Synthesis, Molecular Docking and Anti-cancer Activity of 5-oxopyrazolo[1,5-a]pyrimidine Derivatives.
3.01 Introduction:

Cancer is defined as malignant growth of cells. Most tumors arise from a combinational of genetic mutation in cell. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. Many things are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, obesity and environmental pollutants. Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging.

In the present work, we herein report the synthesis of 5-oxopyrazolo[1,5-a]pyrimidine. Further we have conducted Molecular Docking studied and Anti-cancer activity of 5-oxopyrazolo[1,5-a]pyrimidine using Hep-G2 Cell line.

Heterocyclic ring systems containing the pyrazole ring fused to pyrimidine or quinazoline rings are interesting classe of compounds both chemically and biologically. Reactions of aminopyrazoles with electrophilic reagents give rise to various fused annulated heterocyclic systems, including pyrazolo[1,5-a]pyrimidines. These are synthetic analogs of purines. Pyrazolo[1,5-a]pyrimidine ring system is an important structural template in anticancer research and in drug discovery as antibacterial and histamine releasing agents. Pyrazolo[1,5-a]pyrimidine are considerable chemical and pharmacological importance as purine analogs and many derivatives of pyrazolo[1,5-a]pyrimidines have been reported to exhibit cytotoxic activity.

Molecular docking of Pyrazolo[1,5-a]pyrimidine derivatives using Corticotrophin Releasing Factor (CRF-1) which is 41 amino acid peptide. CRF is the endogenous ligand for two receptors, CRF-1 and CRF-2, both of which belong to the class B subfamily of G-Protein Coupled Receptors (GPCRs). Several different classes
of small molecule CRF-1 antagonists have been reported in the literature. Representative examples are shown in **Fig. 3.01 & fig. 3.02**

CRF-1 antagonists which have been studied in the clinic including R121919 (1), Pexacerfont (2) and Emicerfont (3) attempts to identify CRF-1 antagonists suitable for clinical development. Pyrazolopyrimidine derivatives used as drugs like Zaleplon (4), Ocinaplon (5) & Indiplon(6) use as anxiolytic drugs and hypnotic.
3.02 Literature update for synthesis of Pyrazolopyrimidine Derivatives:

1. *Hala Bakr El-Nassan et al.*\(^{17}\) have reported synthesis of several pyrazolo[1,5-\-a]pyrimidine derivatives, bearing 2-methylsulphanyl group, 3-nitrile groups and 7-amine groups (1-5). (Scheme 1)

![Scheme 1](image)

**Reagents:** a) ArCH=CH(CN)\(_2\); b) CH\(_2\)(CN)\(_2\) triethylamine, ethanol; c) NCCH\(_2\)COOC\(_2\)H\(_5\), Fusion at 160 °C

(Scheme 1)

2. *Xin Jian Song et al.*\(^{18}\) were study pyrazolo[3,4-d]pyrimidine derivatives 8 containing 1,3,4-thiadiazole as potential antitumor agents. Synthesis of pyrazolo[3,4-d]pyrimidine derivatives were prepared from 5-aminopyrazole starting (Scheme 2)

![Scheme 2](image)

(Scheme 2)
3. Madhukar N. Jachak et al.\textsuperscript{19} have studied reaction of 5 amino-1 H-pyrazole-4-carbonitrile \textbf{9} with \(\alpha\)-acetyl-\(\gamma\)-butyrolactone \textbf{10} yielding furnished a mixture of pyrazolo[1,5-a]pyrimidine-3–carbonitrile \textbf{11} and pyrazolo[1,5-a]pyrimidine-3–carbonitrile \textbf{12}. (Scheme 3)

\begin{center}
\includegraphics[width=\textwidth]{scheme3.png}
\end{center}

(Scheme 3)

4. A number of pyrazolopyrimidines were synthesized and tested for their positive allosteric modulation of the HCA\textsubscript{2} receptor (GPR109A) by A. P. IJzerman et al.\textsuperscript{20} (Scheme 4)

\begin{center}
\includegraphics[width=\textwidth]{scheme4.png}
\end{center}

(Scheme 4)

\textbf{Reagents}: (a) EtOH, Reflux, 3h (b) POCl\textsubscript{3}, N,N-dimethylaniline, reflux, 3h (c) NaOAC, 5\% Pd/C, rt, 1h (d) NBS, DCM, 0\(^\circ\)C, 1.5 h, rt, 16h (e) LiOH,\(\text{H}_2\text{O}/\text{MeOH}/\text{THF}, \text{rt, 16h} \) (f)\(R^3\text{NH}_2, \text{EDC.HCl, DCM, rt, 4 h;} \) (g) \(R^1\text{B(OH)}_2\) Microwave, 150 \(^\circ\)C, 2 h.
5. Pyrazolo[1,5-a]pyrimidine Carboxylates 21 and Their Chloride Derivatives were synthesized from 3-carboxy-5-aminopyrazoles 19 by Alexandre V. Ivachtchenko et al.\textsuperscript{21} (Scheme 5)

\begin{center}
\begin{align*}
\text{H}_2\text{N} & \quad \text{R}_1\text{CO} \quad \text{C} \quad \text{O} \quad \text{H}_2\text{N} \\
\text{N} & \quad \text{N} \quad \text{COOH} \\
\end{align*}
\end{center}

(Scheme 5)

6. Pyrazolo[1,5-a]pyrimidine 24 can be formed by the condensation of 3-amin-4,5-diarylpyrazole 22 and a diketone 23 by John A. Katzenellenbogen et al.\textsuperscript{22} (Scheme 6)

\begin{center}
\begin{align*}
\text{Ar}^2 & \quad \text{NH}_2 \\
\text{N} & \quad \text{N} \quad \text{H} \\
\end{align*}
\end{center}

(Scheme 6)

7. Thomas R. Webb et al.\textsuperscript{23} pyrazolopyrimidines 28 from 2,4,6-trichloropyrimidin-5-carbaldehyde 25 (Scheme 7)

\begin{center}
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \quad \text{Cl} \\
\text{O} & \quad \text{O} \\
\text{H}_2\text{N} & \quad \text{R}_1 \quad \text{NH}_2 \\
\text{N} & \quad \text{N} \quad \text{H} \\
\text{Cl} & \quad \text{Cl} \\
\end{align*}
\end{center}

(Scheme 7)

**Reagent and Condition:**
- (a) R\textsubscript{1}NH\textsubscript{2}, KHCO\textsubscript{3}, TBAL, CH\textsubscript{2}Cl\textsubscript{2}
- (b) R\textsubscript{2}NH\textsubscript{2}, KHCO\textsubscript{3}, TBAL, CH\textsubscript{2}Cl\textsubscript{2}, rt
- (c) R\textsubscript{3}NHNH\textsubscript{2}, THF reflux
8. N-N Bond-Forming cyclization for the One-Pot Synthesis of N-Aryl[3,4]-pyrazolopyrimidines 31 were study by Keith Jones et al.\textsuperscript{24} (Scheme 8)

(Scheme 8)

3.03 Hypothesis:-

The search for new anticancer chemotherapeutic agents continues to be an area of research in many research institutes worldwide.\textsuperscript{25,26} Last decade pyrazolopyrimidine derivatives have received considerable attention due to their wide-range of applications such as anti-inflammatory, anti-tumor, antimycobacterial, antifungal and anti-viral activities.\textsuperscript{27,28} Pyrazolo[4,3- d]pyrimidine analogue and pyrazolo[1,5- a ] pyrimidine derivatives were reported as inhibitors of tyrosine kinase and cyclin dependent kinases (CDK) which involved in mediating the transmission of mitogenic signals and numerous other cellular events,\textsuperscript{29,30} including cell proliferation, migration, differentiation, metabolism and immune response. It was also found that many of these derivatives may block proliferation of various cancer cell lines.\textsuperscript{31}

Above pharmacological importance of Pyrazolopyrimidine promote us for synthesis and anticancer activity using Hep-G2 Cell line for new functionalised pyrazolopyrimidine derivatives.
3.04 Present Work:-

This chapter includes the introduction, Literature update for synthesis of 5-oxopyrazolo[1,5-a]pyrimidine derivatives, Hypothesis. The synthesis of 5-oxopyrazolo[1,5-a]pyrimidine derivatives have been attempted. The target molecules were prepared using 3-(4-Aryl)-1H-pyrazol-5-amine I as starting compound. Reaction of 3-(4-chlorophenyl)-1H-pyrazol-5-amine I with active methylene containing ester II afforded the N-(3-(4-Aryl)-1H-pyrazol-5-yl) substituted amide III. Finally target molecule 2-(4-Aryl)-6-substituted-7-substitutedpyrazolo[1,5-a]pyrimidin-5(4H)-one IV synthesized by reaction of N-(3-(4-Aryl)-1H-pyrazol-5-yl)substituted amide III with trimethyl substituted ester in acetic anhydrides solvent.

Reagents and Conditions: - (1) Ammonium Acetate/ 80°C,
(2) Trimethyl Orthosubstituted ester / acetic anhydride at 90°C

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Reagents and Conditions:  
(1) Ammonium Acetate/ 85°C  
(2) TriethylOrthoester/Ac2O 180°C

3.05 Experimental

3.05.1 General remarks:-

Melting points of all the synthesized compounds were determined by open capillary method and are uncorrected. The monitoring of reaction and checking of purity of the product were done using pre-coated silica gel plates and visualized using iodine chamber/UV lamp. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. ¹H-NMR spectra were scanned on a (Varian Mercury) on YH-300 MHz FT NMR in CDCl₃ and CDCl₃ using tetramethylsilane as an internal standard. Mass spectra were recorded from an HP 1100 LC/MSD mass spectral instrument (positive and negative APCI ion source, 50-200 V, nitrogen). All the chemicals and solvents used were of synthetic grade (Sd. Fine, chemicals, Mumbai, India).
3.05.2 Experiment No. 1

_Synthesis of N-(3-(4-Aryl)-1H-pyrazol-5-yl)substituted amide (IIIa-f)_

$$
\begin{align*}
\text{Ia-c} & \quad + \quad \text{IIa-b} \\
& \quad \xrightarrow{\text{Ammonium Acetate, 80°C}} \quad \text{IIIa-f}
\end{align*}
$$

Mixture of 3-(4-Aryl)-1H-pyrazol-5-amine _Ia-c_ (0.01 mol), active methylene containing ester _II_ (0.01 mol) in ammonium acetate (20 gm) was heated to 80-90°C for 2 h. (TLC monitor). After completion of reaction the solution was poured in to cold water (50 mL) and stirred. The separated solid was filtered, washed with cold water (100 mL) and dried, afforded N-(3-(4-Aryl)-1H-pyrazol-5-yl)substituted amide _IIIa-f_ in very good yield. The crude compounds which were crystalised using appropriate solvent.

_N-(3-(4-chlorophenyl)-1H-pyrazol-5-yl)-3-oxobutanamide (III-a)_

_Yield (%): 71 \quad M. P. (°C) : 149 \quad Molecular Formula: C_{13}H_{12}ClN_{3}O_{2}\_

_IR (cm\(^{-1}\)) (KBr) : 1620(C=C), 1664(C=O Amide), 1726(C=O), 3255(N-H).\_

_1H-NMR (δ, ppm) : 2.18(3H, s, CH\(_3\)), 3.15(2H, s, CH\(_2\)), 6.93(1H, s, Ar-H), 7.64(2H, d, Ar-H), 7.82(2H, d, Ar-H), 10.52(1H, s, N-H), 12.81(1H, bs, N-H).\_

_N-(3-(4-chlorophenyl)-1H-pyrazol-5-yl)-2-cyanoacetamide (III-b)_

_Yield (%): 81 \quad M. P. (°C) : 187 \quad Molecular Formula: C_{12}H_{9}ClN_{4}O\_

_IR (cm\(^{-1}\)) (KBr) : 1605(C=C), 1676(C=O Amide), 2245(CN), 3302(N-H).\_

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$^1$H-NMR (δ, ppm): 3.25(2H, s, CH$_2$), 6.88(1H, s, Ar-H), 7.56(2H, d, Ar-H), 7.80(2H, d, Ar-H), 10.47(1H, s, N-H), 11.87(1H, s, N-H).

(CDCl$_3$)

**N-(3-(4-bromophenyl)-1H-pyrazol-5-yl)-3-oxobutanamide (III-c)**

Yield (%): 86

M. P. (°C): 170

Molecular Formula: C$_{13}$H$_{12}$BrN$_3$O$_2$

IR (cm$^{-1}$) (KBr): 1610(C=C), 1679(C=O Amide), 1711(C=O), 3360(N-H).

$^1$H-NMR (δ, ppm): 2.19(3H, s, CH$_3$), 3.22(2H, s, CH$_2$), 6.87(1H, s, Ar-H), 7.61(2H, d, Ar-H), 7.80(2H, d, Ar-H), 10.42(1H, s, N-H), 11.86(1H, bs, N-H).

(CDCl$_3$)

**N-(3-(4-bromophenyl)-1H-pyrazol-5-yl)-2-cyanoacetamide (III-d)**

Yield (%): 90

M. P. (°C): 196

Molecular Formula: C$_{12}$H$_9$BrN$_4$O

IR (cm$^{-1}$) (KBr): 1616(C=C), 1670(C=O Amide), 2256(CN), 3345(N-H).

$^1$H-NMR (δ, ppm): 3.21(2H, s, CH$_2$), 6.81(1H, s, Ar-H), 7.51(2H, d, Ar-H), 7.87(2H, d, Ar-H), 10.67(1H, s, N-H), 12.91(1H, s, N-H).

(CDCl$_3$) Fig. 3.06

**3-oxo-N-(3-p-tolyl-1H-pyrazol-5-yl)butanamide (III-e)**

Yield (%): 62

M. P. (°C): 187

Molecular Formula: C$_{14}$H$_{15}$N$_3$O$_2$

IR (cm$^{-1}$) nujol: 1606(C=C), 1677(C=O Amide), 1723(C=O), 3247(N-H).

$^1$H-NMR (δ, ppm): 2.05(3H, s, CH$_3$), 2.35 (3H, s, CH$_3$), 3.21(2H, s, CH$_2$), 6.94(1H, s, Ar-H), 7.60(2H, d, Ar-H), 7.64(2H, d, Ar-H), 10.11(1H, s, N-H), 11.80(1H, bs, N-H).

(CDCl$_3$)

**2-cyano-N-(3-p-tolyl-1H-pyrazol-5-yl)acetamide (III-f)**

Yield (%): 86

M. P. (°C): 209

Molecular Formula: C$_{13}$H$_{12}$N$_4$O

IR (cm$^{-1}$) (KBr): 1611(C=C), 1681(C=O Amide), 2240(CN), 3294(N-H).

$^1$H-NMR (δ, ppm): 2.11(3H, s, CH$_3$), 3.21(2H, s, CH$_2$), 6.71(1H, s, Ar-H), 7.53(2H, d, Ar-H), 7.87(2H, d, Ar-H), 10.40(1H, s, N-H), 11.64(1H, s, N-H).

(CDCl$_3$)

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3.05.3 Experiment No. 2

Synthesis of 2-(4-Aryl)-6-substituted-7-substitutedpyrazolo[1,5-a]pyrimidin-5(4H)-one (IV a-r)

A solution of N-(3-(4-Aryl)-1H-pyrazol-5-yl) substituted amide III (0.01 mol), Trimethyl Orthosubstituted ester (0.01 mol) in acetic anhydride (20 gm) was refluxed at 90°C for 5 h. monitored by TLC. Then the reaction mixture was poured in to cold water (50 mL) and stirred. The obtained solid was filtered, washed with cold water (100 mL) and dried. 2-(4-Aryl)-6-substituted-7-substitutedpyrazolo[1,5-a]pyrimidin-5(4H)-one IV was obtained in very good yield. The crude compound was recrystallized from appropriate solvent.

6-acetyl-2-(4-chlorophenyl)pyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-a)

Yield (%): 60  M. P. (°C): 169  Molecular Formula: C_{14}H_{10}ClN_{3}O_{2}

IR (cm⁻¹) (KBr) : 1600 (C=C), 1660(C=O Amide), 1665( C=O), 3350(N-H).

¹H-NMR (δ, ppm) : 2.21(3H, s, CH₃), 6.94(1H, s, Ar-H), 7.25(1H, s, Ar-H), 7.50(2H, d, Ar-H), 7.97(2H, d, Ar-H), 10.64(1H, s, N-H).

Mass (M/Z) : 287, 193
6-acetyl-2-(4-chlorophenyl)-7-methylpyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-b)

Yield (%): 74  M. P. (°C): 184  Molecular Formula: C₁₅H₁₂ClN₃O₂

IR (cm⁻¹) (KBr): 1610 (C=C), 1666 (C=O Amide), 1675 (C=O), 3330 (N-H).

¹H-NMR (δ, ppm) (CDCl₃): 1.87 (3H, s, CH₃), 2.21 (3H, s, CH₃), 7.25 (1H, s, Ar-H), 7.51 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 10.21 (1H, s, N-H).

Mass (M/Z): 193, 301.

6-acetyl-2-(4-chlorophenyl)-7-ethylpyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-c)

Yield (%): 86  M. P. (°C): 198  Molecular Formula: C₁₆H₁₄ClN₃O₂

IR (cm⁻¹) (KBr): 1595 (C=C), 1665 (C=O Amide), 1672 (C=O), 3315 (N-H).

¹H-NMR (δ, ppm) (CDCl₃): 1.23 (3H, t, CH₃), 2.14 (2H, m, CH₂), 10.55 (1H, s, N-H), 7.85 (2H, d, Ar-H), 7.53 (2H, d, Ar-H), 7.33 (1H, s, Ar-H), 2.45 (3H, s, CH₃).


2-(4-chlorophenyl)-4,5-dihydro-7-methyl-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-d)

Yield (%): 91  M. P. (°C): 201  Molecular Formula: C₁₃H₇ClN₄O

IR (cm⁻¹) (KBr): 1620 (C=C), 1676 (C=O Amide), 2235 (CN), 3210 (N-H).

¹H-NMR (δ, ppm) (CDCl₃): 7.15 (1H, s, Ar-H), 7.20 (1H, s, Ar-H), 7.73 (2H, d, Ar-H), 7.33 (1H, s, Ar-H), 2.84 (2H, d, Ar-H), 9.84 (1H, s, N-H).

Mass (M/Z): 193, 270.

2-(4-chlorophenyl)-4,5-dihydro-7-methyl-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-e)

Yield (%): 83  M. P. (°C): 188  Molecular Formula: C₁₄H₉ClN₄O

IR (cm⁻¹) (KBr): 1605 (C=C), 1672 (C=O Amide), 2255 (CN), 3342 (N-H).

(Fig. 3.07) ¹H-NMR (δ, ppm) (CDCl₃) (Fig. 3.08): 2.20 (3H, s, CH₃), 7.21 (1H, s, Ar-H), 7.45 (2H, d, Ar-H), 7.92 (2H, d, Ar-H), 10.49 (1H, bs, N-H).
2-(4-chlorophenyl)-7-ethyl-4,5-dihydro-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-f)

Yield (%): 78  
M. P. (°C): 149  
Molecular Formula: C_{15}H_{11}ClN_{4}O

IR (cm\(^{-1}\)) (KBr): 1600(C=C), 1678 (C=O Amide), 2244(CN), 3260(N-H).

\(^1\)H-NMR (δ, ppm): 1.13(3H, t, CH\(_3\)), 2.10(2H, m, CH\(_2\)), 7.35(1H, s, Ar-H), (CDCl\(_3\)) 7.51(2H, d, Ar-H), 7.84(2H, d, Ar-H), 11.07(1H, s, N-H).

6-acetyl-2-(4-bromophenyl)pyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-g)

Yield (%): 74  
M. P. (°C): 159  
Molecular Formula: C_{13}H_{10}BrN_{3}O_{2}

IR (cm\(^{-1}\)) (KBr): 1615 (C=C), 1660(C=O Amide), 1681( C=O), 3380(N-H).

\(^1\)H-NMR (δ, ppm): 2.02(3H, s, CH\(_3\)), 7.14(1H, s, Ar-H), 7.35(1H, s, Ar-H), (CDCl\(_3\)) 7.51(2H, d, Ar-H), 7.88(2H, d, Ar-H), 11.23(1H, s, N-H).

6-acetyl-2-(4-bromophenyl)-7-methylpyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-h)

Yield (%): 65  
M. P. (°C): 176  
Molecular Formula: C_{13}H_{12}BrN_{3}O_{2}

IR (cm\(^{-1}\)) (KBr): 1620 (C=C), 1665(C=O Amide), 1670( C=O), 3400(N-H).

\(^1\)H-NMR (δ, ppm): 1.89(3H, s, CH\(_3\)), 2.20(3H, s, CH\(_3\)), 7.21(1H, s, Ar-H), (CDCl\(_3\)) 7.47(2H, d, Ar-H), 7.91(2H, d, Ar-H), 11.02(1H, s, N-H).

(Fig. 3.09)

6-acetyl-2-(4-bromophenyl)-7-ethylpyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-i)

Yield (%): 76  
M. P. (°C): 163  
Molecular Formula: C_{16}H_{14}CBrN_{3}O_{2}

IR (cm\(^{-1}\)) (KBr): 1630 (C=C), 1655(C=O Amide), 1685( C=O), 3378(N-H).

\(^1\)H-NMR (δ, ppm): 1.20(3H, t, CH\(_3\)), 2.16(2H, m, CH\(_2\)), 2.36(3H, s, CH\(_3\)), (CDCl\(_3\)) 7.30(1H, s, Ar-H), 7.73(2H, d, Ar-H), 7.87(2H, d, Ar-H), 11.01(1H, s, N-H).

Mass (M/Z): 361, 237

2-(4-bromophenyl)-4,5-dihydro-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile(IV-j)

Yield (%): 90  
M. P. (°C): 162  
Molecular Formula: C_{13}H_{2}BrN_{4}O

IR (cm\(^{-1}\)) (KBr): 1610(C=C), 1668 (C=O Amide), 2240(CN), 3340(N-H).

\(^1\)H-NMR (δ, ppm): 7.19(1H, s, Ar-H), 7.26(1H, s, Ar-H), 7.70(2H, d, Ar-H), (CDCl\(_3\)) 7.79(2H, d, Ar-H), 10.84(1H, s, N-H).

Mass (M/Z): 237, 315

(Fig. 3.10)
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2-(4-bromophenyl)-4,5-dihydro-7-methyl-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-k)

Yield (%): 82  M. P. (°C) : 188  Molecular Formula: C_{14}H_{9}BrN_{4}O
IR (cm\(^{-1}\)) (KBr) : 1618(C=C), 1660 (C=O Amide), 2255(CN), 3280(N-H).

\(^1\)H-NMR (δ, ppm): 2.34(3H, s, CH\(_3\)), 7.11(1H, s, Ar-H), 7.41(2H, d, Ar-H),
(CDCl\(_3\)) 7.72(2H, d, Ar-H), 10.35(1H, bs, N-H).

2-(4-bromophenyl)-7-ethyl-4,5-dihydro-5-oxopyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-l)

Yield (%): 77  M. P. (°C) : 180  Molecular Formula: C_{13}H_{11}BrN_{4}O
IR (cm\(^{-1}\)) (KBr) : 1602(C=C), 1680 (C=O Amide), 2250(CN), 3276(N-H).

\(^1\)H-NMR (δ, ppm): 1.17(3H, t, CH\(_3\)), 2.16(3H, m, CH\(_2\)), 7.31(1H, s, Ar-H),
(CDCl\(_3\)) 7.47(2H, d, Ar-H), 7.78(2H, d, Ar-H), 11.17(1H, s, N-H).

6-acetyl-2-p-toly pyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-m)

Yield (%): 76  M. P. (°C) : 200  Molecular Formula: C_{15}H_{13}N_{3}O_{2}
IR (cm\(^{-1}\)) (KBr) : 1621(C=C), 1662(C=O Amide), 1678( C=O), 3355(N-H),
\(^1\)H-NMR (δ, ppm): 1.94(3H, s, CH\(_3\)), 2.35(3H, s, CH\(_3\)), 7.01(1H, s, Ar-H),
(CDCl\(_3\)) 7.25(1H, s, Ar-H), 7.58(2H, d, Ar-H), 7.92(2H, d, Ar-H),
10.51(1H, s, N-H).

6-acetyl-7-methyl-2-p-toly pyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-n)

Yield (%): 71  M. P. (°C) : 201  Molecular Formula: C_{16}H_{15}N_{3}O_{2}
IR (cm\(^{-1}\)) (KBr) : 1616 (C=C), 1671(C=O Amide), 1685( C=O), 3340(N-H),
\(^1\)H-NMR (δ, ppm): 1.81(3H, s, CH\(_3\)), 2.26(3H, s, CH\(_3\)), 2.36(3H, s, CH\(_3\)),
(CDCl\(_3\)) 7.20(1H, s, Ar-H), 7.61(2H, d, Ar-H), 7.84(2H, d, Ar-H),
11.21(1H, s, N-H).

6-acetyl-7-ethyl-2-p-toly pyrazolo[1,5-a]pyrimidin-5(4H)-one (IV-o)

Yield (%): 70  M. P. (°C) : 179  Molecular Formula: C_{17}H_{17}N_{3}O_{2}
IR (cm\(^{-1}\)) (KBr) : 1616 (C=C), 1669(C=O Amide), 1685( C=O), 3322(N-H).
H-NMR (δ, ppm) : 1.21(3H, t, CH₃), 2.10(2H, m, CH₂), 2.36(3H, s, CH₃),
(CDCl₃) 2.45(3H, s, CH₃), 7.31(1H, s, Ar-H), 7.73 (2H, d, Ar-H),
7.89(2H, d, Ar-H), 10.57(1H, s, N-H).

4,5-dihydro-5-oxo-2-p-tolylpyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-p)
Yield (%): 88  M. P. (°C) : 167  Molecular Formula: C₁₄H₁₀N₄O
IR (cm⁻¹) (KBr) : 1590(C=C), 1669 (C=O Amide), 2240(CN), 3332(N-H).
H-NMR (δ, ppm) : 2.26(3H, s, CH₃), 7.16(1H, s, Ar-H), 7.28(1H, s, Ar-H),
(CDCl₃) 7.40(2H, d, Ar-H), 7.82(2H, d, Ar-H), 10.44(1H, bs, N-H).

4,5-dihydro-7-methyl-5-oxo-2-p-tolylpyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-q)
Yield (%): 68  M. P. (°C) : 155  Molecular Formula: C₁₅H₁₂N₄O
IR (cm⁻¹) (KBr) : 1605(C=C), 1679 (C=O Amide), 2245(CN), 3287(N-H).
H-NMR (δ, ppm) : 2.23(3H, s, CH₃), 2.39(3H, s, CH₃), 7.26(1H, s, Ar-H),
(CDCl₃) 7.66(2H, d, Ar-H), 7.97(2H, d, Ar-H), 9.87(1H, bs, N-H).

7-ethyl-4,5-dihydro-5-oxo-2-p-tolylpyrazolo[1,5-a]pyrimidine-6-carbonitrile (IV-r)
Yield (%): 66  M. P. (°C) : 176  Molecular Formula: C₁₆H₁₄N₄O
IR (cm⁻¹) (KBr) : 1615(C=C), 1675 (C=O Amide), , 2250(CN), 3300(N-H).
H-NMR (δ, ppm) : 1.16(3H, t, CH₃), 2.24(2H, m, CH₂), 2.36(3H, s, CH₃),
(CDCl₃) 7.35(1H, s, Ar-H), 7.51(2H, d, Ar-H), 7.81(2H, d, Ar-H),
11.50(1H, s, N-H).

3.06 Molecular Docking:-
Molecular docking work was performed with the Hex molecular modeling
package version 6.3. The three dimensional crystal structure of Corticotrophin
Releasing Factor (CRF-1) (PDB code: 2NPU) was used as the receptor throughout the
work. The ligands were converted to 2D and 3D energy-minimized conformations
using Hex 3D Ultra 6.0. respectively and visualize the conformation by using Accelrys
Discovery Studio 3.1 Client.

Molecular docking study the binding models of the enzyme active site of
Corticotrophin Releasing Factor (CRF-1) were depicted in **Fig 3.03** respectively. All
the ligands are cased between different amino acids. Among IVa-r synthesized
pyrazolopyrimidine derivatives IVc this derivative show more binding site with
amino acid i.e. THR 180, ASN 178, ARG 166 and TYR 184.
Fig. 3.03 Interaction of IVc with active site of CRF-1 protein

3.07 Anti-tumor cytotoxicity bioassay in vitro:

HepG2 (hepatocellular carcinoma cell line) were obtained from National Center for Cell Science, Pune. Cells were maintained in DMEM medium with 10% foetal calf serum, sodium pyruvate, 100 U/ml penicillin and 10 mg/ml streptomycin at 37°C and 5% CO₂. Potential cytotoxicity of IVa, IVb, IVc, IVg, IVh, IVi, IVm, IVp and IVr were tested using the method of Skeha et al. Briefly, 10⁴ cells/well were plated onto 96-well dishes overnight before the treatment with the tested compounds to allow the attachment of cells to the wall of the plate. Different concentrations of each tested compound (10, 20, 40 and 8010µg/ml) were added to the cell monolayer; triplicate wells were used for each individual dose. Monolayer cells were incubated with the tested agent(s) for 48 h at 37°C and 5% CO₂. At the end of the incubation period, the cells were fixed and stained with sulforhodamine B dissolved in acetic acid. Unbound stain was removed by washing four times with 1% acetic acid and the protein bound dye was extracted with tris EDTA buffer. Absorbance was measured in Spectrophotometrically. The inhibition rate of cell growth was calculated by the following formula:

Mean value of {((control group - treated group)/control group) × 100%.

Triplicate wells were analyzed per each concentration.
Three experiments were carried out for IVa, IVb, IVe, IVg, IVh, IVi, IVm, IVp and IVr and Adriamycin (ADR) as positive control drug in different concentration summarized in Table No. 1.

### Table No. 1

<table>
<thead>
<tr>
<th>Drug Concentration (µg/ml)</th>
<th>% Control Growth</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>IVa 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>IVb 96.2</td>
<td>95.9</td>
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<tr>
<td>IVe 96.1</td>
<td>94.3</td>
</tr>
<tr>
<td>IVg 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>IVh 94.3</td>
<td>92.5</td>
</tr>
<tr>
<td>IVi 98.7</td>
<td>97.1</td>
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<tr>
<td>IVm 85.1</td>
<td>84.9</td>
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<tr>
<td>IVp 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>IVr 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>ADR 19.0</td>
<td>8.4</td>
</tr>
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</table>

### 3.08 Result and Discussion:

For evaluation of anti-tumor cytotoxicity of the synthesized compounds (IVa, IVb, IVe, IVg, IVh, IVi, IVm, IVp, IVr and ADR), human cancer cell lines were used: HepG2 (liver carcinoma cell line). Cytotoxicity of 5-oxopyrazolo[1,5-a]pyrimidine derivatives against above human cancer cell lines is shown in fig.3.04. The graph % control growth Vs concentration of different synthesized compounds.

All tested compounds observed above 80 % control Growth, if this value is observed below 80 % then such compound may be active toward Human Hepatoma cell line HEPG2. All the tested compounds were inactive on Human Hepatoma cell line HEPG2 in assay system used compared with Adriamycin (ADR) as positive control drug.
Three parameters were calculated from graph i.e. LC50, TGI and GI50 these values are represented in Table 2.

<table>
<thead>
<tr>
<th>HEPG2</th>
<th>LC50</th>
<th>TGI</th>
<th>GI50</th>
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<tbody>
<tr>
<td>IVa</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td>IVb</td>
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<td>IVe</td>
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<tr>
<td>ADR</td>
<td>64.1</td>
<td>32.2</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Table 2
3.09 Conclusion:-

We have synthesized and characterized series of various Pyrazolo[1,5-a]pyrimidine derivatives. All synthesized Pyrazolo[1,5-a]pyrimidine derivetives were poor anti Cancer activity using human cancer cell lines HepG2 (liver carcinoma cell line). But when Molecular docking of Pyrazolo[1,5-a]pyrimidine derivatives were carried out using Corticotrophin Releasing Factor (CRF-1), it were observed that all ligands bind with maximum amino acid of Proteins.
3.10 Spectra:

![Image of 1H-NMR Spectrum of compound (II-a)](image-url)
Fig. 3.06: $^1$H-NMR Spectrum of compound (III-d)
Fig. 3.07: IR Spectrum of compound (IV-e)
Fig. 3.08: $^1$H-NMR Spectrum of compound (IV-e)
Fig. 3.09: $^1$H-NMR Spectrum of compound (IV-h)
Fig. 3.10: Mass Spectrum of compound (IV-\(j\))
3.11 References:


18 Xin Jian Song, Yu Shao, Xing Gao Dong, *Chinese Chemical Letters*, 22, 1036, **2011**.


