INTRODUCTION
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The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties, and their quantitative structure-activity relationships (QSAR). Whereas pharmaceutical chemistry being concerned primarily with modification of structures having known physiological or pharmacological effects and with analysis of drugs. It is focused on quality aspects of medicines and aims to assure fitness for the purpose of medicinal products.

The pharmacologically active compound from which the synthetic analogues are developed is known as the lead compound. The work of medicinal chemist is based on the discovery of new lead compound with specific medicinal properties and improving existing drug by increasing their potency, duration of action and decreasing their side effects. This is achieved through organic synthesis and pharmacological testing of hundreds of compounds before a suitable compound is produced. It is currently estimated that for every 100,000 compounds synthesized, only one is suitable for medicinal use. Currently sophisticated computer programs are used to simulate the structure, properties and behavior of molecules. Predictions based on such theoretical approaches are amazingly close to what is actually observed (such as QSAR, CADD, Bioisosterism etc.) but best known before they to plan their synthesis to improve as few steps as possible, consistent with the highest overall yield.

Pharmaceutical discovery and development is an evolving cascade of extremely complex and costly research encompassing many facets. Starting from therapeutic target identification and bioinformatics study through candidate drug discovery and optimization; to pre-clinical organism-level evaluations and beyond to extensive clinical trial assessing effectiveness and safety of new medicines. In general, the process of generating new therapeutics consists of two stages:

- Drug discovery
- Drug development.

The discovery stage includes target selection, lead identification and preclinical studies, while the development stage includes clinical trials, manufacturing and product life cycle management.
Fig 1 summarizes the events which paved the evolution of a drug reaching the market.
The drug discovery process is responsible not only for the introduction of many new drugs to the market place but also for marked improvements in the therapy of many diseases. These include drugs to treat diseases such as cancer, hypertension, cardiac disease, stroke, bronchial asthma, rheumatoid arthritis, diabetes, bacterial and viral infections, and neurological and psychiatric diseases.

Cancer is one of the most formidable diseases of the world. It has been recognized as a disease of aberrant cellular proliferation, affecting different organs and systems of the body. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. WHO estimates that 84 million people will die of cancer between 2005 and 2015 without intervention. According to the edition of the World Health Organization's (WHO) World Cancer Report, the global cancer burden doubled in the last thirty years and is estimated to double again between 2000 and 2020 and nearly triple by 2030.

THE CLINICAL-MOLECULAR INTERFACE

- Clinical

Cancer is the common term for any of a group of diseases characterized by abnormal cellular growth that typically produces malignant tumors. Tumors that may potentially cancerous are categorized as neoplasms. A neoplasm may be either benign or malignant (cancerous). A neoplasm is malignant when it is a fast-growing, non-encapsulated, infiltrative, erosive growth of cells capable of being transported from its site of origin and being implanted in other sites (this cellular relocation process being referred to as metastasis). Malignant tumors have poorly formed and poorly differentiated cellular elements (i.e., subcellular structures have altered morphology and do not resemble the typical nucleus and organelles of normal cells called anaplastic). Neoplasms can be as either simple or compound; simple neoplasms are composed of one single neoplastic cell type, whereas compound neoplasms contain more than one neoplastic cell type. Both simple and compound neoplasms may be either benign or malignant. Malignant simple neoplasms are of two types: carcinomas and sarcomas. Carcinoma is derived from an epithelial cell line including the skin, gastrointestinal tract, genitourinary tract,
respiratory tract, and the associated glands like mammary glands and structures
derived there from. The type of carcinoma reflects the organ of origin: liver
(hepato carcinoma), skin (squamous cell carcinoma), hair follicle (basal cell
carcinoma), respiratory tract (bronchogenic carcinoma), and urinary bladder
(transitional cell carcinoma). When the carcinoma arises from the epithelium that
lines a gland, is called an adenocarcinoma. A malignant sarcoma is a neoplasm
derived from a mesenchymal cell line i.e. portion of the body that produces the
muscles, connective tissues (e.g., tendons, cartilage, bone, fat), fluid distribution
systems (i.e., blood and lymph vessels), and blood cells (e.g., erythrocytes [red
blood cells], leukocytes [white blood cells: granulocytes, monocytes]). The type of
sarcoma reflects the organ of origin: fat (liposarcoma), bone (osteogenic sarcoma),
lymph vessel (lymphangiosarcoma), muscle (rhabdomyosarcoma), blood cell
(leukemia).

Compound tumors are frequently derived from uncommitted cells which are
totipotential or pluripotential, i.e., capable of differentiating into a variety of
different cell types. Compound neoplasms characteristically develop within
embryonic cells or cells in gonads and ovaries. The cancer derived from embryonic
tissue is called blastoma. Carcinomas are more frequently spread by the lymphatic
system, while sarcomas typically are disseminated by embolization through blood
vessels. The spread of a breast carcinoma through the lymphatic system to the
lymph nodes of the axilla (armpit) is a well-recognized mode of spread. Organs
that receive a voluminous blood supply are frequent sites for blood-borne
metastatic spread for either carcinomas or sarcomas. Secondary tumors are thus
common in the liver, lungs, and brain.

Clinically depending on the site of origin of the neoplasm

- primary tumors
- secondary tumors

Primary tumors are found in an easily observed area and may produce symptoms
by virtue of its location (primary brain tumor causing seizures) or by means of its
erosive, infiltrative behaviour (pulmonary adenocarcinoma eroding a blood
vessel), causing bleeding into the lungs and hemoptysis (coughing up blood). If the
tumors are found in a hidden area and may grow undetected and be diagnosed only
when metastatic spread produces secondary tumors in other more easily detected anatomical regions. Alternatively, symptoms may arise from the secondary metastatic tumors being present in vital organs such as brain or liver. Metastases to bone are common and can cause the bone to break (pathological fractures) or can cause severe pain.

- **Molecular mechanism of Cancer Development**

Cancer is the result of a multistep process in which cells acquire features that enable them to control uncontrollably and to metastatize. Crucial steps in transformation of normal cells into malignant cells are the ability of the cells to be self sufficient in growth signals and to be intensive to growth inhibitory signals. As a consequence the cell cycle will be deregulated in favour of continuous growth.

Cancer is a fundamental disease of cells and of the macromolecular constituents of cells. Since cancer is characterized by an alteration in the control mechanisms that govern cell proliferation and differentiation, the nucleic acids (DNA, RNA) are the central players in the molecular cascade of cancer.

Mechanism of carcinogenesis is that the process proceeds through multiple discernible stages:

- **Tumor initiation**
- **Tumor promotion**
- **Tumor progression**

**Tumor Initiation**: A neoplastic transformation is related to genes alteration i.e. the genes implicated in malignancies are often modified forms of human genes or oncogene activation, (activation of protooncogenes to oncogenes may contribute to malignancy) and tumor suppressor gene repression. Mutations can also convert protooncogenes into carcinogenic oncogenes. In normal individuals oncogenes are genes which promote cell growth and reproduction and tumor suppressor genes are genes which inhibit cell division and survival.

These tumor suppressor genes or antioncogenes could inhibit the evolution and growth of tumor cells. Mutations that delete their function would enhance tumor
formation. Indeed the deletion of such genes appears to be involved in certain human tumors including retinoblastoma, Wilm's tumor, lung cancer, colon cancer, and other cancers\textsuperscript{13}. In addition to the above genes, \textit{transcriptional regulatory sequences} (transcriptional enhancer and promoter DNA sequences), may also be critical targets during carcinogenesis. Agents that initiate the carcinogenic process known as \textit{carcinogens} often converts protooncogenes to produce activated oncogenes and thus lead to abnormalities in growth control and differentiation by damaging cellular DNA. Carcinogens also cause deletions in critical growth suppressor genes, can also act on elements that specifically regulate transcription and by inducing complex changes in the genome (e.g., \textit{DNA amplification or transposition}) or by combinations of such mechanisms. Tumor initiation requires only a single exposure to a carcinogen and appears to involve DNA damage.

Classical example of an ubiquitous carcinogen, benzopyrene, that is representative of a large number of polycyclic aromatic hydrocarbon carcinogens\textsuperscript{14}. The compound is not active as such but undergoes metabolism by the microsomal cytochrome P-450 monoxygenase system to yield the highly reactive metabolite benzopyrene-7,8-diol-9,10-epoxide (BPDE)\textsuperscript{15}.

Carcinogens include:

- **Environmental Pollution**
  - Chlorination by products such as trihalomethanes - bladder cancer.
  - Petrochemicals and combustion products, including motor vehicle exhaust and polycyclic aromatic hydrocarbons - cancers of the bladder, lung, and skin.
  - Reactive chemicals such as vinyl chloride - liver cancer and soft tissue sarcoma.
  - Solvents such as benzene - leukemia and non-Hodgkin's lymphoma; tetrachloroethylene - bladder cancer; and trichloroethylene - Hodgkin's disease, leukemia, and kidney and liver cancers.
  - Environmental tobacco smoke - cancers of the breast and lung.
  - Metals such as arsenic - cancers of the bladder, lung, and skin.
 Ionizing Radiations
- Ultraviolet Radiation - leukemia, lymphoma, thyroid cancers.
- Radon, radium, and uranium - Gastric cancers.
- X-ray - breast cancer.

Lifestyle factors
- Tobacco and cigarette smoking - lung cancer.
- Excessive alcohol consumption - cancers of the liver, pancreas, mouth, oesophagus.
- Poor diet - Heavy consumption of red meat, heterocyclic amines produced during the cooking of meat - cancers of gastrointestinal tract, colorectal and prostate.

Obesity
- Cancers of breast, endometrium, kidneys and oesophagus.

Infectious Agents

In addition to this, reactive oxygen species (ROS) such as super oxide radical, hydrogen peroxide, singlet oxygen, hydroxyl radical are cytotoxic and have been implicated in the etiology of cancer. Various carcinogens may partly exert their effect by generating reactive oxygen species during their metabolism. Oxidative damage to the cellular DNA can lead to mutations; therefore play an important role in the initiation and progression of cancer.

Tumor promotion:
Promotion involves multiple exposure to agents known as tumor promoters that do not damage DNA directly. Tumor promoters can be defined as compounds which have very weak or no carcinogenic activity when tested alone but markedly enhance tumor yield when applied repeatedly following a low or suboptimal dose of a carcinogen (initiator).

Classical example is a phorbol ester tumor promoter; 12-O-tetradecanoylphorbol-13-acetate (TPA) does not bind to DNA but instead act by binding to membrane-
associated receptors and thus produce their initial effects at the epigenetic level. It is reported that TPA activates the enzyme Protein Kinase (PKC) and subsequent studies indicating that PKC is the major cellular receptor for TPA have merged research on tumor promotion with that on growth factors, signal transduction, and the action of specific oncogenes\textsuperscript{18-19}.

- Tumor progression

Progression involves the conversion of benign to malignant tumors and can be considered an open-ended process since tumors often continue to increase in their degree of malignancy and heterogeneity. A further mutation in an oncogene might cause the cell to reproduce more rapidly and more frequently than its normal counterparts. A further mutation may cause loss of a tumor suppressor gene, disrupting the apoptosis signalling pathway and resulting in the cell becoming immortal. Once cancer has begun to develop, this ongoing process, termed clonal evolution drives progression towards more invasive stages\textsuperscript{20}. Schematic representation of mechanism of carcinogenesis is depicted in Fig 2.
CANCER DRUG DESIGN AND CHALLENGES

Recognizing that nucleic acids (DNA and RNA) constitute the clinical–molecular interface in cancer represents a major challenge in drug design.

Three fundamental processes which are central targets in drug design:

1. **Replication**: the process by which identical copies of DNA can be made so that information is preserved and handed down from cell to cell.
2. **Transcription**: the process by which the genetic information contained in DNA is read and carried out of the nucleus on RNA.
3. **Translation**: the process by which the genetic information being carried by RNA is decoded and used to build proteins.

Nucleic acids are crucial not only to the growth of the tumor but also to the overall wellbeing of the patient in general. Drugs engineered to attack tumor nucleic acid targets will correspondingly also target (in principle) all other healthy cells within the host’s body. This introduces immense complexities when endeavoring to design cancer chemotherapeutic agents with optimal cancer killing efficacy but minimal toxicity. Since the toxicities are primarily mediated by the therapeutic mechanism of action, separation of pharmacophore from toxicophore becomes a seemingly insurmountable problem. The most reasonable approach for addressing this dilemma is to exploit differences in cell growth kinetics between cancer cells and host cells.

A hallmark of cancerous cell growth is the rapidity of its cellular proliferation. At any given time, a malignant tumor should have more cells undergoing mitosis and replication than other tissues in the host. This observation opens a window of opportunity, enabling a partially selective targeting of tumor cells in preference to host cells. By designing agents which attack nucleic acids at particular times during the cell cycle, it is possible to devise molecules with improved specificity for tumor cells. Given the importance of nucleic acids to heredity and to the control of cellular protein synthesis, drug design that targets nucleic acids are reserved for cancer. On the basis of molecular mechanisms, drugs that act upon nucleic acids can be classified in the following way:
1. **Drugs interfering with DNA replication**
   
a. Intercalating cytostatic agents
   - Actinomycyes
   - Anthracyclines

b. Alkylating cytostatic agents
   - Bis(chloroethyl)amines (nitrogen mustards)
   - Nitrosoureas
   - Aziridines
   - Alkylsulphonates

c. Antimetabolites interfering with DNA synthesis
   - Folate antagonists
   - Purine antimitabolites
   - Pyrimidine antimitabolites

d. Antibacterial agents interfering with DNA Topoisomerase

2. **Drugs interfering with transcription and translation**
   
a. Cytostatic platinum complexes and bleomycin
   b. Antisense oligomers

3. **Drugs interfering with mitosis**
   
a. Vinca alkaloids
   b. Taxane alkaloids

**Emerging Trends in Cancer Drug Design**

The diverse compounds discussed above as putative therapies for cancer tend to exhibit significant toxicities that arise from their inability to differentiate between the DNA of the tumor and the DNA of the host patient. Accordingly, several new directions in cancer chemotherapy drug design are exploiting non-nucleic acid strategies. These new directions include:

1. Angiogenesis inhibitors
2. Proteases
3. Signal transduction inhibitors
4. Hormonal manipulation
5. Photodynamic therapy
6. Immunotherapy
These new directions can be used in combination with traditional anti-nucleic acid approaches.

From the beginning of medicinal chemistry, heterocycles have constituted one of the largest areas of research. It is well known that a number of heterocyclic compounds containing nitrogen and sulphur exhibited a wide variety of biological activities. The heterocyclic molecules have received much attention for their strong electron-withdrawing capabilities, due to which the hetero aromatic rings can be exploited to enhance the donor-acceptor effects in the chromophores21-22.

Pyrimidines have a long and distinguished history extending from their days of discovery as important constituent of nucleic acids to their current use in chemotherapy of cancer. Pyrimidines represent one of the most active classes of compounds possessing wide spectrum of biological activities such as anti-cancer23, diuretic24, anti-viral25, anti-HIV26, anti-hypertensive27, anti-convulsant28, anti-tubercular29, anti-bacterial30, anti-fungal31 and anti-epileptic32 properties and many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use33.

ROLE OF PYRIMIDINES IN CANCER THERAPY

Within the past decade our understanding of malignant cell growth and the regulation of the cell cycle machinery has offered several new opportunities for targeted cancer therapy and the promise of a broader therapeutic window. In medicinal chemistry Pyrimidines and its Benz fused derivatives viz quinazolines have been very well known for their therapeutic applications. Presence of a pyrimidine base in thymine, cytosine, and uracil which are the essential building blocks of nucleic acids; this could be the possible reason for their activity.

Mechanism of Action of Pyrimidines as Anticancer Agent

Pyrimidine scaffold was reported to have significant anticancer effects against various tumour cells by acting as alkylating agents or antimetabolites.

The term alkylating agents in its widest sense denotes those compounds capable of replacing a hydrogen atom in another molecule by an alkyl radical, and this of course involves electrophilic attack by the alkylating agent34. The alkylating agents

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exhibit a diversity of pharmacological properties including the capacity to interfere with mitosis, to cause mutations, and to initiate and promote malignant tumors. First-order nucleophilic substitution (SN1) and second-order nucleophilic substitution (SN2) are generally accepted basic mechanisms of alkylation. Since cancer cells, in general, proliferate faster and with less error-correcting than healthy cells, cancer cells are more sensitive to DNA damage such as being alkylated. The alkyl group is attached to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. Nimustine and Uramustine are used as alkylating agents.

An antimetabolite is a chemical that inhibits the use of a metabolite, which is another chemical that is part of normal metabolism. The presence of antimetabolites can have toxic effects on cells, such as halting cell growth and cell division as they interfere with DNA production, so these compounds are used in chemotherapy for cancer. Since cancer cells spend more time in dividing than other cells, inhibiting cell division harms tumor cells more than other cells. 5-Fluorouracil, Thiouracil, 6-Mercaptopurine, 6-Thioguanine, Raltitrexed, Tegafur, Trimetrexate, Mopidamol, Gemcitabine, 1-β-D-Arabinosyl cytosine (Ara-C) are very well known antimetabolites.

In addition to the anti cancer activity the importance of pyrimidine as diuretics in the management of hypertension is a topic of interest.

Diuretics and hypertension: Diuretics have been used effectively to treat millions of hypertensive patients during the past four decades and act primarily by inhibiting the reabsorption of sodium ions from the renal tubules in the kidney. The application of diuretics in the management of hypertension and congestive heart failure has outstripped their use in edema. They reduce both systolic and diastolic blood pressures in the great majority of hypertensive patients. They are as effective as most other antihypertensive drugs and also enhance the antihypertensive efficacy of multidrug regimens and can be useful in achieving blood pressure control. Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 6) guidelines, which were issued in 1997, and (JNC 7) guidelines, which were issued in 2003, both recommend diuretics as first-line drugs in the treatment of uncomplicated hypertension. Diuretics should...
be used as initial therapy for most patients with hypertension either alone or in association with one of the others classes or in presence of oedema due to heart failure (HF) or other causes. They demonstrated to be beneficial in a series of cases in patients with severe congestive HF for reducing fluid retention and to be beneficial in a large randomized clinical trial in hypertensive patients for the prevention of HF. The current diuretic regimen is associated with adverse effects like hypokalemia, hyperuricemia, and glucose intolerance and raises concern over their safety and oral activity profile, so there exist urgent clinical requirements for novel, selective diuretics. It should not only an effective diuretic, should be useful in the treatment of hypertension by themselves or in combination with other antihypertensive drugs. Pyrimidines are least explored compounds for diuretic profile although few promising diuretic drugs possess this ring.
REFERENCES


References

34. GP Warwi, Cancer Res, 23, 1963, 1315.
LITERATURE REVIEW
PYRIMIDINE
Pyrimidine is a six-membered aromatic heterocyclic compound that contains two nitrogen atoms, separated by a carbon atom, in the ring. It is the most important member of diazines viz pyridazine and pyrazine. The diazines are six-membered cyclic systems, in which two of the carbon atoms have been replaced by nitrogen atoms. It occurs in three isomeric forms 1, 3-diazine (I), 1, 4-diazine (II) and 1, 2-diazine (III).

- The three isomers are:

```
   I
           N
           |
           |
   II       N
           |
           |
            \__\__

   III
```

Our focus is on pyrimidines, which are the most important member of all diazines as this ring system occurs widely in living organisms.

Nucleic acids, DNA and RNA, contain substituted purines and pyrimidines. Cytosine (IV), uracil (V) and thymine (VI) are just a few of the biologically significant modified pyrimidine compounds, which are components of the nucleic acids. Uracil and thymine may be considered to contain the neutral urea unit or the acidic imide moiety.

```
   IV  
O     |    NH2
     |     H
     |     |
   V  
O     |    H
     |     |
   VI  
O     |    CH3
     |     H
```

Current nomenclature of pyrimidines have been derived and officially recommended by the International Union ofPure and Applied Chemistry (IUPAC)/International Union of Biochemistry and Molecular Biology (IUBMB).
The number 1-6 indicates the position of a substituent group in the pyrimidine nucleus. Numbering commences with the nitrogen atom and proceeds in an anticlockwise direction to the second nitrogen atom at the position 3 of the six membered ring.

In 1818 Gasper Brugnatelli\(^1\) isolated the first pyrimidine derivative, Alloxan (VII), by oxidation of uric acid with nitric acid. The landmark in pyrimidine chemistry was the synthesis by Grimaux\(^2\), in 1878 of barbituric acid (VIII) from malonic acid and urea. Our knowledge of the pyrimidine series is largely due to the work of Pinner\(^3\), who first applied the name pyrimidine to the unsubstituted parent body in 1883. Barbitone (IX), the first barbiturate hypnotic sedative and anticonvulsant was introduced into medicine by Emil Fischer\(^4\) and Joseph von Mering in 1903.

Barbituric acid is a fairly strong acid with a pKa of 4.12, but upon substitution at the 5 position, the pKa rises dramatically, the 5,5-disubstituted barbiturates react with sodium hydroxide to form a salt that is quite water soluble.

Pyrimidine is symmetrical about the line passing C-2 and C-5, the positions C-4 and C-6 are equivalent and so are N-1 and N-3. If a hydroxyl or amino group is present at the 2, 4, or 6, position then they are tautomeric with oxo and imino respectively.
The pyrimidine is a colorless crystalline compound with a characteristic odour, soluble in alcohol, melts at 20-22°C, boils at 124°C, having molecular formula C₄H₄N₂ and molecular weight of 80.08. Pyrimidines are weakly basic (pKa 1.3) as compared to pyridine (pKa 5.2) or imidazole (pKa 7.2). The decrease in the basicity is due to electron-withdrawing effect of the second nitrogen atom present in the ring. Moreover, the addition of the proton does not increase the probability for mesomerism and hence the resonance energy. Presence of alkyl groups enhances the basicity, thus 4-methylpyrimidine has pKa 2.0 while 4,6-dimethylpyrimidine has a value of 2.8. The 2 and 4-aminopyrimidines are more basic with pKa 3.54 and 5.71 respectively. In these two compounds more resonance structures are possible in the cation than in the neutral molecule.

The close relationship of pyrimidine with benzene suggests the former is also highly aromatic and the ring is virtually planar. The following canonical structures contribute to the resonance hybrid. But pyrimidine ring is less aromatic compared to pyridine and benzene.

This view is corroborated by the resonance energies which are benzene (36 Kcal/mole), pyridine (31 Kcal/mole) and pyrimidine (26 Kcal/mole).

A. SYNTHESIS / METHODS OF PREPARATION

The most general and widely used route to synthesize pyrimidines involves the combination of a reagent containing the N-C-N skeleton with C-C-C unit. Urea, thiourea and guanidine are the most commonly used N-C-N agents and 1,3-
diketones and diesters are the common agents to provide the C-C-C unit. These synthesis are typical examples of the bis-nucleophilic plus bis-electrophilic method of constructing heterocycles. Both the nitrogen atoms of the N-C-N reagents acts as nucleophiles and both the terminal carbon atoms of C-C-C reagents are electrophiles.

1. **Pinner Pyrimidine Synthesis**

   The condensation of 1,3-dicarbonyl compounds with amidines catalyzed by acids or bases to give pyrimidine derivatives.

   ![Chemical structure of Pinner Pyrimidine Synthesis](image)

2. **Condensation of Urea with oxaloaceticester**

   Initial product is a hydantoin, which rearranges to pyrimidine on treatment with alkali.

   ![Chemical structure of Condensation of Urea with oxaloaceticester](image)

3. **From α, β- Unsaturated Ketones**

   Benzamidine condenses with compounds of the type C₆H₅CH=CHCOR in which R does not contain an α-hydrogen to yield 2, 4, 6-trisubstituted pyrimidines. The dihydropyrimidine first formed is dehydrogenated by unreacted unsaturated ketone.
4. From Ethyl Crotonate
Following condensation of urea with unsaturated compounds, dihydropyrimidine formed initially readily oxidised to the corresponding pyrimidine derivative.

5. Condensation of Formamide with Acetophenone
Formamide reacts with compounds containing active methylene group to form $\beta$-enamino ketone, which cyclises with excess formamide to form pyrimidines.
6. **By Dechlorination**

Dechlorination of 2,4-dichloropyrimidine yield pyrimidine itself\(^\text{11}\).

\[ \text{Cl} \quad \text{H}_2 \text{Pd-C} \quad \text{Cl} \]

7. **Claisen condensation**

Reaction between an ortho ester and a reactive methylene compound\(^\text{12}\).

\[ \text{H}_3 \text{C}^{\text{O}} \text{OEt} + \text{H}_3 \text{C}^{\text{N}} \text{H}^+ \cdot \text{HCl} \rightarrow \text{HO}^{\text{N}} \text{C} \quad \text{CN} \quad \text{NC} \]

8. **Base catalyzed condensation**

Base catalyzed reaction between 1,3-dicarbonyl compound and an amidine\(^\text{13}\).

\[ \text{O} \quad \text{O} \quad \text{+} \quad \text{NH} \quad \text{R} \quad \text{NH}_2 \quad \text{EtO}^- \quad \text{R} \quad \text{N} \quad \text{Y} \quad \text{R} = \text{H, OH, NH}_2, \text{SH} \quad \text{X and Y} = \text{H, OH or NH}_2 \]

9. **Biginelli Reaction**

It involves one-pot reaction between aldehyde, 1,3-dicarbonyl and urea or thiourea in the presence of an acidic catalyst to afford pyrimidine\(^\text{14}\).

\[ \text{R} = \text{Alkyl, Aryl, Heteroaryl} \quad \text{R}_2 = \text{Ester, Amide, Acyl} \quad \text{R}_3 = \text{Alkyl} \quad \text{X} = \text{O, S} \]
10. *From other Heterocycles*

*a. From Pyrazoles*

Phenyl pyrazolone with methyl iodide yield phenyl pyrimidinone\(^{15}\).

![Reaction scheme for phenyl pyrazolone with methyl iodide yielding phenyl pyrimidinone.](image)

*b. From Oxadiazoles*

Reductive fission of 1,2,4-oxadiazole bearing a suitable chain at 3\(^{rd}\) position yield pyrimidine derivatives\(^{16}\).

![Reaction scheme for reductive fission of 1,2,4-oxadiazole yielding pyrimidine derivatives.](image)

c. *From 4-Methoxy-6-methyl 2H-pyran-2-one*

Reacts with β-ketoester and thiourea in ethanolic ethoxide yielded thioxopyrimidines\(^{17}\).

![Reaction scheme for thioxopyrimidines.](image)
d. From Hydroxy Iminopyrole

On treatment with phosphorus pentachloride in ether, two intermediate products are isolated. The former on heating gives hydroxy substituted pyrimidines and the latter on reduction gives amino substituted pyrimidines.

11. Frankland and Kolbe Synthesis from nitriles and alkali metals

Nitriles having CH₂ group adjacent to the cyano group undergoes trimerization in the presence of alkali metals yielded pyrimidine derivatives.
B. CHEMICAL REACTIONS

✧ Addition Reactions

a) Bromination
Bromine adds to 2-methylmercaptopyrimidine via an unstable dibromide which on heating under pressure converted to 5-bromo derivative.

\[
\begin{align*}
\text{H}_3\text{C} & \text{S} \quad \text{Br}_2 \quad \text{CCl}_4 \quad \text{H}_3\text{C} \\
\text{N} & \quad \text{H} \quad \text{Br} \\
\text{H}_3\text{C} & \text{S} \quad \text{N} \quad \text{H} \quad \text{Br}
\end{align*}
\]

b) Formation of an addition product
Same dihydropyrimidine derivative is formed by addition of nitric acid to 5-bromouracil and by addition of hypobromous acid to 5-nitouracil.

\[
\begin{align*}
\text{OH} & \quad \text{OH} \quad \text{Br} \\
\text{NO}_2 & \quad \text{OBr} \\
\end{align*}
\]

✧ Reaction with Acids

Protonation of pyrimidine N-hydrogen
Pyrimidine is a weaker base than pyridine because of the presence of the second nitrogen. Though a weak base, it can be protonated in the presence of acids. Its conjugate acid is a much stronger acid (pKₐ=1.0).

\[
\begin{align*}
\text{N} & \quad \text{H} \\
\text{N} & \quad \text{H} + \quad \text{H}^+ \\
\end{align*}
\]

✧ Substitution Reactions

➢ Electrophilic Substitution
Pyrimidine is resistant to electrophilic substitution. The attack at positions 2, 4 and
6 are particularly retarded because of electron deficiency at these positions. The second nitrogen in the aromatic ring makes it less reactive towards electrophilic substitutions. Electrophilic substitution at position-5 is easy if one or more electron releasing groups are present on the ring.

a) Nitration

Pyrimidines undergo straightforward nitration at C-5.

Nitration of uracil and 6-methyluracil yielded 5-nitro pyrimidine derivatives.

The 6-methyluracil nitrates with accompanying oxidation of the methyl to carboxyl.

b) Nitrosation
When 3 electron releasing groups are present, nitroso derivative formed at 5\textsuperscript{th} position.

\[
\begin{align*}
\text{NH}_2 & \quad \text{NO} \\
\text{HS} & \quad \text{OH}
\end{align*}
\]
\[
\text{NH}_2 & \quad \text{NO} \\
\text{HS} & \quad \text{OH}
\]

\text{aq.CH}_3\text{COOH} \quad \text{NaNO}_3/ \quad \text{room temp}

\text{NH}_2 \\
\text{HS} \\
\text{OH}

\text{c) Halogenation}

\[
\begin{align*}
\text{N} & \quad \text{F} \\
\text{AgF}_2 & \quad \text{Triperfluoro butylamine}
\end{align*}
\]

Chloropyrimidines can be prepared by the action of phosphorous pentachloride on hydroxypyrimidines\textsuperscript{21}.

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{K} & \quad \text{Cl}
\end{align*}
\]

\text{d) Sulphonation}

\[
\begin{align*}
\text{HO}_3\text{S} & \quad \text{NH}_2 \\
\text{OH} & \quad \text{NH}_2
\end{align*}
\]

\text{e) Alkylation}

Chloromethylation of 1,4-dimethyluracil and 6-methyluracil with formaldehyde results in the formation of 5-chloromethyl\textsuperscript{22} and 5-methylol\textsuperscript{23} derivatives respectively.
f) Amidation

\[
\begin{align*}
\text{NH}_2 & \quad \xrightarrow{\text{Amidation}} \quad \text{H}_2\text{NOC} \\
\text{HO} & \quad \text{HO} \\
\text{N} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{NH}_2 & \quad \xrightarrow{\text{Amidation}} \quad \text{H}_2\text{NOC} \\
\text{HO} & \quad \text{HO} \\
\text{N} & \quad \text{N} \\
\end{align*}
\]

g) Reimer-Tiemann reaction

Introduction of an aldehydic group into the 5-position of pyrimidines already having 2 or 3 electron releasing groups\(^{24}\).

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{CHCl}_3} \quad \text{OHC} \\
\text{H}_3\text{C} & \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{CHCl}_3} \quad \text{OHC} \\
\text{H}_3\text{C} & \quad \text{N} \\
\end{align*}
\]

h) Diazotization

Pyrimidines with OH or NH\(_2\) groups in the 2,4; 4,6; 2,4,6; positions couple with diazonium salts to give 5-phenylazo derivatives\(^{25}\).

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{C}_6\text{H}_5\text{N}_2\text{Cl}} \quad \text{N} = \text{C}_6\text{H}_5 \\
\text{HO} & \quad \text{OH} \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{C}_6\text{H}_5\text{N}_2\text{Cl}} \quad \text{N} = \text{C}_6\text{H}_5 \\
\text{HO} & \quad \text{OH} \\
\end{align*}
\]

⇒ Nucleophilic Substitution

The attack of a nucleophile takes place easily on pyrimidine ring at positions 2, 4 and 6.
a) With Hydrazine

In boiling hydrazine, pyrimidine undergoes rearrangement to pyrazolo, via a ring opened intermediate.\(^{26}\)

\[\text{H}_2\text{N},\text{H}_2\text{NH}_2 \xrightarrow{aq \text{NH}_2,\text{NH}_2} 130 \degree \text{C} \xrightarrow{} \text{NH},\text{N} \xrightarrow{} \text{N}-\text{H} \xrightarrow{} \text{H} \xrightarrow{} \text{H} \]

\[\text{N} \quad + \quad \text{H}_2\text{N},\text{NH} \]

b) With Grignard reagent

Readily adds to the 3,4-bond of the pyrimidines to yield 4-phenyl pyrimidine.\(^{27}\)

\[\text{H} \quad \text{C}_6\text{H},\text{MgBr} \xrightarrow{} \text{H} \quad \text{CH}_3,\text{MgBr} \xrightarrow{} \text{H} \quad \text{C}_6\text{H},\text{MgBr} \xrightarrow{} \text{H} \quad \text{H}_2\text{O} \xrightarrow{} \text{H} \quad \text{K}_2\text{MnO}_4 \xrightarrow{} \text{H} \quad \text{Acetone} \xrightarrow{} \text{H} \quad \text{C}_6\text{H}_5 \]

\(\text{Reaction with Oxidising and Reducing Agents}\)

Pyrimidine gives N-oxide on oxidation with a peracid.

\[\text{CH}_3 \quad \text{H}_2\text{O}_2, \text{CH},\text{COOH} \xrightarrow{} \text{CH}_3 \quad \text{CH}_3 \quad \text{N} \quad \text{O} \quad - \]

2-Cyano-4-methoxypyrimidine is formed by the reaction with sodium cyanide and benzoyl chloride under alkaline conditions.
Thio-Claisen Rearrangement

5-Allyl-3-methyl-4-thiouracil is formed from substituted thiopyrimidine derivatives.

C. BIOLOGICAL ACTIVITY / RECENT ADVANCEMENT

The chemistry of heterocyclic compounds has been an interesting field of study since long time. Among the heterocyclic compounds, Pyrimidines have a long and distinguished history extending from their days of discovery as important constituent of nucleic acids to their current use in chemotherapy of cancer. Presence of a pyrimidine base in thymine, cytosine, and uracil which are the essential building blocks of nucleic acids; could be the possible reason for their activity. The chemistry of pyrimidine with N-C-N bond considered to be the key factor. The broad and potent activity of pyrimidine and their derivatives has established them as pharmacologically significant scaffolds. Therefore syntheses of novel pyrimidine derivatives and investigation of their chemical and biological behaviour have gained more importance for therapeutic purposes and have also found application in agricultural and industrial chemicals.

Activity: Diuretic

Ukrainets & Co workers (2008) reported the synthesis and biological screening of a series of structurally related 2-hydroxy-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylicacid N-R-amides to increase the diuretic kidney function. The meta isomer was found to be most active.
Kumaraswamy & Co workers (2006) reported synthesis and pharmacological evaluation of 2-mercapto-4-acylnaphtho[2,1-b]furo[3,2-d]pyrimidines (4a-d) and S-(4-acylnaphtho[2,1-b]furo[3,2-d]pyrimidine)mercaptoacid (5a-d) as diuretic agents. All the synthesized compounds were evaluated for in vivo diuretic activity on Wistar rats. Among the compounds studied, compound 4c was the most potent.

\[
\begin{align*}
(4a-d) & \quad R = \text{C}_6\text{H}_5, \text{CH}_3, 4-\text{Cl}\text{C}_6\text{H}_4, 4-\text{OH}\text{C}_6\text{H}_4 \\
(5a-d) & \quad R = \text{C}_6\text{H}_5, \text{CH}_3, 4-\text{Cl}\text{C}_6\text{H}_4, 4-\text{OH}\text{C}_6\text{H}_4
\end{align*}
\]

**Activity: Anticancer**

Rashad & Co workers (2011) reported synthesis of pyrazolo[3,4-d]pyrimidine-5(4H)-thione starting with pyrimidinone derivative. Their in vitro cytotoxicity against human breast adenocarcinoma (MCF-7) cell lines have been investigated and most of the tested compounds exploited potent cytotoxic activity against MCF-7 cell lines comparable to the activity of the commonly used anticancer drug cisplatin. Among the synthesized compounds, compound (4-ethylsulfanyl)-1-(9-methyl-5,6-dihydronaphtho[1’,2’:4,5]thieno[2,3-d]pyrimidin-11-yl)-1H-pyrazolo [3,4-d]pyrimidine revealed the highest anticancer activity.
Ghorab & Co workers (2010) reported synthesis of a series of novel 2-substituted-3-cyano-4-phenyl-pyrrole and 5-phenyl-pyrrolo[2,3-d]pyrimidine derivatives bearing either sulfathiazole or sulfapyridine. All the newly synthesized compounds were evaluated for their in vitro cytotoxicity against liver and breast cancer cell line (HEPG2 and MCF7). Most of the screened compounds showed interesting cytotoxic activities compared with the used reference drug (doxorubicin) where as among pyrrolopyrimidine derivatives, the compound 22 (IC50 = 3.9 μM) was found to be the best candidate. 

![Chemical Structure](image)

Tangeda & Co workers (2010) reported new pyrrolo[2,3-d]pyrimidines (6a-c) with heteroaryl substitution at 5th position through sulfur linker synthesized by incorporating putative pharmacophoric moieties like benzimidazole and benzothiazole, pyridyl, substituted oxadiazoles as heteroaryl groups. Cytotoxic effect of all the compounds was carried out by trypan blue exclusion assay on HCT116 colon cancer cell lines. Compounds 2-Amino-6-methyl-5-[20-(50-nitrobenzimidazolyl)sulfanyl]-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d] pyrimidine (6c) and 2-Amino-6-methyl-5-[20-pyrimidinyl)sulfanyl]-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d] pyrimidine (6h) with nitrobenzimidazole and pyrimidyl heterocycles attached at 5th position via sulfur were the most potent of all with IC50 values of 17.6 μM.
Abdel-Mohsen & Co workers (2010) reported synthesis and antitumor activity of newer series of 2-((1Hbenzo[d]imidazol-2-yl)methylthio)-4-(substituted)-6-phenylpyrimidine-5-carbonitriles. The synthesized compounds were evaluated for their in vitro cytotoxic activity using MTT assay according to Mosmann’s method against twelve cell lines namely, Cervical carcinoma (KB), Ovarial carcinoma (SKOV-3), CNS cancer (SF-268), Non small lung cancer (NCI H460), Colonadenocarcinoma (RKOP27), Leukaemia (HL60, U937, K562), Melanoma (G361, SK-MEL-28) and Neuroblastoma (GOTO, NB-1) revealed their marked potency when compared with known anticancer drugs.

Horiuchi & Co workers (2009) reported a new analogues of thieno[2,3-d]pyrimidin-4-yl hydrazone compounds as cyclin D1/CDK4 inhibitors and evaluated their enzyme inhibitory activity and antiproliferative activity. The potency, selectivity profile, and structure–activity relationship trends of this class of compounds were discussed.
Xie & Co workers (2009) reported a series of novel 2, 4, 5-substituted pyrimidine derivatives and evaluated for inhibition against the human hepatocellular carcinoma BEL-7402 cancer cell line. Structure–activity relationships for this class of compounds at the 2 and 5-position of the pyrimidine scaffold have been elucidated. Alkylation of 3-(4-hydroxyphenyl)-4H-chromen-4-one with various alkyl halides followed by condensation with guanidine yielded O-alkylated derivatives (7ia-7im). Among them 7ia was the most active compound in the series, with IC\(_{50}\) of 0.24 µM. Overall the N-methyl ethyl substituted compound 7gc showed excellent inhibitory activity with an IC\(_{50}\) of 0.024 µM for the BEL-7402 cancer cell line and found to be the most active compound.

Singh & Co workers (2009) designed and synthesized by combining the structural features of indole and barbituric acid, new hybrid molecule. Evaluations of these molecules over 60 cell line panel of human cancer cells have identified two molecules with significant anticancer activities. Dockings of two active molecules...
in the active sites of COX-2, thymidylate synthase and ribonucleotide reductase indicate their strong interactions with these enzymes.

\[
\text{G}_{1/2} \text{ for:} \\
\text{IGROVI} = 0.06 \mu M \\
\text{MDA-MB-468} = 0.02 \mu M \\
\text{A 498} = 0.03 \mu M \\
\text{MDA-MB-468} = 0.1 \mu M
\]

Nguyen & Co workers (2009) described the synthesis and SAR of a series of 6-chloro-4-fluoroalkylamino-2-heteroaryl-5-(substituted)phenylpyrimidines as anticancer agents\textsuperscript{38}. For the 2-heteroaryl group, the best activity is obtained when the heteroaryl group has a nitrogen atom at the ortho-position to the pyrimidyl core. The lead compounds in this series are more potent than the corresponding triazolopyrimidines \textit{in vitro} and \textit{in vivo} and was selected to advance to preclinical development. Compound 21 (PTI-868) showed tumor growth inhibition in several nude mouse xenograft models, and was selected to advance to preclinical development.
Gangjee & Coworkers (2008) synthesized N-{4-[(2-Amino-6-methyl-4-oxo-3,4-
dihydrothieno[2,3- d]pyrimidin-5-yl)sulfanyl]benzoyl}-L-glutamic acid (4) and
nine nonclassical analogues (5-13) as potential dual thymidylate synthase (TS) and
dihydrofolate reductase (DHFR) inhibitors. Compound 4 was the most potent dual
inhibitor of human TS (IC$_{50}$ = 40 $\mu$M) and human DHFR (IC$_{50}$ = 20 $\mu$M)$^{39}$.

Azam & Co workers (2008) reported synthesis of a series of 5-[(4,6-disubstituted
pyrimidine-2-yl)thio]methyl-N-phenyl-1,3,4-thiadiazole-2-amines (6a-c). It
involves the reaction between chalcones and thiourea followed by condensation
with hydrazine hydrate yielded 2-[(4,6-disubstituted pyrimidine-2-yl) thio]
acetohydrazides. Further condensation of these intermediates with phenyl
isothiocyanate concentrated sulphuric acid afforded titled compounds and
evaluated for their anticancer activities. Compound 6b exhibited significant
antitumor activity against human breast cancer MCF 7 cell line. Apart from
anticancer activity the compounds synthesized were evaluated for antioxidant
activity; compound 6b exhibited moderate antioxidant activity$^{40}$.

Nersesyan & Co workers (2008) reported two novel compounds DGB-100
(4,5,7-trimethyl-2-phenylpyrazol[1,5a]pyrimidine iodide) and DGS-618 (2,4,5-
trimethyl-7-aminopyrazol [1,5a] pyrimidineiodide) and the assessment of the
micronucleus and antitumor activities were carried out on mice. Both compounds
substantially increase the micronucleus as well as the antitumor activities of
cyclophosphamide$^{41}$.
Amr & Co workers (2006) reported synthesis and anticancer activities of 10-nitro-4-(substitutedphenyl)-1,3,4,5,6,7-hexahydro-2H-benzo[6,7]-cyclohepta[1,2-d]pyrimidin-2-thione derivatives. The antitumor activities of the synthesized compounds were evaluated utilizing 59 different human tumor cell lines. Some of the tested compounds exhibited better in vitro antitumor activities at low concentration (log_{10} GI_{50} = -4.7) against the used human tumor cell lines.

Chauhan & Co workers (2005) reported synthesis of some novel heterocycles as potential anticancer agents. 6-(6,7-Dimethyl-4-oxo-3,4-dihydro-pteridin-2-y1-sulfanyl methyl)-1H-pyrimidine-2,4-dione was synthesized by the reaction between 2-mercapto-4-hydroxy-6,7-dimethylpteridine dissolved in methanolic sodium hydroxide and 2-chloromethyl uracil. The study was related with in vitro anticancer screen to identify agents which can serve as novel templates for the anticancer chemotherapy and can serve as leads in cancer chemotherapy. At its primary anticancer assay, a 3-cell panel consisting of NCI-H460 (Lung), MCF-7 (Breast) and SF-268 (CNS) have been used; 48 hr continuous drug exposure protocol was used to estimate cell viability or growth.
Cocco & Co workers (2001) reported new series of 6-thioxopyrimidines, 6-oxo and pyrimidine-2,4-diones and found that most of them displayed antitumor activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines. Whereas the N'-substituted-4-dialkylamino-6-thioxopyrimidine-5-carboxylate (N' bearing benzyl group) was found to be the best candidate with highest cytostatic activity.\(^{44}\)

\[
\begin{align*}
X & \quad R \\
\text{CN} & \quad \text{CH}_3 \\
\text{CN} & \quad \text{C}_6\text{H}_5 \\
\text{CN} & \quad \text{C}_6\text{H}_5-\text{CH}_2 \\
\text{COOC}_2\text{H}_5 & \quad \text{CH}_3 \\
\text{COOC}_2\text{H}_5 & \quad \text{C}_6\text{H}_5 \\
\text{COOC}_2\text{H}_5 & \quad \text{C}_6\text{H}_5-\text{CH}_2 \\
\end{align*}
\]

**Activity: Anti hypertensive**

Alam & Co workers (2010) reported design, synthesis, characterization and anti hypertensive activity of 5-(4-substituted phenyl)-2-(substituted benzylsulfanyl)-4-(substituted phenyl)-6-methyl-1,4-dihydro-5-pyrimidine carboxamides.\(^{45}\) All the synthesized compounds were tested for antihypertensive activity by non-invasive blood pressure (NIBP) measurements (tail-cuff method) in rats. Almost all the tested compounds displayed considerable decrease in the blood pressure as compared to control.
Singh & Co workers (2009) has been found that selective Ni-alkylation of 3,4-dihydropyrimidine-2 (1H)-ones can be achieved under solvent-less, mild phase transfer catalytic (PTC) conditions with tetrabutylammonium hydrogen sulfate and 50% aqueous NaOH as the catalyst and base, respectively. The procedure is tolerant to substitutional variation at key diversity points on the pyrimidinone moiety. Ni-Substituted DHPM derivatives show moderate calcium channel blocking activity\textsuperscript{46}.

Sehon & Co workers (2008) recently studied using known Rho-associated Kinase isoform-1 (ROCK1) inhibitors along with cellular and molecular biology data have revealed a pivotal role of this enzyme in many aspects of cardiovascular function. Here a series of ROCK1 inhibitors which were originally derived from a dihydropyrimidinone were reported\textsuperscript{47}. 

\[
\text{R = F, Br, NO}_2 \\
\text{R}_1 = 2,4-\text{Cl, 4-Br, 3-NO}_2, 4-\text{NO}_2, 3,4-\text{OCH}_3 \\
\text{R}_2 = \text{H, 2-Cl, 4-Cl, 4-F}
\]
Activity: Anti bacterial

Solankee & Co workers (2009) synthesized and screened for antimicrobial activity of 2,4-bis-ethylamino-6-[4'-(5'-{5''-(substituted phenyl / 2''''-furanyl)-pyrazolin-3''-yl}-phenyl amino]-s-triazine derivatives and 2,4-bis-ethylamino-6-[4'-2''-amino-6''-(substituted phenyl / 2''''-furanyl)-pyrimidin-4''-yl]-phenylamino]-s-triazine derivatives.

\[
\begin{align*}
\text{R} = 3,4-(OCH_3)_2C_6H_3 & \\
\text{R} = 2,4-(Cl)_2C_6H_3 & \\
\text{R} = 2,6-(Cl)_2C_6H_3 & \\
\text{R} = 2-Furanyl & \\
\end{align*}
\]

Akhari & Co workers (2008) reported some new N-(4-chlorophenyl)-6-methyl-4-aryl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxamide 4(a-h) and N-(4-chlorophenyl)-7-methyl-5-aryl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxamide 5(a-h) were synthesized by the reaction of N-(4-chlorophenyl)-3-oxobutanamide, thiourea and 1,2-dibromoethane which were evaluated for their antimicrobial activities.

Zhi & Co workers (2005) synthesized and screened 3-substituted-6-(3-ethyl-4-methylanilino) uracils (EMAU) for their capacity to inhibit the replication-specific bacterial DNA polymerase IIIC (pol IIIC) and the growth of Gram+ bacteria in culture. Certain intermediates, e.g. the 3-(iodoalkyl) compounds, were converted to a variety of (3-substituted-alkyl)-EMAUs by displacement. Several compounds protected mice from lethal intraperitoneal infections with S. aureus when given by the intraperitoneal route.
Activity: Anti Tubercular

Bacelar & Co workers (2010) reported synthesis and in-vitro antimycobacterial activity of novel pyrimido[5,4-d]pyrimidines against Mycobacterium tuberculosis strain H37Rv. Most of the new compounds showed high potency and promising antitubercular activity, as is the case of N-[8-[(4-fluorophenyl)amino]-4-iminopyrimido[5,4-d]pyrimidin-3(4H)-yl]isonicotinamide with an IC$_{90}$ = 3.58 $\mu$g/mL, and regarded as new hits for further development as a novel class of antitubercular agents.$^5$

Sharma & Co workers (2009) reported a series of oxo linked and amino linked pyrimidine via condensation of chalcones with guanidine hydrochloride and evaluated for antitubercular along with antimalarial activities. As far as antitubercular activity is concerned, compound N-(4-(2-amino-6-(4-(methylthio)phenyl)pyrimidin-4-yl)phenyl)-7-chloroquinolin-4-amine (54) and N-(4-(2-amino-6-(4-isopropylphenyl)pyrimidin-4-yl)phenyl)-7-chloroquinolin-4-
amine (56) have shown antitubercular activity of minimum inhibitory concentration of 1μg/mL.52.

\[ X \quad R \\
O \quad C_6H_5 \\
O \quad 4-CH_3-C_6H_4 \\
O \quad 4-OCH_3-C_6H_4 \\
O \quad 3,4,5-(OCH_3)_3C_6H_2 \\
O \quad 2,3,4-(OCH_3)_2C_6H_2 \\
O \quad Furan-2yl \\
O \quad 2,3-(OCH_3)_2C_6H_2 \\
NH \quad C_6H_5 \\
NH \quad 4-CH_3-C_6H_4 \\
NH \quad 4-OCH_3-C_6H_4 \\
NH \quad 3,4,5-(OCH_3)_3C_6H_2 \\

Virsodia & Co workers (2008) reported the synthesis, biological evaluation and 3D-QSAR analysis of a set of substituted N-phenyl-6-methyl-2-ooxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamides as antitubercular agents. Substituted acetoaceta nilides were reacted with various aromatic aldehydes and urea yielded the tetrahydropyrimidine derivatives with a phenyl carbamoyl group at C-5 position, and with various substitutions on the 4-phenyl and the N-phenyl aromatic rings. Newly synthesized compounds were tested in vitro for their antitubercular activity against *Mycobacterium tuberculosis* H37RV.

Among the synthesized compounds, compound N-(2,3-Dimethylphenyl)-6-methyl-2-ooxo-4-(3-phenoxypyphenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxamide (3c) was found to be more active agent against *M. tuberculosis* H37RV with % inhibition of 65μg/mL.53.
Activity: Antimalarial

Bello & Co workers (2008) investigated the structure-activity relationships of various C₆ derivatives of UMP. 6-Cyano, 6-azido, 6-amino, 6-methyl, 6-N-methylamino, and 6-N,N-dimethyl amino derivatives of uridine were evaluated against *P. falciparum*. The mononucleotides of 6-cyano, 6-azido, 6-amino, and 6-methyl uridine derivatives were studied as inhibitors of plasmodial orotidine 5'-monophosphate decarboxylase (ODCase). 6-Methyluridine exhibited weak antimalarial activity against *P. falciparum* 3D7 isolate.

\[
\begin{align*}
\text{CN} & \\
\text{N₃} & \\
\text{NH₂} & \\
\text{CH₃} & \\
\text{NHCH₃} & 
\end{align*}
\]

Agarwal & Co workers (2005) reported synthesis of new 2,4,6-trisubstituted pyrimidines by cyclization of chalcones with imine hydrochlorides in the presence of sodium isopropoxide and evaluated for antimalarial activity against *P. falciparum*. Most of the compounds have shown MIC in the range of 0.25-2μgm/mL and are several fold active than standard pyrimethamine *in vitro*.
Tarnchompoo & Co workers (2002) reported the reduced binding of pyrimethamine to Ser108Asn (S108N) mutants of parasite Dihydrofolate Reductase (DHFR), which forms the basis of resistance of *P. falciparum* to pyrimethamine. Removal of the *p*-Cl or replacement with *m*-Cl led to better binding with the mutant DHFRs. Some of these compounds show good antimalarial activities against pyrimethamine resistant *P. falciparum* containing the mutant DHFRs with low cytotoxicity to three mammalian cell lines.\(^6\)

**Activity: Antifilarial**

Katiyar & Co workers (2005) synthesized series of compounds of tri substituted pyrimidine derivatives and evaluated for their *in vitro* Topoisomerase II inhibitory activity against filarial parasite *Setaria cervi*. Five compounds exhibited 70–80% inhibition at 10 μg/mL concentration and three compounds have shown 40–60% inhibition at 5 μg/mL concentration. All the mentioned compounds have shown better Topoisomerase II inhibitory activity than standard antifilarial drug (DEC) and standard Topoisomerase II inhibitors (Novobiocin, Nalidixic acid).\(^7\)
Activity: Antileishmanial

Sunduru & Co workers (2009) have been synthesized a series of 2,4,6-trisubstituted pyrimidines and 1,3,5-triazines and screened for their \textit{in vitro} and \textit{in vivo} antileishmanial activity against \textit{Leishmania donovani}. Three compounds 13, 32 and 33 with good selectivity index (S.I.) were screened for their \textit{in vivo} activity in golden hamsters (\textit{Mesocricetus auratus}) infected with MHOM/IN/80/Dd (8) strain of \textit{L. donovani}.$^{38}$

Activity: Anti inflammatory

Lebsack & Co workers (2009) have identified and synthesized 2,7-diamino-thiazolo[5,4-\textit{d}]pyrimidines as TRPV1 antagonists. An exploration of the structure-activity relationships at the 2, 5, and 7-positions of the thiazolo [5,4-\textit{d}]pyrimidine led to the identification of several highly potent TRPV1 antagonists, including 3. TRPV1 knockout mice demonstrate an impaired ability to develop inflammatory
thermal hyperalgesia, suggesting that TRPV1 has an important role in transmitting inflammatory pain signals. The most active compound \( N^2-(2,6\text{-dichlorophenyl})-N^2-[4\text{-}(\text{trifluoromethyl})\text{phenyl}]1,3\text{thiazolo}[5,4-d]\text{pyrimidine-2,7-diamine} \) was orally bioavailable and afforded a significant reversal of carrageenan-induced thermal hyperalgesia\(^9\).

\[ \text{Structure Image} \]

**Panda & Co workers** (2008) reported anti-inflammatory, antioxidant and antibacterial activities of 4-(indol-3-yl)-6-(4-substitutedphenyl)-2-substituted pyrimidines via chalcones prepared by Vilsemier Haack reaction of indole-3-aldehyde with 4-substituted acetophenone. As far as anti-inflammatory activity is concerned, compound 4-(indol-3-yl)-6-(4 aminophenyl)-2-thiopyrimidine was found to have significant anti-inflammatory activity by carageenan-induced rat paw oedema method\(^60\).

\[ \text{Structure Image} \]

**Activity: Antifungal**

**Singh & Co workers** (2008) have synthesized dihydropyrimidinones (80–96\% yields) by the Biginelli reaction. Copper (II) chloride in the absence of any solvent, efficiently catalyses the six compounds were selected and examined their antifungal activities against the radial growth of three fungal species viz., *Trichoderma hammatum*, *Trichoderma koningii* and *Aspergillus niger*\(^61\).
Literature Review

\[ X = \text{O, S}; R_2 = \text{Et, Isopropyl, } R_3 = (4-	ext{Cl})\text{Ph, (4-OMe)Ph, (2,4-OMe)Ph} \]

**Activity: Antiparkinsonism**

*Gillespie & Co workers (2009)* have identified a novel series of antagonists of the human \( A_{2A} \) receptor and have been shown to display good potency and high degrees of selectivity over other receptor sub-types. Displaying *in vivo* potency in commonly used disease models and high oral bio-availability, this class of compounds may serve as clinically useful treatments for the relief of the symptoms associated with Parkinson’s disease\(^6\).

**Activity: Antidiabetic**

*Lee & Co workers (2005)* reported the synthesis and antidiabetic activity of novel substituted pyrimidines having thiazolidinedione moiety. All the synthesized compounds were evaluated for their glucose and lipid lowering activity in genetically diabetic KKA\(^Y\) mice. From the results, the compounds, 5-(4-{2-[methyl-(6-phenoxy pyrimidin-4-yl)amino]ethoxy}benzyl)thiazolidine-2,4-dione (5c) and 5-(4-{2-[6-(4-methoxyphenoxy)pyrimidin-4-yl]methylaminoethoxy} benzyl)thiazolidine-2,4-dione (5g), exhibited considerably more potent biological activity than that of the reference compounds, pioglitazone and rosiglitazone, respectively\(^6\).
Activity: Antiplatelet and antithrombotic

Bruno & Co workers (2006) reported the synthesis of new 2-substituted benzo pyran[4,3-d]pyrimidin-4-cycloamines and 4-amino/cycloamino-benzopyran[4,3-d]pyrimidin-5-ones endowed with in vitro anti-aggregating activity. Some tested compounds showed a large-spectrum antiplatelet activity in vitro, and are more potent than aspirin as antithrombotics in vivo.

\[
\begin{align*}
R &= \text{OC}_3\text{H}_3, \\
R &= \text{OCH}_3\text{C}_6\text{H}_4
\end{align*}
\]

Activity: Antiviral

Donghi & Co workers (2009) reported a new class of inhibitors of HIV-1 Integrase which has been optimized to provide selective and highly efficient strand transfer inhibition.

\[
\begin{align*}
X &= \text{CO, R = H, NH}_2, \text{Cycloamines; R} \_1 = \text{H, NH}_2, \text{OCH}_3, \text{SCH}_3
\end{align*}
\]

Radi & Co workers (2008) synthesized a small family of 5-DABOC cytosine analogues (5-DABOCs) and biologically evaluated as HIV-1 inhibitor both on wild
type and drug-resistant mutants. An interesting compound (5d) has been identified which showed a predicted pharmacokinetic profile similar to that of anti-HIV drugs on the market. Molecular modelling studies have been finally performed in order to rationalize the results.

![Chemical Structure](image)

**Activity: CCR5 Antagonist**

Yang & Co workers (2009) reported the development of new agents for the treatment of HIV-1/AIDS which remains a necessity, particularly due to the ongoing problem of resistance development to current therapies. Efforts at Roche have led to the discovery of a series of potent CCR5 antagonists represented by structure 1. They developed a convenient synthesis of 9-benzyl-1-butyl-3,9-diazaspiro[5.5]undecane 4 and undecan-2-one 5 via Michael addition of the lithium enolate 2 to the tetrasubstituted olefin acceptor.

![Chemical Structure](image)

**Patents Available on Pyrimidine**

Q₁ & Q₂ = bears (Ia) substituent formula independently.

Qi & Q₂ = Aryl, Heteroaryl, N-(C₄₋₅-alkyl)amino, N,N-di-(C₄₋₅-alkyl)amino,
Phenyl, Phenoxyl, C₃₋₄-alkyl, C₃₋₄-alkoxy.

G = -O- or -NR².
X = -CH₂-, -O-, -NH-, -S-
Y = H, C₄₋₅-alkyl
Y' = H or alkyl
R₁ = H, Halo, OH, NO₂, NH₂, N-(C₄₋₅-alkyl)amino, N,N-di-(C₄₋₅-alkyl)amino,
Cyano, Sulphamoyl, C₃₋₅-alkynyl, Carbamoyl.
R₂ = H, C₃₋₅-alkynyl, C₃₋₅-alkenyl.

Pease et al. (2006) synthesized a series of 2,4-di(hetero)arylamino-(oxy)-5-

Pease et al. (2006) synthesized a series of pyrimidine derivatives as selective inhibitors of Cell Cycle Kinase which were found to be potent anti-proliferative agents. Patent No: US,7,153,964,B2.

\[ \text{Formula 1 (Ia)} \]

\[ \text{Formula 1 (Ia')} \]

Q\(_1\) & Q\(_2\) = bears (Ia) & (Ia') substituent formula independently.

G = -O- or -S-

R\(_1\) = H, Halo, OH, NO\(_2\), NH\(_2\), N-(C\(_1,3\)alkyl)amino, N,N-di-(C\(_1,3\)alkyl)amino.

Y = NHS(O)\(_2\), S(O)\(_2\)NH, S(O)\(_2\)

Z = Phenyl, Heteroaryl

n = 0 or 1

m = 1,2,3

N = (C\(_1,3\)alkyl)amino, Cyanotrifluoromethyl, trichloromethyl, N,N-di-(C\(_1,3\)alkyl)amino, C\(_1,3\)alkoxy, C\(_1,3\)alkysulphanyl.

Pease et al. (2004) synthesized a series of pyrimidine derivatives as selective inhibitors of Cell Cycle Kinase which were found to be potent anti-proliferative agents. Patent No: US,6710,052, B2.

Q₁ & Q₂ = bears (Ia) substituent formula independently.
G = -O- or -S-
X = -CH₂⁻, -O-, -NH-, -S-
Y₁ = H, C₅alkyl
Y₂ = H or alkyl
R₃ = H, Halo, OH, NO₂, NH₂, N-(C₅alkyl)amino, N,N-di-(C₅alkyl)amino.
Z = nitrogen linked heteroaryl
n = 1, 2, 3
m = 1, 2, 3

![Pyrimidine derivative](image)

Q₁ & Q₂ = Phenyl, Naphthyl, Indanyl, 1,2,3,4-tetrahydronaphthyl, Morpholino, benzyl, 2-Phenylethyl, (3-5C)Alkenyl independently.

R₁ = H, Halo, OH, NO₂, NH₂, N-(C₁₅, alkyl)amino, N,N-di(C₁₅, alkyl)amino.


![Guanidinopyrimidine derivative](image)

R & R₂ = guanidino

R₁ = H, Halo, F, Cl, Br, I, amino


![ThiazoloPyrimidine derivative](image)

R₁ = H, F, Cl, Br, I, alkyl (upto 3 carbon atom), dimethylamino

R₂ = H, F, Cl, Br, I, alkyl (upto 3 carbon atom)

n = 0,1,2

Q = divalent moiety of the formula -CH₂-, -CH₂CH₂-, 

-CH₂CH₂CH₃.
REFERENCES

1. Brugnatelli, Ann Chim, 8, 1818, 201.
2. Grimaux, Ber, 12, 1879, 378.
3. Pinner, Ber, 17, 1884, 2519.
18. Ajello, Gazz chim ital 70, 1940, 504.
22. Schmedes, Ann, 441, 1925, 192.
23. Kircher, Ann, 385, 1911, 293.


References


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CLINICALLY USED DRUGS
## CLINICALLY USED DRUGS CONTAINING PYRIMIDINE NUCLEUS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
<td>Nimustine</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Anticancer (leukemia)</td>
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<tr>
<td>Uramustine</td>
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<td>Anticancer (Non-Hodkin's lymphoma)</td>
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<td>Tegafur</td>
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<tr>
<td>Raltitrexed</td>
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<td>Sedative Hypnotic &amp; Anti-convulsant</td>
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<table>
<thead>
<tr>
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RESEARCH
ENVISAGED
The selectivity of anticancer drugs towards cancerous cells without harming the normal cells is a serious problem. In addition to these, the narrow therapeutic index, various side effects and obstacles of multi-drug resistance necessitated the search for new anticancer pharmacophore.

There are large number of anticancer agents possessing pyrimidine ring viz Cytarabine, Fludarabine, Cladribine, Raltitrexed and Caepectabine (antimetabolites) and Nimustine and Uramustine (alkylating agents).

Cladribine, Cytarabine, Gemcitabine and Nimustine, are effective agents against lymphoproliferative diseases; having free amino group at the 4th position of the pyrimidine ring in their chemical structures which might have responsible for pronounced anticancer properties.

Therefore it was aimed to synthesize a new series of pyrimidines having free amino group at the 2nd position in order to investigate the effect of structural variation on the anticancer activity.

Apart from these, diuretics are the most frequently prescribed therapeutic agents for the treatment of edema, hypertension and other cardiac ailments.

Current diuretic regimens are associated with various unpleasant side effects as well as lack of oral activity which necessitates the design and synthesis of newer diuretics.

As evident by literature survey, compounds having atleast one guanidino substituent at the 2nd or 4th position of the pyrimidine ring as well as mercapto group present at the 2nd position of the pyrimidine ring were found to possess significant diuretic activity.

These findings prompted us to synthesize pyrimidine derivatives having 2-mercapto and 2-amino substituents so as to observe the effect of substitution on diuretic activity.
REFERENCES


PLAN OF WORK
PLAN OF WORK

The plan of work was meant to synthesize different series of pyrimidine derivatives. The synthesized compounds were characterized by modern analytical techniques (FT-IR, $^1$H NMR & Mass) and screened for in vitro anticancer and in vivo diuretic activity.

The plan of work comprises of:

- Exhaustive literature survey of various series of pyrimidine derivatives which were already synthesized and tested for biological activity.
- Procurement of chemicals and Synthesis of intermediates and final products as per the reaction scheme.
- Determination of physico-chemical properties of the synthesized compounds i.e. melting point, solubility profile and TLC.
- Determination of elemental analysis for C, H and N.
- Structure elucidation of synthesized compounds by FT-IR, $^1$H-NMR spectroscopy and Mass spectrometry.
- Biological screening of synthesized compounds:
  - Evaluation of in vitro anticancer activity of the synthesized compounds.
  - Evaluation of in vivo diuretic activity.
  - Assessment of Acute toxicity studies (hepatotoxicity) of potent compounds:
    - Alkaline Phosphatase (AP)
    - Serum glutamic oxaloacetic transaminase (SGOT)
    - Serum glutamic pyruvic transaminase (SGPT)
    - Total Protein (TP)
    - Total Bilirubin (TB)
EXPERIMENTAL WORK
EXPERIMENTAL WORK

The experimental work is divided into two parts:

A. Reaction Schemes and Synthetic Work

Various newer acetophenones were reacted with different aromatic aldehydes by Claissen Schmidt Condensation. It is a carbon-carbon bond forming reaction that occurs in the presence of a base resulting in the formation of α,β-unsaturated compounds. Cyclization of these chalcones with guanidine hydrochloride, urea and thiourea resulted in the formation of various aminopyrimidines, hydroxypyrimidines and thiopyrimidines respectively.

This part of work deals with the detailed study of steps involved in the synthesis of various new substituted pyrimidine derivatives as per the reaction schemes (Scheme I, II, III, IV and V). The data of the structural conformation of the synthesized compounds by modern analytical techniques like FT-IR, 1H-NMR and Mass spectrometry were given.

B. Biological Evaluation

Biological evaluation part deals with the *in vitro* anticancer activity (Scheme I, II and III) of newly synthesized compounds by SRB assay method. The compounds of (Scheme-IV and V) were screened for *in vivo* diuretic activity by adopting the Lipschitz method. The potent compounds obtained from *in vitro* anti cancer screening as well as *in vivo* diuretic activity were evaluated for hepatotoxicity studies to investigate any toxicity to the liver compared to control and standard.
Scheme- I (Anti cancer activity)

\[
\begin{align*}
\text{Scheme- I (Anti cancer activity)}
\end{align*}
\]

Ar = Phenyl, 4-Methoxyphenyl, 4-Fluorophenyl, 2-Furyl, 2-Chlorophenyl, 3, 4-Dimethoxyphenyl, 2-Hydroxyphenyl, 2,6- Dichlorophenyl, 3-Methoxy-4-hydroxyphenyl, 4-Dimethylaminophenyl, 3-Nitrophenyl, 3- Indolyl
Scheme II (Anti cancer activity)

\[ \text{Scheme} \]

\[ \begin{align*}
\text{Phenyl ketone} + \text{Aldehyde} & \xrightarrow{\text{NaOH/EtOH}} \text{Product} \\
\text{NaOH/EtOH} & \xrightarrow{\text{Phosphine}} \text{Final Product}
\end{align*} \]

\( R = \text{H, 4-Methoxy, 2-Chloro, 3, 4-Dimethoxy, 4-Fluoro, 2, 6-Dichloro, 4-Dimethylamino, 2-Hydroxy, 3-Nitro.} \)
Scheme- III (Anti cancer activity)

R = H, 4-Fluoro, 2-Chloro, 4-Methoxy, 4-Dimethylamino, 3,4-Dimethoxy, 3-Methoxy-4-hydroxy, 3,4,5-Trimethoxy, 2,6-Dichloro, 3-Nitro, 2-Hydroxy, 3-Bromo, 2,3,4-Trimethoxy.
Scheme- IV (Diuretic activity)

\[
\text{HO-CHO} + \text{Cl-CHOH} \xrightarrow{\text{KOH}} \text{HO-CO-CHO} \\
\text{(LXXXV)}
\]

\[
\text{KOH} \xrightarrow{\text{CH}_3\text{OH}} \text{R-CH}_2\text{O} \\
\text{(LXXXVI - XCI)}
\]

\[
\text{alc. KOH} \xrightarrow{\text{dry Dioxane}} \text{H}_2\text{N-CONH}_2 \\
\text{(XCVI - XCVII)}
\]

R= H, 4-Fluoro, 2-Hydroxy, 4-Methoxy, 2, 6- Dichloro, 2,4-Dihydroxy
Scheme- V (Diuretic activity)

\[ \text{Scheme} \]

\[
\begin{align*}
\begin{array}{c}
\text{R} = \text{H, 4-Methoxy, 4-Fluoro, 2-Chloro, 2,6-Dichloro, 3,4-Dimethoxy} \\
\end{array}
\end{align*}
\]
B. SYNTHETIC WORK

MATERIALS AND METHODS

Reagents and solvent

The chemicals used for experimental work were procured from various chemical units viz. Sigma-Aldrich (India), E. Merck (Germany) and Qualigen. The solvent and reagents were of AR grade and purified before use.

The silica gel-G (160-120 mesh) used for chromatography (TLC) was obtained from E. Merck India Ltd. The solvent system used for TLC were Petroleum ether: Ethyl acetate (7:3), Toluene: Ethyl Acetate: Formic acid (5:4:1) and Benzene: Acetone (9:1). The Whatman no.1 filter paper was used for vacuum filtration.

Instrument and Equipment

Melting points were determined by open tube capillary method and were uncorrected. Thin layer chromatography (TLC) plates prepared by silica gel-G were used to monitor the reactions as well as to confirm the purity of the synthesized compounds. Iodine chamber and UV lamp were used for visualization of TLC spots.

All the Fourier Transform Infra Red (FT-IR) spectra were recorded on Perkin-Elmer 1720 FT-IR spectrophotometer using KBr Pellets; \( \nu_{\text{max}} \) values were given in cm\(^{-1}\). \(^1\)H NMR spectra were recorded on Bruker AC 300/400 MHz using trimethylsilane (TMS) as internal standard in CDCl\(_3\) / DMSO-\(d_6\) as solvents. Chemical shifts were given in \( \delta \) (ppm) scale and coupling constants (\( J \) values) were expressed in Hz. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. The \( m/z \) values of the more intense peaks were mentioned. Solubility of the synthesized compounds was checked in different solvents at room temperature (28°-38°C). Elemental analysis was carried out on Perkin-Elmer Model 240 Elemental Analyzer and values were found within ±0.4% of the theoretical values.
Experimental

**SCHEME-I**

**Synthesis of 1-(1H-Benz[d]imidazol-2-yl)ethanol (I)**

o-Phenylenediamine was allowed to reflux in excess of lactic acid for four h. After completion of the reaction the content was poured on to the crushed ice. The excess of acid was neutralized with ammonia and the crystalline product was filtered, washed with water, dried and recrystallized to get shiny pure crystals of compound.

$$\begin{align*}
\text{NH}_2 & + \text{HOOC} - \text{CH}_3 \\
\text{H}_2\text{N} & \text{OH} \\
\text{Reflux} & \\
\text{NH} & \text{CH}_3
\end{align*}$$

FT-IR (KBr) cm⁻¹: 3350 (O-H), 3300 (N-H), 3025 (Ar-H), 2880 (C-H), 1606 (C=N), 1325 (C-N);¹H-NMR (DMSO-d₆) ppm: 2.18 (3H, d, CH₃), 3.65 (1H, s, OH), 4.90 (1H, m, CH), 7.45-7.67 (4H, m, Ar-H), 12.19 (1H, s, NH-benzimidazole).

**Synthesis of 2-Acetyl benzimidazole (II)**

Aqueous solution of chromium trioxide (0.002 M) was added dropwise to the acidic solution of compound I (0.015 M) keeping the temperature 90°C. Once the addition was completed, the temperature was raised upto 100°C and the reaction mixture was heated at the same temperature for 5 min. There after the cooled contents were poured into a pool of 500 mL of water with constant stirring. The solution was filtered off and a flocculant precipitate was discarded. From the filtrate the product was extracted in chloroform and solid/precipitate was obtained after filtration under reduced pressure. The recrystallization was done from benzene (Caution!).

$$\begin{align*}
\text{OH} & \text{CH}_3 \\
\text{CH}_3\text{COOH} & \\
\text{Chromium trioxide} & \\
\text{N} & \text{CH}_3
\end{align*}$$

---

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FT-IR (KBr) cm⁻¹: 3290 (N-H), 3046 (Ar-H), 2910 (C-H), 1675 (C=O), 1579 (C=N), 1314 (C-N); ¹H-NMR (DMSO-d₆) ppm: 2.82 (3H, s, CH₃), 7.33-7.88 (4H, m, Ar-H) 12.86 (1H, s, NH-benzimidazole).

General procedure for synthesis of 1-(1H-benzimidazol-2-yl)-3-(substituted phenyl) prop-2-en-1-one (III-XIV)

2-Acetylbenzimidazole (II) was allowed to react with substituted aromatic aldehydes in 10% ethanolic NaOH solution to obtain the desired chalcones derivatives (III-XIV).

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<td>Phenyl</td>
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<tr>
<td>IV</td>
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<td>V</td>
<td>4-Fluorophenyl</td>
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<td>VI</td>
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<td>3-Nitrophenyl</td>
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<tr>
<td>XIV</td>
<td>3-Indolyl</td>
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</table>
Spectral data of the synthesized compounds (III-XIV)

1-(1H-Benzimidazol-2-yl)-3-phenylprop-2-en-1-one (III)

![Chemical structure of 1-(1H-Benzimidazol-2-yl)-3-phenylprop-2-en-1-one (III)](image)

**FT-IR (KBr) cm⁻¹**: 3305 (N-H), 3028 (Ar-H), 1661 (C=O), 1635 (C=C), 1594 (C=N), 1329 (C-N); **¹H-NMR (DMSO-d₆) ppm**: 6.68-7.50 (9H, m, Ar-H), 6.92 (1H, d, J = 16.8 Hz, H₆), 7.11 (1H, d, J = 16.5Hz, H₅), 13.32 (1H, s, NH-benzimidazole); Anal. Calcd. for C₁₆H₁₂N₂O: C: 77.40, H: 4.87, N: 11.28. Found: C: 77.12, H: 4.88, N: 11.25.

1-(1H-Benzimidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (IV)

![Chemical structure of 1-(1H-Benzimidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (IV)](image)

**FT-IR (KBr) cm⁻¹**: 3301 (N-H), 3076 (Ar-H), 2890 (C-H), 1653 (C=O), 1620 (C=C), 1576 (C=N), 1330 (C-N); **¹H-NMR (DMSO-d₆) ppm**: 3.63 (3H, s, OCH₃), 6.46 (1H, d, J = 16.4 Hz, H₆), 6.92-7.11 (4H, m, Anisyl), 7.34 (1H, d, J = 16.0 Hz, H₅), 7.42-7.85 (4H, m, Ar-H), 13.41 (1H, s, NH-benzimidazole); Anal. Calcd. for C₁₇H₁₄N₂O₂: C: 73.37, H: 5.07, N: 10.07. Found: C: 73.52, H: 5.06, N: 10.08.

1-(1H-Benzimidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (V)

![Chemical structure of 1-(1H-Benzimidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (V)](image)

**FT-IR (KBr) cm⁻¹**: 3292 (N-H), 3039 (Ar-H), 1660 (C=O), 1600 (C=C), 1577 (C=N), 1335 (C-N), 1117 (C-F); **¹H-NMR (DMSO-d₆) ppm**: 6.42 (1H, d, J = 15.6 Hz, H₆), 7.22 (1H, d, J = 15.4 Hz, H₅), 7.43-7.89 (8H, m, Ar-H), 13.10 (1H, s, NH-benzimidazole); Anal. Calcd. for C₁₆H₁₁N₂OF: C: 72.17, H: 4.16; N: 10.52. Found: C: 71.98; H: 4.15; N: 10.51.
Experimental

Synthetic Work

1-(1H-Benzimidazol-2-yl)-3-(furan-2-yl)prop-2-en-1-one (VI)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \ 3310 (N-H), 3069 (Ar-H), 1670 (C=O), 1640 (C=C), 1589 \\
& \ (C=N), 1338 (C-O-C); \ \text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm: 6.34 (1H, d, } J = \\
& \ 15.6 \text{ Hz, H'}), 6.58-7.32 (7H, m, Ar-H, furan), 7.51 (1H, d, } J = 15.6 \text{ Hz, H}_2; \\
& \ 13.20 (1H, s, NH-benzimidazole); \ \text{Anal. Caled. for C}_{14}H_{10}N_2O_2: C: 70.58, H: 4.23, N: 11.76. \ \text{Found: C:70.26, H: 4.22, N: 11.72.}
\end{align*}
\]

1-(1H-Benzimidazol-2-yl)-3-(2-chlorophenyl)prop-2-en-1-one (VII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \ 3286 (N-H), 3035 (Ar-H), 1653 (C=O), 1610 (C=C), 1578 \\
& \ (C=N), 1332 (C-N), 1073 (C-Cl); \ \text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm: 6.32 (1H, d, } J = \\
& \ 16.0 \text{ Hz, H'}), 7.21-7.75 (8H, m, Ar-H), 7.89 (1H, d, } J = 16.2 \text{ Hz, H}_2; \\
& \ 13.15 (1H, s, NH-benzimidazole); \ \text{Anal. Caled. for C}_{16}H_{11}N_2OCl: C: 67.97, H: 3.92, N: 9.91. \\
& \ \text{Found: C:68.12, H: 3.91, N: 9.90.}
\end{align*}
\]

1-(1H-Benzimidazol-2-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (VIII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \ 3321 (N-H), 3035 (Ar-H), 2935 (C-H), 1653 (C=O), 1598 \\
& \ (C=C), 1508 (C=N), 1330 (C-N); \ \text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm: 3.5 (6H, s, 2 \times \\
& \ OCH_3), 6.26 (1H, d, } J = 16.4 \text{ Hz, H'}), 6.68-7.14 (3H, m, Ar-H), 7.23-7.46 (4H, m, \\
& \ Ar-H), 7.59 (1H, d, } J = 16.0 \text{ Hz, H}_2; \\
& \ 12.91 (1H, s, NH-benzimidazole); \ \text{Anal. Caled. for C}_{18}H_{16}N_2O_3: C: 70.12, H: 5.23, N: 9.09. \ \text{Found: C:70.05, H: 5.24, N: 9.09.}
\end{align*}
\]
Experimental Synthetic Work

1-(1H-Benzimidazol-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (IX)

\[
\text{FT-IR (KBr) cm}^{-1}: \quad 3354 (\text{O-H}), \quad 3311 (\text{N-H}), \quad 3019 (\text{Ar-H}), \quad 1683 (\text{C=O}), \quad 1610 (\text{C=C}), \quad 1512 (\text{C=N}), \quad 1336 (\text{C-N}); \quad ^1\text{H-NMR (DMSO-\text{d}_6) ppm: } 5.30 (1\text{H, s, OH}), 6.12 (1\text{H, d, } J = 15.6 \text{ Hz, H}_\alpha), \quad 6.62-7.57 (8\text{H, m, Ar-H}), \quad 7.73 (1\text{H, d, } J = 15.4 \text{ Hz, H}_\beta), \quad 12.45 (1\text{H, s, NH-benzimidazole}); \quad \text{Anal. Calcd. for } C_{16}H_{12}N_2O_2: C: 72.72, H: 4.58, N: 10.60. \quad \text{Found: C: 72.43, H: 4.56, N: 10.59.}
\]

1-(1H-Benzimidazol-2-yl)-3-(2,6-dichlorophenyl)prop-2-en-1-one (X)

\[
\text{FT-IR (KBr) cm}^{-1}: \quad 3298 (\text{N-H}), \quad 3019 (\text{Ar-H}), \quad 1653 (\text{C=O}), \quad 1600 (\text{C=C}), \quad 1518 (\text{C=N}), \quad 1332 (\text{C-N}), \quad 1069 (\text{C-Cl}); \quad ^1\text{H-NMR (DMSO-\text{d}_6) ppm: } 6.19 (1\text{H, d, } J = 16.4 \text{ Hz, H}_\alpha), \quad 7.41-7.60 (4\text{H, m, Ar-H}), \quad 7.69 (1\text{H, d, } J = 16.4 \text{ Hz, H}_\beta), \quad 7.72-7.89 (3\text{H, m, Ar-H}), \quad 13.19 (1\text{H, s, NH-benzimidazole}); \quad \text{Anal. Calcd. for } C_{16}H_{10}Cl_2N_2O: C: 60.59, H: 3.18, N: 8.83. \quad \text{Found: C: 60.57, H: 3.17, N: 8.83.}
\]

1-(1H-Benzimidazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (XI)

\[
\text{FT-IR (KBr) cm}^{-1}: \quad 3310 (\text{O-H}), \quad 3281 (\text{N-H}), \quad 3010 (\text{Ar-H}), \quad 1683 (\text{C=O}), \quad 1590 (\text{C=C}), \quad 1512 (\text{C=N}), \quad 1336 (\text{C-N}); \quad ^1\text{H-NMR (DMSO-\text{d}_6) ppm: } 3.76 (3\text{H, s, OCH}_3), 5.67 (1\text{H, s, OH}), \quad 6.13 (1\text{H, d, Hz, } J = 16.2 \text{ Hz, H}_\alpha), \quad 6.33-7.46 (7\text{H, m, Ar-H}), \quad 7.82
\]
Experimental Synthetic Work

(1H, d, J = 16.0 Hz, H_d). 13.23 (1H, s, NH-benzimidazole); Anal. Caled. for C_{17}H_{14}N_{2}O_{3}: C: 69.38, H: 4.79, N: 9.52. Found: C: 69.61, H: 4.78, N: 9.50.

1-(1H-Benzimidazol-2-yl)-3-[4-(dimethylamino)phenyl] prop-2-en-1-one (XII)

\[
\text{FT-IR (KBr) cm}^{-1}: 3261 (N-H), 3023 (Ar-H), 2932 (C-H), 1653 (C=O), 1600 (C=C), 1523 (C=N), 1338 (C-N); ^1\text{H-NMR (DMSO-}d_6\text{) ppm: 2.92 (6H, s, 2} \times \text{CH}_3, 6.22 (1H, d, J = 16.4 Hz, H_d), 6.51-7.79 (8H, m, Ar-H), 7.91 (1H, d, J = 16.2 Hz, H_b), 13.23 (1H, s, NH-benzimidazole); Anal. Caled. for C_{18}H_{17}N_{3}O: C: 74.20, H: 5.88, N: 14.42. Found: C:73.90 , H: 5.85, N: 14.38.
\]

1-(1H-Benzimidazol-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (XIII)

\[
\text{FT-IR (KBr) cm}^{-1}: 3284 (N-H), 3014 (Ar-H), 1657 (C=O), 1609 (C=C), 1579 (C=N), 1531, 1325 (N=O)_{2}, 1372 (C-N); ^1\text{H-NMR (DMSO-}d_6\text{) ppm: 6.17 (1H, d, J = 16.0 Hz, H_d), 7.09-7.51 (4H, m, benzimidazolyl), 7.63 (1H, d, J = 16.4 Hz, H_b), 7.77-7.99 (4H, m, Ar-H), 13.18 (1H, s, NH-benzimidazole); Anal. Caled. for C_{16}H_{11}N_{3}O_{3}: C : 65.53, H: 3.78, N :14.33. Found: C: 65.28, H: 3.76, N: 14.29.
\]

1-(1H-Benzimidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (XIV)
Experimental

Synthetic Work

FT-IR (KBr) cm⁻¹: 3310 (N-H), 3024 (Ar-H), 1657 (C=O), 1594 (C=C), 1572 (C=N), 1334 (C-N); ¹H-NMR (DMSO-d₆) ppm: 6.23 (1H, d, J = 15.6 Hz, Hα), 6.99-7.13 (4H, m, indolyl), 7.33-7.69 (4H, m, benzimidazolyl), 7.78 (1H, d, J = 15.4 Hz, Hβ), 9.28 (1H, s, CH-indolyl), 10.66 (1H, s, NH-indolyl), 13.23 (1H, s, NH-benzimidazole); Anal. Calcd. for C₁₈H₁₃N₃O: C: 75.25, H: 4.56, N: 14.63. Found: C: 75.25, H: 4.54, N: 14.59.

General procedure for synthesis of 4-(1H-benzimidazol-2-yl)-6-(substitutedphenyl)-1,6-dihydropyrimidin-2-amine (XV-XXVI)

To a solution of guanidine hydrochloride (1.1 M) in 50 mL of isopropanol, sodium metal was added (sodium isopropoxide generated in situ). The reaction mixture was refluxed for 2 h and then different chalcones (III-XIV, 1.0 M) were added to it and refluxed for 8 h. The solvent was removed from the reaction mixture under reduced pressure. Water was added and the aqueous phase was extracted with chloroform. The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was purified by crystallization from methanol or ethanol.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Ar</th>
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<tbody>
<tr>
<td>XV</td>
<td>Phenyl</td>
</tr>
<tr>
<td>XVI</td>
<td>4-Methoxyphenyl</td>
</tr>
<tr>
<td>XVII</td>
<td>4-Fluorophenyl</td>
</tr>
<tr>
<td>XVIII</td>
<td>2-Peryl</td>
</tr>
<tr>
<td>XIX</td>
<td>2-Chlorophenyl</td>
</tr>
</tbody>
</table>
Spectral data of the synthesized compounds (XV-XXVI)

4-(1H-Benzimidazol-2-yl)-6-phenyl-1,6-dihydropyrimidin-2-amine (XV)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3461, 3389 (1^\circ-N-H), 3320 (2^\circ-N-H), 3089 (Ar-H), 1640 (C=N), 1333 (C-N); \\
\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm:} & \quad 4.91 (1H, d, CH), 5.30 (2H, br s, NH_2), 5.75 (1H, d, NH-CH), 6.98-7.43 (9H, m, Ar-H), 7.69 (1H, s, NH-pyrimidine), 13.21 (1H, s, NH-benzimidazole); \\
\text{Mass m/z:} & \quad M^+ 289, M^{+1} 290; \\
\text{Anal. Calcd. for C}_{17}\text{H}_{15}\text{N}_{5}:} & \quad \text{C: 70.57, H: 5.23, N: 24.21. Found: C: 70.29, H: 5.21, N: 24.22.}
\end{align*}
\]

4-(1H-Benzimidazol-2-yl)-6-(4-methoxyphenyl)-1,6-dihydropyrimidin-2-amine (XVI)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3459, 3390 (1^\circ-N-H), 3344 (2^\circ-N-H), 3055 (Ar-H), 2925 (C-H), 1610 (C=N), 1332 (C-N); \\
\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm:} & \quad 3.52 (3H, s, OCH_3),
\end{align*}
\]
Experimental

4.95 (1H, d, CH), 5.19 (2H, br s, NH₂), 5.82 (1H, d, NH-CH), 7.04-7.32 (4H, m, Anisyl), 7.60 (1H, s, NH-pyrimidine), 7.90-8.18 (4H, m, Ar-H), 13.12 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 319, M⁺⁺ 320; Anal. Calcd. for C₁₈H₁₇N₃O: C: 67.70, H: 5.37, N: 21.93. Found: C: 67.43, H: 5.36, N: 21.93.

4-(1H-Benzimidazol-2-yl)-6-(4-fluorophenyl)-1,6-dihydropyrimidin-2-amine (XVII)

FT-IR (KBr) cm⁻¹: 3472, 3376 (1°-N-H), 3338 (2°-N-H), 3095 (Ar-H), 1611(C=N), 1330 (C-N), 1110 (C-F); ¹H-NMR (DMSO-d₆) ppm: 4.77 (1H, d, CH), 5.24 (2H, br s, NH₂), 5.78 (1H, d, NH-CH), 6.88-7.13 (8H, m, Ar-H), 7.71 (1H, s, NH-pyrimidine), 13.29 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 307, M⁺⁺ 308; Anal. Calcd. for C₁₇H₁₄FN₃: C: 66.44, H: 4.59, N: 22.79. Found: C: 66.20, H: 4.57, N: 22.69.

4-(1H-Benzimidazol-2-yl)-6-(furan-2-yl)-1,6-dihydropyrimidin-2-amine (XVIII)

FT-IR (KBr) cm⁻¹: 3466, 3382 (1°-N-H), 3329 (2°-N-H), 3086 (Ar-H), 1614 (C=N), 1328 (C-N), 1092 (C-O-C); ¹H-NMR (DMSO-d₆) ppm: 4.69 (1H, d, CH), 5.27 (2H, br s, NH₂), 5.68 (1H, d, NH-CH), 6.58-7.62 (7H, m, Ar-H, furan), 7.63 (1H, s, NH-pyrimidine), 13.31 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 279, M⁺⁺ 280; Anal. Calcd. for C₁₉H₁₃N₃O: C: 64.51, H: 4.69, N: 25.07. Found: C: 64.28, H: 4.69, N: 24.97.
4-(1H-Benzimidazol-2-yl)-6-(2-chlorophenyl)-1,6-dihydropyrimidin-2-amine (XIX)

FT-IR (KBr) cm⁻¹: 3480, 3379 (1°-N-H), 3310 (2°-N-H), 3089 (Ar-H), 1609 (C=N), 1330 (C-N), 1072 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 4.74 (1H, d, CH), 5.18 (2H, br s, NH₂), 5.73 (1H, d, NH-CH), 7.11-7.69 (8H, m, Ar-H), 7.73 (1H, s, NH-pyrimidine), 13.29 (IH, s, NH-benzimidazole); Mass m/z: M⁺ 323, M⁺² 325; Anal. Caled. for C₁₇H₁₄ClN₅: C: 63.06, H: 4.36, N: 21.63. Found: C: 62.80, H: 4.34, N: 21.55.

4-(1H-Benzimidazol-2-yl)-6-(3,4-dimethoxyphenyl)-1,6-dihydropyrimidin-2-amine (XX)

FT-IR (KBr) cm⁻¹: 3488, 3374 (1°-N-H), 3328 (2°-N-H), 3058 (Ar-H), 2928 (C-H), 1609 (C=N), 1326 (C-N); ¹H-NMR (DMSO-d₆) ppm: 3.61 (6H, s, 2 × OCH₃), 4.79 (1H, d, CH), 5.25 (2H, br s, NH₂), 5.71 (1H, d, NH-CH), 6.71-6.83 (3H, m, Ar-H), 7.19-7.59 (4H, m, Ar-H), 7.67 (1H, s, NH-pyrimidine), 13.21 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 349, M⁺² 350; Anal. Caled. for C₁₉H₁₉N₅O₂: C: 65.32, H: 5.48, N: 20.04. Found: C: 65.05, H: 5.46, N: 19.98.
2-[2-Amino-6-(1H-benzimidazol-2-yl)-3,4-dihydropyrimidin-4-yl]phenol (XXI)

\[
\begin{aligned}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3488, 3374 (\text{N-H}), 3353 (\text{O-H}), 3328 (\text{N-H}), 3053 (\text{Ar-H}), 1616 (\text{C=N}), 1330 (\text{C-N}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_6) ppm}: & \quad 4.71 (1\text{H, d, CH}), 5.15 (2\text{H, br s, NH}_2), 5.59 (1\text{H, d, NH-CH}), 5.92 (1\text{H, s, OH}), 6.99-7.45 (8\text{H, m, Ar-H}), \\
& \quad 7.53 (1\text{H, s, NH-pyrimidine}), 13.22 (1\text{H, s, NH-benzimidazole}); \\
\text{Mass m/z: } & \quad M^+ 305, M^{+1} 306; \text{Anal. Calcd. for C}_{17}H_{15}N_5O: \text{C: 66.87, H: 4.95, N: 22.94. Found: C: 66.61, H: 4.94, N: 22.84.}
\end{aligned}
\]

4-(1H-Benzimidazol-2-yl)-6-(2,6-dichlorophenyl)-1,6-dihydropyrimidin-2-amine (XXII)

\[
\begin{aligned}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3472, 3389 (\text{N-H}), 3320 (\text{N-H}), 3078 (\text{Ar-H}), 1615 (\text{C=N}), 1333 (\text{C-N}), 1069 (\text{C-Cl}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_6) ppm}: & \quad 4.82 (1\text{H, d, CH}), 5.30 (2\text{H, br s, NH}_2), 5.72 (1\text{H, d, NH-CH}), 7.35-7.49 (4\text{H, m, Ar-H}), 7.61 (1\text{H, s, NH-pyrimidine}), 7.67-7.91 (3\text{H, m, Ar-H}), 13.26 (1\text{H, s, NH-benzimidazole}); \\
\text{Mass m/z: } & \quad M^+ 358, M^{+2} 360; \text{Anal. Calcd. for C}_{17}H_{13}Cl_2N_5: \text{C: 57.00, H: 3.66, N: 19.55. Found: C: 56.78, H: 3.66, N: 19.47.}
\end{aligned}
\]
4-[2-Amino-6-(1H-benzimidazol-2-yl)-3,4-dihydropyrimidin-4-yl]-2-methoxy phenol (XXIII)

![Structure of compound XXIII]

FT-IR (KBr) cm⁻¹: 3480, 3370 (1°-N-H), 3349 (O-H), 3323 (2°-N-H), 3089 (Ar-H), 2942 (C-H), 1640 (C=N), 1333 (C-N); ¹H-NMR (DMSO-d₆) ppm: 3.26 (3H, s, OCH₃), 4.64 (1H, d, CH), 5.26 (2H, br s, NH₂), 5.66 (1H, d, NH-CH), 5.84 (1H, s, OH), 6.41-7.73 (7H, m, Ar-H), 7.78 (1H, s, NH-pyrimidine), 13.31 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 335, M⁺⁺ 336; Anal. Caled. for C₁₈H₁₇N₅O₂: C: 64.47, H: 5.11, N: 20.88. Found: C: 64.21, H: 5.10, N: 20.79.

4-(1H-Benzimidazol-2-yl)-6-[4-(dimethylamino)phenyl]-1,6-dihydropyrimidin-2-amine (XXIV)

![Structure of compound XXIV]

FT-IR (KBr) cm⁻¹: 3482, 3369 (1°-N-H), 3329 (2°-N-H), 3082 (Ar-H), 2930 (C-H), 1619 (C=N), 1328 (C-N); ¹H-NMR (DMSO-d₆) ppm: 2.79 (6H, s, 2 x CH₃), 4.73 (1H, d, CH), 5.29 (2H, br s, NH₂), 5.61 (1H, d, NH-CH), 7.08-7.46 (8H, m, Ar-H), 7.59 (1H, s, NH-pyrimidine), 13.19 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 332, M⁺⁺ 333; Anal. Caled. for C₁₉H₂₀N₆: C: 68.65, H: 6.06, N: 25.28. Found: C: 68.39, H: 6.04, N: 25.17.
4-(1H-Benzimidazol-2-yl)-6-(3-nitrophenyl)-1,6-dihydropyrimidin-2-amine (XXV)

![Structure of compound XXV]

FT-IR (KBr) cm⁻¹: 3490, 3380 (1°-N-H), 3337 (2°-N-H), 3058 (Ar-H), 1516, 1342 (N=O), 1615 (C=N), 1333 (C-N); ¹H-NMR (DMSO-d₆) ppm: 4.82 (1H, d, CH), 5.20 (2H, br s, NH₂), 5.48 (1H, d, NH-CH), 7.38-7.58 (4H, m, benzimidazolyl), 7.65 (1H, s, NH-pyrimidine), 7.73-8.08 (4H, m, Ar-H), 13.32 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 334, M⁺⁺ 335; Anal. Calcd. for C₁₇H₁₄N₆O₂: C: 61.07, H: 4.22, N: 25.14. Found: C: 60.82, H: 4.20, N: 25.04.

4-(1H-Benzimidazol-2-yl)-6-(1H-indol-3-yl)-1,6-dihydropyrimidin-2-amine (XXVI)

![Structure of compound XXVI]

FT-IR (KBr) cm⁻¹: 3476, 3362 (1°-N-H), 3312 (2°-N-H), 3089 (Ar-H), 1609 (C=N), 1330 (C-N); ¹H-NMR (DMSO-d₆) ppm: 4.71 (1H, d, CH), 5.33 (2H, br s, NH₂), 5.49 (1H, d, NH-CH), 7.12-7.29 (4H, m, indolyl), 7.46 (1H, s, NH-pyrimidine), 7.51-7.63 (4H, m, benzimidazolyl), 9.33 (1H, s, CH-indolyl), 10.69 (1H, s, NH-indolyl), 13.19 (1H, s, NH-benzimidazole); Mass m/z: M⁺ 328, M⁺⁺ 329; Anal. Calcd. for C₁₉H₁₈N₆: C: 69.50, H: 4.91, N: 25.59. Found: C: 69.22, H: 4.92, N: 25.50.
General procedure for synthesis of [4-(1H-benzimidazol-2-yl)-6-(substitutedphenyl)-1,6-dihydropyrimidin-2-yl]carbamic chloride (XXVII-XXX)

The aminopyrimidines (XVI, XVII, XIX, XX) were refluxed with equimolar proportions of chloro acetyl chloride in the presence of triethylamine and benzene to afford the chloroacetyl derivatives of pyrimidines.

\[
\text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

\[
\text{Ar}
\]

\[
\text{Cl}
\]

\[
\text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

\[
\text{Ar}
\]

\[
\text{Cl}
\]

\[
\text{Triethyl amine}
\]

\[
\text{Benzene}
\]

(XXVII - XXX)

<table>
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<tr>
<th>Compound No.</th>
<th>Ar</th>
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<tr>
<td>XXVII</td>
<td>4-Methoxyphenyl</td>
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<tr>
<td>XXVIII</td>
<td>4-Fluorophenyl</td>
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<td>XXIX</td>
<td>2-Chlorophenyl</td>
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<tr>
<td>XXX</td>
<td>3,4-Dimethoxyphenyl</td>
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</table>

Spectral data of the synthesized compounds (XXVII-XXX)

[4-(1H-Benzimidazol-2-yl)-6-(4-methoxyphenyl)-1,2-dihydropyrimidin-2-yl] carbamic chloride (XXVII)

\[
\text{OCH}_3
\]

FT-IR (KBr) cm\(^{-1}\): 3472 (N-H), 3315 (2°-N-H), 3089 (Ar-H), 2942 (C-H), 1644 (C=O), 1573 (N-H bending), 1493 (C=N), 710 (C-Cl); \(^1\text{H-NMR (DMSO-d}_6\)) ppm: 3.65 (3H, s, OCH\(_3\)), 4.20 (2H, s, CH\(_2\)), 4.59 (1H, d, CH), 6.13 (1H, d, NH-
Experimental

Synthetic Work

C\(\text{H}\), 7.09-7.52 (8H, m, Ar-H), 7.87 (1H, s, NH-pyrimidine), 8.79 (1H, s, NH), 12.50 (1H, s, NH-benzimidazole); Mass \(m/z\): \(M^+ 395, M^{+2} 397\); Anal. Calcd. for C\(_{20}\)H\(_{18}\)CIN\(_5\)O\(_2\): C: 60.68, H: 4.58, N: 17.69. Found: C: 60.45, H: 4.56, N: 17.63.

[4-(1H-Benzimidazol-2-yl)-6-(4-fluorophenyl)-1,2-dihydropyrimidin-2-yl] carbamic chloride (XXVIII)

\[
\text{O} \quad \text{H} \quad \text{N} \quad \text{N} \\
\begin{array}{ccccccccc}
\text{F} & \text{C} & \text{N} & \text{C} & \text{N} & \text{C} \\
\end{array}
\]

\text{FT-IR (KBr) cm}^{-1}: 3212 (2°-N-H), 3076 (Ar-H), 2941 (C-H), 1649 (C=O), 1569 (N-H bending), 1548 (C=N), 1110 (C-F), 694 (C-Cl); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 4.22 (2H, s, CH\(_2\)), 5.06 (1H, d, CH), 6.02 (1H, d, NH-CH\(_2\)), 7.12-7.72 (8H, m, Ar-H), 7.78 (1H, s, NH-pyrimidine), 8.83 (1H, s, NH), 12.65 (1H, s, NH-benzimidazole); Mass \(m/z\): \(M^+ 383, M^{+2} 385\); Anal. Calcd. for C\(_{19}\)H\(_{15}\)ClF\(_5\)N\(_2\): C: 59.46, H: 3.94, N: 18.25. Found: C: 59.22, H: 3.91, N: 18.18.

[4-(1H-Benzimidazol-2-yl)-6-(2-chlorophenyl)-1,2-dihydropyrimidin-2-yl] carbamic chloride (XXIX)

\[
\text{O} \quad \text{H} \quad \text{N} \quad \text{N} \\
\begin{array}{ccccccccc}
\text{Cl} & \text{C} & \text{N} & \text{C} & \text{N} & \text{C} \\
\end{array}
\]

\text{FT-IR (KBr) cm}^{-1}: 3478 (N-H), 3218 (2°-N-H), 3079 (Ar-H), 2960 (C-H), 1658 (C=O), 1511 (C=N), 1562 (N-H bending), 689 (C-Cl); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 4.29 (2H, s, CH\(_2\)), 5.09 (1H, d, CH), 6.09 (1H, d, NH-CH\(_2\)), 6.94-7.76 (8H, m, Ar-H), 7.88 (1H, s, NH-pyrimidine), 8.89 (1H, s, NH), 12.79 (1H, s, NH-benzimidazole); Mass \(m/z\): \(M^+ 400, M^{+2} 402\); Anal. Calcd. for C\(_{16}\)H\(_{15}\)Cl\(_2\)N\(_2\): C: 57.07, H: 3.78, N: 17.50. Found: C: 56.85, H: 3.76, N: 17.43.
Experimental

[4-(1H-Benzimidazol-2-yl)-6-(3,4-dimethoxyphenyl)-1,2-dihydropyrimidin-2-yl]carbamic chloride (XXX)

FT-IR (KBr) cm⁻¹: 3482 (N-H), 3218 (2°N-H), 3085 (Ar-H), 2959 (C-H), 1653 (C=O), 1580 (N-H bending), 1564 (C=N), 698 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 3.80 (6H, s, 2 x OCH₃), 4.27 (2H, s, CH₂), 4.68 (1H, d, CH), 6.05 (1H, d, NH-CH), 6.98-7.89 (7H, m, Ar-H), 7.93 (1H, s, NH-pyrimidine), 8.94 (1H, s, NH), 12.72 (1H, s, NH-benzimidazole). Mass m/z: M⁺ 425, M⁺² 427; Anal. Calcd. for C₂₁H₂OClN₅O₃; C: 59.23, H: 4.73, N: 16.44. Found: C: 58.99, H: 4.72, N: 16.37.

SCHEME-II

General procedure for synthesis of 3-(substituted phenyl)-1-phenylprop-2-en-1-one (XXXI-XXXIX)

To an alkaline alcoholic solution of acetophenone (0.01 mL) various aromatic aldehydes (0.01 M) were added in small amounts with continuous stirring for about 2 h. The mixtures were kept in the refrigerator for 24 h. The product formed were filtered, dried and recrystallized from ethanol.

Ph.D Thesis

91

Jamia Hamdard
Experimental

Synthetic Work

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<td>3-Nitro</td>
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</table>

Spectral data of the synthesized compounds (XXXI- XXXIX)

1,3-Diphenylprop-2-en-1-one (XXXI)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3035 (\text{Ar-H}), 1663 (\text{C=O}), 1492 (\text{C=C}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm:} & \quad 6.85 (1\text{H}, \text{d}, J = 15.3 \text{ Hz}, \text{H}_a), 7.29-7.78 (10\text{H}, \text{m}, \text{Ar-H}), 7.88 (1\text{H}, \text{d}, J = 15.1 \text{ Hz}, \text{H}_b); \\
\text{Anal. Calcd. for C}_{15}\text{H}_{12}\text{O}: & \quad \text{C: 86.51, H: 5.81. Found: C: 86.16, H: 5.80.}
\end{align*}
\]

3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (XXXII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3035 (\text{Ar-H}), 2922 (\text{C-H}), 1655 (\text{C=O}), 1500 (\text{C=C}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) ppm:} & \quad 3.60 (3\text{H}, \text{s}, \text{OCH}_3), 6.68 (1\text{H}, \text{d}, J = 15.4 \text{ Hz}, \text{H}_a), 6.98-7.19 (4\text{H}, \text{m}, \text{Anisyl}), 7.34-7.71 (5\text{H}, \text{m}, \text{Ar-H}), 7.91 (1\text{H}, \text{d}, J = 15.2 \text{ Hz}, \text{H}_b); \\
\text{Anal. Calcd. for C}_{16}\text{H}_{14}\text{O}_2:} & \quad \text{C: 80.65, H: 5.92. Found: C: 80.35, H: 5.90.}
\end{align*}
\]
3-(2-Chlorophenyl)-1-phenylprop-2-en-1-one (XXXIII)

![Chemical structure of XXXIII]

FT-IR (KBr) cm⁻¹: 3032 (Ar-H), 1651 (C=O), 1510 (C=C), 1072 (C-Cl); ¹H-NMR (DMSO-δ₆) ppm: 6.69 (1H, d, J = 15.5 Hz, H₆), 7.15-7.76 (9H, d, Ar-H), 7.89 (1H, d, J = 15.6 Hz, H₇); Anal. Calcd. for C₁₅H₁₁ClO: C: 74.23, H: 4.57. Found: C: 73.95, H: 4.57.

3-(3,4-Dimethoxyphenyl)-1-phenylprop-2-en-1-one (XXXIV)

![Chemical structure of XXXIV]

FT-IR (KBr) cm⁻¹: 3029 (Ar-H), 2930 (C-H), 1682 (C=O), 1523 (C=C); ¹H-NMR (DMSO-δ₆) ppm: 3.59 (6H, s, 2 × OCH₃), 6.66 (1H, d, J = 15.2 Hz, H₆), 6.69-6.92 (3H, m, Ar-H), 7.18-7.62 (5H, m, Ar-H), 7.83 (1H, d, J = 15.2 Hz, H₇); Anal. Calcd. for C₁₇H₁₆O₃: C: 76.10, H: 6.01. Found: C: 75.80, H: 5.99.

3-(4-Fluorophenyl)-1-phenylprop-2-en-1-one (XXXV)

![Chemical structure of XXXV]

FT-IR (KBr) cm⁻¹: 3043 (Ar-H), 1655 (C=O), 1520 (C=C), 1160 (C-F); ¹H-NMR (DMSO-δ₆) ppm: 6.69 (1H, d, J = 16.2 Hz, H₆), 7.22-7.79 (9H, m, Ar-H), 7.88 (1H, d, J = 16 Hz, H₇); Anal. Calcd. for C₁₅H₁₁FO: C: 79.63, H: 4.90. Found: C: 79.31, H: 4.91.
Experimental Synthetic Work

FT-IR (KBr) cm⁻¹: 3039 (Ar-H), 1684 (C=O), 1499 (C≡C), 1086 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 6.63 (1H, d, J = 15.3 Hz, Hₐ), 7.27-7.49 (5H, m, Ar-H), 7.69-7.81 (3H, m, Ar-H), 7.91 (1H, d, J = 15.6 Hz, Hₖ); Anal. Calcd. for C₁₅H₁₀Cl₂O: C: 65.01, H: 3.64. Found: C: 65.78, H: 3.62.

3-[4-(Dimethylamino) phenyl]-1-phenylprop-2-en-1-one (XXXVII)

![Chemical structure of XXXVII](image)

FT-IR (KBr) cm⁻¹: 3019 (Ar-H), 2962 (C-H), 1692 (C=O), 1529 (C=C); ¹H-NMR (DMSO-d₆) ppm: 2.85 (6H, s, 2 x CH₃), 6.59 (1H, d, J = 16.3 Hz, Hₐ), 7.25-7.81 (9H, m, Ar- H), 7.88 (1H,d, J = 16.1 Hz, Hₖ); Anal. Calcd. for C₁₇H₁₇NO: C: 81.24, H: 6.82, N: 5.57. Found: C: 80.91, H: 6.80, N: 5.54.

3-(2-Hydroxyphenyl)-1-phenylprop-2-en-1-one (XXXVIII)

![Chemical structure of XXXVIII](image)

FT-IR (KBr) cm⁻¹: 3419 (O-H), 3022 (Ar-H), 1687 (C=O), 1506 (C=C); ¹H-NMR (DMSO-d₆) ppm: 4.78 (1H, s, OH), 6.58 (1H, d, J = 15.1 Hz, Hₐ), 7.44-7.78 (9H, d, Ar- H), 7.82 (1H,d, J = 15.1 Hz, Hₖ); Anal. Calcd. for C₁₇H₁₆O₂: C: 80.34, H: 5.39. Found: C: 80.04, H: 5.37.

3-(3-Nitrophenyl)-1-phenylprop-2-en-1-one (XXXIX)

![Chemical structure of XXXIX](image)

FT-IR (KBr) cm⁻¹: 3058 (Ar-H), 1655 (C=O), 1570, 1376 (N-O), 1520 (C=C); ¹H-NMR (DMSO-d₆) ppm: 6.63 (1H, d, J = 15.3 Hz, Hₐ), 7.29-7.59 (5H, m, Ar-
Experimental Synthesis Work


General procedure for synthesis of 6-(substituted phenyl)-4-phenyl-1,6-dihydro pyrimidin-2-amine (XL - XLVIII)

To a mixture of various chalcones (0.01 M), guanidine HCl (0.03 M) in 20 mL of ethanol, 5 mL of sodium hydroxide (0.02 M) was added and the mixture was refluxed for approximately 6 h. The reaction was monitored with TLC using ethyl acetate: petroleum ether (2:1). After the completion of the reaction, the reaction mixture was poured in 50 mL of 10 % HCl solution. The product was filtered out, washed with water and recrystallized with benzene-ethanol mixture.

![Chemical structure](image)

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<td></td>
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</table>
Spectral data of the synthesized compounds (XL - XLVIII)

4,6-Diphenyl-1,6-dihydropyrimidin-2-amine (XL)

![Chemical structure of 4,6-Diphenyl-1,6-dihydropyrimidin-2-amine (XL)]

FT-IR (KBr) cm⁻¹: 3479, 3363 (1°-N-H), 3310 (2°-N-H), 3096 (Ar-H), 1610 (C=N); ¹H-NMR (DMSO-d₆) ppm: 4.70 (1H, d, CH), 5.21 (2H, br s, NH₂), 5.45 (1H, d, NH-CH), 6.89-7.21 (10H, m, Ar-H), 7.53 (1H, s, NH); Mass m/z: M⁺ 249, M⁺⁺ 250; Anal. Caled. for C₁₆H₁₅N₃: C: 77.08, H: 6.06, N: 16.85. Found: C: 76.80, H: 6.04, N: 16.80.

6-(4-Methoxyphenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLI)

![Chemical structure of 6-(4-Methoxyphenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLI)]

FT-IR (KBr) cm⁻¹: 3492, 3376 (1°-N-H), 3339 (2°-N-H), 3055 (Ar-H), 2918 (C-H), 1571 (C=N); ¹H-NMR (DMSO-d₆) ppm: 3.55 (3H, s, OCH₃), 4.68 (1H, d, CH), 5.28 (2H, br s, NH₂), 5.79 (1H, d, NH-CH), 6.89-6.97 (4H, m, Anisyl), 7.14-7.39 (5H, m, Ar-H), 7.63 (1H, s, NH); Mass m/z: M⁺ 279, M⁺⁺ 280; Anal. Caled. for C₁₇H₁₇N₃O: C: 73.10, H: 6.13, N: 15.04. Found: C: 72.81, H: 6.11, N: 15.00.

6-(2-Chlorophenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLII)

![Chemical structure of 6-(2-Chlorophenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLII)]
Experimental

Synthetic Work

FT-IR (KBr) cm⁻¹: 3487, 3378 (1°-N-H), 3329 (2°-N-H), 3089 (Ar-H), 1609 (C=N), 1084 (C-Cl); ¹H-NMR (DMSO-d₄) ppm: 4.57 (1H, d, CH), 5.33 (2H, br s, NH₂), 5.51 (1H, d, NH-CH), 7.12-7.36 (9H, m, Ar-H), 7.61 (1H, s, NH); Mass m/z: M⁺ 283, M⁺² 285; Anal. Caled. for C₁₆H₁₄CIN₃: C: 67.72, H: 4.97, N: 14.81. Found: C: 67.47, H: 4.97, N: 14.77.

6-(3,4-Dimethoxyphenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLIII)

\[ \text{OCH₃} \]
\[ \text{OCH₃} \]
\[ \text{NH₂} \]

FT-IR (KBr) cm⁻¹: 3464, 3387 (1°-N-H), 3332 (2°-N-H), 3059 (Ar-H), 2919 (C-H), 1571 (C=N); ¹H-NMR (DMSO-d₄) ppm: 3.64 (6H, s, 2 × OCH₃), 4.73 (1H, d, CH), 5.34 (2H, br s, NH₂), 5.86 (1H, d, NH-CH), 6.49-6.67 (3H, m, Ar-H), 7.18-7.41 (5H, m, Ar-H), 7.82 (1H, s, NH); Mass m/z: M⁺ 309, M⁺² 310; Anal. Caled. for C₁₆H₁₇N₃O₂: C: 69.88, H: 6.19, N: 13.58. Found: C: 69.62, H: 6.19, N: 13.54.

6-(4-Fluorophenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLIV)

\[ \text{F} \]
\[ \text{NH₂} \]

FT-IR (KBr) cm⁻¹: 3473, 3385 (1°-N-H), 3339 (2°-N-H), 3095 (Ar-H), 1611 (C=N), 1021 (C-F); ¹H-NMR (DMSO-d₄) ppm: 4.98 (1H, d, CH), 5.28 (2H, br s, NH₂), 5.58 (1H, d, NH-CH), 6.95-7.28 (9H, m, Ar-H), 7.63 (1H, s, NH); Mass m/z: M⁺ 267, M⁺² 268; Anal. Caled. for C₁₆H₁₆FN₃: C: 71.89, H: 5.28, N: 15.72. Found: C: 71.90, H: 5.28, N: 15.66.
6-(2,6-Dichlorophenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLV)

FT-IR (KBr) cm⁻¹: 3496, 3382 (1°-N-H), 3329 (2°-N-H), 3089 (Ar-H), 1609 (C=N), 1089 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 4.78 (1H, d, CH), 5.34 (2H, br s, NH₂), 5.73 (1H, d, NH-CH), 6.78-7.09 (5H, m, Ar-H), 7.48-7.63 (3H, m, Ar-H), 7.68 (1H, s, NH); Mass m/z: M⁺ 318, M²⁺ 320; Anal. Calcd. for C₁₆H₁₃Cl₂N₂: C: 60.39, H: 4.12, N: 13.21. Found: C: 60.16, H: 4.11, N: 13.16.

6-[(Diethylamino)phenyl]-4-phenyl-1,6-dihydropyrimidin-2-amine (XLVI)

FT-IR (KBr) cm⁻¹: 3473, 3382 (1°-N-H), 3327 (2°-N-H), 3069 (Ar-H), 2972 (C-H), 1610 (C=N); ¹H-NMR (DMSO-d₆) ppm: 2.85 (6H, s, 2 × CH₃), 4.69 (1H, d, CH), 5.21 (2H, br s, NH₂), 5.93 (1H, d, NH-CH), 7.15-7.55 (9H, m, Ar-H), 7.72 (1H, s, NH); Mass m/z: M⁺ 292, M²⁺ 293; Anal. Calcd. for C₁₈H₂₀N₄: C: 73.94, H: 6.89, N: 6.89. Found: C: 73.64, H: 6.89, N: 6.91.

2-(2-Amino-6-phenyl-3,4-dihydropyrimidin-4-y1)phenol (XLVII)
Experimental

Synthetic Work

FT-IR (KBr) cm⁻¹: 3479, 3388 (1°-N-H), 3462 (O-H), 3310 (2°-N-H) 3099 (Ar-H), 1610 (C=N); ¹H-NMR (DMSO-d₆) ppm: 4.78 (1H, d, CH), 4.97 (1H, s, OH), 5.22 (2H, br s, NH₂), 5.79 (1H, d, NH-CH), 6.61-7.25 (9H, m, Ar-H), 7.63 (1H, s, NH); Mass m/z: M⁺ 265, M⁺ 266; Anal. Calcd. for C₁₆H₁₅N₃O: C: 72.43, H: 5.70, N: 15.84. Found: C: 72.16, H: 5.67, N: 5.64.

6-(3-Nitrophenyl)-4-phenyl-1,6-dihydropyrimidin-2-amine (XLVIII)

FT-IR (KBr) cm⁻¹: 3488, 3376 (1°-N-H), 3337 (2°-N-H), 3095 (Ar-H), 1614 (C=N), 1496, 1340 (N=O)₂; ¹H-NMR (DMSO-d₆) ppm: 4.53 (1H, d, CH), 5.33 (2H, br s, NH₂), 5.69 (1H, d, NH-CH), 7.13-7.29 (5H, m, Ar-H), 7.51 (1H, s, NH), 7.69-8.09 (4H, m, Ar-H); Mass m/z: M⁺ 294, M⁺ 295; Anal. Calcd. for C₁₆H₁₄N₄O₂: C: 65.30, H: 4.79, N: 19.04. Found: C: 65.05, H: 4.78, N: 18.98.

SCHEME-III

Synthesis of 4-Hydroxy-3-methyl acetophenone

A mixture of o-cresol and acetic anhydride was heated in presence of zinc chloride on flame at a temperature of 140-145° C. After the completion of reaction and usual work up followed by column chromatography pure light brown crystals of the acetophenone derivative was obtained.
General procedure for synthesis of 1-(4-hydroxy-3-methylphenyl)-3-(substituted phenyl)-2-propen-1-one (XLIX – LXI)

A mixture of 4-hydroxy-3-methylacetophenone (0.01 M) and appropriate substituted aromatic aldehydes (0.01 M) in absolute ethanol was stirred in presence of base (sodium hydroxide, 30%, 5 mL) till completion of the reaction. The resulting solution was allowed to stand overnight and then poured into ice-cold water followed by neutralization with HCl. The solid separated was filtered off, dried and recrystallized with hot ethanol to obtain the desired chalcones derivatives (XLIX – LXI).

\[
\begin{align*}
\text{HO} & \quad \text{CH}_3 \\
\text{O} & \quad \text{CH}_3 \\
\text{C} & \quad \text{R} \\
\text{CHO} & \\
\end{align*}
\]

\[
\begin{align*}
\text{R} & \quad \text{NaOH} \\
\text{C}_2\text{H}_5\text{OH} & \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{CH}_3 \\
\text{O} & \quad \text{CH}_3 \\
\text{C} & \quad \text{R} \\
\text{CHO} & \\
\end{align*}
\]

(XLIX - LXI)

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Experimental

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<th>No.</th>
<th>Compound</th>
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<td>10.</td>
<td>LVIII</td>
<td>3-Nitro</td>
</tr>
<tr>
<td>11.</td>
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<td>12.</td>
<td>LX</td>
<td>3-Bromo</td>
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<tr>
<td>13.</td>
<td>LXI</td>
<td>2,3,4-Trimethoxy</td>
</tr>
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</table>

Spectral data of the synthesized compounds (XLIX – LXI)

1-(4-Hydroxy-3-methyl-phenyl)-3-phenyl-2-propen-1-one (XLIX)

\[
\text{HO} \quad \text{CH}_3 \quad \text{C}_6 \quad \text{O} \\
\]

FT-IR (KBr) cm\(^{-1}\): 3436 (O-H), 3058 (Ar-H), 2960 (C-H), 1690 (C=O), 1522 (C=C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.33 (3H, s, CH\(_3\)), 6.80 (1H, d, \(J = 8.28\) Hz, H\(_a\)), 6.93 (1H, s, OH), 7.40 (1H, d, \(J = 6.70\) Hz, H\(_b\)), 7.69-7.83 (8H, m, Ar-H); Anal. Calcd. for C\(_{16}\)H\(_{14}\)O\(_2\): C: 80.65, H: 5.92. Found: C: 80.31, H: 5.90.

1-(4-Hydroxy-3-methylphenyl)-3-(4-fluorophenyl)prop-2-en-1-one (L)

\[
\text{HO} \quad \text{CH}_3 \quad \text{F} \quad \text{C}_6 \quad \text{O} \\
\]

FT-IR (KBr) cm\(^{-1}\): 3420 (O-H), 3042 (Ar-H), 2950 (C-H), 1708 (C=O), 1530 (C=C), 1229 (C-F); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.27 (3H, s, CH\(_3\)), 6.88 (1H, d, \(J = 7.24\) Hz, H\(_a\)), 6.97 (1H, s, OH), 7.53 (1H, d, \(J = 7.89\) Hz, H\(_b\)), 7.71-7.83 (7H, m, Ar-H); Anal. Calcd. for C\(_{16}\)H\(_{13}\)FO\(_2\): C: 74.99, H: 5.11. Found: C: 74.67, H: 5.13.
1-(4-Hydroxy-3-methylphenyl)-3-(2-chlorophenyl)prop-2-en-1-one (LI)

![Chemical Structure of LI]

FT-IR (KBr) cm⁻¹: 3390 (O-H), 3056 (Ar-H), 2948 (C-H), 1669 (C=O), 1522 (C=C), 1063 (C-Cl); ¹H-NMR (DMSO-d₄) ppm: 2.18 (3H, s, CH₃), 6.89 (1H, d, J = 7.99 Hz, Hₐ), 6.97 (1H, s, OH), 7.34 (1H, d, J = 8.35 Hz, Hₖ), 7.67-7.88 (7H, m, Ar-H); Anal. Calcd. for C₁₆H₁₃ClO₂: C: 70.46, H: 4.80. Found: C: 70.72, H: 4.78.

1-(4-Hydroxy-3-methylphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (LII)

![Chemical Structure of LII]

FT-IR (KBr) cm⁻¹: 3378 (O-H), 3072 (Ar-H), 2958 (C-H), 1676 (C=O), 1549 (C=C); ¹H-NMR (DMSO-d₄) ppm: 2.67 (3H, s, CH₃), 3.87 (3H, s, OCH₃), 6.79 (1H, d, J = 7.50 Hz, Hₐ), 6.89 (1H, s, OH), 7.58 (1H, d, J = 8.51 Hz, Hₖ), 7.66-7.87 (7H, m, Ar-H); Anal. Calcd. for C₁₆H₁₈O₃: C: 76.10, H: 6.01. Found: C: 75.78, H: 5.98.

1-(4-Hydroxy-3-methylphenyl)-3-(4-dimethylaminophenyl)prop-2-en-1-one (LIII)

![Chemical Structure of LIII]
Experimental

1-(4-Hydroxy-3-methylphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (IIV).

![Structure of IIV]

FT-IR (KBr) cm⁻¹: 3423 (O-H), 3067 (Ar-H), 2948 (C-H), 1684 (C=O), 1520 (C=C); ¹H-NMR (DMSO-d₆) ppm: 2.23 (3H, s, CH₃), 3.11 (6H, s, 2 × CH₃-N), 6.70 (1H, d, J = 7.61 Hz, Hₐ), 6.82 (1H, s, OH), 7.58 (1H, d, J = 7.93 Hz, Hₙ), 7.66-7.83 (7H, m, Ar-H); Anal. Calcd. for C₁₉H₁₅NO₃: C: 76.84, H: 6.81, N: 4.98. Found: C: 76.54, H: 6.80, N: 4.97.

1-(4-Hydroxy-3-methylphenyl)-3-(4-chloro-3-methoxyphenyl)prop-2-en-1-one (LV).

![Structure of LV]

FT-IR (KBr) cm⁻¹: 3389 (O-H), 3069 (Ar-H), 2965 (C-H), 1686 (C=O), 1552 (C=C); ¹H-NMR (DMSO-d₆) ppm: 2.27 (3H, s, CH₃), 3.94 (6H, s, 2 × OCH₃), 6.82 (1H, d, J = 7.45 Hz, Hₐ), 6.93 (1H, s, OH), 7.58 (1H, d, J = 7.89 Hz, Hₙ), 7.62-7.80 (3H, m, cresyl), 7.84-7.93 (3H, m, Ar-H); Anal. Calcd. for C₁₉H₁₅ClO₃: C 72.47, H 6.08. Found: C: 72.73, H: 6.08.

1-(4-Hydroxy-3-methylphenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (LV).

![Structure of LV]

FT-IR (KBr) cm⁻¹: 3379 (O-H), 3064 (Ar-H), 2956 (C-H), 1710 (C=O), 1563 (C=C); ¹H-NMR (DMSO-d₆) ppm: 2.23 (3H, s, CH₃), 3.84 (3H, s, OCH₃), 6.88 (1H, d, J = 7.51 Hz, Hₐ), 6.92 (2H, s, OH), 7.63 (1H, d, J = 8.50 Hz, Hₙ), 7.60-7.875 (6H, m, Ar-H); Anal. Calcd. for C₁₉H₁₆O₄: C 71.82, H 5.67. Found: C: 71.52, H: 5.65.
Experimental

Synthetic Work

1-(4-Hydroxy-3-methylphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (LVII).

\[
\text{OCH}_3
\]

FT-IR (KBr) cm\(^{-1}\): 3386 (O-H), 3059 (Ar-H), 2968 (C-H), 1680 (C=O) 1542 (C=C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.27 (3H, s, CH\(_3\)), 3.94 (9H, s, 3 \times \text{OCH}_3), 6.84 (1H, d, \(J = 7.55\) Hz, H\(_a\)), 6.94 (1H, s, OH), 7.43 (1H, d, \(J = 7.97\) Hz, H\(_b\)), 7.51-7.63 (3H, m, Ar-H), 7.88-7.98 (2H, m, Ar-H); Anal. Calcd. for C\(_{19}\)H\(_{20}\)O\(_5\): C: 69.50; H: 6.14. Found: C: 69.77; H: 6.11.

1-(4-Hydroxy-3-methylphenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one (LVII).

\[
\text{Cl}
\]

FT-IR (KBr) cm\(^{-1}\): 3388 (O-H), 3069 (Ar-H), 2950 (C-H), 1698 (C=O), 1566 (C=C), 1059 (C-Cl); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.28 (3H, s, CH\(_3\)), 6.88 (1H, d, \(J = 8.41\) Hz, H\(_a\)), 6.99 (1H, s, OH), 7.43 (1H, d, \(J = 8.96\) Hz, H\(_b\)), 7.54-7.89 (6H, m, Ar-H); Anal. Calcd. for C\(_{18}\)H\(_{12}\)Cl\(_2\)O\(_2\): C: 62.56; H: 3.94. Found: C: 62.29; H: 3.92.

1-(4-Hydroxy-3-methylphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (LVIII).

\[
\text{NO}_2
\]
Experimental Synthetic Work

FT-IR (KBr) cm$^{-1}$: 3379 (O-H), 3059 (Ar-H), 2968 (C-H), 1684 (C=O), 1559 (C=C), 1531, 1325 (N=O)$_2$; $^1$H-NMR (DMSO-$d_6$) ppm: 2.24 (3H, s, CH$_3$), 6.82 (1H, d, $J = 7.68$ Hz, H$_a$), 6.95 (1H, s, OH), 7.49 (1H, d, $J = 8.24$ Hz, H$_b$), 7.68-7.74 (3H, m, Ar-H), 7.81-8.09 (4H, m, Ar-H); Anal. Calcd. for C$_{16}$H$_{13}$NO$_4$: C: 67.84, H: 4.63. Found: C: 68.11, H: 4.63.

1-(4-Hydroxy-3-methylphenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one (LIX).

\[
\text{HO} \begin{array}{c} \text{HO} \\ \text{CH$_3$} \\ \text{C} \end{array} \]

FT-IR (KBr) cm$^{-1}$: 3398 (O-H), 3076 (Ar-H), 2949 (C-H), 1681 (C=O), 1549 (C=C); $^1$H-NMR (DMSO-$d_6$) ppm: 2.43 (3H, s, CH$_3$), 6.83 (1H, d, $J = 7.64$ Hz, H$_a$), 6.91 (2H, s, OH), 7.53 (1H, d, $J = 8.28$ Hz, H$_b$), 7.66-7.80 (7H, m, Ar-H); Anal. Calcd. for C$_{16}$H$_{13}$O$_3$: C: 75.57, H: 5.55. Found: C: 75.81, H: 5.54.

1-(4-Hydroxy-3-methylphenyl)-3-(3-bromophenyl)prop-2-en-1-one (LX).

\[
\text{HO} \begin{array}{c} \text{CH$_3$} \\ \text{Br} \\ \text{C} \end{array} \]

FT-IR (KBr) cm$^{-1}$: 3388 (O-H), 3076 (Ar-H), 2954 (C-H), 1693 (C=O), 1562 (C=C), 698 (C-Br); $^1$H-NMR (DMSO-$d_6$) ppm: 2.63 (3H, s, CH$_3$), 6.80 (1H, d, $J = 7.68$ Hz, H$_a$), 7.01 (1H, s, OH), 7.43 (1H, d, $J = 8.24$ Hz, H$_b$), 7.67-7.89 (7H, m, Ar-H); Anal. Calcd. for C$_{16}$H$_{13}$BrO$_2$: C: 60.59, H: 4.13. Found: C: 61.01, H: 4.13.
Experimental

1-(4-Hydroxy-3-methylphenyl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (LXI).

FT-IR (KBr) cm\(^{-1}\): 3392 (O-H), 3065 (Ar-H), 2949 (C-H), 1682 (C=O), 1570 (C=C); \(^1\)H- NMR (DMSO-\(d_6\)) ppm: 2.31 (3H, s, CH\(_3\)), 3.83 (9H, s, 3 × OCH\(_3\)), 6.90 (1H, d, \(J = 7.56\) Hz, H\(_a\)), 6.98 (1H, s, OH), 7.38 (1H, d, \(J = 7.97\) Hz, H\(_b\)), 7.49-7.57 (3H, m, Ar-H), 7.85-8.01 (2H, m, Ar-H); Anal. Calc'd. for C\(_{19}\)H\(_{20}\)O\(_5\): C: 69.50, H: 6.14. Found: C: 69.76, H: 6.14.

General procedure for synthesis of 4-{2-amino-6\{(substituted) phenyl\}-1,6-dihydro-1H-pyrimidine-4yl}-2 methyl phenol (LXII-LXXIV)

A mixture of chalcone (1 M), guanidine hydrochloride (1.5 M) and sodium hydride (3.0 M) in DMF (100 mL) was refluxed for 6-8 h. The reaction mixture was poured into ice cold water, a solid was separated out. The separated solid was filtered, washed and recrystallized with ethanol.

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<th>Compound No.</th>
<th>R</th>
</tr>
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<td>4-Fluoro</td>
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### Experimental Synthetic Work

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<td>3,4,5-Trimethoxy</td>
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<td>2,3,4-Trimethoxy</td>
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Spectral data of the synthesized compounds (LXII – LXXIV)

4-(2-Amino-6-phenyl-3, 4-dihydropyrimidin-4-yl)-2-methylphenol (LXII)

![Chemical Structure](image)

FT-IR (KBr) cm\(^{-1}\): 3468, 3378 (1\(^\circ\)-N-H), 3366 (O-H), 3319 (2\(^\circ\)-N-H), 3096 (Ar-H), 2976 (C-H), 1640 (C=N). \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.36 (3H, s, CH\(_3\)), 4.61 (1H, d, \(J = 8.0\) Hz, CH), 5.44 (2H, br s, NH\(_2\)), 5.93 (1H, d, \(J = 12.0\) Hz, NH-CH\(_2\)), 6.94 (1H, s, OH), 7.07-7.49 (8H, m, Ar-H), 7.81 (1H, s, NH); Mass \(m/z\): M\(^+\) 279, M\(^{+1}\) 280; Anal. Calcd. for C\(_{17}\)H\(_{17}\)N\(_3\)O: C: 73.10, H: 6.13, N: 15.04. Found: C: 72.78, H: 6.15, N: 15.08.
Experimental Synthesis Work

4-[2-Amino-6-(4-fluorophenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXIII)

\[
\text{FT-IR (KBr) cm}^{-1}: 3476, 3389 (\text{N-H}), 3368 (\text{O-H}), 3330 (\text{N-H}), 3095 (\text{Ar-H}), 2964 (\text{C-H}), 1584 (\text{C=N}), 1280 (\text{C-F}); \quad \text{'H-NMR (DMSO-\text{d}_6) ppm: 2.98 (3H, s, CH}_3), 4.69 (1H, d, J = 8.8 Hz, CH), 5.21 (2H, br s, NH}_2), 5.96 (1H, d, J = 12.4 Hz, NH-CH), 6.97 (1H, s, OH), 7.22-7.70 (7H, m, Ar-H), 7.89 (1H, s, NH); \quad \text{Mass (m/z): } M^+ 297, M^{+1} 298; \quad \text{Anal. Calcd. for } C_{17}H_{16}FN_3O: C: 68.67, H: 5.42, N: 14.13. \quad \text{Found: C: 68.38, H: 5.39, N: 14.06.}
\]

4-[2-Amino-6-(2-chlorophenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXIV)

\[
\text{FT-IR (KBr) cm}^{-1}: 3476, 3385 (\text{N-H}), 3320 (\text{O-H}), 3262 (\text{N-H}), 3089 (\text{Ar-H}), 2962 (\text{C-H}), 1546 (\text{N=C}), 1042 (\text{C-Cl}); \quad \text{'H-NMR (DMSO-\text{d}_6) ppm: 2.91 (3H, s, CH}_3), 4.63 (1H, d, J = 8.4 Hz, CH), 5.16 (2H, br s, NH}_2), 5.96 (1H, d, J = 12.8 Hz, NH-CH), 6.89 (1H, s, OH), 6.98-7.62 (7H, m, Ar-H), 7.76 (1H, s, NH); \quad \text{Mass (m/z): } M^+ 313, M^{+2} 315; \quad \text{Anal. Calcd. for } C_{17}H_{15}ClN_3O: C: 65.07, H: 5.14, N: 13.39. \quad \text{Found: C: 65.31, H: 5.14, N: 13.33.}
\]
4-[2-Amino-6-(4-methoxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXV)

\[
\begin{align*}
\text{FT-IR (KBr) } & \text{ cm}^{-1}: 3482, 3377 (\text{O-H}), 3359 (\text{O-H}), 3298 (2\text{-N-H}), 3066 (\text{Ar-H}), 2942 (\text{C-H}), 1584 (\text{C=N}), 1174 (\text{C-O-C}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_6) } & \text{ ppm}: 2.72 (3\text{H}, \text{s, CH}_3), 3.86 (3\text{H}, \text{s, OCH}_3), 4.71 (1\text{H}, \text{d, } J = 8.0 \text{ Hz}, \text{CH}), 5.21 (2\text{H}, \text{br s, NH}_2), 5.20 (1\text{H}, \text{d, } J = 12.0 \text{ Hz}, \text{NH-CH}), 6.92 (1\text{H}, \text{s, OH}), 7.22-7.69 (7\text{H}, \text{m, Ar-H}), 7.82 (1\text{H}, \text{s, NH}); \\
\text{Mass (m/z): } M^+ & 309, M^{1+} 310; \text{ Anal. Calcd. for } C_{18}H_{19}N_3O_2: \text{ C} 69.88, \text{ H} 6.19, \text{ N} 13.58. \text{ Found: } \text{C} 69.58, \text{ H} 6.16, \text{ N} 13.61.
\end{align*}
\]

4-[2-Amino-6-[4-(dimethylamino)phenyl]-3,4-dihydropyrimidin-4-yl]-2-methyl phenol (LXVI)

\[
\begin{align*}
\text{FT-IR (KBr) } & \text{ cm}^{-1}: 3499, 3386 (\text{1°-N-H}), 3375 (\text{O-H}), 3296 (2\text{-N-H}), 3088 (\text{Ar-H}), 2969 (\text{C-H}), 1412 (\text{C=N}); \text{ \textsuperscript{1}H-NMR (DMSO-\text{d}_6) ppm}: 2.60 (3\text{H}, \text{s, CH}_3), 2.96 (6\text{H}, \text{s, } [2 \times \text{CH}_3] \text{N}), 4.64 (1\text{H}, \text{d, } J = 8.6 \text{ Hz, CH}), 5.31 (2\text{H}, \text{br s, NH}_2), 5.61 (1\text{H}, \text{d, } J = 12.4 \text{ Hz, NH-CH}), 6.96 (1\text{H}, \text{s, OH}), 7.29-7.66 (7\text{H}, \text{m, Ar-H}), 7.83 (1\text{H}, \text{s, NH}); \\
\text{Mass (m/z): } M^+ & 322, M^{1+} 323; \text{ Anal. Calcd. for } C_{19}H_{22}N_4O: \text{ C} 70.78, \text{ H} 6.88, \text{ N} 17.38. \text{ Found: } \text{C} 71.04, \text{ H} 6.86, \text{ N} 17.31.
\end{align*}
\]
Experimental

Synthetic Work

4-[2-Amino-6-(3,4-dimethoxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXVII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & 3489, 3376 (\text{1}^{\circ}-\text{N-H}), 3369 (\text{O-H}), 3340 (\text{2}^{\circ}-\text{N-H}), 3066 (\text{Ar-H}), 2956 (\text{C-H}), 1419 (\text{C=N}), 1170 (\text{C-O-C}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_6) ppm: } & 2.36 (3H, s, \text{CH}_3), 3.67 (6H, s, 2 \times \text{OCH}_3), 4.63 (1H, d, J = 8.4 \text{ Hz, CH}), 5.25 (2H, br s, \text{NH}_2), 5.69 (1H, d, J = 12.0 \text{ Hz, NH-CH}), 6.81-6.94 (3H, m, \text{Ar-H}), 7.01 (1H, s, \text{OH}), 7.43-7.64 (3H, m, cresyl), 7.93 (1H, s, NH); \\
\text{Mass (m/z): } & M^+ 339, M^{+1} 340; \\
\end{align*}
\]

4-[2-Amino-6-(4-hydroxy-3-methoxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methyl phenol (LXVIII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & 3492, 3378 (\text{1}^{\circ}-\text{N-H}), 3369 (\text{O-H}), 3342 (\text{2}^{\circ}-\text{N-H}), 3076 (\text{Ar-H}), 2954 (\text{C-H}), 1412 (\text{C-N}), 1176 (\text{C-O-C}); \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_6) ppm: } & 2.86 (3H, s, \text{CH}_3), 3.86 (3H, s, \text{OCH}_3), 4.61 (1H, d, J = 8.40 \text{ Hz, CH}), 5.21 (2H, br s, \text{NH}_2), 5.69 (1H, d, J = 12.40 \text{ Hz, NH-CH}), 6.89 (2H, s, \text{OH}), 7.13-7.29 (6H, m, \text{Ar-H}), 7.89 (1H, s, NH); \\
\text{Mass (m/z): } & M^+ 325, M^{+1} 326; \\
\text{Anal. Calcd. for } C_{18}H_{16}N_3O_3: & \text{C: 66.45, H: 5.89, N: 12.91. Found: C: 66.17, H: 5.87, N: 12.85.}
\end{align*}
\]
Experimental

4-[2-Amino-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methyl phenol (LXIX)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3489, 3378 (1^\circ-N-H), 3367 (O-H), 3338 (2^\circ-N-H), 3076 (Ar-H), 2947 (C-H), 1429 (C-N), 1176 (C-O-C); \\
^1\text{H-NMR (DMSO-}d_6) \text{ ppm:} & \quad 2.38 (3H, s, CH_3), 3.89 (9H, s, 3 \times OCH_3), 4.58 (1H, d, J = 8.40 Hz, CH), 5.27 (2H, br s, NH_2), 5.99 (1H, d, J = 12.40 Hz, NH-CH), 6.68-6.87 (2H, m, Ar-H), 6.93 (1H, s, OH), 7.48-7.77 (3H, m, Ar-H), 7.90 (1H, s, NH); \\
\text{Mass (m/z):} & \quad M^+ 353, M^{+1} 354; \\
\text{Anal. Calcd. for C}_{20}\text{H}_{23}\text{N}_3\text{O}_4:} & \quad C: 65.03, H: 6.28, N: 11.37. \text{Found: C: 64.73, H: 6.28, N: 11.39.}
\end{align*}
\]

4-[2-Amino-6-(2,6-dichlorophenyl)-3,4-dihydropyrimidin-4-yl]-2-methyl phenol (LXX)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3498, 3388 (1^\circ-N-H), 3369 (O-H), 3336 (2^\circ-N-H), 3046 (Ar-H), 2968 (C-H), 1448 (C=N), 1084 (C-Cl); \\
^1\text{H-NMR (DMSO-}d_6) \text{ ppm:} & \quad 2.89 (3H, s, CH_3), 4.57 (1H, d, J = 8.0 Hz, CH), 5.26 (2H, br s, NH_2), 5.91 (1H, d, J = 12.0 Hz, NH-CH), 6.97 (1H, s, OH), 7.47-7.78 (6H, m, Ar-H), 7.93 (1H, s, NH); \\
\text{Mass (m/z):} & \quad M^+ 348, M^{+2} 350; \text{Anal. Calcd. for C}_{17}\text{H}_{15}\text{Cl}_2\text{N}_3\text{O}:} & \quad C: 58.63, H: 4.34, N: 12.07. \text{Found: C: 58.85, H: 4.32, N: 12.10.}
\end{align*}
\]
4-[2-Amino-6-(3-nitrophenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol

(LXXI)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3494, 3386 (\text{N-H}), 3367 (\text{O-H}), 3339 (\text{N-H}), 3049 (\text{Ar-H}), 2939 (\text{C-H}), 1458 (\text{C=N}), 1506, 1301 (\text{N=O}) ; \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_{6}) ppm:} & \quad 2.39 (3\text{H}, \text{s}, \text{CH}_3), 4.53 (1\text{H}, \text{d}, J = 8.0 \text{ Hz}, \text{CH}), 5.28 (2\text{H}, \text{br s}, \text{NH}_2), 5.69 (1\text{H}, \text{d}, J = 12.0 \text{ Hz}, \text{NH-CH}), 6.93 (1\text{H}, \text{s}, \text{OH}), 7.68-7.74 (3\text{H}, \text{m}, \text{Ar-H}), 7.89 (1\text{H}, \text{s}, \text{NH}), 7.92-8.10 (4\text{H}, \text{m}, \text{Ar-H}); \\
\text{Mass (m/z):} & \quad \text{M}^+ 324, \text{M}^{15} 325; \text{Anal. Calcd. for C}_{17}\text{H}_{15}\text{N}_{4}\text{O}_{2}; \text{C: 62.95, H: 4.97, N: 17.27. Found: C: 62.69, H: 4.97, N: 17.22.}
\end{align*}
\]

4-[2-Amino-6-(2-hydroxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol

(LXXII)

\[
\begin{align*}
\text{FT-IR (KBr) cm}^{-1}: & \quad 3496, 3388 (\text{N-H}), 3369 (\text{O-H}), 3328 (\text{N-H}), 3052 (\text{Ar-H}), 2946 (\text{C-H}), 1461 (\text{C=N}) ; \\
\text{\textsuperscript{1}H-NMR (DMSO-\text{d}_{6}) ppm:} & \quad 2.43 (3\text{H}, \text{s}, \text{CH}_3), 4.66 (1\text{H}, \text{d}, J = 8.4 \text{ Hz}, \text{CH}), 5.13 (2\text{H}, \text{br s}, \text{NH}_2), 5.78 (1\text{H}, \text{d}, J = 12.8 \text{ Hz}, \text{NH-CH}), 6.91 (2\text{H}, \text{s}, \text{OH}), 7.08-7.49 (7\text{H}, \text{m}, \text{Ar-H}), 7.97 (1\text{H}, \text{s}, \text{NH}); \\
\text{Mass (m/z):} & \quad \text{M}^+ 295, \text{M}^{15} 296; \text{Anal. Calcd. for C}_{17}\text{H}_{17}\text{N}_{3}\text{O}_2; \text{C: 69.14, H: 5.80, N: 14.23. Found: C: 69.39, H: 5.80, N: 14.26.}
\end{align*}
\]
4-[2-Amino-6-(3-bromophenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXXIII)

\[
\text{FT-IR (KBr) cm}^{-1}: 3491, 3387 (1\text{-N-H}), 3366 (\text{O-H}), 3328 (2\text{-N-H}), 3050 (\text{Ar-H}), 2945 (\text{C-H}), 2945 (\text{C-H}), 2860 (\text{C-H}), 1458 (\text{C-N}), 1358 (\text{C-Br})
\]

\[\text{^1H-NMR (DMSO-}d_6\text{) ppm: 2.37 (3H, s, CH}_3\text{), 4.62 (1H, d, J = 8.0 Hz, CH), 5.14 (2H, br s, NH}_2\text{), 5.91 (1H, d, J = 12.0 Hz, NH-CH), 6.97 (1H, s, OH), 7.61-7.86 (7H, m, Ar-H), 7.92 (1H, s, NH)}\]

\[\text{Mass (m/z): M}^+ 358, \text{M}^{+2} 360; \text{Anal. Calcd. for C}_{17}\text{H}_{16}\text{BrN}_3\text{O: C: 57.0, H: 4.50, N: 11.73. Found: C: 56.77, H: 4.49, N: 11.76.}\]

4-[2-Amino-6-(3,4-trimethoxyphenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol (LXXIV)

\[
\text{FT-IR (KBr) cm}^{-1}: 3487, 3376 (1\text{-N-H}), 3369 (\text{O-H}), 3331 (2\text{-N-H}), 3063 (\text{Ar-H}), 2952 (\text{C-H}), 1438 (\text{C=N}), 1182 (\text{C-O-C})
\]

\[\text{^1H-NMR (DMSO-}d_6\text{) ppm: 2.38 (3H, s, CH}_3\text{), 3.92 (9H, s, 3 x OCH}_3\text{), 4.58 (1H, d, J = 8.4 Hz, CH), 5.26 (2H, br s, NH}_2\text{), 5.99 (1H, d, J = 12.8 Hz, NH-CH), 6.49-6.61 (2H, m, Ar-H), 6.89 (1H, s, OH), 7.65-7.83 (3H, m, Ar-H), 7.95 (1H, s, NH)}\]

General procedure for synthesis of 2-chloro-N-[6-(4-hydroxy-3-methylphenyl)-4-(substituted phenyl)-1,6-dihydropyrimidin-2-yl]acetamide (LXXV- LXXVIII)

The aminopyrimidines (LXII, LXIV, LXV, LXVII) were refluxed with equimolar proportions of chloro acetyl chloride in the presence of triethylamine and benzene to afford the chloroacetyl derivatives of pyrimidines.

\[ \text{HO} \quad \text{Cl} \quad \text{R} \quad \text{O} \quad \text{Cl} \]

\[ \text{HN} \quad \text{Cl} \quad \text{HN} \quad \text{Cl} \]

S. No. | Compound No. | R
--- | --- | ---
1. | LXXV | H
2. | LXXVI | 2-Chloro
3. | LXXVII | 4-Methoxy
4. | LXXVIII | 3,4-Dimethoxy

Spectral data of the synthesized compounds (LXXV- LXXVIII)

2-Chloro-N-[6-(4-hydroxy-3-methylphenyl)-4-phenyl-1,6-dihydropyrimidin-2-yl]acetamide (LXXV)
Experimental

Synthetic Work

FT-IR (KBr) cm⁻¹: 3472 (N-H), 3420 (O-H), 3366 (2°-N-H), 3042 (Ar-H), 2958 (C-H), 1642 (C=O), 1573 (N-H bending), 1449 (C=N), 769 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 2.63 (3H, s, CH₃), 4.27 (2H, s, CH₂), 4.82 (1H, d, CH), 5.97 (1H, d, NH-CH), 6.68 (1H, s, OH), 6.81-7.76 (8H, m, Ar-H), 7.96 (1H, s, NH-pyrimidine), 8.49 (1H, s, NH); Mass (m/z): M⁺ 355, M⁺² 357; Anal. Calcd. for C₁₀H₁₀ClN₂O₂: C: 64.13, H: 5.10, N: 11.81. Found: C: 63.87, H: 4.90, N: 12.77.

2-Chloro-N-[4-(2-chlorophenyl)-6-(4-hydroxy-3-methylphenyl)-1,6-dihydro pyrimidin-2-yl]acetamide (LXXVI)

2-Chloro-N-[6-(4-hydroxy-3-methylphenyl)-4-(4-methoxyphenyl)-1,6-dihydro pyrimidin-2-yl]acetamide (LXXVII)

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Experimental Synthesis Work

FT-IR (KBr) cm⁻¹: 3478 (N-H), 3426 (O-H), 3303 (2°-N-H), 3094 (Ar-H), 2970 (C-H), 1649 (C=O), 1586 (C=N), 1570 (N-H bending), 785 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 2.78 (3H, s, CH₃), 3.84 (3H, s, OCH₃), 4.21 (2H, s, CH₂), 4.62 (1H, d, CH), 5.94 (1H, d, NH-CH), 6.59 (1H, s, OH), 6.98-7.81 (7H, m, Ar-H), 7.79 (1H, s, NH-pyrimidine), 8.66 (1H, s, NH); Mass (m/z): M⁺ 385, M⁺² 387; Anal. Calcd. for C₂₀H₂₀ClN₃O₃: C: 62.26, H: 5.22, N: 10.89. Found: C: 62.03, H: 5.19, N: 10.85.

2-Chloro-N-[6-(4-hydroxy-3-methylphenyl)-4-(3,4-dimethoxyphenyl)-1,6-dihydro pyrimidin-2-yl]acetamide (LXXVIII)

![Chemical structure image]

FT-IR (KBr) cm⁻¹: 3481 (N-H), 3329 (O-H), 3204 (2°-N-H), 3096 (Ar-H), 2963 (C-H), 1664 (C=O), 1560 (N-H bending), 1416 (C-N), 787 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 2.72 (3H, s, CH₃), 3.81 (6H, s, 2 x OCH₃), 4.31 (2H, s, CH₂), 4.63 (1H, d, CH), 5.89 (1H, d, NH-CH), 6.57 (1H, s, OH), 6.83-7.86 (6H, m, Ar-H), 7.90 (1H, s, NH-pyrimidine), 8.62 (1H, s, NH); Mass (m/z): M⁺ 415, M⁺² 417; Anal. Calcd. for C₂₁H₂₂ClN₃O₄: C: 60.65, H: 5.33, N: 10.10. Found: C: 60.43, H: 5.30, N: 10.07.

General procedure for synthesis of 4-(4-hydroxy-3-methylphenyl)-6-(substituted phenyl)-1,6-dihydropyrimidin-2-ol (LXXIX - LXXXIV)

A mixture of various chalcones (0.001 M), urea (0.001 M) and 0.1 g of sodium hydroxide in 25 mL of 80% dilute ethanol was refluxed for 15 h, cooled, filtered and recrystallized from hot methanol.
Experimental

Synthetic Work

\[
\begin{align*}
&\text{CH}_3 \\
&\text{ HO..} \\
&\text{--------} \\
&\text{NaOH} \\
&\text{dil 80\% C}_2\text{H}_5\text{OH} \\
&\text{(LXXIX - LXXXIV)}
\end{align*}
\]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound No.</th>
<th>R</th>
</tr>
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<td>LXXX</td>
<td>2-Chloro</td>
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<tr>
<td>3.</td>
<td>LXXXI</td>
<td>4-Methoxy</td>
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<td>4.</td>
<td>LXXXII</td>
<td>3,4-Dimethoxy</td>
</tr>
<tr>
<td>5.</td>
<td>LXXXIII</td>
<td>4-Fluoro</td>
</tr>
<tr>
<td>6.</td>
<td>LXXXIV</td>
<td>2,6-Dichloro</td>
</tr>
</tbody>
</table>

4-(4-Hydroxy-3-methylphenyl)-6-phenyl-1,6-dihydropyrimidin-2-ol (LXXIX)

\[
\begin{align*}
&\text{FT-IR (KBr) cm}^{-1}: 3401 (\text{OH}), 3310 (\text{N-H}), 3065 (\text{Ar-H}), 2956 (\text{C-H}); 1^1\text{H-NMR} \\
&(\text{DMSO-}d_6) \text{ ppm: } 2.54 (3\text{H}, \text{s, CH}_3), 4.49 (1\text{H}, \text{d, CH}), 5.97 (1\text{H}, \text{d, NH-CH}), 6.96 \\
&(2\text{H}, \text{s, OH}), 7.31-7.79 (8\text{H, m, Ar-H}), 7.92 (1\text{H}, \text{s, NH}); \text{Mass} (m/z): \text{M}^+ 280, \text{M}^{+1} 281; \text{Anal. Calcd. for C}_{17}\text{H}_{16}\text{N}_2\text{O}_2: C: 72.84, \text{H: 5.75, N: 9.99. Found: C: 72.57, H: 5.73, N: 9.95.}
\end{align*}
\]
6-(2-Chlorophenyl)-4-(4-hydroxy-3-methylphenyl)-1,6-dihydropyrimidin-2-ol (LXXX)

![Chemical structure of LXXX]

FT-IR (KBr) cm⁻¹: 3399 (OH), 3329 (N-H), 3072 (Ar-H), 2967 (C-H), 1062 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 2.83 (3H, s, CH₃), 4.48 (1H, d, CH), 5.94 (1H, d, NH-CH), 6.92 (2H, s, OH), 6.89-7.79 (7H, m, Ar-H), 7.88 (1H, s, NH); Mass (m/z): M⁺ 314, M⁺² 316; Anal. Caled. for C₁₇H₁₅CIN₂O₂: C: 64.87, H: 4.80, N: 8.90. Found: C: 64.61, H: 4.79, N: 8.88.

4-(4-Hydroxy-3-methylphenyl)-6-(4-methoxyphenyl)-1,6-dihydropyrimidin-2-ol (LXXXI)

![Chemical structure of LXXXI]

FT-IR (KBr) cm⁻¹: 3405 (OH), 3332 (N-H), 3076 (Ar-H), 2958 (C-H); ¹H-NMR (DMSO-d₆) ppm: 2.90 (3H, s, CH₃), 3.72 (3H, s, OCH₃), 4.46 (1H, d, CH), 5.92 (1H, d, NH-CH), 6.86 (2H, s, OH), 7.41-7.82 (7H, m, Ar-H), 7.93 (1H, s, NH); Mass (m/z): M⁺ 310, M⁺² 311; Anal. Caled. for C₁₈H₁₆N₂O₃: C: 69.66, H: 5.85, N: 9.03. Found: C: 69.38, H: 5.85, N: 9.05.

6-(3,4-Dimethoxyphenyl)-4-(4-hydroxy-3-methylphenyl)-1,6-dihydropyrimidin-2-ol (LXXXII)

![Chemical structure of LXXXII]
Experimental

Synthetic Work

FT-IR (KBr) cm⁻¹: 3435 (OH), 3368 (N-H), 3052 (Ar-H), 2957 (C-H); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.57 (3H, s, CH₃), 3.33 (6H, s, 2 × OCH₃), 4.98 (1H, d, CH), 6.15 (1H, d, NH-CH), 6.26 (2H, s, OH), 6.39-6.95 (6H, m, Ar-H), 7.60 (1H, s, NH); Mass (m/z): M⁺ 340, M⁺⁺ 341; Anal. Calcd. for C₁₉H₂₀N₂O₄: C: 67.05, H: 5.23, N: 8.23. Found: C: 66.80, H: 5.89, N: 8.20.

6-(4-Hydroxy-3-methoxyphenyl)-4-(4-hydroxy-3-methylphenyl)-1,6-dihydropyrimidin-2-ol (LXXXIII)

\[ \text{FT-IR (KBr) cm}^{-1}: \text{3421 (OH), 3340 (N-H), 3079 (Ar-H), 2964 (C-H), (C-F) 1210;} \]
\[ \text{\(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.6 (3H, s, CH₃), 4.98 (1H, d, CH), 5.41 (1H, d, NH-CH), 6.84 (2H, s, OH), 6.84-7.78 (7H, m, Ar-H), 7.91 (1H, s, NH); Mass} \]
\[ \text{(m/z): M}^+ \text{ 298, M}^{+\_2} \text{ 299; Anal. Calcd. for C}_{17}\text{H}_{15}\text{FN}_{2}\text{O}_{2}: C: 68.45, H: 5.07, N: 8.56. Found: C: 68.17, H: 5.06, N: 8.52.} \]

6-(2,6-Dichlorophenyl)-4-(4-hydroxy-3-methylphenyl)-1,6-dihydropyrimidin-2-ol (LXXXIV)

\[ \text{FT-IR (KBr) cm}^{-1}: \text{3388 (OH), 3337 (N-H), 3085 (Ar-H), 2973 (C-H), 1059 (C-Cl);} \]
\[ \text{\(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.63 (3H, s, CH₃), 4.91 (1H, d, CH), 5.91 (1H, d, NH-CH), 6.93 (2H, s, OH), 6.79-7.85 (6H, m, Ar-H), 7.94 (1H, s, NH); Mass} \]
\[ \text{(m/z): M}^+ \text{ 349, M}^{+\_2} \text{ 351; Anal. Calcd. for C}_{17}\text{H}_{14}\text{Cl}_{2}\text{N}_{2}\text{O}_{2}: C: 58.47, H: 4.04, N: 8.02. Found: C: 58.24, H: 4.03, N: 7.99.} \]

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Experimental

SCHEME-IV

Synthesis of 2-(4-formyl-2-methoxyphenoxy)acetic acid (LXXXV)

A mixture of 3-methoxy-4-hydroxybenzaldehyde and chloroacetic acid was refluxed in presence of sodium hydroxide solution (2 M) for 3-4 h. After the completion of reaction, the mixture was cooled, acidified with conc. HCl and allowed to stand overnight and precipitate was filtered and recrystallized with hot water.

\[
\begin{align*}
\text{HO} & - \text{CHO} + \text{Cl} & \text{KOH} & \rightarrow \text{HO} & - \text{O} & - \text{CHO} \\
\text{H}_2\text{CO} & & & & & \text{H}_2\text{CO}
\end{align*}
\]

(LXXXV)

FT-IR (KBr) cm\(^{-1}\): 3362 (OH), 3076 (Ar-H), 2969 (C-H), 1689 (C=O); \(^1\text{H}-\text{NMR (DMSO-}d_6\text{)}\) ppm: 3.62 (3H, s, OCH\(_3\)), 4.77 (2H, s, CH\(_2\)), 6.89-7.65 (3H, m, Ar-H), 9.93 (1H, s, CHO), 12.89 (1H, s, COOH).

General procedure for synthesis of 4-[3-(substituted phenyl)-3-oxoprop-1-enyl]-2-methoxyphenoxyacetic acid (LXXXVI - XCI)

Equimolar quantities of 2-(4-formyl-2-methoxyphenoxy)acetic acid (LXXXV) and substituted acetophenones in oxygen-free ethanol were stirred at room temperature in the presence of base (aqueous solution of potassium hydroxide 30%, 5 mL) till completion of the reaction. The reaction mixture was allowed to stand overnight and then poured into ice-cold water followed by neutralization with HCl. The solid separated was filtered, dried and recrystallized from absolute ethanol. The purity of the chalcone was checked by TLC using solvent system petroleum ether: ethyl acetate (7:3).

\[
\begin{align*}
\text{HO} & - \text{O} & - \text{O} & - \text{CHO} & \text{KOH} / \text{CH}_2\text{OH} & \rightarrow \text{HO} & - \text{O} & - \text{O} & - \text{CHO}
\end{align*}
\]

(LXXXV)

(LXXXVI - XCI)
Experimental

SYNTHETIC WORK

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<td>H</td>
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<td>6.</td>
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<td>2,4-Dihydroxy</td>
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</table>

Spectral data of the synthesized compounds (LXXXVI - XCI)

2-Methoxy-4-[3-oxo-3-phenylprop-1-en-1-yl]phenoxyacetic acid (LXXXVI)

\[
\begin{align*}
\text{HO} & \text{O} \\
\text{C} & \text{H}_3 \\
\end{align*}
\]

FT-IR (KBr) cm\(^{-1}\): 3358 (br O-H), 3068 (Ar-H), 2976 (C-H), 1682, 1699 (C=O), 1572 (C=C), 1192 (C-O-C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 3.61 (3H, s, OCH\(_3\)), 4.13 (2H, s, CH\(_2\)), 6.42 (1H, d, \(J = 8.4\) Hz, H\(_a\)), 6.5-7.13 (8H, m, Ar-H), 7.34 (1H, d, \(J = 8.9\) Hz, H\(_b\)), 12.73 (1H, s, COOH\(_a\)); Anal. Calcd. for C\(_{18}\)H\(_{16}\)O\(_3\): C: 69.22, H: 5.16. Found: C: 68.94, H: 5.14.

4-[3-(4-Fluorophenyl)-3-oxoprop-1-en-1-yl]-2-methoxyphenoxyacetic acid (LXXXVII)

\[
\begin{align*}
\text{HO} & \text{O} \\
\text{C} & \text{H}_3 \\
\end{align*}
\]

FT-IR (KBr) cm\(^{-1}\): 3348 (O-H), 3058 (Ar-H), 2965 (C-H), 1704, 1695 (C=O), 1575 (C=C), 1210 (C-F), 1197 (C-O-C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 3.46 (3H, s, OCH\(_3\)), 4.34 (2H, s, CH\(_2\)), 6.64 (1H, d, \(J = 8.6\) Hz, H\(_a\)), 6.73-7.26 (7H, m, Ar-H), 7.34 (1H, d, \(J = 8.9\) Hz, H\(_b\)), 12.73 (1H, s, COOH\(_a\)).
7.39 (1H, d, J = 8.7 Hz, H_6), 12.33 (1H, s, COOH); Anal. Caled. for C_{18}H_{18}F_{10}: C: 65.45, H: 4.58. Found: C: 65.18, H: 4.56.

4-\{3-(2-Hydroxyphenyl)-3-oxoprop-1-en-1-yl\}-2-methoxyphenoxyacetic acid

(LXXXVIII)

\[
\text{HO} \quad \text{O} \\
\text{OCH}_3 \\
\text{HO} \\
\]

FT-IR (KBr) cm\(^{-1}\): 3359 (br O-H), 3339 (O-H), 3079 (Ar-H), 2983 (C-H), 1719, 1702 (C=O), 1589 (C=C), 1212 (C-O-C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 3.73 (3H, s, OCH\(_3\)), 4.28 (2H, s, CH\(_2\)), 5.89 (1H, s, OH), 6.46 (1H, d, J = 8.5 Hz, H\(_a\)), 6.66-7.20 (7H, m, Ar-H), 7.31 (1H, d, J = 8.8 Hz, H\(_b\)), 12.44 (1H, s, COOH); Anal. Caled. for C_{18}H_{16}O\(_6\): C: 65.45, H: 4.58. Found: C: 65.20, H: 4.57.

4-\{3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl\}-2-methoxyphenoxy acetic acid

(LXXXIX)

\[
\text{HO} \quad \text{O} \\
\text{OCH}_3 \\
\text{HO} \\
\]

FT-IR (KBr) cm\(^{-1}\): 3349 (br O-H), 3081 (Ar-H), 2964 (C-H), 1712, 1699 (C=O), 1576 (C=C), 1189 (C-O-C); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 3.98 (6H, s, 2 \times OCH\(_3\)), 4.76 (2H, s, CH\(_2\)), 6.11 (1H, d, J = 8.5 Hz, H\(_a\)), 6.50-7.18 (7H, m, Ar-H), 7.21 (1H, d, J = 8.8 Hz, H\(_b\)), 12.35 (1H, s, COOH); Anal. Caled. for C_{19}H_{18}O\(_6\): C: 66.66, H: 5.30. Found: C: 66.39, H: 5.29.

4-\{3-(2,6-Dichlorophenyl)-3-oxoprop-1-en-1-yl\}-2-methoxyphenoxyacetic acid

(XC)
FT-IR (KBr) cm⁻¹: 3359 (br O-H), 3082 (Ar-H), 2968 (C-H), 1706, 1691 (C-0),
1551 (C=C), 1210 (C-O-C), 1067 (C-Cl); ¹H-NMR (DMSO-d₆) ppm: 3.39 (3H, s,
OCH₃), 4.59 (2H, s, CH₂), 6.62 (1H, d, J = 8.7 Hz, H₆), 6.79-7.31 (6H, m, Ar-H),
7.41 (1H, d, J = 8.8 Hz, H₅), 12.32 (1H, s, COOH); Anal. Calcd. for C₁₈H₁₄Cl₂O₅:
C: 56.71, H: 18.60. Found: C: 56.48, H: 18.54.

4-[3-(2,4-Dihydroxyphenyl)-3-oxoprop-l-en-1-yl]-2methoxyphenoxycetic
acid (XCI)

FT-IR (KBr) cm⁻¹: 3369 (br OH), 3352 (O-H), 3087 (Ar-H), 2968 (C-H), 1704,
1686 (C-0), 1595 (C=C), 1198 (C-O-C); ¹H-NMR (DMSO-d₆) ppm: 3.57 (3H, s,
OCH₃), 4.46 (2H, s, CH₂), 6.33 (2H, s, OH), 6.47 (1H, d, J = 8.4 Hz, H₆), 6.77-
7.49 (6H, m, Ar-H), 7.63 (1H, d, J = 8.8 Hz, H₅), 12.83 (1H, s, COOH); Anal.

General procedure for synthesis of 4-[6-(substitutedphenyl)-2-sulfanyl-1,2-
dihydro pyrimidin-4-yl]-2-methoxyphenoxycetic acid (XClII - XCVII)

A mixture of chalcones (LXXXVI - XCI) and thiourea (0.01 M, 0.78 g) in the
presence of dry dioxane and alcoholic potassium hydroxide was refluxed for 10 h.
The excess solvent was distilled off and the residue obtained was recrystallized
with ethanol.
Spectral data of the synthesized compounds (XCII - XCVII)

2-Methoxy-4-(6-phenyl-2-sulfanyl-1,2-dihydropyrimidin-4-yl)phenoxyacetic acid (XCII)

![Chemical Structure](attachment:image.png)

FT-IR (KBr) cm⁻¹: 3345 (br OH), 3288 (N-H), 3077 (Ar-H), 2969 (C-H), 2538 (S-H), 1686 (C=O), 1598 (C=C), 1197 (C=O-C), 670 (C=S); ^1^H-NMR (DMSO-d₆) ppm: 2.99 (IH, s, SH), 3.6 (3H, s, OCH₃), 4.70 (2H, s, CH₂), 4.91 (1H, d, CH), 5.82 (1H, d, NH-CH), 6.91-7.48 (8H, m, Ar-H), 7.78 (1H, s, NH), 12.43 (1H, s, COOH); Mass (m/z): M⁺ 370, M⁺¹ 371; Anal. Calcd. for C₁₉H₁₈N₂O₄S: C: 61.61, H: 4.90, N: 7.56. Found: C: 61.36, H: 4.89, N: 7.54.

4-[6-(4-Fluorophenyl)-2-sulfanyl-1,2-dihydropyrimidin-4-yl]-2-methoxy phenoxyacetic acid (XCIII)

![Chemical Structure](attachment:image.png)
Experimental

Synthetic Work

FT-IR (KBr) cm\(^{-1}\): 3351 (br OH), 3292 (N-H), 3086 (Ar-H), 2982 (C-H), 2546 (S-H), 1711 (C=O), 1578 (C=C), 1216 (C-F), 1188 (C-O-C), 682 (C=S); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.92 (1H, s, SH), 3.70 (3H, s, OCH\(_3\)), 4.62 (2H, s, CH\(_2\)), 4.86 (1H, d, CH), 5.92 (1H, d, NH-CH), 7.11-7.68 (7H, m, Ar-H), 7.79 (1H, s, NH), 12.67 (1H, s, COOH); Mass (m/z): \(M^+\) 388, \(M^{1+}\) 389; Anal. Calcd. for C\(_{19}\)H\(_{17}\)FN\(_2\)O\(_4\)S: C: 58.75, H: 4.41, N: 7.21. Found: C: 58.51, H: 4.39, N: 7.18

4-[6-(2-Hydroxyphenyl)-2-sulfanyl-1,2-dihydropryrimidin-4-yl]-2-methoxy phenoxyacetic acid (XCV)

\[
\text{HO} - \begin{array}{c}
\text{OCH}_3 \\
\text{SH} \\
\text{OCH}_3 \\
\text{SH}
\end{array}
\]

FT-IR (KBr) cm\(^{-1}\): 3361 (br OH), 3353 (O-H), 3279 (N-H), 3069 (Ar-H), 2977 (C-H), 2561 (S-H), 1689 (C=O), 1588 (C=C), 1187 (C-O-C), 683 (C=S); \(^1\)H-NMR (DMSO-\(d_6\)) ppm: 2.89 (1H, s, SH), 3.36 (3H, s, OCH\(_3\)), 4.45 (2H, s, CH\(_2\)), 4.90 (1H, d, CH), 6.07 (1H, d, NH-CH), 6.39 (1H, s, OH), 7.15-7.71 (7H, m, Ar-H), 7.89 (1H, s, NH), 12.74 (1H, s, COOH); Mass (m/z): \(M^+\) 386, \(M^{1+}\) 387; Anal. Calcd. for C\(_{19}\)H\(_{18}\)N\(_2\)O\(_5\)S: C: 59.06, H: 4.70, N: 7.25. Found: C: 58.82, H: 4.68, N: 7.23.

4-[6-(4-Methoxyphenyl)-2-sulfanyl-1,2-dihydropryrimidin-4-yl]-2-methoxy phenoxyacetic acid (XCV)

\[
\text{HO} - \begin{array}{c}
\text{OCH}_3 \\
\text{SH} \\
\text{OCH}_3 \\
\text{SH}
\end{array}
\]

FT-IR (KBr) cm\(^{-1}\): 3357 (br OH), 3291 (N-H), 3078 (Ar-H), 2973 (C-H), 2567 (S-H), 1707 (C=O), 1589 (C=C), 1206 (C-O-C), 681 (C=S); \(^1\)H-NMR (DMSO-\(d_6\))
Experimental

Synthetic Work

ppm: 2.93 (1H, s, SH), 3.85 (6H, s, 2 × OCH₃), 4.63 (2H, s, CH₂), 4.92 (1H, d, CH), 5.78 (1H, d, NH-CH), 7.07-7.74 (7H, m, Ar-H), 7.78 (1H, s, NH), 12.80 (1H, s, COOH); Mass (m/z): M⁺ 400, M⁺² 401; Anal. Calcd. for C₂₀H₂₀N₂O₅S: C: 59.99, H: 5.03, N: 7.00. Found: C: 59.75, H: 5.01, N: 6.98.

4-[6-(2,6-Dichlorophenyl)-2-sulfanyl-1,2-dihydropyrimidin-4-yl]-2-methoxy phenoxyacetic acid (XCVI)

FT-IR (KBr) cm⁻¹: 3359 (br OH), 3308 (N-H), 3091 (Ar-H), 2974 (C-H), 2561 (S-H), 1693 (C=O), 1578 (C=C), 1188 (C-O-C), 1076 (C-Cl), 681 (C-S); ¹H-NMR (DMSO-d₆) ppm: 2.95 (1H, s, SH), 3.27 (3H, s, OCH₃), 4.23 (2H, s, CH₂), 4.88 (1H, d, CH), 5.97 (1H, d, NH-CH), 6.98-7.63 (6H, m, Ar-H), 7.89 (1H, s, NH), 12.82 (1H, s, COOH); Mass (m/z): M⁺ 439, M⁺² 441; Anal. Calcd. for C₁₉H₁₆Cl₂N₂O₄S: C: 51.95, H: 3.67, N: 6.38. Found: C: 51.74, H: 3.67, N: 6.36.

4-[6-(2,4-Dihydroxyphenyl)-2-sulfanyl-1,2-dihydropyrimidin-4-yl]-2-methoxy phenoxyacetic acid (XCVII)

FT-IR (KBr) cm⁻¹: 3367 (br OH), 3344 (OH), 3298 (N-H), 3076 (Ar-H), 2983 (C-H), 2579 (S-H), 1711 (C=O), 1599 (C=C), 1193 (C-O-C), 686 (C=S); ¹H-NMR (DMSO-d₆) ppm: 2.88 (1H, s, SH), 3.58 (3H, s, OCH₃), 4.53 (2H, s, CH₂), 5.02 (1H, d, CH), 5.99 (1H, d, NH-CH), 6.84 (2H, s, OH), 7.11-7.78 (6H, m, Ar-H), 7.96 (1H, s, NH), 12.79 (1H, s, COOH); Mass (m/z): M⁺ 402, M⁺² 403; Anal.

Ph.D Thesis

Jamia Hamdard

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Experimental


**SCHEME-V**

Synthesis of 3-(substituted phenyl)-1-(pyridin-2-yl)prop-2-en-1-one (XCVIII – CIII)

A mixture of 2-acetylpyridine (0.01 M) and various aryl aldehydes (0.01 M) were stirred in ethanol (25 mL). To this mixture an aqueous solution of NaOH (40%, 10 mL) was added at once and the reaction mixture was stirred for 40 min at room temperature. The mixture was kept overnight at room temperature, poured into crushed ice and then acidified with dil HCl. The solid separated, was filtered, washed with water until neutral. The resulting chalcone was purified by recrystallization with ethanol.

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<td>6.</td>
<td>CIII</td>
<td>3,4-Dimethoxy</td>
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</table>
Experimental

Synthetic Work

Spectral data of the synthesized compounds (XCVIII – CIII)

3-Phenyl-1-(pyridin-2-yl) prop-2-en-1-one (XCVIII)

![Chemical structure of 3-Phenyl-1-(pyridin-2-yl) prop-2-en-1-one](image)

FT-IR (KBr) cm⁻¹: 3077, 3029 (Ar-H), 1716 (C=O), 1648 (C=N), 1589 (C=C), 1670-1555 (skeletal vibrations); 'H-NMR (CDCl₃) ppm: 7.23 (1H, d, J = 8.4 Hz, H₂), 7.76 (2H, d, Ar H-3',5'), 7.86 (1H, d, J = 8.9 Hz, H₆), 7.94 (1H, m, Ar H-4'), 8.01 (2H, d, Ar H-2,6), 8.41 (1H, m, Ar H-4), 7.94 (1H, d, Ar H-6'), 8.84 (2H, d, Ar H-3,5). Anal. Calcd. for C₁₅H₁₃NO: C: 71.98, H: 5.64, N: 22.38. Found: C: 71.37, H: 5.44, N: 22.24.

3-(4-Methoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (XCIX)

![Chemical structure of 3-(4-Methoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one](image)

FT-IR (KBr) cm⁻¹: 3068, 3034 (Ar-H), 2988 (C-H), 1714 (C=O), 1635 (C-N), 1578 (C=C), 1679-1565 (skeletal vibrations), 1186 (C-O-C); 'H-NMR (CDCl₃) ppm: 3.84 (3H, s, OCH₃), 6.94 (1H, d, J = 8.6 Hz, H₂), 7.95 (1H, d, J = 8.7 Hz, H₆), 8.04 (2H, d, Ar H-3',5'), 8.10 (2H, d, Ar H-2,6), 8.44 - 8.52 (2H, m, Ar H-3,5), 8.65 (1H, m, Ar H-4'), 9.10 (1H, d, Ar H-6'). Anal. Calcd. for C₁₅H₁₃NO₂: C: 75.30, H: 5.48, N: 5.85. Found: C: 75.28, H: 5.38, N: 5.57.

3-(4-Fluorophenyl)-1-(pyridin-2-yl) prop-2-en-1-one (C)

![Chemical structure of 3-(4-Fluorophenyl)-1-(pyridin-2-yl) prop-2-en-1-one](image)
Experimental Work

FT-IR (KBr) cm⁻¹: 3073, 3015 (Ar-H), 1710 (C=O), 1639 (C=N), 1582 (C=C), 1679-1565 (skeletal vibrations), 1187 (C-F); ¹H-NMR (CDCl₃) ppm: 7.25 (1H, d, J = 8.5 Hz, H₆), 7.36 (2H, d, Ar H-3',5'), 7.61 (1H, m, Ar H-5), 7.82 (1H, m, Ar H-4'), 8.15 (1H, s, Ar H-2), 8.24 (1H, d, J = 8.8 Hz, H₆), 8.65 (2H, d, Ar H-3, 6), 8.75 (1H, d, Ar H-6'); Anal. Calcd. for C₁₄H₁₀FNO: C: 74.00, H: 4.44, N: 6.16. Found: C: 73.76, H: 4.17, N: 6.08.

3-(2-Chlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (Cl)

FT-IR (KBr) cm⁻¹: 3082, 3029 (Ar-H), 1714 (C=O), 1642 (C=N), 1588 (C=C), 1656-1679 (skeletal vibrations), 1094 (C-Cl); ¹H-NMR (CDCl₃) ppm: 7.02 (2H, d, Ar H-4,6), 7.42 (1H, d, J = 8.5 Hz, H₆), 7.52 (2H, d, Ar H-3,5'), 7.66 (1H, m, Ar H-4'), 7.68 (1H, d, J = 8.6 Hz, H₆), 7.92 (2H, d, Ar H-3,5), 8.76 (1H, d, Ar H-6'); Anal. Calcd. for C₁₄H₁₀ClNO: C: 69.00, H: 4.14, N: 5.75. Found: C: 68.69, H: 4.06, N: 5.32.

3-(2,6-Dichlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (CII)

FT-IR (KBr) cm⁻¹: 3069, 3022 (Ar-H), 1729 (C=O), 1632 (C=N), 1589 (C=C), 1630-1565 (skeletal vibrations), 1096 (C-Cl); ¹H-NMR (CDCl₃) ppm: 7.46 (2H, d, Ar H-3,5'), 7.36 (1H, d, J = 8.7 Hz, H₆), 7.56 (1H, d, Ar H-4), 7.84 (2H, d, Ar H-3,5), 7.98 (1H, m, Ar H-4'), 8.34 (1H, d, J = 8.8 Hz, H₆), 8.78 (1H, d, Ar H-6'). Anal. Calcd. for C₁₄H₉Cl₂NO: C: 60.46, H: 3.26, N: 5.04. Found: C: 60.23, H: 3.08, N: 5.02.
3-(3,4-Dimethoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (CIII)

![Chemical Structure of CIII](image)

FT-IR (KBr) cm⁻¹: 3083, 3014 (Ar-H), 1728 (C=O), 1638 (C=N), 1565 (C-C), 1610-1500 (skeletal vibrations), 1182 (C-O-C); ¹H-NMR (CDCl₃) ppm: 3.87 (6H, s, 2 × OCH₃), 7.11 (1H, d, J = 8.4 Hz, H₇), 7.32 (2H, d, Ar H-5, 6), 7.64 (1H, s, Ar H-2), 7.86 (1H, d, J = 8.5 Hz, H₆), 8.11 (2H, d, Ar H-3',5'), 8.37 (1H, m, Ar H-4'), 9.14 (1H, d, Ar H-6'). Anal. Calcd. for C₁₆H₁₅NΟ₃: C: 71.36, H: 5.61, N: 5.20. Found: C: 71.18, H: 5.46, N: 5.12.

Synthesis of 6-(substitutedphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (CIV - CIX)

A mixture of chalcone (1 M), guanidine hydrochloride (1.5 M) and sodium hydride (3.0 M) in DMF (100 mL) was refluxed for 6-8 h. The reaction mixture was poured into ice cold water, a solid was separated out. The separated solid was filtered, washed with water and recrystallized with methanol.

![Synthesis Reaction](image)

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<th>S. No.</th>
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<td>3.</td>
<td>CVI</td>
<td>4-Fluoro</td>
</tr>
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</table>
Spectral data of the synthesized compounds (CIV - CIX)

*6-Phenyl-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (CIV)*

\[
\text{FT-IR (KBr) cm}^{-1}: 3487, 3355 (1^\circ\text{-N-H}), 3331 (2^\circ\text{-N-H}), 3056 (\text{Ar-H}), 1568 (\text{C=N}), 1565 (\text{C=C}), 1684-1510 (\text{skeletal vibrations}); \quad \text{\textsuperscript{1}H-NMR (DMSO } d_6) \text{ ppm:}
\]

- 4.61 (2H, br s, NH$_2$), 5.81 (1H, d, CH), 5.93 (1H, d, NHCH), 7.82 (1H, s, NH), 7.11-7.92 (3H, m, Ar H-3',4',5' ring-B), 8.12 (5H, m, Ar-H ring-A), 8.83 (1H, d, Ar H-6' ring-B); Mass (m/z): $M^+$ 250, $M^{11}$ 251; Anal. Calcd. for C$_{13}$H$_{14}$N$_4$: C: 71.98, H: 5.64, N: 22.38. Found: C: 71.74, H: 5.49, N: 22.37.

*6-(4-Methoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (CV)*

\[
\text{FT-IR (KBr) cm}^{-1}: 3482, 3377 (1^\circ\text{-N-H}), 3341 (2^\circ\text{-N-H}), 3072 (\text{Ar-H}), 1568 (\text{C=N}), 1561 (\text{C=C}), 1684-1510 (\text{skeletal vibrations}), 1189 (\text{C-O-C}); \quad \text{\textsuperscript{1}H-NMR (DMSO } d_6) \text{ ppm:}
\]

- 3.80 (3H, s, OCH$_3$), 4.60 (2H, br s, NH$_2$), 5.81 (1H, d, CH), 5.92 (1H, d, NHCH), 6.6 (4H, m, Ar-H ring-A), 7.81 (1H, s, NH), 8.09-8.68 (3H, m, Ar H-3',4',5' ring-B), 9.12 (1H, d, Ar H-6' ring-B); Mass (m/z): $M^+$ 280, $M^{11}$ 281.
Experimental

Synthetic Work


6-(4-Fluorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (CVI)

![Chemical Structure](Image)

FT-IR (KBr) cm\(^{-1}\): 3476, 3369 (\(\text{r-N-H}\)), 3337 (\(\text{2°-N-H}\)), 3068 (Ar-H), 1547 (C=N), 1561 (C=C), 1610-1520 (skeletal vibrations), 1196 (C-F); \(^1\text{H-NMR}\) (DMSO \(d_6\)) ppm: 4.61 (2H, br s, NH\(_2\)), 5.82 (1H, d, CH), 5.94 (1H, d, NHCH\(_3\)), 6.70 (4H, m, Ar-H ring-A), 7.83 (1H, s, NH), 8.01-8.62 (3H, m, Ar H-3',4',5' ring-B), 9.10 (1H, d, Ar H-6' ring-B); Mass (m/z): M\(^+\) 268, M\(^{+1}\) 269; Anal. Calcd. for C\(_{15}\)H\(_{13}\)FN\(_4\): C: 67.59, H: 6.03, N: 19.70. Found: C: 67.26, H: 6.01, N: 19.58.

6-(2-Chlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (CVII)

![Chemical Structure](Image)

FT-IR (KBr) cm\(^{-1}\): 3488, 3373 (\(\text{1°-N-H}\)), 3329 (\(\text{2°-N-H}\)), 3059 (Ar-H), 1547 (C=N), 1561 (C=C), 1610-1539 (skeletal vibrations), 1089 (C-Cl); \(^1\text{H-NMR}\) (DMSO \(d_6\)) ppm: 4.51 (2H, br s, NH\(_2\)), 5.81 (1H, d, CH), 5.92 (1H, d, NHCH\(_3\)), 6.82 (4H, m, Ar-H ring-A), 7.83 (1H, s, NH), 7.51-7.90 (3H, m, Ar H-3',4',5' ring-B), 8.72 (1H, d, Ar H-6' ring-B); Mass (m/z): M\(^+\) 285, M\(^{+1}\) 286; Anal. Calcd. for C\(_{15}\)H\(_{13}\)ClN\(_4\): C: 63.27, H: 4.60, N: 19.68. Found: C: 63.14, H: 4.42, N: 19.32.
6-(2,6-Dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine
(CVIII)

\[
\text{FT-IR (KBr) cm}^{-1}: 3492, 3369 (\text{N-H}), 3334 (\text{N-H}), 3067 (\text{Ar-H}), 1553 (\text{C=N}), 1576 (\text{C=C}), 1598-1520 (\text{skeletal vibrations}), 1094 (\text{C-Cl}); \ ^1\text{H-NMR (DMSO} \text{d}_6) \text{ ppm: 4.61} (2\text{H, br s, NH}_2), 5.82 (1\text{H, d, CH}), 5.91 (1\text{H, d, NHCH}), 7.40 (3\text{H, m, Ar-H ring-A}), 7.48-7.79 (3\text{H, m, Ar H-3',4',5' ring-B}), 7.87 (1\text{H, s, NH}), 8.42 (1\text{H, d, Ar H-6' ring-B}); \text{ Mass (m/z): M}^{+1} 320, M^{+2} 321; \text{ Anal. Calcd. for C}_{15}\text{H}_{12}\text{Cl}_2\text{N}_4: C: 56.44, H: 3.79, N: 17.55. \text{ Found: C: 56.23, H: 3.32, N: 17.24.}
\]

6-(3,4-Dimethoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine
(CIX)

\[
\text{FT-IR (KBr) cm}^{-1}: 3492, 3379 (\text{N-H}), 3336 (\text{N-H}), 3069 (\text{Ar-H}), 1579 (\text{C=N}), 1563 (\text{C=C}), 1648-1510 (\text{skeletal vibrations}), 1189 (\text{C=O-C}); \ ^1\text{H-NMR (DMSO} \text{d}_6) \text{ ppm: } 3.93 (6\text{H, s, 2 x OCH}_3), 4.51 (2\text{H, br s, NH}_2), 5.81(1\text{H, d, CH}), 5.92 (1\text{H, d, NHCH}), 6.40 (3\text{H, m, Ar-H ring-A}), 7.84 (1\text{H, s, NH}), 7.11-7.92 (3\text{H, m, Ar H-3',4',5' ring-B}), 8.74 (1\text{H, d, Ar H-6' ring-B}); \text{ Mass (m/z): M}^{+1} 310, M^{+1} 311; \text{ Anal. Calcd. for C}_{17}\text{H}_{18}\text{N}_4\text{O}_2: C: 65.79, H: 5.85, N: 18.05. \text{ Found: C: 65.57 H: 5.71, N: 18.01.}
\]

Synthesis of 6-(substituted phenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (CX – CV)

A mixture of chalcone (0.01 M), thiourea (0.012 M) and sodium methoxide (0.025 M) in ethanol (30 mL) was refluxed for 3-6 h. The reaction mixture was
Experimental

Synthetic Work

concentrated and cooled. The solid separated out was filtered and recrystallized from DMF/ water.

\[
\begin{align*}
\text{R} & \quad \text{Sodium Methoxide} \\
H_2N & \quad \text{NH}_2 \\
\text{SH} & \\
\end{align*}
\]

\((\text{CXVIII} - \text{CIII})\)

\[
\begin{align*}
\text{H}_\alpha & \quad \text{H}_\beta \\
\end{align*}
\]

\((\text{CX} - \text{CXV})\)

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</tr>
</tbody>
</table>

Spectral data of the synthesized compounds (CX – CXV)

6-Phenyl-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (CX)

\[
\begin{align*}
\text{SH} & \\
\end{align*}
\]

FT-IR (KBr) cm\(^{-1}\): 3201 (N-H), 3120 (Ar-H), 2591 (S-H), 1520 (C-N), 1640 (C=O), 1593 (C=C); \(^1\)H-NMR (CDCl\(_3\)) ppm: 7.74 (1H, s, NH), 6.7-8.1 (11H, m, Ar-H), 9.72 (1H, s, SH); Mass (m/z): M\(^+\) 267, M\(^{13+}\) 268; Anal. Calcd. for C\(_{15}\)H\(_{13}\)N\(_3\)S: C: 67.39, H: 4.90, N: 15.72. Found: C: 67.25, H: 4.56, N: 15.32.
6-(4-Methoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (CXI)

FT-IR (KBr) cm⁻¹: 3334 (N-H), 3119 (Ar-H), 2959 (C-H), 2585 (S-H), 1516 (C-N), 1651 (C=N), 1582 (C=C); ¹H-NMR (CDCl₃) ppm: 3.72 (3H, s, OCH₃), 7.79 (1H, s, NH), 6.81-8.32 (10H, m, Ar-H), 9.68 (1H, s, SH); Mass (m/z): M⁺ 291, M⁺ 298; Anal. Caled. for C₁₆H₁₃N₂OS: C: 64.62, H: 5.08, N: 14.13. Found: C: 64.29, H: 5.04, N: 14.10.

6-(4-Fluorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (CXII)

FT-IR (KBr) cm⁻¹: 3325 (N-H), 3082 (Ar-H), 2598 (S-H), 1524 (C-N), 1635 (C=N), 1580 (C=C), 1215 (C-F); ¹H-NMR (CDCl₃) ppm: 7.72 (1H, s, NH), 6.52-8.18 (10H, m, Ar-H), 9.13 (1H, s, SH); Mass (m/z): M⁺ 285, M⁺ 286; Anal. Caled. for C₁₅H₁₂FN₃S: C: 63.14, H: 4.24, N: 14.73. Found: C: 63.04, H: 4.16, N: 14.48.

6-(2-Chlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (CXIII)

FT-IR (KBr) cm⁻¹: 3316 (N-H), 3122 (Ar-H), 2540 (S-H), 1526 (C-N), 1618 (C=N), 1598 (C=C), 1058 (C-Cl); ¹H-NMR (CDCl₃) ppm: 7.76 (1H, s, NH) 6.6-
Experimental

Synthetic Work

8.8 (10H, m, Ar-H), 9.99 (1H, s, SH); Mass (m/z): M^+ 302, M^+^2 303; Anal. Caled. for C_{13}H_{12}ClN_{2}S: C: 59.70, H: 4.01, N: 13.92. Found: C: 59.56, H: 4.01, N: 13.66.

6-(2,6-Dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-thiol (CXIV)

FT-IR (KBr) cm^-1: 3296 (N-H), 3125 (Ar-H), 2540 (S-H), 1522 (C-N), 1614 (C=C), 1592 (C=C), 1063 (C-Cl); ^1H-NMR (CDCl_3) ppm: 6.24-8.64 (9H, m, Ar-H), 7.70 (1H, s, NH), 9.71 (1H, s, SH); Mass (m/z): M^+ 337, M^+^2 338; Anal. Caled. for C_{13}H_{11}ClN_{3}S: C: 53.58, H: 3.30, N: 12.50. Found: C: 53.36, H: 3.28, N: 12.37.

6-(3,4-Dimethoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-thiol (CXV)

FT-IR (KBr) cm^-1: 3316 (N-H), 3086 (Ar-H), 2949 (C-H), 2543 (S-H), 1520 (C-N), 1585 (C=C), 7.76 (1H, s, NH), 6.15-8.23 (9H, m, Ar-H), 9.56 (1H, s, SH); Mass (m/z): M^+ 327, M^+^2 328; Anal. Caled. for C_{17}H_{17}N_{3}O_{2}S: C: 62.36, H: 5.23, N: 12.83. Found: C: 62.28, H, 5.16 N: 12.69.
BIOLOGICAL SCREENING
Cytotoxicity assays are widely used to screen the anticancer or anti-proliferative activity of any compound. Cytotoxicity can also be measured by different techniques:

- SRB assay
- MTT or MTS assay
- WST assay
- Clonogenic assay

In order to overcome the frequent adverse effects of the anticancer drugs including lack of selective cytotoxicity and subsequent organ damages the drugs need to be synthesized at large scale in the laboratory and be subjected to rapid assay methods such as 60-cell screen developed by NIH-NCI to zero onto the effective chemical compounds\(^1\)-\(^2\).

**IN VITRO ANTICANCER SCREENING**

Evaluation of anti cancer activity was performed on the synthesized compounds at the National Cancer Institute (NCI) utilizing 60 different human tumor cell lines representing leukemia, melanoma and cancers of lung, colon, brain, ovary, breast, prostate as well as kidney following the standard procedure. Each compound is tested at a minimum of five concentrations at ten fold dilutions against every cell line in the panel. Growth inhibition experiments were done to assess the chemosensitivity to anticancer drugs. A 48 h continuous drug exposure protocol is used, and a *Sulforhodamine B (SRB) protein assay* is used to estimate the cell viability or growth.

**SULFORHODAMINE-B ASSAY**

**PRINCIPLE**

The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue culture plates by trichloroacetic acid (TCA). SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino-acid residues under mild acidic conditions, and dissociate under basic conditions, as the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass. The strong intensity of SRB staining allows the assay to be carried out in a 96 well format. The assay is most sensitive and superior to other protein staining methods. SRB staining is independent of cell
metabolic activity; therefore fewer steps are required to optimize assay conditions for specific cell lines.

CLINICAL SIGNIFICANCE

It is well suited to large screening applications as well as research. This assay has been widely used for drug-toxicity testing against different types of cancerous and non-cancerous cell lines. This method has been shown to be effective for in vitro testing of cancer cell sensitivity to radiation, and for the study of interactions between radiotherapy and chemotherapy, with sensitivity comparable to that of the standard clonogenic assay.

PROCEDURE

The human tumor cell lines of the cancer-screening panel were grown in RPMI 1640 medium containing 5% foetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T₀). Experimental drugs were solubilised in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/mL gentamycin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 100µL of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5% CO₂, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle
addition of 50 µL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 µL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilised with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µL of 80% TCA (final concentration, 16% TCA).

**CALCULATION**

Using the seven-absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition calculated as:

\[
\frac{(Ti-Tz)}{(C-Tz)} \times 100 \text{ for concentrations for which } Ti \geq Tz \\
\frac{(Ti-Tz)}{Tz} \times 100 \text{ for concentrations for which } Ti < Tz
\]

Three dose response parameters (GI50, TGI, LC50) were calculated for each experimental agent.

GI50 = concentration of the compound causing 50% decrease in net cell growth

TGI = concentration of the compound resulting in total growth inhibition

LC50 = concentration of the compound causing net 50% loss of initial cell at the end of the incubation period 48 h.

GI50 was calculated from \([ (Ti-Tz)/(C-Tz) ] \times 100 = 50\), (as measured by SRB staining) in control cells during the drug incubation. TGI was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from \([ (Ti-Tz)/Tz] \)
Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

**IN VIVO DIURETIC ACTIVITY**

The application of diuretics in the management of hypertension and congestive heart failure has outstripped their use in edema. Evaluation of diuretic activity was performed on the synthesized compounds by *Lipschitz method*.

**LIPSCHITZ METHOD**

**PRINCIPLE**

The test is based on water and sodium excretion in test animals and compared to group treated with a high dose of urea. Lipschitz value is the quotient between excretion by test animals and excretion by the urea control.

**CLINICAL SIGNIFICANCE**

Excretion of electrolyte is as important as excretion of water for the treatment of peripheral edema and ascites in congestive heart failure as well as for the treatment of hypertension. Diuretics are the most frequently prescribed therapeutic agents for the treatment of edema, hypertension and other cardiac ailments. Lipschitz method has been proven to be a standard method and a very useful tool for screening of potential diuretics.

**PROCEDURE**

Diuretic activity was measured on healthy adult albino rats weighing 180-200 g according to the Lipschitz method. Each group was comprised of six animals (n=6). They were housed in standard environmental conditions (temperature: 25-30°C). The rats are fed with standard diet (Altromin® pellets) and water ad libitum. Fifteen h prior to the experiment food and water are withdrawn. Diuretic activity was measured by collecting total excreted urine of rat kept in metabolic cages designed to separate the urine and faeces. The cages together with the funnel and measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine with minimum loss. Each
animal was placed in metabolic cage provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves were placed in the funnel to retain feces and to allow the urine to pass. Rats were placed in metabolic cages individually as soon as the treatments started. The urine sample was collected for a total period of 5 h (urine collected initially of 20 min was discarded). All the doses were administered with the aid of an oral dosing needle. The test compounds were administered orally at a dose of 45 mg/kg body weight in 5 mL of (0.5% carboxy methyl cellulose + 0.9% NaCl solution). Control group received 5 mL of 0.9% NaCl solution per kg body weight. The test compounds are compared with two standard diuretics, Urea (1 g/kg body weight in 5 mL of 0.5% carboxy methyl cellulose + 0.9% NaCl solution) and Acetazolamide (45 mg/kg body weight in 5 mL of 0.5% carboxy methyl cellulose + 0.9% NaCl solution). The excreted urine was collected, measured and studied for cumulative urine output, diuretic action, diuretic activity, \(Lipschitz\) value and electrolyte excretion (Na\(^+\), K\(^+\) & Cl\(^-\)). Sodium and potassium is estimated by using lab model Mediflame photometer. Chloride was estimated by titrating the urine by Volhard's method.

**CALCULATION**

\[
\text{Urinary excretion} = \frac{\text{Total Urinary Output}}{\text{Normal Saline Intake}} \times 100
\]

\[
\text{Diuretic action} = \frac{\text{Urinary excretion of treated group}}{\text{Urinary excretion of control group}}
\]

\[
\text{Diuretic activity} = \frac{\text{Diuretic action of treated group}}{\text{Diuretic action of control group}}
\]

**ACUTE TOXICITY STUDIES**

Acute toxicity studies can provide preliminary information on the toxic nature of a material for which no other toxicology information is available. Determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.
To deal with cases of accidental ingestion of a large amount of the material (e.g., for poison control information).

To determine possible target organs that should be scrutinized and/or special tests that should be conducted in repeated-dose toxicity tests.

To select doses for short-term and subchronic toxicity tests when no other toxicology information is available.

To determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD50/ED50).

To determine the absolute dose for a species.

The greater the index, safer is the compound.

HEPATOTOXICITY STUDIES

Wistar strains of albino rats (120-180 g) of either sex were used. Rats were divided into control, toxic control, standard and test groups. The serums collected from the groups of the albino rats were used for the estimation of biochemical parameters to determine the functional state of the liver.

Assessment of Liver Function Test

- Alkaline phosphatase (ALP)
- Serum glutamic oxaloacetic transaminase (SGOT)
- Serum glutamic pyruvic transaminase (SGPT)
- Total Protein (TP)
- Total Bilirubin (TB)

ALKALINE PHOSPHATASE (ALP)

PRINCIPLE

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red colored complex, which can be measured colourimetrically. The colour intensity is proportional to the enzyme activity.

The reaction can be represented as:
**CLINICAL SIGNIFICANCE**

The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. In addition to liver, bile duct, or gallbladder dysfunction, an elevated serum alkaline phosphatase can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. An increased serum alkaline phosphatase may be due to oral contraceptives, obstructive pancreatitis, hepatitis/mononucleosis/CMV, congestive heart failure, parasites, malignancy involving liver, Paget's Disease, herpes zoster (Shingles), hyperthyroidism, over-activity of the parathyroid glands (primary hyperparathyroidism, secondary hyperparathyroidism from kidney disease, osteomalacia.

A decreased serum alkaline phosphatase may be due to zinc deficiency, hypothyroidism, vitamin C deficiency/scurvy, folic acid deficiency, excess vitamin D intake, low phosphorus levels (hypophosphatasia), celiac disease, malnutrition with low protein assimilation (including low stomach acid production/hypochlorhydria).

**SAMPLE**: Serum 0.05 mL

**REAGENTS**

Reagent 1: Buffered substrate, pH 10.0

Reagent 2: Chromogen reagent

Reagent 3: Phenol standard, 10 mg%

**PREPARATION OF WORKING SOLUTION**

One vial of buffered substrate was reconstituted with 2.2 mL of purified water.
PROCEDURE

<table>
<thead>
<tr>
<th>Pipette Into</th>
<th>Blank (B)</th>
<th>Standard (S)</th>
<th>Control (C)</th>
<th>Test (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube marked</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Working</td>
<td>1.5 mL</td>
<td>1.5 mL</td>
<td>1.5 mL</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>Buffered</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mixed well and incubated at 37°C for 3 min.

| Serum          | -       | -           | -           | 0.05 mL  |
| Reagent 3      | -       | 0.05 mL     | -           | -        |

Mixed well and incubated at 37°C for 15 min.

| Reagent 2      | 1.0 mL  | 1.0 mL      | 1.0 mL      | 1.0 mL   |
| Serum          | -       | -           | 0.05 mL     | -        |

Mixed well after addition of each reagent and the O. D. of Blank (B), Standard (S), Control (C) and Test (T) against purified water using a green filter were measured.

CALCULATION

Serum Alkaline Phosphatase activity in IU/L Units

\[ \frac{O.D. \text{ Test} - O.D. \text{ Control}}{O.D. \text{ Standard} - O.D. \text{ Blank}} \times 10 \]

SGOT (AST)

ASSAY PRINCIPLE

Aspartate aminotransferase (AST) catalyses the transamination of L-aspartate and α-ketoglutarate (α-KG) to form oxaloacetate and L-glutamate. Oxaloacetate so formed is coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown colored complex in alkaline medium and this can be measured colourimetrically.

\[ \alpha \text{-Ketoglutarate} + \text{L-Aspartate} \rightarrow \text{Oxaloacetate} + \text{L-Glutamate} \]

\[ \text{Oxaloacetate} + \text{2,4-DNPH} \rightarrow \text{Corresponding Hydrazone} \text{ (brown color)} \]
Reitman and Frankel method is an end point colorimetric method for the estimation of enzyme activity. To obtain accurate results, method has been standardized with Kinetic method (Standard Karmen Unit Assay).

CLINICAL SIGNIFICANCE

Serum AST and ALT levels are elevated in viral and other forms of liver diseases associated with hepatic necrosis. ALT is more liver specific enzyme, while AST activity increases relatively more during myocardial infarction.

SAMPLE: Serum 0.05 mL

REAGENTS

<table>
<thead>
<tr>
<th>Reagent No.</th>
<th>Reagents</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Buffered Aspartate-α-KG Substrate, pH 7.4</td>
<td>Phosphate Buffer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Aspartic Acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-KG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stabiliser</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td>2.</td>
<td>2,4-DNPH colour reagent</td>
<td>2,4-Dinitrophenyl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrazine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stabiliser</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium Hydroxide, 4N</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>4.</td>
<td>Working Pyruvate Standard, 6mM (114 IU/L)</td>
<td>Sodium Pyruvate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stabiliser</td>
</tr>
</tbody>
</table>

WORKING REAGENT PREPARATION

Solution I: 1 mL of 4 N sodium hydroxide was diluted upto 10 mL with purified water, mixed well and O.D. against purified water in a colorimeter using a green filter or on photometer at 505 nm, within 15 min was measured.
PROCEDURE

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mixed well and incubated at 37°C for 60 min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Deionised Water</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Mixed well and allowed to stand at room temperature (15-30°C) for 20 min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution I</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

AST(SGOT) = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times \text{Conc. of Stand.}

SGPT (ALT)

ASSAY PRINCIPLE

Alanine aminotransferase (ALT) catalyzes the transamination of L-Alanine and α-Ketoglutarate (α-KG) to form pyruvate and L-glutamate. Pyruvate so formed is coupled with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown colored complex in alkaline medium and this can be measured colorimetrically.\textsuperscript{10-11}

\[ \text{α-Ketoglutarate} + \text{L-Alanine} \rightarrow \text{Pyruvate} + \text{L-Glutamate} \]

\[ \text{Pyruvate} + 2,4-\text{DNPH} \rightarrow \text{Corresponding Hydrazone} \]

(brown color)

Reitman and Frankel method is an end point colorimetric method for the estimation of enzyme activity. To obtain accurate results, the method has been standardized with Kinetic method (Standard Karmen Unit Assay).
CLINICAL SIGNIFICANCE

Serum AST and ALT levels are elevated in viral and other forms of liver diseases associated with hepatic necrosis. ALT is present in liver cells in much higher concentration than any other organ.

SAMPLE: Serum 0.05 mL

REAGENTS

<table>
<thead>
<tr>
<th>Reagent No.</th>
<th>Reagents</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Buffered Alanine-α-KG Substrate, pH 7.4</td>
<td>L- Alanine α-KG, Phosphate Buffer, Preservative, Stabiliser</td>
</tr>
<tr>
<td>2.</td>
<td>2,4-DNPH colour reagent</td>
<td>2,4-Dinitrophenyl Hydrazine, Preservative, Stabiliser</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium Hydroxide, 4N</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>4.</td>
<td>Working Pyruvate Standard, 8 mM (150 IU/L)</td>
<td>Sodium Pyruvate, Preservative, Stabiliser</td>
</tr>
</tbody>
</table>

WORKING REAGENT PREPARATION

Solution I: 1 mL of 4 N sodium hydroxide was diluted upto 10 mL with purified water.

PROCEDURE

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Mix well and incubate at 37°C for 30 min.

<table>
<thead>
<tr>
<th>Reagent 2</th>
<th>0.25</th>
<th>0.25</th>
<th>0.25</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionised Water</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix well and allow to stand at room temperature (15-30°C) for 20 min.

| Solution 1 | 2.5 | 2.5 | 2.5 | 2.5 |

Mixed well and the O.D. against purified water in a colorimeter using a green filter or on photometer at 505 nm, within 15 min was measured.

**CALCULATION**

\[
\text{ALT(SGPT) Activity (in IU/L)} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times \text{conc. of Stand.}
\]

**TOTAL PROTEIN**

**ASSAY PRINCIPLE**

The peptide bonds of proteins react with cupric ions in alkaline solution to form a blue-purple colored chelate, the absorbance of which is measured at 578 nm. The biuret reagent contains sodium-potassium tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final color is proportional to the concentration of total protein in the sample. \(^{12-13}\)

\[
\text{Protein} + \text{Cu}^{++} \xrightarrow{\text{Alkaline pH}} \text{Cu-Protein Complex}
\]

**CLINICAL SIGNIFICANCE**

Total protein estimation is useful for monitoring gross changes in protein levels caused by various disease states.

Increased concentration: dehydration, monoclonal diseases (myeloma, macroglobulinemia, cryoglobulinemia) and some chronic polyclonal diseases, (liver cirrhosis, sarcoidosis, systemic lupus erythematosis). Decreased concentration: over hydration, protein loss through kidneys (nephrotic syndrome), from skin (severe burns), starvation, protein malnutrition, severe non-viral liver
cell damage.

SAMPLE: Serum 10 \( \mu \)L

REAGENTS

<table>
<thead>
<tr>
<th>Reagent No.</th>
<th>Reagent</th>
<th>Composition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Biuret Reagent</td>
<td>Copper Sulphate; Sodium Hydroxide; Sodium-Potassium Tartrate; Surfactant</td>
<td>7 mM/L; 200 mM/L; 20 mM/L; q.s.</td>
</tr>
<tr>
<td>2.</td>
<td>Protein Standard</td>
<td>BSA; Preservative</td>
<td>6.5 g/dL; q.s.</td>
</tr>
</tbody>
</table>

ASSAY PARAMETERS

<table>
<thead>
<tr>
<th>Mode</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>578 nm (550-580 nm)</td>
</tr>
<tr>
<td>Flow Cell Temperature</td>
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</tr>
<tr>
<td>Optical Path Length</td>
<td>1 cm</td>
</tr>
<tr>
<td>Blanking</td>
<td>Reagent Blank</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>10 ( \mu )L</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>1000 ( \mu )L</td>
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<tr>
<td>Incubation Time</td>
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</tr>
<tr>
<td>Concentration of Standard</td>
<td>6.5 g/dL</td>
</tr>
<tr>
<td>Stability of Final Color</td>
<td>2 h</td>
</tr>
<tr>
<td>Permissible Reagent Blank Absorbance</td>
<td>&lt; 0.2 AU</td>
</tr>
<tr>
<td>Linearity</td>
<td>Upto 20 g/dL</td>
</tr>
<tr>
<td>Units</td>
<td>g/dL</td>
</tr>
</tbody>
</table>

PROCEDURE

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/ Plasma</td>
<td>-</td>
<td>-</td>
<td>10 ( \mu )L</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>10 ( \mu )L</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1000 ( \mu )L</td>
<td>1000 ( \mu )L</td>
<td>1000 ( \mu )L</td>
</tr>
</tbody>
</table>


Ph.D Thesis 149

Jamia Hamdard
1. Mixed well and incubated at 37°C for 5 min.
2. Analyser was programmed as per the assay parameters.
3. Blank reagent absorbance was taken.
4. Absorbance of the standard was measured followed by the test at 578 nm.
5. Results were calculated as per the given calculation formula.

**CALCULATION**

\[
\text{Serum Total Protein Concentration (g/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 6.5
\]

Globulins = Total Protein – Albumin

**Conversion Factor**

Total protein concentration in (g/L) = Total protein concentration in (g/dL) × 10

**TOTAL BILIRUBIN**

**ASSAY PRINCIPLE**

Direct: Conjugated bilirubin couples with diazotized sulfanilic acid, forming azobilirubin, a red-purple colored product in acidic medium.

Indirect: Unconjugated bilirubin is diazotized only in the presence of its dissolving solvent (methanol). Thus the red-purple colored azobilirubin produced in presence of methanol originates from both direct and indirect fractions and thus represents total bilirubin concentration. The difference of total and direct bilirubin gives indirect unconjugated bilirubin\(^{14-16}\).

The intensity of red-purple color so developed above is measured colorimetrically and it is proportional to the concentration of the appropriate fraction of bilirubin. This reaction can be represented as:

\[
\text{H}^+ \\
\text{Bilirubin} + \text{Diazotized Sulphanilic Acid} \xrightarrow{\text{H}^+} \text{Azobilirubin} \\
\downarrow \text{H}^+ \\
\text{Red-Purple color}
\]
CLINICAL SIGNIFICANCE

Increase in serum bilirubin levels may be found in obstructive jaundice, hemolytic jaundice, neonatal jaundice, and hepatitis. In hemolytic jaundice and in neonatal jaundice, primarily there is an increase in the unconjugated indirect bilirubin fraction. Both conjugated and unconjugated bilirubin is increased in hepatitis.

SAMPLE: Serum 0.1 mL

REAGENTS

Reagent 1: Diazo - A
Reagent 2: Diazo - B
Reagent 3: Diazo blank
Reagent 4: Methanol
Reagent 5: Artificial standard (10 mg% bilirubin)

PREPARATION OF WORKING SOLUTION

Diazo Reagent: 1.0 mL of Reagent 1 was mixed with 0.030 mL of Diazo-B.

PROCEDURE

<table>
<thead>
<tr>
<th>Pipette into tube marked</th>
<th>T₁</th>
<th>T₂</th>
<th>D₁</th>
<th>D₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/Plasma, mL</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Distilled Water, mL</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Reagent 3: Diazo Blank, mL</td>
<td>--</td>
<td>0.25</td>
<td>--</td>
<td>0.25</td>
</tr>
<tr>
<td>Diazo Reagent, mL</td>
<td>0.25</td>
<td>--</td>
<td>0.25</td>
<td>--</td>
</tr>
<tr>
<td>Distilled Water, mL</td>
<td>--</td>
<td>--</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Reagent 4: Methanol, mL</td>
<td>1.25</td>
<td>1.25</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Mixed well and the tubes T₂ and T₁ were kept in the dark at room temperature for 30 min and O.D. against distilled water was measured on a colorimeter with a yellow green filter.

Mixed well and O.D. of the contents of tubes D₂ and D₁ after one minute were measured on a colorimeter using a yellow green filter, against distilled water.
Standard: O.D. of Reagent 5 [Artificial standard (10 mg % bilirubin)] against distilled water was measured in both colorimetric and spectrophotometric procedures.

**CALCULATION**

Bilirubin concentration in mg/100 mL:

\[
\text{Total (A)} = \frac{\text{O.D. } T_1 - \text{O.D. } T_2}{\text{O.D. Standard}} \times 10
\]

\[
\text{Direct (B)} = \frac{\text{O.D. } D_1 - \text{O.D. } D_2}{\text{O.D. Standard}} \times 10
\]

Indirect = (A) - (B)
REFERENCES


RESULTS

&

DISCUSSION
The present work is divided into two parts:

SECTION A: Synthetic work
   a. Aminopyrimidines
   b. Hydroxypyrimidines
   c. Thiopyrimidines

SECTION B: Pharmacological evaluation of pyrimidine derivatives
   a. *In vitro* anticancer activity (Scheme I, II and III)
   b. *In vivo* diuretic activity (Scheme IV and V)
   c. Hepatotoxicity Studies (Potent compounds)

SECTION A: SYNTHETIC WORK

66 New substituted pyrimidine derivatives including 49 intermediates were synthesized by following five different schemes (I-V).

The synthesis of various new chalcones from substituted benzaldehydes and acetophenones were carried out according to the Claisen-Schmidt condensation. The 4,6-diarylsubstituted-1,6-dihydropyrimidine-2-amines, 4,6-diarylsubstituted-1,6-di hydropyrimidine-2-hydroxy derivatives and 4,6-diarylsubstituted-1,6-di hydropyrimidine-2-thiol derivatives were synthesized from chalcones using guanidine hydrochloride, urea and thiourea respectively. The key reactions involved the formation of chalcones and subsequent addition of guanidine hydrochloride, urea and thiourea to form cyclised pyrimidine derivatives.

The structures of newly synthesized compounds were established by FT-IR, \(^1\)H NMR, mass spectral studies and elemental analysis. Both analytical and spectral data (\(^1\)H NMR, FT-IR and Mass) of the synthesized compounds were in full agreement with the proposed structures. In the nuclear magnetic resonance spectra (\(^1\)H NMR) the signals of the respective protons of the final title compounds were verified on the basis of their chemical shifts and multiplicities. All the phenyl protons were observed at the expected chemical shifts and integral values. The mass spectrum of the final compounds showed the molecular ion peak consistent with molecular weight of the compounds. The elemental analysis results were within ±0.4% of the theoretical values.
4,6-diaryl substituted 1,6-dihydro pyrimidine-2-amine (Scheme I, II and III)

- 4,6-diaryl substituted 1,6-dihydro pyrimidine-2-amine derivatives were synthesized according to schemes I, II and III and the physicochemical data of all the synthesized compounds were given in (Table 1, 2, 3 and 4).

- IR spectra of chalcones (intermediates) exhibited characteristic band in the region of 1720-1647 cm\(^{-1}\) (very strong) which indicates the presence of C=O group and aliphatic (C=C) stretching vibration was appeared at 1640-1720 cm\(^{-1}\) and 1492-1599 cm\(^{-1}\).

- The synthesized 4,6-diaryl substituted-1,6-dihydropyrimidine-2-amine exhibited characteristic bands in the region of 3400-3300 cm\(^{-1}\) which indicates the presence of primary amine (NH\(_2\)) which showed 2 sharp bands at 3500-3450 cm\(^{-1}\) and 3390-3380 cm\(^{-1}\) which are due to asymmetric and symmetric vibrations of (NH\(_2\)) groups whereas the bands due to secondary amines appeared at 3350-3310 cm\(^{-1}\).

- The stretching peak of C=N was appeared at 1600-1510 cm\(^{-1}\). The absorption band at 855-800 cm\(^{-1}\) indicates characteristic aromatic bending.

- Other bands appeared at 3200-3440 cm\(^{-1}\) (OH) group, 1089 cm\(^{-1}\) (C-O-C in furan), 1450-1220 cm\(^{-1}\) (C=C) aromatic stretching, 1061 (C-Cl), 1117 (C-F).

- Two bands are observed at 1550-1500 cm\(^{-1}\) and 1360-1290 cm\(^{-1}\) which are due to asymmetric and symmetric vibrations of (N=O)\(_2\) group.

- The final pyrimidine derivatives does not show any absorption in the region of 1700-1647 cm\(^{-1}\) which indicates the absence of C=O groups.

- Further derivatization of pyrimididine-2-amines was done with chloroacetyl chloride. The IR spectra of chloroacetyl derivatives exhibited characteristic band for secondary amide in the region of 3330-3090 cm\(^{-1}\). The amide-I band and amide-II band were appeared at 1644-1664 cm\(^{-1}\) (for C=O st.) and 1560-1580 cm\(^{-1}\) (for N-H bending) respectively.
The structures of the chalcones and its cyclization products were further confirmed by the corresponding $^1$H NMR spectra.

$^1$H NMR spectra of chalcones exhibited two characteristic doublets for $H_a$ (lower $\delta$ value) and $H_b$ (higher $\delta$ value).

The pyrimidine derivatives obtained from their respective chalcones were characterized by the appearance of a broad singlet near about $\delta$ 5.18-5.34 which was assigned for amino protons. A distinct and characteristic singlet about $\delta$ 7.46-7.95 was assigned for the N-H protons of pyrimidine confirming the cyclization. The presence of two doublets, with $\delta$ values 4.48-4.91 and 5.75-5.94 were assigned for each of the one proton of pyrimidine at C-5 and C-6 respectively. All other aromatic protons appeared as a multiplet with $\delta$ values 6.05-8.18.

The N-H of benzimidazole derived pyrimidine derivatives (Scheme I) showed a singlet at $\delta$ values 12.45-13.45.

The chloroacetyl derivatives of pyrimidine-2-amines were further characterized by the disappearance of broad singlet about $\delta$ values 5.18-5.34 for amino protons (1° amine) and appearance of a sharp singlet of amide protons at $\delta$ values 8.66-8.94.

4,6-diaryl substituted 1,6-dihydro pyrimidine-2-ol (Scheme III)

4,6-diaryl substituted 1,6-dihydro pyrimidine-2-ol derivatives were synthesized according to scheme III and the physicochemical data of all the synthesized compounds were given in (Table 5).

The IR spectra of 4,6-diaryl substituted 1,6-dihydro pyrimidine-2-ol exhibited characteristic O-H bands at 3421-3401 cm$^{-1}$.

The $^1$H NMR spectra of these derivatives afforded a singlet for the O-H protons at $\delta$ value 6.84-6.96.

A distinct and characteristic singlet about $\delta$ value 7.60-7.94 was assigned for the N-H protons of pyrimidine.

The presence of two doublets, with $\delta$ values 4.46-4.91 and 5.91-6.15 were assigned for each of the one proton of pyrimidine at C-5 and C-6 respectively.
4,6-diaryl substituted 1,6-dihydro pyrimidine-2-thiol (Scheme IV and V)

- 4,6-diaryl substituted 1,6-dihydro pyrimidine-2-thiol derivatives were synthesized according to schemes IV and V and the physicochemical data of all the synthesized compounds were given in (Table 6 and 7).

- The IR spectra of the chalcones showed the disappearance of \( \text{C} = \text{C} \) (olefinic) and exhibited characteristic stretching vibrations (\( \text{C} = \text{O} \)) at 1735-1710 cm\(^{-1}\) and \( \text{CH} = \text{CH} \) at 1658-1640 cm\(^{-1}\).

- The IR spectra of 4,6-diaryl substituted 1,6-dihydro pyrimidine-2-thiol derivatives afforded the characteristic S-H stretching at 2598-2538 cm\(^{-1}\). The stretching peak of \( \text{C} = \text{N} \) and N-H appeared at 1651-1614 cm\(^{-1}\) and 3480-3180 cm\(^{-1}\) respectively. The absorption band at 855-800 cm\(^{-1}\) was characteristic of aromatic C-H bending vibrations. The \( \text{C} = \text{N} \) stretching and \( \text{C} = \text{C} \) stretching were observed at 1561-1593 cm\(^{-1}\) respectively.

- A broad peak was observed at about 3495-2550 cm\(^{-1}\) which confirms the presence of O-H stretching of the COOH (Scheme IV).

- In the IR spectrum, the presence of band at 2598-2538 cm\(^{-1}\) (S-H) and the absence of band due to (\( \text{C} = \text{O} \)) confirmed the formation of pyrimidine-2-thiol moiety was further confirmed by \( ^1\text{H} \) NMR spectra.

- In the \( ^1\text{H} \) NMR spectra, the appearance of a singlet due to SH at \( \delta \) value 9.13-9.99 (CDCl\(_3\) as solvent) and at \( \delta \) value 2.89-3.04 (DMSO as solvent) confirmed the formation of pyrimidine-2-thiol. The aromatic protons appeared as multiplet at 6.2-8.1.

- In the phenoxyacetic acid derived thiopyrimidines (Scheme IV), a distinct singlet was appeared at \( \delta \) values 12.43-12.80. The methylene protons appeared as a singlet having \( \delta \) values 4.13-4.76.

- In the \( ^1\text{H} \) NMR spectra, a distinct and characteristic doublet was observed for olefinic protons of chalcones (\( \text{H}_a \) and \( \text{H}_b \)) with a higher \( \delta \) value for \( \text{H}_b \) due to the deshielding effect. For \( \text{H}_a \) and \( \text{H}_b \) \( \delta \) value were found to be 6.11-6.92 and 7.31-7.91 respectively.
The reaction schemes were bifurcated for anticancer screening and evaluating the diuretic profile i.e. Schemes I, II and III for in vitro anticancer screening and Schemes IV and V for in vivo diuretic activity.

**IN VITRO ANTICANCER ACTIVITY**

**Primary single high dose (10^{-5} M) full NCI 60 Cell Panel in vitro assay**

The selected compounds of scheme I, II and III were evaluated for their in vitro anticancer activity. The structures of the final compounds were submitted to National Cancer Institute (NCI), USA, and were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their in vitro anticancer activity.

Primary in vitro one dose anticancer assay was performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. One dose mean graph of all the compounds are attached (Graph 1-21) whereas growth percent of all the compounds of specific series is represented in (Tables 8-11).

**Interpretations of one dose mean graph data**

The one dose data represented as mean graph of the percent growth of treated cells. The number reported for the one dose assay is growth relative to the no-drug control, and relative to time zero number of cells. The value between 0 and 100 indicates growth inhibition whereas a value less than 0 indicate lethality. For example, a value of 100 means no growth inhibition. A value of 40 means 60% growth inhibition. A value of 0 means no net growth over the course of the experiment.

Compound which satisfied pre-determined threshold inhibition criteria selected for 5 dose assay.
In vitro 5 dose anticancer assay

For the selected compounds, all the cell lines (about 60), representing nine tumor subpanels representing leukemia, melanoma and cancers of lung, colon, brain, ovary, breast, prostate as well as kidney, were incubated at five different concentrations (0.01, 0.1, 1, 10 & 100 μM). The outcomes were used to create log concentration versus % growth inhibition curves and three response parameters ($G_{50}$, TGI and $L_{C50}$) were calculated for each cell lines. The $G_{50}$ value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and $L_{C50}$ value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h.

a. One Dose Assay

Compounds which reduces the growth of any of the cell lines by 32% or less (negative number indicate cell killing) is considered as in vitro active, i.e. growth inhibition of 68%.

Scheme-I

As per the protocol of NCI, nine representative compounds of the series were selected and granted NSC code viz; XV (NSC D-753871), XVI (NSC D-753872), XVII (NSC D-753882), XVIII (NSC D-753883), XIX (NSC D-753884), XX (NSC D-753885), XXI (NSC D-753886), XXIV (NSC D-753888), XXVI (NSC D-753889) and screened in vitro for antiproliferative activity at a single high dose ($10^{-5}$ M) in full NCI 60 cell panel.

The percentage growth inhibitions of the selected benzimidazole derived pyrimidine derivatives at $10^{-5}$ M concentration over the full cell lines panel are illustrated in (Table 8 and 9) and one dose mean graph of all the nine compounds are attached (Graph 1-9).

- The compound XXIV containing electron releasing N,N-dimethylamino group exhibited strong inhibition (99.11%) against SR cell lines of leukemia. This compound with mean percent growth of 75.22 is the most efficacious of all the compounds among this series.
Apart from this, halogen substituted derivatives also exhibited remarkable growth inhibition. Among halogen substituted compounds, 2-chlorophenyl derivative (XIX) displayed 75.41 % and 73.38 % inhibition against MDA-MB-468 cell lines of breast cancer and SK-MEL-5 cell lines of melanoma respectively. The compound XIX have also shown 79.99 % growth inhibition of NCI-H522 cell lines of human lung carcinoma and 62.73 % growth inhibition against SR cell lines of leukemia.

The compound XXI have shown 81.64% growth inhibition against NCI-H522 cell lines of human lung carcinoma. This compound also exhibited 78.12% inhibition against SR cell lines of leukemia and the activity may be due to the presence of OH substituent.

The mean growth inhibition was weak in the rest of the compounds viz; XV, XVI, XVII, XVIII, XX and XXVI.

Therefore out of the three compounds, 4-(1H-benimidazo[2-yl]-6-[4-(dimethylamino)phenyl]-1,6-dihydropyrimidin-2-amine (XXIV) exhibited remarkable activity amongst tested compounds but failed to satisfy overall selection criterion set by NCI for full 5-dose anticancer screening assay.

We here concluded the role of dimethylamino substituted phenyl ring with pyrimidine for anticancer activity, although the compound did not exhibit very good activity but remarkable superiority within the series was clear when electron donating substituent was present. Hence it is presumed worth to synthesize some more derivatives with some similarity to verify these assumptions.

Scheme-II

As per the protocol of NCI, five representative compounds of the series were selected and granted NSC code viz; XLI (NSC D-753873), XLII (NSC D-753874), XLIV (NSC D-753875), XLVI (NSC D-753869), XLVII (NSC D-753868) and screened in vitro for antiproliferative activity at a single high dose (10⁻⁵ M) in full NCI 60 cell panel. The one dose mean graphs for anticancer activity are represented on (Graph 10-14) and growth percent is represented in (Table 10).
In this series none of the selected compounds have shown any remarkable growth inhibition against any cell lines.

It was found that the mean growth inhibition was weak in all the compounds which may be due to unsubstituted phenyl group in the 4th position of pyrimidine ring. Even though phenyl group at the 6th position of pyrimidine ring is substituted, this series failed to produce remarkable in vitro anti-cancer activity.

The in vitro activity data reveals that none of the candidate of the series was able to satisfy the predetermined threshold inhibition criterion of NCI. Therefore no further five dose evaluation was recommended for any candidate of the series.

Scheme-III

As per the protocol of NCI, seven representative compounds of the series were selected and granted NSC code viz; LXII (NSC D-753876), LXIII (NSC D-753879), LXIV (NSC D-753880), LXV (NSC D-753877), LXVII (NSC D-753878), LXVIII (NSC D-753881), LXX (NSC D-753870) and screened in vitro for antiproliferative activity at a single high dose (10^-5 M) in full NCI 60 cell panel. The data is represented as one dose mean graph on (Graph 15-21) for compounds LXII, LXIII, LXIV, LXV, LXVII, LXVIII & LXX respectively and percentage growth in (Table 11).

On the basis of single dose screening, compound LXII, LXIV, LXV, LXVIII have shown 70.62%, 65.17%, 50.56% and 87.49% growth inhibition respectively against CCRF-CEM (leukemia) cell lines. Significant growth inhibition of 90.29% and 94.39% against RPMI-8226 (leukemia) cell lines was exhibited by LXII and LXVIII. LXII have also shown 77.38% growth inhibition against SR cell lines of leukemia. LXIV has shown remarkable growth inhibition of 98.89% against HL-60-TB cell lines of leukemia. Hence LXII, LXIV and LXVIII was considered in vitro active against leukemia cell lines.
Similarly the compounds LXIV and LXV have shown 91.16% and 97.61% growth inhibition against NCI-522 (lung cancer) also indicating in vitro activity against lung cancer.

Along with LXII, LXIV, LXV and LXVIII, the compound LXX has shown significant growth inhibition of 88.17%, 98.3%, 97.91%, 75.89% and 81.39% respectively against MDA-MB-468 (breast cancer) cell lines and all of them found to be promising against breast cancer.

Both LXIV and LXV exhibited significant in vitro anticancer activity against ovarian cancer. LXIV and LXV have shown 92.37% and 96.63% growth inhibition against OVCAR cell lines and NCI/ADR-RES cell lines respectively.

Apart from this LXIV have shown remarkable growth inhibition of 91.92% against SNB-75 cell lines of CNS cancer.

Overall the compound LXIV (2-amino pyrimidine having o-chlorophenyl at its 6\textsuperscript{th} position) showed the most potent inhibitory effect of 98.89% on the growth of HL60-TB (leukemia) cell lines.

The compounds LXIII and LXVII have not shown significant growth inhibition against any of the cancer cell lines.

Furthermore the mean of growth percent for the compounds LXII, LXIV, LXV, LXVIII and LXX were found to be 49.98%, 42.42%, 73.56%, 66.28% and 76.82% respectively.

The compound LXIV with mean percent growth of 42.42% was found to be the most efficacious among the compounds investigated here (Graph 17). Only significant results have been reported and presented as percentage growth inhibition (Table 12).

b. Multiple Dose Assay

Within these seven candidates of the series, compounds LXII, LXIV, LXV, and LXVIII showed remarkable anticancer activity in one dose assay and was selected for 5 dose assay. The overall results of 5 dose assay for compounds LXII, LXIV, LXV, and LXVIII are reported as mean graph on (Graph 22-25) and their numerical data in (Table 13, 14, 16, 17, 19, 20, 22 and 23).
• All the 4 compounds LXII, LXIV, LXV, and LXVIII exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels (leukemia, melanoma and cancers of lung, colon, brain, ovary, breast, prostate and kidney) with GI₅₀ values between “0.81 to 33.6 μM”. The results of in vitro studies in terms of growth inhibition of 50% (GI₅₀) have been given in (Table 25) and the data of log concentrations resulting in growth inhibition of 50% (log₁₀ GI₅₀) of in vitro human tumor cell lines is given in (Table 15, 18, 21 and 24).

• The compound LXII was found to be active against all leukemia cell lines (GI₅₀ value ranging from 1.38-3.62 μM). It seems that the cytostatic activity was associated with the presence of unsubstituted phenyl group at the 6th position of the pyrimidine ring.

• A chlorine atom (an electron withdrawing group) at the ortho position of the phenyl moiety was highly effective in inducing antiproliferative activity as in LXIV which was evident from the satisfactory sensitivity against individual cell lines. This compound showed highest growth inhibitory activity against MDA MB-435 (melanoma) cell lines with GI₅₀ of 0.81 μM.

• The compound LXV exhibited moderate to significant inhibitory effects on leukemia and breast cancer cell lines due to the presence of methoxy (electron donating group) substituent on the phenyl ring.

• Whereas compound LXVIII having phenyl ring with substituents m-OCH₃ (electron donating groups) & p-OH (electron withdrawing group) was found to be the most popular among the series (38 cell lines over 60) with wide range of sensitivity profile towards leukemia (GI₅₀ 0.82-3.16 μM), CNS cancer (GI₅₀ 1.97-4.48 μM) and breast cancer (GI₅₀ 2.43- 4.96 μM). The obtained data for this compound also revealed that an obvious sensitivity profile towards leukemia subpanel, least for K-562 and maximum for CCRF-CEM cell lines. The compound LXVIII proved to be sensitive towards all the tested cell lines with not more than 14.0 μM concentration except for the SK-MEL-2 Melanoma cell lines (GI₅₀-17.3 μM).

• The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines
towards the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel towards the test agent). Ratio between 3-6 refers to moderate selectivity, ratio greater than 6 indicate high selectivity towards the corresponding cell lines, while compounds not meeting either of these criteria rated as non-selective. As per the criterion for the selectivity of a compound, the compound LXII was found to be moderate selective towards leukemia subpanel only.

The preliminary in vitro anti cancer activity suggested the following structure activity relationships (SAR):

From the in vitro study, the pyrimidine analogues can serve as new leads in cancer chemotherapy. Therefore, a suitable combination of the groups and their appropriate placement in the molecule significantly control the function of the molecule. Here we can suggest that the anti cancer activity may be due to:

- The presence of nitrogen heterocycle.
- Presence of free amino group in the pyrimidine ring.
- The correlation of phenyl substitution at 6th position of the pyrimidine ring and anti-proliferative activity.
- The difference in the activity between the compounds may be due to the indicated substituents in the phenyl group of the molecule.
- Role of the electro negative chlorine atom in inducing antiproliferative activity and effect of electron donating groups in the phenyl ring for enhancing the anti-cancer activity.

From in vitro anticancer data of compounds LXII, LXIV, LXV and LXVIII; it was concluded that

- Compounds LXIV and LXVIII was found that the most potent proliferation inhibitors of the series. The compound LXIV having o-chlorophenyl at the 6th position of pyrimidine ring showed highest growth inhibitory activity against MDA MB-435 (melanoma) cell lines with GI50 value of 0.81 μM. The compound LXVIII with phenyl ring having m-methoxy and p-OH
substituents; exhibited potent growth inhibitory activity against CCRF-CEM cell lines of leukemia with GI_{50} value of 0.82 μM.

- From the multiple dose study it was concluded that 4-[2-amino-6-(phenyl)-3,4-dihydropyrimidin-4-yl]-2-methylphenol [(LXII), (NSC 753876)] was found to be the most selective analogue of the series against NCI's 60 human cancer cell lines.

- To conclude, LXIV was found to be the most cytotoxic (GI_{50} 0.81 μM) among the series towards MDA MB-435 (melanoma) cell lines. But the compound LXII seems to be more promising. Although it was not so cytotoxic to the cancer cells as LXIV, the compound was still quite potent (GI_{50} 1.38 μM). More importantly, it has a favourable \textit{in vitro} selectivity index and found to be selective towards leukemia. Further optimization of these pyrimidine analogues may possibly lead to more active molecules against cancer.

\textbf{IN VIVO DIURETIC ACTIVITY}

Diuretic activity was evaluated on healthy adult albino rats (body weight 180-200 g) according to an adaptation of the method of Lipschitz. The \textit{in vivo} diuretic activity of all the synthesized compounds from scheme IV and V were performed and the results are summarized in (Table 26-29). Comparison of % Urinary Excretion data of all the compounds was presented in (Graph 26-29).

\textbf{Scheme-IV}

In the present scheme, the normal Albino rats (body weight 180-200 g) were used for the screening of synthesized compounds.

The \textit{in vivo} diuretic activity as well as saluretic and kaliuretic effect of the synthesized compounds in albino rats of scheme-IV is summarized in (Table 26-27).

- The excreted urine was collected, measured and studied for Cumulative Urine Output, Diuretic action, Diuretic activity, Lipschitz value and Electrolyte excretion (Na^+, K^+ & Cl^-). Sodium and potassium is estimated by using lab
model Mediflame photometer. Chloride was estimated by titrating the urine by Vollhard’s method. Statistical analysis of control and test data was determined by ANOVA. Simple one-way analyses of variance were used for different doses within a group. A probability value of $p<0.001$ and $p<0.05$ was considered statistically significant.

- Cumulative urine excreted during 0-5 h for each group (6 albino rats) is a measure of urinary excretion. After 5 h of screening, none of the compounds have shown good cumulative urine output comparable with urea and standard acetazolamide.

- Percentage Urinary excretion of all the compounds was found to be less when compared to standard. Comparatively better results were observed with compound XCVI which was found to be 88.02%, where as for urea and standard acetazolamide percentage urinary excretion was found to be at 138.09% and 184.77% respectively.

- Diuretic action was measured as the ratio (%) of the total volume of urine excreted during the 5 h following the administration of the test drug at a dose of 45 mg/kg to the volume of urine excreted by the saline control. None of the compounds have showed remarkable diuretic action in comparison to urea (1.2) and acetazolamide (1.6). Among this series compound XCVI exhibited highest value of 0.79.

- Diuretic activity was evaluated in terms of daily diuresis. Diuretic activity was measured as the ratio of the diuretic action of the treated groups to the diuretic action of the standard (acetazolamide) group. Compound XCVI exhibited a weak diuretic activity of 0.49, whereas the compounds XCIII and XCV both showed a value of 0.45.

- The Lipschitz value (the ratio $T/U$, in which $T$ is the response of the test compound and $U$, that of urea treatment, indices of 1.0 and higher are regarded as a positive effect in terms of diuretic activity) shows that any of the synthesized compounds in this series fails to meet this criterion. Comparatively remarkable value was shown by the compound XCVI, which
may be due to substitution of chlorine atoms at the ortho and para positions of the phenyl ring.

Apart from diuretic activity all the compounds (LXXXVI- XCI) and (XCII-XCVII) were also tested for saluretic and kaliuretic effects in \textit{albino} rats.

- After 0-5 h all the compounds showed a very minimal increase in sodium excretion, which was very low when compared to standards, i.e. urea (2.74±0.154) and acetazolamide (3.36 ± 0.294). A relatively significant increase in sodium excretion has shown by XCIII (1.98±0.768) and XCVI (1.56±0.056), which may be due to presence electron withdrawing groups like fluoro and chloro respectively attached to the phenyl moiety.

- As far as kaliuretic property is concerned, compound XCIII have shown relatively better kaliuretic effect of 1.02±0.290, which was very much less than acetazolamide (1.523±0.118).

- With regard to Na\textsuperscript{+}/ K\textsuperscript{+} ratio, it was observed that LXXXVII and XCIII is a moderate kaliuretic.

- Chloride was estimated by titrating the urine by Volhard's method. Chloride excretion was mildly increased to similar extent of sodium.

- To conclude, thiopyrimidine derivatives derived from phenoxy acetic acid fails to meet the criteria for effective diuretic. Comparative increase in the diuretic activity was exhibited by compounds possessing electron withdrawing groups like chlorine and fluorine attached to the phenyl moiety at the 6\textsuperscript{th} positions of the pyrimidine ring.

- It was also presumed that phenoxy acetic acid group attached to the phenyl moiety at the 4\textsuperscript{th} position of the pyrimidine ring fails to contribute for the diuretic activity.

\textbf{Scheme-V}

The \textit{in vivo} diuretic activity as well as saluretic and kaliuretic effect of the synthesized compounds in \textit{albino} rats of scheme V is summarized in (Table 28-29).
Cumulative urine excreted during 0-5 h for each group (6 albino rats) is a measure of urinary excretion. After 5 h of screening, the compounds CXIV, CXIII, CVIII, CVII and CXII showed good cumulative urine output.

The urinary output of compound CXIV was highly significant 15.09±0.540 (P<0.01) i.e. increased by 300% with respect to control.

Urinary excretion of CXIV was 301.80% and that of CXIII, CVIII, CVII and CXII lies in between % urinary excretion of urea (138.09%) and acetazolamide (184.77%).

The Diuretic action of CXIV and CXIII was found to be 2.62 and 2.03 in comparison to 1.2 and 1.6 for urea and acetazolamide respectively.

As far as diuretic activity is concerned, compound CXIV and CXIII was found to be 1.63 and 1.25 while CVIII, CVII, and CXII were calculated as 0.93, 0.87 and 0.81 respectively (Table 28).

The Lipschitz value (the ratio T/U, in which T is the response of the test compound, and U, that of urea treatment, indices of 1.0 and higher are regarded as a positive effect in terms of diuretic activity) shows that CXIV is twice potent than urea and as far as urinary output is concerned; compounds CXIII, CVIII, CVII and CXII were nearly equal to urea (>1.0, means positive effects) (Table 28).

Apart from diuretic activity all the compounds (CIV-CIX) and (CX-CXV) were also tested for saluretic and kaliuretic effects in albino rats.

After 0-5 h compounds CXIV, CVIII and CXIII showed a significant increase in sodium excretion (p<0.01) i.e. 3.68±0.284, 2.99±0.150 and 2.83±0.429, respectively, which was either almost similar or more than standards, i.e. urea (2.74± 0.154) and acetazolamide (3.36 ± 0.294). The Na" excretion of CVII was also significant as compared to urea (P<0.05) i.e. 2.56±0.278 and 2.74±0.154 respectively. (Table 29)

Compounds CXIV and CVIII were also found to have significant kaliuretic property (P<0.01) i.e. 1.72±0.156 and 1.480±0.405 respectively which similar
to acetazolamide (1.523±0.118) and with regards to Na⁺/ K⁺ ratio, it was observed that CXIV is a stronger kaliuretic.

- Table 29 showed that compounds CX, CV and CXV were potassium sparing (3.6, 3.4, and 3.3), while in rest of the compounds Na⁺/ K⁺ ratio lies between 1.8-2.1 i.e. more kaliuretic than potassium sparing.

- Chloride was estimated by titrating the urine by Volhard’s method. Chloride excretion was also increased to similar extent of sodium.

The structure activity relationships of pyrimidine diuretics may be rationalized by assuming that the newly synthesized compounds may possess an important site which involves a basic centre of the drug, which may be N-1, N-3 of pyrimidine nucleus or both. Groups that decrease the basic strength of pyrimidine nucleus reduce the diuretic activity. The other site involves the substituted phenyl ring at 6th position which may be hydrophobic in nature. To conclude, the structure activity relationships based on the observed results indicated that, the type of aryl group substitution attached to the 6th position of pyrimidine nucleus plays a significant role for diuretic activity. It has been noticed that, substitution of the phenyl group at the 6th position of pyrimidine heterocycle, with a chlorine atom seems more favourable for an active diuretic agent than a methoxy residue.

The preliminary in vivo diuretic studies suggested the following structure activity relationships (SAR),

- Compound CXIV, prototype of this series possess strong aquaretic and saluretic activity when given orally in a single dose.

- Substitution at 6th position of pyrimidine ring system with electron withdrawing groups (Cl, F, Br) increases significant urinary excretion.

- Compounds containing electron releasing groups such as CH₃ or OCH₃ at any position of pyrimidine ring reduces diuresis.

- Substitution with heteroaryl moiety (pyridine) at the 6th position of the pyrimidine ring may also contribute to diuretic activity.

In short the diuretic activity may be increased by incorporating strong electron withdrawing and large size substituent on the phenyl ring.
The compounds CXIV and CXIII showed diuretic properties more than that of standard (acetazolamide).

Overall, the compound CXIV 6-(2,6-dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol was found to be most promising candidate of the series.

HEPATOTOXICITY STUDIES

Liver is vulnerable to drug-induced toxicities because of its role as a primary organ of drug elimination and its subsequent exposure to potential toxins, the need of toxicity studies are significant.

Among the synthesized compounds evaluated for in vitro anticancer screening (scheme I, II and III), the compounds LXII, LXIV, LXX and LXXVIII were found to have significant anticancer effects and were evaluated for hepatotoxicity studies.

The serum collected from the groups of albino rats were used for estimation of biochemical parameters to determine the functional state of the liver.

The extent of hepatic damage was assessed by the level of various biochemical parameters in circulation.

Table 30 shows the results of liver function test with reference to selected compounds. The estimation revealed that there was no significant increase in, SGOT, SGPT, alkaline phosphatase, bilirubin and total protein level in serum as compared to the control and standard.

The hepatotoxicity studies are significant for diuretics also, since diuretic agents like furosemide and triamterine have been reported for their hepatotoxocities.34

Among the synthesized compounds evaluated for in vivo diuretic activity (scheme IV and V), the compounds CVII, CVIII, CXIII and CXIV were found to have excellent diuretic profiles which were evaluated for hepatotoxicity studies.
Table 31 shows the results of liver function tests with reference to selected compounds. The estimation revealed that there was no significant increase in, SGOT, SGPT, alkaline phosphatase, bilirubin and total protein level in serum as compared to the control and standard.

Statistical Analysis: Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference which was analyzed separately and one-way analysis of variance (ANOVA) was carried out. Dunnet's test was used for individual comparisons.

It was clearly indicated from the results obtained (Table 30 and 31) that none of the compound showed any toxicity to the liver as compared to control and standard.