DESIGN AND SYNTHESIS OF NEW POTENTIAL ANTICANCER AGENTS

A Thesis Submitted to Gujarat Technological University

For the Award of

Doctor of Philosophy

in

PHARMACY

(PHARMACEUTICAL CHEMISTRY)

by

SHAH ASHISH PRASENJITKUMAR

[149997390003]

Under supervision of

Dr. CHHAGANBHAI N. PATEL

Professor & Principal

Shri Sarvajanik Pharmacy College

GUJARAT TECHNOLOGICAL UNIVERSITY

AHMEDABAD

[JUNE-2021]

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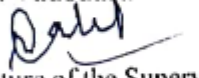

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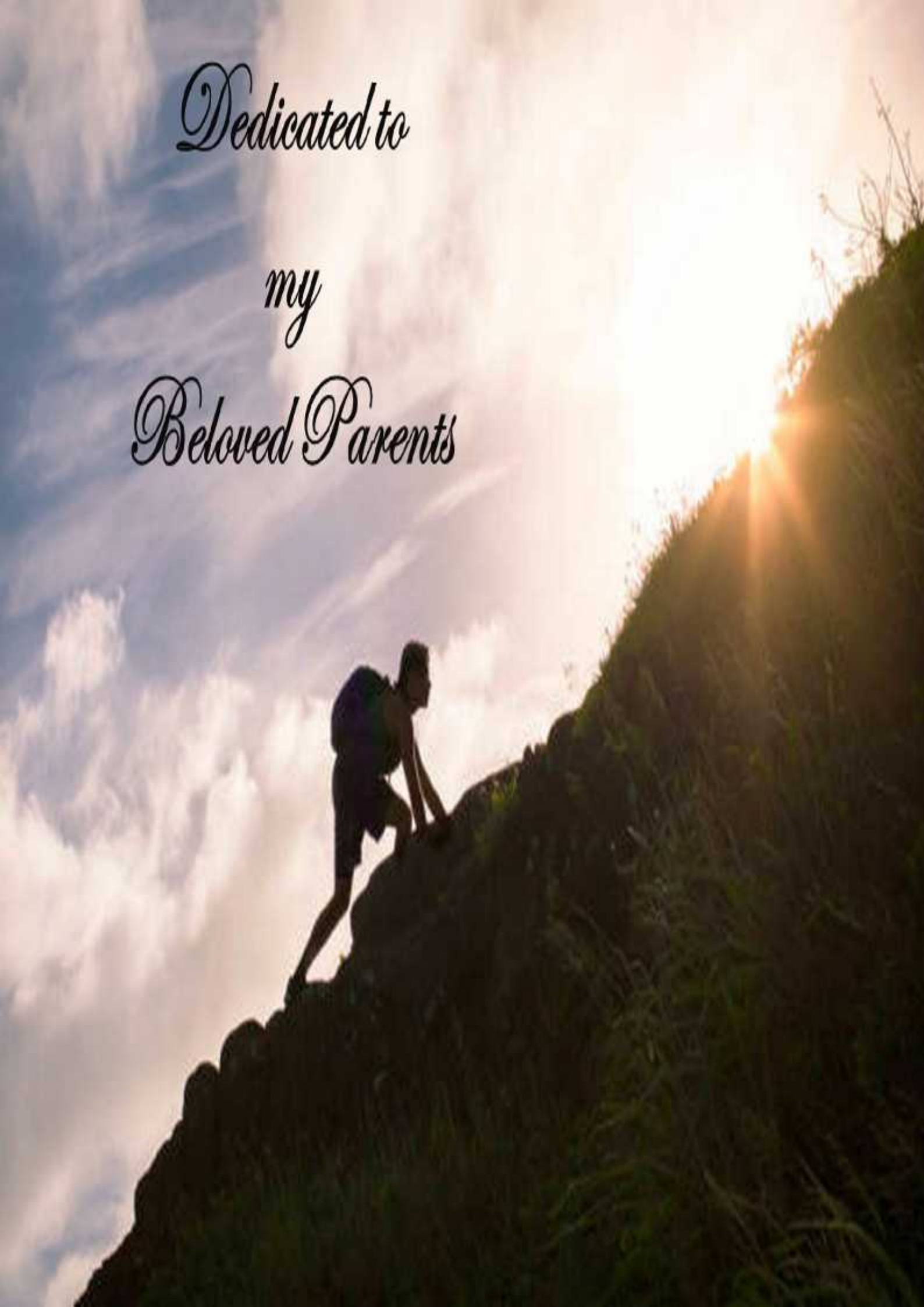
Abstract

Identification of a specific target is very important for safe and effective cancer chemotherapy. The tyrosine kinase (TK) family is considered as one of the important family members of the kinase family due to its important role in various cellular processes. Overexpression and dysfunction of tyrosine kinase receptors lead to the development of malignancy; thus, they are considered as one of the important targets for the development of anti-cancer molecules. Src is a prototypical member of the tyrosine kinase family who plays a critical role in the various cellular process like signal transduction, proliferation, survival, differentiation, etc. Mutation in Src leads to the development of different types of cancer. Three different series of benzothiazole-thiadiazol analogs (1a-7a, 1sa-7sa, and 1ib-4ib) were efficiently synthesized and evaluated for anti-cancer activity. The anti-cancer activities of all synthesized compounds were evaluated against two different cancer cell lines Caco-2 (colon) and MCF-7 (breast). Further validation of target was done by in-vitro enzyme inhibition study and insilico molecular docking studies. We found eight compounds namely **1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib** which were potent in anticancer evaluation, enzyme inhibition and also get high binding affinity in molecular docking studies. These results suggest that the potency of these compounds due to inhibition of the target Src-kinase. Finally, we want to conclude that these compounds should be further explored as Src inhibitors and they may have the potential to target Src mutated cancers.

Keywords: Molecular hybridization, benzothiazole, thiadiazole, NSAIDs, tyrosine kinase, Src, Cancer


Dedicated to
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Beloved Parents

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Place: VADODARA **SHAH ASHISH PRASENJITKUMAR**

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ABSTRACT

Identification of a specific target is very important for safe and effective cancer chemotherapy. The tyrosine kinase (TK) family is considered as one of the important family members of the kinase family due to its important role in various cellular processes. Overexpression and dysfunction of tyrosine kinase receptors lead to the development of malignancy; thus, they are considered as one of the important targets for the development of anti-cancer molecules. Src is a prototypical member of the tyrosine kinase family who plays a critical role in the various cellular process like signal transduction, proliferation, survival, differentiation, etc. Mutation in Src leads to the development of different types of cancer. Three different series of benzothiazole-thiadiazol analogs (1a-7a, 1sa-7sa, and 1ib-4ib) were efficiently synthesized and evaluated for anti-cancer activity. The anti-cancer activities of all synthesized compounds were evaluated against two different cancer cell lines Caco-2 (colon) and MCF-7 (breast). Further validation of target was done by in-vitro enzyme inhibition study and insilico molecular docking studies. We found eight compounds namely **1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib** which were potent in anticancer evaluation, enzyme inhibition and also get high binding affinity in molecular docking studies. These results suggest that the potency of these compounds due to inhibition of the target Src-kinase. Finally, we want to conclude that these compounds should be further explored as Src inhibitors and they may have the potential to target Src mutated cancers.

Keywords: Molecular hybridization, benzothiazole, thiadiazole, NSAIDs, tyrosine kinase, Src, cancer

CHAPTER NO. 1

INTRODUCTION

1 INTRODUCTION

In the field of science and technology, medicinal chemistry has been a fascinating subject. The rapid development in the last seven decades has been truly challenging and very exciting. Medicinal chemistry according to Burger, ***tries to be based on the ever-increasing hope that biochemical rationales for drug discovery may be found.***

Medicinal chemistry is the branch of science, which deals with synthesis of novel drugs with intense therapeutic activity. Medicinal chemistry concerned with discovery, development, identification, and interpretation of mode of action of biologically active compounds at molecular level(1) .

Human body made up of trillions of cells; they grow divides and dies in conventional manner. Normal/healthy cell controls their growth and when they become unhealthy, destroys by themselves. Sometimes due to dysfunction or error uncontrolled cell growth occurs, which leads to cancer. The cancer cells combine and form extra mass tissue known as tumour. It may not possible to find out the exact reason for cancer as cancer cells are modulated by culture condition and extracellular microenvironment condition. In worldwide nearly 7.6 million deaths are seen due to lung, stomach, liver, breast cancer mainly. Recently National Cancer Institute showed that the person who exposed to solvent, grease and oils, those who work in textile and plastic industries have high risk of cancer (2, 3).

1.1 TYPES OF CANCER

Carcinoma:

Arises from the epithelial cell linings of the internal surface of various organs (e.g. mouth, oesophagus, uterus)

Sarcoma:

Arises from the mesodermal cells constituting the various connective tissues (e.g. fibrous tissue, bone)

Lymphoma, myeloma and leukaemia:

Arising from the cells of the bone marrow and immune system**(4)**

Table 1 Types, causes and symptoms of cancer

Carcinoma			
Type	Example	Causes	Symptoms
Adenocarcinoma	Lung cancer	Cigarette smoking, Tobacco and alcohol intake, Air pollution, high levels of arsenic intake, radon gas etc	chest pain, joint pains, difficulty in breathing, loss of appetite, bone pains, blood in cough, difficulty in swallowing, weight loss suddenly, weakness, jaundice etc
Squamous cell carcinomas	Oral Cancer	Poor oral hygiene, taking immune-suppressants, Smoking, tobacco using and alcohol intake etc	Mouth ulcers, crack in the edge of the mouth, pale yellow, Difficulty in chewing, mouth sores, tongue problems, difficulty while speaking etc
Transitional cell carcinoma	Bladder Cancer	Cigarette smoking, polluted environment, workers of the industry like aluminium, rubber, leather, pesticide etc	Abdominal pain, blood in urine, weight loss etc
Basal cell carcinomas	Skin cancer	Exposure of UV, X-ray, heavy metals. Working in mines, industry of plastic etc	Change in colour size and shape of the skin, formation of ulcer etc
Sarcoma			
Example		Causes	Symptoms
Soft tissue sarcomas		Radiation therapy for long time, industry workers (like pesticide, heavy metals, certain chemicals)	Lumps found anywhere in the body. Pain in nerves and muscles.
Lymphoma			
Cancer of lymph cell in immune system		Age, gender, family history, exposure to certain chemicals and drugs, Autoimmune disease etc.	Chills, weight loss, fatigue, chest pain etc.

1.2 CHARACTERISTICS OF THE CANCER

1.2.1 Tumor suppresser genes:

In normal conditions if a cell starts divide excessively then to stop this neighbour will sent inhibiting factors that controls excess division. Tumor suppressor gene inhibits the signals of inhibiting factors. Due to this excess cell division will occur which will allow to grow tumor cells.

1.2.2 Angiogenic genes:

Angiogenesis is the process in which tumor blood vessels will develop separately. These blood vessels supply oxygen and nutrients and remove waste products from the cancer cells. Angiogenesis stars when cancer cells release molecules which send signals to surrounding normal host tissue. This signal activates the certain genes that encourage the growth of new blood vessels.

1.2.3 Proto-oncogenes:

Mutation turn normal gene into oncogene. Oncogene can activate signalling molecules like growth factors which will enhance the growth of cancer cells (5, 6).

1.3 SOME GENES INVOLVES COMMONLY IN CANCER:

- **XRCC1:**

This gene is involved in DNA repair process. They do complexes with DNA ligase-III enzyme and protein involved in repair of sing strand breaks of DNA. Single strand breaks are caused by mainly alkylating agents and radiation therapy. These gene bind with DNA ligase-III, polymerase and poly (ADP-ribose) polymerase and initiate the DNA repair process via base excision repair pathway.

- **EFGR:**

Epidermal growth factor receptors (EGFRs) are the cell surface receptors of EGF family of extracellular protein ligands. This family is closely related to the tyrosine kinase. Mutation in EGFR activity also causes cancer.

- **KRas:**

KRas protein belongs to Ras family and play important role in signal transduction pathways. KRas usually located in cell membranes. The protein formed by KRas play important role in normal cell signalling. Mutation KRas can also cause cancer.

- **P53:**

P53 is a tumor suppressor gene encoded by TP53 gene. P53 plays important role in cell cycle of multicellular organisms. P53 involved in preventing cancer. P53 gene is known as guardian of genome as it plays very important role in preventing genome mutation. Mutation or altering the function of P53 gene causes cancer.

- **BRCA1:**

BRCA is tumor suppressor gene present in human. BRCA plays important role in cell division process. BRCA gene keeps the cell in a normal condition and controls the rapid uncontrolled growth. Protein synthesized from BRCA gene directly involve in DNA repair process. Inhibition or alteration in the function of BRCA also causes the cancer.

1.4 TREATMENT OF CANCER

TABLE 16.2 Preventing Cancer through Diet and Lifestyle

Type of Cancer	Factors that Decrease Risk	Factors that Increase Risk
Breast	Engage in physical activity for at least 4 hours per week; consume lots of fruits and vegetables	Obesity and weight gain; alcohol consumption; hormone replacement therapy
Colorectal	Engage in regular, moderate physical activity; consume lots of fruits and vegetables	High intake of red meat; smoking; alcohol consumption; obesity
Lung	Consume at least 5 servings of fruits and vegetables daily	Tobacco use; some occupations
Oral/Throat	Consume at least 5 servings of fruits and vegetables daily; engage in regular, moderate physical activity	Tobacco use; obesity; alcohol consumption; salted foods
Prostate	Consume at least 5 servings of fruits and vegetables daily	High intake of red meat and high-fat dairy products
Stomach	Consume at least 5 servings of fruits and vegetables daily; refrigerate food	Salted foods; *Helicobacter pylori* bacteria

Here are some additional tips issued by a panel of cancer researchers:

- Avoid being underweight or overweight, and limit weight gain during adulthood to less than 11 pounds.
- If you don't get much exercise at work, take a 1-hour brisk walk or similar exercise daily, and exercise vigorously for at least 1 hour a week.
- Eat 8 or more servings a day of cereals and grains (such as rice, corn, breads, and pasta), legumes (such as peas), roots (such as beets, radishes, and carrots), tubers (such as potatoes), and plantains (including bananas).
- Limit consumption of refined sugar.
- Limit alcoholic drinks to less than 2 a day for men and 1 for women.
- Limit intake of red meat to less than 3 ounces a day, if eaten at all.
- Limit consumption of salted foods and use of cooking and table salt. Use herbs and spices to season foods.

Sources: World Cancer Research Fund, American Institute for Cancer Research, "Food, Nutrition and the Prevention of Cancer," www.wecf-uk.org; American Cancer Society, "The Complete Guide: Nutrition and Physical Activity," www.cancer.org.

Figure 1 Causes of Cancer

1.4.1 Anticancer agents:

There are three major groups of anticancer drugs:

1) Cytotoxic Drugs (largest group)

- Alkylating agents
- Antimetabolites
- Antitumor antibiotics
- Plant alkaloids
- Miscellaneous cytotoxic drugs

2) Hormones and hormone antagonists

These are among the best-tolerated chemotherapeutics because they target specific receptors, and thus only specific cell types e.g. Tamoxifen

3) Immunomodulators

- Immunostimulants, including interferons and interleukins
- Immunosuppressant

Alkylating Agents:

Mechanism of action:

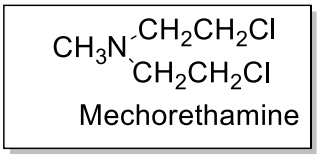
- These drugs work by alkylation with nucleophilic substitution. They alkylate a variety of cellular constituents, such as cell membranes, proteins, and most importantly DNA. More specifically, the nitrogenous bases of DNA are what get alkylated.
- The drugs start off as pro-drugs that become activated when a chlorine atom is extracted. A carbonium ion is thus formed. This "carbonium ion" is very electrophilic and will then attack any free pair of electrons (i.e. on the N7 of guanine). This electrophilic attack results in a bond being formed between the drug and the guanine of DNA. As a result of this "alkylation", there are a few consequences:

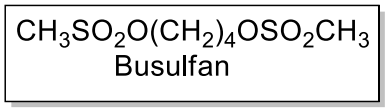
  1) **Miscoding (In transcription)**
  2) **Cross linking**- This only occurs if the drug is bifunctional

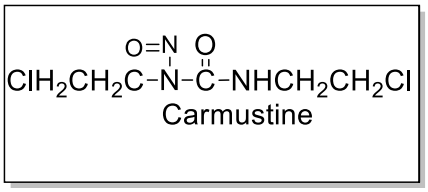
<u>Subgroups of Alkylating Agents</u>

- Nitrogen mustards: Ex. Mechlorethamine, cyclophosphamide, melphalan & chlorambucil
- Nitrosoureas: Ex. Carmustine, lomustine
- Alkyl sulfonates: Ex. Busulfan

- Aziridines: Ex. Benzotepa, thiotepa





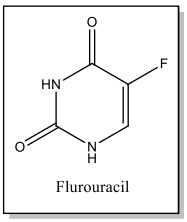


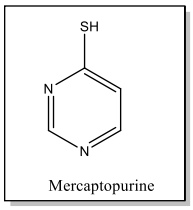
Anti-metabolites:

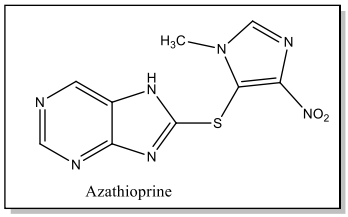
- An antimetabolite is a chemical with a similar structure to a metabolite required for normal biochemical reactions, yet different enough to interfere with the normal functions of cells, including cell division.
- All anti-metabolites are used in cancer treatment, as they interfere with DNA production and therefore cell division and the growth of tumors (mainly in S-phase specific).

They are classified into:

1. Folic acid analogues: Ex. Methotrexate
2. Purine analogues: Ex. Mercaptopurine
3. Pyrimidine analogues: Ex. Cyterabine, 6-flurouracil







Antibiotics:

Doxorubicin (adriamycin)

Mechanism of action

- Doxorubicin is anthracyclin antibiotic interferes with the cells' production of DNA and RNA by inserting itself between adjacent base pair causing local uncoiling thus blocking DNA and RNA synthesis.
- Also its antitumor effect is related to its inhibition of topoisomerase II enzyme (responsible for DNA repair).
- CYTP 450 catalyzes the conversion of Dox. into semiquinone free radicals which produce superoxide ion & H2O2 that mediate single strand scission in DNA

Mitomycin−C

- Mitomycin−C is an antitumor antibiotic. Mechanistically however, it belongs to DNA alkylating agents.
- Upon bioactivation inside the cell, it preferentially alkylates of guanine base in DNA leading to cross linking of DNA.
- It also degrades DNA through formation of free radicals.

Uses:

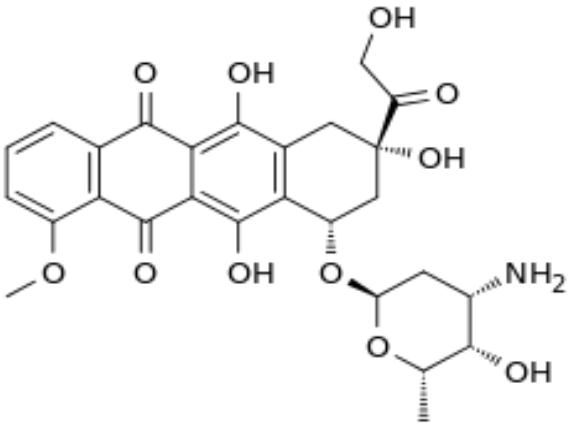
- Multiple cancers including breast, bone, ovarian &leukemia.
- Acute lymphocytic leukaemia (ALL).
- Non−Hodgkin's lymphoma

Bleomycin

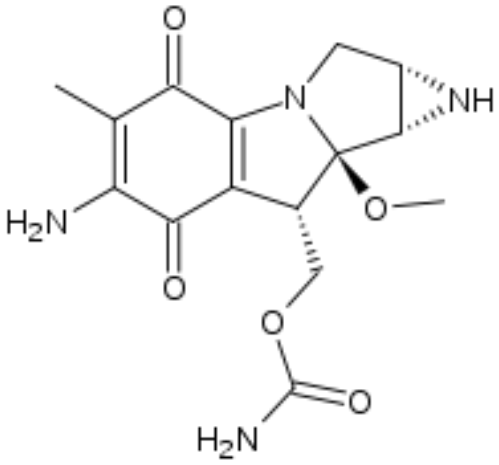
- It is cytotoxic in any phase of the cycle even on G0 phase
- Bleomycin degrades performed DNA causing chain fragmentation and release of free bases through the formation of free radicals (superoxide and hydroxyl radicals).

Uses

Bleomycin is used in the treatment of a number of different cancers, including cancer of the head and neck, skin, esophagus, lung, testis, and genitourinary tract. In addition, it is used in the treatment of Hodgkin's disease and non−Hodgkin's lymphomas.



Doxorubicin



Mitomycin

Natural Products:

The vinca alkaloids

Ex. Vincristine & vinblastine (M-phase)

Mechanism of action

Tubulin is a structural protein which polymerises to form microtubules. The cell cytoskeleton and mitotic spindle, amongst other things, are made of microtubules. *Vincristine* binds to tubulin inhibiting polymerization of microtubule structures. Disruption of the microtubules arrests mitosis in metaphase. The vinca alkaloids therefore affect all rapidly dividing cell types including cancer cells, but also intestinal epithelium and bone marrow.

Uses: Non-Hodgkin's & Hodgkin's disease, malignant lymphomas and leukemia.

Taxanes: Paclitaxel &docetaxel

It is used for treatment of lung, ovarian and breast cancer.

Mechanism of action

- Paclitaxel hyper-stabilizes microtubule structure (freeze them). Paclitaxel binds to the β subunit of tubulin, the resulting microtubule/paclitaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules is necessary for their function.

- Further research has indicated that paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function**.**

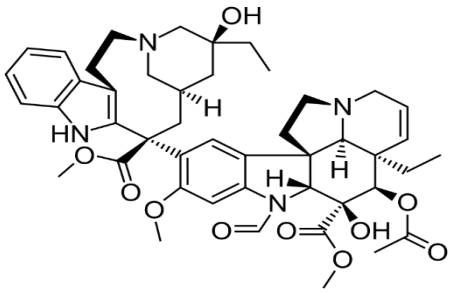
Etoposide

- Chemically it is derived from podophyllotoxin, a toxin found in the mandrake root.
- An inhibitor of the enzyme topoisomerase II which breaks in the DNA inside the cancer cells and prevent them from further dividing and multiplying. Then the cells die.

Uses

- It has been useful for treatment of testicular cancer and small cell lung cancer.

Vincristine:



1.4.2 Drugs acting on Hormones:

It involves the manipulation of the endocrine system through exogenous administration of specific hormones, particularly steroid hormones, or drugs which inhibit the production or activity of such hormones.

Because steroid hormones are powerful drivers of gene expression in certain cancer cells, changing the levels or activity of certain hormones can cause certain cancers to cease growing, or even undergo cell death.

Corticosteroids:

Corticosteroids are strong anti-inflammatory drugs.

They are used to reduce swelling that causes cancer pain.

1.5 TARGET BASED CANCER THERAPY

Researchers have identified some of the differences in cancer cells (in comparison with normal cell) and this difference (biomarker) help cancer cells to grow. The special activity of that biomarker is considered as target for cancer therapy. The researcher develops

specific drug which targets specific biomarker responsible for cancer. Treatment with these drugs is called *targeted therapy*. Targeted therapy is a special type of chemotherapy which has an advantage to difference normal and cancer cells. Targeted therapy is sometime used alone or with combination therapy.

Targeted therapy drugs are used to treat different types of cancer so these drugs consider as chemotherapeutic drugs but the way of working is different than standard chemotherapeutic drugs. These drugs have different side effects than standard chemotherapeutic drugs. For example, many targeted drugs go after the cancer cells' inner workings – the programming that makes them different from normal, healthy cells, while leaving most healthy cells alone.

1.5.1 How does targeted cancer therapy work?

Most of the standard chemotherapeutic drugs kill cells in the body which grow and divide fast as the cancer cell grow and divide quickly these drugs are effective. The problem of standard chemo drugs are they also kills normal body cells that grow and divide quickly which produce sometimes serious side effects. Target therapy has overcome this problem as the way of working is different. Cancer cells have many changes in their genes which are not observed in normal cells. These types of genetic changes are responsible for development of cancer cells. The mechanisms for formation of cancer are different and due to this all cancer are not same. For example, colon cancer and breast cancer have different genes that help them to grow. Sometimes different genes are responsible for the development of same type of cancer.

Targeted drugs can work to:

- **Block or turn off chemical signals** that tell the cancer cell to grow and divide
- **Change proteins** within the cancer cells so the cells die
- **Stop making new blood vessels** to feed the cancer cells
- **Trigger your immune system** to kill the cancer cells
- **Carry toxins to the cancer cells** to kill them, but not normal cells

Some targeted drugs are more "targeted" than others. Some might target only a single change in cancer cells, while others can affect several different changes. Others boost the way your body fights the cancer cells (7).

CHAPTER NO. 2

LITERATURE SURVEY

2 LITERATURE SURVEY

2.1 ROLE OF TYROSINE KINASE IN CANCER:

Protein phosphorylation is the most common process occurs in cell cycle. Phosphorylation is controlled by various specific kinase and phosphates that may add or remove phosphates (8). The signalling networks which work though phosphorylation have significant action on cellular function. Protein kinases are second most important drug targets after G-protein couple receptor (9). Abnormal kinase activities observed in various diseases like cancer, rheumatoid arthritis, cardiovascular neurological disorders, asthma and psoriasis. Kinase specific pathways have important role on proliferation, survival, motility, metabolism and angiogenesis of tumor cells (10).

Kinases are involved in almost every cellular process like signal transduction, regulation, proliferation, apoptosis etc. The major function of kinase is to catalyse the process which transfers the gama-phosphate group of ATP to the substrate. Kinase receptors are located at key position in the cell form where they maintain intracellular and extracellular communication. This communication is regulated by various receptor and non-receptor tyrosine kinases. Extra cellular communication regulated by specific signalling receptors and intra cellular communication regulated through secondary signalling molecules. Kinases play important role in signalling transmission, termination and communication(8-11).

2.2 BIOCHEMICAL ACTION OF TYROSINE KINASE:

Tyrosine kinases are group of enzymes that are engaging in selectively phosphorylation of tyrosine substrates which are activated by binding of ligand with their respective extracellular domain(12). This extracellular domain includes various signalling molecules like EGFR, FGFR, PDGFR, VEGFR, HGFR etc. Ligands bind with receptor and form ligand receptor complex in combination with 1:2 (one ligand binds with two receptors) or 2:2 (two ligands binds simultaneously with two receptors. e.g. VEGF and VEGFR. The receptor dimerization is also stabilized by receptor–receptor interactions. Some ligand receptor is not sufficient for some complex and is stabilized by accessory molecules e.g. FGFs are unable to activate FGFR complex and is stabilized by heparin sulfatoglycans (HSPG). Ligand binding to the extracellular domain stabilizes the formation of active dimmers and consequently protein tyrosine kinase activation.

Studies suggest that catalytic domain of several RTKs, based on the evidence of biochemical and kinetic studies of receptor phosphorylation provides acceleration of oligomerization process which increase the concentration of RTKs leads to efficient transphosphorylation of tyrosine residues for the activation of the loop in the catalytic domain. Activation of loop provides open conformation that gives access of ATP and substrates and transfer ATP to tyrosine residue form Mg-ATP and this process involve in signal transduction. The ATP binding sites act as a docking site for proteins which contains Src homology-II and protein tyrosine binding domains. These proteins provide molecules having different domains like having SH2, SH3, PTB and Pleckstrin homology (PH) domain. Overall, this process activates receptors and membrane that drives the signals which leads to activation of various genes which gives biological response (13, 14).

2.1.1 Receptor tyrosine kinase:

Growth factors present in cell membrane or cytoplasm and have role in cell growth, angiogenesis and metastasis. Amplification of growth factors may responsible for anti-apoptosis process and resistance of chemotherapeutic drugs. There are various types of GFRS which is responsible various biological processes and abnormalities of GFRs lead to development of various types of cancers(15, 16).

2.2.1.1 Epidermal growth factor receptor

The epidermal growth factor receptor contains four different types ErbB/EGFR-1 to 4. These receptors expressed mainly in cell surface and gives tyrosine kinase activities. The basic structure of EGFR contains three different domains: an extracellular domain (ligand binding site), transmembrane domain and intracellular domain with kinase activity. The receptors forms homo or hetero dimmers upon binding with ligand and promotes signalling for cell proliferation, survival migration, differentiation etc and thus playing important role in development of cancer. Overexpression of EGFR found in different cancer like breast, lung, bladder etc (17, 18).

2.2.1.2 Vascular endothelial growth factor receptor

The Vascular endothelial growth factor receptor contains three domains VEGFR 1-3 and mainly expressed in endothelial cells. On extracellular site VEFGR contains seven immunoglobulins like domains and in the intracellular it contains two split tyrosine kinase domains. They bind with homodimer or heterodimer of VEGFR and placenta growth factors (PIGF 1and 2) and form homo or heterodimer of VEGFR-1 and VEGFR-2 which gives the signal inside. The signals transduced by VEGFR are different based on the receptors. For

example, VEGFR2 induces mitogen-activated protein kinases (MAPK)- dependent cell proliferation whereas VEGFR1does not induce cell growth. VEGFR plays important role in angiogenesis. Overexpression of VEGFR found in different cancer like brain, breast, colon, gastric etc(19-21).

2.2.1.3 Fibroblast growth factor receptor

This growth factor family consists of four transmembrane proteins FGFR 1-4. All these proteins have different isoforms and due to this they have different ligand specificity. Out of four proteins; three are located in extra cellular domain and one in intracellular domain with kinase activity at carboxy terminal. Eighteen different ligands that can bind with different FGF receptors and upon binding dimerization is occur which leads to autophosphorylation and kinase activation. These phosphorylated proteins serve as binding site for many effectors and upon binding they play important role in cellular process like cell proliferation, growth, differentiation, migration etc. FGF/R plays important role in formation of tumor by giving anti-apoptosis signals. FGFRs have also important role in angiogenesis process involving in both paracrine and autocrine process. It may also cause the resistance of several anticancer drug due to mutation of FGFRs especially the drugs which targets EGFR, VEGFR and PDFGR. Amplification and mutations in *FGFR* genes that lead to constitutive activation/up-regulation of receptors are found indifferent types of malignancies, including breast, ovarian, gastric and lung cancers (22-24).

2.2.1.4 Platelet derived growth factor receptor

These family consist of two types PDGFR-α and PDGFR-β encoded by two different genes which are expressed on the membranes of different types of cells. In extracellular domain five Ig like receptor protein and tyrosine kinase domain present in the intracellular domain on one single chain of protein. For phosphorylation process conformational changes occurs due to dimerization process upon receptor binding with ligand. The whole process depends on the signal received via MAPK or PI3K pathways and due to this play important role in regulation of cell proliferation, differentiation, growth, migration, and survival. PDGFR have also role in angiogenesis and thus supporting in tumor growth(25, 26). Mutation in PDGFR may responsible for diverse types of cancers like glioblastoma, GI tumors and chronic leukaemia(27, 28).

2.2.1.5 Insulin-like growth factor receptor

This family consists of two different types IGF1R and IGF2R. IGF1R forms hetero-dimer with insulin receptor has higher binding affinity as compared to IGF2R to elicit the growth signal required for foetal and postnatal development. After the translation process IGF1R modified and contains one alpha and one beta chain that connected by disulfide bond and expressed on the cell surface. The mature IGF1R is a homodimer comprising the α2 and β2 chains linked by disulfidebonds(29). The tyrosine kinase activity of intracellular domain which auto phosphorylates the receptor and other binding proteins (30).Overexpression of *IGF1R* gene is implicated in cellular proliferation, transformation and metastasis in several carcinomas. Amplification of *IGF1R* gene in breast cancer and melanoma and overexpression of *IGF1R* gene in paediatric cancer has been reported (31).

2.2.1.6 Transforming growth factor-beta receptor

The receptor family comprises of three membrane receptors namely TβRI, TβRII and TβRIII which are expressed in various cells and have diverse types of cellular response based on the signal transduction process. TβRI and TβRII are serine/threonine kinase which contains N-terminal ectodomain and C-terminal kinase domain. TβRIII protein doesn't possess any intracellular domain. They bind with TβRII ligands and initiate transphosphorylation of TβRI which activates SMAD (homologues of the Drosophila protein proteins) (32). SMAD proteins translocated into nucleus and function as transcription a factor which regulates cell proliferation, survival, migration and differentiation. Under the physiological conditions TGF-β receptors prevent cell growth, stimulates apoptosis or differentiation while in tumorigenesis conditions receptor mediated signals promotes cell growth due to genetic mutations (33). For example, down-regulation of *TGF-βRII* gene leads to development of breast and lung cancer and different mutations responsible for colon and pancreatic cancer (34).

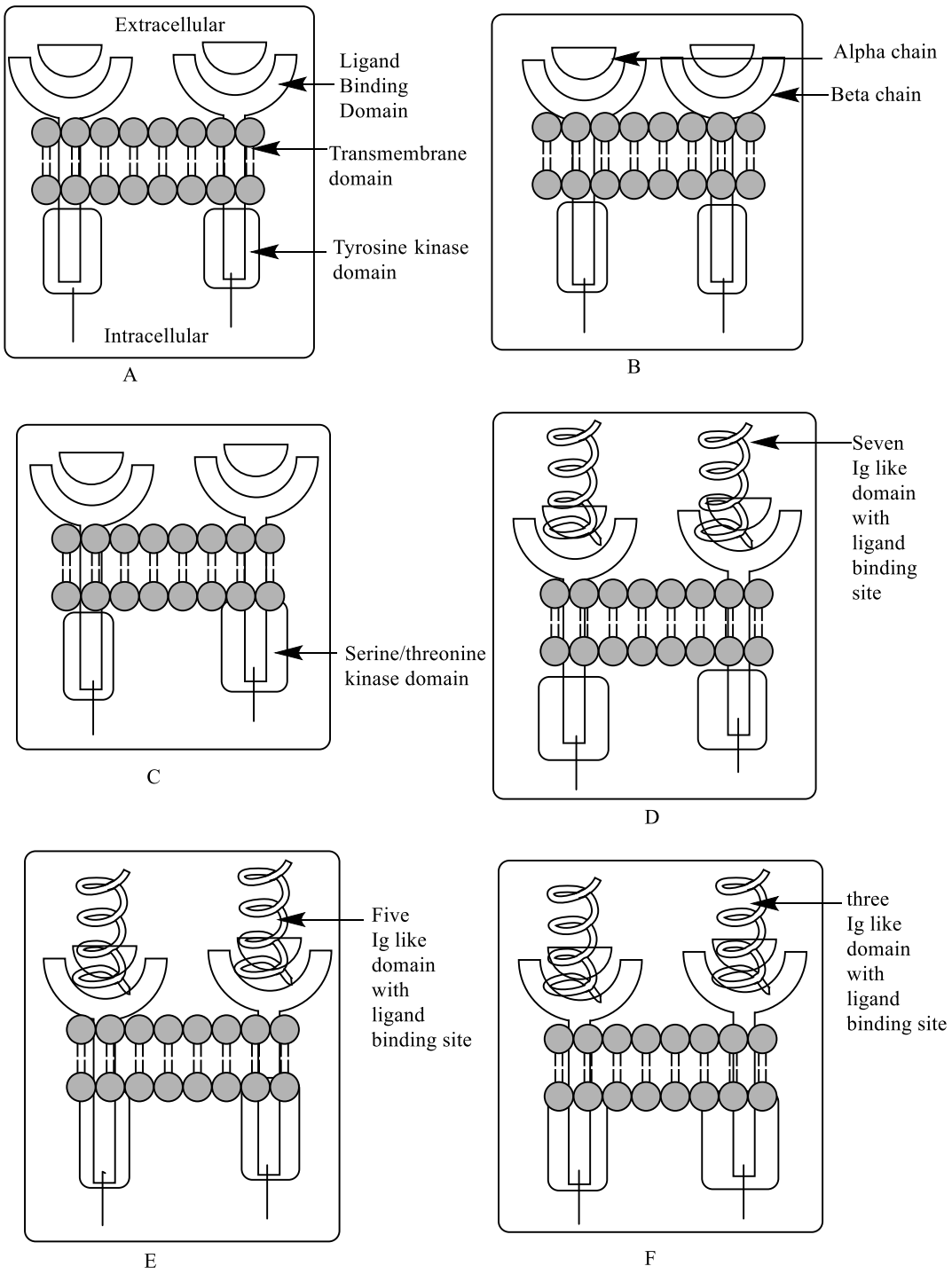


Figure 2 Domain organization of the major families of RTKs

2.2.2 Non-Receptor tyrosine kinase (NRTKs):

NRTKs are subdivided into nine main families based on structural domain features which includes Src homology -1 (SH1), p-Tyr binding Src homology-2 (SH2), protein–protein interaction Src homology-3 (SH3).

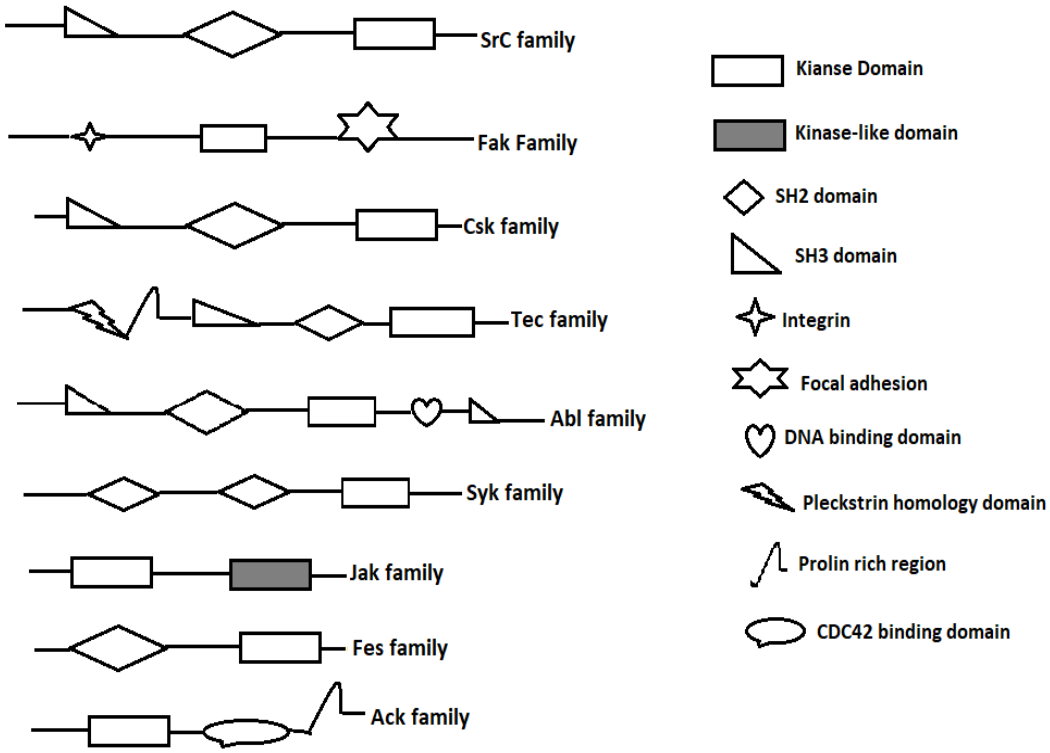


Figure 3 Domain organization of the major families of NRTKs

2.2.2.1 Src family:

Src is prototypical member of tyrosine kinase family, discovered before four decades from the rous avian sarcoma virus as first oncogene. c-src belongs to family of non-receptor tyrosine kinase which is about 60KDa in size. Src plays critical role in various cellular process like signal transduction, proliferation, survival, differentiation etc(35, 36). The structure of c-Src contains seven different parts which includes: SH4 domain, SH3 domain, SH2 domain, SH1 catalytic domain, SH2-SH3 linker, unique domain and C-terminal negative regulatory region. SH1 domain is called as catalytic domain as it is autophoshorylated by active c-Src. Conformational changes occur in active and inactive state of Src protein. Catalytic and non-catalytic domain activity is important in signal transduction process. The active conformation of Src is formed by assembling in between SH3 and SH2 domain. The inactive conformation formed intramolecular bonding of SH2 with C-terminal of c-Src(37, 38). Src plays important role in tumor metastasis, as it has role in cell migration, adhesion

and invasion and as a result of this tumor microenvironment is maintained(39). In hypoxic condition, Src activation stimulates endothelial growth factor (VEGF), matrix metallopeptidase (MMPs) and interleukin-8 (IL-8) expression and due to this angiogenesis rate is increases(39, 40). Src-mediated VEGF secretion elicits angiogenicsignaling in endothelial cells and Src activation in osteoclasts facilitates osteolytic bone metastasis(40).

2.2.2.2 Csk family:

C-terminal Src kinase (Csk) family served as negative regulator of Src family tyrosine kinse (SFKs) which phosphorylates the negative regulatory sites of SFKs and suppress oncogenic potential. It is regulated through SH2 domain which relies on membrane translocation of Csk by binding with various scaffold proteins(41). The binding of scaffold protein with SH2 domain can increase the kinase activity(42). Upregulation of Csk observed in different types of cancers, such as hepatocellular, colorectal and non-small lung cancer(42-44).

2.2.2.3 Fak family

Focal adhesion kinase (Fak) family consist of two kinase namely; proline rich NRTKs-Fak and related adhesion protein kinase (Raftk) which have closely related structure without SH2 and SH3 domain with molecular masses in between 110 -125 kDa. Fak found in almost all tissues while Raftk generally more found in central nervous system and hematopoietic cells. The signalling of Fak and Raftk observed in various cell stimuli such as hormones, growth factors, cytokines etc.(45, 46).

2.2.2.4 Tec family

Tec family kinase family have five different members namely; Bruton's tyrosine kinase (Btk), inducible T-cell kinase (Itk), Tec (tyrosine kinase expressed in hepatocellular carcinoma), bone marrow – expressed kinase (Bmx); epithelial and endothelial tyrosine kinase (Etk) and resting lymphocyte kinase (Rlk). This family distinguishes from other NRTKs based on two different properties; proline-rich region and a pleckstrin homology domain(47, 48). This family specifically expressed in liver and kidney and involved in regulation of multiple cellular process. The activation of Tec receptor done by downstream of various receptors which includes RTKs, cytokine receptors, antigen receptors, integrins and G-protein-coupled receptors(48). Evidence suggest that Tec kinase can affect variety of cellular signalling pathways which includes controlling actin reorganization, cell adhesion and migration, transcriptional regulation, or apoptosis (49). The activation of Tac receptors occurring in two steps: first is translocation of Tec to the cell membrane and second is

phosphorylation of tyrosine in Tec domain. The first step is catalysed by interaction of PH domain with phospholipids and second step is catalysed by Src family kinase (50, 51).

2.2.2.5 Abl family:

Abelson tyrosine kinase family has two members namely; c-Abl and Arg which are localized in cell membrane, cytosol, cell membrane and actin cytoskeleton. In the normal cell function of this protein includes cell adhesion, motility, DNA damage response, microbial pathogenic response etc. Mutation of c-Abl kinase may lead to several types of cancer which includes breast, colon and non-small cell lung cancer (52, 53). Upon phosphorylation of C-Abl oncogenic signalling pathways are activated by activation ofERK5, Rac/Jnk, and Stat 1/3 pathways. It can also form myeloid leukaemia via oncogenic fusion of protein with Bcr via translocation of chromosome 9 to 22(54). Imatinib is FDA approved drug which inhibits Abl via blocking kinase activation by binding with ATP-pocket of Abl(55).

2.2.2.6 Syk family

Spleen tyrosine kinase widely expressed in the cell involved in the cells related to the immune system such as T cells, B cells and macrophages. The protein is about 72-kDa in size and involved in signalling of the immune receptors which includes T-cell receptors, B-cell receptor and integrins. These immune receptors require tyrosine-based activation for signalling (56, 57). Recently, it was found that Sy3/PI3K dependent pathways involved in the survival of the diffuse large B-cell lymphoma which on overactivation stimulated NF-kB activity to stimulate the anti-apoptotic signals which escapes tumour cell death(58). The discovery of Fostamatinib an FDA approved drug opens newer direction to discover small molecules which target Syk for the treatment of lymphomas (59).

*2.1.2.7***Jak family:**

Jak family consist of four members Jak1, Jak2, Jak3 and tyrosine kinase-2 (tyk2). All of the receptors have similar structure which characterized by two kinase domains out of that one is catalytically inactive. Downstream signalling of insulin receptor, tyrosine kinase receptor and G-protein coupled receptor were found in the presence of Jak. Their action also found in activation of signal transduction and transcription (stat) factors. Through the stat pathway Jak activates several cellular processes like cell differentiation, proliferation and cell stress by providing signalling from outside the cell through the cell membrane to the promotor gene(60, 61). Jak family phosphorylates the stat and dimerizes to translocate into the cell nucleus(62). Jaks are important biomarkers in several biological process like

inflammation, maintenance of immunity and haematopoiesis and mutation in Jak/stat pathways lead to development of rheumatoid arthritis, psoriasis, polycythaemia vera, and myeloproliferative diseases. Mutation in gene leads to over activation of tyrosine kinase signalling which provide signals against the cell apoptosis. Jak2 bind with growth hormone and forms a complex which activates several signalling pathways that includes Stat and MAP kinase pathways(63, 64). Complex of Jak2 with leptin required for the activation of Stat3 and Stat5 signalling. Tyk2 mutation responsible for increased in susceptibility of microbial infection, allergic asthma and hyper IgE syndrome. Overexpression of Tyk2 receptors leads development of various types of cancers such as prostate, breast and cervical(65, 66).

2.2.2.7 Fes family:

Fes family consists of two members: Feline sarcoma (Fes) and its homologues related protein (Fer). Activation of Fes give downstream signal to several classed of receptors which involved in hematopoietic cell development, inflammatory mediator release, survival, migration, and angiogenesis(67). From the studies it was found that Fes play important role in regulation of cytoskeletal rearrangements provide outside signals that produce cell-cell, receptor ligand and cell-matrix interactions. It was found Src and Fer together play important role in angiogenesis(68). In primary activated myeloid leukaemia (AML) both Fes and Fer found in activated stage and this activation done by binding with Flt3 (fms-like tyrosine kinase 3). Flt3 in activated stage, dimerize and auto phosphorylated which producing signals that increase cell growth and inhibit apoptosis. Mutation in Flt3 produces ligand- independent dimerization which activates downstream signalling pathways such as MAP kinase, PI3-kinase, p38, LYN and Stat5 which leads to cytokine independent proliferation. In concluding remarks Fer requires for cell cycle transition and Fes necessary for cell survival and both are activated in downstream signalling of Fit3-ITD (internal tandem duplication-ITD)(69-71).

2.2.2.8 Ack family:

Ack family is considered as very unique in their structural features and properties which includes Tnk1 and Tank2 receptors. Human Ack1 (activated Cdc42-associated kinase, also known as Tnk2) is majorly expressed spleen thymus and brain. This protein is activated by signalling through growth factors and integrin mediated cell adhesion. Overexpression of ack1 found in breast, prostate and renal cancers(72, 73). Activation of Act1 can be possible in presence of EGF which binds with EGFR via EGFR binding domain. Tnk1 is type of non-receptor tyrosine kinase which is activated in negative cell regulation condition by inhibition

of Ras signalling pathways. For this reason, in an interesting contrast to Tnk2/Ack1, Tnk1/Kos1 is believed to have tumour suppressor activity(74, 75).

2.2.3 Tyrosine kinase inhibitors:

Tyrosine kinase inhibitors are the group of the drugs which majorly targets intracellular signalling pathways which includes active site of kinase and preventing phosphorylation of intracellular targets(76, 77). As of now there are more than 40 RTK inhibitors were approved by FDA to treat the different types of cancers(78). These agents majorly divided into type-I, type-II, type-III, type-IV and type-V inhibitors. Type-I inhibitors binds either reversibly or irreversibly with tyrosine kinase and inhibit the phosphorylation process. Reversible inhibitors bind covalently near ATP binding site while irreversible binds non covalently and they are ATP competitive inhibitors. Type-II inhibitors bind to a site adjacent to the ATP site of inactive kinases and maintain their inactive conformation. This type of inhibitor is usually nonselective. Type-III inhibitors are allosteric inhibitor which binds selectively near to the allosteric site, remote from ATP site and hinge region. Type-IV inhibitors are subtract-directed inhibitors which targets subtract binding site in irreversible manner(79). Type-V are covalent kinase inhibitor which binds irreversibly with kinase active site(80).

RTKIs are used in treatment of various types of cancer which includes non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), breast cancer, hepatocellular carcinoma, colorectal cancer and myeloid leukemia. Drug like erlotinib and gefitinib reported as first-generation EGFR inhibitors to treat the NSCLC patients which act as reversible inhibitors of ATP-binding sites while drug like afatinib and dacomitinib are second generation RTKIs which act as irreversible inhibitors with specificity for EGFR kinase domain(81). Drug like sunitinib and pazopanib used in the treatment of patients suffering from RCC(82). The systemic therapy of hepatocellular carcinoma focused on multitarget kinase inhibitors(83). Drug like sorafenib and Lenvatinib (multi kinase inhibitors) used to treat hepatocellular carcinoma.These drugs are also useful as first line therapy of thyroid cancers resistant to radioactive therapy(84).

Table 2 List of FDA approved tyrosine kinase inhibitors

SR. No.	Name of Drug	Target	Use	Year of Approval
1	Imatinib	BCR-Abl	Philadelphia chromosome-positive CML or ALL, aggressive systemicmastocytosis, chronic eosinophilicleukemias, dermatofibrosarcomaprotuberans, hypereosinophilic syndrome,gastrointestinalstromaltumors,myelodysplastic/ myeloproliferative disease	2001
2	Gefitinib	EGFR	Non-small cell lung cancer	2003
3	Erlotinib	EGFR	Non-small cell lung cancer, pancreatic cancers	2004
4	sorafenib	VEGFR	Hepatocellular carcinomas, renal cell carcinomas, thyroid cancers	2005
5	Sunitinib	VEGFR	Gastrointestinal stromal tumors, pancreatic neuroendocrine tumors, renal cell carcinomas	2006
6	Dasatinib	BCR-Abl	Chronic myelogenousleukemias	2006
7	Lapatinib	EGFR	HER-2 positive breast cancers	2007
8	Linotinib	BCR-Abl	Philadelphia chromosome-positive CML	2007
9	Pazopanib	VEGFR	Renal cell carcinomas, soft tissue sarcomas	2009
10	Vandetanib	VEGFR	Medullary thyroid cancer	2011
11	Vemurafenib	B-raf	B-Raf mutant melenomas	2011
12	Crizotinib	ALK	ALK or ROS1-postive NSCLC	2011
13	Ruxolitinib	JAK 1/2/3 and Tyk2	Myelofibrosis, polycythemiavera	2011
14	Axitinib	VEGFR	Advance renal cell carcinomas	2012
15	Bosutinib	BCR-Abl	Chronic myelogenousleukemias	2012
16	Regorafenib	VEGFR	Colorectal cancers	2012
17	tofacitinib	JAK 1/2/3 and Tyk2	Rheumatoid arthritis	2012
18	cabozantinib	RET	Advanced medullary thyroid cancers	2012
19	Ponatinib	BCR-Abl	Philadelphia chromosome-positive CML or ALL	2012
20	Trametinib	MEK1/2	Melanomas	2013
21	Dabrafenib	B-Raf	BRAF mutation-positive melanomas and NSCLC	2013
22	Afatinib	EGFR	Non-small cell lung cancer	2013
23	Ibrutinib	BTK	Chronic lymphocytic leukemias, mantle cell lymphomas, marginal zone lymphomas, graft vs. host Disease	2013

24	Ceritinib	ALK	ALK-positive NSCLC resistant to crizotinib	2014
25	Idelalisib	PI3K	Chronic lymphocytic leukemia	2014
26	Nintedanib	FGFR	Idiopathic pulmonary fibrosis	2014
27	Palbociclib	CDK4/6	Estrogen receptor- and HER2-positive breast cancers	2015
28	Lenvatinib	VEGFR/RAT	Differentiated thyroid cancers	2015
29	Abemaciclib	CDK4/6	Combination therapy and monotherapy for breast cancers	2017
30	Acalabrutinib	BTK	Mantle cell lymphomas	2017
31	Brigatinib	ALK	ALK-positive NSCLC	2017
32	Midostaurin	Flt3	Acute myelogenousleukemias, mastocytosis, mast cell leukemias	2017
33	Neratinib	ErbB2	HER2-positive breast cancers	2017
34	Ribociclib	CDK4/6	Combination therapy for breast cancers	2017
35	Baricitinib	JAK 1/2/3	Rheumatoid arthritis	2018
36	Binimetinib	MEK1/2	Melanomas	2018
37	Dacomitinib	EGFR	EGFR-mutant NSCLC	2018
38	Fostamatinib	Syk	Chronic immune thrombocytopenia	2018
39	Lorlatinib	ALK	ALK-positive NSCLC	2018
40	Tamatinib	SYK	Chronic immune thrombocytopenia	2018

2.3 INFLAMMATION AND CANCER:

In 1863 the first clue between inflammation and cancer was identified by Rudolf Ludwig Carl Virchow that inflammatory process is one of the conditions for the development of cancer cells (85). Chronic inflammation triggers the growth of the tumor cells by accelerating production if growth factors as well as reactive oxygen and nitrogen which interacts with DNA and produce mutations (86). There are different inflammatory modulators like chemokines, cytokines, growth factors, free radicals, prostaglandins and proteolytic enzymes that favors the development of the tumor cells. These inflammatory modulators synthesized from different types of cells such as fibroblasts, adipocytes, dendritic cells, natural killer cells,lymphocytes, neutrophils, macrophages etc. Some of these modulators directly act on tumor cells by supporting proliferation, oncogenic mutation and inhibiting the cell death (87, 88). Some of the modulators act as prototumorogenic agents which act on the components of tumor microenvironment. Literature data says that 20% of the cancers are produced from chronic inflammatory disease (89, 90). Inflammation is the common process

of various cancer risk factors which includes smoking, alcohol consumption, obesity, several types of infection etc. The goal of primary, secondary or tertiary cancers prevention is to reduce the exposure of risk factors of the cancers. Avoiding exposure of primary carcinogenic factors has potential to reduce 30% cancer deaths (91, 92).

Prevention of cancer can be possible by two ways; first is primary prevention by reducing exposure of risk factors of cancer and second is by immunoprevention and chemoprevention. The aim of immune prevention is to control development (or initiation) of cancer cells by immune system while chemo preventive effect focused on to suppress or prevent conversion of malignant to invasive cancer type (93, 94). The inflammatory modulators which promote the growth of oncogenes are discussed as follows.

2.3.1 TNF-alpha:

TNF-alpha is multicellular kinase which plays important role in various cellular events like cell differentiation, survival and death. There are two types of this receptor; first is TNFR1 that express in all over the cell and second is TNFR2 which is mainly express in immune cells(94-96). TNFR1 receptor contains intracellular domain, transmembrane domain and extracellular domain and it is considered as one of the important members of death receptor family as it is mainly involved in cell death program. Due to this it is also considered as death domain (DD) receptor. TNFR2 doesn't contain DD domain its action mediates through TNFR1(97). Biological function of TNF executes through activating several signalling pathways like NF-κB and c-Jun N-terminal kinase (JNK). NF-κB produce cell survival signal that produce anti-apoptosis effect. There are various approaches like transgenic models, gene deletion, use of antibodies have been adopted to check the role of TNF-alpha in cancer. and data suggest that this receptor have important role in development of malignant cells. In the skin cancer study, it was found that TNFR1 mediated signalling activates NF-κB; that provides signals which helps tumor cells to escape from apoptosis(98, 99).

2.3.2 Interleukins:

Interleukins functioned as like intercellular hormones which have ability to alter the cellular functions. There several types of interleukins (IL) that includes IL-1, IL-6, IL-8 and IL-18 play important role in cancer development(100, 101). IL-1α promotes cervical carcinoma and also induces anchorage independence in embryo fibroblasts. IL-1β also increases the cancer cell growth and mainly associated with for developing chemoresistance in pancreatic carcinoma(101, 102). Production of IL-6 linked with p53; upon mutation of p53

produce higher level of IL-6 and mainly involved in the cancers like multiple myeloma, non-Hodgkin's lymphoma, bladder cancer, colorectal cancer, and renal cell carcinoma (RCC)(103, 104). Cytokine IL-8 has been reported to promote growth and metastasis of wide variety of tumors. For tumor associated inflammation Ras dependent IL-6 production is required. Expression of IL-8 by human melanoma cells and human ovarian cancer cells correlates with their metastatic potential. IL-8 has been detected in astrocytoma, anaplastic astrocytoma, glioblastomas, and central nervous system cervical carcinoma metastasis(105-107).

2.3.3 Chemokines:

Chemokines family has four different members based on cystein residues they are classified as C, CC, CX3C and CXC. Chemokines plays either beneficial or non-beneficial for the cancer patients. Recruitment of mature dendritic and/or effectors cell provide beneficial effect while chemokine mediated recruitment of immature dendritic cell can increase tumor cell tolerance. In the cancer cell development, chemokines play important role in process like angiogenesis, inflammation, cell migration etc(108). The main role of chemokine in inflammation is by trafficking leucocytes at inflammation site. CC chemokines play important role in macrophage and lymphocyte infiltration in melanoma and carcinoma associated with different cancers such as ovary, breast, cervix and glioma(109). The concentration of chemokine receptor CXC4 and CCR7 found higher in breast cancers(110, 111). CXC play important role in inflammation, wound healing, cellular cycle regulation, angiogenesis, tumorigenesis etc. Presence of CXCR4 was found in ovarian cancer while upregulation of CCR4 found in renal cell carcinoma. CXCR4 activates EGFR in ovarian cancer(111).

2.3.4 Matrix metalloproteinases (MMPs):

Matrix metalloproteins are involved in various biological process like inflammation, wound healing, cellular migration, skeletal formation and cancer(112). MMP9 upregulation found in various stages of tumorigenesis. MMP9 transferred by bone marrow play crucial role in skin carcinogens; supported by the evidence that transgenic mice lacking MMP9 have reduced hyperproliferation and invasiveness(113). In the breast cancer patient 70 genes were identified for their poor prognosis; out of that two genes are MMP-1 and MMP-9. In the recent study it was found that out of the 95 gene; MMP1 is second most important gene which have potential of breast cancer to produce lung metastases(114, 115).

2.3.5 Cyclooxygenase (COX)

There are three isoforms of COX have been identified. A) COX-1 mainly involved in tissue homeostasis, platelet aggregation, renal blood flow and maintenance of gastric mucosa. B) COX-2 found in inflamed and neoplastic tissues. C) COX-3 mainly express in brain and spinal cord(116, 117). The COX pathway by PGH2 synthetase produces PGG2 which is unstable and PGH2 in the presence of PGH2 synthase and peroxidase enzyme. This convert in to various PGs and TxA2(116). COX-2level in cancer can be elevated by cytokines, growth oncogene and other factors. COX-2 responsible for the development and growth of variety of cancers(118-120). Expression of COX-2 regulated by NF-κB. In the colon carcinoma, COX enzyme induces angiogenesis by two way; first way is modulation of angiogenic factors by COX-2 and COX-1 regulates angiogenesis in endothelial cells(121). COX-2 found in higher level in epithelial cells of invasive breast cancer(122). COX-2 also play important role in the growth of human lung adenocarcinoma(123). Physiological effect of PGs and TXA2 mediated through G protein coupled proteinoid receptors which are divided into nine different types(124, 125). PGE2 gives biological response through four different receptors; EP1 to EP4. EP4 modulate PGE2 which involved in proliferation of colon cancer cells(126). Around 93% of melanomas are express with COX-2 with expression ratio around 68%. This overexpression plays important role in development and growth of malignant epithelial cancer cells (127, 128). In pathogenesis of oesophageal cancer involvement of both COX-1 and COX-2 have been detected due to their link with VEGF-A and VEGF-C which are important modulator of angiogenesis(129).

2.3.6 Lipoxygenase

The metabolic process for conversion of arachidonic acid to leukotrienes requires presence of 5-lipooxygnese enzyme which is key factor for this metabolic process. Leukotrienes play important role in allergic and inflammatory conditions. Apart from they are also involved in pathophysiological functions of brain like, cerebral ischemia, brain edema, and increased permeability of the blood-brain barrier in brain tumors(130). In the nude mice xenograft model treated with colon cancer with cigarettes smoke extract; the inhibition of the enzyme COX-2 and 5-LOX reduce the tumor size(131, 132). The evidence is further confirmed by the experiment in which cigarettes smoke without filter increase the expression of 5-LOX. This overexpression stimulates MMP and VEGF which are key factors in angiogenesis process. 5-LOX inhibitors decrease the colon adenoma formation and also

decrease the expression of VEGF and MMP in tumor cells, ultimately angiogenesis rate will be decreased(133).

2.3.7 NF-κB:

Regulation of TNF, COX, LOX, MMPs done by the transcription factor NF-κB which is normally present in inactivated state in most cells but in cancer cells NF-κB found in activated state. The activation of NF-κB induce inflammation and tumorigenesis(134). The activation of NF-κB response, triggered in presence of pro inflammatory cytokines and infectious agents. NF-κB heterodimer trapped in cytoplasm when it is bound to IkB which upon phosphorylation induces the cytokines which result in activation of NF-κB. It was found that in inflammation response of NF-κB is triggered through TNFα which produce anti-apoptosis signals. By enhancing signalling of NF-κB, cancer cells increase the invasiveness. The cancer associated TNFα overexpression; Inhibition of TNFα suppress the response of NF-κB and by this way produce apoptosis process(135). Since last few decades extensive research going on NF-κB and it was found that over production of this receptor increase the resistance of chemotherapy as well as γ-radiation therapy. Inhibition of NF-κB can sensitize the cancer cells and therefore NF-κB considered as one of the important targets for development of chemotherapeutic agents(136).

2.3.8 Hypoxia inducible factor-1 (HIF-1)

Hypoxia inducible factor contains two subunit alpha and beta; receptor present in heterodimeric complex. Alpha subunit is less stable as compared to beta subunit. Recent literature data have shown that inflammation (via inflammatory mediators) can also active HIF-1 in normoxic condition (137). Various cytokines, hormones have ability to increase the expression of HIF-1. Cytokines like IL-1β and TNF-α were reported for stimulation of HIF-1α expression. HIF-1α can stimulate the expression of several genes that includes, COX-2, iNOS, vascular endothelial growth factor receptor (VEGF), glucose transporter etc(138, 139). Overactivation of HIF-1α have been demonstrated in various types of cancer as it provides favourable conditions for development and growth of tumor cells (137).

2.3.9 Inducible nitric oxide synthase (iNOS)

There arethree different enzymes required for the synthesis of nitric oxide and iNOS is one of them(140). Cytokines like IL-1β, TNF-α and IFN-γ have ability stimulate this enzyme. The expression of iNOS is regulated by transcription factors including NF-kB, activator protein 1, signal transducer and activator of transcription, 1α interferon regulatory protein 1, nuclear factor interleukin-6, and high motility group I (γ) protein(141). iNOS mediated cellular

changes can produce malignancy, metastasis, angiogenesis of cancer cells which is involved in variety of cancer types such as melanoma, prostate, bladder and colorectal(142).

2.3.10 Jak/STAT pathway:

STAT family includes seven members which are involved in different cellular process like survival, cell proliferation and angiogenesis. Out of the seven members the important member involved in cancer is STAT3 transcription factor. The expression of STAT3 stimulated by mainly IL6, IL-11 and other members of cytokine family as well as growth factors. Upon activation, STAT3 phosphorylation followed by homodimerization is occur. This dimer shift into the nucleus, binds with DNA and stimulate the transcription of some genes involved in oncogenic activation such as Bcl-2, CDK1, VEGF etc. Due to this STAT3 found highly expressed in several cancers like multiple myeloma, leukaemia, prostate cancer, lymphoma, breast cancer, squamous cell carcinoma of head and neck(143).

2.3.11 Other Pathways:

The other pathways which are directly or indirectly activated by inflammation or inflammatory receptors includes mitogen activated protein kinase (MAPK), Phosphoinositide-3-Kinase (PI3K), CREB (cAMP-response element binding protein) Signalling pathway and Wnt/Beta Catenin Pathway. MAPKs are involved in various cellular process like cell growth, differentiation, survival and various immune and stress related responses(144-146). It is mainly activated and regulated by various cytokines through phosphorylation process(147). PI3K mainly involved in immune response of cancer cells and found highly expressed in pancreatic cancer due to mutation of K-Ras in patient of pancreatic cancer(148, 149). CREB play important role in cell survival, differentiation of neuron and in metabolism. The process like reverse phosphorylation of serine by various kinase can increase the transcription activity of CREB(150). The high expression of CREB found in various types of cancer which includes myeloid leukaemia(151), non-small cell lung carcinoma(152), melanoma(153), mammary carcinoma(154) etc. Wnt/Beta Catenin Pathway involved in various biological process like cell polarity, cell proliferation and cell fate determination during embryonic development and tissue homeostasis(155). Wnt pathway have ability to interact with many other pathways including HIF-1α, NF-Kβ and Notch, stimulate the function of this pathway(156). Mutation of Wnt pathway can produce various types of cancer which includes, stomach, liver, intestine, pancreas and ovaries(156) .

Table 3 Inflammatory receptor involved in different types of cancer

Cancer type	Inflammatory receptor involved
Breast cancer	CXCR4(110), CCR7(110), COX-2(122, 157), MMP1(114), MM9(115)
Cervical carcinoma	IL-1α(158), TNF(158), COX-1(159)
Ovarian tumors	TNF(97), IL-8(160), CXCR4/CXCL12(107), CXCR4(111), SDF1(111), COX-2(161), iNOS(162)
Glioma	TNF(163), IL-8(164), COX-2(165)
Prostate cancer	IL-8(166), CXCL14(167), COX-2(168)
Melanoma	IL-8 (105), IL-18, CXCR4(110), CCR7(110), CCR10, COX-2(127, 128)
Oesophageal adenocarcinoma	COX-2(169)
Oesophageal SCC and AC	COX-2(170)
Urinary bladder	COX-1, COX-2(171)
Pancreatic carcinoma	IL-1α(172), IL-1β(102), MIP-3a(173), CCR6(173), COX-2(174)
Head and neck SCC	COX-2(175)
Lung carcinoma	IL-1α(176), IL-1β(177), COX-2(101, 123), CXC, CXCL5 and CXCL8(123)
Gastric carcinoma	IL-8(178), COX-2(179)
Colorectal cancer	IL-6(180), COX-2(181)
Brain tumors	5-LOX(130)
Colon cancer	IL-6, COX-2(132), 5LOX(132), MMP7(182)
Skin cancer	TNF(183), 5LOX(184), MMP9(113)
Bladder cancer	IL-6(104)
Renal Cell carcinoma	IL-6(185), CCR3(186)
Leukaemia	TNF(187, 188)

2.4 ROLE OF ANTI-INFLAMMATORY AGENTS IN CANCER

2.4.1 Non-steroidal anti-inflammatory drugs:

The main role of NSAIDS as anticancer agents is due to their role inhibition of COX1/2 enzyme which requires for the biosynthesis of prostaglandins and leukotrienes. Elevate level of PGE2 observed in different types of cancer production(189, 190). They increase the cancer cell production by favouring angiogenesis, tumor growth, metastasis and inhibiting apoptosis. PGE2 have ability to activate the several cellular pathways like MAPK, PI3K/AKT and NF-Kβ which further activates VEGF, Bcl-2, EGFR and MMPS and increase the rate of tumorigenesis. The possible mechanism for rationale use of aspirin for cancer prevention involved inhibition of various targets like inhibition of COX1/2, certain pro-inflammatory cytokines, modulation on immune response, effect on PI3K signalling,

maintenance of cancer stem cell homeostasis, and decreased glycolytic rate in cancer cells(191, 192). The long-term use of NSAIDS may lead development of several side effects like renal failure, GI problems like acidity, ulcer and intestinal inflammation which cause perforation and strictures in small and large intestines. This factor can also induce the risk of cancer. To reduce the side effect of NSAIDs, they can combine with 5-LOX inhibitors so that synthesis of prostaglandins and leukotrienes are blocked(193).

The investigation of link between NSAIDs and cancer was done by kune *et al*; the report suggests that the patient taking NSAIDS had significantly lower incidence of cancer. The positive results if NSAIDS in cancer prevention open a new direction in cancer research. The study was done on >1 million subjects with over 30 epidemiological studies; results suggest that NSAIDs can be prototypical agents in prevention of cancer(194). Aspirin is very widely used drug in the world whose major role in cardiovascular disease. Multiple trial using aspirin for evaluation of anticancer properties have been done; data suggest that aspirin have approximately 20 to 25% ability to reduce incidence and mortality of several types of cancers(195, 196). The major beneficial effects were found in stomach, oesophageal and colorectal cancer(196, 197).

2.4.2 Corticosteroids

Corticosteroids used as anti-emetic agents to prevent nausea and vomiting driven by cancer chemotherapeutic agents. They are also effective as anti-inflammatory agents in various chronic inflammatory diseases. In the xenograft or experimental model of breast(198), colorectal(199), glioma(200) and lung cancer. It was observed that pre-treatment with dexamethasone increase the effectiveness of chemotherapy. The dexamethasone was also found to decrease the incidence of lung tumor(201). The combination of dexamethasone with carfilzomib and lenalidomide had advantageous effect in multiple myeloma patients(202).

2.4.3 Statins, metformin and embelin:

Statins are used as anti-hyperlipidaemic agents(203), which are also reported for anti-inflammatory properties(204). The mechanism involved for anti-inflammatory activity is due to reduction of pro-inflammatory cytokine, macrophage infiltration and C-reactive protein(205). Due to additional anti-inflammatory properties statins have ability to reduce the risk of several cancers which includes colorectal(206), HCC and breast(207). Metformin, a drug used as oral hypoglycaemic agent, also reported for reducing the risk of several caners which includes, colon(208), breast, lung, prostate, ovarian, and pancreatic cancers. Its

antineoplastic effect is mediated through activation of the AMPK pathway, which counteracts the protumorigenic effect of hyperinsulinemia, the reduction of systemic glucose concentration, which counteracts Warburg effect and through its anti-inflammatory properties(209). Embelin which is isoquinoline derivative, reported for anti-inflammatory properties due to its interference in arachidonic acid metabolic pathway and has ability block 5-LOX and PGEs(210).

Table 4 Role of Anti -inflammatory agents in the treatment of cancer

Drug	**Preventive effect on cancer**	**Anti-cancer effect**
Aspirin	Bladder(211), breast(212), colorectal(213), oesophageal (214)and lung(215)	Gastric(216) and colon(217) cancer
Celecoxib	Bladder(218), breast(219), cervix(220), colorectal(221), lung(222), prostate(223)	Prostate(224), liver(225) and colon(226)
Ibuprofen	Colon adenoma(227)	Breast cancer(212)
Sulindac	Breast cancer(228)	Colon cancer(229)
Piroxicam	Colorectal cancer(230)	Colon cancer(231)
Dexamethasone	Breast(232), rectal(199)	Multiple myeloma(233)

2.4.4 Natural Products:

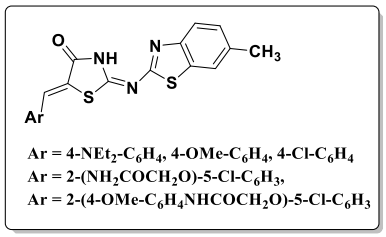
Some natural products and food have anti-inflammatory effects which includes grapes (reservatrol), garlic, curry powder (curcumin). These compounds have also shown anti-cancer properties due to induction of apoptosis. These compounds have anti-inflammatory action due to inhibition of the target NF-κB, MAPK, JNK, VEGF and COX and due to this they have role in anticancer activity(234-237). Literature data also suggest that combination of natural products with chemotherapeutic agents gives beneficial effects. For example, combination of curcumin with 5-flurouracil(235) gives synergistic while in another study ginseng saponins were reported for increase the response of cancer cells to chemotherapeutic agents and reduce haematological toxicity after radiation therapy(236). Berberine act as anti-inflammatory agent and also having anti-cancer activity. Activity is due to inhibition of NF-κB and COX-2 with IC50 value around 0.3μM(238).

2.5 BIOLOGICAL IMPORTANCE OF BENZOTHIAZOLE:

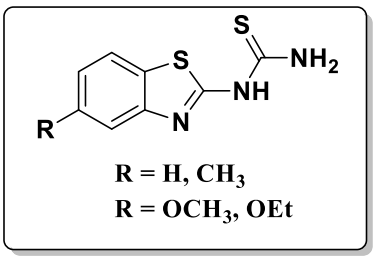
Benzothiazole is a privileged bicyclic ring system. Due to its potent and significant biological activities it has great pharmaceutical importance; hence, synthesis of this compound is of considerable interest. The small and simple benzothiazole nucleus if present in compounds involved in research aimed at evaluating new products that possess interesting biological activities. 2-substitued benzothiazole has emerged in its usage as a core structure in the diversified therapeutically applications. The studies of structure–activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity. Among those 2-substituted benzothiazole derivatives with fluorine substituted molecules have already received considerable attention due to their potential bioactivities. Since most of the benzothiazole derivatives were reported for their diversified activity viz., antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antifungal, a topical carbonic anhydrase inhibitor and an antihypoxic.

2.5.1 Anticancer activity

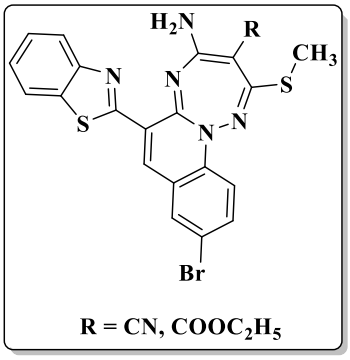
1. Dmytro Havrylyuk *et al*., reported synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety (239).



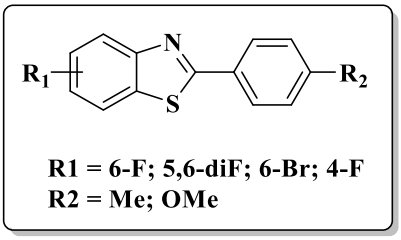
2. Fatemeh Eshkil *et al*., reported benzothiazole thiourea derivatives as anticancer agents:design, synthesis, and biological screening(240).



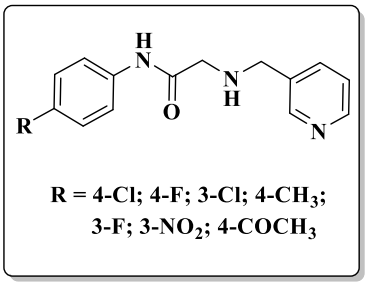
3. Aisha Y. Hassan *et al*., reported design, synthesis, and anticancer activity of novel benzothiazole analogues(241).



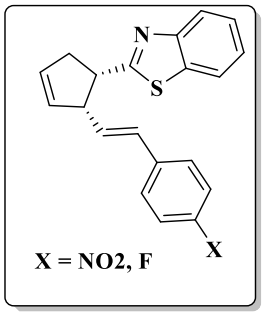
4. Hemal A. Bhuva*et al*., reported synthesis, anticancer activity and docking of some substituted benzothiazoles as tyrosine kinase inhibitors(242).



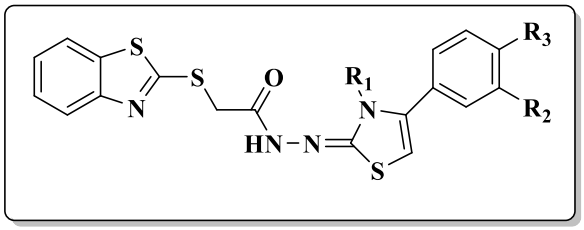
5. Nirav Shah *et al*., reported design, synthesis and evaluation of antimicrobial and anticancer activity of Novel 3-aminomethyl pyridine derivatives(243).



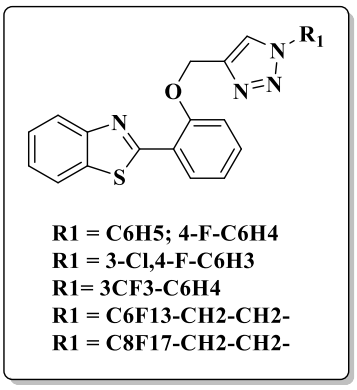
6. Nuray Uremis *et al*., reported Synthesis of 2-substituted benzothiazole derivatives and their in vitro anticancer effects and antioxidant activities against pancreatic cancer cells (244).



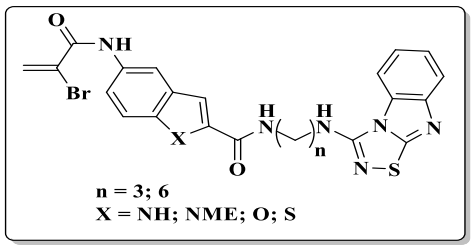
7. Derya Osmaniye *et al*., reported synthesis and anticancer activity of some novel benzothiazole-thiazolidine derivatives(245).



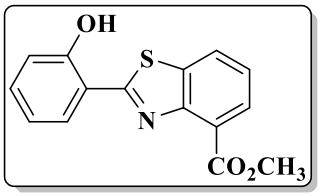
8. Ravindra M. Kumbhare *et al*., reported synthesis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl benzothiazole as potential anticancer agents(246).



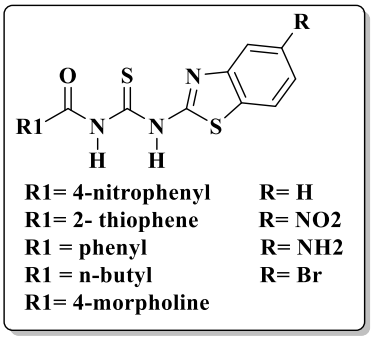
9. Romeo Romagnoli *et al*., reported hybrid molecules containing benzo [4,5] imidazo-[1,2-d] [1,2,4] thiadiazole and a-bromoacryloyl moieties as potent apoptosis inducers on human myeloid leukaemia cells(247).



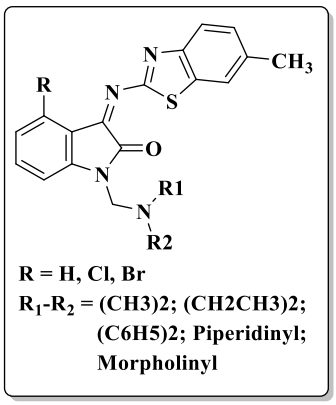
10. Shu-Ting Huang *et al*., reported Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoxazoles), and benzothiazoles(248).



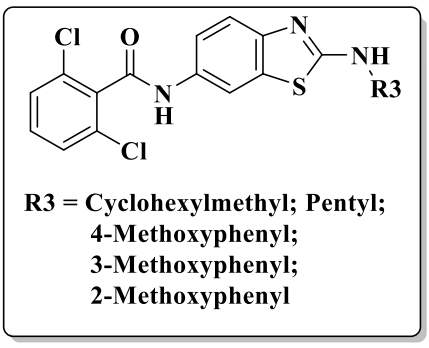
11. Sohail Saeed *et al*., reported synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents(249).



12. V. Raja Solomon *et al*., reported hybrid pharmacophore design and synthesis of isatin–benzothiazole analogues for their anti-breast cancer activity(250).



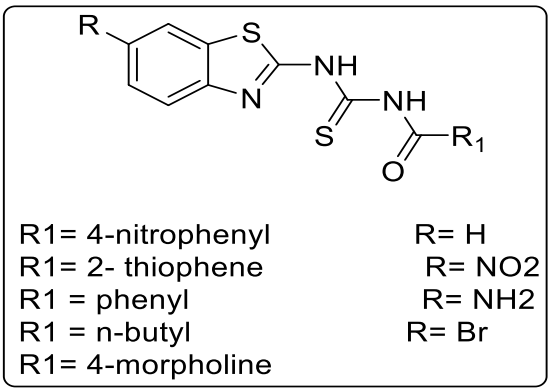
13. Masao Yoshida *et al*., reported synthesis and biological evaluation of benzothiazole derivatives as potent antitumor agents(251).



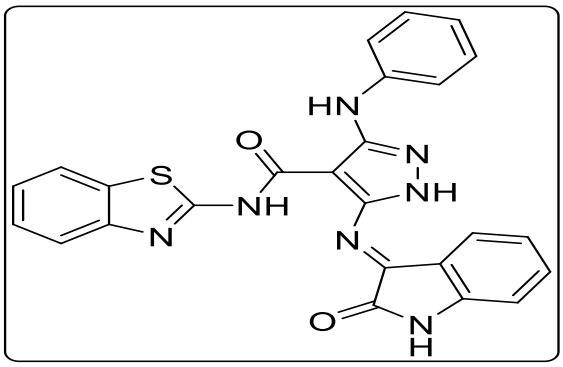
2.5.2 Other activities:

Antibacterial activity:

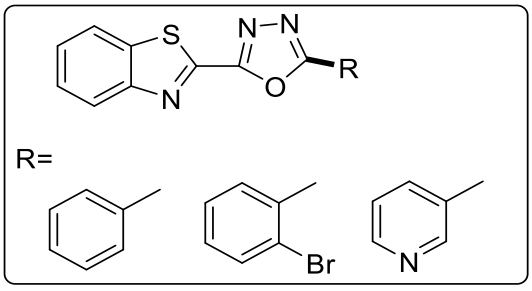
Sohail Saeed.*et al*., reported synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents(249).



Samir Bondock.*et al*., reported synthesis and antimicrobial activity of some new thiazole, thiophene and pyrazole derivatives containing benzothiazole moiety(252).

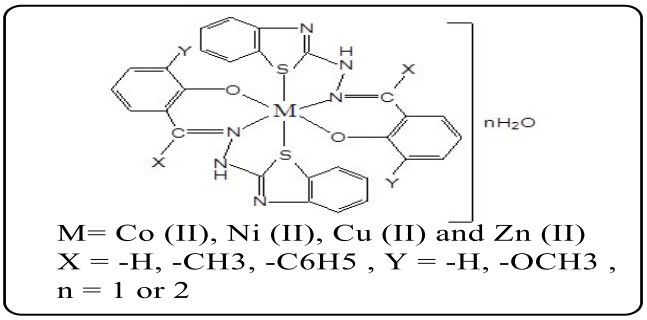


B. Rajeeva.*et al*., reported synthesis and antimicrobial activity of some new 2-substituted benzothiazole derivatives(253).

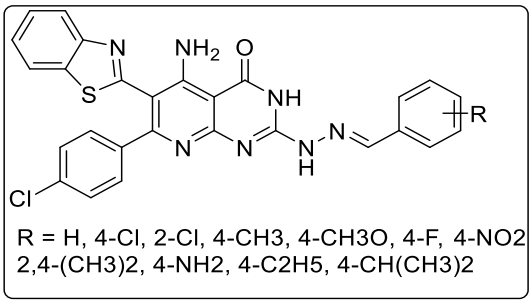


Antifungal activity:

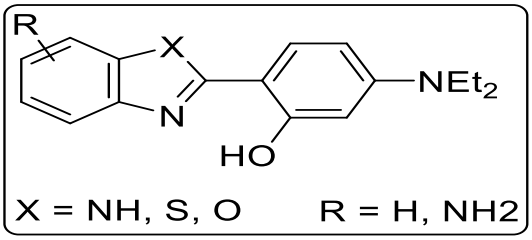
Ranjan K. Mohapatra.*et al*.[9], reported synthesis, structural investigations, DFT, molecular docking and antifungal studies of transition metal complexes with benzothiazole based Schiff base ligands(254).



Suresh Maddila.*et al*.[10], reported synthesis, antibacterial and antifungal activity of novel benzothiazole pyrimidine derivatives(255).

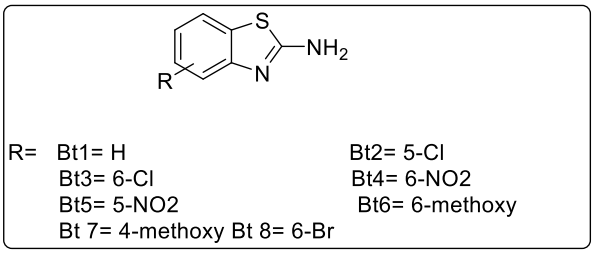


Vikas S. Padalkar.*et al*.[11], reported synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives(256).



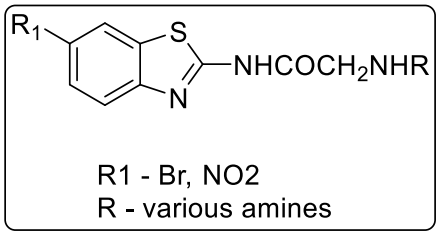
Anti-inflammatory activity:

Venkatesh P. *et al*.[13], reportedsynthesis, characterization and anti-inflammatory activity of some 2-amino benzothiazole derivatives(257).



Anthelmintic activity:

GawareVinayak M.*et al*.[14], reported synthesis and evaluation of benzothiazole derivatives for anthelmintic activity(258).

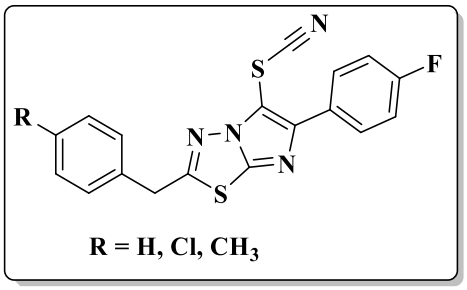


2.6 BIOLOGICAL IMPORTANCE OF THIADIAZOLE

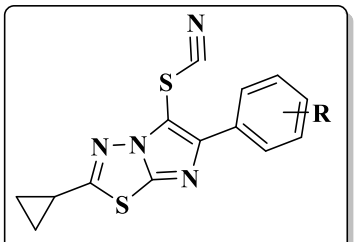
There are four types of thiadiazole: 1,3,4-, 1,2,4-, 1,2,5- and 1,2,3-thiadiazole (Figure 1 a), the most fully investigated of these being the 1,2,4- and 1,3,4-thiadiazoles. It has been widely reported that compounds bearing thiadiazole rings exhibit anticancer, anti-inflammatory, antibacterial, antifungal, antiviral, anticonvulsant and antiparasitic activities. The 1,3,4-thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei, which is a common and integral feature of a variety of natural products and medicinal agents.

2.6.1 Anticancer activity

1. Subhas S. Karki*et al*., reported Synthesis and biological evaluation of novel 2-aralkyl-5-substituted-6-(40-fluorophenyl)-imidazo[2,1-b] [1,3,4] thiadiazole derivatives as potent anticancer agents(259).

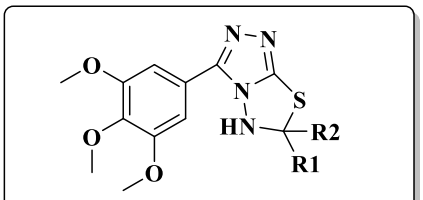


2. Malleshappa N. Noolvi *et al*., reported Synthesis and anticancer evaluation of novel 2-cyclopropylimidazo[2,1-b] [1,3,4]-thiadiazole derivatives(260).



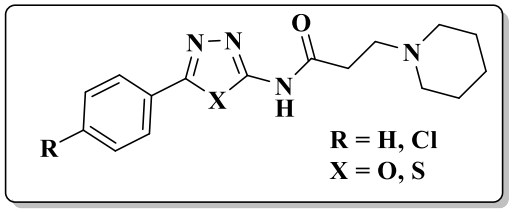
R = Hb, 4-Cl, 4-Br
R = 4-F, 2,4-di-Cl
R = 2,4-di-OH
R = 3-NH2, 4NH2
R = 3-NO2, 4-NO2
R = 2-OH

3. Pei-Liang Zhao *et al*., reported synthesis and cytotoxicity of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazoles and novel 5,6-dihydro- [1,2,4] triazolo[3,4-b] [1,3,4]thiadiazole derivatives bearing 3,4,5-trimethoxyphenyl moiety(261).



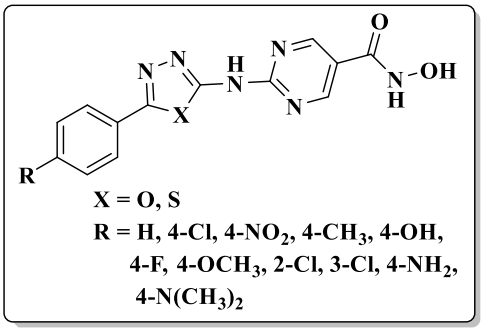
R1 = C_6H_5, 2,4-(MeO)2C6H3
R2 = Me, $C_6H_5CH_2$, 4-$FC_6H_4CH_2$, 2-$ClC_6H_4CH_2$, $EtCO_2CH_2$, 4-MeC6H4COCH2

4. Azza T. Taher*et al*., reported novel 1,3,4-heterodiazole analogues: Synthesis and in-vitro antitumor activity(262).

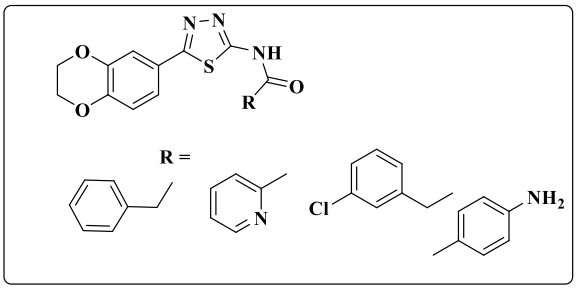


R = H, Cl
X = O, S

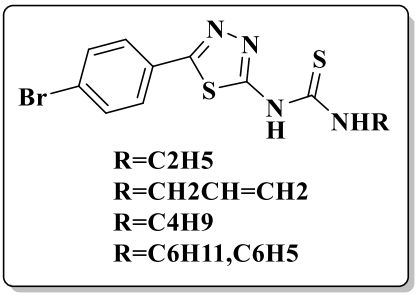
5. Harish Rajak*et al*., reported 2,5-disubstituted-1,3,4-oxadiazoles/thiadiazole as surface recognition moiety: design and synthesis of novel hydroxamic acid-based histone deacetylase inhibitors(263).



6. Juan Sun *et al*., reported synthesis, biological evaluation and molecular docking studies of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan as potential antitumor agents(264).



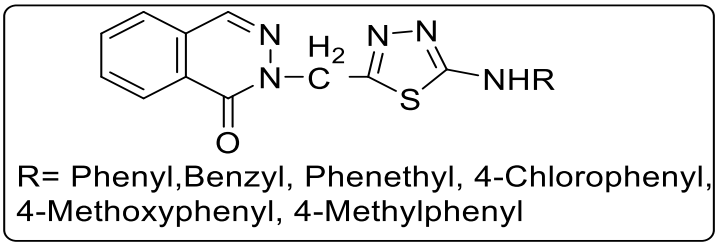
7. Doaa E. Abdel Rahman *et al*., reported synthesis of novel 1,3,4-thiadiazole analogues with expected anticancer activity(265).



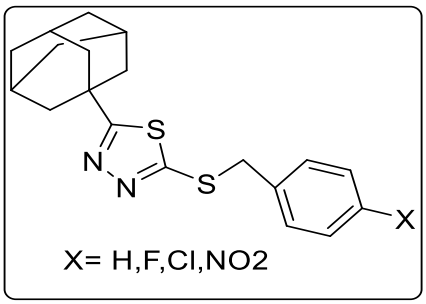
Other Activity

Antimicrobial

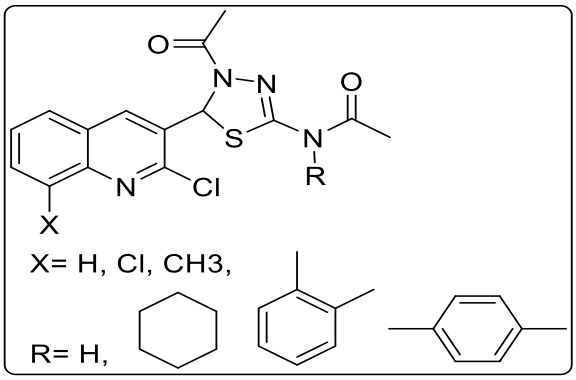
Tijeno N.*et al*., reported synthesis and antimicrobial activity of new 1,2,4-triazole and 1,3,4-thiadiazole derivatives (266).



Adnan A. Kadi.*et al*., reported synthesis, antimicrobial and anti-inflammatory activities of novel 5-(1-adamantyl)-1,3,4-thiadiazole derivatives(267).

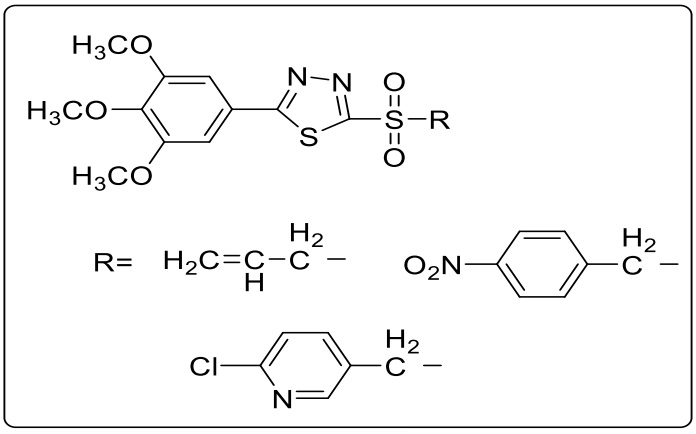


Abdul R. Bhat.*et al*.[18], reported 3-(1,3,4-Thiadiazole-2-yl) quinoline derivatives: synthesis, characterization and anti-microbial activity(268).

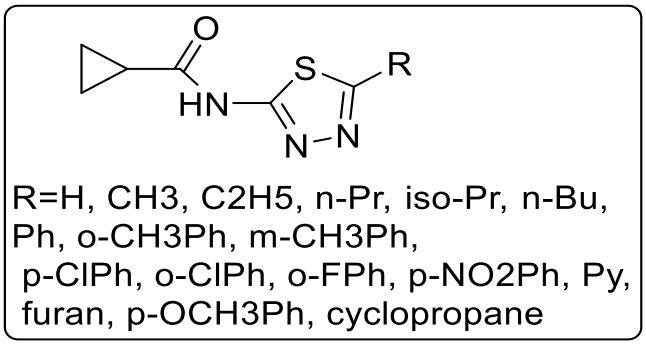


Antifungal activity:

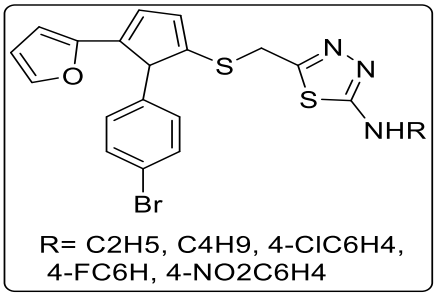
Cai-Jun Chen.*et al*., reported synthesis and antifungal activities of 5-(3,4,5 trimethoxy phenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives(269).



Xing-Hai Liu.*et al*., reported synthesis, antifungal activities and 3D-QSAR study of N-(5-substituted-1,3,4-thiadiazol-2-yl) cyclopropanecarboxamides(270).

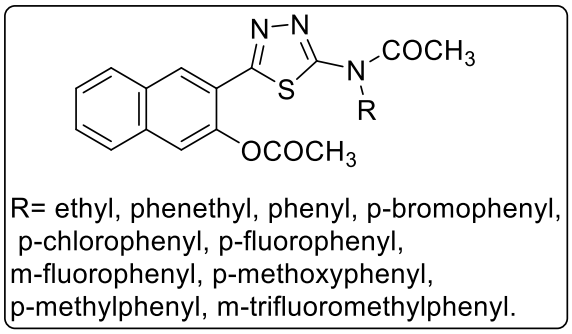


NalanTerzioğlu Klip.*et al*., reported synthesis, structure, and antifungal evaluation of some novel 1,2,4-triazolylmercaptoacetylthiosemicarbazide and 1,2,4-triazolylmercaptomethyl-1,3,4-thiadiazole Analogues(271).



Anticonvulsant activity:

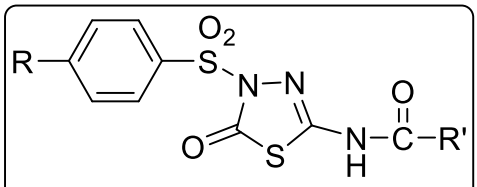
Hatice N. Dogan.*et al*., reported synthesis of new 2,5-disubstituted-1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities(272).



R= ethyl, phenethyl, phenyl, p-bromophenyl, p-chlorophenyl, p-fluorophenyl, m-fluorophenyl, p-methoxyphenyl, p-methylphenyl, m-trifluoromethylphenyl.

Anti-inflammatory activity:

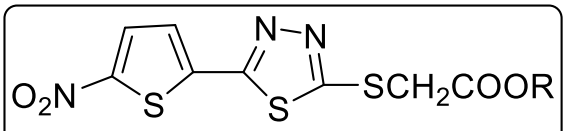
Silvia Schenone.*et al*., reported new 1,3,4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities(273).



R= H, CH3
R'= Phenyl, 4-Methylphenyl, 4-Methoxyphenyl, 4-Chlorophenyl, etc.

Antitubercular activity:

Alireza Foroumadi.*et al*., reported antituberculosis agents VIII synthesis and in vitro antimycobacterial activity of alkyl a-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetates(274).



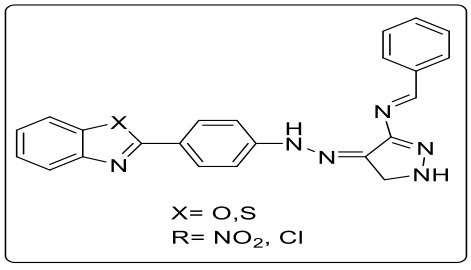
R= Methyl, Ethyl
n -Propyl, n -Buthyl,
Benzyl

2.7 IMPORTANCE OF MOLECULAR HYBRIDIZATION TECHNIQUE IN MEDICINAL CHEMISTRY:

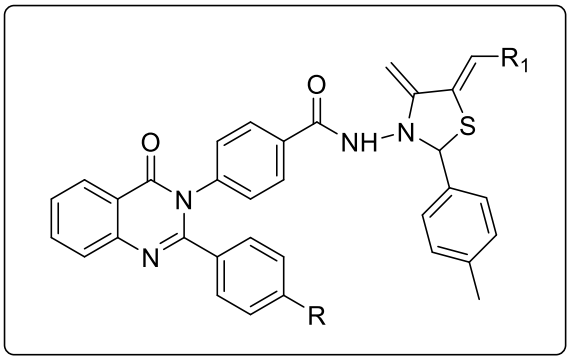
In the current medical era, molecular hybridization (MH) approach has stood out as a valuable and important structural modification tool useful for the discovery and development of better therapies for diverse human diseases, mostly for cancer. The growing endeavors to discover hybrid drugs resulting from the combination of two or more haptophoric moieties of different bioactive substances have brought a new hope for the treatment of multifactorial disorders in recent years. Moreover, hybrid drugs can potentially overcome most of the pharmacokinetic drawbacks encountered by conventional anticancer drugs as well as provide combination therapies in a single multifunctional therapeutic agent at the target molecule conferring a more powerful, selective and safer drug compared to conventional classic treatments.

The molecular hybridization (MH) is a strategy of rational design of new leads based on molecular structure of two or more known bioactive derivatives which, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.The molecular hybridization strategy is particularly interesting for the development of new leads for the diseases whose treatment is restricted to few commercial drugs or in cases when bioactive compounds are discovered but present high toxicity or pharmacokinetic and pharmacodynamic restrictions. Hybridization of two different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action has showed synergistic effects. Following examples are molecular hybrids reported in literature with good pharmacological activity(275).

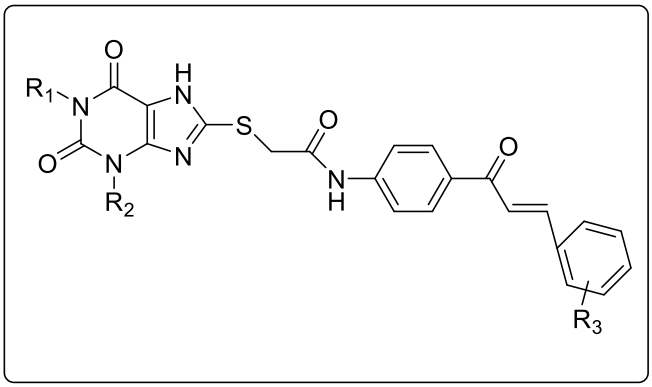
Belal *et al* reported hybrids of benzothiazole/benzoxazole-pyrazole as potential COX-II inhibitors(276).



Nara et al reported hybrids of quinazolin-4(3H)-one as anticancer agents(277).



Abou-Zied *et al* reported hybrids of xanthine derivatives carrying chalcone moiety as EGFR inhibitors(278).

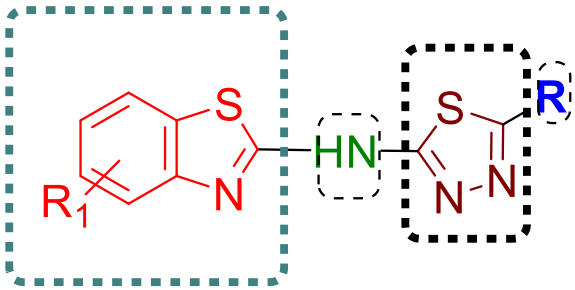


CHAPTER NO. 3

RATIONALE OF WORK

3 RATIONALE OF WORK:

- Based on these prior observations, we postulated that a compound containing **NSAIDs, benzothiazole and thiadiazole** pharmacophores could be very effective for anticancer activity.
- Hybridization of different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action has shown **synergistic effects**.
- The synthesis of NSAIDs, benzothiazole and thiadiazole conjugates could be possible by a pharmacophore **hybrid approach**.
- Therefore, aim of the work is to design and synthesize new anti-cancer compounds by linking the main structural unit of the NSAIDs with the benzothiazole and thiadiazole ring system as anticancer agents.



Hybrid Pharmacophore

CHAPTER NO. 4

PLAN OF WORK

4 PLAN OF WORK

- To establish novelty of work and compounds to be synthesized.
- Insilco drug designing and find out best lead compounds for synthesis
- Synthesizing new substituted new potential anticancer agents and their derivatives using appropriate approach.
- To characterize the structure of the newly synthesized compounds by physical data (M.P., TLC) and spectral data (IR, NMR and MASS).
- To evaluate the anticancer activity of the newly synthesized compounds and perform enzyme inhibition study

CHAPTER NO. 5

MATERIALS and METHODS

5 MATERIAL AND METHODS

5.1 CHEMICALS AND REAGENTS

The chemicals used in this work were of SD Fine, E. Merck and Ranchem grade. The chemicals were used without further purification.

Table 5 List of chemicals

2-amino benzothiazole	Carbon disulphide
2-methyl aniline	4-methyl aniline
2-methoxy aniline	4-methoxy aniline
2-nitro aniline	4-nitro aniline
2-cholro aniline	4-cholro aniline
Aniline	Ethanol
Ethlyacetate	Hydrazine hydrate
Aspirin	Ibuprofen
Salicylic acid	Phosphorous oxychloride
Ammonium thiocyanate	Potassium thiocyanate
n-hexane	Glacial acetic acid

5.2 ANALYTICAL TECHNIQUES

Physical data:

All the melting points were determined in open capillaries and are uncorrected.

Thin Layer Chromatography (TLC):

Purity of the compounds was checked by thin layer chromatography using prepared silica Gel G slides (2x7.5cm) as a stationary phase and various combinations of ethyl acetate: n-hexane as mobile phase. The spots resolved were visualized by using iodine chamber and ultra violet chamber.

Instrumentation for Characterization

The techniques employed for the characterization of the synthesized compounds were IR, 1H-NMR and Mass spectral analysis.

Infrared spectra:

The IR spectra of synthesized compounds were recorded on a fourier-transform IR spectrometer (model-DRS-8400, Shimadzu) in the range of 400-4000 cm-1 using KBr pellets and values of υ_{max} are reported in cm^{-1}.

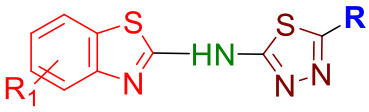
NMR spectra:

The ^{1}H-NMR and ^{13}C NMR spectra of synthesized compounds were recorded on Bruker advance–III NMR-400MHz FT-NMR (TOPSPIN 1.3 Version) spectrophotometer by Brüker Biospin, Switzerland.

Mass spectra:

Mass spectrums were recorded by SHIMADZU QP – 2010, GC-MS/MS (SHIMADZU) instrument.

5.3 DESIGN AND INSILICO SCREENING:



R		R1	
Substitution	Code	Substitution	code
2-hydroxy phenyl (salicylic acid)	sa	6-CH_3	1
2-acetoxy phenyl (aspirin)	a	4-CH_3	2
1-isobutyl-4-isopropylbenzene (ibuprofen)	ib	6-OCH_3	3
2-ethyl-6-methoxynaphthalene (Naproxen)	na	4- OCH_3	4
4-isopropylphenyl)(phenyl)methyl)-l-oxidane (fenoprofen)	fp	6-Cl	5
(4-ethylphenyl)(phenyl)methanone (Ketoprofen)	kp	4-Cl	6
		6-NO_2	7
		4-NO_2	8
		H	9

Initially we had designed 54 novel molecules by taking different substitution at R (different NSAIDs) and R1 position on benzothiazole and thiadiazole conjugates and screened using Insilico approach. In silico screening was done using different techniques like drug likeness screening and molecular docking approach. Based on Literature data we had rationally selected three different targets; TNF-alpha, COX-II and protein kinase (dual specific tyrosine and serine/threonine kinase) to perform molecular docking studies. Screened compound were synthesized by appropriate synthetic methods.

5.3.1 Procedure for molecular docking studies

Step: I Preparation of Ligands using Ligprep of Schrodinger:

All the structures were prepared from 2D sketcher from Schrodinger. After this all the structure were exported to LigPrep. All the structures were energy minimized; 3D geometry corrected. There was no change made in ionization state. Then output file of LigPrep was directly used for docking and calculation of ADME properties. ADME properties were screened using QuikProp module.

Step: II Protein preparation and Receptor grid generation:

Protein structure was exported from Protein Data Bank (PDB id: 4MXO)(279). This structure was directly used in protein preparation wizard were missing chain residue addition, H-bond addition, water molecules removed and finally energy minimized. The output file used for receptor grid generation. After refinement of protein receptor grid was generated at site of ligand present in protein which defines docking site. The size of grid was similar to size to the workspace of ligand which was selected by default 20A^o. The output of glide grid file was used for docking.

Step: III Ligand docking with extra precision mode (XP):

Glide grid file and LigPrep file was used as input for ligand docking process. The mode of docking was extra precision mode (XP) which provides flexible docking. At the end of process docking score, H-bond interaction, hydrophobic interaction etc details were collected.

5.4 SYNTHETIC PROCEDURE

Synthetic Scheme:

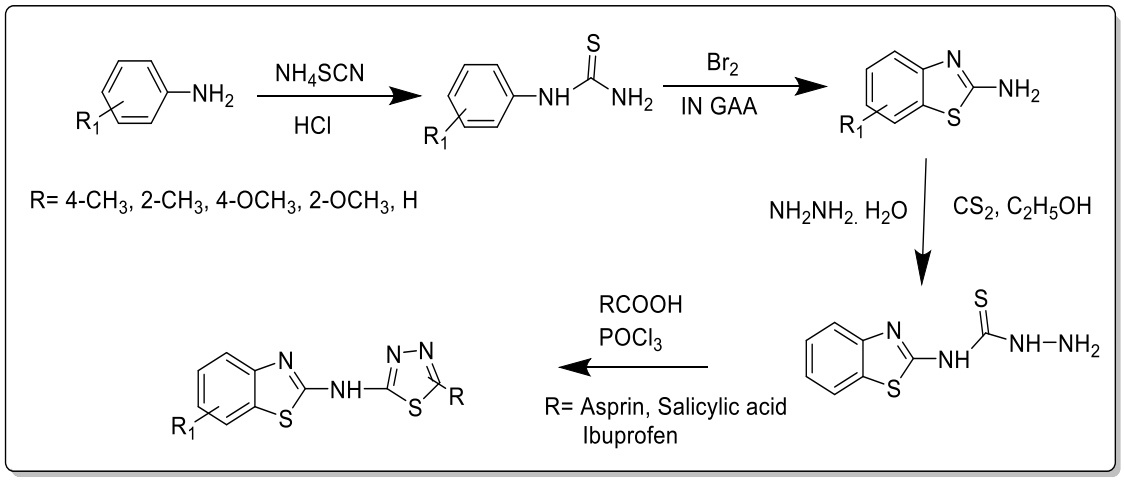
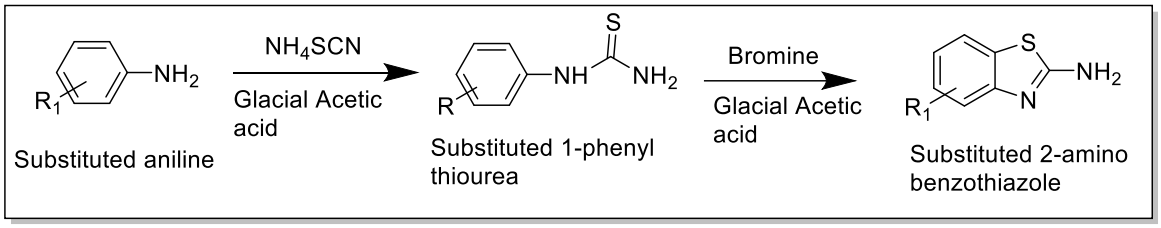


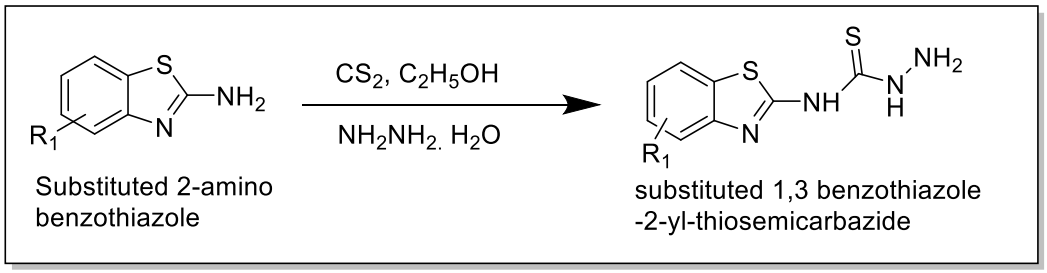
Figure 4 Synthetic scheme

Step-1: Synthesis of substituted 2-amino benzothiazole



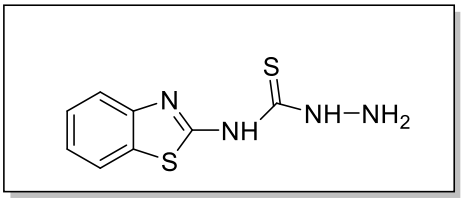
Aryl amines (0.01mol) and ammonium thiocyanate (0.01mol) in glacial acetic acid (10%) were cooled and stirred. During stirring, cold bromine in glacial acetic acid (0.01mol, in 10ml) was added drop wise. During addition temperature was maintained around 100°C. Stirring was continued for an additional 3 hr at 10-150°C. The separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water, neutralized with aqueous ammonia solution (25%), and filtered off. Finally, the products were washed with cold water, dried, and recrystallized from ethanol.

Step-II Synthesis of substituted 1,3 benzothiazole-2-yl thiosemicarbazide (INT)

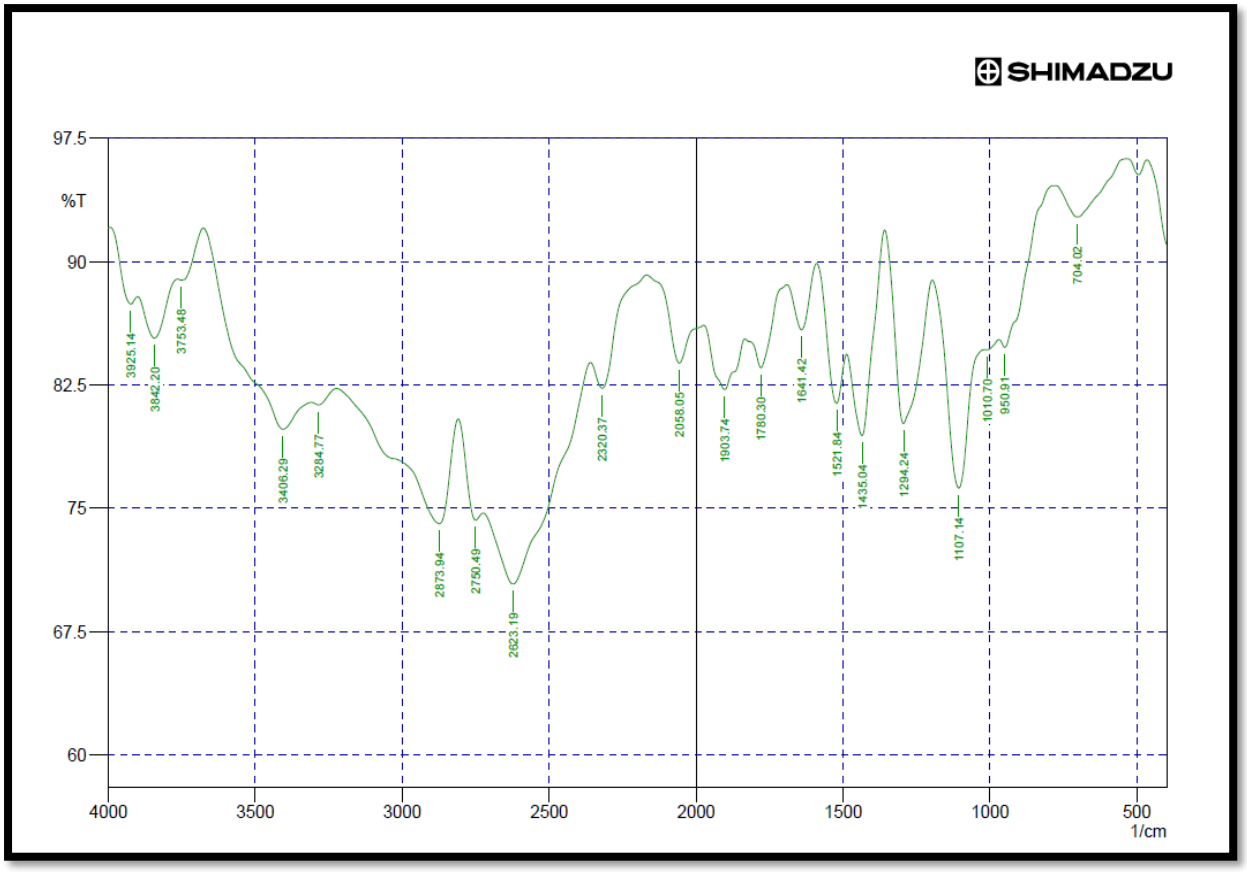


Substituted 2-amino benzothiazole (0.10mol) was dissolved in ethanol and ammonia solution (20ml). Then carbon disulfide (20ml) was added slowly within 15min with shaking and then solution was allowed to stand for 1 hr. Then sodium chloroacetate (0.1mol) and 50% hydrazine hydrate (20ml) was added. The reaction mixture was warmed gently, filtered and evaporated to its half volume and kept overnight, the separated solid was filtered and recrystalised from ethanol.

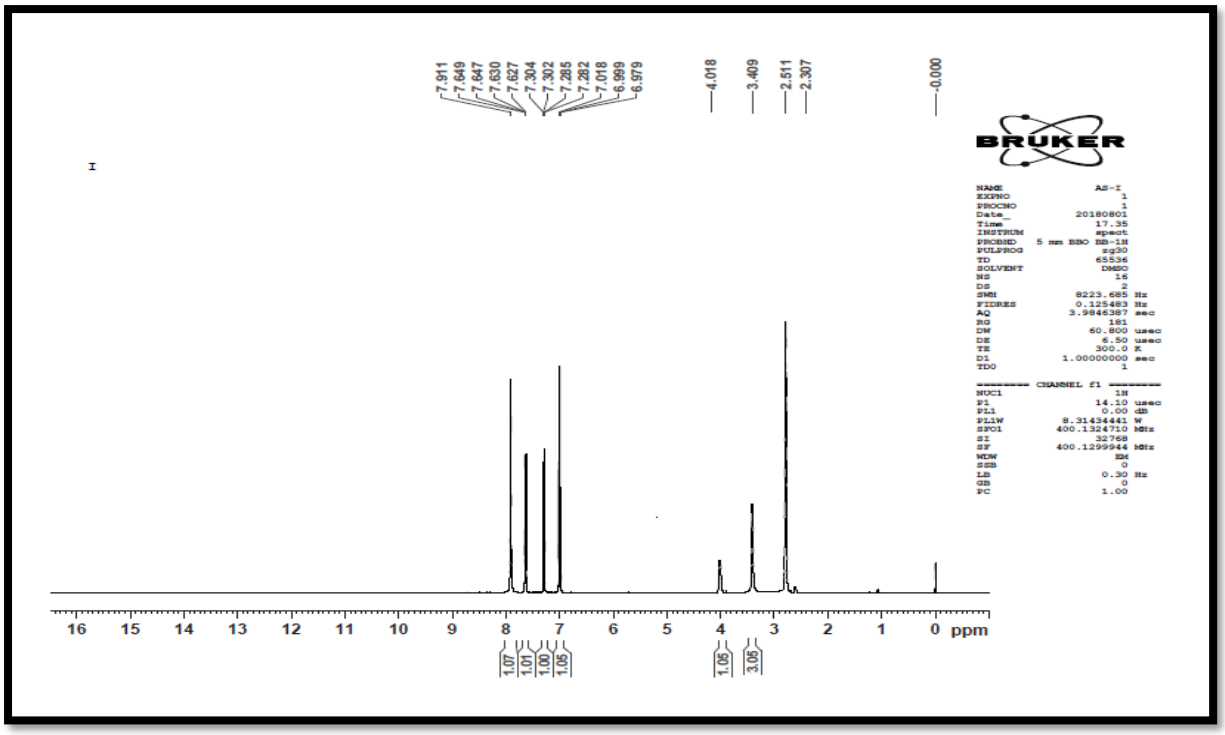
N-(benzo[d]thiazol-2-yl) hydrazinecarbothioamide (INT1)



Molecular Formula	$C_8H_8N_4S2$
Molecular Weight (g/mol)	224.30
Melting Point (ºC)	182-184ºC
Yield (%w/w)	62
Recrystallisation solvent	Ethanol
TLC	Rf = 0.73 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3406 (NH), 1537 (C=N), 1641 (C=C),1294 (C-N), 1107 (C=S)
^{1}H NMR (DMSO)	6.9-7.9 (m, 4-ArH), 4.0 (s, 1H, NH), 3.4 (s, 3H, NH-NH_2)

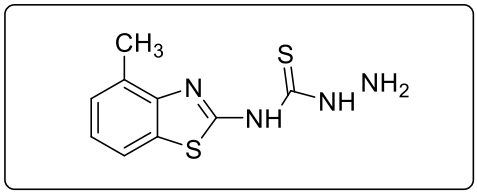


Spectra 1: IR spectra of 1INT1

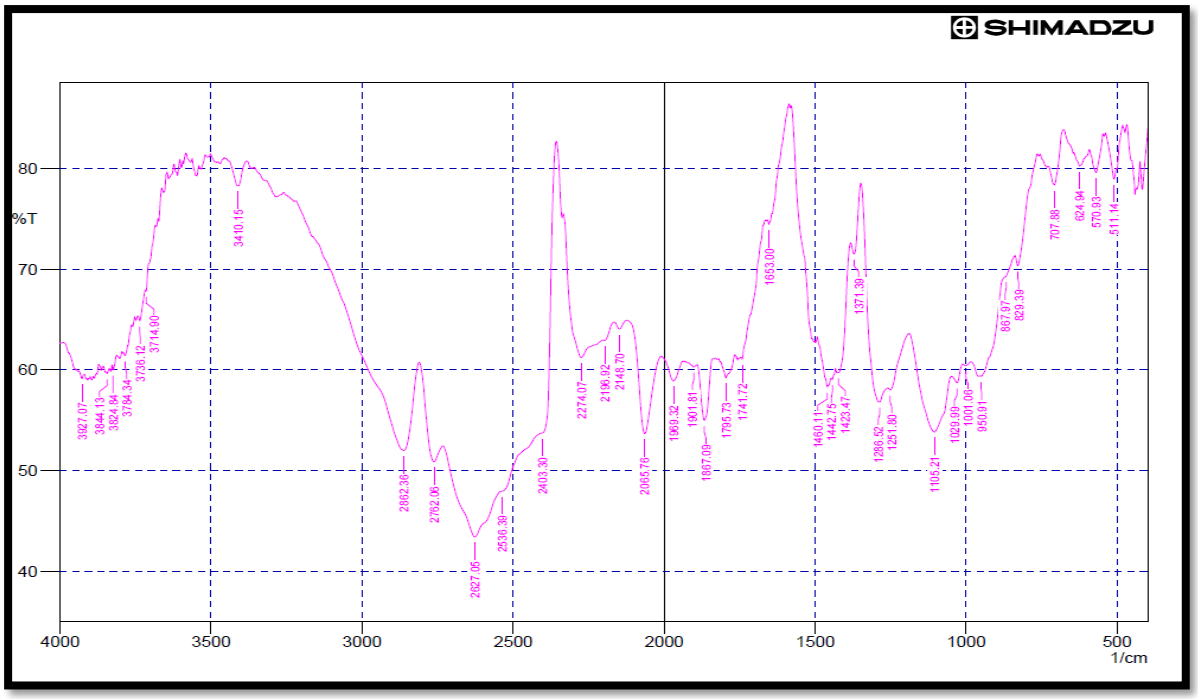


Spectra 2: ^{1}H NMR spectra of INT1

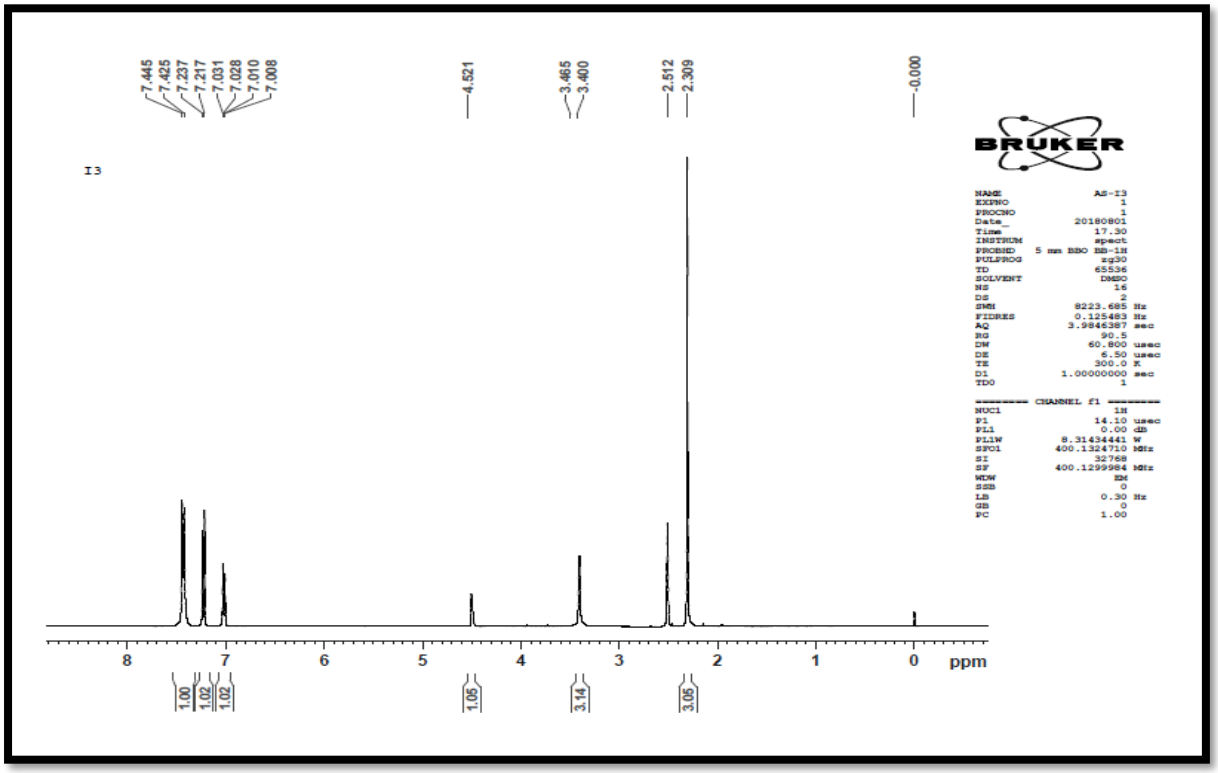
N-(4-methylbenzo[d]thiazol-2-yl)hydrazinecarbothioamide (INT2)



Molecular Formula	$C_9H_{10}N_4S_2$
Molecular Weight (g/mol)	238.33
Melting Point (ºC)	172-174ºC
Yield (%w/w)	54
Recrystallisation solvent	Ethanol
TLC	Rf = 0.68 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3444 (NH), 2870 (CH_3), 1645 (Ar C=C), 1523 (C=N), 1261 (C-N), 1095(C-S)
^{1}H NMR (DMSO)	6.89-7.04 (m. Ar-3H), 4.5 (s, 1H-NH), 3.4 (S, 3H, NH-NH_2)2.4 (s, 3H, Ar-CH_3)

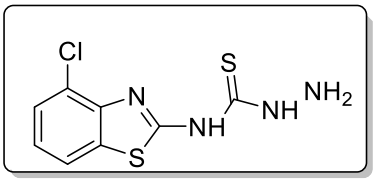


Spectra 3: IR Spectra of INT2

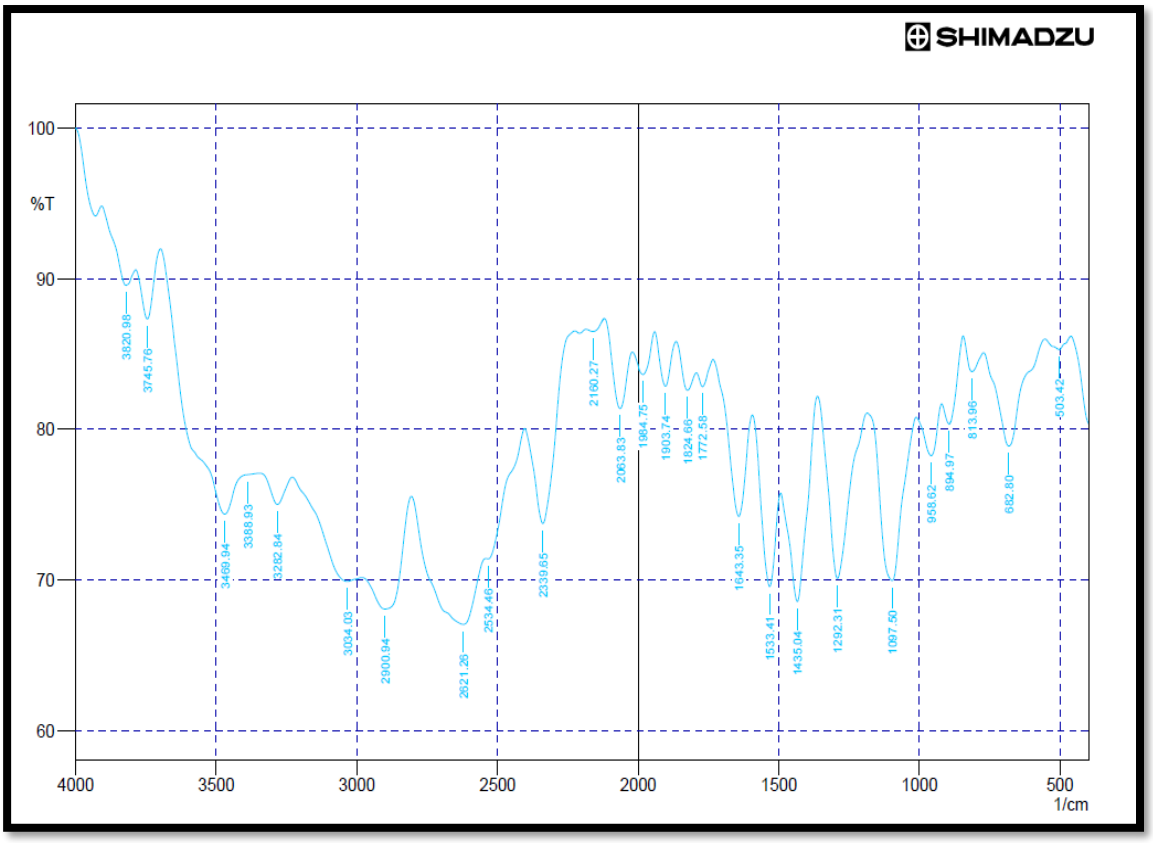


Spectra 4: ^{1}H NMR spectra of INT2

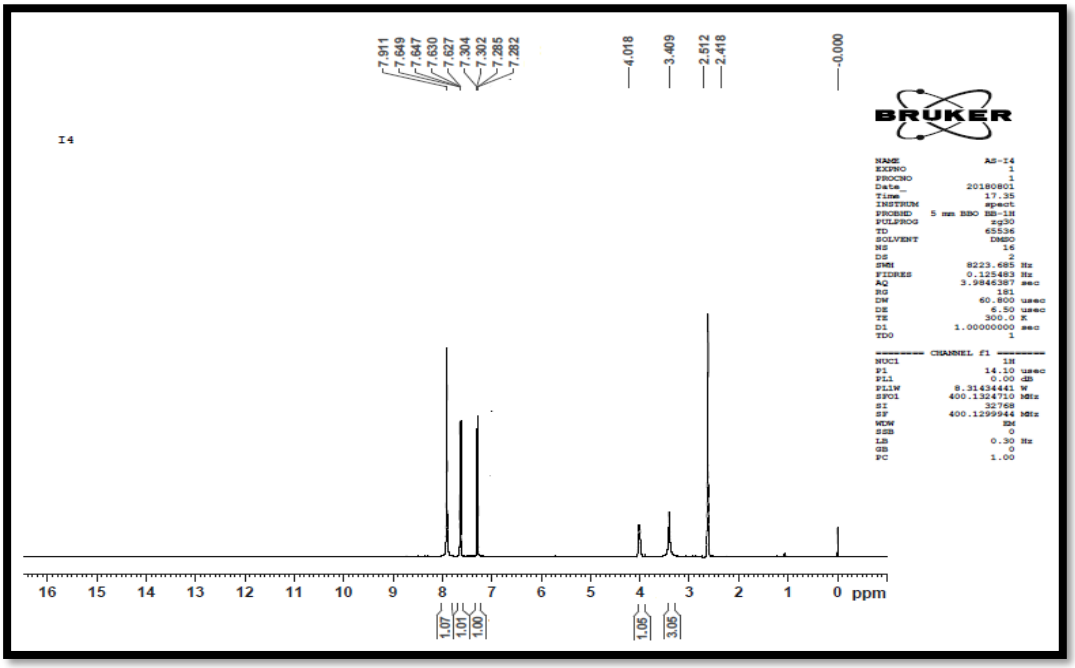
N-(4-chlorobenzo[d]thiazol-2-yl)hydrazinecarbothioamide (INT3)



Molecular Formula	$C_8H_7ClN_4S_2$
Molecular Weight (g/mol)	258.75
Melting Point (ºC)	192-194ºC
Yield (%w/w)	48
Recrystallisation solvent	Ethanol
TLC	Rf = 0.65 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3469 (NH), 1643 (C=C), 1533 (C=N), 1292 (C-N), 1001(C-S), 682 (C-Cl)
^{1}H NMR (DMSO)	7.2-7.9 (m,Ar-3H), 4.0 (s, 1H-NH), 3.4 (S, 3H, NH-NH_2)

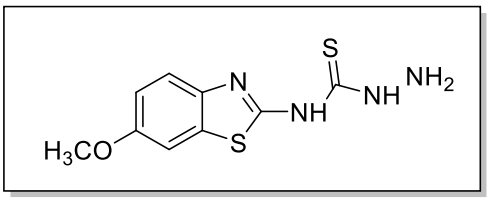


Spectra 5: IR Spectra of INT3

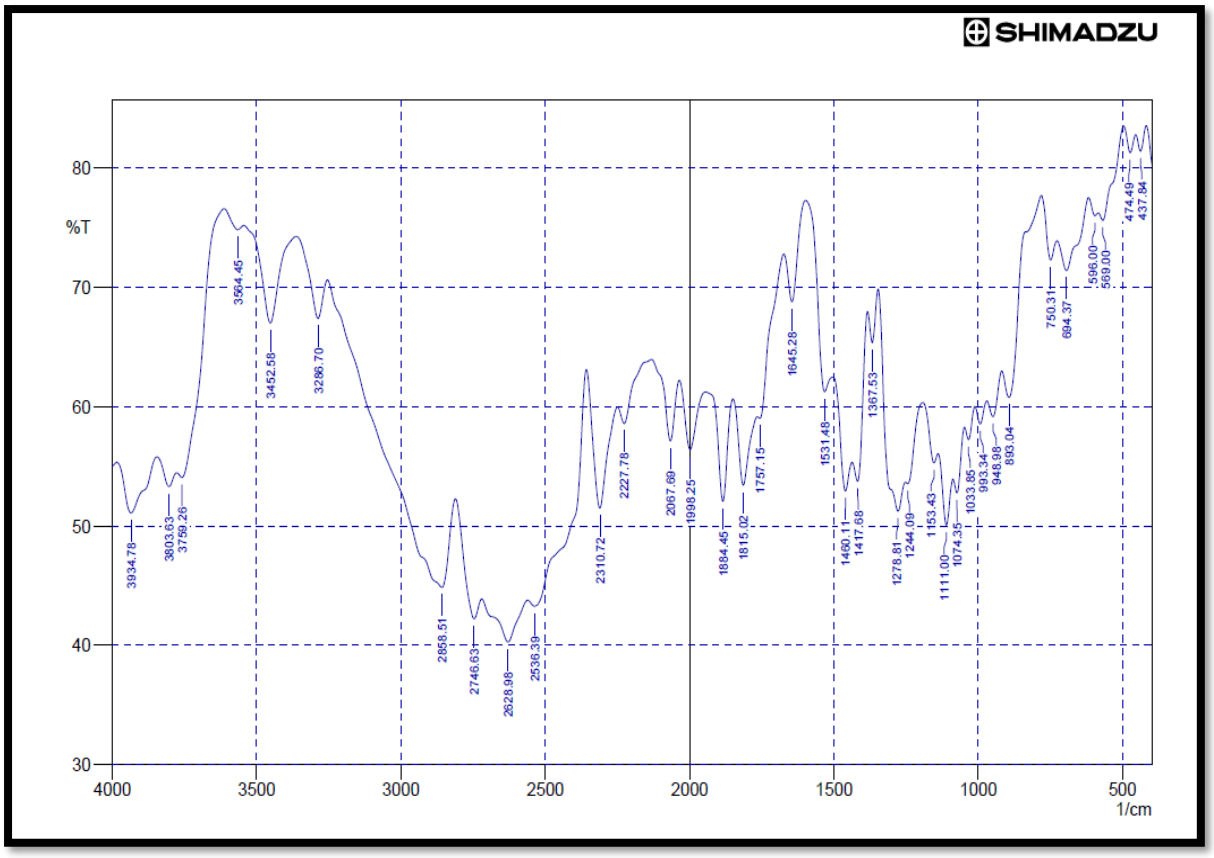


Spectra 6: ^{1}H NMR spectra of INT3

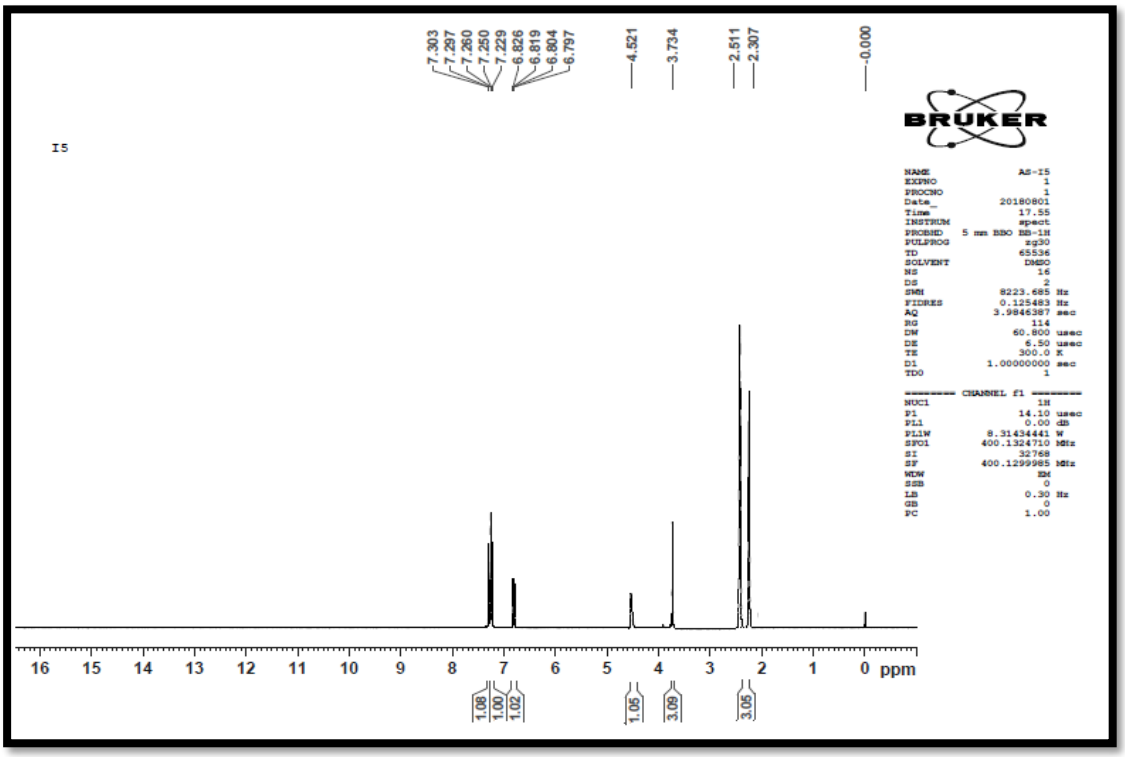
N-(6-methoxybenzo[d]thiazol-2-yl)hydrazinecarbothioamide (INT4)



Molecular Formula	$C_9H_{10}N_4OS_2$
Molecular Weight (g/mol)	254.33
Melting Point (ºC)	202-204ºC
Yield (%w/w)	76
Recrystallisation solvent	Ethanol
TLC	Rf = 0.58 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3452 (NH), 2858 (CH3), 1645(C=C), 1531 (C=N), 1005(C-S),
^{1}H NMR (DMSO)	6.7-7.3 (m. Ar-3H), 4.5 (s, 1H-NH), 3.7 (S, 3H, NH-NH_2) 2.3 (s, 3H, Ar-CH_3)

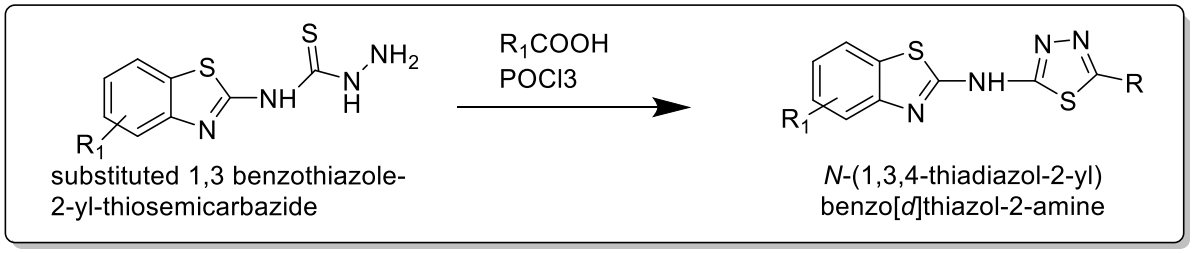


Spectra 7: IR Spectra of INT4



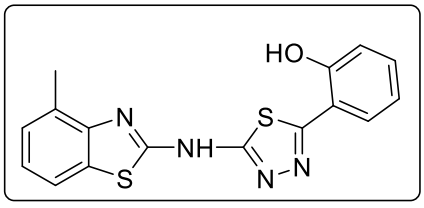
Spectra 8: ^{1}H NMR Spectra of INT4

Step-III: Synthesis of substituted N-(1,3,4-thiadiazol-2-yl)benzo[d]thiazol-2-amine

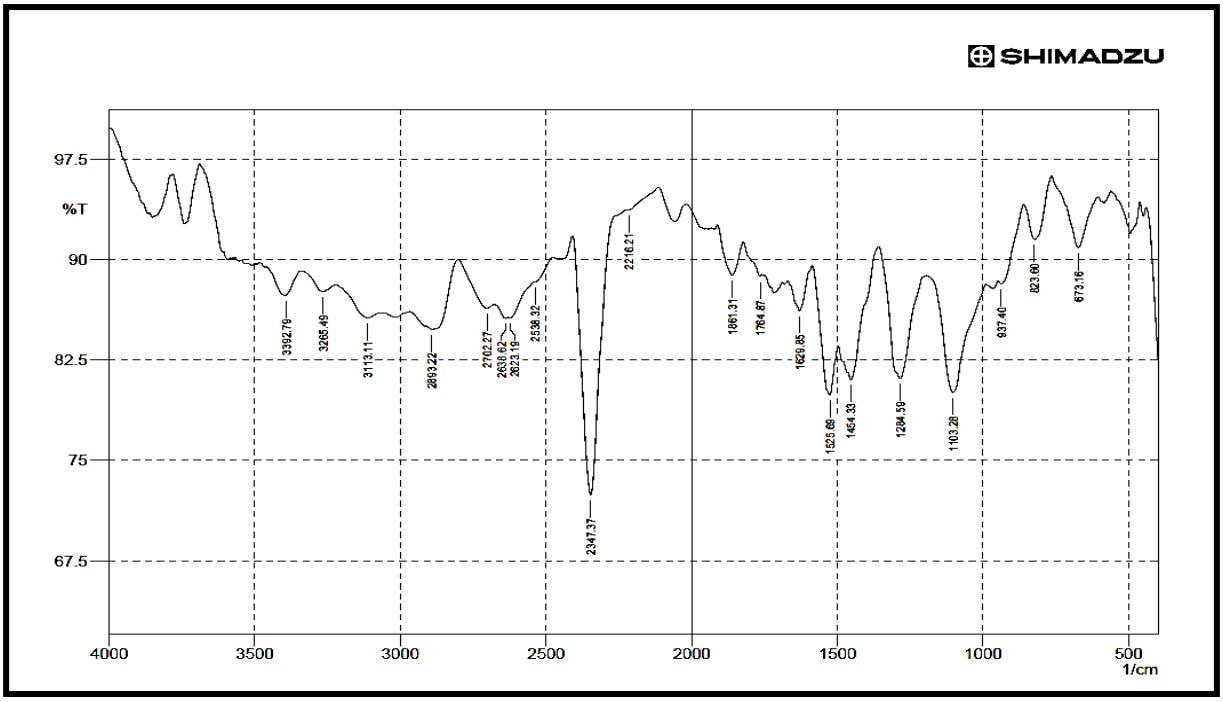


A mixture of, 3 benzothiazole-2-yl-thiosemicarbazides (0.01mol), an aromatic acid (0.01mole) and phosphorous oxychloride 5ml was refluxed for 18-22hr. After cooling at room temperature mixture was slowly poured into crushed ice and kept overnight. The solid thus was separated was filtered, wash with water, dried and recrystallized from ethanol.

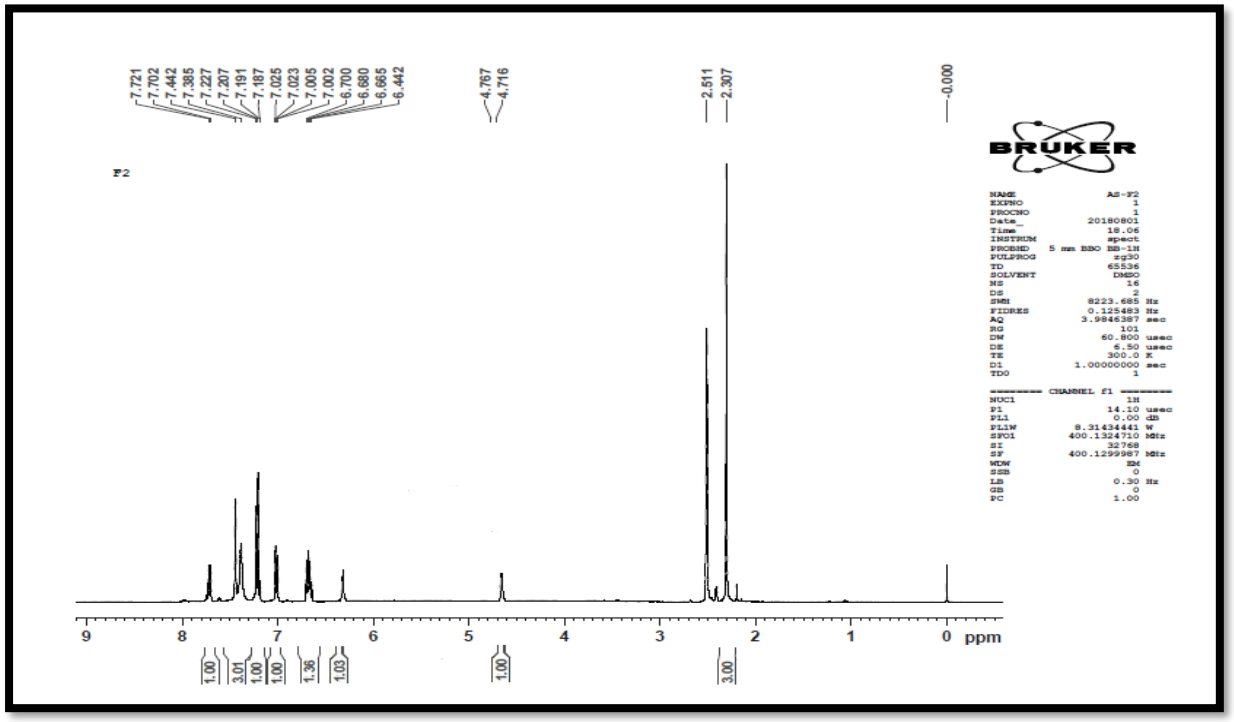
2-(5-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (1SA)



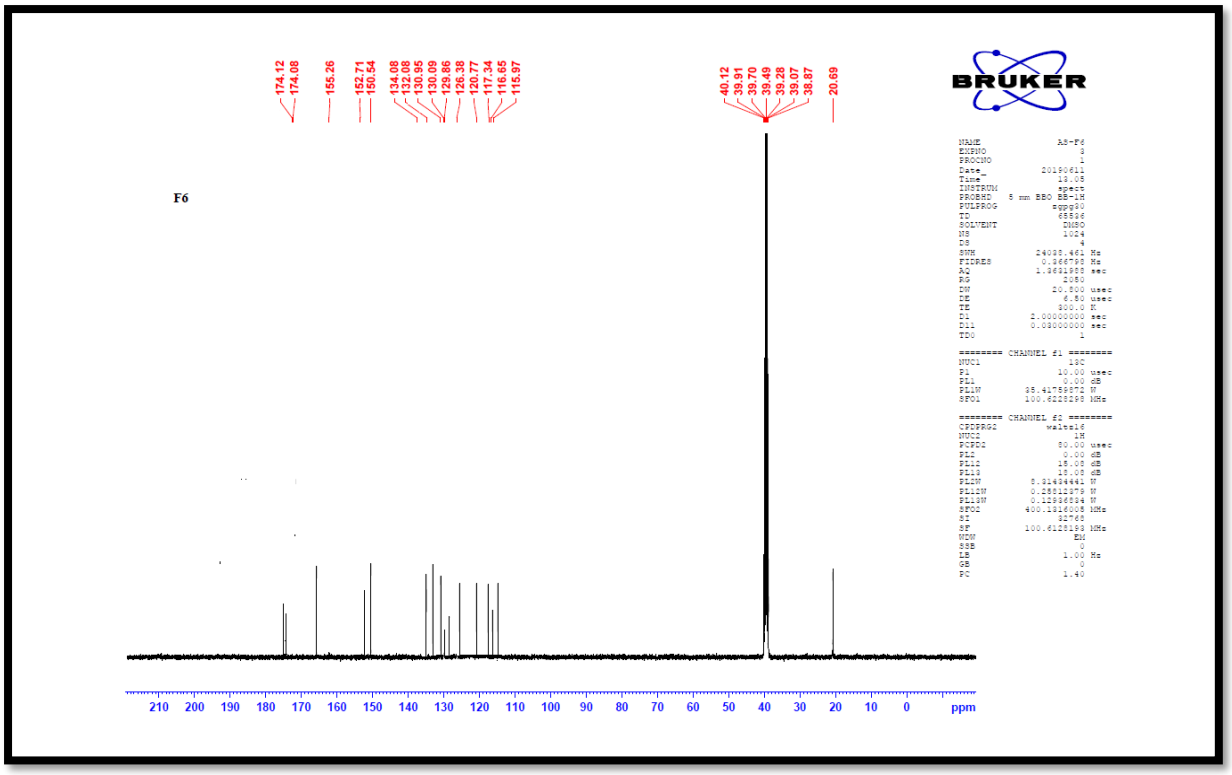
Molecular Formula	$C_{16}H_{12}N_4OS_2$
Molecular Weight (g/mol)	340.42
Melting Point	240-242ºC
% Yield	33
Recrystallisation solvent	Ethanol
TLC	Rf = 0.48 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3392 (NH), 1629 (C-C), 1525 (C=N), 1284 (C-N), 1103(C-S)
^{1}H NMR (DMSO)	6.7-7.7 (m. Ar-7H), 6.4 (S, 1H, Ar-OH),4.7 (s, 1H-NH), 2.3 (S,3H, Ar-CH3)
^{13}C NMR (DMSO)	174.08,174.12(C=N); 155.26(C-O); 150.54,152.71(C-N); 20.69(CH_3); 115.97,116.65,117.34,120.77,126.38, 129.86, 130.09, 130.95,132.08,134.08
MS (70eV) m/z (%)	340



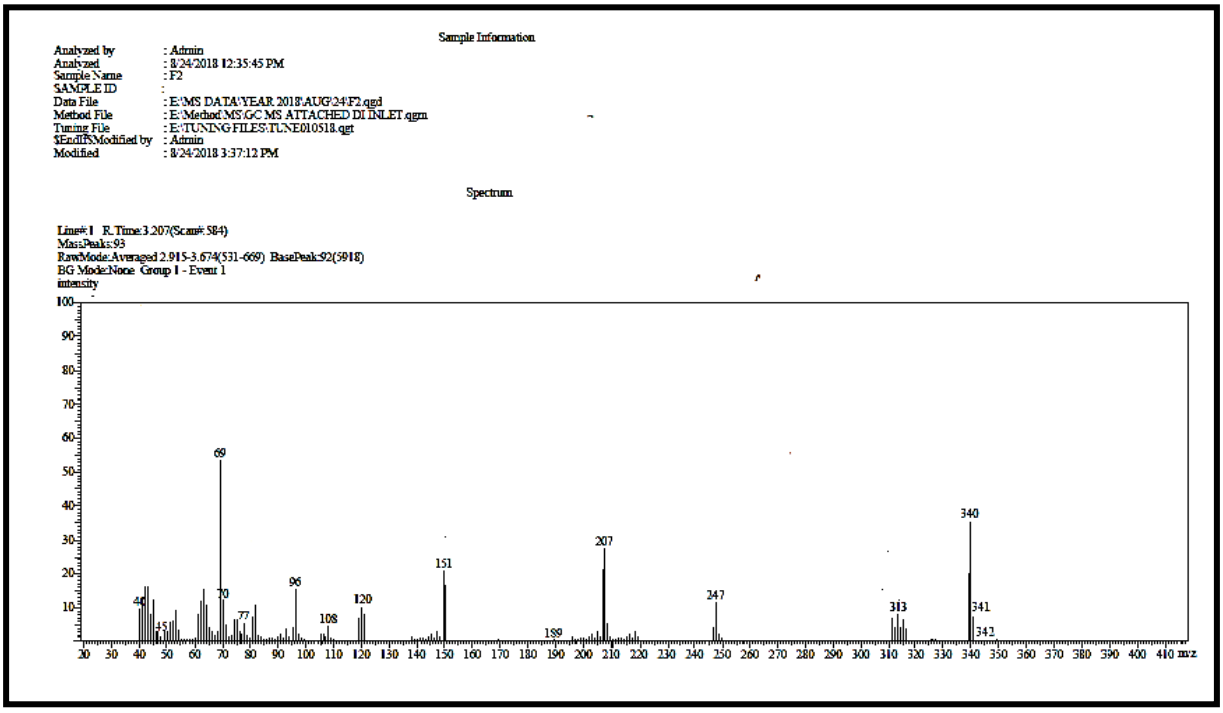
Spectra 9: IR Spectra of Compound 1sa



Spectra 10: ^{1}H NMR spectra of Compound 1sa

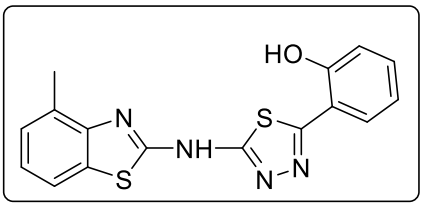


Spectra 11: ^{13}C NMR spectra of Compound 1sa

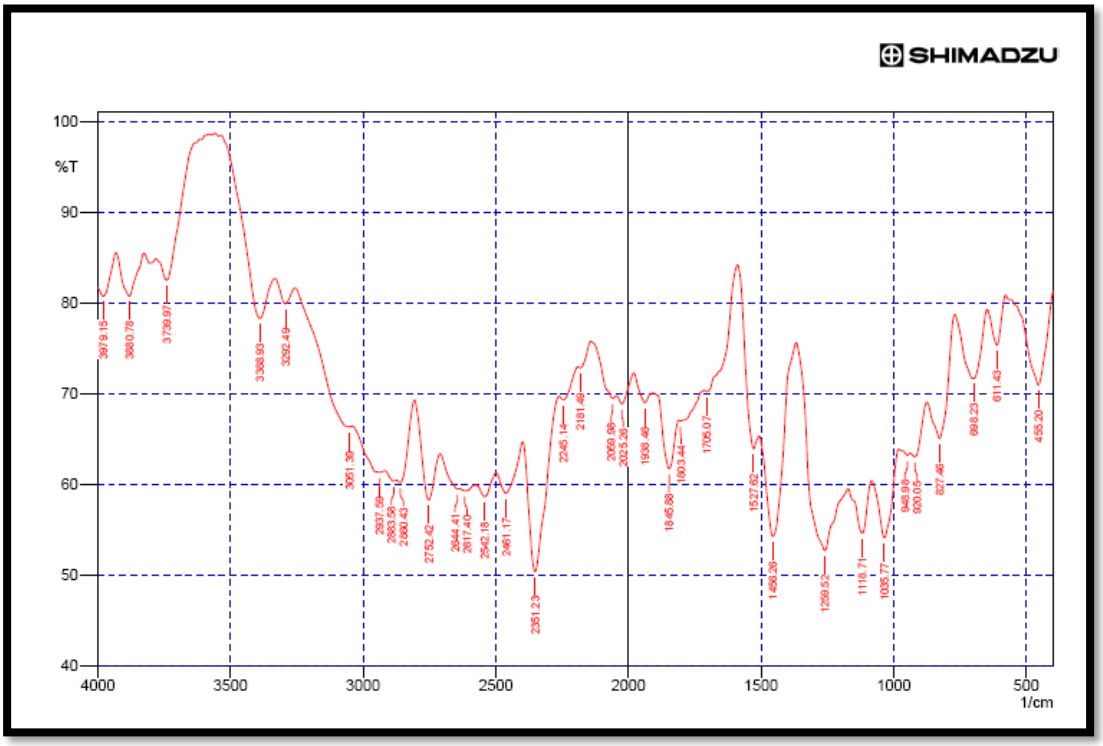


Spectra 12: MASS spectra of Compound 1sa

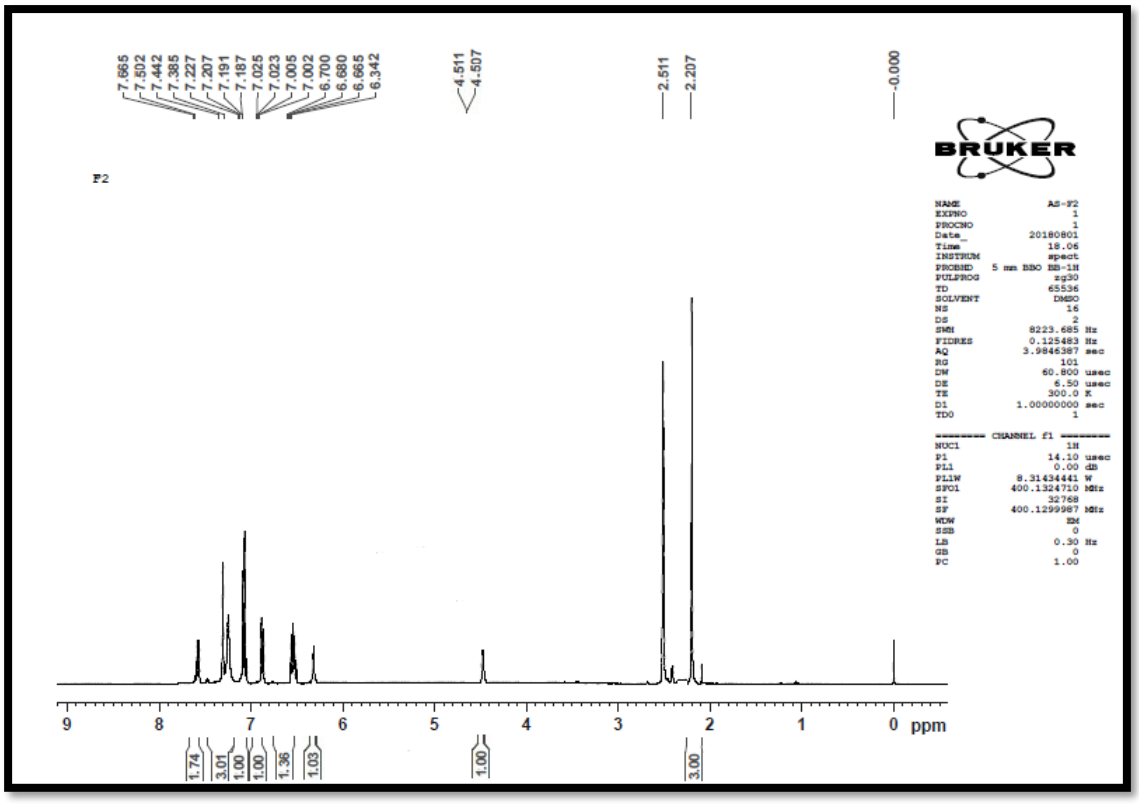
2-(5-((4-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (2sa)



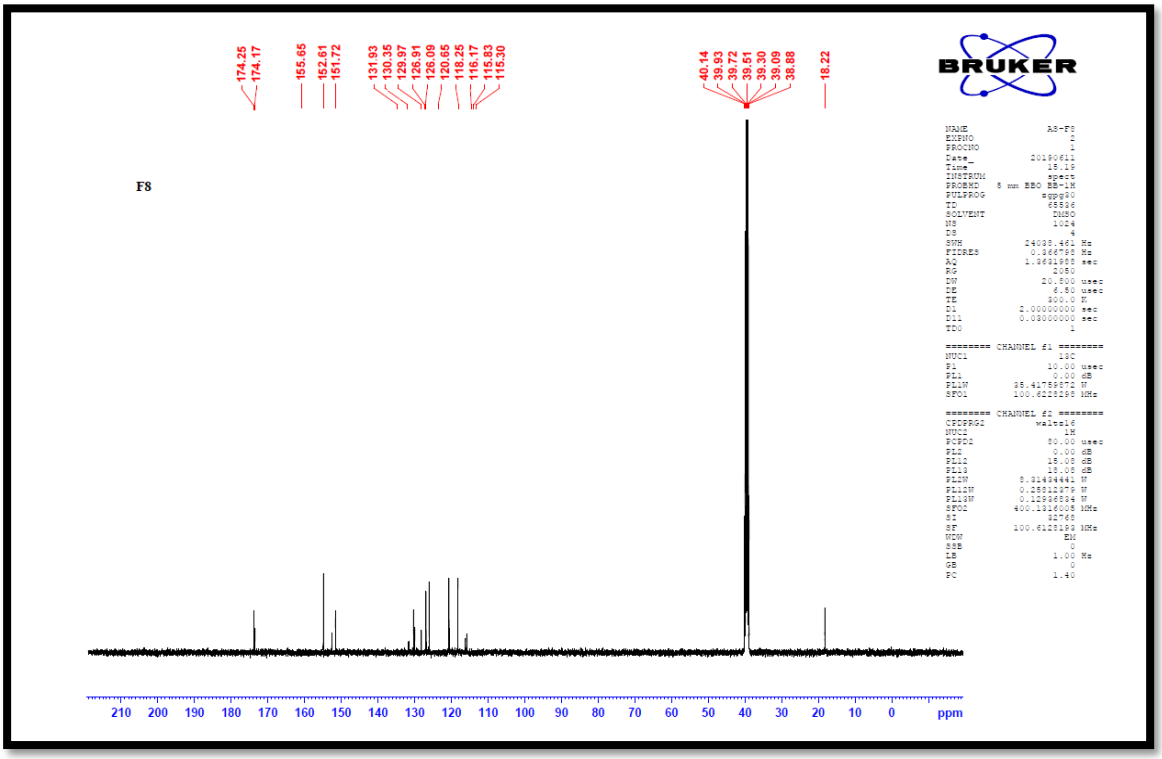
Molecular Formula	$C_{16}H_{12}N_4OS_2$
Molecular Weight (g/mol)	340.42
Melting Point	236-238ºC
% Yield	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.58 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3388 (NH), 2752 (Ar-CH3), 1525 (C=N), 1259 (C-N), 1118(C-S)
^{1}H NMR (DMSO)	6.6-7.7 (m. Ar-7H), 6.3 (S, 1H, Ar-OH),4.5 (s, 1H-NH), 2.2 (S,3H, Ar-CH3)
^{13}C NMR (DMSO)	174.17,174.25(C=N); 155.65C-O); 152.61,151.72C-N);18.22(CH_3); 131.93, 130.35, 129.97, 126.91, 126.09, 120.65, 118.25, 116.17, 115.83, 115.30
MS (70eV) m/z (%)	340



Spectra 13 IR spectra of compound 2sa

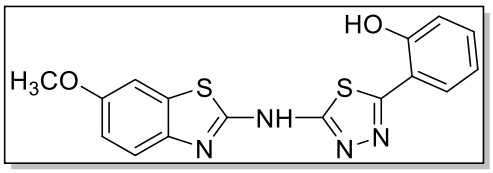


Spectra 14: ^{1}H NMR spectra of Compound 2sa

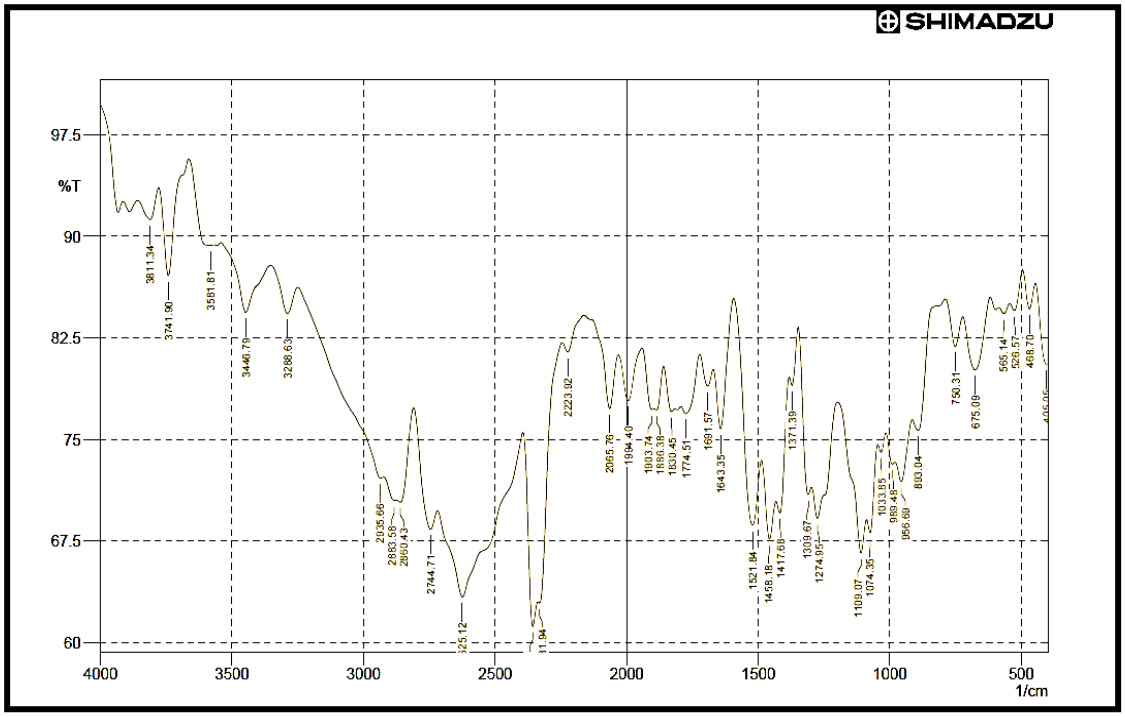


Spectra 15: MASS spectra of Compound 2sa

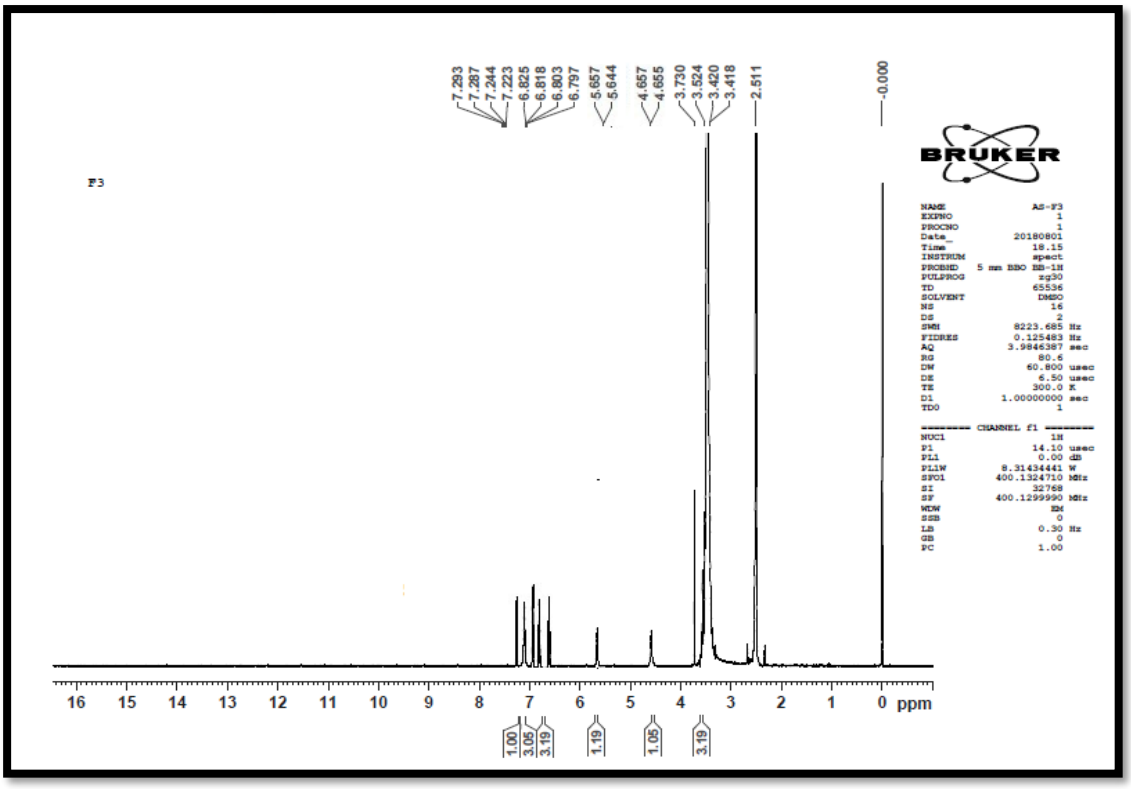
2-(5-((6-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (3SA)



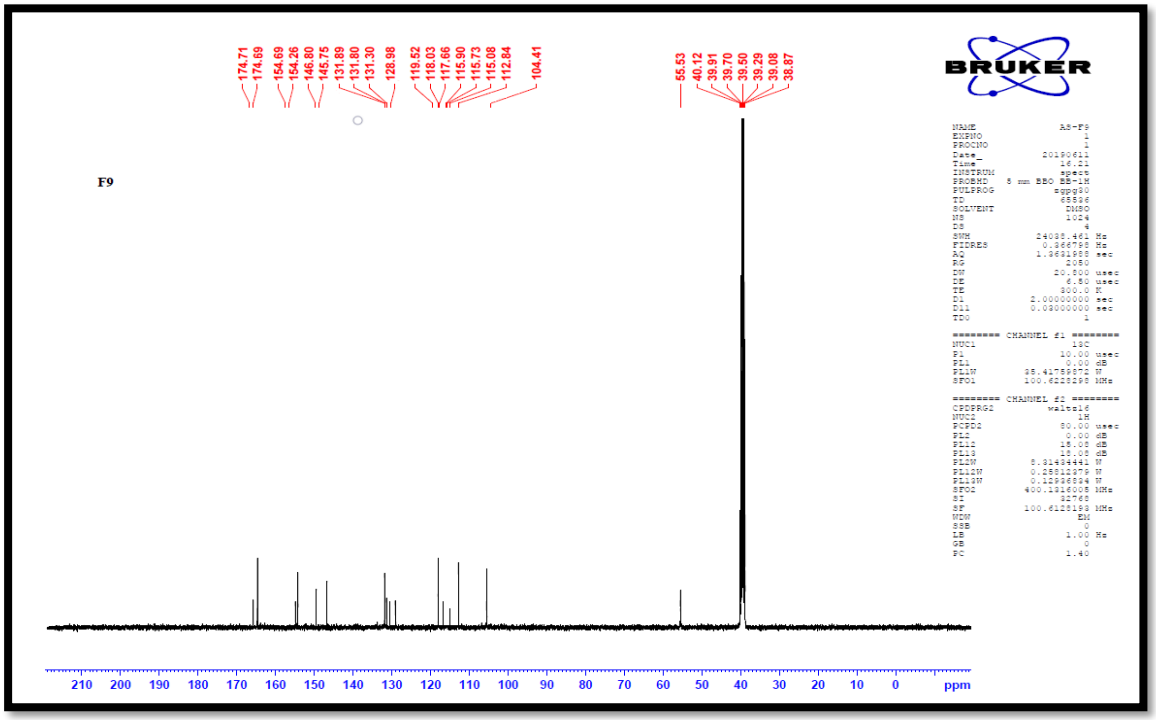
Molecular Formula	$C_{16}H_{12}N_4O_2S_2$
Molecular Weight (g/mol)	356.42
Melting Point	254-256ºC
% Yield	56
Recrystallisation solvent	Ethanol
TLC	Rf = 0.63 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3811 (Ar-OH), 3446 (NH), 1643 (C-C), 1521 (C=N), 1274 (C-N) 1109(C-S)
^{1}H NMR (DMSO)	6.7-7.29 (m. Ar-7H), 5.6 (S, 1H, Ar-OH),4.6 (s, 1H-NH), 3.7 (S,3H, Ar-OCH_3)
^{13}C NMR (DMSO)	174.71,174.69(C=N);154.69,154.26(C-O); 55.53(CH_3); 145.75,146.80(C-N); 131.89, 131.30, 128.98, 119.52, 118.03, 115.08,115.73, 115.90,104.41
MS (70eV) m/z (%)	356



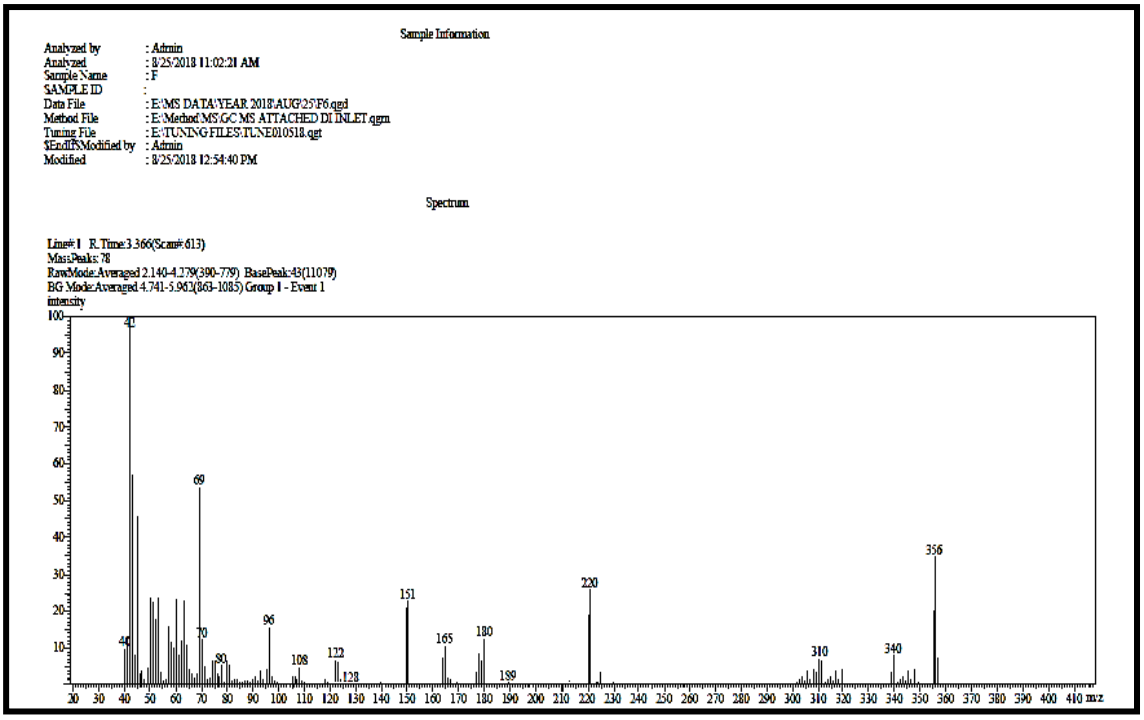
Spectra 16: IR spectra of compound 3sa



Spectra 17: 1H NMR spectra of Compound 3sa

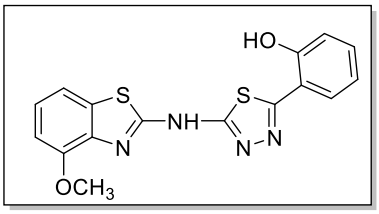


Spectra 18: ^{13}C NMR spectra of Compound 3sa

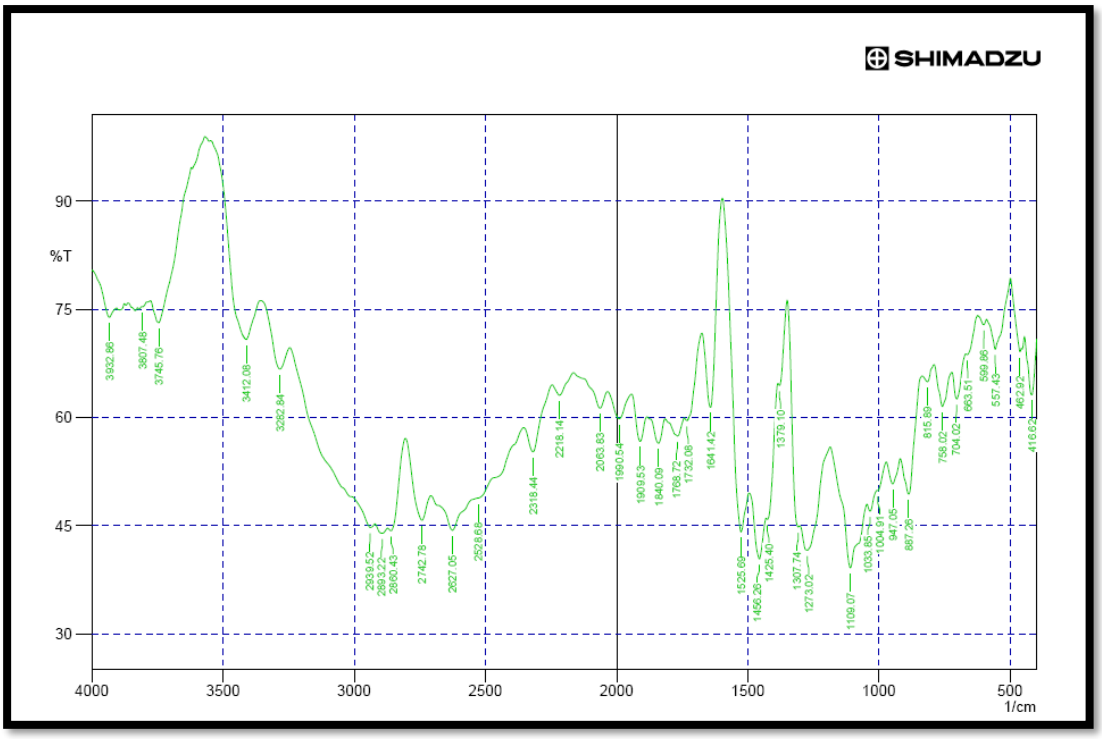


Spectra 19: Mass Spectra of compound 3sa

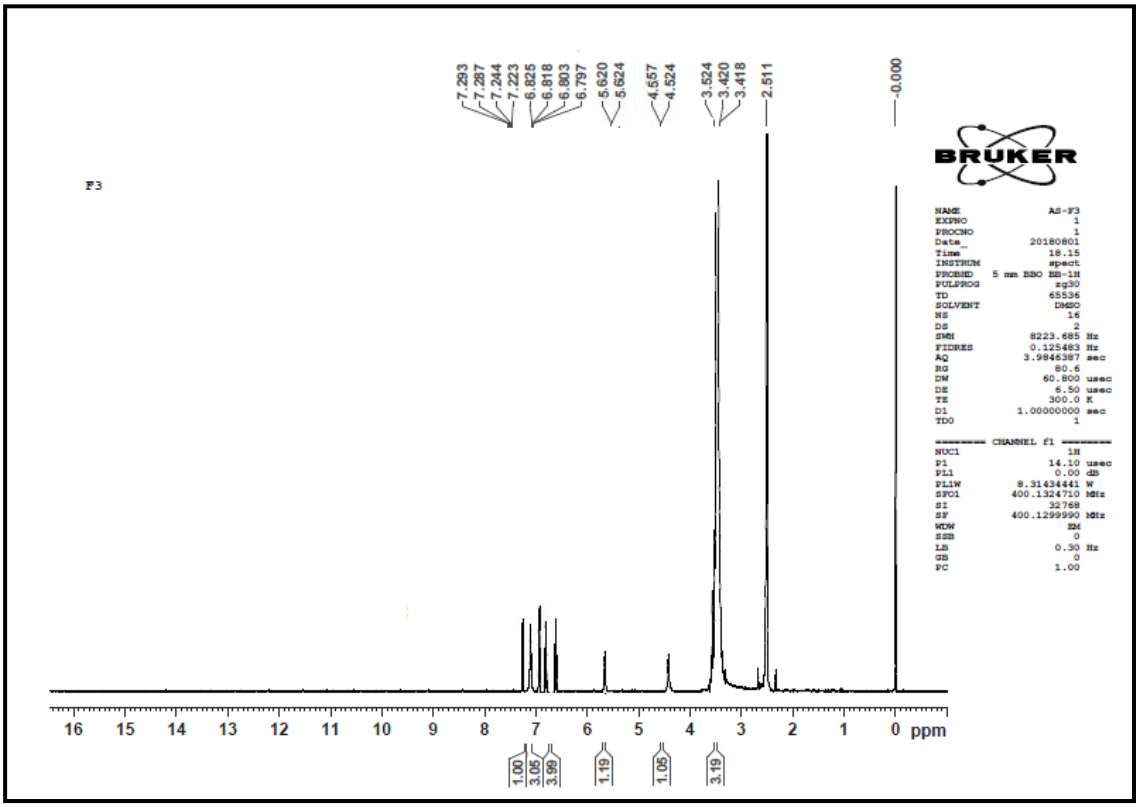
2-(5-((4-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (4SA)



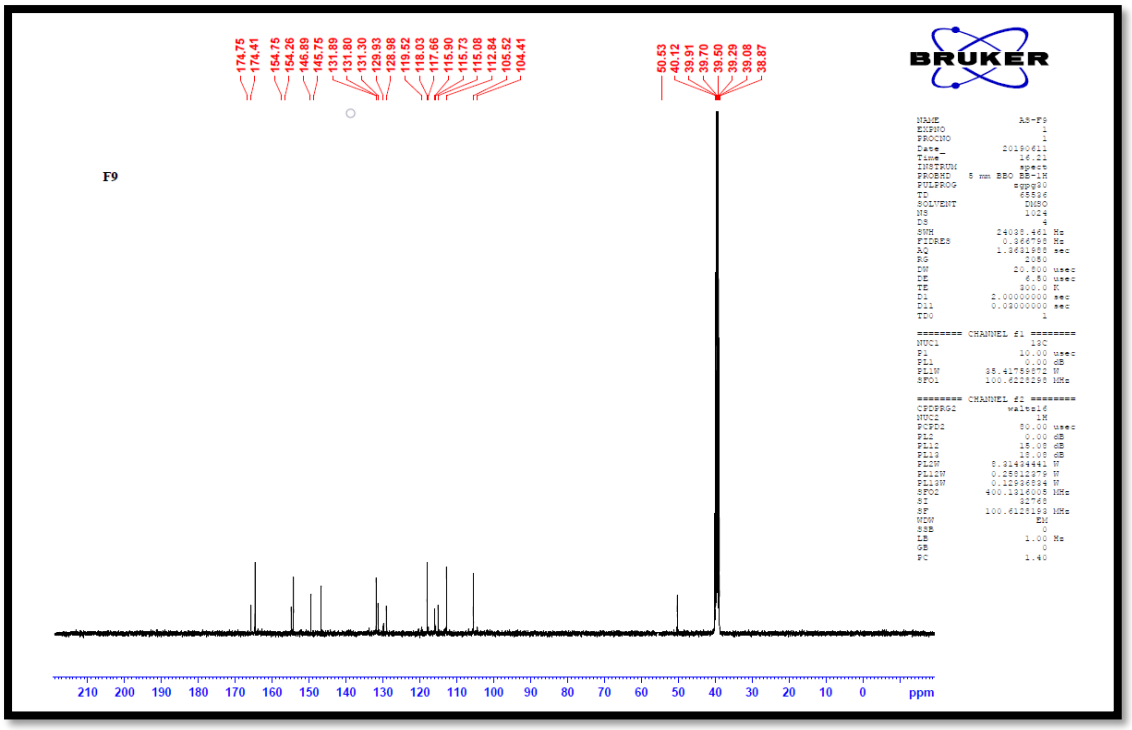
Molecular Formula	$C_{16}H_{12}N_4O_2S_2$
Molecular Weight (g/mol)	356.42
Melting Point	246-248ºC
% Yield	48
Recrystallisation solvent	Ethanol
TLC	Rf = 0.67 n-hexane: ethyl acetate (8:2)
IR (ν, cm^{-1})	3807 (Ar-OH), 3412 (NH), 1641 (C-C), 1525 (C=N), 1273 (C-N) 1109(C-S)
^{1}H NMR (DMSO)	6.7-7.29 (m. Ar-7H), 5.6 (S, 1H, Ar-OH),4.5 (s, 1H-NH), 3.5 (S,3H, Ar-OCH_3)
^{13}C NMR (DMSO)	174.75,174.41(C=N);154.75,154.26(C-O);50.53(CH_3); 145.75, 146.89(C-N);104.41,105.52,112.84,115.08, 118.03,119.52,128.98,129.93,131.80
MS (70eV) m/z (%)	356



Spectra 20:IR spectra of compound 4sa

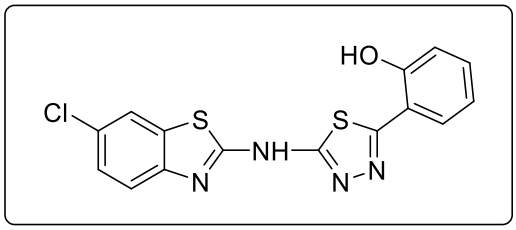


Spectra 21:^{1}H NMR spectra of Compound 4sa

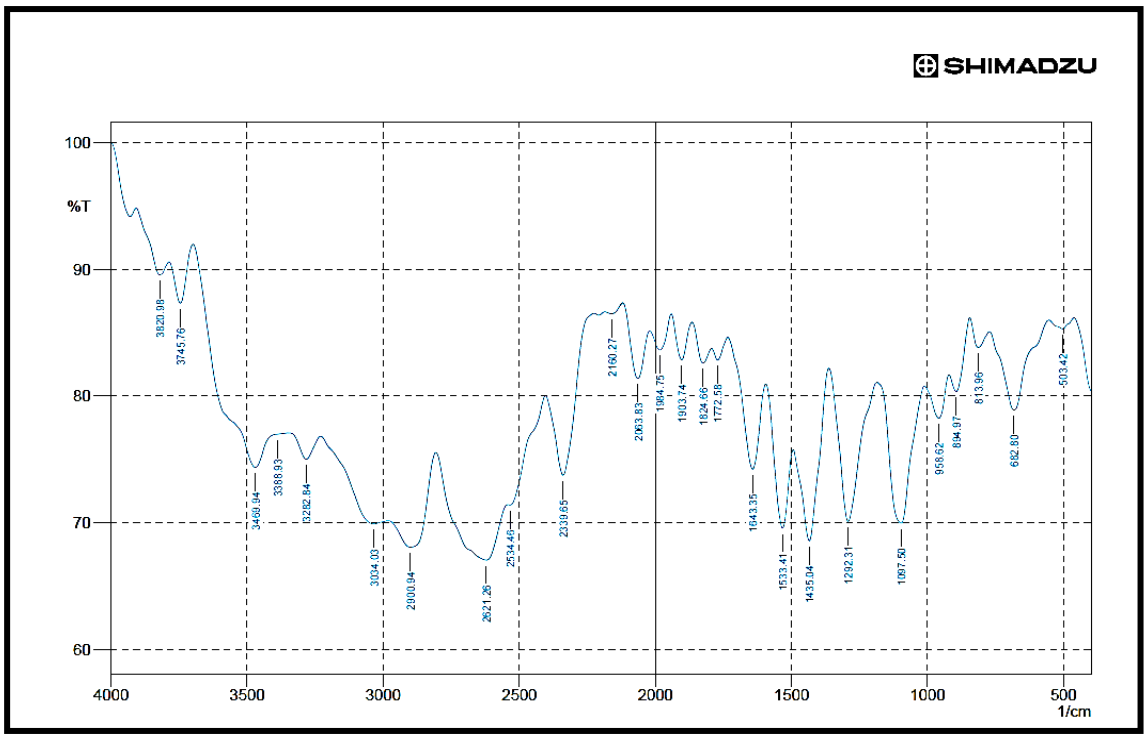


Spectra 22 13C NMR spectra of Compound 4sa

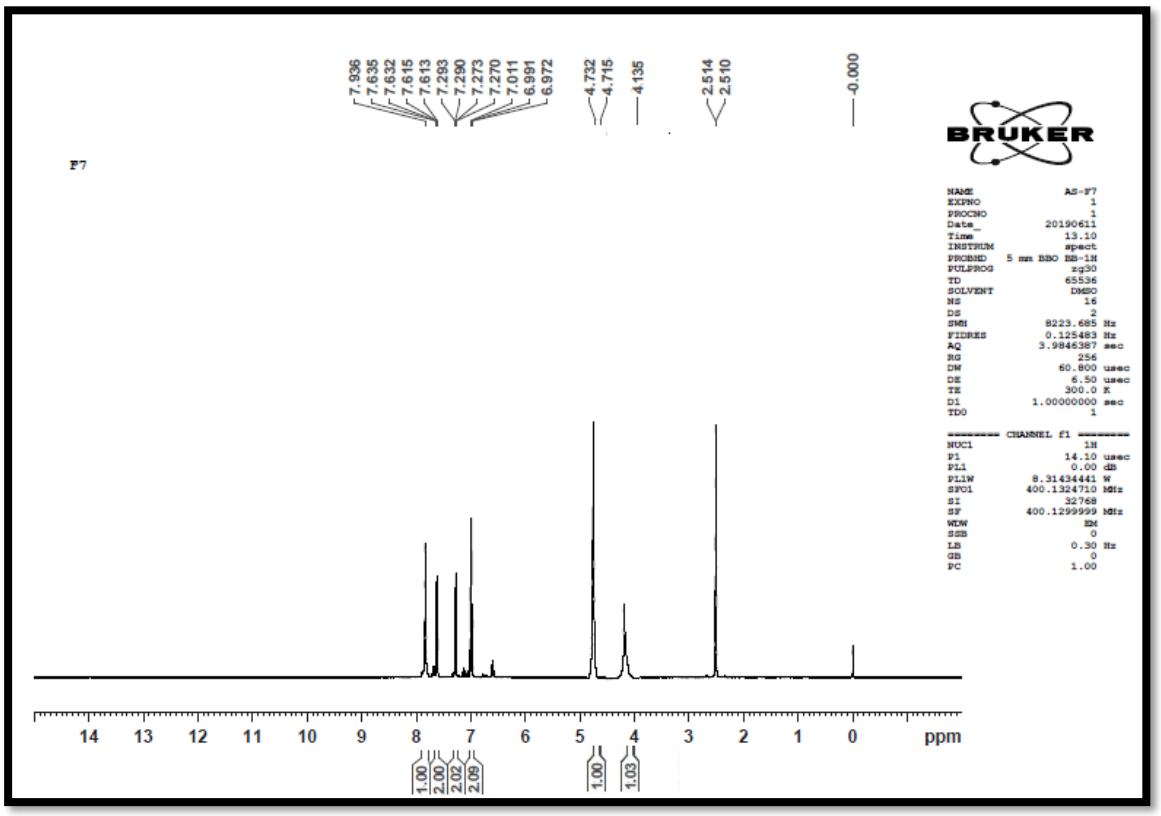
2-(5-((4-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (5SA)



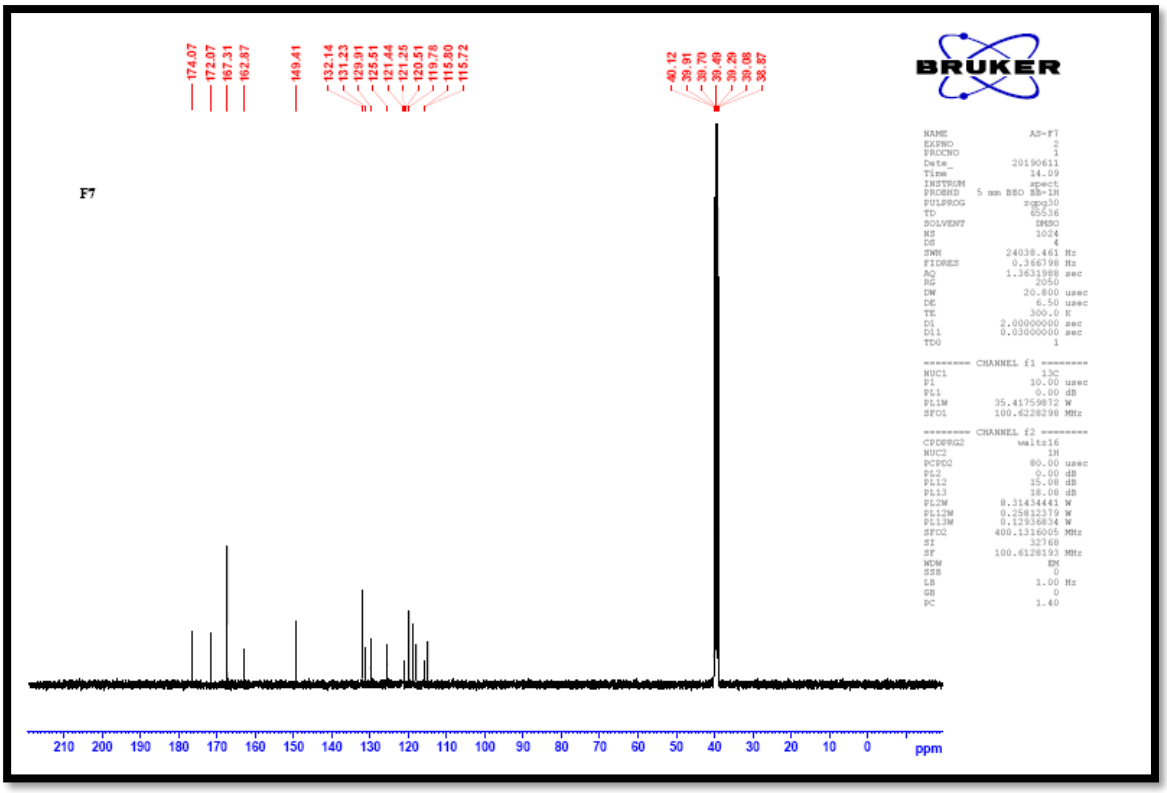
Molecular Formula	$C_{15}H_9ClN_4OS_2$
Molecular Weight (g/mol)	360.83
Melting Point	244-246ºC
% Yield	40
Recrystallisation solvent	Ethanol
TLC	Rf = 0.60 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3820(Ar-OH), 3388(NH), 1435(C=N), 1292(C-N) 1097(C-S)
^{1}H NMR (DMSO)	6.97-7.93 (m, Ar-7H), 4.76 ($_S$, 1H, Ar-OH), 4.1 (s, 1H-NH)
^{13}C NMR (DMSO)	172.07,174.07(C=N); 162.87,167.31(C-N); 149.42(C=C); 115.72, 115.80, 119.78, 120.51,121.25,121.44,125.51,129.91, 131.23,132.14
MS (70eV) m/z (%)	360



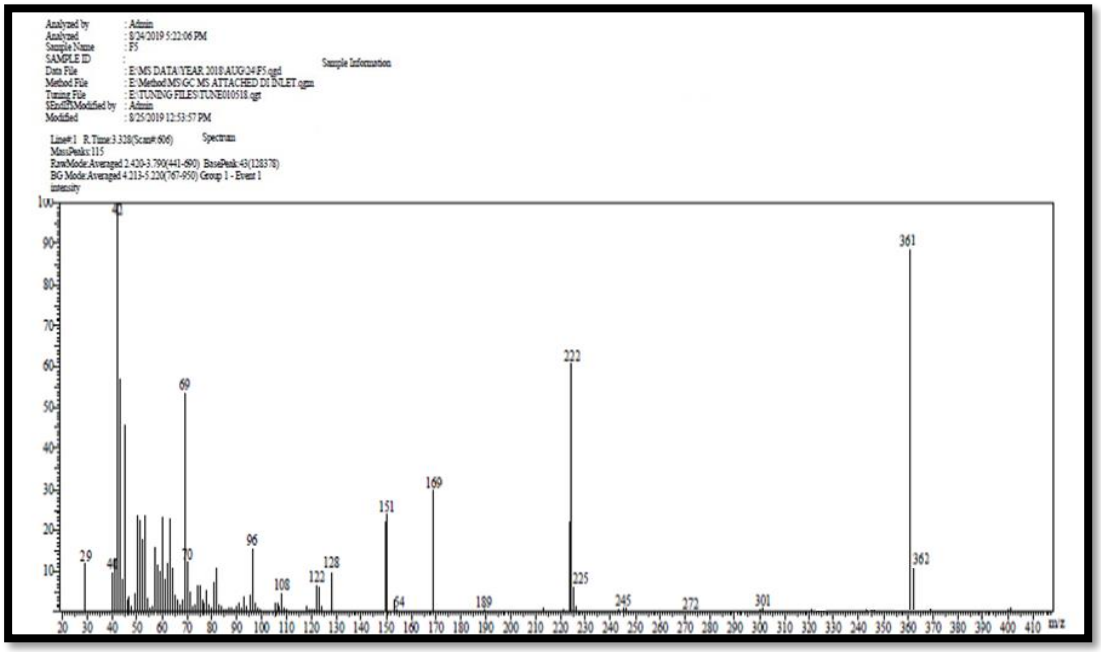
Spectra 23: IR spectra of compound 5SA



Spectra 24:^{1}H NMR spectra of Compound 5sa

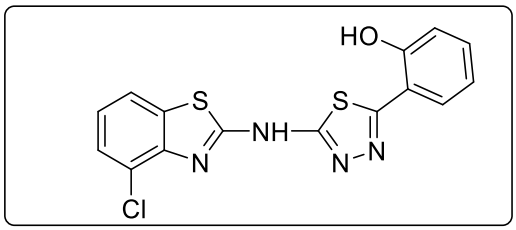


Spectra 25: ^{13}C NMR spectra of Compound 5sa

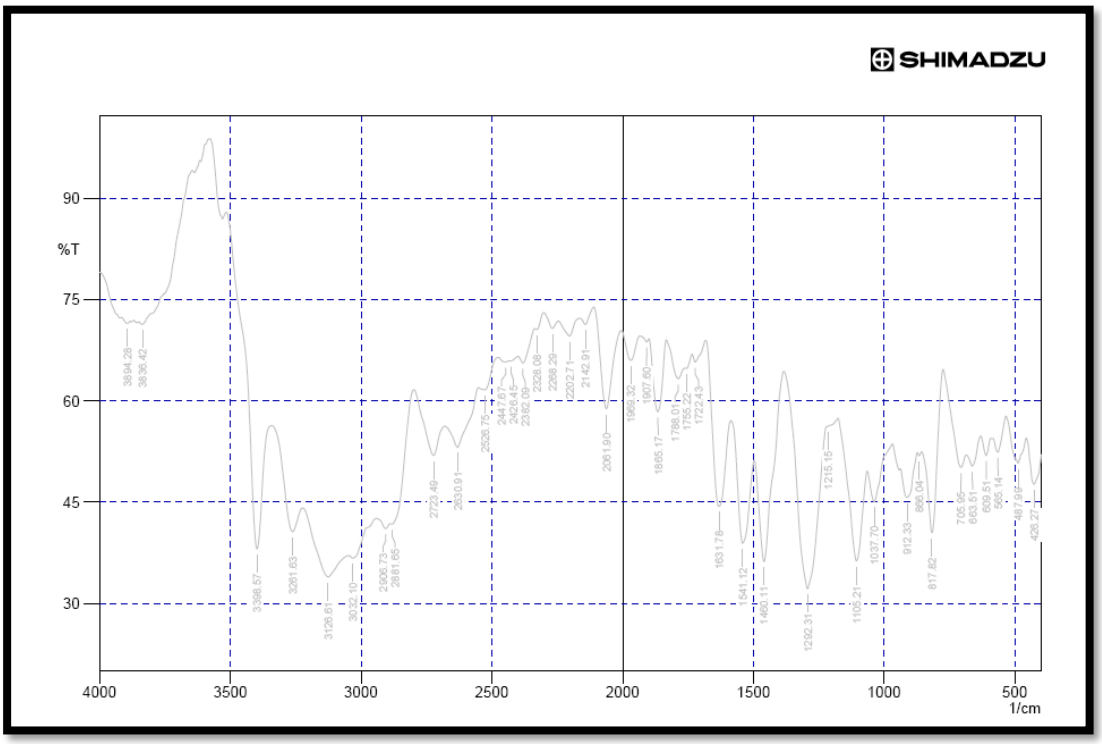


Spectra 26: MASS spectra of compound 5sa

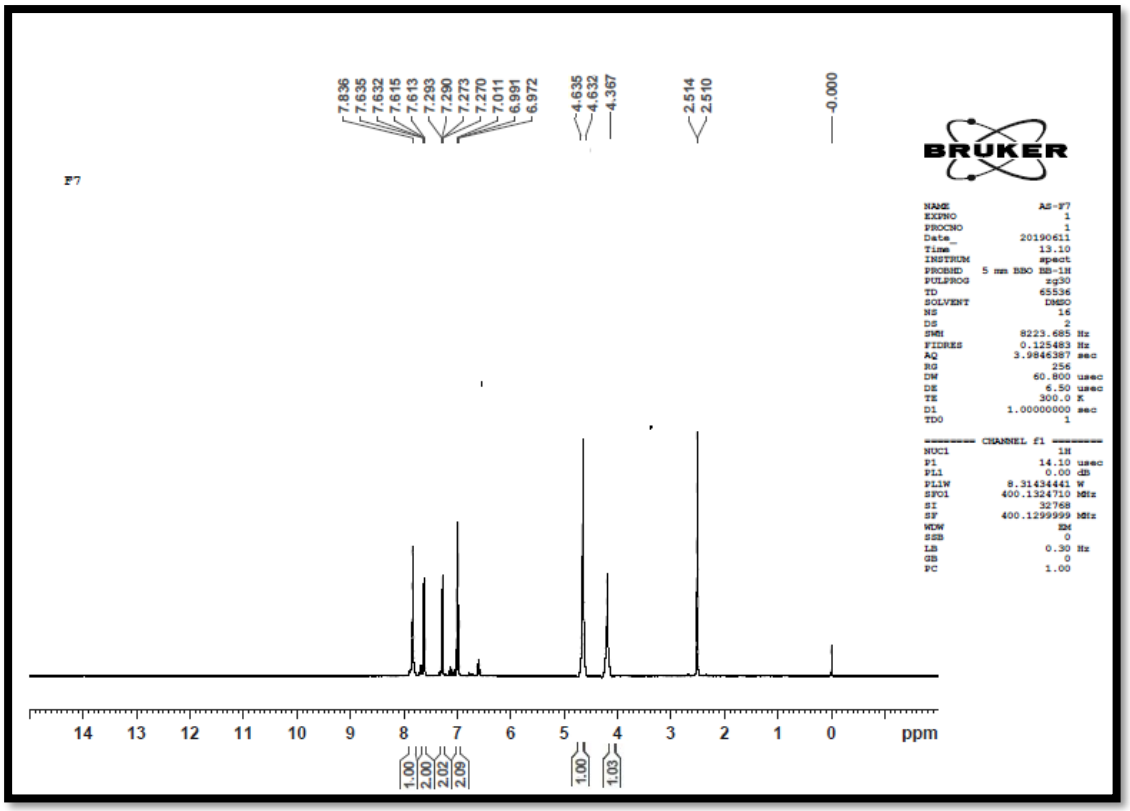
2-(5-((4-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (6sa)



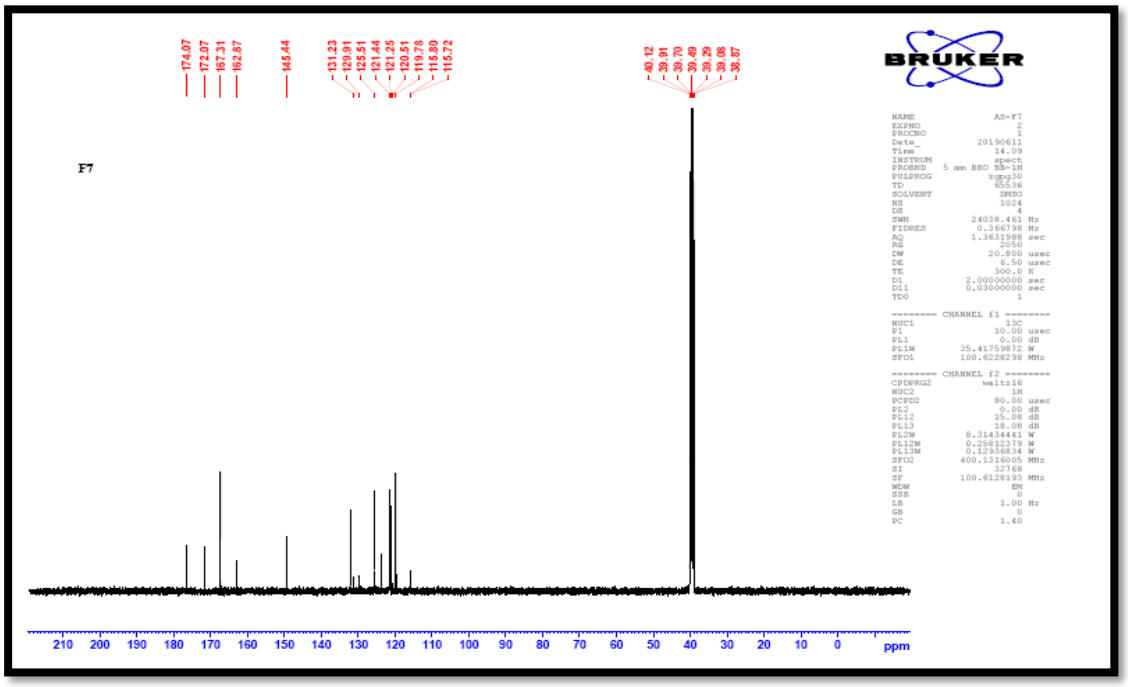
Molecular Formula	$C_{15}H_9ClN_4OS_2$
Molecular Weight (g/mol)	360.83
Melting Point	260-262ºC
% Yield	40
Recrystallisation solvent	Ethanol
TLC	Rf = 0.54 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3894(Ar-OH), 3396(NH), 1460(C=N), 1292(C-N) 1037(C-S)
^{1}H NMR (DMSO)	6.97-7.83 (m, Ar-7H), 4.76 ($_S$, 1H, Ar-OH), 4.36 (s, 1H-NH)
^{13}C NMR (DMSO)	172.07,174.07(C=N); 162.87,167.31(C-N); 149.42(C=C); 115.72,115.80,119.78,120.51,121.25,121.44,125.51,129.91, 131.23,132.14
MS (70eV) m/z (%)	360



Spectra 27: IR Spectra of compound 6sa

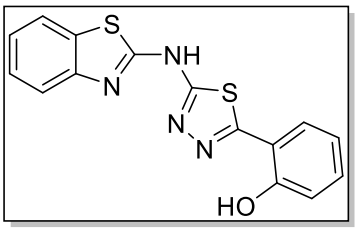


Spectra 28: ^{1}H NMR spectra of Compound 6sa

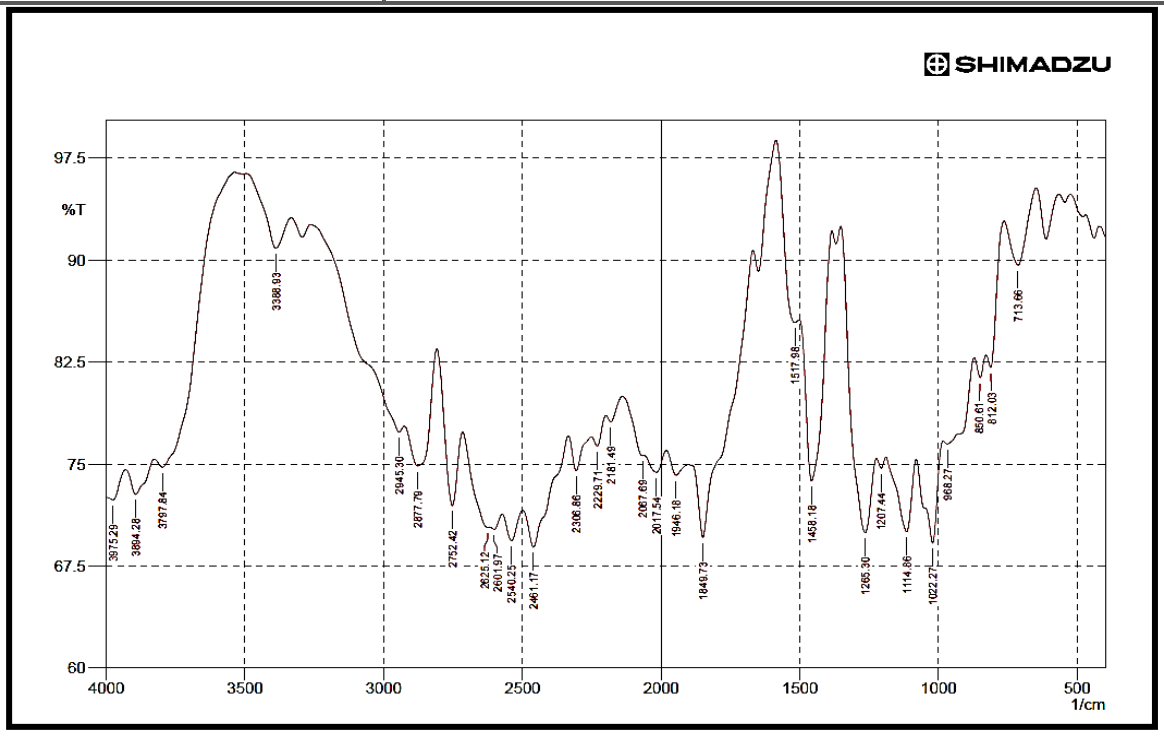


Spectra 29: ^{13}C NMR spectra of Compound 6sa

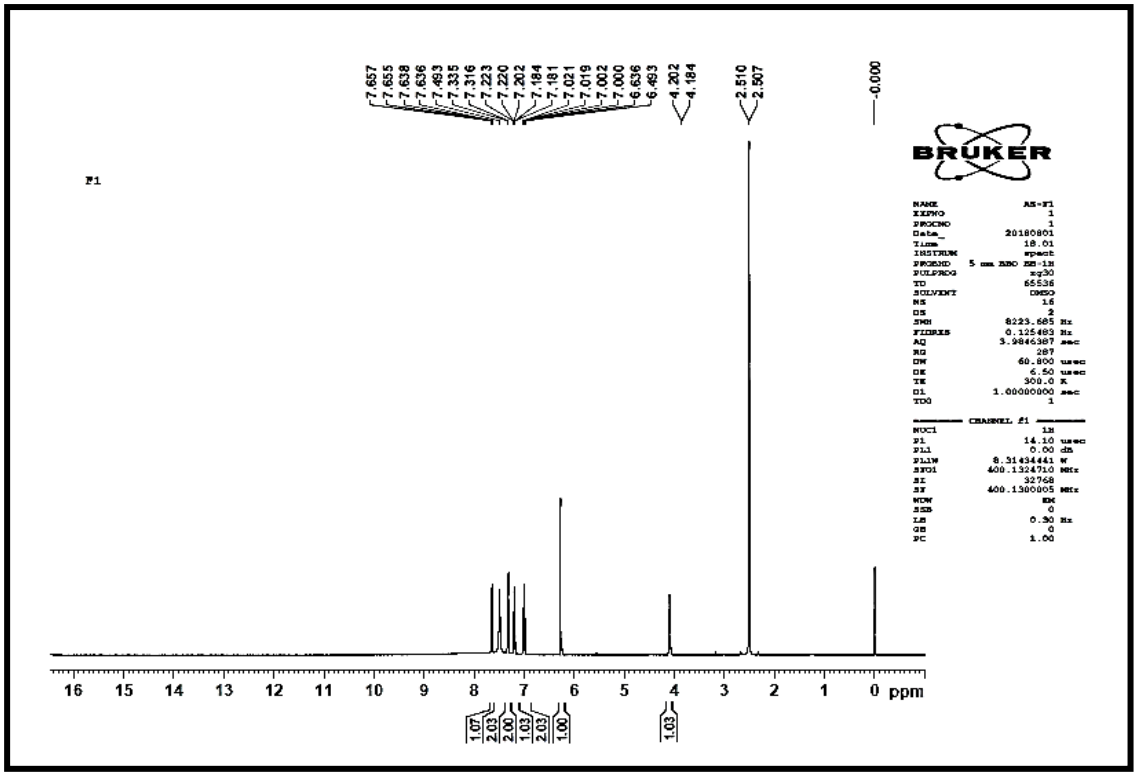
2-(5-(benzo[d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl) phenol (7SA)



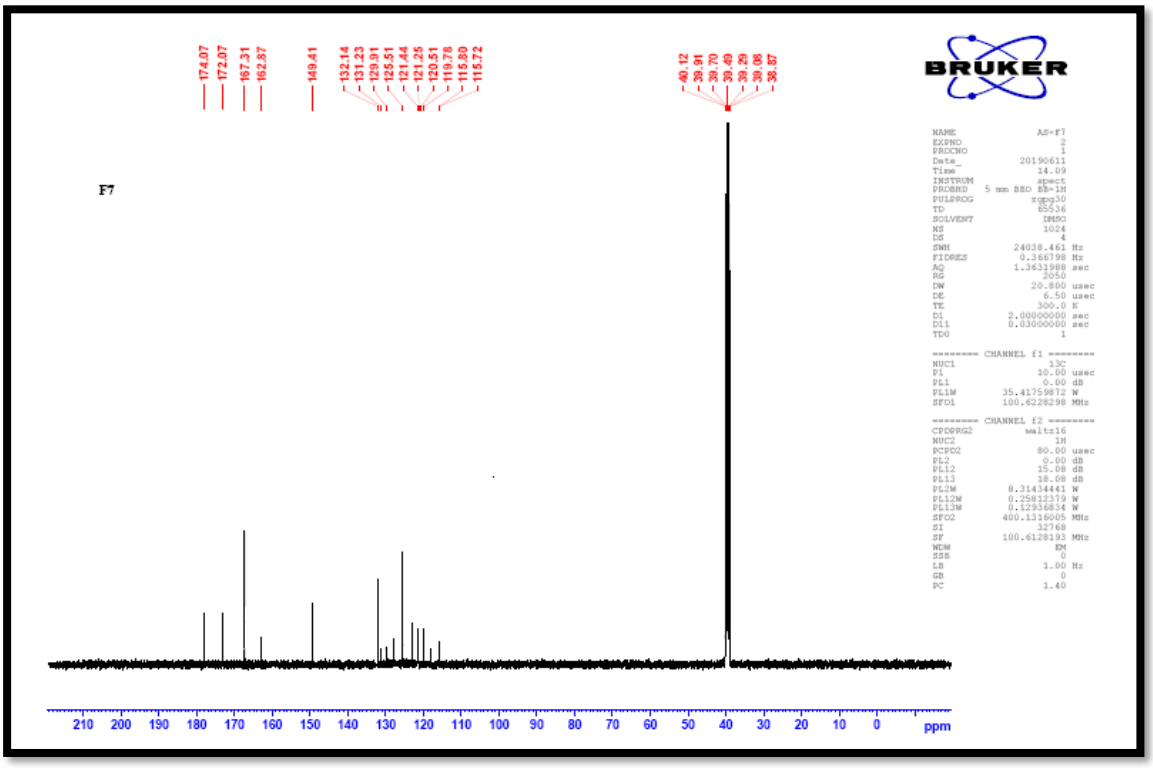
Molecular Formula	$C_{15}H_{10}N_4OS_2$
Molecular Weight (g/mol)	326.40
Melting Point	234-236ºC
% Yield	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.55 n-hexane: ethyl acetate (8:2)
IR (ν, cm^{-1})	3894 (Ar-OH), 3388 (NH), 1458 (C=N), 1207 (C-N) 1114(C-S)
^{1}H NMR (DMSO)	6.49-7.65 (m, Ar-8H), 6.5 (S, 1H, Ar-OH),4.2 (s, 1H-NH)
^{13}C NMR (DMSO)	174.09,172.07(C=N);162.87,167.31(C-N);149.41(C-N); 115.72, 115.80, 119.78, 120.51, 121.25, 121.44, 125.51, 129.91, 131.23,132.14
MS (70eV) m/z (%)	326



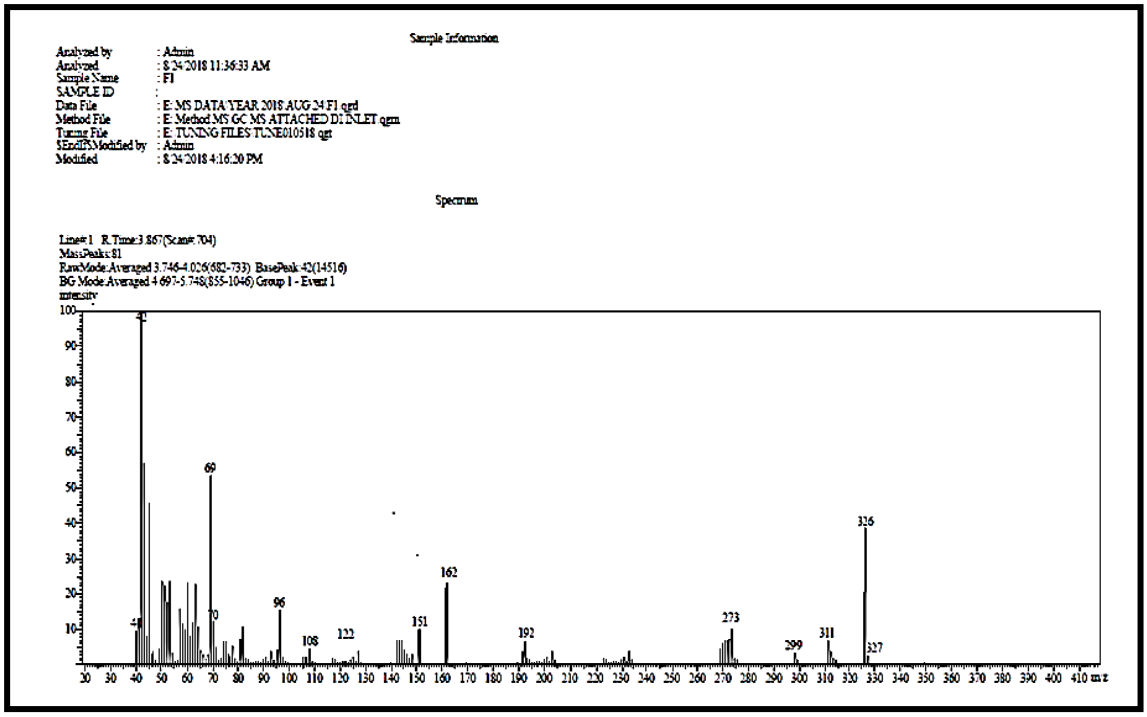
Spectra 30: IR spectra of compound 7sa



Spectra 31: ^{1}H NMR spectra of Compound 7sa

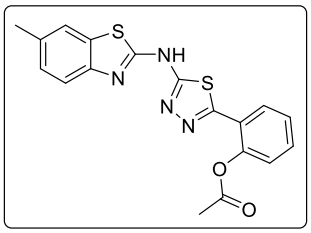


Spectra 32: ^{13}C NMR spectra of Compound 7sa

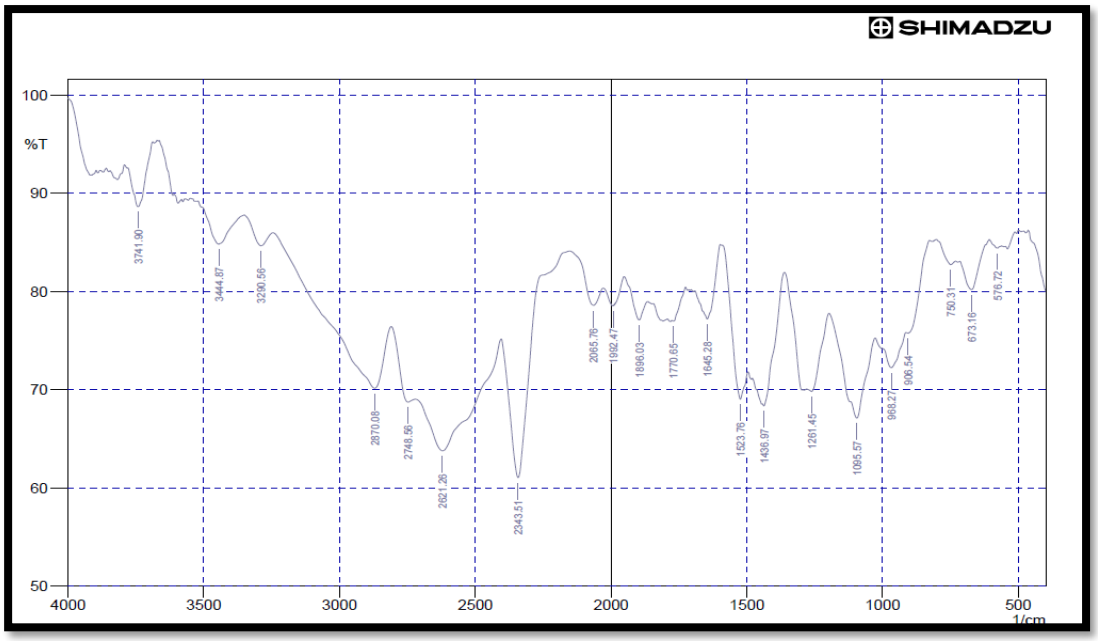


Spectra 33: MASS spectra of compound 7sa

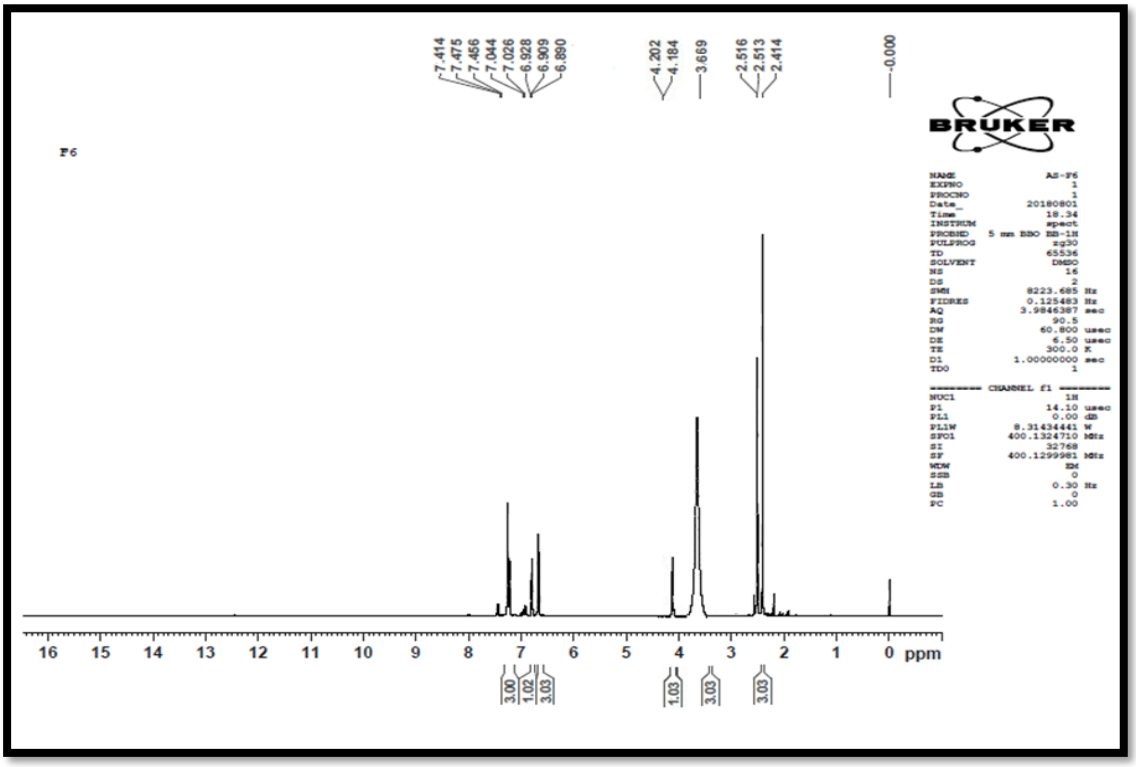
2-(5-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (1a)



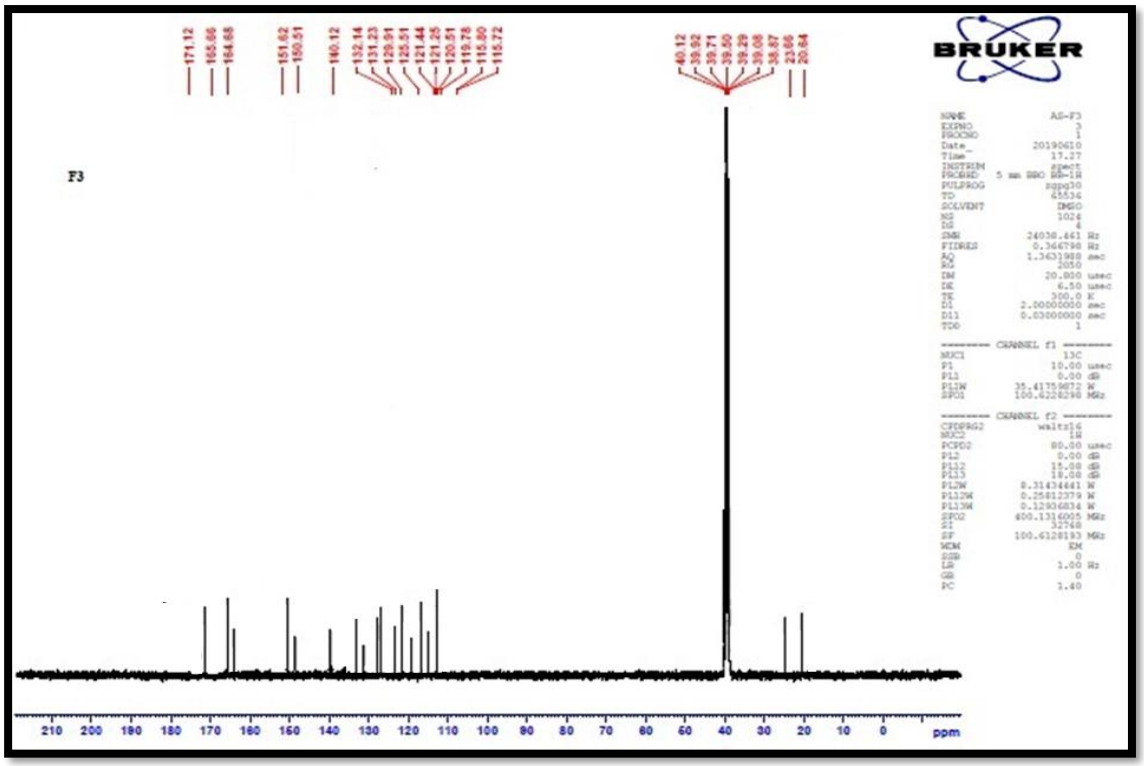
Molecular Formula	$C_{18}H_{14}N_4O_2S_2$
Molecular Weight (g/mol)	382.46
Melting Point	248-252ºC
Yield (%w/w)	52
Recrystallisation solvent	Ethanol
TLC	Rf = 0.58 n-hexane: ethyl acetate (8:2)
IR (ν, cm^{-1})	3444(NH), 2343(C-N), 1770 (C=O),1645(C=C), 1523(C=N), 1095(C-S)
^{1}H NMR (DMSO)	6.89-7.4 (m. Ar-7H), 4.2 (S, 1H, Ar-NH), 3.66 (S,3H, Ar-CH_3), 2.4 (S,3H,OCOCH3)
^{13}C NMR	171.12(OCOCH3),165.72(C=N),164.66((C=N), 151.62(C-O), 150.51(C-N), 140.12(C-N), 132.14, 131.23, 129.91, 125.5, 121.44, 121.25, 120.51, 119.78, 115.80, 115.72, 23.66(OCOCH3), 20.64(CH3),
MASS (70eV) m/z (%)	382



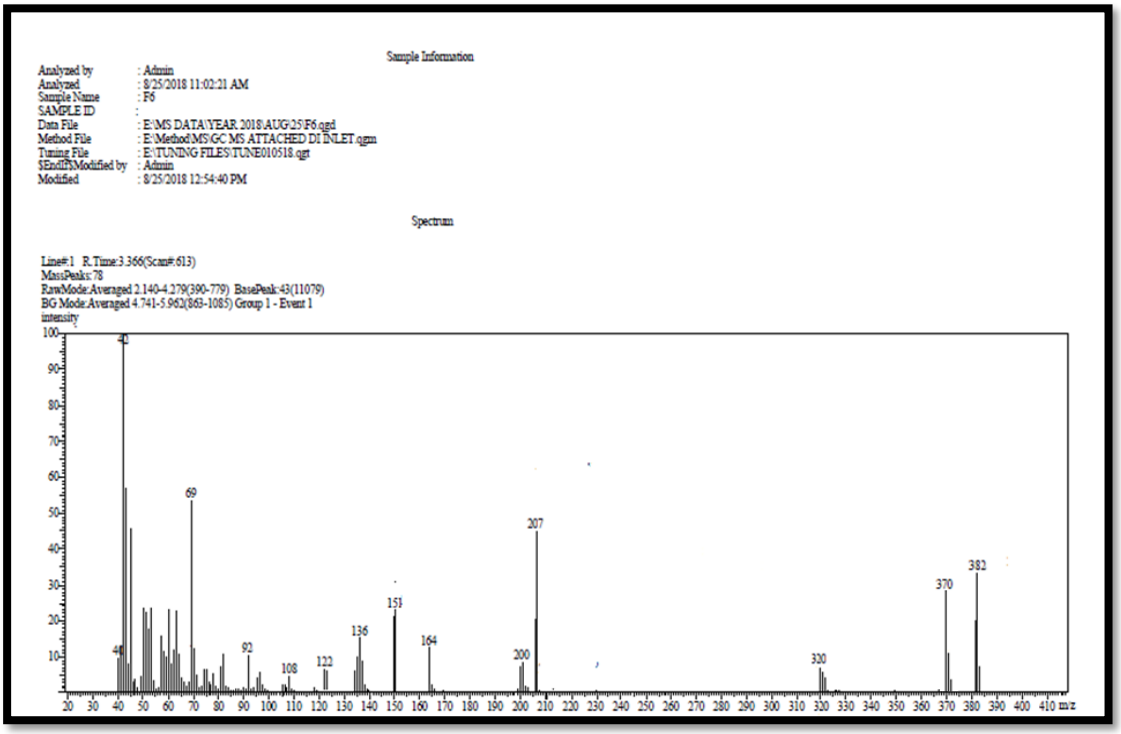
Spectra 34: IR spectra of compound 1a



Spectra 35: ^{1}H NMR spectra of Compound 1a

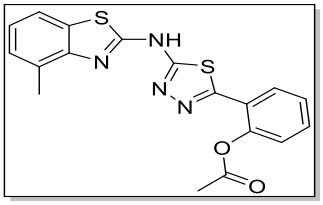


Spectra 36: ^{13}C NMR spectra of Compound 1a

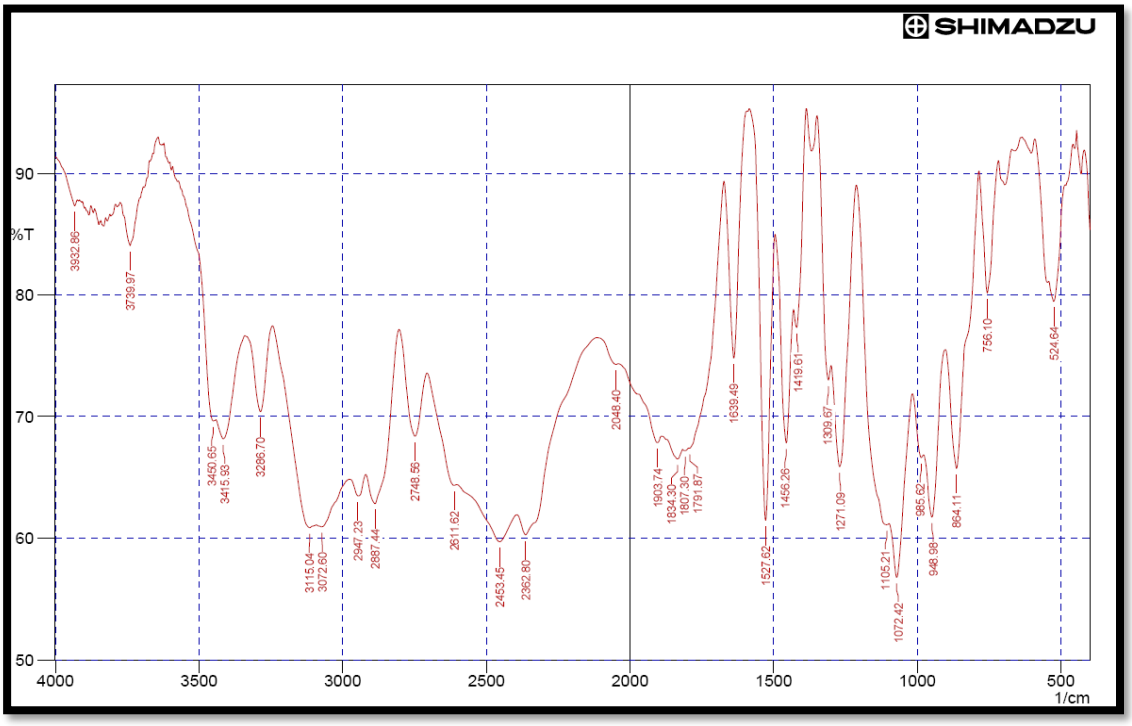


Spectra 37: Mass Spectra of compound 1a

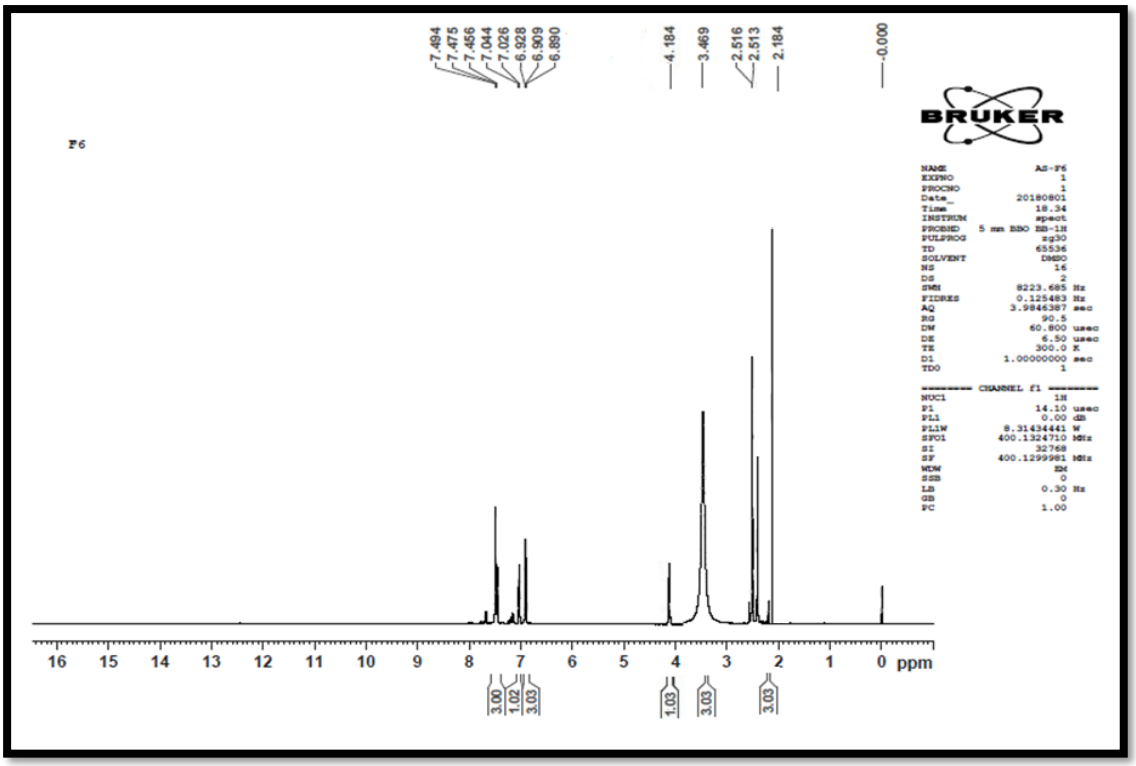
2-(5-((4-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (2a)



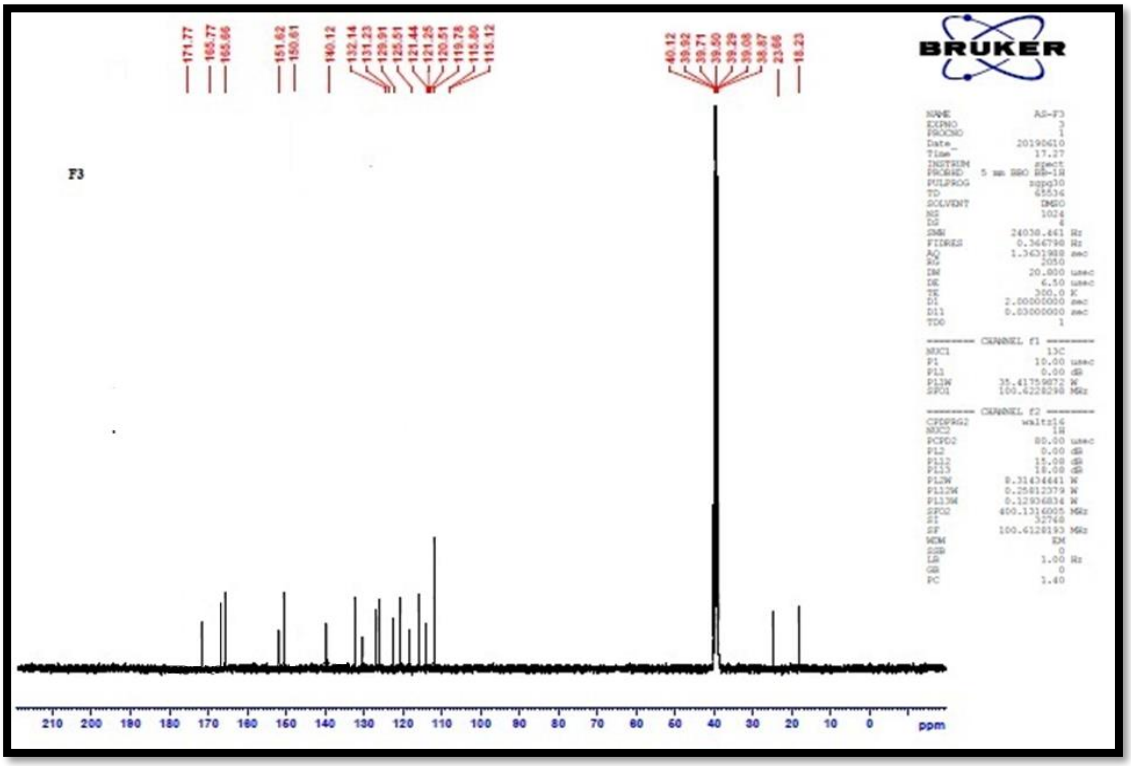
Molecular Formula	$C_{18}H_{14}N_4O_2S_2$
Molecular Weight (g/mol)	382.46
Melting Point	256-258ºC
Yield (%w/w)	46
Recrystallisation solvent	Ethanol
TLC	Rf = 0.49 n-hexane:ethyl acetate (8:2)
IR (ν, cm^{-1})	3415(NH), 1791(C=O), 1639(C=C), 1527(C=N), 1072(C-S)
^{1}H NMR (DMSO)	6.89-7.49 (m. Ar-7H), 4.1 (S, 1H, Ar-NH), 3.46 (S,3H, OCOCH3), 2.18 (S,3H, Ar-CH_3)
^{13}C NMR	171.77(OCOCH3), 165.77(C=N), 165.66(C=N), 150.61(C-O), 151.62(C-N), 140.12(C-N), 23.66(CH3), 18.23(CH3), 115.12, 115.80, 119.78, 121.25, 120.51, 121.44, 125.51, 129.91, 131.23, 132.14
MASS (70eV) m/z (%)	382



Spectra 38 IR spectra of compound 2a

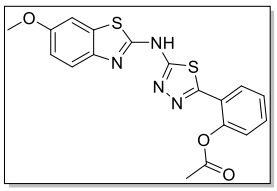


Spectra 39: ^{1}H NMR spectra of Compound 2a

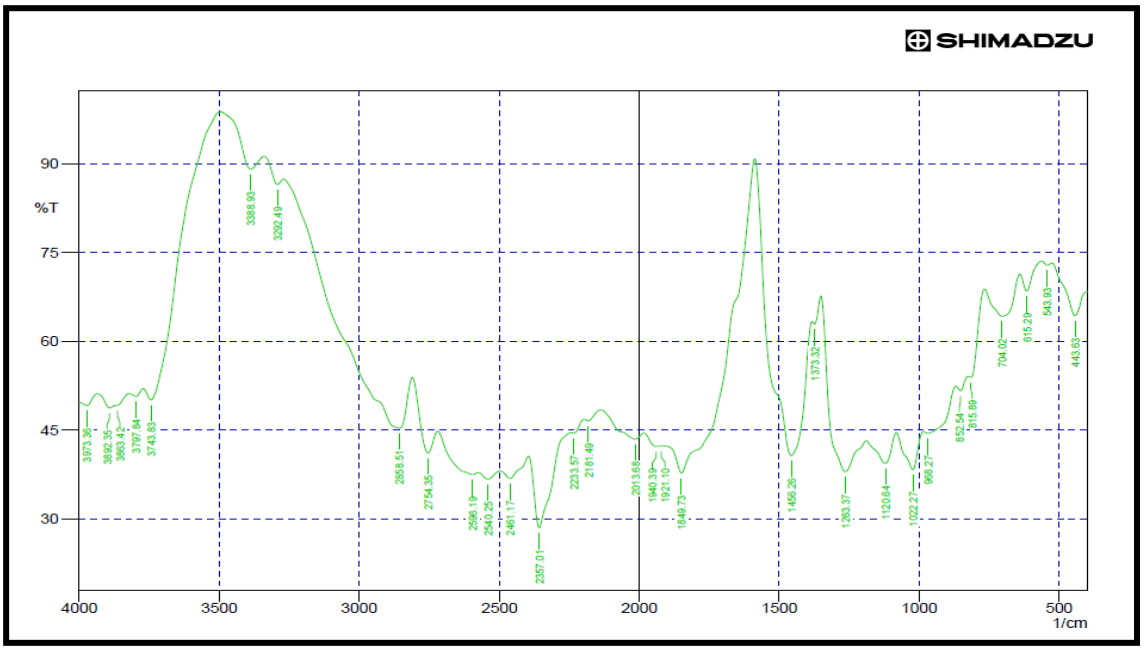


Spectra 40: ^{13}C NMR spectra of Compound 2a

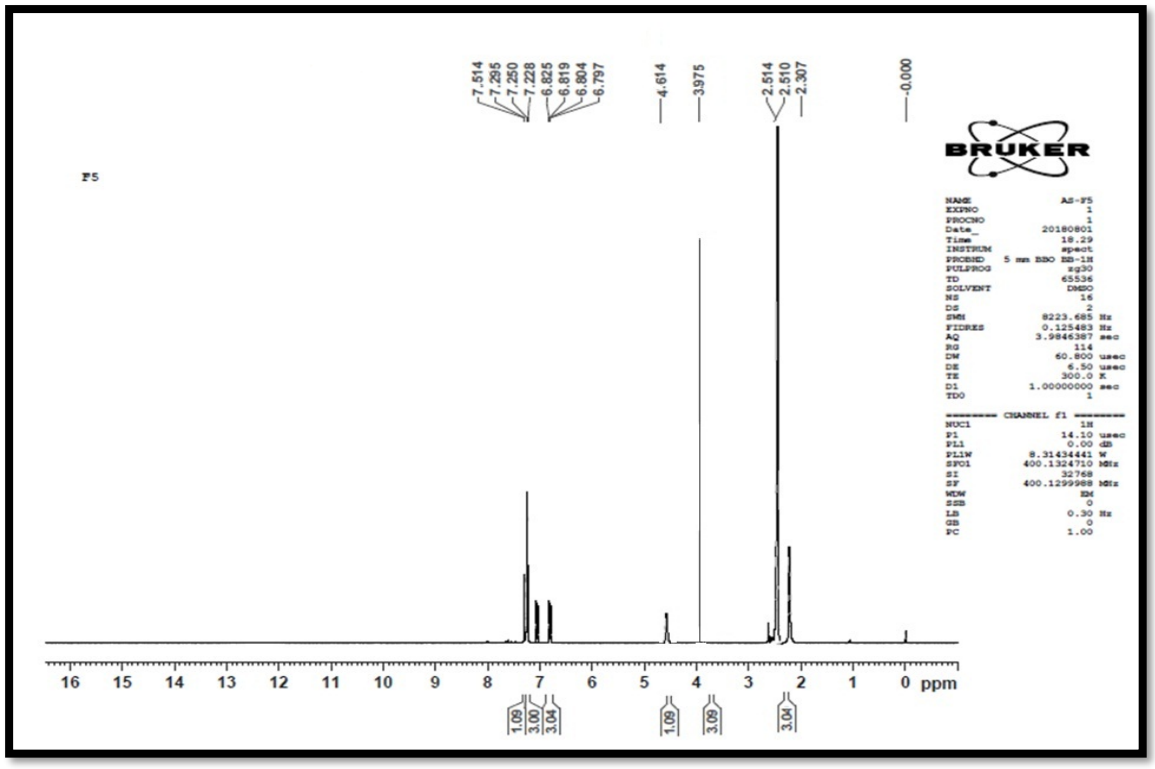
2-(5-((6-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (3a)



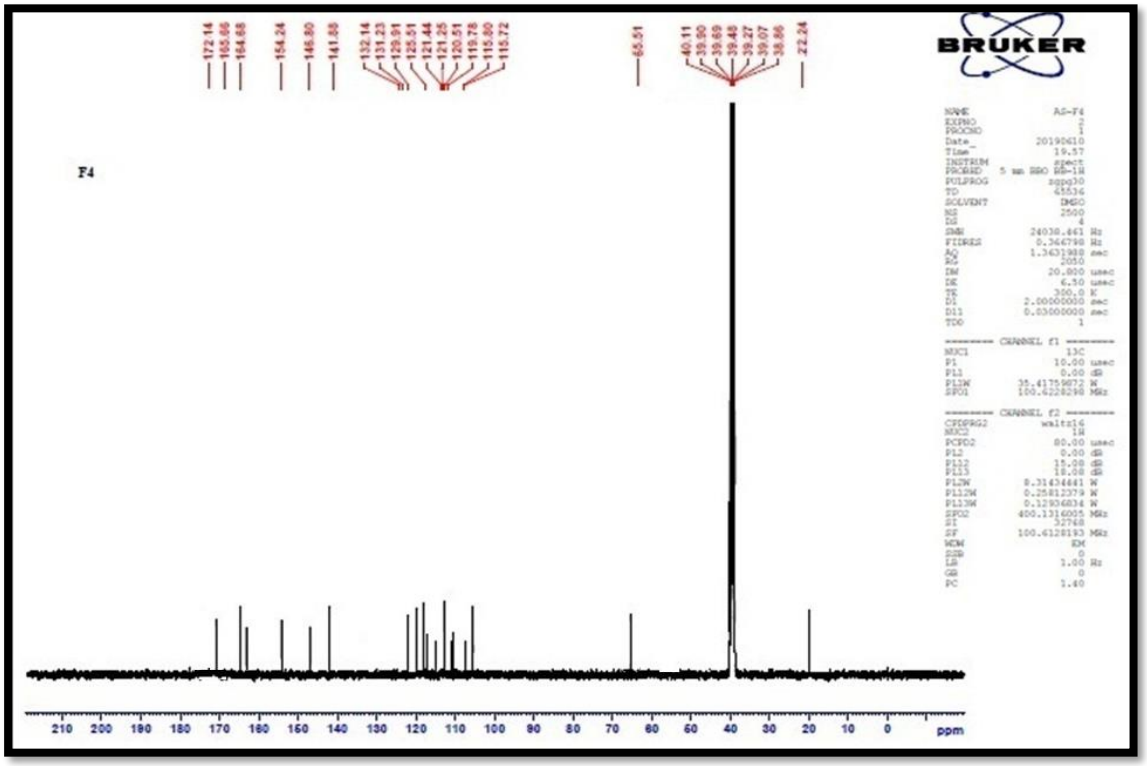
Molecular Formula	$C_{18}H_{14}N_4O_3S_2$
Molecular Weight (g/mol)	398.05
Melting Point	262-264ºC
Yield (%w/w)	48
Recrystallisation solvent	Ethanol
TLC	Rf = 0.56 n-hexane:ethyl acetate (7:3)
IR (ν, cm^{-1})	3388(NH), 2357(C-N), 1849 (C=O), 1122(C-S), 1263 (C-O)
^{1}H NMR (DMSO)	6.7-7.5 (m. Ar-7H), 4.6 (S, 1H, Ar-NH), 3.9 (S,3H, Ar-OCH_3), 2.3 (S,3H,OCOCH3)
^{13}C NMR	172.14(OCOCH3),165.66,164.66(2C=N),154.24(C-O), 146.80(C-N),141.88(C-N),65.51(OCH3), 22.24 (CH3), 132.14, 131.24, 129.91, 125.51, 121.44, 121.25, 120.41, 119.78, 115.80, 115.72
MASS (70eV) m/z (%)	398



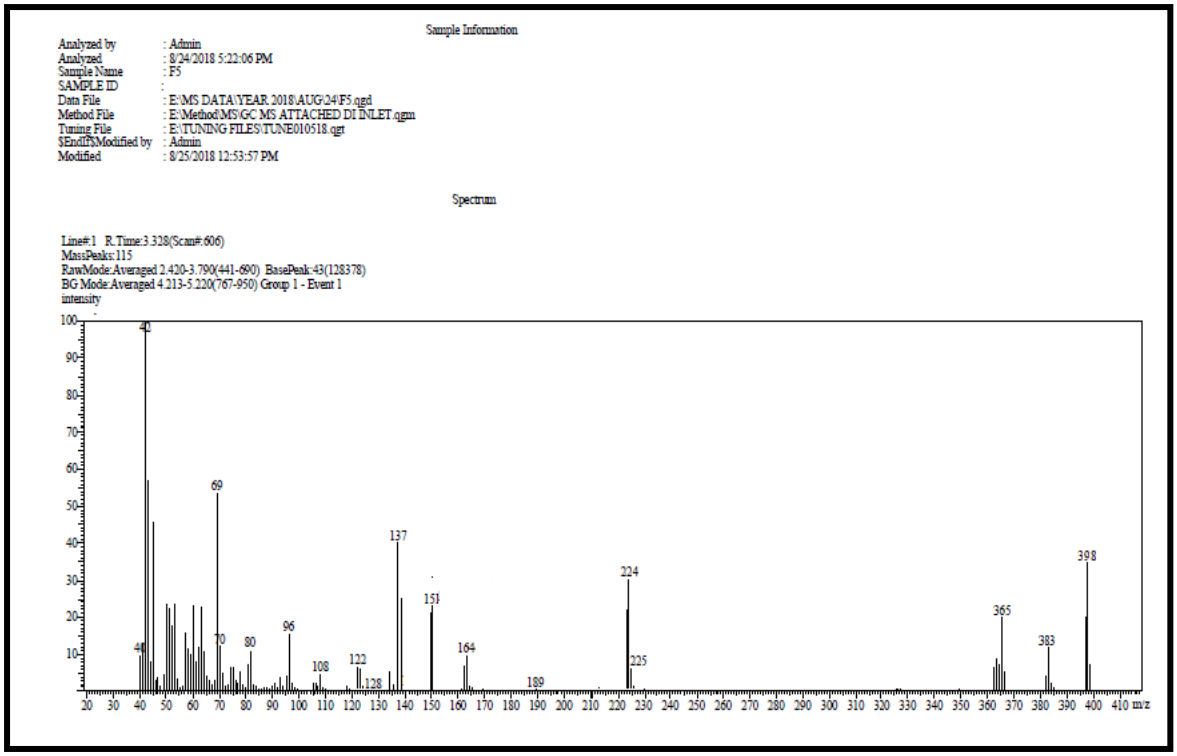
Spectra 41: IR spectra of compound 3a



Spectra 42 ^{1}H NMR spectra of Compound 3a

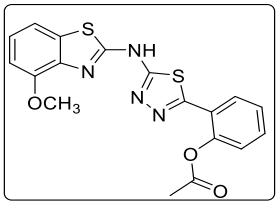


Spectra 43: ^{13}C NMR spectra of Compound 3a

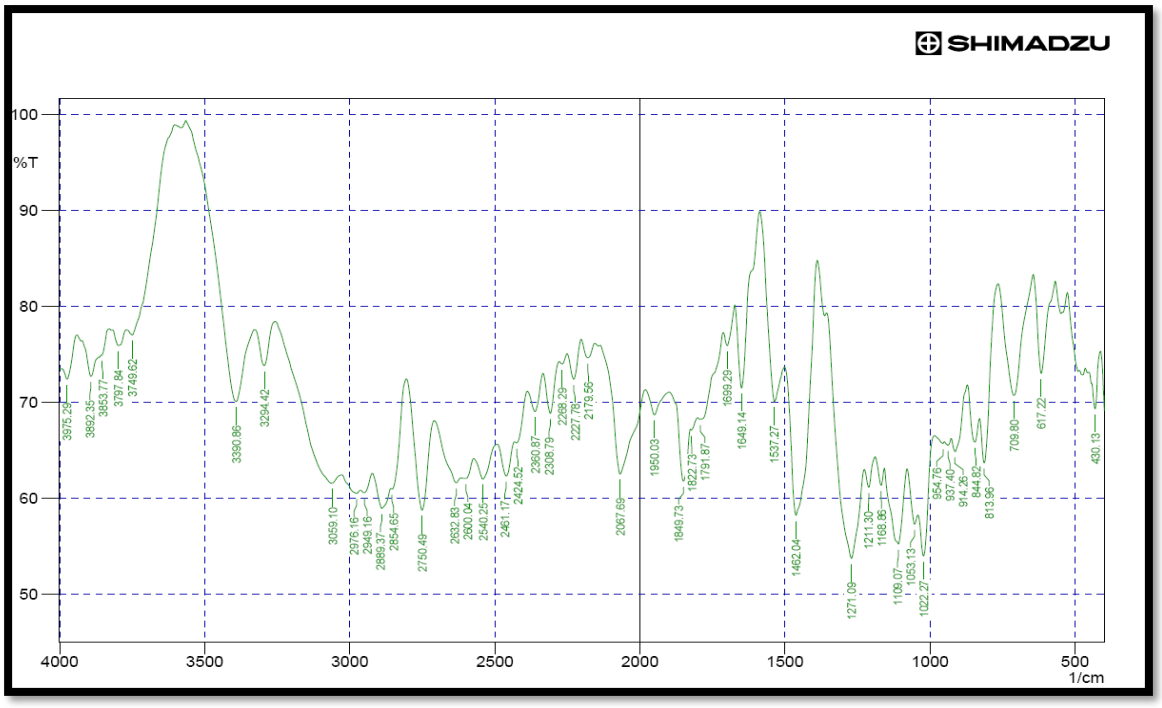


Spectra 44: MASS spectra of compound 3a

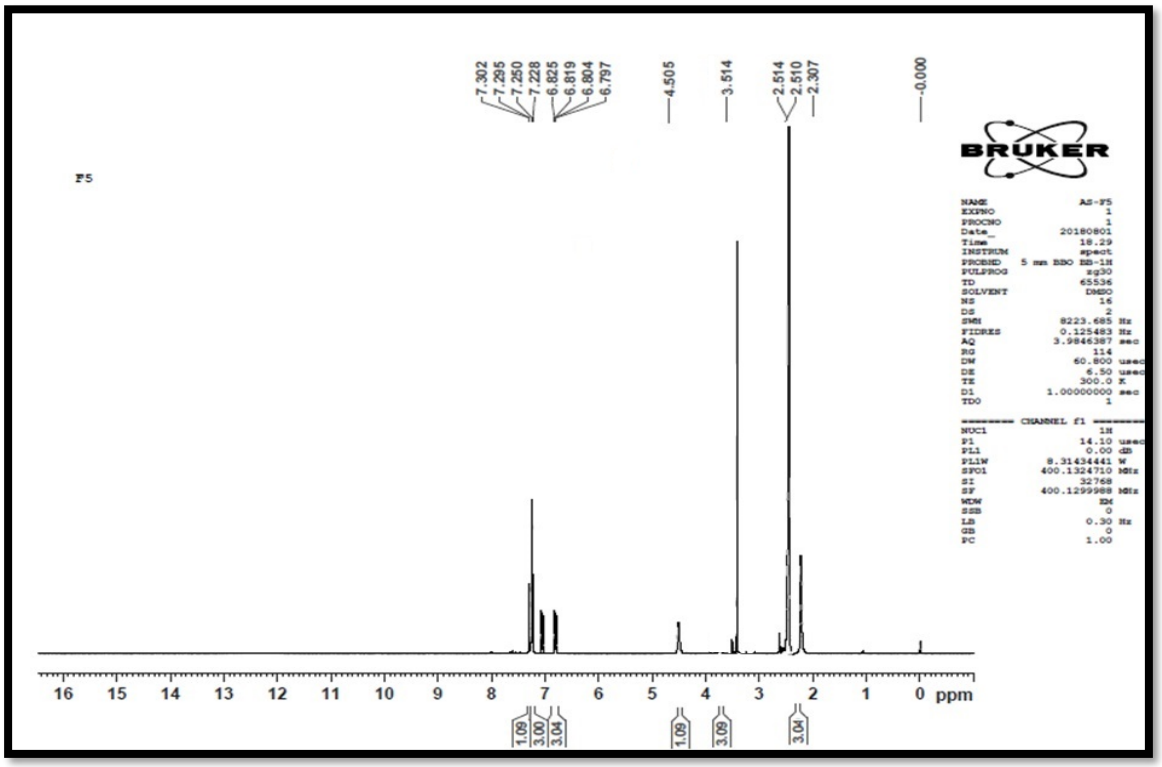
2-(5-((4-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (4a)



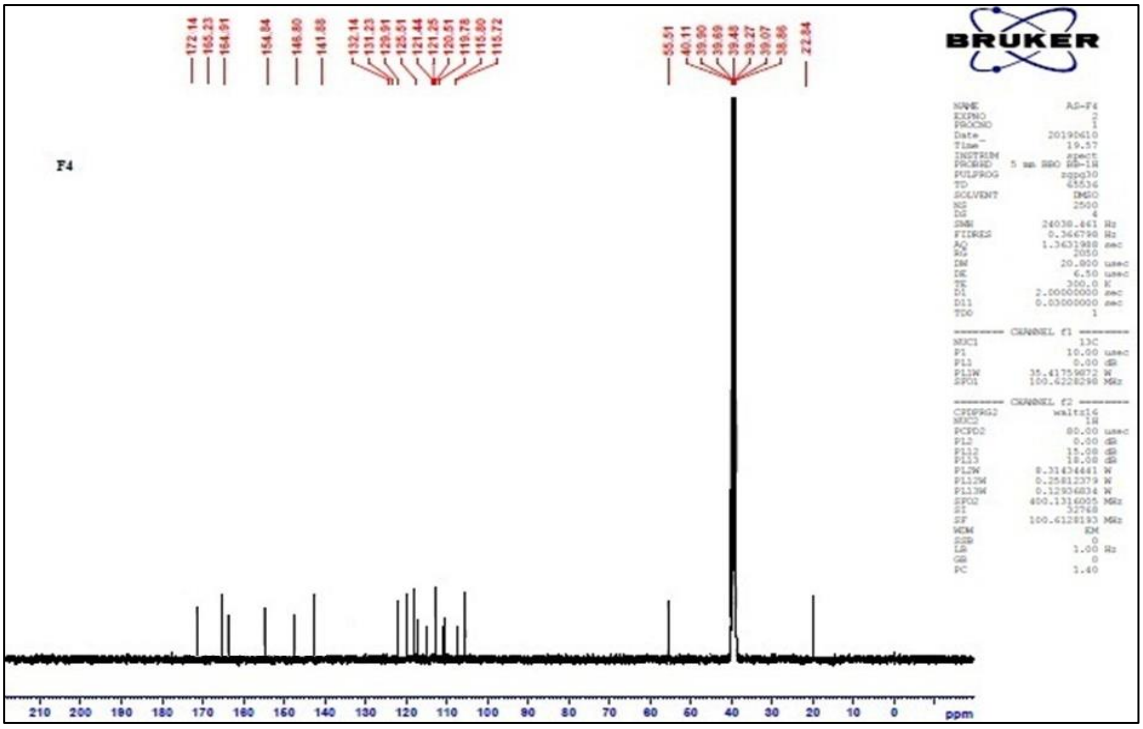
Molecular Formula	$C_{18}H_{14}N_4O_3S_2$
Molecular Weight (g/mol)	398.05
Melting Point	268-272ºC
Yield (%w/w)	42
Recrystallisation solvent	Ethanol
TLC	Rf = 0.46 n-hexane:ethyl acetate (6:4)
IR (ν, cm-1)	3309(NH), 1699 (C=O),1537(C=N), 1109(C-S), 1649 (C=C)
1H NMR (DMSO)	6.7-7.3 (m. Ar-7H), 4.5 (S, 1H, Ar-NH), 3.5 (S,3H, Ar-OCH3), 2.3 (S,3H,OCOCH3)
13C NMR	172(OCOCH3),165.23,164.91(2C=N),154.84(C-O),146.80(C-N),141.88(C-N),55.51(OCH3), 22.24 (CH3), 132.14,131.24, 129.91, 125.51, 121.44, 121.25,120.41, 119.78, 115.80, 115.72
MASS (70eV) m/z (%)	398



Spectra 45: IR spectra of compound 4a

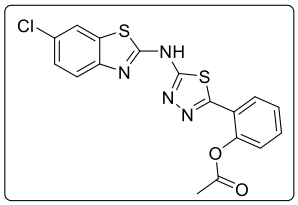


Spectra 46: ^{1}H NMR spectra of Compound 4a

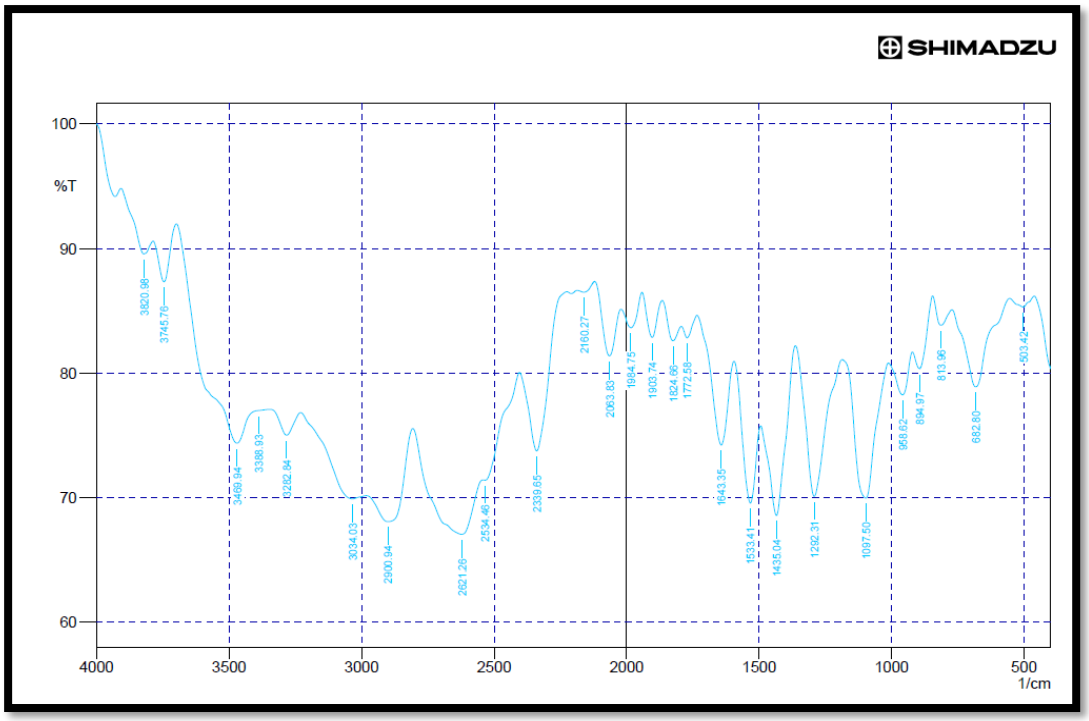


Spectra 47: ^{13}C NMR spectra of Compound 4a

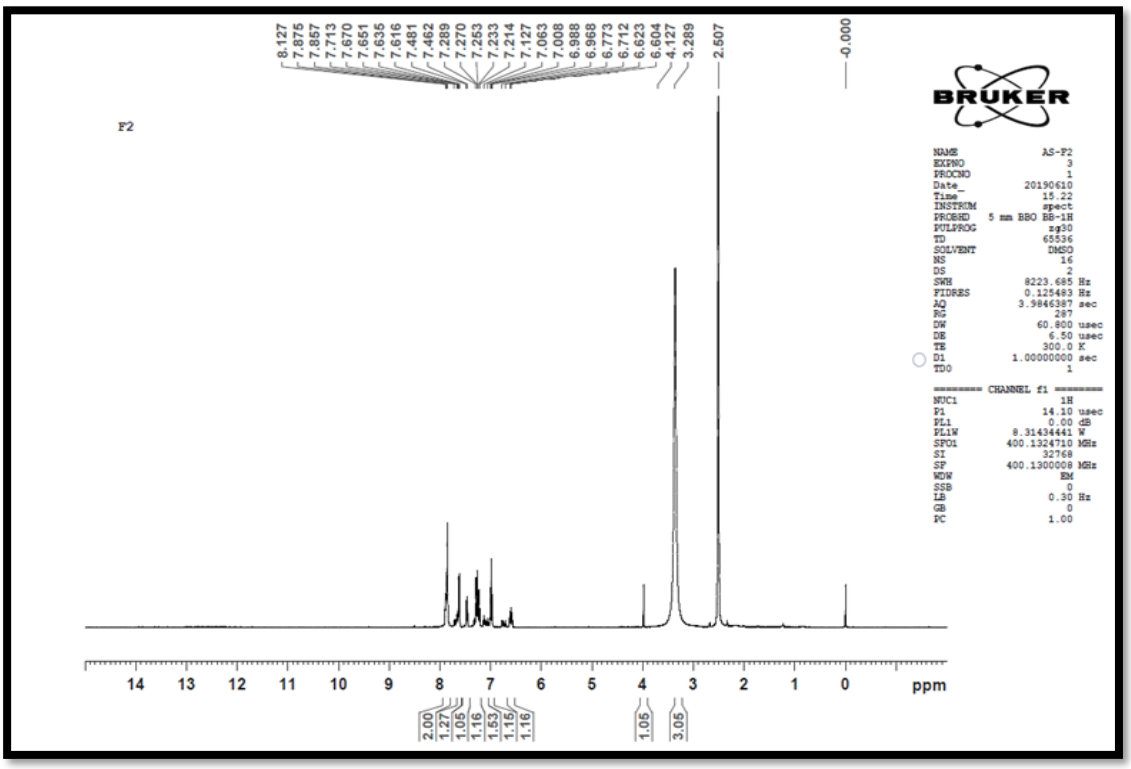
2-(5-((6-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (5a)



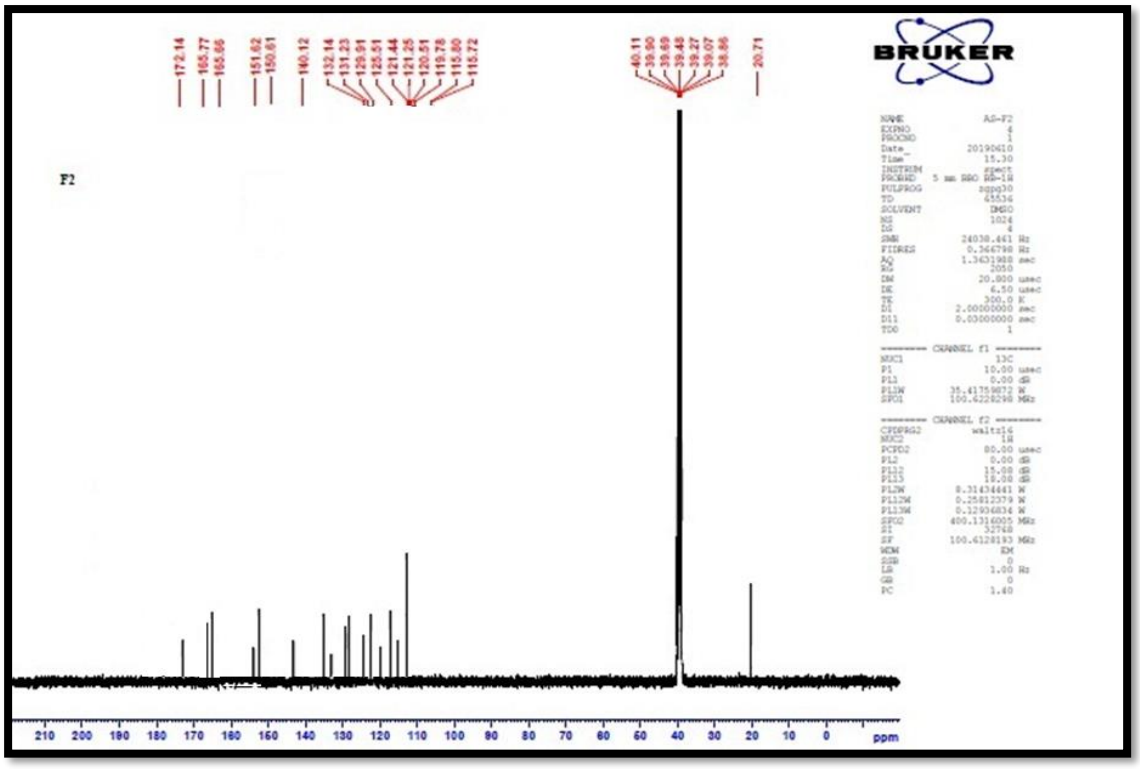
Molecular Formula	$C_{17}H_{11}ClN_4O_2S_2$
Molecular Weight (g/mol)	402.83
Melting Point	260-262ºC
Yield (%w/w)	52
Recrystallisation solvent	Ethanol
TLC	Rf = 0.58 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3469(NH), 2339(C-N), 1772 (C=O) 1643(C=C), 1533(C=N), 1097(C-S), 682 (C-Cl)
^{1}H NMR (DMSO)	6.6-8.1 (m. Ar-7H), 4.17 (S, 1H, Ar-NH), 3.2 (S,3H,OCOCH3)
^{13}C NMR	172.14(C=O), 165.77 (C=N), 165.66(C=N), 151.62 (C-N), 150.61(C-N), 20.71(-CH3), 140.12 (C-O), 132.14, 131.23, 129.91, 125.51, 121.44, 121.25, 119.78, 115.80, 115.72
MASS (70eV) m/z (%)	403



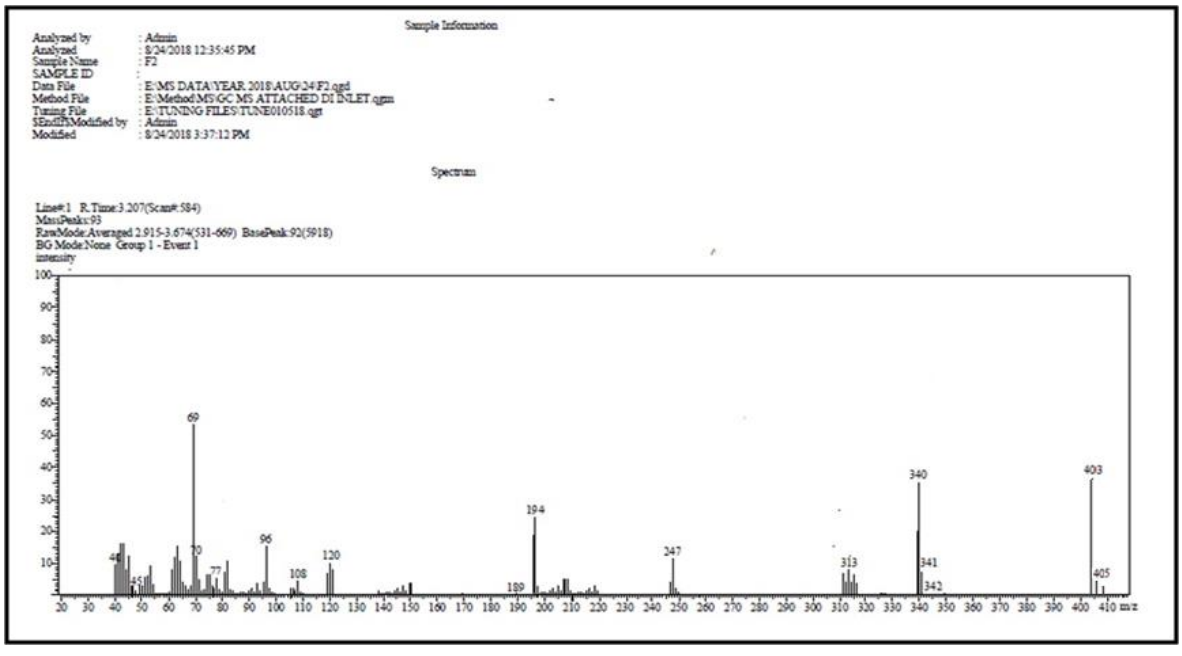
Spectra 48: IR spectra of compound 5a



Spectra 49: ^{1}H NMR spectra of Compound 5a

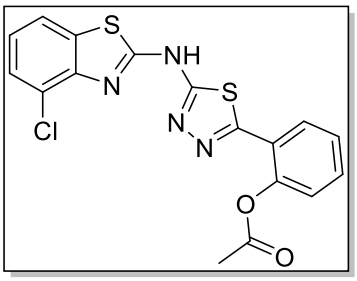


Spectra 50: ^{13}C NMR spectra of Compound 5a

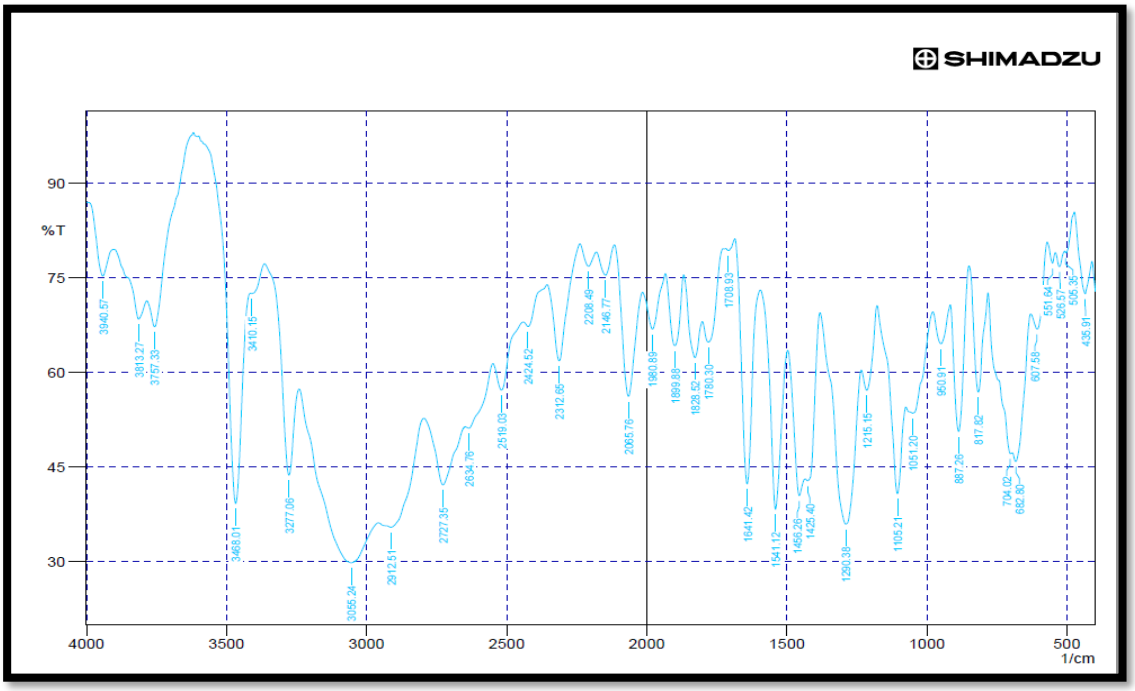


Spectra 51: MASS spectra of compound 5a

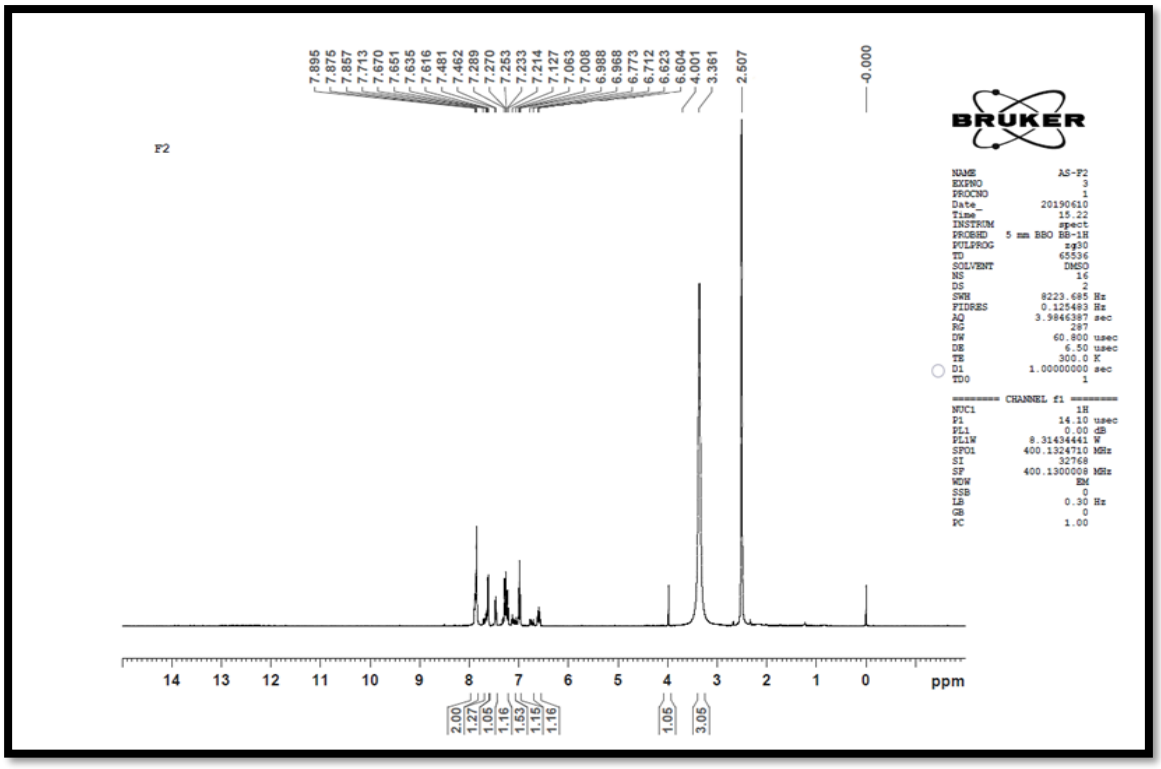
2-(5-((4-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenyl acetate (6a)



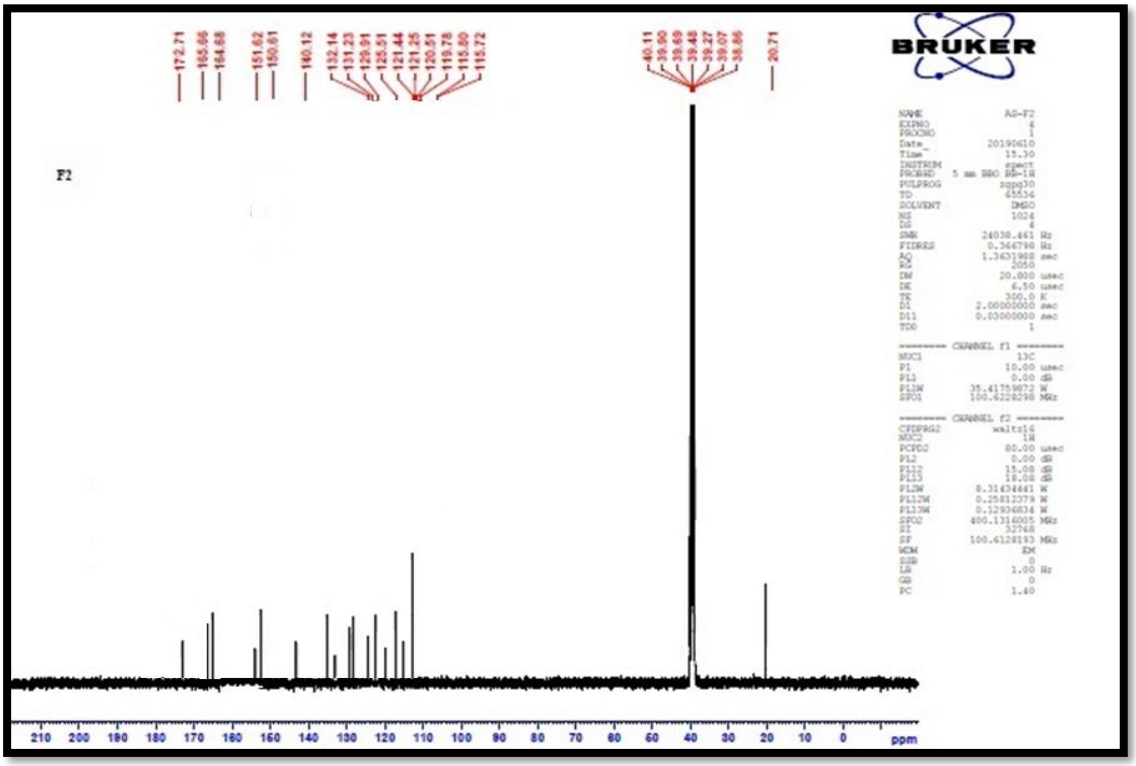
Molecular Formula	$C_{17}H_{11}ClN_4O_2S_2$
Molecular Weight (g/mol)	402.83
Melting Point	266-268 ºC
Yield (%w/w)	41
Recrystallisation solvent	Ethanol
TLC	Rf = 0.47 n-hexane:ethyl acetate (7:3)
IR (ν, cm^{-1})	3468(NH), 2312(C-N), 1780 (C=O), 1641(C=C), 1541(C=N), 1105(C-S), 682 (C-Cl)
^{1}H NMR (DMSO)	6.5-7.8 (m. Ar-7H), 3.67 (S, 1H, Ar-NH), 2.1 (S,3H,OCOCH3)
^{13}C NMR	172.71(C=O), 165.66 (C=N), 164.68(C=N), 151.62 (C-O), 150.61(C-N), 20.71(-CH3), 140.12 (C-N), 132.14, 131.23, 129.91, 125.51, 121.44, 121.25, 119.78, 115.80, 115.72
MASS (70eV) m/z (%)	403



Spectra 52: IR spectra of 6a

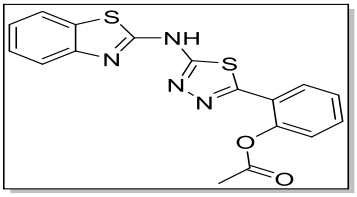


Spectra 53: ^{1}H NMR spectra of Compound 6a

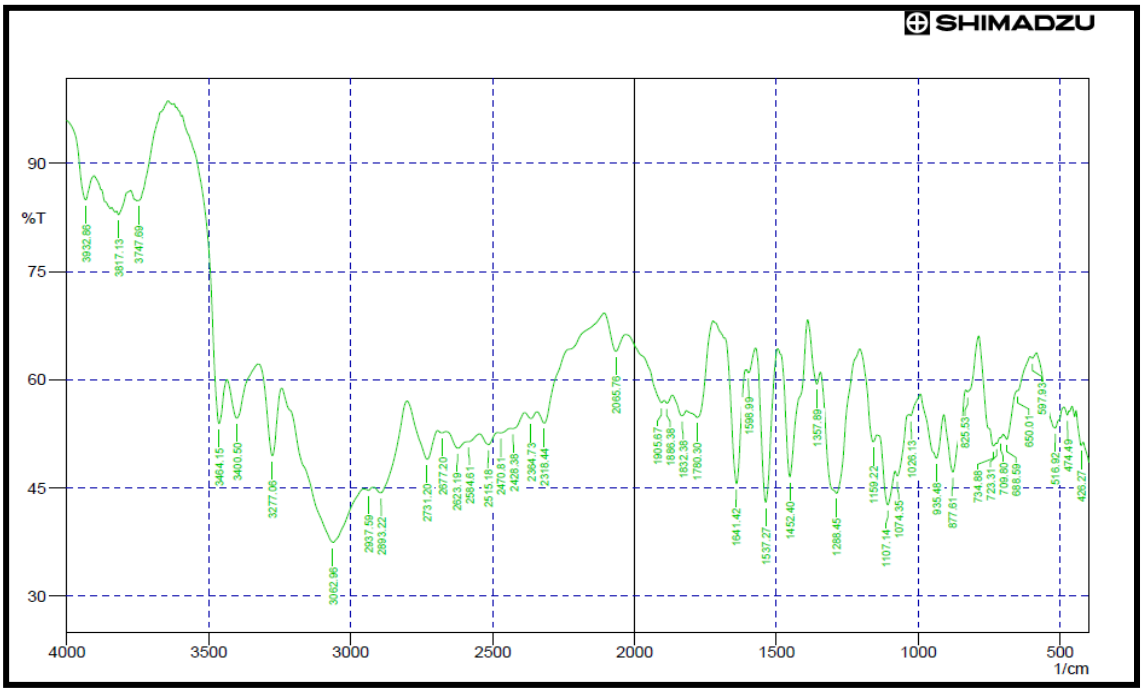


Spectra 54: ^{13}C NMR spectra of Compound 6a

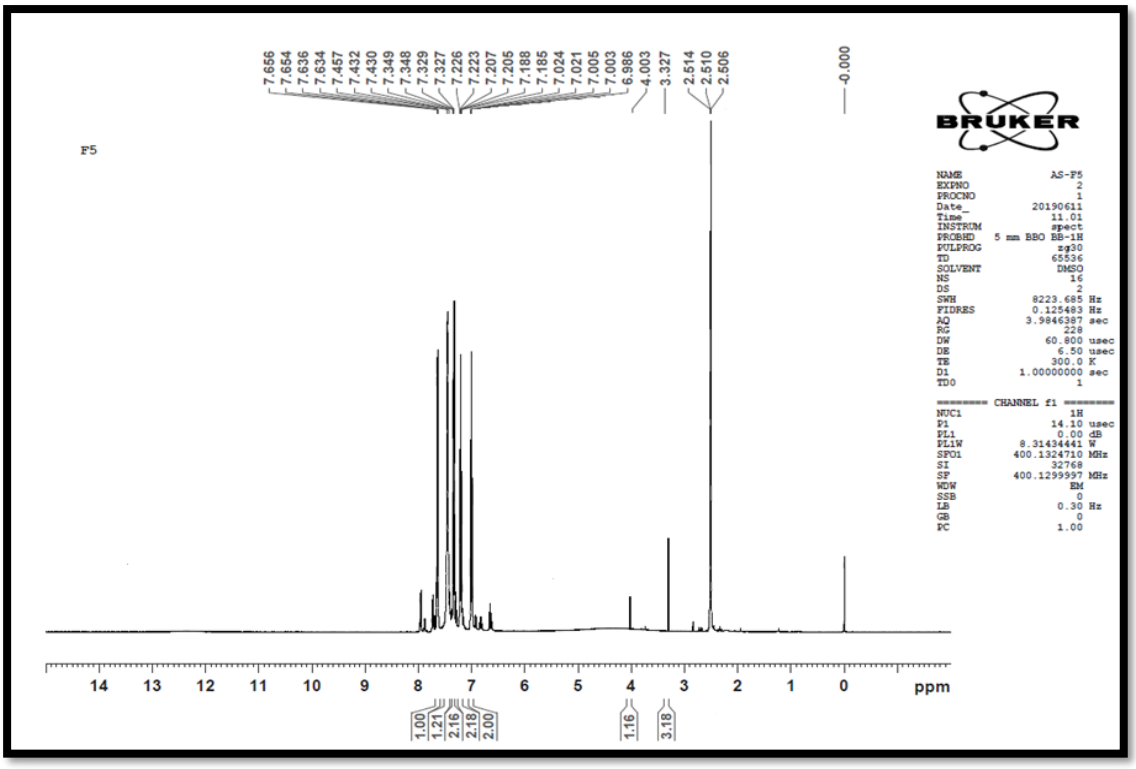
N-(1, 3, 4- thiadiazol-2-yl) benzo[d]thiazol-2-amines (7a)



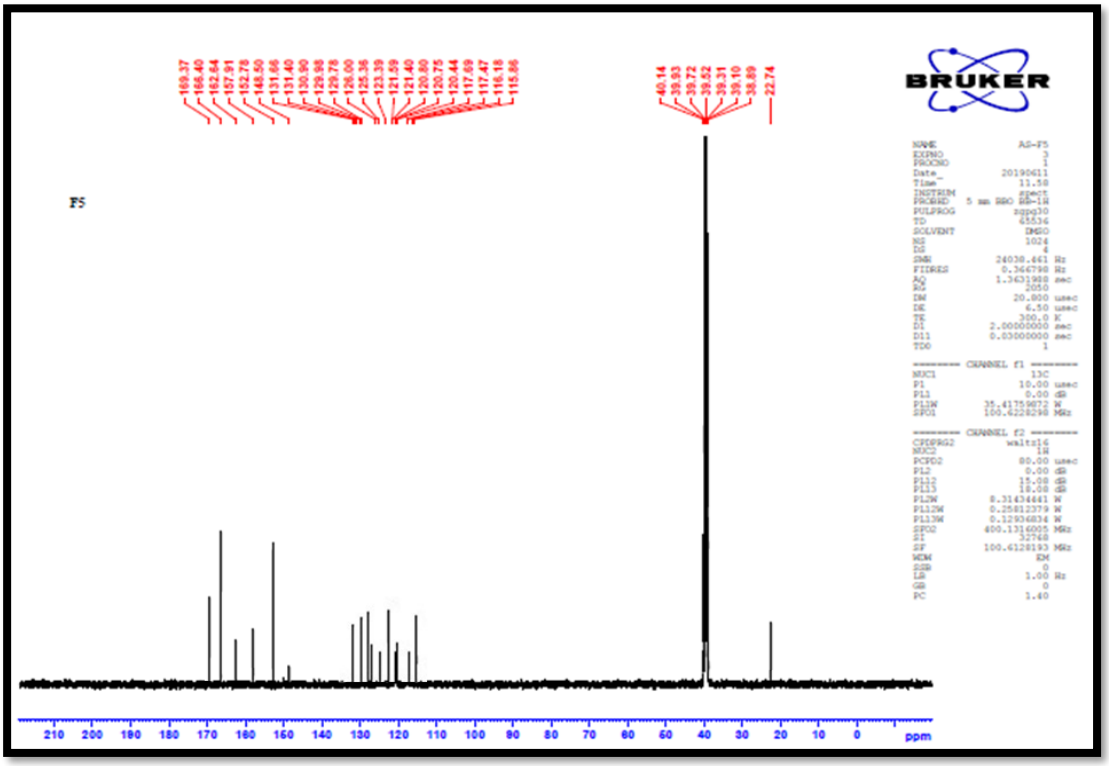
Molecular Formula	$C_{17}H_{12}N_4O_2S_2$
Molecular Weight (g/mol)	368.43
Melting Point	266-268 ºC
Yield (%w/w)	52
Recrystallisation solvent	Ethanol
TLC	Rf = 0.52 n-hexane:ethyl acetate (8:2)
IR (ν, cm^{-1})	3464(NH), 2318(C-N), 1780 (C=O), 1641(C=C), 1537(C=N), 1107(C-S)
^{1}H NMR (DMSO)	6.9.-7.6 (m. Ar-8H), 4.0 (S, 1H, Ar-NH), 3.32 (S,3H,OCOCH3)
^{13}C NMR	169.37(C=N),166.40(C=N),162.64(C=O),157.91(C-O),152.78(C-N),148.50(C-N), 22.74(CH3), 131.66, 130.90, 129.93, 126, 123.39, 121.59, 120.80, 117.69, 116.18, 115.88
MASS (70eV) m/z (%)	369



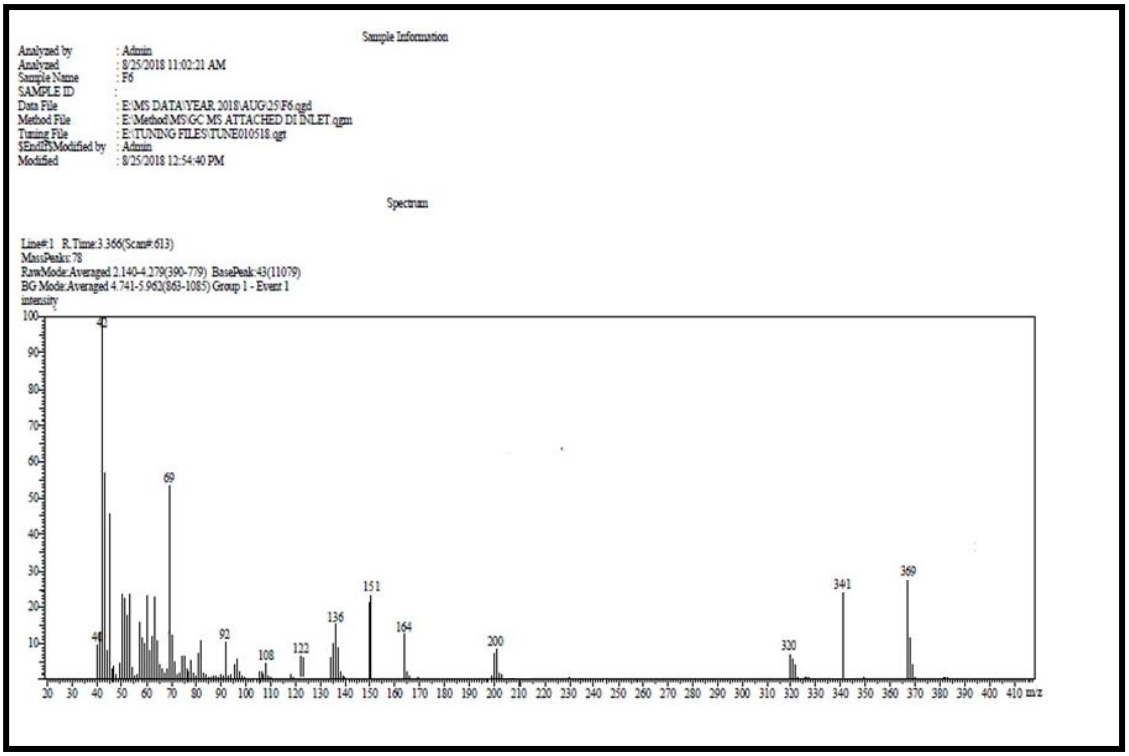
Spectra 55: IR spectra of 7a



Spectra 56: ^{1}H NMR spectra of Compound 7a

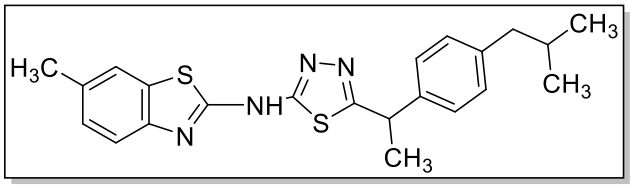


Spectra 57: ^{13}C NMR spectra of Compound 7a

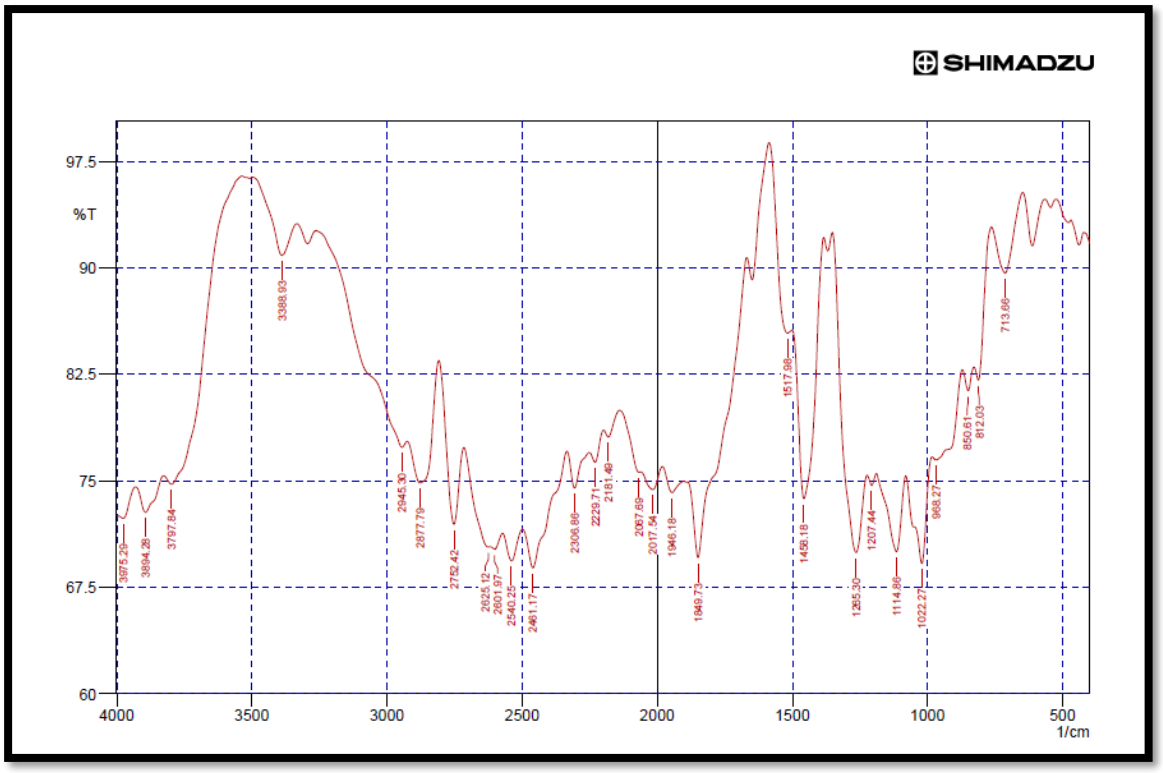


Spectra 58: MASS spectra of 7a

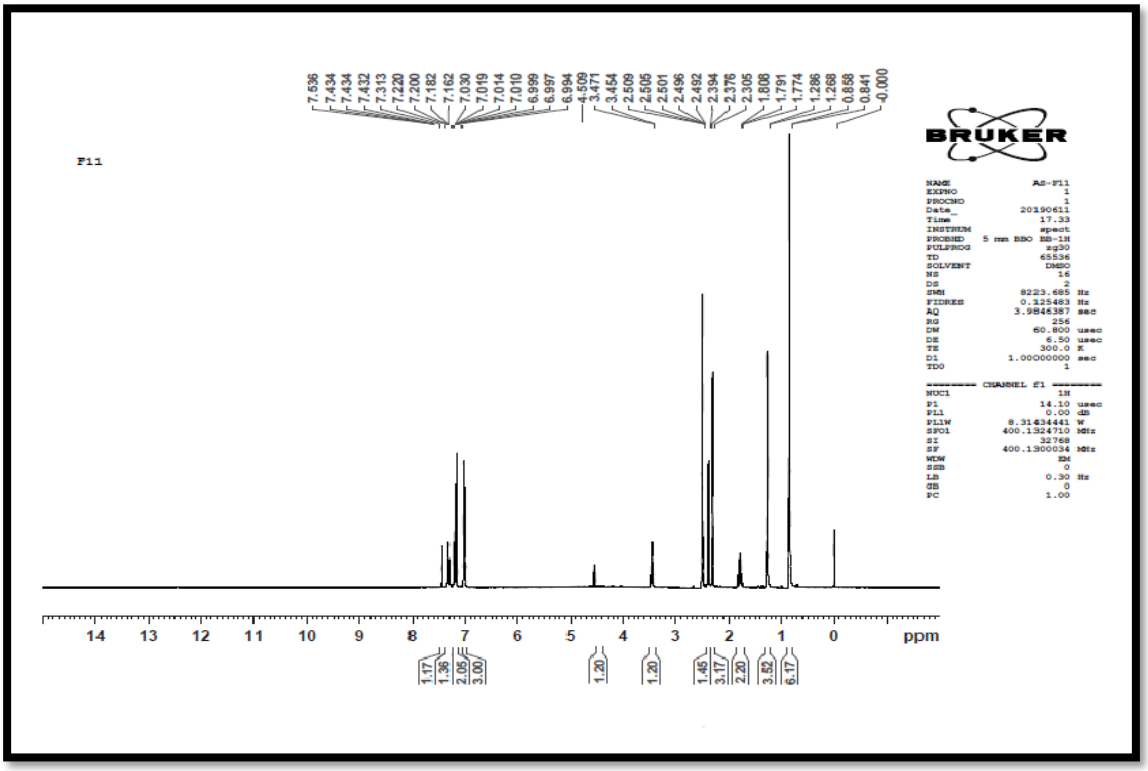
N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)-6 methylbenzo[d]thiazol-2-amine (1ib)



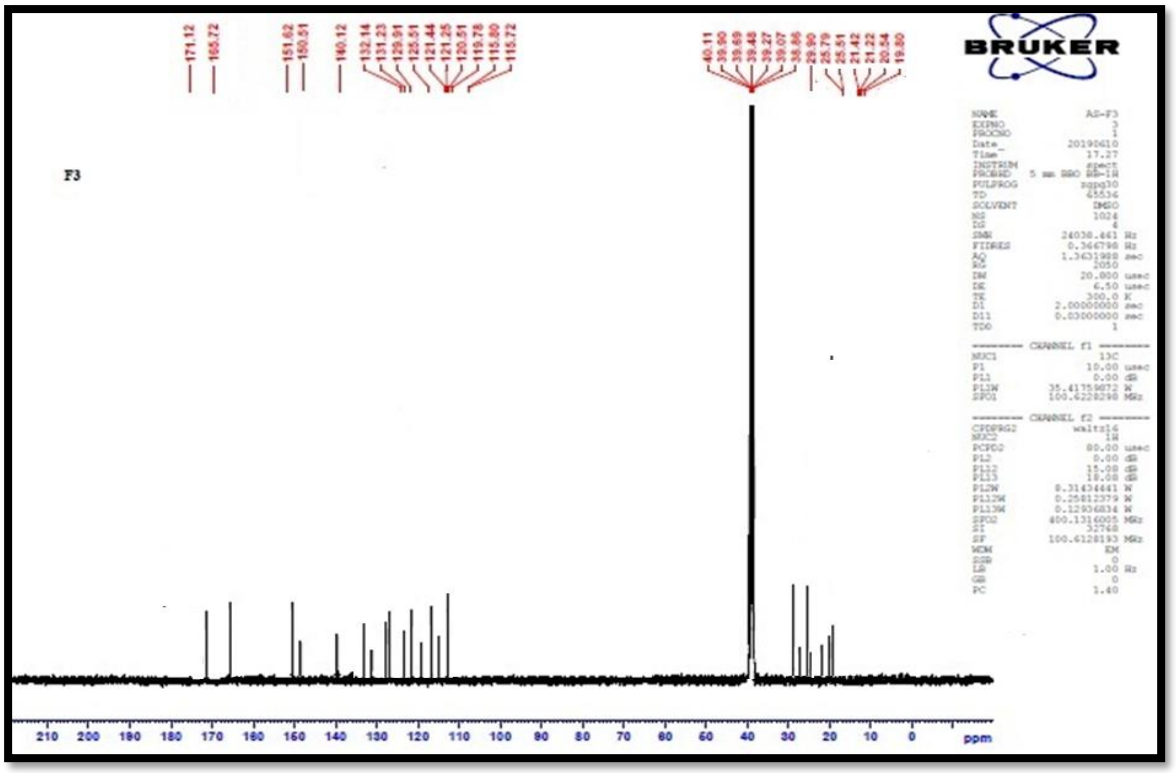
Molecular Formula	$C_{22}H_{24}N_4S_2$
Molecular Weight (g/mol)	408.14
Melting Point	280-284 ºC
Yield (%w/w)	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.42 n-hexance:ethylacetate:Glacial acetic acid (7:2:1)
IR (ν, cm^{-1})	3388(NH), 2306(C-N), 1517(C=N), 1114(C-S)
^{1}H NMR (DMSO)	6.9.-7.5(m.,Ar-7H), 4.5 (S, 1H, Ar-NH), 3.47 (q, 1H, CH), 2.4 (q, 1H, CH2 -CH), 2.3 (S,3H, Ar-CH3),`1.8 (s, 2H, CH2), 1.2 (d, 3H,CH3), 0.84 (s, 6H, (CH3)2)
^{13}C NMR	171.12(C=N),165.72(C=N),151.62(C-N),150.51(C N), 140.12, 132.14, 132.23, 129.91, 125.51, 121.55, 121.44, 120.51, 119.78, 115.80, 115.72 (11 Ar-C), 19.8-29.90 (7-C)
MASS (70eV) m/z (%)	408.58



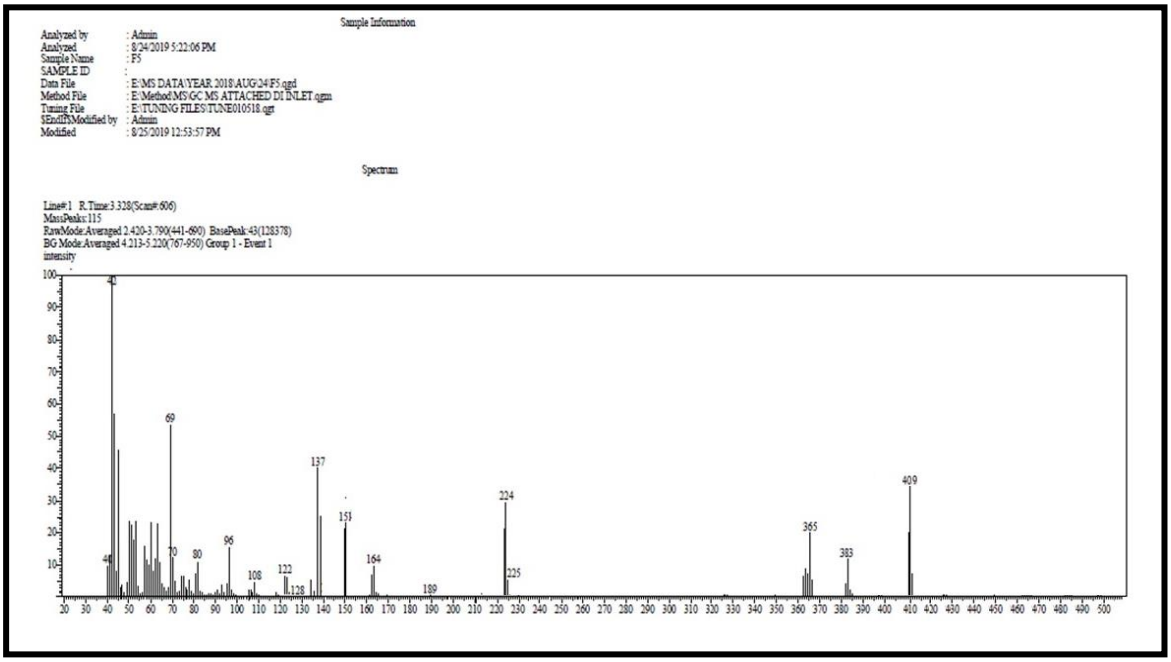
Spectra 59 IR Spectra of compound 1ib



Spectra 60: ^{1}H NMR spectra of Compound 1ib

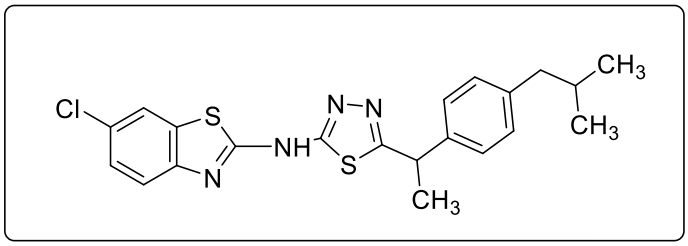


Spectra 61: ^{13}C NMR spectra of Compound 1ib

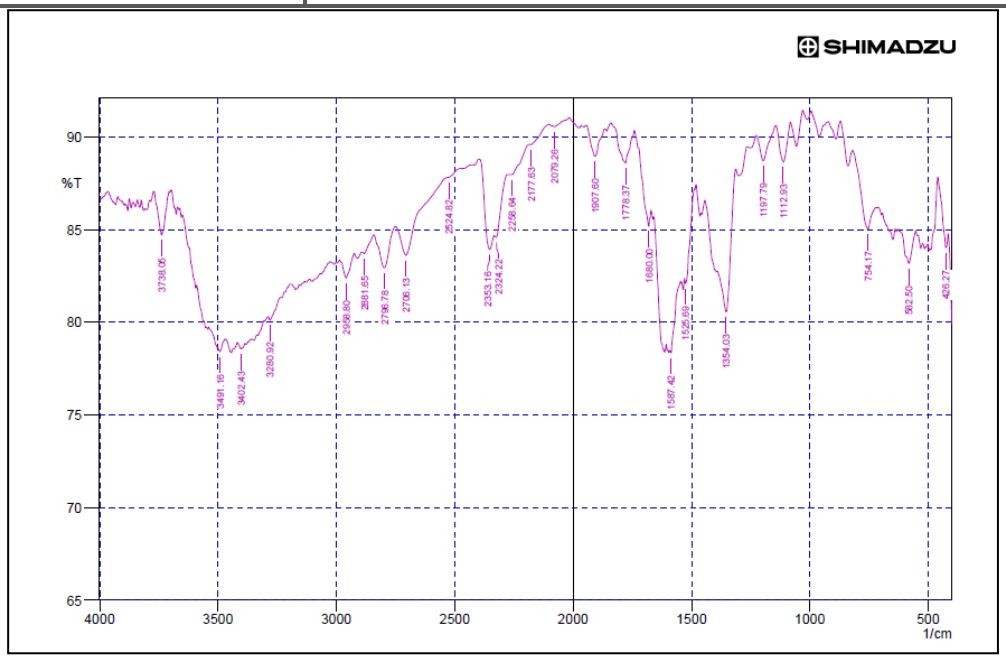


Spectra 62: MASS spectra of compound 1ib

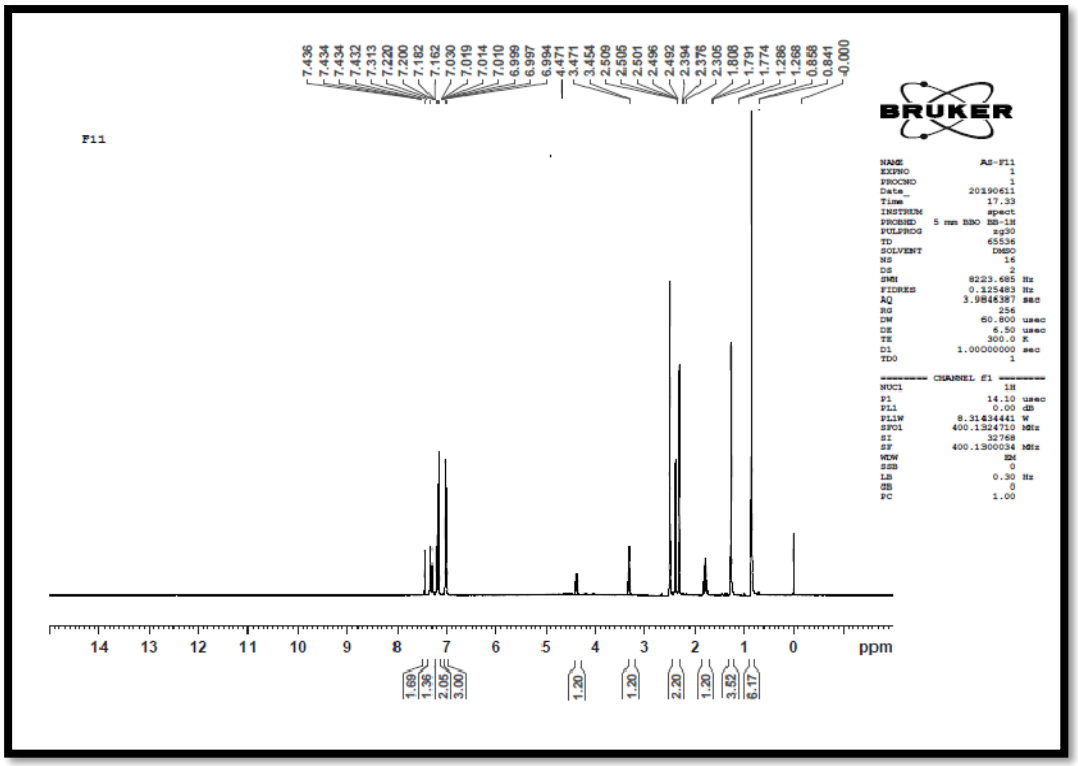
6-chloro-N-(5-(1-(4-isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl)benzo[d]thiazol-2-amine (3ib)



Molecular Formula	$C_{21}H_{21}ClN_4S_2$
Molecular Weight (g/mol)	428.14
Melting Point	290-292 ºC
Yield (%w/w)	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.38 n-hexance:ethylacetate:Glacial acetic acid (6:2:2)
IR (ν, cm^{-1})	3402(NH), 2324(C-N), 1680(C=C), 1587(C=N), 1112(C-S)
^{1}H NMR (DMSO)	6.9.-7.45(m.,Ar-7H), 4.4 (S, 1H, Ar-NH), 3.47 (1H, CH), 2.4(m,2H, CH2),`1.8 (m, 1H, CH), 1.2 (s, 3H,CH3), 0.84 (s, 6H, (CH3)2)
^{13}C NMR	165.66, 162.14 (C=N), 151.62, 150.01 (C-N), 140.12 (C-Cl), 130.34-115.12 (Ar-10C), 19.90-25.79 (6-C)
MASS (70eV) m/z (%)	429



Spectra 63:IR Spectra of compound 3ib



Spectra 64: ^{1}H NMR spectra of Compound 3ib

5.5 MTT ASSAY:

Procurement of cell line:

The Caco-2 and MCF-7 cell line with passage number 45 was procured from NCCS, Pune.

Media Preparation:

Pour 20-30 ml MEM media in centrifuge. To this add 10ml of bovine serum, 0.5 ml antibiotic solution, 1.25ml HEPES and make up volume up to 50ml by appropriate media. Mix it and store at 208^{o}C (for up to 4 weeks)

Sub culturing cells:

Take above solution and remove the media and wash with PBS. Remove PBS and add 1ml trypsin-EDTA solution. Incubate the flask at 37^{o}C in CO_2 incubator

Table 6 Protocol for MTT assay

Day 1 **(Cells Suspended)**	**Day 2** **(Drug Treatment)**	**Day 3** **(MTT Assay)**
Washing with PBS	Removed the plate form incubator to remove the media from each well	25 µl of freshly prepared MTT added in each well
Trypsinization	Drug treatment (10, 25, 50,100,200 and 300µM)	Keep in nncubator at 37°C for 2-3h
Cell Counting	Untreated / DMSO	Media was removed
Filled 96 well Plate (10000 cells/well)	Incubation (24h)	DMSO (100 µl) was added
Incubation (24h)		Kept the plate in Incubator at 37°C for overnight and recorded plate at 570nm in ELISA plate reader

5.6 ENZYME INHIBITION ASSAY:

The enzyme inhibition study of synthesized compounds was performed in micro plate (96 well plates) using Takara Universal Tyrosine Kinase Assay Kit (Cat. # MK410, Takara Bio.Inc., Shiga, Japan). This assay kit measures the percentage inhibition of substrate of tyrosine kinase against the inhibitors. The assay was performed according to the manufacturer's protocol. The activity can be measured by inhibition of Src gene (already present in phosphate substrate) or EGFR (by adding A431 cells).

Procedure:

(1) Suspension and dilution of cells: Wash 1 - 5 x 106 cells with PBS and spin at room temperature for 5 min at 300 X g, then recover the cells as pellet. · Add 1 ml of extraction buffer, then suspend the pellet by vortex gently. Spin at 4℃ for 10 min at 10,000 X g, recover the supernatant as sample. Dilute the prepared sample (as mentioned in VII-3. Sample preparation (1), (2), (3) with Kinase Reacting Buffer by more than 5 times. When extracts of cultured cells are used, dilute by 15 - 100 times. Prepared sample is stable at - 80℃ for few days before dilution. The diluted sample is unstable and the activity should be measured on the same day.

(2) Phosphorylation: Add 40 µl of serial dilutions of PTK control or samples into each well with micro pipette in duplicate. Add 10 µl of 40 mM ATP-2Na solution into each well and mix well. Incubate it for 30 min at 37℃ Addition of ATP solution starts phosphorylation of tyrosine. If reaction time is over 45 min, non-specific binding of PTK to a plate may occur and may cause high background.

(3) Blocking: Remove sample solution and wash the wells 4 times with washing buffer; between each washing step, empty out the remaining solution by vigorously tapping microtiter plate onto paper towel, especially after the last washing. · Add 100 µl of Blocking solution into each well and incubate it for 30 min at 37℃.

(4) Addition of antibody· Discard blocking solution and empty out washing buffer fully on a paper towel. (On this step washing procedure is not needed.) Add 50 µl of Anti-phosphotyrosine (PY20) - HRP solution into each well and incubate it for 30 min at 37℃.

(5) Substrate reaction· Discard the antibody solution and wash each well 4 times with washing buffer. Empty out washing buffer fully on a paper towel. Add 100 µl of HRP

substrate solution (TMBZ) into each well. Incubate it at 37℃. (15 min is recommended as standard reaction time.)

(6) Stop colouring development: Add 100µl of stop solution into each well in the same order as HRP substrate solution.

(7) Measurement: 1. Measure the absorbance at 450 nm with a plate reader. 2. Construct a standard curve by plotting the absorbance on the y-axis against the activity of PTK control on the x-axis. 3. Calculate PTK activity of sample on the basis of the prepared standard curve.

CHAPTER NO. 6

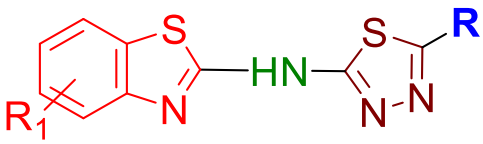
RESULTS and DISCUSSION

6 RESULT AND DISCUSSION

In the present work we had synthesized eighteen novel hybrid anticancer agents by combining benzothiazole, thiadiazole and NSAIDs using appropriate synthetic approach. In the first step various substituted 2-amino benzothiazole derivates were synthesized as per reported procedure. In the second step substituted 1,3 benzothiazole-2-yl thiosemicarbazide were synthesized by treating substituted 2-aminobenzothiazole with carbon disulphide and hydrazine hydrate in presence of ethanol. In the third step various substituted N-(1,3,4-thiadiazol-2-yl) benzo[d]thiazol-2-amine (final product) were synthesized by treating it with various NSAIDs in presence of phosphorous oxychloride.

TLC studies were carried out for all the derivatives, as they gave single spot after the development of chromatogram that clearly indicates the purity and homogeneity of the synthesized compounds. All the derivatives were characterized by melting point determination and thin layer chromatography. The purity of all derivatives was checked by thin layer chromatography and structures were characterized by IR, ^{1}H-NMR, ^{13}C NMR and mass spectral studies.

6.1 INSILICO STUDIES



R		R1	
Substitution	Code	Substitution	code
2-hydroxy phenyl (salicylic acid)	sa	6-CH_3	1
2-acetoxy phenyl (aspirin)	a	4-CH_3	2
1-isobutyl-4-isopropylbenzene (ibuprofen)	ib	6-OCH_3	3
2-ethyl-6-methoxynaphthalene (Naproxen)	na	4- OCH_3	4
4-isopropylphenyl)(phenyl)methyl)-l-oxidane (fenoprofen)	fp	6-Cl	5
(4-ethylphenyl)(phenyl)methanone (Ketoprofen)	kp	4-Cl	6
		6-NO_2	7
		4-NO_2	8
		H	9

6.1.1 Virtual screening

We had designed 54 hybrid NSAIDS which includes different derivatives of salicylic acid, aspirin, ibuprofen, naproxen, fenoprofen and ketoprofen. Molecular hybridization approach was applied to design new compounds by linking the main structural unit of the NSAIDs with the benzothiazole and thiadiazole ring. Structure based virtual screening was performed by applying ADMET filtration and Glide docking in SP mode to find out best effective compounds. The docking studies were performed on three different receptors; TNF-alpha, COX-II and protein kinase (dual specific tyrosine and serine/threonine kinase) to predict the binding affinity based on the docking score as compared to standard/native ligand. Based on docking studies we found protein kinase as most relevant target. To predict specific kinase binding affinity molecular docking studies were performed on different receptors which includes EGFR, VEGFR, FGFR, HGFR and Src (table 2). We found higher binding affinity for the target Src and EGFR tyrosine kinase. Finally, we screened 25 compounds based on insilico studies which further taken for synthesis using appropriate synthetic approach. The other derivatives were not screened due to unfavourable properties for drug likeness (due to violations of Lipinski rule of five) and their molecular interactions are not favourable due to more electrostatic charges and rotational penalties (due to more rotatable bonds) of the compounds with the targeted proteins.

6.1.1.1 Docking studies of screened compounds with the target TNF-α

The glide score with TNF-α receptor was found in the range of -5 to -7 with good H-bond interactions. The docking score of screened compounds were compared with the original ligand present in protein structure. The original ligand bound to the target site exhibits glide score -8.17. It was observed that screened compounds have good binding affinity (less docking score) as compared to standard ligand. GLY349, GLU406, LEU348, ALA439 and HIS405 were identified as interacting residues for original (standard) ligand. For the screened compound ASN389, GLU406, HIS405, ALA351, LEU350, TYR352, ILE438, GLY349 and ALA439 were identified as common interacting residues (figure 6a).

6.1.1.2 Docking studies of screen compounds with the target COX-II

The glide score with COX-II receptor was found in the range between -6.1 to -7.9 with good H-bond interactions. The docking score screened compound were compared with the original ligand (mefenamic acid) present in protein structure. The original ligand bound to the target site exhibits glide score -8. It was observed that screened compounds have good binding affinity (less docking score) as compared to standard ligand. ASN389, GLU406,

HIS415 and ALA351were identified as interacting residues for original (standard) ligand. For the screened compounds PHE518, ALA516, VAL523, MET522, SER353, TYR355 and PHE357were identified as common interacting residues (figure 6a).

6.1.1.3 Docking studies of screened compounds with the target Protein Kinase

The glide score with MPS1 receptor was found in the range between -6.2 to -8.84 with good H-bond interactions. The docking score of screened compounds were compared with the original ligand present in protein structure. The original ligand bound to the target site exhibits glide score -8.7. It was observed that screened compounds have good binding affinity (less docking score) as compared to standard ligand. GLY605, ILE586, ALA551, VAL536, ILE663, ASP608, PRO673 and ILE607 were identified as interacting residues for original (standard) ligand. For the screened compound GLY605, ASN606, ILE531, ALA551, ILE5869, MET602, LEU654, VAL539 and PRO673were identified as common interacting residues (Fig. 6a).

6.1.2 Insilico ADMET study

All the screened compounds pass the Lipinski rule of five and show no violations. Oral absorption of all screened compounds was found in the range of 72-100 percentages. It means that compounds may be orally active. Drug like properties was found in between 0 to 1 star which indicated screened compounds have drug like properties. Results of insilico ADMET study in comparisons with standard ligands using QuikProp are mentioned in table 6a.

To predict specific kinase binding affinity molecular docking studies were performed on different receptors which includes EGFR, VEGFR, FGFR, HGFR and Src (table 2). We found higher binding affinity for the target Src and EGFR tyrosine kinase. Finally, we screened 25 compounds based on *Insilico* studies which further taken for synthesis using appropriate synthetic approach. The other derivatives were not screened due to unfavourable properties for drug likeness (due to violations of Lipinski rule of five) and their molecular interactions are not favourable due to more electrostatic charges and rotational penalties (due to more rotatable bonds) of the compounds with the targeted proteins.

Table 7 ADME properties of Screened Compounds

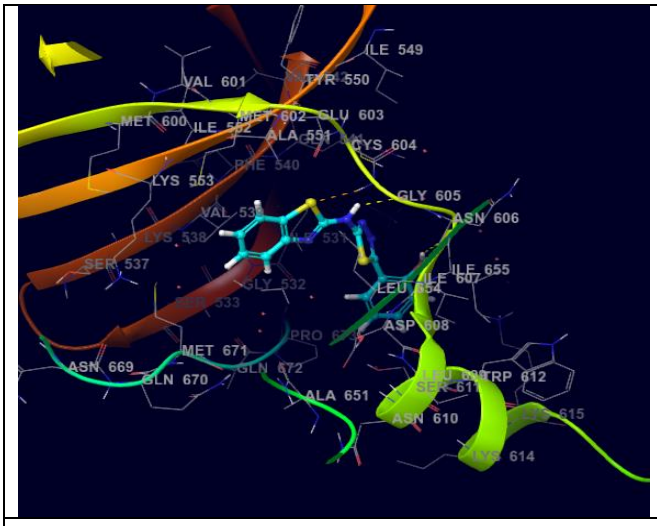
Compound	S	MW	HBd	HBa	QPlogPo/w	QP logS	QP PCaco	PHOA	RO5
1a	0	382.45	1	6.5	3.485	-5.598	790.186	100	0
1Sa	0	340.41	2	4.75	3.105	-5.037	523.385	93.785	0
2a	0	382.45	1	6.5	3.523	-5.698	790.223	100	0
2Sa	0	340.41	2	4.75	3.149	-5.152	524.325	94.06	0
3a	0	398.45	1	7.25	3.271	-5.345	774.263	100	0
3Sa	0	356.41	2	5.5	2.895	-4.646	523.157	92.554	0
4a	0	398.45	1	7.25	3.314	-5.364	791.324	100	0
4Sa	0	346.42	2	6.45	2.547	-4.209	451.161	89.365	0
5a	0	402.87	1	6.5	3.608	-5.744	774.095	100	0
5Sa	0	360.83	2	4.75	3.286	-5.219	524.905	94.868	0
6a	0	402.87	1	6.5	3.706	-5.864	790.215	100	0
6SA	0	350.84	2	5.7	2.916	-4.654	450.814	91.521	0
7a	0	413.42	1	7.5	2.499	-5.15	108.706	78.025	0
7Sa	1	371.38	2	5.75	2.163	-4.689	72.071	72.858	0
8a	1	413.42	1	7.5	2.518	-5.304	594.279	77.027	0
8SA	1	371.38	2	5.75	2.181	-4.843	562.503	71.859	0
9a	0	368.42	1	6.5	3.219	-5.137	789.17	100	0
9Sa	0	326.39	2	4.75	2.833	-4.602	523.9	92.201	0
1ib	0	408.58	1	3	4.92	-7.061	529.242	64.187	0
2ib	0	408.58	1	3	4.95	-7.092	530.423	63.172	0
3ib	1	429.12	1	3	5.14	-7.38	510.123	60.231	1
4ib	1	429.12	1	3	5.17	-7.34	509.923	60.129	1
4na	1	432.55	1	4.75	5.677	-6.58	532.557	78.127	1
7kp	1	476.99	1	6	5.689	-6.43	132.324	65.123	1
9fp	1	474.59	2	6.45	5.351	-6.12	156.127	72.143	1

S (STARS) = Number of property/descriptor values falling outside the 95% range of similar values for known drugs. Recommended value 0-5 QPlogS = Predicted aqueous solubility, log S. Recommended values –6.5 – 0.5 QPPCaco = Predicted apparent Caco-2 cell permeability in nm/sec. Recommended values <25 poor, >500 great. PHOA= Predicted human oral absorption on 0 to 100% scale Recommended values >80% is high <25% is poor

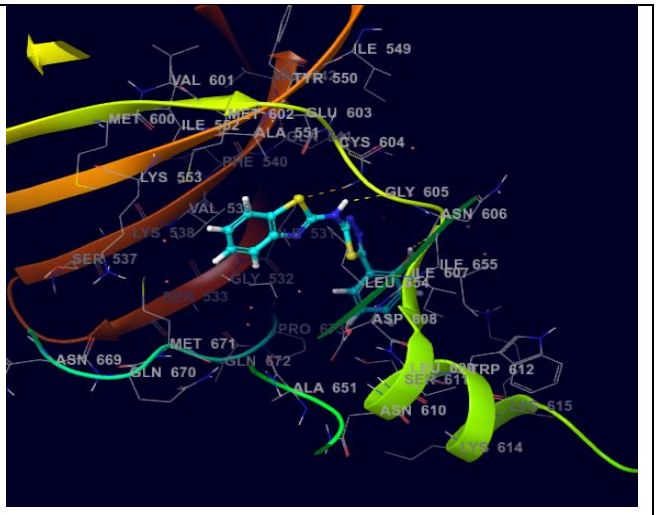
Table 8 Docking score of screened compounds

	Docking Score (Kcal/mol)							
	TK	**TNF-α**	**COX-II**	Tyrosine Kinase				
PDB ID	5EHO	1ZXC	5IKR	**EGFR**	**VEGFR**	**FGFR**	**HGFR**	**Src**
				1XKK	2XIR	4F65	5GRN	4MXO
Code								
1a	-7	-6.26	-6.4	-7.5	-6.4	-6.6	-8.2	-7.5
1sa	-8.6	-6.14	-6.8	-7.2	-6.8	-6.4	-4.1	-7.1
2a	-7.73	-6.52	-6.9	-6.7	-6.3	-6.6	-5	-6.3
2sa	-7.66	-6.25	-6.6	-6.5	-6.6	--6.4	-4.6	-5.2
3a	-7.72	-5.37	-6.2	-7.4	-6.5	-6.8	-6.9	-7.1
3sa	-7.92	-5.45	-6.1	-6.7	-6.9	-6.2	-5.1	-7.5
4a	-7.22	-5.74	-6.4	-5.8	-6.3	-6.8	-5.3	-5.7
4sa	-7.81	-5.09	-6.3	-7	-7.1	-6.8	-3.3	-5.1
5a	-6.92	-5.12	-6.1	-6.8	-7.5	-6.7	-4.7	-6.9
5sa	-7.21	-5.34	-6.6	-6.9	7.5	-6.2	-4.9	-5
6a	-8	-5.12	-6.2	-6.6	-6.9	-6.4	-6.1	-6.3
6sa	-8.68	-5.3	-7	-8	-7.3	-6.4	-4.8	-8.9
7a	-7.49	-5.48	-6.1	-6.9	-6	-5.4	-4.9	-5.4
7sa	-8.23	-5.74	-6.5	-7.1	-7.6	-6.4	-2.1	-5.
8a	-6.92	-5.42	-6.2	-6.9	-7.7	-5.5	-5.5	-5.2
8sa	-8.84	-6.54	-6.5	-6.5	-7.2	-6.3	-4.2	-5.6
9a	-6.73	-5	-6.4	-6.4	-6.5	-6.5	-5.3	-6.17
9sa	-8.78	-5.68	-6.3	-6.7	-6.9	-5.7	-4.8	-7.29
7kp	-6.86	-7	-7.9	-6.9	-7.5	-5.6	-4.8	-6.5
9fp	-8.92	-6.52	-6.14	-6.2	-7.8	-6.4	-2.1	-6..1
4na	-8	-5.22	-6.22	-6.26	-6.8	-6.1	-6.1	-6.2
1ib	-6.3	-6.6	-7.8	-6.9	-7.5	-6.4	-4.7	-7.3
2ib	-7.3	-6.46	-7.89	-7	-6.3	-6.1	-4.9	-7.2
5ib	-6.4	-6.3	-6.23	-6.9	-6	-5.5	-5.7	-7.4
6ib	-6.23	-6.49	-6.1	-6.9	-7.7	-5.4	-4.9	-7.1
STD	-8.7	-8.6	-8	-8.1	-8.4	-8.2	-8.2	-7.78

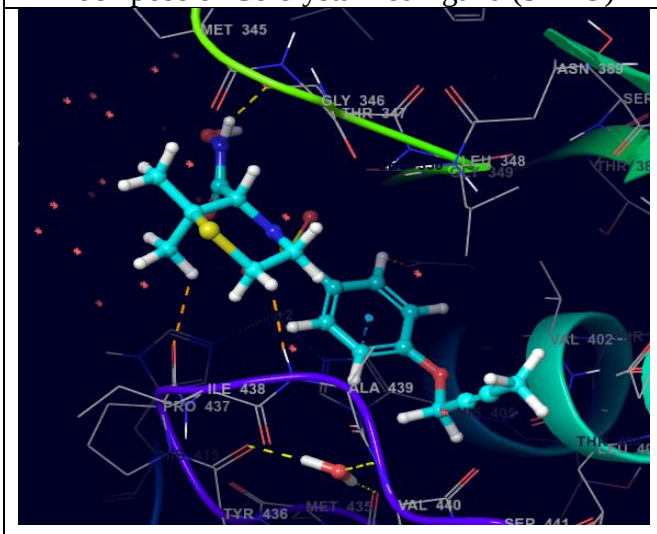
STD= standard. For standard we had used co-crystallized ligands (drugs) present in crystal structure of protein



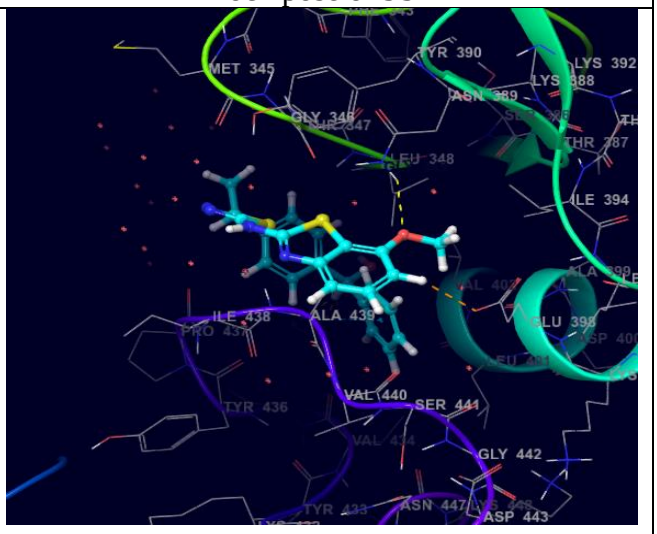
Dock pose of Co crystalized ligand (5EHO)



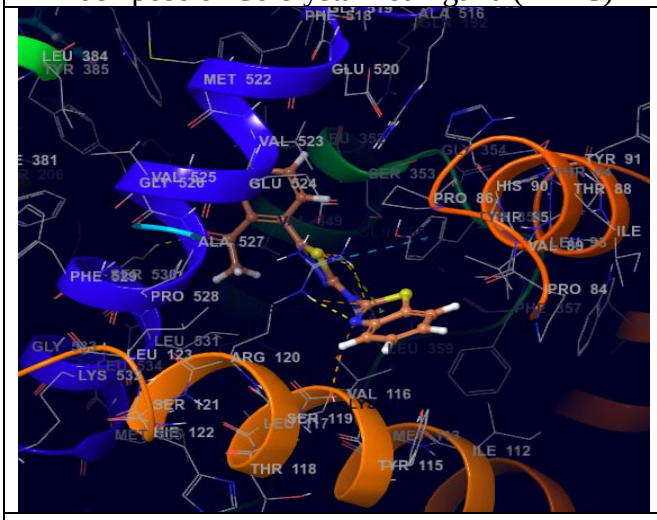
Dock pose of 9SA



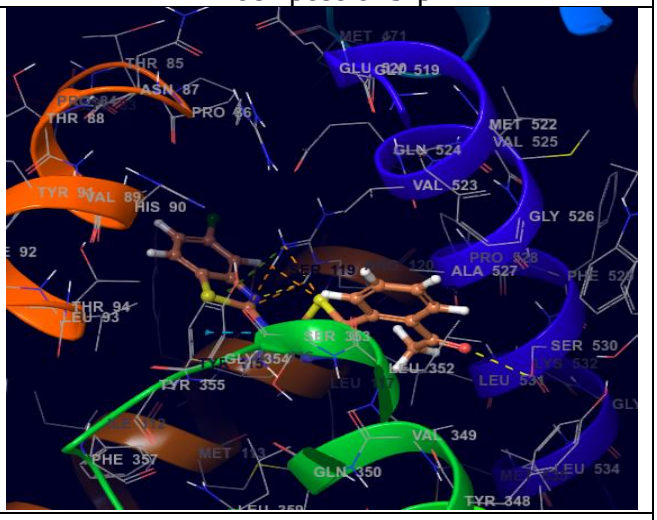
Dock pose of Co crystalized ligand (1ZXC)



Dock pose of 9fp



Dock pose of Co crystalized ligand (1ZXC)



Dock pose of 9a

Figure 5 Molecular interactions of compounds 9Sa, 9fp and 9a in comparison with standard co-crystalized ligand.

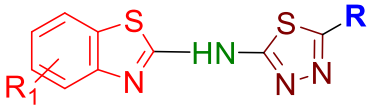
6.2 ANTICANCER ACTIVITY:

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high throughput screening.

Measuring the uptake of radioactive substances, usually tritium-labelled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this coloured solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in colour.

Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves(280).

Table 9 Results of cytotoxicity studies



Code	R	R1	MCF-7	CaC0-2
1a	2-acetoxy phenyl	6-CH_3	**22.15 ± 0.98**	**18.51± 0.22**
2a	2-acetoxy phenyl	4- CH_3	58.62 ± 0.45	55.15± 0.54
3a	2-acetoxy phenyl	6-OCH_3	**29.32 ± 0.21**	**31.44± 0.21**
4a	2-acetoxy phenyl	4-OCH_3	62.84 ± 0.58	58.56± 0.89
5a	2-acetoxy phenyl	6-Cl	60.14 ± 0.65	59.12± 0.78
6a	2-acetoxy phenyl	4-Cl	60.45 ± 0.44	58.59± 0.68
7a	2-acetoxy phenyl	H	**30.21 ± 0.21**	**25.61± 0.11**
1sa	2-hydroxy phenyl	6-CH_3	**32.47 ± 0.11**	**34.18± 0.12**
2sa	2-hydroxy phenyl	4- CH_3	55.56 ± 0.25	59.15± 0.45
3sa	2-hydroxy phenyl	6-OCH_3	**26.45 ± 0.32**	**24.88± 0.25**
4sa	2-hydroxy phenyl	4-OCH_3	62.78 ± 0.87	65.32± 0.98
5sa	2-hydroxy phenyl	6-Cl	59.41 ± 0.44	57.21± 0.45
6sa	2-hydroxy phenyl	4-Cl	**22.2 ± 0.14**	**25.61± 0.14**
7sa	2-hydroxy phenyl	H	66.34 ± 0.21	57.21± 0.25
1ib	1-isobutyl-4-isopropylbenzene	6-CH_3	**32.48± 0.13**	**22.15± 0.27**
2ib	1-isobutyl-4-isopropylbenzene	4- CH_3	65.23± 0.22	55.12± 0.12
3ib	1-isobutyl-4-isopropylbenzene	6-Cl	75.11± 024	65.48± 0.23
4ib	1-isobutyl-4-isopropylbenzene	4-Cl	**22.36± 0.18**	**20.45± 0.42**
Bosutinib			**5.4± 0.42**	**3.2± 0.17**

Anticancer activities of synthesized compounds were performed using MTT assay. We had evaluated anticancer activity on two different cell line; MCF-7 (breast cancer) and CaCo-2 (colon cancer). Out of 18 synthesized compounds we found eight potent compounds namely; **1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib and 3ib** which have IC50 value less than 50µM. Structure activity relationship revels that; for compound **1a-7a** substitution in the benzothiazole ring (R1) at 6th position of benzothiazole with CH3, OCH3 and H had more potency as compared to 4th position. For compound **1sa-7sa** substitution in the benzothiazole ring (R1) at 6th position with CH3 (1sa) and OCH3 (3sa) group and 4th position with Cl (6sa) group found to be more effective than others. For the series of 1ib-4ib substitution in the benzothiazole ring (R1) at 6th position with CH3 and 4th position with Cl found to be more potent.

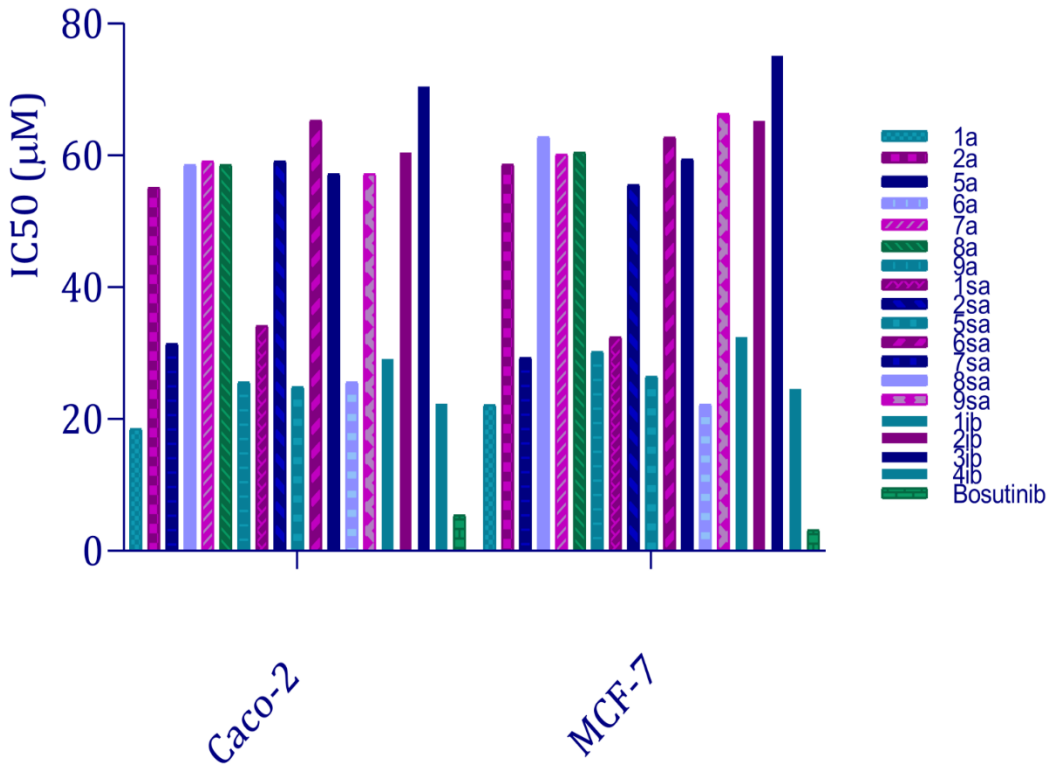
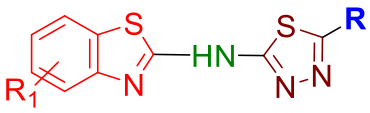


Figure 6 Graphical representation of IC50 values of synthesized compounds against the cell line Caco-2 and MCF-7

6.3 ENZYME INHIBITION ASSAY

Results of *Insilico* and Invitro enzyme inhibition study

Table 10 Enzyme inhibition activity of synthesized compounds with their docking scores



Code	R	R1	Src-TK IC50 µM	Docking score
1a	2-acetoxy phenyl	6-CH_3	**25.45± 1.12**	**-7.52**
2a	2-acetoxy phenyl	4- CH_3	99.31± 1.23	-6.34
3a	2-acetoxy phenyl	6-OCH_3	**35.38± 2.45**	**-7.18**
4a	2-acetoxy phenyl	4-OCH_3	99.6± 1.02	-5.72
5a	2-acetoxy phenyl	6-Cl	95.65± 0.25	-6.97
6a	2-acetoxy phenyl	4-Cl	83.33± 0.89	-6.32
7a	2-acetoxy phenyl	H	**30.12± 2.26**	**-7.29**
1sa	2-hydroxy phenyl	6-CH_3	**36.89± 0.45**	**-7.13**
2sa	2-hydroxy phenyl	4- CH_3	78.56± 3.56	-5.23
3sa	2-hydroxy phenyl	6-OCH_3	**32.54 ± 0.47**	**-7.51**
4sa	2-hydroxy phenyl	4-OCH_3	89.98± 2.54	-5.12
5sa	2-hydroxy phenyl	6-Cl	84.56± 1.89	-5.04
6sa	2-hydroxy phenyl	4-Cl	**16.14± 2.01**	**-8.09**
7sa	2-hydroxy phenyl	H	52.55± 1.45	-6.17
1ib	1-isobutyl-4-isopropylbenzene	6-CH_3	**22.15± 01.12**	**-7.64**
2ib	1-isobutyl-4-isopropylbenzene	4- CH_3	55.12± 0.24	-7.24
3ib	1-isobutyl-4-isopropylbenzene	6-Cl	65.48± 2.21	-7.38
4ib	1-isobutyl-4-isopropylbenzene	4-Cl	**20.45± 1.13**	**-8.41**
Bosutinib			**12.41± 0.02**	**-7.78**

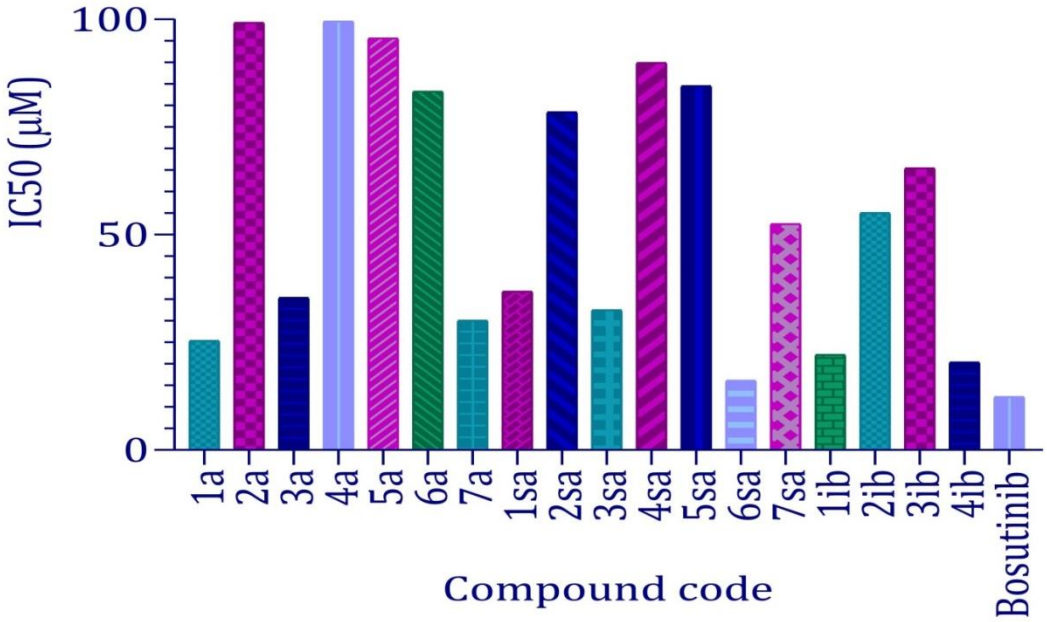


Figure 7 Graphical representation of enzyme inhibition (IC50) values of synthesized compounds

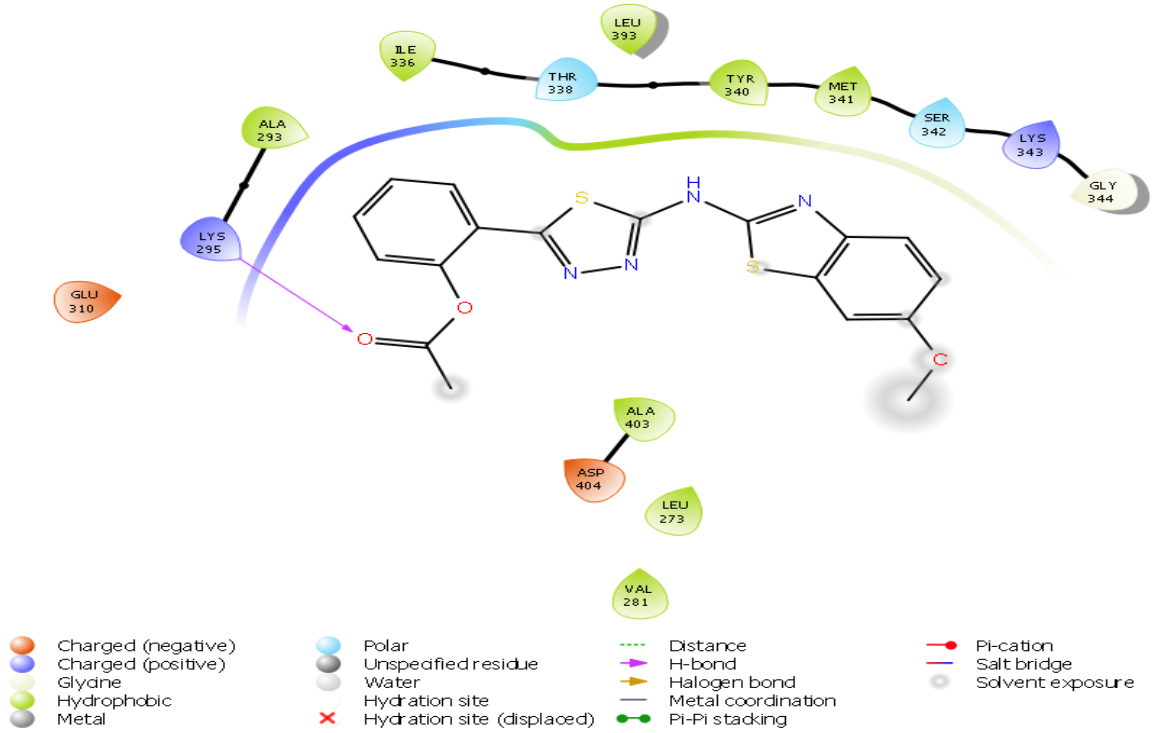


Figure 8 Molecular interactions of compound 3a with Src Kinase

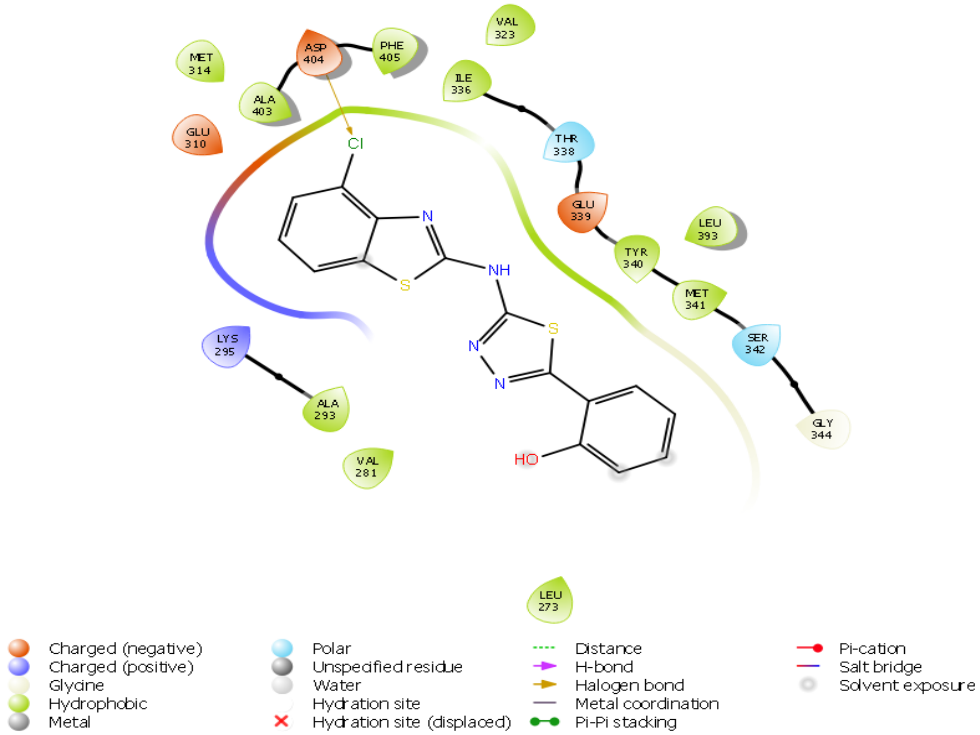


Figure 9 Molecular interactions of compound 6sa with Src Kinase

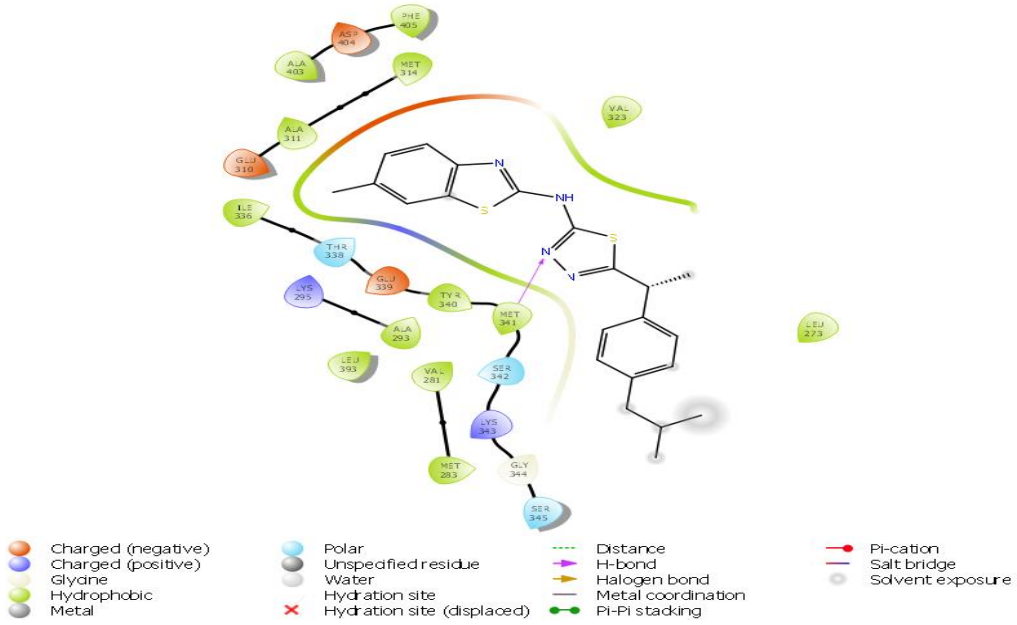


Figure 10 Molecular interactions of compound 1ib with Src Kinase

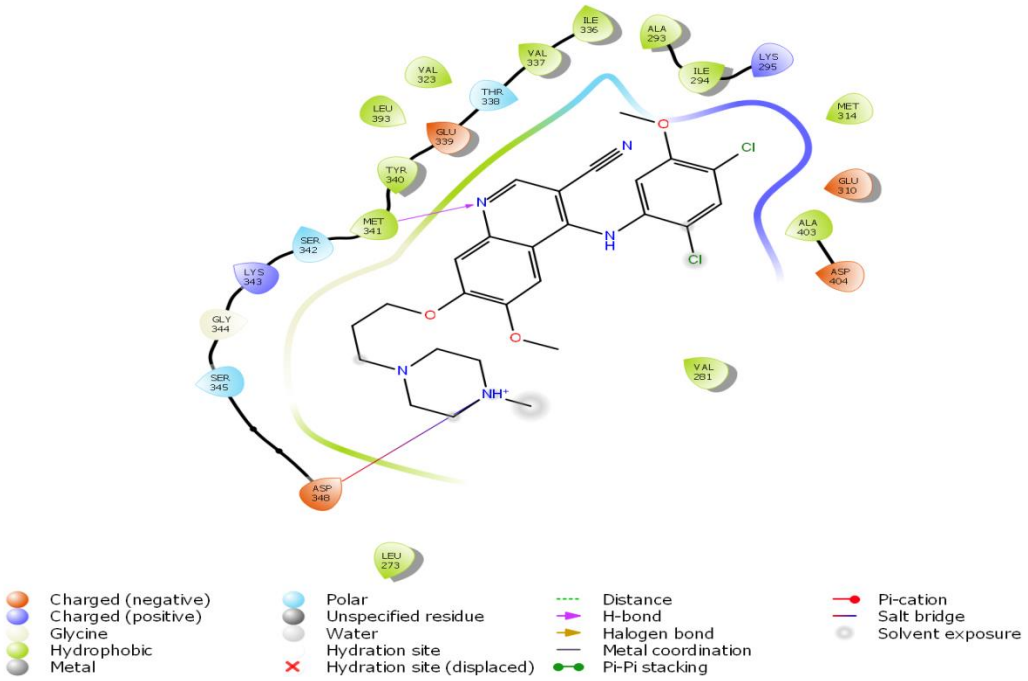


Figure 11 Molecular interactions of bosutinib with Src Kinase

Out of 18 synthesized compounds we found eight potent compounds namely; 1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib and 3ib which have IC50 value less than 50µM in enzyme inhibition study. These compounds also showed higher binding affinity as compared to others. Docking score of eight screened compounds were found in the range between -7.13 to -8.41. Reference drug bosutinib had docking score -7.78. As Compared to reference drug two compounds 6sa and 1ib had higher binding affinity. All the screened compounds had shown good H-bond and hydrophobic intersections. THR338, GLU339, TYR340, MET341, LEU273, LYS295, GLY344 and LEU393 were identified as common interacting residues in the active site.

Structure activity relationship between synthesized compounds and Src- enzyme inhibition study reveals that for compound **1a-7a** substitution in the benzothiazole ring (R1) at 6th position of benzothiazole with CH3, OCH3 and H had more potency as compared to 4th position. For compound **1sa-7sa** substitution in the benzothiazole ring (R1) at 6th position with CH3 and OCH3 group and 4th position with Cl group found to be more effective than others. For the series of **1ib-4ib** substitution in the benzothiazole ring (R1) at 6th position with CH3 and 4th position with Cl found to be active than others.

CHAPTER NO. 7

SUMMARY and CNCLUSION

7 SUMMARY AND CONCLUSION

The tyrosine kinase (TK) family is considered as one of the important family members of the kinase family due to its important role in various cellular processes. Src is the prototypical member of the tyrosine kinase family who plays a critical role in various cellular processes like signal transduction, proliferation, survival, differentiation etc. Mutation in Src leads to the development of different types of cancer. Therefore Src considers an important target for cancer therapy. Molecular hybridization (MH) is a strategy of rational design of new leads based on the molecular structure of two or more known bioactive derivatives which, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates. The molecular hybridization strategy is particularly interesting for the development of new leads for diseases whose treatment is restricted to few commercial drugs or in cases when bioactive compounds are discovered but presents high toxicity or pharmacokinetic and pharmacodynamic restrictions.

Using the molecular hybridization approach we had designed our molecules using three different moieties; benzothiazole, thiadiazole, and NSAIDs. Initially, the library of 54 different molecules was prepared and Insilico evaluation was done to find out best lead compounds. The insilico studies were performed using various tachniques like molecular docking, ADME, and toxicity screening. Based on our hypothesis we had performed structure-based virtual screening using three different receptors namely; TNF-alpha, Tyrosine kinase, and COX-II. Based on the molecular docking studies we found that for our designed molecules, tyrosine kinase could be a suitable target. Further to find out specific tyrosine kinase targets we had again performed molecular docking studies on the various receptor and non-receptor tyrosine kinase targets. The EGFR and Src tyrosine kinase were found most prominent based on molecular docking studies. Finally, we found 25 best hints from that we had successfully synthesized 18 compounds. Compounds were characterized using various methods like; TLC, melting point determination, IR, ^{1}H-NMR, ^{13}C NMR, and mass spectral data. Compliance with spectra data proves the structures of synthesized compounds.

These synthesized compounds were evaluated for anticancer activity. Screening of anticancer activity was done by performing an MTT assay using two different cell lines MCF-7 (breast cancer) and CaCo2 (colon cancer). Out of the 18 synthesized compounds, we found eight potent compounds namely; **1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib** which had IC50 values less than 50 µM. To validate the target, we performed the enzyme inhibition study using a universal tyrosine kinase kit. Based on data Src inhibition was found most

prominent by the compounds. In the enzyme inhibition study, we found eight compounds 1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib which had IC50 value less than 50 µM. In the molecular docking studies, the same compounds had good binding affinity as compared to standard drug bosutinib.

Finally based on insilico and in-vitro data we want to conclude that these compounds should be further explored as **Src inhibitors** and they may have the potential to target Src mutated cancers.

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LIST OF ABBREVIATIONS

˚C : Degree Centigrade

^{1}H: Proton Nuclear Magnetic Resonance

IR: Infra Red Spectra

SAR: Structure Activity Relationship

TLC : Thin Layer Chromatography

Mol. Wt. : Molecular weight

M: Molar

gm: Gram

mg: Milligram

µg: Microgram

ml: Millilitre

m/e : Mass to charge ratio

s : Singlet

d : Doublet

m: multiplet

Hr: hour

Ar : Aromatic

R_f: Retention factor

cm^{-1}: per centimeter

DMSO: Dimethyl sulfoxide

KBr: Potassium bromide

Fig.: Figure

%: Percent

COX: cyclooxygenase

TK: tyrosine kinase

EGFR: epidermal growth factor receptor

VEGFR: Vascular endothelial growth factor receptor

FGFR: Fibroblast growth factor receptor

PDGFR: Platelet derived growth factor receptor

IGFR: Insulin like-growth factor receptor

NRTKs: Non-Receptor tyrosine kinase

Fak: Focal adhesion kinase

Abl: Abelson tyrosine kinase

Syk: Spleen tyrosine kinase

Fes: feline sarcoma

TNF- tumor necrosis factor

IL: interlukins

MMPs: Matrix metalloproteinases

LOX: Lipooxygenase

iNOS: Inducible nitric oxide synthase

HIF: hypoxia inducible factor

PI3K: Phosphoinositide-3-Kinase

MAPK: mitogen activated protein kinase

LIST OF PUBLICATIONS

1. **Shah AP,** Patel CN. Virtual Screening of Novel Hybrid Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): Exploring Multiple Targeted Cancer Therapy by an In Silico Approach. Current Cancer Therapy Reviews. 2020 Mar 1;16(1):70-7.
2. **Shah AP,** Patel CN. *In silico* Discovery of Novel Benzothiazole-Thiadiazole hybrid analogues as Src Kinase inhibitors, International Journal of Pharmaceutical Research. 2020 Apr-June; 12(2), 2064-2070
3. **Shah AP**, Patel CN, Sureja DK, Sanghavi KP. A review on DNA repair inhibition by PARP inhibitors in cancer therapy. Folia medica. 2018 Mar 1;60(1):39-47.

LIST OF PAPER PRESENTED (CONFERENCE PROCEEDINGS)

1. Design and molecular Docking Studies of Some new Hybrid NSAIDs as Potential Anticancer agents" in INTERNATIONAL CONFERENCE ON DRUG DESIGN, 7-9 April 2017, JNU, New Delhi
2. "*In silico* Design, Synthesis and Anticancer Activity of Novel Benzothiazole-Thiadiazole Hybrid Analogues as MPS1 Inhibitors" INTERNATIONAL CONFERENCE ON DRUG DESIGN AND DISCOVERY (ICDD), Bits Pillani, Hyderabad, 29th Feb-2nd March

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RESEARCH ARTICLE

Virtual Screening of Novel Hybrid Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): Exploring Multiple Targeted Cancer Therapy by an *In Silico* Approach

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Abstract: ***Background*:** Dual-targeting/Multi-targeting of oncoproteins by a single drug molecule represents an efficient, logical and alternative approach to drug combinations. *In silico* methods are useful tool for the search and design of selective multi-target agents.

***Objective*:** The objective of the present study was to design new hybrid compounds by linking the main structural unit of the NSAIDs with the benzothiazole and thiadiazole ring and to discover new hybrid NSAIDs as multi targeted anticancer agents through *in silico* approach.

***Methods*:** Structure-based virtual screening was performed by applying ADMET filtration and Glide docking using Virtual screening Workflow. The docking studies were performed on three different types of receptors TNF-α, COX-II and protein kinase. Bioactivity prediction of screened compounds were done using Molinspiration online software tool.

***Results*:** Out of the 54 designed compounds eighteen were screened on the basis of binding affinity on various receptors and ADMET filtration. Bioactivity prediction reveals that screened compounds may act through kinase inhibition or enzyme inhibition. Compounds 2sa, 5sa, 6sa and 7sa showed higher binding affinity with all three receptors.

***Conclusion*:** The study concluded that compound 2sa, 5sa, 6sa, and 7sa could be further explored for multiple targeted cancer therapy.

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1. INTRODUCTION

Cancer is a major human health issue in the world. The discovery of new target based therapy opened a new window for the treatment of cancer. Major problems in the current cancer chemotherapy are the development of resistance and severe toxic effects. Resistance may occur through several different cellular mechanisms. Therefore, the challenge is to identify new less toxic drug and to improve existing cancer therapy. Active research going on to identify targets whose expression or activation increases cancer growth. Since the last century, there have been major developments in our understanding of cancer at the molecular level [1]. Various growth factors, hormones, cytokines, oncogenes, viruses, bacteria, and carcinogens have been identified that initiate and promote cancer. Many sub-cellular mechanisms promote the growth of cancer cells. Many biomarkers of cancer like hypoxia inducible factor (HIF), CYP450, TNF-α, COX-II, protein kinase, *etc.* have been discovered whose overexpression/dysfunction causes cancer [2-8].

It is well admitted that the link between chronic inflammation and cancer involves cytokines and mediators of inflammatory pathways, which activate several cell survival pathways, which escape the death of tumor cells. The most well known is the case of TNF-*α,* produced by tumor and immune cells, which leads to the survival of cancer cells. [9-11]. TNF-*α* function can be inhibited in two stages: **1**) inhibition of TNF-*α* converting enzyme (TACE), which inhibits Pro TNF-*α* processing. **2**) Inhibition of nuclear factor (NF)-*κB* which inhibits pro TNF-*α* synthesis. TACE inhibitors have been investigated as a means to prevent/limit inflammation by blocking the release of TNF in inflammatory diseases. TMI-005 and BMS-561392 were tested in Phase-II clinical trial but they were unsuccessful due to systemic toxicity and lower efficacy. Toxicities observed due to the inhibition of MMPs, including MMP1, MMP2, and MMP13. Therefore, there is a need to develop specific TACE inhibitors which are non specific target for MMPs to reduce toxicities. Currently, specific TACE inhibitors have shown great

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Research Article

Insilico Discovery of Novel Benzothiazole-Thiadiazole hybrid analogues as Src Kinase inhibitors

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ABSTRACT
Identification of specific target is very important for safe and effective cancer chemotherapy. Protein tyrosine kinase is considered as one of the important target for cancer. Src is prototypical member of tyrosine kinase family who plays critical role in various cellular process like signal transduction, proliferation, survival, differentiation etc. Mutation in Src leads to development of different types of cancer. Therefore Src consider as important target for cancer therapy. We designed benzothiazole-thiadiazole analogues targeting Src kinase using Insilico approach. Structure based virtual screening was performed to find out the novel compounds. Validations of ligands were done using ADME filtration and bioactivity prediction. All the designed compounds have passed Lipinski rule of five. Compounds 8a, 4a, 9a, 2a, 4sa, 3a and 3sa had higher binding affinity (low docking score) as compared to standard drug. Bioactivity prediction reveals that screened compounds may act through kinase inhibition or enzyme inhibition. The promising insilico results of compounds 8a, 4a, 9a, 2a, 4sa, 3a and 3sa opens newer directions to be explored as Src inhibitors.

Keywords: Src kinase, Virtual screening, benzothiazole, thiadiazole, Cancer

INTRODUCTION
Identification of specific target is very important for safe and effective cancer chemotherapy. At present, medicinal chemists are more focusing on discovery of novel target specific anticancer molecules in order to achieve safe and effective anticancer therapy. Protein tyrosine kinase is considered as one of the important target for cancer. Protein tyrosine kinase involved in variety of cellular functions such as cell growth, cell differentiation, cell motility, cell proliferation etc[1, 2]. Active research is going since few decades to discover novel protein tyrosine kinase inhibitors and new small molecules approved by varying chemical and biological approach[3]. It was reported that out of 300 cancer genes about 10% are protein kinases. Protein tyrosine kinasecatalyse the phosphorylation of tyrosine by transferring phosphoryl group form ATP to hydroxyl group of specific tyrosine (Tyr) residues in protein[4].There are about 100 types of PTKs which are broadly divided in to two categories: receptor and non receptor tyrosine kinase. Receptor tyrosine kinases (RTKs) include various subfamilies such as epidermal growth factor receptor, fibroblast growth factor receptors, vascular endothelial growth factor receptor, hepatocyte growth factor receptor and insulin-like growth factor receptor. Various subfamilies of RTKs are used target for cancer therapy[5].Src is prototypical member of tyrosine
kinase family, discovered before four decades from the rous avian sarcoma virus as first oncogene. c-src belongs to family of non receptor tyrosine kinase which is about 60KDa in size. Src plays critical role in various cellular process like signal transduction, proliferation, survival, differentiation etc[6, 7]. The structure of c-Src contains seven different parts which includes: SH4 domain, SH3 domain, SH2 domain, SH1 catalytic domain, SH2-SH3 linker, unique domain and C-terminal negative regulatory region. SH1 domain is called as catalytic domain as it is autophoshorylated by active c-Src[8, 9]. Conformational changes occur in active and inactive state of Src protein. Catalytic and non catalytic domain activity is important in signal transduction process. The active conformation of Src is formed by assembling in between SH3 and SH2 domain. The inactive conformation formed intramolecular bonding of SH2 with C-terminal of c-Src[9, 10]. Src plays important role in tumor metastasis, as it has role in cell migration, adhesion and invasion and as a result of this tumor microenvironment is maintained. In hypoxic condition, Src activation stimulates endothelial growth factor (VEGF), matrix metallopeptidase (MMPs) and interleukin-8 (IL-8) expression and due to this angiogenesis rate is increases. Src-mediated VEGF secretion elicits angiogenic signaling in endothelial cells and Src activation in osteoclasts facilitates osteolytic bone metastasis[11, 12].Tumor migration and adhesion are the primary steps of metastasis that requires formation of actin based cell membrane. Actin based cell membrane promotes directional migration and extracellular matrix (ECM) degradation. Epithelial mesenchymal transition (EMT) and metastasis promoted by Twist1 transcription factor.

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REVIEW

A Review on DNA Repair Inhibition by PARP Inhibitors in Cancer Therapy

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The DNA repair process protects the cells from DNA damaging agent by multiple pathways. Majority of the cancer therapy cause DNA damage which leads to apoptosis. The cell has natural ability to repair this damage which ultimately leads to development of resistance of drugs. The key enzymes involved in DNA repair process are poly(ADP-ribose) (PAR) and poly(ADP-ribose) polymerases (PARP). Tumor cells repair their defective gene via defective homologues recombination (HR) in the presence of enzyme PARP. PARP inhibitors inhibit the enzyme poly(ADP-ribose) polymerases (PARPs) which lead to apoptosis of cancer cells. Current clinical data shows the role of PARP inhibitors is not restricted to BRCA mutations but also effective in HR dysfunctions related tumors. Therefore, investigation in this area could be very helpful for future therapy of cancer. This review gives detail information on the role of PARP in DNA damage repair, the role of PARP inhibitors and chemistry of currently available PARP inhibitors.

INTRODUCTION

Cancer is one of the major human health problems worldwide. The discovery of new target based therapy has opened a new window in the treatment of cancer. One major problem in current cancer chemotherapy is resistance development and severe toxic effects. Resistance may occur through several different cell mechanisms. Therefore, the challenge is to identify new less toxic drugs and to improve the existing cancer therapy. There is active research going on to identify targets whose expression or activation increases cancer growth. The cell DNA repair mechanism may provide important information on the above stated problem.[1] PARP is the enzyme which is involved in the DNA damage repair process. Inhibition of PARP may induce apoptosis **(Fig. 1)**. PARP enzymes are important for many cellular functions[2] which includes inflammatory gene[3] in smooth muscles to response tumor necrosis factor (TNF).[4]

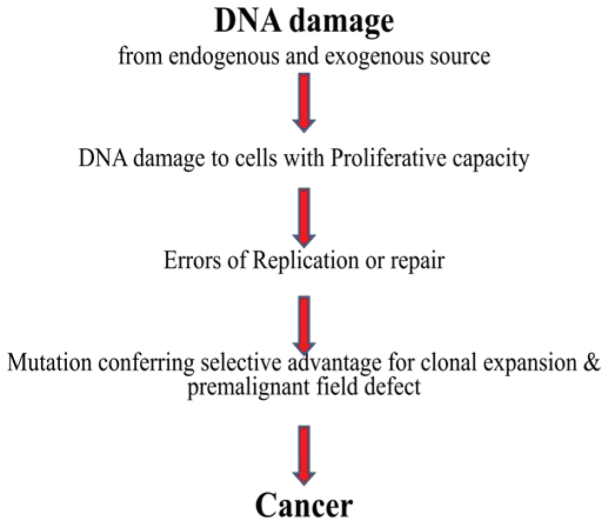

Figure 1. Link between DNA damage repair and cancer.

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