 Docking with Glutathione S-Transferase(PDB ID:1GNW)

The three-dimensional structure of Glutathione S-Transferase was downloaded from PDB database with PDB ID: 1GNW with crystallographic resolution 2.20 Å⁰ (Fig. 1). The protein chain consists of two polypeptide chain A and B with total 211 amino acids and has a molecular weight of 47860.6 Daltons. The active site of protein interacting with the standardised ligand molecules was selected as the binding site.

Table 6. Characterization of synthesized compounds

Sl.No	Compounds	Molecular formula	Mol.wt	M.P. ^o C	% Yield	Rf Value
1	T-IV-B	C ₂₁ H ₁₇ NO ₂ S	347.45	151	65	0.44
2	T-IV-C	C ₂₀ H ₁₅ O ₃ SN	349.42	153	59	0.36
3	T-IV -F	C ₂₀ H ₁₅ O ₂ NS	333.15	147	71	0.38
4	T-IV -H	C ₂₀ H ₁₄ O ₄ N ₂ S	378.43	159	58	0.33
5	T-IV -I	C ₂₀ H ₁₄ O ₄ N ₂ S	378.43	159	66	0.30
16	T-IV-J	C ₁₈ H ₁₃ O ₃ NS	323.38	143	64	0.29

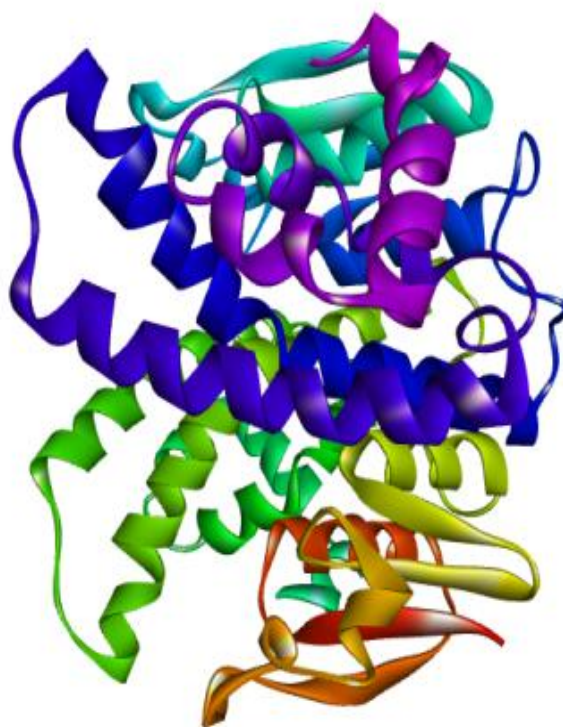


Fig. 1. Crystallographic structure of target from PDB database

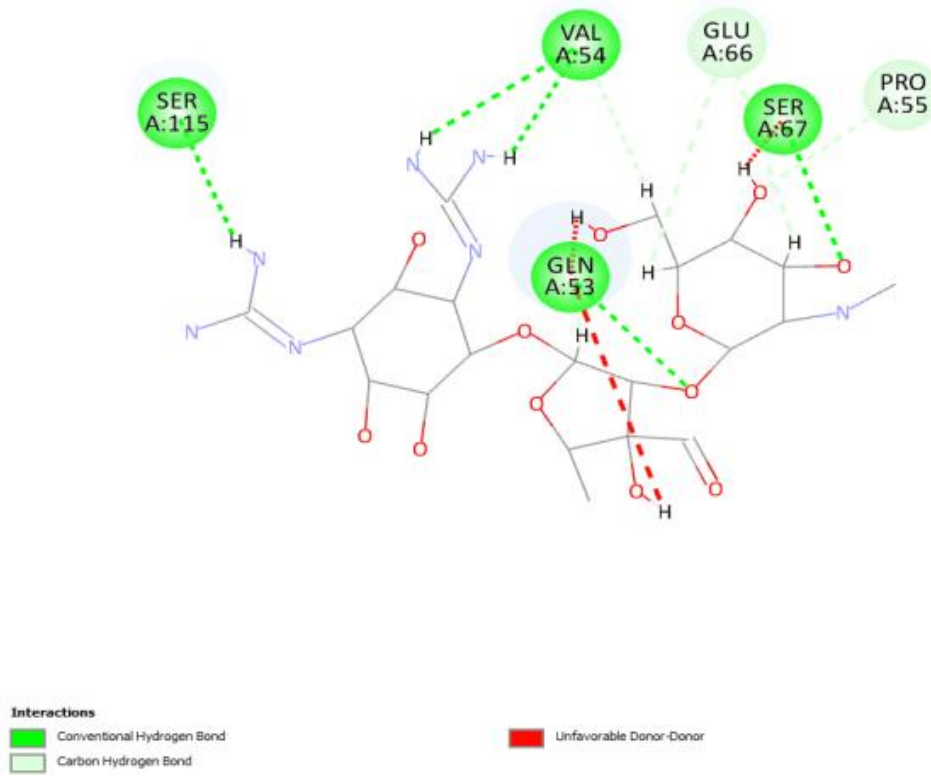


Fig. 2. Interactions between target and ligand Steptomycin

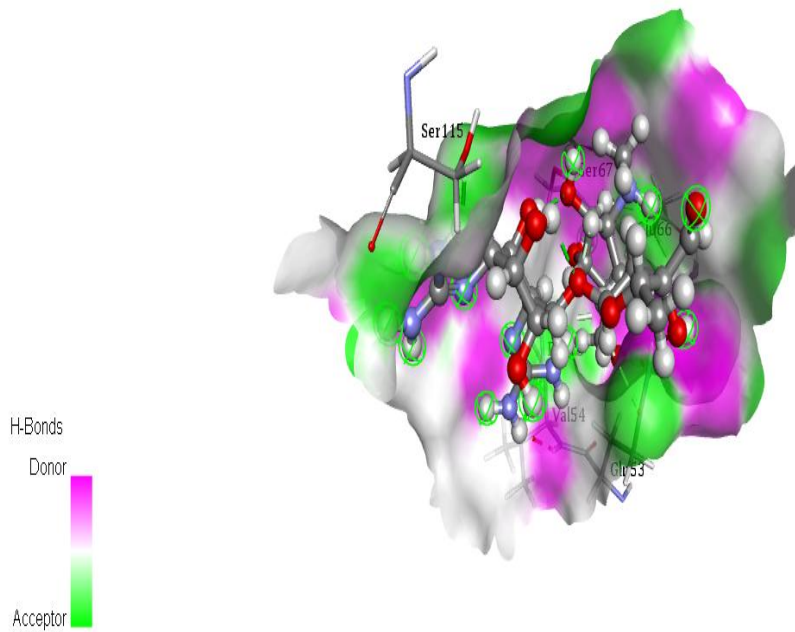


Fig. 3. Docking image of Steptomycinwith 1GNW

Table 7. Interactions between target and ligand Streptomycin

Ligand	LibDock Score	Interacting Residue	Bond Distance	Nature of Bonding
Streptomycin (Pubchem ID: 19649)	137.09	A:GLN53:HE21 - 19649:O3	2.0144	Hydrogen Bond
		A:SER67:HG - 19649:O9	2.01601	Hydrogen Bond
		19649:H43 - 19649:O9	2.26087	Hydrogen Bond
		19649:H44 - 19649:O1	2.31986	Hydrogen Bond
		19649:H44 - 19649:O7	2.33702	Hydrogen Bond
		19649:H50 - A:VAL54:O	2.74177	Hydrogen Bond
		19649:H52 - A:VAL54:O	2.17354	Hydrogen Bond
		19649:H53 - A:SER115:OG	1.95762	Hydrogen Bond
		A:PRO55:HD1 - 19649:O10	1.96641	Hydrogen Bond
		19649:H59 - A:GLN53:OE1	2.41303	Hydrogen Bond
		19649:H61 - 19649:O2	2.11697	Hydrogen Bond
		19649:H67 - 19649:O1	2.3044	Hydrogen Bond
		19649:H68 - A:GLU66:OE2	2.33391	Hydrogen Bond
		19649:H70 - A:GLU66:OE2	2.93707	Hydrogen Bond
		19649:H75 - A:VAL54:O	2.08244	Hydrogen Bond

Table 8. Interactions between target and ligand T-IV-C

Ligand	LibDock Score	Interacting Residue	Bond Distance	Nature of Bonding
T-IV-C	99.4459	A:ASN49:HD22 - T-IV-C:O18	1.87007	Hydrogen Bond
		A:ARG68:HH12 - T-IV-C:O11	1.76843	Hydrogen Bond
		A:ARG68:HH22 - T-IV-C:O11	2.67981	Hydrogen Bond
		A:GLU66:OE2 - T-IV-C	3.47069	Electrostatic
		A:ILE12:HN - T-IV-C	2.74928	Hydrogen Bond
		A:SER11:HA - T-IV-C	2.41645	Hydrophobic
		A:GLU66:HA - T-IV-C	2.71497	Hydrophobic
		T-IV-C - A:ILE12	4.88906	Hydrophobic
		T-IV-C - A:ALA13	3.67637	Hydrophobic
		T-IV-C - A:ILE12	4.97354	Hydrophobic

Table 9. Interactions between target and ligand T-IV-F

Ligand	LibDock Score	Interacting Residue	Bond Distance	Nature of Bonding
T-IV-F	92.2231	A:ILE12:HN - T-IV-F:S24	1.92784	Hydrogen Bond
		A:ARG68:HH12 - T-IV-F:O11	2.05865	Hydrogen Bond
		T-IV-F - A:ILE12	5.45599	Hydrophobic
		T-IV-F - A:ALA13	3.30132	Hydrophobic
		T-IV-F - A:PRO55	5.44559	Hydrophobic
		T-IV-F - A:ILE12	5.20394	Hydrophobic

Table 10. Interactions between target and ligand T-IV-I

Ligand	LibDock Score	Interacting Residue	Bond Distance	Nature of Bonding
T-IV-I	108.335	T-IV-I:N20 - A:GLU66:OE2	4.18342	Electrostatic
		A:ASN49:HD22 - T-IV-I:O22	2.15575	Hydrogen Bond
		A:GLN53:HE21 - T-IV-I:O21	2.48605	Hydrogen Bond
		A:ARG68:HH12 - T-IV-I:O11	1.87358	Hydrogen Bond
		B:GLN109:HE21 - T-IV-I:O21	2.1217	Hydrogen Bond
		A:GLU66:OE2 - T-IV-I	3.60154	Electrostatic
		A:ILE12:HN - T-IV-I	2.59346	Hydrogen Bond
		A:ALA13:HB2 - T-IV-I	2.29107	Hydrophobic
		T-IV-I - A:ILE12	5.42287	Hydrophobic
		T-IV-I - A:ILE12	4.74983	Hydrophobic

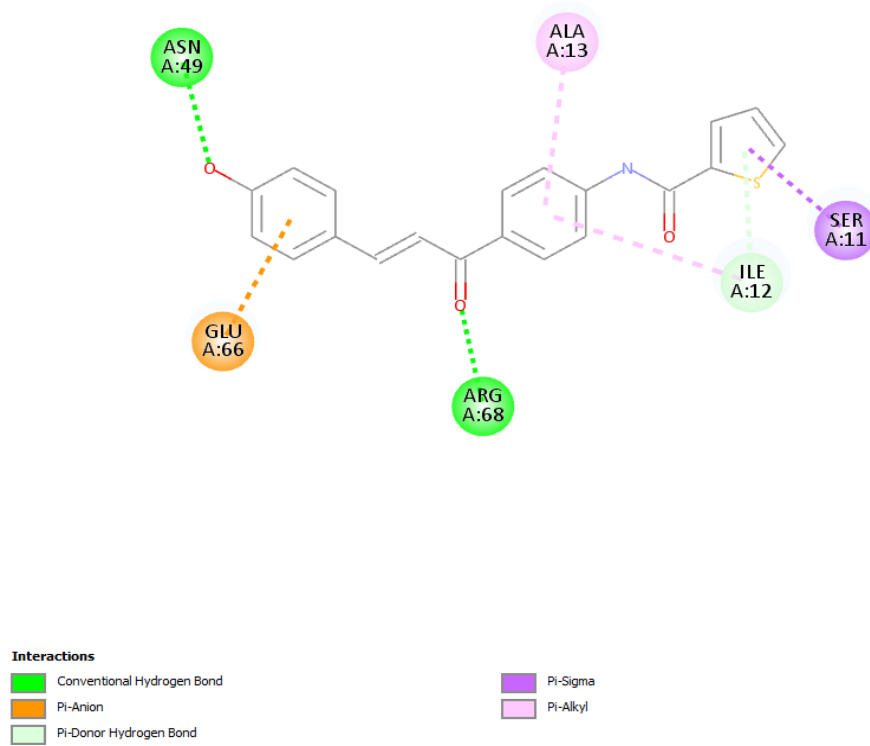


Fig.4. Interactions between target and ligand T-IV-C

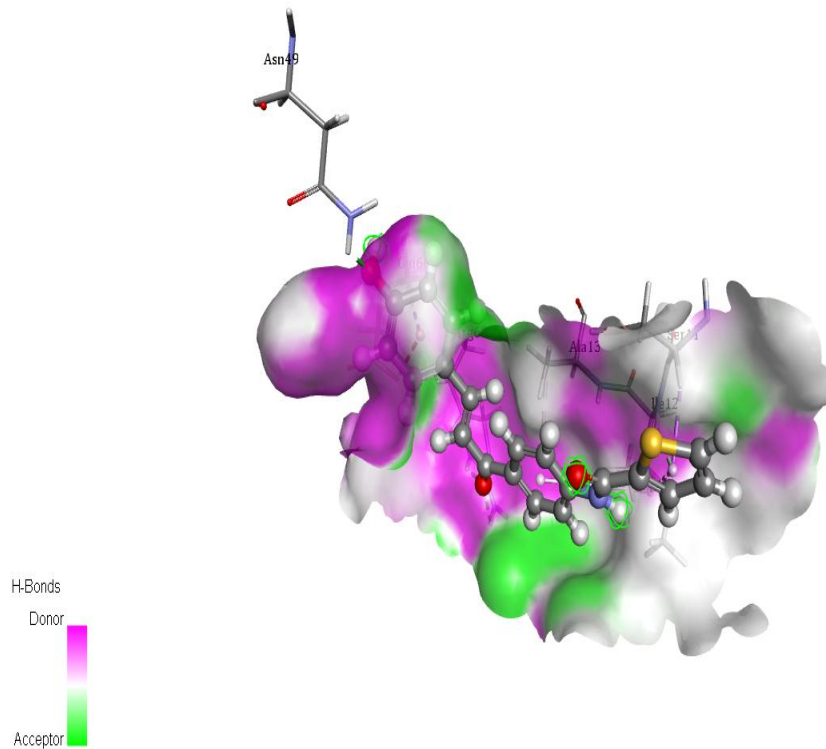


Fig.5. Docking image of T-IV-C with 1GNW

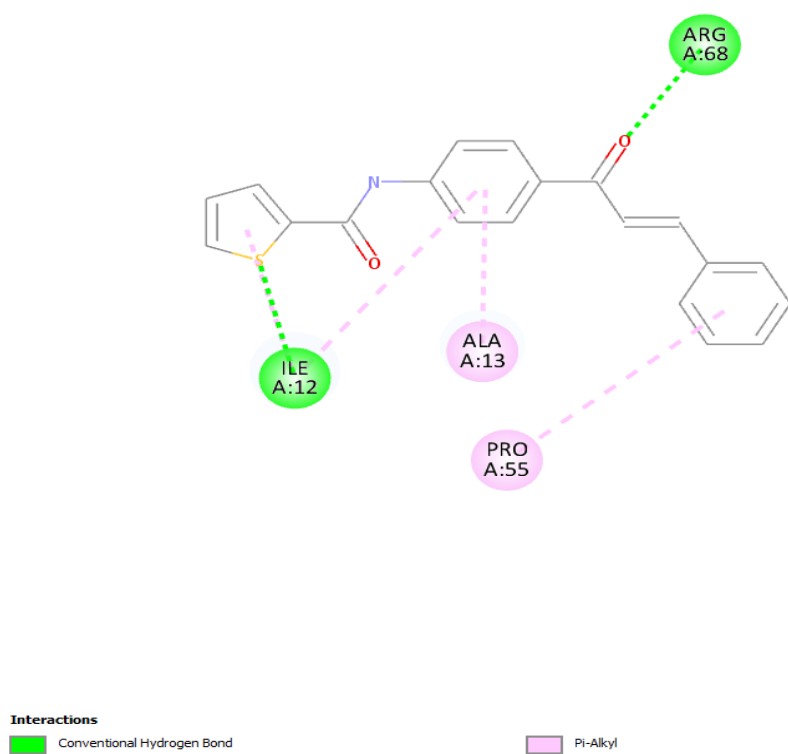


Fig.6. Interactions between target and ligand T-IV-F

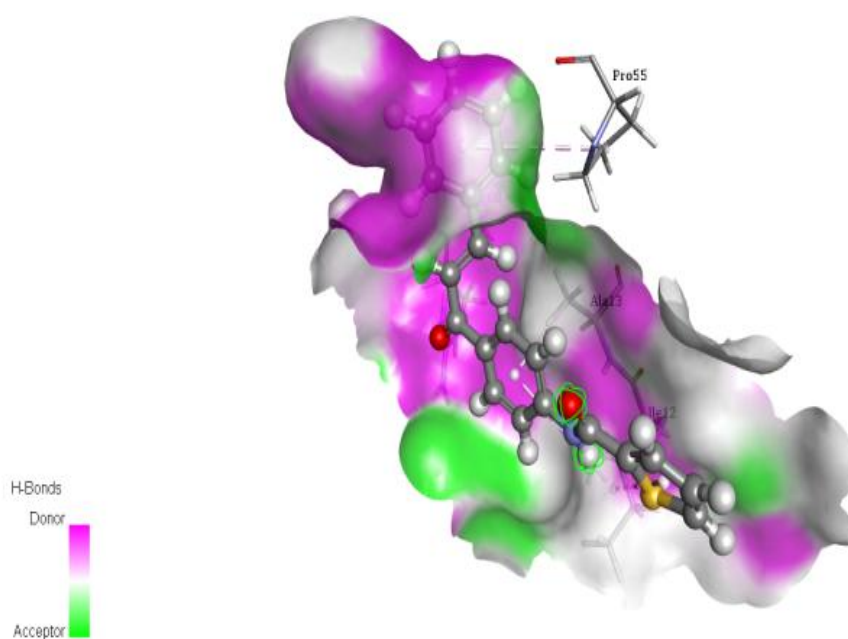


Fig. 7. Docking image of T-IV-F with 1GNW

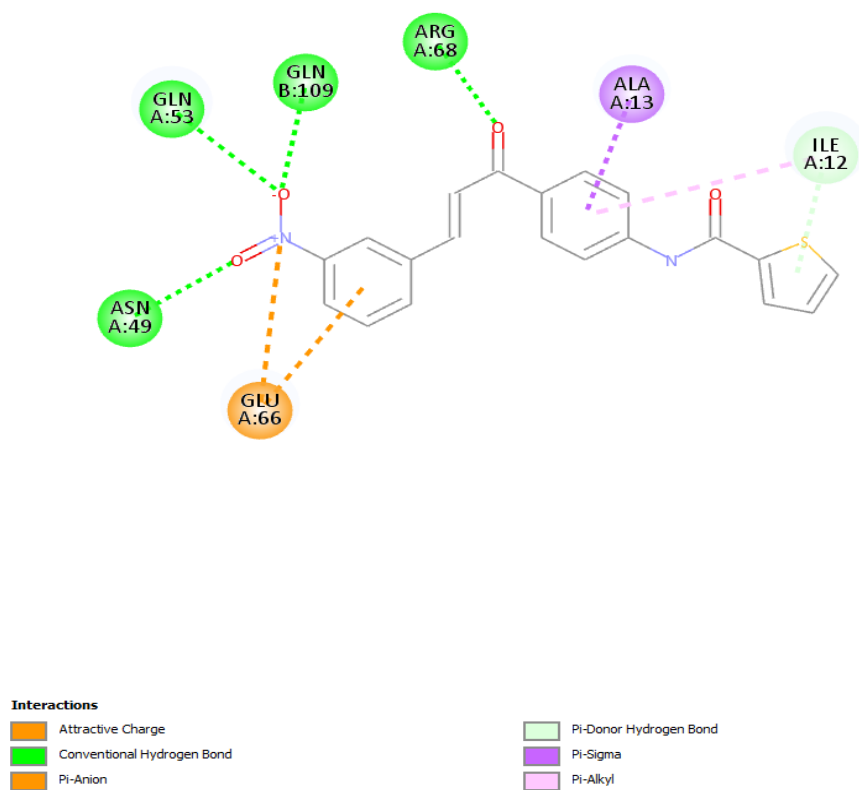


Fig. 8. Interactions between target and ligand T-IV-I

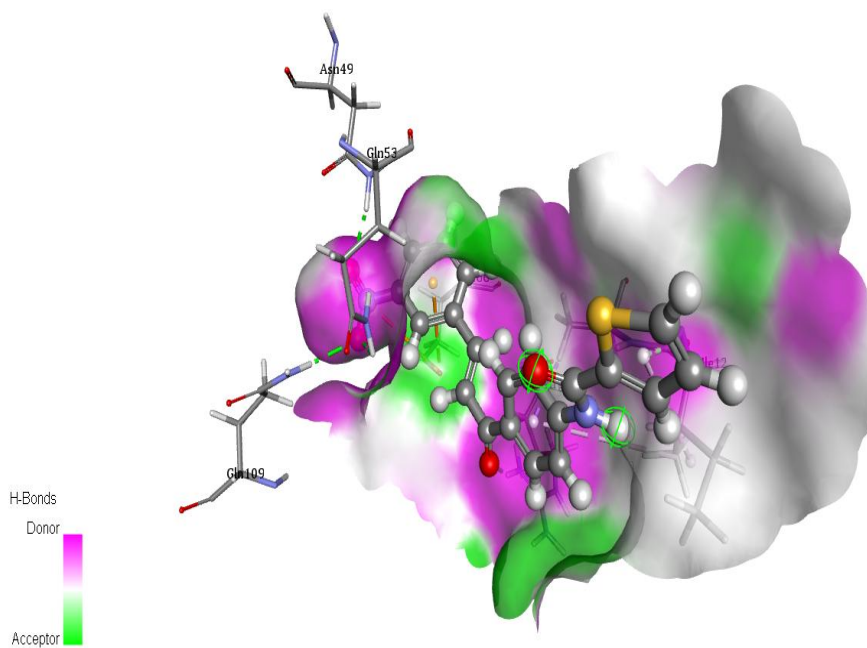


Fig.9. Docking image of T-IV-I with 1GNW

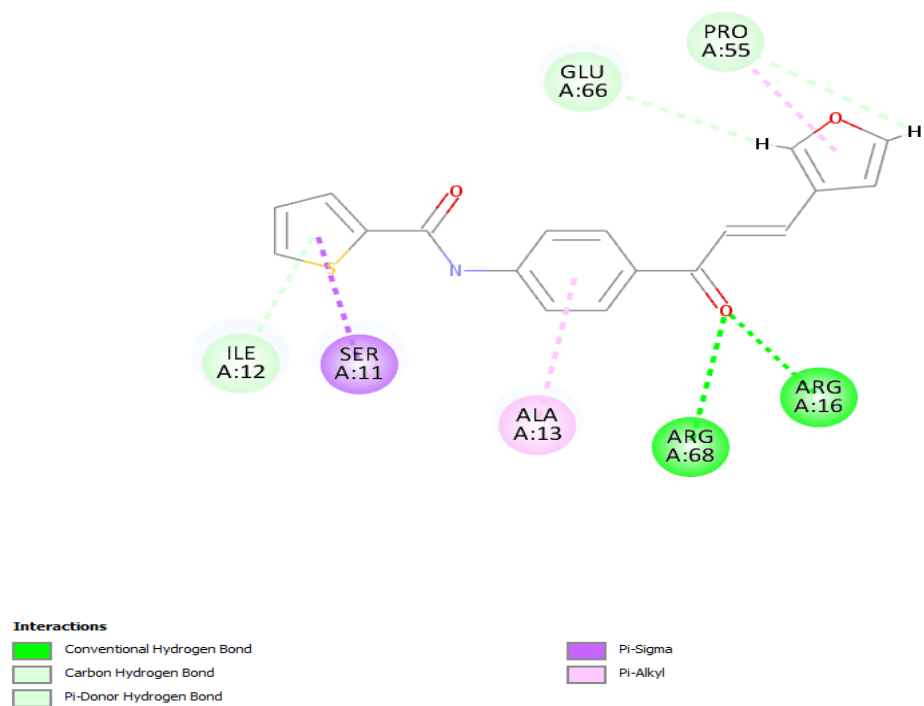


Fig. 10. Interactions between target and ligand T-IV-J

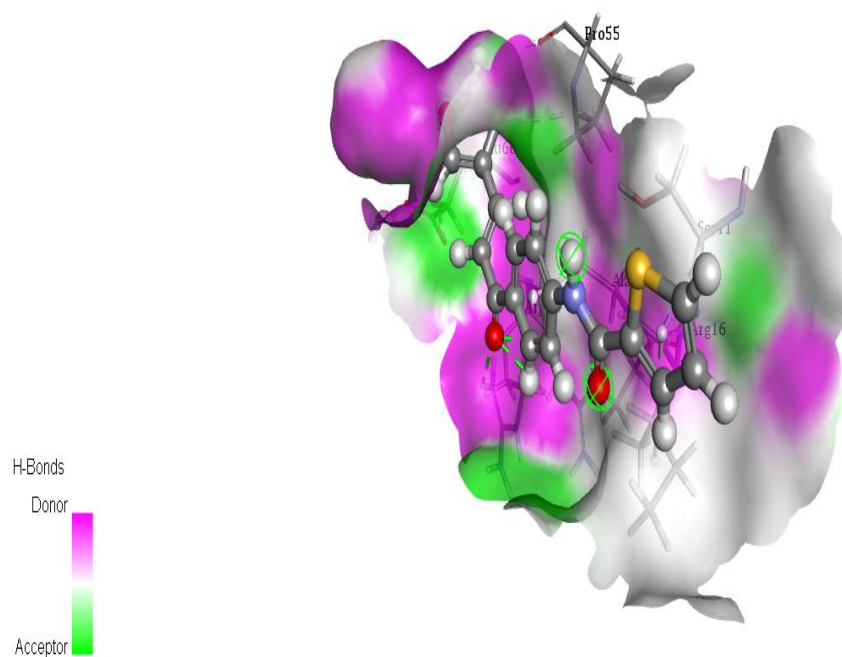
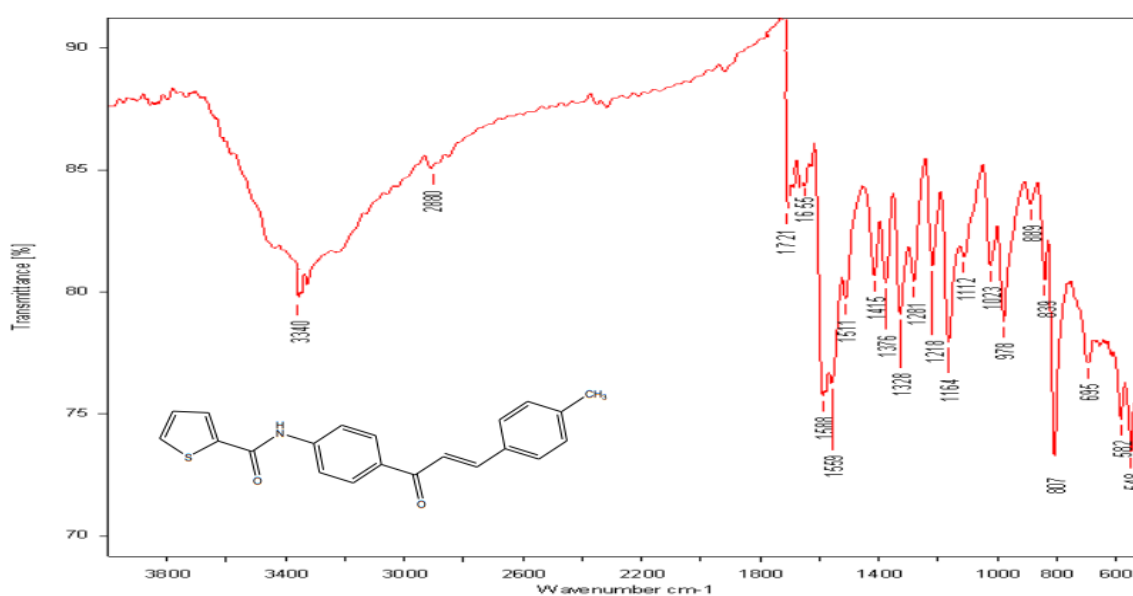


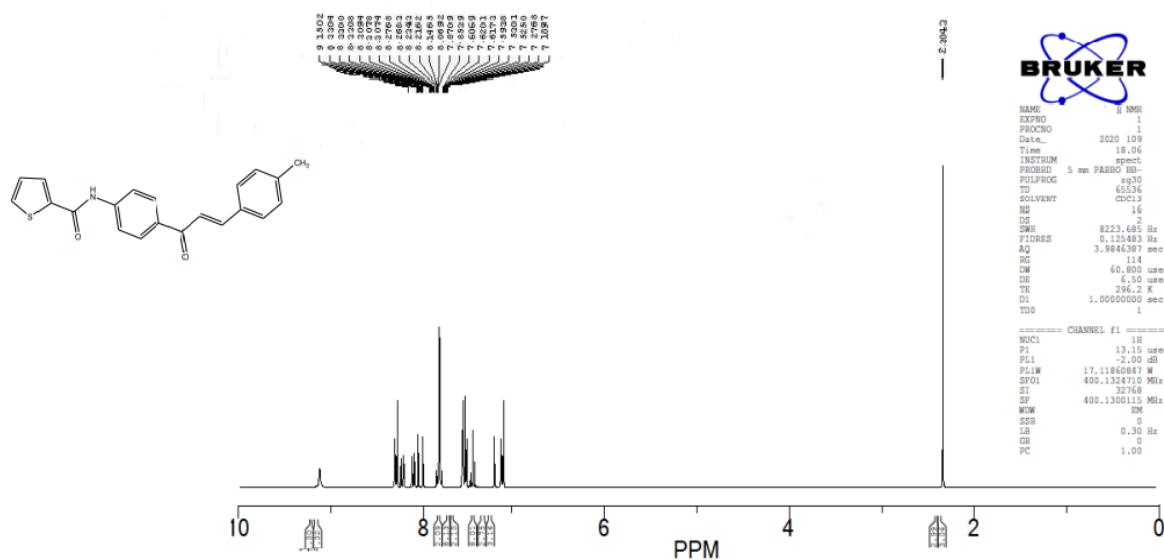
Fig. 11. Docking image of T-IV-J with 1GNW

Table 11. Interactions between target and ligand T-IV-J

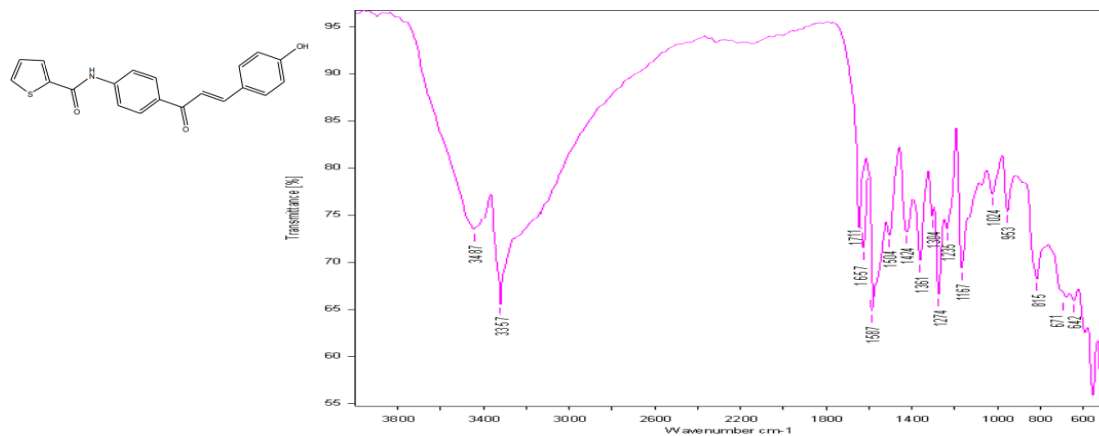
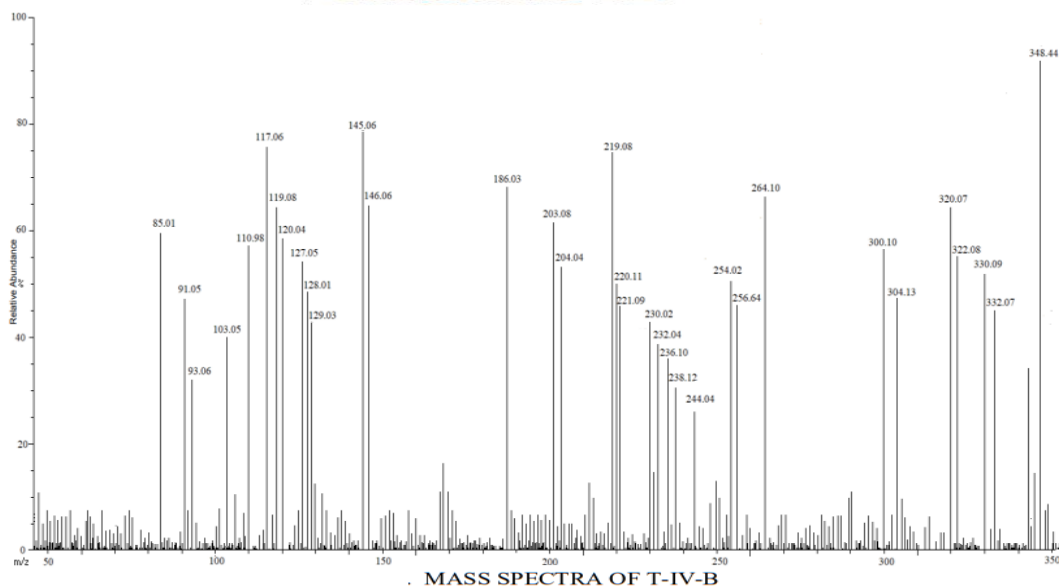
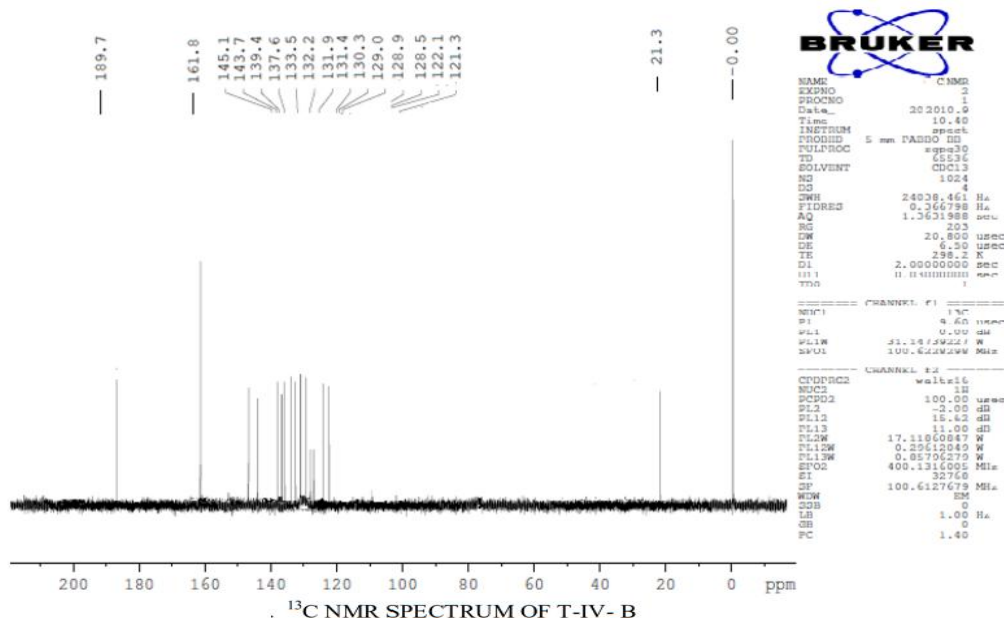
Ligand	LibDock Score	Interacting Residue	Bond Distance	Nature of Bonding
T-IV-J	99.4675	A:ARG16:HH21 - T-IV-J:O11	2.89866	Hydrogen Bond
		A:ARG16:HH22 - T-IV-J:O11	2.79546	Hydrogen Bond
		A:ARG68:HH12 - T-IV-J:O11	2.41164	Hydrogen Bond
		T-IV-J:H32 - A:PRO55:O	2.75122	Hydrogen Bond
		T-IV-J:H33 - A:GLU66:OE2	2.6823	Hydrogen Bond
		A:ILE12:HN - T-IV-J	2.87628	Hydrogen Bond
		A:SER11:HA - T-IV-J	2.50115	Hydrophobic
		T-IV-J - A:ALA13	3.1151	Hydrophobic
		T-IV-J - A:PRO55	5.40317	Hydrophobic
		T-IV-J - A:ILE12	5.05287	Hydrophobic



IR SPECTRA OF T-IV-B

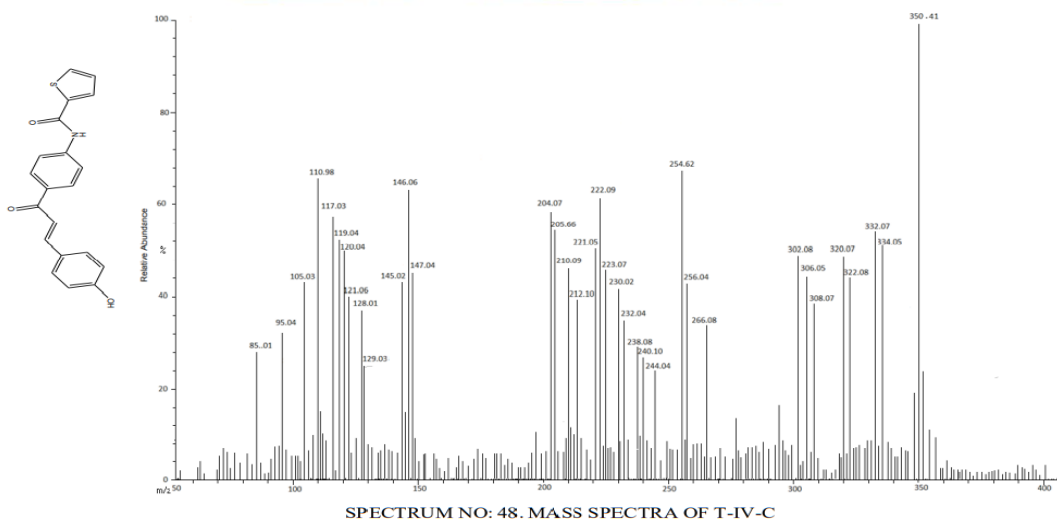
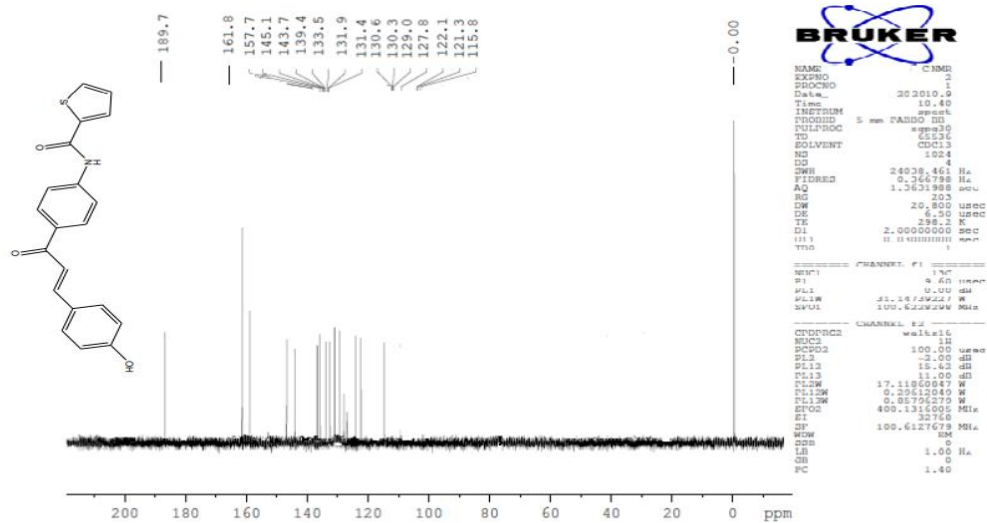
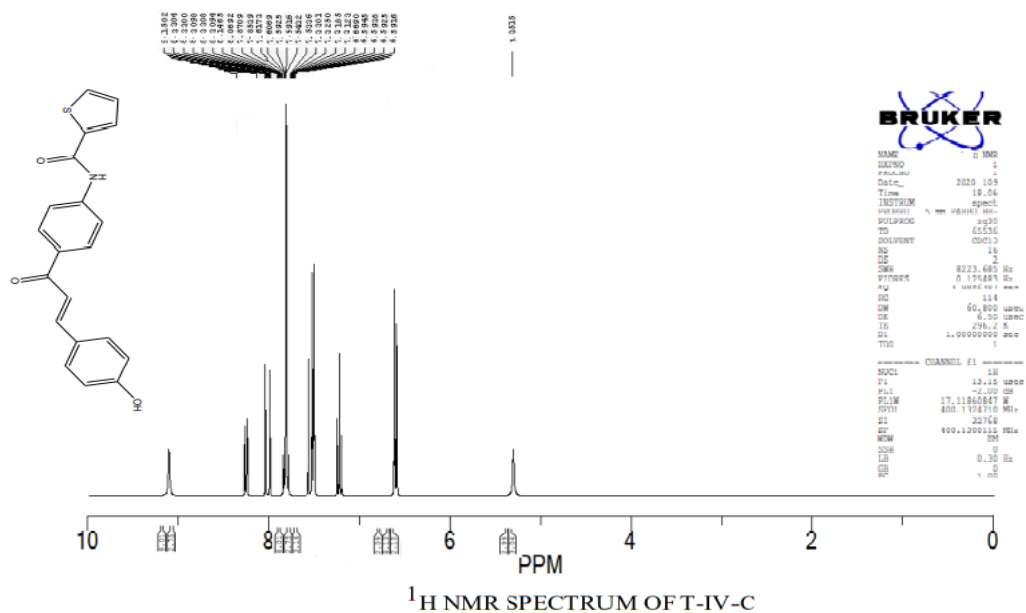


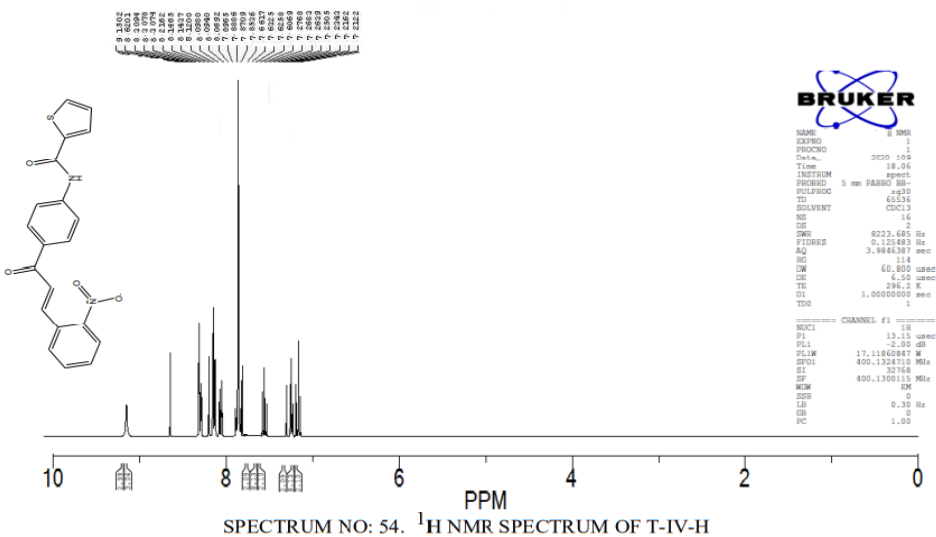
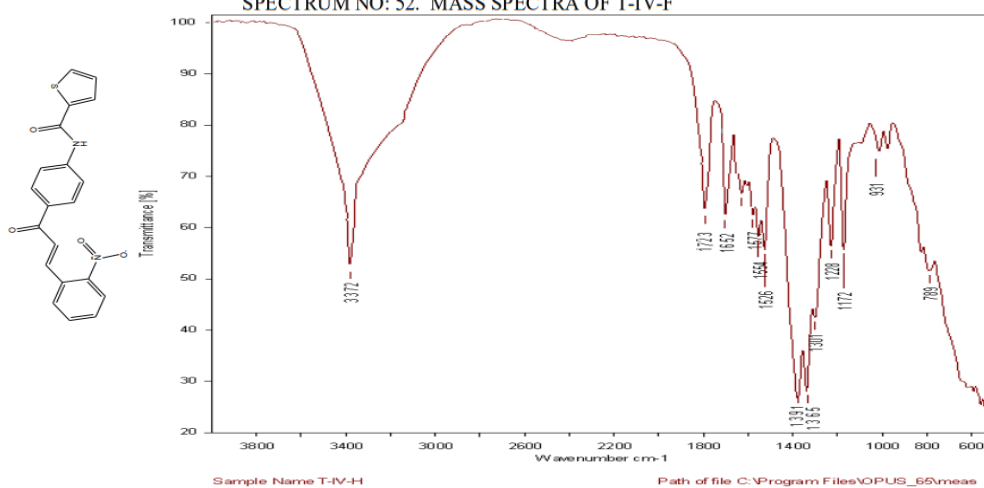
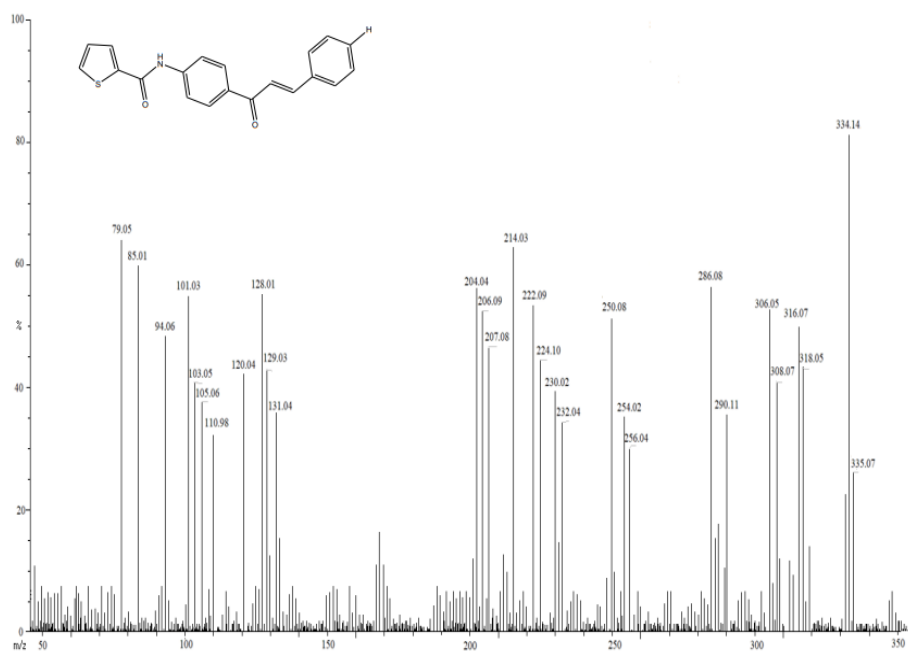
¹H NMR SPECTRUM OF T-IV-B

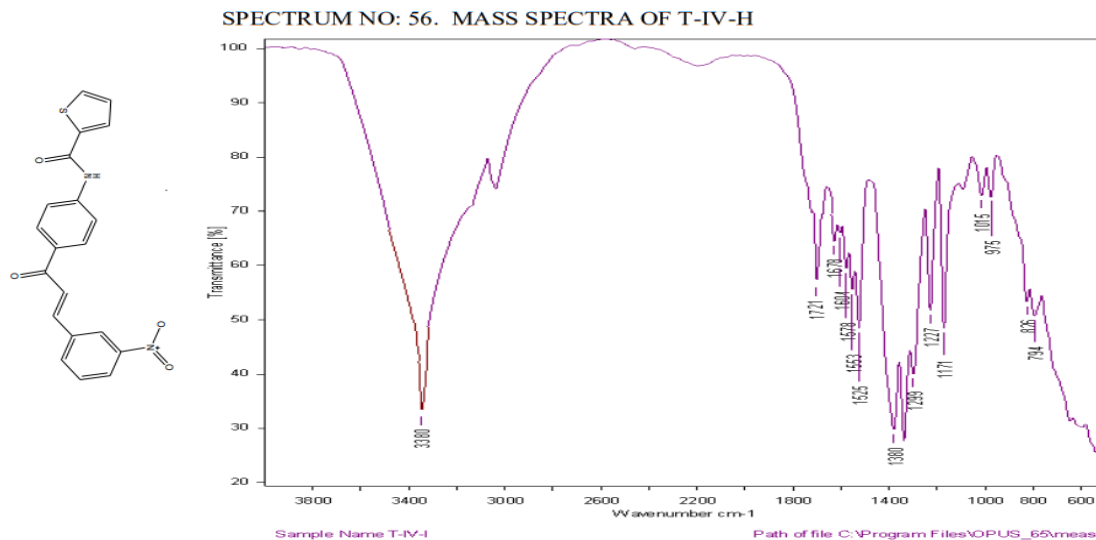
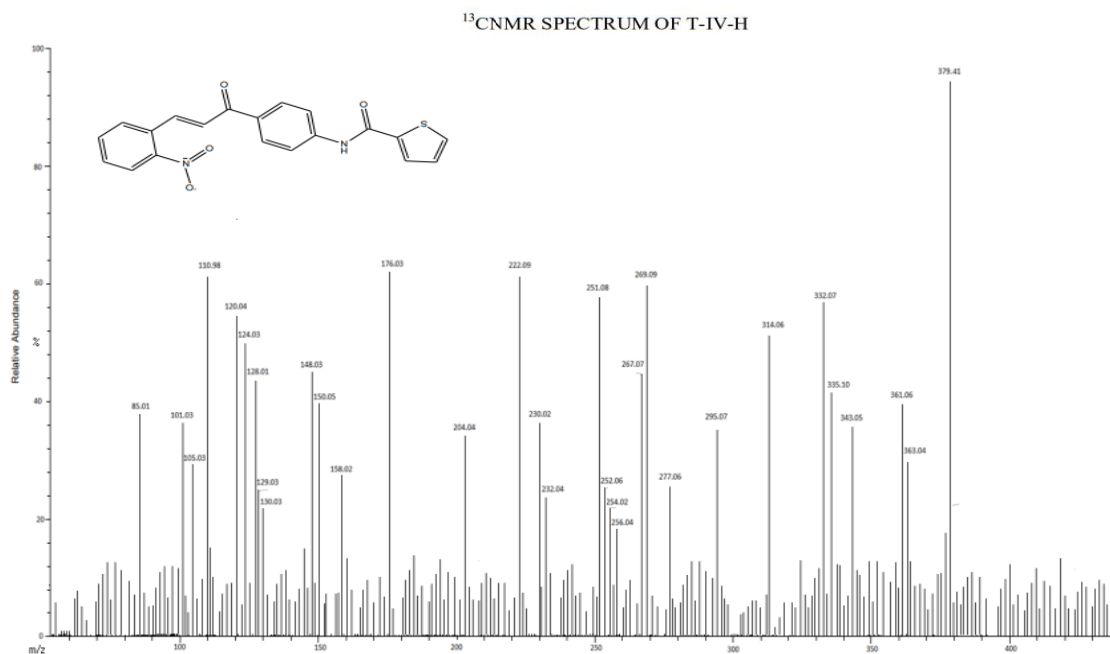
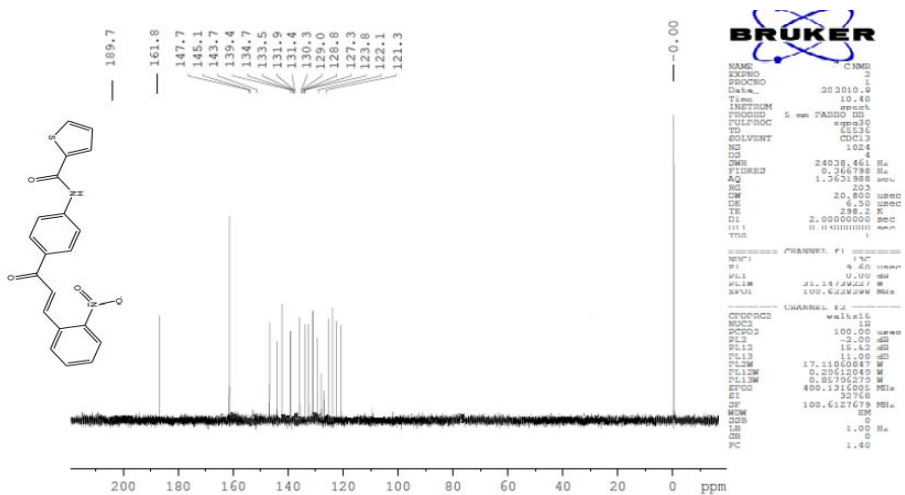


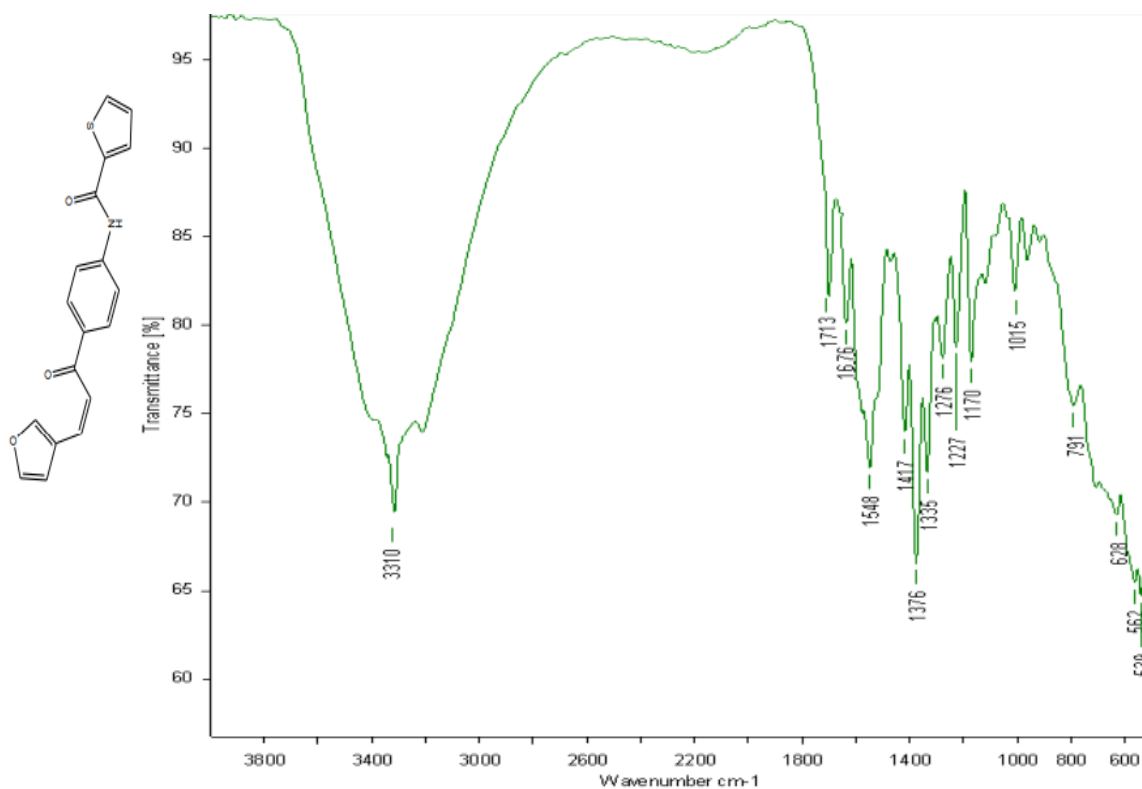
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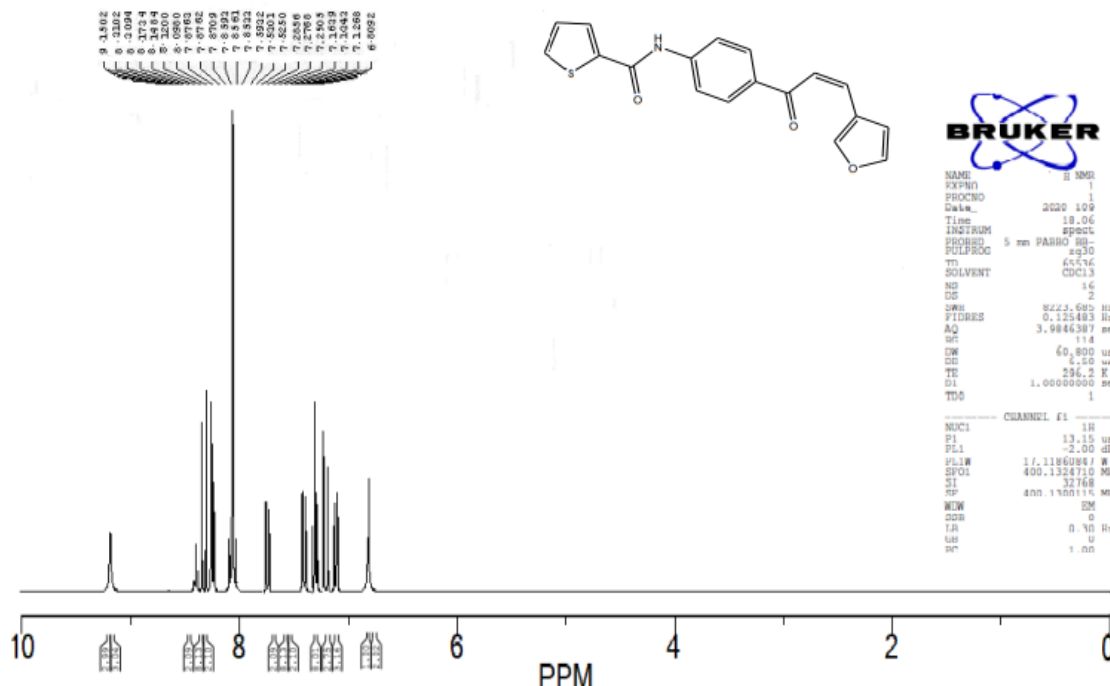




Sample Name T-IV-J

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IR SPECTRA OF T-IV-J



BRUKER

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PROCNO: 1
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PULPROG: zgpg30
TD: 65536
SOLVENT: CDCl3
NS: 16
DS: 2
SWH: 8223.680 Hz
FIDRES: 0.125483 Hz
AQ: 3.9844387 sec
RG: 114
EW: 60.800 usec
CE: 4.50 usec
TE: 296.2 K
D1: 1.00000000 sec
TD0: 1

===== CHANNEL f1 =====
NUC1: 1H
P1: 13.15 usec
PL1: -2.00 dB
SFO1: 400.1324710 MHz
SI: 32768
SF: 400.1304110 MHz
WDW: EM
SSB: 0
LR: 0.10 Hz
GB: 0
PC: 1.00
    
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¹H NMR SPECTRUM OF T-IV-J

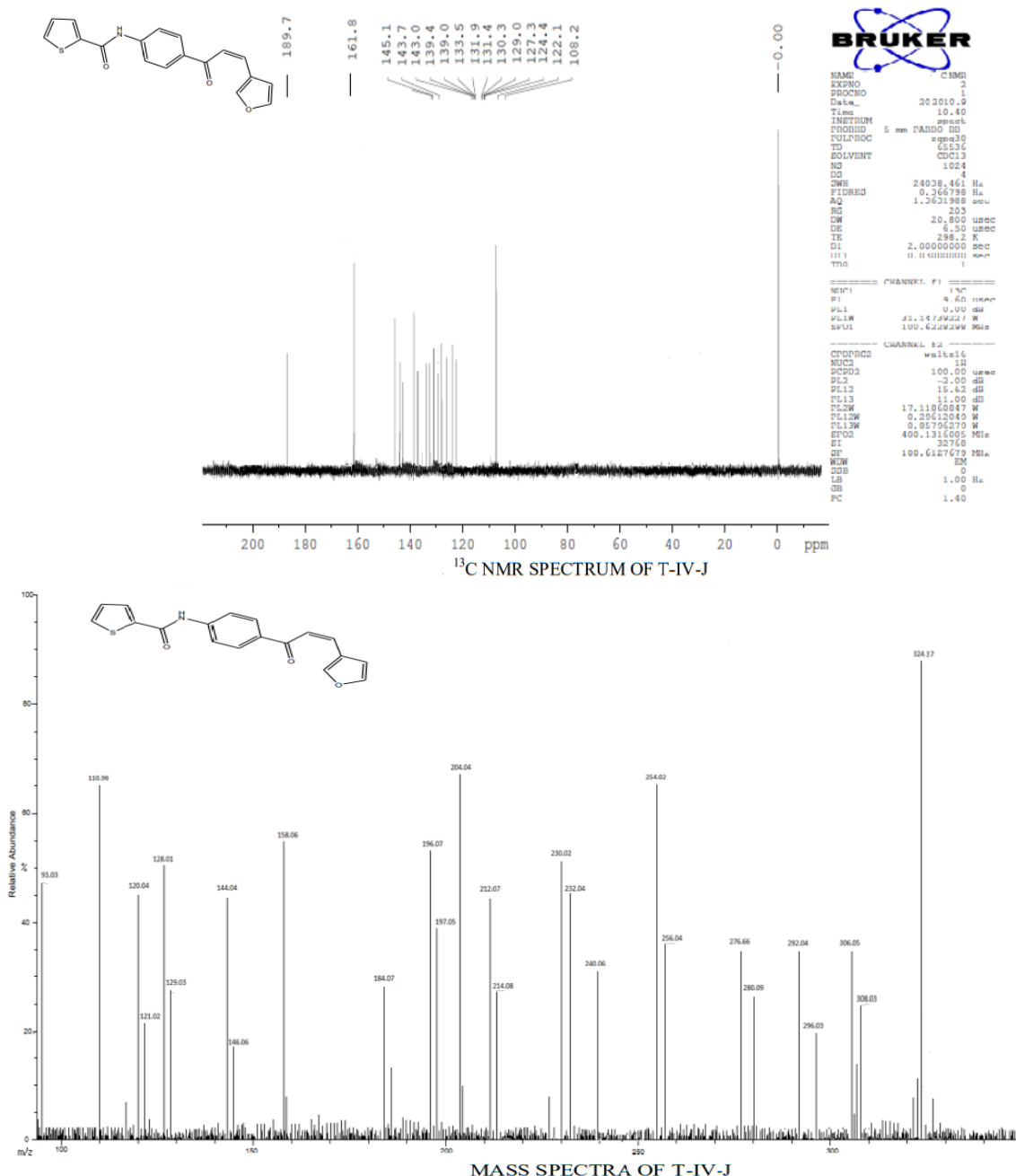


Fig. 12. Spectral images of all synthesized compounds

The result showed that T-IV-I have high binding affinity to target compared to other ligands. All the docked complexes were analysed to study non-bond interactions between the target and the ligand molecule. The results are summarized in the Table 10. The results revealed that all the ligands bind the same active site.

5.2 *In vitro* Antibacterial Activity

The selected synthesized thiophene derivatives of the present investigation were screened for

their anti-bacterial activity by subjecting the compounds to standard procedures [8]. The antibacterial activity was performed by cup and plate method (diffusion technique). The fresh culture of bacteria was obtained by inoculating bacteria in nutrient broth media and incubated at $37 \pm 2^\circ\text{C}$ for 18 – 24 hrs. This fresh culture was mixed with nutrient agar media and poured into sterile petri-plates by pour plate method, by following aseptic technique [9]. After solidification of the media, six bores were made at equal distance by using sterile steel cork borer (8 mm

diameter). Into this cup 100µg/ml, 200µg/ml concentration solution of standard drug and synthesized compounds were introduced. Dimethyl formamide [10] was used as a control. After introduction of standard drugs and synthesized compounds, the plates were placed for proper diffusion of drug into the media for about 2 hrs. After 2hrs. The plates were incubated in BOD incubator and maintained at $37 \pm 0.5^\circ \text{C}$ for 18-24 hrs. After the incubation period, the plates were observed for zone of inhibition [11] by using Hi-antibiotic zone reader. Results were evaluated by comparing the zone of inhibition shown by synthesized compounds with the standard drug. [12] the results were the average value of zone of inhibition measured in millimeter of three sets. The standard drug was dissolved in distilled water and the synthesized compounds were dissolved [13] in minimum quantity of DMF and diluted with water to get desired concentrations [14]. All data's were summarized in Table 12.

The Procaine penicillin, was used as standard drug against *Staphylococcus aureus* and *Streptococcus aureus* (gram+^{ve}) and Streptomycin was used against *Escherichia coli* and *P.aeruginosa* [15] (gram-^{ve} organism).

6. DISCUSSION

The present study can be summarized as the designing of novel thiophene substituted chalcones as Glutathione-S-Transferase (PDB ID: 1GNW) selective inhibitors and analysis of the compounds through ADMET filters and molecular docking studies. From a library of designed compounds were chosen which had binding energy more could serve as lead compound for the development of newer potent anti-bacterial agents. *In vitro* study of synthesized compounds T-IV B and T-IV-H shows good antibacterial activity against *Streptococcus aureus* compared to docking result because docking is a hypothetical methodology. Sometimes experimental result may change with docking report.

Table 12. Anti-bacterial activity of synthesized compounds various strains of gram Positive and gram negative organisms in different concentration

Compounds	%Zone of inhibition in mm (200 µg/ml)			
	<i>Streptococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
T-IV-B	15	12	11	11
T-IV-C	13	13	15	14
T-IV-F	11	12	13	12
T-IV-H	14	13	11	12
T-IV-I	15	10	14	15
T-IV-J	12	13	15	14
Procaine penicillin,	18	19	-	-
Streptomycin	-	-	19	20
Control(DMF)	00	00	00	00

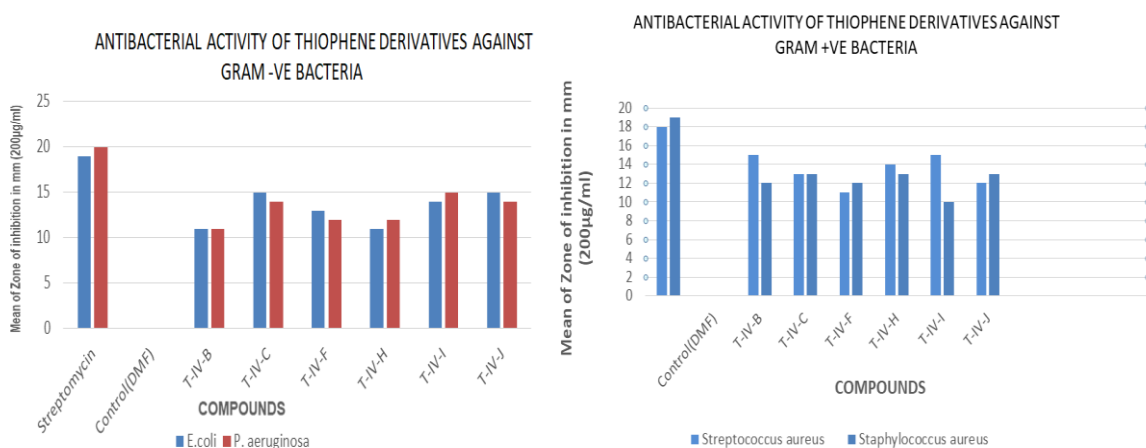


Fig. 13. Antimicrobial activity of thiophene derivatives against different microorganisms

7. CONCLUSION

Among the synthesized thiophene substituted chalcones T-IV-I and thiophene T-IV-B displayed remarkable antimicrobial activity against *Streptococcus aureus*. Compounds T-IV-J, T-IV-H and T-IV-C shows better activity against *Staphylococcus aureus*. Moreover T-IV-C and T-IV-J exhibits good antibacterial activity against *E.coli* and *Pseudomonas aeruginosa*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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