Molecular docking studies of benzimidazopyrimidine and coumarin substituted benzimidazopyrimidine derivatives: As potential human Aurora A kinase inhibitors

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Abstract: Protein kinases are important drug targets in human cancers, inflammation and metabolic diseases. Docking studies was performed for all the benzimidazopyrimidine and coumarin substituted benzimidazopyridimine derivatives with human Aurora A kinase target (3FDN) employing flexible ligand docking approach by using AutoDock 4.2. All the compounds were found to have minimum binding energy ranging from -6.26 to -9.29 kJ/mol. Among the molecules tested for docking study, 10-(6-Bromo-2-oxo-2H-chromen-4-ylmethyl)-2-isopropyl-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2k) showed minimum binding energy (-9.29 kJ/mol) with ligand efficiency of -0.31. All the ligands were docked deeply within the binding pocket region of 3FDN showing hydrogen bonds with Ala 213 and Asn 261. The docking study results showed that these derivatives are excellent inhibitor of human Aurora A kinase target; and also all these docked compounds have good inhibition constant, vdw + Hbond + desolv energy with best RM value.

Key Words: Benzimidazopyrimidine and coumarin substituted benzimidazopyridimine derivatives, Single crystal structure, Aurora A, Docking studies.

Background: Benzimidazopyrimidine belonging to the fused heterocyclic system have three nitrogen atoms. The most prominent benzimidazole moiety in nature is N-ribosyl -dimethyl benzimidazole, which serves as an axial ligand for cobalt in vitamin B₁₂ [1]. Literature survey revealed that benzimidazole derivatives play a vital role in biological activities such as antifungal, kinase inhibitor, anti-hepatitis-C-virus, antidepressant and anticancer [2-8]. Also, heterocycles containing an imidazolone moiety exhibits various biological activities such as antibacterial and antifungal activities [9-11]. Coumarins are an important class of widely distributed heterocyclic natural products exhibiting several biological properties. 4-Amino-3-(2-methylbenzyl)coumarin derivatives [12] exhibited potent estrogenic activity on the estrogen receptor positive (ER⁺) human MCF-7 breast cancer cell line. Benzothiazolyl coumarin acetamide derivatives [13] exhibited strong in vitro anti-HIV effect against the wild-type HIV-1 cell
line. The *in vitro* antioxidant activities of 4-schiff bases-7-benzyloxy coumarin derivatives [14] revealed that DPPH and ABTS' radical scavenging activities were better than that of the commercial antioxidant BHT.

Human protein kinases are attractive targets for the development of new therapeutics because of their involvement in processes associated with the progression of cancers and metabolic diseases [15, 16]. Aurora kinase family of serine/threonine kinase regulates some important events during mitosis. They play key roles in centrosome maturation and separation, mitotic spindle assembly and chromosome segregation [17]. The role of Aurora A in the cell cycle and tumorgenesis suggested that the inhibition of the kinase activity have remarkable value for the development of small molecular therapeutics for cancer treatment. Based on the current success of Aurora kinase inhibitors in the development of kinase-based cancer therapy, we have initiated a virtual screening program for the identification of Aurora kinase inhibitors.

In our previous paper [20], we have reported the synthesis, *in vitro* antimicrobial and anticanccer activities of novel coumarin substituted dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-ones. In continuation to this, we study herewith, the crystal structure (2g) and molecular docking studies of benzimidazopyrimidine and coumarin substituted benzimidazopyrimidine derivatives with human Aurora A kinase target to evaluate their potential value for the treatment of cancers.

**Methodology:**

*Preparation of ligands*

The 2D structures (.mol) of all the thirteen compounds as tabulated in Table 1 (see supplementary material) were drawn and the structure was analyzed by using ChemDraw Ultra 12.0. All the compounds (2a-2k) are converted to 3D structure (.pdb) using Openbable software tool. The 3D coordinates (.pdb) of each molecule were loaded on to Dundee PRODRG server for energy minimization [21]. The structure of the benzimidazopyrimidine and coumarin substituted benzimidazopyrimidines are shown in Figure 1.

*Preparation of macromolecule*

The protein target, which is retrieved from the RCSB Protein Data Bank (PDB code 3FDN) serves as docking receptor [22]. All the bound ligands and water molecules were removed from the active site of the receptor. For docking target, crucial amino acids of the active site were identified using data in pdbsum as shown in Figure 2 [23].

*X-ray structure determination*

A single crystal of the compound (2g) with dimensions of 0.30 × 0.25 × 0.20 mm was chosen for X-ray diffraction studies. The data were collected on a Bruker SMART APEX II X-ray diffractometer with graphite monochromated MoKα radiation, operating at 50 kV and 30 mA. Raw data was processed and reduced by using APEX2 and SAINT [24]. The structure was solved by direct methods using SHELXS-97 [25]. All non-hydrogen atoms were revealed in the first Fourier map itself. Full-matrix least squares refinement was carried out using SHELXL-97 [25]. Anisotropic refinement of non-hydrogen atoms was started at this stage. Subsequent refinements were carried out with anisotropic thermal parameters for non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms which were placed at chemically acceptable positions. CCDC- 990917 contains the supplementary crystallographic data of molecule 2g [26]. The X-ray structure of this compound (2g) was used for the docking studies.

**Figure 1.** Structure of (A) Bennzimidazopyrimidine (B) Coumarin substituted benzimidazopyrimidine derivatives.

**Figure 2.** Ligplot results of Aurora A (3FDN), showing the binding of ligand Mmh 1(A) with amino acid residues present in an active site pocket.
bonds were set by the AutoDock tools and all torsions were allowed to rotate. Polar hydrogen atoms were added and Kollman charges were assigned to the protein using AutoDock tools (ADT). The grid map was centered at the active site pocket of the protein by Autogrid. The grid map which was centered at the following residues of the protein (Thr 217, Arg 220, Arg 137, Leu 139, Pro 214, Ala 213, Gly 216, Tyr 212, Gly 140, Leu 263, Val 147, Glu 211, Leu 194, Leu 210, Ala 160) were predicted from the ligplot (Figure 2). In all the cases, we have used grid maps with a grid box size of 55×55×55 Å³ points with a grid-point spacing of 0.375 Å. During docking, centre grid parameters were specified for x, y and z axis as -2.0, -32.0 and 7.0, respectively. The Lamarckian genetic algorithm, the pseudo-Solis and Wets methods were applied for minimization using default parameters. Binding energy, torsional energy, intermolecular energy, number of H-bonds and RMS value were recorded in each ligand bound conformations. The details of dock score results of the different benzimidazopyrimidine derivatives are given in Table 2 (see supplementary material).

Results & Discussion:
The crystal structure analysis showed that the compound (2g) crystallizes in monoclinic system under the space group P2₁/n with cell parameters, a = 9.3819(12) Å, b = 8.0471(10) Å, c = 26.808(4) Å, β = 93.592(7)° and Z=4. The crystal structure was solved by direct methods and refined by full-matrix least squares on F² to a final residual value of R₁ = 0.0486.

Automated docking was used to assess the binding modes and conformation of the ligand molecules. Docking studies was performed for all the benzimidazopyrimidine and coumarin substituted benzimidazopyrimidine derivatives. In docking study, all the compounds were found to have minimum binding energy ranging from -6.26 to -9.29 kJ/mol. Among the docked molecules, 2h, 2i, 2j and 2k revealed minimum binding energy of -8.8, -9.1, -8.9 and -9.29 kJ/mol, with ligand efficiency of -0.29, -0.29, -0.3 and -0.31, respectively. These molecules were completely wrapped by active site amino acid residues at the active site pocket region (as shown in Figure 3).

The protein (3FDN) comprises of fifteen active site residues, which are promiscuous to the ligands. Out of which only two (Ala 213 and Asn 261) residues are directly interacting with the ligands. Most of the residues that are in close proximity to the inhibitor are hydrophobic in nature. All the ligands were docked deeply within the binding pocket region of 3FDN. The ligand molecules 2e, 2f, 2h and 2k were found to show hydrogen bond interaction with active site amino acid residues Ala 213 and Asn 261 at a distance of (1.923 and 2.203), (1.909 and 1.925), (2.234 and 1.928) and (2.024 and 2.104) Å, respectively (Figure 4). The ligands 2a, 2b, 2g and 2j showed only one hydrogen bond interaction with protein, where as ligand 2c and 2i has no interaction with the protein. The docking results for all ligand molecules against protein target showed minimum intermolecular energy, ligand efficiency, binding energy, inhibition constant and vdW + Hbond + desolv energy with best RMSD value. From this study, we have predicted ligands 2h, 2i, 2j and 2k may act as potential inhibitors of human Aurora A kinase enzyme.

Figure 3: Enfolding of molecules 2e, 2f, 2h and 2k in the active site pocket

Figure 4: H-bond interaction of ligand molecules (2e, 2f, 2h and 2k) with 3FDN

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### Table 1: Different groups of benzimidazopyrimidine and coumarin substituted benzimidazopyrimidine derivatives

| Compounds | R  | R1     |
|-----------|----|--------|
| 2a        | ipr| -      |
| 2b        | 4-CF3C6H4 | -    |
| 2c        | 3-FC3H4  | -      |
| 2d        | CF3     | 6-OCH3 |
| 2e        | CF3     | 6-F    |
| 2f        | CF3     | 6-CH5  |
| 2g        | CF3     | 6,8-(OCH3)2 |
| 2h        | ipr     | 6-F    |
| 2i        | ipr     | 6-OCH5 |
| 2j        | ipr     | 6-Cl   |
| 2k        | ipr     | 6-Br   |

### Table 2: The dock score results of the different benzimidazopyrimidine and coumarin substituted benzimidazopyrimidine derivatives

| Compounds | Binding Energy (kJ mol⁻¹) | Ligand Efficiency | Inhibition Constant | vdW+H-bond+desolv energy | No. of H-bonds | Bonding residues | Bond Length (Å) |
|-----------|--------------------------|-------------------|--------------------|--------------------------|----------------|-----------------|-----------------|
| 2a        | -6.26                    | -0.37             | 25.94              | -6.55                    | 1              | 3FDN:A:ALA213:HN | 1.778           |
| 2b        | -7.3                     | -0.3              | 4.47               | -7.59                    | 1              | 3FDN:A:ALA213:HN | 1.959           |
| 2c        | -7.28                    | -0.35             | 4.58               | -7.48                    | -              | -               | -               |
| 2d        | -8.03                    | -0.25             | 1.3                | -9.23                    | 2              | 3FDN:A:ALA213:HN | 2.063           |
| 2e        | -8.13                    | -0.26             | 1.1                | -8.89                    | 2              | 3FDN:A:ALA213:HN | 1.923           |
| 2f        | -8.39                    | -0.27             | 706.74             | -9.33                    | 2              | 3FDN:A:ALA213:HN | 2.090           |
| 2g        | -8.67                    | -0.27             | 412.37             | -9.49                    | 1              | 3FDN:A:ALA213:HN | 1.938           |
| 2h        | -8.8                     | -0.29             | 354.57             | -9.69                    | 2              | 3FDN:A:ALA213:HN | 2.234           |
| 2i        | -9.1                     | -0.29             | 214.1              | -10.34                   | -              | -               | -               |
| 2j        | -8.9                     | -0.3              | 301.24             | -9.79                    | 1              | 3FDN:A:ALA213:HN | 2.18            |
| 2k        | -9.29                    | -0.31             | 158.7              | -10.23                   | 2              | 3FDN:A:ALA213:HN | 2.024           |