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Identification of potential CDK 8 inhibitor from pyrimidine derivatives via *In-Silico* approach

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ABSTRACT

Pyrimidinesare six-membered heterocyclic scaffold present naturally in nucleic acid components and are promising leads for the synthesis of medicinally important compounds. Cyclin-dependent kinases (CDKs) with a serine/threonine catalytic core are important druggable targets for cancer therapy and the binding of regulatory subunits controls them. In the present study series of virtually designed pyrimidine derivatives were screened using molecular docking techniques against the cyclin-dependent kinase-8 (CDK8) as a targeted protein. The density functional theory calculation of compounds having good binding affinity was done to estimate the orbital energy. The molecular dynamics simulation of the best-docked compound with the CDK8 was simulated to estimate the effect of mobility on the interactions.Themolecular docking provided insights regarding the binding ability of the designed compounds with the targeted CSK8 structure. As a result, the docked compounds exerted good interactions with the CDK8, and the compound PB129 showed the highest negative binding affinity of -12.4 kcal/mol with the formation of two hydrogen bonds. The results of the simulation study indicated that the complex of CDK-8 and PB129 hasa tight binding with constant hydrogen contacts. Moreover, the density functional theory indicated that PB129 has strong orbital energy and this compound will show tight interactions by either donating or accepting the electron with protein structure. Studied compounds showed good results for the docking study by exerting tight binding with the CDK-8 (PDB 6T41). Compound PB129 showed stable confirmation over the simulation run and has good orbital energies. Compound PB129 may act as a lead against the CDK8. .

Keywords: Cancer, Cyclin-dependent kinases-8, In-silico approach, Pyrimidine derivatives.

INTRODUCTION

Cancer is the second main cause of mortality in the world and cancer is a significant diagnostic challenge, followed by therapeutic effectiveness [1]. According to Global Cancer Observatory (GLOBOCAN), cancer death and prevalence are predicted to increase to 29.5 million and 16.3 million, respectively, by the year 2040 [2]. Variations in both the incidence rates and death rates of cancers have an impact on the future burden of these diseases, in addition to increases in cancer deaths carried on by demographic changes [3]. The occurrence of obesity, alcohol addiction, cigarette smoking, the influence of hereditary factors, physical inactivity, and poor nutrition are all risk factors for cancer [4]. India comes under the nations with a National Cancer Control Program, which is supported by the World Health Organization (WHO). WHO primarily contributes to the fight against the use of tobacco [5]. The most prevalent cancers produced by persistent infections are those of the uterine cervix, stomach, and liver caused by HPV, Helicobacter pylori, HBV, and Hepatitis C (HCV) virus, respectively [6]. The selective therapies are based on a better understanding of the biology and molecular genetics in the tumor progression used for the prospective treatments, in addition to common cancer treatments like surgery, radiation therapy, chemotherapy,

combination therapy, and laser therapy [7]. The primary focus of research is on cancer therapy strategies that target the different aspects of the disease [8]. The concept that targeted therapies specifically inhibit the growth of cancer cells or kill them gives them an advantage over both chemotherapy and radiation treatments [9].The discovery of new cancer treatments and their development is considered to be an extremely time and money-consuming process [10].

The cell cycle cyclin-dependent kinases (CDKs) are essential for regulating both cellular transcription and the change between cell cycle stages [11]. Based on respective homologous sequences, 21 CDKs, and 5 CDK-like genes have been found in the human genome [12]. Some members of the CDK family have not played a direct role in controlling the cell cycle and those are CDK7, CDK8, and CDK9, which are involved in the control of transcription [13]. Since it was established that CDK-8 plays crucial roles in oncogenesis, it has received considerable interest recently [14]. Numerous substrates involved in transcription, DNA repair, and metabolic activities were revealed to be phosphorylated by CDK-8 [15]. A crucial aspect of the mediator complex is CDK-8 and specifically, CDK-8 is closely linked to the transcription of genes involved in the oncogenesis of several cancers, including colorectal, breast, prostate, and hematological malignancies [16]. Utilizing CDK8 inhibitors may have two main effects: first, directly targeting cancer cells; and second, indirectly stimulating natural killer (NK) cells to more effectively lysis cancer cells [17]. CDK8 was discovered to be an oncogene that frequently amplifies or is over expressed in colorectal cancer (CRC) and plays a significant role in colorectal carcinogenesis [18]. CDK8 binds to cyclin C (CCNC), which regulates several signaling pathways for the formation and progression of cancer and it plays a role in the regulation of tumor stress, energy supply, and drug resistance mechanisms [19]. Epithelial-to-mesenchymal transition (EMT) is essential for the invasion and metastasis of breast cancer cells, which is promoted by CDK-8 [20].

MATERIALS AND METHODS
Computational methods
Ligand preparation
ACD/ChemSketch software was used to draw the chemical structures and SMILES of designed compounds and saved them in mol2 file format as shown in Table 1.Hydrogen atoms were added to designed chemical structures via BIOVIA Discovery Studio to correct the ionization and tautomeric state [21].Further, the protonated chemical structures were energy minimized using the Open Babel plugin of the PyRx 0.8 program [22]. The MMFF94 force field with the steepest descent algorithm was applied to minimize the energy of newly designed compounds. Energy-minimized compounds were then converted to pdbqtfile format for docking study.

Protein preparation
The previously reported 3D crystal structure of CDK8 (PDB 6T41) havinga resolution of 2.45 Å was acquired from the online RCSB Protein data bank (Available at https://www.rcsb.org/) [23,24]. The downloaded protein was refined for docking study by removing all the HETATM. Further,polar hydrogen atoms were added to protonate and correct ionization as well as tautomeric states of amino acids of the refined protein structures. The protein refinement step was performed using BIOVIA Discovery Studio [21].

Molecular docking
A molecular docking study of a virtually designed ligand library with CDK8 (PDB 6T41) was achieved using various modules of the PyRx 0.8 program [25,26]. Initially, energy-minimized ligands and structure of CDK8 were imported in PyRx and selected in the AutoDOck Vina wizard module of PyRx 0.8.[27,28]. The blind docking protocol was used to explore the entire protein surface for binding ability with the docked compounds. The exhaustiveness was set to default at 8.[29] The best-docked pose with the highest negative binding affinity was saved and binding interactions were analyzed with the help of BIOVIA Discovery Studio [21].

Density functional theory assessment
The DFT study of the designed compounds was done with the help of the Orca 4.2.1 package [30]. The input files for the orca were generated via the orca-enhanced version of Avogadro and the same tool was used to visualize the frontier molecular orbitals (FMO)[31].The B3LYP functional was implemented to optimize the compound structure [32–34]. The def2-SVP basis set was used to perform the final DFT calculation [35]. The FMO analysis and global chemical reactivity descriptors of synthesized compounds were estimated according to the previously reported equations of Koopmans' theory [36,37].

Molecular dynamics simulation
Molecular dynamics (MD) simulation study was carried outrushing GROMACS with the GROMOS96 43a1 force field [38–40]. The PRODRG2 server was utilized to generate the ligand topologies [41].Solvation of the entire protein-ligand complex was done using a simple point charge (SPC) water model with a triclinic box [42]. The energy minimization (EM) of the complex systems was achieved with 10,000 steps of the steepest descent algorithm. The present MD simulation study was carried out in the presence of 0.15 M NaCl.[28,43] Equilibration of the simulated complex systems was performed with canonical (NVT) and isothermal–isobaric (NPT) ensembles after the completion of each step of EM[44–46].The temperature was kept constant at 310K using the Nosé-Hoover thermostat approach while pressure on the system was kept constant at 1.0 bar using the Parrinello-Rahmanbaro stat approach to control the simulated complex systems [47–49]. The prepared complex systemwas simulated for 50 ns and the output MD trajectory was used further for statistical analysis of deviation, fluctuation, and formation of the number of hydrogen contacts [50].

Table 1: List of Pyrimidine derivatives studied

Code	SMILES
PB1	Clc1nc2ccccc2cc1c1cc(nc(n1)N1N=C(C)CC1=O)c1ccccc1
PB2	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB3	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB4	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB5	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB6	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB7	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB8	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB9	[O-][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB10	[O-][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB11	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB12	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB13	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB14	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB15	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB16	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB17	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB18	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB19	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB20	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB21	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB22	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB23	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB24	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB25	[O-][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB26	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB27	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB28	Cc1cc(C)cc(C)c1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB29	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccccc2nc1Cl
PB30	Nc1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1
PB31	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB32	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB33	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB34	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB35	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB36	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB37	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB38	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB39	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB40	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB41	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB42	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB43	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB44	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB45	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB46	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB47	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB48	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB49	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB50	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB51	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB52	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB53	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB54	[O-][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB55	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB56	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB57	Cc1cc(C)cc(C)c1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB58	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(N)ccc2nc1Cl
PB59	Brc1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1
PB60	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB61	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB62	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB63	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB64	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB65	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB66	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB67	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB68	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB69	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB70	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB71	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB72	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB73	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB74	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB75	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB76	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB77	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB78	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB79	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB80	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB81	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB82	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB83	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB84	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB85	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB86	Cc1cc(C)cc(C)c1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB87	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Br)ccc2nc1Cl
PB88	Oc1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1

PB89	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB90	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB91	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB92	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB93	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB94	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB95	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB96	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB97	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB98	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB99	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB100	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB101	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB102	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB103	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB104	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB105	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB106	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB107	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB108	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB109	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB110	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB111	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB112	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB113	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB114	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB115	Cc1cc(C)cc(C)c1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB116	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(O)ccc2nc1Cl
PB117	Clc1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1
PB118	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB119	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB120	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB121	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB122	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB123	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB124	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB125	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB126	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB127	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB128	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB129	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB130	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB131	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB132	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB133	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB134	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB135	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB136	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB137	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB138	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB139	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB140	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB141	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB142	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB143	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB144	Cc1cc(C)cc(C)c1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB145	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(Cl)ccc2nc1Cl
PB146	[O-][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1
PB147	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3N)c(Cl)nc2cc1
PB148	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(N)c3)c(Cl)nc2cc1
PB149	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(N)cc3)c(Cl)nc2cc1
PB150	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(Br)cc3)c(Cl)nc2cc1
PB151	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)cc3O)c(Cl)nc2cc1
PB152	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(O)c3)c(Cl)nc2cc1
PB153	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)cc3)c(Cl)nc2cc1
PB154	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB155	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB156	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(SC)cc3)c(Cl)nc2cc1
PB157	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB158	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB159	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB160	[O[N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(OC)c(O)c(OC)c3)c(Cl)nc2cc1
PB161	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(C)c3)c(Cl)nc2cc1
PB162	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3)c(Cl)nc2cc1
PB163	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(F)cc3)c(Cl)nc2cc1
PB164	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(OC)c(OC)c(OC)c3)c(Cl)nc2cc1
PB165	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(C)cc3C)c(Cl)nc2cc1
PB166	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3OC)c(Cl)nc2cc1
PB167	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(Cl)ccc3Cl)c(Cl)nc2cc1
PB168	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)ccc3OC)c(Cl)nc2cc1
PB169	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(Cl)c(Cl)c3)c(Cl)nc2cc1

ID	SMILES
PB170	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(ccc2nc1Cl)[N+]([O-])=O
PB171	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(C)ccc3O)c(Cl)nc2cc1
PB172	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3)c(Cl)nc2cc1
PB173	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3c(C)cc(C)cc3C)c(Cl)nc2cc1
PB174	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)c(OC)c3)c(Cl)nc2cc1
PB175	[O-][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2c1
PB176	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3N)c(Cl)nc2c1
PB177	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(N)c3)c(Cl)nc2c1
PB178	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(N)c3)c(Cl)nc2c1
PB179	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(Br)cc3)c(Cl)nc2c1
PB180	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)cc3O)c(Cl)nc2c1
PB181	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(O)c3)c(Cl)nc2c1
PB182	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)cc3)c(Cl)nc2c1
PB183	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB184	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB185	[O][N+](=O)c1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(SC)cc3)c(Cl)nc2c1
PB186	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB187	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB188	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB189	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(OC)c(O)c(OC)c3)c(Cl)nc2c1
PB190	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cccc(C)c3)c(Cl)nc2c1
PB191	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3)c(Cl)nc2c1
PB192	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(F)cc3)c(Cl)nc2c1
PB193	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(OC)c(OC)c(OC)c3)c(Cl)nc2c1
PB194	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(C)cc3C)c(Cl)nc2c1
PB195	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3OC)c(Cl)nc2c1
PB196	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(Cl)ccc3Cl)c(Cl)nc2c1
PB197	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(OC)ccc3OC)c(Cl)nc2c1
PB198	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(Cl)c(Cl)c3)c(Cl)nc2c1
PB199	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2ccc(cc2nc1Cl)[N+]([O-])=O
PB200	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3cc(C)ccc3O)c(Cl)nc2c1
PB201	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(OC)cc3)c(Cl)nc2c1
PB202	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3c(C)cc(C)cc3C)c(Cl)nc2c1
PB203	[O][N+](=O)c1ccc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccc(O)c(OC)c3)c(Cl)nc2c1
PB204	Cc1cc2cc(c3cc(nc(n3)N3N=C(C)CC3=O)c3ccccc3)c(Cl)nc2cc1
PB205	Nc1ccccc1c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB206	Nc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB207	Nc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB208	Brc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB209	Oc1ccc(c(O)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB210	Oc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB211	Oc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB212	[O][N+](=O)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB213	[O][N+](=O)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB214	CSc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB215	FC(F)(F)c1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB216	FC(F)(F)c1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB217	FC(F)(F)c1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB218	COc1cc(cc(OC)c1O)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB219	Cc1cccc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB220	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB221	Fc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB222	COc1cc(cc(OC)c1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB223	Cc1ccc(c(C)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB224	COc1ccc(c(OC)c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB225	Clc1cc(c(Cl)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB226	COc1cc(c(OC)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB227	Clc1ccc(cc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB228	[O][N+](=O)c1cc(ccc1Cl)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB229	Cc1cc(c(O)cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB230	COc1ccc(cc1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB231	Cc1cc(C)cc(c1)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl
PB232	Oc1ccc(cc1OC)c1cc(nc(n1)N1N=C(C)CC1=O)c1cc2cc(C)ccc2nc1Cl

RESULTS AND DISCUSSION

Molecular docking

The anticancer potential of designed compounds was estimated with the help of a molecular docking study. docking study was accomplished using the Auto Dock Vina module of PyRx 0.8 and the designed compounds were docked on the Cyclin-Dependent Kinase 8 (CDK8) (PDB 6T41). A total of 232 pyrimidine derivatives were used in this study. Canonical SMILES of designed compounds are represented in Supplementary Table S1. The results of the docking study represented that all the docked compounds have a binding affinity of more than -8.7 kcal/mol with strong binding interactions with targeted CDK8 (PDB 6T41) structures. The binding affinity and interactions of all the docked compounds with CDK8 were represented in Supplementary Table S2. The binding affinity of docked compounds ranged between -8.7 to -12.4 kcal/mol. Compound PB71 showed a binding affinity of -12.2 kcal/mol and the interactions were seen between LEU158, VAL35, HIS106, ALA155, and LEU359 with formation Pi-Pi T shaped, Alkyl, and Pi-Alkyl type of interactions. The 2D and 3D visualization of binding interactions formed between compound PB71 and CDK8 (PDB 6T41) are represented in Figure 1a-1b. Compound PB71 formed a single conventional hydrogen bond with

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the TYR32 residue. Whereas the compound PB129 showed the highest negative binding affinity of -12.4 kcal/mol compared to the other docked compounds, the compound PB129 showed good binding affinity. The binding interactions LEU158, VAL35, LEU359, ALA155, and HIS106 showed hydrophobic interactions while TYR32 and ARG356 showed hydrogen bonding with the docked compound PB129. Oxygen and chlorine functional groups present in the Compound PB129 are responsible for the formation of hydrogen bonds with the targeted protein. Hydrogen bonds play a crucial role in the stability of the docked protein-ligand complex, though the docking study was performed with the static condition and the dynamic behavior of the complex may affect the formation of hydrogen bonds. The 2D and 3D binding interactions between the compound PB129 and CDK8 are represented in Fig. 1c-1d

Density functional theory

The DFT calculations were performed to study the molecular geometry and electron distribution in the designed compounds. According to the frontier molecular orbital theory, the Lowest Unoccupied Molecular Orbitals (LUMOs) and Highest Occupied Molecular Orbitals (HOMOs) signify active sites and chemical reactivity of compounds. FMO also has a great influence on the biological potential of compounds as the activity depends on the transfer of electrons between protein and ligand complex [51].The negative chemical potential of the compounds dictates the non-spontaneous decomposition. The HOMO-LUMO energy gap gives information on the electrical transport properties of molecules. The LUMO energy is parallel to the electron affinity (EA) while HOMO energies correspond to the ionization potential (IP) of the compounds [52,53]. The chemical reactivity of the compounds depends on the gap between HOMO-LUMO energies.

The high gap represents low chemical reactivity while the low gap indicates higher chemical reactivity. In Fig. 2, the red colour indicates the positive electron density of the compounds while the negative electron density was represented with blue colour. Compounds PB71, PB86, PB129, PB223, and PB 231 showed a good binding affinity with the targeted CDK8, and the DFT study of these compounds indicated the possibility of higher reactivity and interactions with the targeted proteins. HOMOs of compound PB129 formed tight interactions with the targeted protein structure as shown in Fig 1. The global chemical reactivity descriptors estimated as per the equations of Koopman's theorem for the compounds are represented in Table 2.

Figure 1 a) 3D and b) 2D interactions of compounds PB71and c) 3D and d) 2D interactions of compounds **PB129** with CDK8 (PDB 6T41).
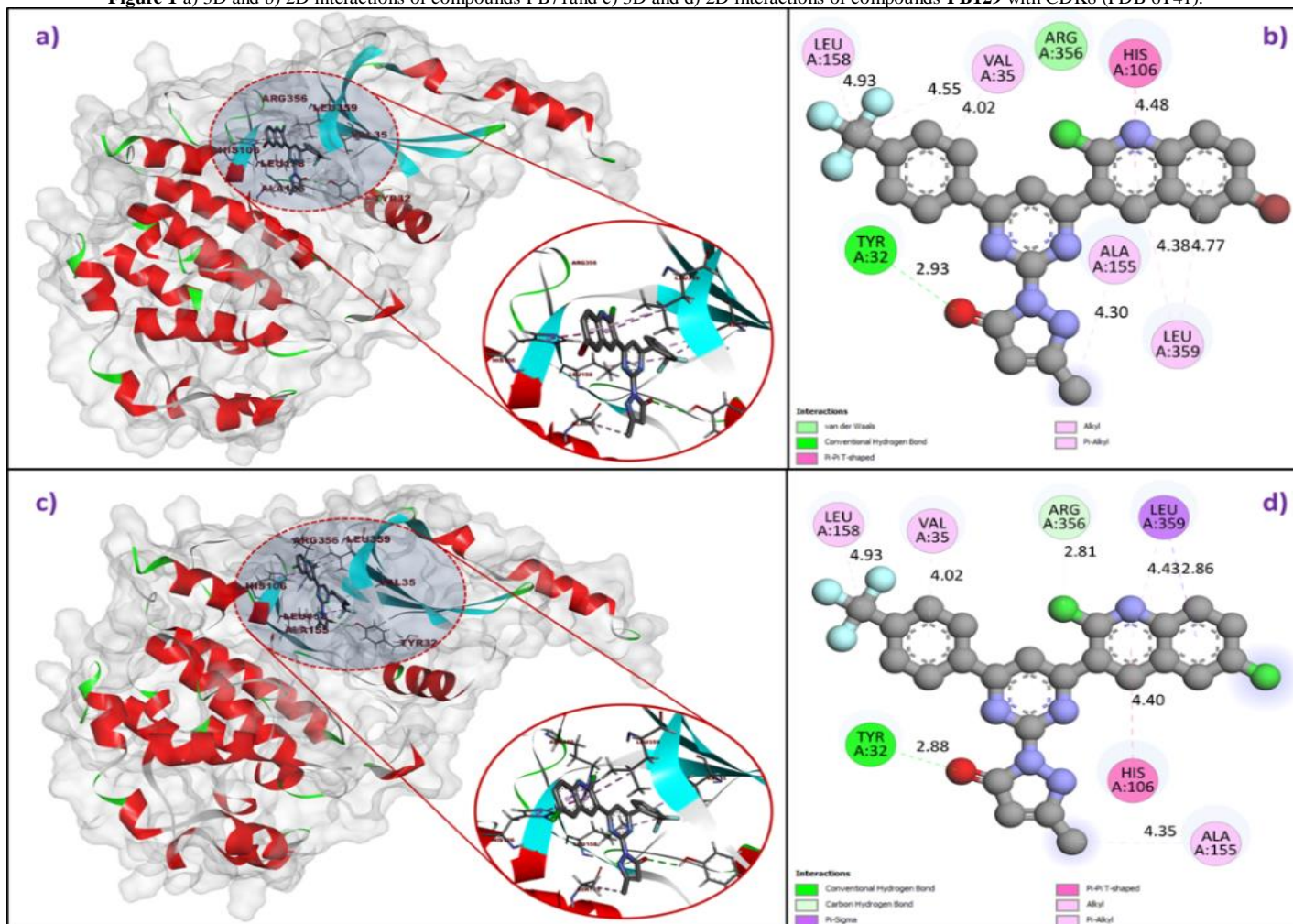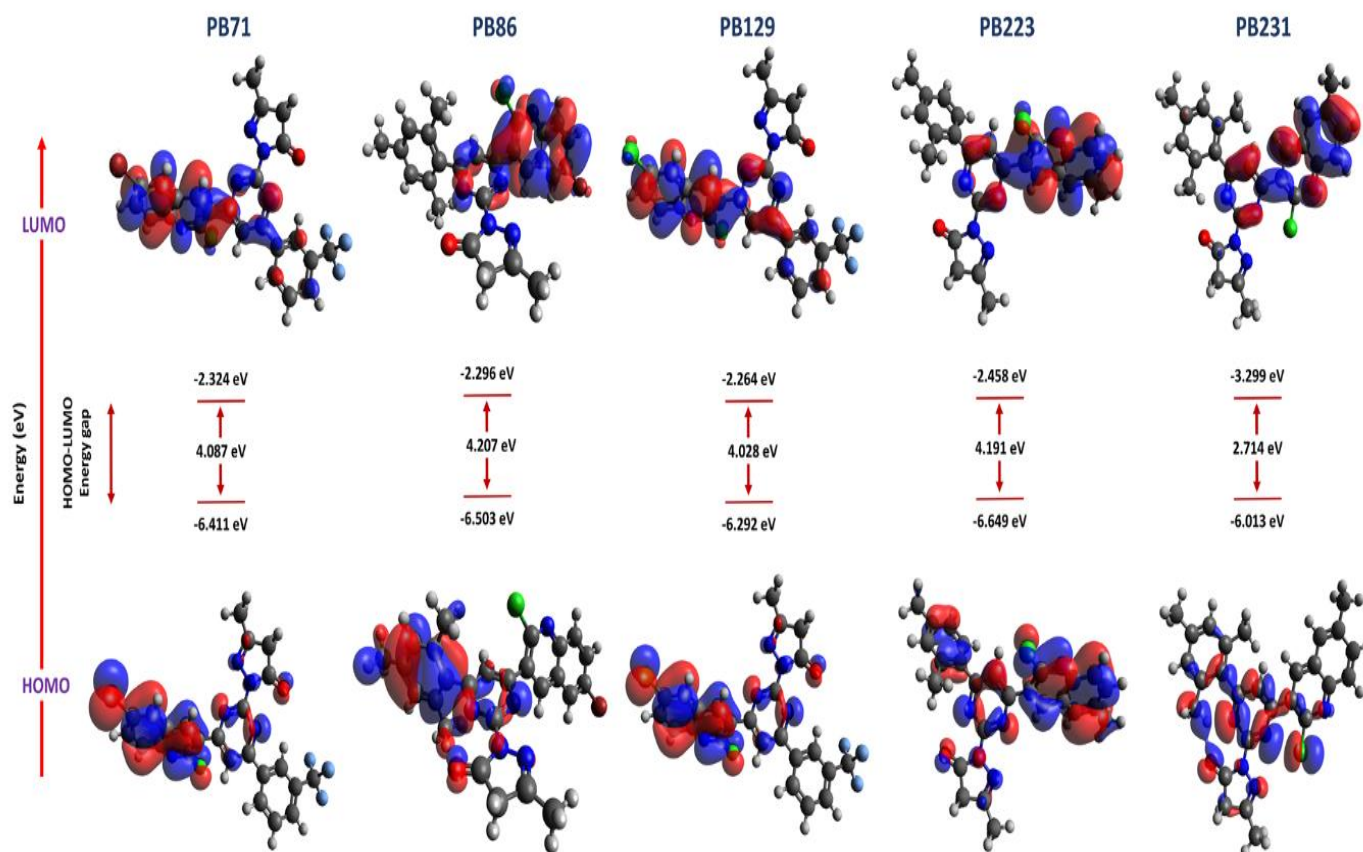
Figure 2: FMO for the designed compound showing the highest negative binding affinity in the dockingg study

Table 2: Frontier orbital energies and theoretical molecular descriptors calculated for the designed compound showing the highest negative binding affinity in the docking study.

Code	HOMO (eV)	LUMO (eV)	HLG (eV)	DM (Debye)	IP (eV)	EA (eV)	χ (eV)	μ (eV)	η (eV)	ω (eV)
PB71	-6.508	-2.395	4.113	4.20031	6.508	2.395	4.4515	-4.4515	2.0565	4.8178
PB86	-6.281	-2.275	4.006	2.95165	6.281	2.275	4.278	-4.278	2.003	4.5684
PB129	-6.498	-2.372	4.126	4.31393	6.498	2.372	4.435	-4.435	2.063	4.7671
PB223	-6.175	-1.974	4.201	4.39198	6.175	1.974	4.0745	-4.0745	2.1005	3.9518
PB231	-5.789	-2.004	3.785	1.26583	5.789	2.004	3.8965	-3.8965	1.8925	4.0112

Molecular dynamics simulation

Molecular docking study of the designed compounds provided information regarding the binding orientations with the CDK8. Compound PB129 showed tight binding with the targeted protein and showed two hydrogen bonds. However, the docked complex needs to undergo the MD simulation study to estimate the stability of the formed interactions between compound PB129 and CDK8 (PDB 6T41). Hence, the molecular dynamics (MD) simulation of the docked protein-ligand complex was done in the mobile phase with an artificial physiological atmosphere. MD simulation of protein-ligand complex systems was done for 50ns. The statistical MD trajectory was done with the parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), and hydrogen bonds (HBs). RMSD was studied to determine the devotions showed by complex during the simulation period of 50ns. Overall,

Complex RMSD ranged between 0.2nm to 0.6nm as shown in Figure 3a. Complex system showed scattering in the final RMSD plot throughout the simulation run. RMSD plot represents the maximum deviations in the complex system between 15ns to 35ns. RMSF of the entire simulated complex showed major fluctuations in the initial amino acid present in CDK8 (PDB 6T41). RMSF of the simulated complex ranged between 0.1nm to 1nm as shown in Figure 3b. Correlation between RMSF and RMSD helped to determine that fluctuated amino acid residues of the targeted protein influenced the deviation in the RMSD. LYS0, ASP1, ASP2, MET1, TYR3, ASP4, PHE5, LYS6, VAL7, and LYS8 are the residues that showed fluctuations during the simulation study. None of the above-mentioned residues showed direct contact with the PB129 in both dockings as well as MD study. Most of the regions of the RMSD and RMSF plots evolved in correlation to each other indicating the stability of the protein-ligand complex.

Figure 3: a) RMSD and b) RMSF of the simulated MD trajectory of 50ns.
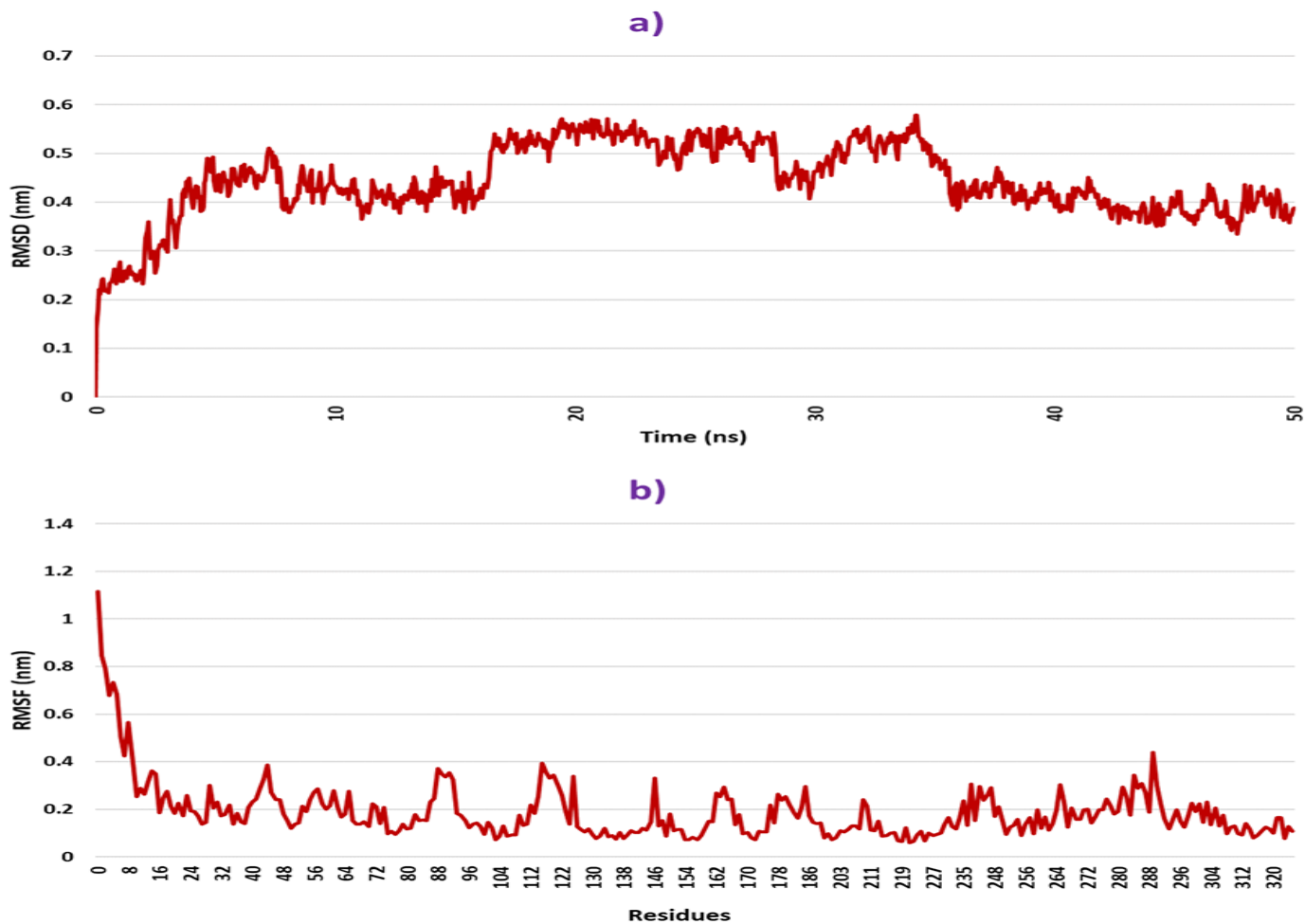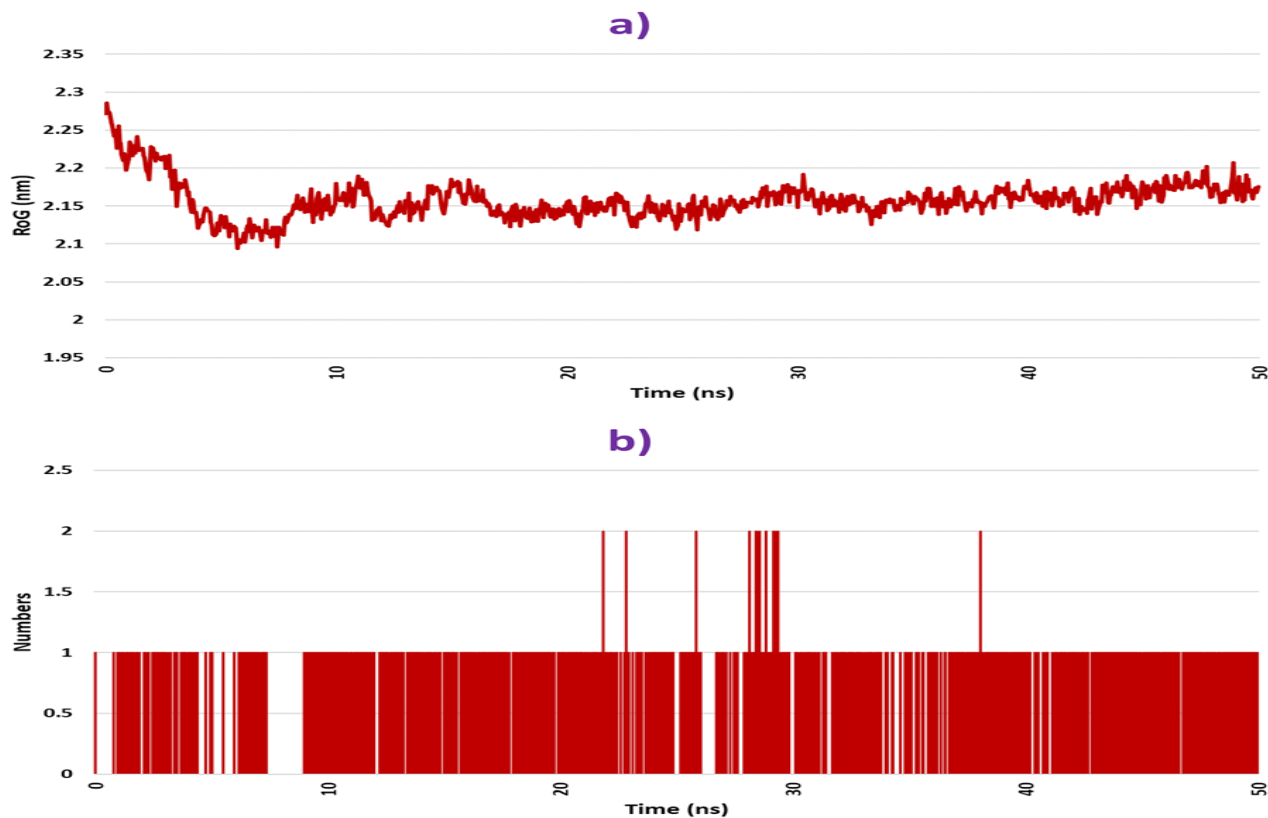

Figure 4: a) RoG and b) number of HBs estimated using the simulated MD trajectory of 50ns

Moreover, the RoG was calculated to study the compactness of the protein-ligand complex system during MD simulation. RoG study helps to determine the folding and unfolding of the complex during the simulation study. A higher RoG value reflects less compactness and unfolding of the complex. The RoG values of the complex system showed consistency with minimum fluctuation in the plot (**Fig 4a**). RoG values stabilized after 10ns indicating the folded state of the complex. Finally, the number of hydrogen bonds (HB) formed during the MD simulation was plot trends shown in **Fig 4b**. The complex system showed one consistent hydrogen bound throughout the simulation run and between 20ns to 30ns two hydrogen contacts were observed between the simulated protein-ligand complexes. Hydrogen bonds between complexes are important to stabilize the protein-ligand complex. Based on the overall statistical analysis of the MD trajectory for compound **PB129** with the CDK8 (PDB 6T41), it is confirmed that the complex system has stable confirmation throughout the simulation with minimum deviation in the RMSD plot and fluctuations in the RMSF plot.

CONCLUSION

The molecular docking study under static conditionsof designed pyrimidine compounds againstCyclin-Dependent Kinase 8 (CDK8) (PDB 6T41) was doneto investigate the anticancer potential and ideal leads against CDK8. The results of the docking study indicated that designed compounds have a good binding affinity (≥-8.7 kcal/mol) and the binding interactions were also found to be appropriate for the formation of hydrogen bonds. Compound **PB129** showed good binding affinity (-12.4 kcal/mol) as compared to the other docked compounds. Further dynamics study of the PB129 will give more insights regarding the behavior of the docked protein-ligand complex. The molecular dynamics study indicated conformational stability with the minimum deviation in the interactions and fluctuations in the protein structure. DFT calculations revealed that all five compounds have good orbital energy and a HOMO-LUMO energy gap. Transfer of electrons between protein-ligand will be present while biological screening. Though the in-silico investigation provided important information regarding the designed compounds, the final confirmation of the anticancer activity needs to be screened with in-vitro or in-vivo models.

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