European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07 Issue 11, 2020

Original Research Article

HETEROCYCLIC AND PHARMACOPHORIC FRAGMENT BASE DRUG DISCOVERY

Rita D. Chakole[1], Manoj S. Charde[2]*, Rahul A. Sachdeo[3], Nikita M. Gaikwad[4]

[1]Associate Professor, Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad.

[2]*Assistant Professor, Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad.

[3]Ph. D. Research Scholar, Krishna Vishwa Vidyapeeth, Karad.

[4]Assistant Professor, Department of Pharmaceutics, Modern College of Pharmacy, Nigdi, Pune

***Corresponding Author-** Manoj S. Charde

*Assistant Professor, Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad.

Abstract:

Cancer has scrolled up to the second position and has become the leading cause of deaths worldwide responsible for 9.6 million of deaths in 2018 across the globe. Colon cancer is the cancer of alimentary canal that starts with the development of abnormal projection of tissue or mucous membrane surrounding the inner lining of the colon and rectum called as polyps. These polyps may be malignant or benign and over time can develop into cancers, which further invade to inner layers resulting in cancerous lesions. The designed methodology is proposed for the design and synthesis to of anti-cancer agents to target colon cancer with the help of heterocyclic fragments to design a clubbed pharmacophoric lead molecule. The two heterocycles reported for anti-cancer activity: thioquinazolinone and benzothiazole were clubbed together to form a new lead molecule which were decorated with different substituents. The designed set of molecules were virtually screened for drug-like properties, toxicity and docking. The synthesized compounds were tested for pharmacological activity with cell viability testing assay with Trypan Blue Exclusion method against Colon Cancer HT29 cell lines of which the compounds C3 and C7 showed highest inhibitory activity compared to others and standard anti-cancer drug. Thus, the study involves design and development of ligands targeting colon cancer parallel with in-silico computational approaches to tackle cancer with fragment-based drug design method rational way towards drug design and development with promising results.

Keywords: Drug Design, anti-cancer, in-silico methods, CADD, colon cancer, molecular hybridization.

Introduction:

The term Cancer refers to a broad group of diseases associated with the rapid generation and proliferation of abnormal cells that can invade tissues and spread from the point of origin to locations far away. Cancer may be of any part of the body's organs and tissues when anomalous cells grow uncontrollably and cross their habitual limits to invade adjacent body parts and/or spread to other organs to cause metastasis. During the conversion of normal cells into cancerous cells, a phase-wise process is followed before the cells become malignant, which must be addressed and monitored before it can lead to serious disease and death. [1]. Cancer has resulted in the world's second leading cause of death, as confirmed by WHO and is subsequent driving reason for 9.6 million deaths in 2018. Around 1 of every 6 passing's overall are because of cancer worldwide [2]. The statistics for 2018 in India show that breast, cervical / uterine and ovarian cancers forms the major cause of increased

mortality rate in women, as stated by the International Agency for Research on Cancer (IARC) while lip/oral cavity, lung and cancers of alimentary canal are leading cause of deaths in men [3]. The risk for development and cause of cancers can be by endogenous factors (such as hereditary mutational changes, hormonal factors, and immune conditions) as well as exogeneous/acquired factors (such as food and diet, tobacco consumption, radiation exposure and infectious organisms). Risk for developing cancer mainly depends on the lifestyle changes and environmental factors. Some of danger signals for cancer specified by Indian Cancer Society are soreness in the oral cavity, appearance of lumps or masses, persistent indigestion or abnormal bleeding and unexplained weight loss, or alteration in bowel habits. [4]

The term Cancer refers to a broad group of diseases associated with the rapid generation and proliferation of abnormal cells that can invade tissues and spread from the point of origin to locations far away. Cancer may be of any part of the body's organs and tissues when anomalous cells grow uncontrollably and cross their habitual limits to invade adjacent body parts and/or spread to other organs to cause metastasis. During the conversion of normal cells into cancerous cells, a phase-wise process is followed before the cells become malignant, which must be addressed and monitored before it can lead to serious disease and death. [1]. Cancer has resulted in the world's second leading cause of death, as confirmed by WHO and is subsequent driving reason for 9.6 million deaths in 2018. Around 1 of every 6 passing's overall are because of cancer worldwide [2]. The statistics for 2018 in India show that breast, cervical / uterine and ovarian cancers forms the major cause of increased mortality rate in women, as stated by the International Agency for Research on Cancer (IARC) while lip/oral cavity, lung and cancers of alimentary canal are leading cause of deaths in men [3]. The risk for development and cause of cancers can be by endogenous factors (such as hereditary mutational changes, hormonal factors, and immune conditions) as well as exogeneous/acquired factors (such as food and diet, tobacco consumption, radiation exposure and infectious organisms). Risk for developing cancer mainly depends on the lifestyle changes and environmental factors. Some of danger signals for cancer specified by Indian Cancer Society are soreness in the oral cavity, appearance of lumps or masses, persistent indigestion or abnormal bleeding and unexplained weight loss, or alteration in bowel habits. [4]

Misfunctioning of normal cellular processes in human body during cell division which is normally in a controlled manner to regulate the functions of body leads to cancer development. When cells become damaged or older, they get replaced by the new cells in a well-defined process. This process is commonly known as apoptosis, but sometimes this process goes wrong. DNA regulates the functions and division of cell, but if it gets damaged causing mutations, these cells do not die and keep dividing and re-dividing causing bulk mass of cells known as tumours [5]. These tumours may be localized or may spread to other parts of body and are thus known as benign or malignant tumours respectively.

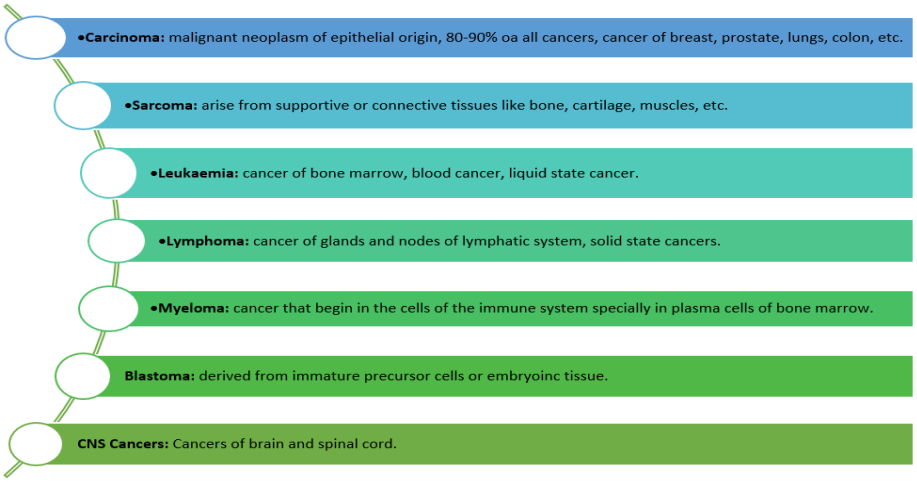


Figure 1: *Classification of Cancers [6]*

ISSN 2515-8260 Volume 07 Issue 11, 2020

Classification of Cancers

Hallmarks of cancer cells as specified in the literature are:
1) Growth signals self-sufficiency,
2) Anti-growth signs insensitivity,
3) Apoptosis evading,
4) Telomerase and telomeres, infinite replicative potential,
5) Angiogenesis is sustained,
6) Invasion and metastasis of the tissue, and
7) Instability of the genome, which is the basis for the distinction between normal and cancerous cells. [7]

When cells become damaged or older, they get replaced by the new cells in a well-defined process. This process is commonly known as apoptosis, but sometimes this process goes wrong. DNA regulates the functions and division of cell, but if it gets damaged causing mutations, these cells do not die and keep dividing and re-dividing causing bulk mass of cells known as tumours [5]. These tumours may be localized or may spread to other parts of body and are thus known as benign or malignant tumours respectively.

Drug Targets for Colon Cancer:

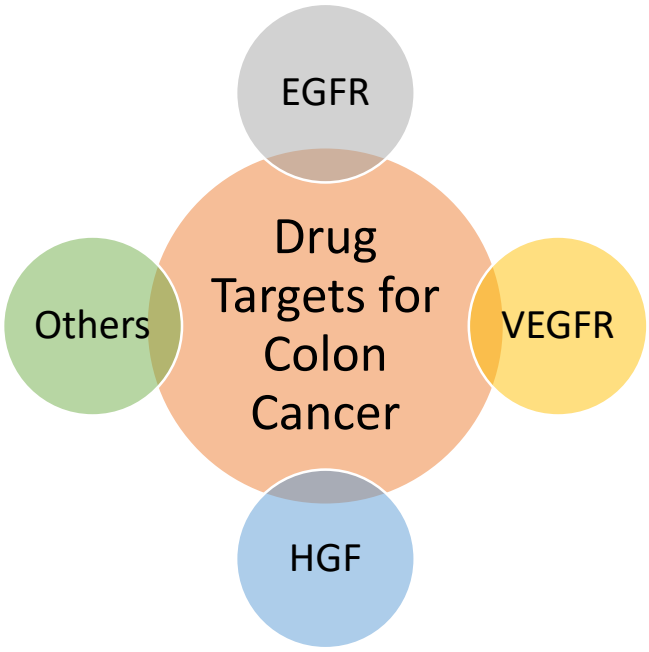


***Figure 1: Major** Drug Targets for Colon Cancer*

[EGFR: Epidermal Growth Factor Receptor, VEGRF: Vascular Endothelial Growth Factor Receptor, HGF: Hepatocyte Growth Factor][18]

1.4 Epidermal Growth Factor Receptor (EGFR)

One of the most encouraging focuses for the therapy of cancers related to colon and rectum is the EGFR, a versatile signal transducer which regulates signalling pathways involved in differentiation of the cells, their proliferation and process of angiogenesis. In 80 percent of colon cancers, EGFR is expressed. [18]

EGFR belongs to the one of the super-family of cell surface receptors known as Receptor Tyrosine Kinase (RTK). These RTKs control several cellular activities such as cell division, cell migration, cellular metabolism, its proliferation and differentiation. EGFR belongs to the RTK family of erythroblastosis oncogene B (ErbB) [19]. The receptor of ErbB family consists of 3 domains

anatomically that are- i) Extracellular Domain (N-terminal ligand binding domain which is cystine-rich and a dimerization arm), ii) Transmembrane Domain (hydrophobic), iii) Cytoplasmic Tyrosine Kinase containing Domain with several phosphorylation sites. ErbB2, ErbB3 and ErbB4 are the other structurally related receptors to EGFR (ErbB1). Excessive ErbB signalling and cascade of downregulation Ras-Raf-MAPK pathway is associated with development of tumours and specifically the immoderate signalling of ErbB1 and ErbB2 are responsible for the malignancy of tumours, increased cell proliferation and inhibition of apoptosis. Colon cancer can be related to EGFR by its overexpression or mutation or increased Epidermal Growth Factor (EGF) which are the ligands that specifically bind to these receptors and results into tyrosine phosphorylation and receptor dimerization. Transactivation of EGFR by various physical stimuli like osmotic stress, UV-radiation or shear stress which bestow the properties like growth, survival and angiogenesis to cancerous cells. Various agents that target EGFR at different levels of cascade signalling pathway with variant modes of action are reported till date like the monoclonal antibodies- Cetuximab and Panitumumab, Tyrosine Kinase Inhibitors (TKIs) such as Gefitinib, Erlotinib, Lapatinib and various other agents. As EGFR is involved in a large signalling network in cellular processes and is implicated in various cancers, targeting EGFR and its various domains helps find better therapeutic leads for cancer treatment. [20-21]

1.5 Drug Design and Synthetic Chemistry:

Drug design aims to design of drugs with various approaches to help target the site of action within minimum time and other resources. Design of drugs with the help of Computer Aided Drug Design (CADD) has helped synthetic chemists to reach a rational way for drug development. Synthesis of compounds which are pre-screened under available in-silico parameters has been helped a lot to find the lead molecules by eliminating the rest of proposed molecules. Various approaches are practised to design the active lead molecule based on target or based on fragment which are commonly known as target-based and fragment-based drug design (FBDD). Virtually screening drugs has helped choose the perfect drug candidate by qualifying various parameters like LogP value, Lipinski's Rule of five, toxicity, etc. Similarly, in case of designing drugs for cancer CADD has helped develop "molecular targets" that help specifically target cancerous cells. In 21st century synthetic chemistry and CADD go hand-in-hand to achieve new therapeutic agents and improve the older ones.

Computer-aided drug design (CADD) explores advanced technologies to accelerate the drug design and development process. Radom screening, extraction, and molecular modifications are the steps that were practiced conventionally to develop and discover drugs. Rational drug discovery involves drug development based upon the known molecular targets such as enzymes, receptors or already discovered chemical moieties.

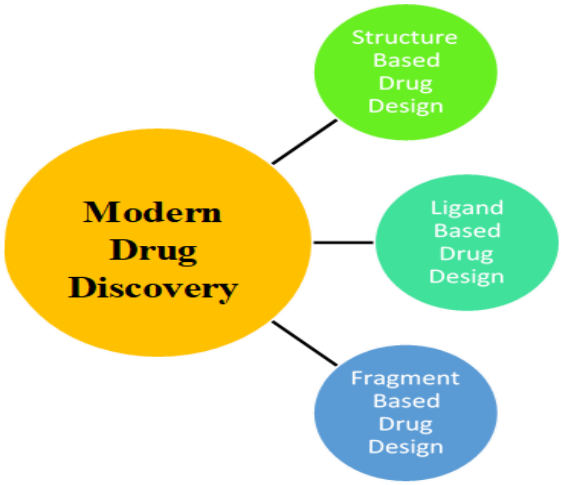


Figure 2 *Illustration: Modern Drug Discovery Approaches*

ISSN 2515-8260 Volume 07 Issue 11, 2020

1.6 Importance of Heterocycles in Drug Design:

Several of the most important advances are being made in treating disease by designing, developing and testing of new structures, which are often heteroaromatic derivatives. Pharmaceutical researchers, inspired by the natural heterocycles and their pharmacological importance, have consistently designed and produced better pharmaceutical products for a better living with semi-synthetic and synthetic chemistry.

Heterocycles are an important category of compounds which makes up more than half of all recognized organic molecules. The have been an area of interest in synthetic pharmaceuticals as they possess outstanding pharmacological actions. Heterocycles are the key structural units in the medicinal chemistry. Relationships between the heterocyclic structure of the scaffold and the substituent groups indicate that the heterocyclic ring is the key source of biological activity. [22] Nitrogen and sulphur-based heterocycles play a significant role not only in the life sciences but also in many other special and fine chemistry industries [23].

1.7 Benzothiazole:

Benzothiazole is a functional aromatic heterocyclic compound used in most of the drugs in use and those under clinical research with a 5-membered 1, 3 thiazole ring fused to the benzene ring. Benzothiazole has been reported to have large spectrum of activities starting from anti-inflammatory, anti-microbial, anthelmintic to anticonvulsant and anti-tumour along with its derivatives. Benzothiazole and its derivatives have been widely studied for their antimicrobial, antitumor, anthelmintic, anticonvulsant, anti-inflammatory activities. Various benzothiazole derivatives have been proposed as inhibitors of fatty acid amide hydrolase (FAAH), Raf kinase (Raf-1) and B-cell lymphoma protein BCL-2. [24-28]

1.8 Quinazolinone:

The heterocyclic chemical compound quinazolinone is a quinazoline with a keto group. Quinazolinone with 4-quinazolinone isomer and the sulphur group attached at second position forms thioquinazolinone. Thioquinazolinones are chemically synthesized for their physiological importance and pharmacological use. Thioquinazolinone analysis indicates that derivatives can be used in a range of biological activities, such as anti-viral, as anticancer, in treatment of fungal and bacterial infections, in coccidiosis, to relieve inflammation, in treatment of CNS related diseases, as anti-malarial and anti-oxidant, etc. [29-30]

Methodology:

The synthetic procedure is divided into three parts as:
PART A: Synthesis of 2-Amino benzoic acid
PART B: Synthesis of 2-Aminobenzothiazole and its derivatives
PART C: STEP-I: Clubbing of two moieties (Intermediate Step)

STEP-II: Cyclization (Final Step)

- Characterization
- Pharmacological Activity Assessment

ISSN 2515-8260 Volume 07 Issue 11, 2020

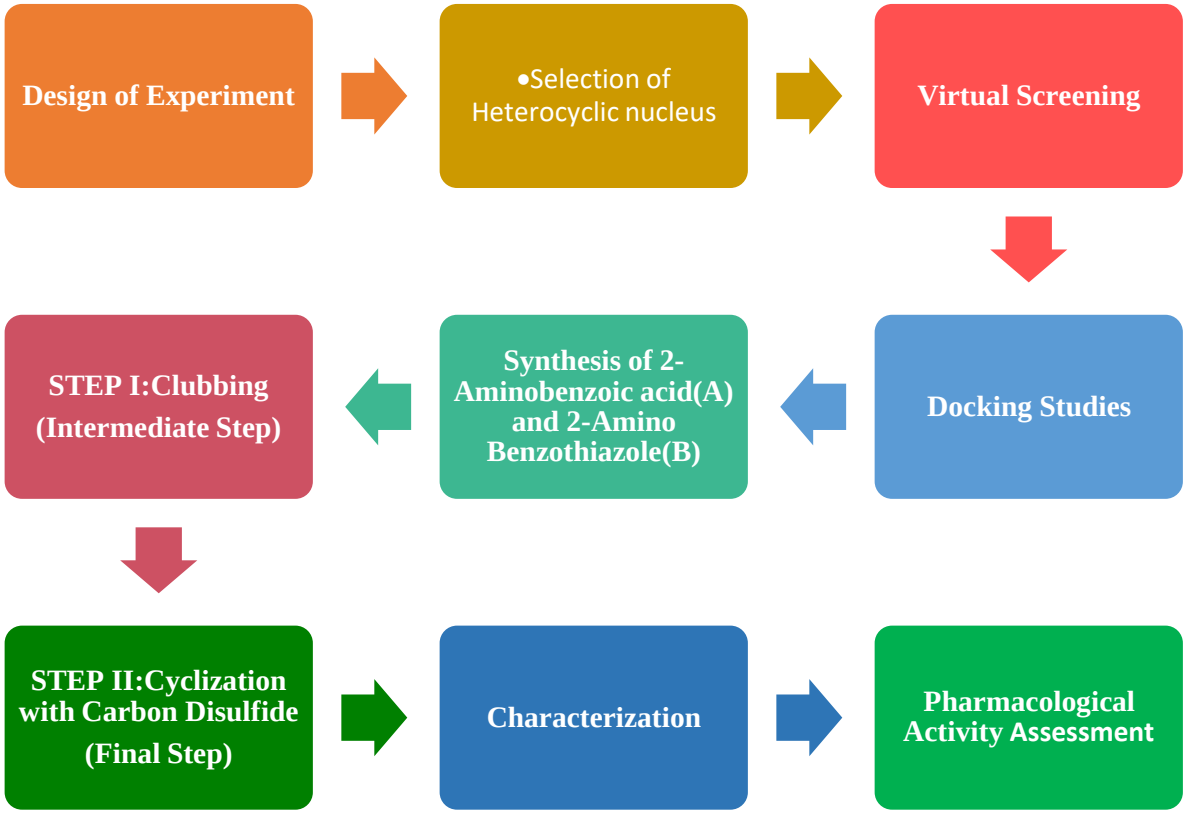


Figure 5: *Diagrammatic representation of experimental work*

6.2 In-Silico Computational Studies:

6.2.1 Selection of Target:

As reported in the literature, EGFR is expressed in colon-cancers for about 80%. The pathological and molecular studies reveal that the EGFR receptor has 8 different domains.

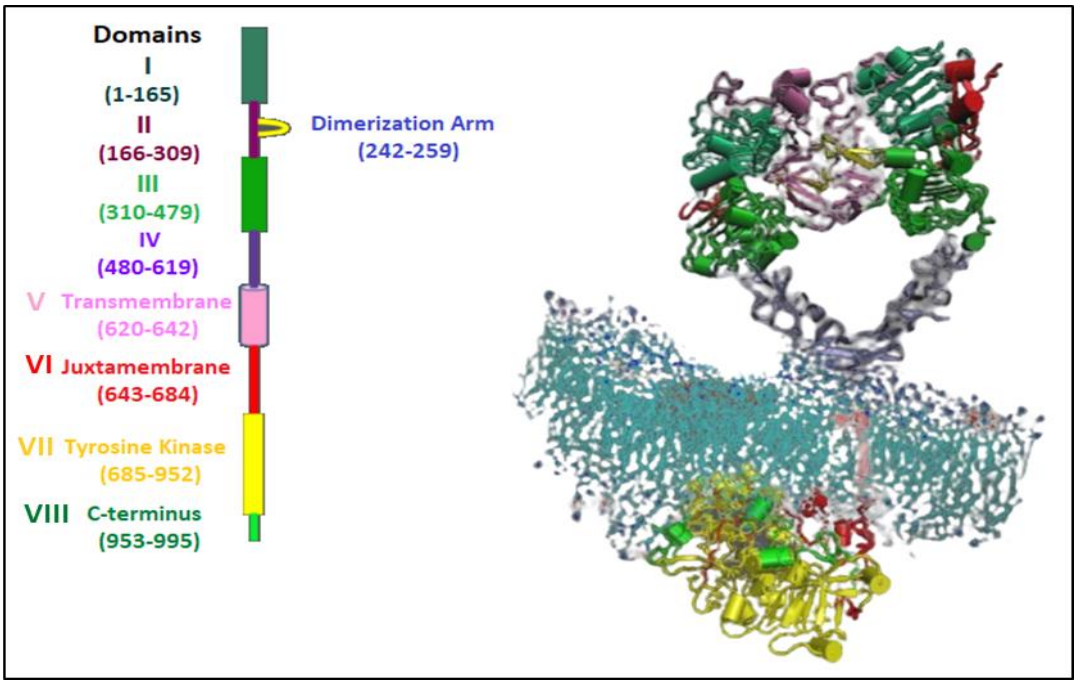


Figure 6*: EGFR molecular structure [49]*

ISSN 2515-8260 Volume 07 Issue 11, 2020

6.2.2 Pocket Modelling:

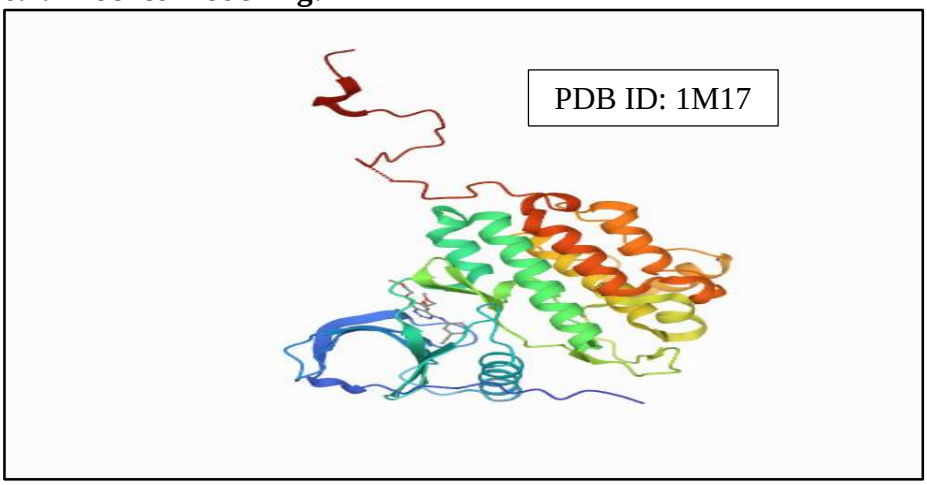


Figure 7: *PDB ID: 1M17 EGFR (tyrosine kinase domain)-Erlotinib Complex*

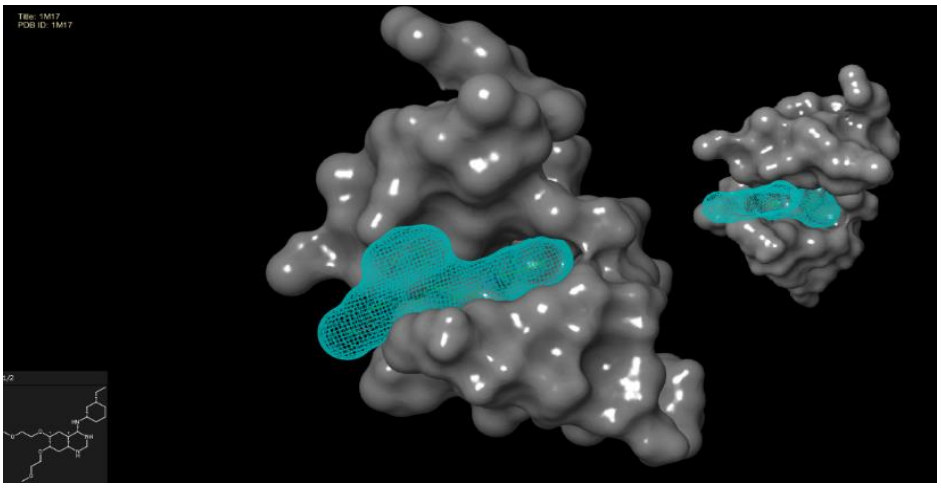


Figure 8: *1M17 Pocket Modelling EGFR (tyrosine kinase domain)-Erlotinib Complex*

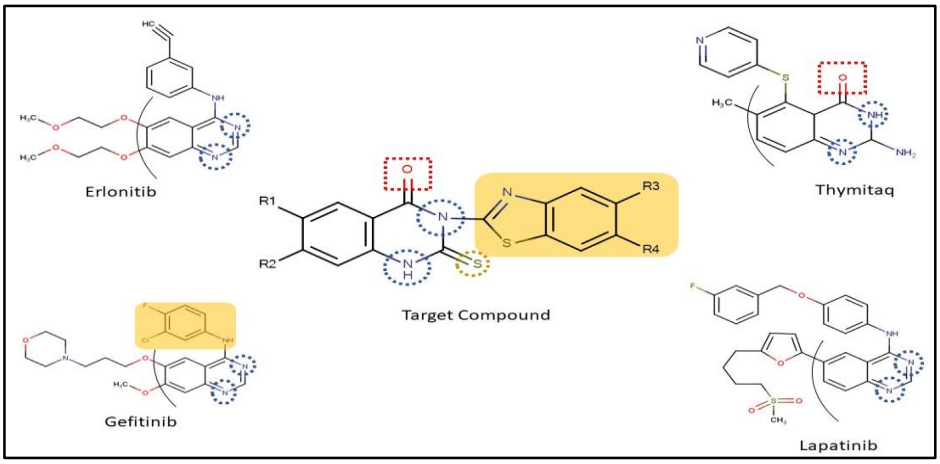


Figure 9: *Design of Lead Molecule [21,36, 50-51]*

6.2.3 Design of Lead:

6.2.4 ADME Prediction:

ADME studies of the designed molecules were performed virtually using swiss ADME software available online. Some of the designed molecules exhibits low violation to the Lipinski rule of five which includes hydrogen bond acceptor, hydrogen bond donor, log P and molecular weight. Using

ISSN 2515-8260 Volume 07 Issue 11, 2020

Swiss ADME portal physicochemical properties, ADME parameters, and pharmacokinetic properties of the designed molecules were computed and thereby serves support to drug discovery and drug design.

6.2.5 Toxicity Prediction:

In silico toxicity assessment of the designed molecules was carried out using lazar toxicity software. Lazar is an online portal which is used by medicinal chemists, toxicologists and chemical informatics.

6.2.6 Docking Studies:

The structures of the molecules were built using Marvin Sketch (Version 15.8.24). The target protein was prepared with protein preparation wizard Maestro 11.5 (Schrödinger software) where the protein was pre-processed for assigning bond orders, create zero-order bonds to metals, create disulphide bonds. The protein residues were reviewed and modified as per the target site. Lastly the protein was refined by optimizing the H-bonds and waters were removed. The energy was minimized by OPLS3 Force Field. The ligands were processed for 2D to 3D conformation and were optimized to generate most probable structures at given pH range using LigPrep wizard. The grid was generated and the docking was performed on XP mode (extra precision mode) using Glide Docking module.

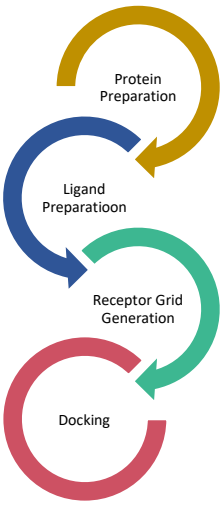


Figure 10: *Diagrammatic representation of Glide Docking*

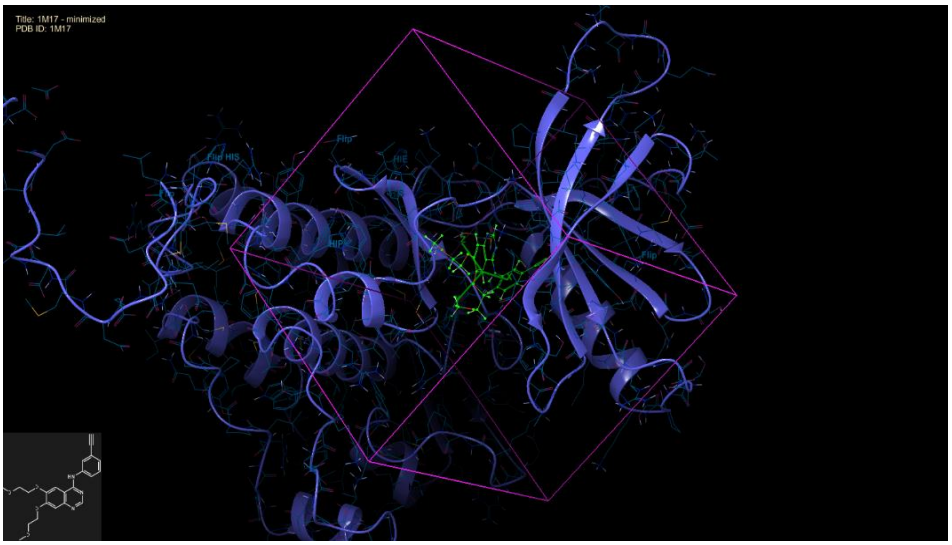


Figure 11: *Receptor Grid Generation*

ISSN 2515-8260 Volume 07 Issue 11, 2020

6.3 Methodology for Synthesis:

6.3.1 Synthetic Scheme:

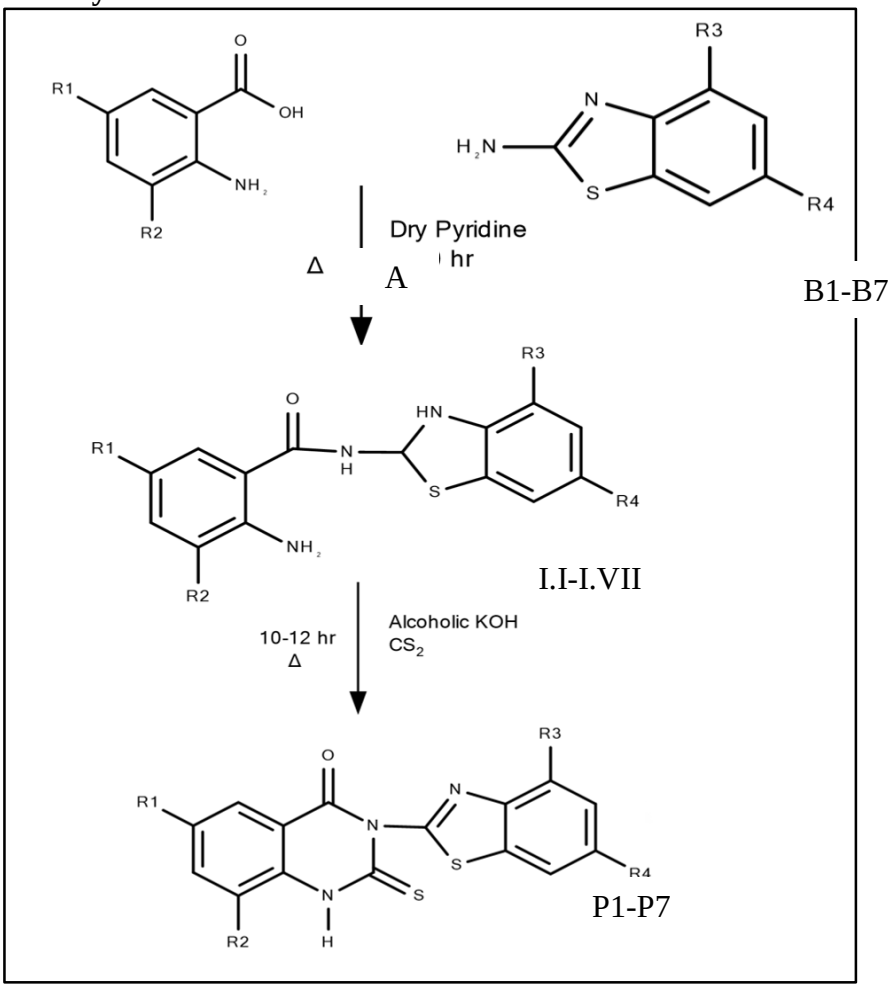
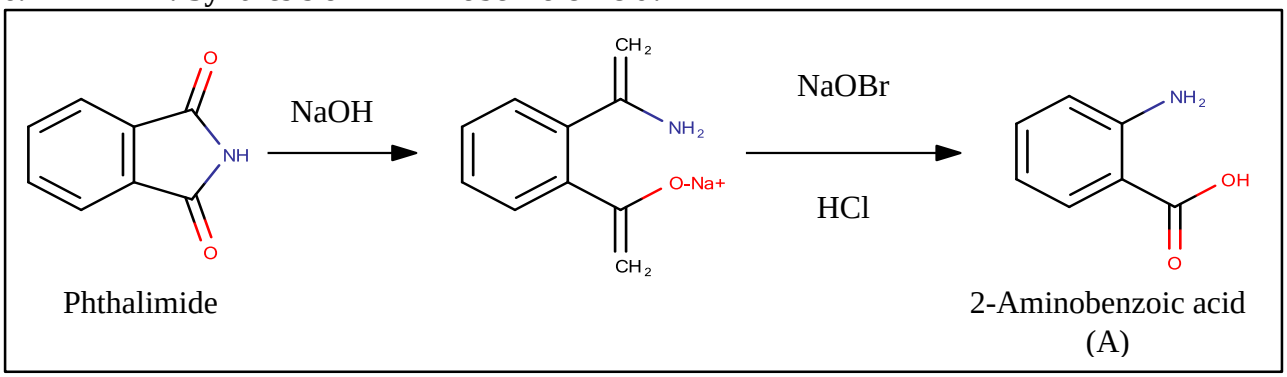


***Table 5**: Compound Codes*

CODE	NAME
A	2-Aminobenzoic acid
B1-B7	2-Aminobenzothiazole and its derivatives
I.I-I.VII	2-Amino-n-(2-benzothiazolyl)benzamide derivatives
P1-P7	3-(1,3-benzothiazol-2-yl)-2-sulfanylidene-1,2,3,4-tetrahydroquinazolin-4-one derivatives

6.4 PART A: Synthesis of 2-Aminobenzoic Acid:



The synthesis involves following steps:

Figure 32: *Synthetic Procedure Part A*

6.5 PART B: Syhthesis of 2-Aminobenzothiazole:

The synthesis of 2-amino benzothiazole and its derivatives was carried out by reported methods, the procedure applied is as follows:

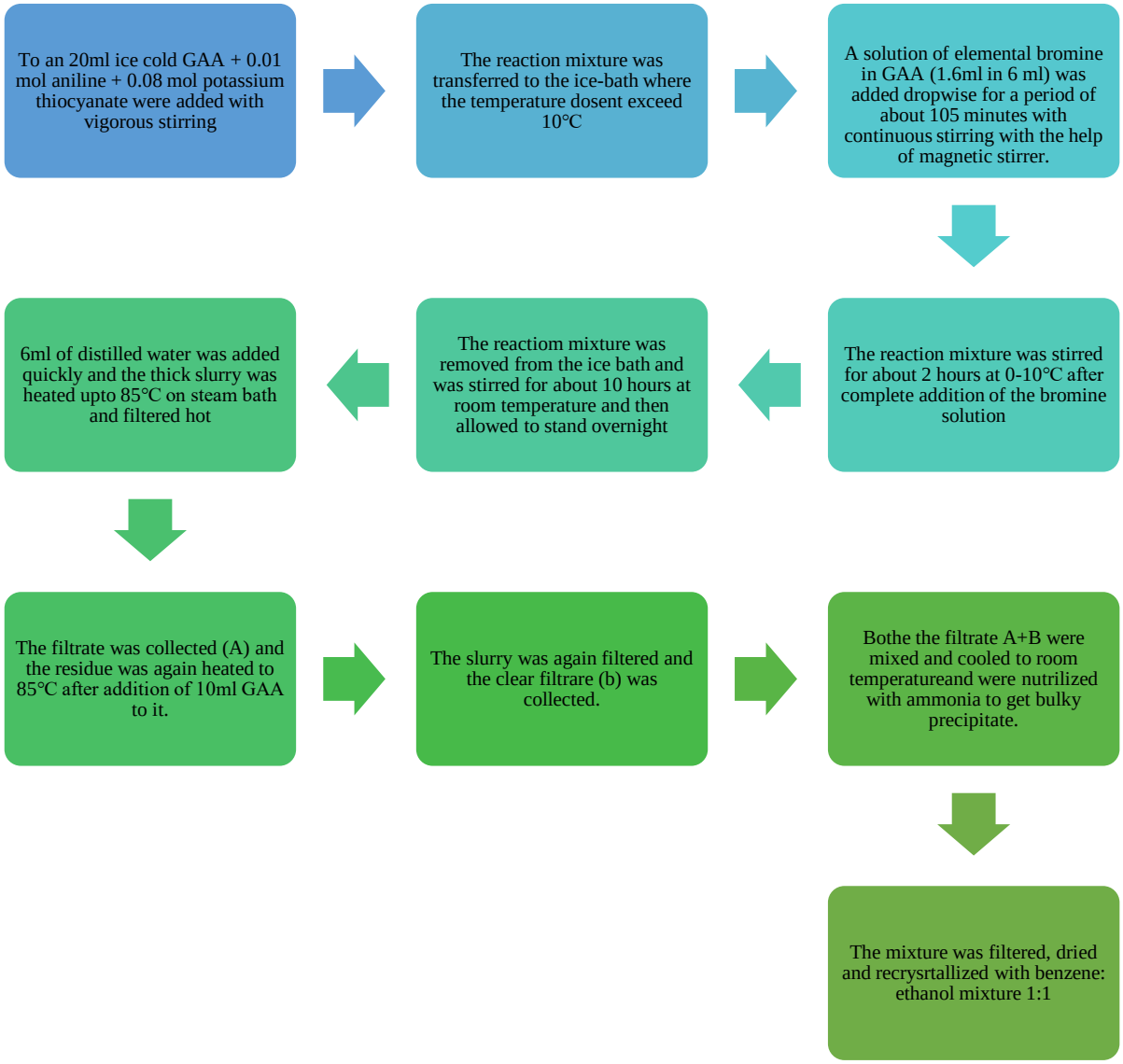
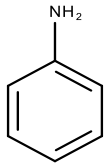
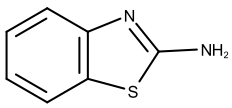
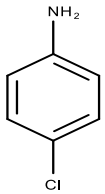
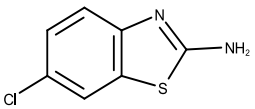
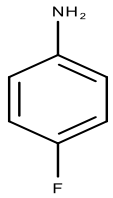
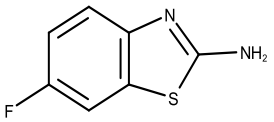
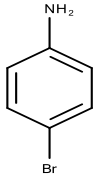
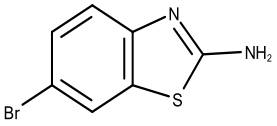
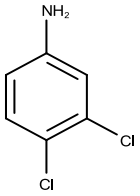
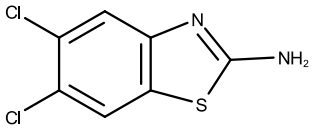
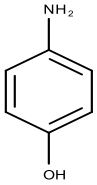
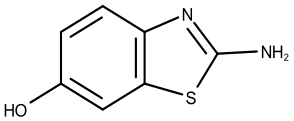
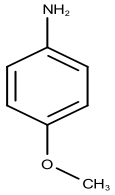
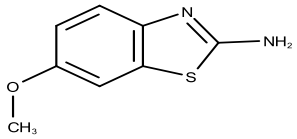


Figure 13: *Synthetic Procedure Part B*

Table 6: *Synthesis Part B*

Code	Aniline Derivative	Substituted 2-Amino Benzothiazole
B1.	 Aniline	 2-Aminobenzothiazole
B2.	 4- Chloroaniline	 2-Amino-6-chlorobenzothiazole

ISSN 2515-8260 Volume 07 Issue 11, 2020

B3.		4- Fluoroaniline	 2-Amino-6-fluorobenzothiazole
B4.		4- Bromoaniline	 2-Amino-6-bromobenzothiazole
B5.		3,4- Dichloroaniline	 2-Amino-5,6-dichlorobenzothiazole
B6.		4- Hydroxyaniline (4- Aminophenol)	 2-Amino-6-hydroxybenzothiazole
B7.		4-Methoxyaniline (p-Anisidine)	 2-Amino-6-methoxybenzothiazole

6.6 PART C:

STEP-I: Synthesis of Substituted Products of 2-amino-N-(2-benzothiazolyl)benzamide

Step-I involves following procedure:

2-Amino benzothiazole and its derivatives (B1-B7) (4g, 0.026 mol) and 2-Amino benzoic (4g, 0.029 mol) acid were dissolved in dry pyridine

The reaction mixture was refluxed for about 8 to 10 hours in sand bath under dry conditions.

The reaction mixture was allowed to cooled at room temperature and was poured in cold water, the separated mass was filtered and dried and was recrystallized with ethanol.

Figure 14: *Synthetic Procedure Part C-I*

ISSN 2515-8260 Volume 07 Issue 11, 2020

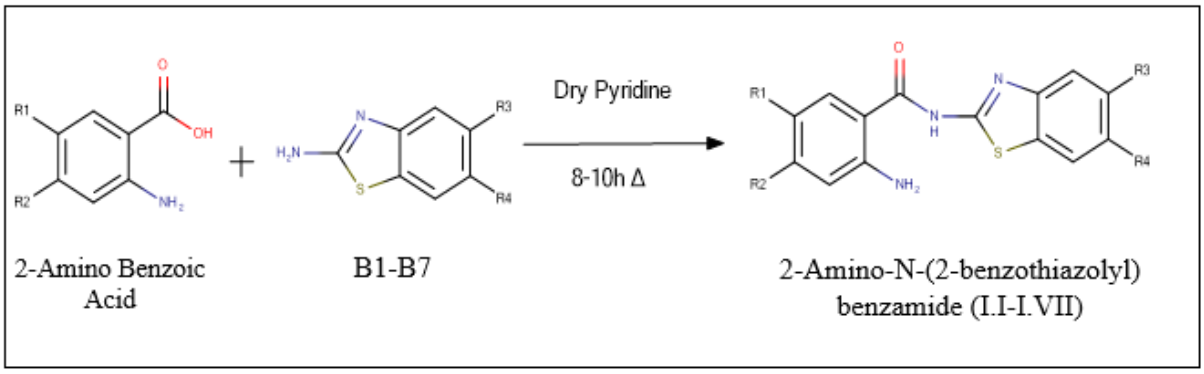
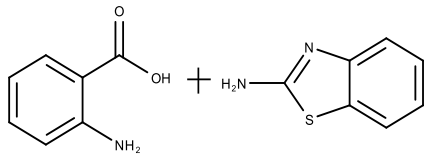
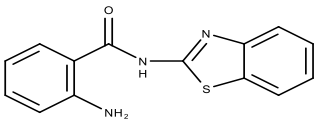
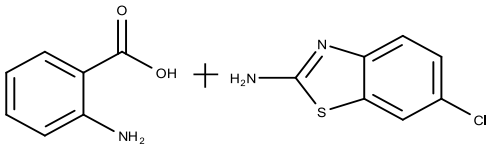
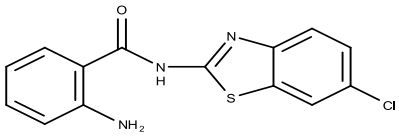
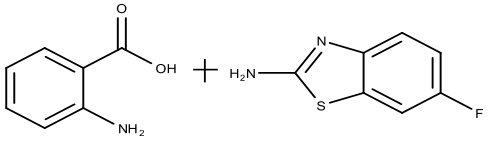
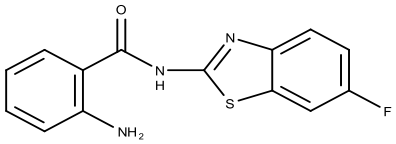
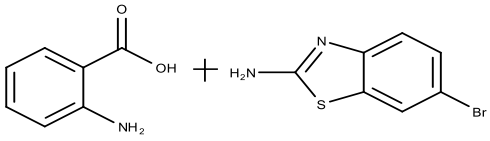
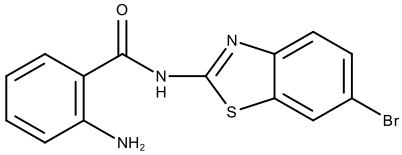
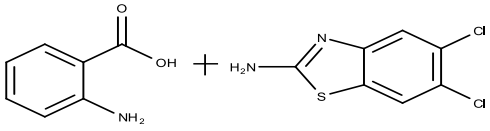
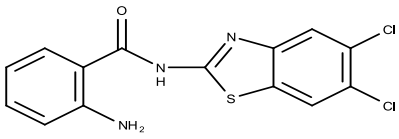
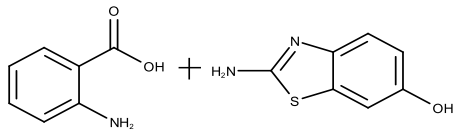
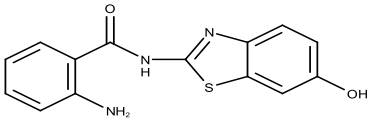
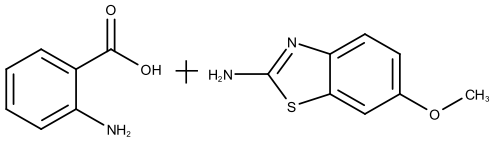
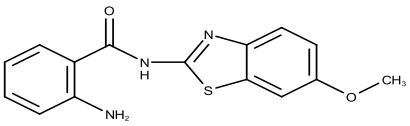


Table 7: *Synthesis Part C-I*

Sr. No.	**Reactants** **2-Amino Benzoic Acid**(R1,R2=H) + **Substituted 2-Aminobenzothiazole**	**2-Amino-n-(2-benzothiazolyl)benzamide derivatives**
I.I	 A B1	 2-Amino-N-(2-benzothiazolyl) benzamide
I.II	 A B2	 2-Amino-N-(4-chloro-2-benzothiazolyl) benzamide
I.III	 A B3	 2-Amino-N-(4-fluoro-2-benzothiazolyl) benzamide
I.IV	 A B4	 2-Amino-N-(4-bromo-2-benzothiazolyl) benzamide
I.V	 A B5	 2-Amino-N-(3,4-dichloro-2-benzothiazolyl) benzamide

I.VI	 A B6	 2-Amino-N-(4-hydroxy-2-benzothiazolyl) benzamide
I.VII	 A B7	 2-Amino-N-(4-methoxy-2-benzothiazolyl) benzamide

STEP: II Synthesis of 2-thio-3-(2-benzothiazolyl)-4(3h)- quinazolinone

Step-II involves following procedure:

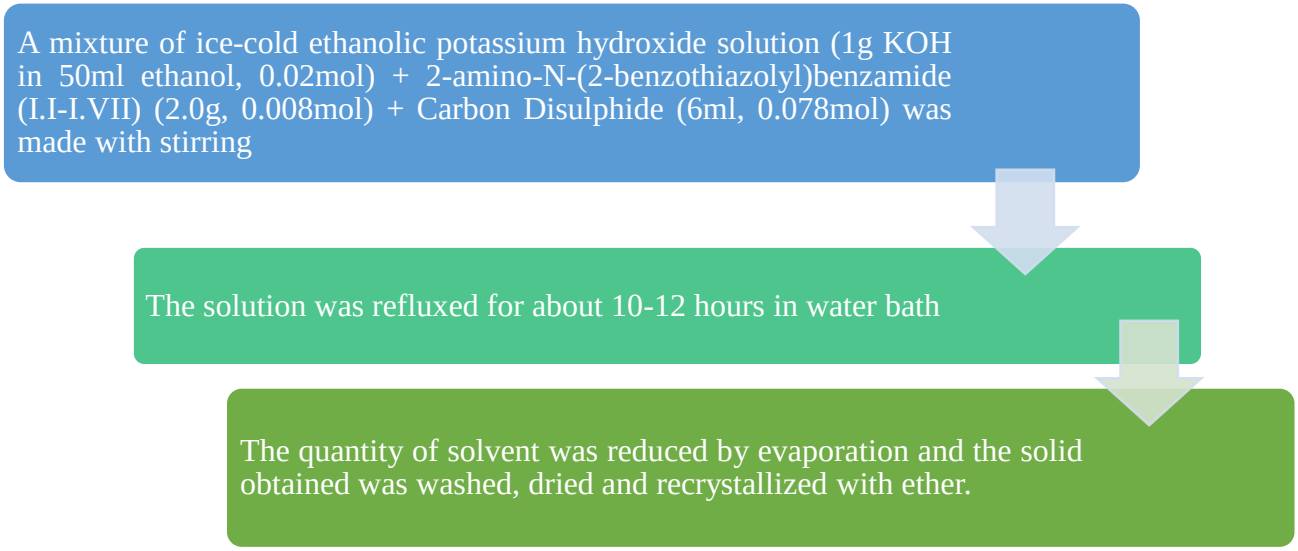


Figure 15: *Synthetic Procedure Part C-II*

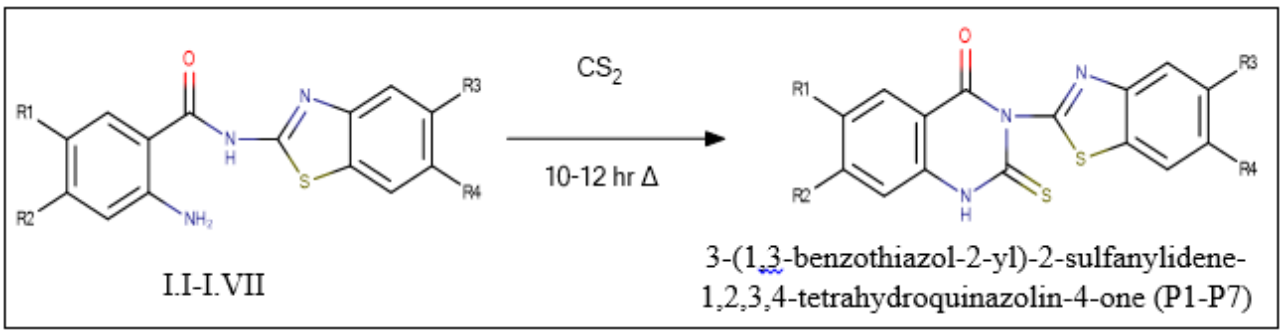
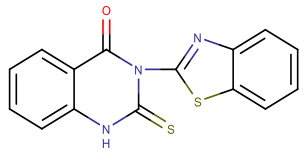
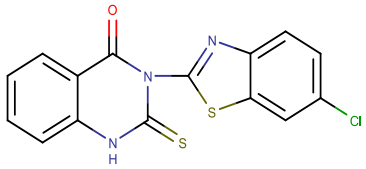
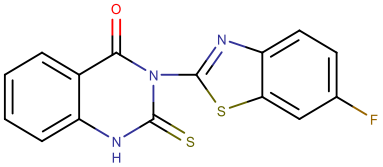
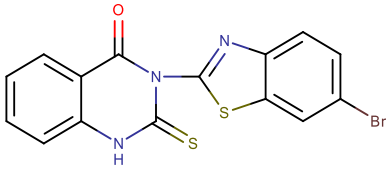
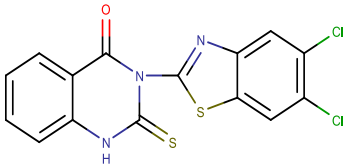
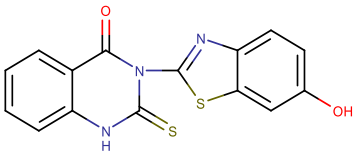
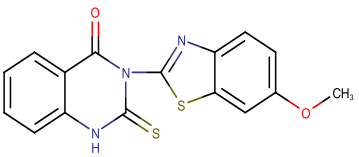


Table 8: *Synthesis Part C-II*

Compound No.	R1	R2	R3	R4	3-(1,3-benzothiazol-2-yl)-2-sulfanylidene-1,2,3,4-tetrahydroquinazolin-4-one Derivatives	CODE

II.I	H	H	H	H		**P1**
II.II	H	H	H	Cl		**P2**
II.III	H	H	H	F		**P3**
II.IV	H	H	H	Br		**P4**
II.V	H	H	Cl	Cl		**P5**
II.VI	H	H	H	OH		**P6**
II.VII	H	H	H	OCH_3		**P7**

6.7 Identification and Characterization:

6.7.1 Physicochemical Studies

- **Preliminary Studies**
- **Melting Point Detection**
- **Thin Layer Chromatography**

Stationary phase: Pre-coated silica gel GF 250
Mobile phase:

ISSN 2515-8260 Volume 07 Issue 11, 2020

Table 9: *TLC Mobile Phase*

Compound	**Mobile Phase**
Compound A	Benzene: Methanol, 4:1
Compounds B1- B7	Benzene: Methanol, 4:1
Compounds I.I- I.VII	Benzene: Ethyl Acetate, 4:1
Compounds P1-P7	Chloroform:Toluene 3.5:1.5

Detection : UV chamber, iodine chamber

6.7.2 Spectral Analysis

- IR Spectroscopy
- NMR Spectroscopy

Figure 16: *Synthesis Setup- Part C-I*

6.8 Bioactivity Evaulation:

6.8.1 Cell Viability Testing with Trypan Blue Exculsion Method:

Principal of Assay:

The Trypan Blue Dye Exclusion Method is used to decide the wide variety of practicable cells current in a cell suspension. It is primarily based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as Trypan blue, Eosin, or Propidium, whereas dead cells do not. The assay is based on the fact that the chromophore is negatively charged and does not have interaction with the cell unless the membrane is broken. When a cell suspension is simply blended with the dye and then visually examined to determine whether cells take up or exclude dye. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. (52-53)

ISSN 2515-8260 Volume 07 Issue 11, 2020

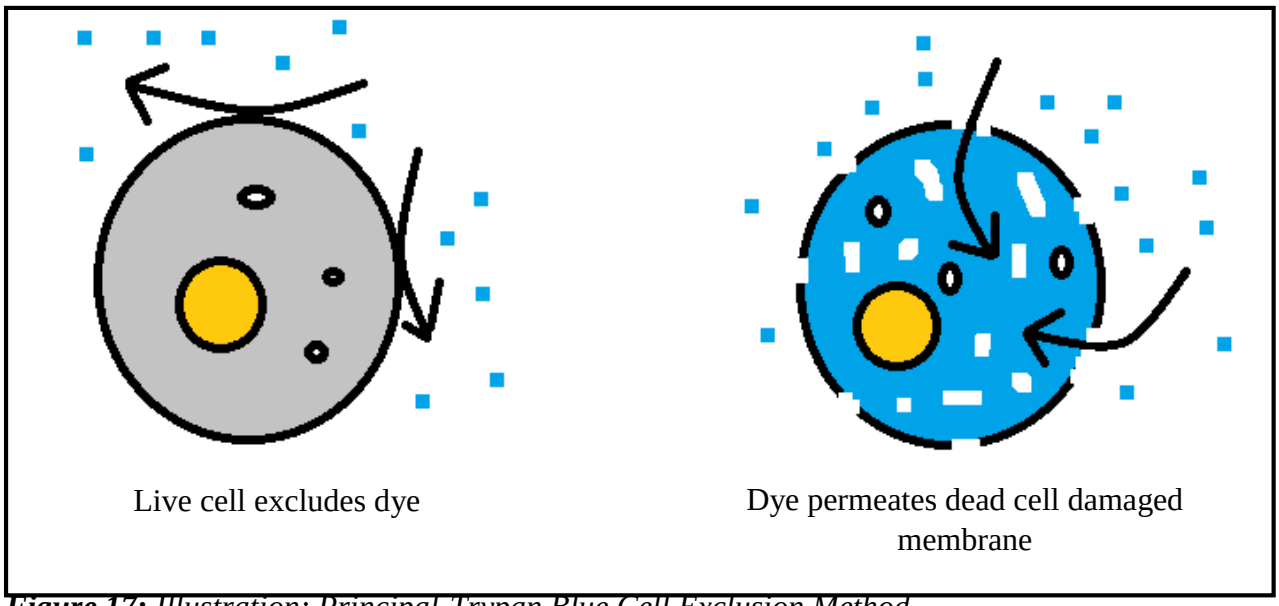


Figure 17: *Illustration: Principal-Trypan Blue Cell Exclusion Method*

Cell Line: HT29 (Colon Cancer, *Homo sapiens*)
Dye Preparation: Prepare 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.4 (Potassium Dihydrogen Phosphate Buffer)

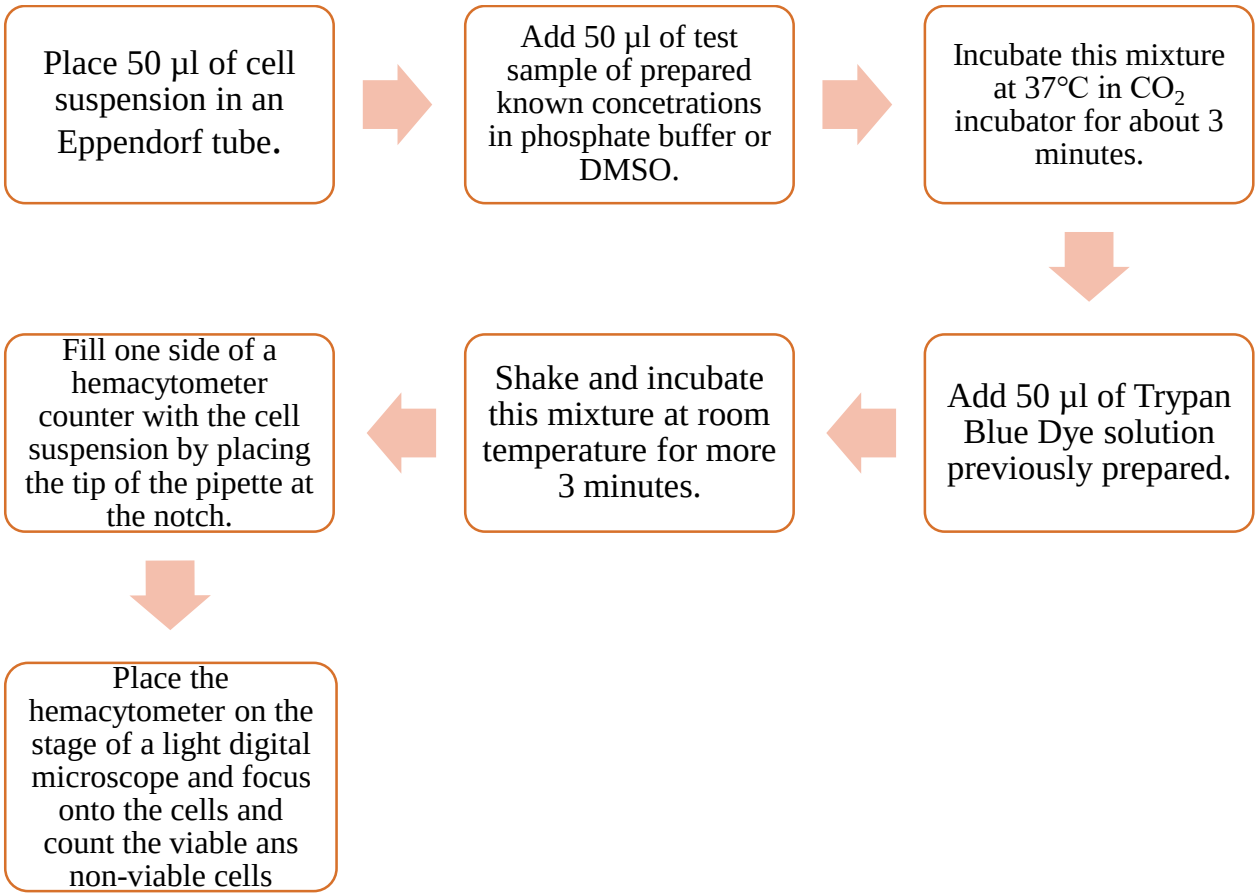


Figure 18: Procedure: Bioactivity Evaluation

ISSN 2515-8260 Volume 07 Issue 11, 2020

6.8.2 Procedure:

6.8.3 Calculations:

Calculate the percentage of viable cells by dividing the number of viable cells by the number of total cells and multiplying by 100 or

% viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100.

7 RESULTS AND DISCUSSION

7.1 Design of molecules for targeting Tyrosine Kinase Domain of Epidermal Growth Factor Receptor:

An array of data obtained from the literature survey states quinazolinones and its derivatives have been widely used for designing novel anticancer compounds. The FDA approved Tyrosine Kinas inhibitors too consists of quinazoline and quinazolinone as heterocyclic nucleus. The shape of binding pocket of Tyrosine Kinase domain on EGFR was identified using pocket modeling studies. The ligand was designed in such a way that it fits the binding pocket of the targeted site. Molecules targeting the specific site must achieve specific conformation in the binding cavity to obtain interaction. The PDB was downloaded from www.rcsb.org Protein Database Bank with PDB ID 1M17 and resolution of 2.60 A˚.

***Table 10**: Synthesized Compounds*

Compound Code	R1	R2	R3	R4
P1	H	H	H	H
P2	H	H	H	Cl
P3	H	H	H	F
P4	H	H	H	Br
P5	H	H	Cl	Cl
P6	H	H	H	OH
P7	H	H	H	OCH3

7.2 ADME prediction and Toxicity assessments of synthesized molecules:

Virtual Screening using in-silico computational approaches was carried out using available online virtual screening portals. The ADME properties of the designed lead molecules were determined, which help determine perfect drug-like candidate amongst the group and follows the Lipinski rule of five which includes hydrogen bond acceptor, hydrogen bond donor, log P and molecular weight. Using Swiss ADME portal physicochemical properties, ADME parameters, and pharmacokinetic properties of the synthesized molecules were computed and thereby serves support to drug discovery and drug design.

***Table 11:** ADME Prediction*

CODE	Molecular Weight (g/mol)	Rotatable bonds	H-bond acceptors	H-bond donors	Log P	Bioavailability Score
P1	311.38	1	2	1	3.50	0.55
P2	345.83	1	2	1	3.98	0.55
P3	329.37	1	3	1	3.77	0.55
P4	390.28	1	2	1	4.07	0.55
P5	380.27	1	2	1	4.51	0.55
P6	327.38	1	3	2	3.07	0.55
P7	341.41	2	3	1	3.46	0.55

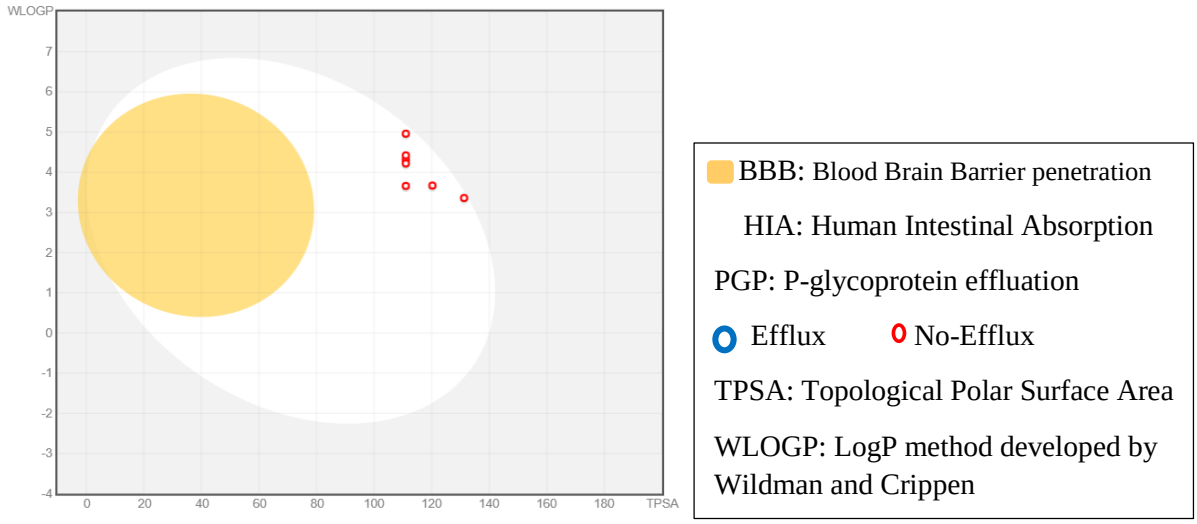


Figure 19: Brain *Or IntestinaL EstimateD permeation method (BOILED-Egg) Illustration for ADME Prediction WLOGP V/S TPSA*

7.3 Toxicity Assessments:

The in-silico toxicity prediction was carried out using Lazar online toxicity prediction portal. The designed lead molecules were assessed for their toxicity virtually to determine the toxicity in respect to acute toxicity, BBB penetration, carcinogenicity, lowest observed adverse level effect, maximum recommended daily dose and mutagenicity where all the synthesized compounds were screened and found as non-toxic.

7.4 Docking Analysis of Synthesized Ligands:

Table 12*: Docking Analysis*

Sr. No.	Compound Code	Docking Score
1.	P1	-5.0
2.	P2	-4.8
3.	P3	-5.3
4.	P4	-4.7
5.	P5	-5.0
6.	P6	-5.1
7.	P7	-6.0

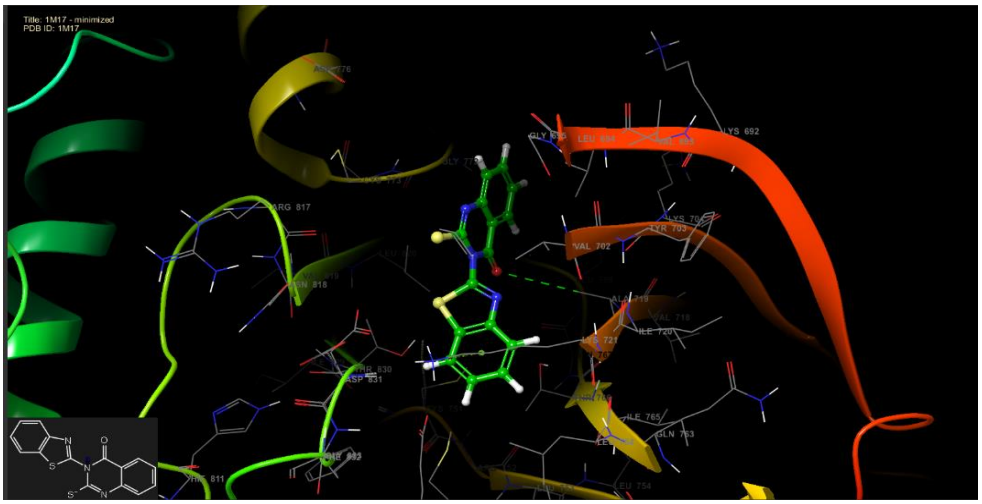


Figure 20*: Docking Pose of Molecule P1 with PDB ID: 1M17*

ISSN 2515-8260 Volume 07 Issue 11, 2020

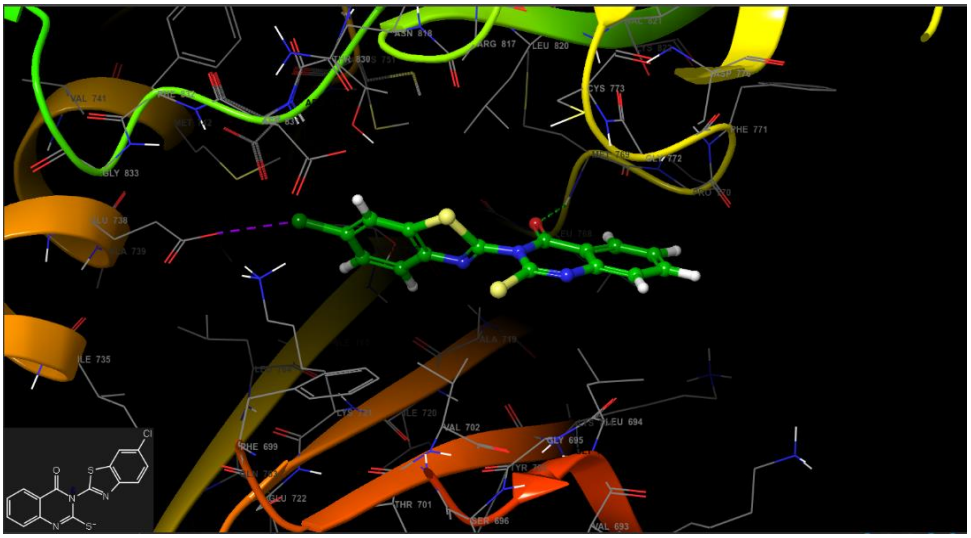


***Figure 21**: Docking Pose of Molecule P2 with PDB ID: 1M17*

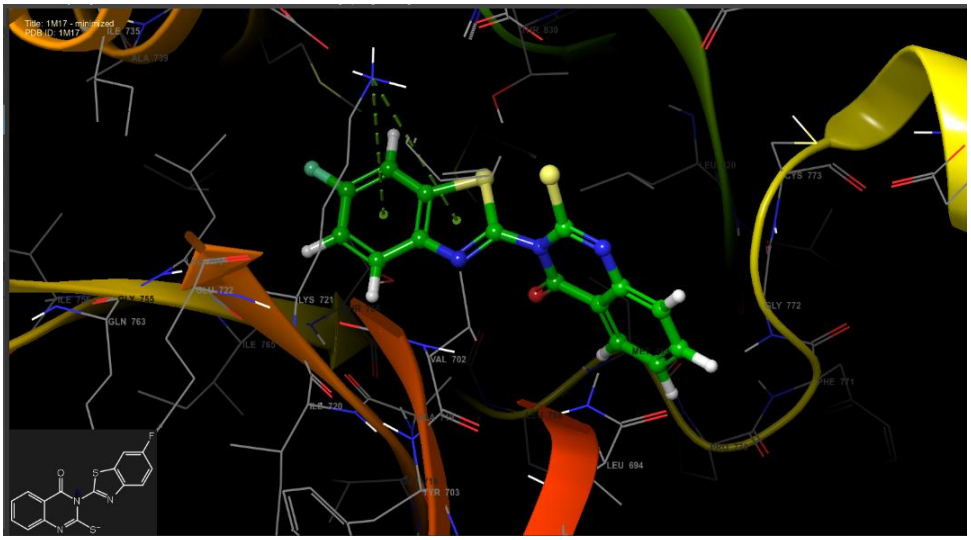


***Figure 22:** Docking Pose of Molecule P3 with PDB ID: 1M17*

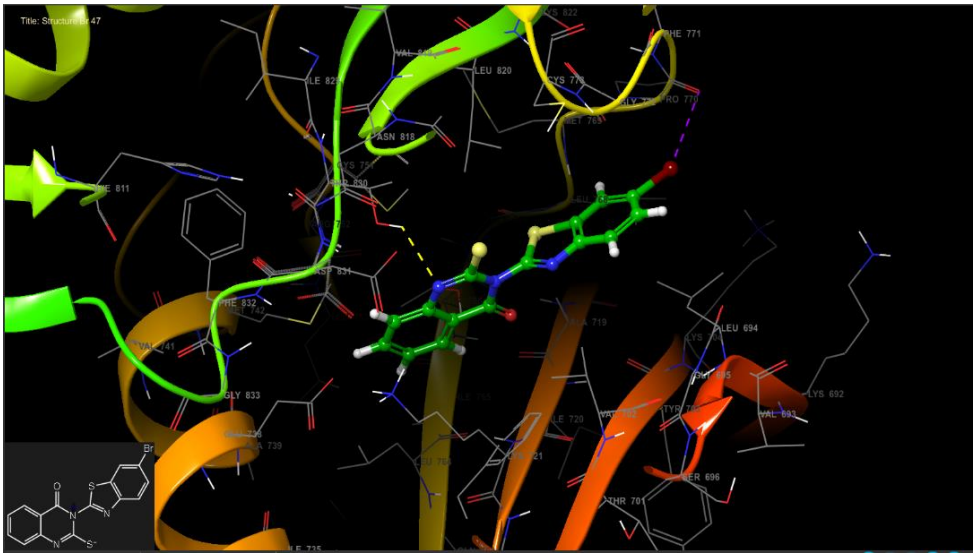


***Figure 23:** Docking Pose of Molecule P4 with PDB ID: 1M17*

ISSN 2515-8260 Volume 07 Issue 11, 2020

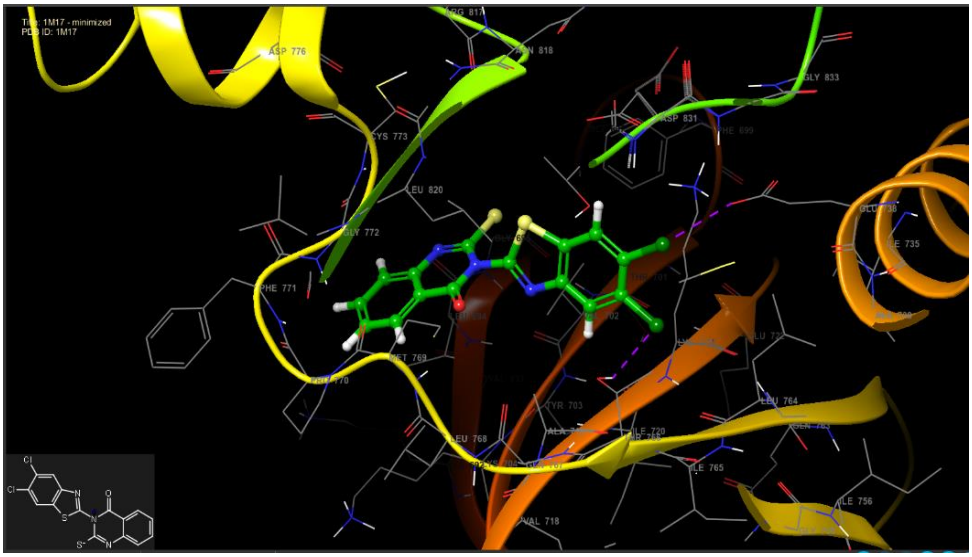


Figure 24: *Docking Pose of Molecule P5 with PDB ID: 1M17*

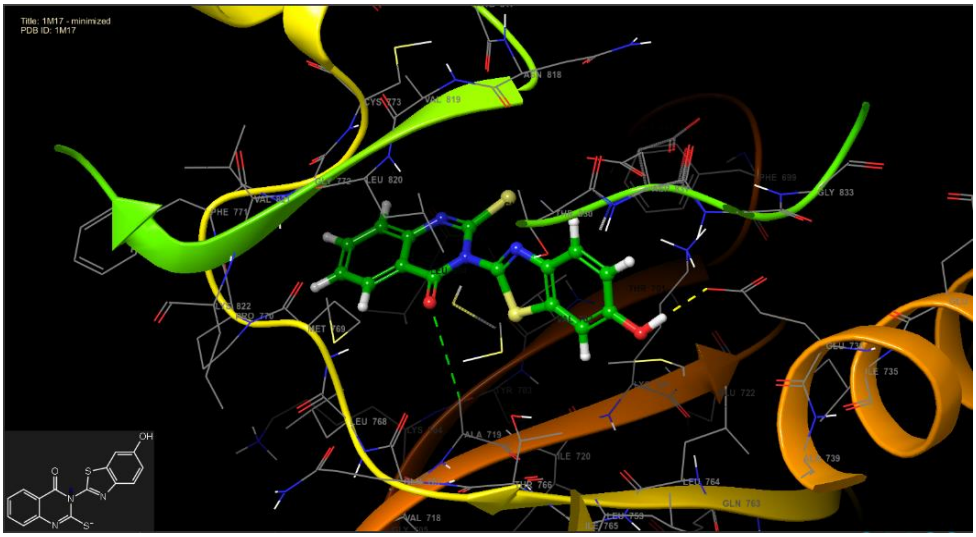


Figure 25*: Docking Pose of Molecule P6 with PDB ID: 1M17*

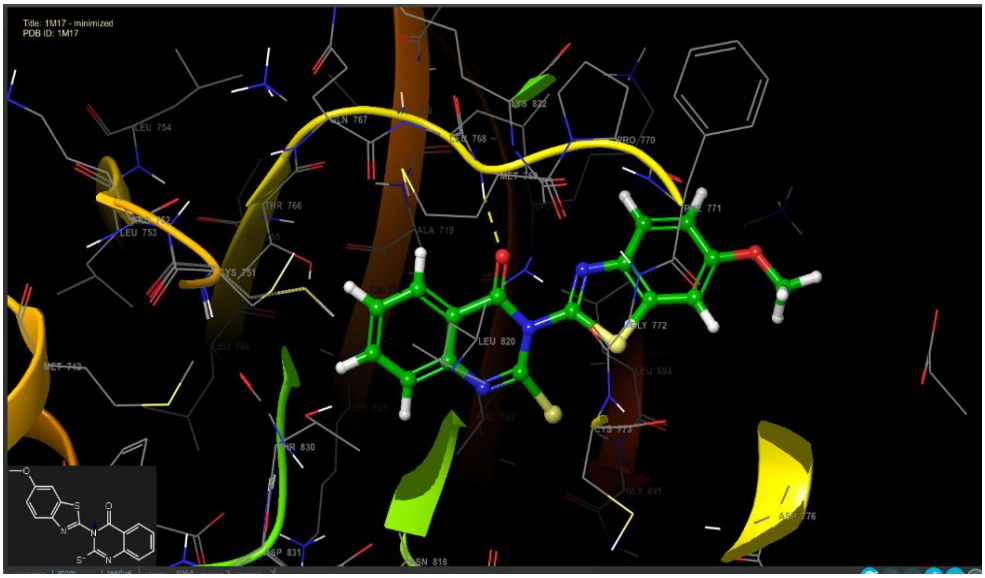
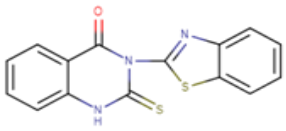
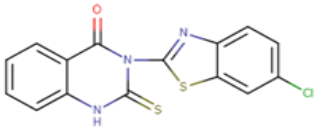
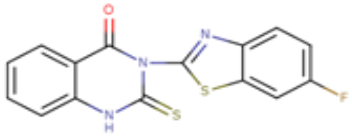
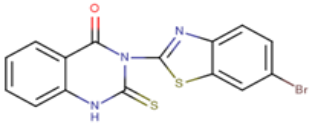
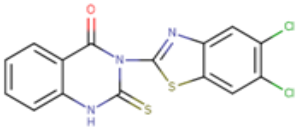
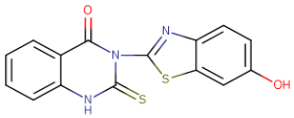
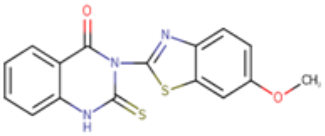


Figure 26: *Docking Pose of Molecule P7 with PDB ID: 1M17*

7.5 Identification and Characterization:

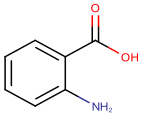
***Table 13**: Final Compounds*

Compound Code	R1	R2	R3	R4	Structure
P1	H	H	H	H	
P2	H	H	H	Cl	
P3	H	H	H	F	
P4	H	H	H	Br	
P5	H	H	Cl	Cl	
P6	H	H	H	OH	
P7	H	H	H	OCH3	

7.5.1 Physicochemical Properties:

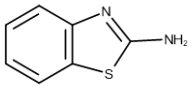
PART A:

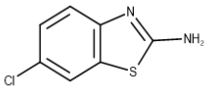
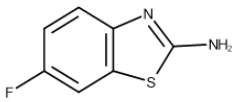
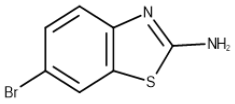
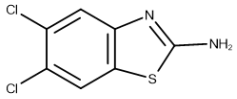
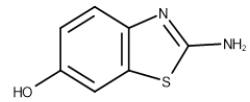
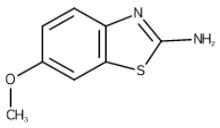
***Table 14:** Physicochemical Characterization (A)*

Compound Code	Structure	Molecular Formula	MW (g/mol)	Colour	MP (°C)	Rf Value	Practical Yield (%)
A		$C_7H_7NO_2$	137.04	Grey-white	138-140	0.64	84.70%

PART B:

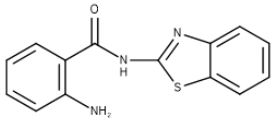
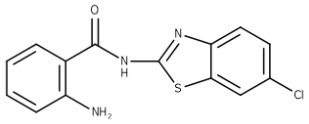
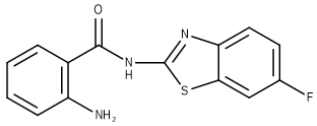
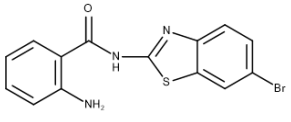
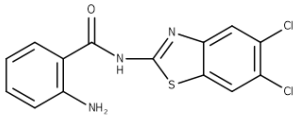
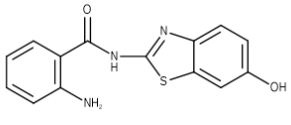
***Table 15:** Physiochemical Characterization (B1-B7)*

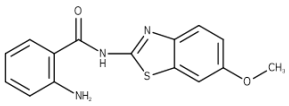
Compound Code	Structure	Molecular Formula	MW (g/mol)	Colour	MP (°C)	Rf Value	Practical Yield (%)
B1		$C_7H_6N_2S$	150.20	Cream	132-134	0.67	82.66

B2		$C_7H_5ClN_2S$	184.65	Cream	196-198	0.72	63.84
B3		$C_7H_5FN_2S$	168.19	Cream	180-182	0.68	83.52
B4		$C_7H_5BrN_2S$	229.10	Pale Green	204-206	0.66	41.04
B5		$C_7H_4Cl_2N_2S$	219.08	Pale Yellow	186-188	0.59	59.41
B6		$C_7H_6N_2OS$	166.20	Cream	232-234	0.48	76.38
B7		$C_8H_8N_2OS$	180.23	Pale Brown	170-172	0.52	62.22

PART C: I

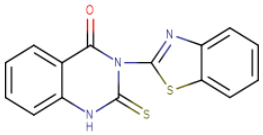
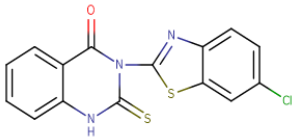
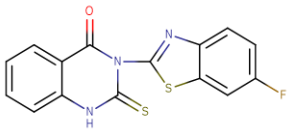
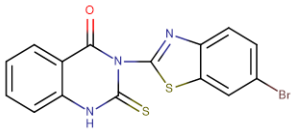
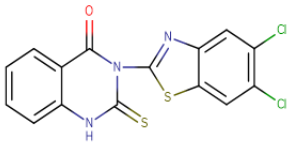
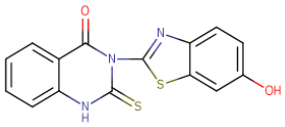
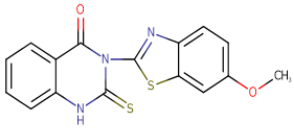
Table 16: *Physiochemical Characterization (I.I-I.VII)*

Code	Structure	Molecular Formula	MW (g/mol)	Colour	MP (°C)	Rf Value	Practical Yield (%)
I.I		$C_{14}H_{11}N_3OS$	269.32	Grey	130-132	0.88	64.66
I.II		$C_{14}H_{10}ClN_3OS$	303.76	Pale Yellow	154-156	0.89	77.65
I.III		$C_{14}H_{10}FN_3OS$	287.31	Pale Yellow	165-167	0.70	84.77
I.IV		$C_{14}H_{10}BrN_3OS$	348.22	Grey	162-164	0.69	78.74
I.V		$C_{14}H_9Cl_2N_3OS$	338.21	Cream	175-177	0.53	81.58
I.VI		$C_{14}H_{11}N_3O_2S$	285.32	Grey	145-148	0.64	56.85

I.VII		$C_{15}H_{13}N_3O_2S$	299.35	Dull Grey	176-178	0.71	78.51

II: Final Compound

Table 17: *Physiochemical Characterization (P1-P7)*

Code	Structure	Molecular Formula	MW (g/mol)	Colour	MP (°C)	Rf Value	Practical Yield (%)
P1		$C_{15}H_9N_3OS_2$	311.38	Off-White	152-154	0.58	42.85
P2		$C_{15}H_8ClN_3OS_2$	345.83	Pale yellow (Buff)	146-148	0.42	53.50
P3		$C_{15}H_8FN_3OS_2$	329.37	Pale grey	142-144	0.53	86.46
P4		$C_{15}H_8BrN_3OS_2$	390.28	Pale green	196-198	0.48	43.75
P5		$C_{15}H_7Cl_2N_3OS_2$	380.27	Deep Yellow	188-190	0.33	58.03
P6		$C_{15}H_9N_3O_2S_2$	327.38	Pale grey	202-204	0.44	51.52
P7		$C_{16}H_{11}N_3O_2S_2$	341.41	Yellowish grey	156-158	0.38	75.31

7.5.2 Characterization:

IR and NMR interpretation of synthesized compounds:

- The IR spectra shows the presence of the functional groups at particular stretching frequencies.
- The spectra are assigned to each functional group after interpretation by comparing to the reported standard values.
- The 1H NMR spectra gives data about the proton and neighbouring proton and depicts change in chemical shifts due to presence of functional groups and substituents.

ISSN 2515-8260 Volume 07 Issue 11, 2020

IR of Compound A:

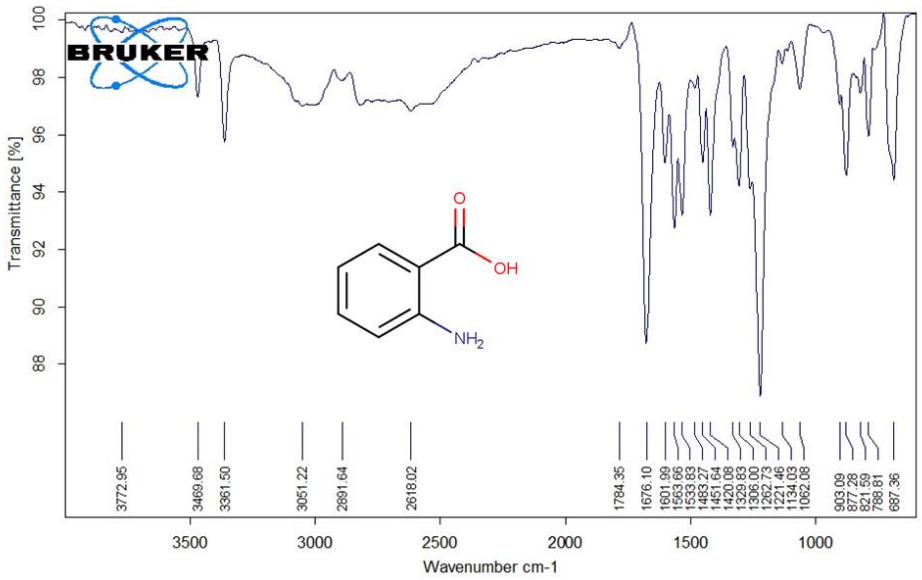
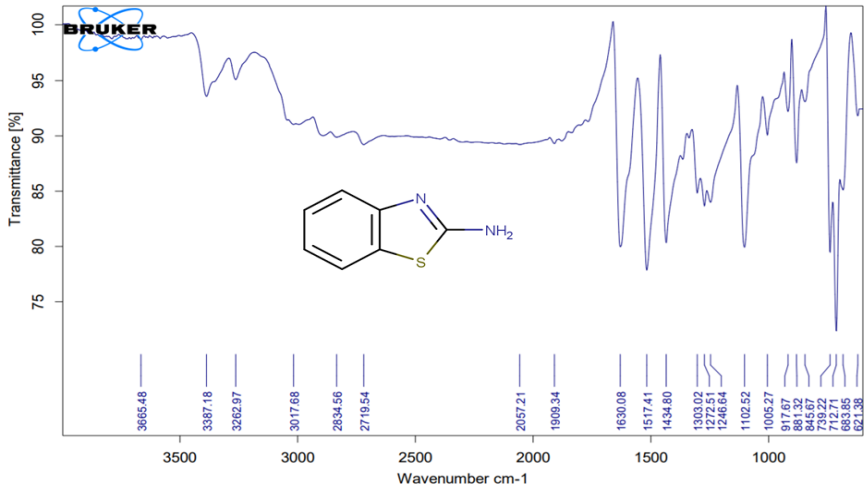


Figure 27: *IR Spectra of Compound A*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
-NH Stretch	3200-3400	3361.50, 3469.68
C=0	1760-1790	1784.35
-OH	2500-3300	3051.22



Compounds B: B1: IR

Figure 28: *IR Spectra of Compound B1*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
-NH Stretch	3200-3450	3262.97, 3387.18
N-H Bend	1580-1650	1630.08
C-N	1260-1342	1272.51
C-S	1215-1275	1246.64

B1: NMR

ISSN 2515-8260 Volume 07 Issue 11, 2020

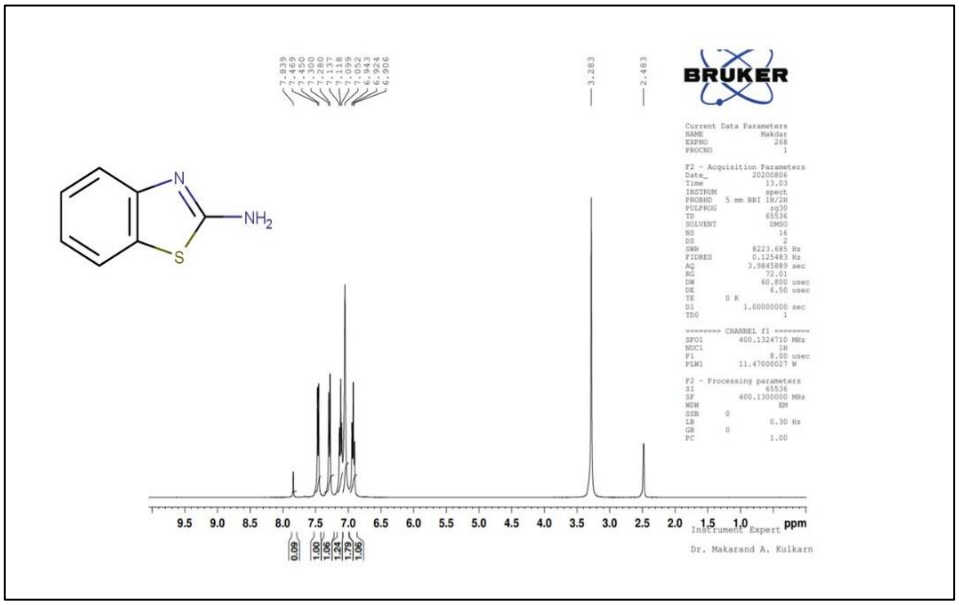


Figure 29: *NMR Spectra of Compound B1*

Proton	Chemical Shift (δ)
Ar-H	6.2-8.5 (6.924, 7.118, 7.450)
Ar-NH2	3-5 (3.28)

B2: IR

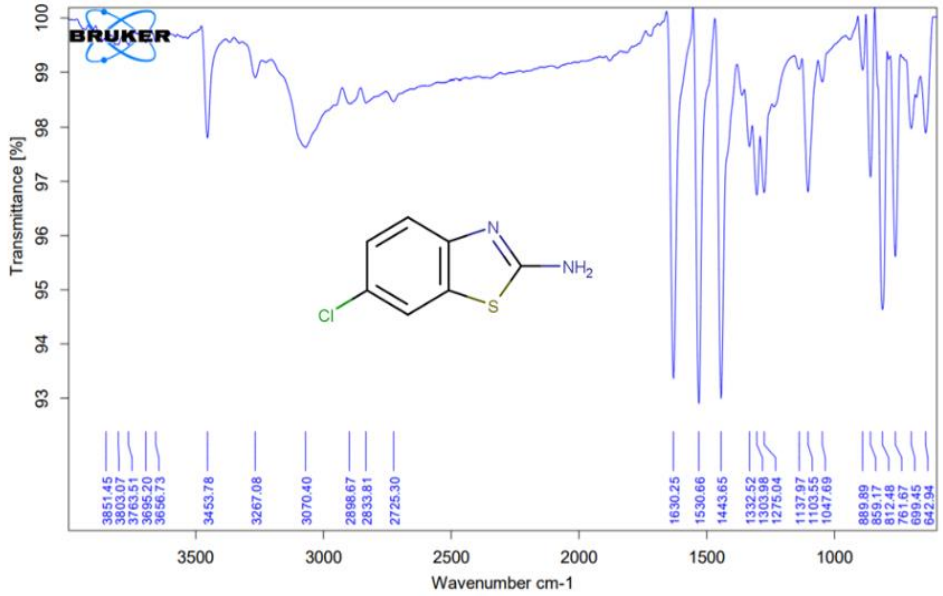


Figure 30: *IR Spectra of Compound B2*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
-NH_2	3200-3450	3267.08, 3453.78
C-N	1260-1342	1303.98
C-S	1215-1275	1275.04
C-Cl	550-850	812.48

B3: IR

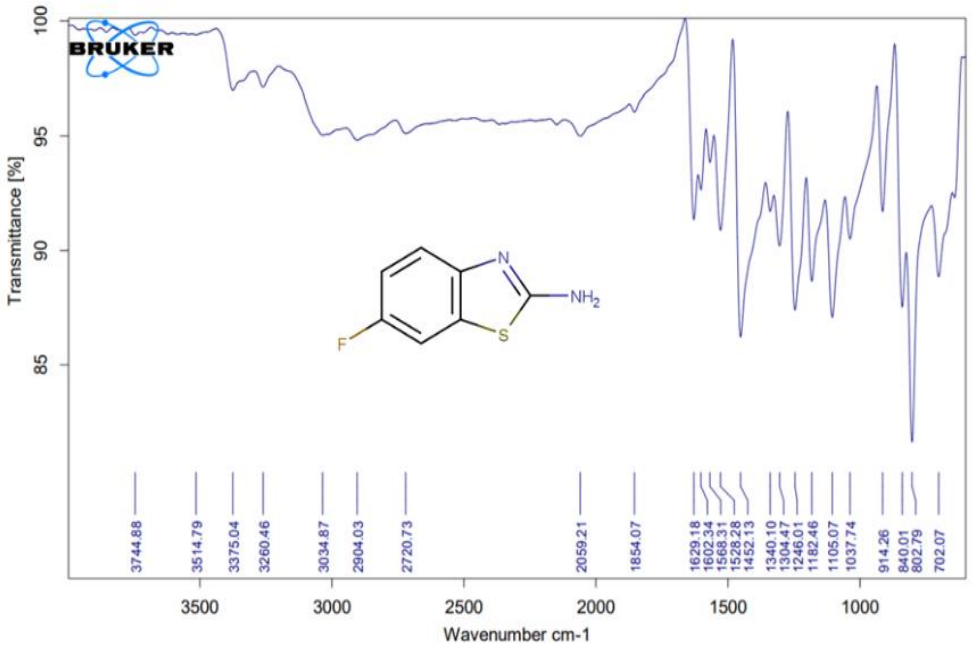


Figure 31: *IR Spectra of Compound B3*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3260.46, 3375.04
C-N	1260-1342	1340.10
C-S	1215-1275	1245.01
C-F	1000-1400	1105.07

B4: IR

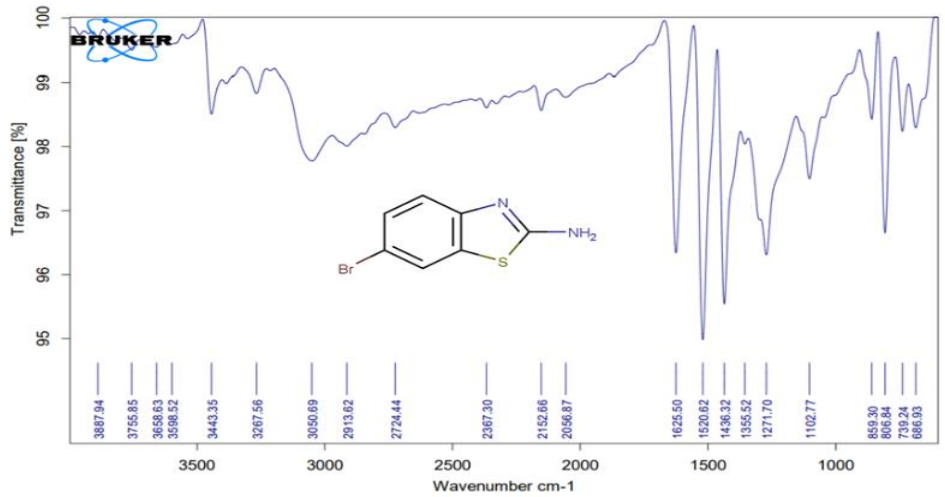


Figure 32: *IR Spectra of Compound B4*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3267.56, 3443.35
C-N	1260-1342	1355.52
C-S	1215-1275	1271.70
C-Br	515-690	686.93

B5: IR

ISSN 2515-8260 Volume 07 Issue 11, 2020

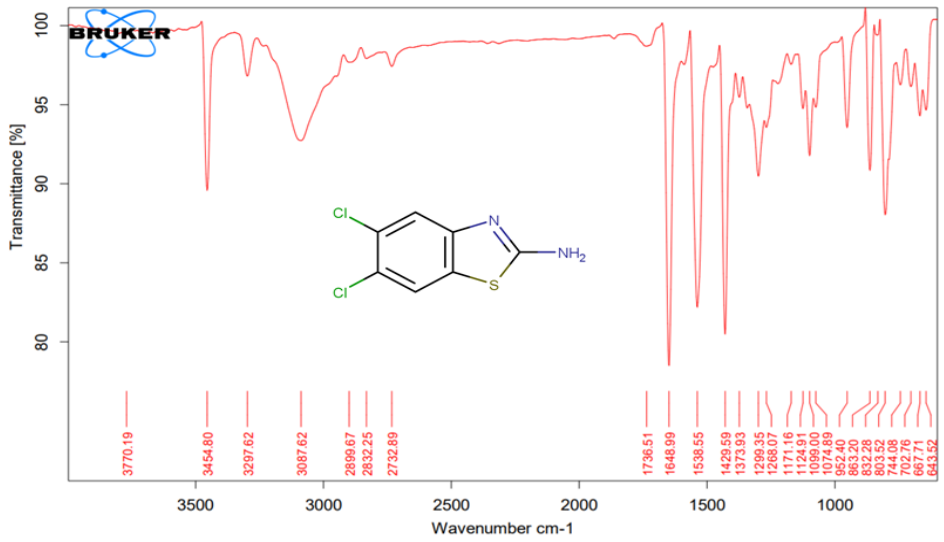


Figure 33: *IR Spectra of Compound B5*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3297.62, 3454.80
C-N	1260-1342	1299.35
C-S	1215-1275	1268.07
C-Cl	550-850	803.52

B6: IR

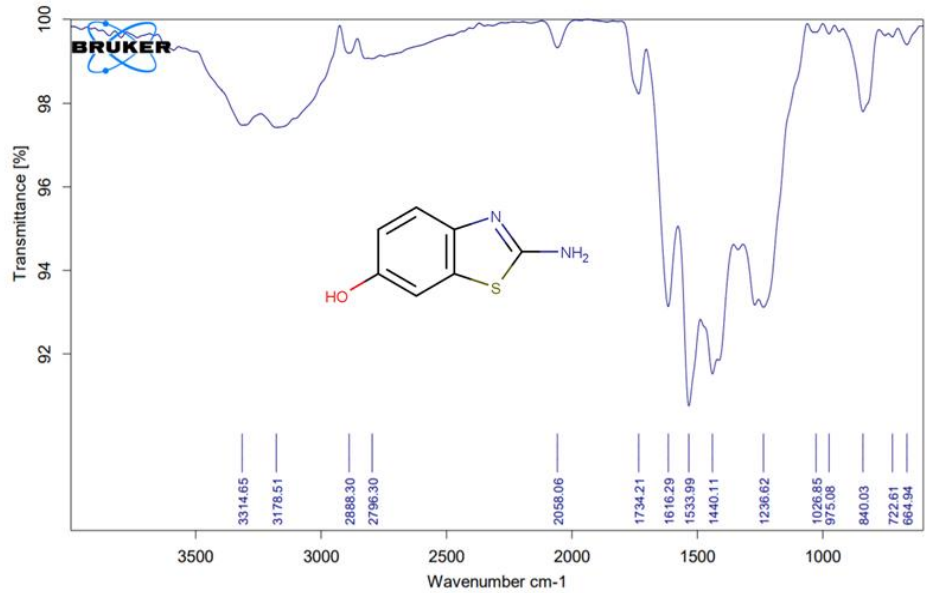


Figure 34: *IR Spectra of Compound B6*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3178.51, 3314.65
C-N	1260-1342	1440.11
C-S	1215-1275	1236.62
OH	3200-3600	3314.65

B7: IR

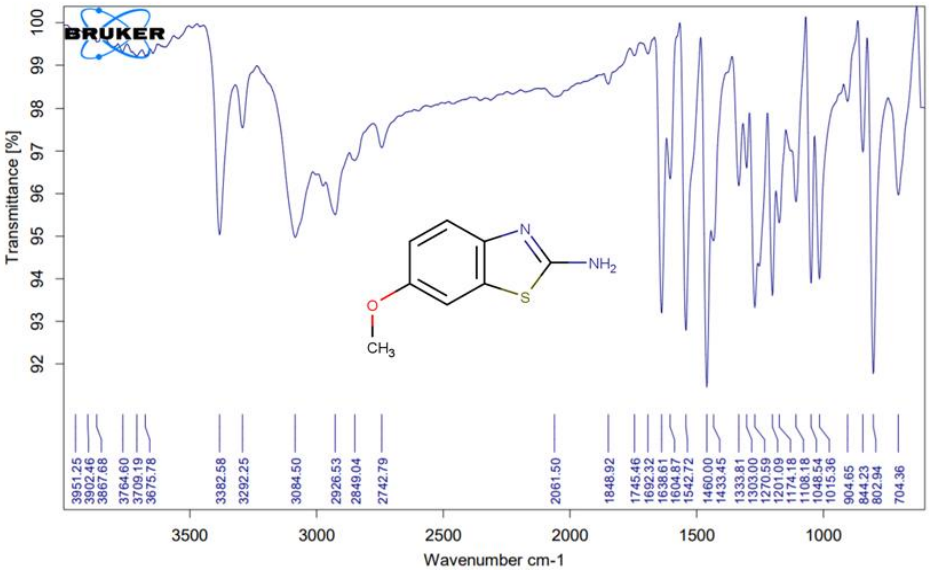


***Figure 35**: IR Spectra of Compound B7*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
-NH2	3200-3450	3292.25, 3382.58
C-N	1260-1342	1303.00
C-S	1215-1275	1270.59
-O-CH_3	2815-2850	2849.04

PART C:
Compound I.I: IR

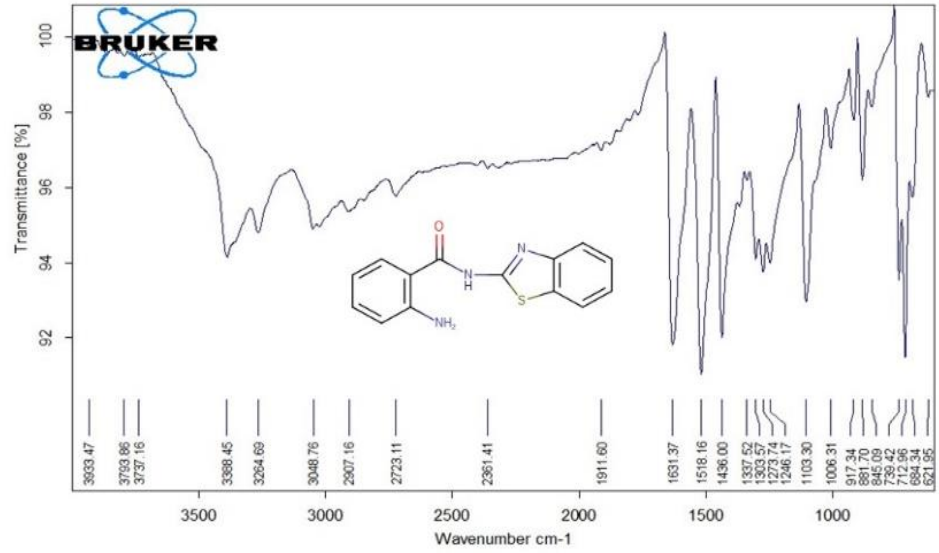


***Figure 36:** IR Spectra of Compound I.I*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3264.69, 3388.45
C=O	1630-1690	1631.37
C-S	1215-1275	1246.17
N-H (amide)	3300-3600	3388.45

Compound I.I: NMR

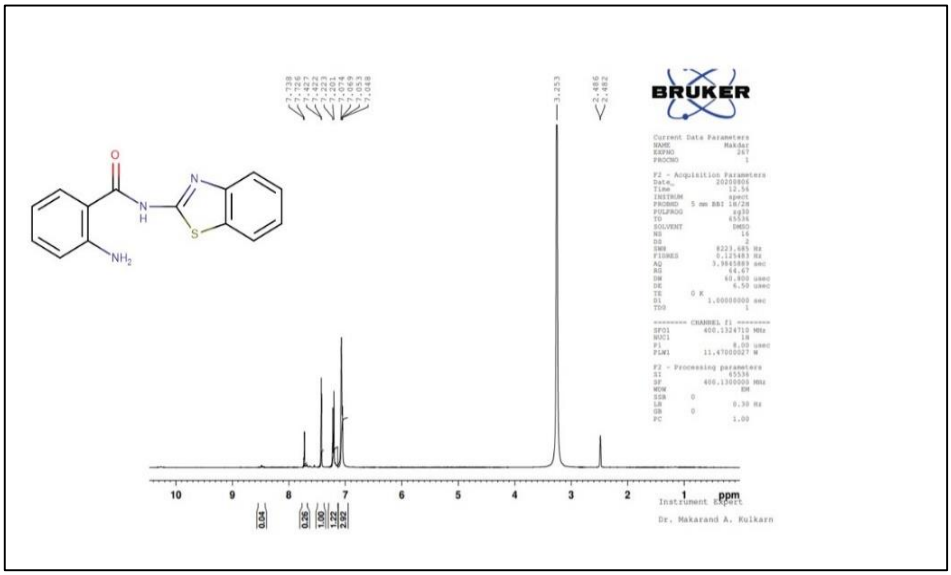


Figure 37: *NMR Spectra of Compound I.I*

Proton	**Chemical Shift (δ)**
Ar-H	6.2-8.5 (7.048, 7.074, 7.427)
Ar-NH_2	3-5 (3.253)
N-H Amide	7-8.5 (7.738)

Compound I.II

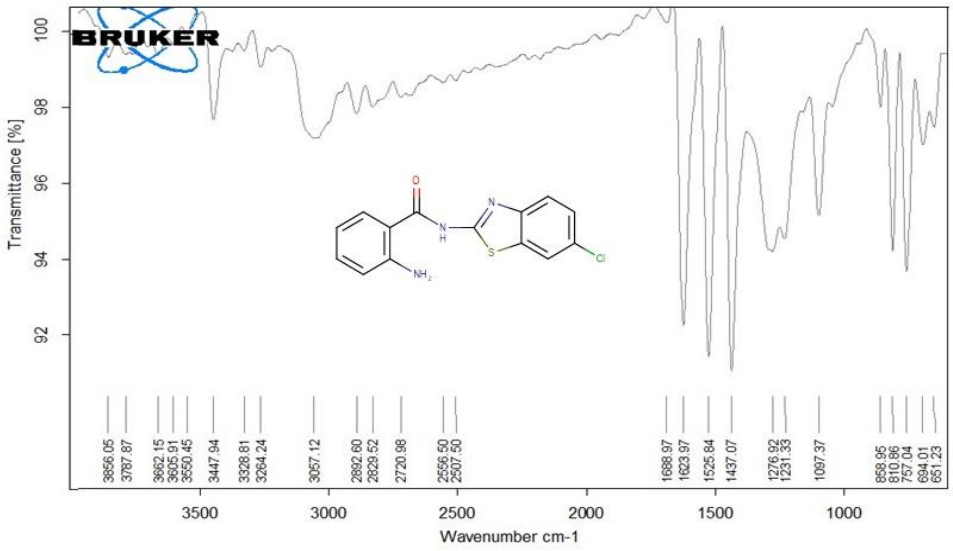


Figure 38*: IR Spectra of Compound I.II*

Functional Group	**Standard Frequency (cm^{-1})**	**Observed Frequency (cm^{-1})**
NH_2	3200-3450	3328.81, 3447.97
C=O	1630-1690	1688.97
C-S	1215-1275	1231.33
N-H (amide)	3300-3600	3550.45
C-Cl	550-850	810.86

Compound I.III

ISSN 2515-8260 Volume 07 Issue 11, 2020

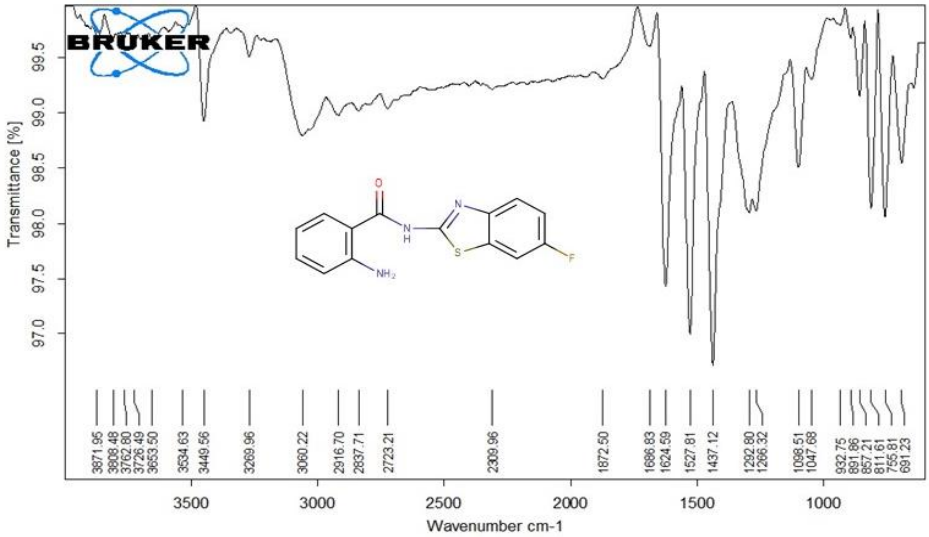


Figure 39: *IR Spectra of Compound I.III*

Functional Group	Standard Frequency(cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3269.96, 3449.56
C=O	1630-1690	1686.86
C-S	1215-1275	1266.32
N-H (amide)	3300-3600	3534.63
C-F	1000-1400	1047.68

Compound I.IV

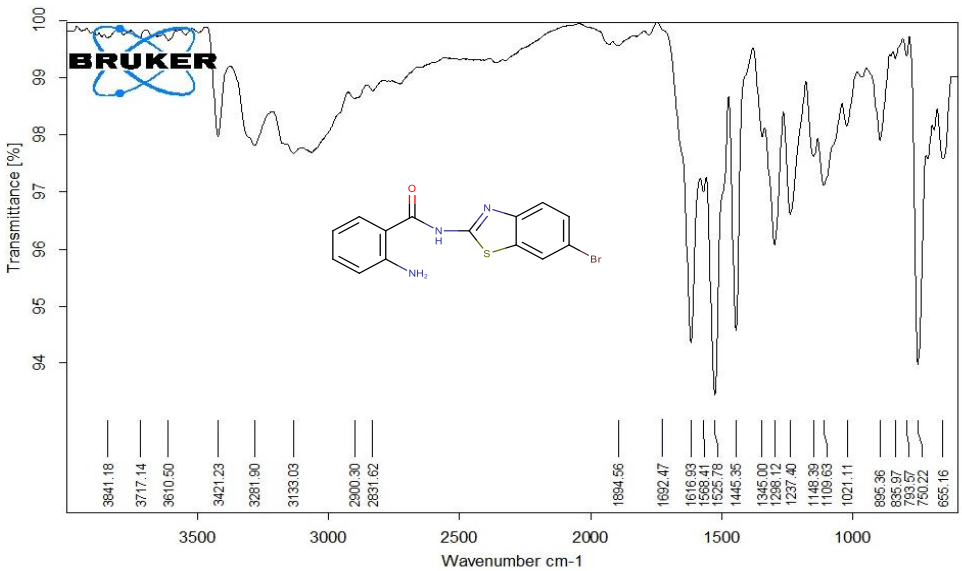


Figure 40: *IR Spectra of Compound I.IV*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3281.90, 3421.23
C=O	1630-1690	1692.47
C-S	1215-1275	1237.40
N-H (amide)	3300-3600	3610.50
C-Br	515-690	655.16

Compound I.V

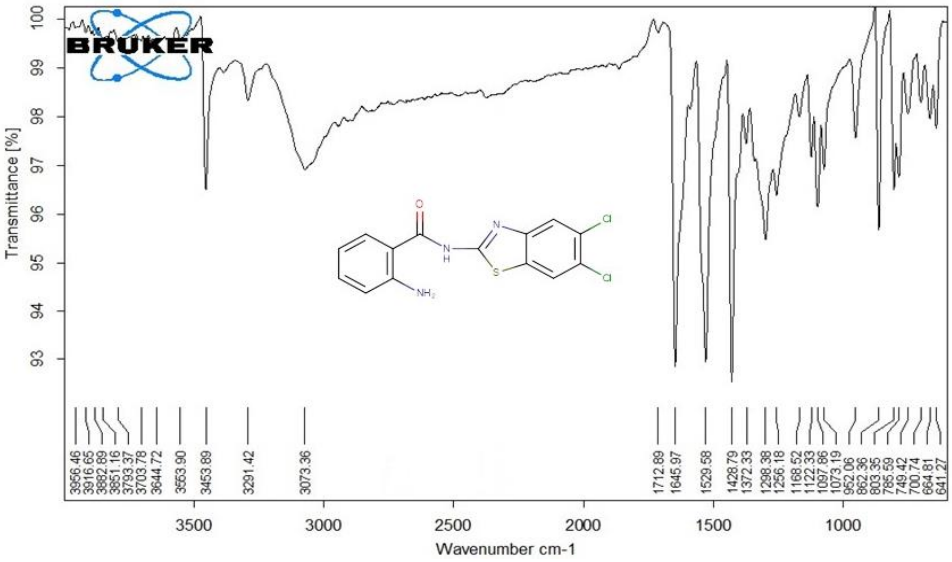


Figure 41: *IR Spectra of Compound I.V*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3391.42, 3453.89
C=O	1630-1690	1645.97
C-S	1215-1275	1256.18
N-H (amide)	3300-3600	3553.90
C-Cl	550-850	803.35

Compound I.VI

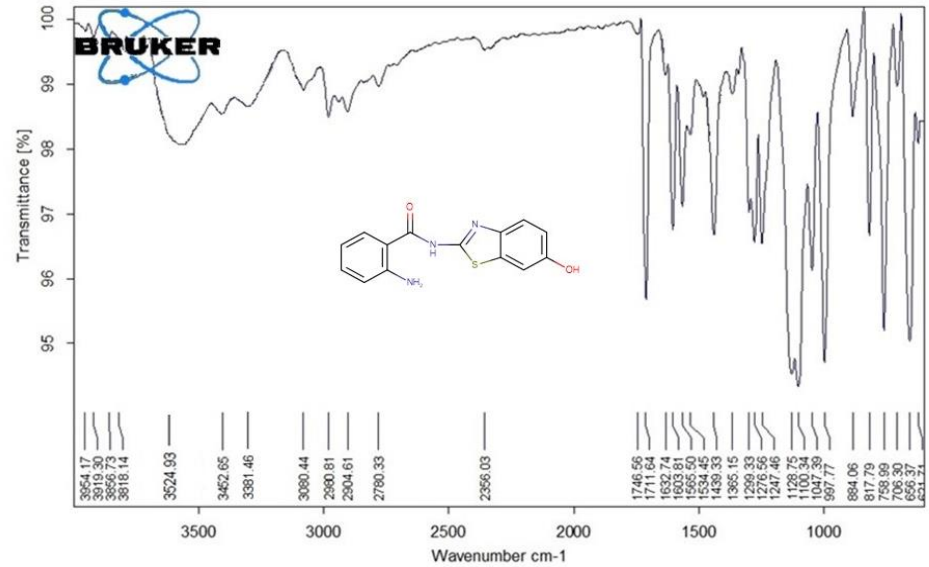


Figure 42: *IR Spectra of Compound I.VI*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3381.46, 3452.65
C=O	1630-1690	1632.74
C-S	1215-1275	1247.46
N-H (amide)	3300-3500	3452.65
OH	3500-3700	3524.93

Compound I.VII

ISSN 2515-8260 Volume 07 Issue 11, 2020

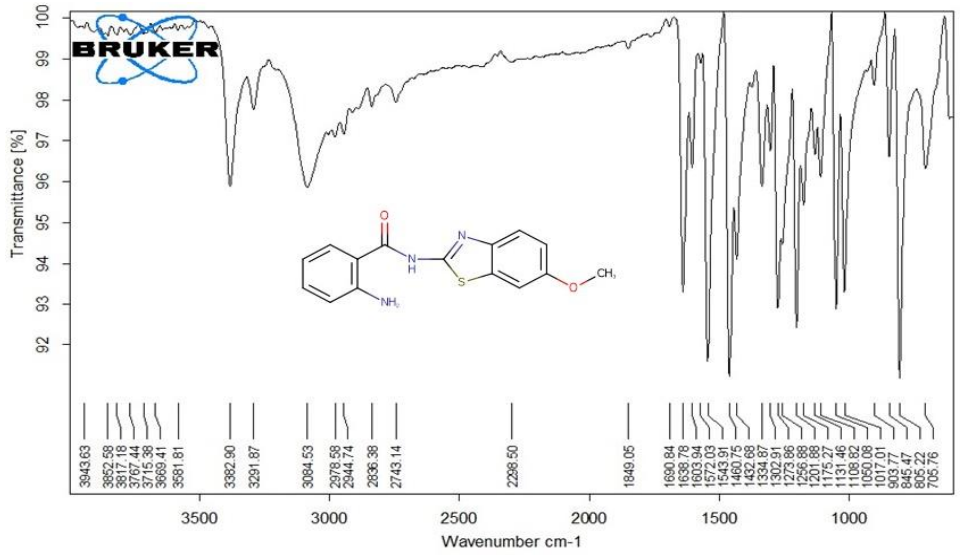


Figure 43: *IR Spectra of Compound I.VII*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
NH_2	3200-3450	3291.87, 3382.90
C=O	1630-1690	1690.84
C-S	1215-1275	1256.88
N-H (amide)	3300-3600	3581.81
O-CH3	2815-2850	2836.38

IR of Compound P1:

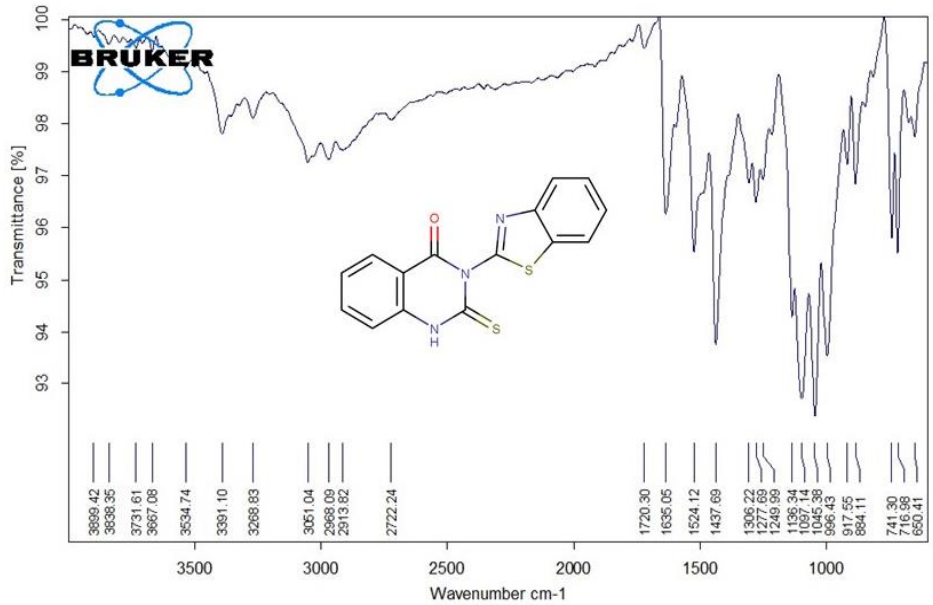


Figure 44: *IR Spectra of Compound P1*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3391.10
N-H wag	665-910	741.30
C=O	1665-1760	1720.30
C=S	1050-1200	1045.38
N-H bend	1500-1640	1524.12

IR of Compound P2:

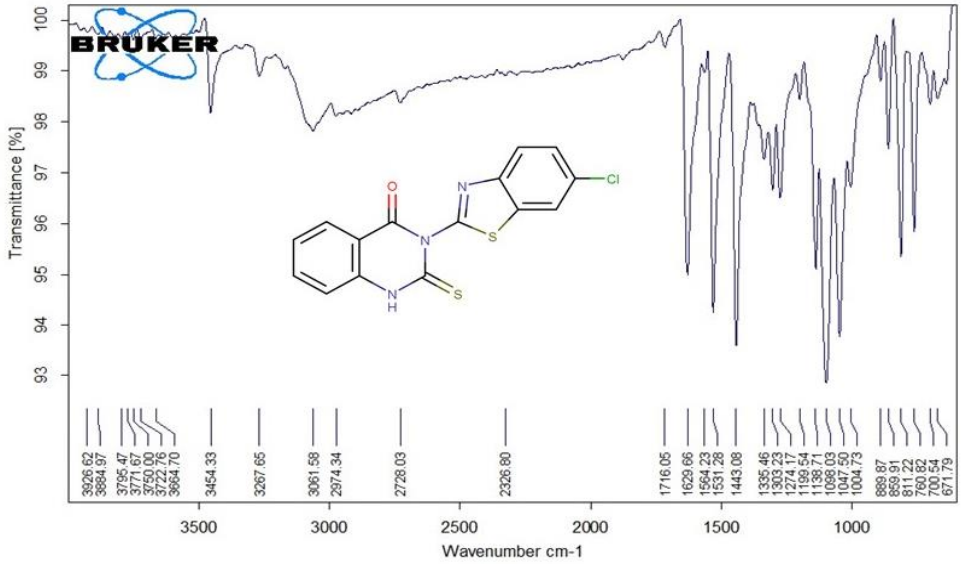


Figure 45: *IR Spectra of Compound P2*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3454.33
N-H bend	1500-1640	1629.66
C=O	1665-1760	1716.05
C=S	1050-1200	1098.03
C-Cl	550-850	811.22

NMR of Compound P2:

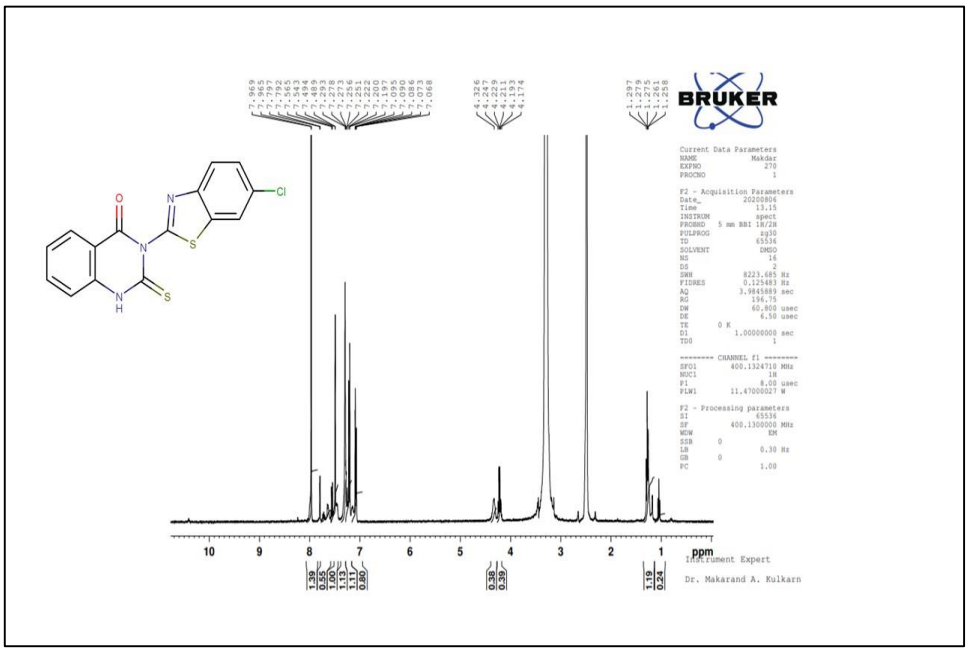


Figure 46: *NMR Spectra of Compound P2*

Proton	Chemical Shift (δ)
Ar-H	6.2-8.5 (7.090, 7.251, 7.293, 7.792, 7.969)
Ar- N-H	3-6.5 (4.211)

IR of Compound P3:

ISSN 2515-8260 Volume 07 Issue 11, 2020

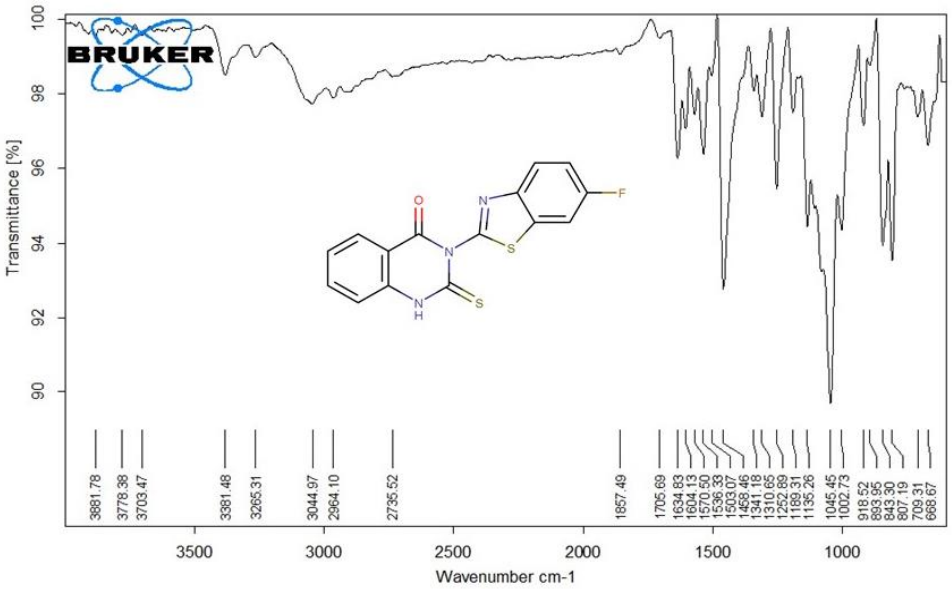


Figure 47: *IR Spectra of Compound P3*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3381.48
N-H bend	1500-1640	1536.33
C=O	1665-1760	1705.69
C=S	1050-1200	1045.45
C-F	1000-1400	1002.73

NMR of Compound P3:

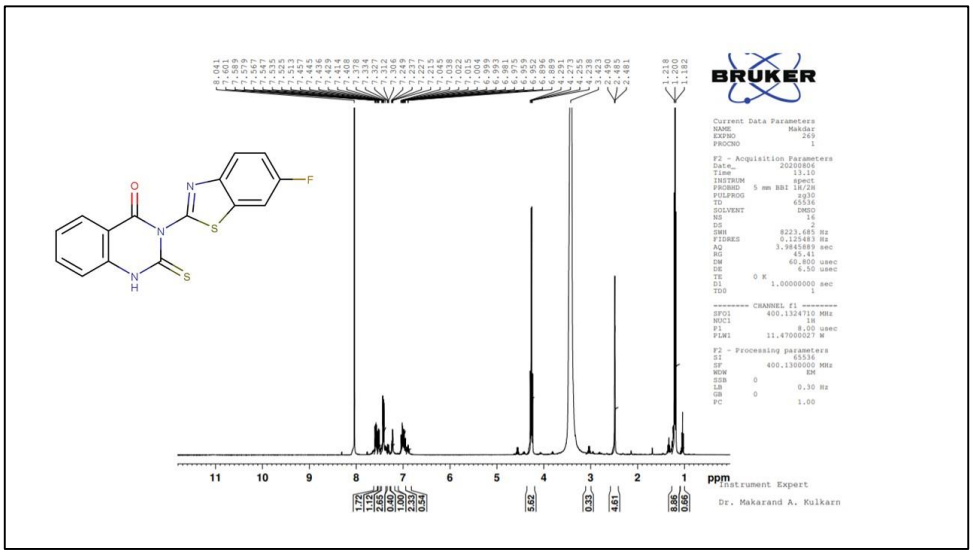


Figure 48: *NMR Spectra of Compound P3*

Proton	Chemical Shift (δ)
Ar-H	6.2-8.5 (7.022, 7.227, 7.334, 8.041)
Ar- N-H	3-6.5 (4.291)

IR of Compound P4:

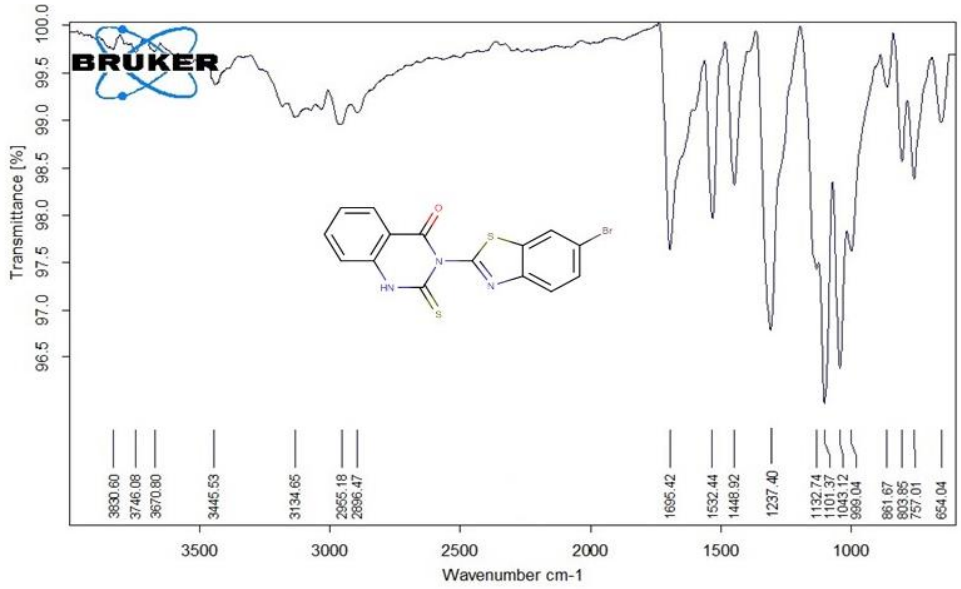


Figure 49: *IR Spectra of Compound P4*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3445.53
N-H bend	1500-1640	1532.44
C=O	1665-1760	1695.42
C=S	1050-1200	1101.37
C-Br	515-690	654.04

IR of Compound P5:

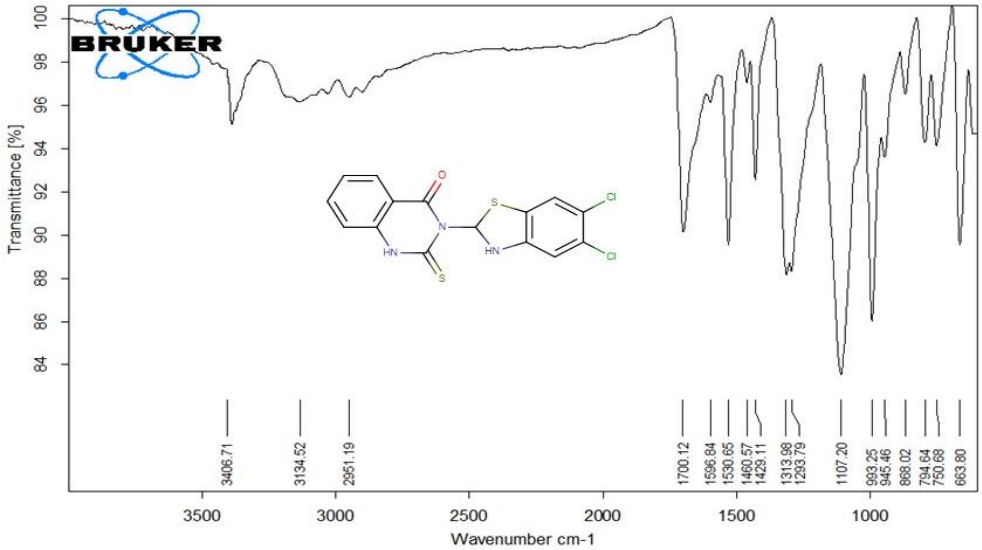


Figure 50: *IR Spectra of Compound P5*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3406.71
N-H bend	1500-1640	1530.65
C=O	1665-1760	1700.12
C=S	1050-1200	1107.20
C-Cl	550-850	663.80

NMR of Compound P5:

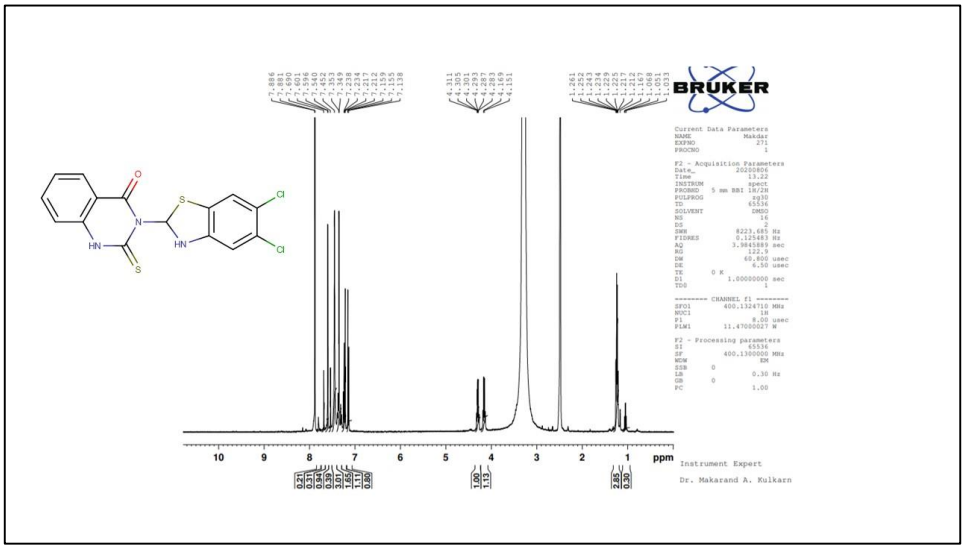


Figure 51: *NMR Spectra of Compound P5*

Proton	Chemical Shift (δ)
Ar-H	6.2-8.5 (7.155, 7.212, 7.238, 7.596, 7.886)
Ar- N-H	3-6.5 (4.293)

IR of Compound P6:

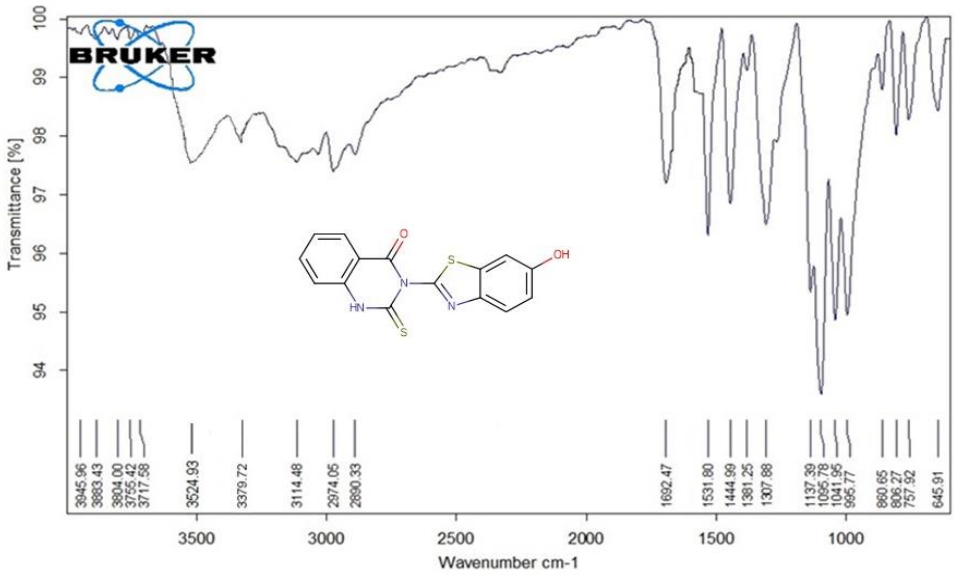


Figure 52: *IR Spectra of Compound P6*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3379.72
N-H bend	1500-1640	1531.80
C=O	1665-1760	1692.47
C=S	1050-1200	1095.78
OH	3500-3700	3524.93

IR of Compound P7:

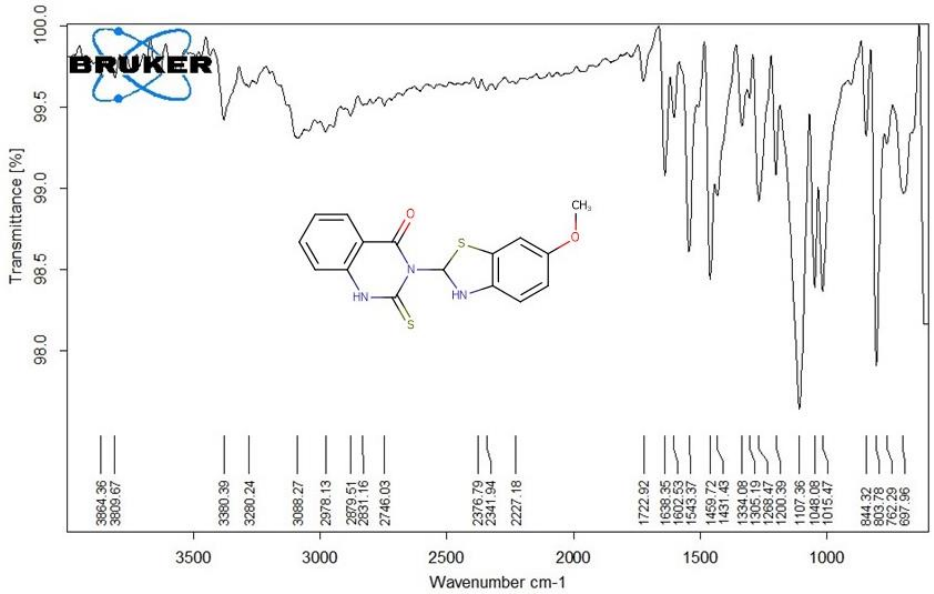


Figure 53: *IR Spectra of Compound P7*

Functional Group	Standard Frequency (cm^{-1})	Observed Frequency (cm^{-1})
N-H stretch	3300-3500	3380.39
N-H bend	1500-1640	1638.35
C=O	1665-1760	1722.92
C=S	1050-1200	1107.36
O-CH3	2815-2850	2831.16

7.6 Pharmacological Assessment:
Anticancer Activity: Cell Cytotoxicity and Cell Viability Assay
Method: Dye Exclusion
Cell-Line: HT29 (Human Colon Cancer Adenocarcinoma)
Dye: Trypan Blue
Standard: 5-Fluorouracil

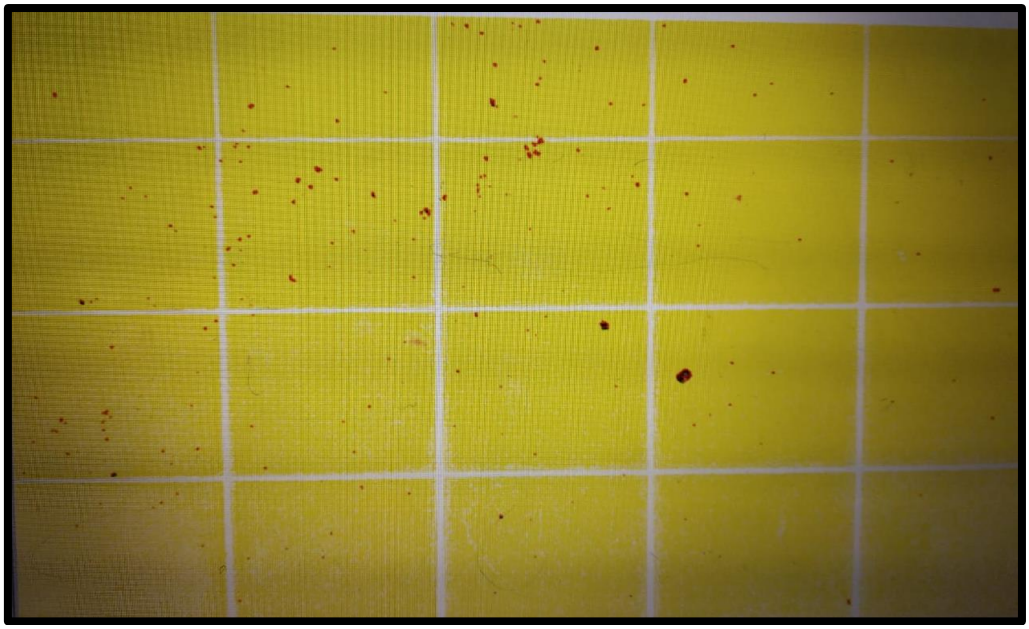


Figure 54: *Image for cell count: Trypan Blue Dye Exclusion Method*

ISSN 2515-8260 Volume 07 Issue 11, 2020

Table 18: *Anticancer Activity of Synthesized Compounds (Conc. 250 µg/ml)*

Compound Code	Concentration 250 µg/ml				
	No. of Viable Cells	No. of Non-Viable Cells	Total	Percent Cell Viability	Percent Cell Inhibition
Standard	11	63	74	14.86	85.13
Control	127	1	128	99.21	0.78
P1	7	55	62	11.29	88.71
P2	15	131	146	10.27	89.73
P3	18	165	183	9.83	90.17
P4	23	123	146	15.75	84.25
P5	11	91	102	10.79	89.21
P6	11	87	98	11.23	88.77
P7	7	71	78	8.97	91.03

Table 19: *Anticancer Activity of Synthesized Compounds (Conc. 500 µg/ml)*

Compound Code	Concentration 500 µg/ml				
	No. of Viable Cells	No. of Non-Viable Cells	Total	Percent Cell Viability	Percent Cell Inhibition
Standard	10	79	89	11.23	88.76
Control	111	1	112	99.10	0.89
P1	7	61	68	10.3	89.7
P2	6	67	73	8.22	91.78
P3	9	109	118	7.63	92.37
P4	23	154	177	12.99	87.01
P5	20	219	239	8.37	91.36
P6	14	124	138	10.14	89.86
P7	18	168	186	9.68	91.32

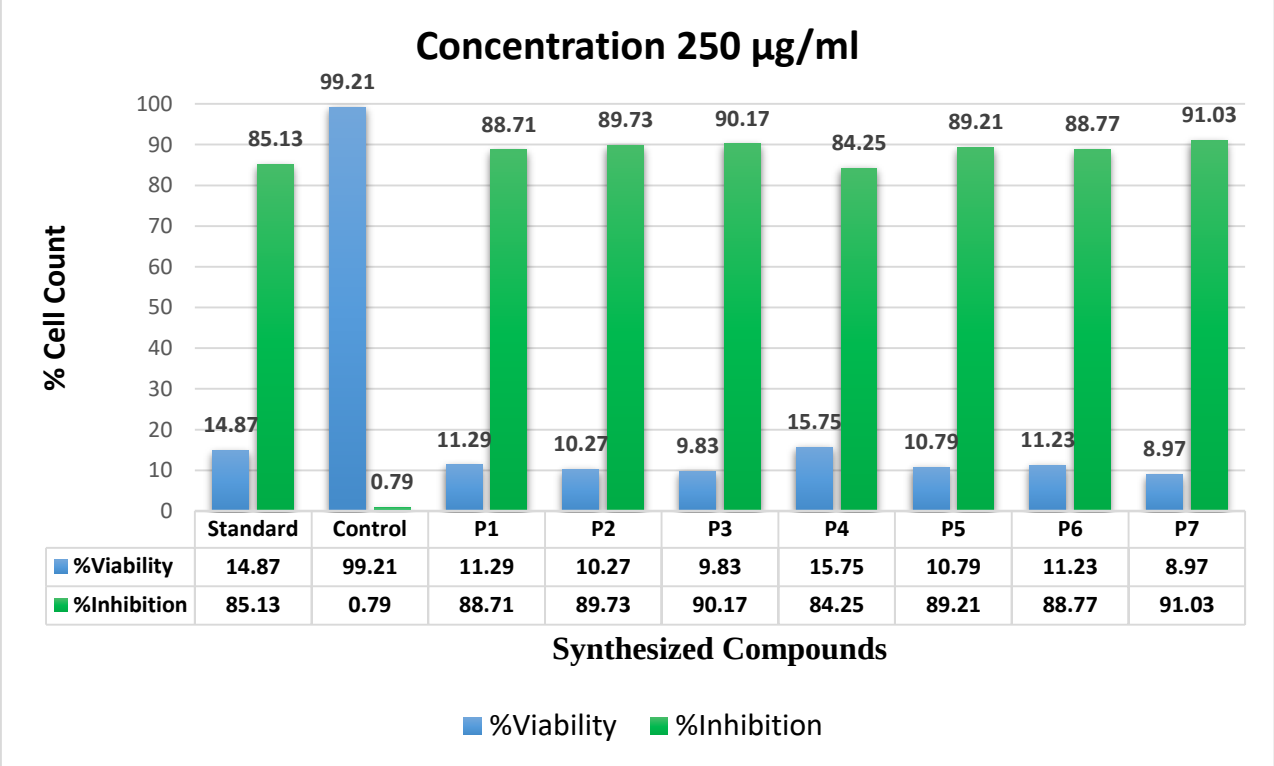


Figure 55: *Graph representing percentage inhibition of compounds (250 µg/ml) against HT29 cell-line via Trypan Blue Cell Exclusion Assay*

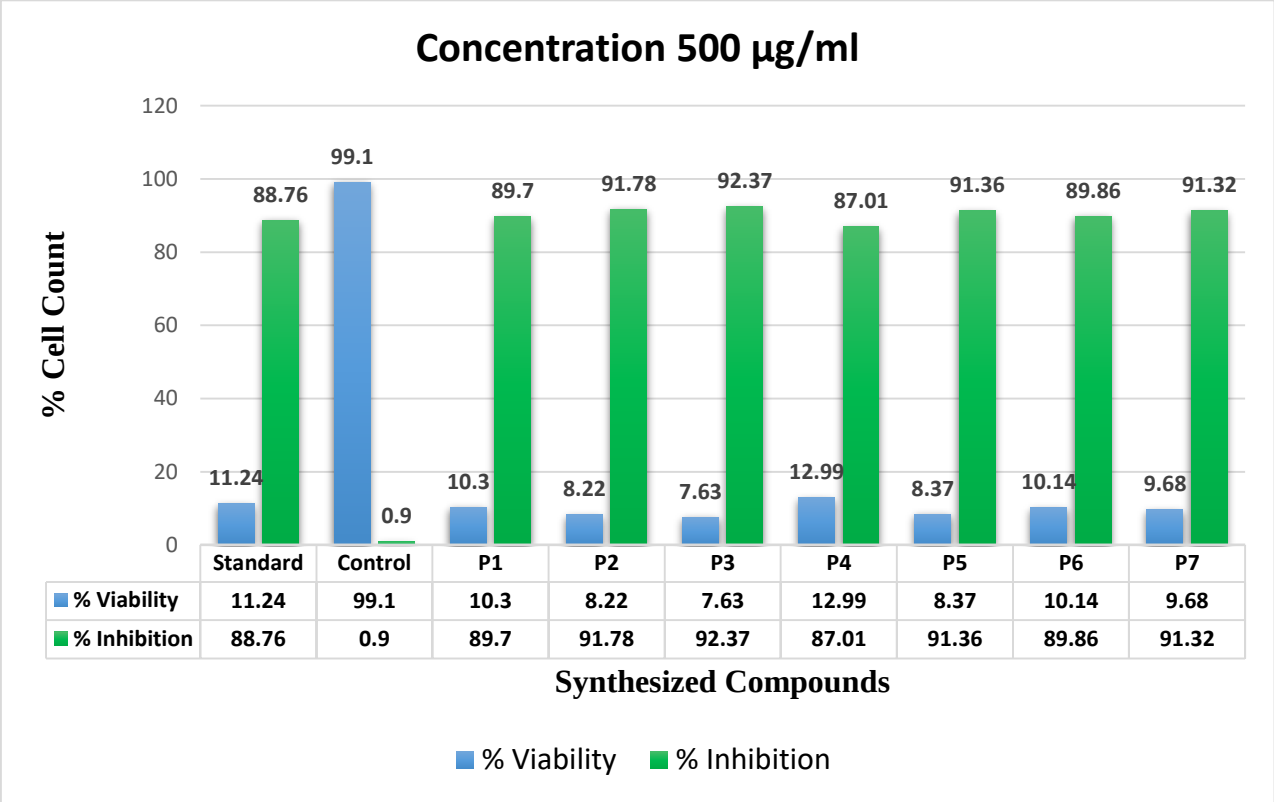


Figure 56: *Graph representing percentage inhibition of compounds (5000 µg/ml) against HT29 cell-line via Trypan Blue Cell Exclusion Assay*

7.7 DISCUSSION

Various targets available for colon cancer treatment were explored and the compounds available in the literature to target colon cancer were studied thoroughly for their active chemical functionalities and their site of action which was found that EGFR is highly expressed in colon cancers and 4-anilino quinazoline is the basic ring structure for the EGFR inhibitors [51]. Based upon the available literature the leads were designed to incorporate the functionalities responsible for the affinity and activity and reaction setup was put forth for chemical synthesis. Further to confirm the structural characters they were assessed by IR and NMR spectroscopy and evaluated for cell cytotoxicity assay.

References:

1. Srivastava A, Creek DJ. Discovery and Validation of Clinical Biomarkers of Cancer: A Review Combining Metabolomics and Proteomics. Vol. 19, Proteomics. Wiley-VCH Verlag; 2019.

2. WHO CANCER [Internet]. Available from: https://www.who.int/health-topics/cancer#tab=tab_2

3. International Agency for Research on Cancer- CANCER TODAY [Internet]. Available from: https://gco.iarc.fr/today/home

4. CANCER INFORMATION [Internet]. Available from: https://www.indiancancersociety.org/cancer-information/

5. Elmore S. Apoptosis: A Review of Programmed Cell Death, Toxicol Pathol. 2007; 35(4): 495–516.

6. NIH. Cancer Classification, National Cancer Institute [Internet]. Available from: https://training.seer.cancer.gov/disease/categories/classification.html.

7. Hanahan D, Weinberg RA. The Hallmarks of Cancer Review evolve progressively from normalcy via a series of pre. Vol. 100, Cell. 2000.

8. Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, et al. Assessment of the evolution of cancer treatment therapies. Vol. 3, Cancers. 2011. p. 3279–330.

9. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, et al. Drug resistance in cancer: An overview. Vol. 6, Cancers. MDPI AG; 2014. p. 1769–92.

10. Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S, Paz-Ares L. Current Challenges in Cancer Treatment. Vol. 38, Clinical Therapeutics. Excerpta Medica Inc.; 2016. p. 1551–66.

11. Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. Vol. 3, Signal Transduction and Targeted Therapy. Springer Nature; 2018.

12. WORLD HEALTH ORGANIZATION: REGIONAL OFFICE FOR EUROPE. WORLD CANCER REPORT: cancer research for cancer development. IARC; 2020.

13. Lauren Byrne, MPHP 439 – Online Textbook Chapter, December 3, 2008 Colorectal Cancer.

14. Rahul P. Phate. Anatomy Physiology And Health Education. Third Edition. Career Publication; 2005. 288–290.

15. Harsh Mohan. Textbook of Pathology. Seventh Edition, editor. Jaypee Brothers Medical Publishers (P) Ltd., New Delhi; 2015. 563–571.

16. American Cancer Society. American Cancer Society. Colorectal Cancer Facts & Figures 2017-2019. [Internet]. Available from: https://www.cancer.org/research/cancer-facts-statistics/colorectal-cancer-facts-figures.html.

17. Sachdeo Rahul A, Charde Manoj S, Chakole Ritu D. Colorectal Cancer: An Overview. Asian Journal of Research in Pharmaceutical Sciences [Internet]. 2020;10(3):2231–5659. Available from: www.anvpublication.orgwww.ajpsonline.com

18. Drug Targets for Colorectal Cancer [Internet]. Available from: https://www.sinobiological.com/research/cancer-drug-targets/colorectal-cancer

19. Epidermal growth factor receptor [Internet]. Available from: https://en.wikipedia.org/wiki/Epidermal_growth_factor_receptor

20. Rajaram P, Chandra P, Ticku S, Pallavi B, Rudresh K, Mansabdar P. Epidermal growth factor receptor: Role in human cancer. Vol. 28, Indian Journal of Dental Research. Medknow Publications; 2017. p. 687–94.

21. Kamath S, Buolamwini JK. Targeting EGFR and HER-2 receptor tyrosine kinases for cancer drug discovery and development. Vol. 26, Medicinal Research Reviews. 2006. p. 569–94.

22. Gupta A. Synthesis of C-6 Methyl Substituted Benzothiazole Derivatives and Antifungal Activity against Aspergillus Niger. Theranostics of Respiratory & Skin Diseases [Internet]. 2018 Jun 19;1(2).

23. Sankar Reddy RD, Sudhakar D. Synthesis, characterization and biological evaluation of some novel 6-fluoro benzothiazole substituted thiazolidinones as anthelmintic activity [Internet]. Vol. 6, Scholars Research Library Der Pharma Chemica. 2014. Available from: www.derpharmachemica.com

24. Havrylyuk D, Mosula L, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety. European Journal of Medicinal Chemistry. 2010 Nov;45(11):5012–21.

25. Venkatesh P, Pandeya SN. SYNTHESIS, CHARACTERISATION AND ANTI-INFLAMMATORY ACTIVITY OF SOME 2-AMINO BENZOTHIAZOLE DERIVATIVES. Vol. 1, International Journal of Chem Tech Research CODEN.

26. Jebur AK, Abdullah W, Saleh HM. Eurasian Journal of Analytical Chemistry Synthesis, characterization and evaluation biological activity of some new Ethyl-2-amino benzothiazole-6-carboxylate derivatives.

27. Ajeet A, Kumar A. Designing of Hybrid form of Benzothiazole-quinazoline as GABA-A Inhibitor with Anticonvulsant Profile: An in-silico Approach. American Journal of Pharmacological Sciences. 2013 Dec 21;1(6):116–20.

28. Yoshida M, Hayakawa I, Hayashi N, Agatsuma T, Oda Y, Tanzawa F, et al. Synthesis and biological evaluation of benzothiazole derivatives as potent antitumor agents. Bioorganic and Medicinal Chemistry Letters. 2005 Jul 15;15(14):3328–32.

29. Mr. Harigopal S Sawarkar. Potential Biological Activities of Thioquinazolinones: Recent Updates. Haya: The Saudi Journal of Life Sciences 2018;3(3):302-5.

30. Hameed A, Al-Rashida M, Uroos M, Ali SA, Arshia, Ishtiaq M, et al. Quinazoline and quinazolinone as important medicinal scaffolds: a comparative patent review (2011–2016). Vol. 28, Expert Opinion on Therapeutic Patents. Taylor and Francis Ltd; 2018. p. 281–97.

31. Allam HA, Aly EE, Farouk AKBAW, el Kerdawy AM, Rashwan E, Abbass SES. Design and Synthesis of some new 2,4,6-trisubstituted quinazoline EGFR inhibitors as targeted anticancer agents. Bioorganic Chemistry. 2020 May 1;98.

32. Abuelizz HA, Marzouk M, Ghabbour H, Al-Salahi R. Synthesis and anticancer activity of new quinazoline derivatives. Saudi Pharmaceutical Journal. 2017 Nov 1;25(7):1047–54.

33. El-Hitt GA, Abdel-Megeed MF, Tarek &, Zied MM. Synthesis and reactions of some 3-aryl-2-thioxoquinazolin-4(3H)-ones. Indian Journal of Chemistry. 2002;41B:1519–22.

34. Cai J, Sun M, Wu X, Chen J, Wang P, Zong X, et al. Design and synthesis of novel 4-benzothiazole amino quinazolines Dasatinib derivatives as potential anti-tumor agents. European Journal of Medicinal Chemistry. 2013; 63:702–12.

35. Cheng C, Yun F, He J, Ullah S, Yuan Q. Design, synthesis and biological evaluation of novel thioquinazolinone-based 2-aminobenzamide derivatives as potent histone deacetylase (HDAC) inhibitors. European Journal of Medicinal Chemistry. 2019 Jul 1;173:185–202.

36. Zayed MF, Rateb HS, Ahmed S, Khaled OA, Ibrahim SRM. Quinazolinone-amino acid hybrids as dual inhibitors of EGFR kinase and tubulin polymerization. Molecules. 2018;23(7).

37. Yu H, Li Y, Ge Y, Song Z, Wang C, Huang S, et al. Novel 4-anilinoquinazoline derivatives featuring an 1-adamantyl moiety as potent EGFR inhibitors with enhanced activity against NSCLC cell lines. European Journal of Medicinal Chemistry. 2016 Mar 3;110:195–203.

38. El-Sayed NNE, Almaneai NM, ben Bacha A, Al-Obeed O, Ahmad R, Abdulla M, et al. Synthesis and evaluation of anticancer, antiphospholipases, antiproteases, and antimetabolic syndrome activities of some 3H-quinazolin-4-one derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry. 2019 Jan 1;34(1):672–83.

39. El-Messery SM, Hassan GS, Nagi MN, Habib ESE, Al-Rashood ST, El-Subbagh HI. Synthesis, biological evaluation and molecular modeling study of some new methoxylated 2-benzylthio-quinazoline-4(3H)-ones as nonclassical antifolates. Bioorganic and Medicinal Chemistry Letters. 2016;26(19):4815–23.

40. Reis RDR, Azevedo EC, de Souza MCBV, Ferreira VF, Montenegro RC, Araújo AJ, et al. Synthesis and anticancer activities of some novel 2-(benzo[d]thiazol-2-yl)- 8-substituted-2H-pyrazolo[4,3-c]quinolin-3(5H)-ones. European Journal of Medicinal Chemistry. 2011 Apr;46(4):1448–52.

41. Xia Y, Yang Z-Y, Hour M-J, Kuo S-C, Xia P, Bastow KF, et al. Antitumor Agents. Part 204: 1 Synthesis and Biological Evaluation of Substituted 2-Aryl Quinazolinones. Bioorganic & Medicinal Chemistry Letters. 2001;11:1193–6.

42. El-Sherbeny MA. Synthesis of Certain Pyrimido[2,1-b]benzo-thiazole and Benzothiazolo[2,3-b]quinazoline Derivatives for in vitro Antitumor and Antiviral Activities. Arzneim-Forsch/Drug Res. 2000;50(II):848–53.

43. El-Azab AS, Abdel-Aziz AAM, Ghabbour HA, Al-Gendy MA. Synthesis, in vitro antitumour activity, and molecular docking study of novel 2-substituted mercapto-3-(3,4,5-trimethoxybenzyl)-4(3H)-quinazolinone analogues. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017 Jan 1;32(1):1229–39.

44. Alafeefy AM. Some new quinazolin-4(3H)-one derivatives, synthesis and antitumor activity. Journal of Saudi Chemical Society. 2011 Oct;15(4):337–43.

45. Mohmed J, Reddy Palreddy R, Rao Gundapaneni R, Purohith DJ, Kumar Gunda S. Three dimensional QSAR analysis of Quinazolinone derivatives as EGFR Inhibitors. International Journal of ChemTech Research. 2016;9(05):887–95.

46. Converso A, Hartingh T, Garbaccio RM, Tasber E, Rickert K, Fraley ME, et al. Development of thioquinazolinones, allosteric Chk1 kinase inhibitors. Bioorganic and Medicinal Chemistry Letters. 2009 Feb 15;19(4):1240–4.

47. Ghorab MM, Alsaid MS, Soliman AM, Al-Mishari AA. Benzo[g]quinazolin-based scaffold derivatives as dual EGFR/HER2 inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry. 2018 Jan 1;33(1):67–73.

48. El-Azab AS, Al-Omar MA, Abdel-Aziz AAM, Abdel-Aziz NI, El-Sayed MAA, Aleisa AM, et al. Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: Molecular docking study. European Journal of Medicinal Chemistry. 2010 Sep;45(9):4188–98.

49. Mitchell RA, Luwor RB, Burgess AW. Epidermal growth factor receptor: Structure-function informing the design of anticancer therapeutics. Vol. 371, Experimental Cell Research. Elsevier Inc.; 2018. p. 1–19.

50. Jin H, Dan HG, Rao GW. Research progress in quinazoline derivatives as multi-target tyrosine kinase inhibitors. Heterocyclic Communications. 2018 Feb 23;24(1):1–10.

51. Ismail RSM, Ismail NSM, Abuserii S, Abou El Ella DA. Recent advances in 4-aminoquinazoline based scaffold derivatives targeting EGFR kinases as anticancer agents. Future Journal of Pharmaceutical Sciences. 2016 Jun;2(1):9–19.

52. NIH. Cell Viability Testing with Trypan Blue Exclusion Method.

53. Strober W. Trypan Blue Exclusion Test of Cell Viability. Current Protocols in Immunology. 2015 Nov 1;111(1):A3.B.1-A3.B.3.