CHAPTER - 3

SYNTHESIS OF ACYCLIC NUCLEOSIDES OF 4-
SUBSTITUTED PYRAZOLO[3,4-\textit{d}]PYRIMIDINE
AND THEIR BIOLOGICAL ACTIVITY
3.1 INTRODUCTION

Adenosine (1), a well-known naturally occurring nucleoside, is an effective coronary vasodilator\(^1,2\), and inhibitor of platelets thrombi\(^3\). It is believed that, when cells are injured, some of their adenosine triphosphate (ATP) breaks down to adenosine diphosphate (ADP), which initiates the aggregation of blood platelets\(^4\). ADP on further dephosphorylation by plasma enzymes\(^5,6\) produces abenosine monophosphate (AMP)\(^7\) or adenosine which exerts its inhibitory action on platelets aggregation\(^8\). The analogs of adenosine are reported to have longer duration of action as compared to adenosine\(^9,10\).

The replacement of ring carbon or nitrogen of purine (2) by a nitrogen or carbon atom gives aza,deaza-purines (3,4) ring system respectively, whereas interchange of nitrogen at position-7 with carbon at position-8 of purine system (2) affords pyrazolo[3,4-d]pyrimidine (5). A number of deaza and aza purine nucleosides have been synthesized as potential antitumor and antiviral agents\(^11,12\).

Pyrazolo[3,4-d]pyrimidine nucleosides have received considerable attention due to their wide spectrum of biological activities. Allopurinol\(^13\) [pyrazolo[3,4-
d]pyrimidine-4-one] (7) an analog of hypoxanthine (6) is an inhibitor of purine catabolic enzyme, xanthine oxidase\textsuperscript{14} and is used for the treatment of hyperurecemia\textsuperscript{15} responsible for gout\textsuperscript{16}. 4-Amino-1H-pyrazolo[3,4-d]pyrimidine ribosides (8) an analog of adenosine (1) is active against a number of species of \textit{Leishmania}\textsuperscript{17-22}. Allopurinol ribonucleoside (9) has been shown to be 300 times more active than allopurinol against \textit{Leishmania donovani} \textit{in vitro}\textsuperscript{21}. Recently, both the 4-amino-1H-pyrazolo[3,4-d]pyrimidine (4-APP) and its ribonucleosides (8) were found to be several times more active than 9 against promastigotes of \textit{Leishmania braziliensis} and \textit{L. mexicana}\textsuperscript{23}.

The antiparasitic properties of allopurinol, 4-APP and their corresponding nucleosides, and the observation that certain analogs of the normal nucleosides, in which the ribose unit was replaced by a truncated acyclic residue, all displayed the potent biological activities as antiviral agents\textsuperscript{24}.

Several varieties of nucleosides have been synthesized in recent years. Some of these nucleosides have shown significant anticancer, antiparasitic and antiviral activities. A few of them are being used as drugs. Ftoraphur\textsuperscript{25} [5-fluoro-1-tetrahydrofuryl]uracil.
(10), 9-[hydroxyethoxymethyl]guanine$^{26}$ (11), 9-[cyclohexyl-2-propoxy]adenine$^{27}$ (12) are very active compounds, which are specific to the infected cells. These compounds 10-12 are not true nucleosides. Although the heterocyclic moiety in these compounds is the same as present in the nucleosides of nucleic acid. However the glucone moiety is not the usual D-ribose or D-deoxy ribose. The tetrahydrofuranyl, cyclohexyl, hydroxyethoxymethyl moieties attached at position N-9 in these compounds, due to their hydrophobic nature may be facilitating the transport of these molecules across the cell membrane. Inside the cell these compounds might be converted into riboside by the action of ribofuranosyl transferase and to nucleosides.

A number of acyclic pyrazolo[3,4-d]pyrimidine nucleosides have been synthesized$^{28}$. Some of the compounds showed highest degree of antileishmanial activity$^{28}$, which yielded a 87% and 96% parasite inhibition.

3.2 PRESENT WORK

The above report prompted us to undertake the synthesis of acyclic nucleosides of 4-hydroxy and 4-amino-1H-pyrazolo[3,4-d]pyrimidines.
In the present chapter the synthesis of 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (23), 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (26), 1-[2-hydroxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (30), 1-[2-hydroxy-1-(aminomethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (34), 1-[hydroxyethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (38), 4-amino-1-[hydroxyethoxy]methyl-1H-pyrazolo[3,4-d]pyrimidine (40) and their antileishmanial activity in vivo are reported.

3.3 SYNTHESIS

3.3.1 Synthesis of 4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (19)

4-Methylthio-1H-pyrazolo[3,4-d]pyrimidine (19) (Scheme 1) the key intermediate with synthesis of 4-substituted pyrazolo[3,4-d]pyrimidine nucleosides had been synthesized by following the method of Robins. Condensation of malononitrile with triethyl orthoformate (13) gave ethoxymethylene malononitrile (14) in quantitative yield. Treatment of 14 with hydrazine hydrate yielded 5-amino-4-cyano-1H-pyrazole (15) in 30% yield. Hydrolysis of 15 with sulphuric acid afforded 5-amino-1H-pyrazole-4-carboxamide (16) in excellent yield. Cyclisation of 16 with formamide gave 4(5H)-oxo-1H-pyrazo-
Reagents:

(i) CH$_2$(CN)$_2$-Ac$_2$O, Pyridine,
(ii) H$_2$NNH$_2$, R.T.
(iii) H$_2$SO$_4$, R.T.
(iv) HCONH$_2$
(v) P$_2$S$_5$, Pyridine
(vi) CH$_3$I, NaOH, R.T.

Scheme - 1
lo[3,4-d]pyrimidine (17) in 58% yield. The mass spectrum, elemental analysis and the IR spectrum of 17 were in accordance with the assigned structure. Treatment of 17 with P2S5 in refluxing pyridine yielded 4(5H)-mercapto-1H-pyrazolo[3,4-d]pyrimidine (18), which on methylation with methyl iodide under basic condition yielded 4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (19) in 52% yield. The pmr spectrum of 19 had a singlet at 2.4 for the methylthio group and two singlets at 8.2 and 8.0 for H-6 and H-3 respectively. The mass spectrum of the compound had the base peak at m/z 167 (M+). Other significant ions in the spectrum were at m/z 120 (C4H4N4) and 93 (C4H3N3).

3.3.2 Synthesis of 1-[2-hydroxy-1-(hydroxymethyl)ethoxyl]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (23)

Method 1

Condensation of 4-methylthio-1H-pyrazolo[3,4-d] pyrimidine (19) (Scheme 2) with 1,3-dibenzylxyloxy-2-chloromethyloxy propane (20) in the presence of Et3N gave 1-[2-benzylxyloxy-1-(benzyloxymethyl)ethoxy]methyl-4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (21) in 70% yield as an oil. The compound 21 analysed for C24H26N4O3S and had molecular ion peak at 450 (M+) in mass spectrum. PMR
Reagents

(i) Et₃N, DMF
(ii) KOH, Dioxane,
(iii) PdCl₂-H₂, R.T.
(iv) HMDS, (NH₄)₂ SO₄,
(v) Benzene, ∆

* Bn = CH₂Ph
spectrum of compound 21 had a singlet at 5.88 for -OCH2N-protons. Compound 21 on reaction with NaOH gave 4(5H)-oxo derivative (22). Hydrogenolysis of 22 with PdCl2 in hydrogen atmosphere furnished the required compound 23 in 55% yield. Compound 23 analysed for C9H12N4O4 and had molecular ion peak at 240 (M+) in its mass spectrum. The ultra violet absorption of compound 23 gave bands at λmax 251, 206 nm. The site of alkyla-
tion in compound 23 was established by correlating the UV absorption pattern (Table-1). Compound 23 shows similar UV absorption pattern as allopurinol-1-riboside, reflecting 23 to be N-1 isomer PMR spectrum of the compound 23 had two singlets at 7.95 and 7.9 for H-6 and H-3 respectively in the heterocyclic moiety and a singlet at 5.75 for -OCH2N-protons suggested the attach-
ment of aglycon moiety.

Method 2

The reaction of 4-hydroxy-1H-pyrazolo[3,4-d] py-
rimidine (17) with hexamethyldisilazane (HMDS) in the presence of (NH4)2SO4 gave silylated derivative (24). The condensation of 24 (Scheme 2) with 1,3-dibenzylxy-
2-chloromethyloxypropane (20) in refluxing benzene gave compound 22, which on hydrogenolysis with PdCl2 furnis-
ed 23. The compound 23 obtained by this procedure was
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**N-2 isomer**

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identical in all respects with the compound made by method-1.

3.3.3 Synthesis of 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (26)

Condensation of 4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (19) (Scheme 3) with 20 gave 21. Compound 21 on reaction with ammonia under pressure at elevated temperature afforded 25 in 50% yield as an oil. Compound 25 analysed for C_{23}H_{25}N_{5}O_{3} had a molecular ion peak at 419 (M^+) in mass spectrum. PMR spectrum of the compound 25 had a two proton singlet at 5.8 for -OCH_{2}N-protons. Compound 25 on hydrogenolysis with PdCl_{2} in hydrogen atmosphere furnished the required compound 26 in 40% yield. The compound 26 analysed for C_{9}H_{13}N_{5}O_{3} had molecular ion peak at 239 (M^+) in mass spectrum. In the infrared spectrum, the N-H stretching was at 3100 cm^{-1}. PMR spectrum of the compound 26 had two singlets at 8.1 for H-6, and at 7.5 for H-3 of the heterocyclic moiety and a singlet at 5.7 for -OCH_{2}N-protons.

3.3.4 Synthesis of 1-[2-hydroxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (30)

Condensation of bis (trimethyl silyl) derivative of 4-hydroxy-1H-pyrazolo[3,4-d]pyrimidine (24) (Scheme 4) with 2-chloromethoxy-1-benzyloxy-3-phthaloylimidopro-
Reagents:

(i) \( \text{Et}_3\text{N}, \text{DMF} \)

(ii) \( \text{NH}_3\cdot\text{MeOH}, 120^\circ \)

(iii) \( \text{PdCl}_2\cdot\text{H}_2, \text{R.T.} \)
Reagents:

(i) Benzene, $\Delta$
(ii) MeOH-$\mathrm{H}_2\mathrm{NNH}_2$, R.T.
(iii) PdCl$_2$-$\mathrm{H}_2$, R.T.

Scheme -4
pane (27) in refluxing benzene gave 1-[2-benzyloxy-1- (phthaloylimidomethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (28). The PMR spectrum of the compound 28 had two singlets at 8.5 for H-6 and 8.2 for H-3 in the heterocyclic moiety, while in the aglycon moiety, two singlets at 7.6-7.8 and at 7.3 stood for aromatic protons. Compound 28 had a singlet at 5.7 for -OCH₂N- protons.

The protecting phthaloyl group was removed by the reaction of 28 with hydrazine hydrate at 0° in EtOH to yield 29 in 40% yield as an oil which on debenzylation with PdCl₂-H₂ to give the required compound 30 in 30% yield. Compound 30 analysed for C₉H₁₄N₅O₃ and had molecular ion peak at 239 (M⁺) in mass spectrum. The ultraviolet absorption of the compound 30 (λmax 250, 206 nm) shows the similar UV absorption pattern as in the case of allopurinol-1-ribosides, reflecting 30 to be N-1 isomer. PMR spectrum of the compound 30 had two singlets at 8.0 for H-6 and 7.8 for H-3 respectively for heterocyclic moiety and had a singlet at 5.8 for -OCH₂N- protons.

3.3.5 Synthesis of 1-[2-hydroxy-1-(aminomethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (34)

Condensation of 4-amino-1H-pyrazolo[3,4-d]pyrimi-
Reagents:

(i) DMF, NaH, R.T.
(ii) EtOH-H$_2$NNH$_2$, R.T.
(iii) PdCl$_2$-H$_2$, R.T.

Scheme - 5
dine (31) (Scheme 5) with 2-chloromethoxy-1-benzyloxy-3-phthaloylimidopropane (27) in dry DMF gave 1-[2-benzyloxy-1-(phthaloylimidomethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (32). The protecting phthaloyl group was removed by the treatment of 32 at 0°C with hydrazine hydrate to yield 33 as an oil in 40% yield. Debenzylation of 33 with PdCl2-H2 in atmosphere afforded the required 34 in 58% yield. The compound 34 analysed for C9H14N4O2 and had molecular ion peak at 238 (M+1). PMR spectrum had two singlets at 8.4 and 8.1 for H-6 and H-3 respectively, and also had a singlet at 5.9 for -OCH2N- protons of aglycon moiety.

3.3.6 Synthesis of 1-[hydroxyethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (38)

Method 1

Condensation of 4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (19) (Scheme 6) with benzoyloxy ethoxymethylene chloride (35) in the presence of Et3N gave 1-[benzoyloxyethoxy]methyl-4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (36) in 60% yield. Deblocking of the protected nucleoside 36 with methanolic ammonia (methanol saturated with ammonia at 0°C) at ambient temperature afforded 37 in 55% yield. Treatment of 37 withaq KOH
Reagents:

1. DMF, Et$_3$N
2. MeOH-NH$_3$, R.T.
3. KOH, Dioxane, $\Delta$
4. Benzene, $\Delta$
5. MeOH-NH$_3$, R.T.

Scheme - 6
gave 1-[hydroxyethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (38) in 30% yield. Compound 38 analysed for \( \text{C}_8\text{H}_{10}\text{N}_4\text{O}_3 \) and had molecular ion peak at \( m/z \) 210 (\( M^+ \)). Ultraviolet absorption of the compound 38 (\( \lambda_{\text{max}} \)

250.6, 207.8 nm) suggested position N-1 the site of alkylation. PMR spectrum of the compound 38 had two singlets at 7.95 and 7.9 for H-6 and H-3 respectively and a singlet at 5.7 for -OCH\(_2\)N- protons.

**Method 2**

Condensation of 24 and 35 in refluxing benzene gave 1-[benzoyloxyethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (39) in 58% yield. Treatment of 39 with methanolic ammonia furnished the required compound 38. The compound 38 obtained by this procedure was identical in all respects with the compound made by the method-1.

3.3.7 Synthesis of 1-[hydroxyethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (40)

Treatment of compound 37 (Scheme 7) with methanolic ammonia (methanol saturated with ammonia at 0°C) at elevated temperature in a steel bomb gave 1-[hydroxyethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (40) in 50% yield. Compound 40 analysed for \( \text{C}_8\text{H}_{11}\text{N}_5\text{O}_2 \) and had
Reagents:

(i) DMF, Et$_3$N
(ii) MeOH-NH$_3$, R.T.
(iii) MeOH-NH$_3$, $\Delta$

Scheme - 7
a molecular ion peak at 209 (M⁺) in the mass spectrum. PMR spectrum of the compound 40 had two singlets at 8.2 and 8.1 for H-6 and H-3 proton respectively and a singlet at 5.7 for -OCH₂N- protons.

3.4 BIOLOGICAL ACTIVITY

Antileishmanial activity

Antileishmanial activity has been carried out in collaboration with Mr. P.Y. Guru and his coworkers in the Parasitology Division of the Central Drug Research Institute, Lucknow.

In vivo tests were carried out against amastigotes of L. donovani in hamsters (M. auralus) infected with Ddg strain of L. donovani obtained in 1980 from a patient in Bihar (India) and maintained in hamsters. Male hamsters weighing 35-40 gm were infected with 1x10⁷ amastigotes, and four weeks later the intensity of infections was assessed by spleen biopsy. Animals having 2 infection were choosen for screening drugs. Usually 2-3 animals were used for each dose schedule of the drug, while 2-3 untreated animals were kept as controls. The list animals were treated with a single daily intraperitoneal injection of the drug suspension for 5 days. The drug suspension employed for
the list was prepared by grinding the accurately weighed drug with distilled water (2-5 ml). In the case of unsoluble drugs, 1-2 drops of tween-80 or alcohol was mixed with water, thus stock solution being suitably diluted for use. The post treatment spleen biopsy was conducted one week after the last day of drug administration inhibition of infection in treated animals was compared with that of the control animals and percentage inhibition was calculated. Allopurinol (17) used as a standard drug.

The activity of the compounds (Series A) is given in (Table 2). 1,5-Dihydroxy pyrazolo[3,4-d]pyrimidine-4-one (allopurinol) (17) exhibited 88% inhibition at 25 mg/kg dose on the 7th day. Substitution of [2-hydroxyethoxy)methyl function which represented (C₅₋C₄₋C₃₋C₂₋C₁) chain of ribose at N₁ of heterocyclic moiety as in 38 rendered the compound inactive. Introduction of [2-hydroxy-1-(hydroxymethyl)ethoxy] function which represented (C₅₋C₄₋C₃₋C₂₋C₁) of ribose at N₁ as in 23 considerably increased the activity 75% inhibition. The activity was reduced drastically when the hydroxy function at 2 of [2-hydroxy-1-(hydroxymethyl)ethoxy] group was replaced by an amino function as in 30. The data thus suggested that not only the nature and chain length of glucone moiety at N-1 is critical for antileishmanial
Table 2

Antileishmanial activity (in vivo) of the nucleosides (series A and B) at 25 mg/kg on 7th day, against amastigotes of Leishmania donovani in hamster

Series A

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<td>88</td>
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<tr>
<td>38</td>
<td>CH$_2$OCH$_2$CH$_2$OH</td>
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</tr>
<tr>
<td>39</td>
<td>CH$_2$OCH$_2$CH$_2$OCOPh</td>
<td>82</td>
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<tr>
<td>30</td>
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<td>75</td>
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Series B

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<td>CH$_2$OCH$_2$CH$_2$OH</td>
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activity of the alicyclic nucleosides (Series A) but also the nature of the functional groups present in it.

The antileishmanial activity of nucleosides (Series B) is recorded in (Table 2). The activity of the nucleoside 40 was considerable decreased when [2-hydroxyethoxy]methyl function was introduced at N1 of heterocyclic moiety. However, when the hydroxy group in the nucleoside 40 was protected with benzoyloxy function, the corresponding nucleoside 39 exhibited high order of activity. The compound 26 became inactive when the hydroxy function at C2 of [2-hydroxy-1-(hydroxymethyl)ethoxy]moiety was replaced by an amino function. The antileishmanial activity in the series B type of alicyclic nucleosides, thus, revealed that the nature and chain length and also the nature of the functional groups are critical for the activity. Further the high order of activity of the blocked nucleoside 39 indirectly suggested that the compound is probably an inhibitor of some important enzyme involved in the purine salvage process of the parasites.
3.5 EXPERIMENTAL PROCEDURE

1-[2-Benzylolxy-1-(benzyloxymethyl)ethoxy]methyl-4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (23)

A mixture of 4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (19, 3.0 g, 18 mmol), DMF (30 ml) and Et₃N (15 ml) was stirred at ambient temperature. To the mixture was added a solution of 1,3-dibenzylolxy-2-chloromethoxy propane (20, 6 g, 18 mmol) in DMF (10 ml) and the mixture was stirred for 14 hr. The excess of the reagent and solvent were removed at reduced pressure. The residue was taken in EtOAc, washed with H₂O (2×100 ml), dried (Na₂SO₄) and the solvent removed. The product thus obtained was chromatographed on SiO₂ column. Elution of the column with CHCl₃ gave 21 as an oil (3.9 g, 70% yield); MS (m/z): 450 (M⁺); PMR (CDCl₃): 3.7 (s,1H,H-6), 7.9 (s,1H,H-3), 7.2 (m, 10H, Ph-H), 5.88 (s, 2H, H-1'), 4.4 and 4.3 (each s, 4H, 2CH₂Ph), 3.9 (m,1H, H-4'), 3.6-3.3 (m, 4H, H-3', H-5'), 2.6(s, 3H, SCH₃); Found:C, 64.1; H, 5.8; N,12.5; C₂₄H₂₆N₄O₃S requires:C, 64.1; H, 5.9; N, 12.5%.

1-[2-Benzylolxy-1-(benzyloxymethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo [3,4-d]pyrimidine (22)

Method-1

Compound 21 (1.5 g, 3 mmol) in dioxane (30 ml)
was refluxed with aqueous 20% KOH (30 ml) for 12 hr. The resulting mixture was cooled, neutralized with AcOH and the solvent removed under reduced pressure. The residue was taken in CHCl₃, washed with H₂O, dried (Na₂SO₄) and concentrated. The product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃:MeOH (96:4, v/v) gave 22 as an oil (0.4 g, 30% yield); MS (m/z): 420 (M⁺); IR (neat): 1710 (C=O); PMR (CDCl₃): 8.4 (s, 1H, H-6), 8.1 (s, 1H, H-3), 7.2 (m, 10H, ph-H), 5.75 (s, 2H, H-1'), 4.4 (m, 4H, H-3', 5'), 3.7-4.1 (m, 1H, H-4'), 3.3-3.5 (each s, 4H, 2OCH₂); Found: C, 65.7; H, 5.7; N, 13.3; C₂₃H₂₄N₄O₄ requires: C, 65.8; H, 5.8; N, 13.5%.

Method-2

A mixture of pyrazolo[3,4-d]pyrimidine-4(5H)-one (17, 2.0 g, 15 mmol), hexamethyl disilazane (8 ml), dry toluene (50 ml) and (NH₄)₂SO₄ (150 mg) was refluxed for 24 hr. The excess of reagent and solvent from the resulting mixture were removed at reduced pressure to give 24, which was used as such for further reaction without purification. 1,3-Dibenzyloxy-2-chloromethyloxy propane (20, 6.0 g, 19 mmol) was added to 24 in dry benzene and refluxed for 12 hr. It was then cooled and filtered. The solvent from the filtrate was removed
under reduced pressure. The residue was taken in CHCl₃, washed with aq NaHCO₃ (2x100 ml), saturated aq. NaCl solution (2x100 ml), H₂O, dried (Na₂SO₄) and the solvent evaporated. The product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃: MeOH (96:4, v/v) gave 22 (1.19, 55% yield) as an oil.

1-[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (23)

A mixture of 22 (0.4g, 95 mmol), PdCl₂ (50 mg) and MeOH (20 ml) was shaken in hydrogen atmosphere (45 lbs pressure) for 14 hr and filtered. The filtrate was passed through ion exchange resin (IR-45, UH form) and eluted with MeOH. The solvent from the eluate was removed. The product was chromatographed over SiO₂ column. Elution of the column with CHCl₃:MeOH (80:20, v/v) afforded 23 (0.2g, 55% yield); m.p.: 162° (EtOH); MS (m/z): 240 (M⁺); IR (KBr): 1680 (C=O); UV (MeOH): 251, 206 (pH-11); 270, 231 (pH-1); 250, 208 (pH-7); PMR (CDCl₃-DMSO-d₆): 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.75 (s, 2H, H-1'), 3.55-3.7 (m, 1H, H-4'), 3.3-3.5 (m, 4H, H-3', 5'); Found: C, 40.0; H, 5.0; N, 23.3; C₉H₁₂N₄O₄ requires: C, 40.1; H, 5.2; N, 23.4%. 
1-[2-Benzylloxyl-1-(benzyloxy)methyl]ethoxy)methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (25)

Compound 21 (2.0 g, 4 mmol) and methanolic ammonia (Methanol saturated with ammonia at 0° 3 (25 ml) was heated in steel bomb at 120° for 14 hr. Solvent and excess of ammonia were removed and the residue was chromatographed on SiO2 column. Elution with CHCl3: MeOH (96:4, v/v) gave 25 as an oil (1g, 50% yield); MS: 419 (M+); PMR (CDCl3): 8.3 (s, 1H, H-6), 7.8 (s, 1H, H-3), 7.3 and 7.2 (each s, 5H, Ph-H), 5.8 (s, 2H, H-1), 4.5 and 4.4 (each s, 2H, 2OCH2), 4.0 (m, 1H, H-4), 3.7-3.3 (m, 4H, H-3' and H-5'); Found: C, 65.9; H, 6.0; N, 16.7; C23H25N5O3 requires: C, 65.8; H, 6.1; N, 16.8%.

1-[2-Hydroxy-1-(hydroxymethyl)ethoxy)methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (26)

Compound 25 (0.8 g, 1.9 mmol), PdCl2 (100 mg) and MeOH (30 ml) was shaken in H2 atmosphere (45 lbs pressure) for 14 hr and filtered at celite. The filtrate was passed through ion exchange resin (IR-45, OH− form) and eluted with MeOH. The solvent from the eluate was removed on rotary evaporator and the product was chromatographed over SiO2 column. Elution of the column with CHCl3:MeOH (80:20, v/v) gave 26 (0.3 g, 40% yield).
m.p.: 182°; MS (m/z): 239 (M⁺); IR (KBr): 3100 (NH); PMR (CDCl₃-DMSO-d₆): 8.1 (s, 1H, H-6), 7.5 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 4.3 (m, 1H, H-4'), 3.1-3.5 (m, 4H, H-3', 5'); Found: C, 45.2; H, 5.6; N, 29.3; C₉H₁₃N₅O₃ requires: C, 45.2; H, 5.6; N, 28.7%.

1-[2-Benzyl oxy-1-(phthaloylimidomethyl)ethoxy]methyl-4-(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (28)

A mixture of 24 (3.0 g, 20 mmol) and 2-chloromethyl-oxy-1-benzyl-oxy-3-phthaloylimidopropane (27, 9.0 g, 25 mmol) and dry benzene (150 ml) was stirred at ambient temperature for 2 hr and then refluxed for 14 hr. The residue was taken in CHCl₃, washed with NaHCO₃ (2 x 150 ml), NaCl (2 x 100 ml), H₂O dried (Na₂SO₄) and the solvent removed. The product thus obtained was chromato graphed over SiO₂ column. Elution of the column with CHCl₃: MeOH (98:2, v/v) gave 28 as an oil (2.5 g, 52% yield); MS (m/z): 459 (M⁺); PMR (CDCl₃): 8.5 (s, 1H, H-6), 8.2 (s, 1H, H-3), 7.6-7.8 (m, 5H, Ph-H), 7.3 (s, 4H, Ph-H), 5.7 (s, 2H, H-1'), 4.4 (s, 2H, OCH₂Ph), 4-4.2 (m, 1H, H-4'), 3.4-3.8 (m, 4H, H-3', 5').

1-[2-Benzyl oxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (29)

A mixture of 28 (2.0 g, 4 mmol), MeOH (60 ml) and
Hydrazine hydrate (6 ml, 98%) was kept at 0° for 12 hr. The solvent from the resulting mixture was removed at reduced pressure. The residue extracted with CHCl₃ and the solvent removed at reduced pressure. The crude product was chromatographed over SiO₂ column. Elution of the column with CHCl₃: MeOH (95:5, v/v) gave 29 as an oil (0.8 g, 40% yield); MS (m/z): 329 (M⁺); PMR (CDCl₃): 8.2 (s, 1H, H-6), 7.9 (s, 1H, H-3), 7.2 (s, 5H, Ph-H), 5.75 (s, 2H, H-1'), 4.2 (s, 2H, CH₂Ph), 3.9-3.6 (m, 1H, H-4'), 3.4-3.2 (m, 4H, H-3', 5').

1-[2-Hydroxy-1-(aminomethyl)ethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (30)

A mixture of 29 (0.6 g, 1.8 mmol), MeOH (30 ml), PdCl₂ (0.09 g) was shaken under H₂ atmosphere (45 lbs pressure) for 12 hr and filtered through celite. The filtrate was passed through ion exchange resin (IR-45, OH⁻ form). The solvent from the eluate was removed and the product was chromatographed over SiO₂ column. Elution of the column with CHCl₃: MeOH (80:20, v/v) gave 30 (0.2 g, 30% yield); m.p.: 202° (EtOH); MS (m/z): 239 (M⁺); UV (MeOH): 250, 206 (pH-7); 271.6, 211.8 (pH-11); 250.2, 207.2 (pH-1); PMR (CDCl₃-DMSO-d₆): 8.0 (s, 1H, H-6), 7.8 (s, 1H, H-3), 5.8 (s, 2H, H-1'), 3.0-2.5 (m, 4H, H-3', 5'), 2.6-2.4 (m, 1H, H-4'); Found: C, 45.1; H, 5.5; N,
A mixture of 4-amino-1H-pyrazolo[3,4-d]pyrimidine (31, 2.0 g, 0.015 mmol), dry DMF (25 ml) and NaH (0.5 g, 0.02 mmol) was stirred at ambient temperature for 1 hr. To it was added 2-chloromethoxy-1-benzyloxy-3-phthalo-
ylimidoglycerol (27, 8.0 g, 0.02 mmol) in dry DMF (15 ml) and stirred for 2 hr and then refluxed for 12 hr. Resulting mixture was cooled, H₂O was added and extracted with CHCl₃, washed with H₂O (2x150 ml), dried (Na₂SO₄) and concentrated in vacuo. The crude product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃:MeOH (98:2, v/v) gave 32 as an oil (1.9 g, 55% yield); MS (m/z): 458 (M⁺); PMR (CDCl₃): 8.0 (s, 1H, H-6), 7.7 (s, 1H, H-3), 7.25 (s, 5H, Ph-H), 7.4-7.6 (m, 4H, Ph-H), 5.6 (s, 2H, H-1'), 4.9 (s, 2H, OCH₂Ph), 4.1-4.3 (m, 1H, H-4'), 3.4-3.6 (m, 4H, H-3', 5').

A mixture of 32 (1.2 g, 0.03 mmol), MeOH (50 ml) and hydrazine hydrate (5 ml) was kept at 0°C for 12 hr. The solvent and excess of reagent were removed under
reduced pressure. The residue was taken in CHCl₃ (50 ml) and concentrated. The residue was taken in CHCl₃ (50 ml) and concentrated. The crude product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃:MeOH (95:5, v/v) gave 33 as an oil (0.5 g, 40% yield); MS(m/z): 328 (M⁺); PMR (CDCl₃-DMSO-d₆): 8.25 (s, 1H, H-6), 8.15 (s, 1H, H-3), 7.2 (s, 5H, Ph-H), 5.8 (s, 2H, H-1'), 4.35 (s, 2H, CH₂Ph), 4.1-4.3 (m, 1H, H-4'), 3.5-3.6 (m, 4H, H-3', 5'); Found: C, 58.5; H, 6.1; N, 25.6; C₁₆H₂₀N₆O₂ requires: C, 58.6; H, 6.3; N, 25.7%.

1-[2-Hydroxy-1-(aminomethyl)ethoxy]methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (34)

A mixture of 33 (0.4g, 1.2 mmol) and PdCl₂ (60 mg), MeOH (25 ml) was shaken at 45 lbs pressure under H₂ atmosphere. The catalyst was filtered, and the filtrate was neutralized with resin (IR-45, OH form) and the solvent removed. The crude product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃: MeOH (80:20, v/v) afforded 34 (0.2g, 58% yield); MS (m/z): 238 (M⁺+1); PMR (CDCl₃-DMSO-d₆): 8.4 (s, 1H, H-6), 8.1 (s, 1H, H-3), 5.9 (s, 2H, H-1'), 4.0-4.5 (m, 1H, H-4'), 3.6-3.8 (m, 4H, H-3', 5'), 5.0 (bs, 2H, N-H); Found: C, 45.4; H, 5.4; N, 35.3; C₉H₁₄N₆O₂ requires: C, 45.6; H, 5.8; N, 35.4%.
1-[Benzoyloxyethoxy)methyl-4-methy1thio-1H-pyrazolo[3,4-d]pyrimidine (36)

To a stirred mixture of 4-methy1thio-1H-pyrazolo[3,4-d]pyrimidine (19, 3.0g, 18 mmol), Et₃N (20 ml) and dry DMF (50 ml) was added dropwise a solution of benzoyloxyethoxymethylene chloride (6.0 g, 28 mmol) (prepared by passing dry HCl gas into paraformaldehyde and 1-benzoyloxy-2-hydroxyethane mixture in dry CH₂Cl₂ at 0° for 2 hr) and stirring continued for 15 hr. The solvent and excess of reagent were removed at reduced pressure. The residue was extracted with CHCl₃ washed with H₂O, dried (Na₂SO₄) and concentrated in vacuo. The product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃ gave 36 as an oil (2.2 g, 60% yield); MS (m/z): 344 (M⁺); PMR (CDCl₃): 8.65(s,1H,H-6), 7.7-8.1 (m,2H, Ph-H), 7.1-7.5 (m,3H,Ph-H), 5.85(s, 2H, H-1'), 4.1-4.4(m,2H, H-3'), 3.6-3.9(m,2H,H-4'), 2.6(s, 3H,SCH₃); Found: C, 55.8; H,4.7; N, 16.3; C₁₆H₁₆N₆O₃S requires: C,55.7; H, 4.7; N, 16.4%.

1-[Hydroxyethoxy)methyl-4-methylthio-1H-pyrazolo[3,4-d]pyrimidine (37)

A mixture of 36 (2.0g, 6 mmol) and methanolic ammonia (methanol saturated with ammonia at 0°) was kept
at ambient temperature for 24 hr. The solvent and excess of reagent from the resulting mixture was removed and the residue was chromatographed over SiO\textsubscript{2} column. Elution of the column with CHCl\textsubscript{3}:MeOH (96:4, v/v) afforded 37 (1.1g, 55% yield); m.p. : 94°C MS (m/z): 240 (M\textsuperscript{+}); IR(KBr): 3336 (OH); PMR (CDCl\textsubscript{3}-DMSO-d\textsubscript{6}): 8.8 (s,1H H-6), 8.5 (s,1H, H-3), 5.8(s, 2H,H-1'), 3.6 (m, 4H, H-3'), 2.6(s,3H,SCH\textsubscript{3}); Found: C, 45.0; H, 5.0; N, 23.3; C\textsubscript{9}H\textsubscript{12}N\textsubscript{4}O\textsubscript{2}S requires:C, 45.1; H,5.2; N, 23.3%.

1-[Hydroxyethoxy]methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine (38)

Method-1

Compound 37 (0.5g, 2 mmol) in dioxane (60 ml) was refluxed with aq KOH (20%, 15 ml) for 12 hr. The resulting mixture was cooled, neutralized with acetic acid and the solvent removed at reduced pressure. The residue was extracted with CHCl\textsubscript{3}, washed with H\textsubscript{2}O, dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The crude product thus obtained was chromatographed over SiO\textsubscript{2} column. Elution of the column with CHCl\textsubscript{3}:MeOH (90:10, v/v) gave 38 (0.2g, 30% yield); m.p. :138-40°C(EtOH); UV(MeOH): 250.6, 207.8 (pH-7); 221.2, 215.2 (pH-11); 250.8, 212.6 (pH-1); MS (m/z): 210 (M\textsuperscript{+}); PMR (CDCl\textsubscript{3}-DMSO-d\textsubscript{6}): 7.95 (s, 1H, H-6), 7.9 (s, 1H, H-3), 5.7 (s, 2H, H-1'),3.5 (m, 4H, H-
3',4'); Found: C, 45.7; H, 4.8; N, 26.6; C₈H₁₀N₄O₃ requires: C, 45.8; H, 4.9; N, 26.6%.

**Method - 2**

A mixture of 39 (0.8 g, 2.5 mmol) and methanolic ammonia (methanol saturated with ammonia at 0°C) was kept for 24 hr at ambient temperature. The solvent and excess NH₃ were removed at reduced pressure. The crude product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃ : MeOH (92:8, v/v) gave 38 (0.5 g, 60% yield); m.p.: 138-40°C (EtOH).

**1-[Benzoyloxyethoxy)methyl-4(5H)-oxo-1H-pyrazolo[3,4-d]pyrimidine** (39)

The compound 24 (3.0 g, 20 mmol) in benzene (40 ml) was refluxed with 1-benzoyloxy-2-chloromethoxyethane (35, 6.0 g, 28 mmol) for 16 hr. The resulting mixture was cooled, filtered and evaporated. The residue extracted with CHCl₃, washed with NaHCO₃, dried (Na₂SO₄) and concentrated. The product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃:MeOH (98:2, v/v) gave 38 (1.7 g, 58% yield); m.p.: 118-19°C (EtOH); MS (m/z): 314 (M⁺); IR (KBr): 1710 (C=O); PMR (CDCl₃): 8.25 and 8.1 (each s, 2H, H-6, H-3), 8.0-7.8 (m, 2H, Ph-H), 7.4-7.2 (m, 3H, Ph-H), 5.5 (s, 2H, 2,4,5-C₆H₆)
H-1'), 4.5-4.3 (m, 2H, H-3'), 4.1-3.8 (m, 2H, H-4'); Found: C, 57.3; H, 4.8; N, 17.9; C_{15}H_{14}N_{4}O_{4} requires: C, 57.4; H, 4.5; N, 17.7%.

1-[[Hydroxyethoxy]methyl]-4-amino-1H-pyrazolo[3,4-d]pyrimidine (40)

A mixture of 37 (0.5 g, 21 mmol) and methanolic ammonia (methanol saturated with ammonia at 0°C) was heated in a steel bomb at 120°C for 14 hr. The solvent and excess of ammonia from the resulting mixture was removed at reduced pressure. The product thus obtained was chromatographed over SiO₂ column. Elution of the column with CHCl₃: MeOH (90:10, v/v) gave 40 (0.3 g, 50% yield); m.p.: 153-54°C (EtOH); MS (m/z): 209 (M⁺); PMR (CDCl₃-DMSO-d₆): 8.2 (s, 1H, H-6), 8.1 (s, 1H, H-3), 5.7 (s, 2H, H-1'), 3.6 (s, 4H, H-3' and 4'); Found: C, 45.9; H, 5.3; N, 33.5; C_{15}H_{11}N_{5}O_{2} requires: C, 45.8; H, 5.3; N, 33.7%.
3.6 BIBLIOGRAPHY


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