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Research Article

INSILICO APPROACHES TOWARDS THE DRUG TARGET AURORKINASES USING THE ORTHO OR META SUBSTITUTED BENZENE DERIVATIVES IN PYRAZOLES

SOBY DEVASIA*, RANGADURAI.A

Department of chemistry,AVIT,Paiyanoor,Vinayakmission University.Kancheepuram Dist, Chennai 603104.
Email: devasia.soby@gmail.com

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ABSTRACT

Objective: Cancer is the one of the most dreadful in worldwide, the research on different kinds of cancer is still ongoing in many places of world. The most important crucial important of this current study in cancer to check the inhibition of cell growth using the Insilco tools before in vivo study of the chemically synthesized products.

Methods: In this current study ortho or Meta substituted aldehydes derivatives were virtually screened using theoretical drug likeness rule further, synthetically designed ligand and protein were optimized before molecular docking.

Results: The stability energy of the protein was found to be -85.3427Kcal/mol. All the 10 compounds obeys Lipinski's rule of 5, was taken for the receptor-ligand interaction studies. Hence, the interaction was found in between the three compounds such as Cmp2, Cmp3 and Cmp10 with dock score of 90.35, 89.50 and 87.375 respectively.

Conclusion: Thus, among ten substituted aldehyde derivatives only three compounds cmp2, cmp3 with functional group of OCH_3 and cmp10 with functional group Br shows good binding with crucial amino acid in active site of aurokinase.

Keywords: Cancer, cell proliferation, Aurora kinases, aldehydes derivatives.

INTRODUCTION

Aurorakinases are serine/threonine kinases that are essential for cell proliferation. The enzyme helps the dividing cell dispense its genetic materials to its daughter cells. More specifically, Aurora kinases play a crucial role in cellular division by controlling chromatid segregation. Defects in this segregation can cause genetic instability, a condition which is highly associated with tumorigenesis[1]. Aurora family kinases play roles in several mitotic processes, including the G2/M transition, mitotic spindle organization, a regulatory domain in the NH2 terminus and a catalytic domain in the COOH terminus chromosome segregation, and cytokinesis[2-5]. Three Aurora kinases have been identified in mammalian cells to date. Besides being implicated as mitotic regulators, these three kinases have generated significant interest in the cancer research field due to their elevated expression profiles in many human cancers [6]. Aurora kinases comprise mainly two domains. The regulatory domain is diverse largely, whereas the catalytic domain with a short segment of diverse COOH terminus shares >70% homology among Aurora-A, Aurora-B, and Aurora-C. There is a D-Box in the COOH terminus and an A-Box in the NH2 terminus of Aurora kinases, which are responsible for degradation [7-9].Structural and motif based comparison suggested an early divergence of Aurora A from Aurora B and Aurora C [10]. Aurora A, B, &C have been mapped on chromosomes 20q13.2, 17p13.1, and 10q13 respectively [11-13]. Aurorakinases show little variability in their amino acid sequence and this is very important for interaction with different substrates specific for each Aurorakinase and for their different sub cellular localizations.

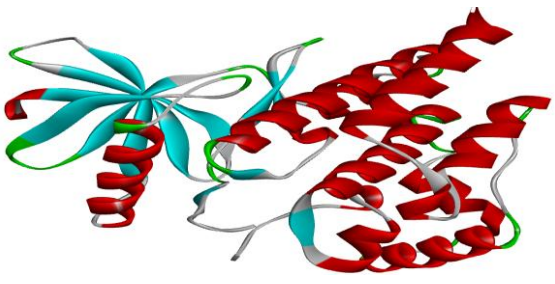


Fig. 1: The secondary structure of the Aurorakinase

Aurorakinases are involved in multiple functions of mitosis. Aurora A is involved in mitotic entry, separation of centriole pairs, accurate bipolar spindle assembly, and alignment of metaphase chromosomes and completion of cytokinesis[14].Aurora proteins in a wide range of tumors including breast [15-16], colon [17–20], pancreas [21], ovary [22], stomach [23], thyroid [24], head and neck [25]. This has increased the possibility of developing new anti-cancer drugs that could target Aurora kinases. Among these inhibitors, AT9283, AZD1152, PHA- 739358, MLN8054, MK-0457 and ZM447439 are of interest with specificities to type of Aurora kinases and are in clinical trials [26].

Aberrant expression of Aurora kinases may disturb checkpoint functions particularly in mitosis and this may lead to genetic instability and trigger the development of tumors. Aurorakinases have gained much attention since they were identified in onco genes. Aurorakinases are over expressed in a variety of tumor cell lines [27-29], suggesting that these kinases might play a role in tumorigenesis. In particular, Aurora-A can transform certain cell lines when over expressed [30, 31, and 32].

The Secondary structure of the Aurorakinase is shown in the fig 1.In this current study a series of chemically synthesized compounds are docked to the active site of the Aurorakinase to screen the compounds bioefficacy for cancer. The parent structure of the chemical moiety is shown in the fig2.The R_1 and R_2 are the functional groups of the parent structure, the various

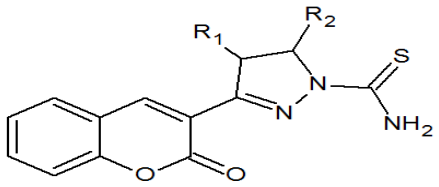


Fig. 2: The parent structure of the chemical moiety

Chemically modified structures are replaced with different functional groups in R_1 and R_2 position were virtually screened using Insilco tools and software's before the in vivo and in vitro studies to reduce the time and cost.

MATERIALS AND METHODS

Retrieval of protein and ligand from database

The structure of the drug target protein AuroraKinase and its X-ray crystallography structure with 2.60Å was retrieved from protein data bank with its Identification number as 2W1I, with selective potent inhibitor4-[(2-{4-[(cyclopropylcarbamoyl)amino]-1h-pyrazol-3-yl}-1hbenzimidazol-6 yl)methyl]morpholin- 4-ium

Protein preparation

The raw protein from protein databank with PDB ID 2W1I named Aurora Kinase alpha is further prepared for docking studies initially, all the Hetatms were removed and subsequently subjected for energy minimization to remove the bad steric clashes using tool smartminimizer for 1000 steps at RMS gradient of 0.1 and 0.03 respectively by applying the suitable force field CHARMm available through Accelrys life science software [33].

Preparation of the ligands

The libraries of compounds were screened for the drug likeness property.the more important filter used commonly to screen the large number of compounds is Lipinski's rule of 5. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules [34-35].The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to insure drug-like physicochemical properties are maintained as described by Lipinski's rule [36].

Molecular docking

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization [37]. In this molecular docking studies the concept of molecular docking can be applied to various methods of drug designing such as structure based drug designing, ligand based drug designing, denova drug designing. In this current study the concept of structure based drug designing is applied.

RESULTS AND DISCUSSION

Stability of the protein

The each amino acid residues in the aurokinase protein is optimized using the charm force filed energy of protein is found as -85.3427 Kcal/mol. vanderwaals energy as -2,297.53 K cal/mol respectively with Maximum stability at pH = 4.40 and pI = 8.87.The structure of the prepared protein is shown in the fig 3 and the compounds screened for the docking studies in the fig 4, the list of compounds and its IUPAC name is shown in Table 1.

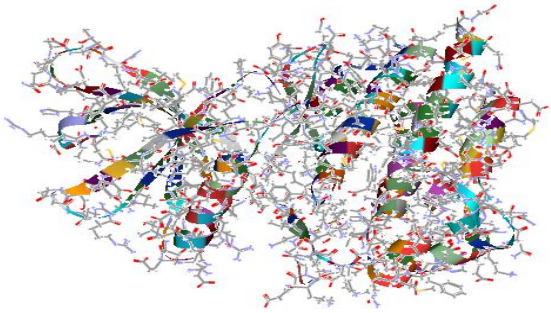


Fig. 3: Aurora Kinase protein after optimized

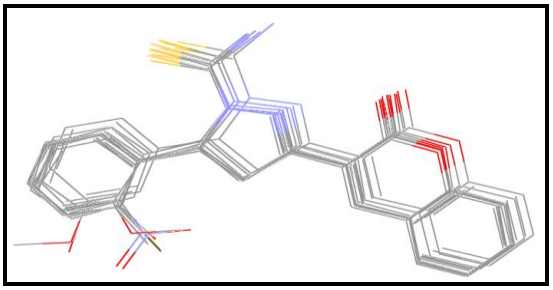


Fig. 4: List of compounds

Table 1: The list of compounds and its IUPAC name

Compound	IUPAC Name of the compound
Cmp1	3-(2-Oxo-2*H*-chromen-3-yl)-5-*o*-tolyl-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp2	5-(2-Methoxy-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp3	5-(3-Methoxy-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp4	5-(2-Hydroxy-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp5	5-(3-Hydroxy-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp6	3-(2-Oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp7	5-(2-Nitro-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp8	5-(2-Fluoro-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp9	5-(2-Chloro-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide
Cmp10	5-(2-Bromo-phenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-pyrazole-1-carbothioic acid amide

Drug likeness screening of the ligand

The 10 compounds are screened for the drug likeness property. Any lead or ligands must undergo this study to check whether the lead can be used as drug candidate. ALOGP as a very effective computational method in the measuring molecular hydrophobicity (lipophilicity), which is usually quantified as logP (the logarithm of 1-octanol/water partition coefficient) [33-38].

Molecular hydrophobicity reflects on the biological and biochemical properties of drugs, including their lipid solubility, absorption, tissue distribution, bioavailability, receptor interaction, metabolism, cellular uptake, and toxicity[40].

The formulation of Lipinski's rule of five is based on the observation that orally active drugs are small in size and have optimal solubility in aqueous and non-polar [38-40]. The Table2 shows drug likeness property of the leads.

Table 2: Drug likeness property of the leads

Sno	AlogP	Molecular weight	Hydrogen bond acceptors	Hydrogen bond donors
Cmp1	4.039	363.433	4	1
Cmp2	3.536	379.432	5	1
Cmp3	3.536	379.432	5	1
Cmp4	3.311	365.406	5	2
Cmp5	3.311	365.406	5	2
Cmp6	3.552	349.406	4	1
Cmp7	3.447	394.404	6	1
Cmp8	3.758	367.397	4	1
Cmp9	4.217	383.851	4	1
Cmp10	4.301	428.302	4	1

Lipinski's rule of five states that a value of ALOGP of ≤5, a molecular weight of ≤500 Daltons, a number of hydrogen bonding acceptor sites (HBA) of ≤10, a number of hydrogen bonding donor sites (HBD) of ≤5 are ideal for a lead to behave as drug candidate [38-40]. All the 10 compounds obeys Lipinski's rule of 5.further, it was taken for the receptor-ligand interaction analysis.

Receptor-ligand interaction

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule, hence docking plays an important role in the rational design of drugs. [40]. the screened compounds are theoretically posse's drug likeness property as per stated rule of Lipinski's.

The concept of shape complementarily between the active site and compounds plays a major role in the docking. On other hand binding the one or more crucial amino acid is important for the bioactivity of the compound. The following fig 5 is taken from the PDBSUM database is set as a reference for the binding between ligands and amino acid. The structure based drug designing is employed when both leads compounds and drug target protein are known for molecular docking. It is an iterative process to dock the lead compounds with specific site of the drug target protein. The active site of the protein is defined the crucial amino acids are Tyr931, Leu932,Glu 930,Leu 983,val 863,Gly856,Leu855,Gln853 among the neighboring amino acids Leu932,Glu 930 are more important.The grid spacing of 0.5 (X),0.5(Y),0.5(Z) in 3D direction respectively. A series of screened compounds are docked to the binding site defined of 42.997 (X), -5.54 (Y), -3.653 (Z) using conformation based docking [41].The fig6 shows the binding site of the protein.

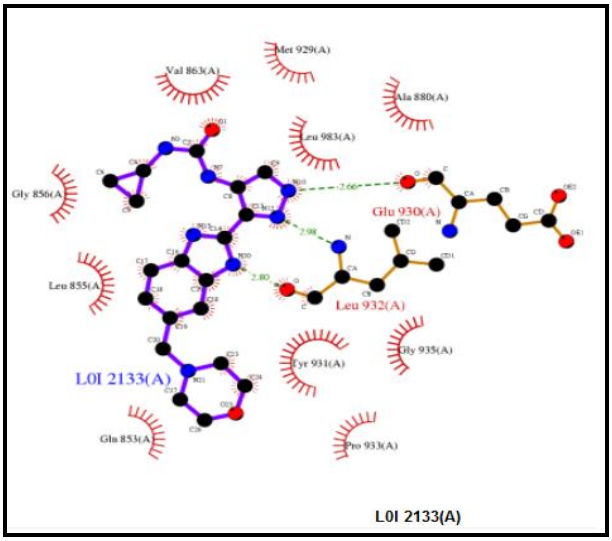


Fig 5: Binding of the L0I 2133 to the A chain active site of Aurorakinase

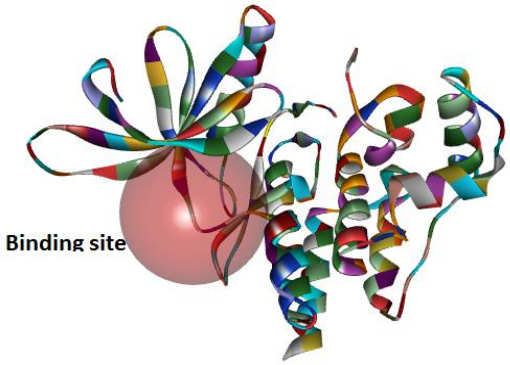
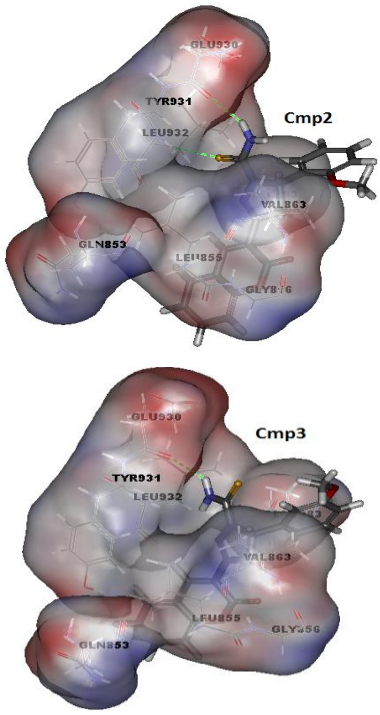


Fig 6: Binding site of the Aurokinase protein

The interaction between the binding site amino acid and chemical lead compounds are shown in the fig7.the shows the interaction with crucial amino acid Glu930 and Leu932



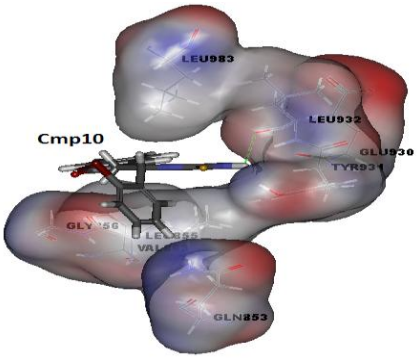


Fig 7: Binding site interaction

Green stacked lines in the fig 7 shows the hydrogen bond interaction between the amino acid and the ligand, among ten compounds the three compounds shows interaction with active site amino acid. The dock score and conformation of the compounds were tabulated in the Table 3.

Table 3: The compounds with dock score and conformation number

S. No.	Dock score	Conformation number
Cmp2	90.35	8
cmp3	89.50	6
Cmp10	87.375	19

CONCLUSION

Hence from this study it is clearly stated that, among 10 compounds of aldehydes, the three compounds such as cmp2,cmp3 and cmp10 shows the interaction with active site amino acid and the dock score is in increasing order of cmp2,cmp 3 and cmp10 respectively. Thus, instead of studying, the large number of compounds for bioactivity studies in vivo and in vitro studies. The screened compounds can be used for further investigation for bioactivity so that it reduces the time and cost of the chemicals and work.

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