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Comprehensive Innovative Design and Synthesis of Targeted Anticancer Compounds for Pathway-Specific Cancer Therapy

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Abstract:
The development of targeted anticancer therapies has emerged as a promising approach to improve the efficacy and specificity of cancer treatment. This study focuses on the innovative design and synthesis of novel anticancer compounds that selectively target specific signaling pathways involved in cancer progression. By utilizing advanced computational modeling and medicinal chemistry techniques, we have identified and synthesized a series of compounds with high affinity for critical molecular targets. In vitro and in vivo evaluations demonstrate that these novel agents effectively inhibit cancer cell proliferation and induce apoptosis with minimal impact on normal cells. The findings underscore the potential of pathway-specific targeting as a strategy for developing safer and more effective anticancer therapies.
Keywords: Signaling pathways, Cancer therapy, Molecular targets.

1.1 INTRODUCTION

Medicinal chemistry is an intriguing area of study in the scientific and technological communities. The previous seventy years have seen both thrilling and very difficult progress. According to Burger, medicinal chemistry is built on the ever-present promise of discovering biological rationales for drug development.

The scientific discipline known as medicinal chemistry focuses on the development of new pharmaceuticals with potent medicinal effects. The branch of medicinal chemistry that studies the molecular mechanisms of action of physiologically active chemicals and focuses on their discovery, development, identification, and interpretation (1).

The human body is composed of billions of cells that undergo the typical processes of growth, division, and death. If a cell is normal and healthy, it can regulate its own development and self-destructs if it is unhealthy. Unchecked cell development may arise as a result of malfunction or mistake, ultimately leading to cancer. When cancer cells clump together, they create a growth called a tumor. Since cancer cells are influenced by both the culture conditions and the extracellular milieu, pinpointing the specific cause of cancer may

be an impossible task. Approximately 7.6 million people lose their lives each year to cancers of the lung, stomach, liver, and breast. People who deal with solvents, grease, and oils, as well as those in the textile and plastic sectors, are at a significant risk of developing cancer, according to recent research from the National Cancer Institute (2, 3).

1.2 TYPES OF CANCER

Carcinoma:

Originating in the epithelial cells that line the inside of the body's organs (such as the uterus, mouth, and oesophagus:)

Sarcoma:

Derived from the embryonic cells that make up the many types of connective tissues (such as bone and fibrous tissue).

Lymphoma, myeloma and leukaemia:

Derived from immune system and bone marrow cells (4)

Table 1.1 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.

1.3 CHARACTERISTICS OF THE CANCER

Tumor suppresser genes:

Normally, when a cell begins to divide too much, its neighbor cells will send inhibitory substances to halt it. One function of tumor suppressor genes is to block the signals that inhibit factors. This causes an overabundance of cell division, which in turn allows tumor cells to proliferate.

Angiogenic genes:

Tumor blood vessels will grow independently during angiogenesis. The cancer cells get oxygen, nutrients, and waste products from these blood arteries, which also eliminate them. When cancer cells secrete chemicals that signal to normal host tissue around them, angiogenesis begins. This signal triggers the expression of certain genes that promote the development of new blood vessel precursors.

Proto-oncogenes:

Once a gene becomes mutated, it becomes an oncogene. In order to promote the proliferation of cancer cells, oncogenes may activate signaling molecules such as growth factors (5, 6).

1.4 SOME GENES INVOLVES COMMONLY IN CANCER:

XRCC1:

The DNA repair process is aided by this gene. They are involved in the repair of single-strand DNA breaks via their complexes with the DNA ligase-III enzyme and protein. Radiation treatment and alkylating chemicals are the most common causes of single strand breakage. Initiating the DNA repair process via the base excision repair route, these genes interact with DNA ligase-III, polymerase, and poly (ADP-ribose) polymerase.

EFGR:

One class of extracellular protein ligands is known as epidermal growth factor (EGF), and the cell surface receptors for this family is called EGFR. The tyrosine kinase is a close relative of this family. Cancer is caused by mutations in EGFR activity as well.

KRas:

An essential component of signal transduction pathways, the KRas protein is a member of the Ras family. It is common for KRas to be found in cell membranes. Normally, cell signaling relies on the protein produced by KRas. One other cancer-causing mutation is KRas.

P53:

P53, which is represented by the TP53 gene, is a tumor suppressor gene. In multicellular creatures, P53 is crucial to the cell cycle. P53 has a role in cancer prevention. As it plays a crucial role in avoiding mutations in the genome, the P53 gene is referred to as the guardian of the genome. Cancer develops when the P53 gene is mutated or its function is changed.

BRCA1:

One of the tumor suppressor genes found in humans is BRCA. The process of cell division is influenced by BRCA. In order to prevent unchecked cell proliferation, the BRCA gene maintains a proper cell environment. The BRCA gene directly contributes to DNA repair via the protein it produces. The cancer is also caused by changes or inhibitions to BRCA function.

1.5 TREATMENT OF CANCER

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 1.1 Causes of Cancer

Anticancer agents:

Medications that combat cancer fall into one of three categories:

Cytotoxic Drugs (largest group)

- Alkylating substances
- Metabolites that inhibit
- Inhibitors of tumor growth
- Anthocyanins made from plants
- Various cytotoxic medications

Hormones and hormone antagonists

Among the most well-tolerated chemotherapeutics, they zero in on certain cell types by targeting their receptors. Taking Tamoxifen

Immunomodulators

- Immunosuppressants
- Immunostimulants,
- interleukins
- interferons

Alkylating Agents:

Mechanism of action:

The alkylation with nucleophilic substitution mechanism is the mechanism by which these medications exert their action. Cell membranes, proteins, and DNA are among the many biological components that they alkylate. This alkylation process mostly affects the nitrogenous bases of DNA.

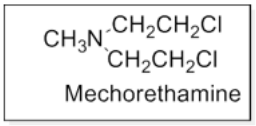
Initial state of the pharmaceuticals is that of pro-drugs, which are activated upon extraction of a chlorine atom. Ions of carbonium are thus produced. Because of its high electrophilicity, this "carbonium ion" will go for any nucleus that has an extra pair of electrons, such as guanine's N7. The drug and DNA's guanine base pair as a consequence of this electrophilic assault. Several effects follow from this "alkylation":

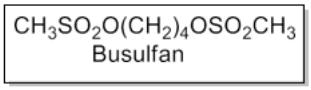
Miscoding (In transcription)

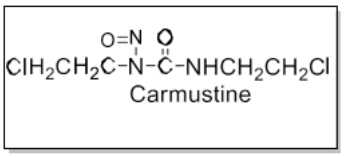
- Only when a medicine serves two purposes can cross-linking take place.
- Various Classes of Alkylating Substances
- Mustards on nitrogen: For instance, melphalan, chlorambucil, cyclophosphamide, and

mechlorethamine

- The nitrosoureas So, for example, carmustine and lomustine
- Stonates of alkyl groups: The aziridines include bensotepa and thiotepa, for example, and busulfan.







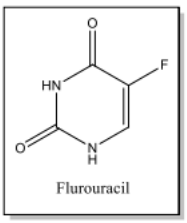
Anti-metabolites:

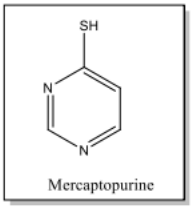
A molecule that mimics the structure of a metabolite needed for biological operations but is structurally distinct enough to disrupt cellular processes, such as cell division, is called an antimetabolite.

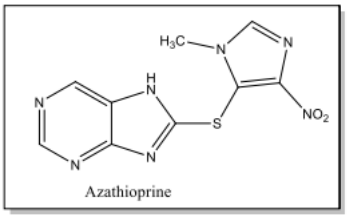
The use of anti-metabolites in cancer therapy stems from their ability to disrupt DNA synthesis, which in turn inhibits cell division and tumor development, particularly during the S-phase.

One way to categorize them is:

- An alternative to folic acid: Purine analogues of methotrexate, for example: Such as mercaptopurine
- Cyterabine and 6-flurouracil are examples of pyrimidine analogues.







Antibiotics:

Doxorubicin (adriamycin)

Mechanism of action

- As an anthracyclin antibiotic, doxorubicin prevents cells from making DNA and RNA by inserting itself between neighboring base pairs, causing local uncoiling and thus halting synthesis.
- Inhibiting the topoisomerase II enzyme, which is responsible for DNA repair, is also associated with its anticancer impact.

- The process of converting dox to semiquinone free radicals, which in turn generate superoxide ions and hydrogen peroxide, is catalyzed by CYTP 450 and mediates DNA single strand scission.

Mitomycin−C

- An antibiotic that targets tumors is mitomycin−C. Nevertheless, from a mechanistic standpoint, it is one of the DNA alkylating agents.
- It bioactivates in the cell by alkylating the guanine base in DNA preferentially, which causes DNA to cross-link.
- Additionally, it causes DNA degradation by creating free radicals.

Uses:

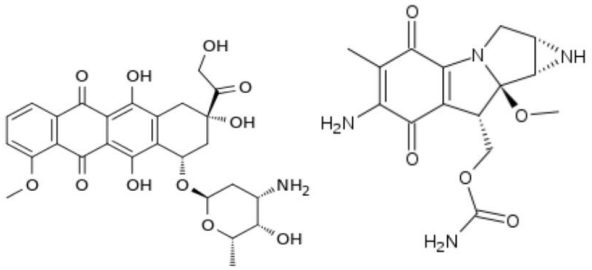
- Various malignancies, including leukemia, bone, ovarian, and breast cancers.
- Myeloid leukemia (ALL).
- Hodgkin's lymphoma-not

Bleomycin

Bleomycin causes DNA degradation, chain breakage, and the release of free bases via the generation of free radicals (superoxide and hydroxyl radicals), making it lethal in any phase of the cell cycle, including G0.

Uses

The genitourinary tract, skin, esophagus, lung, testis, and head and neck malignancies are among those that bleomycin helps treat. Furthermore, it is used for the treatment of non-Hodgkin's lymphomas and Hodgkin's disease.



Doxorubicin Mitomycin

Natural Products:

For example, vincristine and vinblastine (M-phase) are vinca alkaloids.

Mechanism of action

Microtubules are formed when tubulin, a structural protein, polymerizes. In addition to being a component of the mitotic spindle and cell cytoskeleton, microtubules have additional important functions. The polymerization of microtubule structures is inhibited when vincristine binds to tubulin. When the microtubules are disrupted, mitosis stops in metaphase. Since all cells divide quickly, including cancer cells, intestinal epithelium, and bone marrow, the vinca alkaloids have an effect on all of these tissues.

Uses: leukemia, non-Hodgkin's lymphoma, and hodgkin's disease were all treated.

Taxanes: Docetaxel and paclitaxel

Lung, ovarian, and breast cancers are among those that it helps treat.

Mechanism of action

The structure of microtubules is hyper-stabilized, or "frozen," by paclitaxel. After paclitaxel attaches to tubulin's β subunit, the microtubule/paclitaxel complex that forms cannot

disintegrate. Because microtubule function depends on their ability to shorten and lengthen, this has a negative impact on cell function.

New evidence suggests that paclitaxel disrupts the activity of the apoptosis-inducing protein Bcl-2 (B-cell leukemia 2), which in turn causes cancer cells to undergo programmed cell death (apoptosis).

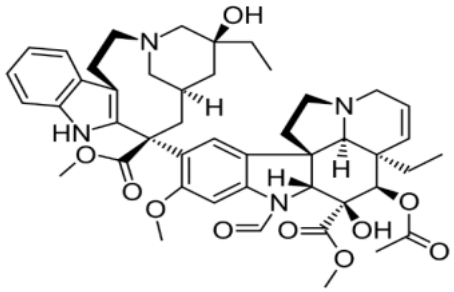
Etoposide

The chemical component comes from the mandrake root toxin podophyllotoxin.

An agent that blocks the enzyme topoisomerase II, which is responsible for repairing damaged DNA inside cancer cells and halting their ability to divide and spread. Death ensues in the cells.

Uses

Testicular cancer and small cell lung cancer have both benefited from its usage in therapy. A medicine called vincristine



Drugs acting on Hormones:

It entails influencing the endocrine system by means of the external delivery of certain hormones, especially steroid hormones, or medications that impede the synthesis or action of these hormones.

Changing the amount or activity of certain hormones may cause some malignancies to stop growing or even suffer cell death. This is because steroid hormones are strong gene expression drivers in certain cancer cells.

Corticosteroids:

- Corticosteroids are powerful medications that reduce inflammation.
- Cancer pain-inducing swelling may be alleviated with their help.

LITERATURE REVIEW

Understanding the hidden causes and creating powerful therapies for cancer introduced huge difficulties because of its mind boggling and diverse nature. The target of this writing survey was to offer a far reaching comprehension of various features of cancer research, going from its essential qualities to the headway of explicit therapies. Cancer alludes to an expansive range of illnesses that include uncontrolled cell development and division. The improvement of cancer is impacted by different elements, including hereditary qualities, climate, and cell processes. Specialists have been exploring different parts of cancer, for example, the contribution of development factors, hereditary drivers, and flagging pathways, to more readily grasp its intricacies. The survey summed up significant exploration studies and examinations that have improved how we might interpret cancer science, worked with drug revelation, and recognized expected focuses for treatment.

Hausman DM (2019) led examination concerning the fundamental request of the meaning of

cancer, offering significant points of view on the complicated idea of this illness. The examination analyzed the different components of cancer, perceiving the troubles in deciding its precise beginnings due to factors like cell breakdown, openness to the climate, and hereditary powerlessness. Hausman stressed the significance of directing broad examination to acquire an extensive comprehension of the various sorts, qualities, and reasons for cancer, featuring its heterogeneity. This survey gave a premise to grasping the intricacies of cancer, laying out the fundamental basis for future exploration on its hereditary and sub-atomic establishments.

Campbell et al. (2018) gave assessment of restorative science comparable to the revelation of anti-cancer drugs. The survey covered a great many years, displaying the noteworthy advancement and obstructions experienced by drug organizations. The significance of restorative science in making strong and successful medications was featured. The creators featured the significance of disentangling biochemical standards to coordinate medication disclosure attempts, highlighting the meaning of appreciating atomic instruments of activity. The survey gave important experiences into the advancement of anti-cancer drug improvement in the drug business, making it a significant asset for those keen on this field.

Sigismund, Avanzato and Lanzetti (2018) analyzed the creating jobs of the Epidermal Development Variable Receptor (EGFR) inside the cancer setting. The examination gave understanding into the different capabilities performed by EGFR during the progression of cancer. The review investigated the sub-atomic components that make sense of how EGFR is associated with various cell cycles and flagging pathways. The meaning of EGFR as a potential restorative objective was underlined in the survey, and its suggestions for cancer therapy were examined. This survey offered significant experiences for scientists and clinicians who are keen on creating designated treatments for EGFR-related malignancies by making sense of the different elements of EGFR in cancer.

Karaman, Leppänen and Alitalo (2018) gave examination concerning the motioning of vascular endothelial development factor (VEGF), looking at its contribution in formative cycles as well as its effect on illness. The audit accentuated the urgent job of VEGF during the time spent angiogenesis, highlighting its importance in giving oxygen and supplements to various tissues, including cancer cells. The review offered significant experiences into the chance of focusing on VEGF in cancer therapy by making sense of the elaborate flagging pathways and sub-atomic systems. The survey gave significant data to scientists and clinicians who are keen on grasping the significance of VEGF in cancer and its true capacity as an objective for treatment.

Zhang and Zhang (2017) zeroed in on distinguishing driver quality sets that are divided between various kinds of cancer as well as those that are extraordinary to explicit sorts of cancer. Bioinformatics and information examination procedures were used in the review to recognize the pivotal qualities answerable for driving cancer improvement in different sorts of cancer. The creators have enlightened the hereditary elements that assumed a part in cancer by ordering those qualities into general and unmistakable gatherings. The survey gave significant data to specialists who are keen on understanding the hereditary underpinning of cancer and the potential for making designated treatments utilizing normal and explicit driver qualities.

Table 2.1 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

MATERIAL AND METHODS

3.1 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 3.1 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

3.2 ANALYTICAL TECHNIQUES

Physical data:

There were no adjustments made to any of the melting points since they were all tested in open capillaries.

Thin Layer Chromatography (TLC):

Thin layer chromatography was used to verify the chemicals' purity. The technique included preparing silica Gel G slides (2x7.5cm) to serve as the stationary phase and experimenting with different ratios of ethyl acetate to n-hexane as the mobile phase. Using an iodine chamber and an ultraviolet chamber, the spots that were resolved could be seen.

Instrumentation for Characterization

Mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance were the methods used to characterise the synthesised chemicals.

Infrared spectra:

A fourier-transform infrared spectrometer (model-DRS-8400, Shimadzu) was used to record the infrared spectra of the synthetic compounds in the 400-4000 cm-1 range using KBr pellets. The values of ϕmax are given in cm-1.

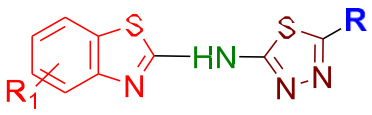
NMR spectra:

Synthesised compounds' 1H-NMR and 13C NMR spectra were captured using a Bruker advance-III NMR-400MHz FT-NMR (TOPSPIN 1.3 Version) spectrophotometer manufactured by Brüker Biospin in Switzerland.

Mass spectra:

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

3.3 DESIGN AND INSILICO SCREENING:



R		R1	
Substitution	Code	Substitution	code
2-hydroxy phenyl (salicylic acid)	sa	6-CH3	1
2-acetoxy phenyl (aspirin)	a	4-CH3	2
1-isobutyl-4-isopropylbenzene (ibuprofen)	ib	6-OCH3	3
2-ethyl-6-methoxynaphthalene (Naproxen)	na	4- OCH3	4
4-isopropylphenyl)(phenyl)methyl)-l-oxidane (fenoprofen)	fp	6-Cl	5
(4-ethylphenyl)(phenyl)methanone (Ketoprofen)	kp	4-Cl	6
		6-NO2	7
		4-NO2	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.

Procedure for molecular docking studies

Step: I Ligand Preparation using Schrodinger's Ligprep:

All of the constructions were created using Schrodinger's 2D sketcher. Following this, LigPrep was consulted for all the structures. We minimised energy use and adjusted the 3D geometry of all the buildings. The ionisation status remained unchanged. Docking and ADME property calculations were then performed directly on the LigPrep output file. The QuikProp module was used to check for ADME attributes.

Step: II Preparing proteins and creating receptor grids:

The 4MXO protein structure (279 amino acids) was extracted from the Protein Data Bank. Protein preparation wizards directly used this structure for adding missing chain residues, forming hydrogen bonds, removing water molecules, and minimising energy. The receptor grid creation output file. A receptor grid was created at the location of the ligand in the protein, which determines the docking site, after the protein had been refined. The grid's dimensions were comparable to those of the ligand workspace, which was set by default at 20Ao. For docking, the glide grid file output was used.

Step: III Docking of ligands using XP mode for enhanced precision:

The ligand docking technique used two files as input: the glide grid file and the LigPrep file. Flexible docking is provided via the extreme precision mode (XP) docking mode. The procedure concluded with the collection of information such as the docking score, H-bond interaction, hydrophobic interaction, etc.

3.4 SYNTHETIC PROCEDURE

Synthetic Scheme:

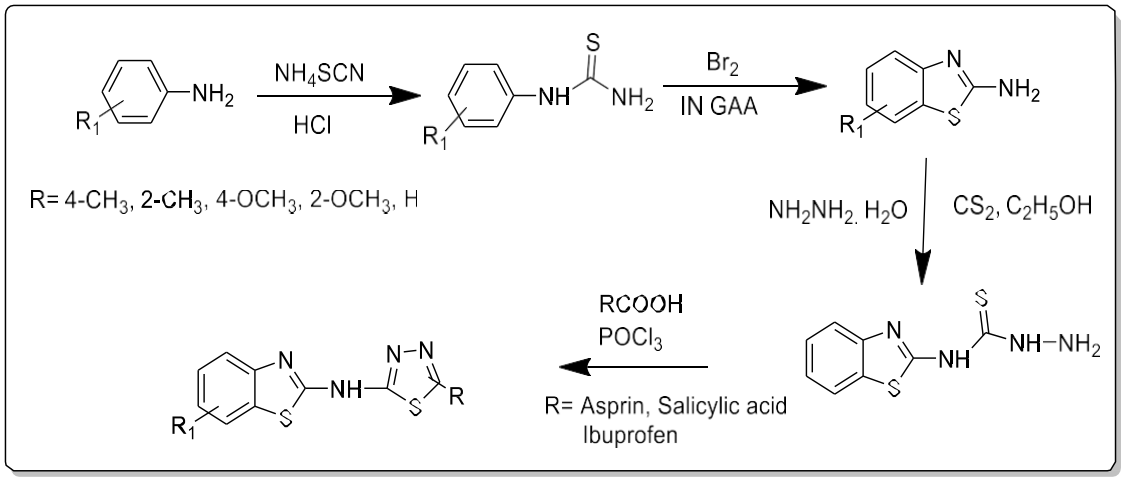
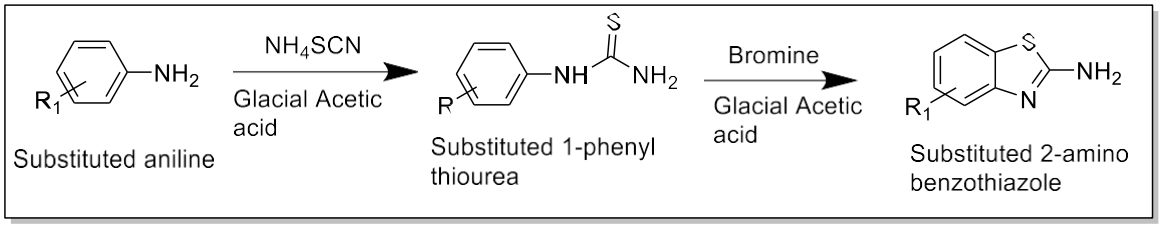


Figure 4 Synthetic scheme

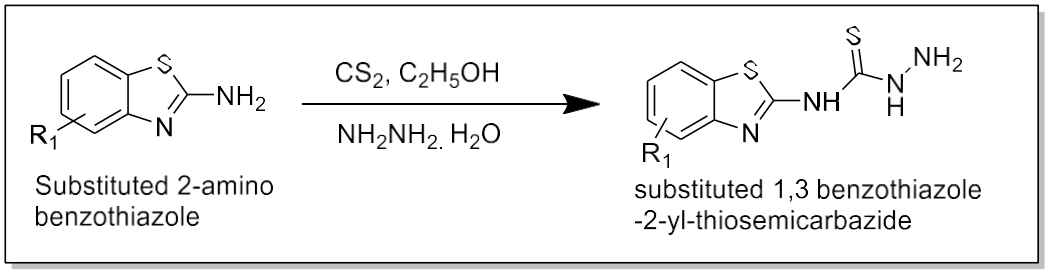
Step-1: Synthesis of substituted 2-amino benzothiazole



A mixture of 0.1 moles of aryl amines and 0.01% ammonium thiocyanate in 10%

glacial acetic acid was chilled and mixed. Add cold bromine in glacial acetic acid drop by drop while stirring; the concentration was 0.01 moles per 10 millilitres. Temperatures about 100°C were maintained throughout the adding process. An further three hours of stirring at 10-150°C was then carried out. Following filtration, the hydrochloride salt that had separated was rinsed with acetic acid. It was then dissolved in hot water and neutralised with a 25% aqueous ammonia solution. Finally, it was filtered out. Lastly, the items underwent a cold water wash, drying, and recrystallization from ethanol.

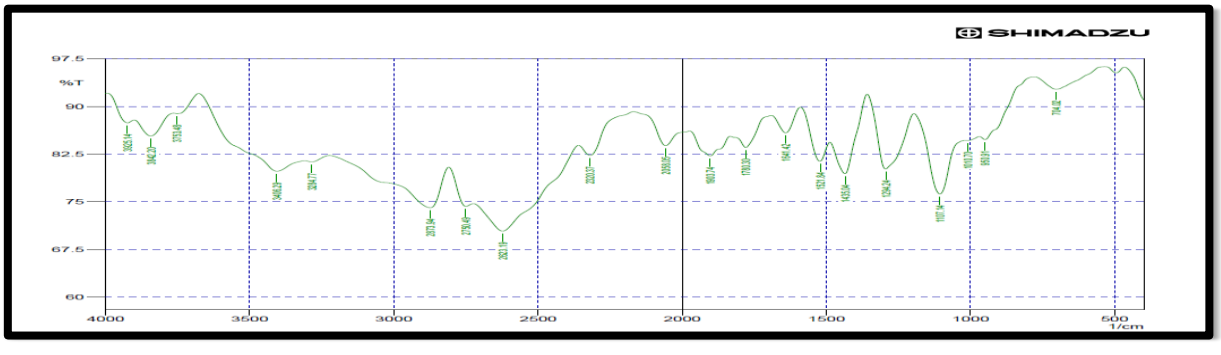
Step-II Synthesis of substituted 1,3 benzothiazole-2-yl thiosemicarbazide (INT)



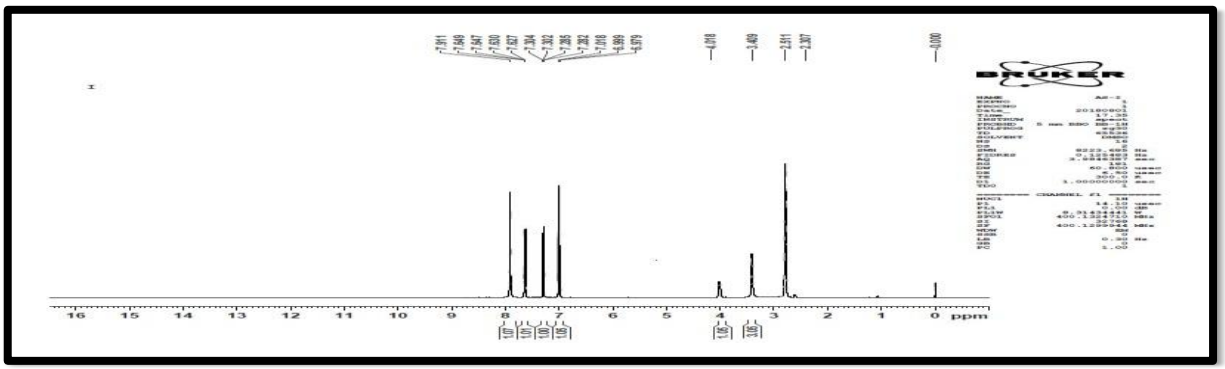
A 20 ml solution of ethanol and ammonia was prepared with 0.10 mol of substituted 2-amino benzothiazole. Then, after 15 minutes of shaking, 20 millilitres of carbon disulfide was incorporated, and the mixture was let to stand for 1 hour. Next, 0.1 mol of sodium chloroacetate and 20 ml of 50% hydrazine hydrate were added. A gentle warming, filtering, and subsequent evaporation of the reaction mixture to half its volume was followed by an overnight period of storage. The resulting solid was then filtered and recrystallized from ethanol.

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

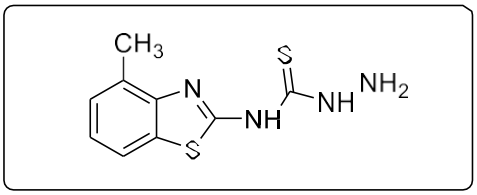
	$C_8H_8N_4S2$
Molecular Formula	
Molecular Weight (g/mol)	224.30
Melting Point (ºC)	182-184ºC
Yield (%w/w)	62
Recrystallisation solvent	Ethanol
TLC	Rf = 0.73 Benzene:ethanol (1:1)
IR (ν, cm^{-1})	3406 (NH), 1537 (C=N), 1641 (C=C),1294 (C-N), 1107 (C=S)
^{1}H NMR (DMSO)	6.9-7.9 (m, 4-ArH), 4.0 (s, 1H,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



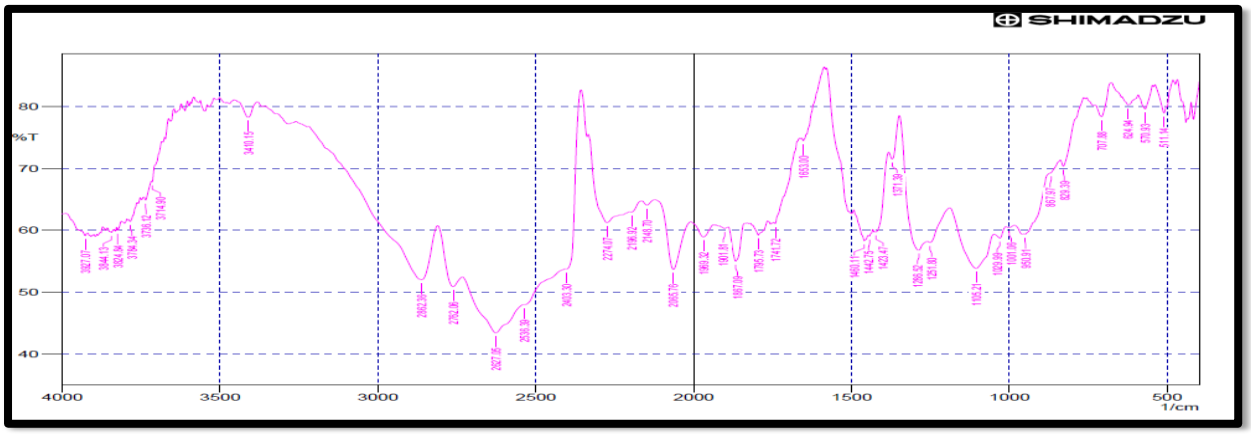
Spectra 1: IR spectra of 1INT1



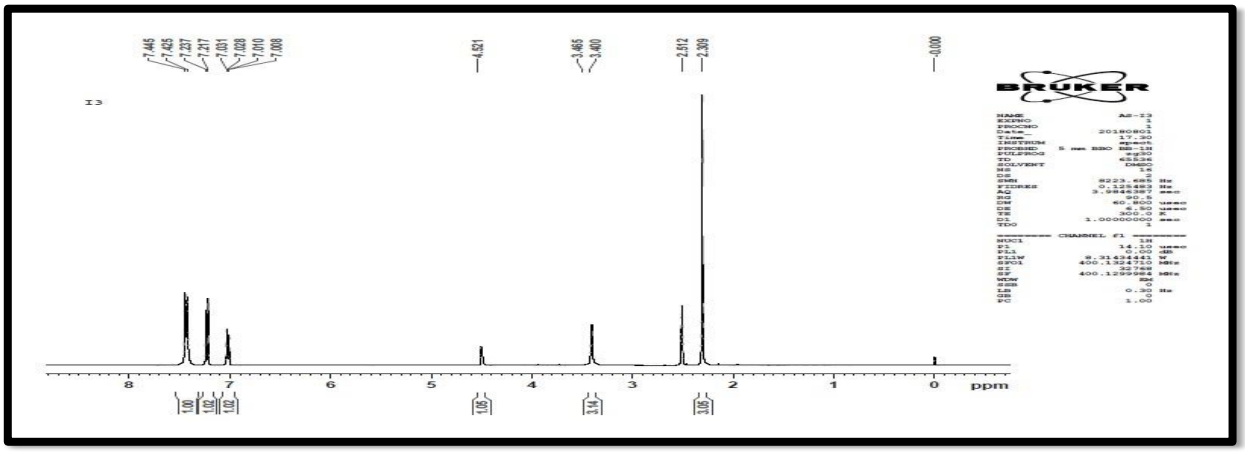
Spectra 2: ^{1}H NMR spectra of INT1 N-(4-methylbenzo[d]thiazol-2-yl)hydrazinecarbothioamide (INT2)



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)

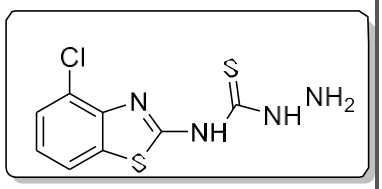


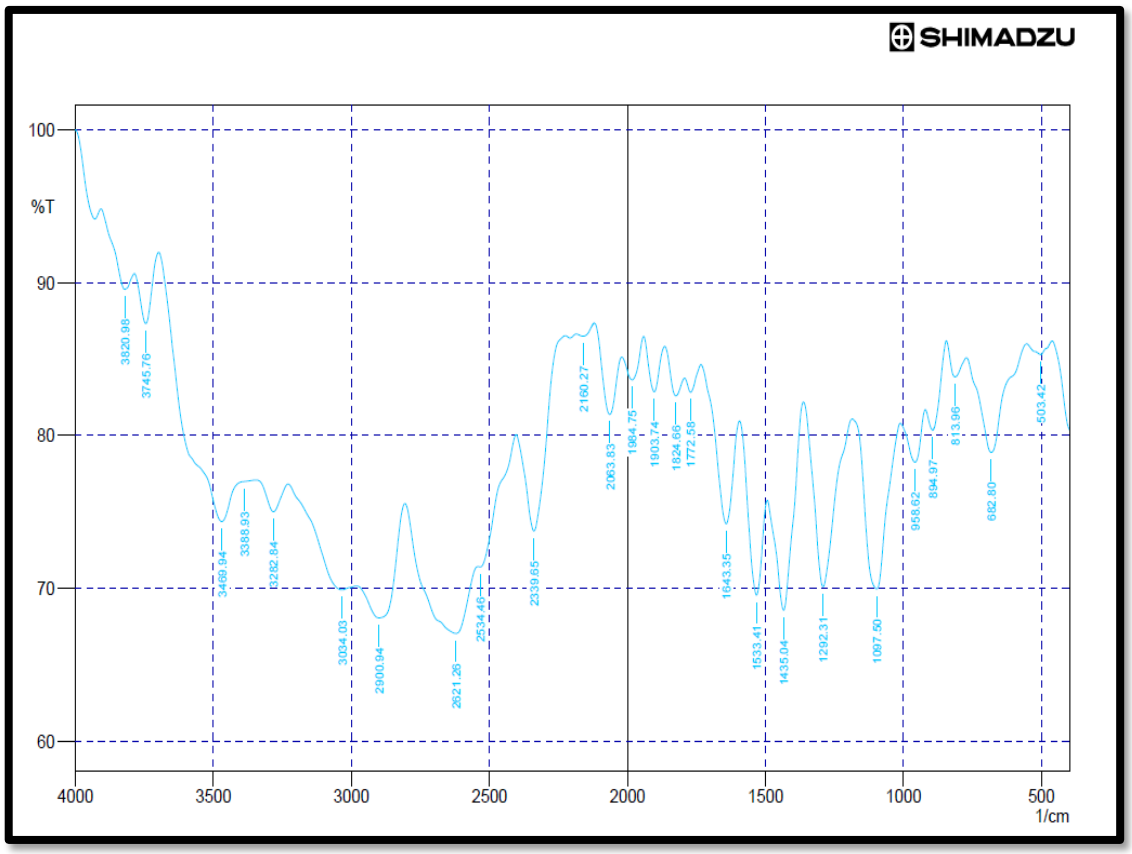
Spectra 3: IR Spectra of INT2



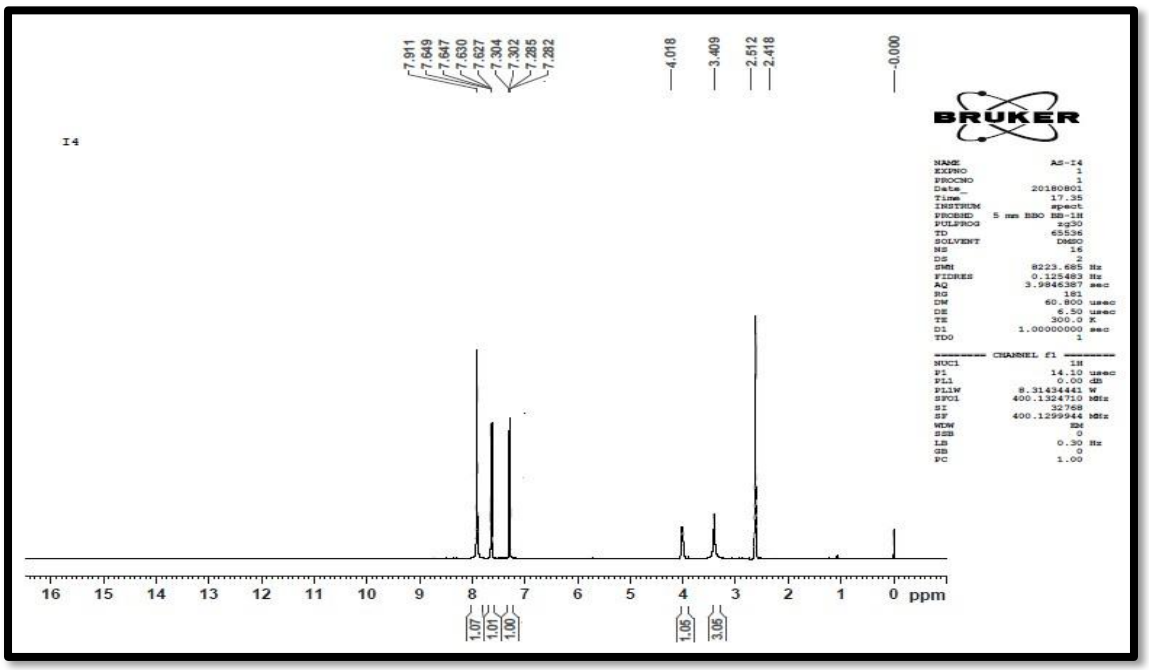
Spectra 4: ^{1}H NMR spectra of INT2

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)

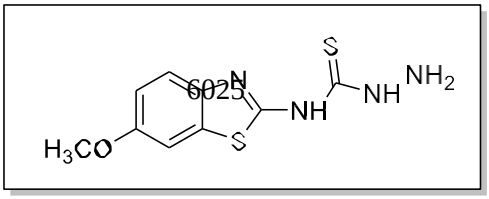


Spectra 5: IR Spectra of INT3

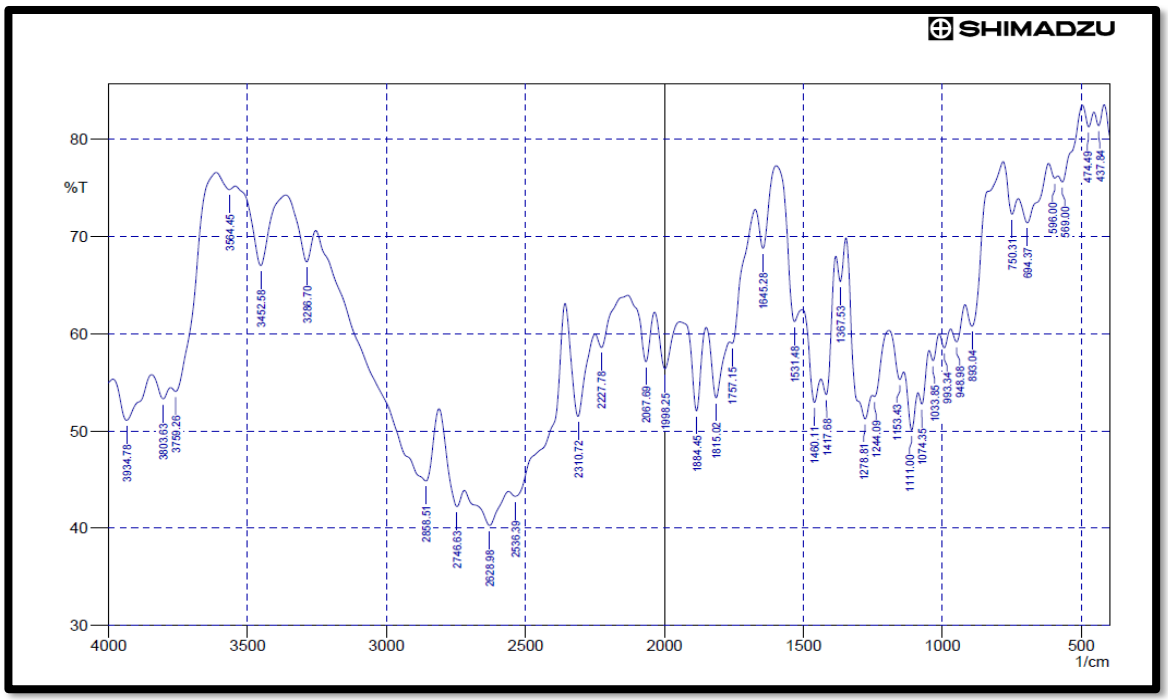


Spectra 6: ^{1}H NMR spectra of INT3

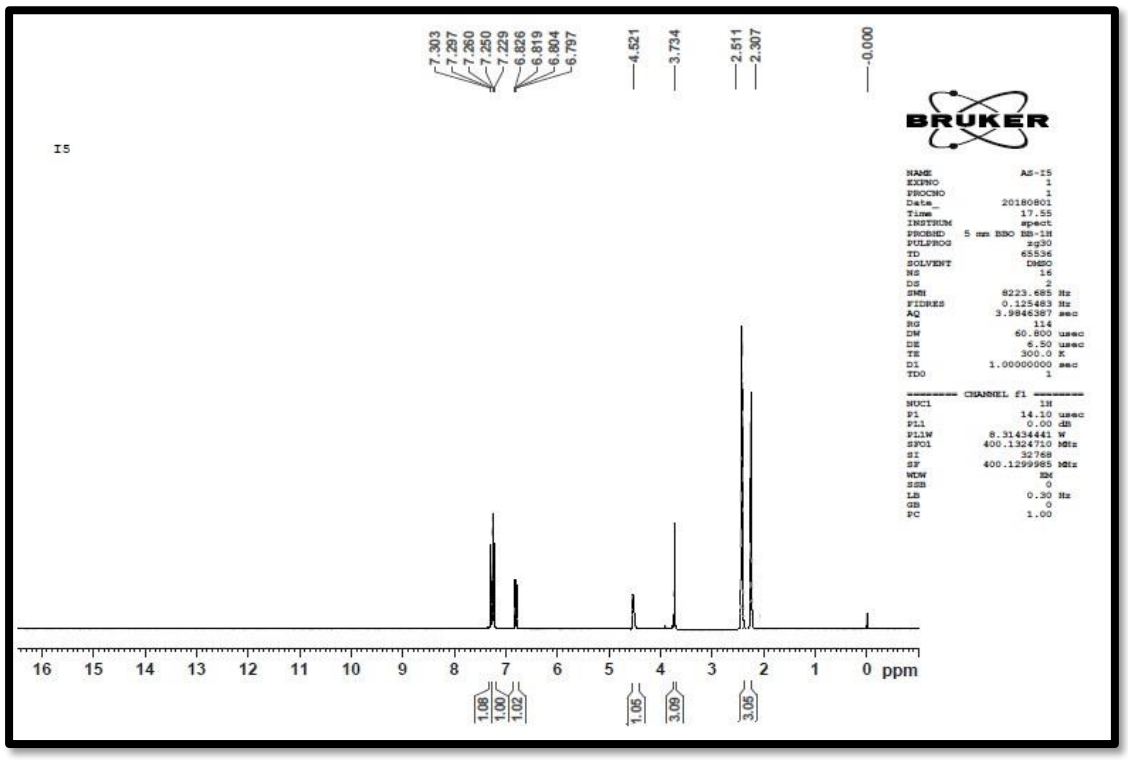
N-(6-methoxybenzo[d]thiazol-2-yl)hydrazinecarbothioamide (INT4)



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),
^1H 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)

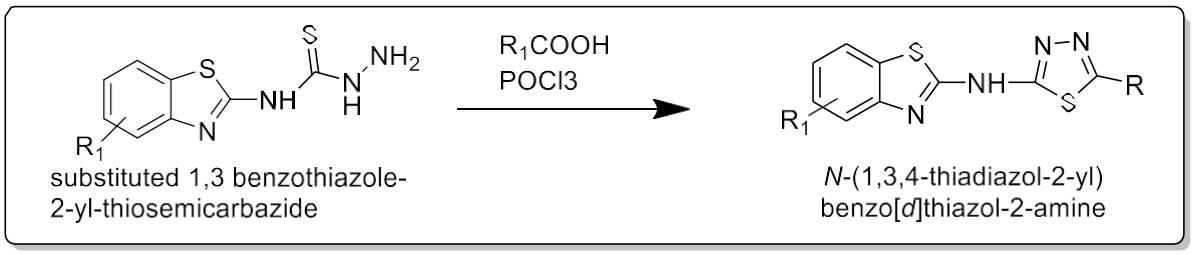


Spectra 7: IR Spectra of INT4



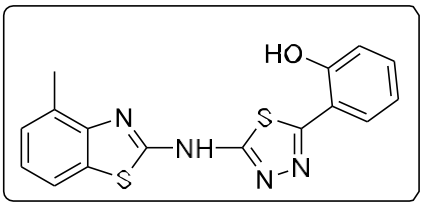
Spectra 8: ^{1}H NMR Spectra of INT4

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

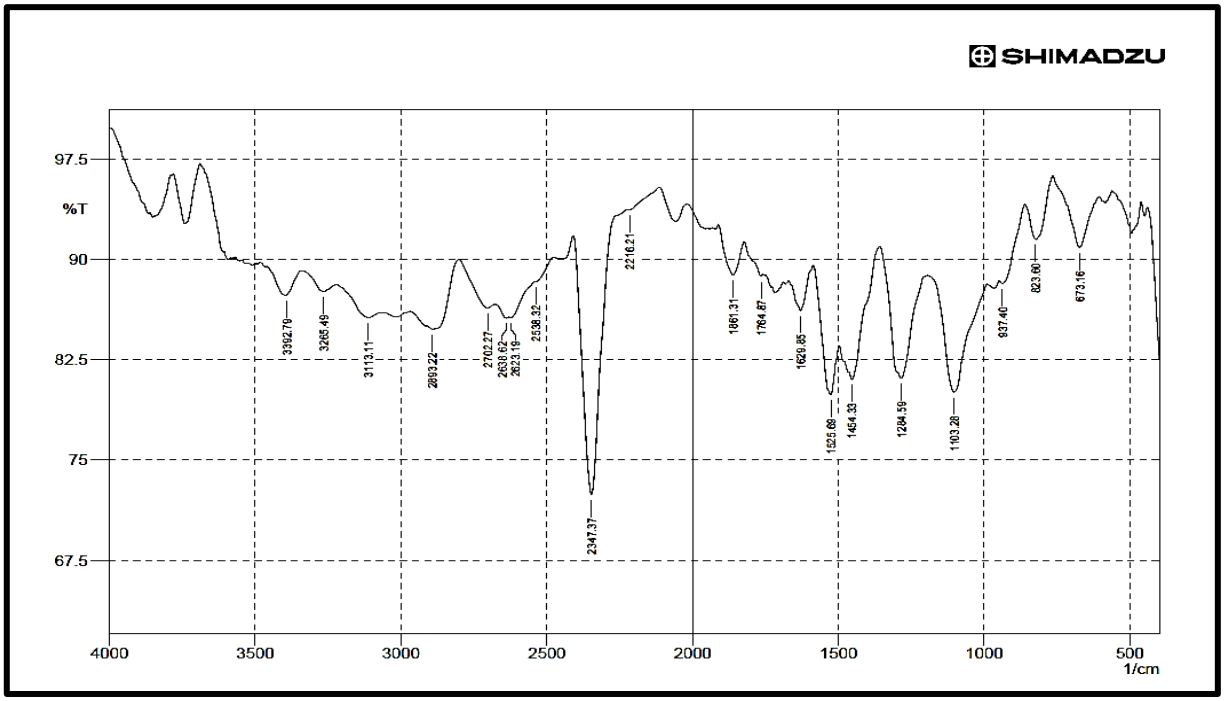


Five millilitres of phosphorous oxychloride, one millilitre of aromatic acid, and three benzothiazole-2-yl-thiosemicarbazides were refluxed for eighteen to twenty-two hours. The liquid was poured carefully into crushed ice after cooling to room temperature and left overnight. After being extracted from the ethanol, the solid was filtered, rinsed with water, dried, and then recrystallized.

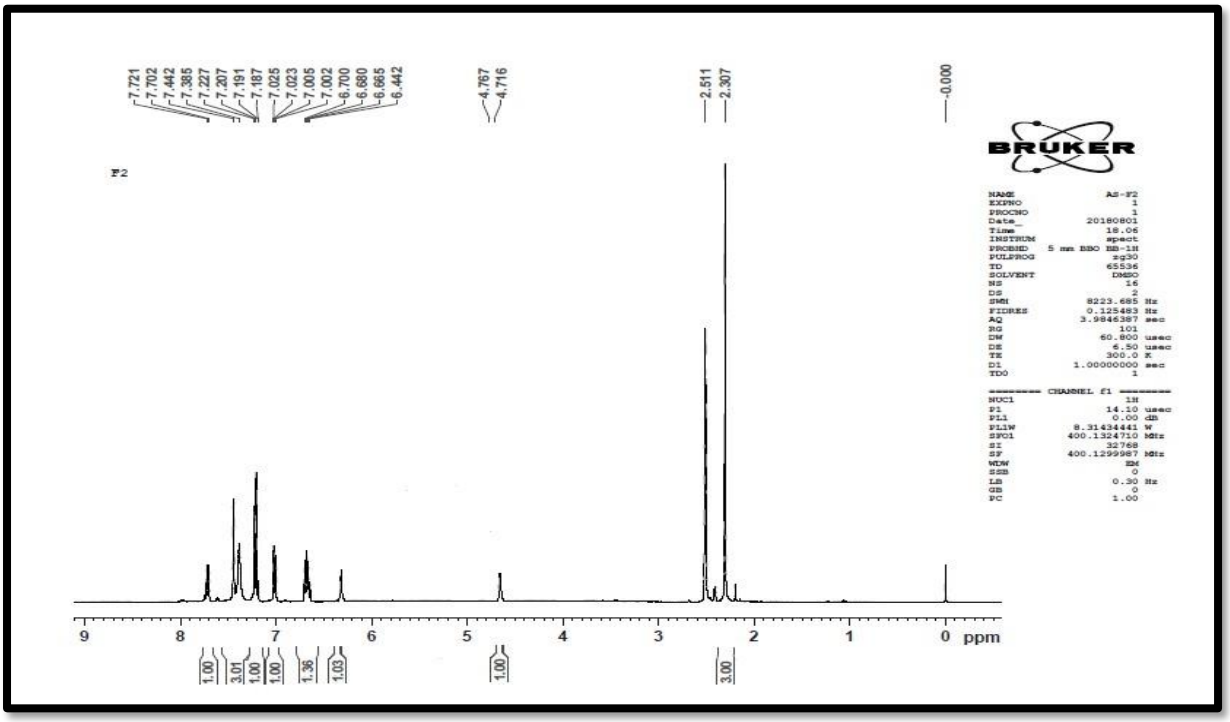
2-(5-((6-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (1SA)



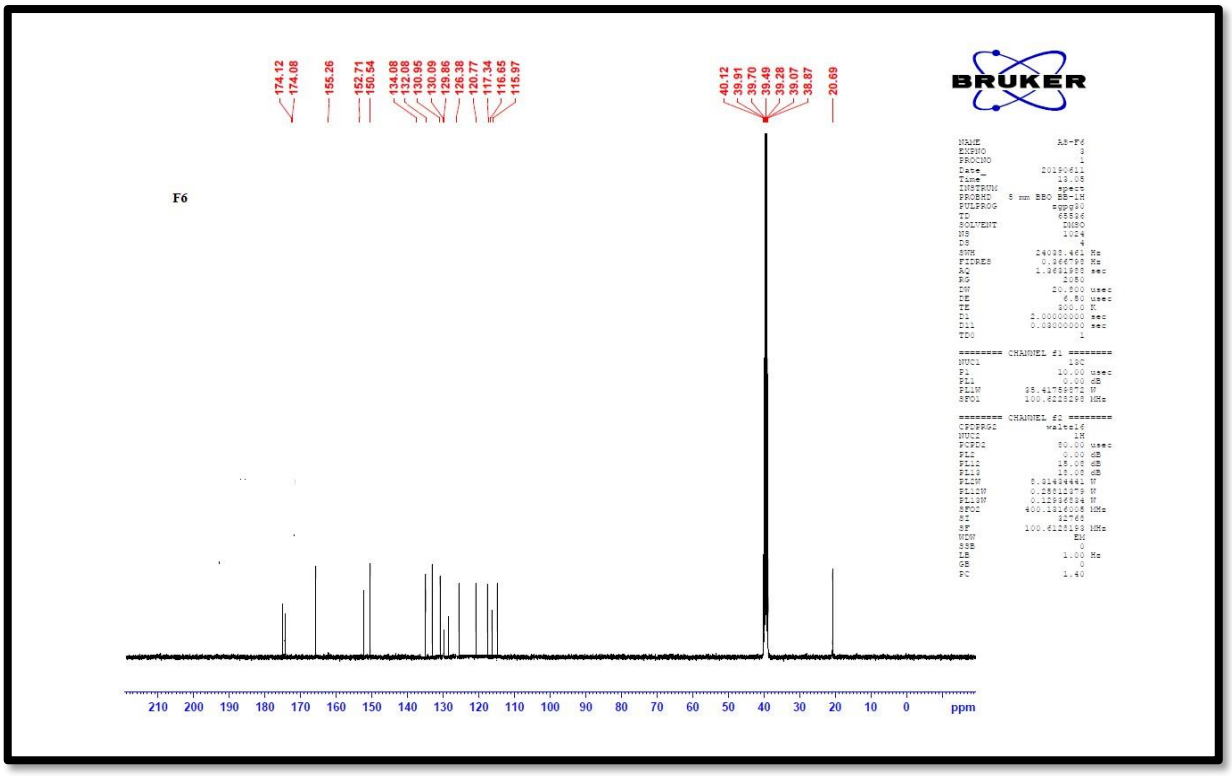
Molecular Formula	$C_{16}H_{12}N_4OS_2$
Molecular Weight (g/mol)	340.42
Melting Point	240-242ºC
% Yield	33
Recrystallisation solvent	Ethanol
TLC	Rf = 0.48 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3392 (NH), 1629 (C-C), 1525 (C=N), 1284 (C-N), 1103(C-S)
^{1}H NMR (DMSO)	6.7-7.7 (m. Ar-7H), 6.4 (S, 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



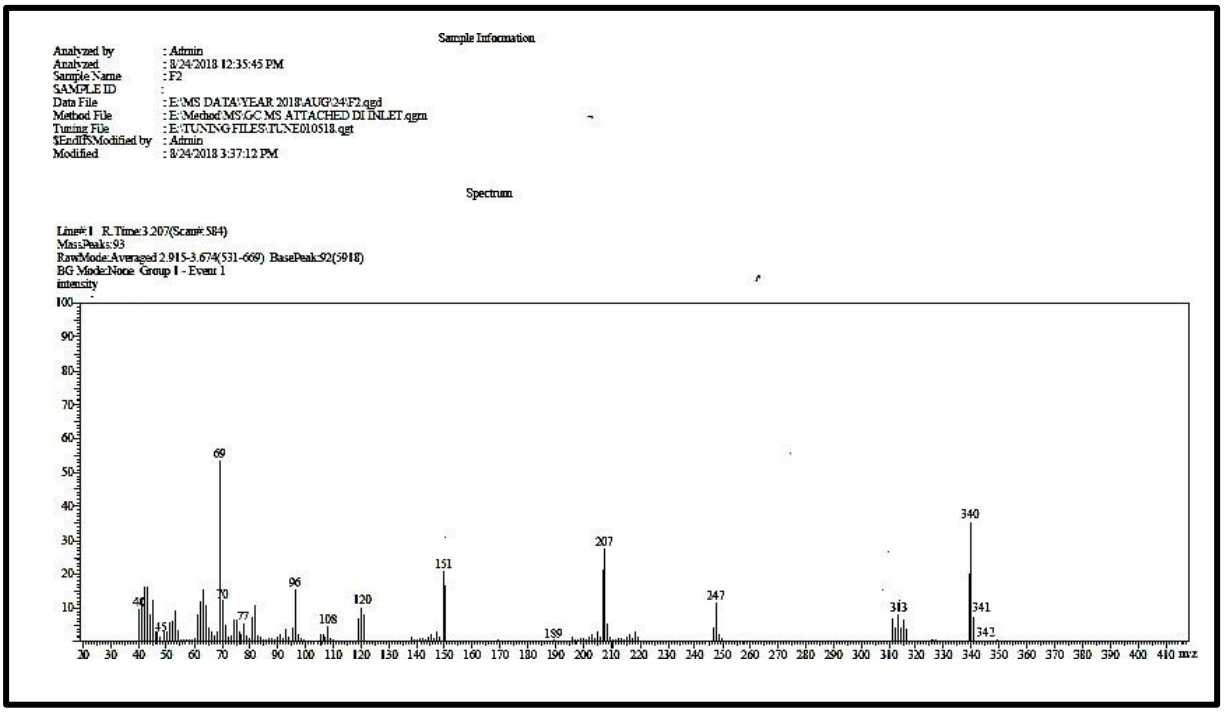
Spectra 9: IR Spectra of Compound 1sa



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

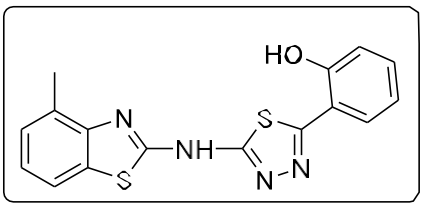


Spectra 11: ^{13}C NMR spectra of Compound 1sa

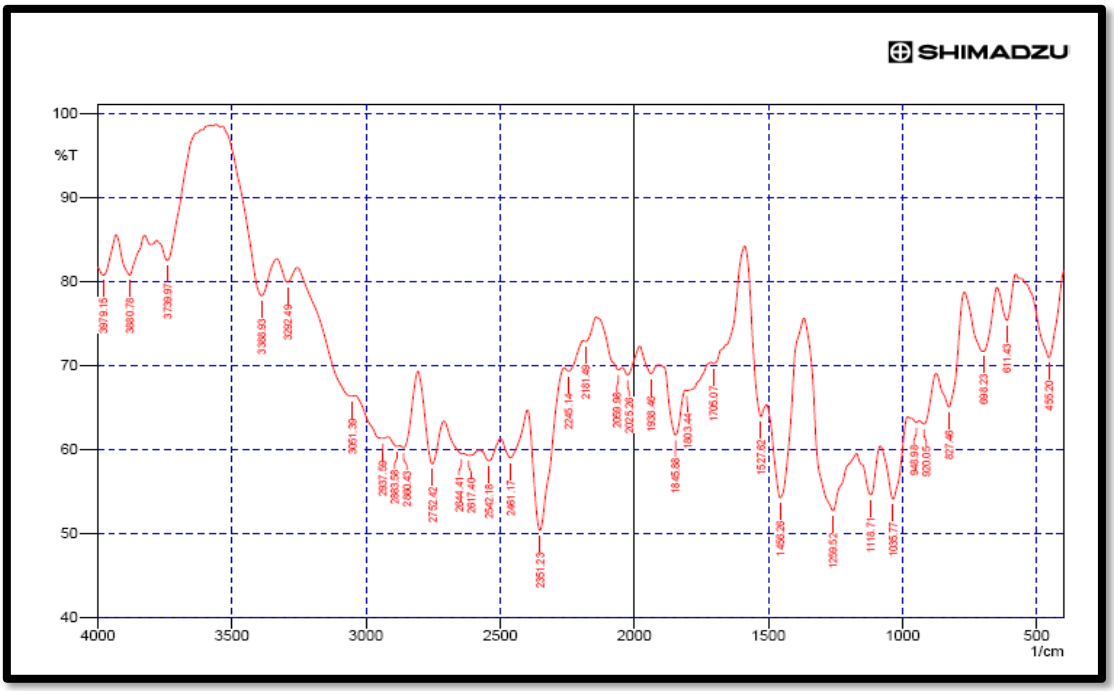


Spectra 12: MASS spectra of Compound 1sa

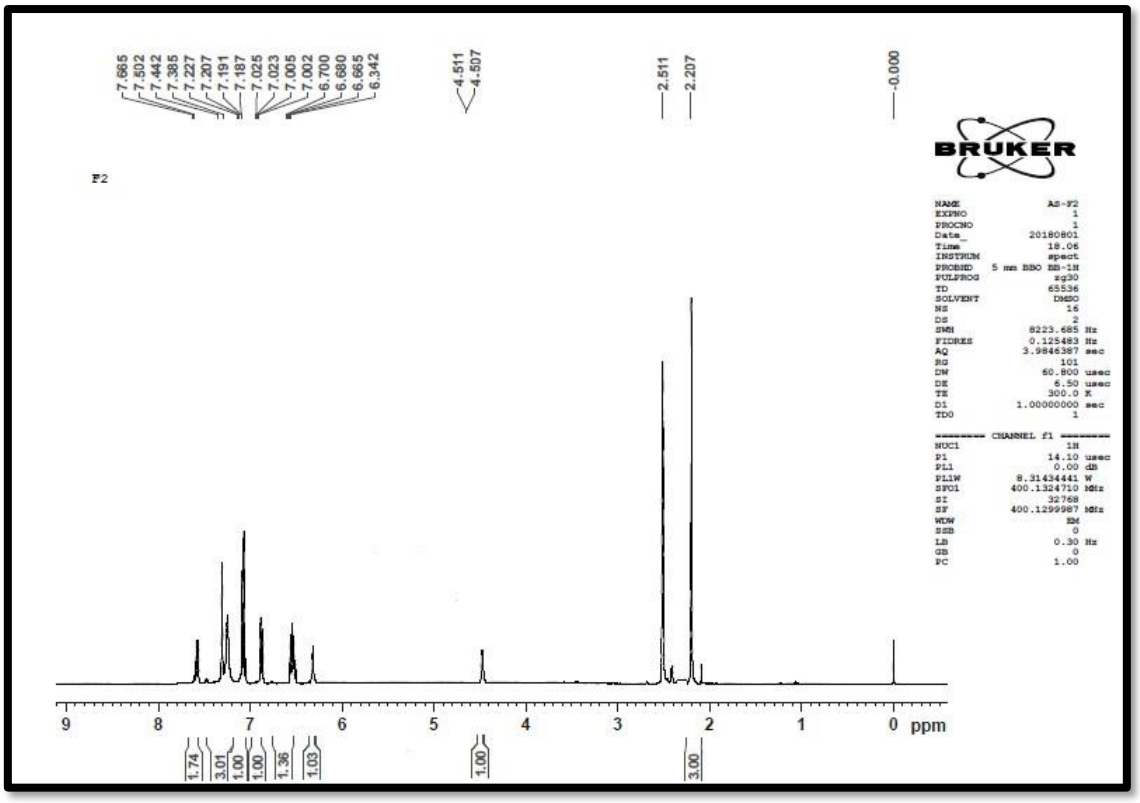
2-(5-((4-methylbenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (2sa)



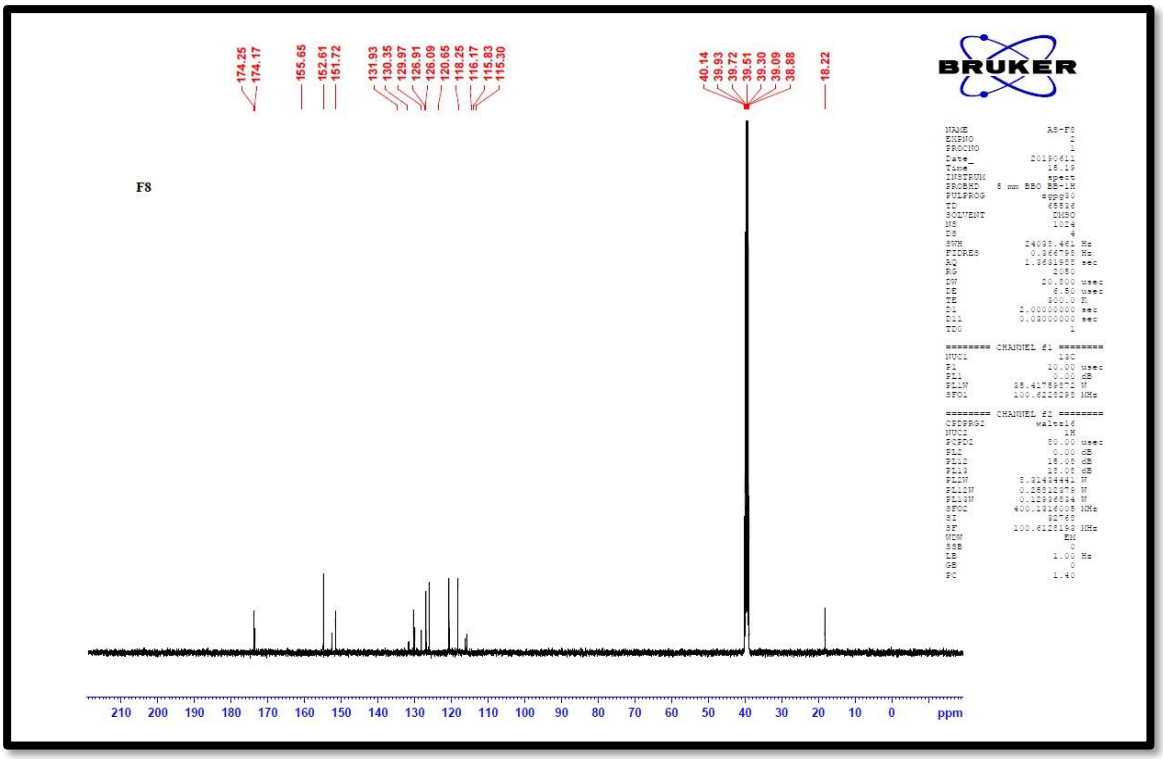
Molecular Formula	$C_{16}H_{12}N_4OS_2$
Molecular Weight (g/mol)	340.42
Melting Point	236-238ºC
% Yield	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.58 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3388 (NH), 2752 (Ar-CH3), 1525 (C=N), 1259 (C-N), 1118(C-S)
^{1}H NMR (DMSO)	6.6-7.7 (m. Ar-7H), 6.3 (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

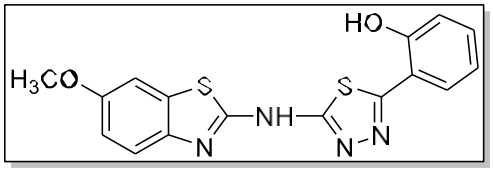


Spectra 14: ^{1}H NMR spectra of Compound 2sa

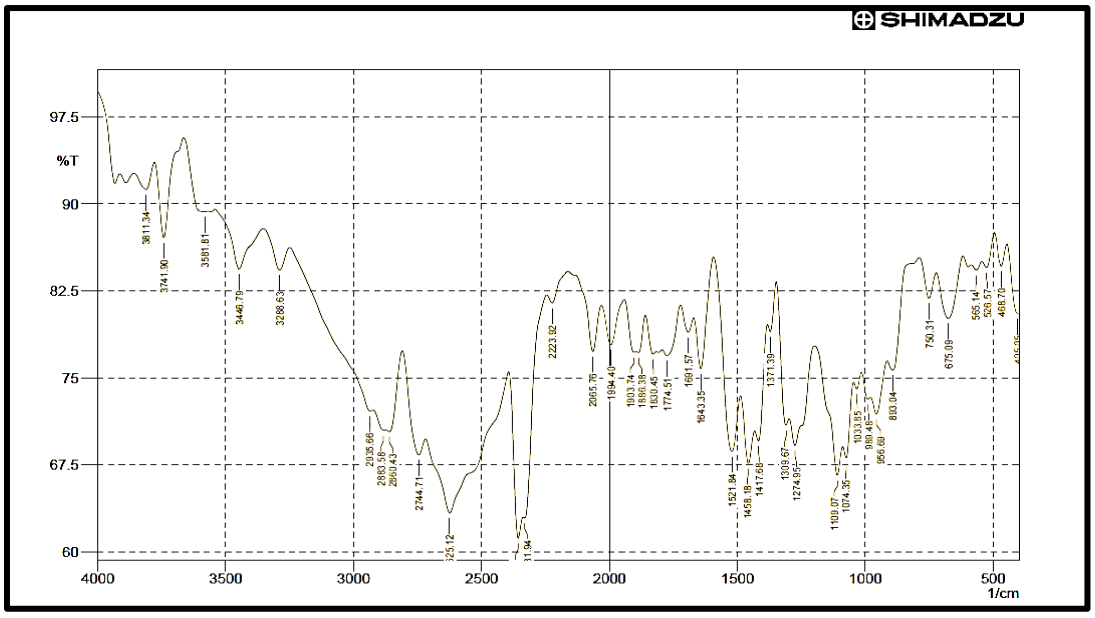


Spectra 15: MASS spectra of Compound 2sa

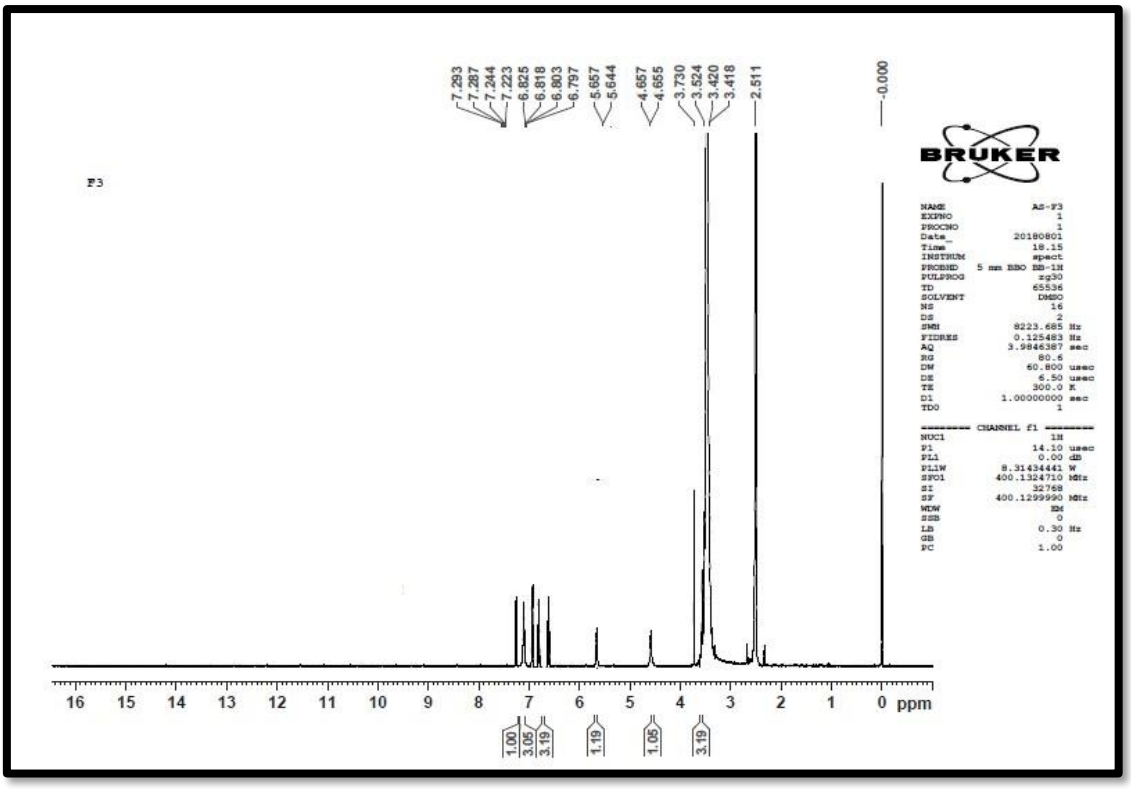
2-(5-((6-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (3SA)



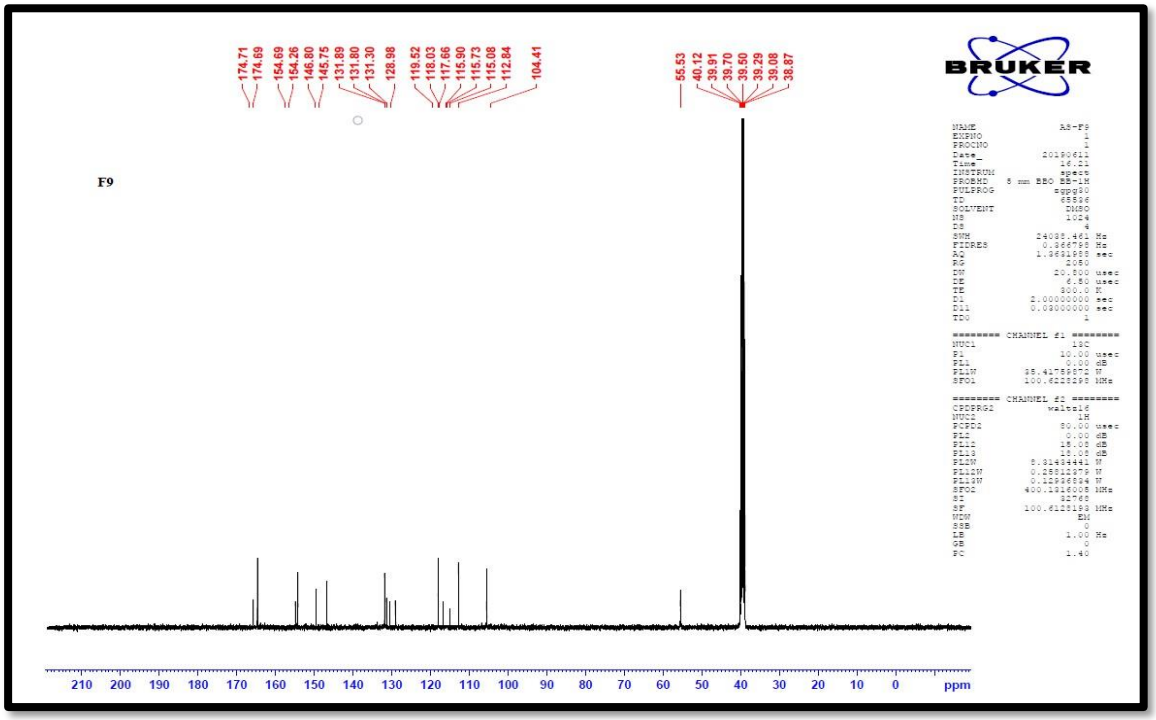
Molecular Formula	$C_{16}H_{12}N_4O_2S_2$
Molecular Weight (g/mol)	356.42
Melting Point	254-256ºC
% Yield	56
Recrystallisation solvent	Ethanol
TLC	Rf = 0.63 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3811 (Ar-OH), 3446 (NH), 1643 (C-C), 1521 (C=N), 1274 (C-N) 1109(C-S)
^{1}H NMR (DMSO)	6.7-7.29 (m. 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



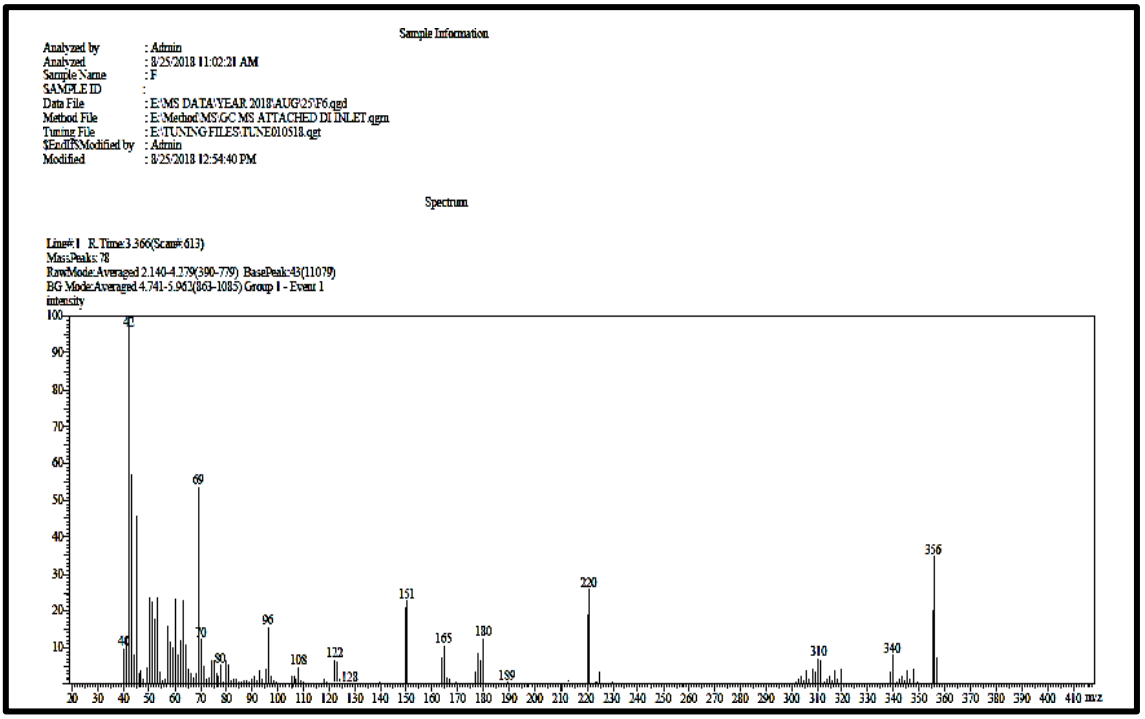
Spectra 16: IR spectra of compound 3sa



Spectra 17: 1H NMR spectra of Compound 3sa

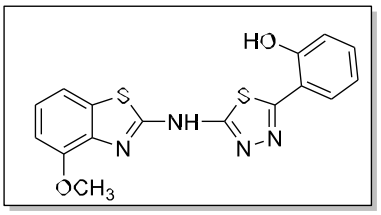


Spectra 18: ^{13}C NMR spectra of Compound 3sa

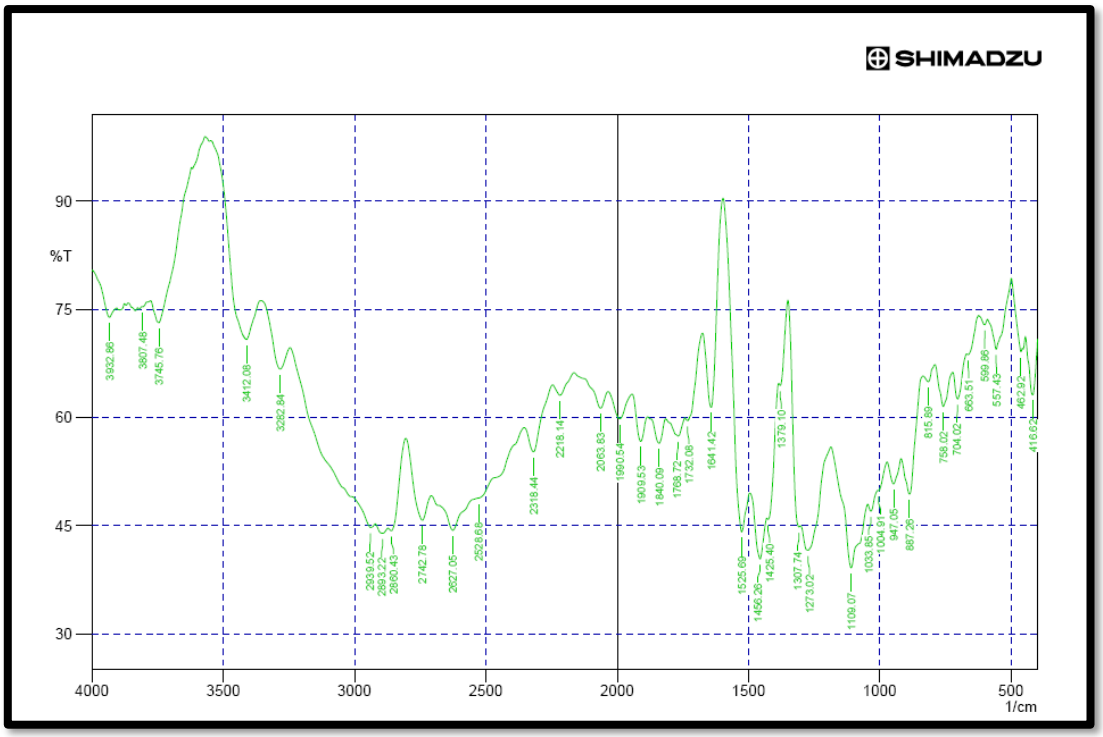


Spectra 19: Mass Spectra of compound 3sa

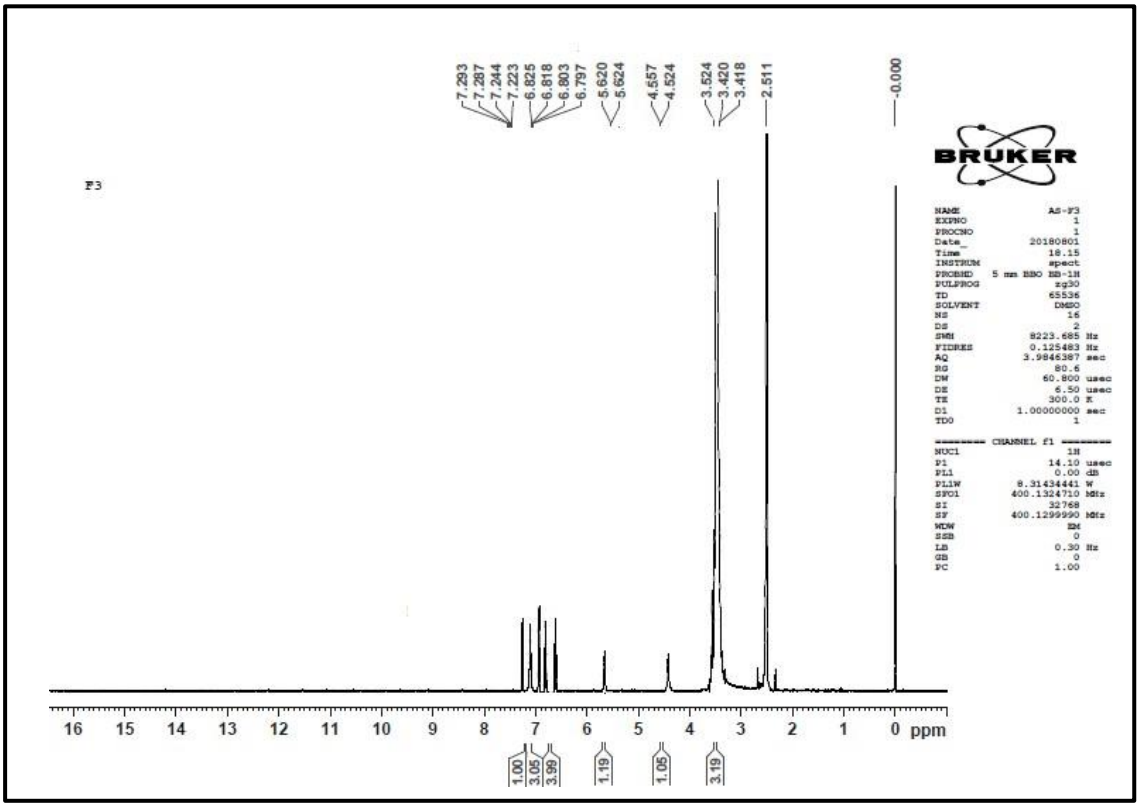
2-(5-((4-methoxybenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (4SA)



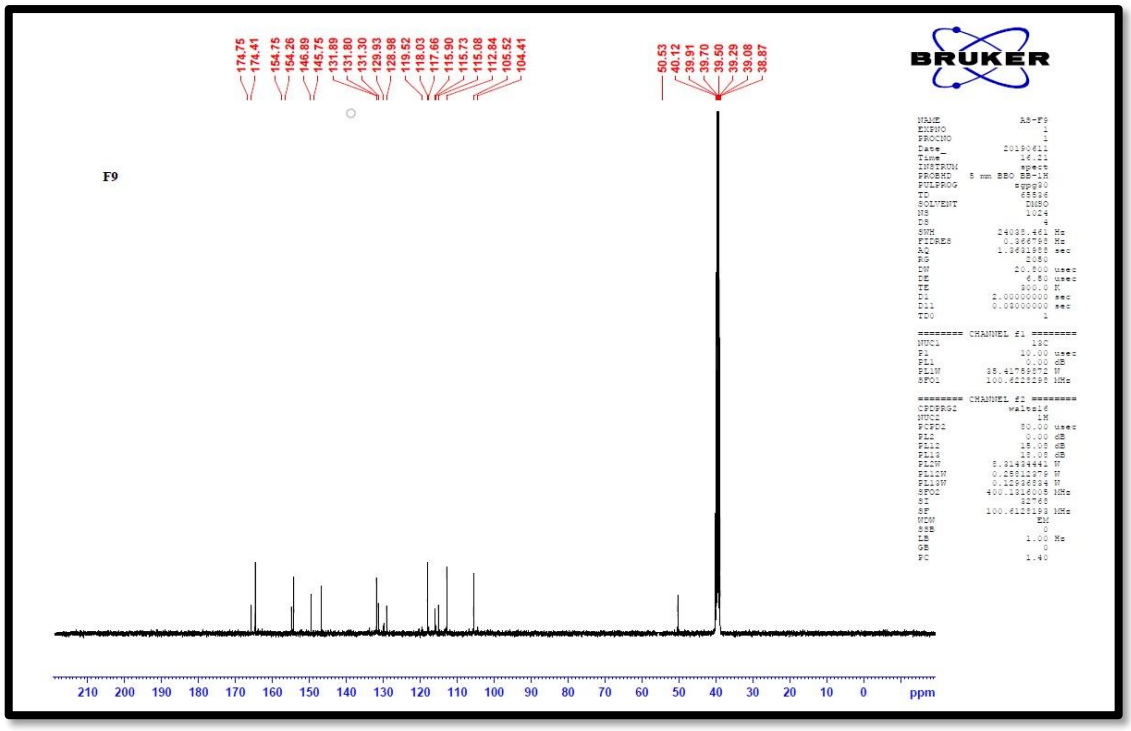
Molecular Formula	$C_{16}H_{12}N_4O_2S_2$
Molecular Weight (g/mol)	356.42
Melting Point	246-248ºC
% Yield	48
Recrystallisation solvent	Ethanol
TLC	Rf = 0.67 n-hexane: ethyl acetate (8:2)
IR (ν, cm^{-1})	3807 (Ar-OH), 3412 (NH), 1641 (C-C), 1525 (C=N), 1273 (C-N) 1109(C-S)
^{1}H NMR (DMSO)	6.7-7.29 (m. 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

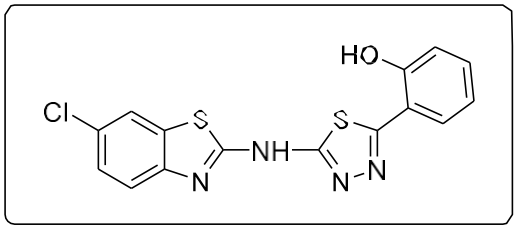


Spectra 21:^{1}H NMR spectra of Compound 4sa

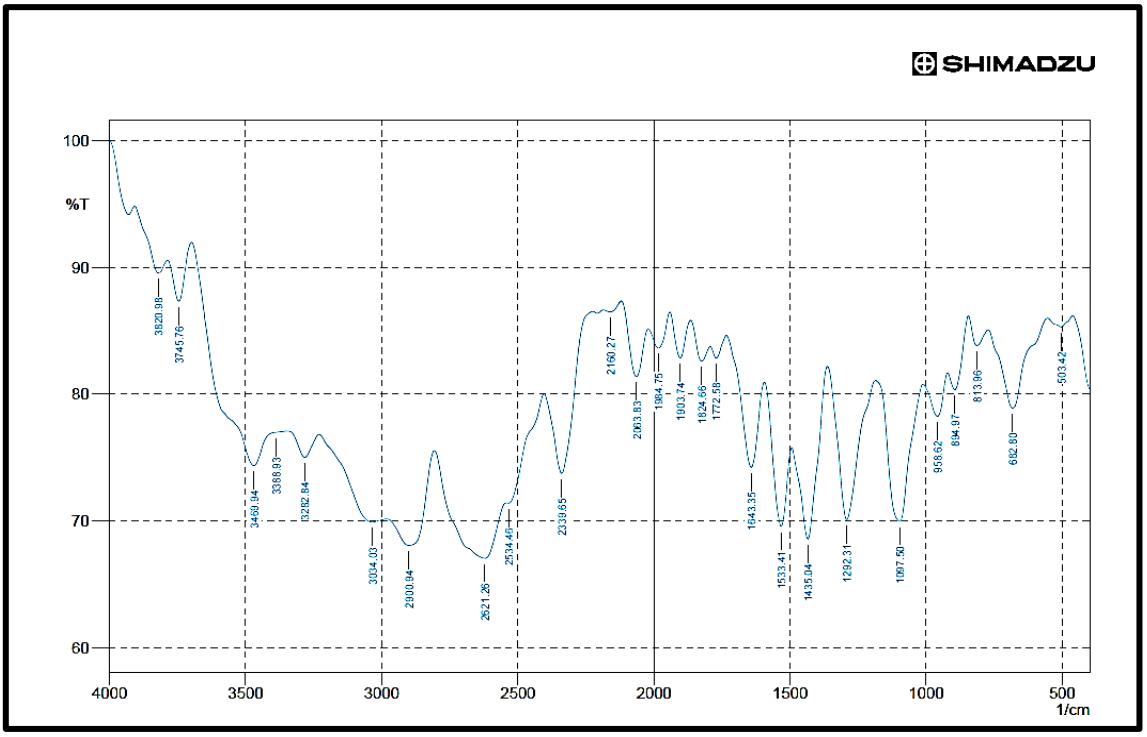


Spectra 22 13C NMR spectra of Compound 4sa

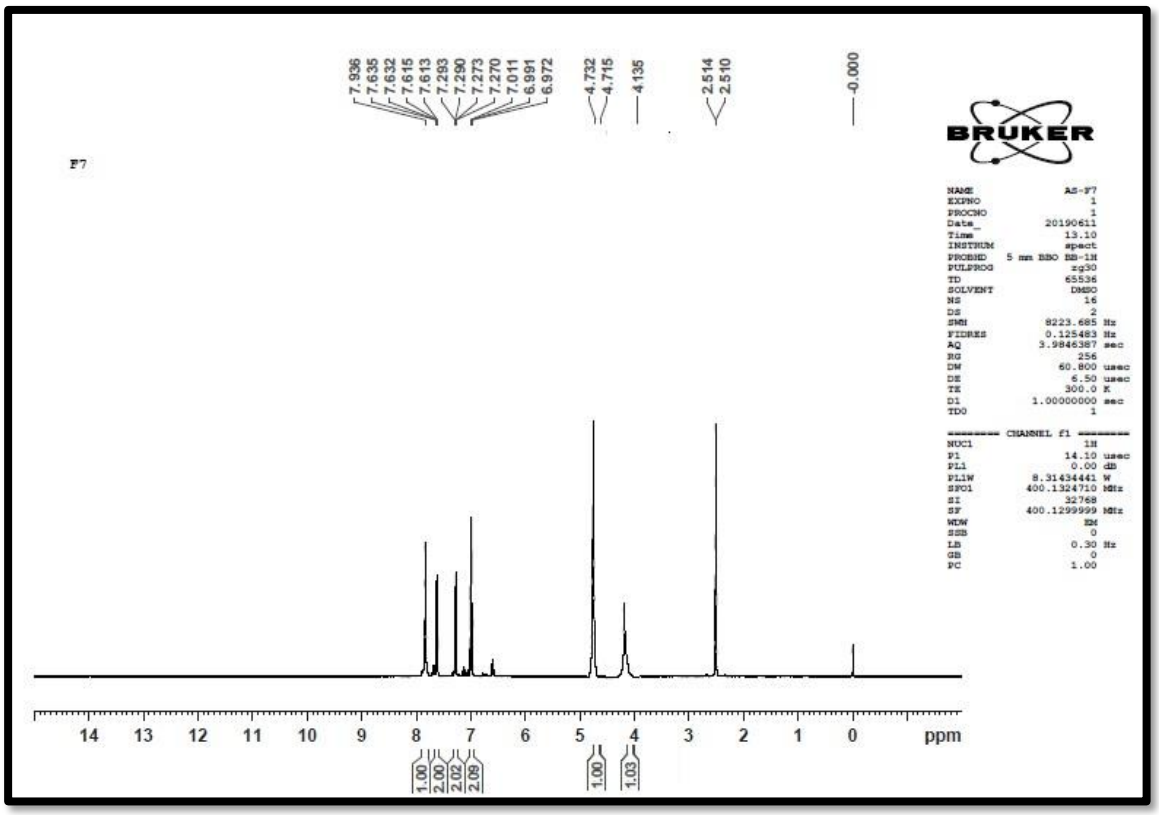
2-(5-((4-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (5SA)



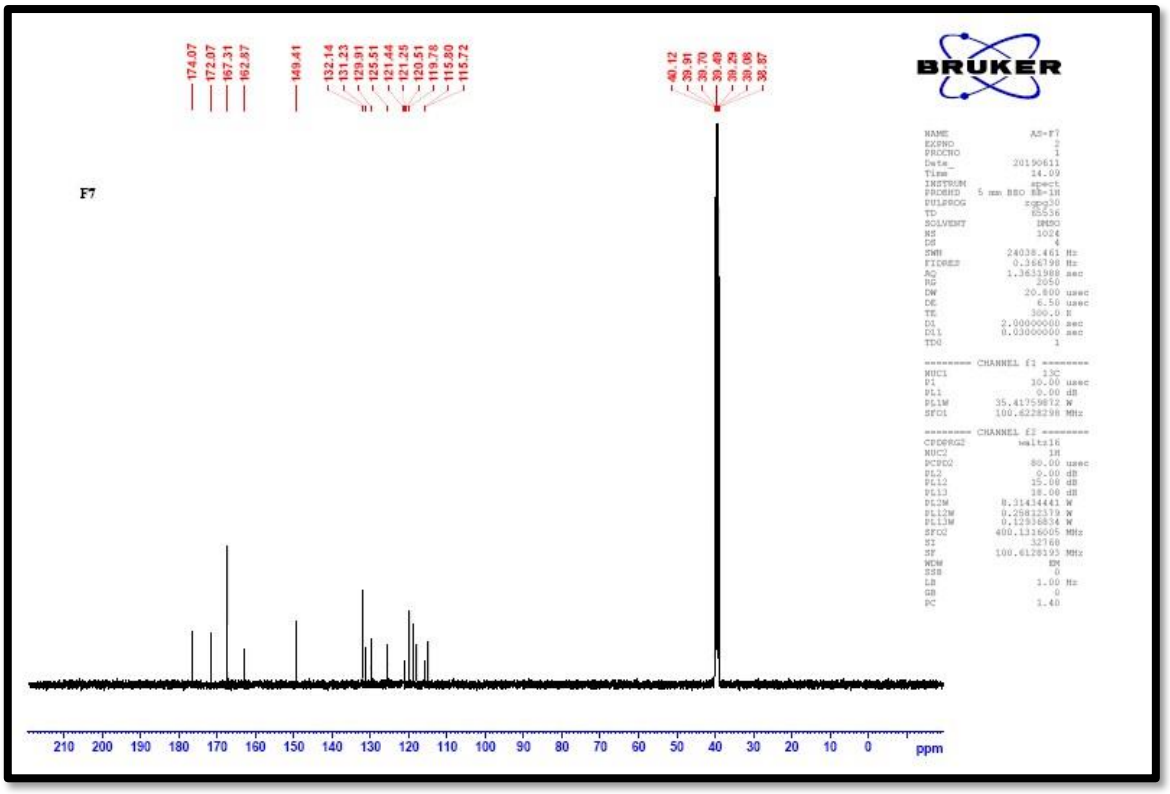
Molecular Formula	$C_{15}H_9ClN_4OS_2$
Molecular Weight (g/mol)	360.83
Melting Point	244-246ºC
% Yield	40
Recrystallisation solvent	Ethanol
TLC	Rf = 0.60 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3820(Ar-OH), 3388(NH), 1435(C=N), 1292(C-N) 1097(C-S)
^{1}H NMR (DMSO)	6.97-7.93 (m, Ar-7H), 4.76 ($_S$, 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



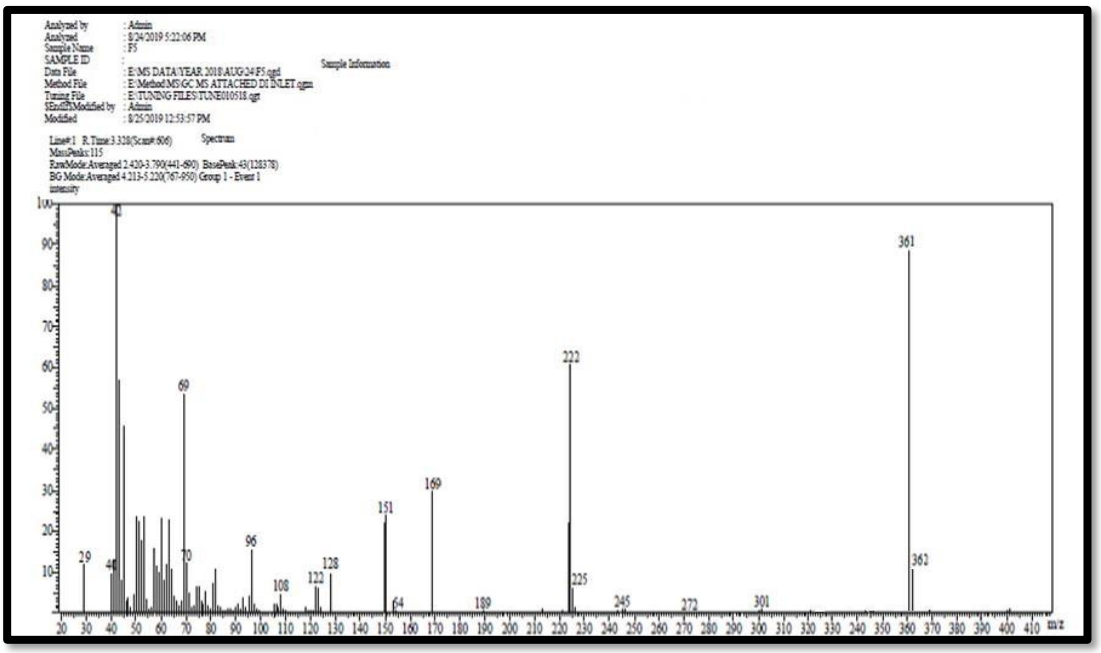
Spectra 23: IR spectra of compound 5SA



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

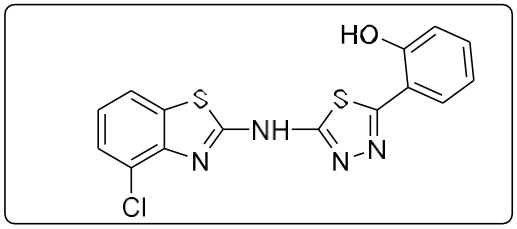


Spectra 25: ^{13}C NMR spectra of Compound 5sa

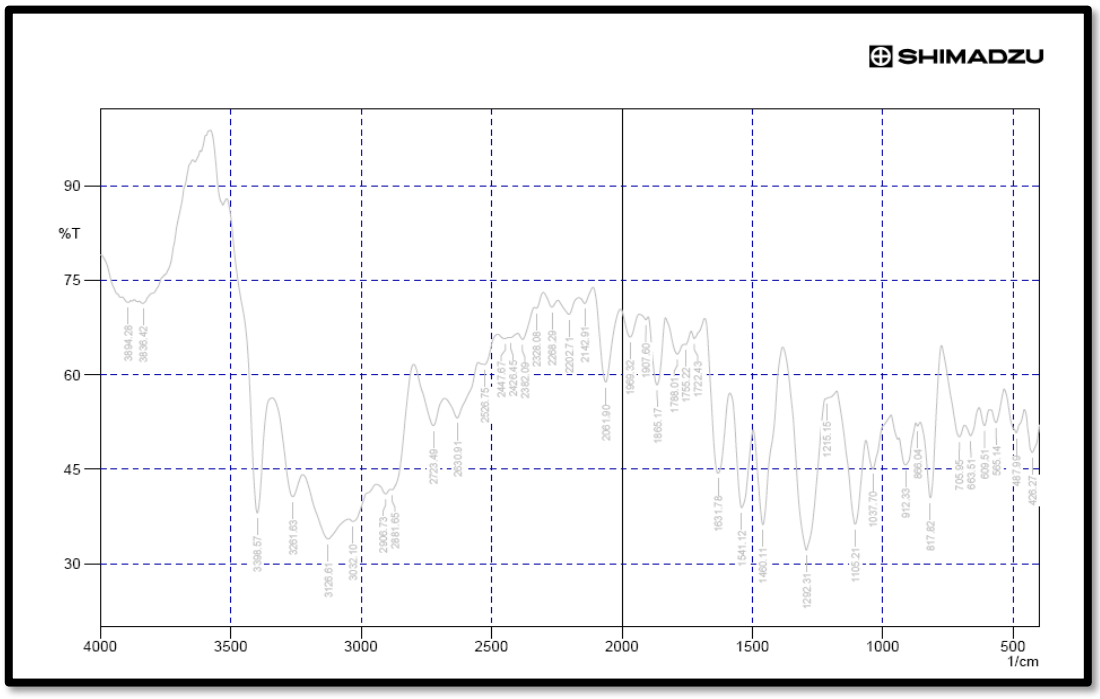


Spectra 26: MASS spectra of compound 5sa

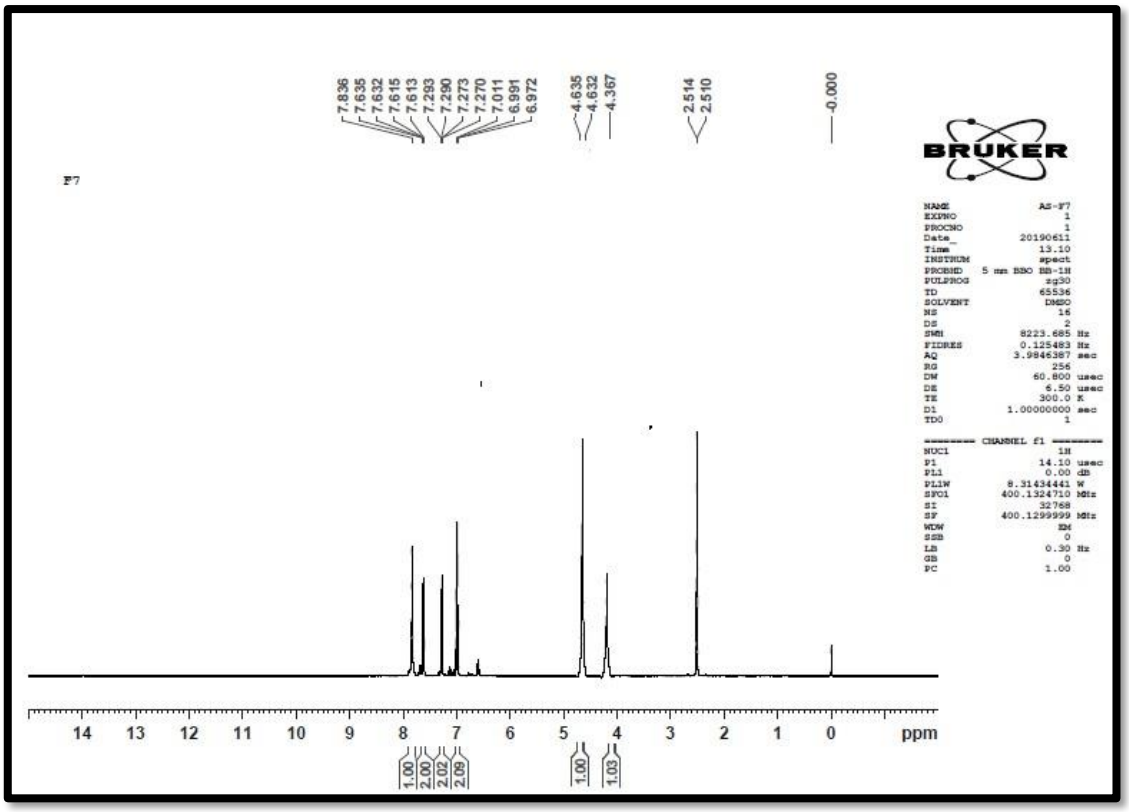
2-(5-((4-chlorobenzo[d]thiazol-2-yl)amino)-1,3,4-thiadiazol-2-yl)phenol (6sa)



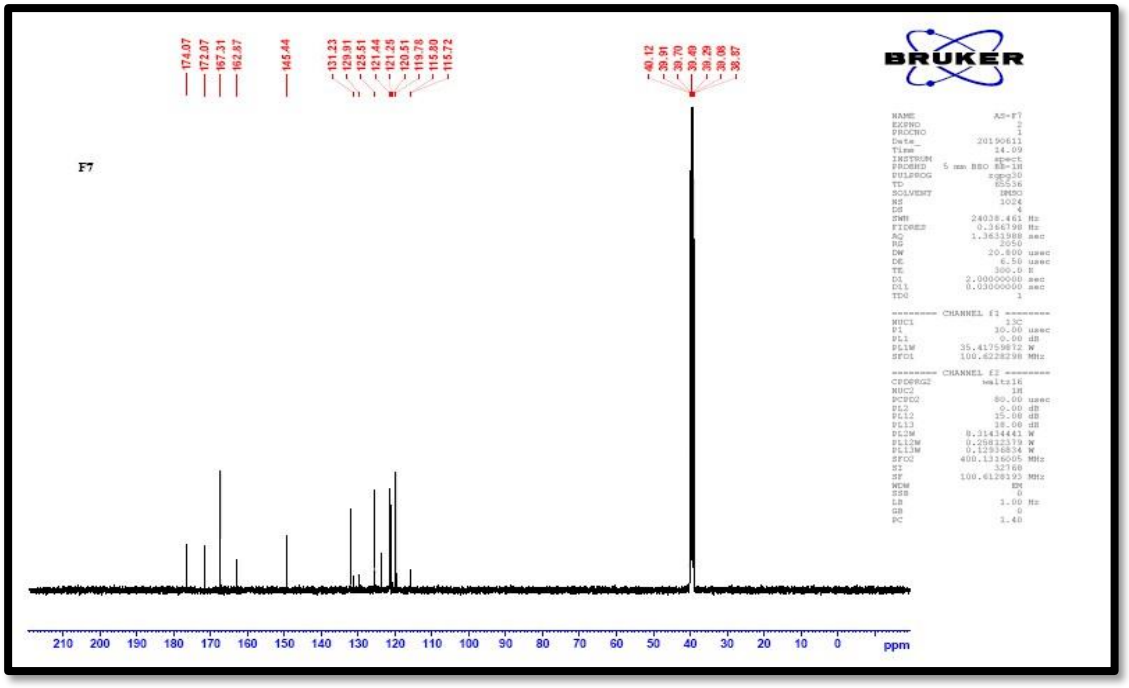
Molecular Formula	$C_{15}H_9ClN_4OS_2$
Molecular Weight (g/mol)	360.83
Melting Point	260-262ºC
% Yield	40
Recrystallisation solvent	Ethanol
TLC	Rf = 0.54 n-hexane: ethyl acetate (7:3)
IR (ν, cm^{-1})	3894(Ar-OH), 3396(NH), 1460(C=N), 1292(C-N) 1037(C-S)
^{1}H NMR (DMSO)	6.97-7.83 (m, Ar-7H), 4.76 ($_S$, 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

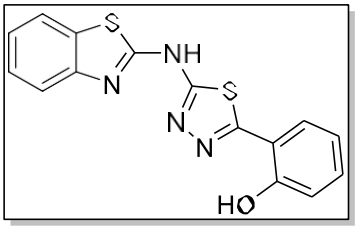


Spectra 28: ^{1}H NMR spectra of Compound 6sa

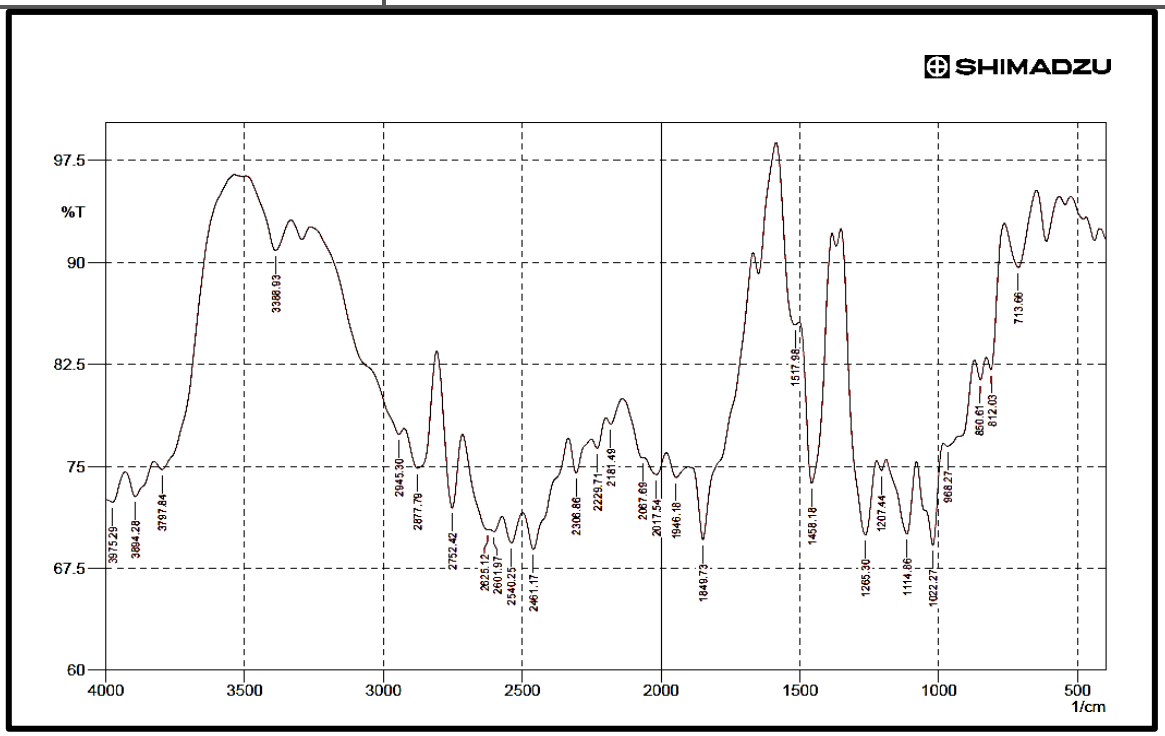


Spectra 29: ^{13}C NMR spectra of Compound 6sa

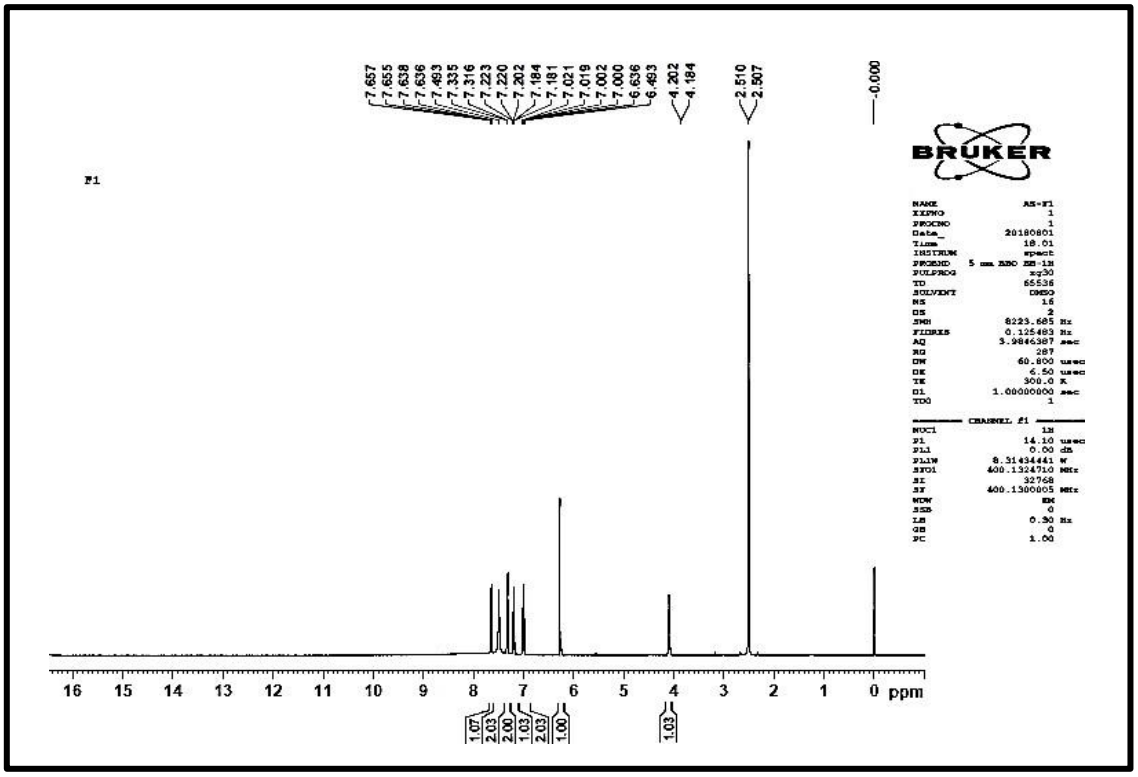
2-(5-(benzo[d]thiazol-2-ylamino)-1,3,4-thiadiazol-2-yl) phenol (7SA)



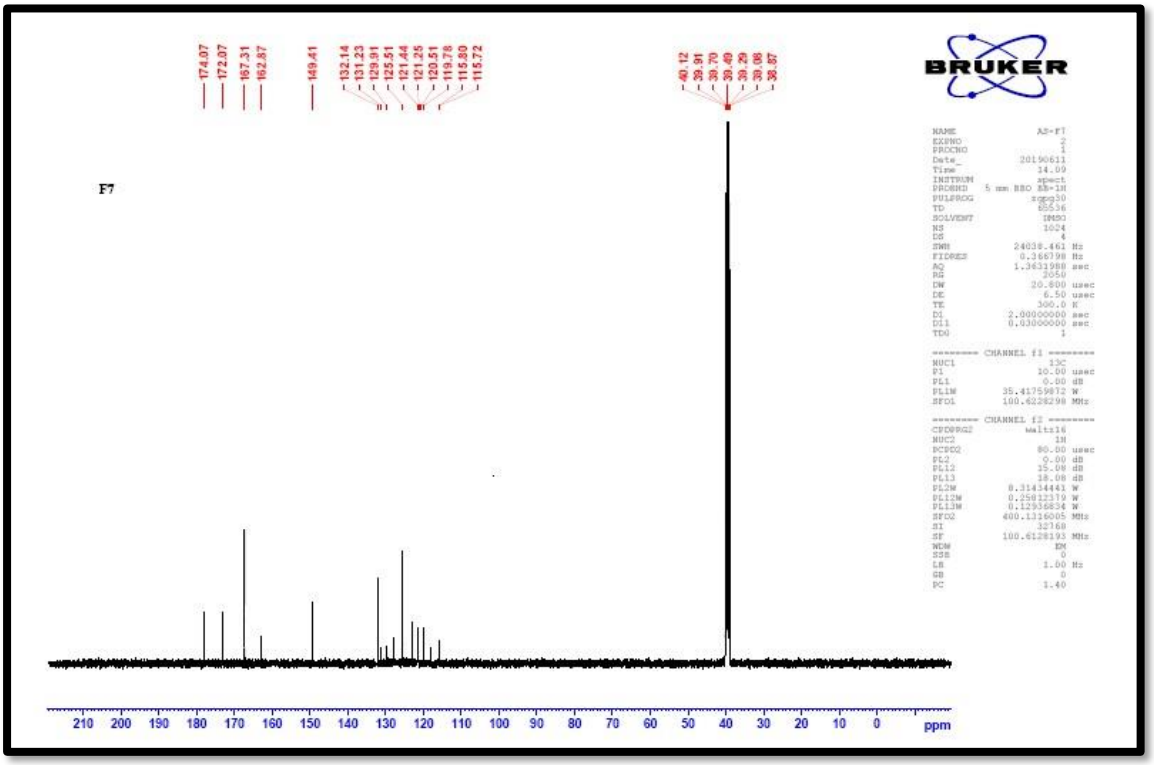
Molecular Formula	$C_{15}H_{10}N_4OS_2$
Molecular Weight (g/mol)	326.40
Melting Point	234-236ºC
% Yield	38
Recrystallisation solvent	Ethanol
TLC	Rf = 0.55 n-hexane: ethyl acetate (8:2)
IR (ν, cm^{-1})	3894 (Ar-OH), 3388 (NH), 1458 (C=N), 1207 (C-N) 1114(C-S)
^{1}H NMR (DMSO)	6.49-7.65 (m, Ar-8H), 6.5 (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



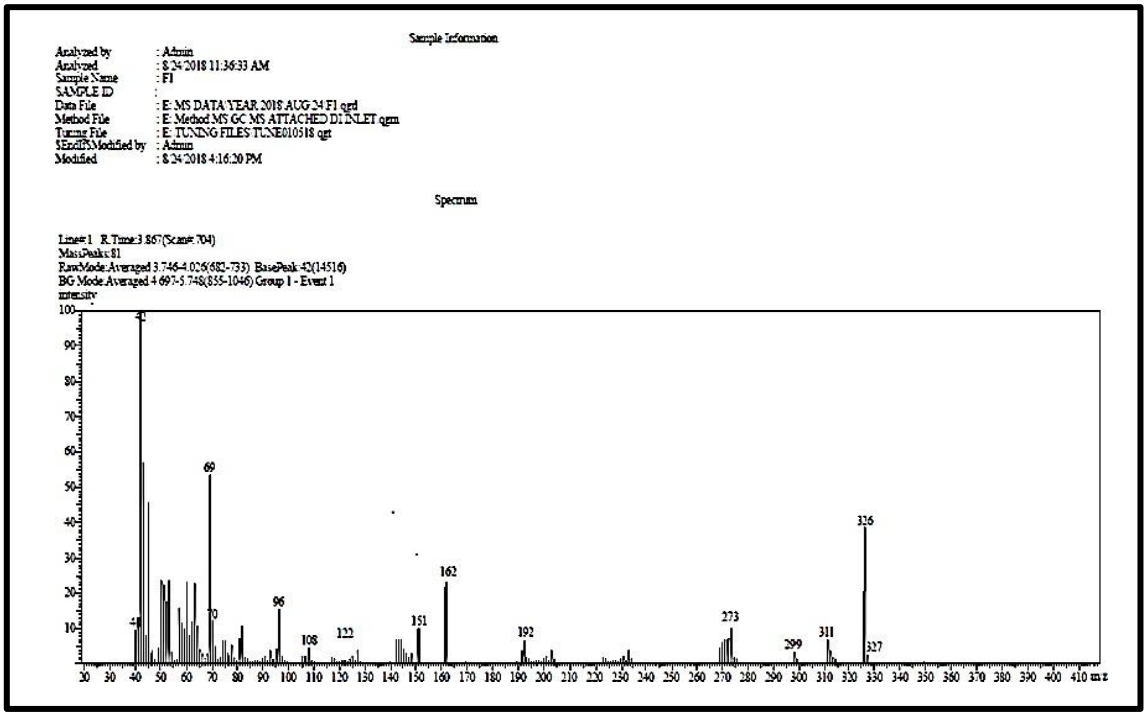
Spectra 30: IR spectra of compound 7sa



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

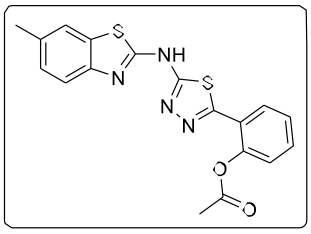


Spectra 32: ^{13}C NMR spectra of Compound 7sa



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

3.6 ENZYME INHIBITION ASSAY

The synthesised compounds were subjected to enzyme inhibition analysis utilising a microplate (96 well plates) and the Takara Universal Tyrosine Kinase Assay Kit (Cat. # MK410, Takara Bio.Inc., Shiga, Japan). This test kit quantifies the degree of inhibition, expressed as a percentage, of the substrate of tyrosine kinase by the inhibitors. The experiment was conducted in accordance with the instructions provided by the manufacturer. The activity may be quantified by assessing the suppression of the Src gene, which is naturally found in the phosphate substrate, or by introducing A431 cells to evaluate the inhibition of EGFR.

Procedure:

- **Cell separation and thinning:** Rinse 1 - 5 x 106 cells with PBS and centrifuge at room temp for 5 min at 300 X g, then collect the cells as pellet. · Include 1 ml of extraction solution, then mix the pellet by gently swirling. Centrifuge at 4℃ for 10 min at 10,000 times gravity, collect the liquid above as sample. Thin the prepared sample (as stated in VII-3). Prepare samples (1), (2), and (3) using Kinase Reacting Buffer in a 5-fold excess. When samples of grown cells are utilised, dilute by 15 - 100-fold. Prepared specimen is stable at -80℃ for a few days prior to dilution. The watered-down sample is shaky 3. and the action should be gauged on the identical day.
- **Phosphorylation:** Add 40 µl of diluted PTK control or samples into each well using a micro pipette, twice. Dispense 10 µl of 40 mM ATP-2Na solution into each well and thoroughly blend. Culture it for 30 min at 37℃ ATP solution initiates tyrosine phosphorylation. If response time exceeds 45 min, indiscriminate attachment of PTK to a plate might happen and could result in elevated background.
- **Blocking:** Discard the control solution and rinse the wells 4 times with washing buffer. After each rinse, remove any remaining solution by tapping the microtiter plate onto a paper towel, especially after the final rinse. · Pipette 100 µl of Block solution into each well and let it incubate for 30 min at 37℃.
- **Inclusion of immune proteins**· Dispose of obstructing substance and drain washing solution completely onto a paper towel. (No need to wash at this stage.) Dispense 50 µl of Anti-PY20-HRP solution into each well and let it incubate for 30 min at 37℃.
- **Substrate response**· Dispose of the antibody solution and rinse each well 4 times with wash buffer. Drain washing solution completely onto a paper towel. Dispense 100 µl of HRP reagent (TMBZ) into each well. Culture it at 37℃. (15 min is suggested as typical response time.)
- **Cease pigmenting progress:** Inject 100µl of cessation solution into each well in the identical sequence as HRP substrate solution.
- **Quantification.** 1. Gauge the intensity at 450 nm using a plate reader. 2. Create a graph by plotting the absorbance on the y-axis against the PTK control's activity on the x-axis. 3. Compute PTK action of sample using the established standard

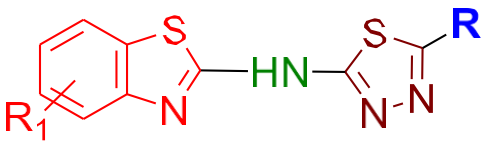
graph.

RESULT AND DISCUSSION

In our latest project, we created eighteen new cancer-fighting compounds by combining benzothiazole, thiadiazole, and NSAIDs using the right methods. In the initial phase, different alternative 2-amino benzothiazole compounds were created following the documented method. In the next phase, replaced 1,3 benzothiazole-2-yl thiosemicarbazide were produced by reacting substituted 2-aminobenzothiazole with carbon disulphide and hydrazine hydrate in the presence of ethanol. In the third phase, different replaced N-(1,3,4-thiadiazol-2-yl) benzo[d]thiazol-2-amine (end result) were produced by reacting it with different NSAIDs in the presence of phosphorous oxychloride.

TLC tests were conducted for all the variations, as they produced a solitary mark after the chromatogram was developed, indicating the purity and uniformity of the created substances. All the derivatives were identified by melting point analysis and TLC. The purity of all derivatives was verified using thin layer chromatography and structures were identified using IR, 1H-NMR, 13C NMR, and mass spectrometry.

4.1 INSILICO STUDIES



Screening using digital technology

We made 54 different NSAIDS including salicylic acid in different forms, as well as aspirin, ibuprofen, naproxen, fenoprofen, and ketoprofen. New compounds were synthesised using the molecular blending technique by linking the NSAIDs' main structural element to the benzothiazole and thiadiazole loops. By employing ADMET filtering and Glide docking in SP mode, ideal compounds were discovered via virtual screening. In order to predict the binding strength, three separate receptors—TNF-alpha, COX-II, and protein kinase—were docked with the native ligand and standard ligand, respectively, in order to calculate the docking score. Protein kinase was shown to be the most relevant target via docking studies. The kinase binding strength was predicted using molecular docking investigations on a number of receptors, including EGFR, VEGFR, FGFR, HGFR, and Src (Table 4.2). We found a stronger binding affinity for the EGFR and Src tyrosine kinase targets. Finally, after conducting computer simulations to assess 25 chemicals, we synthesised them using appropriate procedures. Molecular interactions were not ideal owing to increased electrostatic charges and rotational drawbacks (from having more rotatable bonds) with the targeted proteins, and the remaining variations had unfavourable features for drug similarity (violations of Lipinski rule of five). Consequently, they were not tested.

Studies involving the docking of compounds that have been tested with the TNF-α target

In the region of -5 to -7, with strong H-bond connections, the slide rating was found utilising the TNF-α receptor. Protein structures were used to compare the docking scores of the compounds that were tested with the original ligand. The glide score for the first ligand

bound to the target site is -8.17. When compared to the conventional ligand, the tested compounds showed a high binding affinity, as indicated by a lower docking score. The standard ligand was discovered to interact with GLY349, GLU406, LEU348, ALA439, and HIS405 compounds. Residues ASN389, GLU406, HIS405, ALA351, LEU350, TYR352, ILE438 GLY349, and ALA439 were seen to interact often in Figure 4.6a.

Studying the docking of screen chemicals with the COX-II target

The COX-II receptor-based slide rating, when coupled with strong H-bond connections, was found to be in the -6.1% to -7.9% range. We compared the molecules with the highest docking scores to the protein structure's first ligand, mefenamic acid. The ligand that was first bound to the target location has a glide score of -8. When compared to the conventional ligand, the tested compounds showed a high binding affinity, as indicated by a lower docking score. The original (standard) ligand was discovered to bind with residues ASN389, GLU406, HIS415, and ALA351. As shown in Figure 4.6a, the compounds PHE357, ALA516, VAL523, MET522, SER353, and TYR355 were determined to have similar interaction residues.

Docking studies of candidate chemicals with the target Protein Kinase

The range of slide ratings found utilising the MPS1 receptor, with solid hydrogen bond contacts, was -6.2% to -8.84%. Protein structures were used to compare the docking scores of the compounds that were tested with the original ligand. The glide score is -8.7 for the first ligand bound to the target location. When compared to the conventional ligand, the tested compounds showed a high binding affinity, as indicated by a lower docking score. The original (standard) ligand was discovered to interact with GLY605, ILE586, ALA551, VAL536, ILE663, ASP608, PRO673, and ILE607. Results for the compound GLY605, ASN606, ILE531, ALA551, ILE5869, MET602, LEU654, VAL539, and PRO673 were shown in Fig. 6a, which shows the residues used for common interaction.

Results from the in silico ADME study show that all of the filtered compounds follow the Lipinski rule of five without any violations. When taken orally, all of the drugs exhibited absorption rates between 72% and 100%. What this means is that chemicals may still have an impact when consumed orally. It seems that the chemicals that were tested had drug-like qualities, since compounds with these features were found in ratings that ranged from 0 to 1 star. Using QuikProp, Table 4 lists the results of in silico ADMET study in comparison to ordinary ligands.Part 6a.

The kinase binding strength was predicted using molecular docking investigations on a number of receptors, including EGFR, VEGFR, FGFR, HGFR, and Src (Table 4.2). We found a stronger binding affinity for the EGFR and Src tyrosine kinase targets. Last but not least, we synthesised 25 chemicals after evaluating them using insilico investigations. We did not test the other variants because they exhibited undesirable drug-like properties (they broke the Lipinski rule of five) and their molecular interactions with the target proteins were less than ideal owing to elevated electrostatic charges and rotational defects (there were more rota Table 4.bonds).

Table 4.1 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

Table 4.2 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								

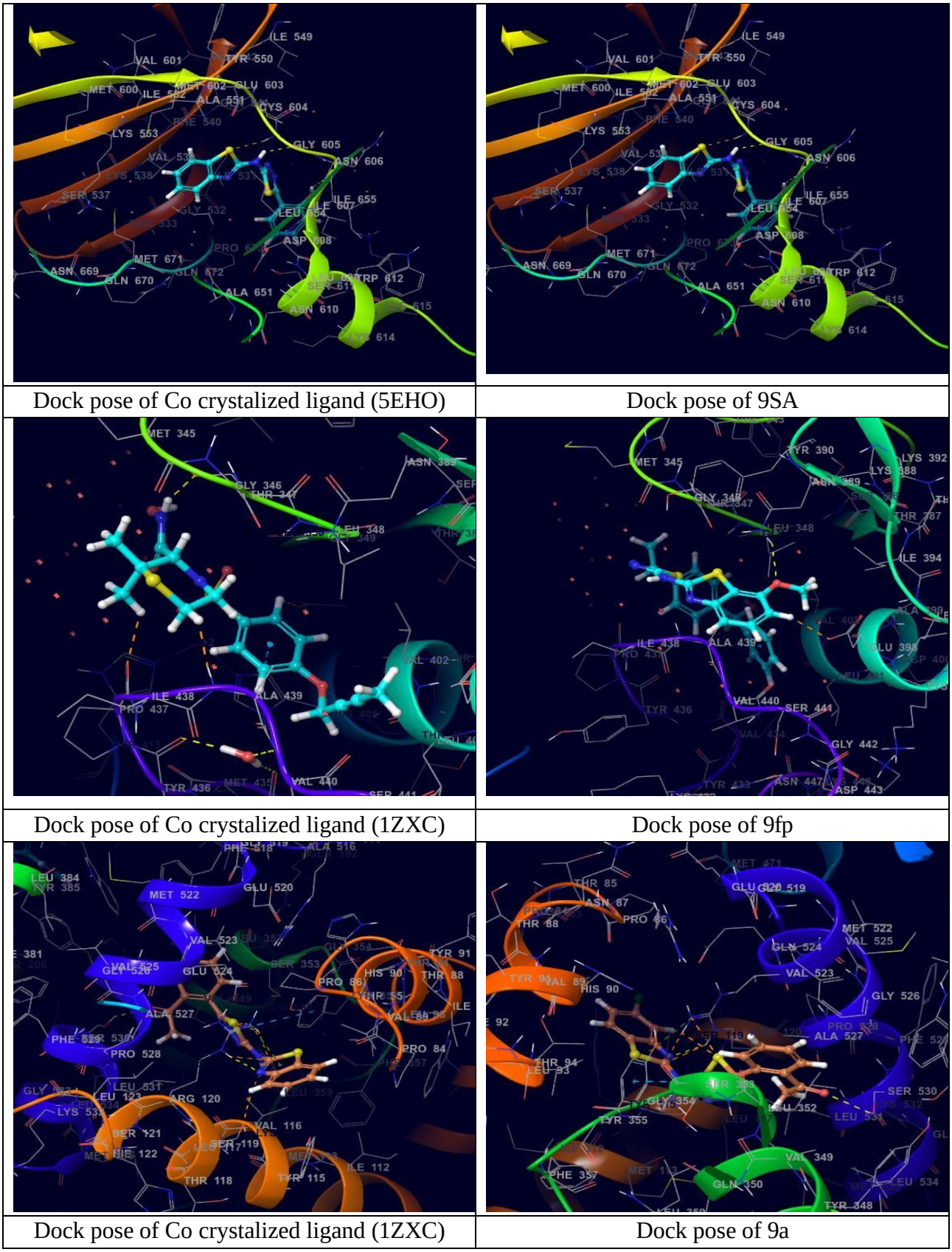


Figure 4.1 Molecular interactions of compounds 9Sa, 9fp and 9a in comparison with standard co-crystalized ligand.

4.2 ANTICANCER ACTIVITY:

It is common practice to count live cells after staining them with a critical dye in order to measure cell proliferation. A number of approaches have been used in the past. The trypan blue staining technique is simple and quick to use for determining whether a cell is growing or dying, however it is not sensitive enough for use in high-throughput screening due to its lack of specificity.

Precise, time-consuming, and radioactive substance-management-intensive assessments of radioactive material absorption are required, most often for tritium-labeled thymidine. In the mitochondria of live cells, the yellow tetrazole MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) transforms into the purple formazan. A spectrophotometer may be used to measure the absorbance of this solution by looking at its colour at a given wavelength, usually between 500 and 600 nm. What determines the peak wavelength is the solvent. There is a clear correlation between the amount of viable cells and conversion since this drop happens only when mitochondrial reductase enzymes are functioning. The influence of a drug on cell death may be deduced by creating a dose-response curve, which compares the amount of violet formazan created by cells exposed to the substance with the amount generated by cells not exposed to the substance. In the absence of phenol red, MTT solutions dissolving in balanced salt solutions or cell culture medium have a yellowish hue.

Crystals of the insoluble purple MTT formazan compound are formed when enzymes produced by mitochondria in live cells hydrolyze the tetrazolium ring. Isopropyl alcohol, an acid, may disintegrate the stones. Spectrophotometry is used for the analysis of the final violet mixture. Both the amount of MTT formazan generated and the absorbance increase as the cell count rises. There are two main issues with using the MTT technique: first, the cells' physiological state, and second, the fact that various cell types have varied levels of mitochondrial dehydrogenase activity. Nonetheless, cell proliferation may be evaluated by using the MTT method for cell determination in cytotoxicity studies, cell growth curve generation, and evaluation of cell growth in response to mitogens, antigens, growth factors, and other chemicals that favour cell proliferation (280).

SUMMARY AND CONCLUSION

The TK family is seen as a vital part of the kinase family because of its crucial role in different cellular processes. Src is the archetypal member of the tyrosine kinase clan who has a vital role in diverse cellular activities such as signal transmission, growth, survival, distinction, etc. Mutation in Src results in the formation of diverse cancer types. Hence, Src is a crucial focus for cancer treatment. Molecular blending (MB) is a method of smartly creating fresh candidates using the molecular makeup of multiple established active variations, resulting in the creation of novel mixed structures that uphold chosen traits of the initial models. The molecular blending method is especially fascinating for creating fresh possibilities for illnesses with limited drug options or when potent compounds have harmful effects or limitations in how they work in the body.

Employing the molecular hybridization method, we crafted our compounds with three distinct components: benzothiazole, thiadiazole, and NSAIDs. At first, a collection of 54 diverse molecules was created and then analysed using computer simulations to identify the most promising lead compounds. The virtual experiments were conducted using different methods

such as molecular docking, ADME, and toxicity screening. Based on our theory, we conducted structure-based virtual screening using three distinct receptors: TNF-alpha, Tyrosine kinase, and COX-II. Based on the molecular docking research, we discovered that tyrosine kinase could be a fitting target for our created molecules. To discover specific tyrosine kinase targets, we conducted additional molecular docking studies on different receptor and non-receptor tyrosine kinase targets. The EGFR and Src TK were identified as the most notable through molecular docking analyses. At last, we discovered 25 top clues from which we synthesised 18 compounds triumphantly. Compounds were identified using different techniques such as TLC, melting point analysis, IR, 1H-NMR, 13C NMR, and mass spectrometry. Adherence to spectral data confirms the formations of synthesised substances.
These synthesised substances were assessed for their anti-tumor effects. Anticancer screening was conducted by MTT assay on two cell lines: MCF-7 (breast cancer) and CaCo2 (colon cancer). Among the 18 created substances, we discovered eight strong compounds, specifically 1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib, with IC50 values below 50 µM. To confirm the goal, we conducted the enzyme blockage test using a versatile tyrosine kinase kit. Based on data, Src inhibition was most notable with the compounds. In the enzyme blockage analysis, we discovered eight substances 1a, 3a, 7a, 1sa, 3sa, 6sa, 1ib, and 3ib with IC50 below 50 µM. In the molecular docking research, the identical compounds exhibited strong binding affinity in comparison to the standard medication bosutinib. Ultimately, using insilico and in-vitro data, we can conclude that these compounds should be further investigated as Src blockers and they might have the ability to target Src mutated tumours.

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