CHAPTER-2

“SYNTHESIS AND CHARACTERIZATION OF 9-METHYL-2-MORPHOLIN-4-YL-8-SUBSTITUTED PHENYL-1H-PURINE DERIVATIVES”
2.1 General Introduction to Purines and Its Derivatives

A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring. Purines, including substituted purines and their tautomers, are the most widely distributed kind of nitrogen-containing heterocycle in nature.

A number of substituted purine derivatives occur in nature; some, as components of nucleic acids and coenzymes, play vital roles in the genetic and metabolic processes of all living organisms. Purines are generally white solids of amphoteric character. They can form salts with both acids and bases. Conjugated double bonds in purines results in aromatic chemical properties, that confers considerable stability, and accounts for their strong ultraviolet absorption spectra. With the exception of the parent compound, most substituted purines have low solubilities in water and organic solvents.

The purine bases, adenine and guanine together with pyrimidines, are fundamental components of all nucleic acids. Certain methylated derivatives of adenine and guanine are also present in some nucleic acids in low amounts. Purine-related compounds have been investigated as potential chemotherapeutic agents. In particular, 6-mercaptopurine in the form of its nucleoside phosphate, inhibits several enzymes required for synthesis of adenosine and guanosine nucleotides, and thus proves useful in selectively arresting the growth of tumors. The
pyrazolopyrimidine has been used in gout therapy. As a purine analog, this agent serves to block the biosynthesis of inosine phosphate, as well as the oxidation of hypoxanthine and xanthine to uric acid. As a result of its use, overproduction of uric acid is prevented and the primary cause of gout is removed.

The quantity of naturally occurring purines produced on earth is huge, as 50 percent of the bases in nucleic acids, adenine and guanine are purines. In DNA, these bases form hydrogen bonds with their complementary pyrimidines thymine and cytosine, respectively. This is called complementary base pairing. In RNA, the complement of adenine is uracil (U) instead of thymine.

Other notable purines are hypoxanthine 4, xanthine 5, theobromine 6, caffeine 7, uric acid 8 and isoguanine 9.
Emil Fischer, nobel laureate in chemistry in 1902, attributed the name purine to the fused imidazo [4, 5- d] pyrimidine compound 1 in 1884 and achieved its synthesis in 1898. He further showed through a series of elegant transformations that the natural substances adenine, xanthine, caffeine, uric acid, and guanine correspond to different hydroxyl and amino derivatives of this fundamental system (Fig. 2.1).

Purines bearing functionality at one or more of the seven peripheral atoms which make up its bicyclic structure can be readily synthesized by
well-established routes from monocyclic precursors.\textsuperscript{3} In order to gain further insight into the structural requirements for active antimycobacterial purines, we needed easy access to 9-arylpurines such as compound 10.

Polyfunctionalized purines 12 substituted at the 2, 6, and 8 positions are also obtained through the reaction of suitably activated purine intermediates 11 with heteroatom and carbon nucleophiles through SNAr-type substitution and transition metal catalyzed coupling reactions.\textsuperscript{4–15} N-alkylation/acylation or Vorbruggen\textsuperscript{16–18} and other electrophile based reactions, can also be used to introduce functionality onto the nitrogen atoms in the purine ring. An example of this methodology is the highly selective N-9 alkylation of purines under Mitsunobu conditions (11–13).\textsuperscript{11, 15, 19, 20}
### 2.2 Biological Importance of Purine Derivatives

#### Table-2.1: Purine moiety containing drugs:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the drug</th>
<th>IUPAC Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nelarabine</td>
<td>(2R,3S,4R,5R)-2-(2-amino-6-methoxy-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol</td>
<td><img src="image" alt="Structure 14" /></td>
</tr>
<tr>
<td>2</td>
<td>Cladribine</td>
<td>5-(6-amino-2-chloro-purin-9-yl)-2-(hydroxymethyl)oxolan-3-ol</td>
<td><img src="image" alt="Structure 15" /></td>
</tr>
<tr>
<td>3</td>
<td>Phthalysulfathiazole</td>
<td>2-[(4-[(1,3-thiazol-2 ylmino) sulfonyl]phenyl]amino) carbonyl]benzoic acid</td>
<td><img src="image" alt="Structure 16" /></td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>Chemical Structure</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------------</td>
<td>---------------------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>Tioguanine</td>
<td><img src="image1.png" alt="Tioguanine" /> 2-amino-7H-purine-6-thiol</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Ribavirin</td>
<td><img src="image2.png" alt="Ribavirin" /> 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(\text{hydroxymethyl})oxolan-2-yl]-1(H)-1,2,4-triazole-3-carboxamide</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>Fludarabine</td>
<td><img src="image3.png" alt="Fludarabine" /> [(2R,3R,4S,5R)-5-(6-amino-2-fluoro-purin-9-yl)-3,4-dihydroxy-oxolan-2-yl] methoxy phosphonic acid</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>$R$-roscovitine</td>
<td>2-($R$)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
2.2.1. Interferon Inducers

A new strategy under consideration for treating virus infections is to induce endogenous IFN synthesis with orally bioavailable small molecular-weight compounds such as imiquimod. New series of 2-substituted 8-hydroxyadenines has been identified, including the purine derivatives 21 and 22, more potent than imiquimod and display excellent bioavailability.\textsuperscript{21-23}

\begin{center}
\includegraphics[width=0.5\textwidth]{imiquimode.png}
\end{center}

2.2.2 Inhibitors of Hsp90

The purine derivatives PU3 23 and Pu24FCl 24 are potent drug-like small molecule inhibitors of Hsp90.\textsuperscript{24, 25} Compound 25 is the most potent and selective purine-based HSP90 inhibitor to date [IC\textsubscript{50} = 30 nM, SI (tumor vs normal cells) = 730- to 3200-fold depending on the cell type].\textsuperscript{26}

\begin{center}
\includegraphics[width=0.5\textwidth]{inhibitors.png}
\end{center}
2.2.3 Antimycobacterial purines

Promising new agents have been identified through the screening of libraries of 6-thio-N9-substituted purines.\textsuperscript{27, 28} In particular, (6-decylsulfanyl-purin-9-yl)-acetic acid ethyl ester 26 and the dodecyl derivative 27 exhibited MIC values of 1.56 and 0.78 \textmu g/mL, respectively, against the Mtb H37Rv strain.\textsuperscript{29} Compounds 28 and 29 were also found highly active against M.tuberculosis (MIC=1.56 and 0.39 \textmu g/mL, respectively) with low toxicity against mammalian cells. Anti-mycobacterium tuberculosis activity has also been reported for Agelasine F 30, a 7, 9-dialkylpurinium salt isolated from marine sponges (Agelas sp.). This activity was accentuated for analogs 31 and 32 obtained by synthesis.\textsuperscript{30}
2.2.4 Inhibitors of leukotriene A4 hydrolase

In a recent study at Pharmacia, a series of imidazopyridines and imidazopyrimidines (purines) like 33 were tested and shown to exhibit improved activities over the known clinical candidates SC-57461A and SC-56938.\textsuperscript{31(85)}

2.2.5 Sulfotransferase inhibitors

A primary screen of a purine library by Bertozzi and coworkers\textsuperscript{32} revealed the 2, 6, 9-trisubstituted purine 34 to be an inhibitor of carbohydrate sulfotransferase Nod-H from Rhizobium meliloti. This led to the discovery of the potent and selective estrogen sulfotransferase (EST) purine inhibitor NG38 35 (IC\textsubscript{50} = 500 nM versus IC\textsubscript{50} = 4000 nM against Cdk1),\textsuperscript{32, 33} and to (2-chloro-9H-purin-6-yl)-naphthalen-1-yl-methyl-amine 36, a specific inhibitor of β-aryl sulfotransferase-IV (β-AST-IV) (IC\textsubscript{50} = 96 nM).
2.2.6  **Inhibitors of the cysteine protease cathepsin K**

In a high-throughput screen of a purine library, researchers from Novartis identified several interesting hits, including compound 37, a novel low nanomolar inhibitor of cathepsin K.

![Cat K: IC50 = 7 nM](image)

2.2.7  **Src tyrosine kinase inhibition**

The purine type Src inhibitor NVP-AAK980 38 has been reported to influence bone remodeling. ARIAD pharmaceuticals has also been active in this domain, demonstrating that bisphosphonate compounds 39 and 40 target bone and are potent Src inhibitors (IC50 = 41 and 10 nM, respectively). The bis phosphonate motif is known to exhibit exceptional affinity for the inorganic component of bone, and its positioning on the para-position of the 6-anilino substituent in 39/40 places it on the easily accessible outside surface of the ATP-binding region. These compounds may find application against osteosarcoma and various bone-related disorders (osteoporosis).
Furthermore, AP23464 41 was very recently shown to strongly inhibit the kinase activity of the D816V activation-loop mutant of C-kit, both in vitro (IC50 = 53 nM) and in vivo, and to induce cell-cycle arrest and apoptosis in cells expressing this mutation.

### 2.2.8 P38α MAP kinase inhibitors

Using database screening and structure-based design strategies Glaxo Smith Kline chemists identified the novel p38α inhibitors 42 and 43 (IC50 = 82 and 16 nM, respectively) in which the C-6 anilino nitrogen is present as a urea. Olomoucine 44, the archetypical Cdk1, 2, and 5 inhibitor, cannot capture these contacts and is consequently inactive against P38α MAP kinase and only weakly active against the closely related MAP kinase ERK2.36, 37, 38
2.2.9 Inhibitors of Inositol-1, 4, 5-trisphosphate-3-kinase.

Schultz and co-workers identified the 2, 6-disubstituted purine 45 as one of several interesting new leads against IP3K (IC50 = 10–200 μM). 39

![Chemical structure of 45](image)

2.2.10 Cyclin-dependent kinase inhibitors

The discovery of olomoucine 40 44 and roscovitine 46 41 triggered intense interest in the development of 2, 6, 9-trisubstituted purines as selective inhibitors of Cdk1, 2, and 5. Purvalanol-A 47, it is one of the most potent compounds in this group, but its pharmacological profile may be inferior to that for roscovitine 46. 42

![Chemical structures of 44, 46, and 47](image)
2.3 Literature Review of Synthesis of Purine derivatives

The purine ring is the most ubiquitous nitrogen-containing heterocycle in nature, since besides the numerous purine derivatives found in various marine organisms and plants, it is the core structure of adenine and guanine in nucleic acids (RNA and DNA). In addition, purines are involved in many metabolic processes as cofactors associated with a great number of enzymes and receptors, notably ATP, GTP, GDP, cAMP, cGMP, AcCoA, NAD, NADP, FAD, PAPS and SAM, which play key roles at different phases of the cell cycle, in cell signalling and other fundamental biological processes. It should be noted that all of these associated proteins contain a purine recognition pocket and, consequently, purine derivatives have long been developed to selectively inhibit or antagonise each of these enzymes and receptors. Indeed, a great variety of di, tri or tetrsubstituted purines described in the literature have been found to be potent inhibitors of chaperone HSP90, protein kinases (MAP, Src and Cdk), sulfotransferases, phosphodiesterases and microtubule assembly, inducers of interferon and dedifferentiation and antagonists of adenosine receptors and corticotropin-releasing hormone receptors. This wide range of biological activities displayed by purines is conferred by a judicious choice of the nature of the substituents that can be combined on the N-1, C-2, N-3, C-6, N-7, C-8 and N-9 centres.
With such an easy access to so much structural diversity, the purine core has become a privileged structure in medicinal chemistry, and an important scaffold in the preparation of combinatorial libraries. In general, two strategies are applied for the preparation of purine libraries. In the first procedure, a preformed purine ring loaded with various reactive functionalities is directly modified, which allows good regiocontrol at C-2, C-6, C-8 and N-9. Alternatively, substituted pyrimidine or imidazole precursors are functionalised, generating the second heterocycle of the purine core in the process with better regiocontrol at N-1, N-3, N-7 and N-9. Following literature is to review recent advances in the synthesis of purine derivatives with particular emphasis on methods that can lead to purine libraries.

2.3.1 Functionalisation at position 6

Wan and co-workers have recently described the synthesis of several 6-aminopurine derivatives and PAH derivatives in one step and high yield from unprotected inosine (Scheme 2.1) by BOP-mediated lamination.

Scheme 2.1:
Using similar reaction conditions, the acetyl-protected inosine 48 led to the corresponding 6-substituted-9-(2, 3, 5-tri-acetyl-β-Dribofuranosyl) -purines 52 (Scheme 2.2).

**Scheme 2.2:**

**Preparation of C-6-arylpurines**

Lakshman and co-workers have synthesised a wide variety of C-6-arylpurine 2-deoxyribosides 54 via the Pd-mediated Suzuki–Miyaura cross-coupling of arylboronic acids with C-6 halonucleosides 53 (Scheme 2.3).

**Scheme 2.3:**
Synthesis of 6-amidopurines

Large libraries of 2, 6, 9-trisubstituted purines bearing an amino substituent at positions 2 and 6 have been synthesised. Recently, the regioselective syntheses of 6-amidopurine derivatives 56 and 58 from 2, 6-dihalogeno purines 55 and 57 respectively have synthesized (Scheme 2.4 & 2.5).

Scheme 2.4:

\[
\begin{align*}
\text{55} & \xrightarrow{\text{RCONH}_2, \text{1 equiv}} \text{Pd}(\text{dba})_3/\text{Xantphos} \quad \text{Cs}_2\text{CO}_3, \text{dioxane, } 100^\circ\text{C} \\
\text{56} & \\
\end{align*}
\]

Scheme 2.5:

\[
\begin{align*}
\text{57} & \xrightarrow{\text{RCONH}_2, \text{3 equiv}} \text{NaH, 3 equiv, DMF} \quad \text{O}^\circ\text{C to rt, 2-4h} \\
\text{58} & \\
\end{align*}
\]

2.3.2 Functionalisation at position 9:

Preparation of 9-substituted purine derivatives

Robins and co-workers reported that the glycosylation of the 6-sodium salts of (imidazol-1-y1)purines 59 with a protected chlorosugar proceeded with high regioselectivity (100%) and stereoselectivity (98%) in high yield (>90%) to afford exclusively the β anomer-N-9 regioisomers 60 (Scheme 2.6). Ammonolysis of the imidazolium salts 61, generated in
situ from 60 with BnCl and NaI, gave high yields of the adenine derivative, Cladribine 62, a clinical anticancer drug.

**Scheme 2.6:**

**Michael addition of adenine**

Highly efficient Michael addition reactions of adenine 63 to α, β-unsaturated esters 64 under microwave irradiation to form 65 have been reported (Scheme 2.7),

**Scheme 2.7:**

**Arylation at N-9 of purines**

Ding and co-workers described one of the first N-9 arylations of purines such as 66 via boronic acid/cupric acetate/NEt₃ in dichloromethane to form 67 (Scheme 2.8).
Bakkestuen and Gundersen later described a regioselective N-9 arylation of purines using arylboronic acids in the presence of Cu(II) and an organic base. Trials revealed phenanthroline to give significantly higher yields of than triethylamine or pyridine (Scheme 2.9).

2.3.3 Functionalisation at N-1, C-2, N-3, N-7 and C-8

Preparation of xanthine derivatives from 6-aminouracils

(Traube synthesis)

Interesting xanthine derivatives have been prepared from 5-bromo-6-aminouracil (Scheme 2.10), 5, 6-diaminouracil such as \( \text{72,63,64} \) and N-1(or N-3) monosubstituted- 5,6-diaminouracil.\(^{65} \)
Pyrimidine precursors of 2, 6, 8, 9-tetrasubstituted purines

A synthesis of tetrasubstituted purines has been reported from 4, 6-dichloro-2-methyl-5-nitropyrimidine 73, itself prepared according to a literature procedure.66, 67 The second point of diversity is introduced by the substitution of one chlorine atom of the 5-aminopyrimidine 74 by various amines.68 Incorporation of an alkylthio group at the 6-position in 75 led to 76, (Scheme 31). Cyclisation with aldehydes in the presence of FeCl₃ afforded 77, which was oxidised to the corresponding sulfone 78 for displacement by various amines to give the third point of diversity 79 (Scheme 2.11). The fourth point of diversity at position C-8 can be introduced with aldehydes (Scheme 2.11).
2.3.4 Functionalisation at position 8

Synthesis of 8, 9-disubstituted adenines

Exchange of the bromo atom of 80 for a sulfur and substitution of the thionoadenine 81 (Scheme 2.12) by the phenyldiazo derivative 82 gave intermediates 83. Further HSP90 inhibitors 86 and 89 can be synthesized according to two related strategies starting from di or triaminopyrimidines 84 or 87 (Schemes 2.13 and 2.14). In the first sequence, the butyl substituent of 86 is introduced prior to the cyclisation of 85 and before amination at C-6 with NH3. Alternatively, the butyl substituent of 89 can be introduced at the last step from the 4, 5, 6-triaminopyrimidine precursor 87,69 via cyclisation of the amido intermediate 88.

**Scheme - 2.12:**

![Scheme 2.12](image)

**Scheme - 2.13:**

![Scheme 2.13](image)
**Scheme-2.14:**

![Chemical structure diagram]

**Synthesis of 2, 8, 9-trisubstituted adenine inhibitors of HSP90**

From the strategies towards the preparation of libraries of functionalized 2-fluorinated purines, from which very potent 8-arylsulfanyl-adenine inhibitors of HSP90 were synthesised.\(^70\) The target 2-fluoroadenines 94 were synthesised from 2-fluoroadenine 92 after alkylation at N-9 with tosylates, bromination at C-8 with N-bromosuccinimide giving 93 and thioarylation at C-8 in DMF/K\(_2\)CO\(_3\) (Scheme 2.15).

**Scheme-2.15:**

![Chemical structure diagram]
Synthesis of 6, 7, 8-trisubstituted purines

Liu and co-workers have recently reported an efficient and regiospecific strategy to prepare N-7-substituted purines (Scheme 2.16).71

Scheme-2.16:
2.4 Our Approach to Synthesis of Purine Derivatives

In continuation of the development of useful synthetic methodologies, we have studied that purine derivatives 6(a-l) can be synthesized efficiently by treatment of N4-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine 5 with substituted aldehydes using polyphosphoric acid (PPA) and DMF as the solvent at room temperature (Scheme-2.17).

Present Scheme:

Scheme-2.17:
2.5 RESULTS & DISCUSSION

N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine 5 is used as starting material for our scheme, which can be prepared from the 5-Nitro Uracil 1 by series of known synthetic reactions. 5-Nitro-1H-pyrimidine-2, 4-dione (5-Nitro Uracil) 1 can be prepared by using uracil upon treatment with fuming nitric acid and H₂SO₄. Yield of the corresponding compound is 95%. 5-Nitro-1H-pyrimidine-2, 4-dione (5-Nitro Uracil) on treatment with phosphorous oxychloride and diisopropyl ethylamine will give 2, 4 Dichloro-5-nitropyrimidine 2 which was further used without purification.

A solution of 2, 4 dichloro-5-nitro pyrimidine 2 (7.26g, 37.4mmol) in THF 100 ml) is cooled to -78°C. A solution of methyl amine (8M in methanol, 9.35 ml, 74.8 mmol) is added drop wise. The mixture is allowed to warm to room temp, and concentrated. The residue is partitioned between ethylacetate and water. The layer is separated and the aqueous layer is extracted with ethylacetate. The combined organic extracts are washed with brine, dried over Na₂SO₄, filterd and concentrated to afford the (2-chloro-5-nitro-pyramidin-4-yl)-methyl-amine 3 (7.0g). The residue is used in the next step without further purification and the product is yellow oil. ¹H NMR (300 MHz, CDCl₃) 8 9.06 (1H, s), 8.43 (1H, br s), 3.25 (3H, d, J=5.1Hz); ESI/MS: 189 (M+H).

A solution of the 2-chloro-4-(methyl amino)-5-nitro pyrimidine (1.00g, 5.30mmol) in acetone (30m) 0°C containing Na₂CO₃ (1.0 eq, 5.16 mmol,
546 mg) was treated drop wise with a solution of Morpholine (1.0 eq, 5.16 mmol, 0.45) in acetone (5ml) and stirred for one hrs at O°C. This showed completion of the reaction to give two products. The acetone was removed in vacuo and the residue partitioned between water and EtOAc, the organic layer dried (MgSO₄) and evaporated in Vacuo. Column chromatography (DCM-EtOAC, 9:1) gave the product Methyl-(2-morpholin-4-yl-5-nitro-pyrimidin-4-yl)-amine 4 (560 mg, 45%) as a pale yellow solid, together with 260mg (17%) of the disubstituted product also as a yellow solid. (¹H NMR 300 MHz, CDCl₃) 8.68 (1H, s, pyrimidine Ar), 3.75-3.71 (4H, m, 2x Morpholine CH₂), 3.57-3.53 (4n, m, 2x Morpholine CH₂).

This Methyl-(2-morpholin-4-yl-5-nitro-pyrimidin-4-yl)-amine on reduction with SnCl₄.2H₂O will give required N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine 5 as a yellow solid. ¹H NMR (300 MHz, CDCl₃) 8.68 (1H, s, pyrimidine Ar), 3.75-3.71 (4H, m, 2x Morpholine CH₂), 3.57-3.53 (4n, m, 2x Morpholine CH₂).

Polyphosphoric acid (PPA), this substance is a powerful dehydrating agent. This can also be used as ring closing reagent, reaction medium and solvent, spinning solvent. It has been used mainly as a ring closing reagent but its scope has not been fully explored. Here it has been applied for oxidative dehydrogenation of the cyclic intermediates formed from the condensation of N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine 5 and substituted aldehydes.
The condensation reaction between, $N^4$-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine 5 and substituted aldehydes is known for practical synthesis of N-substituted amides. Apart from handling these toxic several aldehydes (aromatic, heteroaromatic and aliphatic) underwent the above conversion to form a series of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purines 6(a-1). Aromatic aldehydes containing both electron-donating and electron-withdrawing groups worked well along with the heteroaryl substituted aldehydes. Purine derivatives can be synthesized efficiently by treatment of $N^4$-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine with aldehydes using PPA as an efficient ring closing reagent at room temperature. Similarly, different solvents were tested for the synthesis of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purines. The reaction in DCM, THF gave the corresponding 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purines in low yield and also required prolonged reaction times (entries 1 and 2), whereas the reaction in MeOH, DME, ACN has resulted in slightly improved yields of the product (entries 3-5). However, the reaction in DMF gave 82% of the corresponding 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purines 6(a-1) in 3h. As can be seen from the Table 2.2.

**Effect of solvent on the synthesis of Purine Derivatives.**
Table-2.2: Effect of Solvent on the Synthesis of Purine Derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂Cl₂</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>CH₃OH</td>
<td>2</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>DME</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>CH₃CN</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>H₂O</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>DMF</td>
<td>0.5</td>
<td>82</td>
</tr>
</tbody>
</table>

aReaction conditions: N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4,5-diamine, Substituted aldehydes, PPA, solvent (6 ml).

The method is suitable for the preparation of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purines from an acid sensitive aldehyde such as furfuraldehyde and the sterically hindered aldehyde 2, 3, 4-trimethoxy benzaldehyde. The reaction conditions are mild and the experimental procedure is simple. The products were formed (Table-2.3) in high yields (70–85%). The structures of the products were determined from their spectral (¹H NMR, IR and MS) data.

Table: 2.3: List of Purine Derivatives Prepared
<table>
<thead>
<tr>
<th>S.No</th>
<th>Aldehyde</th>
<th>Diamine</th>
<th>Product</th>
<th>% of yield</th>
<th>Time Mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \text{HOC}_6\text{H}_4\text{NO}_2 )</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>( \text{N}_3\text{H}_2\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>75</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>( \text{O}_3\text{C}_6\text{H}_4 )</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>( \text{N}_3\text{H}_2\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{OH} )</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>( \text{N}_3\text{H}_2\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>84</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{NO}_2 )</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>( \text{N}_3\text{H}_2\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
<td>84</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{O} )</td>
<td>( \text{O}_3\text{C}_6\text{H}_4\text{N}_2\text{H}_2 )</td>
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2.6 Conclusions

This chapter consists of a preparation of 12 purine derivatives covering our results in the development of PPA mediated condensation methodology. In continuation of the development of useful synthetic methodologies, we have observed that purine derivatives can be synthesized efficiently by treatment of N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine with aldehydes using polyphosphoric acid (PPA) and DMF as the solvent at room temperature. N⁴-Methyl-2-morpholin-4-yl-pyrimidine-4, 5-diamine is used as starting material for our scheme, which can be prepared from the uracil by five known synthetic reactions. The reaction conditions are mild and the experimental procedure is simple. Further interdisciplinary studies are now under way searching for other novel types of biologically active compounds. Several types of our compounds are now also applying in chemical biology and bioanalysis.

The methodology of the condensation reactions on purines and nucleosides is now widely used in many laboratories and also the new cytostatic and antiviral compounds are inspiring further design of new compounds with potential biological activity.
2.7 Experimental Section

Preparation of 2, 4-Di Chloro-5-Nitropyrimidine (2):

\[\text{Diisopropyl Ethylamine} \rightarrow \]

25 gm of 5-Nitro urasil is suspended in 490 ml of phosphorous oxychloride for 10 minutes and diisopropyl ethylamine is slowly added to the suspension at room temp. The reaction suspension is refluxed at 130°C for 3 hrs. The solution is concentrated under reduced pressure to be a volume of 100 ml. Then the solution is added drop wise to 500 ml of ice water and stirred for 1 hour, and extracted with diethyl ether.

The organic layer is washed with 500 ml of saturated ammonium chloride and dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Column chromatography on silica gel (ethyl acetate, Hexane=1:5) affords 16.8 gm of the title compound.

Preparation of (2-chloro-5-nitro-pyrimidin-4-yl)-Methyl-amine (3).

\[\text{MeNH}_2\rightarrow\]

A solution of 2, 4-dichloro-5-nitro pyrimidine (7.26g, 37.4mmol) in THF 100 ml) is cooled to -78°C. A solution of methyl amine (8M in methanol, 9.35 ml, 74.8 mmol) is added drop wise. The mixture is
allowed to warm to room temp and concentrated. The residue is partitioned between ethylacetate and water. The layer is separated and the aqueous layer is extracted with ethylacetate. The combined organic extracts are washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated to afford the title product (7.0g)

(Note: The product contains 5% regio-isomer, 4-chloro-2-(methylamino)-5-nitropyrimidine). The residue is used in the next step without further purification. Mp: 86-87°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.06 (1H, s), 8.43 (1H, br s), 3.25 (3H, d, J=5.12 Hz); ESI/MS: 189 (M+H).

**Preparation of Methyl-(2-morpholine-4-yl-5-nitropyrimidine-4yl) amine (4).**

A solution of the 2-chloro-4-(methylamino)-5-nitro pyrimidine(1.00g, 5.30 mmol) in acetone (30ml) 0°C containing Na$_2$CO$_3$ (546 mg) was treated drop wise with a solution of morpholine (1.0 eq, 5.16 mmol) in acetone (5ml) and stirred for one hour at 0°C. TLC (DCM-Hexane, 4:2) showed completion of the reaction to give two products. The acetone was removed in vacuo and the residue partitioned between water and EtOAc, the organic layer dried (MgSO$_4$) and evaporated in vacuo. Column chromatography (DCM-EtOAC, 9:1) gave the product (560 mg, 45%) as a
pale yellow solid, together with 260mg (17%) of the disubstituted product also as a yellow solid. ($^1$H NMR; 300 MHz, CDCl$_3$) 8.68 (1H, s, pyrimidine Ar), 3.75-3.71 (4H, m, 2x Morpholine CH$_2$), 3.57-3.53 (4n, m, 2x morpholine CH$_2$).

**Preparation of N-Methyl-{2-morpholin-4-yl-pyrimidine-4, 5-diamine (5).**

Charge Methyl-{2-morpholine-4-yl-5-nitro pyrimidine-4-yl} amine, 3.76 gm into a RGF, tin (II) chlorides dehydrate (18 gm, 80 mmol) in ethanol (200ml) is heated at 80$^\circ$C. After heating for 2 hours, the reaction mixture is cooled to room temperature and concentrated. Ethyl acetate and celite is added to the residue and the mixture is basified with saturated sodium carbonate solution to a pH of 9-10. The mixture is filtered through a pad of celite and washed with brine, dried over sodium sulphate, filtered and concentrated to get title compound as 2.2 g.

**2.7.1 Typical Experimental Procedure:**

**Preparation of 9-Methyl-2-Morpholin-4-yl-8-Phenyl-9H-Purine Derivatives (6(a-l)):**
**General procedure:**

To the mixture of 2-Morpholin-4-yl-pyrimidine-4, 5-diamine (1 eq) and aldehyde (1.2 eq) in DiMethyl Formamide (DMF) (5ml) under nitrogen atmosphere, polyphosphoric acid (1.5 eq) was added. The mixture was stirred at reflux temperature for about 30-45 mins. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured in ice cold water. The precipitate formed solvent was filtered and dried to afford the desired product as off white or pale yellow solid (yield is 70-90%). The respective compounds are confirmed by NMR, MASS, IR and C\textsuperscript{13} and the spectral data is given below.

**Preparation of 9-Methyl-2-morpholin-4-yl-8-(3-nitro-phenyl)-9H-purine (6a):**

![Chemical Structure of 6a](image)

White solid; mp 154-156°C; m/z=341.20 (M\textsuperscript{+}); \textsuperscript{1}H NMR (400 MHz, DMSO: \textdelta ppm); 8.78(1H, s), 8.68(1H, s), 8.37(1H, J=8.1 Hz, d), 8.21(1H, J=8.2 Hz, d), 7.72(1H, 7.2 Hz, d), 3.77-3.92 (8H, m), 3.48(3H, s); IR (KBr, cm\textsuperscript{-1}); 3341.37, 3241, 2925, 1627.62, 1119.53 (Fig. 2.1, 2.2).
Preparation of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purine (6b):

![Chemical Structure Image]

White solid; mp 162-165°C; m/z: 296.21 (M⁺); ¹H NMR (400 MHz, DMSO: δppm) 8.75(1H, s), 7.80-7.70(2H, m), 7.55-7.70(3H, m), 3.72-3.88(8H, m), 3.75(3H, s); IR (KBr, cm⁻¹); 2863.56, 1615.52, 1463.61, 1117.26 (Fig.No. 2.3, 2.4).

Preparation of 3--9-Methyl-2-morpholin-4-yl-9 H-purin-8-yl phenols (6c):

![Chemical Structure Image]

White solid; mp 116-120°C; m/z: 312.31(M⁺); ¹H NMR (400 MHz, DMSO: δppm) 9.79(1H, s), 8.72(1H, s), 7.27-7.37(2H, m), 6.94-7.26(2H, m), 3.66-3.79(8H, m), 3.32(3H, s); IR (KBr, cm⁻¹); 3156.91, 1618.40, 1432.66, 1273.95 (Fig.No. 2.5, 2.6).

Preparation of 9-Methyl-2-morpholin-4-yl-8-(2-nitro-phenyl)-9H-purine (6d):

![Chemical Structure Image]

White solid; mp 96-98°C; m/z: 341.2(M⁺); ¹H NMR (400 MHz, DMSO: δppm); 8.74(1H, s), 8.23(1H, J=7Hz, d), 7.85-7.96(3H, m), 3.66-3.77(8H,
Preparation of 9-Methyl-2-morpholin-4-yl-8-(2, 3, 4-trimethoxy-phenyl)-9H-purine (6e):

![Chemical Structure]

White solid; mp 102-105°C; m/z: 388.30(M⁺); ¹H NMR (400 MHz, DMSO: δppm); 8.73(1H, s), 7.14(2H, s), 3.65-3.85(8H, m), 3.34(12H, s); ¹³C NMR (100 MHz, CDCl₃) 156.80, 155.14, 153.14, 152.60, 149.68, 148.22, 127.17, 125.12, 106.76, 66.38, 60.52, 56.58, 45.03, 40.05, 30.45, 29.14; IR (KBr, cm⁻¹); 3435.85, 2856.36, 1619.84, 1110.62.

Preparation of 4-Bromo-2-9-methyl-2-morpholin-4-yl-9H-purin-8-yl phenols (6f):

![Chemical Structure]

White solid; mp 120-124°C; m/z: 390.23(M⁺); ¹H NMR (400 MHz, DMSO: δppm); 11(1H, s), 8.74(1H, s), 7.61(1H, s), 7.56(1H, J=8Hz, d), 7.00(1H, 6.8 Hz, d), 3.62-3.82(8H, m), 3.58(3H, s); IR (KBr, cm⁻¹); 3435.85, 2856.36, 1619.84, 1110.62.

Preparation of 8-(2-Bromo-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6g):
White solid; mp 156-158°C; m/z: 374.13(M⁺); ¹H NMR (400 MHz, DMSO: δppm) 8.75(1H, s), 7.80(1H, J=8Hz, d), 7.50-7.72(3H, m), 3.62-3.85(8H, m), 3.60(3H, s); IR (KBr, cm⁻¹); 2853.36, 1619.84, 1241.77, 1110.62 (Fig.No. 2.12, 2.13).

**Preparation of 9-Methyl-2-morpholin-4-yl-8-pyridin-2-yl-9H-purine (6h):**

White solid; mp 132-136°C; m/z: 297.20(M⁺); ¹H NMR (400 MHz, DMSO: δppm); 8.75(1H, s), 8.77(1H, J=7.4Hz, d), 8.22(1H, J=6Hz, t), 8.00(1H, J=6Hz, t), 7.50(1H, J=7.3 Hz, d), 3.62-3.80(8H, m), 3.60(3H, s); IR (KBr, cm⁻¹); 2962.35, 2863.56, 1615.52, 1117.26, 989.65 (Fig.No. 2.14, 2.15).

**Preparation of 8-Cyclooctyl-9-methyl-2-morpholin-4-yl-9H-purine (6i):**
White solid; mp 136-140°C; m/z: 329.22(M⁺); ¹H NMR (400 MHz, DMSO: δ ppm); 1.72(14H, s), 3.20(1H, m), 3.60 (3H, s), 3.62-3.80(8H, m); IR (KBr, cm⁻¹); 2922.94, 1727.86, 1522.67, 991.81 (Fig.No. 2.16).

**Preparation of 9-Methyl-2-morpholin-4-yl-8-thiophen-2-yl-9H-purine (6j):**

White solid; mp 122-124°C; m/z: 301.10(M⁺); ¹H NMR (400 MHz, DMSO: δ ppm) 7.23(1H, s), 7.88(2H, s), 8.78(1H, s), 3.65-3.85(8H, m), 3.98(3H, s) (Fig.No. 2.17).

**Preparation of 9-Methyl-2-morpholin-4-yl-8-m-tolyl-9H-purine (6k):**

White solid; mp 130-132°C(M⁺); m/z: 310.21(M⁺); ¹H NMR (400 MHz, DMSO: δ ppm); 2.41(3H, s), 3.65-3.85(8H, m), 3.80(3H, s), 7.40(2H, d, J=8Hz), 7.62-7.68(2H, m), 7.78(1H, s); ¹³C NMR (100 MHz, CDCl₃); 158.33, 154.55, 152.33, 147.93, 138.03, 130.37, 129.43, 129.24, 128.51, 125.65, 125.64, 65.93, 44.50, 40.25, 39.42, 29.24, 27.98; IR (KBr, cm⁻¹); 3435.22, 2858.74, 1615.88, 1112.68 (Fig.No. 2.18, 2.19, 2.20).
Preparation of 8-(4-Fluoro-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6l):

White solid; mp 134-136°C; m/z: 314.17(M⁺); ¹H NMR (400 MHz, DMSO: δ ppm); 3.65-3.85(8H, m), 3.88(3H, s), 7.42(2H, J=7.2 Hz, t), 7.98(2H, J=7.4 Hz, t), 8.79(1H, s); ¹³C NMR (100 MHz, CDCl₃: δ ppm); 164.69, 161.40, 155.44, 154.69, 151.40, 147.99, 131.30, 131.18, 126.86, 126.14, 125.20, 115.96, 115.67, 65.00, 44.66, 29.94; IR (KBr, cm⁻¹); 3435.27, 2854.94, 1615.73, 1112.48 (Fig.No. 2.21, 2.22, 2.23).
2.8 References


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SPECTRAL REPORTS
Fig. No. 2.1: NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-(3-nitrophenyl)-9H-purine (6.a)

Fig. No. 2.2: Mass Spectrum of 9-Methyl-2-morpholin-4-yl-8-(3-nitrophenyl)-9H-purine (6.a)
Fig. No. 2.3: NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purine (6.b)

Fig. No. 2.4: MASS Spectrum of 9-Methyl-2-morpholin-4-yl-8-phenyl-9H-purine (6.b)
Fig.No.2.5: NMR Spectrum of 3-(9-Methyl-2-morpholin-4-yl-9H-purin-8-yl)-phenol (6.c)

Fig.No.2.6: MASS Spectrum of 3-(9-Methyl-2-morpholin-4-yl-9H-purin-8-yl)-phenol (6.c)
Fig. No. 2.7  NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-(2-nitrophenyl)-9H-purine (6.d)

Fig. No. 2.8  MASS Spectrum of 9-Methyl-2-morpholin-4-yl-8-(2-nitrophenyl)-9H-purine (6.d)
Fig. No. 2.9: C-13 NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-(2, 3, 4-trimethoxy-phenyl)-9H-purine (6.e)
Fig.No.2.10: NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-(2, 3, 4-trimethoxy-phenyl)-9H-purine (6.e)

Fig.No.2.11: MASS Spectrum of 9-Methyl-2-morpholin-4-yl-8-(2, 3, 4-trimethoxy-phenyl)-9H-purine (6.e)
Fig.No.2.12: NMR Spectrum of 8-(2-Bromo-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6.g).

Fig.No.2.13: MASS Spectrum of 8-(2-Bromo-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6.g).
Fig. No. 2.14: NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-pyridin-2-yl-9H-purine (6.h).

Fig. No. 2.15: MASS Spectrum of 9-Methyl-2-morpholin-4-yl-8-pyridin-2-yl-9H-purine (6.h).
Fig.No.2.16: NMR Spectrum of 8-Cyclooctyl-9-methyl-2-morpholin-4-yl-9H-purine (6.i).

Fig.No.2.17: NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-thiophen-3-yl-9H-purine (6.j).
**Fig. No. 2.18:** C13-NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-β-tolyl-9H-purine (6.k).

**Fig. No. 2.19:** NMR Spectrum of 9-Methyl-2-morpholin-4-yl-8-β-tolyl-9H-purine (6.k).
Fig. No.2.20: MASS Spectrum of 9-Methyl-2-morpholin-4-yl-8-m-tolyl-9H-purine (6.k).

Fig. No.2.21 C13-NMR Spectrum of 8-(4-Fluoro-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6l)
Fig. No. 2.22: NMR Spectrum of 8-(4-Fluoro-phenyl)-9-methyl-2-morpholin-4-yl-9H-purine (6l)

Fig. No. 2.23: MASS Spectrum of 8-(4-Fluoro-phenyl)-9-methyl-2-Morpholin-4-yl-9H-purine (6l)