# Antioxidant and cytotoxic effects of novel pyrazolopyrimidines on MCF-7 and HepG-2 Cell lines: ADMET, PASS Prediction and Molecular Docking Studies

**Sathish Kumar Mittapalli[1]*, Ch. Hari Prasad Murthy[2], Jay Prakash Soni[1], Parameshwar Ravula[1], Nimisha Jain[1], Sager Pamu[1]**

[1]Department of Pharmaceutical Chemistry, Amity Institute of Pharmacy, Amity University Madhya Pradesh, Gwalior, Madhya Pradesh-474020, INDIA
[2]Department of Pharmacology, Sri Venkateshwera College of Pharmacy, Hyderabad, Telangana-500081, INDIA
*Corresponding Author E-mail: **sattisuma@gmail.com**

***ABSTRACT:***

*Pyrazolopyrimidines were well established for anticancer activity against many biochemical targets. Cyclin dependent kinase, PIM kinase and Tyrosine kinases are promising targets in the treatment of cancer for its crucial role in regulation of cell cycle. In this research novel pyrazolopyrimidines (7a-7j) were designed and synthesised as mentioned target inhibitors. All the compounds were screened for drug likeness properties and bioactivity score via in-silico ADMET studies also studied molecular docking achieved on designed compounds and confirmed two hydrogen bonds essential with ASP348 and GLU338 in active site and all binding interactions with target proteins resulting glide e-model and docking scores through Schrodinger suite with reference to standards as well as co-crystals. All the compounds evaluated for antioxidant activity and cytotoxic activity by MCF-7 and HepG-2 cell lines. The compound 7b found as good free radical scavenging ability and 7j reported 16.36 where standard 5-FU found as 14.31 µg/ml against MCF-7. In coordination of all experimental and computational studies helped in the structure requirements prediction for the observed antitumor activity.*

***Key words**: Pyrazolopyrimidine, Tyrosine kinase, Scaffold modelling, Antioxidant Activity and Anticancer Activity.*

***Corresponding author:**
**Department of Pharmaceutical Chemistry,**
**Amity Institute of Pharmacy,**
**Amity University, Gwalior,**
**Madhya Pradesh, India. 474020**
**E-mail address:** sattisuma@gmail.com
**Tel: + 919959093754**

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## INTRODUCTION:

Cancer, which is one of the diseases that still people fear the most in the modern world, caused by gene mutations that regulate cell activity in response to certain environmental factors and difficult to cure since it is able to spread distinct organs, can penetrate and circulate in the lymphatic system and then proliferate other sites called metastasis[1]. Pyrazolopyrimidines has diverse pharmacological actions[2–9], especially antioxidant[10–12] and anticancer properties[13–16].

The protein kinases are bio macromolecular structures that are crucial for cell division and control cell cycle progression. The protein kinase family are a sizable collection of structurally related enzymes that are crucial for cell division and control cell cycle progression and are responsible for transferring of phosphate from ATP to substrate and 518 distinct protein kinases known till date, out of which 71 not been reported previously. In several cellular processes these kinases play critical role in the development of diseases like fibrosis, inflammation, neurological diseases and cancer[17]. Kinase mutations can cause oncogenesis and are a major factor in the development of cancer. As a result, during past two decades kinase inhibitors have emerged as a crucial class of oncodrugs[18]. Over 50 kinase inhibitors have been approved for various indications since the first one, imatinib (Gleevec), a BCR-ABL inhibitor used to treat chronic myeloid leukaemia and acute lymphocytic leukaemia, was approved in 2001 and more than 45 inhibitors have been approved for cancer since two decades and nearly 200 molecules under progress for clinical trials for many indications[19]. Reactive oxygen species (ROS), maybe generated in the body during metabolism can cause damage DNA and proteins. Cancer, atherosclerosis, cardiovascular disorders and inflammatory illness are all brought on by these alterations[20].

Pyrazolopyrimidines are one of the most versatile preferred heterocycles studied in the development of small molecules[21]. This scaffold is a bioisostere of adenine and consist of fused pyrazole ring and pyrimidine ring, can simulate the key interactions of ATP with the hinge region of kinase domain of cyclin-dependent kinases (CDK)[22]. To the best of our

knowledge, PP1 and PP2 which are initially shown to operate as kinase inhibitors of the SRC family of non receptor tyrosine kinases in 1996, were the first pyrazolo(3,4-d)pyrimidine kinase inhibitors to be identified.

Years later, in 2013, the FDA approved ibrutinib for treatment of B cell cancers. B cells are key role in immune system and producing antibodies and the drug influenced in signalling pathways[23]. While only three has so far approved as a pyrazolo(3,4-d)pyrimidine conataining kinase inhibitor, at present, a further 3 molecules are in clinical trials; parsaclisib, sapanisertib and umbralisib[24].

Serine-threonine kinase called Cyclin dependent kinases (CDKs) control the cell cycle and regulate cell differentiation and its over expression led to cancer. Due to the excessive expression of cyclin A and E is linked to unchecked CDK2 activation in several cancers. Consequently, CDKs are viewed as crucial targets for the development of novel anticancer molecules[25]. The pyrazolo(3,4-d)pyrimidine has great potential for development of cytotoxic agents and the nucleus as early said purine analog with strong growth inhibitory properties, does via many CDKs such as CDK-1[26], CDK-2[27] and lipoxygenase[28] and additionally focused and strong CDK2/Cyclin A inhibitory properties[25]. Among all known drugs of CDK2 inhibitors are Dinaciclib, ibrutinib and Roscovitine[29]. Pyrazolopyrimidine is a bioisostere of purine received great attention in the designing scaffolds as anticancer agents, evidenced by PKI-166[30], erlotinib[31], Imatinib[32], BIRB796[33] and BAY43-9006[34], tyrosine kinase inhibitors revealed novel binding mechanisms which use extra binding site adjacent to ATP[35].

The aim of the article was to develop and synthesize novel pyrazolo(3,4-d)pyrimidines based on rational drug design and novel derivatives were evaluated for antioxidant activity and *in vitro* anticancer property against breast cell carcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) cell lines and *in silico* evaluation of designed compounds against CDK-2 protein in comparison with many standards.

**Rational design**

The new pyrazolo(3,4-d)pyrimidines were designed based on the lead compounds, Methotrexate, competitive inhibitor of DHFR, which converts DHF to THF[36]. Pemetrexed(PMX) is a dihydropyrrolo[2,3-d]pyrimidin nucleus with ethylene spacer linkage to PABA moiety act by folate anatagonist, and recently reported as cytotoxicity of on BEAS-2B(lung carcinoma) cells and studies revealed that the greater affinity complexation of WP6A and ATP $(5.67 \pm 0.31) \times 10^5$ l/mol, favouring competitive replacement of PMX, was confirmed by NMR and florescence titration[37] and pralatrexate contain diaminopteridin nucleus with methylene spacer linkage to PABA moiety also folate antagonist and was initially found to be an oseltamivir-comparable candidate drug effective against seasonal influenza viruses, exhibited inhibitory effects on the replication of SARS-CoV-2 in Calu-3 cells[38].

Purine scaffold was bioisosterically replaced by the ring system, pyrazolo(2,3-d)pyrimidine in compounds 7a-7j compared the interaction sites at CDKs target site through hydrophobic bonding with GLU81, PHE82, ALA144, LEU134, ASN132, GLN131, ILE10 and GLY11. This is required to place the ligand in the ATP adenine region.

The essential hydrogen bonds in the phosphate binding region was observed with LYS88, LYS89, GLU12, GLU81 and LEU83 in pemetrexed and LYS88, LYS89, ASP145, ILE10 in

Methotrexate was observed and my analogues were designed such a way that pemetrexed has at position 5 on pyrrole ring linked with ethyl spacer substituted with benzamide which nitrogen linked with pentane dioic acid which over all modified with 4,6-dihydro-1H-pyrazol with methylidene amino-phenyl spacer which substituted with various aromatic polar groups. In another target tyrosine kinase of SRC family showed HBD interacting with THR338, MET341, ARG388 while designed analogues ASP348, GLU339, ASN391 and LYS295.

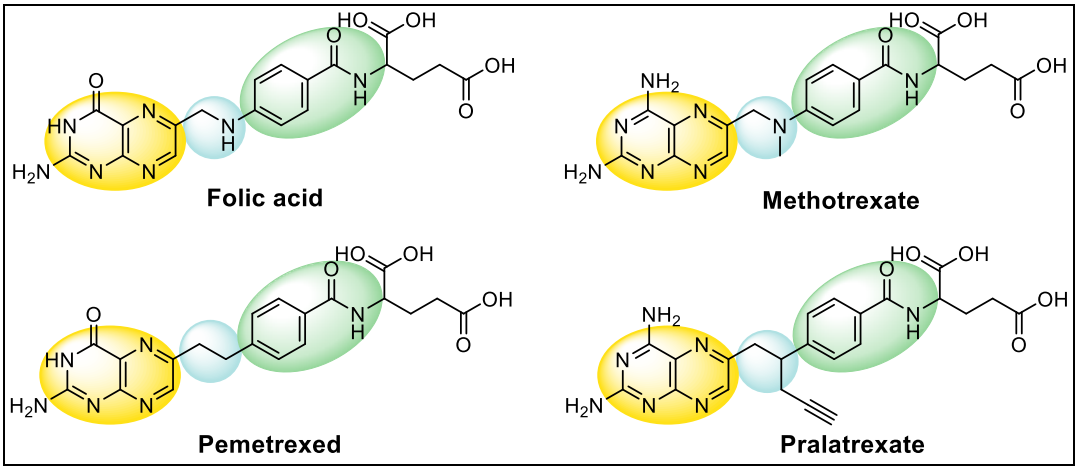



**Figure 1: Structural features of antimetabolites**

## MATERIALS AND METHODS:

All the chemicals were obtained from S.D fine Chemicals and were used without refinement and also some of the chemicals from Aldrich Chemical Company in the United States provided the additional high purity grade chemicals that were utilised. The melting points were identified using an open capillary tube and a melting point apparatus (Stuart Scientific SMP1). Thin Layer Chromatography was performed by ethyl acetate and n-butanol (20:80) using precoated silica plates (silica gel 0.25 mm, Merck, Germany) and results observed through UV lamp to check the compounds purity. Shimadzu-FTIR Infrared spectrometer was used to record the IR spectra in KBr (vmax in $cm^{-1}$). Bruker DPX 300 spectrometers were used to record $^{1}H$ (400 MHz) NMR spectra together with 5 mm PABBO BB-$^{1}H$ tubes and $^{13}C$ (100 MHz). For approximately 0.03 M solutions in DMSO-d6 at 75 MHz or 100 MHz with TMS as an internal standard, NMR spectra were captured. Agilent 1200 series LC and and the mass spectrum was recorded with high-resolution mass spectra on Thermo Finnigan LCQ Ion Trap instrument and were reported in m/z as molecular ion peak. VARIO EL-III (Elementar Analysensysteme GmBH) was used to conduct the elemental analysis.

The human cell lines, MCF-7 (Breast Cancer Cell Lines) and Hep G2 (Hepato Carcinoma Cell Lines) from NCCS (The National Centre for Cell Science), Pune, India. 25 $cm^2$ and 75 $cm^2$ flasks, as well as 96 well plates Purchased from Eppendorf India. All the commercially available solvents and reagents were used without further purification except as indicated.

**Evaluation of the molecular descriptive characteristics**

SwissADME online tool was used to predict the drug-likeness properties, which is the initial stage of drug development process[39]. The primary properties for drug candidate is weight of molecule, absorption, minimal molecular flexibility which measured in terms of number of rotatable bonds and minimal total polar surface area measured in terms of TPSA[40]. Simple molecular data, such as "molecular weight, number of hydrogen bond acceptors and donors, and partition coefficient in a molecule" can be used to determine a compound's permeability and bioavailability[41].

Christopher A. (1997) provided a key guideline for evaluating medication similarity. The Lipinski's Rule of Five, criteria is used to determine if a chemical might be an orally active medicine in the human body based on its biological and pharmacological characteristics[42]. According to this criterion, a molecule qualifies as a drug moiety if it meets the criteria listed below: Five hydrogen bond donors, ten hydrogen bond acceptors, five partition coefficients logP, and 500 Daltons for the molecular weight.

**Calculation Bioactivity Score Using the Molinespiration Toolkit**

Score the proposed compounds for bioactivity using the Molinspiration online toolbox, inhibitory activity against different receptor ligands, inhibitors, and enzymes were calculated[43], and the results are shown in Table 4. A chemical may have significant biological activity if its bioactivity score is greater than 0, moderate biological activity is predicted for scores between -0.50 and 0.00, and inactivity is predicted for scores below -0.50.

**OSIRIS Property Explorer's medication score and toxicity predictions**

Table 5 provides a summary of the data gathered. A compound's drug likeness should be zero or positive. The drug score, which determines whether a chemical qualifies as a drug or not, is a mix of factors including drug resemblance, lipophilicity, solubility, molecular weight, and toxicity concerns[44].

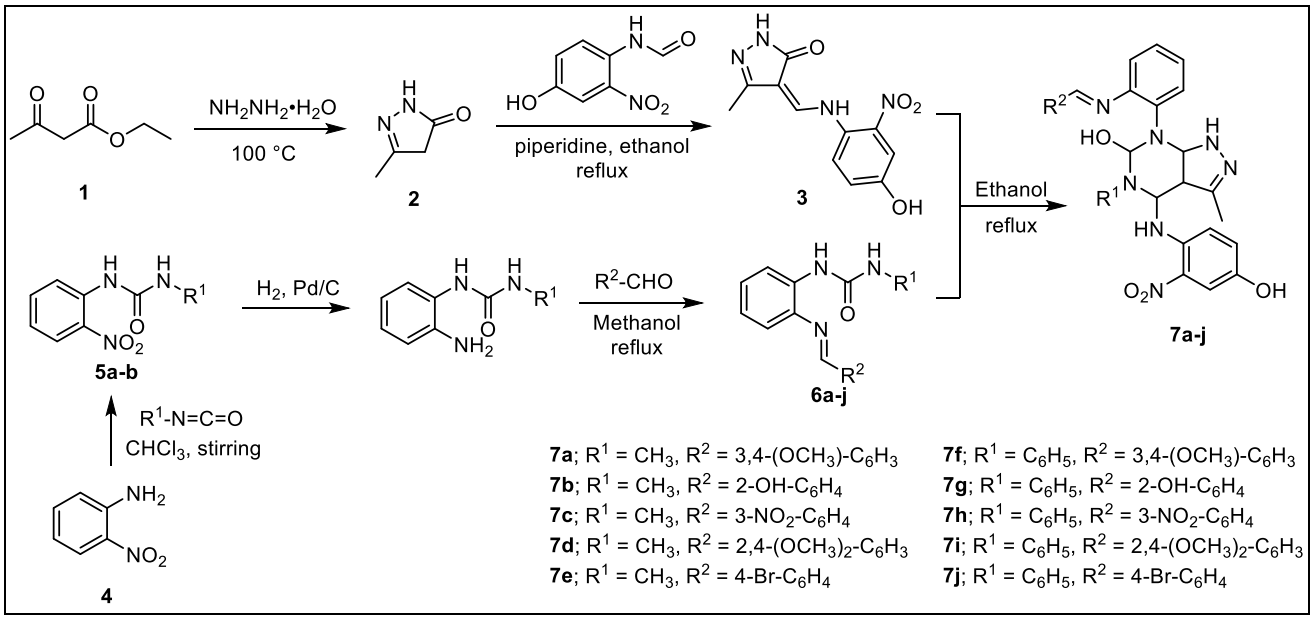
**Experimental details for molecular docking analysis:**

The crystal structure of Cyclin dependent kinases and tyrosine kinasehas been retrieved from Protein Data Bank (PDB ID: 7QHL & 2SRC). The protein preparation tool in Schrodinger suite 20.3 was used to prepare protein structure. The missing atoms were added, peripheral water molecules were removed at a distance of less than 5 Å from the binding pocket, and the structure was energetically minimized through the protein preparation wizard. The grid was generated by picking the active site where the co-crystal locates with a grid box at the centre of bound co-crystal. The designed ligands were drawn using 2D sketcher and subjected to energy minimization, followed by ligand preparation for the generation of different conformers using the LigPrep module of the Schrodinger 20.3. The different conformers thus obtained were subjected to molecular docking with Glide in standard precision (SP) mode. The poses generated were evaluated, and the best-ranked pose was described. It was known that previous reports stated that co-crystal of target CDK receptor binding modes that the three essential hydrogen binding interactions at active site besides hydrophobic interactions are GLU81, ASP86 and LEU83 and validation was confirmed by redocking of co-crystallised structure with target kinase.

## EXPERIMENTAL:

### General Procedure for synthesis of pyazolo(3,4-*d*)pyrimdines

5-methyl-2-phenyl-2, 4-dihydro-3*H*-pyrazol-3-one (2) was prepared by the reaction of ethyl acetoacetate (1) with phenyl hydrazine in ethanol under reflux for 3 hrs. The isolated brown colour solid (2) was treated with *N*-(4-hydroxy-2-nitrophenyl)formamide in acetic anhydride under reflux for 5 hrs to afford 4-[(4-hydroxy-2-nitroanilino)methylidene]-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3). The key intermediates 5a, 5b were obtained by treating ortho nitro aniline (4) with methy isocyanates and phenyl isocyanate respectively under magnetic agitation for 8 hrs in ethanol. The compounds 5a and 5b were undergoes reduction gives amino derivatives, treated with different aldehydes under reflux condition for 5 hrs to obtain the 6a-6j. The final compounds 7a-7j was synthesized by treating the obtained compound 3 with 6a-6j independently under magnetic agitation for 12 hrs in ethanol. The progress of reaction was checked by TLC at every stage of synthesis.




**Scheme for synthesis of 4, 6-dihydro-1H-pyrazolo[3,4-d]pyrimidines**

## BIOLOGICAL EVALUATION:

### DPPH radical scavenging assay

The disclosed method served as the foundation for the DPPH test[45]. Briefly, distilled water was used to dilute the 10 µg/ml DMSO sample of compounds to 4 ml. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added in the amount of 1 ml to this. For 30 minutes, the combined solution was incubated at room temperature. Using a UV-visible spectrophotometer, the absorbance of stable DPPH was measured at 517 nm, and the residual DPPH was estimated. The standard was ascorbic acid. The activity of scavenging free radicals was described as follows:

DPPH Scavenging activity (%) = [Ac-As]/[Ac-Ab], Where Ac was absorbance for control, As for sample, Ab for blank (MeOH) and each sample was measured at a concentration of 10 µg/ml, all tests were done in triplicate and Table 5 displays the RSC as a percentage.

**Cytotoxic activity**

Using the MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, the inhibitory concentration ($IC_{50}$) value is calculated. A 96-well plate with cultured cells ($1\times 10^5$) was placed and incubated for 48 hours in a 5% $CO_2$ incubator at 37°C. After 48 hours, the monolayer was washed with media and 100 µl of samples at various test concentrations (10, 20, 30, 40, and 50 µg/ml) were added. The cells were then incubated again under the same circumstances. Each well received 100 µl of the MTT solution after the culture media was removed and each well was then incubated at 37°C for 4 hours. Following the removal of the supernatant, 100 µl of DMSO was added to each well and incubated for 10 min to dissolve the formazan crystals. The optical density was measured at 590 nm and using a dose response curve, the percentage growth inhibition was calculated, and the results were expressed as $IC_{50}$ values.[46] The percentage growth inhibition was calculated by using the following formula:

$$\% \text{ growth of inhibition} = 100 - \frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control Group}} \times 100$$

The $IC_{50}$ value was determined by using linear regression equation i.e. Y =mx+C. Here, Y = 50, M and C values were derived from the viability graph.

## RESULTS AND DISCUSSION:

### Synthesis and Spectral data of the Synthesized Compounds

**Synthesis of 5-methyl-2, 4-dihydro-3-pyrazol-3-one (2)**

A mixture of ethyl acetoacetate (1.74g, 10 mmol) phenyl hydrazine (5 mmol) in ethanol about 50ml was refluxed for 3 hrs. The precipitate of brown colour solid was formed by cooling, was recrystallized from ethanol to afford 2. Yield 85%; mp 165-167˚C; IR(KBr $cm^{-1}$): 2871 (C-H str.), 1685 (C=O str.), 1595 (C=C str.), 1445 (C-H bending), 1276 (C-O str.); $^1$H-NMR (DMSO-$d_6$): δ 2.07 (s, 3H, CH3), 3.32 (d, 2H, $CH_2$), 7.32(1H, t, CH), 7.51-7.71 (m, 4H, ArH); $^{13}$C-NMR: δ 16.0, 43.1, 122.8, 127.8, 128.2, 138.0, 156.3, 170.6; MS m/z (%): 174.19($M^+$+1, 4.85), 159 ($M^+$, 16.46), 144 (106.87), 95 (100.00), 77 (16.66), 68 (56.30); Anal. Calced for $C_{10}H_{10}N_2O$ (174.19): C (68.95%), H (5.79%), N (16.08%). Found: (174.19): C (68.13%), H (5.12%), N (15.75%).

**Synthesis of 4-[(4-hydroxy-2-nitroanilino)methylidene]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3)**

The compound 2 (3.25g, 10 mmol) was treated with *N*-(4-hydroxy-2-nitrophenyl)formamide (5 mmol) in 50 ml of acetic anhydride under reflux for 5 hrs and mixture was allowed cooled, added in 100 ml ice cold water. Filter off the obtained ppt and recrystallized from ethanol to afford compound 3. Yield 75%; mp 220-222˚C; IR (KBr $cm^{-1}$): 3455 (OH, str.), 3185 (NH, str.), 2865 (C-H str.), 1710 (C=O str.), 1642 (N-O, str.), 1543 (C=C str.), 1358 (C-H bending), 1265 (C-O str.); $^1$H-NMR (DMSO-$d_6$): δ δ 2.32 (s, 3H, $CH_3$), 7.27-7.73 (m, 5H, ArCH, $J$ = 8.0, 7.5, 1.4 Hz), 7.53-7.75 (m, 3H, ArH, $J$ = 7.5, 1.3 Hz); $^{13}$C-NMR: 16.0, 40.7, 101.4, 115.2, 117.7, 122.8, 127.8, 128.2, 137.7, 138.0, 154.8, 157.4, 159.1, 170.0. MS m/z (%):325.29 ($M^+$+1, 4.13), 286 ($M^+$, 18.55), 259 (16.46), 244 (105.25), 186 (95.10), 165 (56.56), 116 (55.50), Anal. Calced for $C_{17}H_{14}N_4O_4$ (325.29): C (60.35%), H (4.17%), N (16.56%). Found: C (59.55%), H (3.97%), N (15.54%).

**Synthesis of N-methyl-N'-(2-nitrophenyl)urea (5a)**

Ortho nitro aniline (1.38g, 10 mmol) was treated with methyl isocyanate (1.25g, 10 mmol) under magnetic agitation for 8 hrs in 50 ml of ethanol. The formed solid product was filtered off, washed with ice cold water, dried and recrystallized from ethanol to afford the compound 5a. The compound treated with palladium to reduced compound. Yield 75%; mp 168-170˚C; IR (KBr $cm^{-1}$): 3135 (NH, str.) 2755(C-H str.), 1741 (C=O str.), 1569 (N-O, str.), 1525 (C=C str.), 1219(C-H bending); $^{1}$H-NMR (DMSO-$d_6$):δ 2.69 (s, 3H, $CH_3$), 6.49-6.73 (m, 2H, ArCH, *J* = 8.0, 7.7, 1.2 Hz), 6.67-7.38 (m, , 2H, ArH, *J* = 7.9, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 26.7, 115.7, 116.7, 128.1, 128.3, 128.2, 131.9, 134.2, 157.3; MS m/z (%):165.19 ($M^+$+1, 8.54), 139($M^+$, 28.25), 115 (19.36), 104 (54.25), 86 (55.16), 65 (52.16); Anal. Calced for $C_8H_{11}N_3O$ (165.19): C (58.17%), H (6.71%), N (25.44%). Found: C (57.57%), H (6.03%), N (24.84%).

**Synthesis of N-phenyl-N'-(2-nitrophenyl)urea (5b)**

Ortho nitro aniline (1.38g, 10 mmol) was treated with phenyl isocyanate (1.28g, 5 mmol) under magnetic agitation for 8 hrs in 50 ml of ethanol. The formed solid product was filtered off, washed with ice cold water, dried and recrystallized from ethanol to afford the compound 5b. The compound treated with palladium to reduced compound. Yield 68%; mp 175-177˚C; IR(KBr $cm^{-1}$): 3254 (NH, str.) 2645(C-H str.), 1756 (C=O str.), 1575 (C=C str.), 1658 (N-O, str.), 1234(C-H bending); $^{1}$H-NMR (DMSO-$d_6$):δ 6.49-6.74 (m, 2H, ArCH, *J* = 8.0, 7.7, 1.2 Hz), 7.01-7.15 (m, 2H, ArH, *J* = 7.9, 1.2, 0.5 Hz), 7.36-8.10 (m, 5H, *J* = 8.2, 1.5, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 115.7, 116.7, 119.9, 127.8, 128.1-128.3, 128.2 , 128.2, 128.2, 131.9, 134.1, 134.3, 134.2, 134.2, 153.0; MS m/z (%): 227.26 ($M^+$+1, 14.14), 189 ($M^+$, 25.54), 154 (21.25), 137 (52.09), 114 (54.65), 98 (50.15); Anal. Calced for $C_{13}H_{13}N_3O$: C (68.70%), H (5.77%), N (18.49%). Found: C (67.54%), H (5.19%), N (18.10%).

**Synthesis of *N*-(2-[(3,4-dimethoxyphenyl)methylidene]amino}phenyl)-*N*'-methylurea (6a)**

The compound 5a (1.65g, 5 mmol) was treated with 3,4-dimethoxy benzaldehyde (2.34g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6a. Yield 82%; mp above 300˚C; IR (KBr $cm^{-1}$): 3435 (NH, str.), 2858 (C-H str.), 1856 (C=O str.), 1668 (C=N str.), 1597 (C=C str.), 1451 (C-H bend), 1361 (C-N bend), 1115 (C-H bending), 1042 (C-O str.); $^{1}$H-NMR: δ 2.71 (s,3H, $CH_3$), 3.78-3.88 (s, 6H, $OCH_3$, *J* = 8.4, 0.5 Hz), 6.97-7.29 (m, 4H, ArCH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 3H, ArH, *J* = 1.8, 0.5 Hz), 8.62 (s, 1H, CH); $^{13}$C-NMR: δ 26.7, 56.0, 56.0, 109.1, 111.2, 115.7, 128.0, 128.1, 128.3, 128.2, 130.1, 131.9, 137.7, 148.2, 148.4, 157.3, 159.0; MS m/z (%):313.35 ($M^+$+1, 9.12), 289 ($M^+$, 25.28), 254 (24.25), 237 (52.12), 214 (53.65), 198 (47.15), 167 (26.35), 115 (21.08); Anal. Calced for $C_{17}H_{19}N_3O_3$: C (65.16%), H (6.11%), N (13.41%). Found: C (65.54%), H (5.80%), N (13.05%).

**Synthesis of *N*-(2-[(2-hydroxyphenyl)methylidene]amino}phenyl)-*N*'-methylurea (6b)**

The compound 5a (1.24g, 5 mmol) was treated with 2-hydroxy benzaldehyde (1.38g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was

filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6b. Yield 82%; mp above 300˚C; IR (KBr $cm^{-1}$): 3474 (OH, str.), 3415 (NH, str.), 2874 (C-H str.), 1871 (C=O str.), 1628 (C=N str.), 1517 (C=C str.), 1415 (C-H bend), 1381 (C-N bend), 1115 (C-H bending), 1103 (C-O str.); $^{1}$H-NMR: δ 2.71 (s, 3H, $CH_3$), 6.97-7.33 (m, 4H, ArH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 4H, ArH, *J* = 1.8, 0.5 Hz), 8.71 (s, 1H, CH), 9.58 (s, 1H, NH), 11.48 (s, 1H, OH), 13.19 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 115.7, 116.8, 118.9, 128.1-128.3, 128.2, 128.2, 128.2, 128.4, 129.4, 131.9, 132.5, 137.7, 157.3, 161.1, 162.2; MS m/z (%): 269.29 ($M^+$+1, 16.15), 217 ($M^+$, 33.18), 204 (24.15), 187 (55.32), 165 (71.65), 148 (47.15), 117 (58.75), 95 (59.55); Anal. Calced for $C_{15}H_{15}N_3O_2$: C (66.90%), H (5.61%), N (15.60%). Found: C (66.12%), H (4.10%), N (14.81%).

**Synthesis of *N*-(2-[(3-nitrophenyl)methylidene]amino}phenyl)-*N*'-methylurea (6c)**

The compound 5a (1.5g, 5 mmol) was treated with 3-nitro benzaldehyde (1.2g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6c. Yield 82%; mp above 300˚C; IR (KBr $cm^{-1}$): 3456 (OH, str.), 3358 (NH, str.), 2714 (C-H str.), 1854 (C=O str.), 1651 (C=N str.), 1558 (N-O, str.), 1416 (C-H bend), 1309 (C-N bend), 1109 (C-H bending), 1030 (C-O str.); $^{1}$H-NMR: δ 2.75 (s, 3H, $CH_3$), 6.97-7.33 (m, 4H, ArH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 4H, ArH, *J* = 1.8, 0.5 Hz), 8.71 (s, 1H, CH), 9.58 (s, 1H, NH), 11.47 (s, 1H, OH), 13.18 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 115.7, 116.8, 118.9, 128.1-128.3, 128.2, 128.2, 128.2, 128.4, 129.4, 131.9, 132.5, 137.7, 157.3, 161.1, 162.2; MS m/z (%): 298.29 ($M^+$+1, 19.05), 267 ($M^+$, 54.17), 214 (68.15), 188 (56.31), 174 (71.54), 148 (54.57), 117 (48.85), 97 (44.15); Anal. Calced for $C_{15}H_{14}N_4O_3$: C (60.40%), H (4.73%), N (18.78%). Found: C (60.40%), H (4.73%), N (18.78%).

**Synthesis of *N*-(2-[(2,4-dimethoxyphenyl)methylidene]amino}phenyl)-*N*'-methylurea (6d)**

The compound 5a (1.5g, 5 mmol) was treated with 2,4-dimethoxy benzaldehyde (1.3g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6d. Yield 82%; mp above 300˚C; IR (KBr $cm^{-1}$): 3416 (NH, str.), 2819 (C-H str.), 1809 (C=O str.), 1616 (C=N str.), 1556 (C=C str.), 1411 (C-H bend), 1461 (C-N bend), 1312 (C-H bending), 1142 (C-O str.); $^{1}$H-NMR: δ 2.73 (s,3H, $CH_3$), 3.78-3.98 (s, 6H, $OCH_3$, *J* = 8.4, 0.4 Hz), 6.97-7.29 (m, 4H, ArCH, *J* = 8.4, 1.8 Hz), 7.08-7.97 (m, 3H, ArH, *J* = 1.8, 0.5 Hz), 8.65 (s, 1H, CH), 9.58 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 56.0, 56.0, 109.1, 111.2, 115.7, 128.0, 128.1, 128.3, 128.2, 130.1, 131.9, 137.7, 148.2, 148.4, 157.3, 159.0; MS m/z (%):313.35 ($M^+$+1, 9.12), 287 ($M^+$, 25.18), 252 (21.15), 234 (52.16), 213 (52.15), 198 (41.16), 165 (22.35), 118 (26.09); Anal. Calced for $C_{17}H_{19}N_3O_3$: C (65.16%), H (6.11%), N (13.41%). Found: C (65.14%), H (5.81%), N (13.15%).

**Synthesis of *N*-(2-[(4-bromophenyl)methylidene]amino}phenyl)-*N*'-methylurea (6e)**

The compound 5a (1.74g, 5 mmol) was treated with 4-bromo benzaldehyde (2.41g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound

6e. Yield 78%; mp above 300˚C; IR (KBr $cm^{-1}$): 3474 (NH, str.), 2944 (C-H str.), 1887 (C=O str.), 1644 (C=N str.), 1558 (C=C str.), 1325 (C-H bend), 1285 (C-N bend), 1215 (C-H bending), 1185 (C-O str.); $^{1}$H-NMR: δ 2.71 (s, 3H, $CH_3$), 6.91-7.33 (m, 4H, ArH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 4H, ArH, *J* = 1.8, 0.5 Hz), 8.71 (s, 1H, CH), 9.58 (s, 1H, NH), 13.18 (s, 1H, NH), 13.19 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 115.7, 116.8, 118.9, 128.1-128.3, 128.2, 128.2, 128.2, 128.4, 129.4, 131.9, 132.5, 137.7, 157.3, 161.1, 162.2; MS m/z (%): 332.19 ($M^+$+1, 11.12), 297 ($M^+$, 31.08), 268 (28.14), 247 (59.32), 165 (71.65), 148 (100.00), 118 (78.75), 98 (54.35); Anal. Calced for $C_{15}H_{14}BrN_3O$: C (54.23%), H (4.25%), N (12.65%). Found: C (54.08%), H (4.19%), N (12.08%).

**Synthesis of *N*-(2-[(3,4-dimethoxyphenyl)methylidene]amino}phenyl)-*N*'-phenylurea (6f)**

The compound 5b (1.65g, 5 mmol) was treated with 3,4-dimethoxy benzaldehyde (1.98g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6f. Yield 71%; mp above 300˚C; IR (KBr $cm^{-1}$): 3384 (NH, str.), 2841 (C-H str.), 1847 (C=O str.), 1615 (C=N str.), 1558 (C=C str.), 1349 (C-H bend), 1254 (C-N bend), 1208 (C-H bending), 1124 (C-O str.).$^{1}$H-NMR: δ 3.78-3.88 (s, 6H, $OCH_3$), 6.98-7.97 (m, 10H, ArH, *J* = 8.4, 1.8 Hz), 8.6 (s, 1H, CH), 8.63 (s, 1H, CH), 9.58 (s, 1H, NH); $^{13}$C-NMR: δ 56.0, 111.2, 115.7, 119.9, 127.8, 128.0, 128.1, 128.3, 128.2, 130.1, 131.9, 134.2, 137.7, 148.2, 148.4, 153.0, 159.0; MS m/z (%): 375.42 ($M^+$+1, 9.12), 287($M^+$, 25.18), 252 (21.15), 234 (52.16), 213 (52.15), 198 (41.16), 165 (22.35), 118 (26.09); Anal. Calced for $C_{22}H_{21}N_3O_3$: C (70.38%), H (5.64%), N (11.19%). Found: C (70.10%), H (5.18%), N (11.84%).

**Synthesis of *N*-(2-[(2-hydroxyphenyl)methylidene]amino}phenyl)-*N*'-phenylurea (6g)**

The compound 5b (1.78g, 5 mmol) was treated with 2-hydroxy benzaldehyde (1.3g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6g. Yield 65%; mp above 300˚C; IR (KBr $cm^{-1}$): 3384 (NH, str.), 3255 (OH, str.), 2829 (C-H str.), 1854 (C=O str.), 1624 (C=N str.), 1509 (C=C str.), 1348 (C-H bend), 1209 (C-N bend), 1155 (C-H bending), 1118 (C-O str.); $^{1}$H-NMR: δ 2.71 (s, 3H, $CH_3$), 6.90-7.39 (m, 4H, ArH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 4H, ArH, *J* = 1.8, 0.5 Hz), 8.71 (s, 1H, CH), 9.58 (s, 1H, NH), 11.45 (s, 1H, OH), 13.18 (s, 1H, NH), 13.19 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 115.7, 116.8, 118.9, 128.1, 128.3, 128.2, 128.2, 128.4, 129.4, 131.9, 132.5, 137.7, 157.3, 161.1, 162.2; MS m/z (%): 331.367 ($M^+$+1, 17.11), 295 ($M^+$, 39.07), 264 (78.14), 247 (59.32), 165 (98.65), 148 (70.00), 114 (78.75), 94 (54.35); Anal. Calced for $C_{20}H_{17}N_3O_2$: C (72.49%), (5.17%), N (12.68%). Found: C (72.19%), H (5.10%), N (12.27%).

**Synthesis of *N*-(2-[(3-nitrophenyl)methylidene]amino}phenyl)-*N*'-phenylurea (6h)**

The compound 5b (1.65g, 5 mmol) was treated with 3-nitro benzaldehyde (1.32g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6h. Yield 65%; mp above 300˚C; IR (KBr $cm^{-1}$): 3254 (NH, str.), 2721 (C-H str.), 1955 (C=O str.), 1651 (C=N str.), 1558 (N-O, str.), 1553 (C=C str.), 1356 (C-H bend), 1201 (C-N bend),

1105 (C-H bending), 908 (C-O str.); $^{1}$H-NMR: δ 2.71 (s, 3H, $CH_3$), 6.90-7.39 (m, 4H, ArH, *J* = 8.4, 1.8 Hz), 7.08-7.96 (m, 4H, ArH, *J* = 1.8, 0.5 Hz), 8.71 (s, 1H, CH), 9.58 (s, 1H, NH), 11.45 (s, 1H, OH), 13.18 (s, 1H, NH), 13.19 (s, 1H, NH); $^{13}$C-NMR: δ 26.7, 115.7, 116.8, 118.9, 128.1, 128.3, 128.2, 128.2, 128.4, 129.4, 131.9, 132.5, 137.7, 157.3, 161.1, 162.2; MS m/z (%): 360.36 ($M^+$+1, 25.11), 295 ($M^+$, 39.47), 264 (78.29), 247 (54.37), 165 (98.65), 138 (75.00), 114 (78.75), 94 (54.35); Anal. Calced for $C_{20}H_{16}N_4O_3$: C (72.49%), (5.17%), N (12.68%). Found: C (72.19%), H (5.10%), N (12.27%).

**Synthesis of *N*-(2-[(2,4-dimethoxyphenyl)methylidene]amino}phenyl)-*N*'-phenylurea (6i)**

The compound 5b (1.35g, 5 mmol) was treated with 2,4-dimethoxy benzaldehyde (1.85g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6i. Yield 68%; mp above 300˚C; IR (KBr $cm^{-1}$): 3474 (NH, str.), 2945 (C-H str.), 1857 (C=O str.), 1676 (C=N str.), 1542(C=C str.), 1349 (C-H bend), 1208 (C-N bend), 1158 (C-H bending), 1103 (C-O str.); $^{1}$H-NMR: δ 3.78-3.88 (s, 6H, $OCH_3$), 6.98-7.97 (m, 10H, ArH, *J* = 8.4, 1.8 Hz), 8.6 (s, 1H, CH), 8.63 (s, 1H, CH), 9.58 (s, 1H, NH). $^{13}$C-NMR: δ 56.0, 111.2, 115.7, 119.9, 127.8, 128.0, 128.1, 128.3, 128.2, 130.1, 131.9, 134.2, 137.7, 148.2, 148.4, 153.0, 159.0; MS m/z (%): 375.42 ($M^+$+1, 9.12), 287 ($M^+$, 25.18), 252 (21.15), 234 (52.16), 213 (52.15), 198 (41.16), 165 (22.35), 118 (26.09); Anal. Calced for $C_{22}H_{21}N_3O_3$: C (70.38%), H (5.64%), N (11.19%). Found: C (70.10%), H (5.18%), N (11.84%).

**Synthesis of *N*-(2-[(4-bromophenyl)methylidene]amino}phenyl)-*N*'-phenylurea (6j)**

The compound 5b (1.36g, 5 mmol) was treated with 4-bromo benzaldehyde (1.85g, 5 mmol) in 25 ml of glacial acetic acid under reflux condition for 5 hrs. The solid product was filtered off washed with ice cold water, dried and recrystallized from ethanol to afford the compound 6j.

Yield 63%; mp above 300˚C; IR (KBr $cm^{-1}$): 3496 (NH, str.), 2827 (C-H str.), 1854 (C=O str.), 1608 (C=N str.), 1557 (C=C str.), 1341 (C-H bend), 1229 (C-N bend), 1217 (C-H bending), 1154 (C-O str.); $^{1}$H-NMR: δ 3.78-3.89 (s, 6H, $OCH_3$), 6.98-7.97 (m, 10H, ArH, *J* = 8.4, 1.8 Hz), 8.7 (s, 1H, CH), 8.91 (s, 1H, CH), 9.58 (s, 1H, NH). $^{13}$C-NMR: δ 56.0, 111.2, 115.7, 119.9, 127.8, 128.0, 128.1, 128.3, 128.2, 130.1, 131.9, 134.2, 137.7, 148.2, 148.4, 153.0, 159.0; MS m/z (%):394.26 ($M^+$+1, 9.12), 287 ($M^+$, 25.18), 252 (21.15), 234 (52.16), 213 (52.15), 198 (41.16), 165 (22.35), 118 (26.09); Anal. Calced for $C_{20}H_{16}BrN_3O$: C (58.71%), H (3.83%), N (11.41%). Found: C (58.11%), H (3.13%), N (11.09%).

**Synthesis of 7-[2-[(3,4-dimethoxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7a)**

A mixture of compound 3 (3.1g, 5 mmol) and compound 6a (2.65g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70$^{o}$C. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7a. Yield 68%; mp above 300˚C; IR (KBr $cm^{-1}$): 3374 (NH, str.), 3275 (OH, str.), 3215 (NH, str.) 2896 (C-H str.), 1957 (C=O str.), 1674 (C=N str.), 1641 (C=C str.), 1312 (C-H bend), 1218 (C-N bend), 1145 (C-H bending), 1102 (C-O str.); $^{1}$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH),

6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-7.04 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.7, 38.2, 53.8, 56.0, 80.5, 100.6, 110.9, 111.2, 115.7, 117.7, 127.3, 128.1, 128.2, 128.3,128.4, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8. MS: m/z (%) 559.57 ($M^+$+1, 19.12), 487 ($M^+$, 25.47), 452 (61.18), 394 (55.16), 342 (65.45), 312 (79.45), 273 (98.15), 258 (67.16), 219 (54.35), 118 (26.09); Anal. Calced for $C_{28}H_{29}N_7O_6$: C (60.10%), H (5.22%), N (17.52%). Found: C (59.54%), H (5.13%), N (16.12%).

**Synthesis of 7-[2-[(2-hydroxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7b)**

A mixture of compound 3 (2.56g, 5 mmol) and compound 6b (2.48g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70$^o$C. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7b. Yield 72%; mp above 300˚C; IR (KBr cm$^{-1}$): 3486 (NH, str.), 3374 (OH, str.), 3295 (OH, str.), 3219 (NH, str.) 2849 (C-H str.), 1967 (C=O str.), 1609 (C=N str.), 1554 (C=C str.), 1252 (C-H bend), 1117 (C-N bend), 1045 (C-H bending), 1002 (C-O str.); $^1$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.37 (s, 2H, CH), 5.23 (s, 1H, CH), 6.19 (s, 1H, CH), 6.51-6.95 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.70-7.19 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.7, 38.8, 53.9, 56.0, 80.7, 100.6, 110.9, 111.2, 115.7, 117.7, 127.3, 128.2, 128.3,128.4, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8; MS: m/z (%) 515.52 ($M^+$+1, 28.12), 491 ($M^+$, 24.17), 455 (60.19), 391 (65.18), 342 (61.45), 308 (79.45), 283 (99.15), 268 (69.16), 210 (34.15), 165 (29.09); Anal. Calced for $C_{26}H_{25}N_7O_5$: C (61.10%), H (5.82%), N (19.52%). Found: C (60.54%), H (5.13%), N (18.12%).

**Synthesis of 7-[2-[(3-nitrophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7c)**

A mixture of compound 3 (2.13g, 5 mmol) and compound 6c (2.36g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70$^o$C. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7c. Yield 85%; mp above 300˚C; IR (KBr cm$^{-1}$): 3418 (NH, str.), 3381 (OH, str.), 3218 (OH, str.), 3196 (NH, str.) 2819 (C-H str.), 1947 (C=O str.), 1671 (C=N str.), 1693 (C=C str.), 1583 (N-O, str.) 1212 (C-H bend), 1206 (C-N bend), 1103 (C-H bending), 1057 (C-O str.); $^1$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH), 6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-7.04 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.8, 38.9, 53.2, 80.2, 100.5, 110.7, 111.3, 115.8, 117.1, 127.4, 128.2, 128.3, 128.5, 128.7, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8; MS: m/z (%) 544.57 ($M^+$+1, 21.12), 495($M^+$, 12.47), 441 (64.18), 385 (51.16), 341 (61.45), 315 (81.45), 271 (98.15), 218 (100.0), 199 (59.35), 118 (26.09); Anal. Calced for $C_{26}H_{24}N_8O_6$: C (61.54%), H (5.75%), N (17.19%). Found: C (60.54%), H (5.13%), N (16.14%).

**Synthesis of 7-[2-[(2,4-dimethoxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7d)**

A mixture of compound 3 (2.85g, 5 mmol) and compound 6d (2.89g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7d. Yield 68%; mp above 300˚C; IR (KBr $cm^{-1}$): 3319 (NH, str.), 3267 (OH, str.), 3269 (NH, str.) 2886 (C-H str.), 1986 (C=O str.), 1671 (C=N str.), 1671 (C=C str.), 1362 (C-H bend), 1288 (C-N bend), 1165 (C-H bending), 1162 (C-O str.).$^{1}$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH), 6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-7.04 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.7, 38.2, 53.8, 56.0, 80.5, 100.6, 110.9, 111.2, 115.7, 117.7, 127.3, 128.1, 128.2, 128.3,128.4, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8; MS: m/z (%) 559.57 ($M^+$+1, 19.12), 487 ($M^+$, 25.47), 452 (61.18), 394 (55.16), 342 (65.45), 312 (79.45), 273 (98.15), 258 (67.16), 219 (54.35), 118 (26.09); Anal. Calced for $C_{28}H_{29}N_7O_6$: C (60.10%), H (5.22%), N (17.52%). Found: C (59.54%), H (5.13%), N (16.12%).

**Synthesis of 7-[2-[(4-bromophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7e)**

A mixture of compound 3 (3.45g, 5 mmol) and compound 6e (2.36g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7e. Yield 61%; mp above 300˚C; IR (KBr $cm^{-1}$): 3356 (NH, str.), 3340 (OH, str.), 3281 (OH, str.), 3209 (NH, str.) 2872 (C-H str.), 1952 (C=O str.), 1694 (C=N str.), 1558 (C=C str.), 1287 (C-H bend), 1137 (C-N bend), 1039 (C-H bending), 982 (C-O str.); $^{1}$H-NMR: δ 2.18 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.37 (s, 2H, CH), 5.23 (s, 1H, CH), 6.19 (s, 1H, CH), 6.51-6.95 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.70-7.19 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH); $^{13}$C-NMR: δ 13.9, 37.8, 56.9, 58.0, 80.7, 101.6, 110.5, 111.9, 119.7, 127.3, 128.2, 128.3,128.4, 129.9, 130.5, 138.8, 148.0, 148.3, 148.4, 151.3, 154.8. MS: m/z (%) 578.41 ($M^+$+1, 11.12), 552 ($M^+$, 14.17), 495 (43.19), 395 (65.18), 335 (56.45), 309 (71.45), 292 (100.0), 268 (69.16), 210 (34.15), 165 (29.09); Anal. Calced for $C_{26}H_{24}BrN_7O_4$: C (60.60%), H (5.89%), N (19.82%). Found: C (60.54%), H (5.13%), N (18.12%).

**Synthesis of 7-[2-[(3,4-dimethoxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7f)**

A mixture of compound 3 (3.25g, 5 mmol) and compound 6f (3.21g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7f. Yield 56%; mp above 300˚C; IR (KBr $cm^{-1}$): 3476 (NH, str.), 3355 (OH, str.), 3317 (NH, str.) 2891 (C-H str.), 1969 (C=O str.), 1658 (C=N str.), 1648 (C=C str.), 1313 (C-H bend), 1256 (C-N bend), 1194 (C-H bending), 1148 (C-O str.); $^{1}$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH), 6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-7.04 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz),

7.11-8.91 (m, 9H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.7, 38.2, 53.8, 56.0, 80.5, 100.6, 110.9, 111.2, 115.7, 117.7, 127.3, 128.2, 128.3,128.4, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8. MS: m/z (%) 621.64 ($M^+$+1, 11.19), 585 ($M^+$, 21.47), 543 (67.11), 492 (54.76), 442 (45.45), 417 (79.45), 387 (99.15), 315 (61.11), 284 (47.35), 218 (54.09); Anal. Calced for $C_{33}H_{31}N_7O_6$: C (63.10%), H (5.22%), N (15.52%). Found: C (63.54%), H (5.13%), N (16.12%).

**Synthesis of 7-[2-[(2-hydroxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)- 3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7g)**

A mixture of compound 3 (3.25g, 5 mmol) and compound 6g (3.54g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7g. Yield 67%; mp above 300˚C; IR (KBr $cm^{-1}$): 3486 (NH, str.), 3315 (OH, str.), 3238 (OH, str.), 3218 (NH, str.) 2849 (C-H str.), 1967 (C=O str.), 1609 (C=N str.), 1554 (C=C str.), 1252 (C-H bend), 1117 (C-N bend), 1045 (C-H bending), 1002 (C-O str.); $^1$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.37 (s, 2H, CH), 5.23 (s, 1H, CH), 6.19 (s, 1H, CH), 6.51-6.95 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.70-8.19 (m, 9H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 15.7, 39.8, 55.9, 59.0, 81.7, 100.6, 111.9, 116.2, 118.7, 119.7, 126.3, 128.2, 128.3,128.4, 129.9, 131.5, 131.8, 132.0, 148.0, 148.3, 148.4, 151.3, 155.8; MS: m/z (%) 577.58 ($M^+$+1, 13.12), 491($M^+$, 28.15), 453 (61.19), 388 (64.18), 342 (60.45), 307 (71.45), 288 (100.0), 268 (69.16), 210 (34.15), 162 (29.09); Anal. Calced for $C_{31}H_{27}N_7O_5$: C (64.10%), H (4.82%), N (16.52%). Found: C (63.54%), H (5.13%), N (17.12%).

**Synthesis of 7-[2-[(3-nitrophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)- 3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7h)**

A mixture of compound 3 (3.65g, 5 mmol) and compound 6h (3.25g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7h. Yield 65%; mp above 300˚C; IR (KBr $cm^{-1}$): 3454 (NH, str.), 3347 (OH, str.), 3349 (NH, str.) 2486 (C-H str.), 1986 (C=O str.), 1671 (C=N str.), 1671 (C=C str.), 1362 (C-H bend), 1288 (C-N bend), 1165 (C-H bending), 1162 (C-O str.); $^1$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH), 6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-8.54 (m, 9H, ArH, *J* = 8.0, 7.5, 1.2 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.7, 38.2, 53.8, 56.0, 80.5, 100.6, 110.9, 111.2, 115.7, 117.7, 127.3, 128.2, 128.3,128.4, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 154.8. MS: m/z (%) 580.55 ($M^+$+1, 13.12), 527 ($M^+$, 27.47), 482 (64.18), 424 (51.16), 362 (100.0), 312 (89.45), 274 (90.15), 258 (64.16), 219 (54.35), 118 (26.09); Anal. Calced for $C_{31}H_{26}N_8O_6$: C (61.10%), H (4.22%), N (18.52%). Found: C (60.54%), H (5.13%), N (17.12%).

**Synthesis of 7-[2-[(2,4-dimethoxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7i)**

A mixture of compound 3 (3.25g, 5 mmol) and compound 6i (3.58g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7i. Yield 78%; mp above 300˚C; IR (KBr $cm^{-1}$): 3457 (NH, str.), 3377 (OH, str.), 3242 (NH, str.) 2858 (C-H str.), 1986 (C=O str.), 1670 (C=N str.), 1587 (C=C str.), 1354 (C-H bend), 1247 (C-N bend), 1148 (C-H bending), 1160 (C-O str.); $^{1}$H-NMR: δ 2.19 (s, 3H, $CH_3$), 2.48 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.35 (s, 2H, CH), 5.22 (s, 1H, CH), 6.17 (s, 1H, CH), 6.53-6.90 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.72-7.04 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-8.91 (m, 9H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.9, 38.5, 53.8, 56.0, 85.5, 100.6, 110.9, 114.2, 115.7, 117.7, 128.3, 128.7, 129.3, 129.9, 130.5, 131.8, 132.0, 131.9, 131.9, 148.0, 148.3, 148.4, 151.3, 161.8; MS: *m/z* (%) 621.64 ($M^+$+1, 13.12), 617 ($M^+$, 15.47), 552 (60.18), 494 (51.16), 442 (65.45), 312 (79.45), 273 (98.15), 258 (67.16), 219 (54.35), 117 (26.09); Anal. Calced for $C_{33}H_{31}N_7O_6$: C (63.10%), H (5.22%), N (15.52%). Found: C (61.54%), H (5.13%), N (16.12%).

**Synthesis of 7-[2-[(4-bromophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7j)**

A mixture of compound 3 (3.45g, 5 mmol) and compound 6j (3.15g, 5 mmol) in 20 ml of ethanol stirred for 12 hrs at 70ºC. The obtained ppt was filtered off and washed with ice cold water. The solid was recrystallized from ethanol and DMF to afford the compound 7j. Yield 65%; mp above 300˚C; IR (KBr $cm^{-1}$): 3457 (NH, str.), 3358 (OH, str.), 3287 (OH, str.), 3241 (NH, str.) 2848 (C-H str.), 1941 (C=O str.), 1687 (C=N str.), 1579 (C=C str.), 1219 (C-H bend), 1145 (C-N bend), 1039 (C-H bending), 982 (C-O str.); $^{1}$H-NMR: δ 2.18 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 3.68-3.85 (s, 6H, $OCH_3$), 4.25-4.37 (s, 2H, CH), 5.23 (s, 1H, CH), 6.19 (s, 1H, CH), 6.51-6.95 (m, 4H, ArH, *J* = 8.0, 1.1, 0.5 Hz), 6.70-7.19 (m, 4H, ArH, *J* = 8.0, 7.5, 1.1 Hz), 7.11-7.91 (m, 4H, ArH, *J* = 8.3, 1.2, 0.5 Hz); $^{13}$C-NMR: δ 13.9, 37.8, 56.9, 58.0, 80.7, 101.6, 110.5, 111.9, 119.7, 127.3, 128.2, 128.3,128.4, 129.9, 130.5, 138.8, 148.0, 148.3, 148.4, 151.3, 154.8; MS: *m/z* (%) 640.48 ($M^+$+1, 14.12), 591 ($M^+$, 12.17), 515 (41.19), 495 (65.18), 391 (54.45), 309 (71.45), 282 (100.0), 289 (69.16), 210 (34.15), 158 (29.09); Anal. Calced for $C_{31}H_{26}BrN_7O_4$: C (58.60%), H (4.89%), N (15.82%). Found: C (59.54%), H (5.13%), N (14.12%).

**Assessment of molecular descriptors and biological targets**

All the designed compounds based of literature the series titled compound 7a-7j, screened through Swissadme reported that 60% target as kinases, primarlly PIM1, Pim kinase, Proto-oncogene that has serine/threonine kinase activity and is important in cell proliferation and survival, giving it a selective advantage in carcinogenesis. Regulation of MYC transcriptional activity, control of cell cycle progression, and phosphorylation and inhibition of proapoptotic proteins are some of the ways that it exerts its oncogenic activity (BAD, MAP3K5 and FOXO3). MYC phosphorylation increases the protein's stability, which in turn increases transcriptional activity.

All the physicochemical properties stated that all the compounds were within the limitations of Lipinski except some like molecular mass which is not validated in many cases and lipophilicity and quantitative estimate of drug likeness reported and compounds 7a, 0.35, 7d 0.69 and 7j 0.73. All the designed series 7a-7j were targeted as Cyclin-dependent kinases (PDB ID: 7QHL), Tyrosine-protein kinase (PDB ID: 2SRC) and all the compounds data was reported in table 1. Score the proposed compounds for bioactivity using the molinspiration online toolbox, inhibitory activity against different receptor ligands, inhibitors, and enzymes were calculate, and the results are shown in Table 2. *In silico* study revealed given in Table 3, that all the compounds binding energies were analysed against selected target proteins in order to predict optimal conformational orientation at active region.

Among all the 7j possess docking score -9.05 k.cal per mole (Figure 2 & 3) and glide emodel score has -94.55 followed by 7b possess docking score -8.66 k.cal per mole and glide emodel score has -96.53 against the epidermal growth factor receptor tyrosine kinase. The 7b possess docking score -8.30 k.cal (Figure 4) per mole and glide emodel score has -73.98 followed by 7g possess docking score -8.15 k.cal per mole (Figure 5) and glide emodel score has -35.84 against the target CDK-2. The standards pemetrexed and doxorubicin reported -7.64, -8.81(Figure 6 & 7), against tyrosine kinase and -8.92 and -8.34 against CDK-28.8.

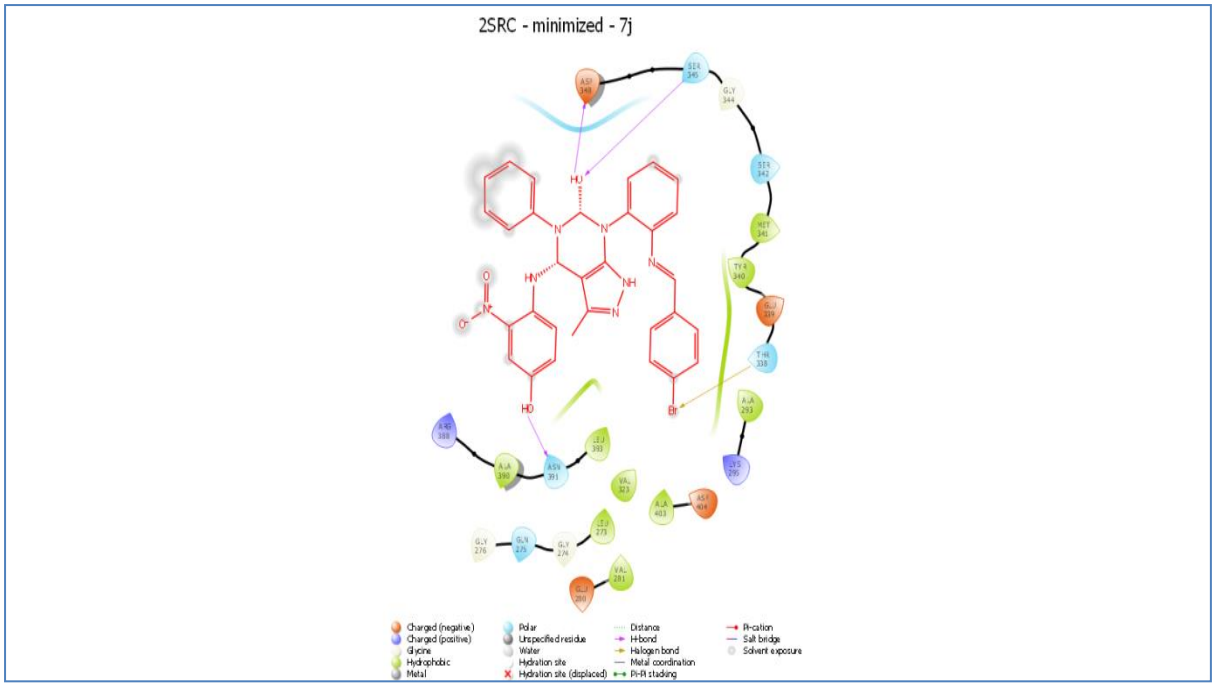



Figure 2: Binding interaction of 7g with 2SRC in 2D mode

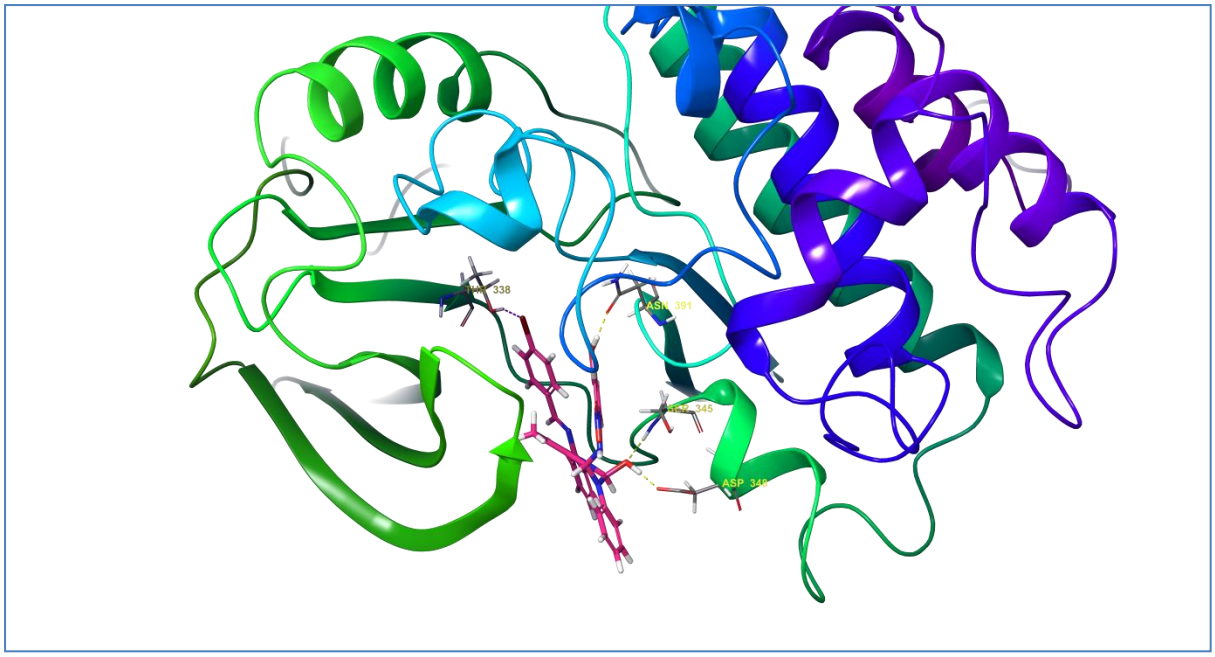



**Figure 3: Binding interaction of 7j with 2SRC in 3D mode**

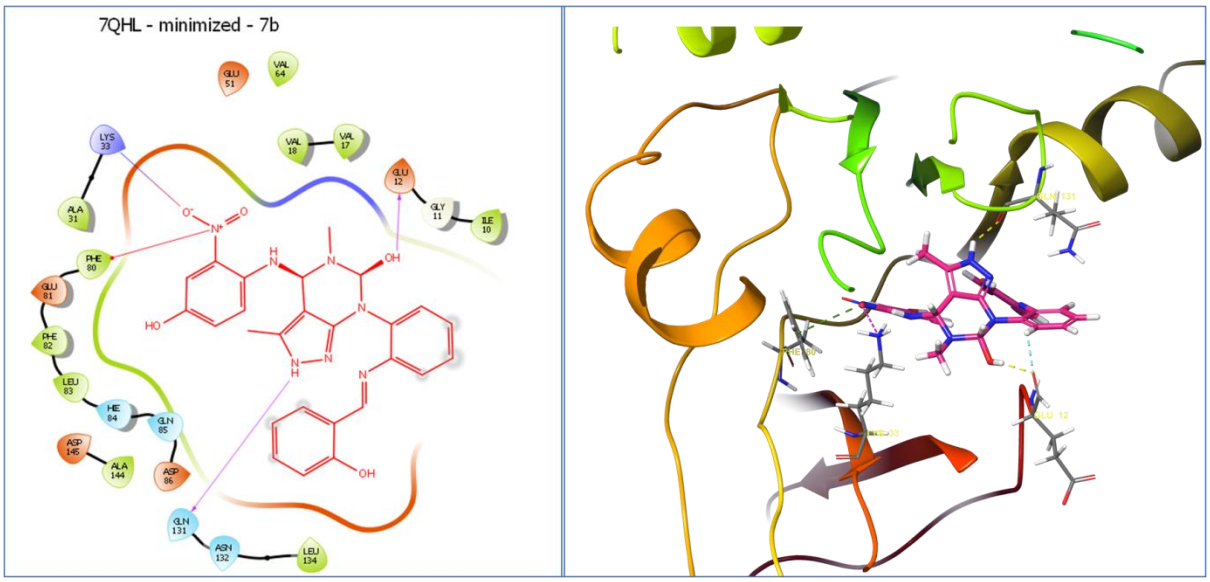



**Figure 4: Binding interaction of 7b with 7QHL in 2D & 3D mode**

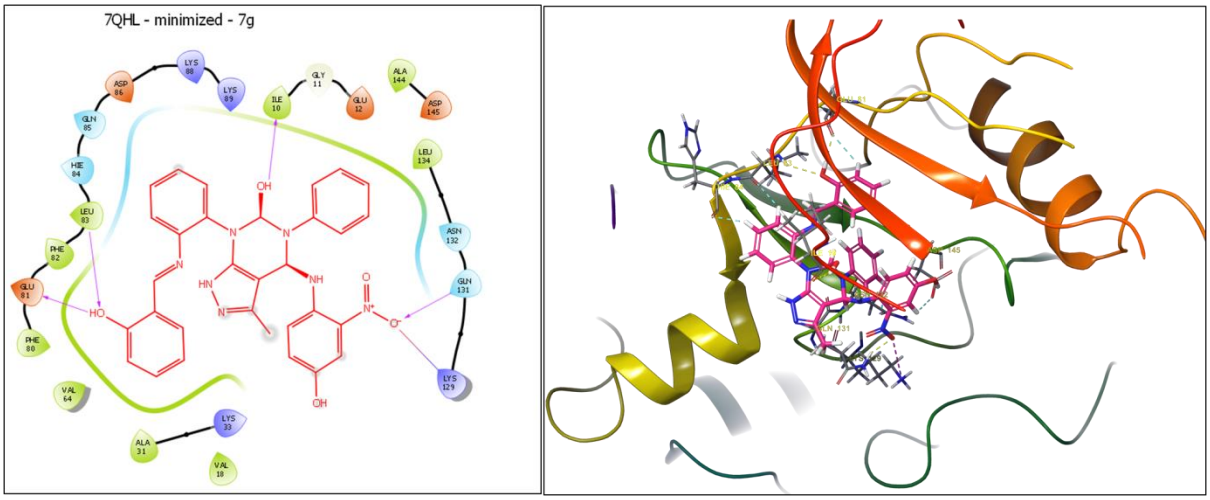



**Figure 5: Binding interaction of 7g with 7QHL in 2D & 3D mode**

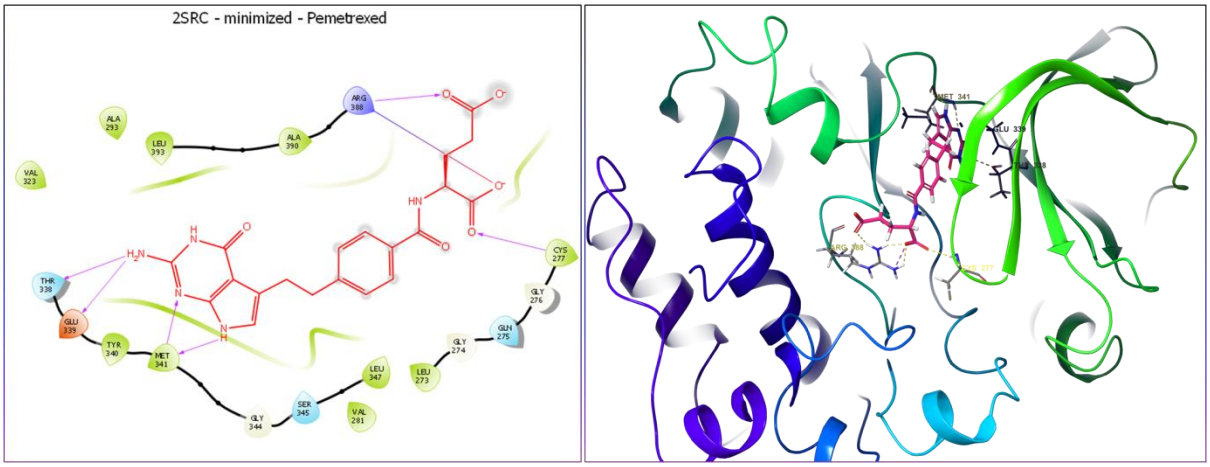



**Figure 6: Binding interaction of pemetrexed with 2SRC in 2D & 3D mode**

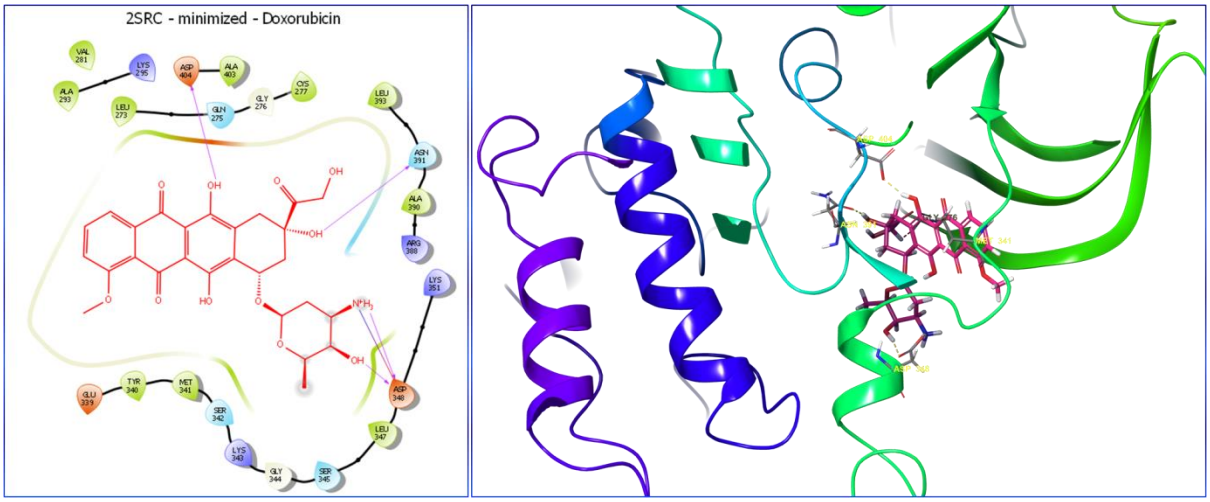



**Figure 7: Binding interaction of doxorubicin with 2SRC in 2D & 3D mode**

Many human diseases are believed that influenced by ROS, due to the free radicals can damage living organisms. The antioxidant effect was accessed by DPPH, a stable free radical, widely used to access antioxidant ability of synthesized compounds to scavenge the free radicals. The compound 7b found as good free radical scavenging ability given percentage, 51.49 ± 1.54, followed by 7j possess 47.28 ± 1.45 and 7g possess 45.26 ± 2.94 in comparison with standard ascorbic acid, found 68.22 ± 1.59. Possibly the groups hydroxyl and dimethoxyl were better scavenging ability among all. MTT assay revealed that 7j, 7c and 7b reported 16.36, 19.61 and 24.67 µg/ml, found as potential anticancer candidates against MCF-7. The compounds 7j and 7b reported 34.65 and 32.36 against Hep G2 cell lines, where standard 5-FU found as 14.31 and 21.36 µg/ml against MCF 7 and Hep-G2 respectively.

**Table 1. Molecular descriptive properties of designed compounds by SwissADME**

| Title | Chemical formula | M.Wt | HBD | HBA | LogP | QED | TPSA[Å²] | nrotb | *RO5* |
|---|---|---|---|---|---|---|---|---|---|
| 7a | $C_{28}H_{29}N_7O_6$ | 559.58 | 5 | 12 | 4.2 | 0.35 | 161.61 | 8 | 2 |
| 7b | $C_{26}H_{25}N_7O_5$ | 520.53 | 5 | 11 | 3.9 | 0.31 | 163.38 | 6 | 3 |
| 7c | $C_{26}H_{24}N_8O_6$ | 544.52 | 4 | 12 | 4.3 | 0.21 | 186.29 | 7 | 2 |
| 7d | $C_{26}H_{25}N_7O_6$ | 531.53 | 5 | 10 | 3.2 | 0.69 | 183.61 | 8 | 2 |
| 7e | $C_{26}H_{24}BrN_7O_4$ | 578.43 | 5 | 10 | 4.9 | 0.16 | 143.15 | 6 | 1 |
| 7f | $C_{33}H_{31}N_7O_6$ | 621.65 | 4 | 13 | 2.7 | 0.07 | 161.61 | 5 | 1 |
| 7g | $C_{31}H_{27}N_7O_5$ | 577.60 | 5 | 12 | 2.8 | 0.07 | 163.38 | 6 | 1 |
| 7h | $C_{31}H_{26}N_8O_6$ | 606.59 | 4 | 14 | 1.8 | 0.1 | 161.61 | 5 | 1 |
| 7i | $C_{33}H_{31}N_7O_6$ | 621.65 | 4 | 13 | 2.6 | 0.07 | 161.61 | 7 | 2 |
| 7j | $C_{31}H_{26}BrN_7O_4$ | 640.49 | 4 | 11 | 3.1 | 0.73 | 143.15 | 6 | 1 |

*M.Wt = Molecular weight; g/mol; HBD = Hydrogen bond donor; HBA = Hydrogen bond acceptor; lipophilicity (expressed as LogP) LogP = implicit logP method; QED = Quantitative Estimate of Drug likeness (Near to 1 good property); TPSA = Topological polar surface area; nrotb = no. of rotatable bonds; RO5 = no. of Lipinski violation*

**Table 2. Bioactivity score of designed compounds by molinspiration**

| Title | GPCR ligand | Ion Channel modulator | Kinase inhibitor | Nuclear Receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| 7a | -0.31 | -0.37 | -0.45 | -0.56 | -0.47 | -0.15 |
| 7b | -0.38 | -0.15 | -0.48 | -0.64 | -0.45 | -0.32 |
| 7c | -0.1 | -0.25 | -0.29 | -0.57 | -0.24 | -0.25 |
| 7d | -0.36 | -0.45 | -0.15 | -0.79 | -0.58 | -0.19 |
| 7e | -0.35 | -0.37 | -0.16 | -0.18 | -0.65 | -0.54 |
| 7f | -0.32 | -0.18 | -0.54 | -0.18 | -0.41 | -0.58 |
| 7g | -0.24 | -0.28 | -0.41 | -0.74 | -0.35 | -0.18 |
| 7h | -0.19 | -0.28 | -0.47 | -0.85 | -0.44 | -0.37 |
| 7i | -0.35 | -0.40 | -0.45 | -0.81 | -0.41 | -0.3 |
| 7j | -0.39 | -0.49 | -0.52 | -0.79 | -0.45 | -0.31 |

**Table 3. Molecular Docking Results targeting 2SRC and 7QHL**

| Compound Code | 2SRC (Tyrosine kinase) | | 7QHL (CDK-2) | |
|---|---|---|---|---|
| | Glide emodel | Docking score | Glide emodel | Docking score |
| **7a** | -83.30 | -8.38 | -81.81 | -7.03 |
| **7b** | -96.53 | -8.66 | -73.98 | -8.30 |
| **7c** | -89.65 | -8.35 | -63.29 | -7.69 |
| **7d** | -96.23 | -8.42 | -77.72 | -7.94 |
| **7e** | -73.80 | -8.26 | -82.92 | -6.42 |
| **7f** | -88.43 | -7.75 | -50.05 | -6.59 |
| **7g** | -65.83 | -8.64 | -35.84 | -8.15 |
| **7h** | -91.4 | -8.50 | -58.95 | -6.42 |
| **7i** | -54.68 | -7.23 | -59.43 | -7.16 |
| **7j** | -94.55 | -9.05 | -62.59 | -7.14 |
| **Doxorubicin** | -95.06 | -8.81 | -89.17 | -8.92 |
| **Pemetrexed** | -84.83 | -7.64 | -88.37 | -8.34 |
| **Co-Crystal** | -139.14 | -9.75 | -99.35 | -10.76 |

**Table 4. DPPH Scavenging Activity**

| S.No | Compound | %DPPH scavenging |
|---|---|---|
| 1 | **7a** | 42.28 ± 2.18 |
| 2 | **7b** | 51.49 ± 1.54 |
| 3 | **7c** | 36.56 ± 2.35 |
| 4 | **7d** | 22.14 ± 2.01 |
| 5 | **7e** | 25.17 ± 2.14 |
| 6 | **7f** | 18.33 ± 1.51 |
| 7 | **7g** | 45.26 ± 2.94 |
| 8 | **7h** | 39.41 ± 1.62 |
| 9 | **7i** | 35.63 ± 2.68 |
| 10 | **7j** | 47.28 ± 1.45 |
| 11 | **Std (Ascorbic acid)** | 68.22 ± 1.59 |

**Table 5. *In-Vitro* Anticancer MTT assay**

| S.No | Compound | $IC_{50}$ (µg) | |
|---|---|---|---|
| | | MCF 7 | Hep G2 |
| 1 | **7a** | 59.65 | 35.85 |
| 2 | **7b** | 24.67 | 32.36 |
| 3 | **7c** | 19.61 | 37.32 |
| 4 | **7d** | >100 | 56.82 |
| 5 | **7e** | >100 | >100 |
| 6 | **7f** | 29.31 | 49.25 |
| 7 | **7g** | 28.34 | 55.37 |
| 8 | **7h** | >100 | >100 |
| 9 | **7i** | 39.08 | >100 |
| 10 | **7j** | 16.36 | 34.65 |
| 11 | **Std (5-Floro U)** | 14.34 | 21.36 |

## CONCLUSION:

In summary, a series of novel pyrazolo(2,3-d)pyrimidine, titled 7a-7j were designed on thes basis of bioisostric replacement of scaffold based on the interaction sites at CDKs and endothelial growth factor tyrosine kinase. All the compounds were screened for various physicochemical parameters in order to predict drug likeliness properties by various computational or web tools and Schrodinger software. All the compounds were synthesized by four step procedures and screened for free radical scavenging assay by DPPH method, revealed that 7b and 7j were potential ligands, may be due to the hydroxyl and bromo group on the benzene ring contributes better scavenging ability. Some of the compounds were emerged as active against the target cancer cells MCF-7 and Hep G2. Among them, 7-[2-[(4-bromophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3-methyl-5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7j) and 7-[2-[(3-nitrophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7c) were showed remarkable effect against MCF-7 cell lines with $IC_{50}$ values of 16.36 and 19.61 µg/ml respectively, which were almost equal to the standard 5-FU, showed 14.34 µg/ml. We also found that 7-[2-[(2-hydroxyphenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3,5-dimethyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7b) and 7-[2-[(4-bromophenyl)methylideneamino]phenyl]-4-(4-hydroxy-2-nitroanilino)-3-methyl, 5-phenyl-4,6-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ol (7j)

were potential compounds against Hep G2 with the $IC_{50}$ values 32.36 and 34.65 µg/ml where, standard 21.36 µg/ml. Taken together, these findings highlight the potential of the series of compounds as completely novel antitumor candidates and additional research concentrating on the derivatives is still being done.

## CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

## ACKNOWLEDGMENTS:

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## Abbreviations:

ATP: Adenosine triphosphate
CDK: Cyclin Dependent Kinase
DMSO: Dimethyl sulphoxide
DNA: Deoxyribonucleic acid
DPPH: 1, 1-diphenyl-2-picryl-hydrazyl
FDA: Food and Drug Administration
Hep G2: Hepato cellular carcinoma
MCF-7: Human breast cancer cell line
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NCCS: The National Centre for Cell Science

NMR: Nuclear magnetic resonance
PABA: Para Amino Benzoic Acid
PMX: Pemetrexed
PP1: Pyrazolopyrimidine
PP2: Pyridopyrimidine
ROS: Reactive oxygen species
SRC: Proto-oncogene tyrosine-protein kinase
TLC: Thin Layer Chromatography

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