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Virtual Screening and Discovery of Novel Aurora Kinase Inhibitors

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Abstract: Cancer remains one of the major contributors to human mortality and a hazard to human growth. The search for a new treatment continues unabated. Aurora kinases play an important role in cell cycle, and thus a potential target for the treatment of cancer. In the present work, we aim to discover potential leads against aurora kinase using various rational methods of drug discovery. The available crystal complexes of AKs were analyzed for their interactions and quantified with glide-extra precision (XP) docking. About 20 crystal pdb were selected from the protein databank based on the resolution factor, R-factor and R-value. And after docking with the native ligands, the RMSD value was calculated, wherein the protein with the least RMSD was found to be 3UOK which was further used for our screening of small molecules from the in-house database by molecular docking. Fragments which were found to possess the best interactions were considered for the synthesis with characterization, and biological activity was carried out against breast cancer and colorectal cancer cell lines to assess the inhibitory capability of synthesized compounds. Molecule with the molecular id IS2 i.e. (3E)-3-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-2H chromene-2,4(3H)-dione was found to possess inhibitory activity with an IC_{50} of 1.324nM and 5.785μM for breast cell line and colorectal cell line studies, respectively.

Keywords: Aurora kinase, cancer, docking, glide score, *in-vitro* cell line studies, PDB.

1. INTRODUCTION

Cancer is just a generic term for more than 200 diseases whose hallmark is an abnormal cell division. Untreated abnormal cell division leads to cancer cells which could end in severe illness and death. It also has the capability of invading the surrounding cells, which normal cells cannot do. Normal cells divide, grow and die, but for cancer cells the process of death is defective as those cells keep growing. Cancer cells may or may not spread and if they spread the process is known as metastasis. Cell cycle consists of many stages which include the mitotic and synthetic phase, gaped with three gap phases. All the stages are controlled by checkpoints, namely, the proteins [1]. Any defect in this process will lead to the abnormal growth of the cell which in turn leads to the formation of cancer. About 7.6 million cancer patients were reported as of 2007 [2], which makes cancer one of the major lifestyle diseases responsible for human mortality.

A lot of studies are in progress on the proteins that act as checkpoints in the cell division process. Proteins that act selectively during the segregation process of the cell division include Polo kinases, Microtubules, Kinesin motors, and the Aurora kinases [3]. Aurora kinase belongs to the serine threonine type protein family. First discovered in 1995 and first found in human cancer in 1998 [4], Aurora kinase was first detected in Drosophila where kinase was found to be responsible for the failure of segregation due to some mutation [5]. There are three types of Aurora kinase: (a) Aurora kinase A, (b) Aurora kinase B, (c) Aurora kinase C [6]. Aurora kinase A acts at the poles during cell division whereas Aurora kinase B acts at the points where the spindle fiber attaches the chromatids, hence the names polar and equatorial kinase, respectively [7]. The Aurora kinase C subtype is far less explored, and its action is a lot less evident. Aurora kinase A plays its role in the proper centromere maturation and its segregation during the cell division process (Fig. **1**), hence its inhibition or loss will lead to the formation of cells of the monospindle type [8,9]. On the other hand, Aurora B is found to act at a stage earlier to the chromosome condensation as well as being found to complete the cytokinesis process. Its loss will lead to errors in the process of chromatid segregation and may also result in early anaphase [10, 11, 12]. Aurora C, which has a similar sequence to Aurora B, shows many similar features to those of Aurora B but is still assumed to be less explored due to a lack of information about its expression pattern [13]. Defect in the

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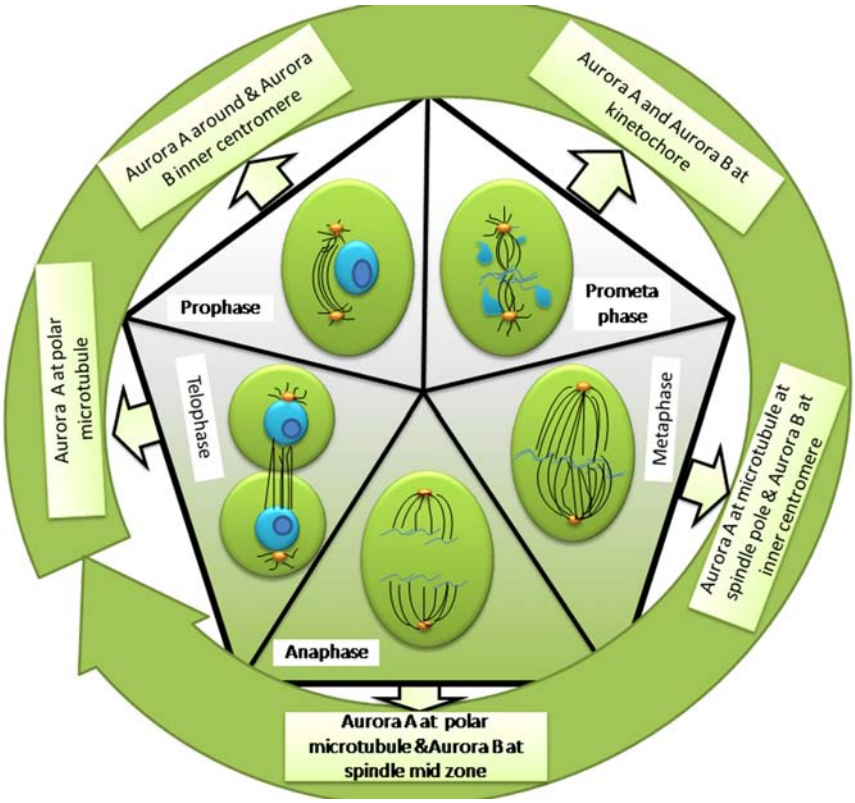


Fig. (1). Cancer cell cycle with contribution of Aurora subtypes in each stage of cell proliferation.

action of these proteins is one of the major causes of tumerogensis (aneuploidy, mitotic arrest, and cell death). Inhibitors of these three subtypes are being looked at in the hope that a better treatment may soon be provided to cancer patients [14].

Aurora kinase is a serine threonine type of protein with amino acids ranging from 309 to 403 depending upon the subtype (A, B or C), and found to be highest for subtype A [15] which has two active regions, one at the end terminal which is regulatory whereas the other C terminal is the catalytic site. The catalytic site has two lobes which are joined by the hinge region with the two lobes as the end terminal, positioning itself to form a pocket for ATP, and the C terminal for the substrate [16,17]. At the hinge region, the Glu 211 and Ala 213 are responsible for the conservation of hydrogen bond interaction with the inhibitors. This lobe conformation determines the kinesis activity. Twenty-six amino acids line up to form the site to which the ATP binds, which is found to be common for all the subtypes with a variation on Aurora A (Leu 215, Thr 217 and Arg 220) [18].

2. AURORA KINASE INHIBITORS AND MAJOR INTERACTIONS

Imidazopyrazinederivatives were found to be active because of their ATP competitive binding behavior. It was found to possess activity of IC_{50} value, ranging from ≤4nM and ≤13nM for Aurora A and B, respectively. They are found to form the H-bond interaction with amino acids Ala213 (hinge region) and Asp 274 in the target site. It is also suggested that the sixth position of Imidazopyrazine derivatives is spread out in the hydrophobic portion of the binding cavity and the eighth is exposed to the solvent which is vital for the inhibition of cell proliferation. These compounds are found to be potent when administered intravenously although found not promising on oral administration. Hence, these molecules were optimized with a fluorine substitution in the core structure to obtain molecules with improved biological activity on oral administration by decreasing the basicity of the nitrogen in the neighboring amines which simultaneously cause a decrease in the pKa value of the nitrogen, there by influencing the pharmacokinetic characteristics [19].

A pentacylic scaffold bears oxothiazole, cycloheptane, pyrrole and indole, where a hydrocarbon ring usually constrains a ring of more than 6 atoms, and when the lactam ring is introduced it raises the potency to about 10 times to a molecule containing a pyrazole and indole as its core. Trans-oriented lactam rings with the substituted methyl group structures were found to be more active than the cis form as they spread over the solvent region to inhibit the solubilizing of the methylpiperzines which are vital as they bind tightly onto the back of the ATP pocket [20].

In the class of Imidazopyridinederivatives, the potent one is the 6-chloro substituted imidazo[4,5-b]pyridine derivative, fused with the piperizine, and thiazole structures were found active against all the subtypes of Aurora kinase, i.e.A, B and C, by showing the IC_{50} values as 0.042, 0.198 and 0.227 nM, respectively. The hit that is Imidazo [4,5-b]pyridine] was obtained via the in-silico technique known as the high throughput virtual screening (HTVS) with the database against the recombinant Aurora kinase A protein. These molecules were found to be establishing bidentate hydrogen

bonds with the Ala213 at the hinge region of the ATP binding site, contributed by the nitrogens of the imdazole (N3) and pyrazole (N4) [21, 22].

Indirubins, which originally were the glycogen synthase kinase-3-α/β -(GSK-3-α/β) inhibitors, were found promising against Aurora kinase inhibitors with the simplest synthetic chemical variation.Instead of the 6-bromo substitution, it was transferred to a 7-bromo substituted molecule which was found to be more potent, in addition to being active against B and C subtypes of Aurora kinase. The amino acids involved in the selective activity established by the above compound were Thr217 for AurA, Glu161 for AurB, Glu127 for AurC. It was also noted that 7-bromo substituted molecules were found to be more dominant for the subtypes B and C than for A [23].

Isoindole derivatives, having the property of forming pseudo rings due to the intra hydrogen bonding, were found to be active against both Aurora kinase and the vascular endothelial growth factor (VEGF). The most potent was the piperizine-1-yl derivative which was found be orally available. And because of the dual action (AKA and VEGF) of these compounds, the activity range of these scaffolds on cancer prophylaxis is more widespread [24].

Tricyclic compounds with pyrazole, pyrrole and pyridine scaffold and p-methoxyhydrazones derivatives were found active against Aurora kinase A, which are obtained as heterocyclic hits by virtual screening of the database on the basis of already existing Aurora kinase A inhibitors. The interaction of the potent tricycle compound, which has the tricyclic scaffold incorporated with the substitution of a benzyl and furan, was found to form hydrogen bonding with the hinge region by the NH group of the central ring of the tricyclic scaffold to the carboxylic group of the Ala 213, and the oxygen atom of the carbonyl group of the pyrrolidine ring with that of the NH group present on the main chain of the Ala 213.The residues involved in the hydrophobic interaction with the potent compound were Leu139, Gly216, and Leu263. The derivatives containing the furanyl and phenyl nucleus provide the extra stability by forming the hydrogen bond, and the hydrophobic bonds with furanyl were interacting with the residues Arg137 and Arg220. However, the phenyl group extended its hydrophobic interaction domain next to the hinge region where the residues involved were found to be Val147, Leu194, Leu210, Leu263, Ala273, and Asp274 [25].

1,4,5,6-tetra pyrrolopyrazole are the type 1 inhibitors of Aurora kinase and these bicyclic ring systems act by targeting the ATP binding site of the Aurora kinase proteins. Identification of these bicyclic rings as Aurora kinase inhibitors was established based on the biological and physicochemical aspects. Derivatives of the bicyclic compound having a methyl piperizine and diethyl benzamide as their substitution were found to be the most potent with the IC_{50} value of 0.027μM. In another study, where the lead, i.e. 5-phenylacetyl 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole was optimized, the compound having the methoxy group was found to be the most potent, and the IC_{50} value for this compound was found to be 13 nM and 79 nM for Aurora A and B, respectively [4, 26, 27].

The amine heterocyclic derivatives, such as the pyrimidine amine, 4-amino thienol, pyrazolo [4, 3-H] quinazoline, N-substituted 2'(amino aryl) benzyl thiazole, bisanilino pyrimidine, cyclohexyl derivative of benzimidazole, pyrimidodiazepine derivative & phthalazinone-pyrazole derivative, were found to be potent inhibitors generally against all the subtypes of Aurora kinase [4, 28, 29, 30, 31, 32] .The different cores that potential against Aurora kinase inhibition is summarised in Fig. (**2**).

The aurora kinase inhibitors, which are now on clinical trials, are reported to be MLN8054 which is the first orally available drug against aurora kinase and the first selective drug to enter into the human clinical trial phase. In the xenograft model, it resulted in the mitotic accumulation and the apoptosis, and in the phas- 1 trials it showed grade 3 which was found to be reversible somnolenc [33, 34]. VX-680, otherwise known as MK-0457, is a pyrimidine derivative with affinity for all the subtypes of the aurora kinase protein where the pre-clinical model of the above molecule showed blocking of the tumor of xenograft growth and its induced regression. Phase 1 clinical trial of the drug proceeded by giving i.v. continuous infusion of the drug solution introduced to previously treated patients for solid tumors for several days, and the principal dose-limiting toxicity (DLT) was given the grade 3 neutropenia while some other side effects were noted [35,36]. PHA-739358, which is active against the pan Auroras, was found to have an established pre-clinical model whereby it inhibits the multiple tumor xenograft model, while the phase-1 clinical trials show neutropenia of 3-4 when i.v. infusion of the drug was done for 6hrs on 1st, 8^{th}, and 15^{th} day on the patient with advanced, pre-treated solid tumor [37]. The most recently discovered pyrazoloquinazoline (AZD1152) showed activity against Aurora B. This molecule is converted into the active drug moiety in the AZD-1152 HQPA which showed inhibition of the human tumor xenograft in its preclinical model. In the pre-clinical trial on patients with advanced, pre-treated solid tumors, they showed grade 3 neutropenia and some other toxicities when given to patients in the i.v. infusion on a weekly basis. ADME studies were also conducted which found that there is rapid conversion into the active metabolite of the AZD-1152 [38, 39].

Computational chemistry is among the most dynamic technologies now being used in drug discovery due to its dual benefits of saving up on time and money as well as being a rational approach to finding new leads. Many drugs, discovered via this technique, are being marketed successfully with better pharmacological action and less side effects [40]. Many techniques are being used by medicinal chemists on the basis of their requirement for drug discovery, which has enabled them to maximize their chances of finding the ideal lead molecule. Some of the technologies that are frequently used in both academia and industry include molecular docking, pharmacophore modelling, and molecular dynamics. Docking is a process whereby better information is procured about the best pose ligand should have to produce its best interaction and become stable [41].

3. INTERACTIONS BETWEEN INHIBITORS AND AURORA KINASE-A AVAILABLE IN PDB

About 50 aurora kinase-A crystal structures are available in PDB, including Apo structures and substrate (ATP)

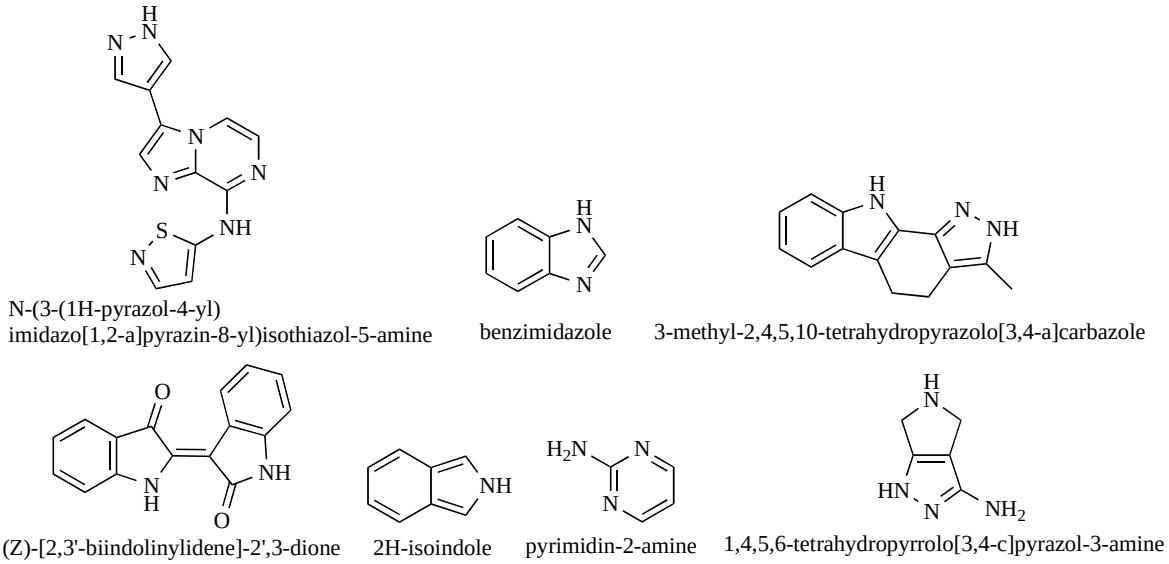


Fig. (2). Different cores still now tested and found potential against Aurora kinase inhibition.

containing structures with inhibitors. After excluding the Apo structures and substrate containing structures, the remaining compounds with diverse co-crystal inhibitors in Aurora kinase A (20 crystal structures) were analyzed for their important interactions. The list of crystal structures and their co-crystal inhibitors, along with their important interactions, is shown in Table **1**. The major interactions formed by inhibitors with aurora kinase A can be categorized as hydrogen bond, hydrophobic, π-π–stacking and electrostatic interactions.

3.1. Hydrogen Bonds

Hydrogen bond interactions with hinge region amino acids of the target kinases are vital for ATP competitive inhibitors. These inhibitors mimic the hydrogen bonds that are normally formed by the adenine ring of ATP [42-43]. Among the 20 crystal structures, all inhibitors were showing strong hydrogen bond interactions with the hinge region amino acids of aurora kinase A. The amino acids Glu211 and Ala213 are involved in the hydrogen bond interactions with the inhibitors. Among the 20 crystal structures, Ala213 was showing hydrogen bond with inhibitors which signifies the importance of this interaction for inhibition. Ala213 forms hydrogen bond with its backbone CO and NH against the inhibitors. Glu211 participates in hydrogen bond only in a few crystal structures along with Ala213. Among the non-hinge amino acids, Arg137 and Arg220 (both exposed to the solvent) were showing predominant hydrogen bond and electrostatic interactions with the inhibitors containing negative ionizable groups like carboxylate and tetrazole. Other amino acids like Asp274, Ser226, Glu181, Lys162 also contribute to the hydrogen bonding with co-crystal inhibitors.

3.2. Hydrophobic Interactions

The heterocyclic ring of the inhibitors that occupy the purine binding site is forming strong hydrophobic interactions with the hinge region amino acids: Tyr212, Ala213, and Pro214. The co-operative hydrophobic and hydrogen bond interactions, formed by these amino acids with the inhibitors, render them well stabilized in the binding pocket. The hydrophobic groups, attached on either side of the heterocyclic ring, form additional hydrophobic interactions. The amino acids Leu139, Val147, Ala160, Leu194, Leu210, Leu263 and Ala273 are also showing strong hydrophobic interaction with the inhibitors.

3.3. Electrostatic Interactions

Electrostatic or columbic interactions are predominant in inhibitors, having charged groups like carboxylate or tetrazole. The negative ionizable group, present in the co-crystal inhibitors of PDBs, 3UOJ, 3UP7, 3UP2, 3UOL, 3UOH and 2X81, show salt bridge interactions with Arg137 and Arg220.

3.4. π-π and Other Weak Interactions

In few crystal structures like 2XE, 2XRU, 3MYG and 3NRM, their inhibitors with aromatic group positioned near the Arg137 are forming and π-π–stacking and NH-π interactions. The inhibitor present in the PDB 3E5A shows π-π–stacking with Phe144. The charged amine group of Lys162 formed cation-π interaction with the aromatic ring of the inhibitor present in the PDBs 3E5A and 3DAJ.

Overall, the hydrogen bond and hydrophobic interactions formed by amino acids Ala213 and Glu211, the hydrogen bond and electrostatic interactions of Arg137 and Arg220, as well as hydrophobic interactions of Tyr212, Pro214, Leu210, Leu139, Val147, Ala160, Leu194, Leu263 and Ala273 are all critical for stabilizing the inhibitors inside the ATP binding pocket of the Aurora kinase receptor. Further, to explore the novel aurora kinase-A inhibitors from our in-house database, we have used these interactions as criteria for selecting the hits obtained from the structure-based virtual screening.

4. VIRTUAL SCREENING OF NOVEL AURORA KINASE-A INHIBITORS

In the past few years, researchers have performed virtual screening on the Aurora kinase A and discovered several inhibitors. In these studies, either ligand-based or structure-based (and sometimes both) methods were used.

Table 1. Docking score for the 20 native ligands of aurora kinase crystal structure.

PDBID	Native Ligands	Important Interactions of Native Ligands with Amino Acids of ATP Binding Site				
		Hydrogen Bonding	Hydrophobic	Positive Ionizable	Negative Ionizable	Polar
3DJ6	AK6	Lys 175, Arg 268, Ala 226	Leu 177, Leu 182, Val 187, trp 290, Leu 221, Val 195, Leu 207, Leu 209, Phe 157, Ala 286, Leu 152, Leu 276, Ala 226	Lys 175, Arg 268, Arg 208	Glu 224, Glu 194	Gln 190, Gln 198
3DJ7	AK7	Lys 175, Lys 156, Glu 194	–Leu 207, Leu 209, Phe 157, Leu 177, Leu 191, Leu 182, Leu 221, Leu 223, Leu 276, Leu 152,Val 187, Ala 286, Val 160, Ala 226, Ala 173, Tyr 225	Lys 175, Lys 156	Glu 224, Glu 194	Gln 190, Thr 230
3E5A	VX6	Ala 213, Phe 144	–Tyr 212, Leu 263, Leu 194, Leu 210, Leu 139, Leu 215, Ala 213, Ala 160, Ala 273, Phe 275, Phe 144, Pro 214, Val 147	Lys 162, Lys 224, Arg 220	Gln 181, Glu 260, Glu 211, Asp 274	Thr 217, Gln 185
3EFW	AK8	Ala 213, Lys 162	Val 147, Leu 263, Leu 139, Leu 194, Leu 210, Leu 208, Leu 178, Leu 164, Leu 169, Tyr 212, Ala 213, Ala 160, Ala 273, Phe 144, Val 174	Lys 162, Lys141, Lys 143	Negative ionizable-Gln 181, Glu 260, Glu 211, Asp 274	Polar- Asn 261, Gln 177
2X6E	YM4	Ala 213 π-π–stacking-Arg 220, Arg 137	Val 147, Val 163, Leu 210, Leu 194, Leu 263, Leu 139, Ala 160, Ala 213, Pro 214, Tyr 212, Phe 144	Lys 162, Lys141, Arg 220, Arg 137	Glu 211	Asn 146, Thr 217
2X81	ZZL	Ala 213, Arg 220, Arg 137 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Ala 160, Ala 213, Ala 273, Pro 214	Lys 162, Arg 220, Arg 137	Glu 211, Glu 260	Asn 261, Thr 217
2XRU	4OO	Ala 213, Glu 211, Arg 137 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Leu 215, Leu 164, Ala 160, Ala 213, Ala 273, Pro 214, Tyr 212	Lys 162, Lys 141, Lys 224, Arg 220, Arg 137	Glu 211, Glu 260, Asp 274	Asn 146, Thr 217
3COH	83H	Ala 213, Glu 211	Val 147, Leu 194, Leu 263, Leu 210, Leu 215, Ala 160, Ala 213, Ala 273, Pro 214, Tyr 212	Lys 162, Arg 220	Glu 211,	Thr 217
3D2K	AK4	Lys 175 Glu 194	Val 187, Leu 276, Leu 152, Leu 223, Leu 207, Leu 221, Leu 191, Leu 182, Leu 177, Val 160, Ala 173, Ala 286, Ala 226, Tyr 255, Phe 157	Lys 175	Glu 194, Glu 224, Asp 287	Gln 190, Thr 330
3DAJ	FXG	Lys 162, Phe 144	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Leu 164, Ala 160, Ala 213, Phe 144, Phe 275	Lys 143, Lys 141, Lys 224	Glu 211	Asn 146, Thr 217
3MYG	EML	Ala 213, ASP 274 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Leu 215, Ala 160, Ala 213, Ala 273, Pro 214, Tyr 212	Lys 162, Lys 224, Arg 220, Arg 137	Asp 274	Thr 217
3NRM	NRM	Ala 213, ASP 274 π-cation–stacking-Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Leu 215, Ala 160, Ala 213, Ala273, Pro 214, Tyr 212	Lys 162, Arg 137	Glu 211 Asp 274	Thr 217
3P9J	P9J	Ala 213 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210,Ala 160, Ala 213, Ala 273, Pro 214, Tyr 212	Lys 162, Lys 141, Arg 137	Glu 211	Thr 217
3UOH	OC4	Ala 213, Arg 137 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Ala 273, Ala 281 Pro 214, Tyr 212	Lys 162, Arg 137	Glu 211 Glu 260	Thr 217, Asn 261, His 280
3UOK	OC6	Ala 213, Arg 137 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Ala 273, Ala 281, Pro 214, Tyr 212	Lys 162, Arg 137, Arg 220	Glu 211 Glu 260	Thr 217, Asn 261, His 280
3UOL	OC7	Ala 213, Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Ala 273, Ala 281, Pro 214, Tyr 212	Lys 162, Arg 137, Arg 220, Val 279	Glu 211 Glu 260	Thr 217, Asn 261, His 280

(Table 1) contd….

PDBID	Native Ligands	Important Interactions of Native Ligands with Amino Acids of ATP Binding Site				
		Hydrogen Bonding	**Hydrophobic**	**Positive Ionizable**	**Negative Ionizable**	**Polar**
3UP2	OC8	Ala 213, Arg 137, Arg 220 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Ala 273, Pro 214, Tyr 212	Lys 141, Arg 137, Arg 220	Glu 211 Glu 260, Asp274	Thr 217, Asn 261
3UP7	OC9	Ala 213, Arg 137, Ash 274, Arg 220	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Pro 214, Tyr 212	Lys 162, Arg 137, Arg 220, Lys 141	Glu 211 Glu 260, Ash 274	Thr 217
2C6E	HPM	Ala 212, Arg 136, Lys 161	Val 146, Leu 193, Leu 262, Leu 138, Leu 209, Leu 207, Leu 195, Leu 214, Leu 177, Ala 159, Ala 212, Ala 272, Phe 274, Trp 276, Val-181	Lys 161, Arg 136	Glu 210 Glu 180, Ash 273	Thr 217, Gln 184
3UOJ	OC5	Ala 213, Arg 137 π-π –stacking- Arg 137	Val 147, Leu 194, Leu 263, Leu 139, Leu 210, Ala 160, Ala 213, Ala 273, Ala 281, Pro 214, Tyr 212, Val 279	Lys 162, Arg 137	Glu 211 Glu 260	Thr 217, Asn 261, His 280

Deng XQ *et al*. have generated a ligand-based 3D pharmacophore model using the known inhibitors and performed virtual screening on specs and china natural products database to find potential hits [44]. Using docking studies further refined the hits obtained. Finally, 39 hits were selected and tested against Aurora kinase A, out of which only two were showing moderate activity.

M.S.Coumar *et al*. used structure-based virtual screening for locating the novel AKA inhibitor containing pyrazole moiety (IC_{50} = 15.1 μM) [45]. Based on the interactions obtained from the X-ray co-crystal structure of this compound complex with Aurora A protein, the compound is further modified by adding hydrophobic substitution to generate the inhibitor with a 450-fold improved Aurora kinase A inhibition potency (IC_{50} = 33 nM). Further, this compound also showed inhibition of Aurora A kinase over Aurora B/C due to the H-bond interaction with non-conserved Thr217 residue of Aurora A. A substructure search of 125,000 compounds using heterocyclic rings of the most frequently reported in kinase inhibitors and five new series of Aurora kinase inhibitors were identified through this approach.

Morshed *et al*. used ligand-based methods for virtual screening of novel Aurora kinase A inhibitors [46]. They generated a 3D pharmacophore model from diverse active Aurora kinase A inhibitors and a recursive partition model to distinguish the actives from the inactives. These two models were used to identify the potential anti-cancer inhibitors. However, they did not test against aurora kinase proteins. Very recently, Barakat *et al.* used a structure-based virtual screening to find the selective ligands toward the mutant variant (Aurora kinase-A T217D) over the wild type protein [47].

Thus, most of the virtual screening experiments were dominated by the ligand-based methods in the attempt to discover the potential AK-A inhibitors. But the limitation of the ligand-based method is its inability to find the diverse ligands when compared with structure-based methods. Among the structure-based experiments, researchers have opted for the protein form PDB based on resolution or randomly from the available crystal structures of AK-A. But one of the important criteria for a successful virtual screening is selecting a representative crystal structure for screening. In order to select the most representative structure of AK-A from the available 20 crystal structures containing inhibitors, we have performed cognate redocking and cross docking into the crystal structures of AK-A (Fig. **3**).

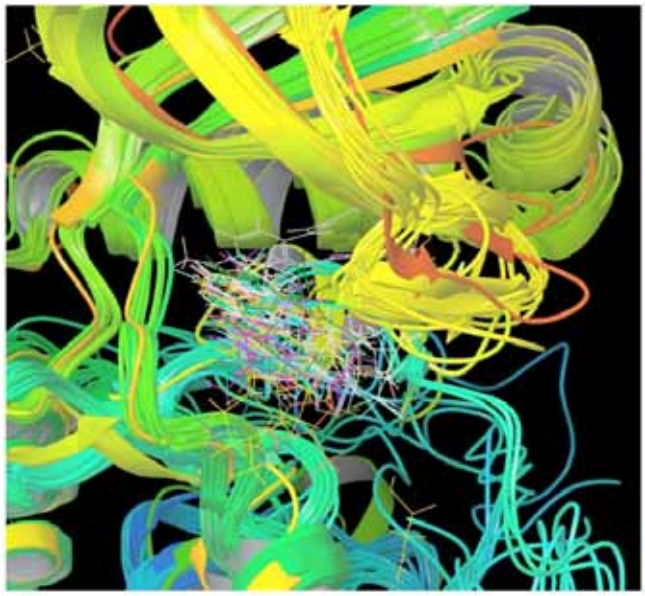


Fig. (3). Protein alignment of all the Aurora pdb files in ribbon form and the native co-crystal ligands (wire form) in the binding site.

4.1. Rigorous Validation and Selection from the Available Crystal Structures for Virtual Screening

To find the representative structure from the available 20AK-A crystal structures in the PDB, we first redocked the compounds into their cognate receptor-structures. Then, we performed cross docking (i.e., non-cognate docking) and evaluated for their reproducibility. Before running the calculations, the AK-A complexes were refined to adjust hydrogens, bond order, deleted water molecules, hydrogen bond network, protonation at pH 7.0, etc., using the Protein Prepa-

ration Wizard (details were provided in the materials and methods section). All the 20 native ligands present in the complexes were sketched and optimized using LigPrep.

These compounds were docked into the binding pocket of cognate and non-cognate proteins using the glide extra precision (XP) method (details were given in the materials and methods section). The top-ranked docking poses, obtained from each protein, were superimposed on to the native ligand and calculated RMSD. The calculated RMSD for cognate and cross docking was tabulated in Table **2**. As shown in that table, most of the ligands exhibited good reproducibility (RMSD <2.5 Å) with their cognate protein with few exceptions. For 3D2K, 3DAJ was showing RMSD ~3.00 Å and for 3P9J the RMSD was about 4.5Å. While in the cross docking experiment, the average RMSD for all the ligands with respect to their crystal pose ranged from 11.9Å to 1.9 Å. The crystal 2C6E shows a least RMSD of 11.9Å; moreover, the compounds did not dock. The crystal 3UOK shows a very good average RMSD of 1.9 Å, and moreover for only three ligands of crystals (3EFW, 2X6E, and 3DJ7), it shows an RMSD of more than 3 Å.

Table 2. Rmsd value (in angstroms) for the best two protein structure for all the twenty ligands. Yellow indicates the cognate docking, green indicates the RMSD >=3 A^0.

PDBID	3UOH	3UOK
2XRU	2.27	2.62
2C6E	3.66	1.78
2X6E	2.14	2.53
2X81	0.45	0.50
3COH	3.44	0.96
3D2K	0.67	2.71
3DAJ	3.50	1.83
3DJ6	7.50	3.45
3DJ7	3.19	0.82
3E5A	2.49	3.00
3EFW	4.88	2.38
3MYG	0.59	1.11
3NRM	1.02	1.04
3P9J	1.93	1.89
3UOH	0.41	0.61
3UOJ	0.33	1.96
3UOK	2.17	1.30
3UOL	0.39	0.50
3UP7	0.75	0.54
3UP2	3.50	4.43
Average	**2.26**	**1.80**

The crystal 3UOH is equally good in reproducing the poses with an RMSD of 2.16 Å; however, for 5 out of 20 crystals, it was showing RMSD of more than 3 Å. This study demonstrates that among the 20 complexes, 3UOK is the most representative structure and can be used for virtual screening in order to find novel compounds. Hence, we have selected this protein for screening of novel compounds from our database.

4.2. Virtual Screening

To further identify the novel heterocyclic small molecules (molecular weight <350 daltons) against the Aurora kinase, we have performed structure-based virtual screening of our in-house database using 3UOK. Diverse 600 heterocyclic compounds with molecular weight of less than 350 daltons, available in our lab, were docked against 3UOK using Glide XP. Since the compounds are performed with low molecular weights, we performed exhaustive sampling (x) using the parameters present in Glide XP. The default initial docking poses per ligand of 5,000 were changed to 50,000,the ''scoring window'' for retaining initial poses of 100.0 kcal/mol was changed to 500 kcal/mol, while the default top-scoring poses per ligand retained for energy minimization on the receptor grid of 800 was increased to 1,000. The obtained hits were further analyzed (Table **3**) based on the binding affinity (glide score) and important interactions that are conserved in the crystallographic structures of the Aurora kinase (details were discussed in the previous session).

When we analyzed the interactions of hit compounds against aurora kinase A, all these hits were showing important hinge region hydrogen bond and electrostatic interactions with Ala213 and 211. The hits also show strong hydrophobic interactions with backbone amino acids Leu139, Gly140, Glu211, Ala213, Pro213, Pro214 and Leu215. And the side-chain interactions, which also help in the affinity of our fragments, are Leu139,Val147, and Leu194. Based on these important interactions, we have selected these 9 hits to further evaluate their biological activity using anti-cancer cell lines. The binding pose of IS2 inside the ATP pocket of Aurora kinase 2 is shown in the Fig. (**4**). Similarly Ligand Interaction pattern with percentage of contacts of the lead and co-crystal ligand against 3UOK & Bar representation of the conserved binding site residues which influenced IS2 and co-crystal ligands against the Aurora receptor 3UOK have been shown in Figs. (**5** & **6**).

5. CHEMISTRY AND SYNTHESIS

Nine molecules, obtained after the process of virtual screening the molecules was synthesized, were found to be with molecule id in the database as D1, D2, D12, D15, D23, D26, IS1, IS2 & IS3. These molecules were synthesized in three schemes (for details see supporting information) due to similarity in their structure. The above-mentioned fragments were synthesized in the lab using three novel synthetic schemes, followed by characterization using Mass, IR and NMR spectroscopy. The percentage yield and melting point were calculated for all the synthesized molecules (Tables **4**, **5** & **6**). The characterization data can be found in the supporting information section.

Table 3. Docking Score and residue Interaction patterns for the Synthesized Compound against Aurora A.

Compounds	Docking Score	Important Interactions with Amino Acids of ATP Binding Site of Aurora Kinase				
		Hydrogen Bonding	Hydrophobic Interaction	Positive Ionizable	Negative Ionizable	Polar Contacts
D1	-6.643	Ala 213, Arg 137	Ala 160, Leu 194, Leu 263, Ala 213, Pro 214,Leu139	Arg 137	Glu 211	Thr 217
D2	-7.973	Ala 213, Arg 137	Ala 160, Leu 194, Leu 263, Ala 213, Pro 214,Leu 139, Val 147, Leu 210	Arg 137,	Glu 211	Thr 217
D12	-5.644	Ala 213, Arg 137	Ala 160, Leu 194, Ala 213, Pro 214,Leu 139, Leu 215,Tyr 212, Leu 210	Arg 137, Arg 220	Glu 211	Thr 217
D15	-6.459	Ala 213, Arg 137	Ala 160, Leu 194, Ala 213, Leu 139, Leu 210, Pro 214, leu 263, val 147	Arg 137, Arg 220, lys 162	Glu 211	Thr 217
D23	-9.581	Ala 213, Arg 137	Ala 160, Ala 213, Pro 214,Leu 139, Leu 215, Leu 210, val 147, leu 263,Val 279, Ala 281	Arg 137, lys 162	Glu 211	Thr 217
D26	-8.716	Ala 213, Arg 137	Ala 160, Ala 213, Pro 214, Leu 210, val 147, Val 279, Ala 281, Tyr 212, Ala 273, Leu 194, Pro 282	Arg 137, Arg 220, lys 162	Glu 211	Thr 217
IS1	-7.821	Ala 213, Lys 162	Ala 160, Ala 213, Pro 214, Leu 210, val 147, Val 279, Ala 281, Tyr 212, Ala 273, Leu 194, Leu 263, Leu 139	Arg 137, lys 162	Glu 211	Thr 217
IS2	-13.005	Ala 213, Glu211	Ala 213, val 147, Ala 281, Tyr 212, Ala 273, Leu 194, Leu 139, Phe 275, Val 279, Tyr 219, Leu 263, Ala 160, Leu210	Lys 162, Arg 220	Glu 260, Glu 211, Asp 274	Thr 217, Lys 162, His 280
IS3	-5.898	Ala 213	Ala 213, Pro 214, val 147, Ala 281, Tyr 212, Leu 194, Leu 139, Lue 210, Leu 263, Ala 160	Arg 137, lys 162	-	Polar - Thr 217

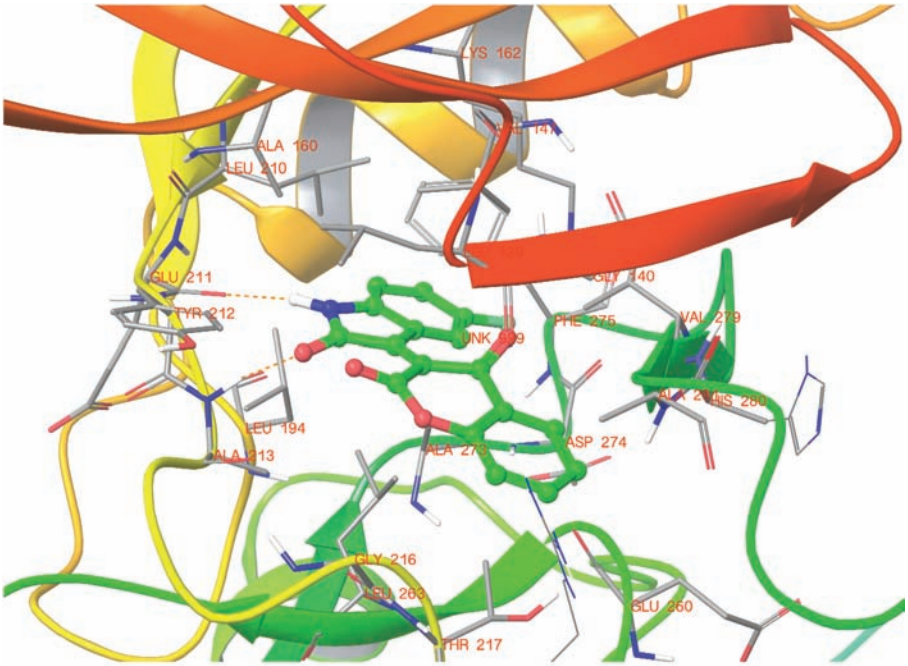


Fig. (4). Binding pose of IS2 inside the ATP binding pocket of Aurora Kinase 2. The compound showing hydrogen bond interactions (yellow color dashed lines) with back bone amino acids (Glu211, Ala2013) of Aurora Kinase2

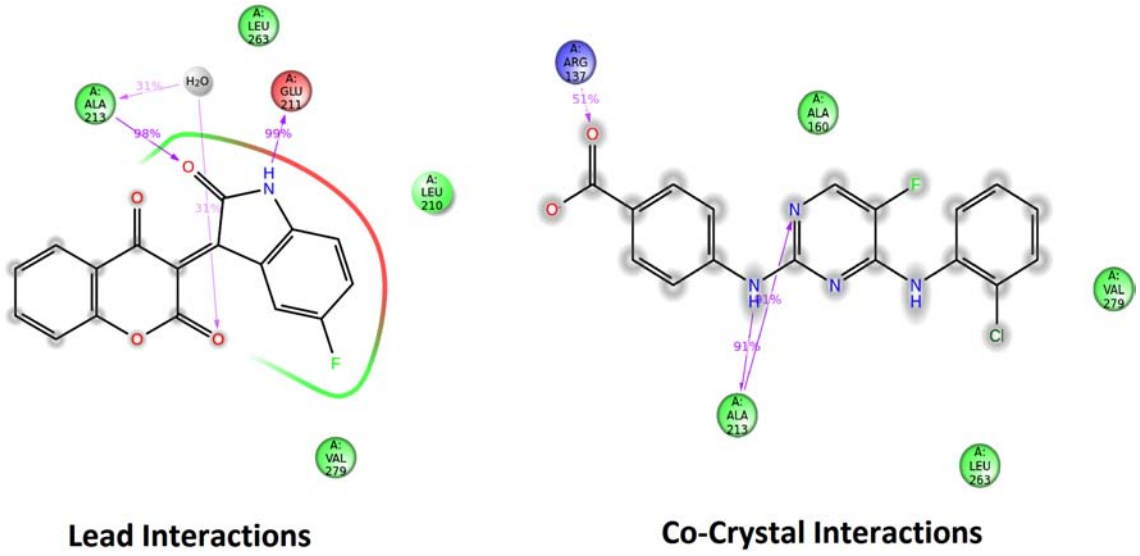


Fig. (5). Ligand Interaction pattern with percentage of contacts of the lead and co-cystal ligand against 3UOK during complete 10ns explicit molecular dynamics simulation.

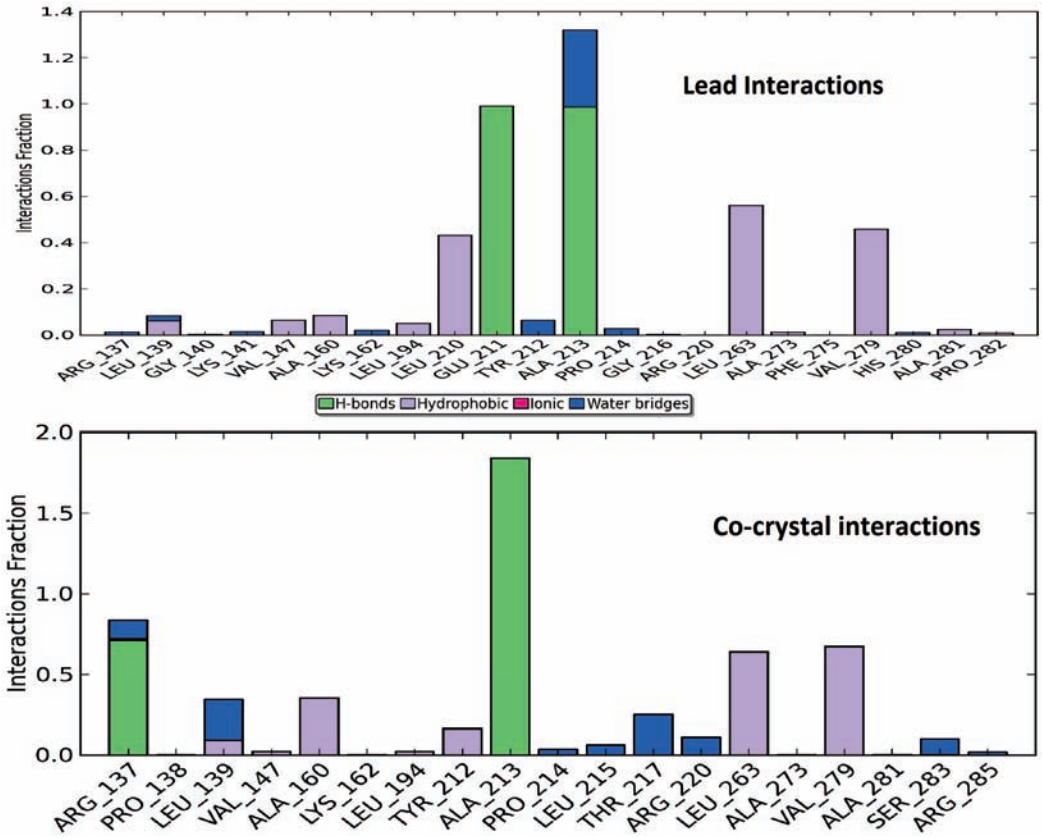


Fig. (6). Bar representation of the conserved binding site residues which influenced IS2 and co-crystal ligands against the Aurora receptor 3UOK during 10ns molecular dynamics simulations.

6. BIOLOGICAL STUDIES

Biological activity was carried out to determine its anti-proliferative activity. Tests were conducted on breast cancer and colorectal cancer cell lines. The standard drug taken was Doxorubicin for breast cancer cell lines and sorafenib for colorectal cell lines. All activities of the 9 hits, as calculated against these cell lines, are shown in Tables **7** & **8**.

Among all these hits, molecule IS2 was showing activity (IC_{50} of 1.32μMand 5.78μM) on breast and colorectal cancer cell lines. Even though this compound is showing activity seven times less than the standard drug doxorubicin against breast cancer cell lines, the compound is very smaller in size and can be used as an active fragment for lead optimization. Interest-ingly enough, molecule IS2 was showing better activity than the standard drug sorafenib on colorectal cancer cell lines.

Table 4. Percentage yield and the M.P determined for scheme 1 compounds.

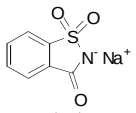
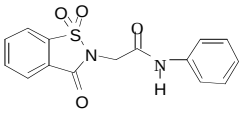
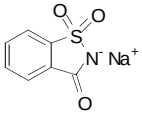
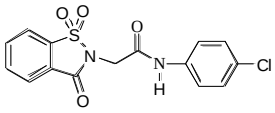
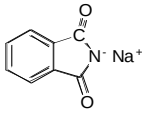
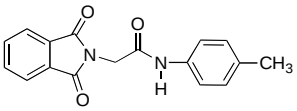
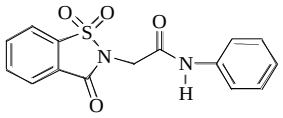
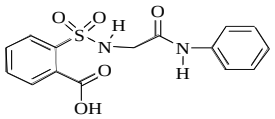
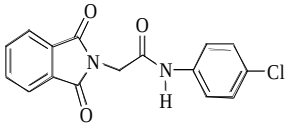
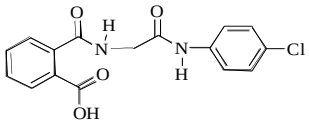
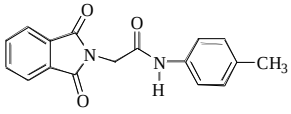
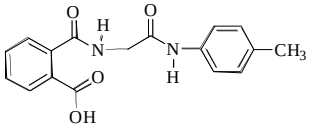
Entry	Starting Material used	Product obtained	Solution Phase (PEG-600)		M.P(^{0}C)
			Time (hr)	Yield (%)	
1	 **(1a)**	 **(D1)**	4	75	142-144
2	 **(1a)**	 **(D2)**	4	78	130-132
3	 **(1b)**	 **(D12)**	4	74	218-220

Table 5. Percentage yield and the M.P determined for scheme 2 compounds.

Entry	Starting Material Used	Product Obtained	Solution Phase (Ethanol)		M.P(^{0}C)
			Time (hr)	Yield (%)	
1	 **(D1)**	 **(D15)**	1	63	180-182
2.	 **(3c)**	 **(D23)**	1	65	>230
3.	 **(3d)**	 **(D26)**	1.5	61	>230

7. MATERIALS AND METHODS

The only software used for all computational calculations is Schrödinger Software [42] with hardware 2x Intel Xeon 1.9 GHz E5-2420/ 6C/15MB Cache RAM 6x4Gb DDR-3 1333MHz ECC RDIMM 4x 500 Gb Graphics Card NvidiaQuadro 600 machine.

7.1. Dataset Preparation

7.1.1. Protein Preparation

X-ray proteins, downloaded from the protein data bank (pdb), are deficient to be used directly in the study. So, they first have to be prepared which is carried out with the help of the protein preparation wizard of Schrödinger known as Prepwiz [48, 49, 50, 51, 52]; by means of a pre-process option, it assigns the bond orders and addition of hydrogen. Excess water was deleted while retaining the required water molecules which were found to be important. The heteroatoms are ionized by epik at biological pH to consider the protein permeability and drug solubility. Optimization of H-Bonds was carried out to relieve the steric clashes by histidine, aspartate, glutamate, and hydroxyl containing amino acids. The final step is to refine the structure which is done to minimize the complete protein to the least possible energy state using the OPLS 2005 force field with convergence criteria of RMSD 0.30Å.

Table 6. Percentage yield and the M.P determined for scheme 3 compounds.

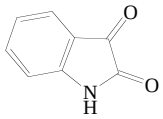
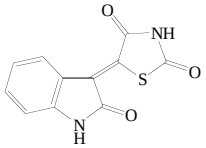
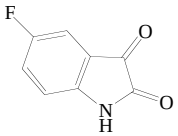
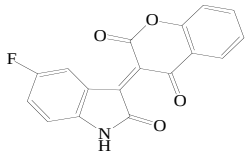
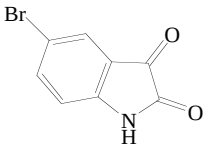
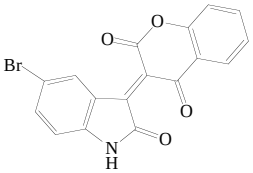
Entry	Starting Material Used	Product Obtained	Solution Phase (Acetic Acid)		M.P(^{0}C)
			Time (hr)	Yield (%)	
1		 **IS1**	2	85	>250
2.		 **IS2**	2	79	>250
3.		 **IS3**	2	81	>250

Table 7: IC_{50} values obtained for the hits from in-house database carried for breast cancer cell line.

Name of the Cell	Name of the Molecule	IC_{50} Values in µM
Breast cancer cell line MDA-MB-361	Doxorubicin	0.330
	IS1	>10
	IS2	1.324
	IS3	>10
	D1	>10
	D2	>10
	D12	>10
	D15	>10
	D23	>10
	D26	>10

Available literature IC_{50} value(reference) for doxorubicin on MDA-MB-361 cell line: 180-250nM

Table 8. IC_{50} values obtained for the hits from in-house database carried for colorectal cell line.

Name of the Cell	Name of the Molecule	IC_{50} Values in µM
Colorectal DLD-1	Sorafenib	6.726
	IS1	>10
	IS2	5.785
	IS3	>10
	D1	>10
	D2	>10
	D12	>10
	D15	>10
	D23	>10
	D26	>10

Available literature IC_{50} value(reference) for sorafenib on DLD-1 cell line: 2-5µM

7.1.2. Ligand Preparation

Ligand molecules sometimes lack 3D coordinates, ionization, stereochemistry, and tautomers, while the least energy state needs to be prepared which is done by ligprep; it is converted from 1D or 2D to 3D, optimized. Finally, it is minimized with the help of the OPLS 2005 force field with default options of 32 stereo isomers, tautomers, and ionization at the biological pH 7±2.

7.1.3. Molecular Docking

For docking, we utilized the prepared protein binding site that used the grid-based energy descriptor for generating the grids which had a default set of options with vanderwaals radius of 1.0, thereby allowing the rotation of the receptor thiol and hydroxyl groups in the receptor site. These grids were then used to calculate the interaction of the individually prepared ligand against the receptor using the xp ligand docking in the glide with nitrogen inversion, flexible ligand

sampling, epik state penalties and ligand vanderwaals scaling radii(0.8) and partial charge cutoffs(0.15).

7.1.4. Identification for the Best Protein pdb for Screening

To identify the best protein to use in the study, we carried out a process whereby we selected 20 co-crystallized Aurora pdb from the protein databank. The crystal ligands were grouped and redocked to the aurora proteins aligned earlier as per binding site to check their reproducibility. The average RMSD of pose reproducibility were calculated with respect to each protein ligand pose. This means that the protein giving the least RMSD with a diverse set of core structures has a binding site which can accommodate a maximum number of diverse molecular structures. This process can help us not to overlook any of the novel molecules, and in identifying the one compound from among the diverse groups as the best inhibitor of cancer prophylaxis.

7.1.5. Virtual Screening

Small molecules from the in-house database were docked with the best protein identified, and the molecules with the best fitness score and docking interactions were considered as hits, with about nine molecules being shortlisted for further steps in order to have them synthesized, characterized and tested for biological activity.

7.1.6. Molecular Dynamics

The explicit molecular dynamics study of the ligand – receptor compounds of the identified lead against the native ligand in the Aurora kinase receptor was carried out using Desmond [54]. First the ligand receptor complex aqueous solvation system was built by system builder with the aid of OPLS 2005 force field by predefined TIP3P solvation of orthorhombic solvation boundary with box volume (8924) A^3that is later followed by ions neutralization with addition of sodium. This system was later minimized with 2000 iterations with convergence criteria of 1 kcal/mol/Å. The minimized explicit solvation complex of ligand receptor complex is simulated for 10 ns using NPT ensemble (temperature of (300 K) and Pressure (1.01325 bar)) with default set of relaxation before simulation.

8. BIOLOGICAL ACTIVITY

The nine molecules were sent for biological activity. Anti-proliferative effects were applied to breast cell and colorectal cell lines. The cell used for the breast cell line was MDA-MB-361, and for colorectal it was DLD-1.

The method followed was MTT assay: briefly, cells were plated at a density of 1,000 to 10,000 cells per well (depending on cell-line growth properties) in 96-well plates and allowed to adhere overnight, incubating at 37°C in 5% CO_2. The following day, the cells were treated with the test compounds and proto drugs, and then allowed to incubate at up to 96 hours. An MTT reagent was then added to the treated cells for an additional 4 hours and then processed according to the standard protocol.

Doxorubicin was used as a reference drug on the breast cancer cell lines, and sorafenib was used as a reference drug on the colorectal cancer cell lines. The concentration of molecules, resulting in 50% inhibition of cell viability (IC_{50}), was calculated and determined from a minimum of three experiments. The IC_{50} was nominally assigned as the highest concentration screened (>10 μmol/L), and these molecules are classified as not potent on specified cell lines.

CONCLUSION

Aurora kinase inhibitors have the attention of medicinal chemists due to their potential as chemotherapeutic agents. We analyzed the established cores against Aurora kinase for the SAR, physiochemical properties, and interaction patterns affecting the biological activity. we considered all the Aurora A x-ray crystallographic structures and analysedthe interaction pattern against the Aurora Kinase A ;The interaction patterns usually found to possess H-bond with the hinge region residues 210-217 and hydrophobic residues 263 and 279 found to play important roles in improving compounds affinity. The cross docking gave us one of the best PDB 3UOK which was able to reproduce the RMSD pose for the diverse set of co-crystal ligands. When we carried our virtual screening of our in-house database by molecular docking with 3UOK, we had identified 9 promising fragment molecules considering the interaction pattern and synthetic feasibility. These compounds were synthesized followed by characterization and biological activity was performed against breast and colorectal cancer cell lines. Molecule with ID IS2 gave IC_{50} of 1.324nM and 5.785μM for breast cell line and colorectal cell line studies. The IS2 could be considered as a lead and we are going to further optimize this fragment to get compounds with better biological response. In order to understand the conserved interaction pattern exhibited by IS2 we carried out explicit molecular dynamics of 10 ns against 3UOK which was compared with the co-crystal interaction patterns where we concluded that our compound was exhibiting the H-Bond with Ala211 and Glu213 and interactions with hydrophobic residues leu 263 and Val 279 against Aurora which was similar to the co-crystal interaction pattern. The shortlisted compounds can serve as useful fragments for further optimization.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIALS

Supplementary material is available on the publishers web site along with the published article.

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