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Molecular docking of 1*H*-pyrazole derivatives to receptor tyrosine kinase and protein kinase for screening potential inhibitors

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Abstract:
Tyrosine kinase receptor and protein kinases drawn much attention for the scientific fraternity in drug discovery due to its important role in different cancer, cardiovascular diseases and other hyper-proliferative disorders. Docking studies of pyrazole derivatives with tyrosine kinase and different serine/threonine protein kinases were employed by using flexible ligand docking approach of AutoDock 4.2. Among the molecules tested for docking study, 2-(4-chlorophenyl)-5-(3-(4-chlorophenyl)-5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazole (1b), 2-(4-methoxyphenyl)-5-(3-(4-methoxyphenyl)-5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazole (1d) and 2-(4-chlorophenyl)-5-(3-(4-chlorophenyl)-5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazole (2b) revealed minimum binding energy of -10.09, -8.57 and -10.35 kJ/mol with VEGFR-2 (2QU5), Aurora A (2W1G) and CDK2 (2VTO) protein targets, respectively. These proteins are representatives of plausible models of interactions with different anticancer agents. All the ligands were docked deeply within the binding pocket region of all the three proteins, showing reasonable hydrogen bonds. The docking study results showed that these pyrazole derivatives are potential inhibitor of all the three protein targets; and also all these docked compounds have good inhibition constant, vdW + Hbond + desolv energy with best RMSD value.

Keywords: Pyrazole derivatives, Tyrosine kinase receptor, Protein kinases inhibitors, Docking studies.

Background:
Pyrazole and its derivatives are a class of five-membered heterocyclic structure with two adjacent nitrogen atoms. These derivatives have drawn more attention in the field of current medicinal and pharmacological research; and reported to have a broad spectrum of biological activities, such as antitumor **[1]**, antimicrobial **[2]**, antioxidant **[3]** and antimalarial activities **[4]**. Several pyrazole derivatives have exhibited potent anticancer activity by the inhibition of the cyclin-dependent kinases (CDKs), which are responsible for eukaryotic cell cycle regulation and they are intensively studied for their cancer implication **[5]**. Recently some aryl pyrozole are reported to have non nucleoside HIV-1 reverse transcriptase inhibitor activities **[6]**. Hence, a systematic investigation of this class of heterocyclic lead containing pharmacoactive agents may play an important role in medicinal and pharmaceutical chemistry.

Cancer is a class of disease that, a group of cells display uncontrolled growth. Strategies to block cell division by affecting the mitotic spindle have been a successful area of research for the advancement of cancer drugs for a long time **[7, 8]**. Since Aurora A, Cyclin-dependent kinases (CDKs) and

Vascular Endothelial Growth Factor Receptor (VEGFR-2) kinases are emerging as a promising molecular drug target for cancer related diseases. These observations have stimulated a great deal of research directed at identifying selective kinase inhibitors as anticancer agents. The VEGFR-2, are attractive targets for the development of anti-cancer agents. Vascular endothelial growth factor belongs to the receptor tyrosine kinase (RTKs) family, play essential roles in all stages of tumor angiogenesis, are able to form autocrine loops which mediate cancer cell growth and survival, and drive hematologic malignancies **[9, 10]**. VEGFR-2 is not only widely distributed in the organization of vascular endothelial cells, but also distributed in some tumor cells; it plays an important role in the cell signaling of VEGFR-2 and tumor proliferation **[11]**. Therefore, inhibition of the VEGFR-2 has become an important research direction in the treatment of cancers **[12]**.

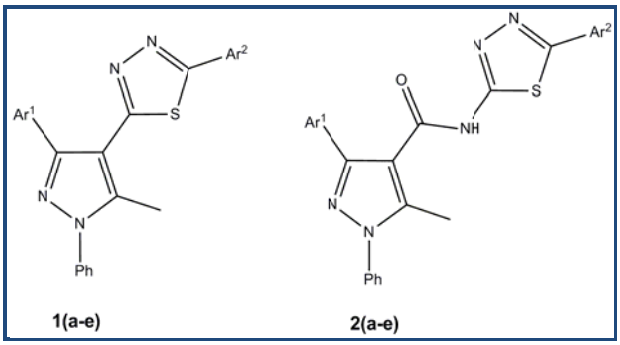

Figure 1: Core structure of pyrazole derivatives.

The Aurora kinases are a family of three highly homologous serine/threonine protein kinases that play a critical role in regulating many of the processes that are pivotal to mitosis **[13]**. Aurora-A kinase has been identified as a colon cancer associated kinase that is overexpressed in a wide range of human tumors such as breast, colorectal, ovarian, as well as glioma **[14-16]**. The role of Aurora A in the cell cycle and tumorogenesis suggested that the inhibition of the kinase activity have remarkable value for the development of small molecular therapeutics for cancer treatment. Thus, targeted inhibition of Aurora-A kinase has become an attractive therapeutic strategy in cancer therapy.

The cyclin-dependent kinases (CDKs) are a family of serine/threonine protein kinases, which are key regulatory elements in cell cycle progression. Inhibition of CDKs activity has turned out to be the most effective strategy for the discovery of novel anticancer agents specifically targeting the cell cycle proteins **[17]**. The importance of CDK2 for cell cycle progression has led to an active pursuit of small molecule inhibitors of this enzyme as a possible treatment against cancer and other hyper-proliferative disorders **[18, 19]**. One significant member of CDKs family, have been proved to participate in the majority of cancer cases mainly due to its vital role during the G1/S transition of the cell cycle when combined with cyclin E. Besides, plenty of reports also illustrated that the inhibition of CDK2 could be an important way for the treatment of cancers **[20, 21]**. In our previous papers **[22, 23]**, we have reported the synthesis and crystal structure of 5-Methyl-1,3-diphenyl-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-4-carboxamide (2a) derivative. In continuation to this, we study herewith, molecular docking studies of pyrazole derivatives with multitargeted kinase (Aurora A, CDK2 and VEGFR2) approach to evaluate their potential value for the treatment of different cancers.

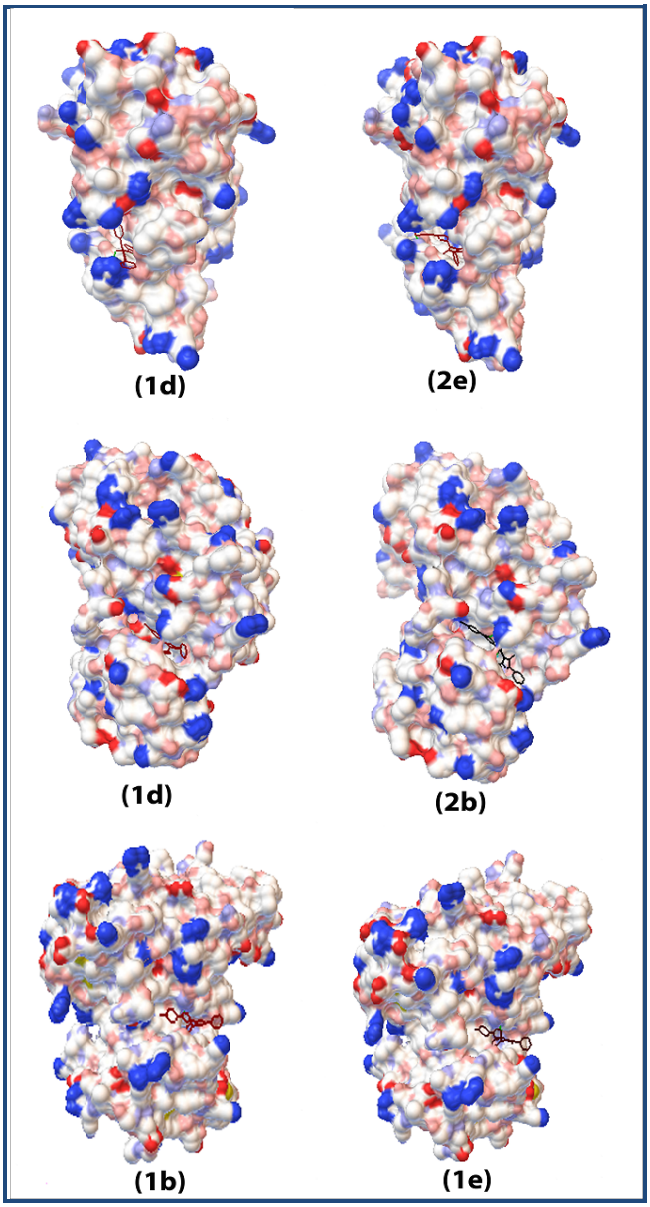

Figure 2: Enfolding of molecules **(1d & 2e), (1d & 2b)** and **(1b, 1e)** in the active site pocket of Aurora A (2W1G), CDK2 (2VTO) and VEGFR-2 (2QU5) inhibitors, respectively

Methodology:

Preparation of ligands and macromolecules

All ligand molecules **Figure 1 (1a-1e and 2a-2e)** were drawn and the structure was analyzed by using ChemDraw Ultra 12.0 **Table 1 (see supplementary material)**. The compounds are converted to 3D structure using Openbable software tool. Energy minimization was performed by employing Dundee PRODRG server **[24]**. The core structure of pyrazole derivatives are shown in **Figure 1**. All three different cancers related proteins (PDB codes 2QU5, 2W1G and 2VTO) were

retrieved from the Protein Data Bank **[25]**. These protein targets were selected based on their best appropriate ligand interactions. The water molecules, co-factors and ligands were removed from the protein structures and then checked for polar hydrogen atom in the macromolecules. For docking study, active site pocket predictions of crucial amino acids of all three proteins are identified from PDB sum **[26]**.

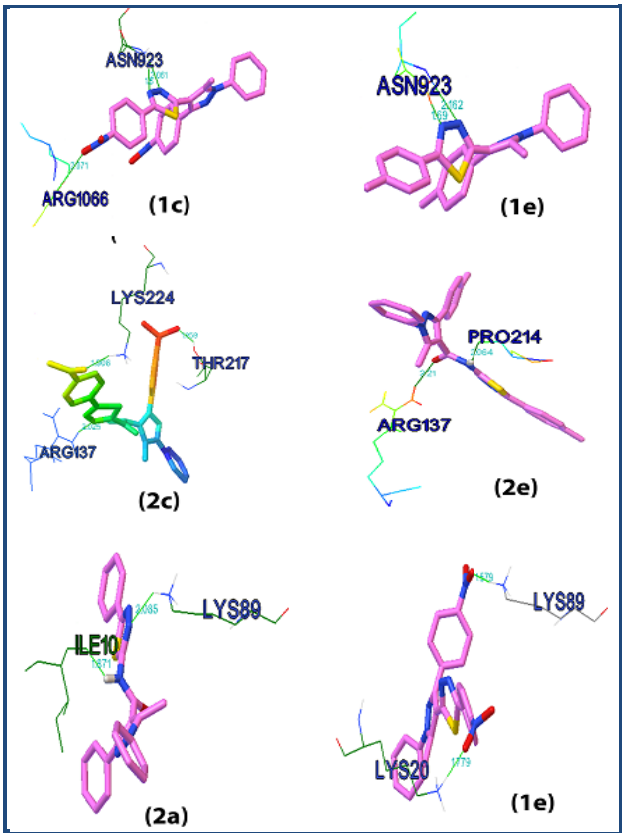

Figure 3: H-bond interaction of ligand molecules **(1c & 1e), (2c & 2e)** and **(2a & 1e)** with VEGFR-2 (2QU5), Aurora A (2W1G) and CDK2 (2VTO) inhibitors, respectively

Molecular docking

Automated docking was used to assess the appropriate binding orientations and conformations of the ligand molecules with different protein inhibitors. A Lamarkian genetic algorithm method implemented in the program AutoDock 4.2, was employed. For docking calculations, Gasteiger charges were added and the rotatable bonds were set by the AutoDock tools and all torsions were allowed to rotate. Polar hydrogen atoms were added and Kollaman charges were assigned to the protein using AutoDock tools (ADT). The grid maps were generated by Autogrid program. Each grid was centered at the active pocket of the proteins (VEGFR-2 (2QU5), Aurora A (2W1G) and CDK2 (2VTO)), and grid parameters were specified separately. In all the cases, we have used grid maps with a grid box size of 55×55×55 **Å**^{3} points with a grid-point spacing of 0.375 **Å**. The Lamarckian genetic algorithm, the pseudo-Solis and Wets methods were applied for minimization using default parameters. The docking protocol for rigid and flexible ligand docking consisted of 10 independent Genetic Algorithm (GA) runs per ligand, population size of 150, maximum number of evaluation 2500000, maximum number of 27000 generation, mutation rate of 0.02 and a crossover rate of 0.8 were used for this study. The docking results for a given macromolecule-ligand pair mainly comprised of the intermolecular interaction energies including inhibition constant, hydrogen bond interaction energy, van der Waals forces, electrostatic energy and ligand efficiency. The lowest binding energy of protein-ligand complex has been considered to be the best. The details of dock score results of the different pyrazole derivatives with protein targets (2QU5, 2W1G and 2VTO) are given in **Table 2-4 (see supplementary material)**.

Results & Discussion:

Docking evaluation for VEGFR-2 inhibitors

Docking studies was carried out for all ten ligand molecules with VEGFR-2 inhibitor (2QU5). Docking of ligands with VEGFR-2 indicated that, all compounds found to have lowest binding energy ranging from -5.92 to -10.09 kJ/mol. Particularly, the ligands 1b and 1e showed minimum binding energy of -10.09 and -9.64 kJ/mol, with ligand efficiency of -0.33 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 2**. The molecular docking of ligand molecules with VEGFR-2 (2QU5) revealed that, maximum numbers of compounds have exhibited hydrogen bond interaction with one or more amino acids in the active pocket region **Figure 3**. The protein (2QU5) comprises of seventeen active site residues, which are promiscuous to the ligands. Out of which, Leu 840, Asn 923, Arg 1066, Cys 919 and Asp 1046, residues are directly interacting with the ligands. The ligands 2d and 2e, has no hydrogen bond interactions with the protein. The details of docked score results of the different pyrazole derivatives with protein VEGFR-2 (2QU5) are given in **Table 2 (see supplementary material)**.

Docking evaluation for Aurora A kinase inhibitors

The active site of Aurora A (2W1G) offers different binding modes for the ligands. All the ligands were docked deeply within the binding pocket region of 2W1G showing considerable hydrogen bonds with Thr 217, Arg 137, Arg 220, Lys 141, Leu 139, Lys 224, Ala 213 and Pro 214. Most of the residues that are in close proximity to the inhibitor are hydrophobic in nature. Ligand molecules 1c and 2a were found to show hydrogen bond interaction with active site amino acid residues (Lys 20 and Lys 89) and (Ile 10 and Lys 89) at a distance of (1.779 and 1.579) and (1.871 and 2.085) Å, respectively **Figure 3**. Almost all the compounds showed promising binding interactions with the receptor. The enzyme surface model was showed in **Figure 2**, which revealed that the molecules 1d and 2e with their binding energy of -8.57 and -8.52 kJ/mol, was well embedded in the active site pocket and likely to be a potent inhibitors of Aurora-A kinase. The ligands with corresponding protein Aurora A (2W1G) interaction details are given in **Table 3 (see supplementary material)**.

Docking evaluation for CDK2 inhibitors

Docking studies was performed for all the pyrazole derivatives with CDK2 (2VTO). Among the molecules tested for docking study, 3-(4-chlorophenyl)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-

carboxamide (**2b**) exhibited minimum binding energy (-10.35 kJ/mol) with ligand efficiency of -0.3. Paul group **[27]** given a detail analysis of the ATP binding site of CDK2 (2VTO) and identified a number of key regions, including a hydrophobic pocket (defined by Ile10, Phe82, Asp86, and Leu134); the relatively small region between the gatekeeper residue (Phe80) and the DFG motif (Asp145) and the solvent accessible region toward Lys89. All the ligands were docked deeply within the binding pocket region of CDK2 (2VTO) showing hydrogen bonds with Ile 10, Lys 20, Lys 89 and Asp 145 **Figure 3)**. The ligand molecules, 1c and 2a showed minimum binding energy of -7.5 and -9.07 kJ/mol, with ligand efficiency of -0.21 and -0.28, respectively. These molecules were completely wrapped by active site amino acid residues at the active site pocket region as shown in **Figure 2**. The details of dock score results of the different pyrazole derivatives with protein (2VTO) are given in **Table 4 (see supplementary material)**.

Conclusion:

Docking studies was performed for all the pyrazole derivatives with protein (VEGFR-2 (2QU5), Aurora A (2W1G) and CDK2 (2VTO) inhibitors) targets. The docking study results showed that these pyrazole derivatives are potential inhibitor of all the three protein targets; and also all these docked compounds have good inhibition constant, vdW + Hbond + desolv energy with best RMSD value. Among the docked molecules, 1b, 1d and 2b revealed minimum binding energy of -10.09, -8.57 and -10.35 kJ/mol with VEGFR-2 (2QU5), Aurora A (2W1G) and CDK2 (2VTO) protein targets, respectively. Thus the docking results provides theoretical framework to rationally design new pyrazole derivatives as cancer inhibitors.

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Supplementary material:

Table 1: Different groups of pyrazole derivatives

Compounds	Ar^1	Ar^2
1a	C_6H_5	C_6H_5
1b	4-Cl-C_6H_4	4-Cl-C_6H_4
1c	4-NO_2-C_6H_4	4-NO_2-C_6H_4
1d	4-OCH_3-C_6H_4	4-OCH_3-C_6H_4
1e	4-CH_3-C_6H_4	4-CH_3-C_6H_4
2a	C_6H_5	C_6H_5
2b	4-Cl-C_6H_4	4-Cl-C_6H_4
2c	4-NO_2-C_6H_4	4-NO_2-C_6H_4
2d	4-OCH_3-C_6H_4	4-OCH_3-C_6H_4
2e	4-CH_3-C_6H_4	4-CH_3-C_6H_4

Table 2: The dock score results of the different pyrazole derivatives with protein VEGFR-2 (2QU5)

Compounds	Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H-bonds	Bond Length (Å)
1a	-8.92	-0.31	290.63	-9.64	2	1.901 2.049
1b	-10.09	-0.33	40.13	-10.76	2	2.238 1.787
1c	-7.62	-0.22	2.59	-8.6	3	2.071 1.8 2.061
1d	-9.06	-0.27	228.21	-10.17	2	2.176 1.913
1e	-9.64	-0.31	86.36	-10.01	2	2.162 1.69
2a	-8.63	-0.27	470.73	-9.86	1	1.984
2b	-9.25	-0.27	164.69	-10.53	1	2.046
2c	-5.92	-0.16	45.5	-7.4	1	2.0
2d	-6.89	-0.19	8.86	-8.97	-	-
2e	-8.24	-0.24	918.24	-9.26	-	-

Table 3: The dock score results of the different pyrazole derivatives with protein Aurora A (2W1G)

Compounds	Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H- bonds	Bond Length (Å)
1a	-7.19	-0.25	5.33	-7.65	-	-
1b	-7.83	-0.25	1.83	-8.92	1	2.1
1c	-6.38	-0.18	20.94	-7.18	2	1.935 2.038
1d	-8.57	-0.26	518.52	-10.17	1	1.911
1e	-8.05	-0.26	1.26	-9.0	-	-
2a	-7.53	-0.24	3.03	-8.28	2	2.208 1.776
2b	-8.06	-0.24	1.24	-8.92	1	2.055
2c	-7.25	-0.19	4.84	-7.81	3	2.025 1.906 1.959
2d	-8.09	-0.22	1.18	-9.38	1	1.789
2e	-8.52	-0.25	571.5	-9.21	2	2.064 2.21

Table 4: The dock score results of the different pyrazole derivatives with protein CDK2 (2VTO)

Compounds	Binding Energy (kJ mol^{-1})	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H- bonds	Bond Length (Å)
1a	-7.75	-0.27	2.09	-8.98	1	2.914
1b	-8.38	-0.27	716.2	-9.69	-	-
1c	-7.5	-0.21	3.2	-7.26	2	1.779 1.579
1d	-9.77	-0.3	68.66	-11.18	1	1.722
1e	-8.29	-0.27	836.94	-9.32	-	-
2a	-9.07	-0.28	223.71	-9.99	2	1.871 2.085
2b	-10.35	-0.3	25.86	-11.15	2	2.126 2.232
2c	-6.94	-0.18	8.13	-8.28	2	2.016 2.009
2d	-8.48	-0.24	611.83	-9.32	1	1.861
2e	-8.58	-0.25	513.29	-10.13	1	1.954