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in silico Binding Mode Analysis (Molecular Docking Studies) and Absorption, Distribution, Metabolism and Excretion Prediction of Some Novel Inhibitors of Aurora Kinase A in Clinical Trials

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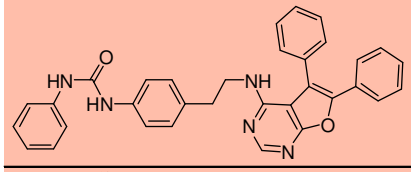
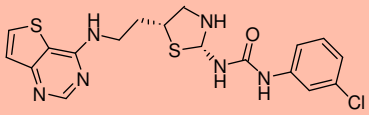
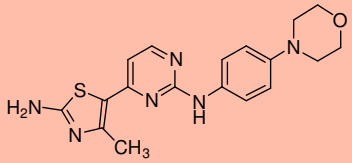
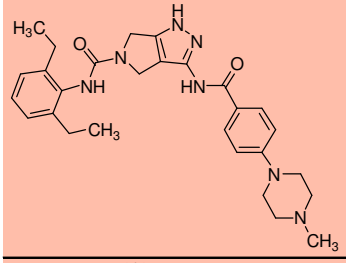
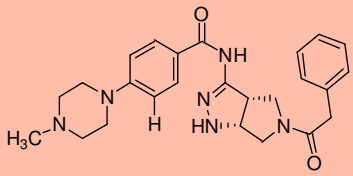
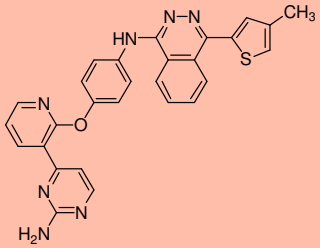
The inhibition of Aurora kinase A is considered to be one of the most promising therapeutic targets for the treatment of cancer. To gain insight into the structural requirements for effective binding and inhibiting the enzyme Aurora kinase A, molecular docking study was carried out by using the potent Aurora kinase A inhibitors (AKAIs) that are currently under clinical trials by employing Glide module of Schrodinger software. Prime MM-GBSA approach was used to study the free energy of binding of these AKIs with the enzyme. Binding mode analysis indicated that a molecule should occupy both ATP binding site (forming essential hydrogen bonding interaction with crucial amino acid residue such as Ala213) as well as allosteric binding cleft (forming hydrogen bonding interaction with amino acid residues Lys162 and Glu181) for exhibiting optimum affinity as well as selectivity towards Aurora kinase A. Further, ADME properties of these study compounds were calculated to get better insight into the physicochemical requirements for effective binding of ligands with Aurora kinase A and also to evaluate their drug-like acceptability which was found to be in the ranges predicted by QikProp module of Schrodinger software for 95 % of known oral drugs. Results confirm the potential of the study which could be useful for the design of new potent inhibitors of Aurora kinase A as possible anticancer agents.

Keywords: Aurora kinase A inhibitors, Docking studies, *in silico* ADME prediction.

INTRODUCTION

The Aurora kinases (AKs) belonging to the serine/threonine kinase family have emerged as potential therapeutic target for cancer chemotherapy[1]. There are three types of Aurora kinases reported in mammals designated as A, B and C, respectively with high amino acid sequence homology, all of which play an essential role in cell division and are primarily active during mitosis[2]. It has been observed that Aurora A is localized at the centrosome from the time of centrosome duplication through to mitotic exit. Aurora B is found to be localized to the centromeres from the prophase to the metaphase-anaphase transition and then it is localized to midzone spindle microtubules during telophase and subsequently to mid body during cell division[2,3]. Aurora C (similar to Aurora B) is also found to be associated with centromeres during the prophase to metaphase and is redistributed to midzone microtubules during anaphase[4]. While Aurora A and B are ubiquitously expressed, Aurora C shows predominant expression in testis suggesting a possible role in meiosis. All three Aurora isoforms exhibit strong similarity in the kinase catalytic domains (sequence identity between Aurora B and C to Aurora A is 75 and 72 %), but they vary in their cellular localization, regulation and substrate specificity. Literature reveals that Aurora kinases are upregulated in wide range of tumors including breast[5], colon[6], pancreas[7], ovary[8], stomach[9], thyroid[10], head and neck[11], respectively. Further, over expression of Aurora kinases has also been observed to impair the functions of tumor suppressor genes, thereby generating aggressive tumors. In particular, when over expressed, Aurora A phosphorylates p53 at Ser215 and inhibits its DNA binding and transcriptional activities[12]. It is postulated that the inhibition of Aurora kinase A may rescue the function of tumor suppressor genes. Thus, Aurora kinases are considered as important new-generation targets for cancer therapy as a result of which more than 30 Aurora kinase inhibitors (AKIs) have entered in various stages of preclinical and clinical studies. Among these AKIs, SNS-314[13], CYC-116[14], PHA-680632[15], PHA-739358[16,17] and AMG-900[18] are of interest with specificities to type of Aurora kinases (Table-1). Hence in the present study, *in silico* binding mode analysis (molecular docking studies) and ADME predictions for these inhibitors have been carried out by using the crystal structures of the enzyme Aurora kinase A (PDB ID: 3M11) to gain insight into their physicochemical and structural requirements for effective binding with the enzyme.

TABLE-1
AURORA KINASE INHIBITORS IN CLINICAL TRIALS

Compound, company, & code	*in vitro* potency (nM)	Preclinical activity	Clinical development
Co-crystal structure of AKA[21] (PDB: 3M11) 	AKA = 43 AKB = 400	--	--
SNS-314,[13]Sunesis 	AKA= 9 AKB= 31 AKC= 3	Solid tumors & leukemias	Phase-I Advanced solid tumors. Six (6) patients displayed stable disease.
CYC116,[14]Cyclacel 	AKA= 44 AKB= 19 AKC = 65	Solid tumors. Reduced tumor weights in various solid tumors & leukemia xenografts	Phase-I Advanced solid tumors
PHA-680632,[15]Nerviano 	AKA= 27 AKB= 135 AKC= 120	Solid tumors and leukemia. 85%TGI resulted in HL60 human xenograft model. Similar results in A2780 and HCT116 xenograft models	--
PHA-73958,[16,17]Nerviano 	AKA= 13 AKB= 79 AKC= 61	Solid tumors & CML	Phase-II Advanced solid and blood cancers. Partial response inone patient. 28 patients displayed stable disease. One CML patient showed complete hematological response.
AMG-900,[18] Amgen 	AKA= 5 AKB= 4 AKC=1	Solid tumors. Activein multidrug resistant xenograft models	Phase -I Advanced solid tumors and acute leukemias.

AKA: Aurora Kinase A; AKB: Aurora Kinase B; AKC: Aurora Kinase C

Results confirm the potential of the study which could be useful for the design of new potent AKAIs.

EXPERIMENTAL

Molecular modeling study: Molecular modeling investigations were carried out by using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4GB RAM, 250 GB hard disk and NVidia Quodro FX 4500 graphics card. Maestro 9.4, GLIDE v5.9 XP docking program (Schrodinger Inc.) was employed for the docking studies[19,20].

Preparation of protein: Crystal structure of Aurora kinase A (PDB ID: 3M11)[21] was downloaded from PDB (www.rcsb.org), refined and prepared by using Schrodinger protein preparation wizard tool (Glide v5.9), which performs the following steps: Assigning of bond orders, addition of hydrogens, optimization of hydrogen bonds by flipping amino side chains, correction of charges and minimization of the protein complex. All the bound water molecules, ligands and cofactors were removed (preprocess) from the proteins which were taken in *.mae* format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization of co-crystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The

TABLE-2
FREE ENERGY OF BINDING OF THE AKIs WITH THE RECEPTOR AURORA KINASE A (PDB ID: 3M11)

Compound	GLIDE XP_Score	IC_{50} (nM)	Prime MMGBSA Complex Energy	Prime MMGBSA Ligand Energy	Prime MMGBSA Receptor Energy	Prime MMGBSA DG bind (ΔG_{bind} = kcal/mol)	Prime MMGBSA DG bind Coulomb	Prime MMGBSA DG bind vdW
[a]Co-crystal structure of AKA	-12.3	43	-11299.591	-93.417	-11073.393	-132.781	-34.994	-75.899
SNS-314	-9.8	9	-11289.977	-113.679	-11073.393	-102.904	-23.101	-55.193
CYC116	-8.7	44	11279.566	-125.412	-11073.393	-80.761	-21.5	-44.054
PHA-680632	-6.1	27	-11113.447	-49.587	-11073.393	-89.641	-30.08	-64.566
PHA-739358	-5.6	13	-11053.736	-62.382	-11073.393	-42.725	189.661	-43.572
AMG-900	-8.3	5	-11263.192	-102.918	-11073.393	-86.881	-23.116	-58.399

AKA: Aurora Kinase A; [a]1-(4-{2-[(5,6-Diphenylfuro[2,3-*d*]pyrimidin-4-yl)amino]ethyl}phenyl)-3-phenylurea

complex obtained was minimized using OPLS_2005 force field[22] with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was terminated either completion of 5,000 steps (or) after the energy gradient converged below 0.05 kcal/mol.

Preparation of ligands: Structures of the ligands (Table-1) were sketched by using built panel of Maestro and taken in *.mae* format. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. Molecular Mechanics Force Fields (OPLS_2005) with default settings were employed for the ligand minimization.

Docking studies: Docking studies were carried out by using the above mentioned prepared proteins (PDB ID: 3M11 and 2VGP) and ligands (Table-1), by employing Glide XP docking program (Schrodinger Inc.) following the reported procedure[19,20].

Calculation of Prime MM-GBSA descriptors: The Prime MM-GBSA approach[23] is used to predict the free energy of binding for a receptor and a set of ligands. MM-GBSA is an acronym for a method that combines OPLS molecular mechanics energies (EMM), an SGB solvation model for polar solvation (GSGB) and a nonpolar solvation term (GNP) composed of the nonpolar solvent accessible surface area and van der Waals interactions. The total free energy of binding is then expressed as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

where

$$G = E_{MM} + G_{SGB} + G_{NP}$$

The ligand in the unbound state is minimized in SGB solvent but is not otherwise sampled. In the calculation of the complex, the ligand is minimized in the context of the receptor. The protein is currently held fixed in all calculations. The following descriptors generated by the Prime MM-GBSA approach:

MM-GBSA_DG_bind	Ligand binding energy, ΔG_{bind}
MM-GBSA_E_complex	Energy of the complex, $G_{complex}$
MM-GBSA_E_protein	Energy of the receptor without the ligand, $G_{protein}$
MM-GBSA_E_ligand	Energy of the unbound ligand, G_{ligand}

To set up the calculation, pose viewer file (generated after docking with Glide) was used to consider the receptor and source of ligands and the program Prime MM-GBSA was run with default options that were chosen to produce reasonable descriptors. The MM-GB/SA scoring along with the experimental binding affinities data of Aurora kinase A is presented in Table-2.

Prediction of ADME properties: The QikProp module of Schrodinger is a quick, accurate, easy-to-use absorption, distribution, metabolism and excretion (ADME) prediction program design to produce certain descriptors related to ADME. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In the present study, QikProp was run in normal processing mode with default options[24]. The selected properties that are known to influence metabolism, cell permeation and bioavailability are presented in Table-4.

RESULTS AND DISCUSSION

The main objective of this work is to carry out the structure-based molecular docking studies for analyzing the binding mode of various potent AKAIs under clinical trials with the enzyme Aurora kinase A to gain insight into their physicochemical and structural requirements for effective binding with the enzyme which can be useful in identifying new potent AKIs. Before carrying out the docking studies, the protocol has been validated by reproducing the bound natural substrate conformation [co-crystalized ligand1-(4-{2-[(5,6-diphenylfuro[2,3-d]pyrimidin-4-yl)amino]ethyl}phenyl)-3-phenylurea] in the crystal structure of Aurora kinase A (PDB ID: 3M11)[21]. The co-crystal natural substrate was taken out of the active site and docked again. The top 3 docking configurations were taken into consideration to validate the results and the RMSD was calculated for each configuration in comparison with the co-crystallized ligand. The acceptable results (RMSD within 0.88-1.25 Å) indicated that the docked configurations have similar binding positions and orientations within the binding site and are similar to the co-crystal structure, which illustrates the fact that the docking protocols used could successfully generate the co-crystal-AKA complex precisely.

Further Prime MM-GBSA approach was used to study the association as well as free energy of binding of the ligands with their receptor (Table-2). Moreover, ADME descriptors were also used to get better insight into the physicochemical requirements for effective binding of ligands with Aurora kinase A (Table 4).

TABLE-3
ELECTROSTATIC INTERACTION (COULOMB) ENERGY (kcal/mol) AND vdW INTERACTIONS ENERGY (kcal/mol) BETWEEN THECOMPOUNDS AND CRUCIAL AMINO ACID RESIDUES OF AURORA KINASE A INVOLVED IN LIGAND RECOGNITION

Compound	LYS 162		GLU 181		GLU 211		ALA 213	
	vdW	Coulomb	vdW	Coulomb	vdW	Coulomb	vdW	Coulomb
[a]Co- AKA	-2.575	-13.341	-1.321	-14.963	-0.984	-0.809	-2.468	-0.991
SNS-314	1.673	-20.402	0.490	-17.888	-0.981	-1.029	-2.094	-2.056
CYC116	-1.056	-1.873	-0.033	-0.644	-0.914	-1.389	-1.815	-3.334
PHA-680832	-2.886	-8.471	-0.894	-0.343	-1.012	0.453	-1.292	-4.353
PHA-739358	-1.718	36.839	-1.072	-19.085	-1.216	0.529	-0.664	-0.028
AMG-900	-1.905	-1.905	-1.877	-1.974	-0.909	-0.885	-1.644	-2.634

[a]Co-crystal structure of Aurora Kinase A

It has been observed that at the ATP binding pocket of Aurora kinase A, Leu139, Val147, Lys162, Glu211 and Ala213 residues are crucial for the potent ligand binding and kinase selectivity. The ATP binding site is commonly referred as front cleft/pocket, while there is another portion/pocket unoccupied by ATP known as back cleft/pocket or allosteric site. Several kinase inhibitors that occupied this allosteric site exhibited enhanced potency as well as selectivity. The binding orientation of co-crystal ligand (Fig. 1a) shows that the furano-pyrimidine core has occupied the ATP binding site forming essential hydrogen bonding interaction with the crucial amino acid residue Ala213 (2.062 Å) and the terminal phenyl moiety forms hydrophobic contacts with the surrounding residues in the allosteric site of the enzyme. The urea carbonyl group (C=O) forms a strong hydrogen bond with Lys162 at a distance of 1.828 Å, while NH groups forms significant hydrogen bonding interaction with the carboxyl group of Glu181 at a distance of 1.765 Å and 2.085 Å respectively. Similarly, in case of the compound SNS-314 (Fig. 1b) the *N*-atom thienopyrimidine core forms essential hydrogen bonding interaction with Ala 213 at a distance of 2.277 Å at the ATP binding site. The *o*-chlorophenyl group occupied the back pocket forming hydrophobic interactions with the surrounding residues and the carbonyl group (C=O) and NH group of urea forms significant hydrogen bonding interaction with Lys162 and Glu181 at a distance of 1.570Å, 1.677Å and 1.823 Å respectively. Interestingly, the compound CYC-116 also occupied the ATP binding site forming strong hydrogen bonding interactions with the crucial amino acid residue Ala213 at a distance of 1.964 Å and 2.190 Å respectively (Fig. 1c). But it could not properly occupy the allosteric binding site and fails to form significant hydrogen bonding interaction with essential amino acid residues Lys162 and Glu181 respectively which could be the reason for its less potency as compared to other compounds. In case of compound PHA-680632, although it occupied the ATP binding site but did not form any significant hydrogen bonding interaction with the most crucial amino acid residue Ala213 which could be the reason for its less potency as compared to other compounds. The 2,6-diethylphenyl group of compound PHA-680632 was found to be anchored comparatively well at the allosteric binding site forming strong hydrophobic interactions with surrounding amino acid residues which could be the reason for its higher selectivity as compared to other compounds. It also formed strong hydrogen bonding interactions with Lys162 (1.651 Å & 2.383 Å) and Glu181 (1.788 Å & 2.326 Å), respectively (Fig. 1d). The compound PHA-73958 (Fig. 1e) on the other hand reasonably occupied both the ATP binding cleft as well as back pocket forming essential hydrogen bonding interaction with all the required amino acid residues, Lys162 (1.878 Å & 1.958 Å), Glu181 (2.515 Å), Ala213 (3.126 Å) and Leu139 (2.939 Å), respectively. The compound AMG-900 (Fig. 1f), anchored firmly at the ATP binding cleft forming strong hydrogen bonding interactions between NH_2 and Glu211 (2.413 Å) and N-atom of pyrimidine moiety with Ala213 (2.181 Å), respectively which might be the reason for its enhanced affinity as compared to other compounds. Although the 4-methyl-thiophene moiety could occupy the allosteric site, it could not form significant hydrogen bonding interactions with essential amino acid residues like Lys161 and Glu181 which could be the reason for its decrease in selectivity. The electrostatic interaction (Coulomb) energy (in kcal/mol) and vdW interaction energy (in kcal/mol) between all the compounds and each single amino acid involved in ligand recognition obtained after docking simulations inside the binding site of Aurora kinase A is presented in Table-3.

Certain molecular properties which could influence the metabolism, cell permeability and bioavailability for all the test compounds under clinical trials were evaluated using QikProp (version 3.6) module of Schrodinger (Table-4). Some of the parameters such as QPlogPo/w and QPlogS are recognized parameters for prediction of drug transport properties. Further, steric and molecular surface descriptors, *e.g.*, solvent accessible surface area (SASA) were calculated. ADME prediction methods were used to assess the bioavailability of the test compounds (Tables 1 and 4). Herein, we calculated the compliance of all the compounds to the Lipinski's 'rule of five'[25] which has been widely used as a filter for predicting the drug-like properties of any molecule. According to this rule, poor absorption or permeation is more likely when there are more than five H-bond donors, ten H-bond acceptors, the molecular weight (MW) is greater than 500 and the calculated Log P (C Log P) is greater than 5. Molecules violating more than one of these rules may have problems with bioavailability. Interestingly, the results of predicted properties for all the compounds are in the ranges predicted by QikProp for 95 % of known oral drugs.

Conclusion

In the present study, *in silico* molecular docking study was carried out by using some of the potent AKIs (under clinical trials) and the crystal structure of Aurora kinase A by

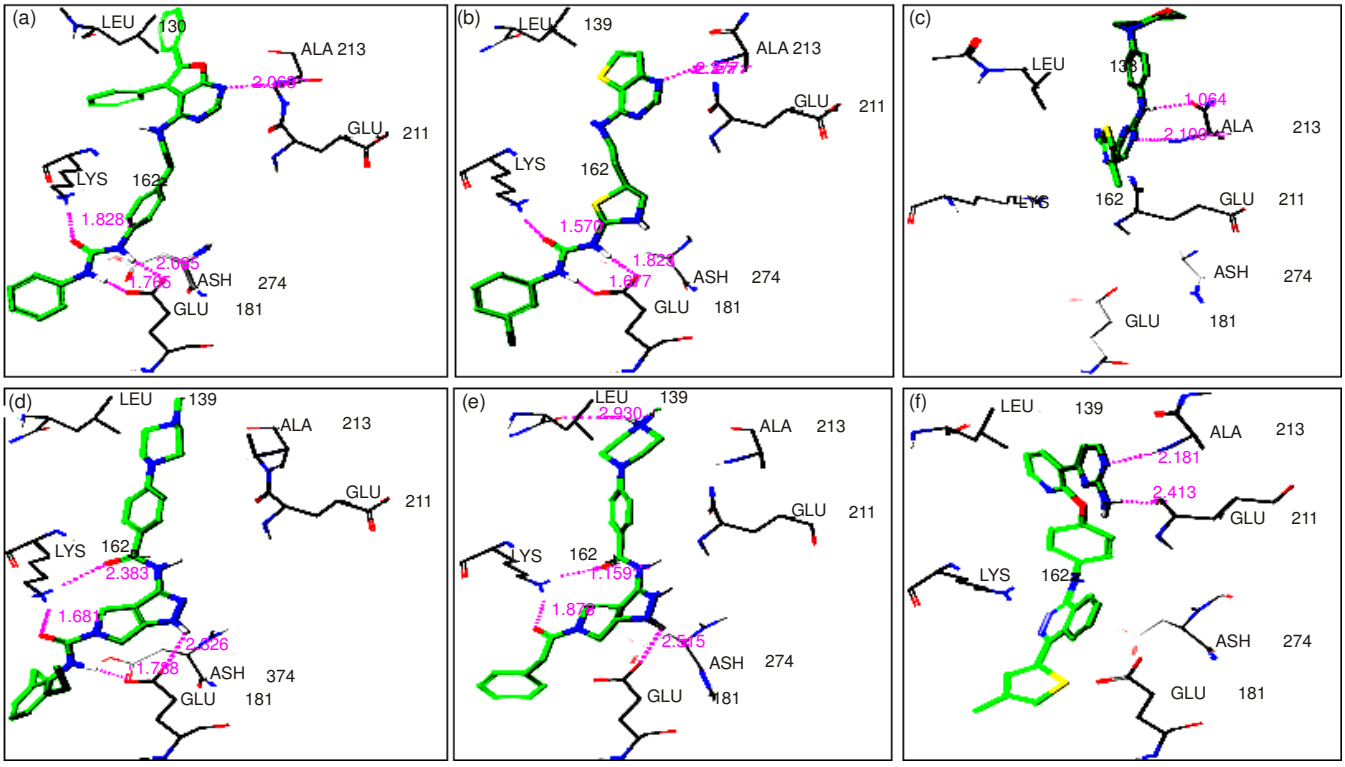


Fig. 1. Binding orientation of all the docked molecules within the binding site of Aurora kinase A (PDB ID: 3M11) showing hydrogen bonding interactions with crucial amino acid residues; (a) Co-crystal ligand, (b) SNS-314, (c) CYC-116, (d) PHA-680632, (e) PHA-73958, (f) AMG-900

TABLE-4
CALCULATION OF VARIOUS ADME PROPERTIES OF AURORA KINASE INHIBITORS (AKIs) IN CLINICAL TRIALS

Molecule	MW[b]	SASA[c]	Donor HB[d]	Accpt HB[e]	QPlogP o/w[f]	QPlogS[g]	QPPCaco[h]	QPlogBB[i]	#Metab[j]	Human Oral Absorption (%)[k]	Rule Of Five[l]
[a]Co- AKA	525.609	917.716	3	5	6.693	-9.042	656.359	-1.104	4	90.642	2
SNS-314	434.961	737.863	3	5	3.55	-5.358	84.77	-0.62	3	82.244	0
CYC116	368.456	692.131	3	7.2	2.94	-5.802	566.474	-0.974	4	93.436	0
PHA-680632	501.63	879.925	2	7.5	4.188	-6.414	59.433	-1.062	6	70.261	1
PHA-739358	446.551	807.799	2	10.5	2.07	-4.3	64.469	-0.918	3	71.447	0
AMG-900	503.58	909.503	3	6.5	5.749	-9.256	327.741	-1.746	4	79.715	2

[a]Co-crystal structure of Aurora kinase A (AKA); [b]Molecular weight;[c] Total solvent accessible surface area (SASA) in square angstromsusing a probe with a 1.4 Å radius, range 95 % of drugs (300-1000); [d]Estimated number of hydrogen bonds that would be donatedby the solute to water molecules in an aqueous solution, range 95 % of drugs (0.0-6); [e]Estimated number of hydrogen bonds that would be acceptedby the solute from water molecules in an aqueous solution, range 95 % of drugs (2-20); [f]Predicted log of the octanol/water partition coefficient, range 95 % of drugs (-2-6.5); [g]Predicted log of aqueous solubility S (mol/L), range 95 % of drugs (-6.5-0.5); [h]Caco2 cell permeability in nm/s, range 95 % of drugs (<25 poor, > 500 great). Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport; [i]Predicted brain/blood partition coefficient, range 95 % of oral drugs (-3-1.2); [j]Number of likely metabolic reactions;range 95 % of drugs (1-8); [k]Predicted human oral absorption on 0 to 100 % scale (> 80 % is high and < 25 % is poor); [l]Number of violations of Lipinski's rule of five. The rules are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB 10. Compounds that satisfy these rules are considered druglike. (The "five" refers to the limits, which are multiples of 5)

employing Glide module of Schrodinger software. Prime MM-GBSA approach was used to study the association as well as free energy of binding of the ligands with the receptor. Binding mode analysis of above mentioned AKIs indicate that a molecule should occupy both ATP binding site forming essential hydrogen bonding interaction with crucial amino acid residue Ala213 as well as allosteric binding cleft (forming hydrogen bonding interaction with residue Lys162 and Glu181) for exhibiting optimum affinity as well as selectivity towards Aurora kinase A. Further, ADME properties of these study compounds were calculated to get better insight into the physicochemical requirements for effective binding of ligands with Aurora kinase A and also to evaluate their drug-like acceptability which was found to be in the ranges predicted by QikProp for 95 % of known oral drugs. Results confirm the potential of the study which could be useful for the design of new potent inhibitors of Aurora kinase A.

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