

**DESIGN AND DEVELOPMENT OF PYRIDINYL TRIAZOLE
DERIVATIVES AS ANTICANCER AGENTS**

Dissertation submitted to

THE TAMIL NADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI – 600032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH-II PHARMACEUTICAL CHEMISTRY

Submitted by

P. BUVANESWARI

261915003

Under the Guidance of

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Associate Professor, Department of Pharmaceutical Analysis



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THORAIPAKKAM, CHENNAI-600097.**

October 2021



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CERTIFICATE

This is to certify that the project entitled “**DESIGN AND DEVELOPMENT OF PYRIDINYL TRIAZOLE DERIVATIVES AS ANTICANCER AGENTS**” was submitted by **BUVANESWARI.P (261915003)** in partial fulfilment for the award of the degree of **Master of Pharmacy** during the academic year 2019–2021 under my guidance.

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Place: Chennai

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DECLARATION

The thesis entitled, “**DESIGN AND DEVELOPMENT OF PYRIDINYL TRIAZOLE DERIVATIVES AS ANTICANCER AGENTS**” was carried out by me in C.L.Baid Metha College of Pharmacy, BUVANESWARI.P hereby declare that this dissertation work has been originally carried out by me during the academic year 2019-2021. The work embodied in this thesis is original and is not submitted in part or full for any other degree of this or any other university.

Date:

Place: Chennai

BUVANESWARI.P

(261915003)

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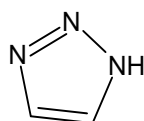
LIST OF ABBREVIATION

EGFR	-	epidermal growth factor receptor
HER-1,2	-	human epidermal growth factor receptor
ErB-2	-	erythroblastic oncogene B
TKI	-	Tyrosine kinase inhibitors
FGFR	-	FGFR: fibroblast growth factor receptor
PDGFR	-	Platelet-derived growth factor
SRC	-	non-receptor tyrosine kinase
FAK	-	Focal adhesion kinase
TGF	-	Transforming growth factor
MCF-7	-	Michigan Cancer Foundation-7
MTT	-	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
DMF	-	Dimethylformamide

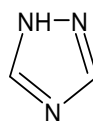
1. INTRODUCTION

TRIAZOLE

Triazoles are an important class of heterocyclic compounds containing three nitrogen atoms in a five-membered ring having molecular formula $C_2H_3N_3$. These exist in two isomeric forms depending upon the position of the nitrogen atom in the heterocyclic ring: 1,2,3-triazoles or 1,2,4-triazoles. Both the structural isomers of triazoles exhibit tautomerism and exist in two tautomeric forms.



1, 2, 3-triazole



1, 2, 4-triazole

Out of the two isomeric forms of triazoles, 1,2,4- triazole derivatives are synthesized in the form of fused ring systems or as heterocyclic or aromatic substitutions [1].

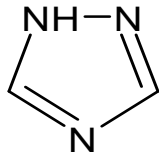
In medicinal chemistry, five-member heterocyclic nitrogen-containing compounds such as triazole are of great importance due to their wide range of biological applications such as anticonvulsant [2,3] antimicrobial, antiviral, antitubercular, antidiabetic, anti-inflammatory, anti-proliferative, antioxidant, anti-urease, and antimalarial activities [4].

1.1 1,2,4 TRIAZOLE

The triazole ring contains three nitrogen atoms and can act as a hydrogen bond acceptor or donor at the active site of the receptors and can modulate their activity accordingly. Being polar in nature, the triazole nucleus can increase the solubility of the ligands and contribute better pharmacokinetic and pharmacodynamic properties [5].

Over the last decades, increased research has been devoted to 1,2,4 triazole drugs [6-8]. Among them, 3-amino-1,2,4-triazoles derivatives have attracted special attention as they demonstrated a broad spectrum of bioactivities, including potential applications against thrombotic disorders [9], fibrotic [10], auto-immune diseases, central nervous

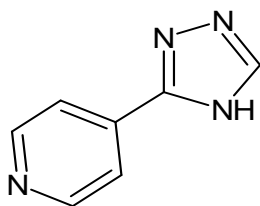
system disorders [11], obesity, diabetes, Alzheimer's disease [12], microbial infections [13-15] cancer [16-19].



Molecular Formula	=	C ₂ H ₃ N ₃
Formula Weight:	=	69.06532
Composition	=	C (34.78%) H(4.38%) N(60.84%)
Molar Refractivity	=	16.86 ± 0.3 cm ³
Molar Volume	=	54.2 ± 3.0 cm ³
Parachor	=	155.1 ± 4.0 cm ³
Index of Refraction	=	1.534 ± 0.02
Surface Tension	=	67.1 ± 3.0 dyne/cm
Density	=	1.274 ± 0.06 g/cm ³
Polarizability	=	6.68 ± 0.5 10 ⁻²⁴ cm ³
RDBE	=	3
Monoisotopic Mass	=	69.032697 Da
Nominal Mass	=	69 Da
Average Mass	=	69.0653 Da
M+	=	69.032149 Da
M-	=	69.033246 Da

1.2 4- PYRIDINYL 1,2,4 TRIAZOLE

4-Pyridinyl 1,2,4 Triazole is a heterocyclic ring with nitrogen at 1,2 and 4 position and pyridine ring at 5 position their derivatives are characterized with a broad spectrum of biological activity, including anti-cancer activity, antiparasitic activity, larvicidal activity, antifungal activity, herbicidal activity, antioxidant activity, cytostatic activity, brassinosteroid biosynthesis inhibitors, antimicrobial activity [20].



Molecular Formula	=	C ₇ H ₆ N ₄
Formula Weight:	=	146.14934
Composition	=	C (57.53%) H (4.14%) N (38.34%)
Molar Refractivity	=	39.55 ± 0.3 cm ³
Molar Volume	=	112.7 ± 3.0 cm ³
Parachor	=	322.6 ± 4.0 cm ³
Index of Refraction	=	1.619 ± 0.02
Surface Tension	=	67.1 ± 3.0 dyne/cm
Density	=	1.296 ± 0.06 g/cm ³
Polarizability	=	15.67 ± 0.5 10 ⁻²⁴ cm ³
RDBE	=	7
Monoisotopic Mass	=	146.059246 Da
Nominal Mass	=	146 Da
Average Mass	=	146.1493 Da
M+	=	146.058698 Da
M-	=	146.059795 Da

1.3 CANCER

Cancer is a condition where some of the cells of the body begin to divide uncontrollably and spread into surrounding parts of the body, destroying healthy neighboring tissues [21]. A cell receives instructions to die so that the body can replace it with a newer cell that functions better. Cancerous cells lack the components that instruct them to stop dividing and to die. Cancerous cells may appear in one area, then spread via the lymph nodes. These are clusters of immune cells located throughout the body [22].

Types of Cancer

There are more than 100 types of cancer, usually named for the organs or tissues where the cancers form. In males, the most common cancers are that of lung, prostate, stomach, colon, and rectum. In females, the most common cancers are that of breast, colon, rectum, lung, and cervix.

Stages of Cancer

Stage means the extent to which the disease has progressed like the size of the tumor and if it has spread to the other parts of the body

Stage 0: Cancer cells are present but have not spread to surrounding tissues

Stage I, II, and III: Higher the number, more advanced the disease in terms of size of the tumor and invasion of surrounding organs and tissues

Treatment of Cancer

The treatment depends on the type and stage of cancer. Treatment includes radiation therapy, chemotherapy, surgery, and target therapy [23].

Cancer statistics

Cancers of oral cavity and lungs account for over 25% of cancer deaths in males and cancer of breast and oral cavity account for 25% of cancers in females.

Breast cancer is the most common cancer in women in India and accounts for 14% of all cancers in women [24].

Table 1: The top five cancers in men and women account for 47.2% of all cancers

MEN	WOMEN
LIP, ORAL CAVITY	BREAST
LUNG	LIP, ORAL
STOMACH	CERVIX
COLORECTAL	LUNG
ESOPHAGUS	GASTRIC

EPIDERMAL GROWTH FACTOR RECEPTOR

The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a transmembrane protein that is a receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands [25].

Different receptor kinases are involved in the signal transduction in mammalian cells. The particularly important one is the epidermal growth factor receptor (EGFR), which is a transmembrane-bound molecule that has important regulatory functions affecting tumor growth and progression. These include cell proliferation, differentiation, migration, apoptosis, and angiogenesis.

EGFR kinase, consisting of ErbB1 and HER1, belongs to the ErbB family of kinases, which also includes human epidermal growth factor receptor-2 (subunits: HER2 and ErbB-2), human epidermal growth factor receptor-3 (subunits: HER3 and ErbB-3), and human epidermal growth factor receptor-4 (subunits: HER4 and ErbB-4).

Food and Drug Administration (FDA) approved drugs targeting this family

- monoclonal antibodies (trastuzumab, cetuximab, panitumumab, and pertuzumab)
- Small-molecule inhibitors or tyrosine kinase inhibitors (TKI)
(gefitinib, erlotinib, lapatinib and afatinib)

EGFR inhibitors may be used in the treatment of cancers that are caused by EGFR up-regulation, such as non-small-cell lung cancer, pancreatic cancer, breast cancer, and colon cancer [26].

Molecular targeting strategies for cancer therapy are distinct from conventional chemotherapy and radiotherapy in their potential to provide increased tumor specificity.

One particular molecular target of high promise in oncology is the epidermal growth factor receptor (EGFR). The EGFR is overexpressed, dysregulated, or mutated in many epithelial malignancies, and EGFR activation appears important in tumor growth and progression [27].

Tyrosine kinases

Tyrosine kinases are important cellular signaling proteins that have a variety of biological activities including cell proliferation and migration.

Multiple kinases are involved in angiogenesis, including receptor tyrosine kinases such as the vascular endothelial growth factor receptor. Inhibition of angiogenic tyrosine kinases has been developed as a systemic treatment strategy for cancer.

Protein kinases phosphorylate proteins, resulting in functional changes of target proteins

The tyrosine kinase group consists of approximately 30 families, for example, the VEGFR family and the fibroblast growth factor receptor (FGFR) family.

Receptor tyrosine kinases are essential for the transduction of extracellular signals into the cell, while non-receptor tyrosine kinases accomplish intracellular communication [28].

Types of tyrosine kinases

Tyrosine kinases can be further subdivided into

- Receptor tyrosine kinases (EGFR, PDGFR, FGFR)
- Non-receptor tyrosine kinases (SRC, ABL, FAK, and Janus kinase)

Oncogenic activation of tyrosine kinase

Normally the level of cellular tyrosine kinase phosphorylation is tightly controlled by the antagonizing effect of tyrosine kinase and tyrosine phosphatases.

The common mechanism of oncogenic activation

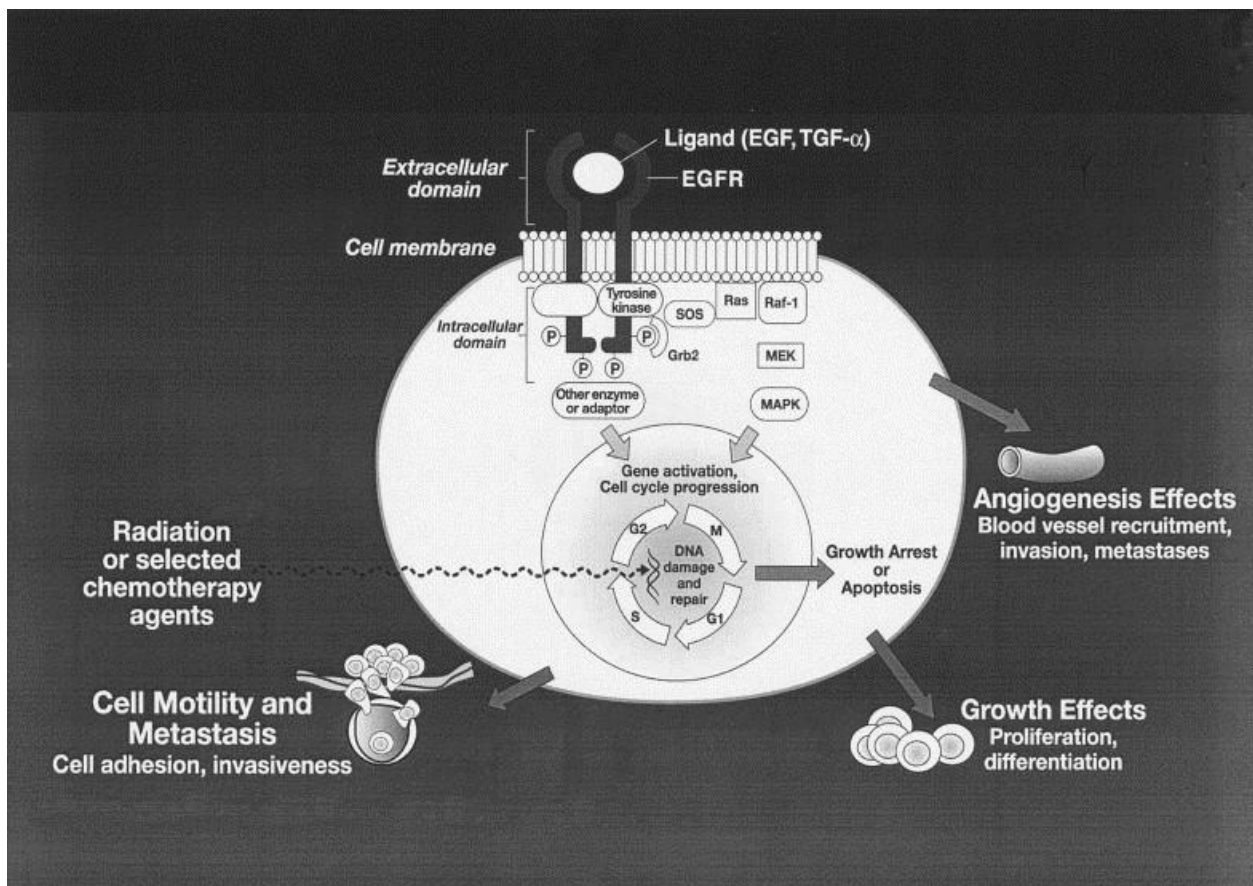
- activation by mutation
- BCR-ABL and human leukemia

Mechanism of action

EGFR tyrosine kinase inhibitors act by inhibiting the EGFR tyrosine kinases by competitive blockade of ATP binding. Eg: Gefitinib, lapatinib

Selectively inhibits EGFR-TK results in blockage of downstream EGFR signal transduction pathways, cell cycle arrest, and inhibition of angiogenesis. [29,30].

Figure 1: EGFR signaling pathway



1.4 DOCKING

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound to form a stable complex.

Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed.

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target-specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy, and stability of complexes. At present, a docking technique is utilized to predict the tentative binding parameters of the ligand-receptor complex beforehand.

The main objective of molecular docking is to attain a ligand-receptor complex with optimized conformation and to possess less binding free energy.

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before the experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, the interaction between small molecules (ligand) and protein target (maybe an enzyme) may predict the activation or inhibition of the enzyme. Such type of information may provide raw material for rational drug design. Some of the major applications of molecular docking are Lead optimization, hit identifications, Drug- DNA interaction.

1.5 DRUG FILTERS

CHEMSKETCH

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers, and Markush structures. It also includes features such as calculation of molecular properties (e.g. molecular weight, density, molar refractivity, etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log P.

SWISS ADME

A free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. It allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

ProTox-II

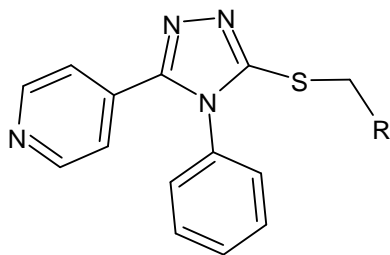
ProTox-II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. ProTox-II incorporates molecular similarity, fragment propensities, most frequent features, and machine-learning, based on a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets.

Pre ADMET

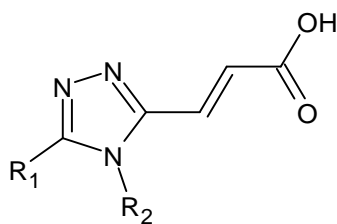
Pre ADMET-is a web-based application for predicting ADME data and building a drug-like library using *in silico* method. It predicts permeability for Caco-2 cell, MDCK cell and BBB, HIA, skin permeability, and plasma protein binding.

2. REVIEW OF LITERATURE

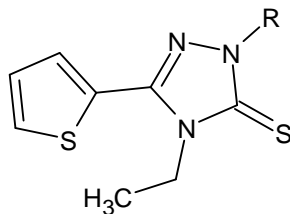
1. Guo-Xiang Sun *et al* were synthesized novel 1,2,4-triazole derivatives containing pyridine moiety under the Microwave Assistant condition and evaluated their fungicidal activities *in vivo* against *Stemphylium lycopersici* (Enjoji) Yamamoto, *Fusarium oxysporum*. sp. cucumebrium, and *Botrytis cinerea*. From the results compound with the electron-donating group at R, 4-BuPh at R (71.43%) and 4-OCH₃Ph (85.12%) at R displayed excellent inhibition activity [6].



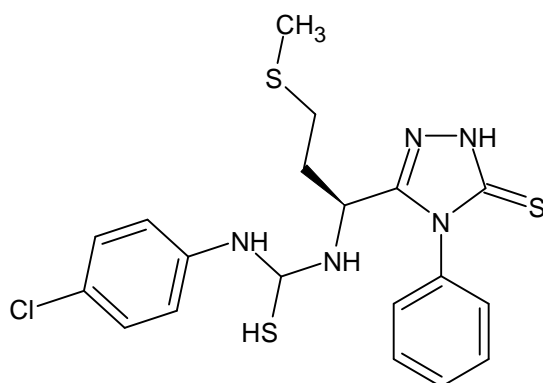
2. Modzelewska-Banachiewicz, B *et al* synthesized New 3-(3,4-diaryl-1,2,4-triazole-5-yl) propenoic acid derivatives (8–14) by condensation of N₃-substituted amidrazones (1–7) with maleic anhydride. From the results compound with 2-methyl pyridine at R₁ and phenyl at R₂ showed anticonvulsive activity and potent antinociceptive action [31].



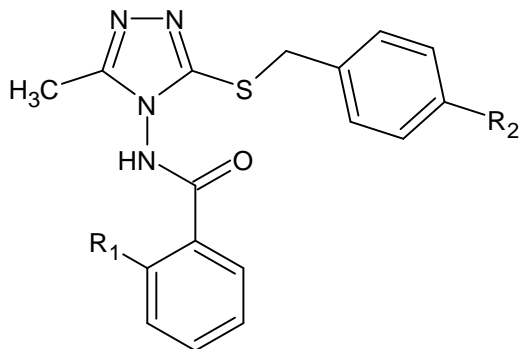
3. Metin Koparir *et al* synthesized novel aminomethyl derivatives of 4-substituted-5-(2-thienyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones and evaluated their biological activity. From the results compound with morpholine and piperazine at R showed effective antifungal activity; 4-methyl piperidine and trifluoromethylphenylpiperazine at R showed the highest antibacterial activity [32].



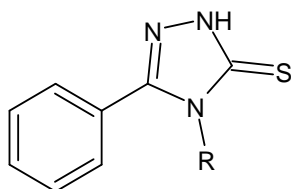
4. Tatar E *et al* synthesized new series of 1,3,4-thiadiazole and 1,2,4-triazole derivatives from L-methionine and screened their antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain. Among screened compounds 1-[4-(4-chloro-(3-trifluoromethyl) phenyl)-3-[3-(methyl-sulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole 3-yl) propyl] thiourea showed the significant antitubercular activity [33].



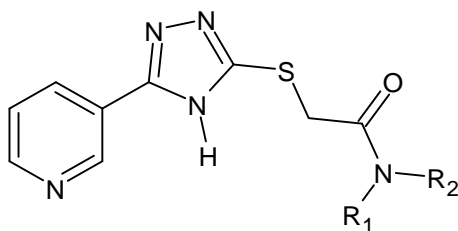
5. Liu XH *et al* synthesized novel fluorinated 1,2,4-triazole derivatives by Microwave-assisted reaction and screened their herbicidal activity. From the results compound with fluorine at ortho and meta position of at R₁, R₂ showed the herbicidal activity against *Brassica campestris* and *Echinochloa crusgalli*. [34].



6. Varvaresou, A *et al* synthesized New indolic derivatives of thiosemicarbazides and some cyclic 1,2,4-triazole-5-thione analogs and evaluated their antimicrobial and antifungal activity. From the results, thiosemicarbazides and cyclic triazole-thien-5-yl analogs showed similarities in the antimicrobial and antifungal activity. α -naphthyl substitution in the non-indolic portion and C5 substitution on the indolic nucleus active against *S. aureus* and *C. Albicans* [35].

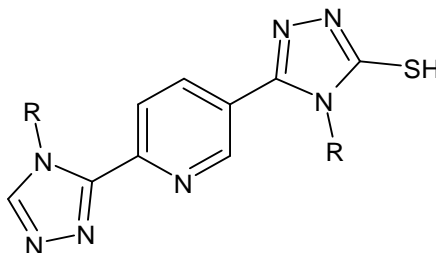


7. R.K. Mali *et al* Synthesized 3, 5-disubstituted-1, 2, 4-triazole derivatives and screened their Anti-fungal activity against *C. albicans* and *A. niger* and anti-tubercular against *Mycobacterium tuberculosis* [36].

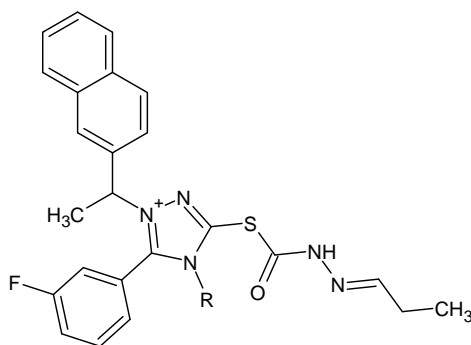


8. Nilufer Bulut *et al* synthesized Some novel derivatives of thiosemicarbazide and 1,2,4-triazole-3-thiol and evaluated their biological activities. From the results among all the

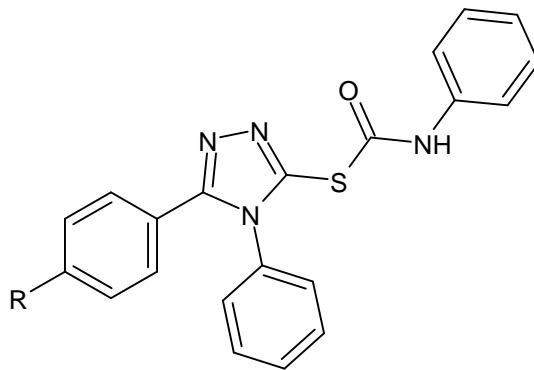
synthesized triazole compounds 5,5' -pyridine-2,5-diylbis(4-phenyl-4H-1,2,4-triazole-3-thiol compound substituted with the aromatic phenyl group at R showed the highest DPPH radical scavenging activity [37].



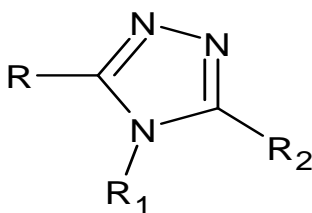
9. Han MI et al synthesized a novel series of 1,2,4-triazole containing hydrazide-hydrazones derived from (S)-Naproxen and evaluated their in vitro anticancer activity by using the MTS method against PC-3, DU-143, and LNC cancer cell lines [38].



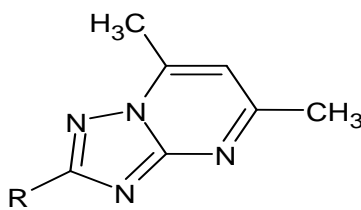
10. Abdallah Turky *et al* synthesized 1,2,4-triazole-N-arylamide hybrids and evaluated their anti-cancer using human breast adenocarcinoma cells (MDA-MB-231). The results showed that two novel C3-linked 1,2,4-triazole-N-arylamide hybrids with antiproliferative potentials [39].



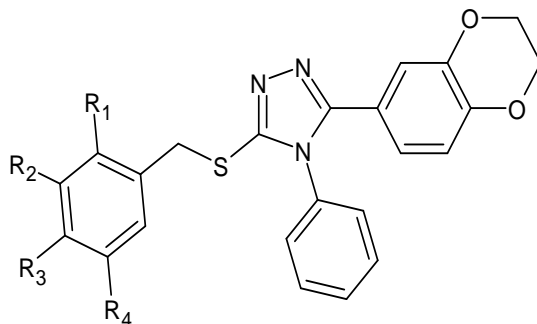
11. G.M. Castanedo *et al* synthesized a highly regioselective one-pot process that provides rapid access to highly diverse 1, 3, 5-trisubstituted 1, 2,4-triazoles from the reaction of carboxylic acids, primary amidines, and monosubstituted hydrazines. Where R is the alkyl and aromatic group [40].



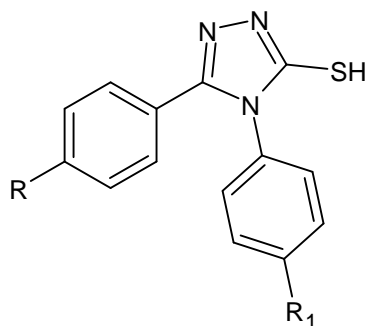
12. V. Ram *et al* synthesized 4- Substituted-5-Aryl-1, 2, 4-Triazoles and screened their Antibacterial and Antifungal Activity and found antibacterial activity. From the results compound with pyridyl at R exhibited antibacterial activity [41].



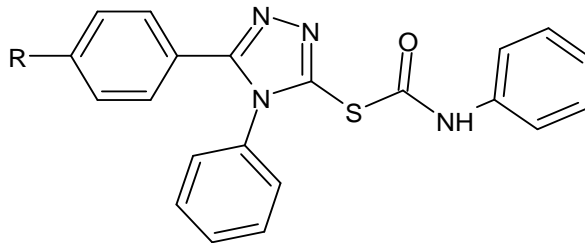
13. Ya-Ping Hou *et al* synthesized new 1,2,4-triazole derivatives containing 1,4-benzodioxan have been synthesized and evaluated for their antitumor activities [42].



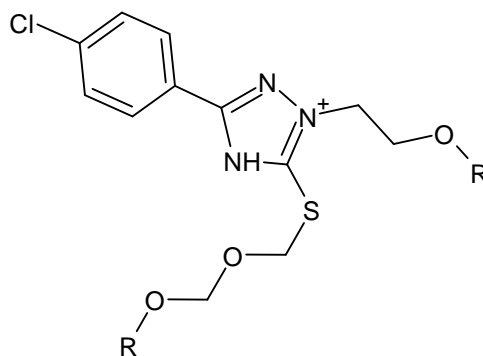
14. A new series of substituted 1,2,4-triazoles as substituted 4,5-diphenyl 4H-1,2,4-triazole-3-thiols have been synthesized and evaluated for their biological activity. From the result synthesized compounds exhibited potent to moderate anti-inflammatory, anticonvulsant and antimicrobial activities [43].



15. Abdallah Turkey *et al* designed and synthesized novel series of 1,2,4-triazole derivatives as potential adenosine A2B receptor antagonists and evaluated in vitro cytotoxicity against a human breast adenocarcinoma cell line (MDA-MB- 231) using the MTT assay method [44].

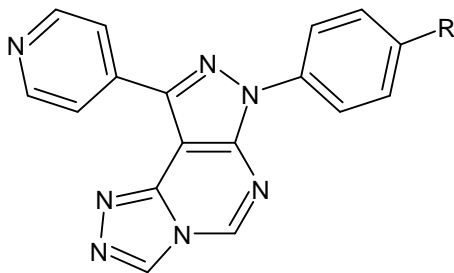


16. Aouad MR *et al* synthesized a novel series of S- and S, N-bis(acyclonucleoside) analogs carrying 5-(2-chlorophenyl)-2,4-dihydro-1,2,4- triazole-3-thione by convenient regioselective method and evaluated their anticancer activity *in vitro* using cancer cell lines [45].



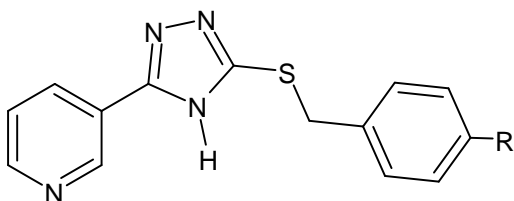
17. Kumar CS *et al* performed docking studies of novel 1, 2, 4- triazole containing 1h-indole-2, 3-dione analogs as anticancer properties. indoleamine 2, 3-dioxygenase 1 (PDB ID: 5ETW), EGFR tyrosine kinase (PDB ID: 5HIC) docking was done by using Autodock software [46].

18. Aliwaini S *et al* designed, synthesized, Three novel pyrazolo-[1,2,4]triazolopyrimidine derivatives and evaluated their antiproliferative activity against tumor cell lines HCC1937 and HeLa cells (EGFR) [47].

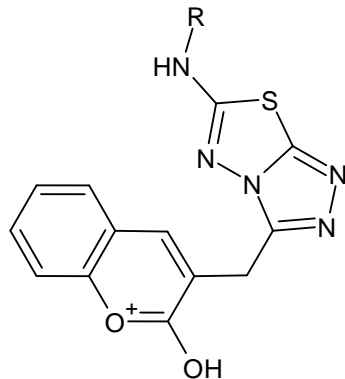


19. Nasab RR *et al* synthesized new series of quinazoline derivatives and explored the interaction between epidermal growth factor receptor tyrosine kinase of the synthesized inhibitors using a combination of *in-silico* and *in-vitro* cytotoxicity methods. EGFR tyrosine kinase (PDB code 1M17) using Autodock 4.2 [48].

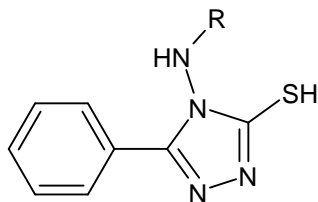
20. Ahirwar J *et al* designed and synthesized Analgesic and Anti-inflammatory Potential of Merged Pharmacophore Containing 1,2,4-triazoles and Substituted Benzyl Groups via Thio Linkage [49].



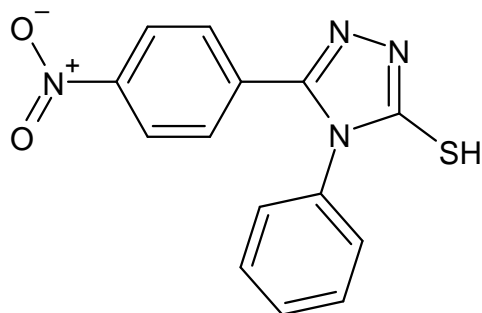
21. Lamy H *et al* Synthesized of Novel 1,2,4-Triazolyl Coumarin Derivatives as Potential Anticancer Agents. Replacement of the thiol group in triazole with an arylamino substituent leading to the 6-arylamino derivatives [50].



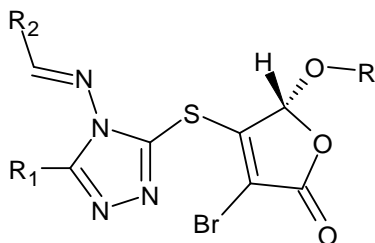
22. Aday HA *et al* synthesized N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl) hydrazine carbothioamide by the condensation of 4- amino-5-phenyl-4H-1,2,4-triazole-3-thiol and thiosemicarbazide [51].



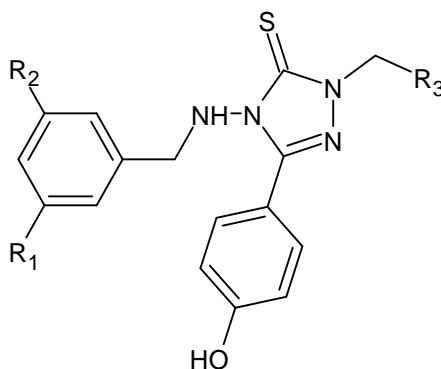
23. Carolin. S *et al* synthesized 5-(4-Nitrophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol from 1-phenyl-4-(4- nitrobenzoyl)thiosemicarbazide and cytotoxicity assay on the rhabdomyosarcoma cell line of heavy metals [52].



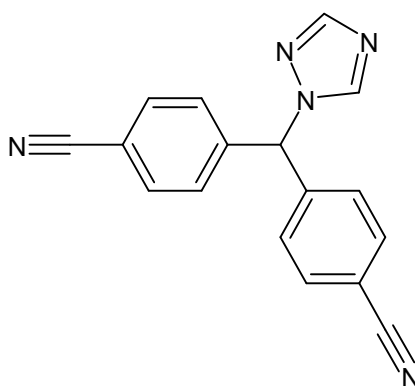
24. Li *et al* synthesized and evaluated in vitro anticancer activity of 12 hybrid 1,2,4- triazole Schiff's bases bearing γ -substituted butenolide moiety [53].



25. Anton Smith *et al.* have been synthesized and evaluated in vitro anticancer activity of 1,2,4-triazole derivatives [54].



26. Mohsen *et al* Designed, synthesized, docking study, and cytotoxic activity evaluation of some novel letrozole analogs on breast cancer cell lines [55].



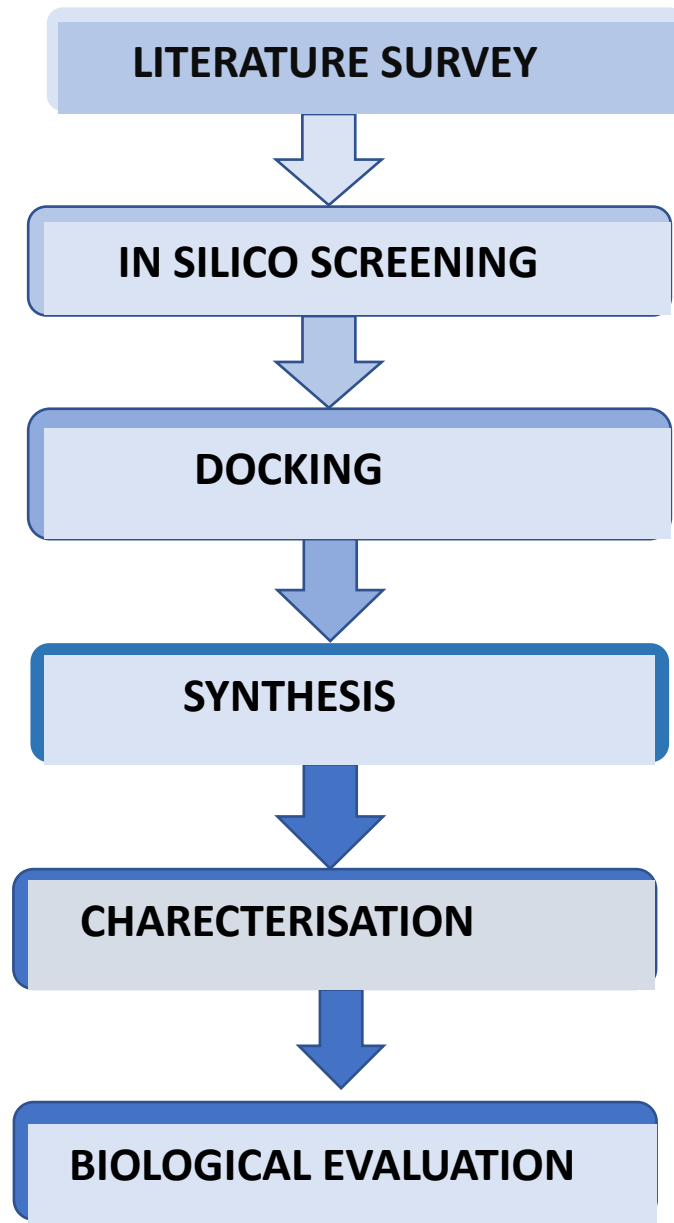
3.AIM AND OBJECTIVE

Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases. It is estimated that 12.5% of the population dies due to cancer. The disease is widely prevalent, and in the west, almost a third of the population develops cancer at some part of time during their life. Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at a molecular level. There is a continuous rise of deaths from various cancer worldwide with an estimated 12 million deaths in 2030. Despite the advancement in the knowledge of biochemical processes associated with carcinogenesis, the successful treatment of cancer remains a significant challenge because of the general toxicity associated with the clinical use of traditional cancer therapeutic agents. Hence, the design and development of new drugs for cancer therapeutics remains to be an important and challenging task for medicinal chemists worldwide.

The work is aimed at

- To design pyridinyl triazole derivatives using chem sketch.
- To perform docking studies for all designed compounds with EGFR tyrosine kinase using Autodock 4.
- To synthesize pyridinyl triazole derivatives based on docking score.
- To characterize the synthesized compounds using IR, NMR, Mass.
- To evaluate the anticancer activity of the synthesized compounds.

4.PLAN OF STUDY



5. MATERIALS AND METHODS

5.1. DESIGN OF COMPOUNDS:

17 compounds were designed using Chemsketch software. Physicochemical properties were calculated for all the compounds.

Molar refractivity

Molar refractivity, A , is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as $A = \frac{4}{3} \pi N_A \alpha$

Where $N_A \approx 6.02210^{23}$ is the Avogadro constant and α is the mean polarizability of the molecule.

Molar Volume

The molar volume, symbol V_m , is the volume occupied by one mole of a substance (chemical element or chemical compound) at a given temperature and pressure. It is equal to the molar mass (M) divided by the mass density (ρ). It has the SI unit cubic meters per mole (m^3/mol), although it is more practical to use the unit's cubic decimeters per mole (dm^3/mol) for gases and cubic centimeters per mole (cm^3/mol) for liquids and solids. The molar volume of a substance can be found by measuring its molar mass and density then applying the relation $V_m = M/\rho$

Index of refraction

The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium.

$$n = c/v$$

Where c is the speed of light in vacuum and v is the phase velocity of light in the medium.

Surface Tension

Surface tension is the elastic force of a fluid surface which makes it acquire the least surface area possible. Surface tension has the dimension of force per unit length, or of energy per unit area.

Polarizability

Polarizability is the ability to form instantaneous dipoles. It is a property of matter. Polarizabilities determine the dynamical response of a bound system to external fields and provide insight into a molecule's internal structure. In a solid, polarizability is defined as the dipole moment per unit volume of the crystal cell.

Parachor

It is an empirical constant for a liquid that relates the surface tension to the molecular volume and that may be used for a comparison of molecular volumes under conditions such that the liquids have the same surface tension and for determinations of partial structure of compounds by adding values obtained for constituent atoms and structural features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the formula $P=Y^{1/4} M/d$

Where: $Y^{1/4}$ is the fourth root surface tension.

M is the molar mass, D is the density.

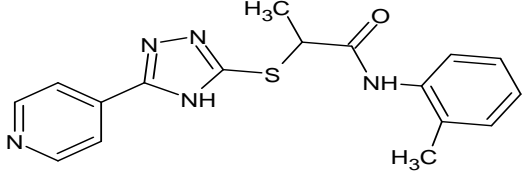
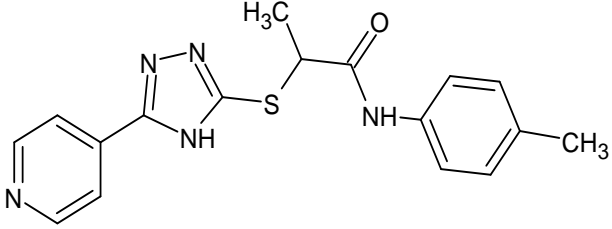
Dielectric constant

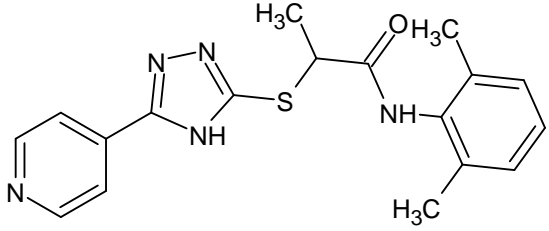
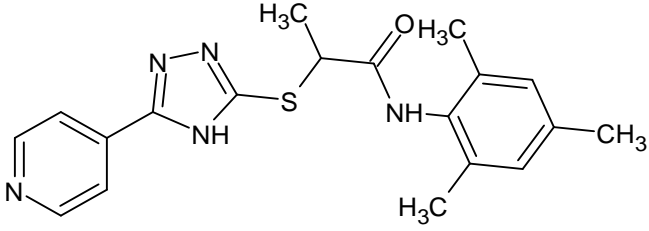
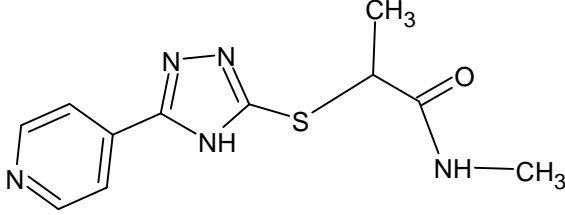
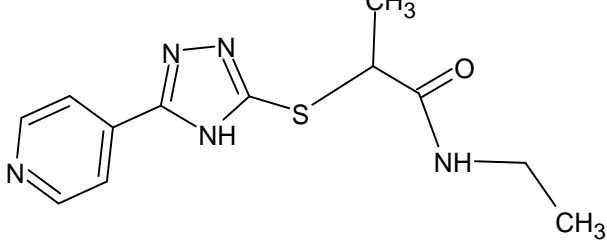
Substances have capacity to produce dipoles in another molecule. Dielectric constant is a measure of this capacity and it is a physical property. It is affected by both the attractive forces that exist between atoms and also molecules. It is denoted by E.

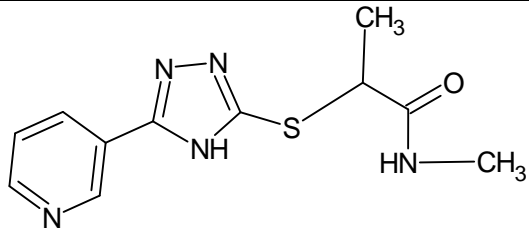
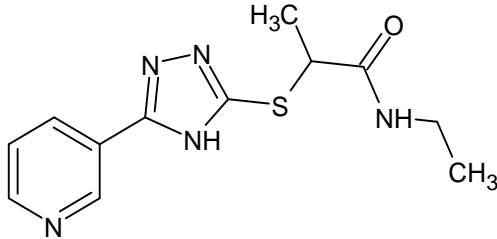
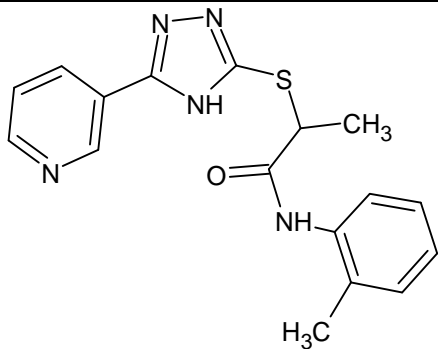
5.1 DESIGN OF COMPOUNDS:

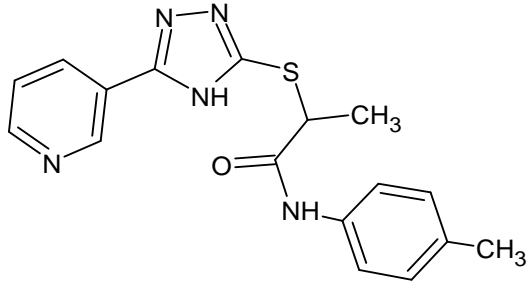
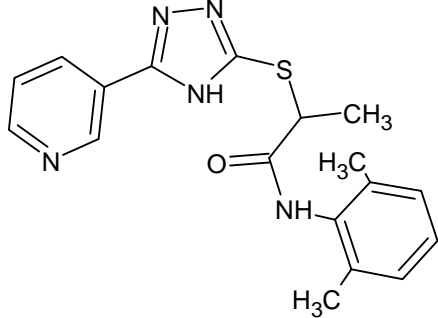
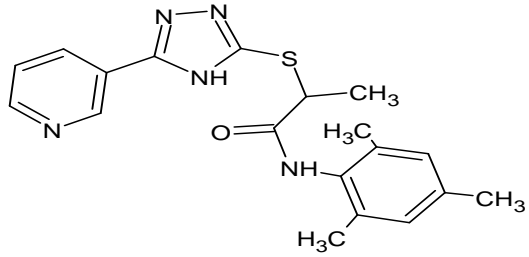
17 compounds were designed using Chems sketch software and physicochemical properties were calculated for all compounds

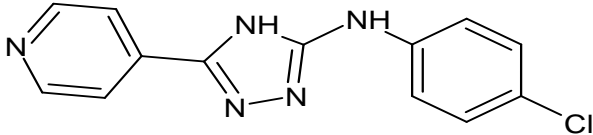
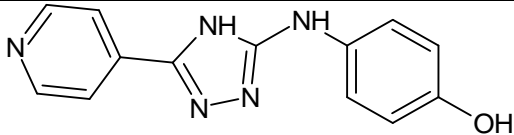
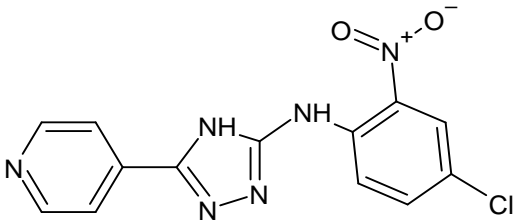
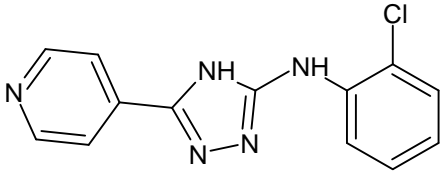
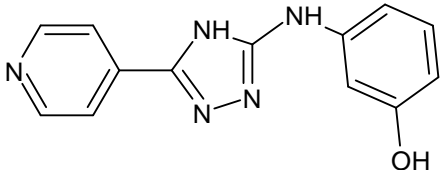
Table: 2 Designed structure of compounds

COMPOUND	DERIVATIVE NAME	STRUCTURE	IUPAC NAME
3a	2-methylaniline		<i>N</i> -(2-methylphenyl)-2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide
3b	4-methylaniline		<i>N</i> -(4-methylphenyl)-2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide

3c	2,6-dimethylaniline		<i>N</i> -(2,6-dimethylphenyl)-2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide
3d	2,4,6-trimethylaniline		2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)- <i>N</i> -(2,4,6-trimethylphenyl)propanamide
3e	methanamine		<i>N</i> -methyl-2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide
3f	ethanamine		<i>N</i> -ethyl-2-([5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide

3g	methanamine		<i>N</i> -methyl-2-([5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide
3h	ethanamine		<i>N</i> -ethyl-2-([5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide
3i	2-methylaniline		<i>N</i> -(2-methylphenyl)-2-([5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl)propanamide

3j	4-methylaniline		<i>N</i> -(4-methylphenyl)-2-[[5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl]propanamide
3k	2,6-dimethylaniline		<i>N</i> -(2,6-dimethylphenyl)-2-[[5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl]propanamide
3l	2,4,6-trimethylaniline		2-[[5-(pyridin-3-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]sulfanyl]- <i>N</i> -(2,4,6-trimethylphenyl)propanamide

4a	4-chloroaniline		<i>N</i> -(4-chlorophenyl)-5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-amine
4b	4-aminophenol		4-[[5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]amino]phenol
4c	4-chloro-2-nitroaniline		<i>N</i> -(4-chloro-2-nitrophenyl)-5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-amine
4d	2-chloroaniline		<i>N</i> -(2-chlorophenyl)-5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-amine
4e	3-aminophenol		3-[[5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]amino]phenol

5.2 *IN SILICO* SCREENING OF DESIGNED COMPOUNDS

In silico is an expression meaning "performed on computer or via computer simulation" in reference to biological experiments. When lead molecules have been identified, they have to be optimized in terms of potency, selectivity, pharmacokinetics (i.e.) absorption, distribution, metabolism and excretion (ADME) and toxicology before they can become candidates for drug development. *In silico* approaches to predict pharmacokinetic parameters (ADME) were pioneered by Lipinski *et al.* By studying the physicochemical properties of >2000 drugs from the WDI (World Drug Index, Derwent Information, London), which can be assumed to have entered Phase II human clinical trials (and therefore must possess drug-like properties), the so-called 'rule-of five' was derived to predict oral bioavailability (intestinal absorption) of a compound that can be considered as the major goal of drug development.

Softwares and Databases used

- Chems sketch
- Swiss ADME
- PreADMET
- ProTox-II
- Molinspiration server
- RCSB protein data bank
- Autodock 4.2 which combines
- Autodock tools
- Python molecule viewer 1.5.6
- Discovery studio visualizer

SwissADME

Lipinski's rule of five

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans.

The rule

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- ✚ No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- ✚ No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- ✚ A molecular mass less than 500 daltons
- ✚ An octanol-water partition coefficient log P not greater than 5

Ghose Filter

This filter defines drug-likeness constraints as follows:

- Calculated log P is between -0.4 and 5.6
- Molecular weight is between 160 and 480
- Molar refractivity is between 40 and 130
- The total number of atoms is between 20 and 70.

Veber Filter

The molecules fitting to these two properties have a high probability of good oral bioavailability.

- ✓ Rotatable bond: max. 12
- ✓ Polar Surface Area: max. 140Å²

Egan Rule

Predicts good or bad oral bioavailability.

- ✓ $0 \geq \text{TPSA} \leq 132$
- ✓ $-1 \geq \log P \leq 6$.

Molar Refractivity

It is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as $A = \frac{4\pi}{3} N_A \alpha$

Where $N_A = 6.022 \times 10^{23}$ is the Avogadro constant and α is the mean polarizability of a molecule

Polar surface area (PSA) or topological polar surface area (TPSA)

It is a measure of apparent polarity of a molecule is defined as the surface sum of overall polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. PSA is commonly used for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes. For molecules to penetrate the blood-brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed. Topological PSA (TPSA, fast 2D calculation).

ADME Guideline

- ✓ $TPSA < 140 \text{ \AA}^2$ good intestinal absorption.
- ✓ $TPSA < 70 \text{ \AA}^2$ good brain penetration.

Lipophilicity

Lipophilicity is the ability of a molecule to mix with an oily phase rather than with water, is usually measured as partition coefficient, P , between the two phases and is often expressed as $\log P$. Lipophilicity has also been found to affect several pharmacokinetic parameters: higher lipophilicity ($\log P > 5$) gives, in general, lower solubility, higher permeability in the gastrointestinal tract, across the blood-brain barrier and other tissue membranes, higher affinity to metabolizing enzymes and efflux pumps, and higher protein binding. Low lipophilicity can also negatively impact permeability and potency and thus results in low BA and efficacy.

Partition coefficient, P

It is defined as a particular ratio of the concentrations of a solute between the two solvents (a biphasic system of liquid phases), specifically for un-ionized solutes, and the logarithm of the ratio is thus **$\log P$** . When one of the solvents is water and the other is a non-polar solvent, then the $\log P$ -value is a measure of lipophilicity or hydrophobicity.

- ✓ $\log P_{\text{oct/wat}} = \log \frac{[\text{solute}]_{\text{unionized octanol}}}{[\text{solute}]_{\text{unionized water}}}$
- ✓ $\log P_{\text{oct/wat}} = \log \frac{C_{\text{O}}}{C_{\text{W}}}$

Lipophilicity not only impacts solubility but also influences permeability, potency, selectivity, absorption, distribution, metabolism, and excretion (ADME) properties and toxicity. A desired $\log P$ value (octanol-water partition coefficient) is no more than 5.

Water Solubility

Water solubility is a measure of the amount of chemical substance that can dissolve in water at a specific temperature. Solubility is a common physicochemical parameter for drug discovery compounds. Determination of the aqueous solubility of the drug candidate is an important analysis as it reflects the bioavailability of the compound.

Log S

- ✓ The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption, and therefore the general aim is to avoid poorly soluble compounds.
- ✓ Log S value is a unit stripped logarithm (base10) of the solubility measured in mol/liter. Log S value should be greater than -4.

Rotatable Bonds

The bioavailability of a drug-like molecule is related with its rotatable bond number. Less than seven rotatable bonds are essential for good bioavailability. Many highly potent molecules carried more than 10 rotatable bonds and still administered through oral route.

Hydrogen bond acceptors and donors

12 or fewer H-bond donors and acceptors will have a high probability of good oral bioavailability.

Drug-Likeliness

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. The most well-known rule relating the chemical structures to their biological activities is Lipinski's rule and it is called the 'rule of five'. Another well-known rule is the Lead-like rule. PreADMET contains drug-likeness prediction module based on these rules.

ADME Prediction

Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2-cell model and MDCK (Madin-Darby canine kidney) cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption. In absorption, this module provides prediction models for *in vitro* Caco2-cell and MDCK cell assay. Additionally, *in silico* HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery.

In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In order to build these QSAR models, genetic functional approximation is used to select relevant descriptors from all 2D descriptors that calculated by Topomol module, followed by Resilient back- propagation (Rprop) neural network to develop successful nonlinear model.

Toxicity prediction

In silico toxicity prediction will have more and more importance in early drug discovery since 30% of drug candidates fail owing to these issues.

ProTox II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments.

ProTox II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity-based CLUSTER cross- validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

5.3 DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

TARGET SELECTION

The present study was focused on EGFR tyrosine kinase inhibition. From the literature review and the current research on EGFR tyrosine kinase enzyme inhibitors, we have selected EGFR tyrosine kinase enzyme as the target for the present study. The PDB structure of EGFR tyrosine kinase (1M17) was downloaded from the RCSB protein data bank.

LEAD SELECTION

The Pyridinyl triazole derivatives were selected based on several literature reviews.

DOCKING STUDIES FOR THE LEAD

Aim : To predict the bioactivity score of the ligands

Database : RCSB protein data bank

Protein selected : EGFR tyrosine kinase enzyme (1M17)

Target proteins were downloaded from RCSB protein data bank and docking studies were performed.

Steps involved in docking studies

- Docking process is done with AutoDock 4.2
- Conversion of refined enzyme into pdb format
- Conversion of pdb format of ligand into pdbqt format
- Preparation of grid box by setting grid parameters
- Docking process by setting docking parameters
- Saving the docked result as dlq file
- Viewing the docked conformation
- Taking snapshots of the interactions

STEP I:**Protein structure refinement**

EGFR tyrosine kinase enzyme was downloaded from RCSB Protein Data Bank and the enzyme was refined before docking. The steps involved are: was downloaded from RCSB Protein Data Bank (PDB) and the enzyme was refined before docking. The steps involved are:

- Discovery studio visualizer
- File → Open → Select the enzyme file downloaded from → RCSB PDB.
- Click View option and then click Hierarchy.
- Click water molecules.
- Click water molecule → select all water molecules → cut.
- Select ligand, which is unnecessary and cut.
- Save the molecule in a desired location

STEP II**Ligand file format conversion**

- The ligands which are desired are drawn in ChemSketch software
- Tools → Click Generate → Click SMILES notation (Simplified Molecular Input Line Entry System, which is a file format).
- Save the SMILES in a word document.
- Open the online smiles translator cactus.nci.nih.gov/services/translate.
- Upload the SMILES.
- By choosing the required file format and save the file in a pdb format (e.g.:ligand.pdb).

The protein and ligand files which are prepared by above said procedures are taken for docking.

STEP III

- Docking with autodock 4.2
- Docking calculation in AutoDock was performed using the refined protein and the desired ligand in pdb format.

Preparation and running a docking programme

Preparing the protein

- Open autodock 4.2
 - Open file → Click read molecule → Choose the particular → refined enzyme file.
 - The elimination of the water is carried by the following steps.
 - Press Select option
 - Click Select → click select from string option
 - Then write “*HOH*” in the Residue line & “*” in the atom line.
 - Click Add → No new selection and then dismiss.
 - Addition of hydrogens is done by,
 - Press Edit option • Click the Hydrogen
 - Then click Add
 - Choose all Hydrogen, No Bond Order, and „yes” to renumbering click Ok
 - . Next click → Edit option → click add the Kollmann Charges.
 - Then save the enzyme molecule as 1ea1refined.pdb
 - Select Edit →Delete → Delete all molecule
-

Preparing the ligand

- Confirm that all the hydrogens are added in the ligand.
- Toggle the Auto Dock Tools button.
- Open the Ligand → Click Input and choose the suitable ligand →file and finally open.
- The torsions are designed by following steps
- In the Ligand option select Torsion Tree
- Select Detect Root option • Click Torsion Tree
- Then select the Choose Torsions option
- Amide bonds should NOT be active.
- After that click the Torsion Tree and select Set Number of Torsions
- Number of rotatable bonds is chosen.
- Finally Save the Ligand files by selecting the Output option (pdbqt file).
- Select Edit → Delete → Delete all molecule.
- Conversion of pdb files of protein into pdbqt file
- Select the Grid option and open the Macromolecule pdb file.
- Auto Dock adds the Charges and itself merges the Hydrogens.
- Save the object as pdbqt in desired area.
- AutoGrid Calculation and creating “gpf” file
- Open the grid and click Macromolecule option and choose the rigid protein then yes to preserve the existing charges.

- The Preparation of grid parameter file is carried out by,
- Open Grid
- Select the Set Map Types
- Choose Ligand
- Accept it.

Setting of grid properties

- Open Grid
- Select the Grid box
- Set the proper Grid Dimensions.
- Adjust the Spacing
- Select the File and click Close Saving Current.
- Save the grid settings as gpf file in the input option (ligand.gpf).
- After running the grid file, the output automatically save as „glg“ file

Auto Dock calculation and creating 'dpf' file:

The rigid molecule specification is carried out by,

- Select the Docking option
- Click the Macromolecule
- Set Rigid File Name.
- The ligand specification is carried out by
- Click the Docking option
- Select the ligand
- And then Accept it. In the next step, click Docking option and select Search Parameters in that click Genetic Algorithm and finally accept it.
- Click Docking options → Select Docking Parameters → Choose the Defaults.

- Click Docking option→Select Output and adds Lamarckian Genetic algorithm (LGA).
- Save the docked settings as „dpf“ file in the input option (ligand.dpf)
- After running the docked file, the output automatically saves as „dlg“ file.

Programming of ‘Auto Grid’ and ‘Auto Dock’execution:

1. Open Cygwin and typed as follows

- cd c:
- cd cygwin
- cd usr
- cd local
- cd bin

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and ligand molecule.

2. Then type as: `./autogrid4.exe <space>-p<space> ligand.gpf -l <space> ligand.glg`

If a ligand gets into the spacing of the grid, then the execution of this command will be; **‘Successful completion’**.

3. Then type as: `./autodock4.exe<space>- p<space>-ligand.dpf<space> – lligang.dlg`

If the ligand binds to the amino acids through 10 different conformations, then the execution of this command will be; **‘Successful completion’**

STEP IV

Viewing docking results

- Reading the docking log file .dlg
- Toggle the AutoDock Tools button
- Click Analyze and Open Dockings.
- In the next step, click Analyze option and Conformations then Load.

- Double click on the conformation for to view it.

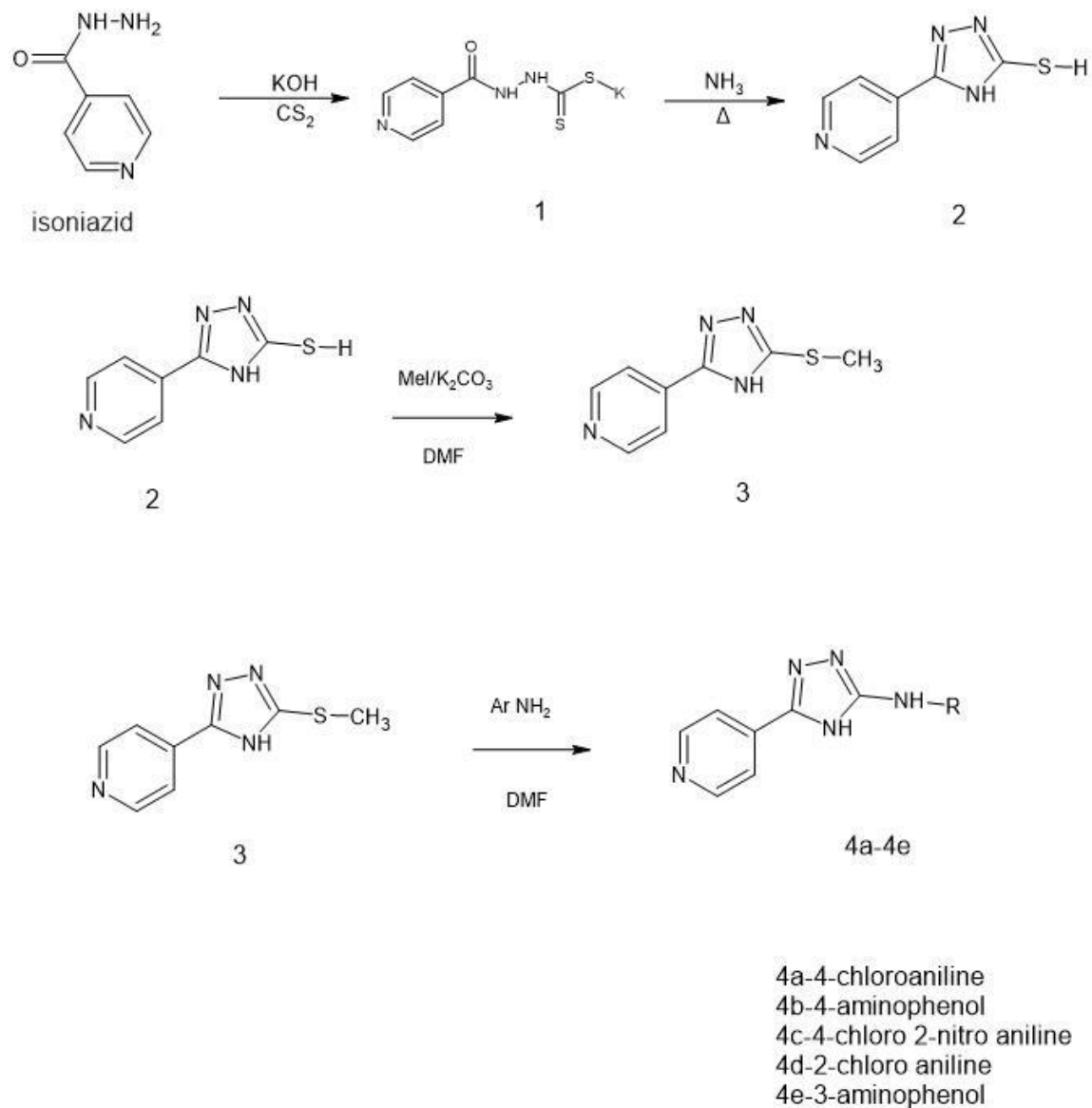
Visualizing docked conformations

- Click Analyze and Dockings then play.
- Load dlg file.
- Choose the suitable conformations.
- In the next step, click Analyze and Docking then Show Interactions.

Obtaining snap shots of docked pose

- Open the File and Read the Molecule
- Open Analyze → Click Dockings and Open dlg file
- Open Analyze → Click Macromolecule and Choose pdbqt file
- Open Analyze → Click Conformations and Load
- Double click the desired conformation
- Click Analyze and Docking then Show Interactions.
- Proteins and ligand interaction will be displayed. Zoom it and increase the contrast by holding right key and ctrl.
- Open File → Save image → cygwin/usr/local/bin as .png
- The above-mentioned steps involved in docking are done for all the ligands.

5.4 SCHEME



General Procedure for Synthesis:**Synthesis of potassium-4-pyridyl-dithiocarbazate 1:**

A solution of 8.4 g (0.15 M) of potassium hydroxide, 13.7 g (0.10 M) of isoniazid, and 11.4 g (0.15 M) of carbon disulfide was prepared in 200 mL absolute ethanol. The mixture was then agitated for 12–16 h. It was then diluted with 200 mL of dry ether and dried at 65°C. The salts, prepared as described earlier, were obtained, were nearly quantitatively yield, and were employed without further purification.

Synthesis of pyridine linked 1,2,4-triazole-3-thiol 2:

A suspension of **1** (24 g, 0.096 M) in 20 mL (0.864 M) Ammonia and water 40 mL was refluxed with stirring for 3 to 4 h. The resulting mixture was poured in ice-cold water (100 mL). Obtained white precipitate was acidified with concentrated HCl, filtered and washed with cold water.

Synthesis of 4-[5-(methylsulfanyl)-4*H*-1,2,4-triazol-3-yl] pyridine 3:

To a solution of 4-[5-(methylsulfanyl)-4*H*-1,2,4-triazol-3-yl] pyridine **2** (1.58 g, 50 mmol) in DMF (10 mL), iodomethane (1.14 g, 80 mM) and anhydrous potassium carbonate (0.69 g, 50 M) were added, and the mixture was stirred at room temperature for 4 hours. Water (15 mL) was added, and the mixture was stirred for further 30 minutes. The precipitated crude product filtered, washed with water, dried, and crystallized from ethanol to yield **3**.

Synthesis procedure for *N*-(4-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-amine (4a):

4-chloroaniline (6.37g ,50 mM) was added to a solution of compound **3** (1.56 g, 50 mM), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off in vacuo, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **4a** reddish-brown crystals; Yield: 71.5%; mp: 120-125°C.

Synthesis procedure for 4-[[5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-yl]amino]phenol (4b):

4-aminophenol (6 ml ,50 mM) was added to a solution of compound **3** (1.56 g, 50 mM), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off in vacuo, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **4b** brown crystals; yield: 65%; M.P-115-118°C

Synthesis procedure for *N*-(4-chloro-2-nitrophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-amine (4c):

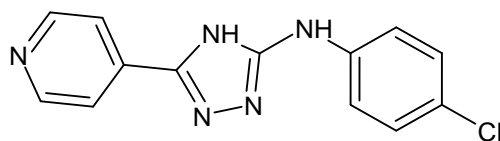
4-chloro 2-nitro aniline (8.6 g,50 mM) was added to a solution of compound **3** (1.56 g, 50 mM), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off in vacuo, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **4c**. yellow crystals; yield:75% M.P-118-122°C.

Synthesis procedure for *N*-(2-chlorophenyl)-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-amine (4d):

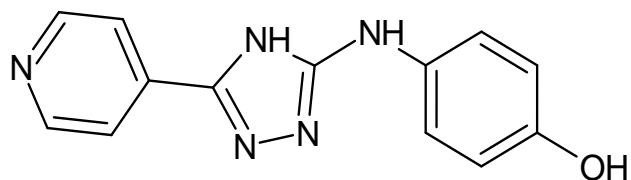
2-chloro aniline (6.35 g,50 mM) was added to a solution of compound **3** (1.56 g, 50 mM), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off in vacuo, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **4d**. Yellowish powder; yield: 70%; M.P-120-125°C.

Synthesis procedure for 3-[[5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl] amino} phenol (4e):

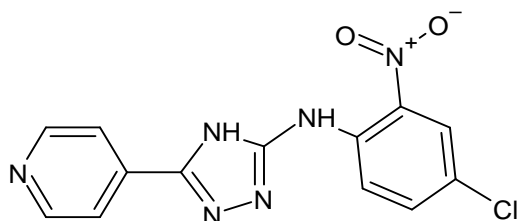
3-aminophenol (5.65 g, 50 mM) was added to a solution of compound **3** (1.56 g, 50 mM), in DMF (10 mL) containing few drops of 37% hydrochloric acid, and the mixture was heated under reflux for 10 hours. The excess DMF was then distilled off in vacuo, and the solid residue was triturated with water (20 mL). The separated solid precipitate was filtered, washed with water, and crystallized from DMF-Ethanol to yield compounds **4e**. reddish crystal; yield: 68%; M.P-128-130°C.

5.5 CHARACTERIZATION:**Compound Code: 4a**

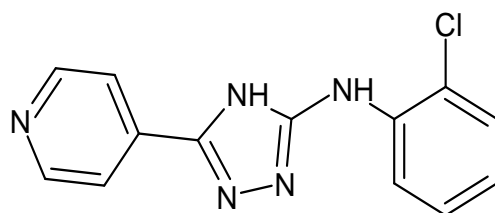
Chemical name	<i>N</i> -(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-amine
IR (KBr, cm⁻¹)	817 (cl), 1490 (C=N stretch), 1527 (Aromatic C-C stretch), 1286 (CN stretch), 1559 (Aromatic C=C stretch), 3198 (Aromatic C-H stretch)
MASS (M⁺)	M ⁺ -271.83, M+2 - 273.4213

Compound Code: 4b

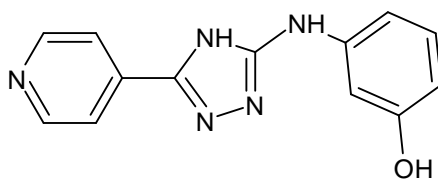
Chemical name	4-[[5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]amino]phenol
IR (KBr, cm⁻¹)	3016 (OH), 3256 (NH stretch), 1496 (C=N stretch), 1567 (Aromatic C=C stretch), 1229 (CN), 2919 (Aromatic CH stretch)
MASS (M⁺)	M ⁺ -253.143

Compound Code: 4c

Chemical name	<i>N</i> -(4-chloro-2-nitrophenyl)-5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-amine
IR (KBr, cm⁻¹)	814 (cl), 3348 (NH stretch), 1497 (C=N stretch) , 1559 (Aromatic C=C stretch), 1243 (CN), 2920 (Aromatic CH stretch), 1365 (NO ₂).
MASS (M⁺)	M ⁺ -316.24, M+2 -318.14

Compound Code: 4d

Chemical name	<i>N</i> -(2-chlorophenyl)-5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-amine
IR (KBr, cm⁻¹)	1527 (C=N stretch), 1141(Aromatic C-C stretch), 1308 (CN), 1595 (Aromatic C=C stretch), 825 (cl), 3378 (NH Stretch)

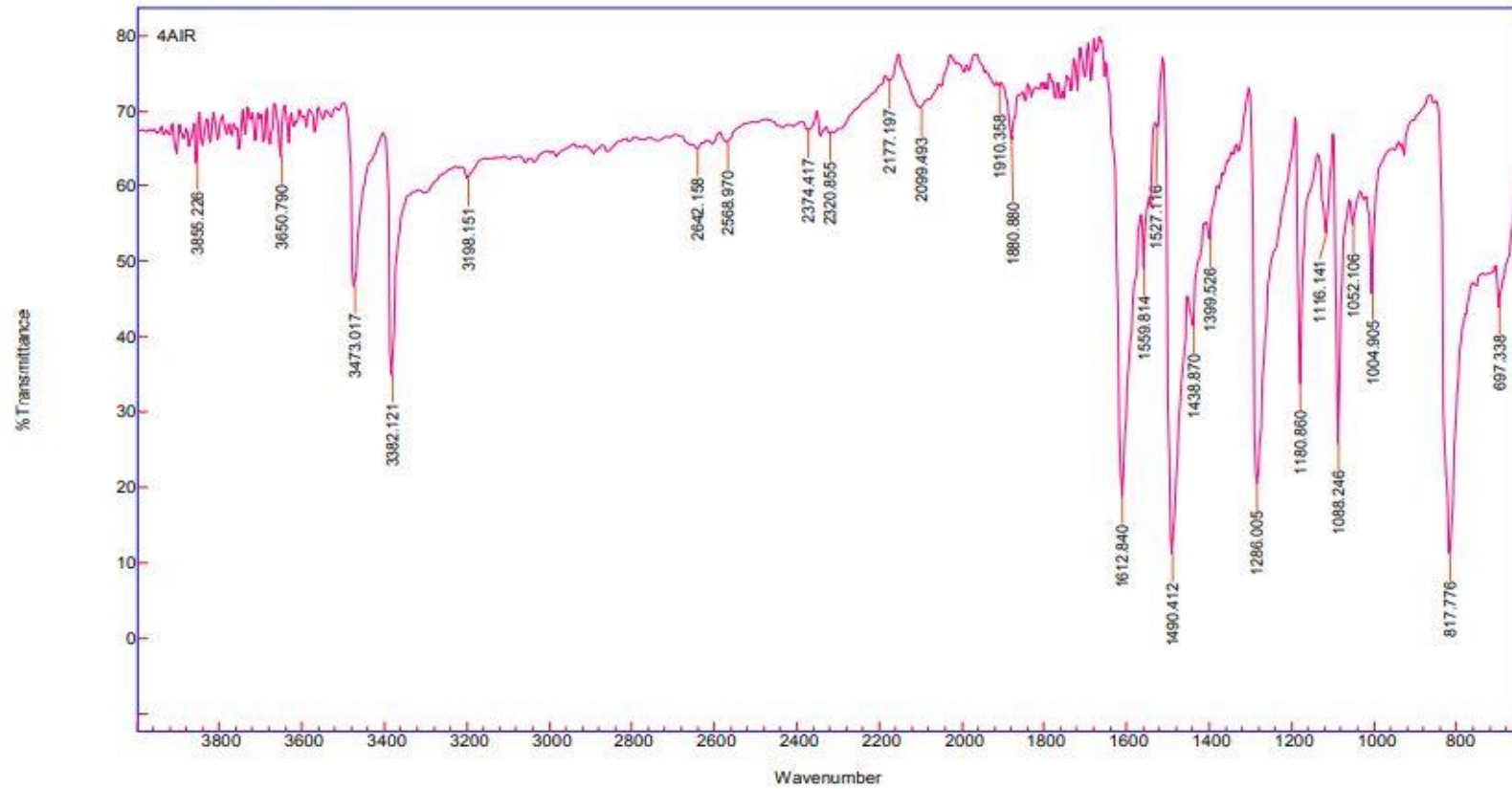
Compound Code: 4e

Chemical name	3-[[5-(pyridin-4-yl)-4 <i>H</i> -1,2,4-triazol-3-yl]amino}phenol
IR (KBr, cm⁻¹)	3205 (OH), 2926 (Aromatic CH stretch), 1660 (Aromatic C=C stretch), 1541 (Aromatic C-C stretch), 1218 (CN), 1490 (C=N)

IR SPECTRA

4a:

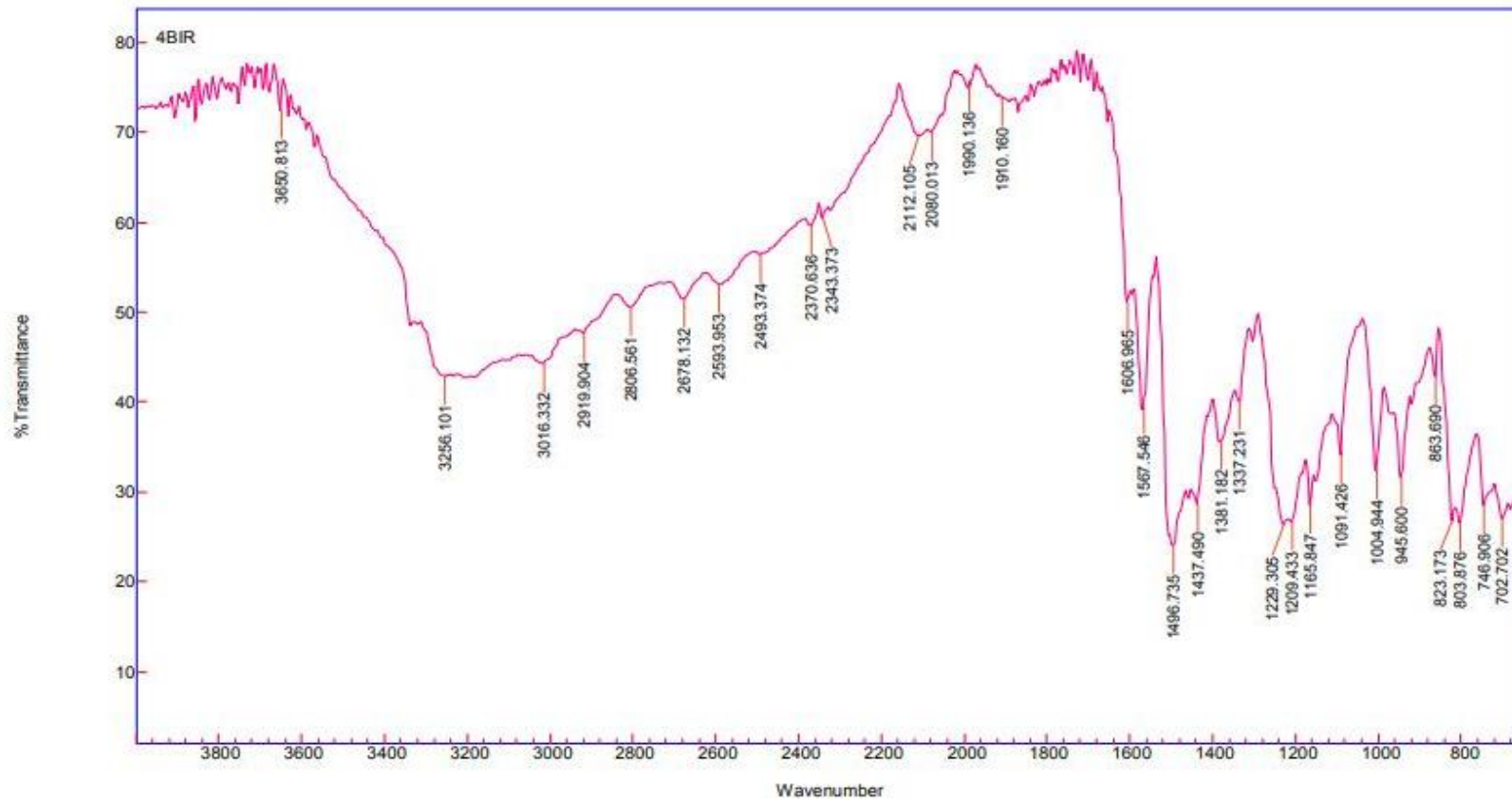
Agilent Resolutions Pro



Name
4AIR

4b:

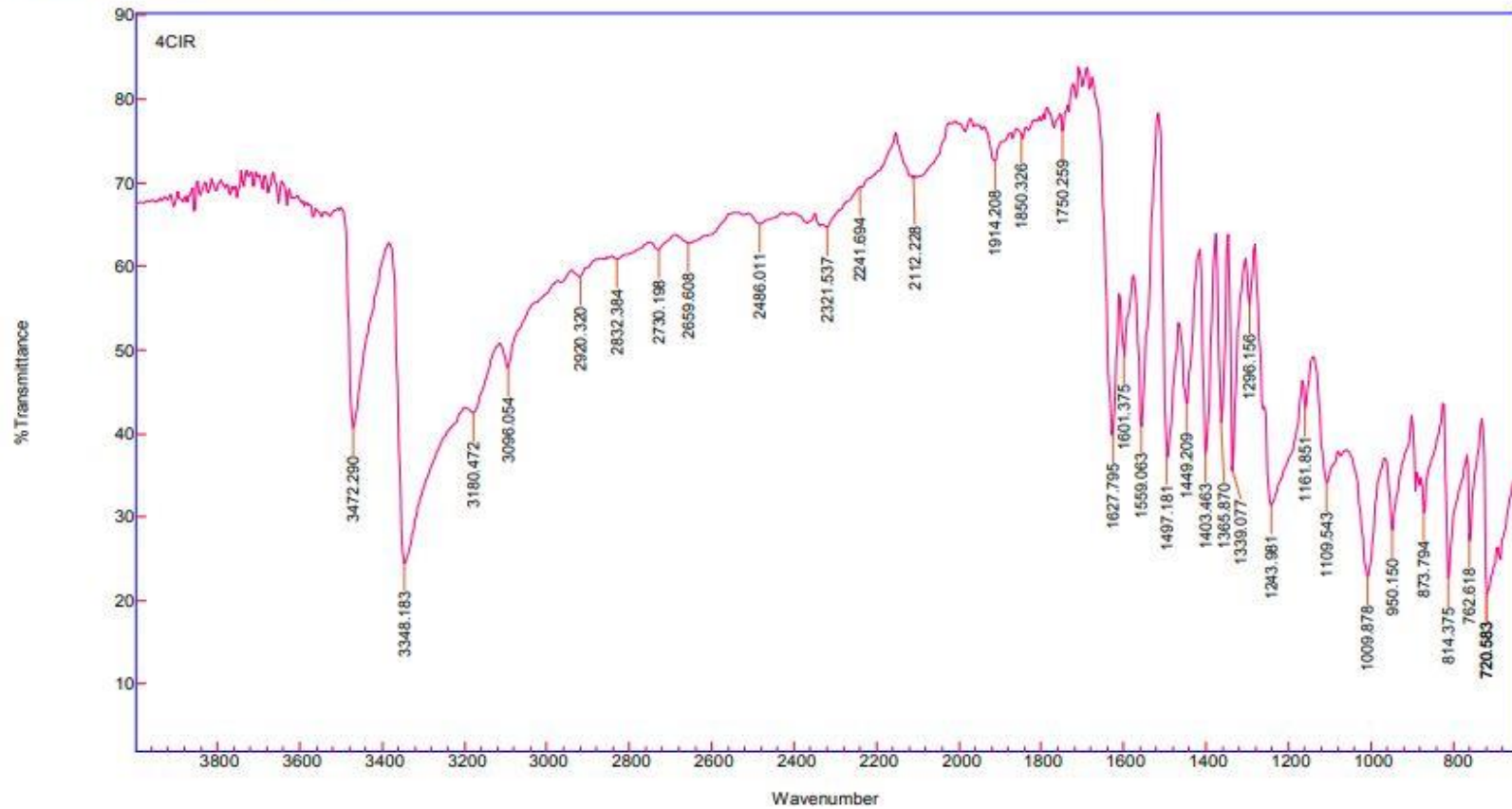
Agilent Resolutions Pro



Name
4BIR

4c:

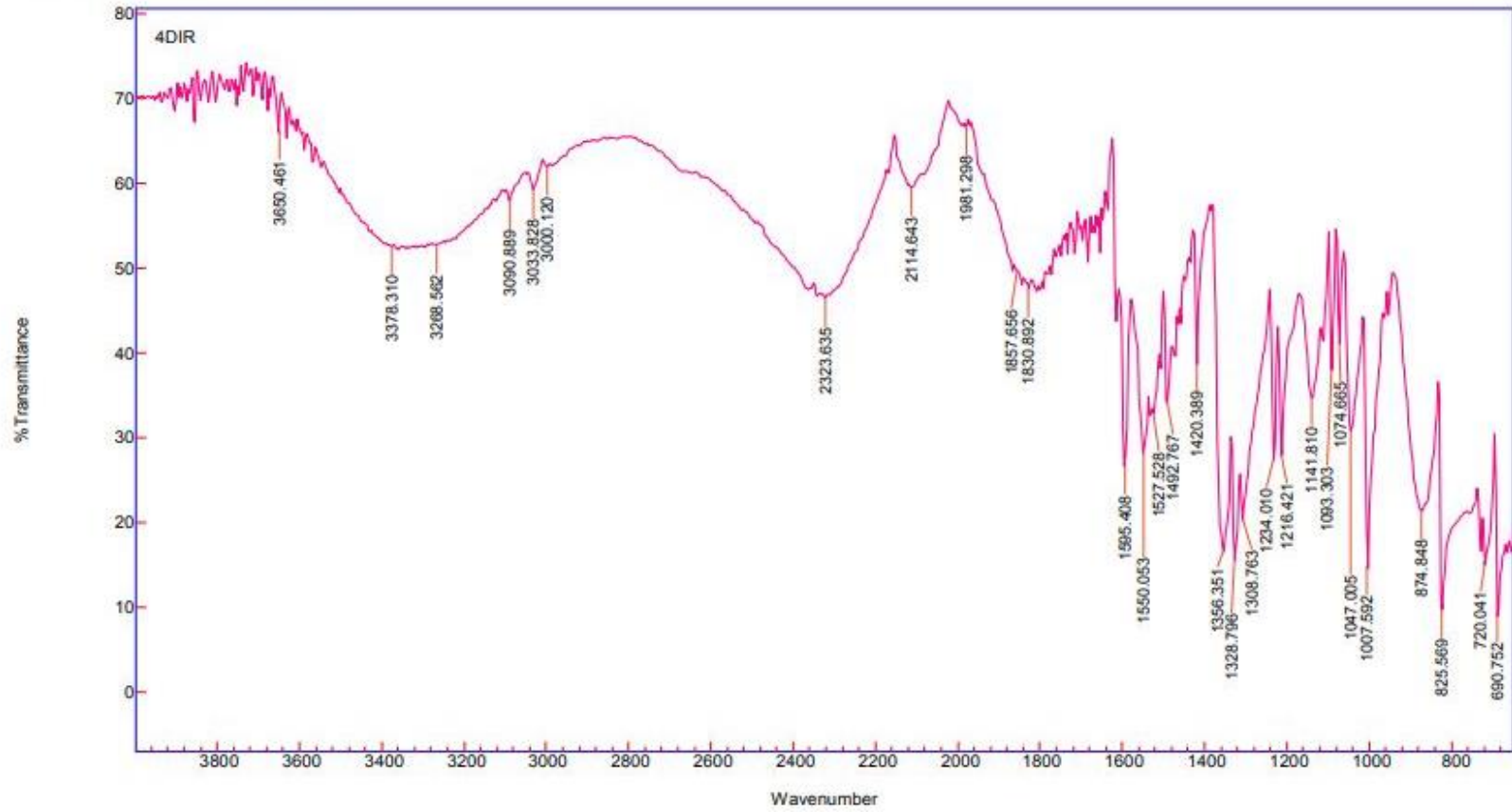
Agilent Resolutions Pro



Name	
4CIR	—

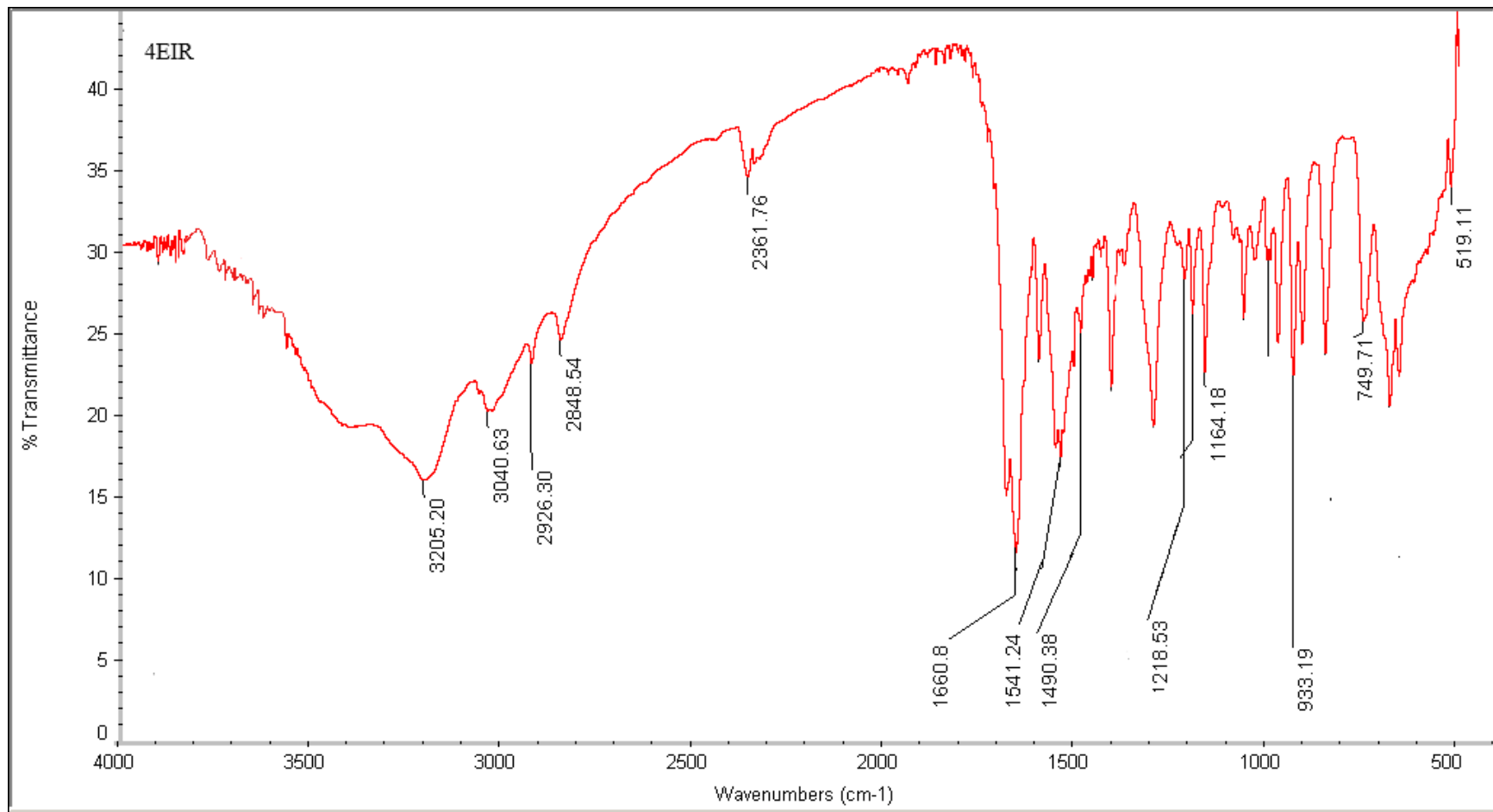
4d:

Agilent Resolutions Pro



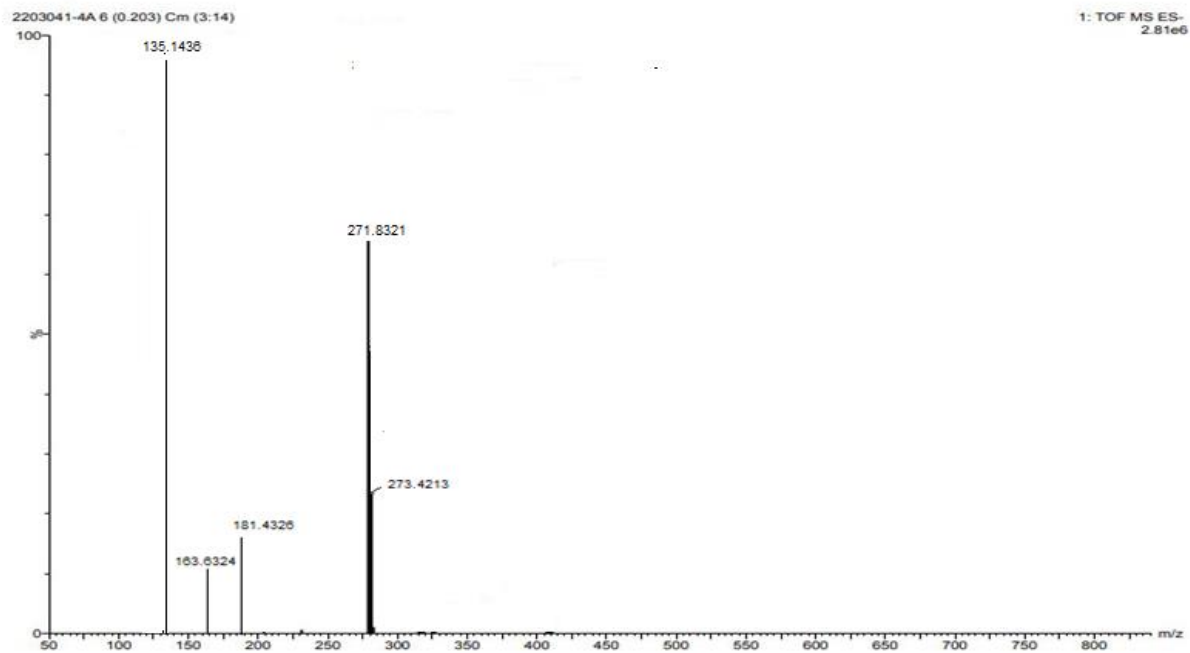
Name
4DIR

4e:

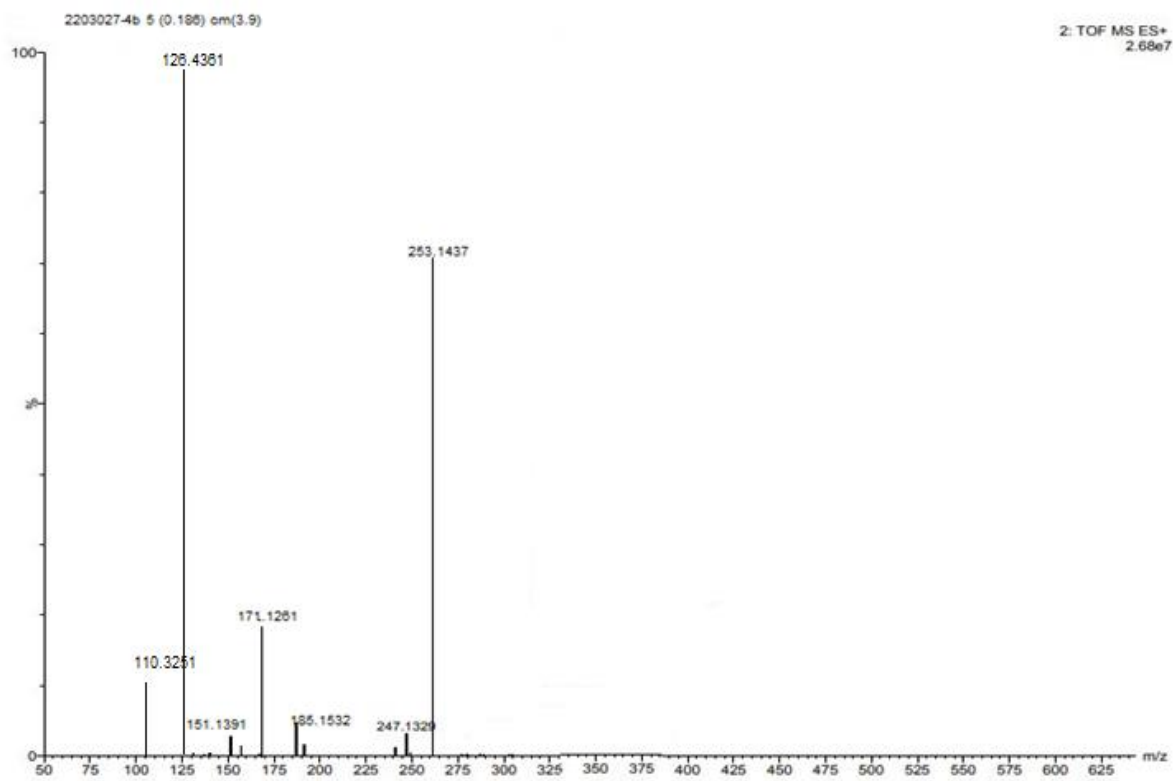


Mass Spectral Data

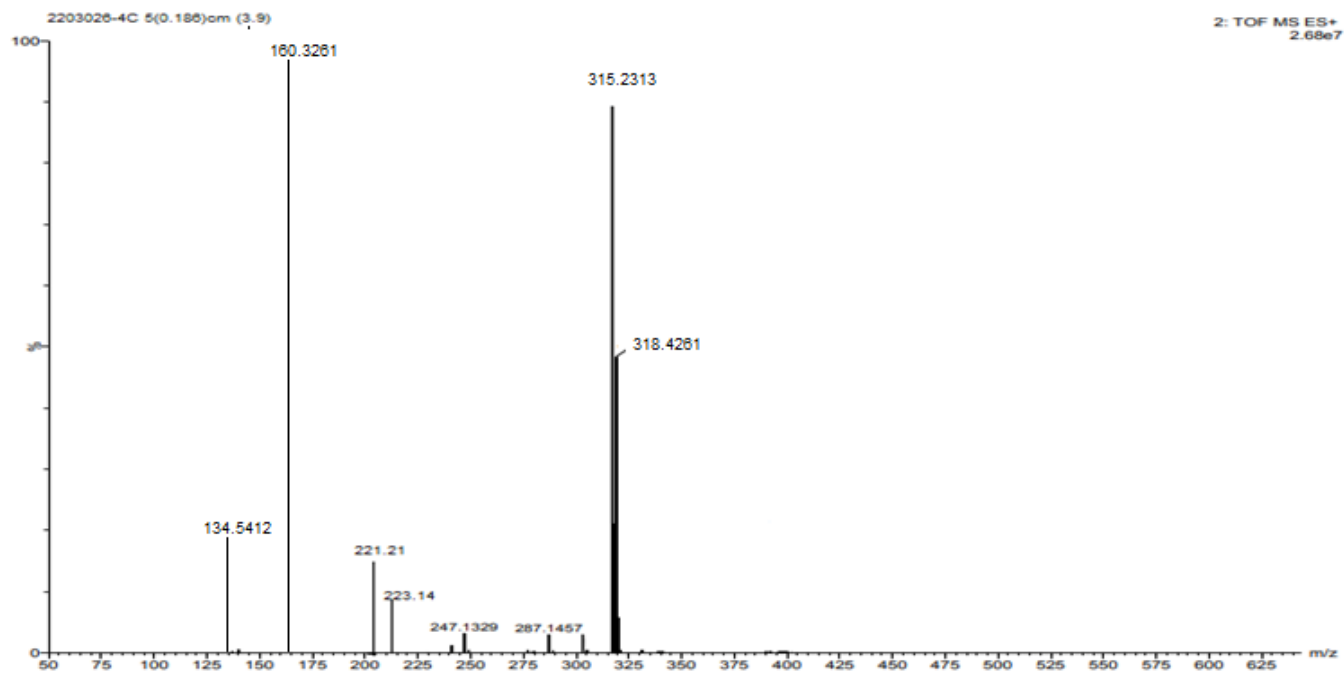
4a



4b



4c



5.6 ANTICANCER ACTIVITY

MTT Cell viability Assay:

Preparation of test solutions

For MTT assay, serial two-fold dilutions (12.5 - 200 μ g/mL) were prepared from this assay.

Cell lines and culture medium

MCF-7 cell line was procured from NCCS, stock cell was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 μ g/mL) in a humidified atmosphere of 5% CO₂ at 37°C until confluent.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 μ L of the diluted cell suspension (1 x 10⁴ cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μ L of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37°C for 24 h in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 20 μ L of MTT (2 mg/mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 μ L of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

The percentage of viability was calculated using the following formula,

$$\% \text{ of viability} = \text{Sample abs/Control abs} \times 100$$

6. RESULTS AND DISCUSSION

6.1 *INSILICO* SCREENING OF DESIGNED COMPOUNDS

The ADME properties of the designed compounds were evaluated using SWISS ADME software and Pre-ADMET software, their results were tabulated (Table no: 4,5)

Toxicity of all the designed compounds were evaluated by using ProTox II software and the results were tabulated (Table No :6). The designed compounds are inactive for mutagenicity, Carcinogenicity, and cytotoxicity.

Anticancer agents generally possess greater number of hydrogen bond acceptors. All the designed compounds have less than 5 hydrogen bond donors and 6-10 hydrogen bond acceptors. This obeys Lipinski's rule of rule. Molecular weight of all the designed compounds was around 500 daltons.

All our designed compounds show log P values between 1- 5. It was found that lipophilicity plays a major role in determining where drugs are distributed within the body after adsorption and, as a consequence, how rapidly they are metabolized and excreted. In the biological system drug disposition depends on the ability to cross membranes, so there is a strong relationship with measures of lipophilicity. So there is a strong lipophilic character of the molecule plays a major role in producing the antimicrobial effect.

TPSA has been used as descriptor for characterizing absorption and passive transportation properties through biological membranes, allowing a good prediction of transport of candidate drugs in the intestines and through the blood-brain barrier. Compounds with TPSA values within the range 140 \AA^2 have good intestinal absorption. TPSA (Total Polar Surface Area) of our designed compounds were found to be in the range of $88\text{-}140 \text{ \AA}^2$. Therefore, it is expected that our compounds might possess good intestinal absorption.

The designed compounds passed Lipinski rule of five, that the drug is suitable for oral administration.

Synthetic accessibility score is normalized between 1 (easy synthesis) and 10 (very difficult synthesis). The designed compounds showed score between 3 to 6. This indicates the designed compounds can be synthesized in ease.

BBB penetration values of all compounds were found to be less than 1. So, these compounds are CNS inactive. Caco2 cell permeability of all compounds lies between 18-25. This range indicates all the designed compounds have moderate permeability. Plasma protein binding of all the compounds was found in the range of 94-100, which indicates the chemicals are strongly bound.

HIA (Human Intestinal Absorption) values of all designed compounds were found between 92-98. This indicates that all the designed compounds are well absorbed in intestine.

Table 3: Chems sketch Results for designed compounds

Compound number	Molecular formula	Molecular Weight (g/mol)	Molar refractivity (cm ³)	Molar volume (cm ³)	Parachor (cm ³)	Number of HBA	Number of HBD	TPSA (Å)
3a	C ₁₇ H ₁₇ N ₅ OS	339.41	93.76	252.0	739.7	4	2	91.77
3b	C ₁₇ H ₁₇ N ₅ OS	339.41	93.76	252.0	739.7	4	2	83.56
3c	C ₁₈ H ₁₉ N ₅ OS	353.44	98.38	267.7	778.0	4	2	83.56
3d	C ₁₉ H ₂₁ N ₅ OS	367.46	103.00	283.4	816.3	4	2	83.56
3e	C ₁₁ H ₁₃ N ₅ OS	263.31	69.05	193.2	567.7	4	2	97.56
3f	C ₁₂ H ₁₅ N ₅ OS	277.34	73.68	209.5	607.7	4	2	93.54
3g	C ₁₁ H ₁₃ N ₅ OS	263.31	69.05	193.2	567.7	4	2	83.56

3h	C ₁₂ H ₁₅ N ₅ OS	277.34	73.68	209.5	607.7	4	2	91.77
3i	C ₁₇ H ₁₇ N ₅ OS	339.41	93.76	252.0	739.9	4	2	96.77
3j	C ₁₇ H ₁₇ N ₅ OS	339.41	93.76	252.0	739.9	4	2	93.56
3k	C ₁₈ H ₁₉ N ₅ OS	353.44	98.38	267.7	778.0	4	2	83.56
3l	C ₁₉ H ₂₁ OS	367.46	103.00	283.4	816.3	4	2	83.56
4a	C ₁₃ H ₁₀ ClN ₅	271.75	73.82	190.7	551.8	3	2	92.56
4b	C ₁₃ H ₁₁ N ₅ O	253.25	70.80	177.1	531.0	4	3	91.77
4c	C ₁₃ H ₉ ClN ₆ O ₂	316.70	80.36	202.5	607.3	5	2	83.56
4d	C ₁₃ H ₁₀ ClN ₅	271.75	73.82	190.7	551.8	3	2	97.45
4e	C ₁₃ H ₁₁ N ₅ O	253.25	70.80	177.1	531.0	4	3	91.77

Table: 4 Swiss ADME results

Compound	Log S ESOL	GI absorption	log P o/w	Lipinski drug- likeness	Synthetic accessibility	Ghose	Vebar	Egan	Muegge	Bioavailability
3a	-3.70	High	1.96	0 violation	3.86	yes	Yes	yes	yes	0.55
3b	-3.70	High	1.89	0 violation	3.85	yes	Yes	yes	yes	0.55
3c	-3.99	High	2.10	0 violation	3.95	yes	Yes	yes	yes	0.55
3d	-4.29	High	2.20	0 violation	4.06	yes	Yes	yes	yes	0.55
3e	-2.01	High	0.97	0 violation	3.67	yes	Yes	yes	yes	0.55

3f	-2-23	High	1.37	0 violation	3.72	yes	Yes	yes	yes	0.55
3g	-2.01	High	0.90	0 violation	3.60	yes	Yes	yes	yes	0.55
3h	-2.23	High	1.17	0 violation	3.66	yes	Yes	yes	yes	0.55
3i	-3.70	High	1.77	0 violation	3.83	yes	Yes	yes	yes	0.55
3j	-3.70	High	1.99	0 violation	3.81	yes	Yes	yes	yes	0.55
3k	-3.99	High	1.90	0 violation	3.92	yes	Yes	yes	yes	0.55
3l	-4.29	High	1.80	0 violation	4.02	yes	Yes	yes	yes	0.55
4a	-3.61	High	1.24	0 violation	2.82	yes	Yes	yes	yes	0.55

4b	-2.88	High	0.70	0 violation	2.80	yes	Yes	yes	yes	0.55
4c	-3.97	High	1.38	0 violation	3.13	yes	Yes	yes	yes	0.55
4d	-3.61	High	1.43	0 violation	2.91	yes	Yes	yes	yes	0.55
4e	-2.88	High	0.80	0 violation	2.85	yes	Yes	yes	yes	0.55

Table: 5 Pre-ADMET results

COMPOUND	BBB	CaCO₂	PLASMA PTROTEIN BINDING	MDCK	HIA
3a	0.375605	18.226	97.780	14.392	90.0494
3b	0.19906	17.9523	98.754	17.910	90.4953
3c	0.567581	19.0135	96.070	0.981	90.8562
3d	0.528786	19.3625	95.081	0.155	91.6408
3e	0.13206	10.8699	44.453	10.328	84.95773
3f	0.154632	15.6032	59.160	16.201	85.8046
3g	0.0446253	10.8417	41.394	14.586	84.9577
3h	0.611783	15.5775	54.840	22.0317	85.8046
3i	0.20089	18.1831	98.800	18.5358	90.4944
3j	0.138276	17.9254	97.948	22.661	90.4952
3k	0.329225	18.9701	95.107	1.319	90.8562
3l	0.359365	19.32832	94.313	0.180	91.2090
4a	1.61676	17.9167	82.49	13.23	90.005

4b	0.42143	19.455	65.87	44.257	80.907
4c	0.114585	15.5653	100.00	6.205	86.907
4d	1.24676	12.203	84.591	27.09	90.005
4e	0.3430	14.5628	67.2458	46.1334	8.90

Table: 6 Pro-Tox II results

Compound	Carcinogenicity	Immuno toxicity	Mutagenicity	Cytotoxicity	Predicted LD₅₀ {mg/kg}
3a	InActive	Inactive	InActive	Inactive	1000
3b	InActive	Inactive	InActive	Inactive	1000
3c	InActive	Inactive	Inactive	Inactive	1000
3d	InActive	Inactive	Inactive	Inactive	1000
3e	InActive	Inactive	Inactive	Inactive	1700
3f	Inactive	Inactive	Inactive	Inactive	1700
3g	Inactive	Inactive	Inactive	Inactive	1000
3h	Inactive	Inactive	Inactive	Inactive	1000

3i	Inactive	Inactive	Inactive	Inactive	1000
3j	Inactive	Inactive	Inactive	Inactive	1000
3k	Active	Inactive	Inactive	Inactive	1000
3l	Active	Inactive	Inactive	Inactive	1000
4a	Inactive	Inactive	Inactive	Inactive	680
4b	Inactive	Inactive	Inactive	Inactive	680
4c	Inactive	Inactive	Inactive	Inactive	680
4d	Inactive	Inactive	Inactive	Inactive	680
4e	Inactive	Inactive	Inactive	Inactive	680

6.2 DOCKING

The docking results of EGFR tyrosine kinase **PDB:1M17** with the 17 designed compounds are reported in the Table 7. The best docked structures should have lower binding energies. 5 compounds of **4a-4e** derivatives with good binding affinity range with reference to standard were selected for synthesis.

TABLE 7: Binding energies of designed compounds

COMPOUND	Binding energy (Kcal/mol)	COMPOUND	Binding energy (Kcal/mol)
3a	-7.0	3j	-7.12
3b	-6.5	3k	-7
3c	-7.1	3l	-6.7
3d	-6.9	4a	-7.16
3e	-6.8	4b	-7.19
3f	-6.5	4c	-7.24
3g	-6.8	4d	-7.57
3h	-6.5	4e	-7.24
3i	-6.6	LETROZOLE	-7.57

Table 8: Energies of designed compounds

COMPOUND NAME	BINDING ENERGY (Kcal/mol)	LIGAND EFFICIENCY	INHIBITION CONSTANT (μM)	INTER MOLECULAR ENERGY	DESOLVATION ENERGY	ELECTROSTATIC ENERGY	TOTAL INTERNAL ENERGY	TORSIONAL ENERGY
4a	-7.16	-0.38	5.68	-8.05	-7.44	-0.61	-0.45	0.89
4b	-7.19	-0.38	5.4	-8.08	-7.52	-0.56	-0.41	0.89
4c	-7.24	-0.33	4.93	-8.43	-7.85	-0.59	-1.76	1.19
4d	-7.57	-0.41	2.81	-8.47	-7.93	-0.53	-0.52	0.89
4e	-7.24	-0.42	1.4	-8.8	-8.33	-0.55	-0.45	0.89
LETROZOLE	-7.57	-0.35	2.22	-8.61	-8.46	-0.15	-0.95	0.89

Table: 9 Amino acids interaction residue

Compound	Enzyme's binding site residue
4a	ILE957A, ILE914A, ASP783A, LYS782A, MET963A, GLU961A.
4b	ILE957A, ILE914A, GLY959A, MET963A, ASN784A, GLU961A
4c	ILE914A, GLN958A, MET963A, ASP783A, LYS782A, GLU961A
4d	ILE957A, ASP783A, MET963A, LYS782A, PHE886A, GLY959A, ASN960A, GLU961A
4e	ILE957A, ILE914A, ASN784A, MET963A, GLU961A, ASP783A, LYS782A, PHE886A, VAL956A, GLN958A, VAL956A
LETROZOLE	ILE957A, ILE914A, MET963A, VAL956A, GLN958A, PHE886A, LYS782A

All synthesized molecules interacted with the active sites of enzyme 1M17 through various bonds like hydrogen, van der Waals, p-alkyl, p-donor hydrogen, alkyl, p-sulfur, carbon-hydrogen bond, and p-sigma. As shown in table 8. The binding energies of the compounds 4a-4e range from -7.16-7.54 kcal/mol and were similar range as compared with the standard drug letrozole -7.57 kcal/mol. The binding energy value of **4d** is similar to standard letrozole binding energy value -7.57 kcal/mol. The ligand efficiency value of **4c** is -0.33 which is close to standard value of -0.35. Intermolecular energy, desolvation energy, torsional energy values of all compounds **4a-4e** comply with a standard value.

Out of 7 amino acids of residue of EGFR tyrosine kinase 1M17, which is responsible for the formation of bonds with letrozole, two were found to be similar in all synthesized compounds **4a-4e** [ILE914A, MET963A] and compound **4e** found 7 amino acid residue to be similar in standard drug letrozole [ILE957A, ILE914A, MET963A, VAL956A, GLN958A, PHE886A, LYS782A]. Compound **4e** showed binding site residue resemblance to that of letrozole with EGFR tyrosine kinase 1M17 in table 9.

Docking conformation of the active compound 4e showed good interactions with the active site residues of this protein. Compound 4e formed two hydrogen bond interactions between amino groups moiety, as it acts as a hydrogen bond donor with the side chain of GLN958A and VAL956A residues.

Molecular docking studies of the active compounds revealed that these compounds might act via inhibition of EGFR tyrosine kinases (1M17).

4a interact with EGFR tyrosine kinase **PDB:1M17**.

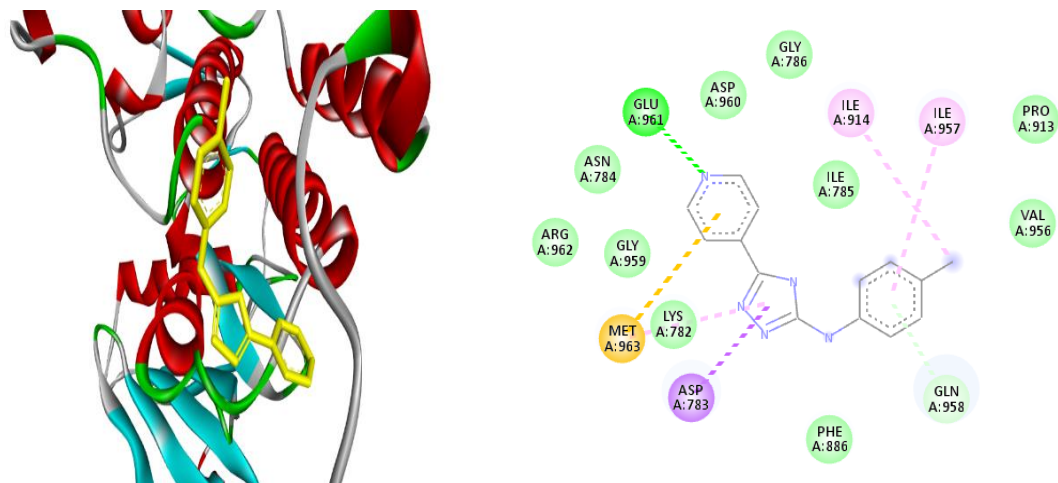


Figure 2: 3D and 2D molecular interaction visualizations of ligand 4a with the active site of 1m17.

4b interact with EGFR tyrosine kinase **PDB:1M17**.

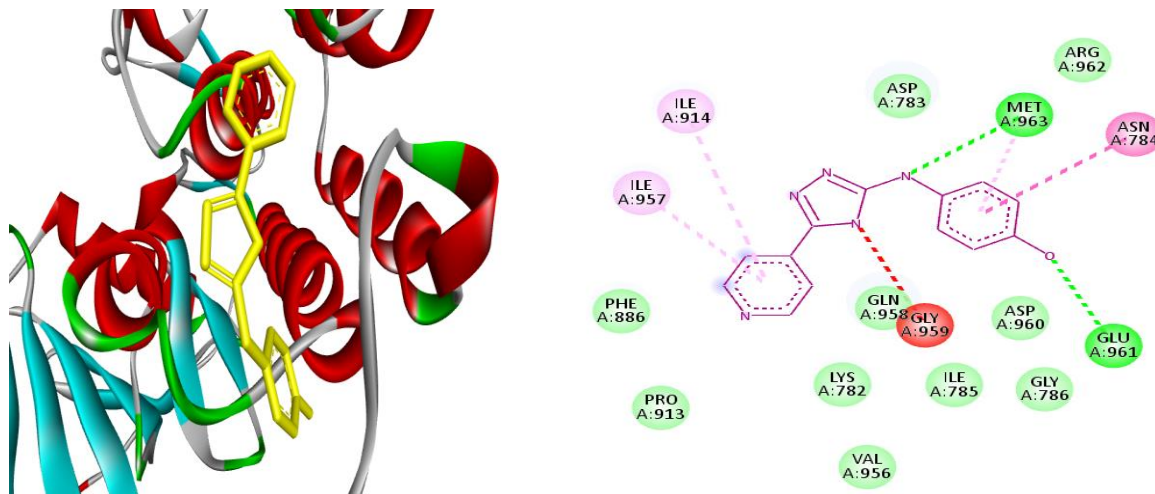


Figure 3: 3D and 2D molecular interaction visualizations of ligand 4b with the active site of 1m17.

4c interact with EGFR tyrosine kinase **PDB:1M17**.

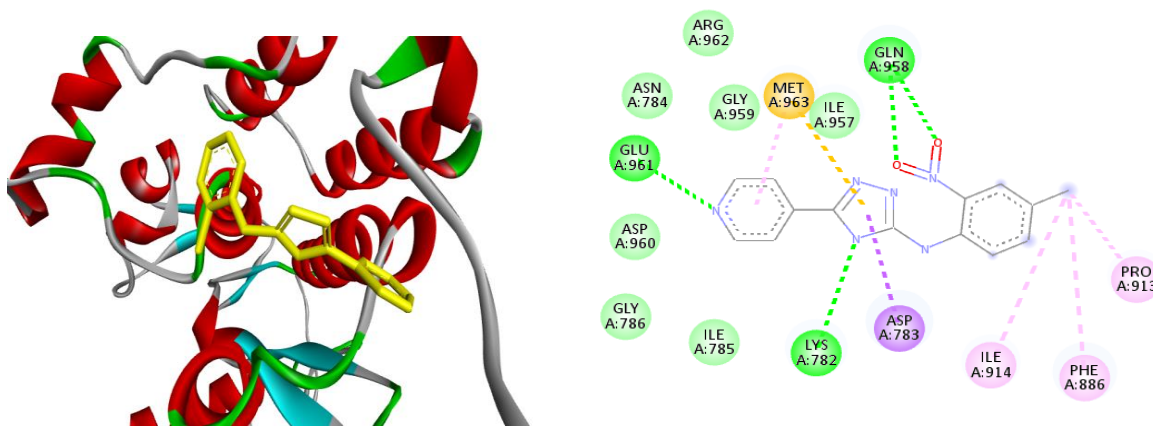


Figure 4: 3D and 2D molecular interaction visualizations of ligand 4c with the active site of 1m17.

4d interact with EGFR tyrosine kinase **PDB:1M17**.

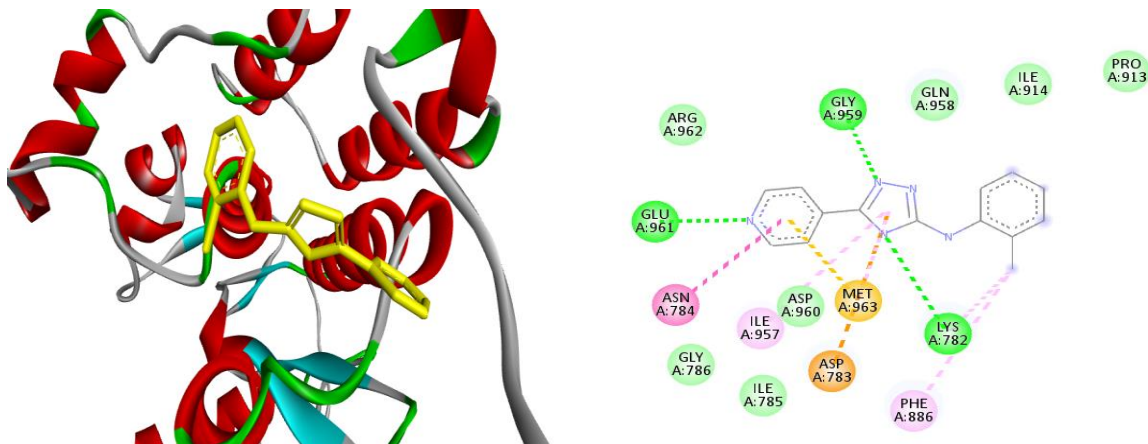


Figure 5: 3D and 2D molecular interaction visualizations of ligand 4d with the active site of 1m17.

4e interact with EGFR tyrosine kinase **PDB:1M17**.

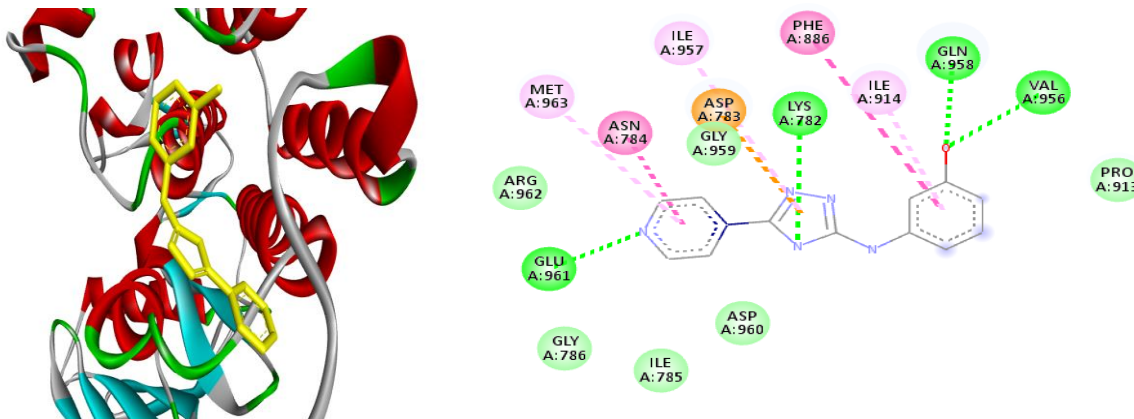


Figure 6: 3D and 2D molecular interaction visualizations of ligand 4e with the active site of 1m17.

LETROZOLE interact with EGFR tyrosine kinase **PDB:1M17**.

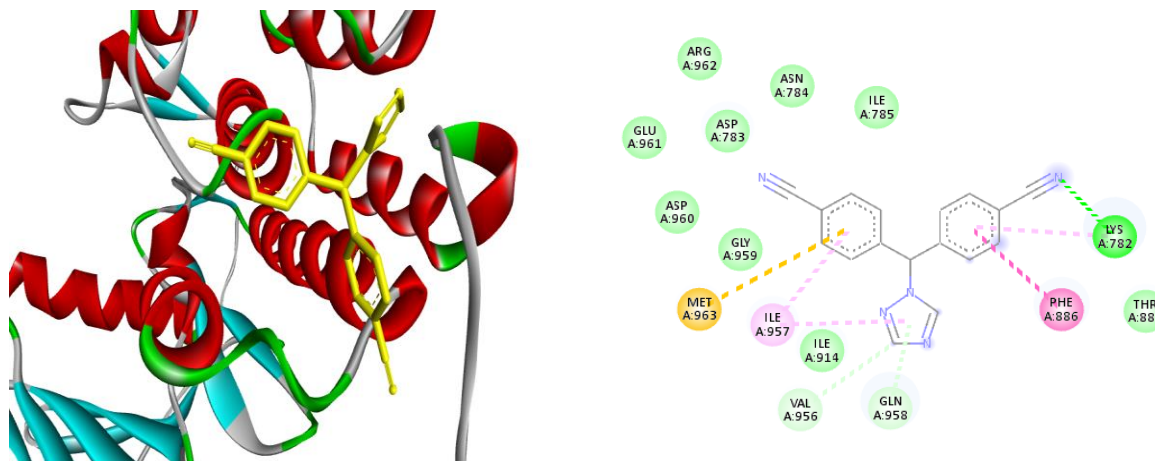


Figure 7: 3D and 2D molecular interaction visualizations of letrozole with the active site of 1m17.

6.3 SYNTHESIS

- Triazole is an attractive bridge group, which could connect two pharmacophores to produce novel bifunctional molecules, while it is almost impossible to be hydrolyzed, oxidized, or reduced. Based on these literature data and we have created a small library by merging the pharmacophore containing 1,2,4-triazoles and substituted benzyl groups via thio linkage.
- The schematic representation for the synthesis of pyridinyl triazole is represented in scheme. pyridine linked 1,2,4-triazole-3-thiol derivative (2) was further reacted with iodomethane in dry N, N-dimethylformamide (DMF), in the presence of anhydrous potassium carbonate to yield the methylthio analogue (3) reaction of the methylthio analogue with different primary aromatic amines via prolonged heating in DMF resulted in replacement of the methylthio group with an arylamino substituent leading to the 6-arylamino derivatives 4a-4e. The yield of the synthesized compounds ranges from 65-70%.
- The schematic representation for the synthesis of pyridinyl triazole is represented in scheme. pyridinyl triazole were synthesized by substitution of different amines (4a-4e) at third position of triazole by conventional method. All the compounds were characterized by FTIR, and MASS.
- The IR spectra of synthesized compounds showed absorption bands due to stretching vibrations of N-H, Aromatic C-H, Aromatic C=C, Aromatic C-C, OH, C=N, and C-N at $3400-3200\text{ cm}^{-1}$, $2900-3200\text{ cm}^{-1}$, $1640-1600\text{ cm}^{-1}$, $1500-1550\text{ cm}^{-1}$, $3000-3600\text{ cm}^{-1}$, $1600-1400\text{ cm}^{-1}$ and $1200-1350\text{ cm}^{-1}$ respectively.
- The mass spectrum showed molecular ion peak which was in agreement with molecular mass of compound while the base peak was observed at 135 (100%).

6.4 INVITRO CYTOTOXICITY STUDIES

- The synthesized pyridine linked triazole derivatives 4a, 4c, 4e were screened for their cytotoxicity studies in MCF-7 cell line using MTT assay and the results were compared to the standard letrozole. The values of compounds 4a,4c,4e was found to be 50,35 and 12.
- 4a ($IC_{50}= 50\pm 0.40$) and 4c ($IC_{50}= 35\pm 0.60$) showed moderate activity against the MCF-7 cell line when compared to the standard letrozole ($IC_{50}= 9\pm 0.23$).
- The compound 4e ($IC_{50}= 12\pm 0.50$) with m-aminophenol substitution was found to demonstrate significant activity against MCF-7 cell line.
- Hence, the overall results showed that the percentage of viable cells, decreased with increasing the dose. The findings indicated that the test compounds showed least to good cytotoxicity against the MCF-7 cell line for anti-breast cancer activity. The anticancer activity results are given in Table 10.

TABLE: 10 The anticancer activity results

S.NO	Compound No	IC ₅₀ (μM) MCF-7
1	4a	50±0.40
2	4c	35±0.60
3	4e	12±0.50
4	Letrozole	9±0.23

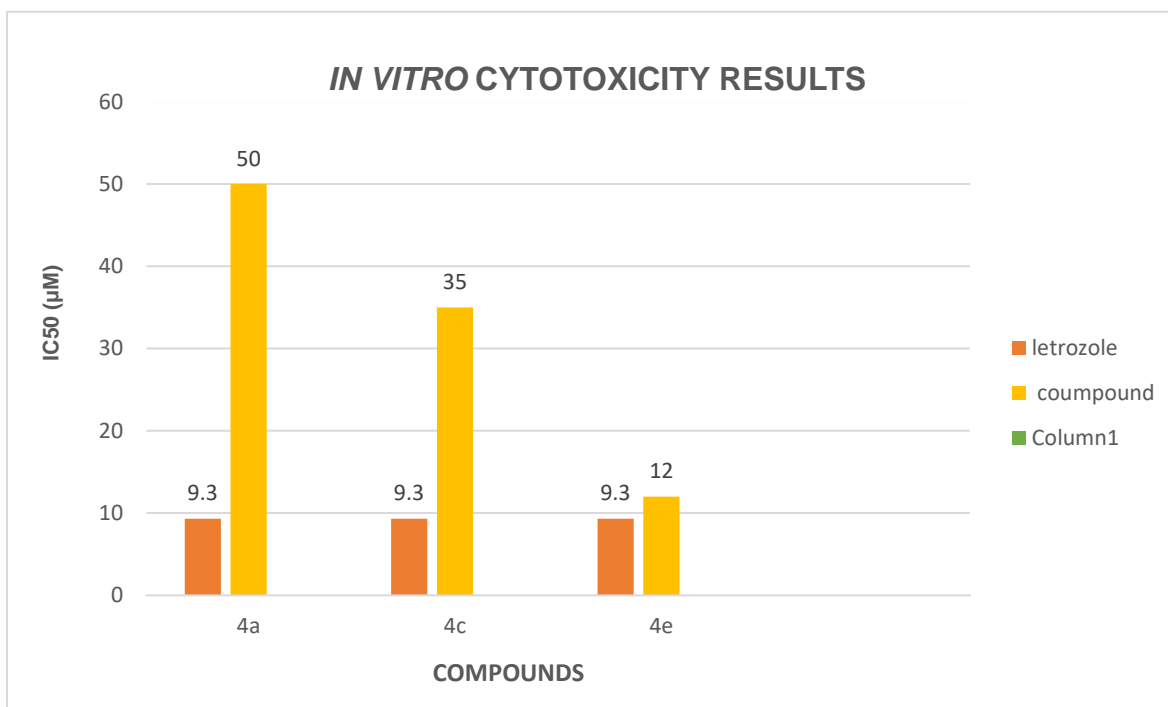


Figure 8: IC₅₀ values of standard and synthesized compounds against MCF-7 cell line

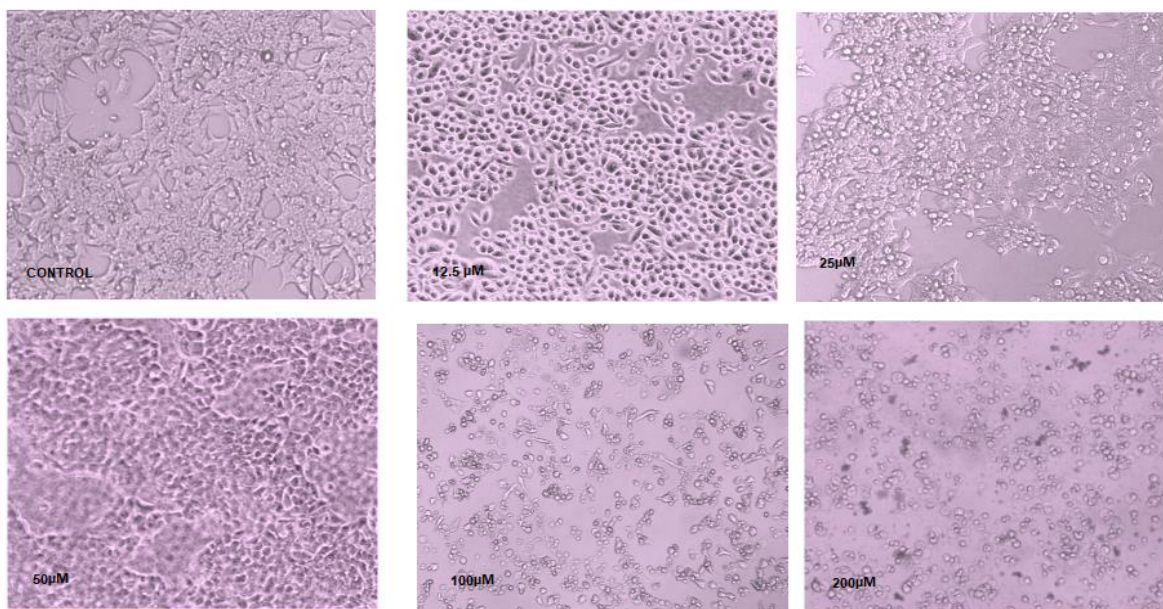


Figure 9: Cell viability images of 4e

6.5 CONCLUSION

- The present study was focused on developing the computational tools which helps in minimizing the process of drug discovery.
- A set of 17 compounds were docked against the target EGFR tyrosine kinase PDB:1M17
- Among the 17 compounds, the top-ranked 5 compounds were synthesized using the conventional Methods.
- Molecular docking studies of the active compounds revealed that these compounds might act via inhibition of EGFR tyrosine kinases (1M17)
- All the synthesized compounds were evaluated for their physiochemical properties such as solubility, melting point analysis and Thin layer Chromatography.
- Characterization of the synthesized compounds was done using Infrared spectroscopy, Mass spectroscopy.
- The synthesized pyridine with triazole derivatives have been screened for their cytotoxicity in MCF-7 cell lines by MTT assay.
- The findings indicated that the test compounds showed least to good activity. Altogether, these results show that the 5-aryl-3-phenylamino-1,2,4-triazole structure possesses fair anticancer activities and that derivative with a 3-amino phenol moiety (4e) seem to be the most promising one among other derivatives.

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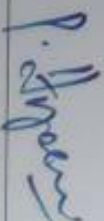
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