GREEN SYNTHESIS, CHARACTERIZATION, *IN-SILICO* MOLECULAR DOCKING STUDY AND *IN-VITRO* ANTI-CANCER ACTIVITY OF 1,2,3-TRIAZOLYL DIHYDROPYRIMIDINE-2-THIONE HYBRIDS

Dissertation submitted to
THE TAMILNADU Dr.M.G.R. Medical University,
Chennai – 600032
in partial fulfilment of the requirements for the award of Degree of

MASTER OF PHARMACY
IN
BRANCH-II PHARMACEUTICAL CHEMISTRY

Submitted by

N.ASHOKKUMAR

REG.NO:261615751

Under the guidance of

Dr.G.UMARANI, M.Pharm., Ph.D.,

Department of Pharmaceutical Chemistry

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,
COLLEGE OF PHARMACY,
MADURAI MEDICAL COLLEGE,
MADURAI - 625 020.

MAY-2018

CERTIFICATE

This is to certify that the dissertation entitled **" GREEN SYNTHESIS, CHARACTERIZATION, *IN SILICO* MOLECULAR DOCKING STUDY AND *IN VITRO* ANTI-CANCER ACTIVITY 1,2,3-TRIAZOLYL DIHYDROPYRIMIDINE-2-THIONE HYBRIDS"** is a bonafide Work done by **Mr.ASHOKKUMAR.N (Reg.No.261615751)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai-625020, in partial fulfilment of The Tamil Nadu Dr.M.G.R Medical University rules and regulations for award of Degree of Master of Pharmacy (II year, Pharmaceutical Chemistry) under my guidance and supervision during the academic year 2017-2018.

Name & Signature of Guide.

Name & Signature of Head of Dept.

Name & Signature of Dean/Principal.

Place:

Date:

Evaluation Certificate

Internal Examiner

External Examiner

ACKNOWLEDGEMENT

"I thank the Almighty, my parents and the helping hands"

I am grateful to express my sincere thanks to **Dr.D.MARUTHUPANDIAN, M.S., FICS., FAIS** Dean, Madurai Medical College, Madurai for giving an opportunity to carry out my project work.

I express my sincere thanks to **Dr.D.DHANALAKSHMI, M.D.,** Vice principal, Madurai Medical College, Madurai for his support and encouragement to carry out the work.

I am thankful to **DR.A.ABDUL HASAN SATHALI., M.Pharm., Ph.D.,** Principal & Head of the Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai, for his support and encouragement of this project work.

I express my deep hearted thanks with respects and regards to **DR.V.NIRAIMATHI., M.Pharm., Ph.D.,** Professor & Head of the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai for her encouragement and support for completion of my project work.

It is my immense pleasure to express my deep hearted thanks with respect to my guide **DR.G.UMARANI., M.Pharm.,Ph.D.,** Associate professor, Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai for her encouragement, support in topic selection, supervision and completion of my project work in a successful manner.

With whole-hearted gladness I thank **Prof.A.PONNUSAMY**, chairperson and Head of the department of Organic chemistry, School of Chemistry, Madurai Kamaraj University, Madurai for the help rendered by him throughout the work.

I convey my sincere thanks to **Mr.S.RAMESH KUMAR.,M.Pharm., Mrs.G.TAMILARASI., M.Pharm., Mr.P.SIVASUBRAMANIAN., M.Pharm.,** Assistant Professors in Department of Pharmaceutical Chemistry, for their encouragement throughout the work.

I hearty thanks to **Dr.M.S.MOHAMED JAABIR**, Associate professor, Department of Biotechnology, National college, Trichy.

I express thanks to **MR.**,**K.LAKSHMANAN B.Sc(Chem)** Lab supervisor **Mrs.R.GEETHA.(DMLT), Mrs.S.RENUKADEVI.(DMLT),** Lab technicians Department of Pharmaceutical Chemistry, College of Pharmacy , Madurai Medical College, Madurai.

I whole heartedly thank **Dr.R.RANGANATHAN,MSc.,M.Phil.,Ph.D., Ms.G.JAABIL.MSc.,B Ed., M.Phil.,** and **Ms.V.ARCHANA, MSc.,** and Department of organic chemistry, school of Chemistry, Madurai Kamaraj University, Madurai for their support and to do this project.

I convey my sincere thanks to my seniors **Ms.BAKYALAKSHMI.M., Ms.GNANAM.D., MR.MOORTHI.R., Ms.SIVASANKARI.K.A.,** and for their encouragement and timingly help to complete my project.

I extend my thanks to my Classmates **Mr.PONNUDURAI.A, Mr.RAJASEKARAN.K,** and **Ms.SWATHI.S** for their kind help. I also express thanks to my juniors **Mr.GOKUL.M., MS.HEMALATHA.P., Mr.KATHIRAVAN.M., Mr.SATHISH.A** in the Department of Pharmaceutical Chemistry.

I also extend my thanks to all the staff members and **P.G.** Students of Department of Pharmaceutics and Pharmacognosy for their co-operation.

DEDICATED TO MY FAMILY MEMBERS AND WELL WISHERS

CONTENTS

DETAILS OF ABBREVIATIONS

%	Percentage
°C	Degree Centigrade
µg	Microgram
µM	Micro Mole
mM	Milli mole
^{1}H-NMR	Proton Nuclear Magnatic Resonance
Ar	Aromatic
Comp.code	Compound code
DMSO	Dimethyl Sulfoxide
E.coli	*Escherichia coli*
Gm	Gram
HRBC	Human Red Blood Cell
Hrs	Hour
IR	Infra-Red
m.p	Melting Point
m/z	Mass/charge
Mg	Milligram
Min	Minutes
Ml	Milliliter
Mol	Mole
MR	Molar Refractivity
Nm	Nanometer
o, m, p	Ortho, Meta, Para
P_C	Critical pressure
p^H	Hydrogen ion Concentration
Ppm	Parts per million
R_f	Retention factor
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S.aureus	*Staphylococcus aureus*
Sec	Seconds
Str	Stretching
TLC	Thin Layer Chromatography
UV	Ultra violet
V_C	Critical volume
Δ	Delta
Kcal/Mol	Kilocalorie/Mole

CHAPTER-I

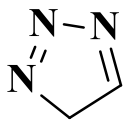
INTRODUCTION

INTRODUCTION

Heterocyclic compounds possess considerable attention in recent years because of their broad range of pharmaceutical activities. Among them, Nitrogen and Sulphur hetero atoms containing five/six membered heterocyclic compounds are found to be of great importance in medicinal applications. Especially, 1,2,3-triazole and dihydropyrimidine-2-thione nucleus is found to possess biological activities such as antifungal,**(Vandana s. Pore *et al*; 2009)** antitubercular **(Vitor F Ferreira *et.al;* 2006)**, antibacterial, **(S.Nagarajan et.al; 2012)**, antiviral **(Chi-Huey wong *et.al.,* 2003)**, antimicrobial **(Kadir dabak *et.al*.,2003),** antiproliferative **(Ahamed ajmal et.al.,2008)** and cytotoxic agents etc. Hence, chemists/biologists are on a continuous pursuit to design and synthesize heterocyclic hybrids over the years essentially because of their medicinal importance.

Computerized conformational analysis used to predict the **3D** structure of drug with the receptor. QSAR used to elucidate the mechanism of action of drugs at the molecular level and physicochemical property like hydrophobicity. (**Burger's)**

1, 2, 3-TRIAZOLE NUCLEUS:

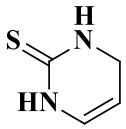


1,2,3-triazole nucleus with three nitrogen atoms and electron rich property has been paid special attention in the development of new drugs due to large medicinal potentiality of triazole-based derivatives. These exciting achievements encourage continuous efforts to develop of 1, 2, 3- triazole compounds for the treatment of infective diseases. Considering the importance of chalcone and

triazole compounds, and as an extension of our researches on bioactive heterocyclic compounds

Triazole moiety is able to easily bind with various enzymes and receptors in organisms through coordination bonds, hydrogen bonds, ion-dipole, cation-p, pep stacking, hydrophobic effect and van der Waals force etc., which helpfully modulate the physicochemical and pharmacokinetics properties.

DIHYDROPYRIMIDINE-2-THIONE:



3,4-dihydropyrimidine-2(1*H*)-thione

On the other hand, dihydropyrimidines has structural resemblance with clinically important Hantzsch pyridines. Also, literature study reveals that the pyrimidines exhibit wide range of biological activities viz. antibacterial **(S.Nagarajan *et.al,* 2012)**, anticancer, antiviral **(Shireesha Boyapati *et al.,*2015)**, antitumor, anti-inflammatory etc. Moreover, Pyrimidine-2-thiones were found to inhibit the synthesis of t-RNA under certain conditions and thus act as anti-tumour and anti-thyroid agents.

1,2,3-TRIAZOLE-PYRIMIDINE-2-THIONE HYBRIDS:

The utilization of simple molecules with different functionalities is a valuable contribution in the chemistry of heterocycles. Particularly, heterocyclic hybrids are single frameworks wherein more than one heterocyclic moieties are being grafted with a view of designing more effective and enhance the bioactive entities.

In view of the above and continuation of our earlier reported work (**S.Nagarajan *et.al.,* 2012**) we planned to synthesize and explore the hybrid

bioactive skeletons *viz*. 1,2,3-triazole linked pyrimidine-2-thione derivatives via green protocol. Furthermore, all the synthesized hybrids were evaluated docking and *in-vitro* anticancer studies.

GREEN CHEMISTRY:

- It is better to prevent waste than to treat or clean up waste after it is formed.
- Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
- Wherever practicable, synthetic methodologies should be designed to use and generate substances that chemical process little or no toxicity to human health and the environment,
- Chemical products should be designed to preserve efficacy of function while reducing toxicity.

- The use of auxillary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
- Energy requirements should be recognised for their environmental and economic impacts and should be minimised. Synthetic methods should be conducted at ambient temperature and pressure.
- A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
- Unnecessary derivatization (blocking group, protection / deprotection, temporary modification of physical/chemical processes) should be avoided wherever possible.
- Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
- Chemical products should be designed so that at the end of their function they do not persist in the environment and breakdown into innocuous degradation products.
- Analytical methodologies need to be developed further to allow for real-time in- process monitoring and control prior to the formation of hazardous substances.
- Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions and fires.

CONDENSED PRINCIPLES OF GREEN CHEMISTRY

P - Prevent wastes

R - Renewable materials

O - Omit derivatization steps

D - Degradable chemical products

U - Use safe synthetic methods

C - Catalytic reagents

T - Temperature, Pressure ambient

I - In-Process Monitoring

V - Very few auxillary substances

E - E-factor, maximise feed in product

L - Low toxicity of chemical products

Y - Yes, it is safe.

The broad scope of the greener methodology involving water as media was established via the synthesis of a library of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids in good to excellent yields (79% to 95%)

MOLECULAR DESIGN:

Various software used in molecular designs are

1. CHEM SKETCH

2. CHEM DOODLE

3.MOLINSPIRATION

SPECTROSCOPY:

Spectroscopy is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.

Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure of organic compounds. (**Y.R.Sharma**)

I.R spectroscopy:

IR spectrum were recorded by absorption of infrared radiation it causes changes in vibrational energy in the ground state of the molecule. (**Robert silverstein)**

NMR spectroscopy:

Nuclear magnetic resonance(NMR) spectroscopy is a technique that permits the transition of a molecule at the level of the individual atom and giving information about the environment of that atom **(Beckett and stanlake)**.

Mass spectroscopy:

Molecules are bombarded with electrons of sufficient energy, loss of an electron and formation of positive ion.It is used to determine the molecular weight of the compounds . **(Y.R.Sharma)**

. **MOLECULAR DOCKING**

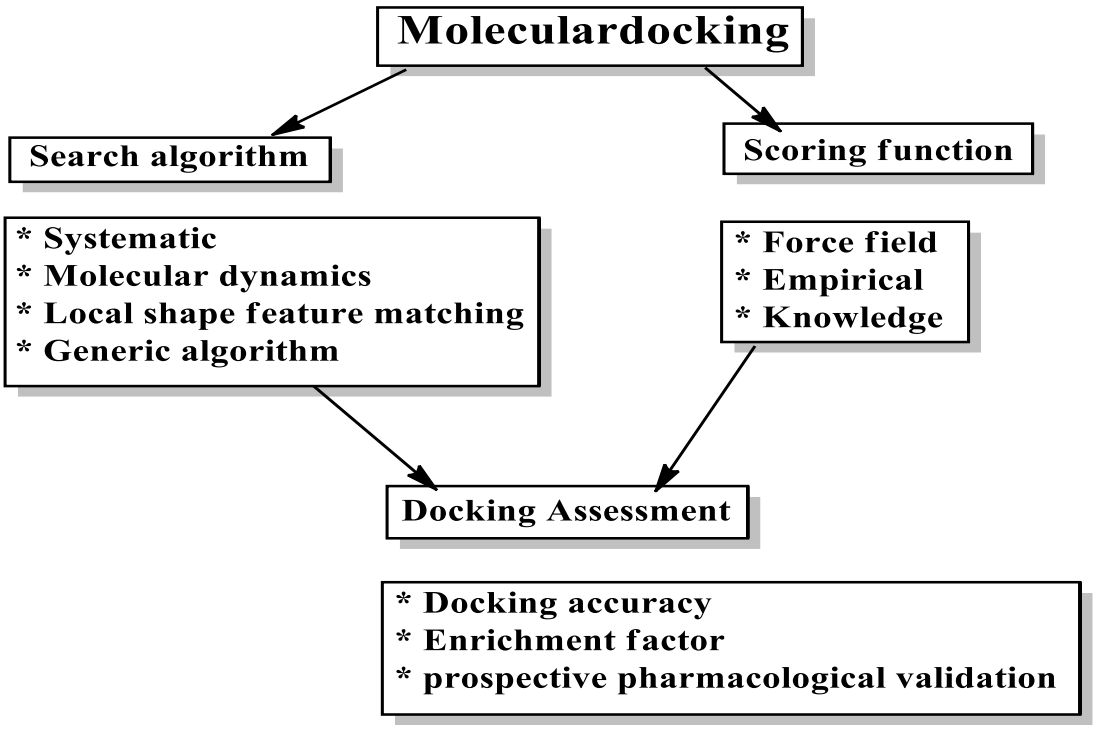
Molecular docking provides useful information about drug receptor interactions. It analyzes the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. **(Mavromoustakosa et.al.,2010)**

Docking is considered to be a powerful simulation of the molecular recognition process. It is used to illustrate the probable molecular interaction of a

designed ligand with the protein of interest, predict the affinity and activity of the ligand, and identify the energy of the interaction between the ligand and protein.

DEFINITION

"Molecular docking may be defined as an optimization problem, which would describe the 'best-fit' orientation of a ligand that binds to a particular protein of interest. However, since both the ligand and the protein are flexible, a 'hand-in-glove' analogy is more appropriate than 'lock-and-key'."



It is an invaluable tool in the field of molecular biology, computational structural biology, computer-aided drug designing, and pharmacogenomics.

Aim of docking studies:

- Accurate structural modeling
- Correct prediction of activity.

Steps of ligand docking:

- Preparation of ligands
- Preparation of proteins
- Setup ligand protein docking calculations
- Evaluation of results

Classification of docking:

Based on the types of ligand, docking can be classified as:

- Protein-small molecule (ligand) docking
- Protein-nucleic acid docking
- Protein-protein docking.

Advantages of docking:

- The application of docking in a targeted drug-delivery system is a huge benefit. One can study the size, shape, charge distribution, polarity, hydrogen-bonding, and hydrophobic interactions of both ligand (drug) and receptor (target site).
- It helps in the identification of target sites of the ligand and the receptor molecule.
- It also helps in the understanding of different enzymes and their mechanism of action.

- The “scoring” feature in docking helps in selecting the best-fit or the best drug from an array of options.
- It has huge advantage when it comes to the study of protein interactions.
- There are a millions of compounds, ligands, drugs, and receptors, the 3D structure of which has been crystallized. Virtual screening of these compounds can be made.

Limitations of docking:

- In protein-small molecule docking, there can be problems in the receptor structure. A reliable resolution value for small- molecule docking is below 1.2 A, while most crystallographic structures have a resolution between 1.5 and 2.5 A increasing the use of homology models in docking should be locked at with care as they have even poorer resolution. Most applications accept and yield good results for structures below 2.2A. All the same, care should be taken while picking a structure.
- The scoring functions used in docking, almost all of them, do not take into account the role played by covalently bound inhibitors or ions.
- The methodology and research in protein-protein docking have to be greatly increased as the success in this field is greatly hampered by many false positives and false negatives.

CANCER :

Cancer is abnormal multiplication of cells. **Cancer** is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumours, which do not spread to other parts of the body. Possible signs and symptoms include a lump, abnormal bleeding,

prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes. Over 100 types of cancers affect humans. **(ASTHOSKAR)**

Tobacco use is the cause of about 22% of cancer deaths. Another 10% are due to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. In the developing world, 15% of cancers are due to infections such as Helicobacter pylori, hepatitis B, hepatitis C, human papillomavirus infection, Epstein–Barr virus and human immunodeficiency virus.

These factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops. Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents. Cancer can be detected by certain signs and symptoms or screening tests. It is then typically further investigated by medical imaging and confirmed by biopsy.

Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat, and avoiding too much sunlight exposure. Early detection through screening is useful for cervical and colorectal cancer.

The benefits of screening in breast cancer are controversial. Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy. Pain and symptom management are an important part of care. Palliative care is particularly important in people with advanced disease. The chance

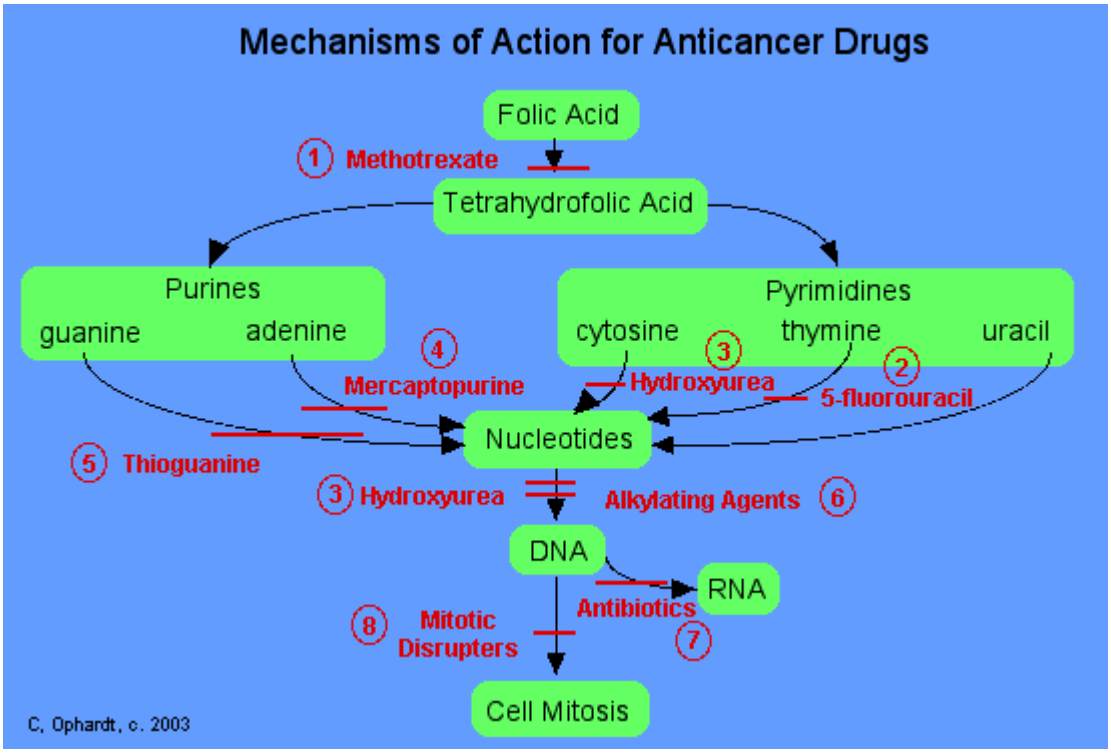
of survival depends on the type of cancer and extent of disease at the start of treatment. In children under 15 at diagnosis the five-year survival rate in the developed world is on average 80%. For cancer in the United States the average five-year survival rate is 66%.

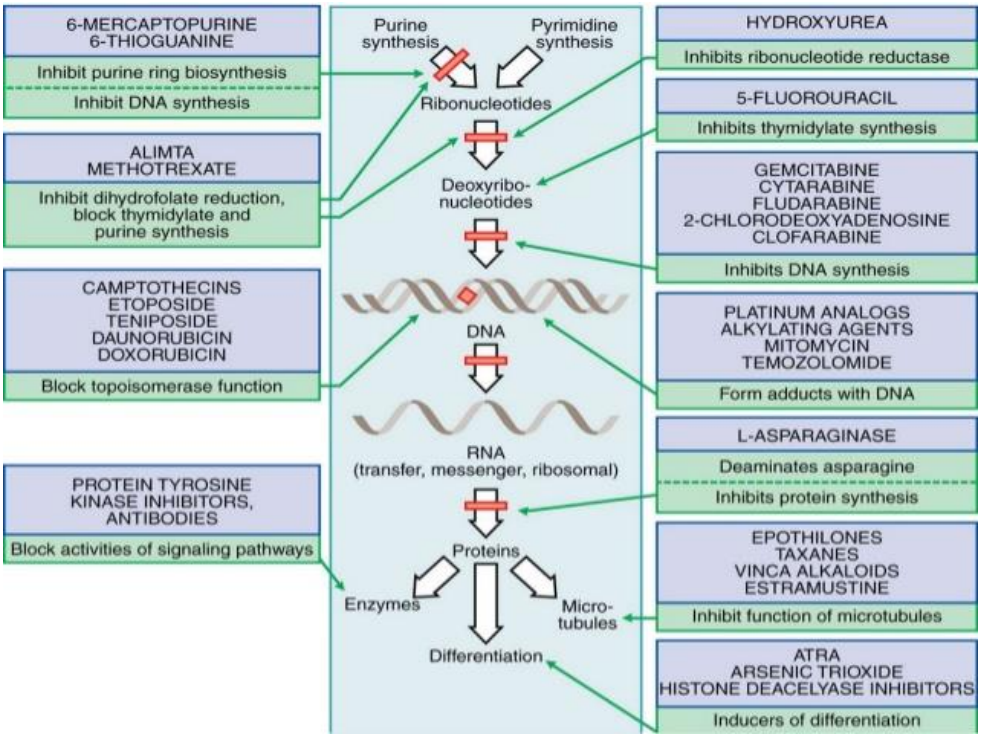
In 2015, about 90.5 million people had cancer. About 14.1 million new cases occur a year (not including skin cancer other than melanoma). It caused about 8.8 million deaths (15.7% of deaths). The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. In females, the most common types are breast cancer, colorectal cancer, lung cancer and cervical cancer.

If skin cancer other than melanoma were included in total new cancers each year, it would account for around 40% of cases. In children, acute lymphoblastic leukaemia and brain tumours are most common except in Africa where non-Hodgkin lymphoma occurs more often. In 2012, about 165,000 children under 15 years of age were diagnosed with cancer.

The risk of cancer increases significantly with age and many cancers occur more commonly in developed countries. Rates are increasing as more people live to an old age and as lifestyle changes occur in the developing world. The financial costs of cancer were estimated at $1.16 trillion USD per year as of 2010. **(K.D.Tripathi)**

Mechanism of anti-cancer drugs:



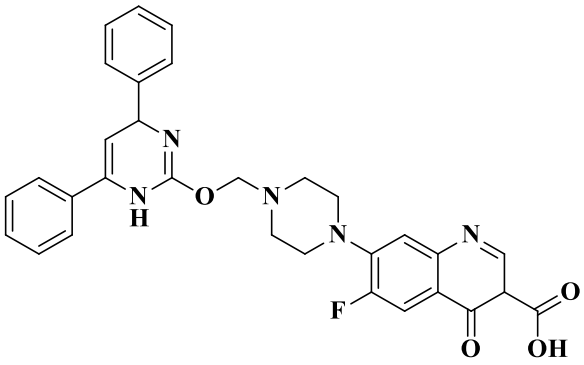


CHAPTER-II

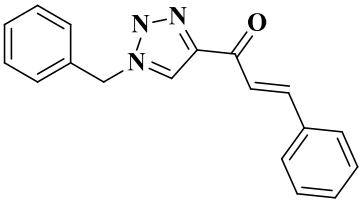
LITERATURE REVIEW

LITERATURE REVIEW

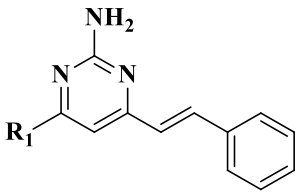
1. **G.Krishnamoorthy *et.al*; (2017)** in-silicco molecular docking of some novel heterocyclic compounds targeting vascular endothelial growth factor receptor-2
2. **Ajmal khan *et.al*; (2016)** dihydropyrimidine based hydrazine dihydrochloride derivatives as potent urease inhibitors for the treatment of peptic ulcer
3. **Shireesha boyapati *et.al*; (2015)** synthesized dihydro pyrimidine-ciprofloxacin mannich bases against various virus strainsand anti-viral activities



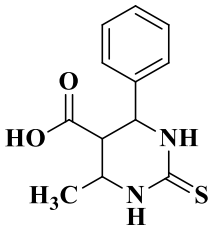
4. **Cheng-he zhou *et.al;* (2014)** synthesized and biological evaluation of α-triazolyl chalcones as a new type of potential anti-microbial agents and their interactions with calf thymus DNA and human serum albumin.



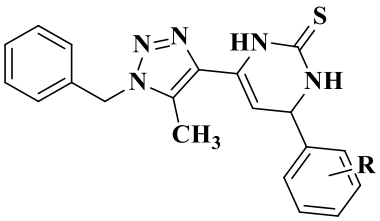
5. **S.Nagarajan *et.al*; (2014)** An eco-friendly water mediated product selective synthesis of 2-Amino pyrimidines and their in-vitro anti-bacterial evaluation.



6. **Farzana latif Ansari *et.al;* (2014)** synthesized ,docking studies and insilico ADMET predictions of some new derivatives of pyrimidine as potential KSP (kinesin spindle protein) inhibitors

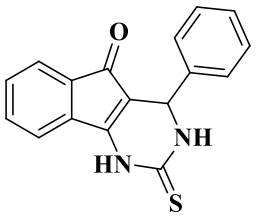


7. **S.Nagarajan *et.al;* (2012)** Facile synthesis of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids – Highly potent anti-bacterial agents.

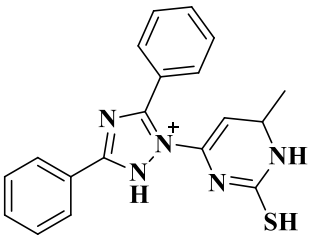


8. **Francesco Caruso *et.al;* (2012)** Ruthenium-Arene complexes of Curcumin: X-ray and density functional theory structure, synthesis and spectroscopic characterization in in-vitro anti-tumor activity of DNA docking studies of (p-cymene) Ru(Curcuminato) chlorinated compounds.

9. **Harrie J.M.Gijsen *et.al;* (2012)** Tricyclic 3,4-Dihydropyrimidine 2thione derivatives as potent TRPA1 Antagonists.



10. **Shantaram Gajanan khanage *et.al* (2012)** synthesis and pharmacological evaluation of some new pyrimidine derivatives containing 1,2,4-triazole derivatives.

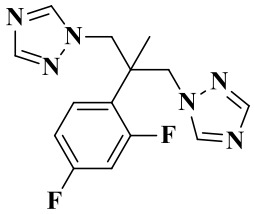


11. **Vandana S. Pore *et.al .,* (2011)** 1,2,3-triazole as pharmacophore

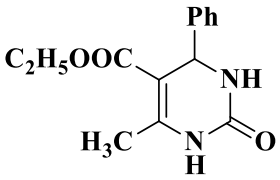
12. **Keun woo lee *et.al;* (2011)** Pharmacophore based virtual screening molecular docking studies to design potent heat shock protein-90 inhibitors.

13. **Thomas Marromoustakos *et. al*; (2010)** Development of accurate binding affinity predictions of novel Renin inhibitors through molecular docking studies.

14. **Vandana S. Pore *et.al;* (2009)** Synthesis and anti-fungal activity of 1,2,3-triazole containing Fluconazole analogues.



15. **Paresh Zalavadiya *et.al;* (2009)** Multicomponent synthesis of Dihydropyrimidines by Iodine catalyst at ambient temperature and in-vitro antimycobacterial activity.



16. **Paulo A Netz *et.al;* (2009)** Docking studies on DNA ligand interactions : Building and application of a protocol to identify the binding mode.

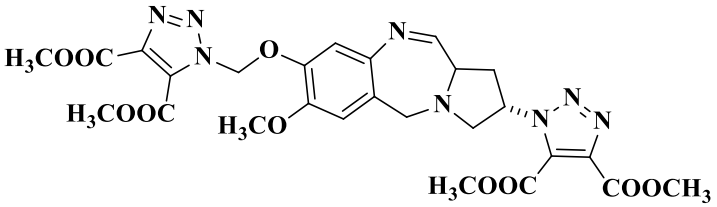
17. **Mahmud Tareq Hassan Khan *et.al*; (2009)** Cholinesterase inhibitory activities of some flavonoid derivatives and chosen Xanthone and their molecular docking studies.

18. **Antonello mai *et.al;* (2008)** Class-II Selective histone deacetylase inhibitors Part 2 : Alignment independent GRIND 3-D QSAR homology and docking studies.

19. **Christian G Hartinger *et.al;* (2008)** Influence of the spacer length on the in-vitro anticancer activity of dinuclear Ruthenium –Arene compounds.\

20. **Christian girard *et.al*; (2008)** Synthesis and antibiotic activity of a small molecules library of 1,2,3-Triazole derivatives

21. **Ahmed ajmal *et.al;* (2008)** Synthesis of 1,2,3-Triazole- linked pyrrolo benzodiazepine conjucates employing click chemistry: DNA Binding affinity and anti-cancer activity.



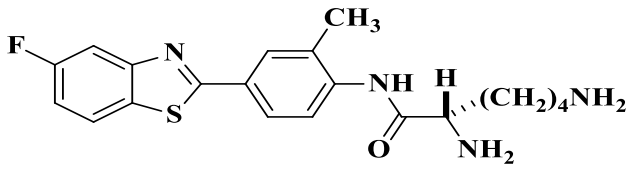
22. **Hashem sharghi *et.al;* (2008)** Copper nano particle on charcoal for multi-component catalytic synthesis of 1,2,3 Triazole derivatives from benzyl halides or alkyl halides, Terminal alkynes and sodium azide in water as a green solvent.

23. **Zheng-Jun Quan *et.al;* (2008)** Efficient synthesis of 5H-Thiazolo (3,2-a) Pyrimidines from reactions of 3,4 dihydropyrimidine-thiones with α-Bromo acetone in aquous media.

24. **Avadhesha Surolia *et.al;* (2006)** Novel diphenyl ethers: Design, docking studies, synthesis and inhibition of enoyl ACP reductase of Plasmodium falciparum and Escherichia coli.

25. **Vitor F Ferreira *et.al*; (2006)** Synthesis tuberculosis inhibitory activity and SAR Study of N-substituted Phenyl 1,2,3-Triazole derivatives.

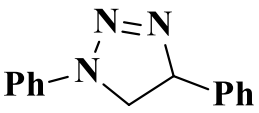
26. **Andrew D.Westwell *et.al;* (2006)** Anti-tumor Benzothiazoles 2-(3,4-dimethoxy phenyl) 5-fluoro benzothiazole (GW610, NSC 721648), a simple fluorinated 2-Aryl benzothiazole shows potent and selective inhibitory activity against lung, colon and breast cancer cell lines.



27. **Hsing-Pang Hsieh *et.al*; (2006)** Indol-1-yl acetic acids as P-PAR agonist : Design synthesis structural biology and molecular docking studies.

28. **Johan Wouters *et.al*; (2005)** Crystal structure of BRL 42715 C_6-CN_1-Methyl-1,2,3- triazolyl methylene Penem in complex with Enterobacter cloacae 908R β-lactamase: Evidence for a stereo-selective mechanism from docking studies.

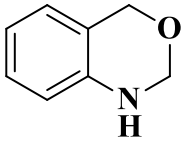
29. **Miguel Yus *et.al;* (2005)** Multicomponent synthesis of 1,2,3-triazoles in water catalysed by Copper nanoparticles on activated carbon.



30. **Q.Ping Dou *et.al*; (2004)** Docking studies and model development of Tea polyphenol Proteasome inhibitors: Applications to rational drug deisgn.

31. Kadir dabak *et.al;* (2003) Synthesis and investigation of tuberculosis inhibition activities of some 1,2,3-triazole derivatives.

32. **Xiaojie XV *et.al;* (2003)** Mapping the binding site of a large set of Quinazoline type EGF-R inhibitors using molecular field analysis and molecular docking studies.



33. **Chi-Huey wong *et.al;* (2003)** 1,2,3-triazole as a peptide surrogate in the rapid synthesis of HIV I Protease inhibitor.

34. **Horst Kessler *et.al;* (2003)** Docking studies on $\alpha_V\beta_3$ integrin ligands: Pharmacophore refinement and implications for drug design.

35. **Todd D.Shenken Berg *et.al;* (1986)** Mitoxantrone- A new anti-cancer drug with significant anti-cancer activity.

36. **Li zhang *et.al;* (2003)** Flavonoids : Promising anti-cancer agents.

37. **Conrad Kunick *et.al;* (1999)** Paullones, a series of cyclin dependent kinase inhibitors : synthesis, evaluation of CDK_1/ cyclin β-inhibition and in-vitro anti-tumor activity.

38. **Maria jose camarasa *et.al;* (1994)** 1,2,3-triazole- (2'5'-Bis-o-(tert-butyl dimethyl silyl)-beta-D-ribofuranosyl)-3'spiro-5''-(4''-amino-1,2''-oxathiole 2'',2''-dioxide) (TSAO) Analogous: Synthesis and anti-HIV-I activity.

39. **Maria jose camarasa *et.al;* (1994)** Regiospecific synthesis and anti-HIV ACTIVITY OF novel 5-substituted N-Alkyl carbomyl and N,N-Dialkyl carbomyl 1,2,3-triazole-TSAO Analogues.

CHAPTER-III

AIM AND OBJECTIVES

AIM AND OBJECTIVES

Generally, Nitrogen and Sulphur hetero atoms containing five/six membered heterocyclic compounds are found to be of great importance in medicinal applications. Especially, 1,2,3-triazole and dihydropyrimidine-2-thione nucleus is found to possess biological activities. The utilization of simple molecules with different functionalities is a valuable contribution in the chemistry of heterocycles. Particularly, heterocyclic hybrids are single frameworks wherein more than one heterocyclic moiety is being grafted with a view of designing more effective and enhance the bioactive entities.

In view of the above and continuation of our earlier reported work (S.Nagarajan et.al.,2012) , we planned to synthesize and explore the hybrid bioactive skeletons *viz*. 1,2,3-triazole linked pyrimidine-2-thione derivatives via green protocol. Furthermore, all the synthesized hybrids were evaluated docking and *in-vitro* anticancer studies.

Objectives of the study:

- ✓ To design the lead molecule of 1,2,3-triazolyl dihydropyrimidine-2-thione and ADMET property.
- ✓ To synthesis the compounds by appropriate methods.
- ✓ To purify the synthesized compound by TLC.
- ✓ To carry out the physical constant like solubility, melting point, etc.
- ✓ To characterize the structures of synthesized compounds by IR, ^{1}HNMR and Mass spectra.
- ✓ To predict the anticancer activity by docking methods.
- ✓ To evaluate the proposed compounds for their in-vitro anti-cancer activity.

CHAPTER-IV

MATERIALS & METHODS

MATERIALS AND METHODS

LISTS OF CHEMICALS USED:

Table no:1.

S.No	Name of Chemicals	Grade	Manufacture/Suppliers
1.	Benzyl azide	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
2.	Acetyl acetone	Analytical Reagent	Avra Synthesis Pvt. Ltd.
3.	Potassium carbonate	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
4.	Ethanol 95%	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
5.	3-methyl benzaldehyde	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
6.	2-chloro benzaldehyde	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
7.	Furfural	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
8.	2-thiophene carbaldehyde	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
9.	Isopropyl benzaldehyde	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
10.	Sodium hydroxide 50%	Analytical Reagent	Avra Synthesis Pvt. Ltd.
11.	Thiourea	Laboratory Reagent	Avra Synthesis Pvt. Ltd.
12.	Potassium hydroxide 10%	Laboratory Reagent	Avra Synthesis Pvt. Ltd.

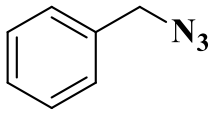
List of instruments used:

Table no: 2

S.No	Name of the Instrument	Model	Manufacturer/supplier
1.	UV-Visible spectrophotometer	1800	Shimadzu
2.	Fourier transform IR Spectrometer	IR-Affinity-1	Shimadzu
3.	KBr press	M-15	Technosearch
4.	Mass Spectrometer	JEOL GC-MATE-II HR	Thermo fisher
5.	NMR Spectrometer	Avance 300 MHz	Bruker
6.	Thermostatically controlled water bath	PIC 108	M.C.Dalal
7.	Electronic Balance	M-D4404420019	Shimadzu
8.	Centrifuge	LABO51	Shimadzu
9.	Autoclave	7441 Fajj 145	Equitron
10.	UV-Chamber	CE102A	Deep vision
11.	Microwave oven	MS-2029UW	Intellowave technology
12.	Incubater	7441 sleudoc	Rays
13.	Melting point apparatus	Ce100	Labtronics

REACTANT PROFILE:

BENZALDEHYDE



Appearance : Flammable liquid and vapour

Molecular formula : $C_7H_7N_3$

Molecular weight : 133.154g/mol

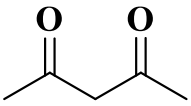
Melting point : 81-83ºC

Density : 1.0655

Solubility : Miscible with ethanol and diethyl ether

Category : Pharmaceutical Aid

ACETYL ACETONE



Appearance : Colourless liquid

Molecular formula : $C_5H_8O_2$

Molecular weight : 100.117g/mol

Boiling point : 284.7 ºF

Melting point : -10.3 ºF

Density : 0.975g/mL at 25 ºC

Solubility : Soluble in water

Category : Pharmaceutical Aid

POTASSIUM CARBONATE

K_2CO_3

Appearance : White or Yellow colour powder

Molecular formula : CK_2O_3

Molecular weight : 138.21g/mol

Melting point : 891 ºC

Density : 2.43g/mL at 25 ºC

Solubility : soluble in water

Category : Pharmaceutical Aid

ETHANOL

C_2H_5OH

Appearance : Colourless liquid

Molecular formula : C_2H_6O

Molecular weight : 46.069g/mol

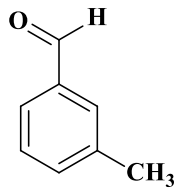
Melting point : 114.1^0C

Density : $789Kg/m^3$

Solubility : Soluble in water.

Category : Pharmaceutical Aid.

3-METHYL BENZALDEHYDE



Appearance : Tan to brown crystalline powder

Molecular formula : C_8H_8O

Molecular weight : 120.151g/mol

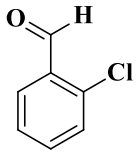
Melting point : 118-120^{0}C

Density : 1.0189g/cu cm at 21^{0}C

Solubility : Slightly soluble in water.

Category : Pharmaceutical Aid.

2-CHLOROBENZALDEHYDE



Appearance : colourless to light yellow colour liquid

Molecular formula : C_7H_5ClO

Molecular weight : 140.57g/mol

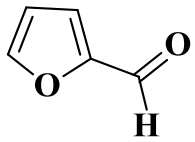
Melting point : 9-11ºC

Density : 1.245g/ml at 25^{0}C

Solubility : Soluble in alcohol, ether, acetone, benzene..

Category : Pharmaceutical Aid

FURFURAL



Appearance : Colourless to Pale yellow oily liquid

Molecular formula : $C_5H_4O_2$

Molecular weight : 96.085g/mol

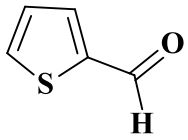
Melting point : -33.7ºF

Density : 1.159 at 68^0C

Solubility : Soluble in water, alcohol, ether.

Category : Pharmaceutical Aid.

2-THIOPHENE CARBALDEHYDE



Appearance : Clear yellow liquid

Molecular Formula : C_5H_4OS

Molecular weight : 112.146g/mol

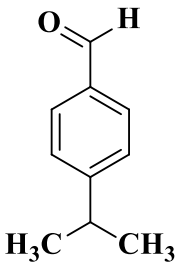
Melting point : <10ºC

Density : 1.29g/ml at 25^0C

Solubility : Insoluble in water

Category : Pharmaceutical Aid.

4-ISOPROPYL BENZALDEHYDE:



Appearance : Clear colourless liquid

Molecular formula : $C_{10}H_{12}O$

Molecular weight : 148.2g/mol

Boiling point : 235-236ºC

Density : 0.977g/ml at 25^0C

Solubility : Soluble in ethanol.

Category : Pharmaceutical Aid

SODIUM HYDROXIDE

NaOH

Appearance : White solid

Molecular formula :NaOH

Molecular weight :39.997g/mol

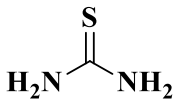
Melting point : 604ºF

Density : 2.13g/cu cm at 25^0C

Solubility : Very Soluble in water, freely soluble in ethanol

Category : Pharmaceutical Aid

THIOUREA:



Appearance : White crystalline solid

Molecular formula :CH_4N_2S

Molecular weight : 76.117g/mol

Melting point : 349 - 352ºF

Density : 1.405 at 68 ºF

Solubility : Soluble in water.

Category : pharmaceutical Aid

POTASSIUM HYDROXIDE:

KOH

Appearance : White solid deliquescent

Molecular formula :KOH

Molecular weight : 56.105g/mol

Melting point : 360ºC

Density : 2.12g/cm^3 at 25 ºC

Solubility : Soluble in water, alcohol, glycerol.

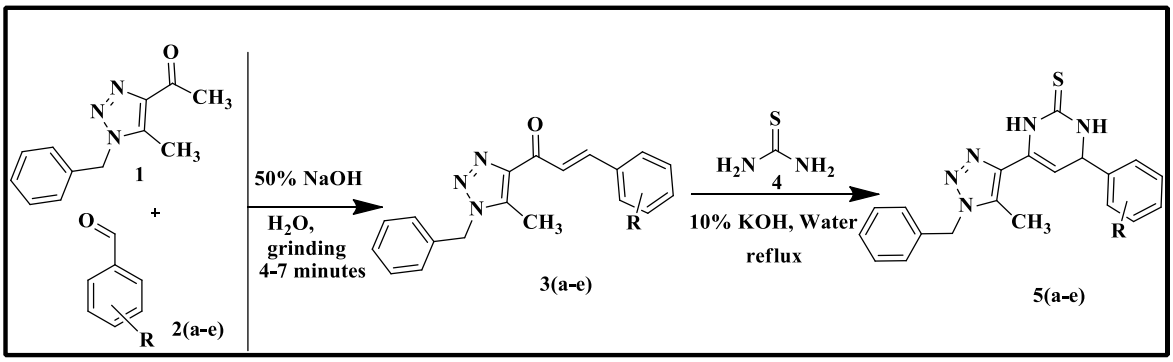
Category : pharmaceutical Aid

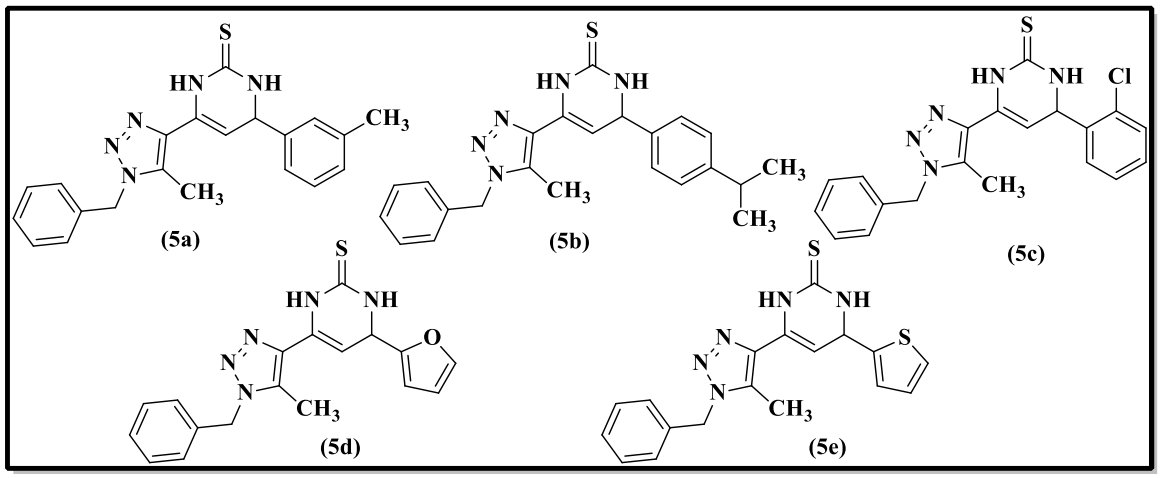
CHAPTER-V

SCHEME OF THE WORK

Scheme of the work

Synthesis of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids





Synthesis of a library of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids **5(a-e)**

CHAPTER-VI

EXPERIMENTAL WORK

EXPERIMENTAL WORK

Molecular design:

The software tools like Chemdoodle, Molinspiration, Chemsketch were used to study the newly synthesized molecules.

A) Chemdoodle:

Lipinski's rule of five also known as the Pfizer's rule of five or simply the Rule of five (RO5) is to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (ADME). However, the rule does not predict if a compound is pharmacologically active.

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

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or More hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons.
- An octanol-water partition coefficient log P not greater than 5.

B) Molinspiration:

Molinspiration, a web based software was used to obtain parameter such as MiLogP, TPSA, drug likeness. MiLogP is calculated by the methodology developed by Molinspiration as a sum of fragment based assistance and correction factors. MiLogP parameter is used to check good permeability across the cell membrane. TPSA is related to hydrogen bonding potential of compound. Number of rotatable bonds measures molecular flexibility**.** Molinspiration helps to conform about the Lipinski's rule of five.It helps to study the Drug likeness of compound, and also helps to know about the Pharmacokinetics profile of the drug entity, that includes absorption, distribution, metabolism and excretion ("ADME").

C) Chemsketch:

It is a software tool used for the prediction of molecular properties such as molecular mass, Log P, molar refractivity, parachor, molar volume, surface tension,polarizability and chemical composition.

D) Chemdraw:

it is a software tool used to draw molecular structure, chemical name, etc. And also designed for creating advanced chemical structures and analysis, you may consider ChemDraw Pro 12.0 or ChemDraw Ultra 12.0. & Advanced drawing application that also includes the ability to draw biological structures and pathways, you will want to refer to ChemBioDraw Ultra 12.0. ChemBioDraw Ultra 12.0 includes all features that are available in the Chem & Bio Draw 12.0 Series and BioDraw Series.

SYNTHETIC PROCEDURE:

General Information

All chemicals, reagents and solvents are commercially high purity grade purchased from Avra Synthesis Pvt. Ltd. and Merck Pvt. Ltd. India. Silica gel (60-120 mesh) was used for column chromatographic isolation and purification of the compounds synthesized. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker Avance 300 MHz spectrometer and the chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane, with coupling constant (*J*) values in Hertz (Hz). In ^{1}H NMR, the abbreviation of splitting refers as s=singlet, d=doublet, and m=multiplet. ^{13}C NMR data are reported with the solvent peak ($CDCl_3$=77.0 MHz) as the internal standard.

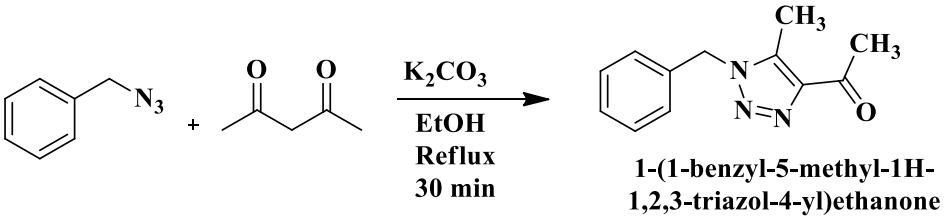
Synthesis of 4-acetyl-1-benzyl-1,2,3-triazole (1)

Chemicals required:

- Benzyl azide
- Acetyl acetone
- Potassium carbonate
- Ethanol 95%

`A mixture of benzyl azide, acetyl acetone, potassium carbonate and absolute ethanol (95%, 15ml) was taken in a round bottomed flask equipped with stirrer. The reaction mixture was stirred at 75°C for 30 minutes. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed

under vacuum and to the residual mass; excess of ice-water was added and neutralized with 10% HCl (20ml). The product was extracted with diethyl ether (20ml) and the extract dried over anhydrous sodium sulphate. Evaporation of the solvent gave the crude product, which was purified by column chromatography using pet.ether: ethylacetate (98:3) as eluent and recrystallized from absolute ethanol, yield 3.97g (82%), m.p. 148ºC.



1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)ethanone

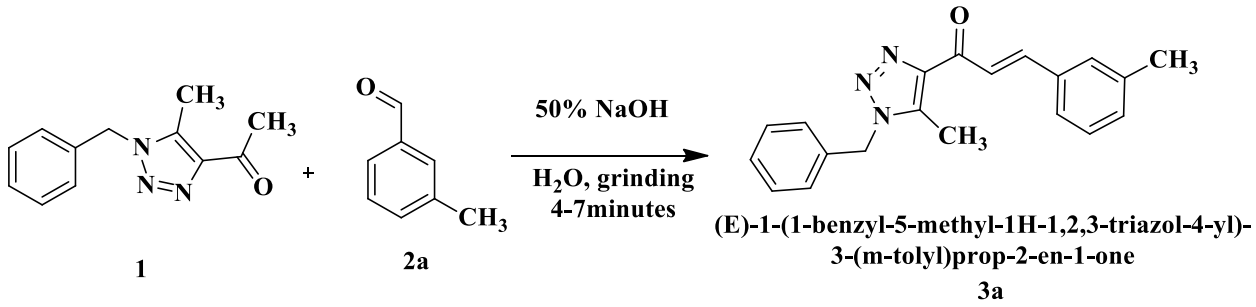
Compound 5a

Step1: Synthesis of 1,2,3-Triazolyl chalcone: (3a)

Chemicals required:

- 4-acetyl 1-benzyl 5-methyl-1,2,3 triazole
- 3-methyl benzaldehyde
- Sodium hydroxide 50%

A mixture of 4-acetyl-1-benzyl-5-methyl-1,2,3-triazole (1) and 3-methyl benzaldehyde (2a) and 50% aqueous sodium hydroxide solution (1 ml) was ground for 4-7 minutes at room temperature and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The chalcone derivatives (3a) precipitated as solid, which were filtered and recrystallized from ethanol. Yield: 97-99%.



1

2a

(E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-3-(m-tolyl)prop-2-en-1-one

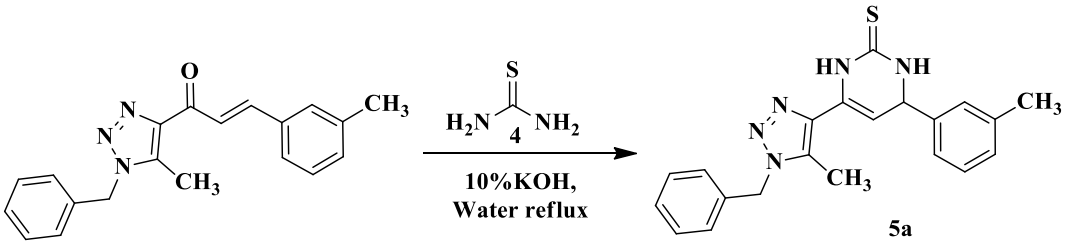
3a

Step-2- Synthesis of 1,2,3-Triazolyl dihydropyrimidine-2-thione hybrid-1: (5a)

Chemicals required:

- 1,2,3 triazolyl chalcone
- Thiourea
- Potassium hydroxide 10%
- Water

A mixture of ((E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)- 3-(m-tolyl)prop-2-en-1-one (3a), thiourea (4) (1.5 equiv.) and 10 % aq. KOH in water or ethanol (10 ml) was refluxed for 30-40 minutes and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The precipitated triazolyl dihydropyrimidine-2-thione derivatives (5a) were filtered and recrystallized from ethanol.



3a

5a

6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione

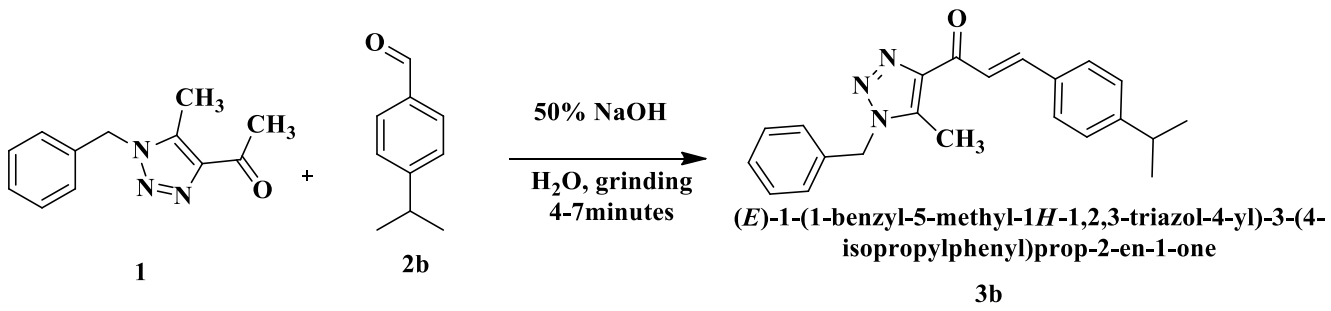
Compound 5b

Step1: Synthesis of 1,2,3-Triazolyl chalcone: (3b)

Chemicals required:

- 4-acetyl 1-benzyl 5-methyl-1,2,3 triazole
- Isopropyl benzaldehyde
- Sodium hydroxide 50%

A mixture of 4-acetyl-1-benzyl-5-methyl-1,2,3-triazole (1) and Isopropyl benzaldehyde (2b) and 50% aqueous sodium hydroxide solution (1 ml) was ground for 4-7 minutes at room temperature and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The chalcone derivatives (3b) precipitated as solid, which were filtered and recrystallized from ethanol. Yield: 97-99%.



1 2b

(*E*)-1-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-(4-isopropylphenyl)prop-2-en-1-one

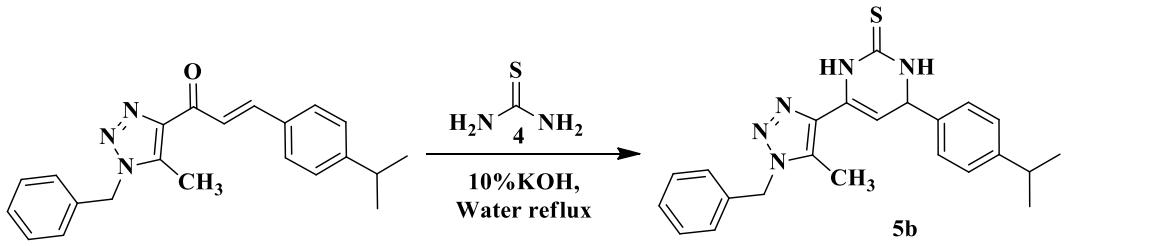
3b

Step-2- Synthesis of 1,2,3-Triazolyl dihydropyrimidine-2-thione hybrid-1: (5b)

Chemicals required:

- 1,2,3 triazolyl chalcone
- Thiourea
- Potassium hydroxide 10%
- Water

A mixture of (E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-3-(4-isopropylphenyl)prop-2-en-1-one (3b), thiourea (4) (1.5 equiv.) and 10 % aq. KOH in water or ethanol (10 ml) was refluxed for 30-40 minutes and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The precipitated triazolyl dihydropyrimidine-2-thione derivatives (5b) were filtered and recrystallized from ethanol.



3b

5b

6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1*H*)-thione

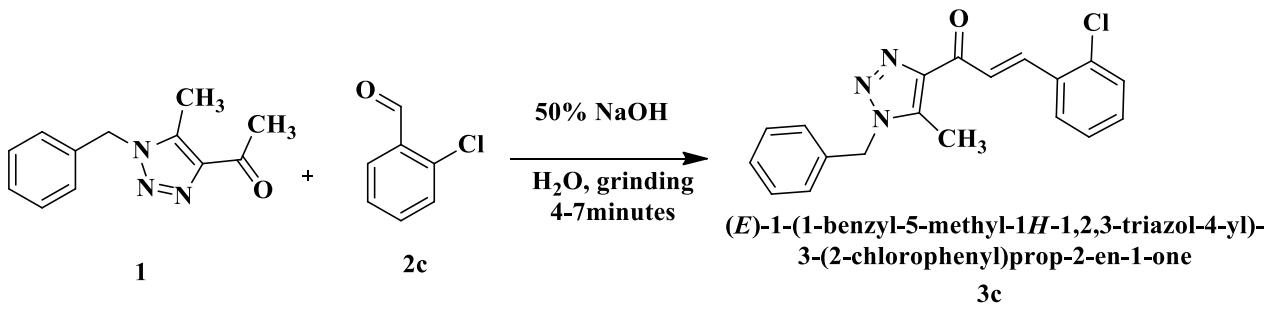
Compound 5c

Step1: Synthesis of 1,2,3-Triazolyl chalcone: (3c)

Chemicals required:

- 4-acetyl 1-benzyl 5-methyl-1,2,3 triazole
- 2-Chloro benzaldehyde
- Sodium hydroxide 50%

A mixture of 4-acetyl-1-benzyl-5-methyl-1,2,3-triazole (1) and 2-Chloro benzaldehyde (2c) and 50% aqueous sodium hydroxide solution (1 ml) was ground for 4-7 minutes at room temperature and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The chalcone derivatives (3c) precipitated as solid, which were filtered and recrystallized from ethanol. Yield: 97-99%.



1 2c

(*E*)-1-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-(2-chlorophenyl)prop-2-en-1-one

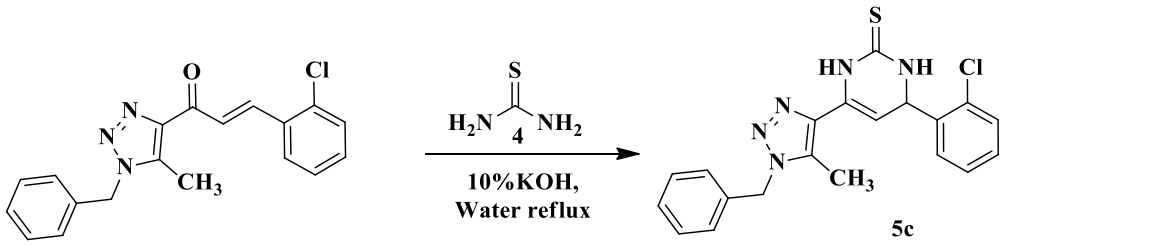
3c

Step-2- Synthesis of 1,2,3-Triazolyl dihydropyrimidine-2-thione hybrid-1: (5c)

Chemicals required:

- 1,2,3 triazolyl chalcone
- Thiourea
- Potassium hydroxide 10%
- Water

A mixture of ((E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-3-(2-chlorophenyl)prop-2-en-1-one (3c), thiourea (4) (1.5 equiv.) and 10 % aq. KOH in water or ethanol (10 ml) was refluxed for 30-40 minutes and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The precipitated triazolyl dihydropyrimidine-2-thione derivatives (5c) were filtered and recrystallized from ethanol.



3c

5c

6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1*H*)-thione

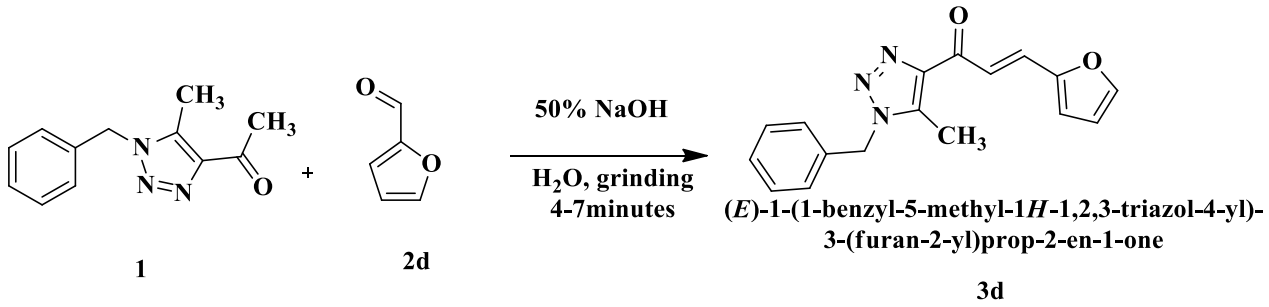
Compound 5d

Step1: Synthesis of 1,2,3-Triazolyl chalcone: (3d)

Chemicals required:

- 4-acetyl 1-benzyl 5-methyl-1,2,3 triazole
- Furfural
- Sodium hydroxide 50%

A mixture of 4-acetyl-1-benzyl-5-methyl-1,2,3-triazole (1) and furfural (2d) and 50% aqueous sodium hydroxide solution (1 ml) was ground for 4-7 minutes at room temperature and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The chalcone derivatives (3d) precipitated as solid, which were filtered and recrystallized from ethanol. Yield: 97-99%.



1 2d

(*E*)-1-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-(furan-2-yl)prop-2-en-1-one

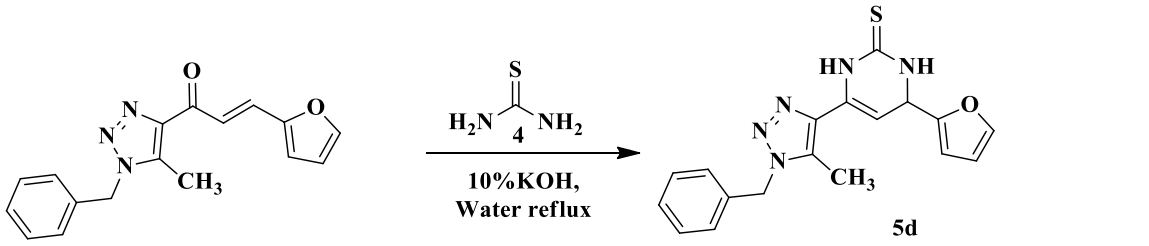
3d

Step-2- Synthesis of 1,2,3-Triazolyl dihydropyrimidine-2-thione hybrid-1: (5d)

Chemicals required:

- 1,2,3 triazolyl chalcone
- Thiourea
- Potassium hydroxide 10%
- Water

A mixture of (E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)- 3-(furan-2-yl)prop-2-en-1-one (3d), thiourea (4) (1.5 equiv.) and 10 % aq. KOH in water or ethanol (10 ml) was refluxed for 30-40 minutes and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The precipitated triazolyl dihydropyrimidine-2-thione derivatives (5d) were filtered and recrystallized from ethanol.



3d

5d

6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

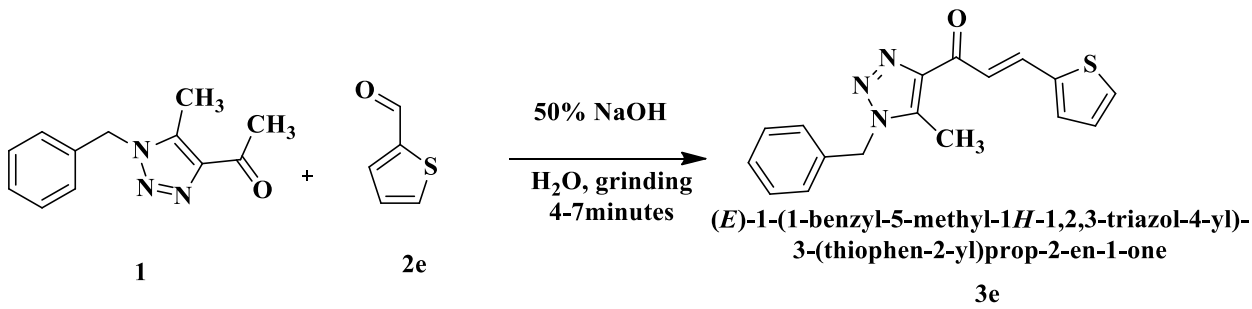
Compound 5e

Step1: Synthesis of 1,2,3-Triazolyl chalcone: (3e)

Chemicals required:

- 4-acetyl 1-benzyl 5-methyl-1,2,3 triazole
- Thiophene 2 carboxaldehyde
- Sodium hydroxide 50%

A mixture of 4-acetyl-1-benzyl-5-methyl-1,2,3-triazole (1) and Thiophene 2 carboxaldehyde (2e) and 50% aqueous sodium hydroxide solution (1 ml) was ground for 4-7 minutes at room temperature and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The chalcone derivatives (3e) precipitated as solid, which were filtered and recrystallized from ethanol. Yield: 97-99%.



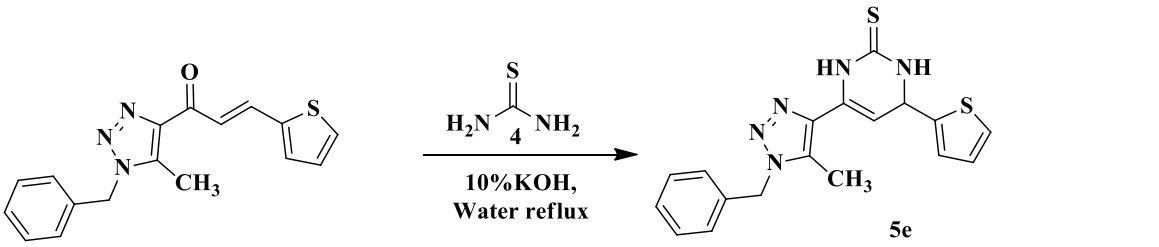
1 2e 3e

Step-2- Synthesis of 1,2,3-Triazolyl dihydropyrimidine-2-thione hybrid-1: (5e)

Chemicals required:

- 1,2,3 triazolyl chalcone
- Thiourea
- Potassium hydroxide 10%
- Water

A mixture of (E)-1-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)- 3-(thiophen-2-yl)prop-2-en-1-one (3e), thiourea (4) (1.5 equiv.) and 10 % aq. KOH in water or ethanol (10 ml) was refluxed for 30-40 minutes and poured onto excess of crushed ice and neutralized with dilute hydrochloric acid. The precipitated triazolyl dihydropyrimidine-2-thione derivatives (5e) were filtered and recrystallized from ethanol.



6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

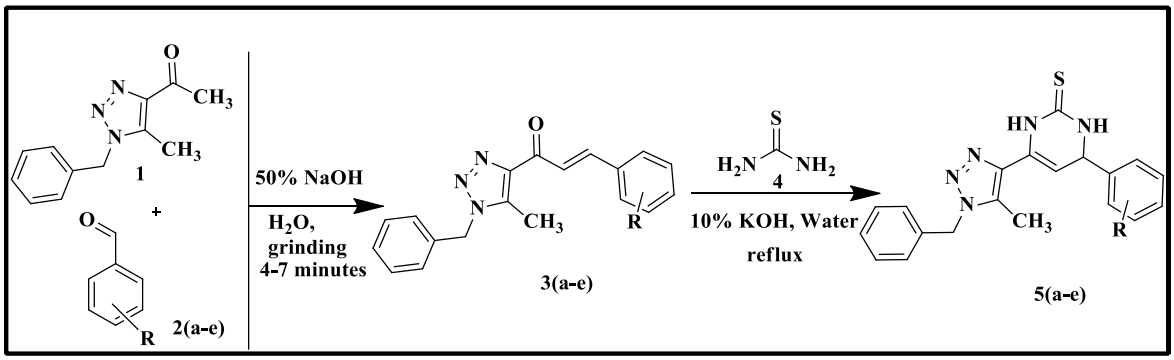
CHAPTER-VII

RESULTS & DISCUSSION

RESULTS AND DISCUSSION

An efficient synthesis of novel 1,2,3 - triazolyl dihydropyrimidine-2-thione hybrids has been described in a green protocol. Furthermore, all the synthesized hybrids were evaluated docking and in-vitro anticancer studies.

Synthesis of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids:



Synthesis of 1,2,3 - triazolyl dihydropyrimidine-2-thione hybrids

Purification:

All the synthesized compounds were purified by thin layer chromatography. The final product was confirmed by TLC using various mobile phases such as N- Hexane: ethyl acetate (8;2) chloroform: methanol (1:9) Petroleum ether : ethyl acetate (7:3).The spots were identified by iodine chamber and UV chamber.

Physical data:

The synthesized compounds physical data such as melting point, solubility were determined. The synthesized compounds were soluble in DMSO, ethanol and methanol. Melting points of the compounds were determined by open capillary tube method with aid of melting point instrument.

Infrared spectroscopy:

The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (Shimadzu) in the range 4000-400 cm^{-1} by KBr pellet technique and the values are reported.

The synthesized compounds show the absorption in the region around 3050-3070 presence of aromatic C=H stretching vibrations. The bands around 3200 - 3500 presence of N-H stretching vibration. The absorption band between 1590 - 1612 indicates the presence of C=N stretching vibrations. The absorption peak present in the range 800-900 indicates the presence of SO_2NH_2. The absorption band around 1220-1280 indicates the presence of C-N stretching vibration.

All the relevant functional group bands were observed for all synthesized compounds. **(Sharma.Y.R.,4th edition).**

Nuclear Magnetic resonance spectroscopy:

The natures of proton of all the synthesized compounds were confirmed by ^{1}HNMRspectroscopy.

The ^{1}H-NMR spectra were recorded on Bruker-NMR 400 MHz using DMSO as Solvent. The data are given in parts per million (ppm) and are referenced to an internal standard of tetra methyl silane (TMS,δ 0.00 ppm). Peak Multiplicity is reported as s (singlet), d (doublet), dd (double doublet), t (triplet), and m (multiplet).

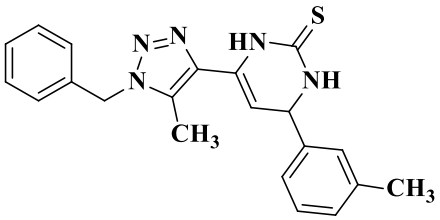
The synthesized compounds of M.D.B1-M.D.B7 show the multiplet peak around 6-8 ppm confirms the presence of Aromatic hydrogen. The chemical shift value around 3-5ppm confirms the N-H proton. The chemical shift value 2-3ppm confirm the presence of aliphatic proton.

The relative functional group proton peaks were observed for all the synthesized compounds. **(A.H.Beckett.,4th edition)**

Mass spectroscopy:

The molecular weight of the synthesized compounds were determined by mass spectroscopy. All the synthesized compounds were shown corresponding molecular ion peak according to their mass.**(Sharma .Y.R,2nd edition)**

ANALYTICAL DATA OF HYBRID-5a



Chemical Formula	:	$C_{21}H_{21}N_5S$
Exact Mass	:	375.15
Molecular Weight	:	375.49
IUPAC Name	:	6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione
Description	:	cream coloured solid
Solubility	:	soluble in dichloromethane, dimethyl formamide, Dimethyl sulfoxide

3D VIEW OF 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione

Chemsketch:

Molecular Formula = $C_{21}H_{21}N_5S$

Formula Weight = 375.48994

Composition = C(67.17%) H(5.64%) N(18.65%) S(8.54%)

Molar Refractivity = 112.26 ± 0.5 cm^3

Molar Volume = 293.0 ± 7.0 cm^3

Parachor = 772.9 ± 8.0 cm^3

Index of Refraction = 1.691 ± 0.05

Surface Tension = 48.3 ± 7.0 dyne/cm

Density = 1.28 ± 0.1 g/cm^3

Dielectric Constant = Not available

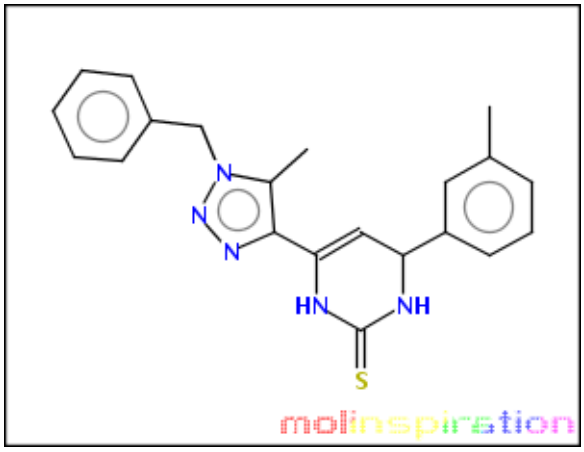
Polarizability = 44.50 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 375.151766 Da

Nominal Mass = 375 Da

Average Mass = 375.4899 Da

Molinspiration:



Molinspiration property engine v2016.10

miLogP	3.97
TPSA	54.77
natoms	27
MW	375.50
nON	5
nOHNH	2
nviolations	0
nrotb	4
volume	339.91

Molinspiration bioactivity score v2016.03

GPCR ligand	- 0.42
Ion channel modulator	- 0.43
Kinase inhibitor	- 0.66
Nuclear receptor ligand	- 0.76
Protease inhibitor	- 0.66
Enzyme inhibitor	- 0.45

Chemdoodle:

Hydrogen bond acceptor : 5

Hydrogen bond donor : 4

Degree of unsaturation : 4

Ring count : 4

Rotatable bonds : 4

Molecular mass : 395.6517 U

Monoisotopic mass : 395.3083 U

Boiling point : 988.82 K

Melting point : 786.24 K

Critical pressure : 15.90 bar

Critical volume : 1166.50 cm^3/mol

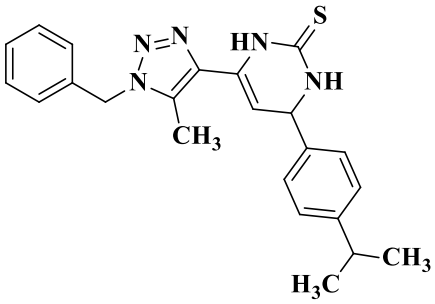
Critical temperature : 1262.17 K

Molar refractivity : 118.574 cm^3/mol

TPSA : 90.160 A^2

$XPlogV_{2.0}$: 4.844

ANALYTICAL DATA OF HYBRID-5b



Chemical Formula : C18H17N5S

Exact Mass : 403.545

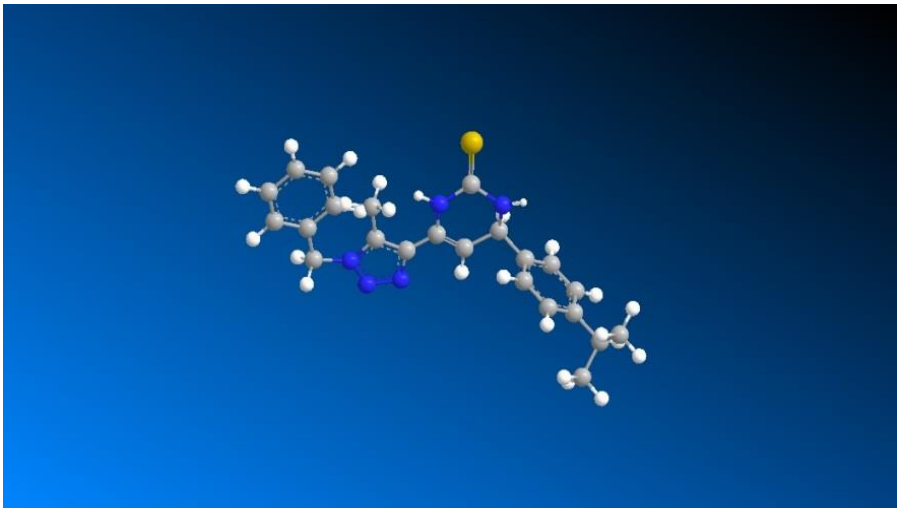
Molecular Weight : 403.54

IUPAC Name : 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(phenyl-4-isopropyl)-3,4-dihydropyrimidine-2(1*H*)-thione

Description : pale brown coloured solid

Solubility : Soluble in dichloromethane, dimethyl formamide, Dimethyl sulfoxide

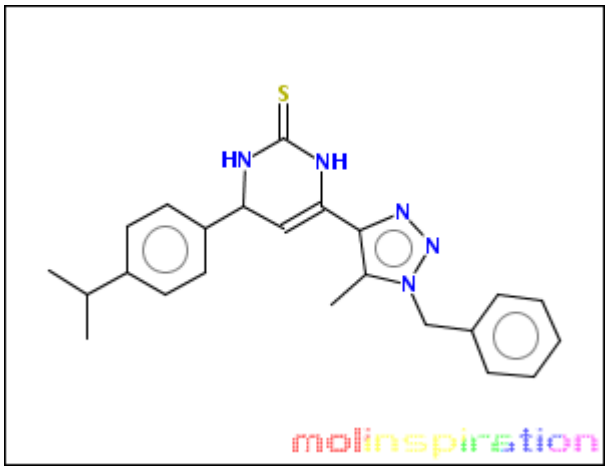
3D view of 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(phenyl-4-isopropyl)-3,4-dihydropyrimidine-2(1*H*)-thione



Chemsketch:

Molecular Formula	= $C_{23}H_{25}N_5S$
Formula Weight	= 403.5431
Composition	= C(68.46%) H(6.24%) N(17.35%) S(7.95%)
Molar Refractivity	= 121.29 ± 0.5 cm^3
Molar Volume	= 324.2 ± 7.0 cm^3
Parachor	= 842.6 ± 8.0 cm^3
Index of Refraction	= 1.671 ± 0.05
Surface Tension	= 45.5 ± 7.0 dyne/cm
Density	= 1.24 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 48.08 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 403.183066 Da
Nominal Mass	= 403 Da
Average Mass	= 403.5431 Da

Molinspiration:



Molinspiration property engine v2016.10

miLogP	5.06
TPSA	54.77
natoms	29
MW	403.56
nON	5
nOHNH	2
nviolations	1
nrotb	5
volume	373.30

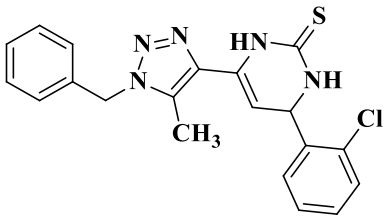
Molinspiration bioactivity score v2016.03

GPCR ligand	-0.37
Ion channel modulator	-0.36
Kinase inhibitor	-0.62
Nuclear receptor ligand	-0.65
Protease inhibitor	-0.60
Enzyme inhibitor	-0.38

Chemdoodle:

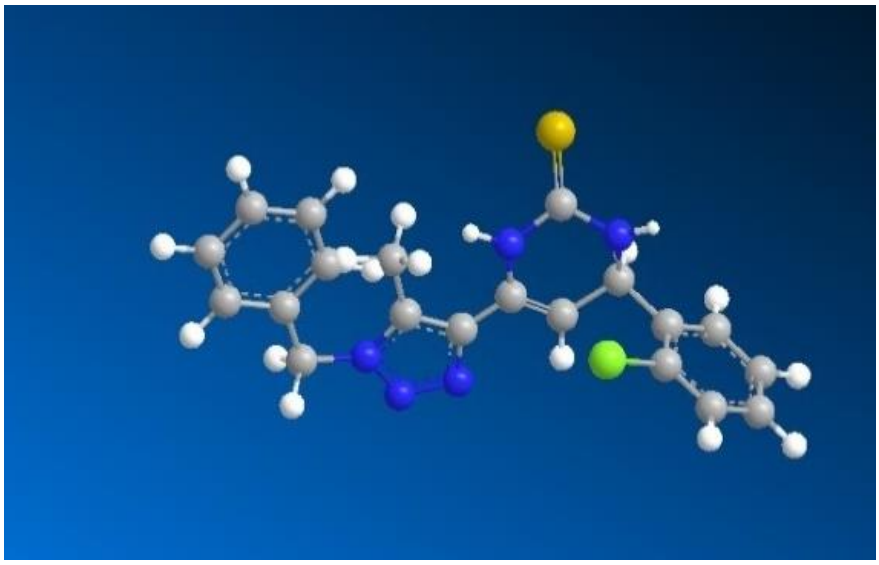
Hydrogen bond acceptor	:	2
Hydrogen bond donor	:	2
Degree of unsaturation	:	14
Ring count	:	4
Rotatable bonds	:	5
Molecular mass	:	403.5451 U
Monoisotopic mass	:	403.1830
Boiling point	:	1040.45 K
Melting point	:	831.25 K
Critical pressure	:	18.52 bar
Critical volume	:	1107.50 cm^3/mol
Critical temperature	:	1319.17 K
Molar refractivity	:	1231.190 cm^3/mol
TPSA	:	86.860 A^2
$XPlogV_{2.0}$	:	3.639

ANALYTICAL DATA OF HYBRID-5c



Chemical Formula	:	C20H18ClN5S
Exact Mass	:	395.9095
Molecular Weight	:	395.91
IUPAC Name	:	6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1*H*)-thione
Description	:	white coloured solid
Solubility	:	soluble in dichloromethane, dimethyl formamide, Dimethylsulfoxide

3D view of 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4 dihydropyrimidine-2(1*H*)-thione



Chemsketch:

Molecular Formula = $C_{20}H_{18}ClN_5S$

Formula Weight = 395.90842

Composition = C(60.67%) H(4.58%) Cl(8.95%) N(17.69%) S(8.10%)

Molar Refractivity = 112.43 ± 0.5 cm^3

Molar Volume = 287.1 ± 7.0 cm^3

Parachor = 770.6 ± 8.0 cm^3

Index of Refraction = 1.711 ± 0.05

Surface Tension = 51.8 ± 7.0 dyne/cm

Density = 1.37 ± 0.1 g/cm^3

Dielectric Constant = Not available

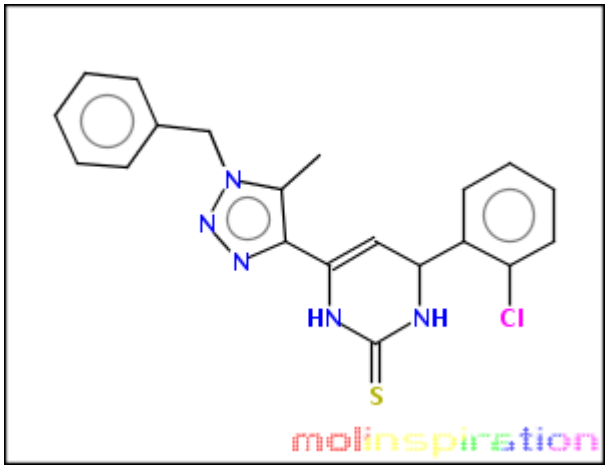
Polarizability = 44.57 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 395.097143 Da

Nominal Mass = 395 Da

Average Mass = 395.9084 Da

Molinspiration:



Molinspiration property engine v2016.10

miLogP	4.18
TPSA	54.77
natoms	27
MW	395.92
nON	5
nOHNH	2
nviolations	0
nrotb	4
volume	336.88

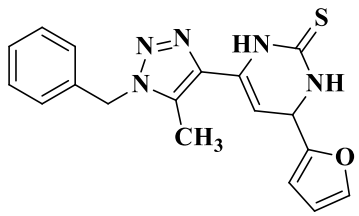
Molinspiration bioactivity score v2016.03

GPCR ligand	- 0.42
Ion channel modulator	- 0.42
Kinase inhibitor	- 0.74
Nuclear receptor ligand	- 0.74
Protease inhibitor	- 0.73
Enzyme inhibitor	- 0.48

Chemdoodle:

Hydrogen bond acceptor	:	2
Hydrogen bond donor	:	2
Degree of unsaturation	:	14
Ring count	:	4
Rotatable bonds	:	4
Molecular mass	:	395.9095 U
Monoisotopic mass	:	395.0970 U
Boiling point	:	1009.68 K
Melting point	:	842.36 K
Critical pressure	:	23.09 bar
Critical volume	:	994.50 cm^3/mol
Critical temperature	:	1300.93 K
Molar refractivity	:	114.190 cm^3/mol
TPSA	:	86.860 A^2
$XPlogV_{2.0}$	:	3.489

ANALYTICAL DATA OF HYBRID-5d



Chemical Formula : C18H17N5OS

Exact Mass : 351.4278

Molecular Weight : 351.43

IUPAC Name : 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

Description : cream coloured solid

Solubility : soluble in dichloromethane, dimethyl formamide, Dimethyl sulfoxide

3D view of 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

Chemsketch:

Molecular Formula = $C_{18}H_{17}N_5OS$

Formula Weight = 351.42548

Composition = C(61.52%) H(4.88%) N(19.93%) O(4.55%) S(9.12%)

Molar Refractivity = 100.00 ± 0.5 cm^3

Molar Volume = 252.2 ± 7.0 cm^3

Parachor = 683.7 ± 8.0 cm^3

Index of Refraction = 1.723 ± 0.05

Surface Tension = 54.0 ± 7.0 dyne/cm

Density = 1.39 ± 0.1 g/cm^3

Dielectric Constant = Not available

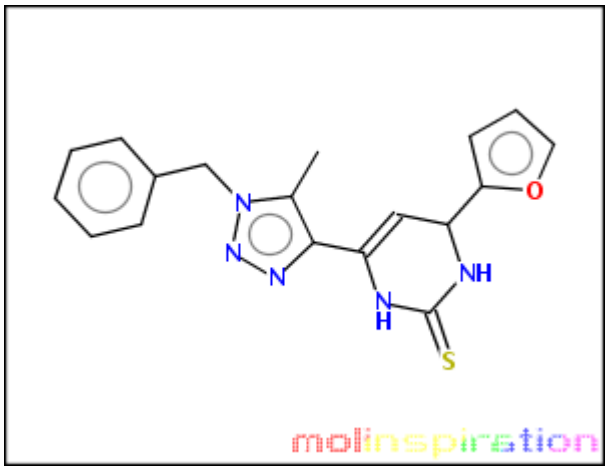
Polarizability = 39.64 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 351.11538 Da

Nominal Mass = 351 Da

Average Mass = 351.4255 Da

Molinspiration:



Molinspiration property engine v2016.10

miLogP	2.80
TPSA	67.91
natoms	25
MW	351.44
nON	6
nOHNH	2
nviolations	0
nrotb	4
volume	304.92

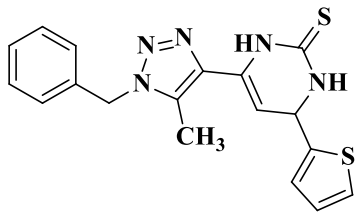
Molinspiration bioactivity score v2016.03

GPCR ligand	-0.51
Ion channel modulator	-0.62
Kinase inhibitor	-0.89
Nuclear receptor ligand	-0.90
Protease inhibitor	-0.89
Enzyme inhibitor	-0.53

Chemdoodle:

Hydrogen bond acceptor	:	3
Hydrogen bond donor	:	2
Degree of unsaturation	:	13
Ring count	:	4
Rotatable bonds	:	4
Molecular mass	:	351.4278 U
Monoisotopic mass	:	351.1153 U
Boiling point	:	945.03 K
Melting point	:	806.71 K
Critical pressure	:	27.50 bar
Critical volume	:	876.50 cm^3/mol
Critical temperature	:	1234.01 K
Molar refractivity	:	101.415 cm^3/mol
TPSA	:	100.000 A^2
$XPlogV_{2.0}$	:	1.771

ANALYTICAL DATA OF HYBRID-5e



Chemical Formula : C18H17N5S2

Exact Mass : 367.4964

Molecular Weight : 367.49

IUPAC Name : 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

Description : Pale yellow coloured solid

Solubility : soluble in dichloromethane, dimethyl formamide, dimethyl sulfoxide

3D view of 6-(1-benzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione

Chemsketch:

Molecular Formula = $C_{18}H_{17}N_5S_2$

Formula Weight = 367.49108

Composition = C(58.83%) H(4.66%) N(19.06%) S(17.45%)

Molar Refractivity = 106.42 ± 0.5 cm^3

Molar Volume = 260.2 ± 7.0 cm^3

Parachor = 714.1 ± 8.0 cm^3

Index of Refraction = 1.753 ± 0.05

Surface Tension = 56.7 ± 7.0 dyne/cm

Density = 1.41 ± 0.1 g/cm^3

Dielectric Constant = Not available

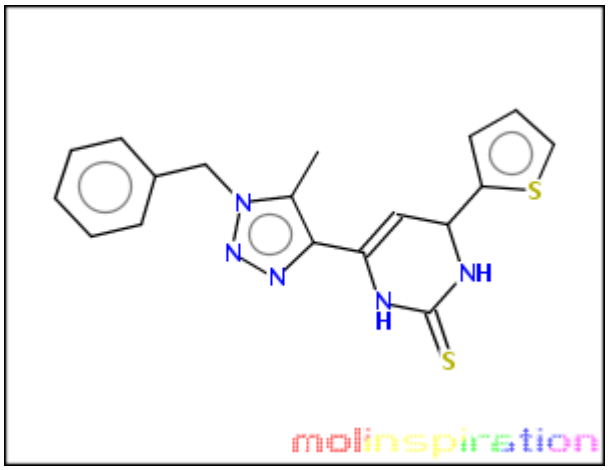
Polarizability = 42.19 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 367.092536 Da

Nominal Mass = 367 Da

Average Mass = 367.4911 Da

Molinspiration:



Molinspiration property engine v2016.10

miLogP	3.44
TPSA	54.77
natoms	25
MW	367.50
nON	5
nOHNH	2
nviolations	0
nrotb	4
volume	314.06

Molinspiration bioactivity score v2016.03

GPCR ligand	- 0.51
Ion channel modulator	- 0.60
Kinase inhibitor	- 0.81
Nuclear receptor ligand	- 0.90
Protease inhibitor	- 0.78
Enzyme inhibitor	- 0.47

Chemdoodle:

Hydrogen bond acceptor	:	2
Hydrogen bond donor	:	2
Degree of unsaturation	:	13
Ring count	:	4
Rotatable bonds	:	4
Molecular mass	:	367.4964 U
Monoisotopic mass	:	367.0925 U
Boiling point	:	965.91 K
Melting point	:	863.59 K
Critical pressure	:	27.58 bar
Critical volume	:	901.50 cm^3/mol
Critical temperature	:	1266.58 K
Molar refractivity	:	107.367 cm^3/mol
TPSA	:	115.100 A^2
$XPlogV_{2.0}$	:	1.560

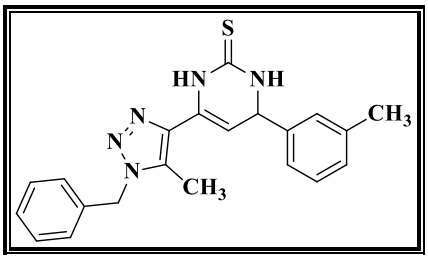
CHAPTER-VII(A)

SPECTRAL DATA

Spectral Data

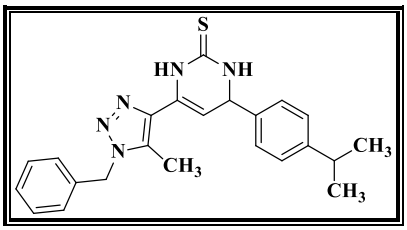
NMR

6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione (5a)



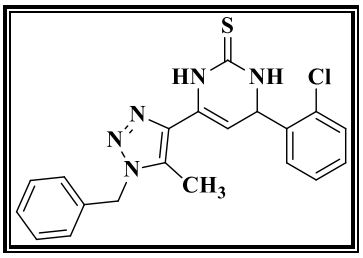
White solid; m.p.176 °C; Yield: 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.48 – 7.01 (m, 9H), 6.73 (s, 1H), 5.50 (s, 2H), 5.22 (d, *J* = 3.3 Hz, 1H), 5.07 (d, *J* = 1.9 Hz, 1H), 2.33 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.33, 139.53, 138.62, 137.43, 134.16, 130.37, 129.83, 129.62, 129.14, 129.05, 128.98, 128.58, 127.32, 127.10, 126.81, 126.11, 99.63, 56.66, 52.18, 21.10, 9.52

6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione (5b)



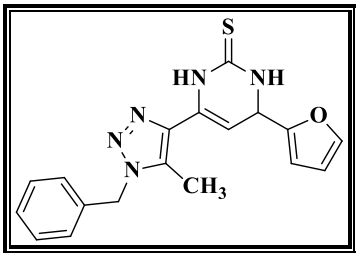
White solid; m.p.173 °C; Yield: 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (s, 1H), 7.38 – 7.30 (m, 3H), 7.30 – 7.22 (m, 4H), 7.18 – 7.11 (m, 2H), 6.65 (s, 1H), 5.52 (s, 2H), 5.24 (d, *J* = 1.8 Hz, 1H), 5.10 (d, *J* = 1.8 Hz, 1H), 2.96 – 2.79 (m, 1H), 2.22 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.85, 149.42, 139.72, 137.32, 136.80, 134.06, 133.92, 130.41, 129.04, 128.92, 128.48, 127.12, 127.03, 126.87, 125.90, 99.61, 56.42, 52.07, 33.76, 23.85, 9.51.

6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione (5c)



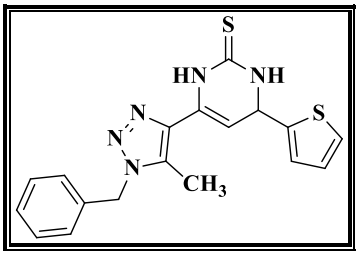
White solid; m.p.198 °C; Yield: 87%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (s, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.41 – 7.23 (m, 6H), 7.21 – 7.10 (m, 2H), 6.88 (s, 1H), 5.72 (d, *J* = 4.0 Hz, 1H), 5.52 (s, 2H), 5.26 (d, *J* = 3.7 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.58, 139.05, 137.06, 133.96, 133.78, 131.20, 130.56, 129.68, 129.41, 128.96, 128.73, 128.67, 128.43, 127.79, 127.01, 126.70, 97.41, 52.93, 51.99, 9.45.

6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5d)

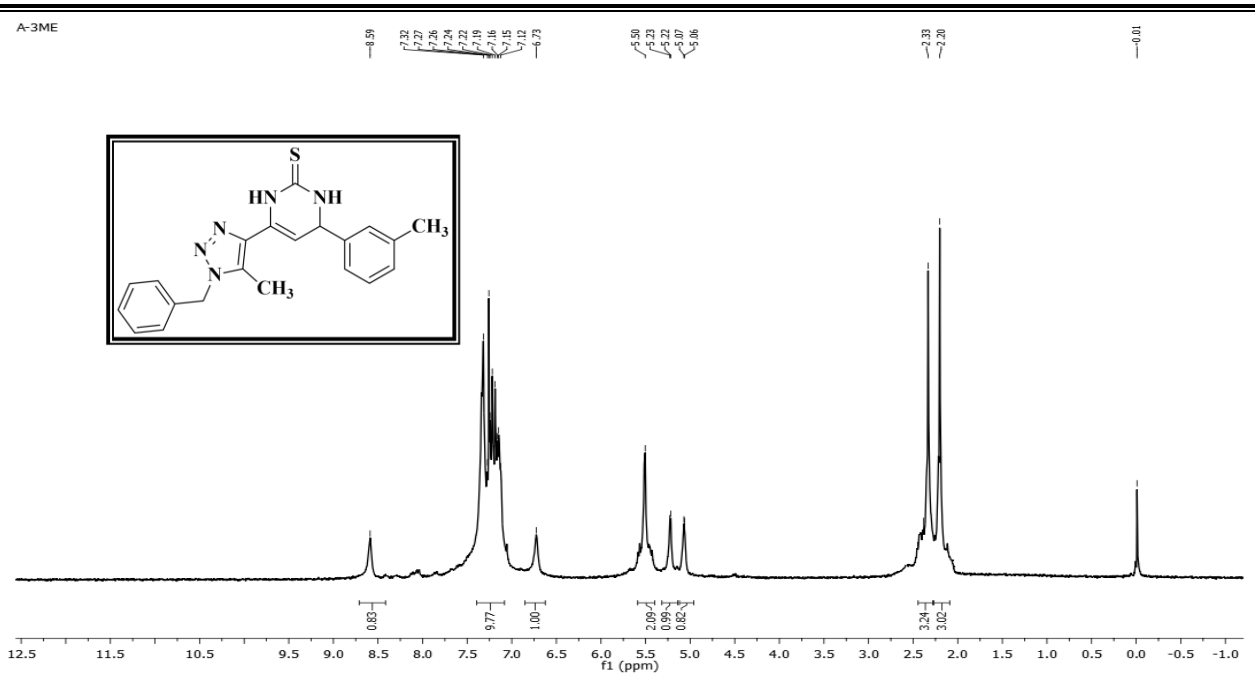


White solid; m.p.186 °C; Yield: 88%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.46 – 7.28 (m, 5H), 7.17 (s, 2H), 6.32 (d, *J* = 12.8 Hz, 2H), 5.53 (s, 2H), 5.40 – 5.13 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.65, 155.21, 153.57, 151.62, 145.53, 134.10, 132.96, 129.12, 128.57, 128.13, 127.12, 124.68, 107.25, 106.13, 95.75, 53.75, 52.16, 9.50.

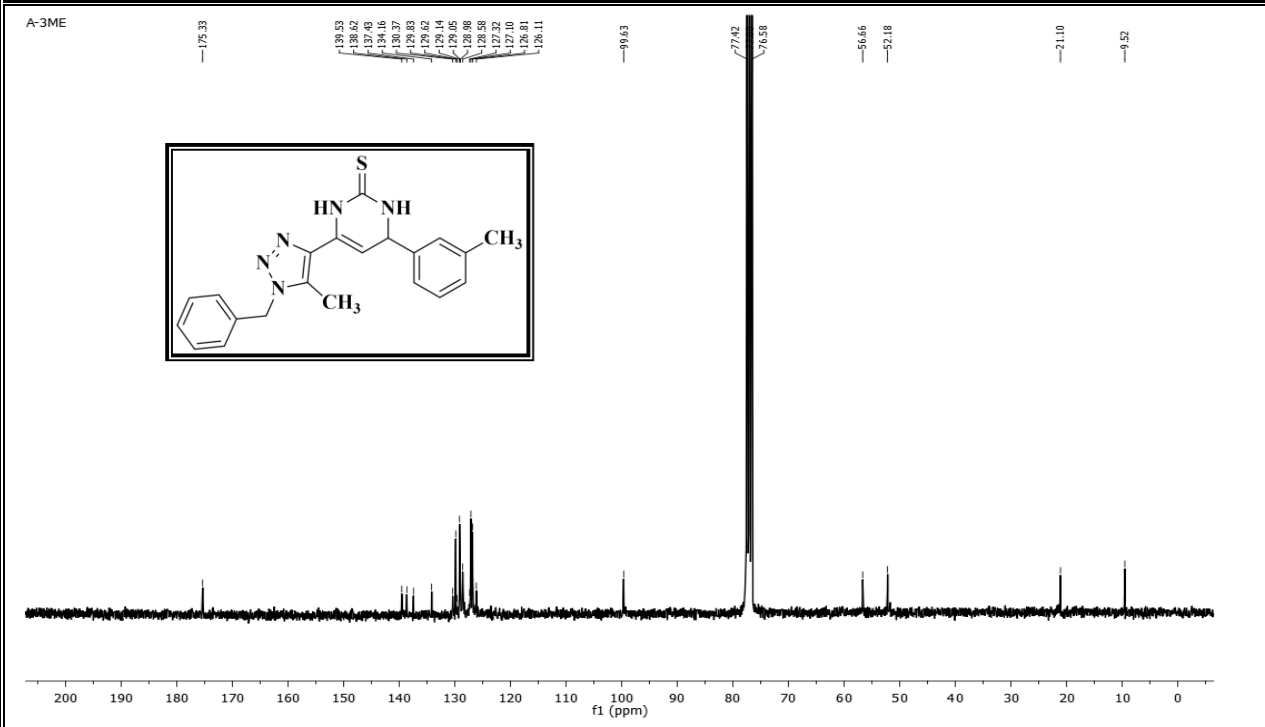
6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5e)



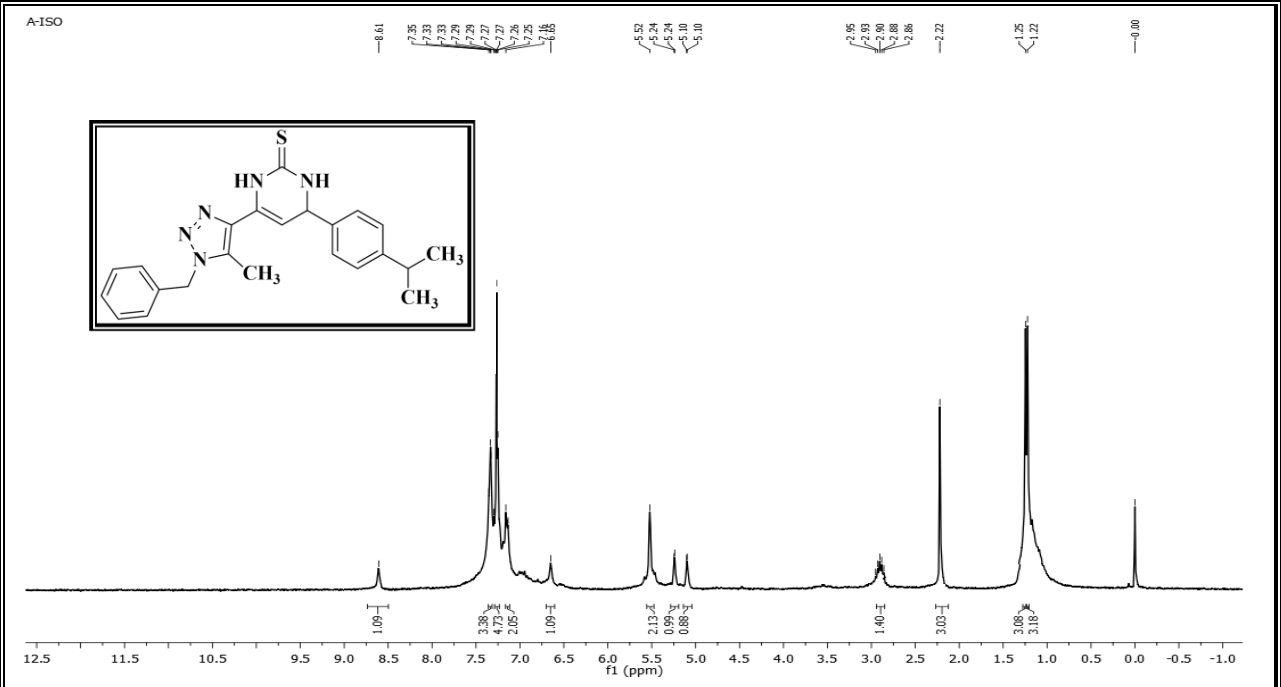
Light yellow; m.p.185 °C; Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.40 – 6.94 (m, 9H), 5.69 – 5.11 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.13, 146.53, 136.86, 133.77, 133.51, 130.51, 128.52, 127.93, 126.64, 126.53, 126.28, 126.16, 125.38, 124.55, 98.75, 51.44, 50.39, 8.86.



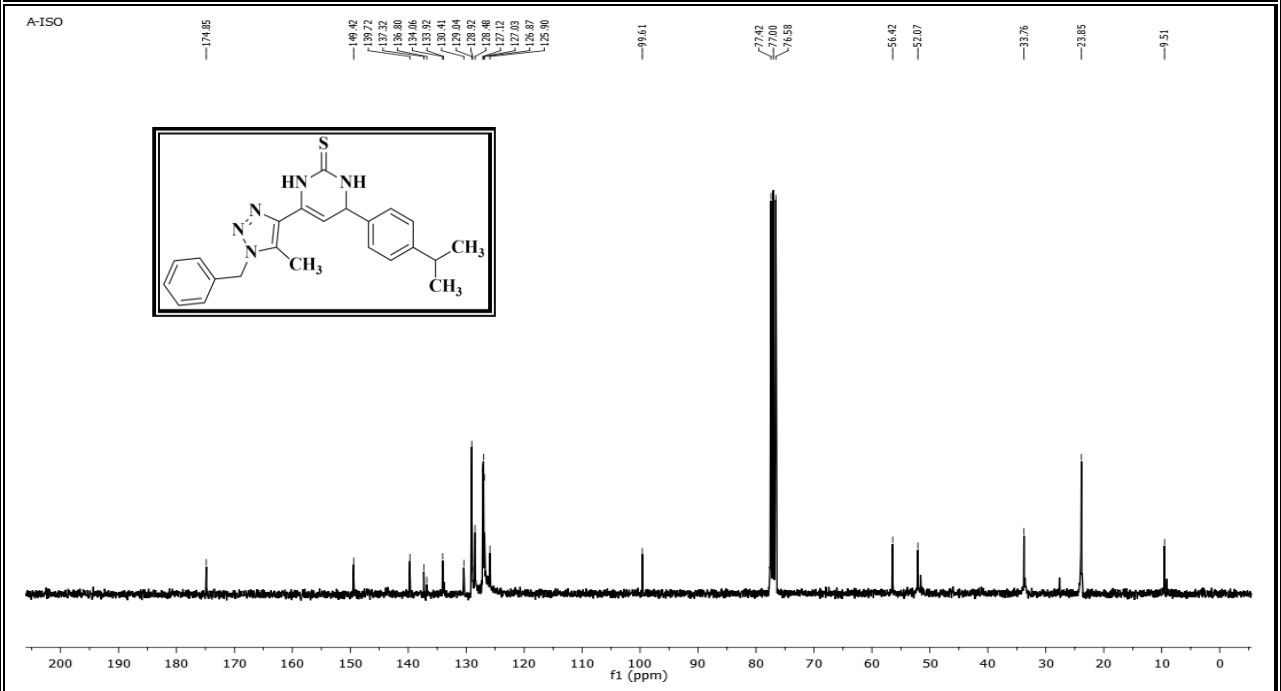
^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione **(5a)**



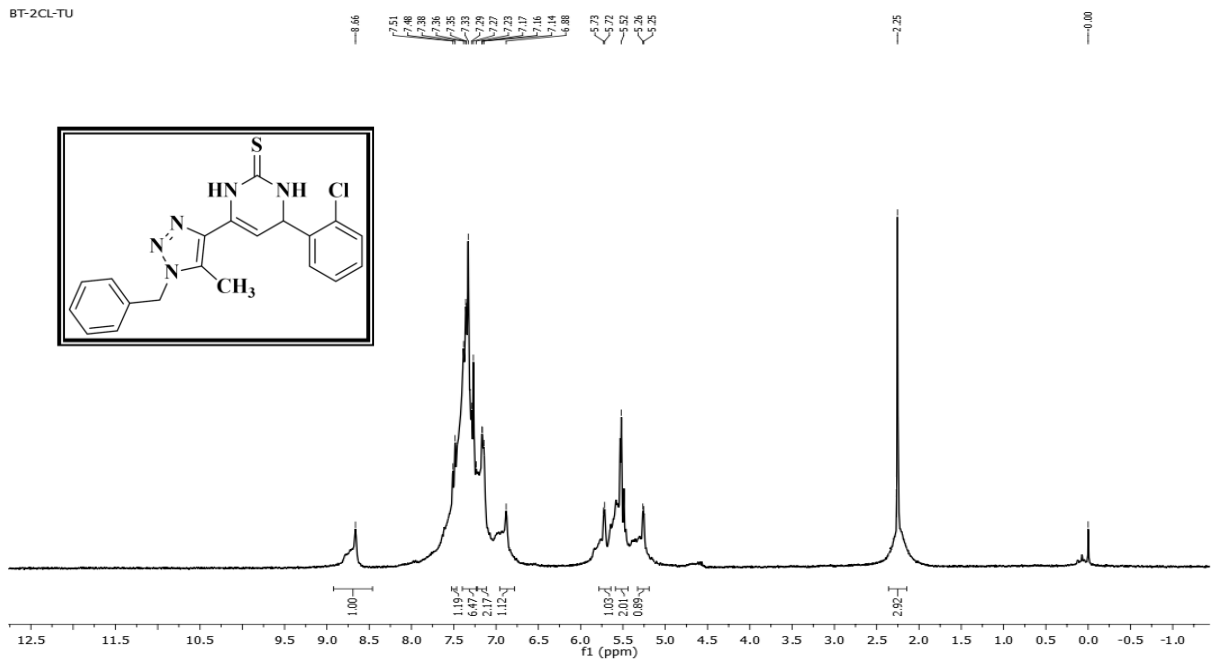
^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione **(5a)**



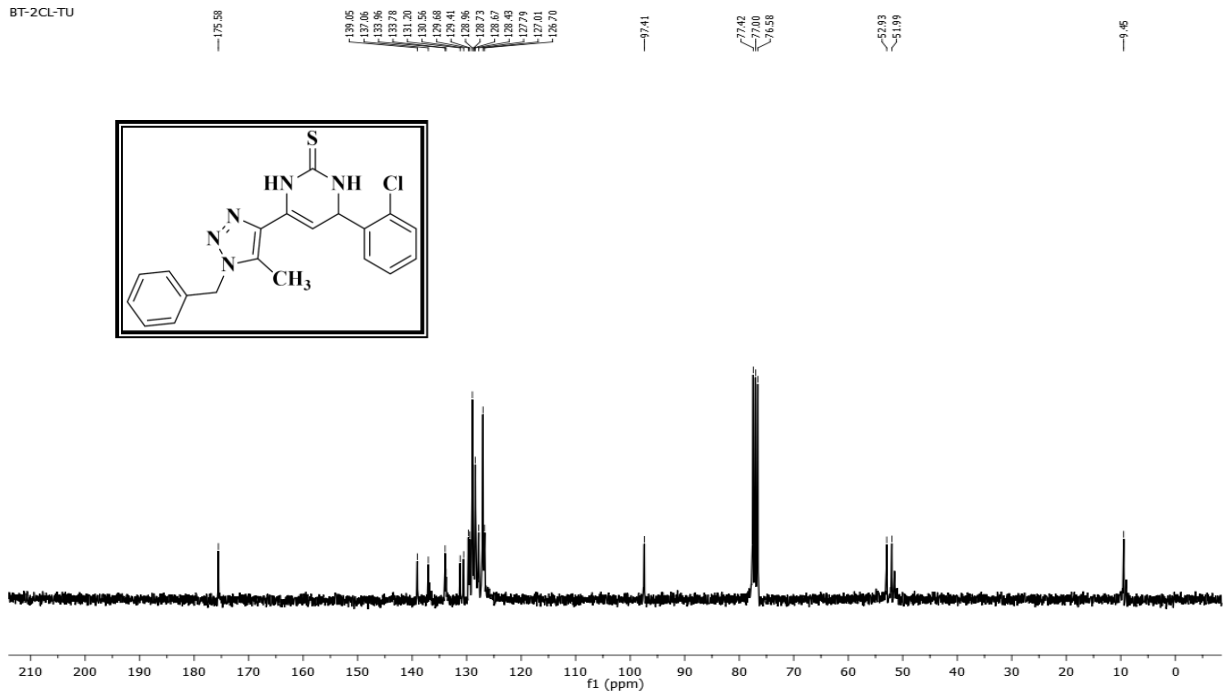
^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione **(5b)**



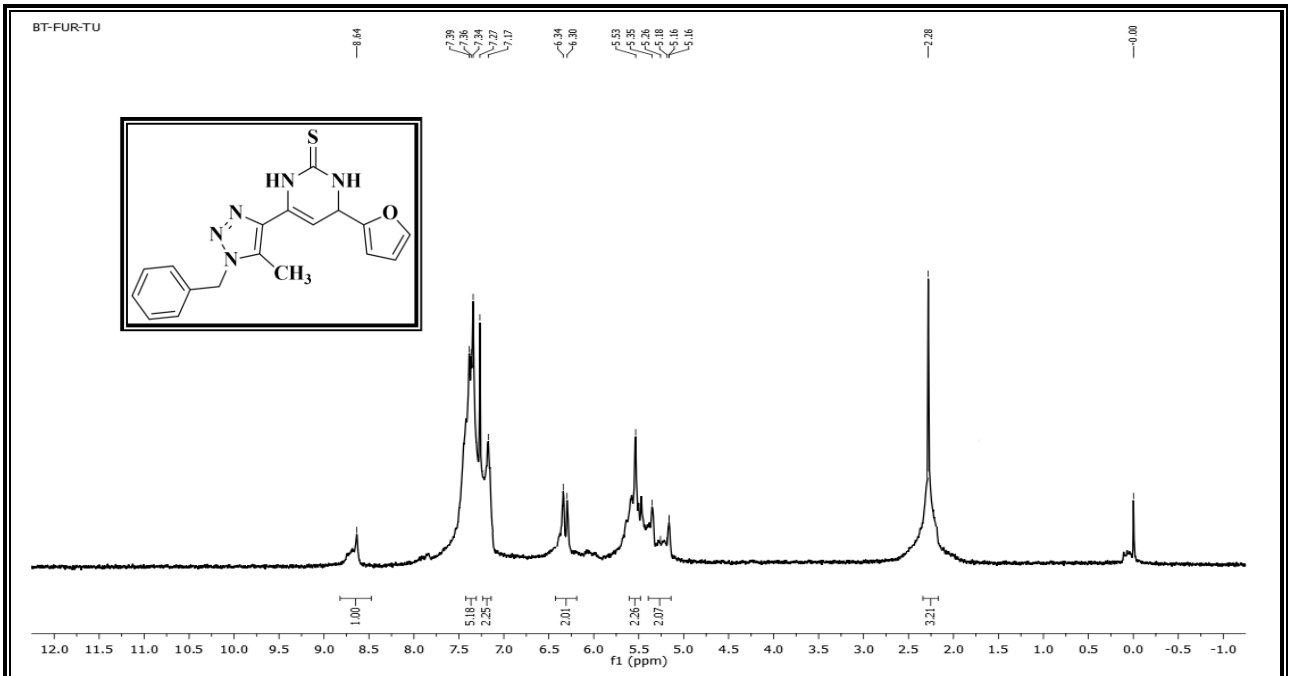
^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione **(5b)**



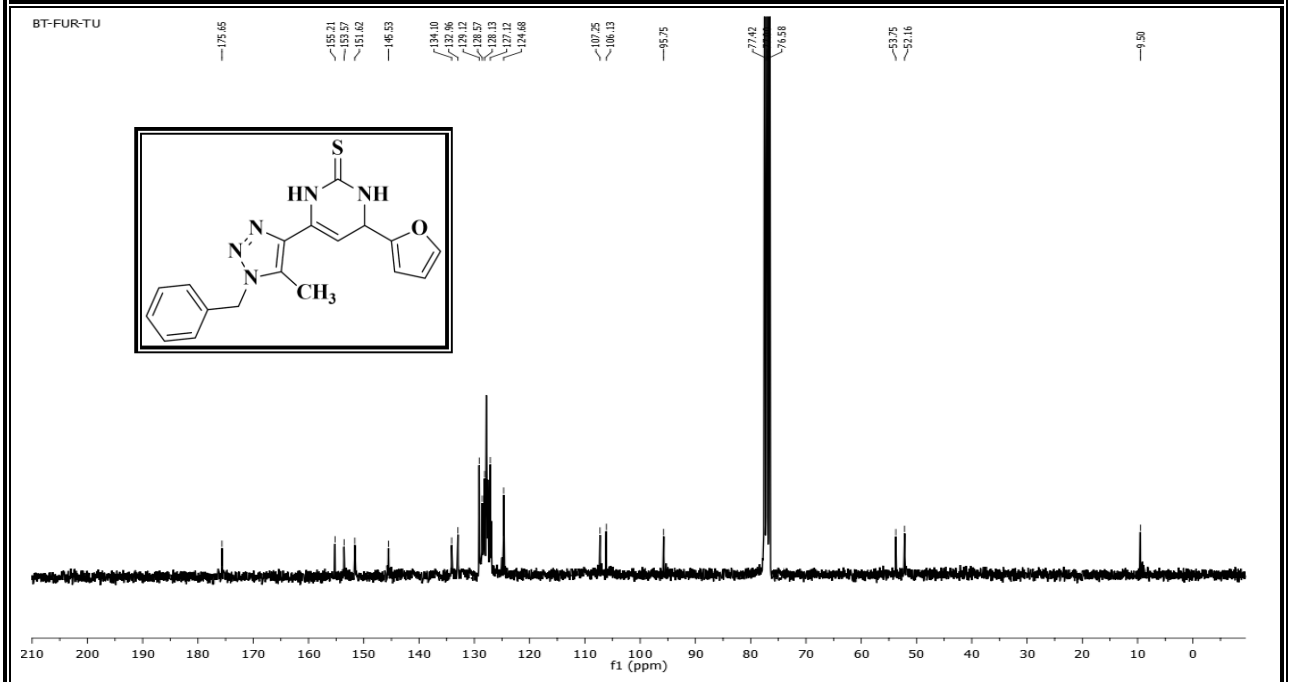
^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione **(5c)**



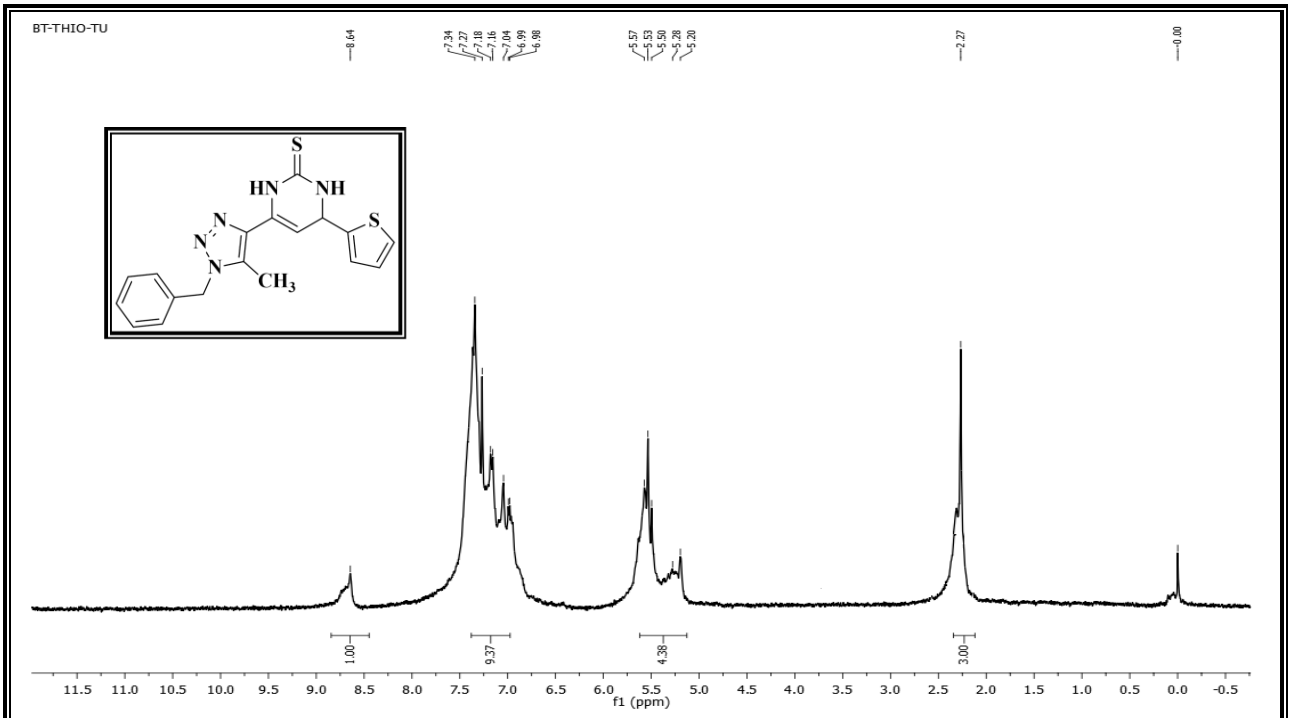
^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione **(5c)**



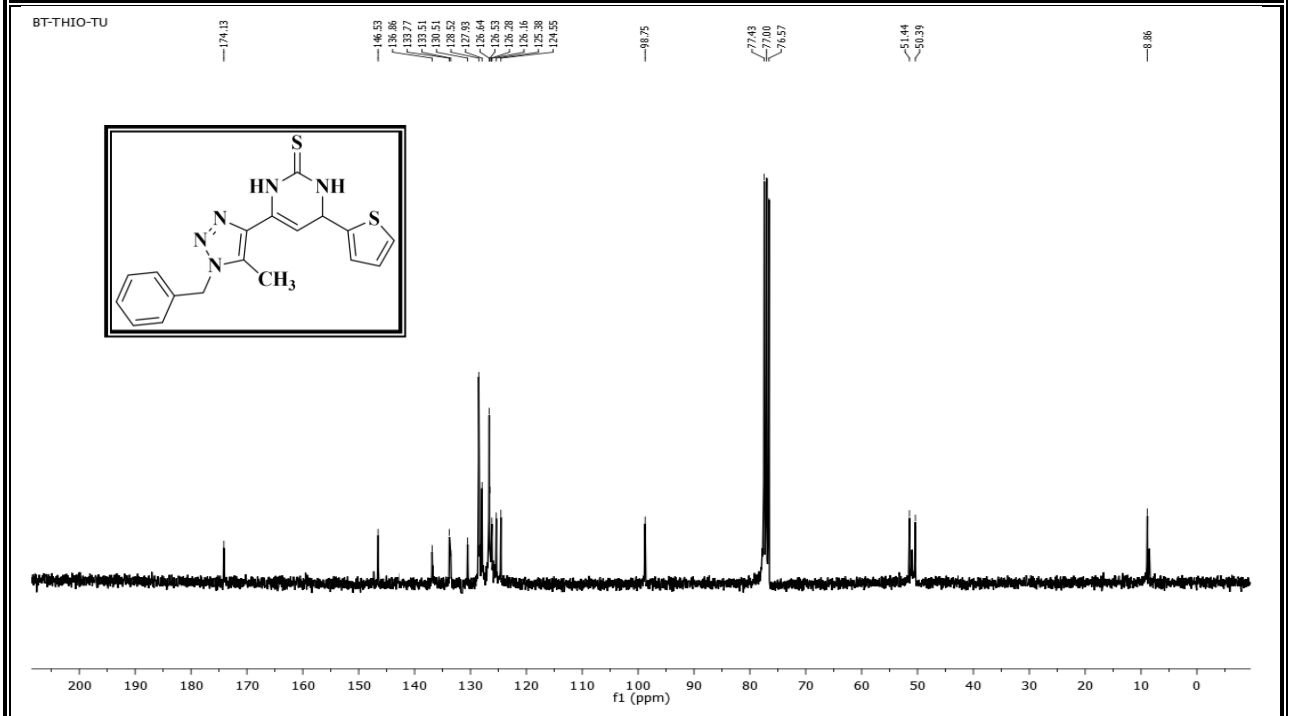
^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione **(5d)**



^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione **(5d)**



^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione **(5e)**



^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione **(5e)**

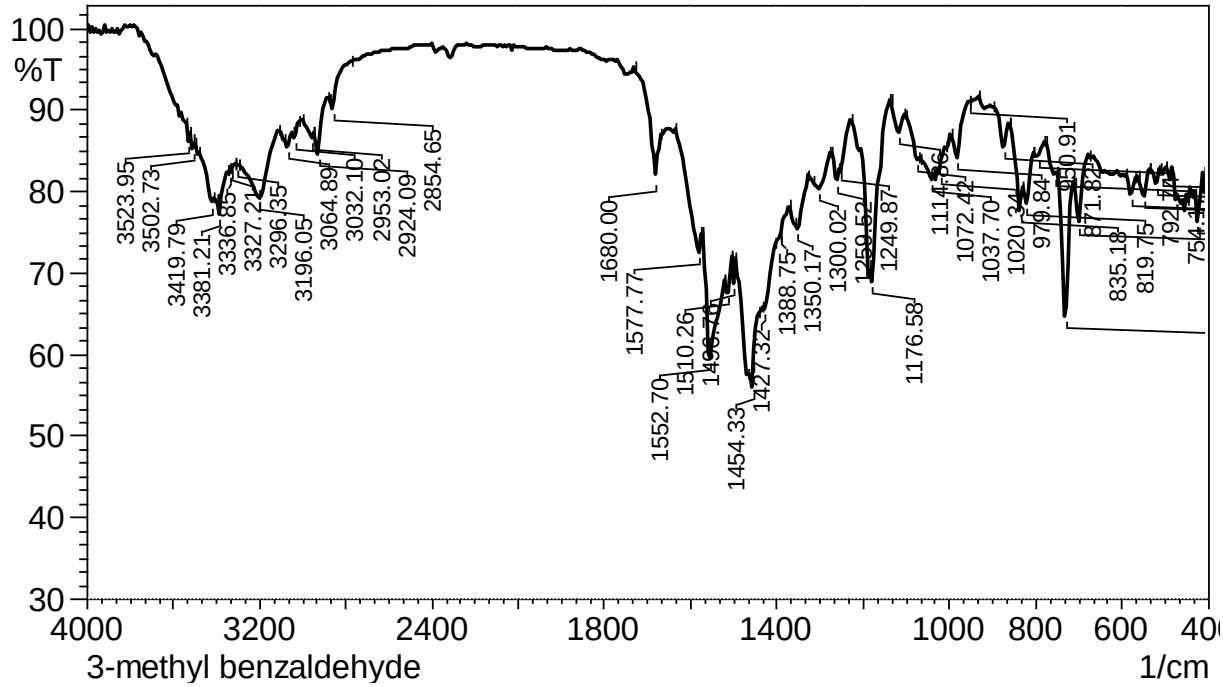
References

(1) Shanmugavelan, P.; Sathishkumar, M.; Nagarajan, S.; Ponnuswamy, A. *J. Chem. Sci.* **2012**, *124*, 941–950.

(2) Sangaraiah, N.; Murugan, S.; Poovan, S.; Raja, R. *Eur. J. Med. Chem.* **2012**, *58*, 464–469.

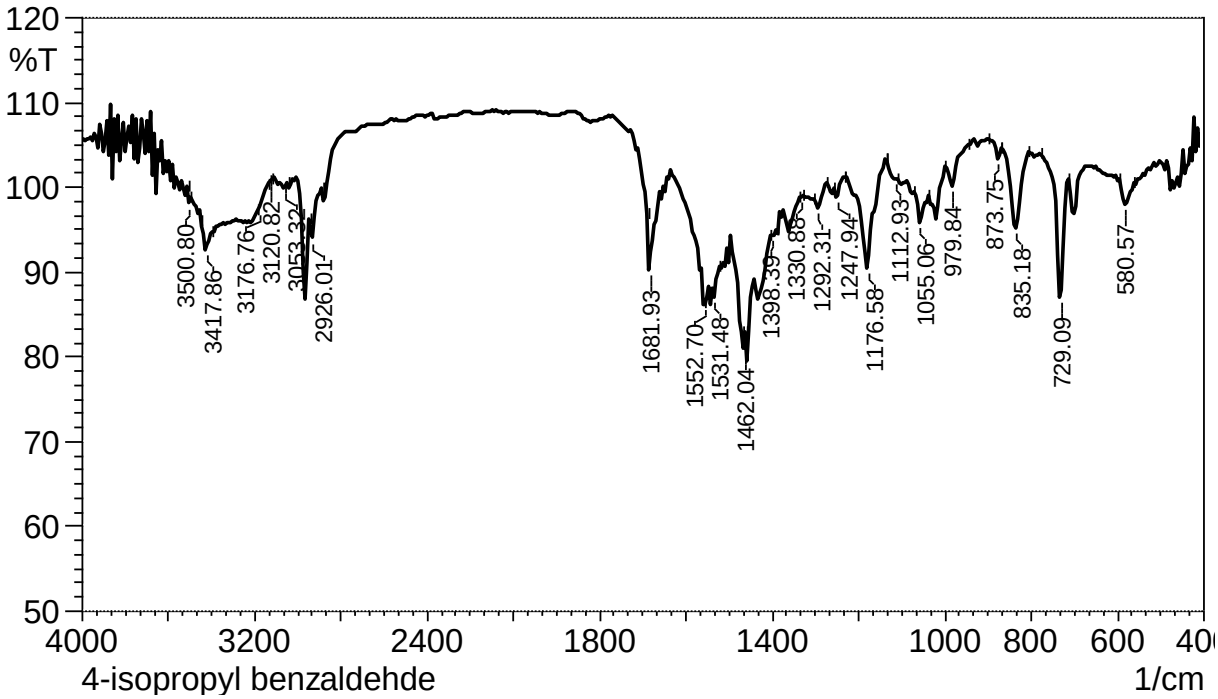
FT-IR

Hybrid-5a



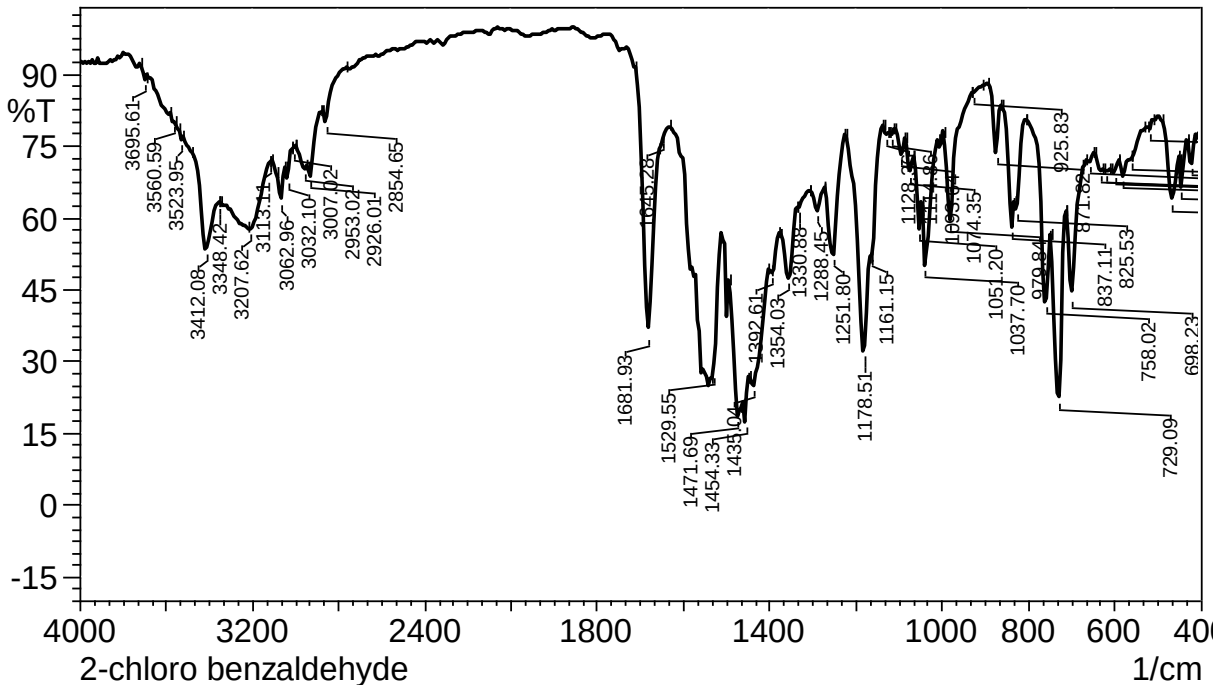
STANDARD REGION (wave number)	OBSERVED REGION (wave number)	FUNCTIONAL GROUP STRETCHING
3500-3100 cm^{-1}	3419 cm^{-1}	N-H Stretching
3150-3020 cm^{-1}	3064 cm^{-1}	C-H Aromatic
3050-2950 cm^{-1}	3032 cm^{-1}	Mono Substituted Benzene
3050-2950 cm^{-1}	3032 cm^{-1}	Di substituted Benzene
3050-2950 cm^{-1}	2953 cm^{-1}	C=CH Stretching
1400-1300 cm^{-1}	1427 cm^{-1}	C=S Stretching
1300-950 cm^{-1}	1072 cm^{-1}	C-N Stretching

Hybrid-5b



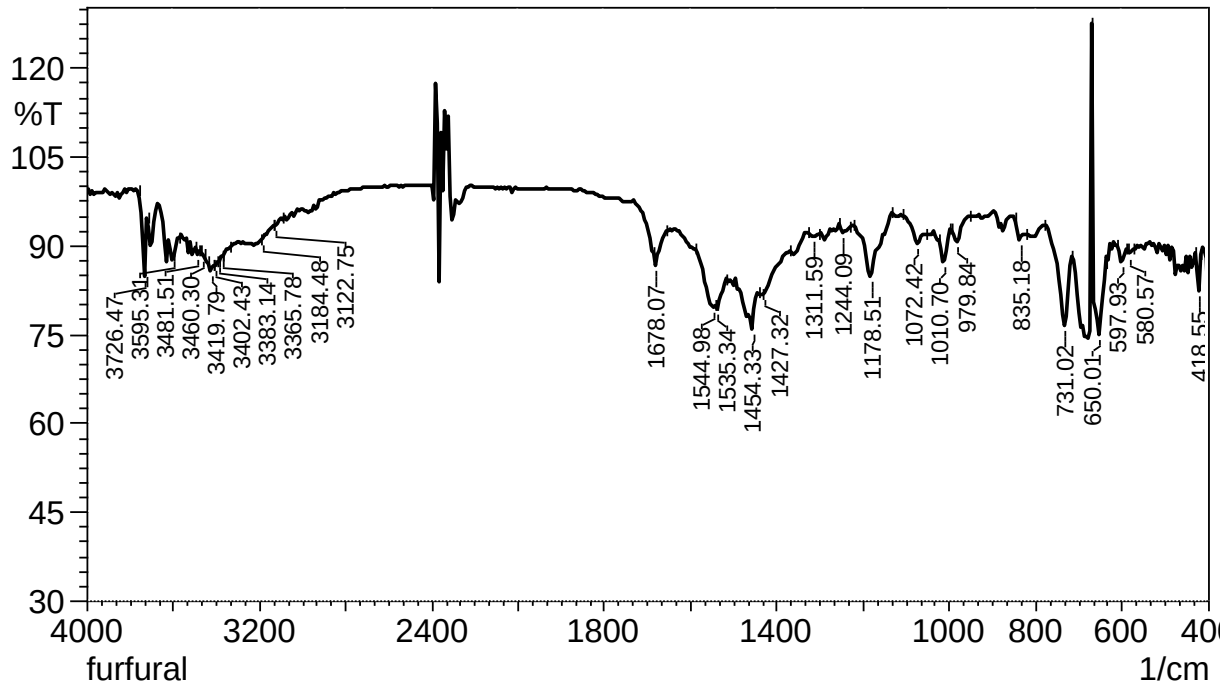
STANDARD REGION (wave number)	OBSERVED REGION (wave number)	FUNCTIONAL GROUP STRETCHING
3500-3100 cm^{-1}	3417 cm^{-1}	N-H Stretching
3150-3020 cm^{-1}	3120 cm^{-1}	C-H Aromatic
3050-2950 cm^{-1}	3053 cm^{-1}	Mono Substituted Benzene
3050-2950 cm^{-1}	2926 cm^{-1}	Di substituted Benzene
3050-2950 cm^{-1}	2926 cm^{-1}	C=CH Stretching
1400-1300 cm^{-1}	1327 cm^{-1}	C=S Stretching
1300-950 cm^{-1}	1176 cm^{-1}	C-N Stretching

Hybrid-5c



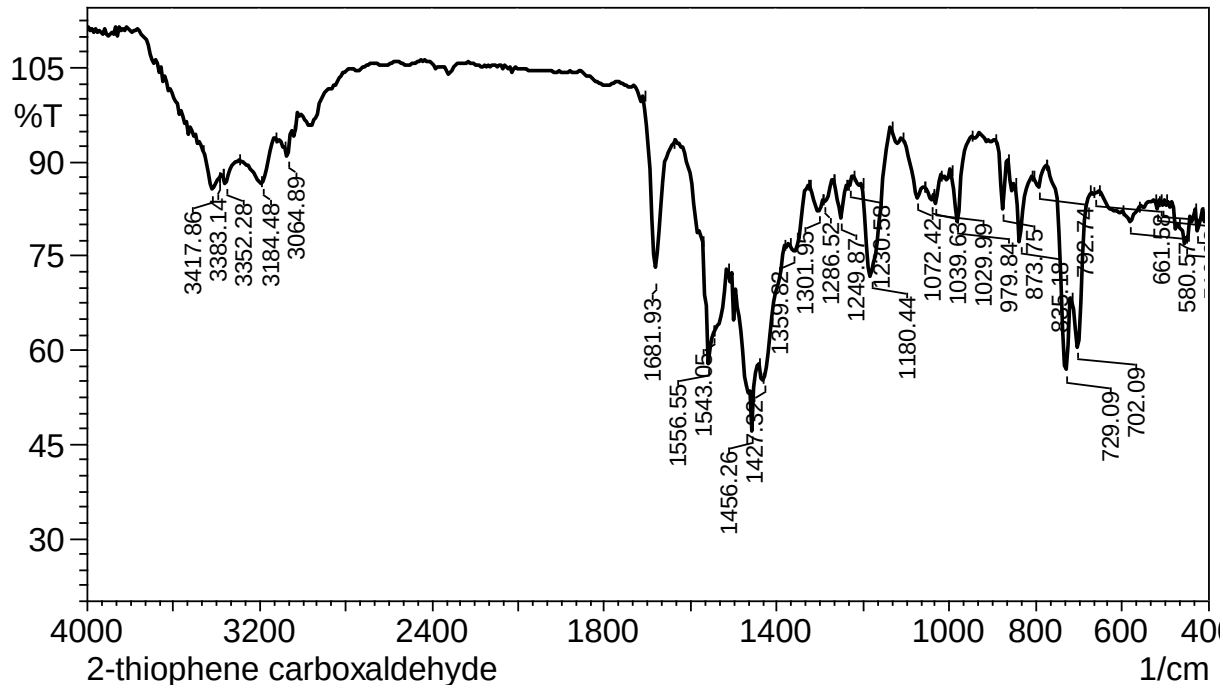
STANDARD REGION (wave number)	OBSERVED REGION (wave number)	FUNCTIONAL GROUP STRETCHING
3500-3100 cm^{-1}	3419 cm^{-1}	N-H Stretching
3150-3020 cm^{-1}	3064 cm^{-1}	C-H Aromatic
3050-2950 cm^{-1}	3032 cm^{-1}	Mono Substituted Benzene
3050-2950 cm^{-1}	3032 cm^{-1}	Di substituted Benzene
3050-2950 cm^{-1}	2953 cm^{-1}	C=CH Stretching
1400-1300 cm^{-1}	1288 cm^{-1}	C=S Stretching
1300-950 cm^{-1}	1251 cm^{-1}	C-N Stretching

Hybrid-5d



STANDARD REGION (wave number)	OBSERVED REGION (wave number)	FUNCTIONAL GROUP STRETCHING
3500-3100 cm^{-1}	3460 cm^{-1}	N-H Stretching
3150-3020 cm^{-1}	3122 cm^{-1}	C-H Aromatic
3050-2950 cm^{-1}	3122 cm^{-1}	Mono Substituted Benzene
3050-2950 cm^{-1}	3122 cm^{-1}	Di substituted Benzene
3050-2950 cm^{-1}	2953 cm^{-1}	C=CH Stretching
1400-1300 cm^{-1}	1311 cm^{-1}	C=S Stretching
1300-950 cm^{-1}	1178 cm^{-1}	C-N Stretching

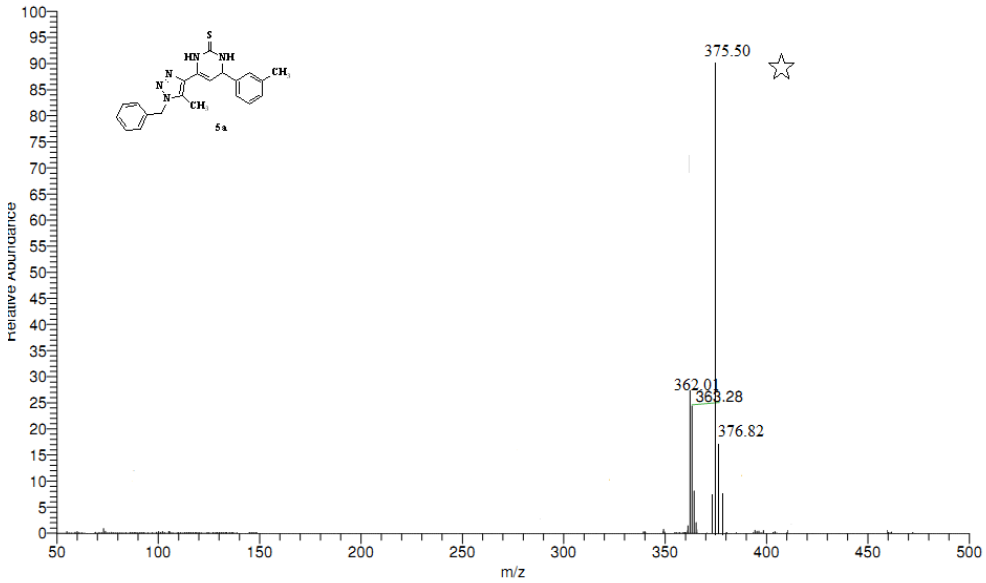
Hybrid-5e



STANDARD REGION (wave number)	OBSERVED REGION (wave number)	FUNCTIONAL GROUP STRETCHING
3500-3100 cm^{-1}	3417 cm^{-1}	N-H Stretching
3150-3020 cm^{-1}	3184 cm^{-1}	C-H Aromatic
3050-2950 cm^{-1}	3184 cm^{-1}	Mono Substituted Benzene
3050-2950 cm^{-1}	3064 cm^{-1}	Di substituted Benzene
3050-2950 cm^{-1}	3064 cm^{-1}	C=CH Stretching
1400-1300 cm^{-1}	1359 cm^{-1}	C=S Stretching
1300-950 cm^{-1}	1286 cm^{-1}	C-N Stretching

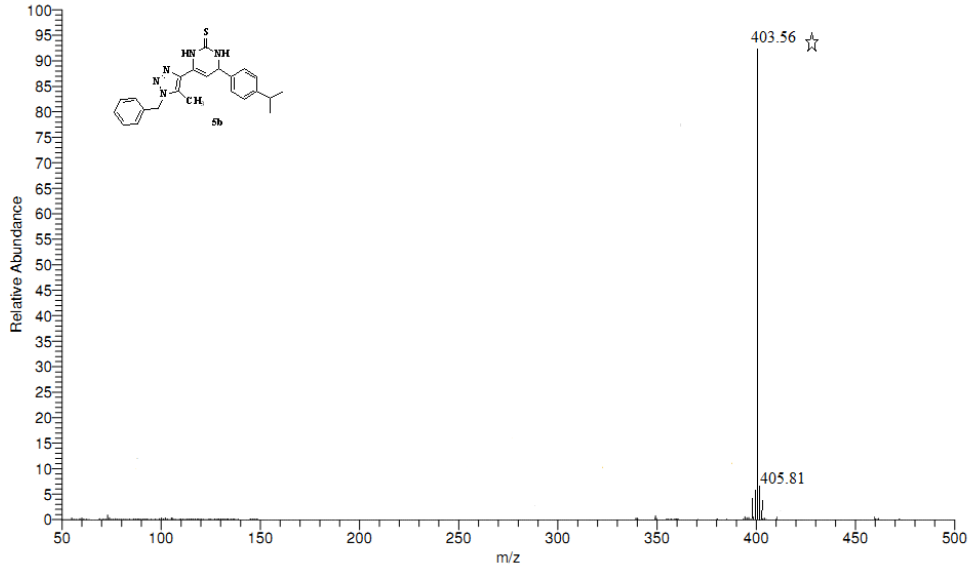
MASS

Hybrid-5a



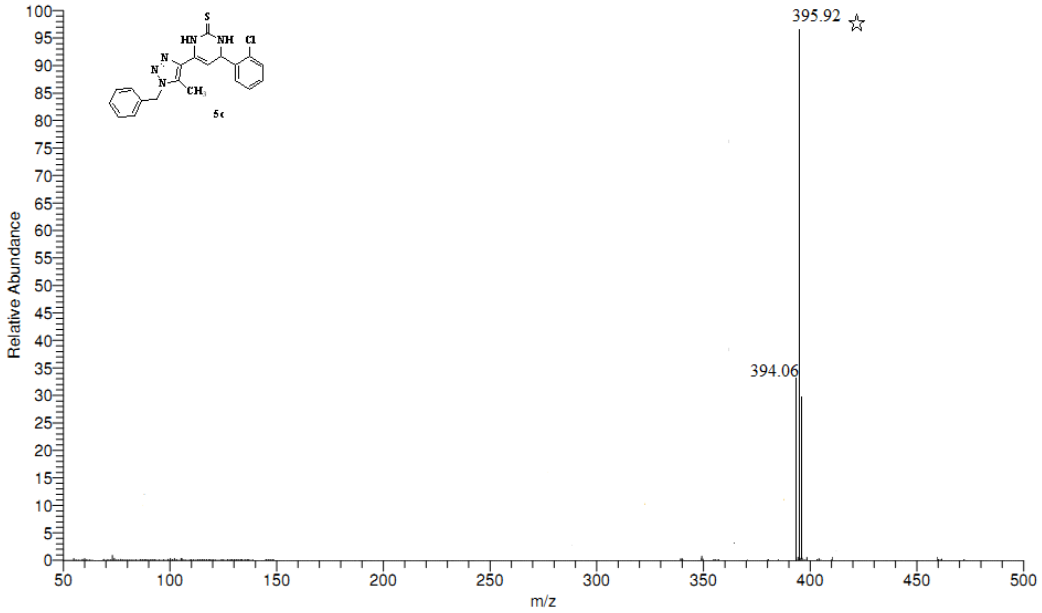
MASS: (m/z value):375.50 M+1 ion peak

Hybrid-5b



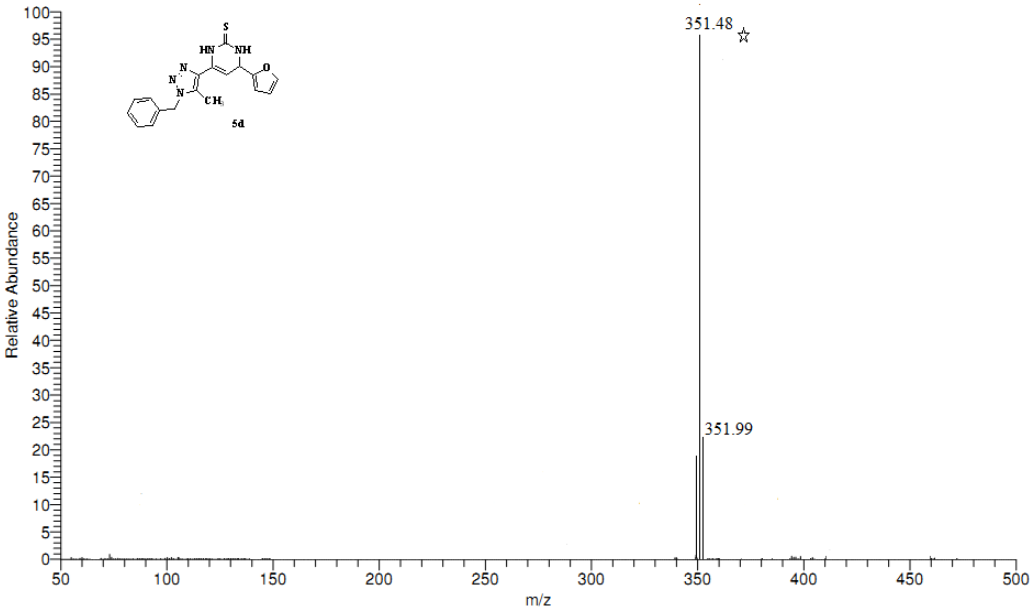
MASS: (m/z value):403.56 M+1 ion peak

Hybrid-5c



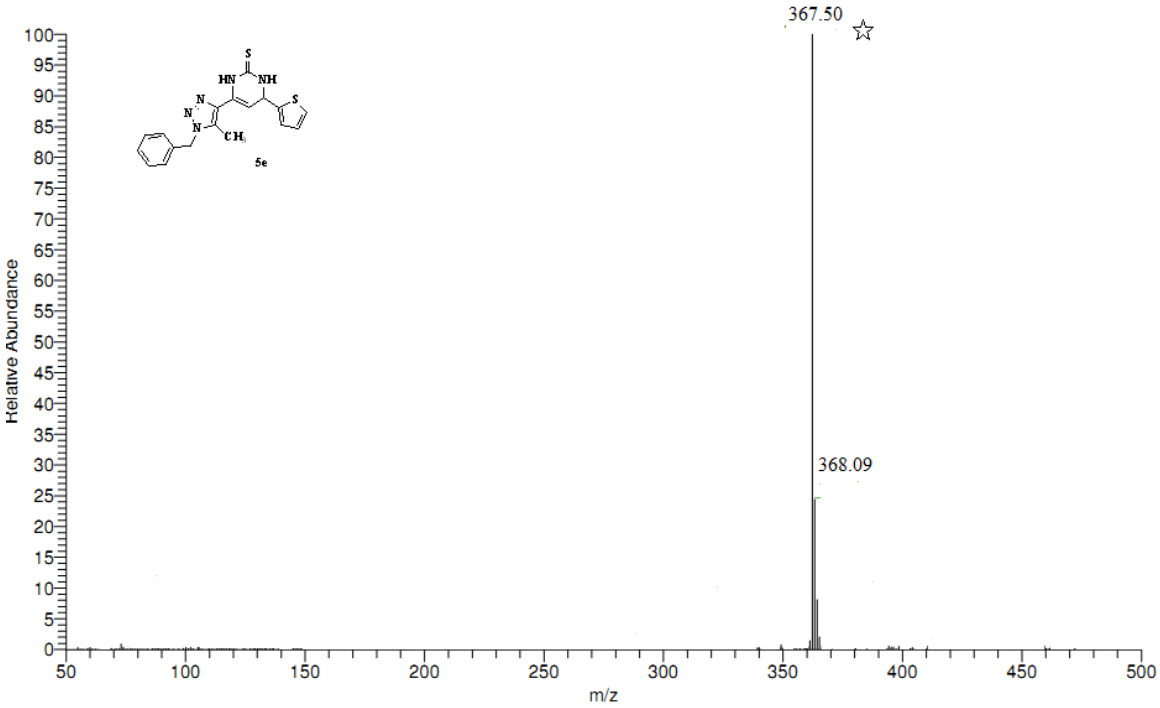
MASS: (m/z value):395.92 M+1 ion peak

Hybrid-5d



MASS: (m/z value):351.48 M+1 ion peak

Hybrid-5e



MASS: (m/z value):367.50 M+1 ion peak

CHAPTER-VII(B)

IN-SILICO MOLECULAR DOCKING

MOLECULAR DOCKING

Insilico Molecular Docking studies

Preparation of Protein structure

Protein target was downloaded from database Protein Data Bank (PDB). DNA Topoisomerase is PDB identification is **rcsb PDB 1JY1** of the target protein. All water molecules were removed and on final stage hydrogen atoms were added to receptor molecule. Protein structure homology modeling was done using Swiss Model.

Preparation of Ligands

Review of Literature show that 1,2,3- triazolyl dihydropyrimidine-2-thione contains wide spectrum of activity. Hence it was decided to design a newer heterocyclic compound of series hybrid (5a-5e) containing 1,2,3-triazolyl dihydropyrimidine-2-thione.

The ligands were drawn in Chemsketch freeware assigned with proper 2D orientation and they are converted in to Three – Dimensional structure using CHEM DRAW. All the compounds from hybrid (5a-5e) were subjected to evaluate their compliance for Lipinski's rule of five.

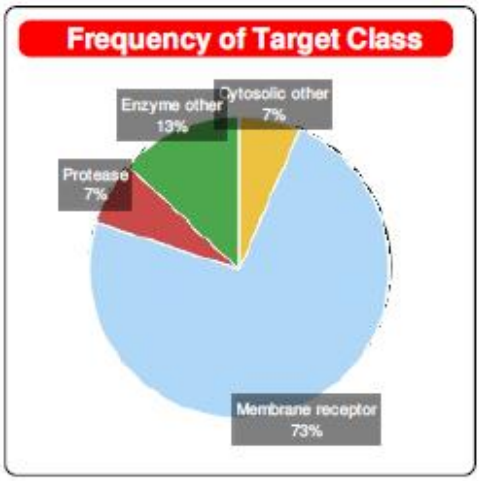
All the newly designed compounds were found in compliance with Lipinski's rule of five recommendations for new chemical entity to have good oral bioavailability with no violations. The miLogP value of all compounds were found below five, suggesting that the molecules have good permeability across the cell membrane which in turn is needed for generation of bioactivity.

Number of violations for all the compounds is zero; it means all newly designed compounds will easily bind to receptors. All the compounds hybrid (5a-5e) are within the limit, that is, 1600A in respect of Topological Polar Surface Area

(TPSA), which showed that molecules are fulfilling the optimal requirement for drug absorption. The values are tabulated in the (Table. 1 and 2) given below. Hence, all the newly designed heterocylic compounds which satisfy Lipinski's rule and drug likeness property has been taken as a lead for anti-cancer drug targeting protein kinase receptor.

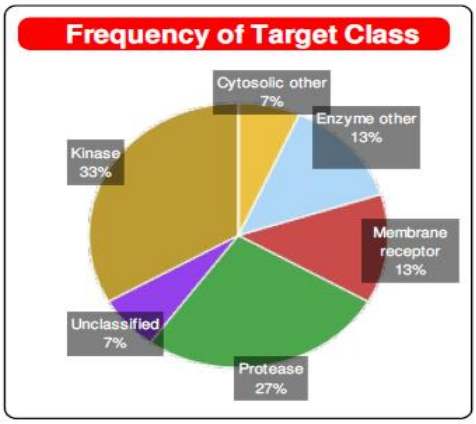
Energy of the molecules was minimized using Dundee PRODRG2 server. The energy minimized compounds were then read as input for AutoDock 4.0, in order to carry out the docking simulation. **(G.Krishnamoorthy et.al 2017)**

Swiss target prediction report of compound 5a



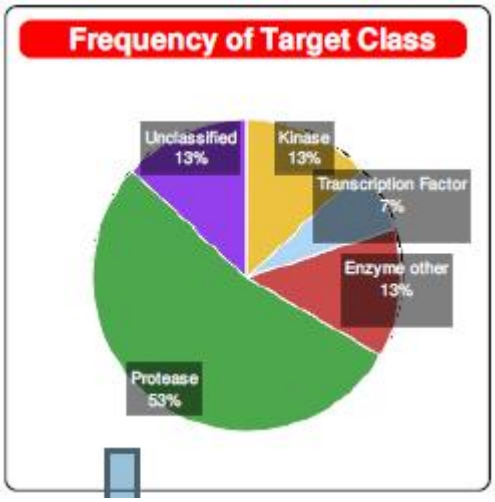
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Kinesin-like protein KIF11	P52732	KIF11	CHEMBL4581		3 / 5	Cytosolic other
5-hydroxytryptamine receptor 6	P50406	HTR6	CHEMBL3371		19 / 0	Membrane receptor
Cathepsin B	P07858	CTSB	CHEMBL4072		3 / 0	Cysteine Protease
Adenosine receptor A1 *(by homology)*	P30542	ADORA1	CHEMBL226		116 / 0	Membrane receptor
Metabotropic glutamate receptor 2	Q14416	GRM2	CHEMBL5137		21 / 0	Membrane receptor
Metabotropic glutamate receptor 4 *(by homology)*	Q14833	GRM4	CHEMBL2736		21 / 0	Membrane receptor
Metabotropic glutamate receptor 8 *(by homology)*	O00222	GRM8	CHEMBL3228		21 / 0	Membrane receptor
Metabotropic glutamate receptor 6 *(by homology)*	O15303	GRM6	CHEMBL4573		21 / 0	Membrane receptor
Metabotropic glutamate receptor 7 *(by homology)*	Q14831	GRM7	CHEMBL3777		21 / 0	Membrane receptor
Metabotropic glutamate receptor 3 *(by homology)*	Q14832	GRM3	CHEMBL2888		21 / 0	Membrane receptor
Adenosine receptor A2a	P29274	ADORA2A	CHEMBL251		46 / 0	Membrane receptor
Adenosine receptor A2b *(by homology)*	P29275	ADORA2B	CHEMBL255		42 / 0	Membrane receptor
Adenosine receptor A3	P33765	ADORA3	CHEMBL256		58 / 0	Membrane receptor
Sterol O-acyltransferase 1	P35610	SOAT1	CHEMBL2782		43 / 0	Enzyme
Sterol O-acyltransferase 2 *(by homology)*	O75908	SOAT2	CHEMBL4465		43 / 0	Enzyme

Swiss target prediction report of compound 5b



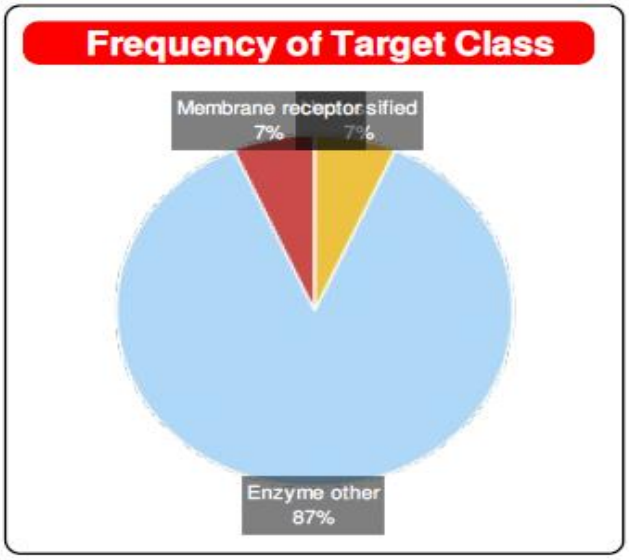
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Kinesin-like protein KIF11	P52732	KIF11	CHEMBL4581		3 / 5	Cytosolic other
Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845	HSD11B1	CHEMBL4235		16 / 0	Enzyme
Hydroxysteroid 11-beta-dehydrogenase 1-like protein *(by homology)*	Q7Z5J1	HSD11B1L			16 / 0	Enzyme
Corticotropin-releasing factor receptor 1	P34998	CRHR1	CHEMBL1800		47 / 0	Membrane receptor
Corticotropin-releasing factor receptor 2 *(by homology)*	Q13324	CRHR2	CHEMBL4069		46 / 0	Membrane receptor
Cathepsin L1 light chain	P07711	CTSL1	CHEMBL3837		10 / 0	Cysteine Protease
Cathepsin S *(by homology)*	P25774	CTSS	CHEMBL2954		10 / 0	Cysteine Protease
Cathepsin K *(by homology)*	P43235	CTSK	CHEMBL268		10 / 0	Cysteine Protease
Cathepsin L2 *(by homology)*	O60911	CTSL2	CHEMBL3272		10 / 0	Cysteine Protease
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		139 / 0	Unclassified
Epidermal growth factor receptor	P00533	EGFR	CHEMBL203		11 / 0	Tyr Kinase
Receptor tyrosine-protein kinase erbB-2 *(by homology)*	P04626	ERBB2	CHEMBL1824		11 / 0	Tyr Kinase
Vascular endothelial growth factor receptor 1	P17948	FLT1	CHEMBL1868		14 / 0	Tyr Kinase
Vascular endothelial growth factor receptor 3 *(by homology)*	P35916	FLT4	CHEMBL1955		14 / 0	Tyr Kinase
Vascular endothelial growth factor receptor 2 *(by homology)*	P35968	KDR	CHEMBL279		14 / 0	Tyr Kinase

Swiss target prediction report of compound 5c



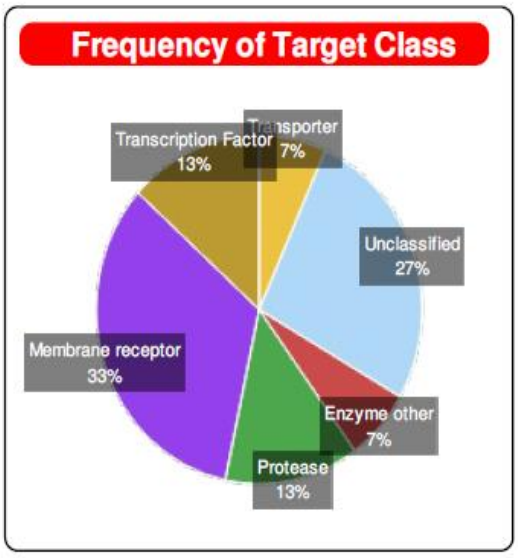
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Glycogen synthase kinase-3 beta	P49841	GSK3B	CHEMBL262		9 / 0	Ser_Thr Kinase
Glycogen synthase kinase-3 alpha *(by homology)*	P49840	GSK3A	CHEMBL2850		9 / 0	Ser_Thr Kinase
Androgen receptor	P10275	AR	CHEMBL1871		9 / 0	Transcription Factor
Hormone-sensitive lipase	Q05469	LIPE	CHEMBL3590		7 / 0	Enzyme
PEX	P08253	MMP2	CHEMBL333		4 / 0	Metallo Protease
67 kDa matrix metalloproteinase-9 *(by homology)*	P14780	MMP9	CHEMBL321		4 / 0	Metallo Protease
22 kDa interstitial collagenase *(by homology)*	P03956	MMP1	CHEMBL332		4 / 0	Metallo Protease
Collagenase 3	P45452	MMP13	CHEMBL280		4 / 0	Metallo Protease
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		92 / 0	Unclassified
Stromelysin-1 *(by homology)*	P08254	MMP3	CHEMBL283		4 / 0	Metallo Protease
Stromelysin-2 *(by homology)*	P09238	MMP10	CHEMBL4270		4 / 0	Metallo Protease
Macrophage metalloelastase *(by homology)*	P39900	MMP12	CHEMBL4393		4 / 0	Metallo Protease
Matrix metalloproteinase-27 *(by homology)*	Q9H306	MMP27			4 / 0	Metallo Protease
Matrix metalloproteinase-20 *(by homology)*	O60882	MMP20	CHEMBL1938226		4 / 0	Enzyme
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			92 / 0	Unclassified

Swiss target prediction report of compound 5d:



Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		941 / 1	Unclassified
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		169 / 1	Enzyme
Monoglyceride lipase *(by homology)*	Q99685	MGLL	CHEMBL4191		24 / 0	Enzyme
Fatty-acid amide hydrolase 1 *(by homology)*	O00519	FAAH	CHEMBL2243		78 / 0	Enzyme
Platelet-activating factor acetylhydrolase	Q13093	PLA2G7	CHEMBL3514		14 / 0	Enzyme
Platelet-activating factor acetylhydrolase 2, cytoplasmic *(by homology)*	Q99487	PAFAH2	CHEMBL4144		13 / 0	Enzyme
Arachidonate 5-lipoxygenase	P09917	ALOX5	CHEMBL215		72 / 0	Enzyme
Arachidonate 15-lipoxygenase *(by homology)*	P16050	ALOX15	CHEMBL2903		65 / 0	Enzyme
Arachidonate 12-lipoxygenase, 12S-type *(by homology)*	P18054	ALOX12	CHEMBL3687		65 / 0	Enzyme
Arachidonate 15-lipoxygenase B *(by homology)*	O15296	ALOX15B	CHEMBL2457		65 / 0	Enzyme
Arachidonate 12-lipoxygenase, 12R-type *(by homology)*	O75342	ALOX12B			64 / 0	Enzyme
Epidermis-type lipoxygenase 3 *(by homology)*	Q9BYJ1	ALOXE3			65 / 0	Enzyme
Cholinesterase	P06276	BCHE	CHEMBL1914		64 / 0	Enzyme
Acetylcholinesterase	P22303	ACHE	CHEMBL220		64 / 0	Enzyme
Endothelin B receptor *(by homology)*	P24530	EDNRB	CHEMBL1785		246 / 0	Membrane receptor

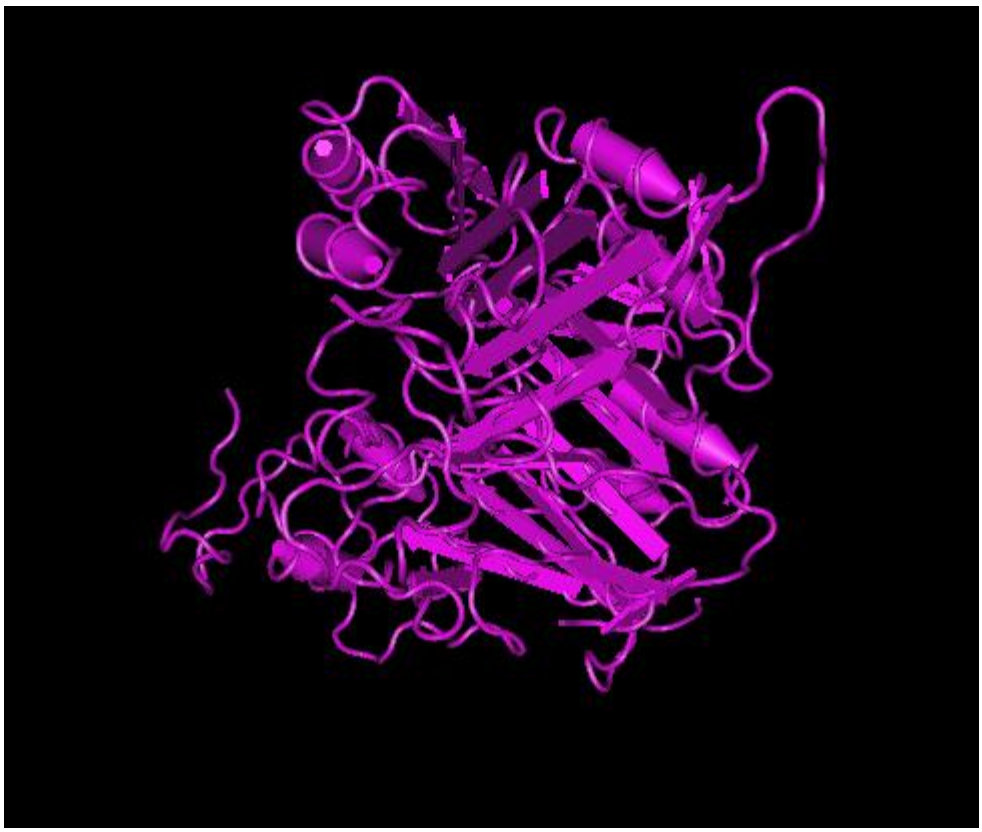
Swiss target prediction report of compound 5e



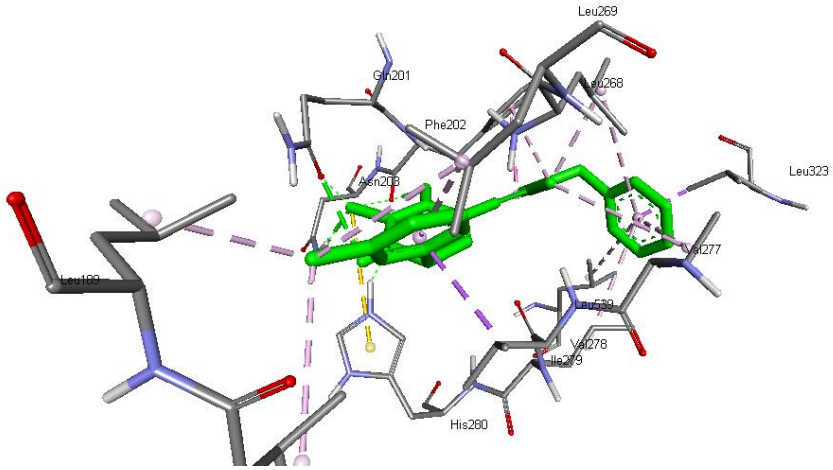
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Multidrug resistance protein 1	P08183	ABCB1	CHEMBL4302		5 / 1	Transporter
Bile salt export pump *(by homology)*	O95342	ABCB11	CHEMBL6020		5 / 1	Unclassified
Multidrug resistance protein 3 *(by homology)*	P21439	ABCB4	CHEMBL1743129		5 / 1	Enzyme
ATP-binding cassette sub-family B member 5 *(by homology)*	Q2M3G0	ABCB5	CHEMBL1772928		5 / 1	Unclassified
Endothelin-converting enzyme 1	P42892	ECE1	CHEMBL4791		8 / 0	Metallo Protease
Endothelin-converting enzyme 2 *(by homology)*	O60344	ECE2	CHEMBL5890		8 / 0	Metallo Protease
Muscarinic acetylcholine receptor M4 *(by homology)*	P08173	CHRM4	CHEMBL1821		107 / 0	Membrane receptor
Muscarinic acetylcholine receptor M5 *(by homology)*	P08912	CHRM5	CHEMBL2035		106 / 0	Membrane receptor
Muscarinic acetylcholine receptor M1 *(by homology)*	P11229	CHRM1	CHEMBL216		121 / 0	Membrane receptor
Muscarinic acetylcholine receptor M3	P20309	CHRM3	CHEMBL245		107 / 0	Membrane receptor
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		421 / 0	Unclassified
Translocator protein *(by homology)*	P30536	TSPO	CHEMBL5742		145 / 0	Unclassified
Glucocorticoid receptor	P04150	NR3C1	CHEMBL2034		143 / 0	Transcription Factor
Mineralocorticoid receptor *(by homology)*	P08235	NR3C2	CHEMBL1994		143 / 0	Transcription Factor
Muscarinic acetylcholine receptor M2	P08172	CHRM2	CHEMBL211		70 / 0	Membrane receptor

3-DIMENSIONAL STRUCTURE OF DNA-TOPOISOMERASE

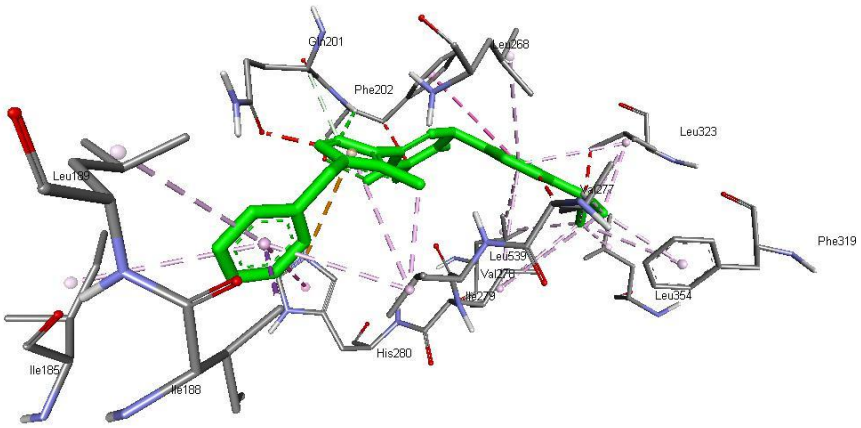
(Protein ID No: rcsb pdb 1JY 1)



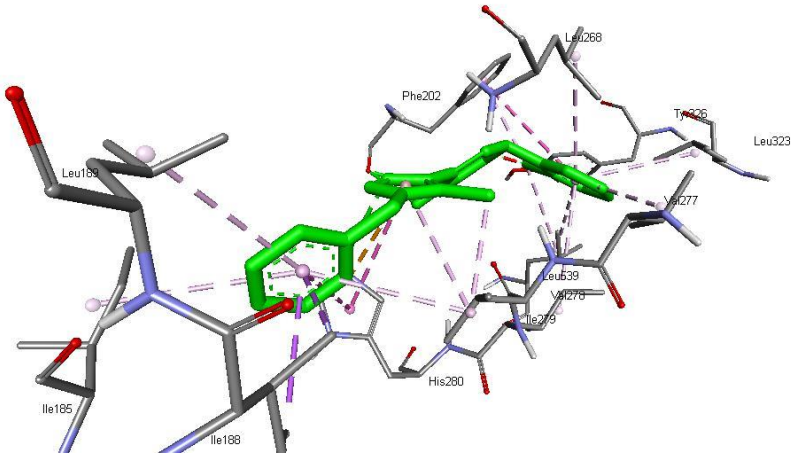
HYBRID-5a



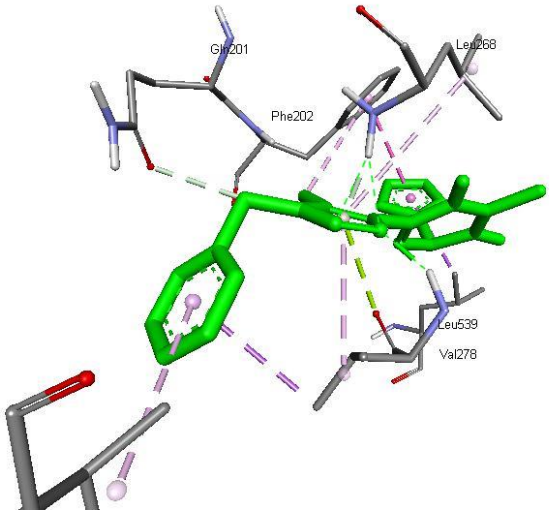
HYBRID-5b



HYBRID-5c



HYBRID-5d



HYBRID-5e

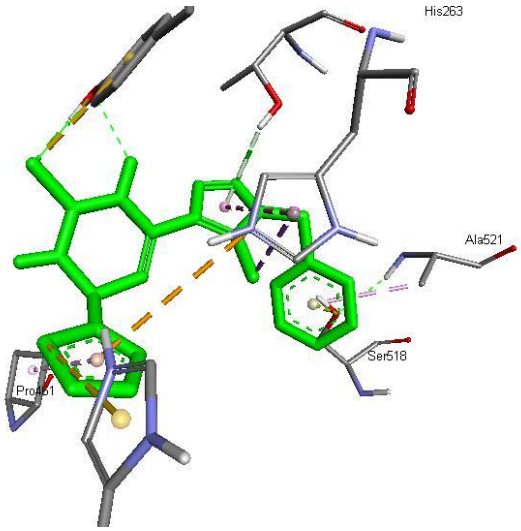


TABLE 1: CALCULATION OF BIOACTIVITY SCORE FOR NEWLY DESIGNED HETEROCYCLIC COMPOUNDS

COMPOUND	GPCR LIGAND	ION CHANNEL MODULATOR	KINASE INHIBITOR	NUCLEAR RECEPTOR LIGAND	PROTEASE INHIBITORS	ENZYME INHIBITORS
HYBRID-5a	-0.42	-0.43	0.66	-0.76	-0.66	-0.45
HYBRID-5b	-0.37	-0.36	-0.62	-0.65	-0.60	-0.38
HYBRID-5c	-0.42	-0.42	-0.74	-0.74	-0.73	-0.48
HYBRID-5d	-0.51	-0.62	-0.89	-0.90	-0.89	-0.53
HYBRID-5e	-0.51	-0.60	-0.81	-0.90	-0.78	-0.47

TABLE 2: CALCULATION OF PHYSIOCHEMICAL PROPERTIES FOR NEWLY DESIGNED HETEROCYCLIC COMPOUNDS

COMPOUND	miLog P	TPSA	MW	nON	nOHNH	nviola	nrot	Volu
HYBRID-5a	3.97	54.77	375.50	5	2	0	4	339.91
HYBRID-5b	5.06	54.77	403.56	5	2	1	5	373.30
HYBRID-5c	4.18	54.77	395.92	5	2	0	4	336.88
HYBRID-5d	2.80	67.91	351.44	6	2	0	4	304.92
HYBRID-5e	3.44	54.77	367.50	5	2	0	4	314.16

TABLE 3: POTENTIAL BINDING SITES OF THE COMPOUND

S.NO	COMPOUND	POTENTIAL BINDING SITES
1.	HYBRID-5a	Leu189, Gln201,Asn203, Phe202, His280, Leu269, Leu268, Leu323, Val277, Leu539, Val278, Ile279.
2.	HYBRID-5b	Ile185, Leu189, Ile188, Gln201, Phe202, His280, Leu268, Leu323, Phe319, Leu354, Val277, Leu539, Val278, Ile279.
3.	HYBRID-5c	Ile185, Leu189, Ile188, Phe202, Leu268, Tyr326, Leu323, Val277, Leu539, Val278, Ile279, His280.
4.	HYBRID-5d	Gln201, Phe202, Leu268, Leu539, Val278.
5	HYBRID-5e	Pro461, His263, Ala521, Ser518.

TABLE 4: BINDING ENERGIES OF THE COMPOUNDS:

S. No	Compound	Binding Energy (-Ve) (Kcal/Mol)
1.	HYBRID-5a	-2.87
2.	HYBRID-5b	+18.61
3.	HYBRID-5c	-2.87
4.	HYBRID-5d	+18.61
5.	HYBRID-5e	+18.61

TABLE 5: INHIBITION CONSTANT OF THE NEWLY DESIGNED COMPOUNDS

S.No	Parameters	Hybrid-5a	Hybrid-5b	Hybrid-5c	Hybrid-5d	Hybrid-5e
1.	Rank	1_1	1_1	1_1	1_1	1_1
2.	Binding Energy	-2.87	18.61	-2.87	18.61	18.61
3.	Inhibition Constant (Ki)	7.84mM	Unavailable	7.84mM	Unavailable	Unavailable
4.	Intermolecular Energy	-3.37	17.85	-3.37	17.85	17.85
5.	Internal Energy	-0.11	-0.43	-0.11	-0.43	-0.43
6.	Torsional Energy	0.55	0.82	0.55	0.82	0.82
7.	Unbound Extended Energy	-0.06	-0.36	-0.06	-0.36	-0.36
8.	Cluster RMS	0.0	0.0	0.0	0.0	0.0
9.	Ref RMS	64.52	79.9	64.52	79.9	79.9

In addition, two other parameters like inhibition constant (Ki) and intermolecular energy were also determined. As shown in **Table 5**, Compounds showed inhibition constant ranging from 7.84mM. The compound hybrids 5a and 5c (7.84mM) showed the lowest inhibition constant. Inhibition constant is directly proportional to binding energy. Thus, the DNA Topoisomerase inhibitory activity of the compounds was proved using molecular simulations. As shown in **Table 5**, the compounds hybrid 5a and 5c showed lesser intermolecular energy (-3.37kcal/mol). This result further indicates that compound hybrid 5a and 5c have better and stronger DNA Topoisomerase inhibitory activity.

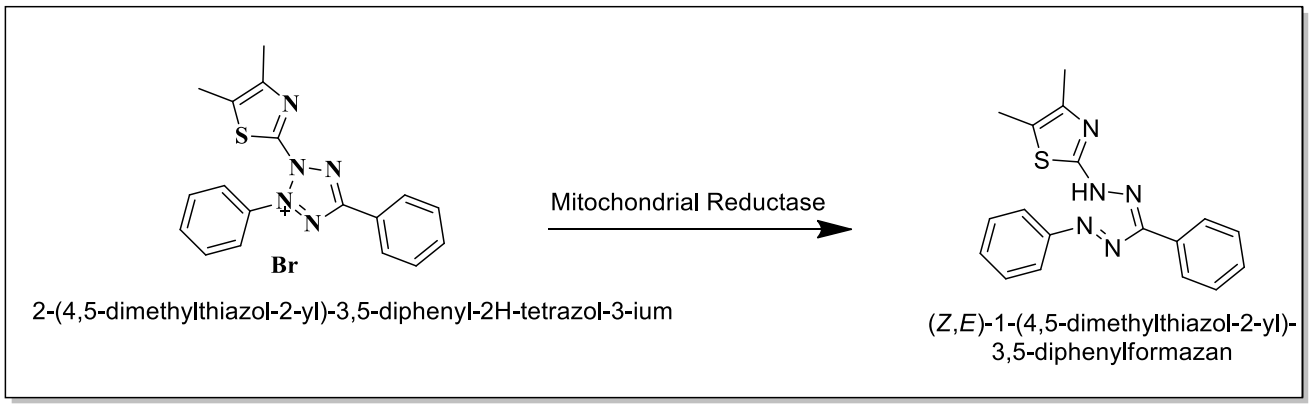
CHAPTER-VII(C)

IN-VITRO ANTI-CANCER ACTIVITY

EVALUATION OF *IN-VITRO* ANTI-CANCER ACTIVITY BY MTT ASSAY METHOD

PRINCIPLE:

MTT, a yellow tetrazole, is reduced to purple formazan in living cells. A solubilization solution usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid is added to dissolve the insoluble purple formazan product into a coloured solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent.



2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2H-tetrazol-3-ium

(*Z*,*E*)-1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan

Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxido reductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and other tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction. It is important to keep in mind that assay conditions can alter metabolic activity and thus tetrazolium dye reduction

without affecting cell viability. In addition, the mechanism of reduction of tetrazolium dyes, *i.e.* intracellular (MTT) *vs.* extracellular (WST-1), will also determine the amount of product

Kit Components

MTT Reagent - 25ml

Detergent reagent - 2 X 125ml

Procedure:

The MTT Reagent is ready to use and stable at 4°C in the dark for up to eighteen months, provided there is no contamination. Care should be taken not to contaminate the MTT Reagent with cell culture medium during pipetting. We recommend that the appropriate volume required for each experiment be removed and aseptically placed into a separate clean tube and the stock bottle returned to 4°C in the dark. .

The Detergent Reagent is supplied ready to use. If the Detergent Reagent has been stored at 4°C, warm the bottle for 5 minutes at 37°C then mix by inverting gently to avoid frothing. The detergent is stable for up to eighteen months at room temperature.

Equipment and Materials Required:

Microtiter plate reader with 650- and 570-nm Filters	Microtiter plate (flat-bottomed)
Inverted microscope	Sterile tubes (5 mL)
Multi-channel pipette	Serological pipettes
37°C incubator	Sterile pipette tips
Laminar flow hood	

BASIC PROTOCOL:

If you are familiar with the procedure and know the cell count to use in your specific assay, you may follow this basic protocol.

STEP	ACTION
1.	Plate cells at 1,000 to 100,000 per well.
2.	Incubate for 6 to 24 hours.
3.	Add 10 µL MTT Reagent.
4.	Incubate for 2 to 4 hours until purple precipitate is visible.
5.	Add 100 µL Detergent Reagent.
6.	Leave at room temperature in the dark for 2 hours.
7.	Record absorbance at 570 nm.

DETERMINING OPTIMAL CELL COUNTS

Use the protocol below to determine the optimal cell count and incubation period for your cell line. This determination should only have to be done once for each cell type. The data will be used thereafter in your experimental system following the protocol.

STEP	ACTION
1.	Harvest suspension cells by centrifugation. Adherent cells should be released from their substrate by trypsinization or scraping.
2.	Re-suspend cells at 1 x 106 per ml.
3.	Prepare serial dilutions of cells in culture medium from 1 x 106 to 1 x 103 cells per ml.
4.	Plate out, in triplicate, 100 µl of the dilutions into wells of a microtiter plate.
5	Include three control wells of medium alone to provide the blanks for absorbance readings.
6.	Incubate the cells under conditions appropriate for the cell line for 6 to 48 hours (to recover from handling). The time required will vary but 12 hours to overnight is sufficient for most cell types
7.	Add 10 µl of MTT Reagent to each well, including controls.
8.	Return plate to cell culture incubator for 2 to 4 hours.
9.	Periodically view the cells under an inverted microscope for presence of intracellular punctate purple precipitate

10.	When the purple precipitate is clearly visible under the microscope add 100 µl of Detergent Reagent to all wells, including controls. Swirl gently do not shake.
11.	Leave plate with cover in the dark for 2 to 4 hours or overnight at room temperature.
12.	Remove plate cover and measure the absorbance in each well, including the blanks, at 570 nm in a microtiter plate reader. [Absorbances can be read with any filter in the wavelength range of 550 - 600 nm. The reference wavelength should be higher than 650 nm. The blanks should give values close to zero (+/- 0.1).]
13.	If the readings are low return the plate to the dark for longer incubation.
14.	Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance against number of cells/mL. The number of cells to use in your assay should lie within the linear portion of the plot and yield an absorbance of 0.75 - 1.25.

DATA INTERPRETATION

The plot of the data obtained in Step 14 on page 3 (absorbance against number of cells) should provide a curve with a linear portion. The optimal number of cells for the assay should fall within the linear portion of the curve and give an absorbance value between 0.75 and 1.25. Then both stimulation and inhibition of cell proliferation can be measured.

To run an assay, select an optimal cell number and follow the MTT Cell Proliferation Assay steps 4 to 13 (page 3) using your experimental system, plating in triplicate. Assays will include:

a) Blank wells containing medium only

b) Untreated control cells

c) Test cells treated with the substance to be assayed

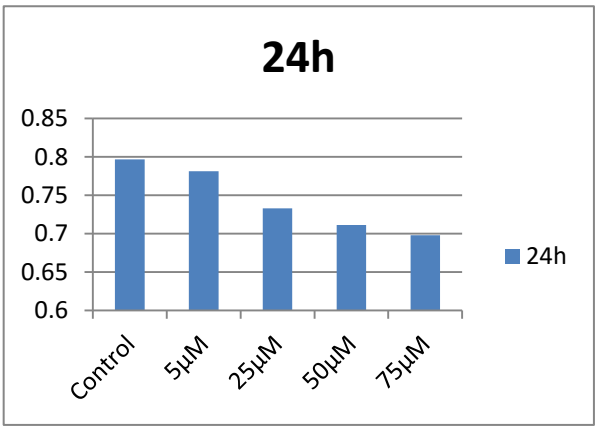
If more than 100 µl of medium is used per well, increase the amount of MTT Reagent accordingly;

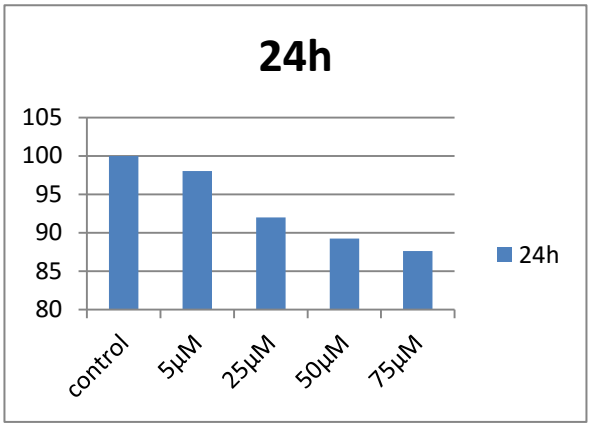
e.g., for 250 µl of medium use 25 µl of MTT Reagent.

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

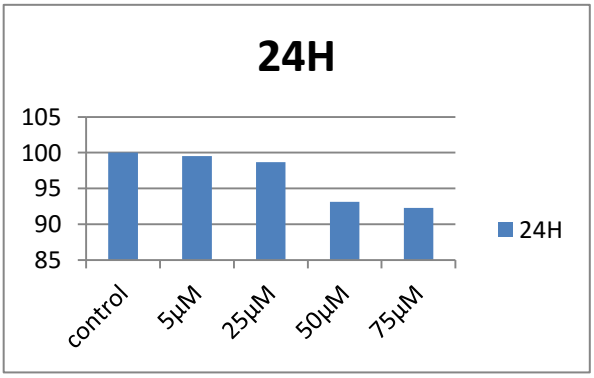
HOURS		AVERAGE VALUE				
	COMPOUND	VARIOUS CONCENTRATION (µg)				CONTROL
		5	25	50	75	
24	Hybrid 5a	0.781	0.733667	0.711	0.698333	0.796667
	PERCENTAGE	98.04	92.01	89.25	87.62	100
	Hybrid 5b	0.793333	0.786	0.742333	0.735	0.796667
	PERCENTAGE	99.55	98,67	93.14	92.26	100
	Hybrid 5c	0.789	0.7686	0.71667	0.722333	0.796667
	PERCENTAGE	99.04	96.40	89.88	90.63	100
	Hybrid 5d	0.762	0.756	0.722	0.609	0.796667
	PERCENTAGE	95.65	94.90	90.63	76.44	100
	Hybrid 5e	0.841667	0.839	0.817	0.783333	0.796667
	PERCENTAGE	105.57	105.32	102.56	98.29	100
48	Hybrid 5a	1.050667	0.973667	0.961333	0.954	1.112
	PERCENTAGE	94.42	87.5	86.42	85.79	100
	Hybrid 5b	1.112333	1.086	1.031667	1.003667	1.112
	PERCENTAGE	100.02	97.66	92.71	90.19	100
	Hybrid 5c	1.106667	1.089333	1.077667	1.051333	1.112
	PERCENTAGE	99.46	97.93	96.85	94.51	100
	Hybrid 5d	1.052	1.048	1.027667	1.023	1.112
	PERCENTAGE	94.60	94.24	93.35	91.99	100
	Hybrid 5e	1.177	1.124667	1.106333	1.086	1.112
	PERCENTAGE	105.84	101.07	99.46	97.66	100

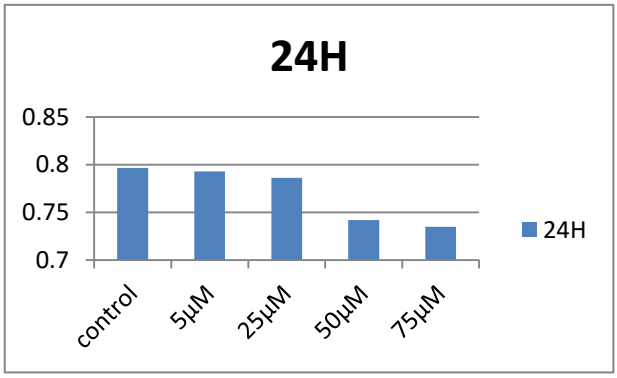
Compound 5a:



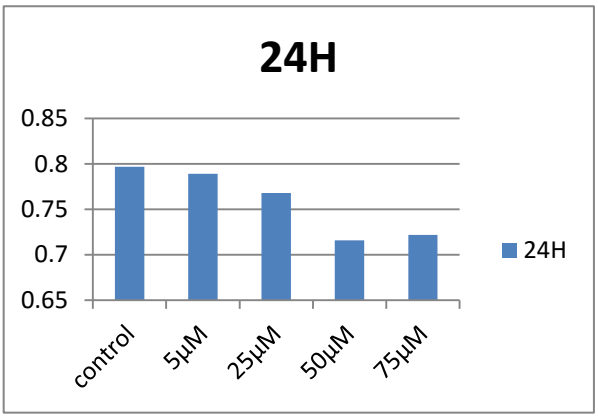


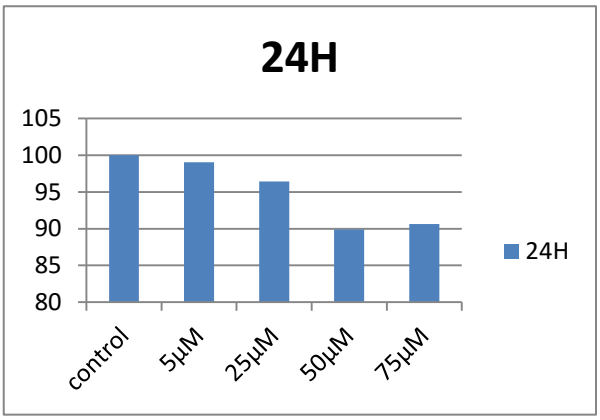
Compound 5b:



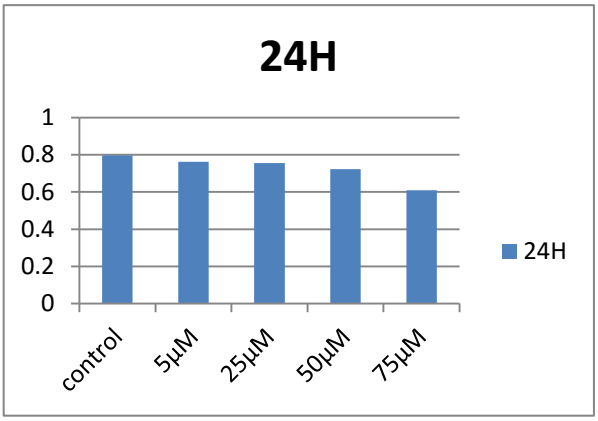


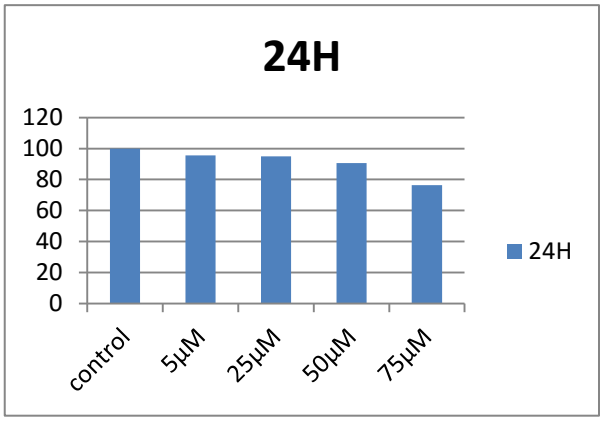
Compound 5c:



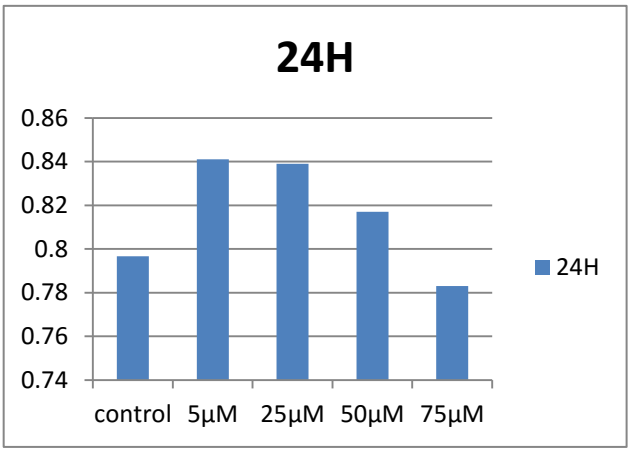


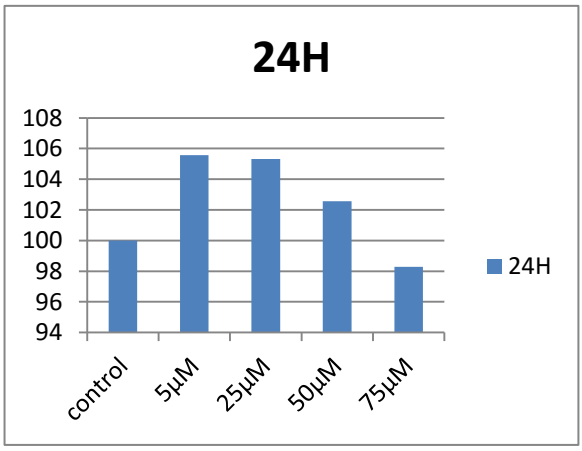
Compound 5d:



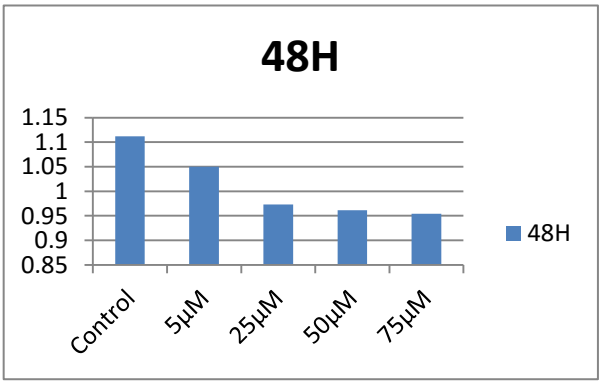


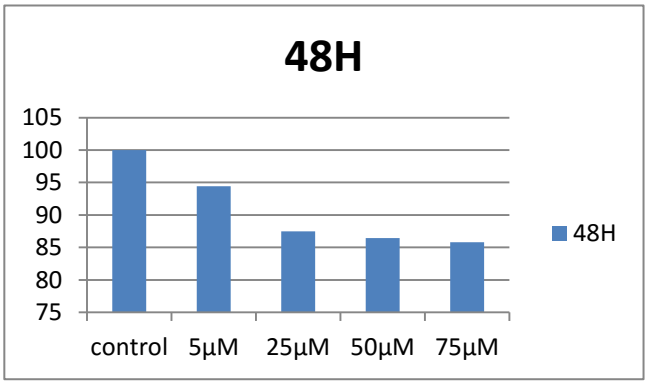
Compound 5e:



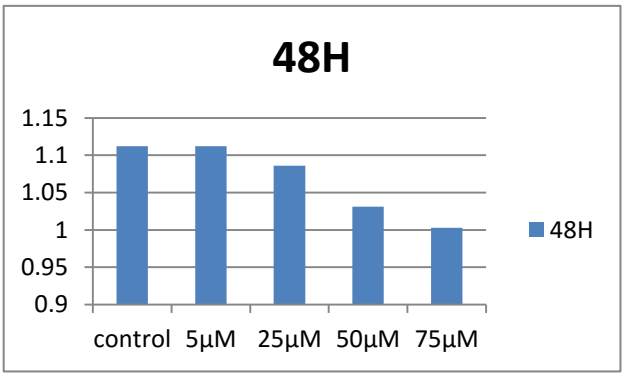


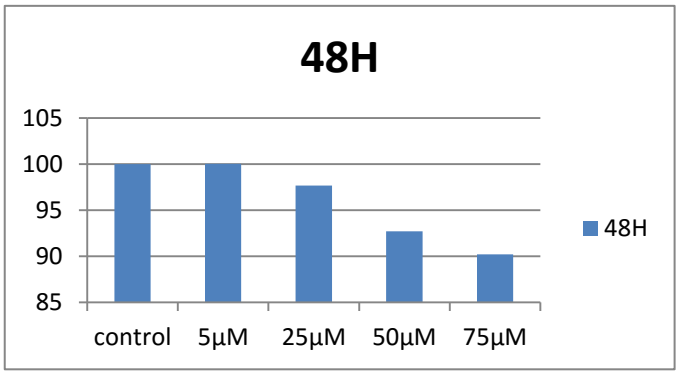
Compound 5a:



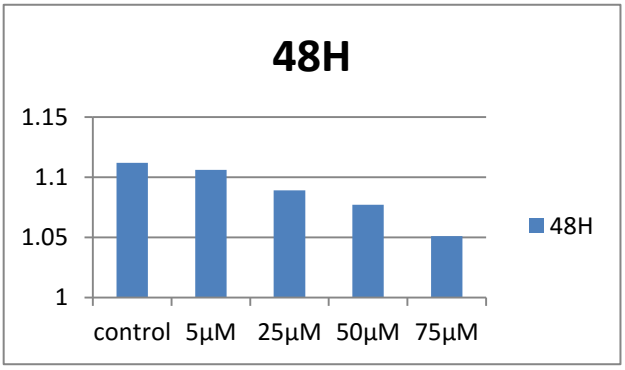


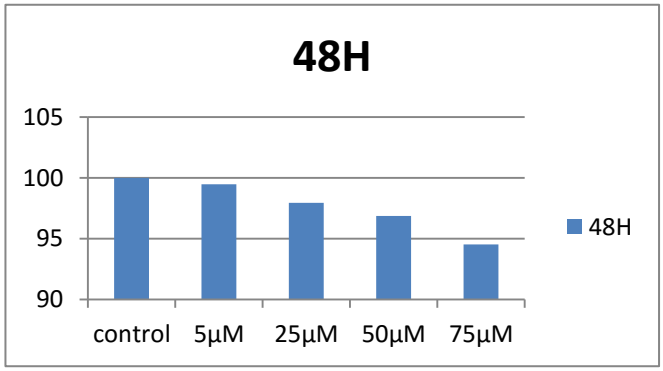
Compound 5b:



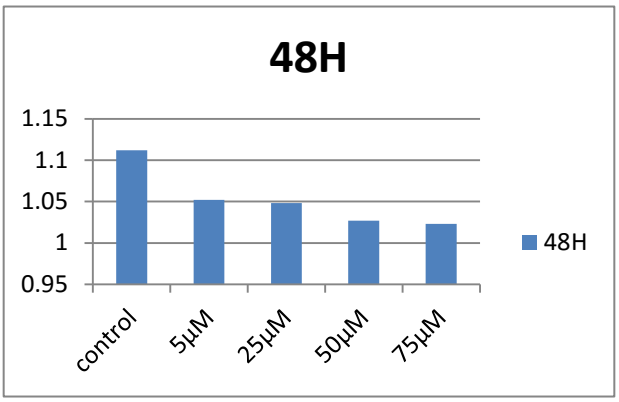


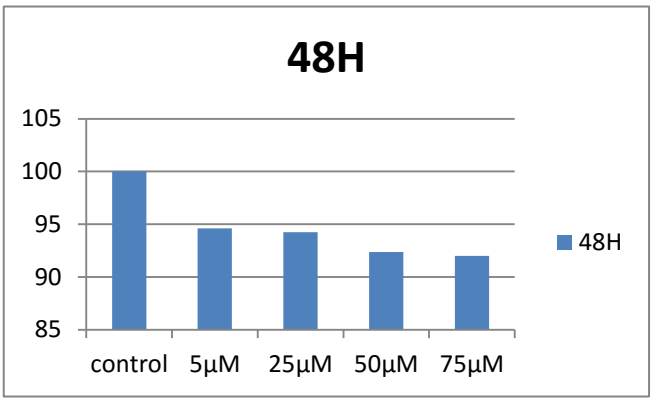
Compound 5c:



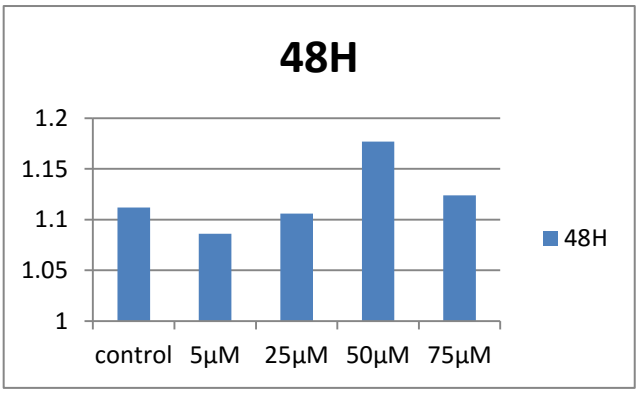


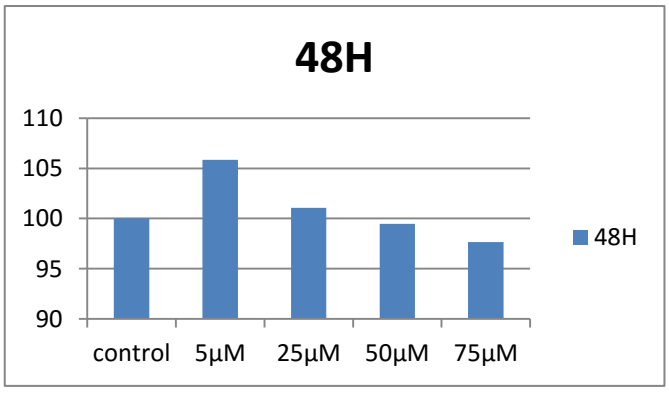
Compound 5d:





Compound 5e:



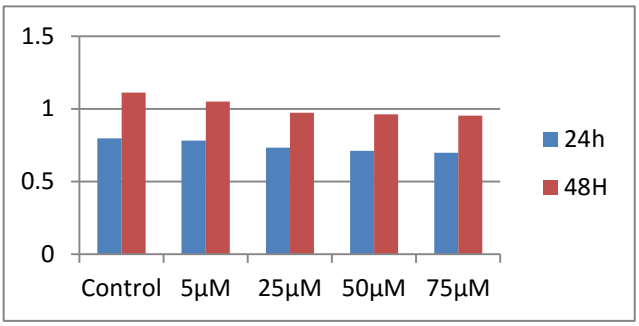


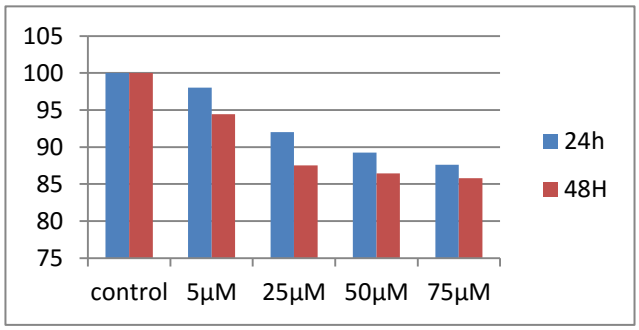
RESULT:

COMPARISION OF CONTROL WITH COMPOUNDS

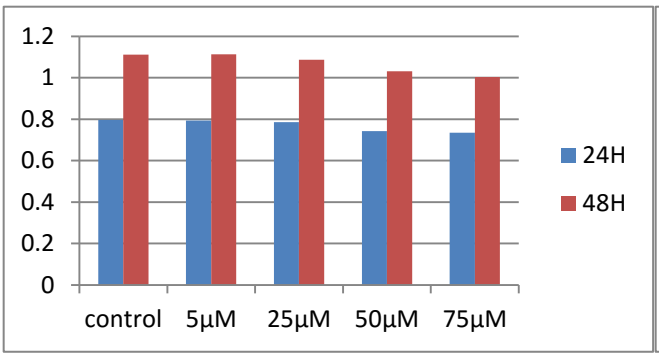
PERCENTAGE OF CELL VIABILITY

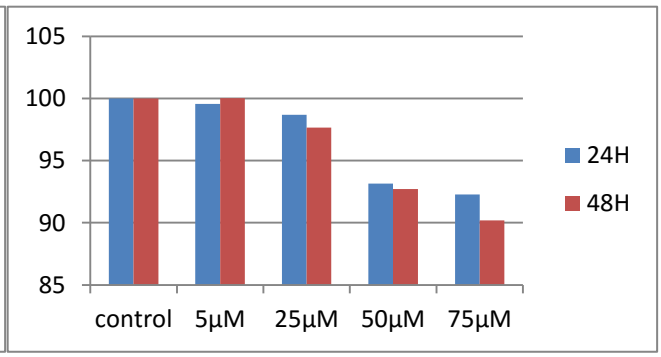
Compound 5a:



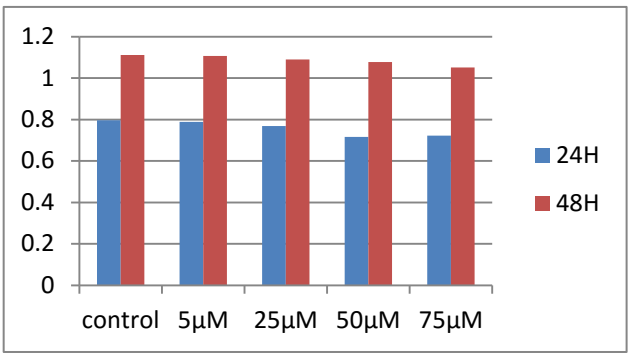


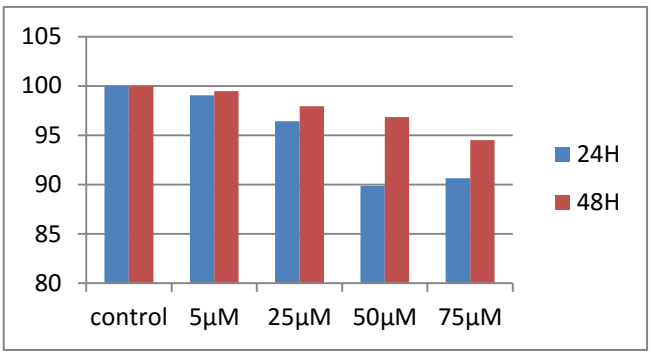
Compound 5b:



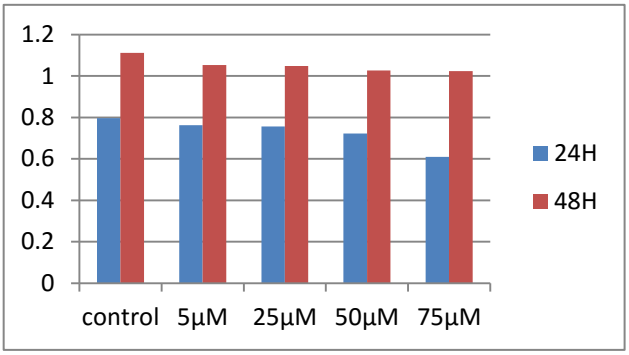


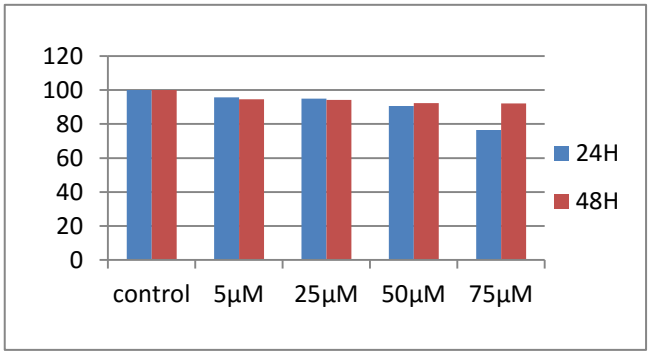
Compound 5c:



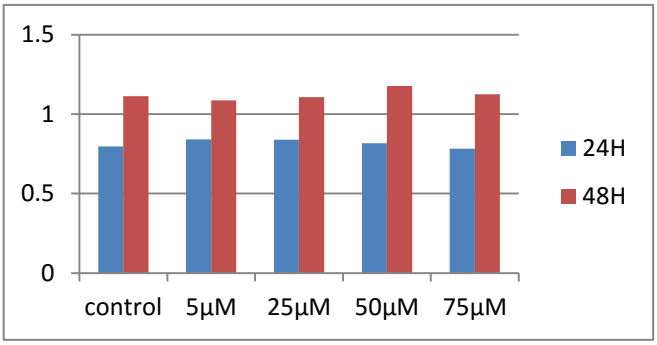


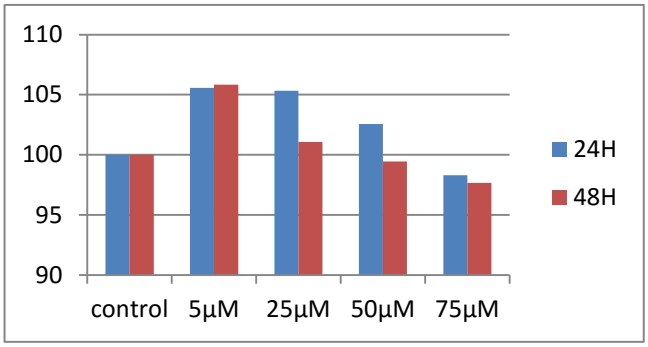
Compound 5d:





Compound 5e:


1.5
1
0.5
0
control
5µM
25µM
50µM
75µM
24H
48H


110
105
100
95
90
control
5µM
25µM
50µM
75µM
24H
48H

CHAPTER-VIII

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present work deals with the green synthesis, characterization, molecular docking and anti-cancer activity of new series of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids.

The study comprises of:

Designed the lead molecule of and 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids assessed ADMET property.

Synthesis of the title compounds by appropriate methods.

Determination of physical properties such as solubility, melting point and TLC.

Characterise the structures of synthesized compounds by IR, ^{1}HNMR and Mass spectra.

Evaluation of the synthesized compounds for their in-vitro anti-cancer activity.

- The compounds were synthesized by reaction between 1,2,3-triazolyl chalcone and aromatic aldehydes in the presence of 50% aqueous sodium hydroxide grinding 4-7 minutes. Then the above product treated with thiourea 10% potassium hydroxide and water reflux to form 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids.
- The method had been optimized to synthesis for the synthetic compounds.
- The compounds were synthesized by appropriate method.
- The synthesized compounds were purified by TLC.
- The synthesized compounds were confirmed by FT-IR, ^{1}H NMR,MASS Spectroscopy.
- The IR data showed relevant bands for the functional groups present in the synthesized compounds.
- The ^{1}H NMR also showed relevant proton peaks for all the synthesized compounds.

- The molecular weights of the compounds were done by MASS Spectrocopy.
- All spectral data coincides with the assigned structure of synthesized compounds.
- The synthesized compounds are analyzed with various steps of molecular docking studies and finally concluded for the activity.
- The synthesized compounds were screened for in-vitro anti-cancer activity.

In-vitro anti-cancer activity:

All the synthesized compounds exposed anti-cancer activity by MTT assay method.

The synthesized compounds **HYBRID-5a, HYBRID-5c** are show good anti-cancer activity. **HYBRID-5b, HYBRID-5d, HYBRID-5e,** shown very poor anti-cancer activity.

CHAPTER-IX

REFERENCES

REFERENCES

1. **Ajmal Khan a, b, Jamshed Hashim a, Nuzhat Arshad a,c, Ijaz Khan a, Naureen Siddiqui a, Abdul Wadood d Muzaffar Ali a, Fiza Arshad a, Khalid Mohammed Khan a, M. Iqbal Choudhary a,** Four series of heterocyclic compounds 4-dihydropyrimidine-2-thiones 7–12 (series A), N,S-dimethyldihydropyrimidines 13–18 (series B), hydrazine derivatives of dihydropyrimidine 19–24 (series C), and tetrazolo dihydropyrimidine derivatives 25–30 (series D), were synthesized and evaluated for in vitro urease inhibitory activity. The series B–D were first time examined for urease inhibition. **Bioorganic Chemistry 64 (2016) 85–96**

2. **Rajasekhar R. Alavala 1, Umasankar Kulandaivelu 1, Pochaiah Bonagiri 1, Shireesha Boyapati 2*, Venkatesan Jayaprakash 3 and Ananda T. Subramaniam 4**, Dihydropyrimidinone is considered as a pharmacophoric skeleton in many drug discovery programmes. We have synthesized a wide range of dihydropyrimidinone analogues using microwave energy in the present investigation. Regioselectivity in these structures is achieved by the influence of weak base like potassium carbonate and synthesized some novel dihydropyrimidine N- and O-Mannich bases of ciprofloxacin (**3a-j**, **4a-g**). All the synthesized molecules have been evaluated for their antiviral activity against hepatitis C, hepatitis B, vaccinia, cowpox, severe acute respiratory syndrome, West Nile, dengue, Venezuelan equine encephalitis viruses, Flu A (H1N1, H3N2 and H5N1) and Flu B viral strains as well as for their cytotoxicity using standard protocols. ***Anti-Infective Agents,* 2015, *researchgate***

3. **Sugunadevi Sakkiah, Sundarapandian Thangapandian 1, Shalini John 1, Keun Woo Lee***, Pharmacophore based virtual screening, molecular docking studies to design potent heat shock protein 90 inhibitors, **European Journal of Medicinal Chemistry 46 (2011) 2937-2947**

4. **TODD D. SHENKENBERG, M.D.; and DANIEL D. VON HOFF, M.D.; San Antonio, Texas** Mitoxantrone: A New Anticancer Drug with Significant Clinical Activity, **Downloaded From: http://annals.org/ by a Penn State University Hershey User on 02/04/2015**

5. **David M. Smith,**1 **Kenyon G. Daniel,**1 **Zhigang Wang,**2 **Wayne C. Guida,**1,3 **Tak-Hang Chan,**2 **and Q. Ping Dou**1* , Docking Studies and Model Development of Tea Polyphenol Proteasome Inhibitors: Applications to Rational Drug Design, **PROTEINS: Structure, Function, and Bioinformatics 54:58–70 (2004)**

6. **Umer Rashid , Syed Fahad Hassan , Samina Nazir , Abdul Wadood , Muhammad Waseem , Farzana Latif Ansari,** Synthesis, docking studies, and in silico ADMET predictions of some new derivatives of pyrimidine as potential KSP inhibitors, **Medicinal chemistry research 2014**

7. **Tingjun Hou, Lili Zhu, Lirong Chen, and Xiaojie Xu*** College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China., Mapping the Binding Site of a Large Set of Quinazoline Type EGF-R Inhibitors Using Molecular Field Analyses and Molecular Docking Studies, ***J. Chem. Inf. Comput. Sci., Vol. 43, No. 1, 2003***

8. **A.H.Buckett.,practical (2007).Pharmaceutical chemistry,**fourth edition

9. **Yeh-Long Chen,* Kuo-Chang Fang, Jia-Yuh Sheu, Shu-Lin Hsu, and Cherng-Chyi Tzeng,** *School of Chemistry, Kaohsiung Medical University, Kaohsiung City 807, Taiwan, Republic of China, and Department of Environmental Engineering & Health, Tajen Institute of Technology, Pingtong 907, Taiwan, Republic of China Received April 3, 2001* Synthesis and Antibacterial Evaluation of Certain Quinolone Derivatives, ***J. Med. Chem.* 2001, *44,* 2374-2377**

10. Junhua Liu,a Min Lei*b and Lihong Hu*a,b Thiamine hydrochloride (VB1): an efficient promoter for the one-pot synthesis of benzo[4,5]imidazo[1,2-a]pyrimidine and [1,2,4]triazolo[1,5-a] pyrimidine derivatives in water medium **Received 23rd November 2011, Accepted 11th January 2012** www.rsc.org/greenchem

11. **Ben-Tao Yin, Cong-Yan Yan, Xin-Mei Peng, Shao-Lin Zhang, Syed Rasheed Rong-Xia Geng*, Cheng-He Zhou*** Synthesis and biological evaluation of a-triazolyl chalcones as a new type of potential antimicrobial agents and their interaction with calf thymus DNA and human serum albumin **European Journal of Medicinal Chemistry 2013**

12. **Aggeliki Politi a, Serdar Durdagib, Panagiota Moutevelis-Minakakisa, George Kokotosa, Thomas Mavromoustakosa,*** Development of accurate binding affinity predictions of novel renin inhibitors through molecular docking studies. **Journal of Molecular Graphics and Modelling 2010**

13. **Sangaraiah Nagarajan a, Poovan Shanmugavelan a, Murugan Sathishkumar a, Ramasamy Selvi a, Alagusundaram Ponnuswamy a,, Hariharan Harikrishnan b, Vellasamy Shanmugaiah b, Saminathan Murugavel,** An eco-friendly and water mediated product selective synthesis of 2-aminopyrimidines and their in vitro anti-bacterial evaluation. **Bioorganic & Medicinal Chemistry Letters2014**

14. **Catriona G. Mortimer, Geoffrey Wells, Jean-Philippe Crochard, Erica L. Stone, Tracey D. Bradshaw, Malcolm F. G. Stevens, and Andrew D. Westwell*** ***J.*** Antitumor Benzothiazoles. 26.1 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a Simple Fluorinated 2-Arylbenzothiazole, Shows Potent and Selective Inhibitory Activity against

Lung, Colon, and Breast Cancer Cell Lines ***Med. Chem.*** **2006, *49,* 179-185 2005**

15. **Nilkanth G. Aher a, Vandana S. Pore a,*, Nripendra N. Mishra b, Awanit Kumar b, Praveen K. Shukla b, Aanchal Sharma c, Manoj K. Bhat c** Synthesis and antifungal activity of 1,2,3-triazole containing fluconazole analogues, **Bioorganic & Medicinal Chemistry Letters 2008**

16. **Seerat Fatima a, Anindra Sharma a, Reshu Saxena b, Rajkamal Tripathi b, Sanjeev K. Shukla c, Swaroop Kumar Pandey d, Renu Tripathi d, Rama P. Tripathi a,*** One pot efficient diversity oriented synthesis of polyfunctional styryl thiazolopyrimidines and their bio-evaluation as antimalarial and anti-HIV agents, **European Journal of Medicinal Chemistry 2012**

17. **Rino Ragno a,*, Silvia Simeoni a, Dante Rotili a, Antonella Caroli a, Giorgia Botta a, Gerald Brosch b, Silvio Massa c, Antonello Mai a,**** Class II-selective histone deacetylase inhibitors. Part 2: Alignment-independent GRIND 3-D QSAR, homology and docking studies, **European Journal of Medicinal Chemistry 43 (2008) 621-632**

18. **Luciana Marinelli, Antonio Lavecchia, Kay-E. Gottschalk, Ettore Novellino, and Horst Kessler*, *J.*** Docking Studies on rvâ3 Integrin Ligands: Pharmacophore Refinement and Implications for Drug Design, ***Med. Chem.*** **2003, *46,* 4393-4404**

19. **Clarisse G. Ricci and Paulo A. Netz* *J.*** Docking Studies on DNA-Ligand Interactions: Building and Application of a Protocol To Identify the Binding Mode**. *Chem. Inf. Model.* 2009, *49,* 1925–1935**

20. **Burger'sMedicinalchemistry.vol-I.**

21. **Wenying Ren, Zhenhua Qiao, Hongwei Wang, Lei Zhu, Li Zhang** Flavonoids: Promising Anticancer Agents, **Medicinal Research Reviews, Vol. 23, No. 4, 519-534, 2003**

22. **Mahmud Tareq Hassan Khana,∗, I. Orhanb, F.S. S¸ enolb, M. Kartalc, B. S¸ enerb, M. Dvorskad, K. ˇSmejkald, T.** Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies **ˇSlapetovad Chemico-Biological Interactions 181 (2009) 383–389**

23. **Kurdeep chatwal**,Instrumental methods of analysis.

24. **Neeraj Mahindroo, Chiung-Chiu Wang, Chun-Chen Liao, Chien-Fu Huang, I-Lin Lu,, Tzu-Wen Lien, Yi-Huei Peng, Wei-Jan Huang, Ying-Ting Lin, Ming-Chen Hsu, Chia-Hui Lin, Chia-Hua Tsai, John T.-A. Hsu, Xin Chen, Ping-Chiang Lyu, Yu-Sheng Chao, Su-Ying Wu,* and Hsing-Pang Hsieh*,** Indol-1-yl Acetic Acids as Peroxisome Proliferator-Activated

Receptor Agonists: Design, Synthesis, Structural Biology, and Molecular Docking Studies **B*J.Med. Chem.* 2006, *49,* 1212-1216**

25. **Francesco Caruso,*, Miriam Rossi,* Aidan Benson, Cristian Opazo, Daniel Freedman,‖ Elena Monti,⊥ Marzia Bruna Gariboldi, Jodi Shaulky, Fabio Marchetti, Riccardo Pettinari, and Claudio Pettinari,** Ruthenium−Arene Complexes of Curcumin: X-Ray and Density Functional Theory Structure, Synthesis, and Spectroscopic Characterization, in Vitro Antitumor Activity, and DNA Docking Studies of (p-Cymene) Ru (curcuminato) chloro, **Journal of medicinal chemistry 2011**

26. **Catherine Michaux, Paulette Charlier, Jean-Marie Fre`re, and Johan Wouters*,** Crystal Structure of BRL 42715, C6-(N1-Methyl-1,2,3-triazolylmethylene) penem, in Complex with Enterobacter cloacae 908R *â*-Lactamase: Evidence for a Stereoselective Mechanism from Docking Studies **JACS COMMUNICATIONS 2004**

27. **Paresh Zalavadiya, Satish Tala, Jignesh Akbari, and Hitendra Joshi Arch** Multi-Component Synthesis of Dihydropyrimidines by Iodine Catalyst at Ambient Temperature and in-vitro Antimycobacterial Activity,. **Pharm. Chem. Life Sci. 2009, 342, 469 – 475**

28. **R.B.Silverman** Textbook of Medicinal Chemistry.

29. **Francisco Alonso,a* Yanina Moglie,a Gabriel Radivoy,b and Miguel Yusa*** Multicomponent Synthesis of 1,2,3-Triazoles in Water Catalyzed by Copper Nanoparticles on Activated Carbon

30. **Manmohan Chhibber,a Gyanendra Kumar,a Prasanna Parasuraman,a T. N. C. Ramya,a Namita Suroliab and Avadhesha Suroliaa,c,*** Novel diphenyl ethers: Design, docking studies, synthesis and inhibition of enoyl ACP reductase of Plasmodium falciparum and Escherichia coli **Bioorganic & Medicinal Chemistry 14 (2006) 8086–8098**

31. **Maria-Grazia Mendoza-Ferri, Christian G. Hartinger,* Rene E. Eichinger, Natalya Stolyarova, Kay Severin, Michael A. Jakupec, Alexey A. Nazarov,*** and Bernhard K. Keppler† Influence of the Spacer Length on the *in Vitro* Anticancer Activity of Dinuclear Ruthenium-Arene Compounds ***Organometallics* 2008, *27,* 2405–2407**

32. **Christiane Schultz, Andreas Link, Maryse Leost, Daniel W. Zaharevitz, Rick Gussio, Edward A. Sausville, Laurent Meijer, and Conrad Kunick*, Paullones,** a Series of Cyclin-Dependent Kinase Inhibitors: Synthesis, Evaluation of CDK1/Cyclin B Inhibition, and in Vitro Antitumor Activity**, *J. Med. Chem.* 1999, *42,* 2909-2919**

33. **Y.R.Sharma,Elementary organic spectroscopy.** Revised edition 2013.S.Chand and company private.ltd

34. **Srinivasa Rao Jada a, Genevieve Suseno Subur b, Charlie Matthews c, Ahmad Sazali Hamzah d, Nordin Haji Lajis e, Mohammad Said Saad f, Malcolm F.G. Stevens c, Johnson Stanslas a,e,***In vitro anticancer activities of andrographolide analogues **Phytochemistry 68 (2007) 904–912**

35. **Doris Dallinger, Alexander Stadler, and C. Oliver Kappe *Institute of Chemistry, Karl-Franzens-University Graz,*** Solid- and solution-phase synthesis of bioactive dihydropyrimidines* ***Austria Pure Appl. Chem.*, Vol. 76, No. 5, pp. 1017–1024, 2004. © 2004 IUPAC 1017**

36. **Meena Kumari a, Jitendra Kumar Maurya a, Upendra Kumar Singh a, Abbul Bashar Khan a, Maroof Ali b, Prashant Singh c, Rajan Patel a,** Spectroscopic and docking studies on the interaction between pyrrolidinium based ionic liquid and bovine serum albumin, **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 124 (2014) 349–356**

37. **Sonika Charaka, Manish Shandilyab, Gunjan Tyagia, Ranjana Mehrotraa,∗,** Spectroscopic and molecular docking studies on chlorambucil interaction with DNA, **International Journal of Biological Macromolecules 2012**

38. **Meena Kumari a, Jitendra Kumar Maurya a, Upendra Kumar Singh a, Abbul Bashar Khan a, Maroof Ali b, Prashant Singh c, Rajan Patel a,** Spectroscopic and docking studies on the interaction between pyrrolidinium based ionic liquid and bovine serum albumin, **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2014**

39. **Principles of Medicinal Chemistry** by **William Foye, 7th edition, ippincott williams & Wilkins, woltes Kluwer (India) Pvt.Ltd, New Delhi.**

40. **Dmytro Havrylyuk a, Ludmyla Mosula a, Borys Zimenkovsky a, Olexandr Vasylenko c, Andrzej Gzella b, Roman Lesyk a,*** Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety **European Journal of Medicinal Chemistry2010**

41. **Ahmed Kamal a,*, S. Prabhakar a, M. Janaki Ramaiah b, P. Venkat Reddy a, Ch. Ratna Reddy a, A. Mallareddy a, Nagula Shankaraiah c, T. Lakshmi Narayan Reddy b, S.N.C.V.L. Pushpavalli b, Manika Pal-Bhadra,** Synthesis and anticancer activity of chalcone-pyrrolobenzodiazepine conjugates linked via 1,2,3-triazole ring side-armed with alkane spacers, **European Journal of Medicinal Chemistry 2011**

42. **Babasaheb P. Bandgar a,⇑, Laxman K. Adsul a, Hemant V. Chavan a, Shivkumar S. Jalde a, Sadanand N. Shringare a, Rafique Shaikh b, Rohan J. Meshram b, Rajesh N. Gacche b, Vijay Masand**, Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1H-2-pyrazolines as potent anti-inflammatory and antioxidant agents, **Bioorganic & Medicinal Chemistry Letters 2012**

43. **Nelilma C. Romeiro a, Gabriela Aguirre b, Paola Hernández b, Mercedes González b, Hugo Cerecetto b, Ignacio Aldana c, Silvia Pérez-Silanes c, Antonio Monge c, Eliezer J. Barreiro a, Lídia M. Lima a,** Synthesis, trypanocidal activity and docking studies of novel quinoxaline-N-acylhydrazones, designed as cruzain inhibitors, **Bioorganic & Medicinal Chemistry 2009**

44. **Xiujie Wang a,□ , Shulan Yuan a, Jing Wang a, Ping Lin a, Guanjian Liu b, Yanrong Lu a, Jie Zhang a, Wendong Wang c, Yuquan Wei d,** Anticancer activity of litchi fruit pericarp extract against human breast cancer in vitro and in vivo, **Toxicology and Applied Pharmacology 215 (2006) 168–178**

45. **Marie Aufort, Jean Herscovici, Pascale Bouhours, Nicole Moreau and Christian Girard*** Synthesis and antibiotic activity of a small molecules library of 1,2,3-triazole derivatives, **Bioorganic & Medicinal Chemistry Letters 18 (2008) 1195–1198**

46. **Principles of Drug Design by Smith**

47. **Ahmed Kamal,a,* N. Shankaraiah,a V. Devaiah,a K. Laxma Reddy,a Aarti Juvekar,b Subrata Sen,b Nisha Kurianb and Surekha Zingdeb,** Synthesis of 1,2,3-triazole-linked pyrrolobenzodiazepine conjugates employing 'click' chemistry: DNA-binding affinity and anticancer activity, **Bioorganic & Medicinal Chemistry Letters 18 (2008) 1468–1473**

48. **Marilia S. Costa,a Nu´bia Boechat,a E´ rica A. Rangel,a Fernando de C. da Silva,b Alessandra M. T. de Souza,b Carlos R. Rodrigues,d Helena C. Castro,e Ivan N. Junior,c Maria Cristina S. Lourenc¸o,c Solange M. S. V. Wardella and Vitor F. Ferreirab,*** Synthesis, tuberculosis inhibitory activity, and SAR study of N-substituted-phenyl-1,2,3-triazole derivatives, **Bioorganic & Medicinal Chemistry 14 (2006) 8644–8653**

49. **Kadir Dabak *, O¨ zkan Sezer, Ahmet Akar, Olcay Anac,** Synthesis and investigation of tuberculosis inhibition activities of some 1,2,3-triazole derivatives, **European Journal of Medicinal Chemistry 38 (2003) 215-218**

50. **Comprehensive Medicinal Chemistry** by **Corwin and Hansch**

51. **Ashraf Brik,[a] Jerry Alexandratos,[b] Ying-Chuan Lin,[c] John H. Elder,[c] Arthur J. Olson,[c] Alexander Wlodawer,*[b] David S. Goodsell,*[c] and Chi-Huey Wong*[a],** 1,2,3-Triazole as a Peptide Surrogate in the Rapid Synthesis of HIV-1 Protease Inhibitors, **Chem BioChem 2005.**

52. **Hashem Sharghi,a,* Reza Khalifeh,a and Mohammad Mahdi**, Copper Nanoparticles on Charcoal for Multicomponent Catalytic Synthesis of 1,2,3-Triazole Derivatives from Benzyl Halides or Alkyl Halides, Terminal Alkynes and Sodium Azide in Water as a “Green” Solvent. **Doroodmanda Adv. Synth. Catal. 2009, 351, 207 – 218**

53. **S Velázquez1, R Alvarez1, C Pérez2, F Gago2, E De Clercq3, J Balzarini3 and M-J Camarasa1*** Regiospecific synthesis and anti-human immuno-deficiency virus activity of novel 5-substituted *N*-alkylcarbamoyl and *N,N*-dialkyl carbamoyl 1,2,3-triazole-TSAO analogues. **Antiviral Chemistry & Chemotherapy 9: 481-489**

54. **Harrie J.M. Gijsen ⇑, Didier Berthelot, Michel A.J. De Cleyn, Ivo Geuens, Bert Brône , Marc Mercken ,** Tricyclic 3,4-dihydropyrimidine-2-thione derivatives as potent TRPA1 antagonists. **Bioorganic & Medicinal Chemistry Letters 2012**

CONFERENCES DELIGATION AND PRESENTATIONS

National Level Seminar on

"RECENT ADVANCES IN ANTIVIRAL DRUG DESIGN AND DISCOVERY AGAINST EMERGING AND RE-EMERGING VIRAL DISEASES"

October 24 & 25, 2017

Certificate

This is to certify that Prof. / Dr. / Mr. / Mrs. / Ms. N. ASHOK KUMAR, M.M.C.....

has actively participated and Presented a paper (oral / poster) entitled Synthesis, characterization and their biological Evaluation of 3[4-Substituted phenyl]-Azo-8-Aldehyda-4-methyl coumarin derivatives *in National Level Seminar on "Recent Advances in Antiviral Drug Design and Discovery Against Emerging and Re-emerging Viral Diseases" held at Kalasalingam University, Krishnankoil on 24 & 25, October 2017.*

" BEST POSTER AWARD "

Organized by
SIR CV RAMAN-K.S.KRISHNAN INTERNATIONAL RESEARCH CENTRE
Kalasalingam University, Krishnankoil - 626 126
in association with
Antiviral Research Society
[REGN NO.144/2016 (TN SOCIETY REGISTRATION ACT)]

Dr. P. Selvam
Organizing Secretary, AVRS

Dr. S. Saravana Sankar
Vice Chancellor

9th NABS National Conference on New Biological Researches: Opportunities and Challenges for Sustainable Development

(Agriculture, Biology, Energy, Environment, Health and Climate Change)

Certificate

This is to Certify that

N. Ashok Kumar

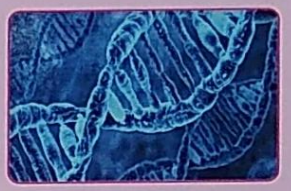
of

Madurai Medical College, Madurai

has presented a paper / poster / participated in the "9th NABS National Conference on New Biological Researches: Opportunities and Challenges for Sustainable Development" (Agriculture, Biology, Energy, Environment, Health and Climate Change) on August 11th & 12th 2016, Organized by School of Energy, Environment & Natural Resources, Madurai Kamaraj University, Madurai - 625 021 and National Academy of Biological Sciences (NABS), Chennai - 600 096.

Prof. Dr. T. Marimuthu
Co-ordinator

Prof. Dr. K. Muthuchelian
Organizing Secretary



FACTs 2017

ALAGAPPA UNIVERSITY

[Accredited with A+ Grade by NAAC (CGPA:3.64) in the Third Cycle]

Karaikudi - 630003, India

DEPARTMENT OF INDUSTRIAL CHEMISTRY

(DST - FIST, DST - PURSE and UGC - SAP Sponsored Department)

CERTIFICATE

This is to certify that Mr/Ms /Mrs/ Dr **N Ashokkumar**, M.Pharm, Student

College of Pharmacy, Madurai Medical College, Madurai

has participated / presented a research paper in the International Conference on **"FRONTIER AREAS IN CHEMICAl TECHNOLOGIES - 2017" (FACTs-2017)** *organized by the Department of Industrial Chemistry, Alagappa University, Karaikudi during 06 - 08 July, 2017.*

Prof. H. GURUMALLESH PRABU
Convener

Prof. G. PARUTHIMAL KALAIGNAN
Chairman

K M C P

K.M. COLLEGE OF PHARMACY

Melur Main Road, Uthangudi, Madurai - 625107

National Seminar on

Advancement of Pharmaceutical Nanotechnology in the Application of Medicinal Field

Certificate

This is to certify that Dr/Mr/Miss/Mrs N. Ashokkumar.

has participated / presented a poster entitled SYNTHESIS & CHARACTERIZATION & THEIR BIOLOGICAL EVALUATION OF 1,2,3-TRIAZOLYL-DIHYDRO-PYRIMIDINE-2-THIONE DERIVATIVES.

in the National Seminar on Advancement of Pharmaceutical Nanotechnology in the Application of Medicinal Field held on 25th November 2017 organized by

K.M. College of Pharmacy, Madurai - 625 107.

Chief Patron Patron Convenor Co - Ordinator

PLATINOFEST CME

PILLS & ILLS

Institute of Pharmacology
Madurai Medical College, Madurai

Certificate of Participation

Certified that **N.ASHOKKUMAR** *has participated as a delegate in the CME Programme on "Pills & Ills" organized by the Institute of Pharmacology, Madurai Medical College, Madurai. under the auspices of The Tamil nadu Dr.MGR Medical University, Chennai.*

on 1st July 2016.

Accreditation

The Tamilnadu Dr.MGR Medical University has awarded 5 credit points under category **II**

DR.M.SHANTHI,M.D.,
Organizing Secretary
Institute of Pharmacology,
Madurai.

DR.R.PARAMESWARI,M.D.,
Director & HOD, Pharmacology,
Madurai Medical College,
Madurai.

DR.M.R.VAIRAMUTHURAJU,M.D.,
The Dean,
Madurai Medical College,
Madurai.

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY

INSTITUTE OF PHARMACOLOGY

Madurai Medical College

Madurai

Certificate of Participation

Certified that **N. ASHOK KUMAR** has participated as a delegate in the CME on Oncopharmacology organized by Institute of Pharmacology, Madurai Medical College, Madurai on 22nd September 2017.

ACCREDITATION

The Tamilnadu Dr.MGR Medical University has awarded 10 credit points under category III

Dr. M.Shanthi, M.D	**Dr. R.Parameswari, M.D**	**Dr. D.Maruthu Pandian, M.S**
Organizing secretary	Director and HOD	Dean
Institute of Pharmacology	Institute of Pharmacology	Madurai Medical College