

Novel Treatment for Cancer by Dual inhibitors of PARP1 and STAT3 enzymes

Submitted In Partial Fulfilment Of The Requirement For The Award Of

Bachelor of Pharmacy

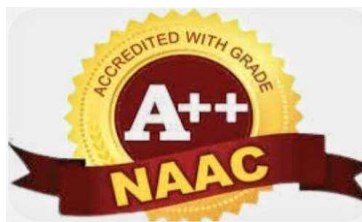
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Sathyabama Institute of Science & Technology

(Institution Deemed to Be University-U/S 3 Of The UGC Act, 1956)

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INSTITUTE OF SCIENCE AND TECHNOLOGY
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SCHOOL OF PHARMACY

Declaration

We Hereby Declare That The Work Presented In The Thesis Entitled “**Novel Treatment for Cancer by Dual inhibitors of PARP1 and STAT3 enzymes**”, Submitted By Us To The Sathyabama Institute Of Science And Technology Chennai – 600119, For The Award Of Bachelor Of Pharmacy (2020-2024) By Research Is An Original Record Of Research Work Carried Out By Us Under The Supervision Of “**Dr Kaviarasan .L, Associate Professor, School Of Pharmacy**”. The Contents Of This Dissertation, In Full Or In Parts, Have Not Been Submitted To Any Other Institute Or University For The Award Of Any Degree, Diploma, Membership, Fellowship, Associate-Ship Etc.. In Keeping With The General Practicing Reporting Scientific Observation, Due Acknowledgement Has Been Made Whenever The Work Described Is Based On The Findings Of Other Investigators.

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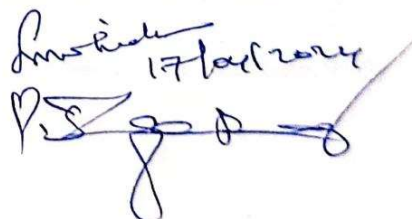
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TABLE OF CONTENTS

S.NO	TITLE	PAGE.NO
	BONAFIDE CERTIFICATE	
	DECLARATION	
	ACKNOWLEDGEMENTS	
	ABSTRACT	
	LIST OF ABBREVIATIONS	
	LIST OF FIGURES	
	LIST OF TABLES	
	CHAPTER - 1	
1	INTRODUCTION	
	1.1 STATICS	
	1.2 DIFFERENCE BETWEEN CANCER CELLS AND NORMAL CELLS	
	1.3 MANAGEMENT AND TREATMENT	
	1.4 LIMITATIONS OF CURRENT TREATMENT OF CANCER	
	CHAPTER - 2	
2	REVIEW OF LITERATURE	
3	AIM AND OBJECTIVES	
	3.1 AIM	

	3.2 OBJECTIVES	
4	MATERIALS AND METHODS	
	4.1 IN-SILICO MOLECULAR DOCKING STUDIES	
	4.2 MOLECULAR DOCKING	
5	RESULTS AND DISCUSSIONS	
	5.1 MOLECULAR DOCKING	
	5.2 SPECTRAL DATA OF SYNTHESIZED COMPOUNDS	
	5.3 CHARACTERIZATION OF SYNTHESIZED COMPOUNDS	
6	SUMMARY AND CONCLUSION	
7	REFERENCES	

CHAPTER – 1

1. INTRODUCTION

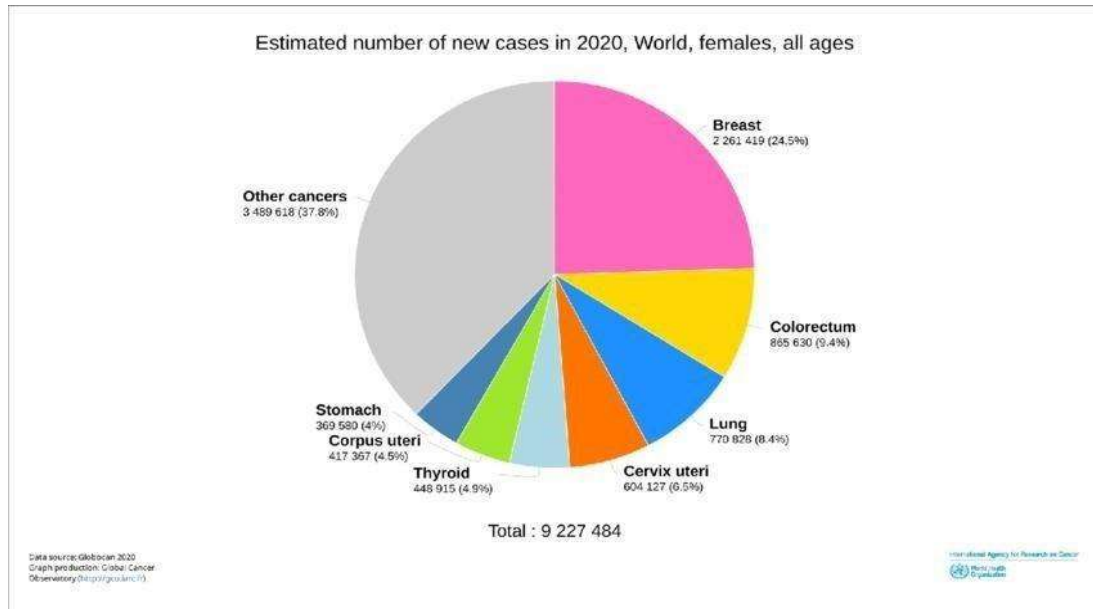
Cancer has emerged as one of several diseases that pose a serious hazard to people worldwide. A terrible threat to people everywhere, cancer has emerged as one. Cancer is a category of diseases characterized by abnormal cell growth that has the potential to infiltrate or spread to other parts of the body.

It is also referred to as a malignant tumour or malignant neoplasm. Tumours are not always cancerous; benign tumours do not spread to other body parts. A succession of molecular events that change the fundamental characteristics of cells lead to cancer. The normal regulatory mechanisms that stop tumour growth and the invasion of other organs are impaired in cancer cells. In the presence of signals that promote cell growth and division, these transformed cells proliferate and expand. They eventually acquire new traits, such as altered cell shape, diminished cell adhesion, and creation of novel enzymes. Even in the presence of other normal cells, which usually prevent the growth of neighbouring cells, these heritable modifications allow the cell and its offspring to divide and grow. The cancer cells can spread and infiltrate other tissues as a result of these modifications.

STATISTICS

- Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths.
- The most common cancers are breast, lung, colon and rectum and prostate cancers.
- Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (1). The most common in 2020 (in terms of new cases of cancer) were:
 - Breast (2.26 million cases);
 - Lung (2.21 million cases);
 - Colon and rectum (1.93 million cases);

- Prostate (1.41 million cases);
- Skin (non-melanoma) (1.20 million cases); and
- Stomach (1.09 million cases).



Differences between Cancer Cells and Normal Cells

Cancer cells differ from normal cells in many ways. For instance, cancer cells:

- Grow in the absence of signals telling them to grow. Normal cells only grow when they receive such signals.
- Ignore signals that normally tell cells to stop dividing or to die (a process known as programmed cell death, or apoptosis).
- Invade into nearby areas and spread to other areas of the body. Normal cells stop growing when they encounter other cells, and most normal cells do not move around the body.

- Tell blood vessels to grow toward tumors. These blood vessels supply tumors with oxygen and nutrients and remove waste products from tumors.
- Hide from the immune system. The immune system normally eliminates damaged or abnormal cells.
- Trick the immune system into helping cancer cells stay alive and grow. For instance, some cancer cells convince immune cells to protect the tumor instead of attacking it.
- accumulate multiple changes in their chromosomes, such as duplications and deletions of chromosome parts. Some cancer cells have double the normal number of chromosomes.
- Rely on different kinds of nutrients than normal cells. In addition, some cancer cells make energy from nutrients in a different way than most normal cells. This lets cancer cells grow more quickly.

Many times, cancer cells rely so heavily on these abnormal behaviors that they can't survive without them. Researchers have taken advantage of this fact, developing therapies that target the abnormal features of cancer cells. For example, some cancer therapies prevent blood vessels from growing toward tumors, essentially starving the tumor of needed nutrients.

MANAGEMENT AND TREATMENT

Healthcare providers may use several different treatments, sometimes combining treatments based on your situation. Common cancer treatments include:

- **Chemotherapy:** Chemotherapy is one of the most common cancer treatments. It uses powerful drugs to destroy cancer cells. You may receive chemotherapy in pill form or intravenously (through a needle into a vein). In some cases, providers may be able to direct chemotherapy to the specific area affected.
- **Radiation therapy:** This treatment kills cancer cells with high dosages of radiation. Your healthcare provider may combine radiation therapy and chemotherapy.

- **Surgery:** Cancerous tumors that haven't spread may be removed with surgery. Your healthcare provider may recommend therapy. This treatment combines surgery with chemotherapy or radiation to shrink a tumor before surgery or to kill cancer cells that may remain after surgery.
- **Hormone therapy:** Sometimes, providers prescribe hormones that block other cancer-causing hormones. For example, men and people assigned male at birth who have prostate cancer might receive hormones to keep testosterone (which contributes to prostate cancer) lower than usual.
- **Biological response modifier therapy:** This treatment stimulates your immune system and helps it perform more effectively. It does this by changing your body's natural processes.
- **Immunotherapy for cancer:** Immunotherapy is a cancer treatment that engages your immune system to fight the disease. The treatment may be called biological therapy.
- **Targeted therapy for cancer:** Targeted therapy is a cancer treatment that targets the genetic changes or mutations that turn healthy cells into cancer cells.
- **Bone marrow transplant:** Also called stem cell transplantation, this treatment replaces damaged stem cells with healthy ones. Autologous transplantation uses your supply of healthy stem cells. Allogeneic transplantation uses stem cells donated by another person.

LIMITATIONS OF CURRENT TREATMENTS OF CANCER:

1. Targeting cancer stem cells (CSCs) is difficult

The growing body of evidence suggests that in many cases cancerous cells originate from a single cell with stem cell characteristics.

These findings should have a profound effect on the treatment of cancer. Traditional cancer treatment is based on the assumption that all somatic cells possess a similar malignant potential.

The most prominent example is Gleevec (Imatinib) used in Chronic Myelogenous

Leukemia(CML). It acts via targeting the BCR-ABL fused protein which has tyrosine kinase activity. This drug interferes with the intracellular growth signaling pathway in cancer cells by blocking the ATP binding site of BCR-ABL and thus prevents it from transducer signaling to its target protein.

2. Drug resistance properties of cancer stem cells make them immune to anticancer drugs

Since normal stem cells have to undergo a repeated process of self-renewal and differentiation in the entire life span of an individual, they have developed certain unique mechanisms by which they can protect themselves from harmful xenobiotic agents.

The most notable example is the drug resistance protein expressed in breast cancer cells - Breast Cancer Resistance protein (BCRP) ABCG2, a specific ATP-binding cassette transporter. This protein was previously identified in a variety of stem cells and is responsible for the drug efflux in breast cancer.

3. Lack of cancer epigenetic profiling and specificity of existing epidrugs

The traditional approach in cancer research was primarily focused on the identification and determining the general patterns of genetic anomalies that result from the mutational or other chromosomal aberration events. But unfortunately only a handful of genetic mutations associated with cancer have been identified as among patients which by no means explains the enormous genetic deviation that eventually manifests in the malignant phenotype of cancer.

Methylation in CpG island residing in the promoter region of the tumour suppressor genes such as Rb, p16 and p53 could have a disastrous effect on cell cycle regulation.

The enzyme responsible for the methylation of CpG dinucleotide is known as DNA methyl transferase (DNMT). This enzyme is the obvious target for reestablishing the normal methylation pattern in the cis elements of the affected gene. In harmony with this notion several DNMT blockers were identified including 5-aza-2'-deoxycytidine which successfully diminished the tumorigenicity in a mouse model of neoplasia .

4. Unavailability of effective biomarkers for cancer diagnosis and prognosis

The unavailability of good biomarkers is another hindrance for cancer treatment. Biomarkers are not only important for diagnostic purposes but can also be of great prognostic value. With the identification of the right biomarker the cancer progression and effect of chemotherapeutic drugs can be evaluated in great detail.

The recently emerged concept of clinical proteomics has shown much promise in identifying effective biomarkers that would enable early detection and the disease progression of cancer.

5. Limitations of conventional chemotherapeutic agents

The existing chemotherapeutic drugs are toxic to all cells including cancer and normal cells. So the administration of these toxic agents kill the rapidly proliferating cancer cells as well as the normal cells which may lead to some serious side effects and may sometimes cause the death of patients.

6. Metastasis poses a huge problem in cancer treatment

One of the main reasons for the difficulties associated with cancer treatment is the metastatic nature of cancer.

The first site where the cancer starts is called the “primary cancer site” whereas the sites in which cancer has spread is known as the “secondary or metastatic site”. In order to spread the cancer cells, primary sites have acquired the ability to invade and colonize a distant site and eventually spread into different parts.

These markers can indicate the presence of cancer spread which is difficult to identify by normal scanning. But unfortunately most of the cancer specific markers have not yet been identified.

2.Poly (ADP-Ribose) Polymerase (PARP)

Poly (ADP-ribose) polymerase is a family of enzymes discovered in 1963 by Chambon and its co-workers, which shares the ability to catalyze the transfer of ADB ribose to target protein. This family of enzymes plays a crucial role in various cellular processes such as transcription, modulation of chromatin structure, replication, recombination and DNA repairs.

The poly (ADP-ribose) polymerases (PARPs) are an emerging family of enzymes that share the ability to catalyze the transfer of ADP-ribose to target proteins (poly ADP-ribosylation). There are at least 18 members of the PARP family that are encoded by different genes, and share homology in a conserved catalytic domain. Although some isoforms including PARP1 and PARP2 are best known for their involvement in DNA repair processes, it is now clear that these and other PARPs have an important role in several cellular processes including cell proliferation and cell death.

PARP1 is the first and best characterized member of the PARP family. PARP2 is most closely related to PARP1 with 69% similarity in its catalytic domain, and was identified on the basis of the persistence of PARP activity in PARP1-deficient cells

Among them, PARP1 is best known for its involvement in DNA repair, cell proliferation and cell death. The pharmacological inhibition of PARP has the potential to enhance the cytotoxicity of certain DNA damaging of anti-cancer drugs, reduce cell necrosis, down-regulate the multiple pathways in inflammatory and injured tissues. Literature has shown that PARP1 action is low in a maximum of normal human cells, whereas it is significantly unregulated in several primary cancerous diseases like lymphomas and cancer of breast, uterus and ovary.

Hence, nowadays the understanding of the function and importance of PARP1 enzyme has been increased due to its vital role in DNA repair, among other things, leading to investigations of specific inhibitors in the drug development process in various pathological conditions including cancer, neurodegenerative disorder, metabolic disorder and various disorders.

3 Signal Transducer and Activator of Transcription 3

Signal transducer and activator of transcription 3 (STAT3) is one of a family of seven proteins, including STAT1, that encodes a transcription factor. STATS 2, 3, 4, 5a, 5b, and 6 are all statistical tests. These are well recognized that proteins are crucial for growth factor production.

and cytokine signaling, which influences immunological responses, cell differentiation, and development. The

Latent cytoplasmic transcription factors known as STAT proteins transport growth factors and cytokines from the cell membrane to the nucleus to control gene expression.

essential for healthy cellular functions like cell differentiation, growth, proliferation, survival, and angiogenesis and immunological performance. The seven human STAT proteins have an amino acid count of between 750 and 850 and are distributed among three chromosomal clusters, with STAT3 being found on chromosome 12. The majority of human cancer cells and tumour tissues, including those from breast cancer, melanoma, lung cancer, pancreatic cancer, prostate cancer, ovarian cancer, leukemia, and lymphomas, frequently include STAT3 genes, one of seven STAT proteins that are continually active. The

target of STAT3 activation prevents tumour development and metastasis both in vitro and in vivo without having any negative effects on normal cells, suggesting that STAT3 may be useful as a molecular target for the therapy of cancer.

CHAPTER - 2

REVIEW OF LITERATURE

A thorough literature survey was carried out on the investigation of the design and development of PARP1 and STAT3 dual inhibitors. The survey was limited to the period of 2011 to 2020.

1. Park et al. (2011) in this study, they examined the efficacy of NVP.BMI20 pan-class I PI3K inhibitor in human gastric cancer cells and hypothesized that the combined inhibition of PI3K and sTAT3 would be synergistic in KRAS mutant Cancer cells. Compound 1 demonstrated anti-proliferative activity in 11 human gastric cancer cell lines by decreasing mTOR downstream signaling.

2. Zeng et al., (2011) studied the Molecular modeling studies on benzimidazole carboxamide derivatives as PARP-1 inhibitors. Using 3D-QSAR and docking in this study, molecular docking and 3D-QSAR studies were carried out not only to explore the interaction mechanism between 145 inhibitors and PARP-1 but also to construct highly accurate and predictive 3D-QSAR models, including the CoMFA (2, 0.899; r^2 , 0.712 and CoMSIA (2, 0.889; r^2 , 0.744, 2) models based on flexible docking alignment, for predicting the biologic activity of new compounds. Molecular docking reveals the detailed structures of PARP-1 binding with the compounds.

3. Ye et al., (2013) described Design, Synthesis and Biological Evaluation of derivatives of Benzo[de][1,7] naphthyridin-7(8H)-ones bearing a functionalised longer chain appendage as novel PARP1 Inhibitors. A series of benzo[de][1,7] naphthyridin-7(8H)-one's derivatives have been designed and evaluated as novel PARP1 inhibitors. Initially, compound 2 bearing a terminal phthalazine-9H-one framework and showing remarkably high PARP1 inhibitory activity (0.31 nM) but only moderate potency in the cell. The further effort generated the second-generation lead 3 showing high potency against both the PARP1.

4. Amin et al., (2013) described the synthesis, cytotoxic evaluation and molecular docking study of novel quinazoline derivatives as PARP-1 inhibitors. There are 16 compounds of

spiro[(2H,3H)-quinazoline-2, 1i-cyclohexane] derivatives that were designed and synthesized in this study. The synthesized compounds evaluated for their in-vitro cellular evaluation against human breast carcinoma cell lines (MCF-7) using doxorubicin as a reference drug. Most of the tested compounds displayed promising cytotoxic activity, especially derivatives 4, 5 and 6 are the most significant ones (35).

5. Deepak et al., (2013) designed a series of fluorinated 3,6-diaryl- 1,2,4]triazolo[3,4-b][1,3,4 thiadiazoles and synthesized. The synthesized Compounds were screened for anticancer activity against three cancerous cell lines MCF7 (human breast cancer), SaOS-2 (human osteosarcoma) and K562 (human myeloid leukemia). Among these, compound 7 showed higher proliferative activity (IC_{50} =22.1, 19 and 15 μ M against MCF7, SaOS-2 and K562 cells, respectively) (36).

6. Zhang et al., (2013) studied a new series of quinazoline derivatives were designed based on the reported synergistic effect between RTK and HDAC inhibitors. These hybrids featured the key moieties of gefitinib or eriotinib as well as the ZBG of HDACi. The inhibitory activities against HDAC, EGFR and HER2 under cell-free conditions were evaluated. Almost all hybrids with hydroxamate segments exhibited potent HDAC inhibitory activity. From the in-vitro studies compound 8 showed similar anti-HDAC activities against HDAC1 (IC_{50} =0.16 μ m), HDAC3 (IC_{50} =0.18 μ m) and HDAC6 (IC_{50} = 0.56 μ m) (37).

7. Nan et al, (2014) was shown here that 2-[(aminocarbonyl)amino]-5 4- fluorophenyl]-3-thiophene carboxamide (9), a previously reported inhibitor of I κ B kinases (IKK), blocked STAT3 recruitment to upstream kinases by docking into SH2 domain of STAT3 and attenuated STAT3 activity induced by cytokines and cytoplasmic tyrosine kinases. TPCA-1 is an effective inhibitor of STAT3 phosphorylation, DNA binding, and transactivation in vivo. It selectively To suppress the proliferation of NSCLC cells with constitutive STAT3 activation. Objectively, in this work. the authors identified 9 as a direct dual inhibitor for both IKKs and STAT3 (38).

8. Daka et al., (2015) was reported the design, synthesis and evaluation of a novel class of

small-molecule inhibitors, that is, 10 and its analogs, as promising leads for further development of STAT3 inhibitors. Preliminary SARs was established for 10 and its derivatives; and the binding modes were predicted by molecular docking. Lead compounds with IC₅₀ as low as 6.5 pM in breast cancer cell lines and 7.6 uM in pancreatic cancer cell lines were identified (39)

9. Patel et al., (2015) studied the Discovery and Structure-Activity Relationship of Novel 2,3 Dihydrobenzofuran-7-carboxamide and 2,3-Dihydrobenzofuran-3(2H)-one-7-carboxamide Derivatives as PARP-1 Inhibitors. Novel substituted 2,3-dihydro benzofuran-7-carboxamide (DHBF-7-carboxamide) and 2,3-dihydro benzofuran-3(2H)-one-7-carboxamide (DHBF-3-one-7-carboxamide) derivatives were synthesized and evaluated as inhibitors of poly (ADP-ribose) polymerase- 1 (PARP-1). A structure-based design strategy resulted in lead compound 11 (DHBF-7-carboxamide: IC₅₀ = 9.45 uM), Substituents larger than fluorine at the position of the DHBE scaffold were found to be detrimental for PARP-1 W PARP.1 inhibition. The 2-position methyl substitution is well tolerated in the DHBF-7- carboxamide scaffold 12 (IC₅₀= 0.531 M), yielding enantiomers that bind AD differently in the active site.

10. Wang et al. NH, (2016) studied ONH, 12 novel PARP-1 Inhibitors Based on a 1H-thieno[3,4-d] Imidazole 4-Carboxamide Scaffold In this study, the author was designed 1H-thieno(3,4-d] imidazole-4- carboxamide moiety and synthesized it. These synthesized compounds were undergone cellular evaluation and it indicated that the anti-cancer activities of 13, 14. 15 and 16 against BRCA. -deficient cell lines were similar to that of olaparib, while the design, synthesis, and biological evaluation of toxicities of 3 and 4 toward human normal cells were lower.

11. Yang et al., (2016) Herein the authors describe a pharmacophore merging strategy combining the JAK2/FLT3 inhibitor pacritinib with the pan-HDAC inhibitor, vorinostat, to create bispecific single molecules with both JAK and HDAC targeted inhibition. A preferred ether hydroxamate, 17 inhibits JAK2 and HDAC6 with low nanomolar potency, is <100nM potent against HDACs 2 and 10, sub-micromolar potent against HDACs 1, 8 and 11, and >50 fold selective for JAK2 in a panel of 97 kinases. Broad cellular

antiproliferative potency is supported by the demonstration of JAK-STAT and HDAC pathway blockade in several hematological cell lines, inhibition of colony formation in HEL cells, and analysis of apoptosis.

CHAPTER - 3

AIM AND OBJECTIVES

AIM

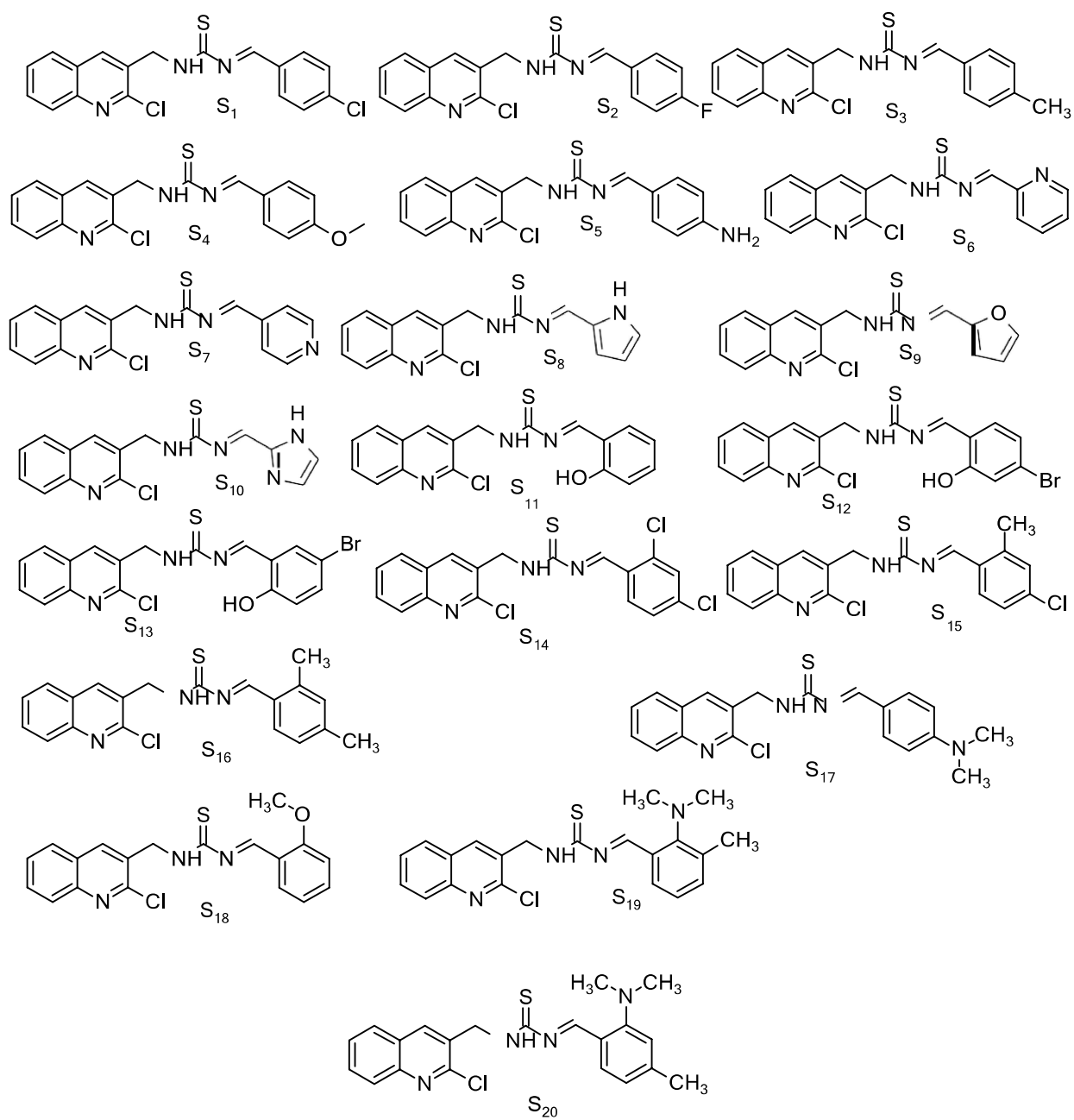
To develop a dual inhibitor for PARP1 and STAT3 enzymes and evaluation for their anti- cancer potential.

Objective

- Designing of dual inhibitors based on DML approaches of known inhibitors of PARP1 and STAT3.
- In silico ADMET prediction and molecular docking studies.
- the designed compounds will be synthesized in the laboratory by the conventional methods
- synthesized compounds will be characterized by IR, H¹ NMR, C¹³ NM, LC-MS, and through elemental analysis

CHAPTER – 4

MATERIALS AND METHODS



In-silico molecular docking studies

Devices and materials

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

Preparation of protein

By utilizing the offline program protein data bank (PDB), we take the PARP1 (PDB ID: 4ZZZ) and STAT3 (6NJS) 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.biotech.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

We used 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 Å of bound crystallographic ligand atom. Default settings were used for all the calculations. Docking was performed using a grid resolution.

***In-silico* ADMET prediction**

A computational study was conducted to predict the pharmacokinetic properties (ADMET) of designed compounds using swiss ADME prediction. Here we calculated molecular volume (MV), molecular weight (MW), number of acceptors of hydrogen bonds (n-ON), number of donors of hydrogen bonds (n-OH/NH), total CNS activity, % of oral absorption for humans, polar surface area (PSA), 1-Octyl alcohol-water distribution constant (log P o/w), and BBB penetration. The described properties help to understand the ADME properties of any drug/synthesized molecule. The drug likeness, rule-of-five and rule-of-three violations were also ascertained. For a molecule to be administered via oral route, it should possess distribution constant 5, molecular mass 500, number of donors of H-bonds 5 and number of acceptors of H-bonds 10 and only one violation of the above criteria is acceptable.

Chemistry

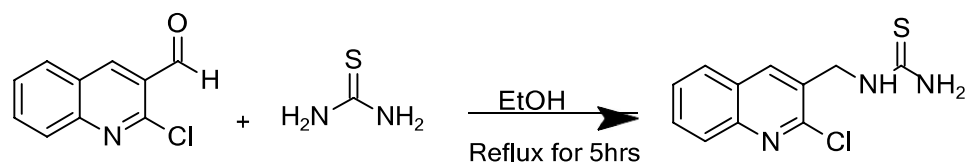
Step 1: synthesis of (E)-1-((2-chloroquinolin-3-yl) methylene) thiourea

The compound 2-chloroquinoline-3-carbaldehyde (2) was added with carbonyldiamide (3) in ethanol and was refluxed at 80-90°C for 5 hr to yield (E)-1-((2-chloroquinolin-3-yl) methylene) thiourea derivative.

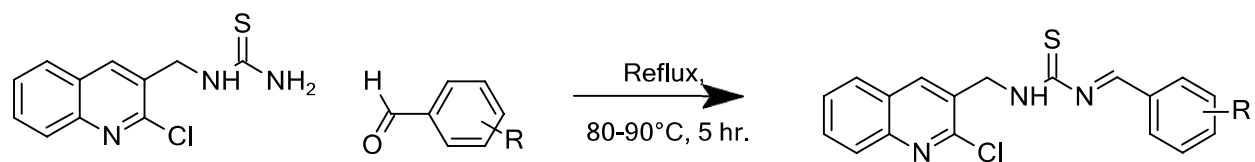
Step 2: Synthesis of 3-[(2-chloroquinolin-3-yl)methyl]-1-[(1E)-phenylmethylidene]thiourea derivatives

The compound (E)-1-((2-chloroquinolin-3-yl) methylene) urea (4) was added with substituted aromatic aldehydes (5) and was refluxed at 80-90°C for 5 hr to yield the corresponding title compounds.

Step 1



Step 2



CHAPTER – 5

RESULTS AND DISCUSSIONS

Molecular docking

The molecular docking studies for the designed compounds were carried out through PyRx molecular docking software to determine the free energy binding towards targeted enzymes. The docking pose for the ligand enzyme interaction was visualized with discovery studio. The binding free energy for all the ligands was tabulated in table. From the results it clearly shows that, all the compounds have promising interaction with targeted enzyme PARP1. The interaction is mainly due to the presence of lipophilic factor of aromatic heterocyclic ring. From the docking results, compound S7 (-9.3 kcal/mol) shows highest binding affinity toward PARP1 enzyme compared to standard drug olaparib. 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 PARP1 and also play important role in the inhibition of the ligand-binding domain of PARP1 inhibitors. These non-covalent interactions, van der Waals, columbic interaction, π - π interaction, and hydrogen interaction, are shown in **Figure**. The table 3 shows the binding energy of studied compounds.

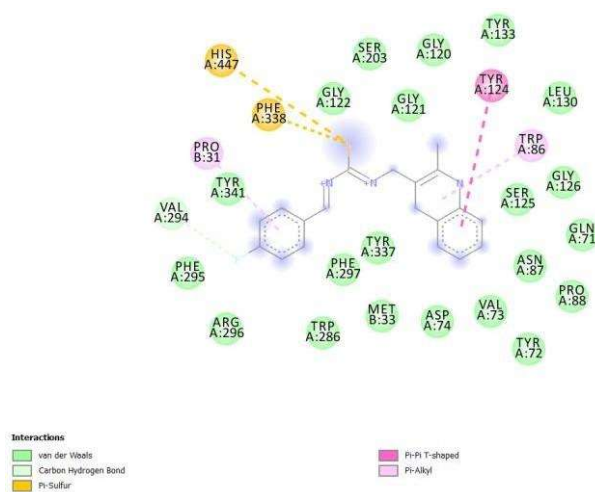


Figure 3. 2D docking interaction of compound S2 against PARP1 enzyme

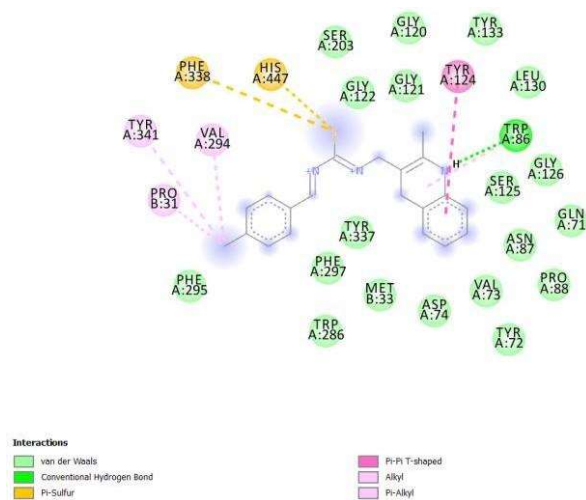


Figure 4. 2D docking interaction of compound S3 against PARP1 enzyme

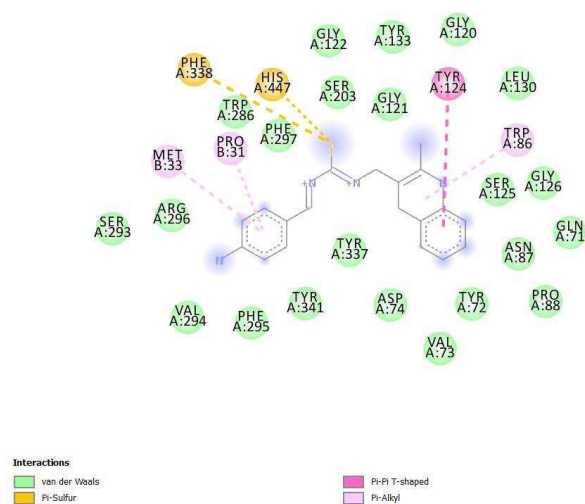


Figure 5. 2D docking interaction of compound S5 against PARP1 enzyme

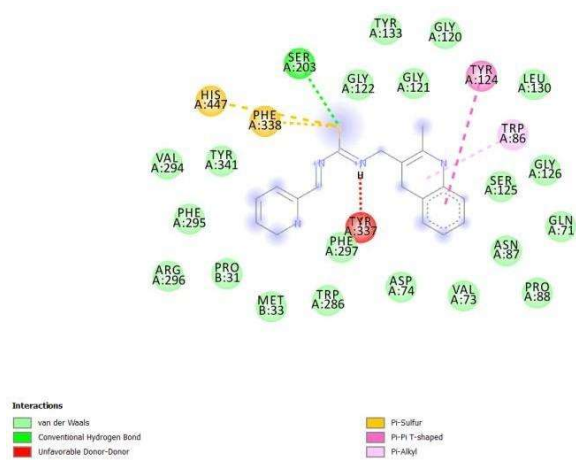


Figure 6. 2D docking interaction of compound S6 against PARP1 enzyme

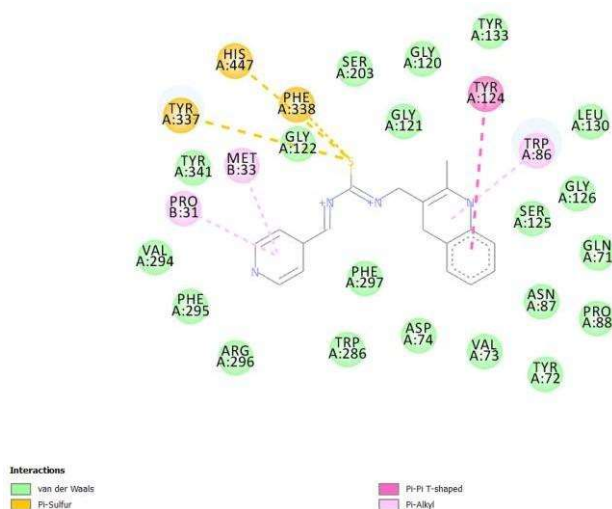


Figure 7. 2D docking interaction of compound S7 against PARP1 enzyme

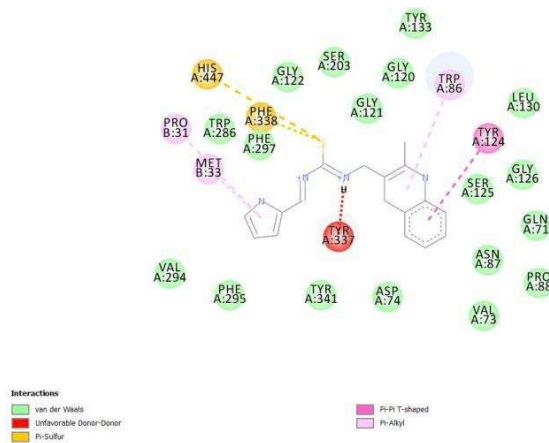


Figure 8. 2D docking interaction of compound S8 against PARP1 enzyme

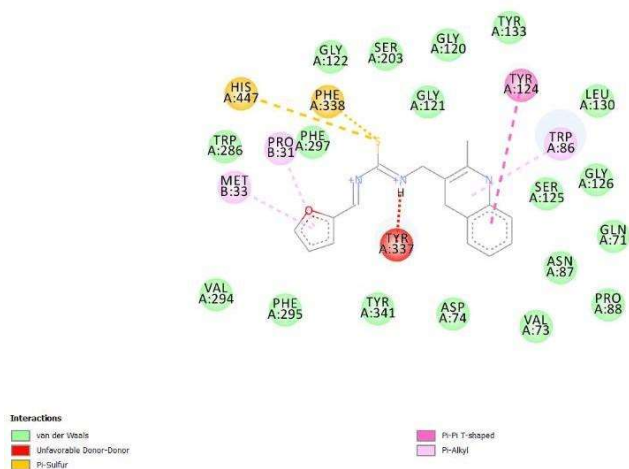


Figure 9. 2D docking interaction of compound S9 against PARP1 enzyme

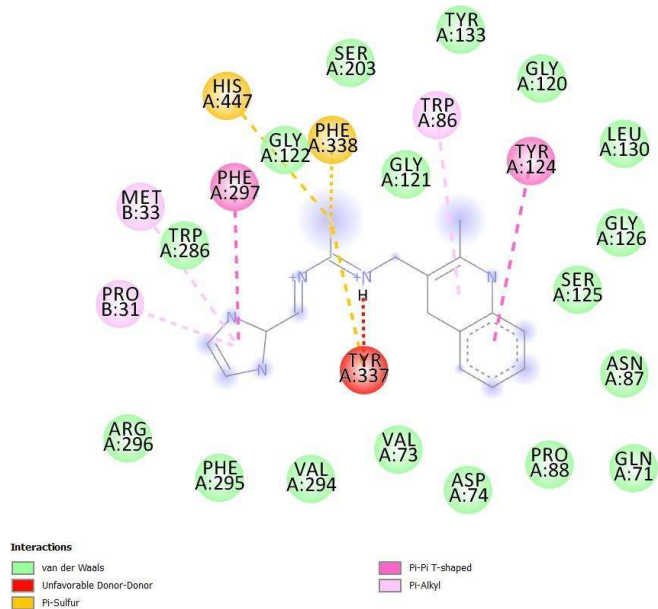


Figure 10. 2D docking interaction of compound S10 against PARP1 enzyme

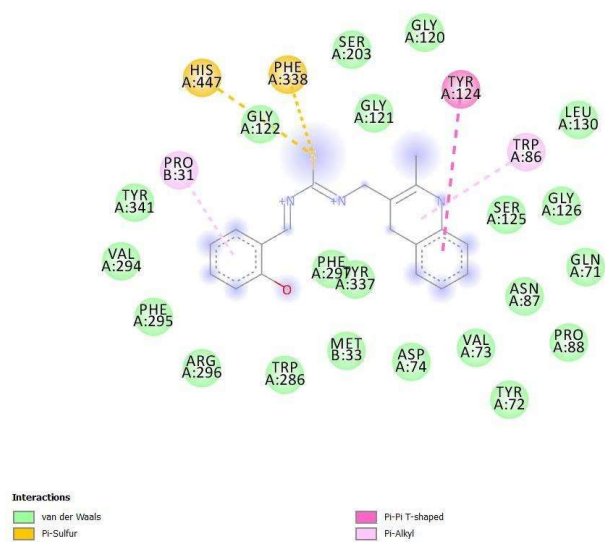


Figure 11. 2D docking interaction of compound S11 against PARP1 enzyme

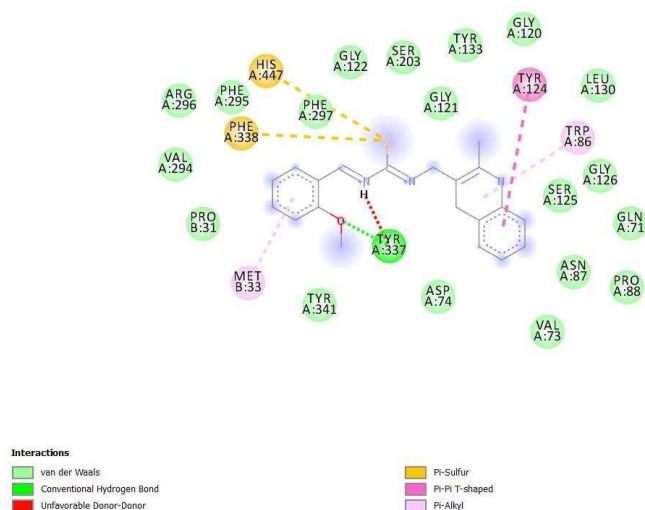


Figure 12. 2D docking interaction of compound S18 against PARP1 enzyme.

Table. Binding energy of studied compounds.

Ligand	Binding Affinity
S1	-7.1
S2	-7.4
S3	-8.8
S4	-7.7
S5	-8.6
S6	-9.2
S7	-9.3
S8	-9.1
S9	-8.1
S10	-8.3
S11	-9.2
S12	-7.6
S13	-7.3
S14	-7.7
S15	-8.1

S16	-8.1
S17	-7.4
S18	-8.2
S19	-6.7
S20	-8.1
Olaparib	-11.2

From the docking results for STAT3, compound 11 (9 kcal/mol) shows highest binding affinity toward STAT3 compared to standard drug niclosamid.

This compound produced one conventional hydrogen bonds between sulfur with residues of Gln 833. The following amino acids such as Leu 801, Leu 832, and Phe 858 are interacting with ligand through hydrophobic bond. This interaction due to the aromatic character of ligands. The remaining the entire studied compound shows good to moderate binding affinities to the selected enzymes. These amino acids have been repeatedly implicated during ligand interaction with the STAT3 enzyme and also play important role in the inhibition of the ligand-binding domain of STAT3 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 S3, S6, S7, S8, and S11 are selected for the conventional synthesis and it was further evaluated for the cytotoxicity studies against the MCF-7 cells.

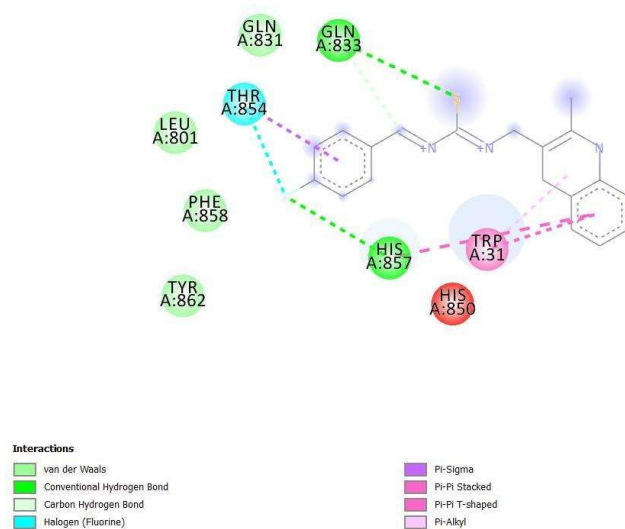


Figure 3. 2D docking interaction of compound S2 against STAT3 enzyme

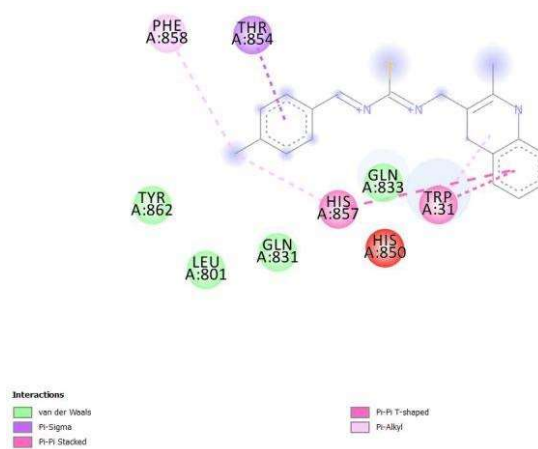


Figure 4. 2D docking interaction of compound S3 against STAT3 enzyme

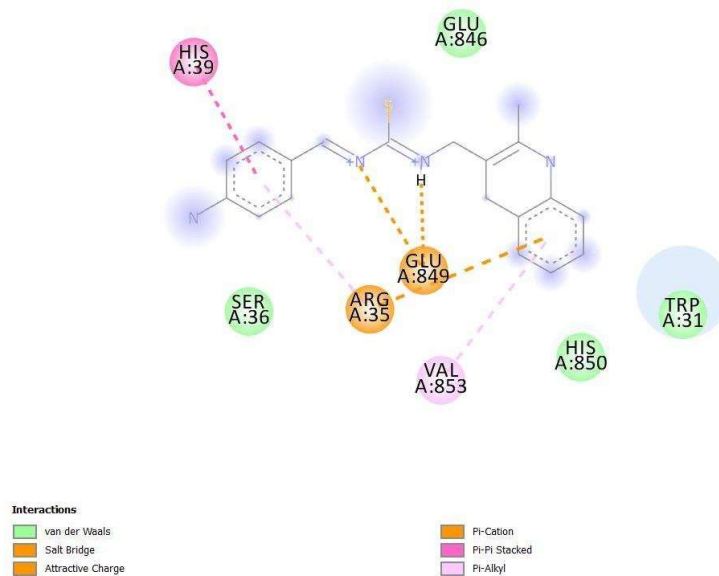


Figure 5. 2D docking interaction of compound S5 against STAT3 enzyme

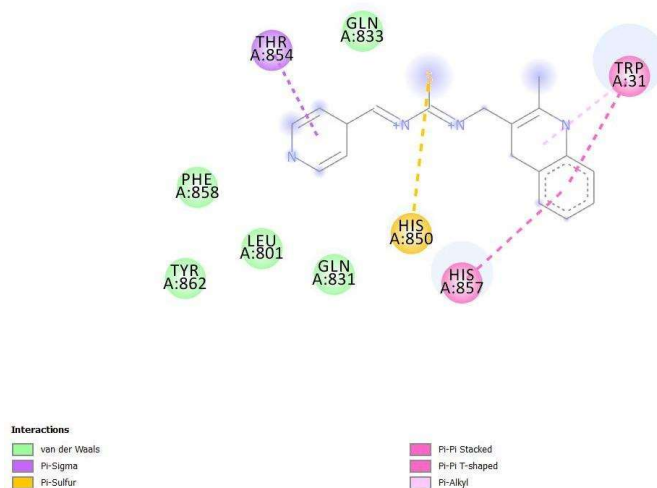


Figure 6. 2D docking interaction of compound S6 against STAT3 enzyme

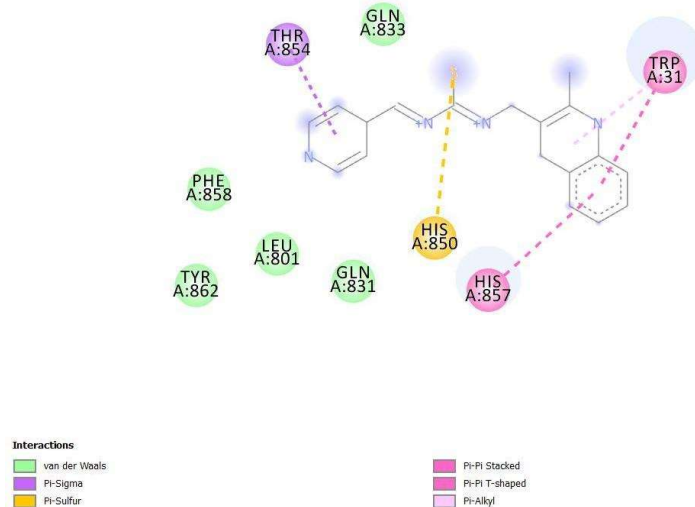


Figure 7. 2D docking interaction of compound S7 against STAT3 enzyme

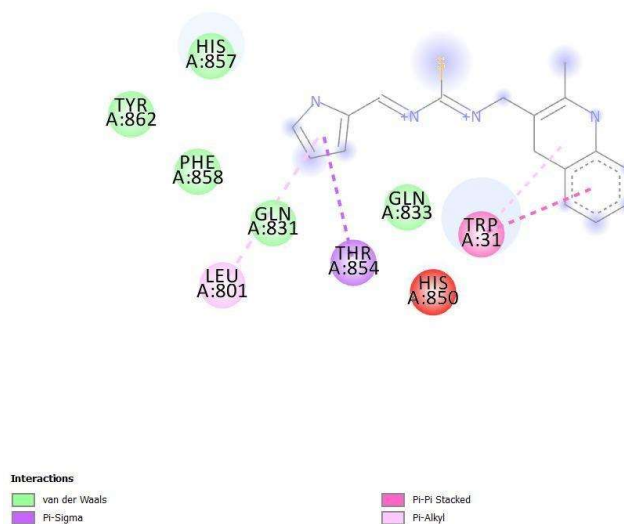


Figure 8. 2D docking interaction of compound S8 against STAT3 enzyme

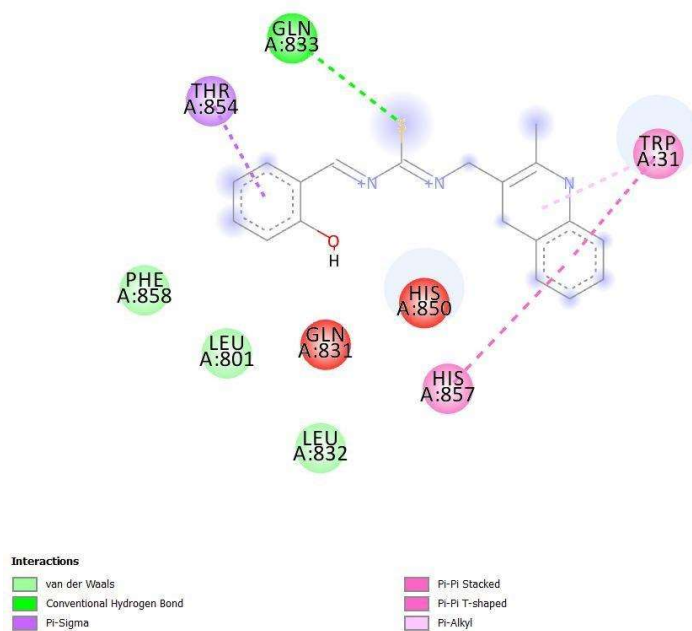


Figure 9. 2D docking interaction of compound S11 against STAT3 enzyme

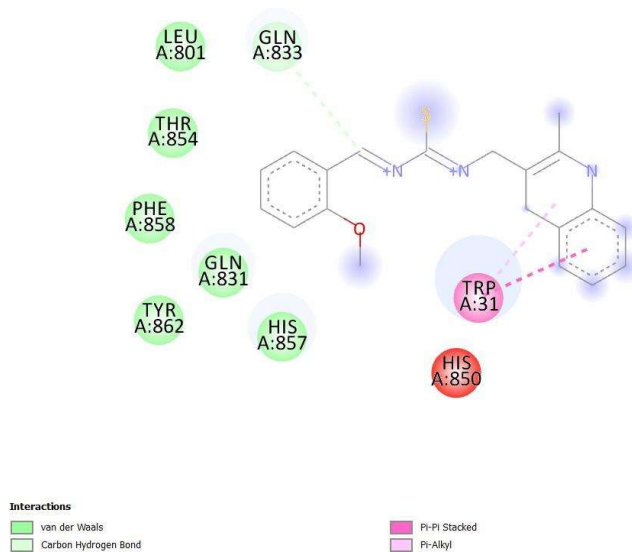


Figure 10. 2D docking interaction of compound S18 against STAT3 enzyme

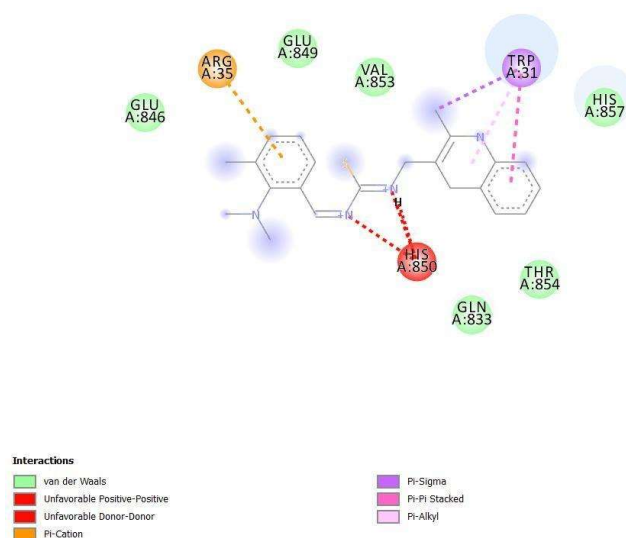


Figure 11. 2D docking interaction of compound S19 against STAT3 enzyme

Table 1. Binding energy of studied compounds.

Ligand	Binding Affinity
S1	-7.4
S2	-8.6
S3	-8.6
S4	-7.7
S5	-8.5
S6	-8.5
S7	-8.3
S8	-8.2
S9	-7.6
S10	-7.9
S11	-9
S12	-7.9
S13	-7.5

S14	-7.4
S15	-7.8
S16	-7.3
S17	-7.9
S18	-8.3
S19	-8.1
S20	-6.9
Niclosamide	10.4

Chemistry

The final derivatives of derivatives were achieved by two step process. The compound 2-chloroquinoline-3-carbaldehyde (2) was added with carbonyldiamide (3) in ethanol and was refluxed at 80-90°C for 5 hr to yield (E)-1-((2-chloroquinolin-3-yl) methylene) thiourea derivative. The compound (E)-1-((2-chloroquinolin-3-yl) methylene) urea (4) was added with substituted aromatic aldehydes (5) and was refluxed at 80-90°C for 5 hr to yield the corresponding title compounds. The completion of the reaction and purity of synthesized compounds were analyzed by TLC using ethylacetate and n-hexane as mobile phase and the synthesized derivatives was subjected to melting point determination. The structure of synthesized compounds was elucidated by various spectral analyses. From the spectral analysis, it evident that all the compounds showed a corresponding signals in all the spectral data. The spectral data for all the compounds are given below:

Spectral Data of synthesized compounds

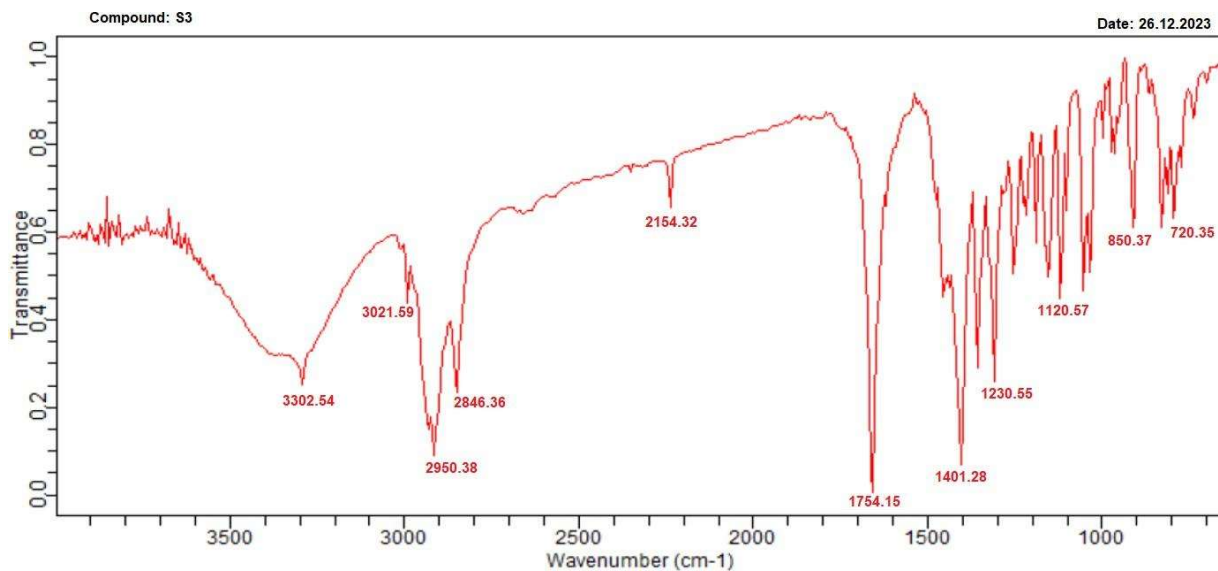


Figure 17: IR Spectra for compound S3

001371 #4 RT: 0.02 AV: 1 SB: 269 12.24-14.09 , 14.29-17.15 NL: 6.54E5
T: FTMS - p ESI Full ms [100.0000-1000.0000]

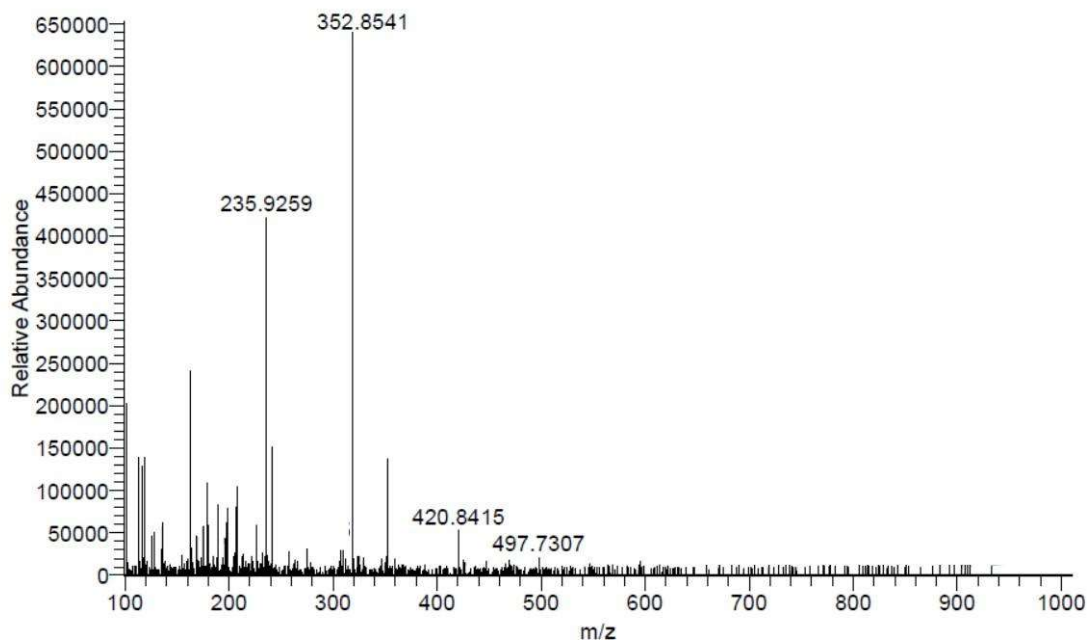


Figure 18: Mass Spectra for compound S3

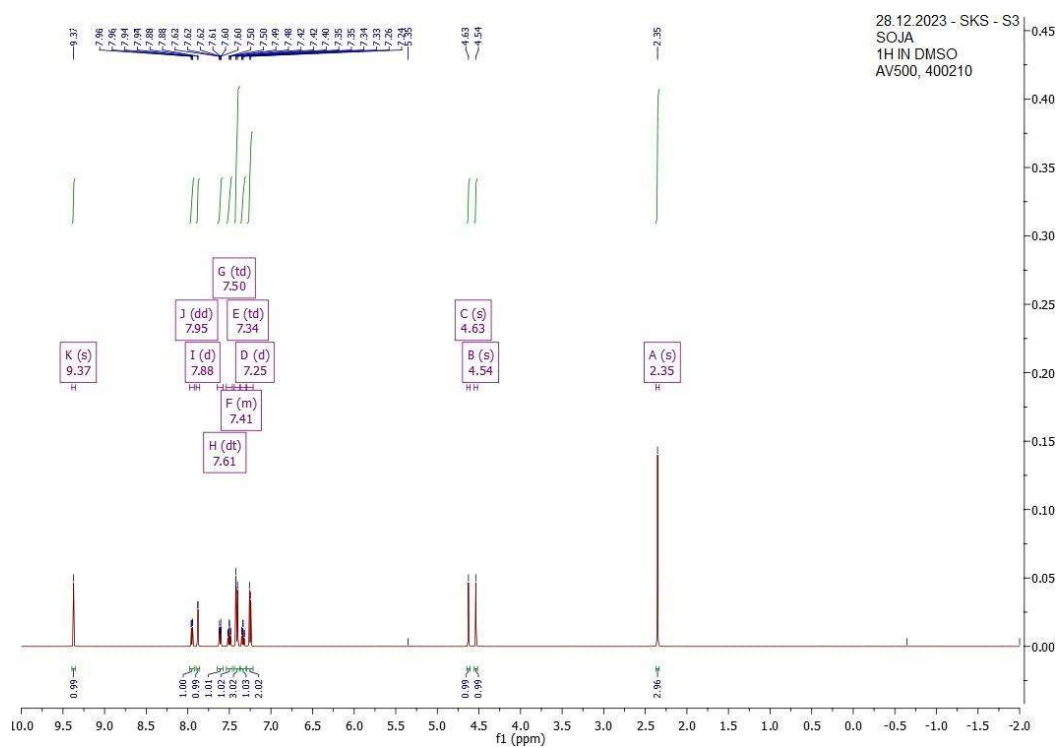


Figure 19: ^1H NMR Spectra for compound S3

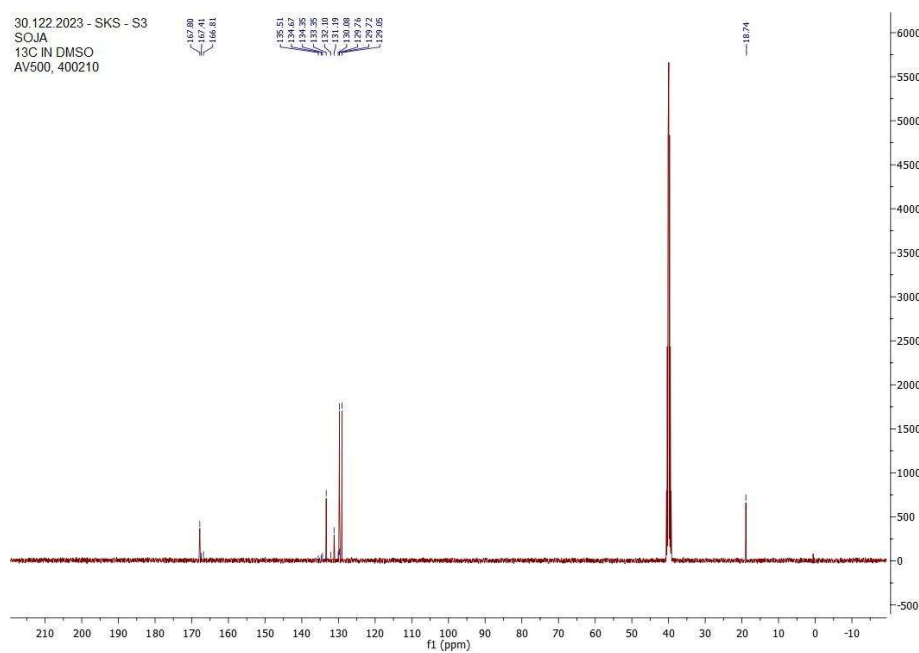


Figure 20: ^{13}C NMR Spectra for compound S3

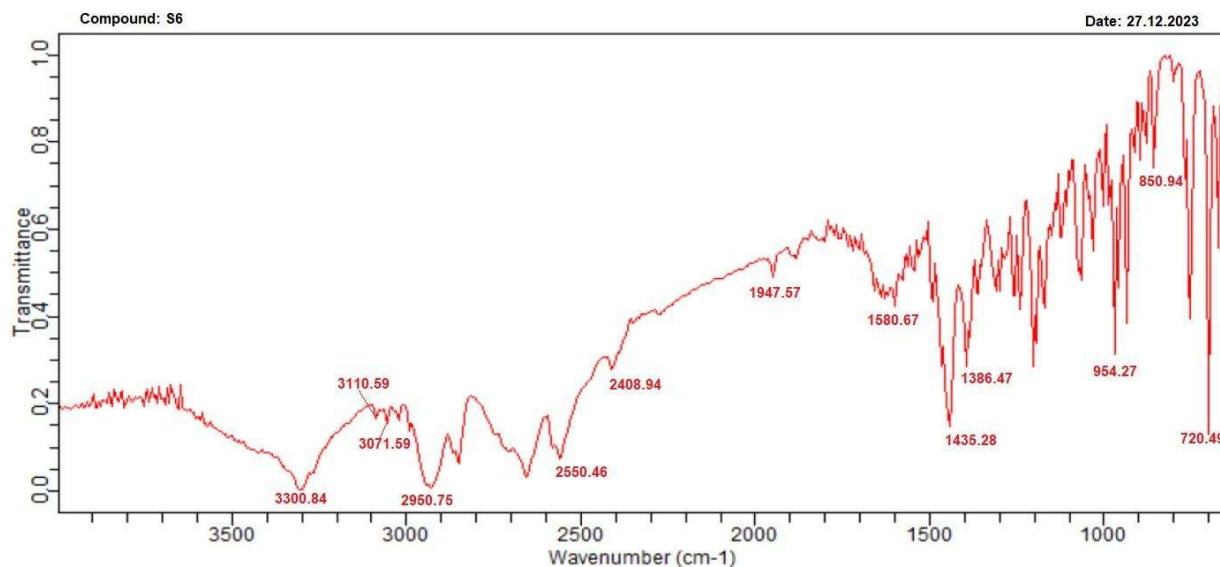


Figure 25: IR Spectra for compound S6

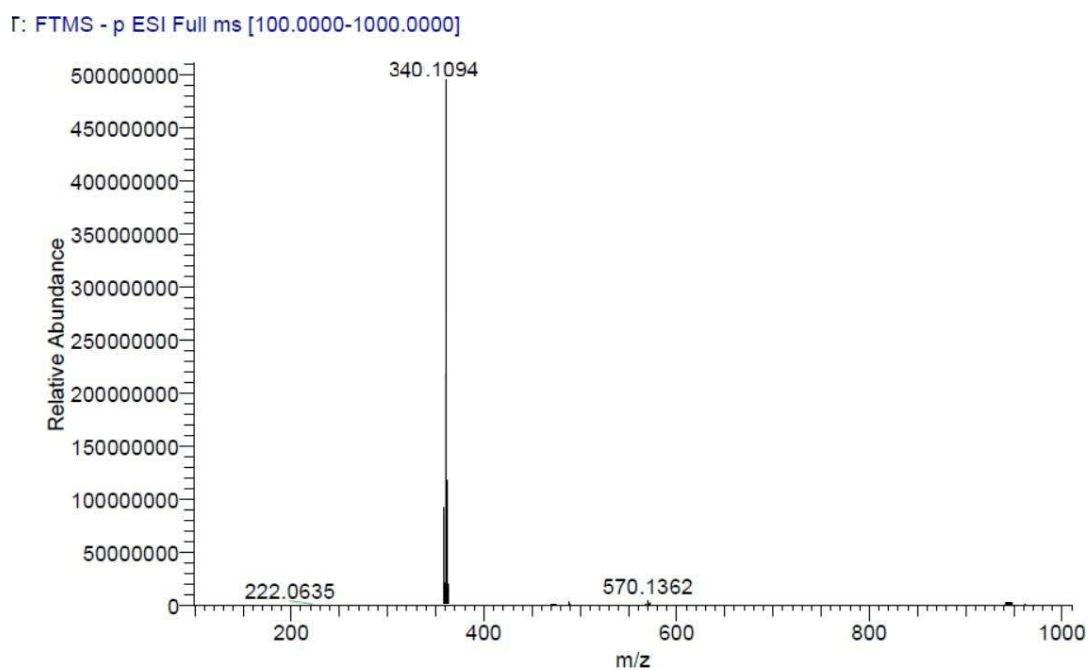


Figure 26: Mass Spectra for compound S6

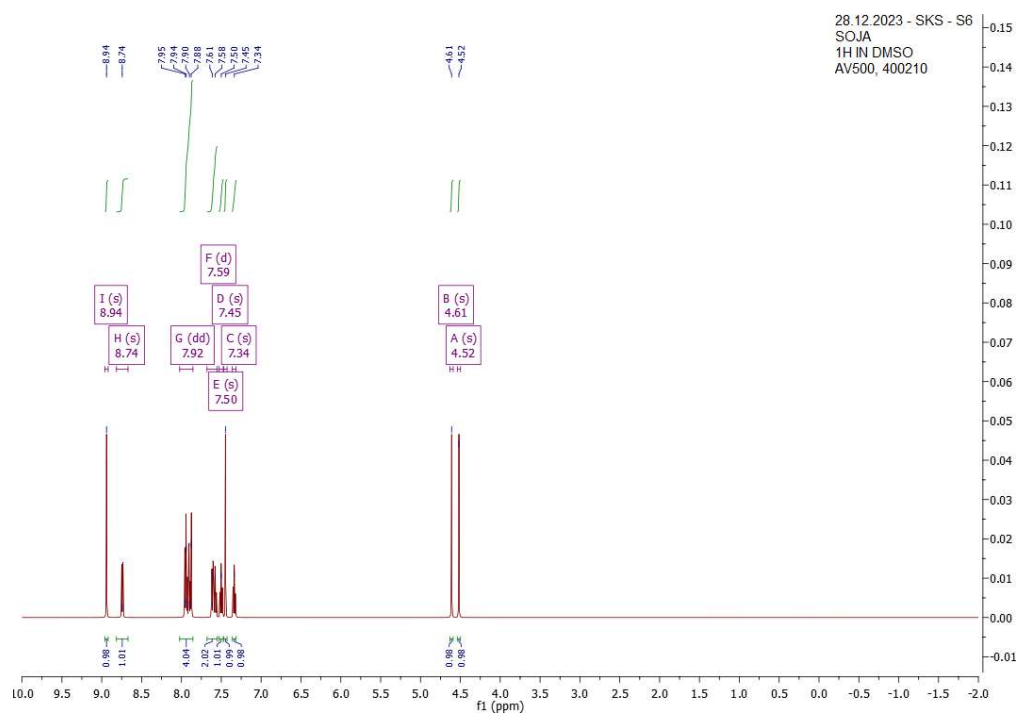


Figure 27: ^1H NMR Spectra for compound S6

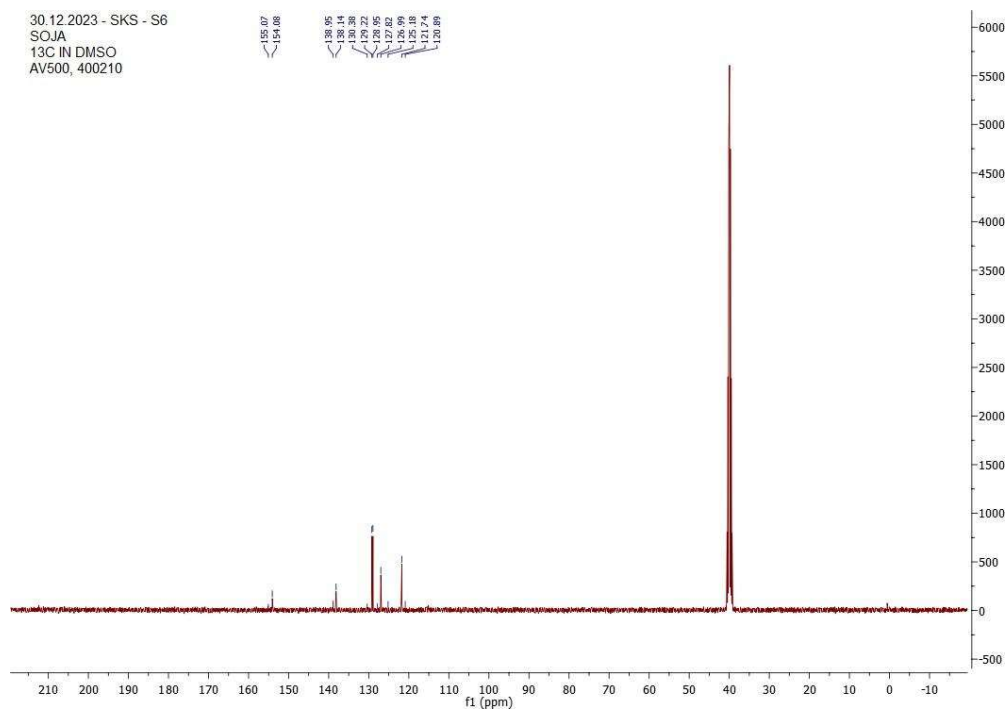


Figure 28: ^{13}C NMR Spectra for compound S6

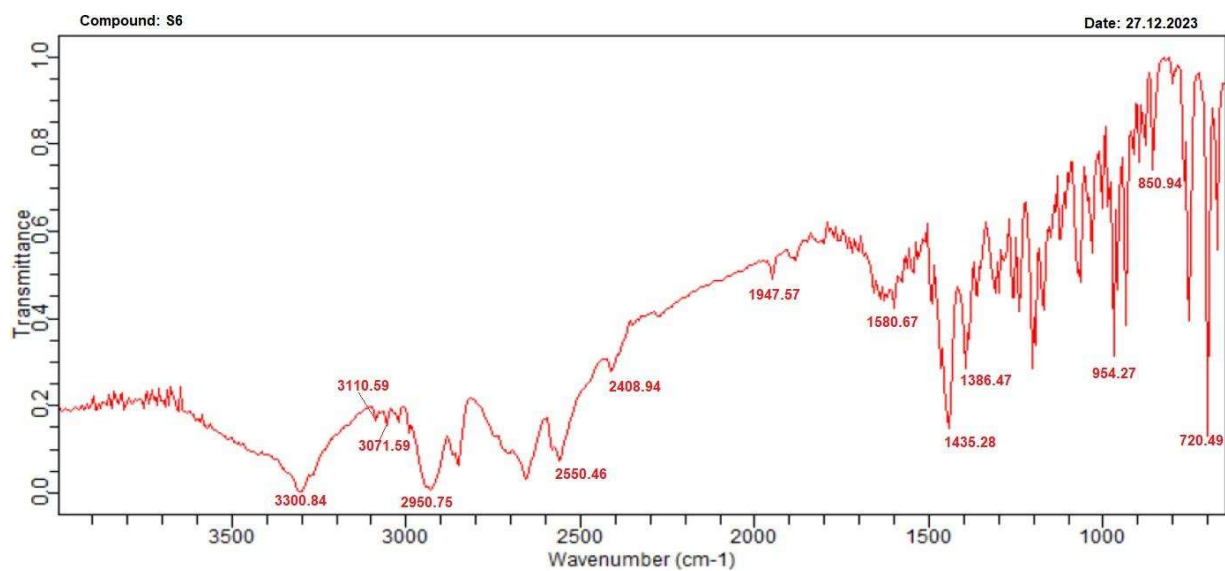


Figure 29: IR Spectra for compound S7

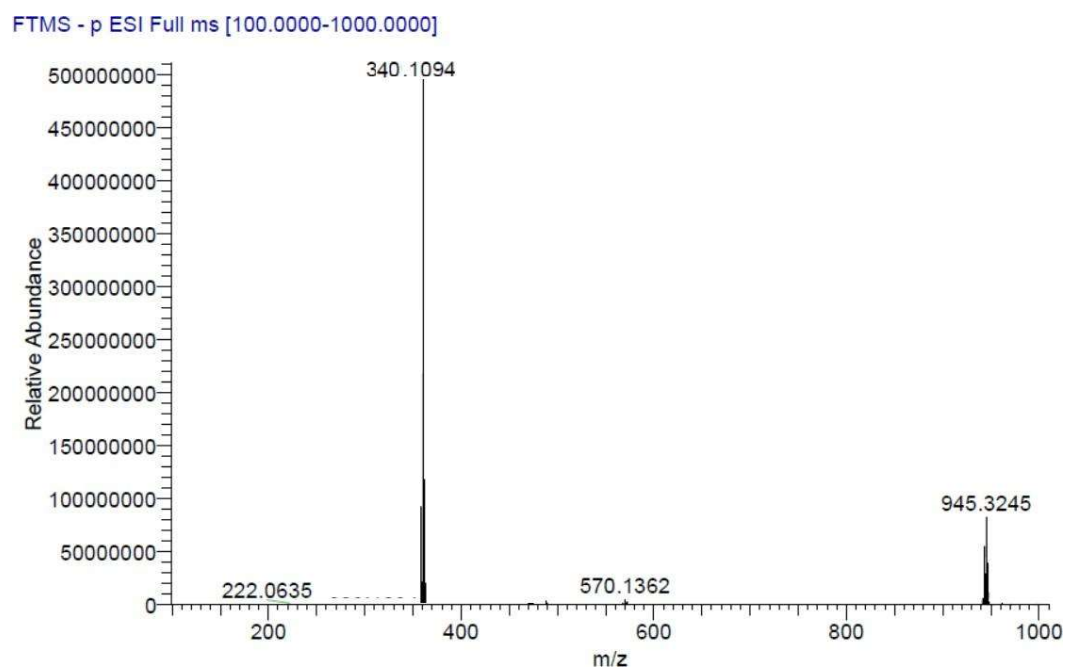


Figure 30: Mass Spectra for compound S7

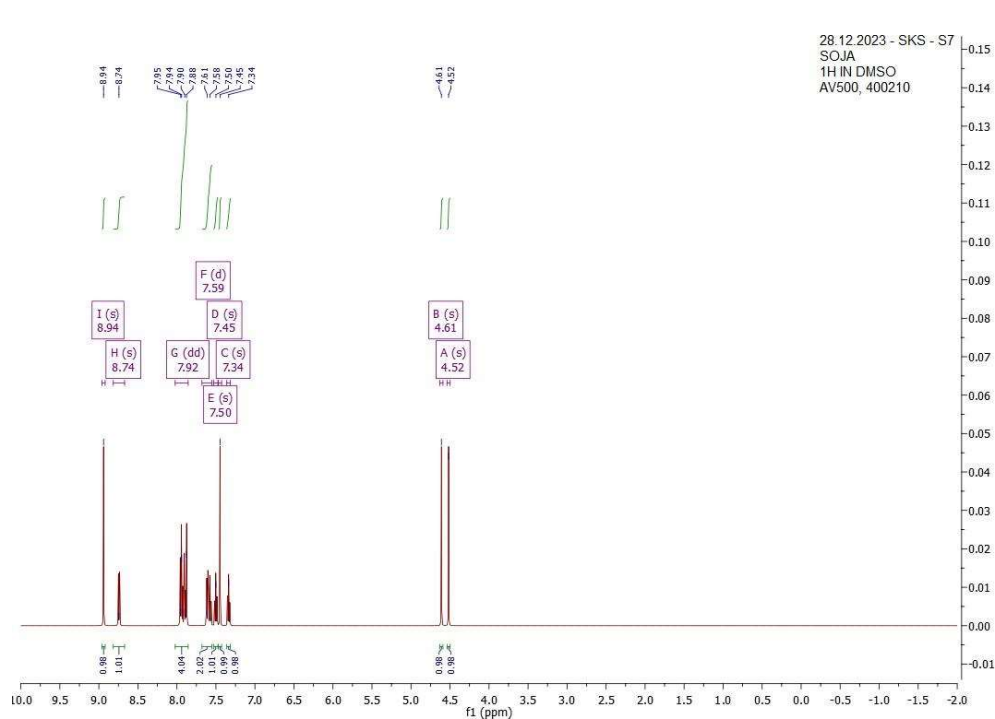


Figure 31: ^1H NMR Spectra for compound S7

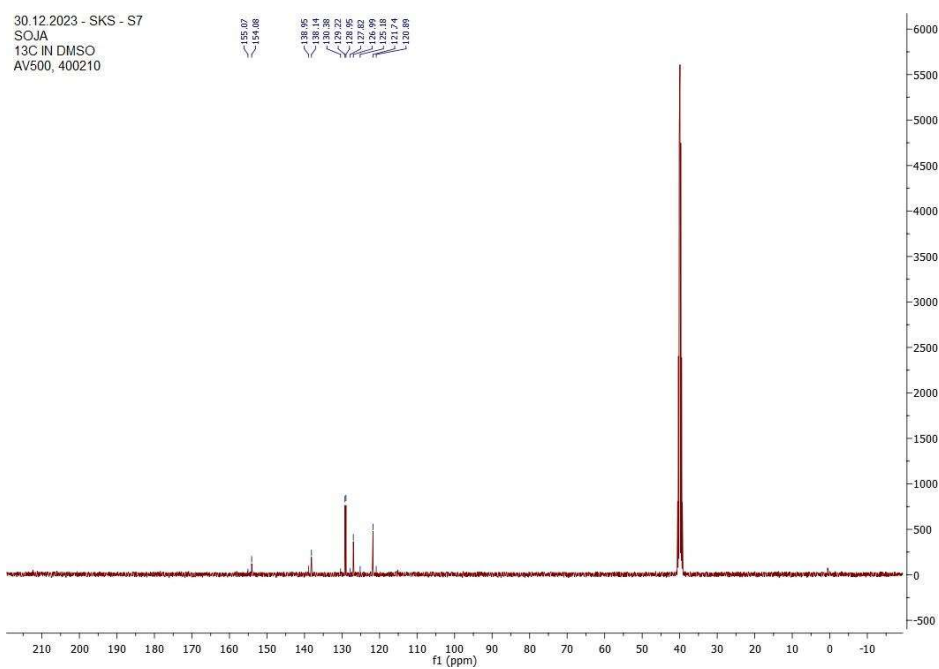


Figure 32: ^{13}C NMR Spectra for compound S7

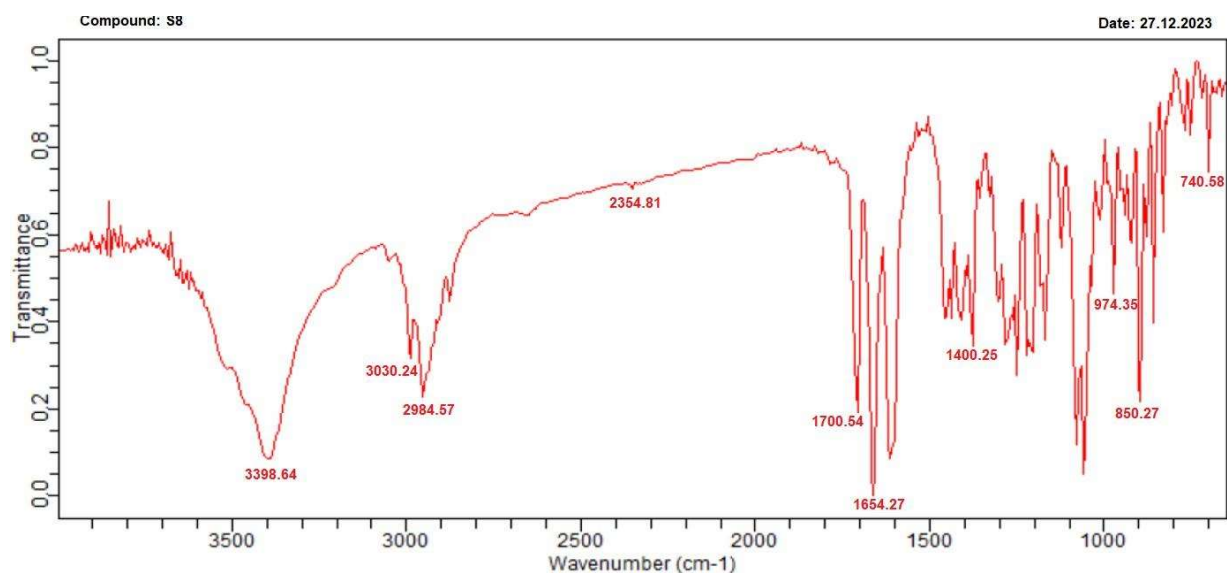


Figure 33: IR Spectra for compound S8

FTMS - p ESI Full ms [100.0000-1000.0000]

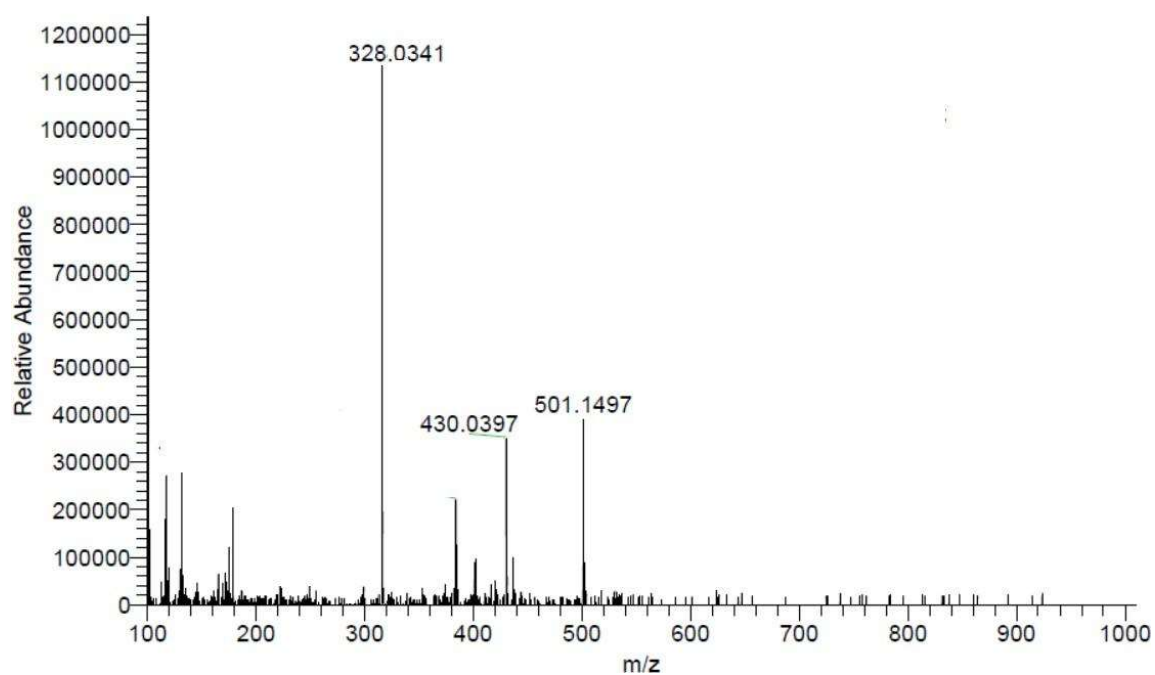


Figure 34: Mass Spectra for compound S8

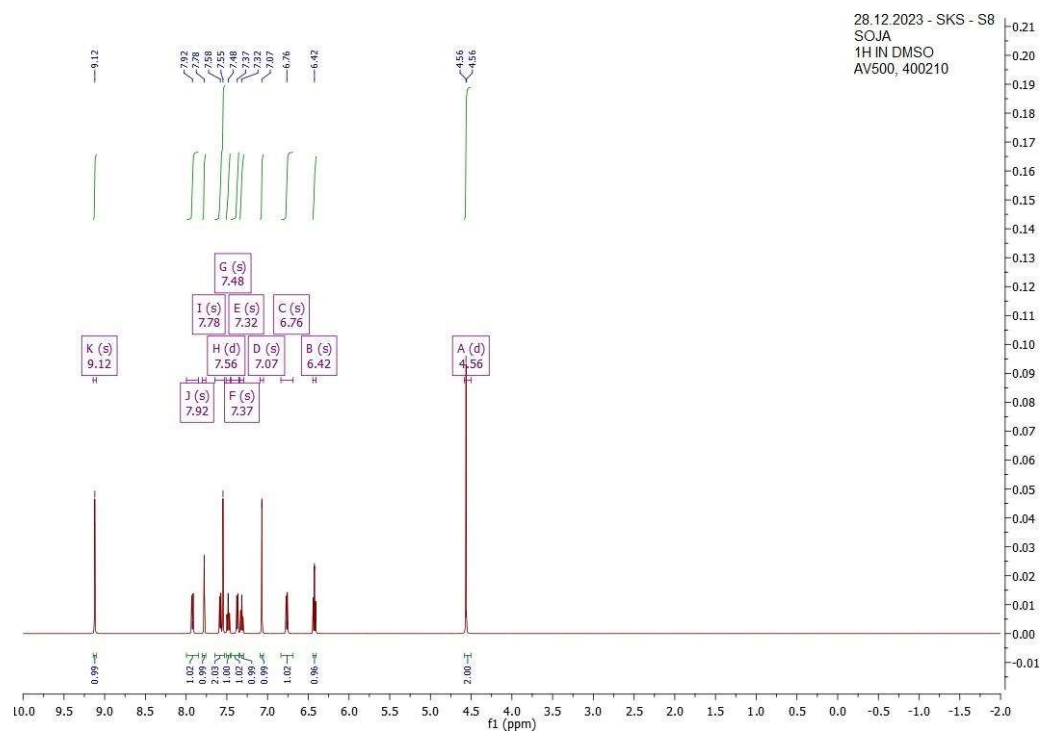


Figure 35: ^1H NMR Spectra for compound S8

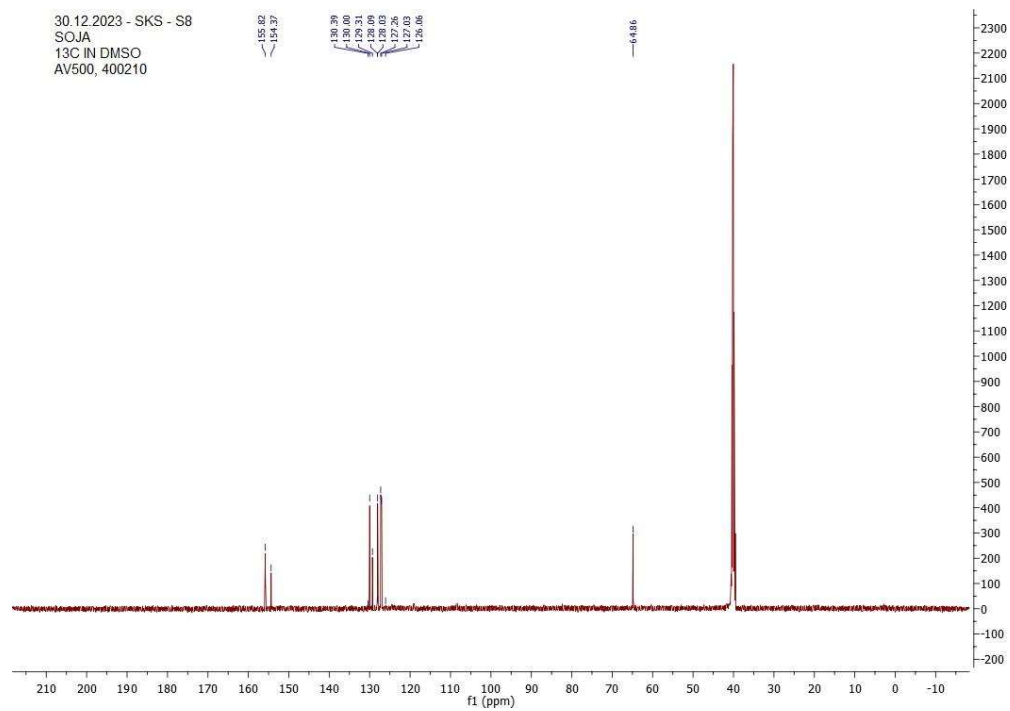


Figure 36: ^{13}C NMR Spectra for compound S8

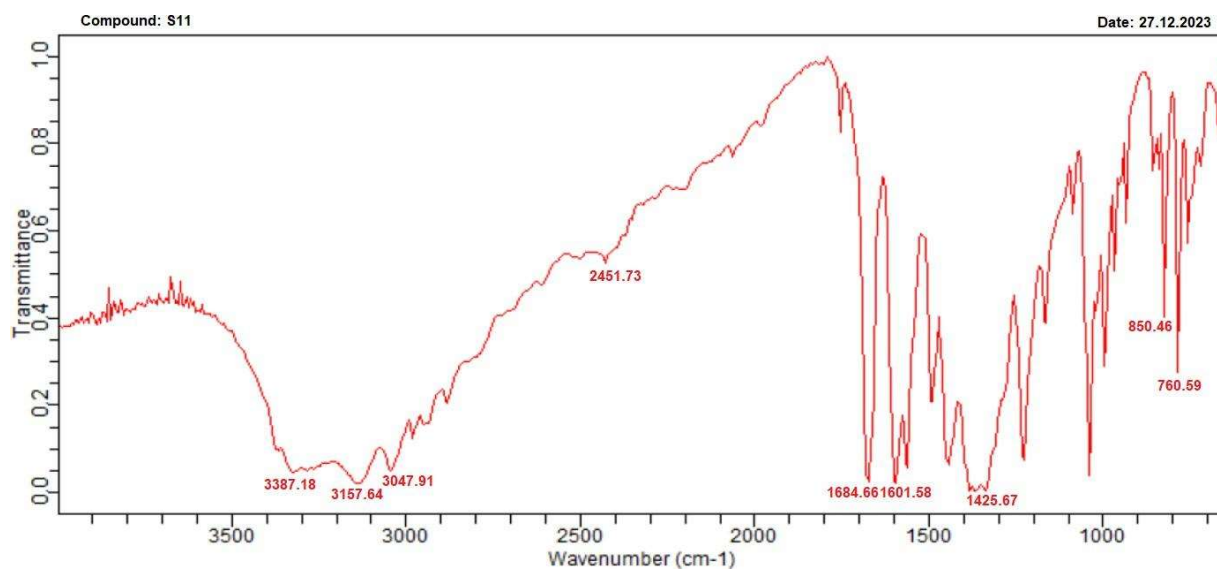


Figure 45: IR Spectra for compound S11

FTMS - p ESI Full ms2 530.1389@hcd15.00 [56.1762-561.7617]

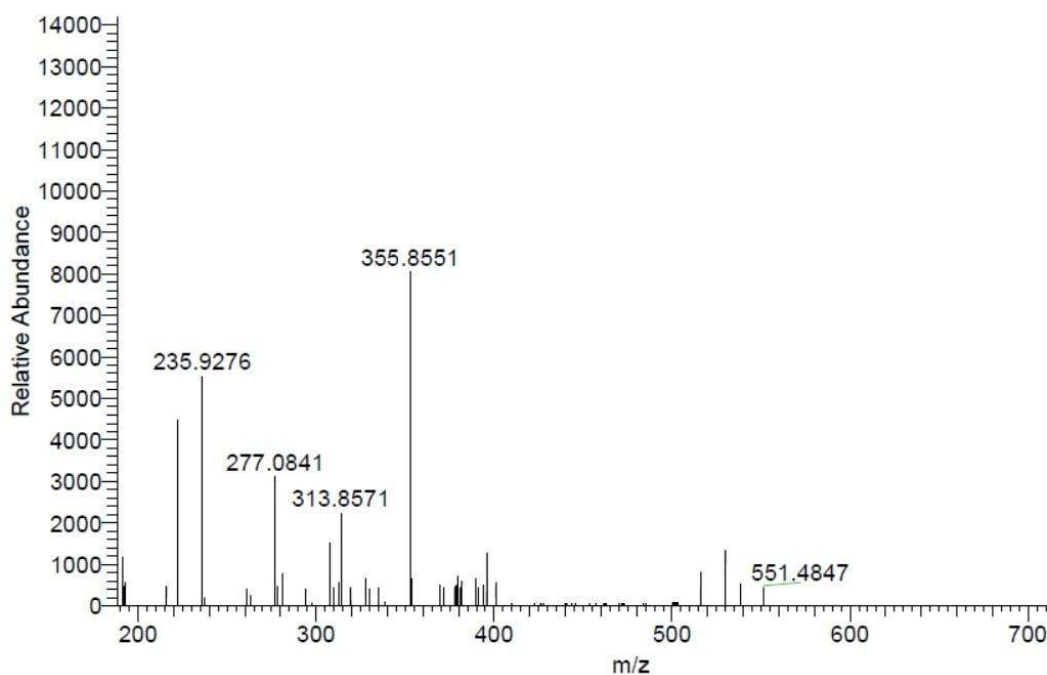


Figure 46: Mass Spectra for compound S11

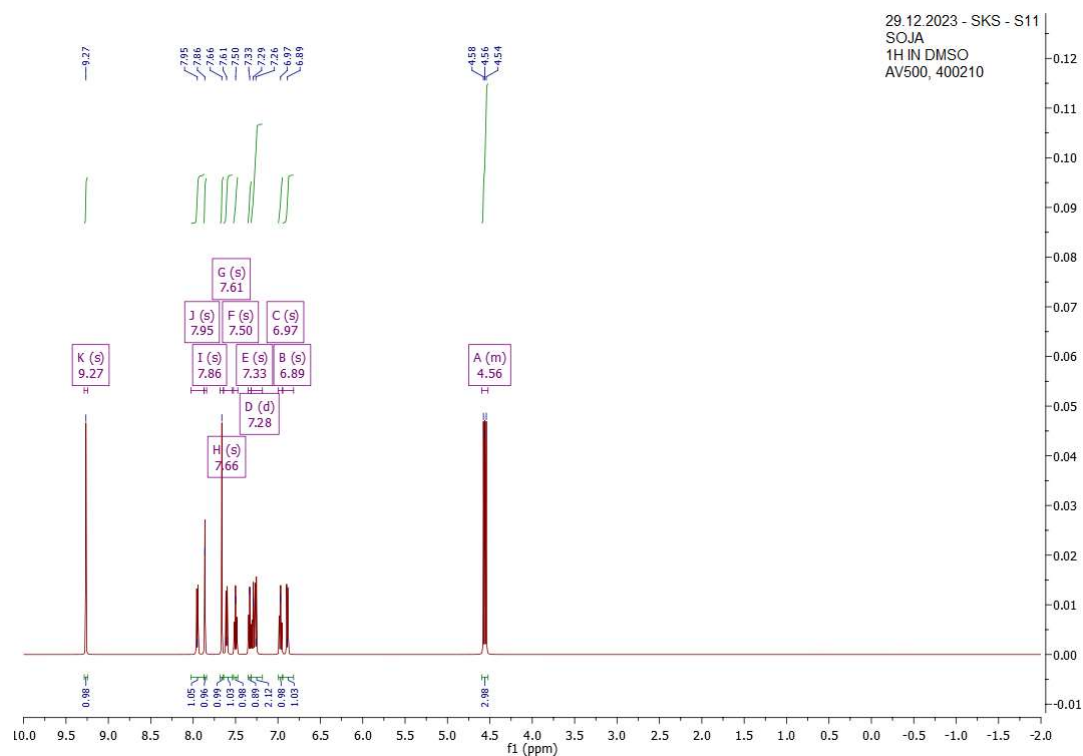


Figure 47: ^1H NMR Spectra for compound S11

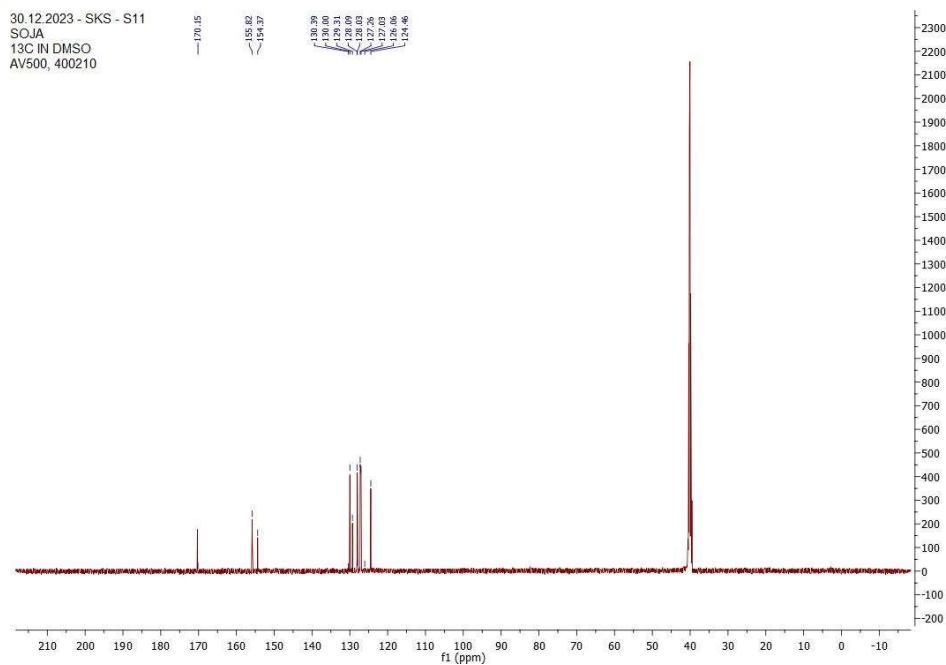


Figure 48: ^{13}C NMR Spectra for compound S11

Characterization of synthesized compounds

(E)-1-((2-chloroquinolin-3-yl)methyl)-3-(4-methylbenzylidene)thiourea (S3)

C₁₉H₁₆ClN₃S; Yellow colour solid; MP: 104 – 107°C; yield: 71%; Rf: 0.59; IR (KBr) cm⁻¹: 3320 (NH str amine), 3021 (CH str CH=CH), 2950 (CH str CH₂), 2154 (CH str aromatic), 1403 (C-N bending), 850 (Aromatic ring), 720 (C-Cl str) ; ¹H NMR (500 MHz, DMSO) δ 9.37 (s, 1H), 7.95 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.88 (d, *J* = 1.4 Hz, 1H), 7.61 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.50 (td, *J* = 7.5, 1.5 Hz, 1H), 7.44 – 7.37 (m, 3H), 7.34 (td, *J* = 7.5, 1.5 Hz, 1H), 7.25 (d, *J* = 7.5 Hz, 2H), 4.63 (s, 1H), 4.54 (s, 1H), 2.35 (s, 3H); ¹³C NMR (101 MHz, DMSO) δ 167.80, 167.41, 166.81, 135.51, 134.67, 134.35, 133.35, 132.10, 131.19, 130.08, 129.76, 129.72, 129.05, 18.74; Mass: Actual: 353; Found: 352 m/z (M-1) .

5.3.2(E)-1-((2-chloroquinolin-3-yl)methyl)-3-(pyridin-2-ylmethylene)thiourea (S6)

C₁₇H₁₃ClN₄S; white colour solid; MP: 106 – 109°C; yield: 63%; Rf: 0.59; IR (KBr) cm⁻¹: 3300 (NH str amine), 3071 (CH str CH=CH), 2950 (CH str CH₂), 2408 (CH str aromatic), 1435 (C-N bending), 850 (Aromatic ring), 720 (C-Cl str) ; ¹H NMR (500 MHz, DMSO) δ 8.94 (s, 1H), 8.74 (s, 1H), 7.92 (dd, *J* = 28.4, 8.5 Hz, 4H), 7.59 (d, *J* = 16.3 Hz, 2H), 7.50 (s, 1H), 7.45 (s, 1H), 7.34 (s, 1H), 4.61 (s, 1H), 4.52 (s, 1H); ¹³C NMR (101 MHz, DMSO) δ 155.07, 154.08, 138.95, 138.14, 130.38, 129.22, 128.95, 127.82, 126.99, 125.18, 121.74, 120.89; Mass: Actual: 340; Found: 340 m/z.

(E)-1-((2-chloroquinolin-3-yl)methyl)-3-(pyridin-4-ylmethylene)thiourea (S7) C₁₇H₁₃ClN₄S; white colour solid; MP: 115 – 119°C; yield: 62%; Rf: 0.57; IR (KBr) cm⁻¹: 3300 (NH str amine), 3071 (CH str CH=CH), 2950 (CH str CH₂), 2408 (CH str aromatic), 1435 (C-N bending), 850 (Aromatic ring), 720 (C-Cl str) ; ¹H NMR (500 MHz, DMSO) δ 8.94 (s, 1H), 8.74 (s, 1H), 7.92 (dd, *J* = 28.4, 8.5 Hz, 4H), 7.59 (d, *J* = 16.3 Hz, 2H), 7.50 (s, 1H), 7.45 (s, 1H), 7.34 (s, 1H), 4.61 (s, 1H), 4.52 (s, 1H); ¹³C NMR (101 MHz, DMSO) δ 155.07, 154.08, 138.95, 138.14, 130.38, 129.22, 128.95, 127.82, 126.99, 125.18, 121.74, 120.89; Mass: Actual: 340; Found: 340 m/z.

(E)-1-((1H-pyrrol-2-yl)methylene)-3-((2-chloroquinolin-3-yl)methyl)thiourea (S8)

C₁₆H₁₃ClN₄S; Brown colour solid; MP: 121 – 123°C; yield: 64%; Rf: 0.53; IR (KBr) cm⁻¹: 3398 (NH str amine), 3030 (CH str CH=CH), 2984 (CH str CH₂), 2354 (CH str aromatic), 1400 (C-N bending), 850 (Aromatic ring), 740 (C-Cl str); ¹H NMR (500 MHz, DMSO) δ 9.12 (s, 1H), 7.92 (s, 1H), 7.78 (s, 1H), 7.56 (d, *J* = 16.4 Hz, 2H), 7.48 (s, 1H), 7.37 (s, 1H), 7.32 (s, 1H), 7.07 (s, 1H), 6.76 (s, 1H), 6.42 (s, 1H), 4.56 (d, *J* = 2.6 Hz, 2H); ¹³C NMR (126 MHz, DMSO) δ 155.82, 154.37, 130.39, 130.00, 129.31, 128.09, 128.03, 127.26, 127.03, 124.46, 64.86; Mass: Actual: 328; Found: 328 m/z.

(E)-1-((2-chloroquinolin-3-yl)methyl)-3-(2-hydroxybenzylidene)thiourea (S11)

C₁₈H₁₄ClN₃OS; White colour solid; MP: 115 - 117°C; yield: 74%; Rf: 0.61; IR (KBr) cm⁻¹: 3387 (NH str amine), 3047 (CH str CH=CH), 2985 (CH str CH₂), 2451 (CH str aromatic), 1425 (C-N bending), 850 (Aromatic ring), 760 (C-Cl str); ¹H NMR (500 MHz, DMSO) δ 9.27 (s, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.66 (s, 1H), 7.61 (s, 1H), 7.50 (s, 1H), 7.33 (s, 1H), 7.28 (d, *J* = 15.2 Hz, 2H), 6.97 (s, 1H), 6.89 (s, 1H), 4.60 – 4.52 (m, 3H); ¹³C NMR (126 MHz, DMSO) δ 155.82, 155.82, 154.37, 134.50, 130.39, 130.00, 130.00, 129.31, 128.09, 128.03, 128.03, 127.26, 127.03, 126.06, 124.46, 124.46, 119.01, 108.40, 64.86; Mass: Actual: 355; Found: 355 m/z.

5.4 *In-vitro* anticancer activity

Results of anticancer activity of the compounds were expressed as IC₅₀ 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 IC₅₀ values were calculated by taking average of triplicate experimental results. The results of *in-vitro* anti-cancer activity expressed in IC₅₀ (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 **S6** have shown a significant IC₅₀ value (44.57 nM) in the tested cell lines. The **Table 3** shows the results for *in vitro* MTT assay of cell viability of the compounds.

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

Compound	IC ₅₀ (nM)
----------	-----------------------

S3	58.48
S6	44.57
S7	58.34
S8	49.29
S11	46.37
Olaparib	12.40
Niclosamide	23.54

CHAPTER – 6

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 both the targeted enzyme in molecular docking studies. 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. Compounds **S6** 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.

CHAPTER - 7

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