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Research Article

MOLECULAR DOCKING STUDIES OF PHENYLAMINOPYRIMIDINE AND PYRAZOLYLAMINOPYRIMIDINE DERIVATIVES AS JANUS KINASE 2 (JAK2) INHIBITORS

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ABSTRACT

Background: Janus kinase 2 (JAK2) plays a crucial role in the myeloproliferative disorders, autoimmune diseases and many other diseases. Somatic mutation of JAK2 (V617F) results in a constitutively active JAK2 tyrosine kinase. Thus inhibition of JAK2 kinase activity is of significant therapeutic importance in the treatment of myeloproliferative disorders.

Objective: The aim of this study is to reveal the binding mode and hydrogen bond interactions of the phenylaminopyrimidine and pyrazolylaminopyrimidine derivatives with Janus kinase 2

Methods: In the present study, we describe the molecular docking studies for a series of 55 phenylaminopyrimidine and pyrazolylaminopyrimidine derivatives against JAK2 using Glide tool by Flexible Docking Method.

Results: The docking results indicated that most of the compounds have shown hinge hydrogen bond interactions with Leu932, Glu930 and hydrophobic contacts with Asp939, Arg980, Leu855 and Asp994. The clinical development ligands TG101348 and AZD-1480 have shown hinge hydrogen bond interactions with Leu932 and Arg980.

Conclusion: Hence, the above docking study results indicate that the binding affinity and hydrogen bond interactions of these molecules with respect to amino residues can be supportive evidence to carry out further studies in designing structure-based newer molecules with JAK2 inhibitory activity.

Keywords: Janus kinase 2 (JAK2), Phenylaminopyrimidine, Pyrazolylaminopyrimidine, Docking, Glide.

INTRODUCTION

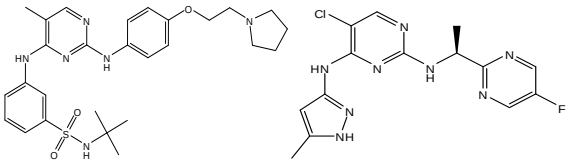
Janus kinase 2 (JAK2) is a nonreceptor tyrosine kinase corresponding to a member of the Janus kinase family found to be essential in many cellular signaling pathways [1, 2]. The Janus kinase (JAK) family consists of four non-receptor tyrosine kinases (JAK1, JAK2, JAK3 and TYK2), and are the key elements in many aspects of cytokines, growth factor mediated signaling pathways [3-6] and several intracellular signaling pathways, including the eponymous JAK/STAT pathway [7-10].

The members of the JAK family consist of seven regions of JAK homology (JH1-JH7) domains [10,11]. The C-terminus of JAKs is enclosed with a catalytic kinase domain (JH1) and a pseudokinase domain (JH2), while the JH2 domain exposes to retain an important regulatory role in the tyrosine kinase activity of the JH1 domain [12, 13]. The role of SH2 domain (JH3-4) remains ambiguous [14-16]. The N-terminal domain contains a FERM (Band-4.1, ezrin, radixin and moesin) domain (JH4-7), essential for interaction of the JAKs with their receptors, regulatory proteins and also in some cases to the receptor cell-surface [17-19].

JAK2 is an essential enzyme in mammalian development. A single somatic mutation in pseudokinase (JH2) domain of JAK2 results in JAK2V617F mutation producing a constitutively active JAK2 tyrosine kinase.

The JAK2V617F mutation has been recently identified in patients with myeloproliferative disorders [20-23], cardiovascular disease, autoimmune diseases, essential thrombocytosis, etc. [24, 25]. Thus inhibition of JAK2 kinase activity became significantly and therapeutically important.

TG101348 and AZD-1480 are the two compounds showing JAK2 inhibitory activity [26-29], under clinical development [26-29], consisting of Phenylaminopyrimidine and Pyrazolylaminopyrimidine moieties respectively. This concludes that JAK2 inhibitors will offer a new targeted therapy for patients with myeloproliferative disorders [30-32].



TG101348AZD-1480

Fig. 1: Chemical structure of the JAK2 inhibitors, TG101348 and AZD-1480

The aim of the present study is to conduct the molecular docking studies on a series of known Phenylaminopyrimidine and Pyrazolylaminopyrimidine derivatives as JAK2 inhibitors. The interest of the molecular docking studies is to know the binding mode of the ligands on JAK2 kinase and the structural requirements important for JAK2 inhibition. Molecular docking studies were carried out to a data set of 55 molecules using GLIDE 5.5. [33].

MATERIALS AND METHODS

The Dataset

A series of 55 Phenylaminopyrimidine and Pyrazolylaminopyrimidine derivatives having JAK2 inhibitory activity (IC50 < 1 μM) was collected from the literature [34-38] as shown in Table 1 and Table 2. The molecules were sketched using ISIS Draw and then applied into a LigPrep module for further preparations.

Ligand Preparation

LigPrep module [39] was used to convert the 2D structures to 3D and for hydrogen addition, the unfavourable bond lengths and bond angles were corrected by subjecting each ligand to a full length

minimization using OPLS-2005 (Optimized Potential for Liquid Simulations-2005) force field. We performed the ionization of a molecule in the pH range of 5-9 with the help of Epik (Sophisticated algorithm and performs ionization and tautomerization together) in LigPrep.

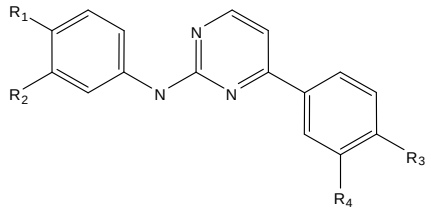
Tautomeric forms and stereoisomeric forms of a ligand were generated, where one of the isomeric forms will have strong interactions with the binding sites. Finally, the lowest energy conformation as one per ligand was generated.

Protein preparation

The X-ray crystal structure of the JAK2 protein (PDB ID: 3KRR) was obtained from protein data bank and further modified for Glide docking calculations.

The Janus kinase 2 was prepared by employing the Protein Preparation Wizard of the Schrödinger suite 2009. H-atoms were added to the protein and the co-crystallized ligands, water molecules were identified and removed from the structure. Further minimization was performed by applying an OPLS-2005 as force field and RMSD value of 0.30 Å.

Table 1a: Compounds 1-32 (Phenylaminopyrimidine) selected for docking studies



Comp.	R_1	R_2	R_3	R_4
1	morpholinyl	H	CN	H
2	morpholinyl	H	H	H
3	morpholinyl	H	$CONH_2$	H
4	morpholinyl	H	H	NH_2
5	morpholinyl	H	N-(furan-2-ylmethyl)carbamoyl	H
6	morpholinyl	H	SO_2NH_2	H
7	morpholinyl	H	(2-methyl-1H-imidazol-1-yl)methyl	H
8	morpholinyl	H	$NHC=OCH_3$	H
9	morpholinyl	H	NH_2	OMe
10	morpholinyl	H	OH	OMe
11	H	pyrrolidin-1-ylmethyl	$NHSO_2CH_3$	H
12	piperidin-1-ylmethyl	H	$NHSO_2CH_3$	H
13	H	morpholinomethyl	$NHSO_2CH_3$	H
14	H	(4-hydroxypiperidin-1-yl)methyl	$NHSO_2CH_3$	H
15	morpholinomethyl	H	$NHSO_2CH_3$	H
16	H	(4-methylpiperazin-1-yl)methyl	$NHSO_2CH_3$	H
17	(4-methylpiperazin-1-yl)methyl	H	$NHSO_2CH_3$	H
18	morpholinyl	H	$NHSO_2CH_3$	H
19	morpholinyl	H	H	N-(cyanomethyl)carbamoyl
20	morpholinyl	H	H	$NHC=OCH_2CN$
21	morpholinyl	H	H	CH_2NHCH_2CN
22	morpholinyl	H	$NHC=OCH_2CN$	H
23	morpholinyl	H	N-(cyanomethyl)carbamoyl	OMe
24	morpholinyl	H	(2-cyanopropan-2-yl)carbamoyl	H
25	morpholinyl	H	N-methyl-(cyanomethyl)carbamoyl	H
26	morpholinyl	CF_3	N-(cyanomethyl)carbamoyl	H
27	3-(diethylamino)propoxy	H	N-(cyanomethyl)carbamoyl	H
28	morpholinomethyl	H	N-(cyanomethyl)carbamoyl	H
29	morpholinyl	F	N-(cyanomethyl)carbamoyl	H
30	H	morpholinyl	N-(cyanomethyl)carbamoyl	H
31	thiomorpholinyl	H	N-(cyanomethyl)carbamoyl	H
32	(4-ethylpiperazin-1-yl)methyl	H	N-(cyanomethyl)carbamoyl	H

Receptor grid generation

After protein preparation, the receptor energy grid was built, generated by the Receptor Grid Generation panel. The Janus kinase 2 (3KRR) was associated with a ligand (NVP-BSK805), the ligand then picked to determine the active site position and size of the active site.

A docking energy grid was developed by the center determined by ligand, enclosing the residues within 10 Å from their centroid. The dataset of 55 Phenylaminopyrimidine and Pyrazolylamino pyrimidine derivatives was docked in the kinase domain of JAK2, using Glide 5.5 module in extra precision mode (XP) by the application of MCSA (Monte Carlo Based Simulated Algorithm) based minimization.

Docking Validation

For validating the Glide dock program, the protein (JAK2) was redocked with the native ligand and the clinical trial ligands (TG101348 and AZD-1480).

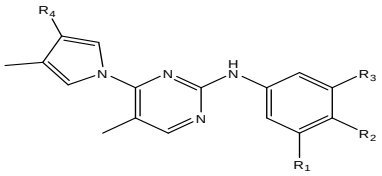
Finally, the results of the reference ligands and the molecules (Phenylaminopyrimidine and Pyrazolylaminopyrimidine derivatives) of the current dataset were compared as mentioned in Table 3.

RESULTS AND DISCUSSION

Binding mode analysis by molecular docking

A molecular docking technique was performed by using Glide, in order to investigate the detailed intermolecular interaction between ligands and the Janus kinase 2 enzyme. Flexible docking simulations were performed for understanding the binding mode of the ligands on JAK2 kinase as well as to obtain information for further structural development. All the docking results are shown in Table

Table 1b: Compounds 33-39 (Phenylaminopyrimidine) selected for docking studies



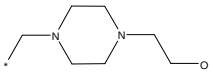
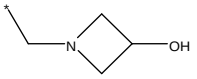
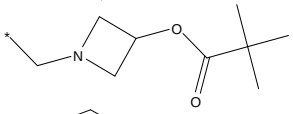
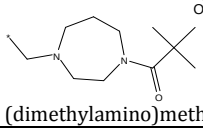
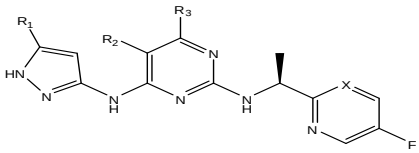
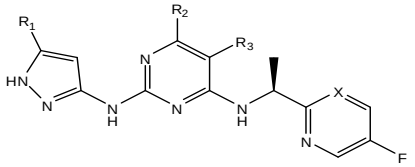
Comp.	R_1	R_2	R_3	R_4
33	OMe	OMe	OMe	CH_2OH
34	H	pyrrolidin-3(S)-ol-1-yl	Me	CH_2OH
35	OMe	OMe	OMe	
36	OMe	OMe	OMe	
37	OMe	OMe	OMe	
38	OMe	OMe	OMe	
39	Me	$O(CH_2)_2OH$	Me	(dimethylamino)methyl

Table 2a: Compounds 40-51 (Pyrazolylaminopyrimidine) selected for docking studies



Comp.	R_1	R_2	R_3	X
40	OMe	H	3-methoxyphenyl	CH
41	OMe	H	4-fluorophenyl	CH
42	OMe	H	1H-indol-5-yl	CH
43	OMe	Cl	H	N
44	Me	Me	H	N
45	Me	Br	H	N
46	dimethylamino	Cl	H	N
47	dimethylamino	F	H	N
48	Me	Cl	morpholinyl	N
49	OMe	H	morpholinyl	N
50	OMe	F	morpholinyl	N
51	OMe	Cl	morpholinyl	N

Table 2b: Compounds 52-55 (Pyrazolylaminopyrimidine) selected for docking studies



Comp.	R_1	R_2	R_3	X
52	OMe	morpholin-4-yl	F	N
53	OMe	morpholin-4-yl	H	CH
54	OMe	1-methylpiperazin-2-one-4-yl	H	CH
55	OMe	2-oxa-5-azabicyclo[2.2.1]heptan-5-yl	H	CH

Table 3: Glide extra-precision (XP) results of the compounds along with the standards against Janus kinase 2 protein

Comp.	IC50 (μM)	Poses[a]	G. score[b]	G. energy[c]	H[d]	Interacting Residues
1	0.533	2	-8.54	-47.96	2	Leu932 (2)
2	0.467	2	-8.86	-45.52	2	Leu932 (2)
3	0.326	2	-9.56	-51.01	3	Leu932 (2), Asp994
4	0.322	2	-8.96	-45.72	3	Leu932 (2), Asp994
5	0.202	2	-8.91	-61.02	2	Leu932 (2)
6	0.083	1	-8.63	-54.45	3	Leu932 (2), Asp994
7	0.081	2	-10.07	-56.06	2	Leu932 (2)
8	0.067	2	-8.87	-53.7	3	Leu932 (2), Asp994
9	0.016	1	-8.57	-49.85	3	Leu932 (2), Asp994
10	0.003	2	-9.1	-43.21	3	Leu932 (2), Asp994
11	0.006	1	-8.14	-52.03	2	Leu932 (2)
12	0.011	1	-8.55	-56.58	2	Leu932 (2)
13	0.008	3	-9.39	-61.32	3	Leu932 (2), Asp994
14	0.012	2	-7.74	-65.17	3	Leu932 (2), Asp994
15	0.009	2	-8.82	-60.02	3	Leu932 (2), Asp994
16	0.014	5	-9.65	-59.97	2	Leu932 (2)
17	0.018	3	-10.09	-57.31	3	Leu932 (2), Gln853
18	0.004	4	-9.1	-49.03	3	Leu932 (2), Asp994
19	0.545	2	-8.99	-50.86	3	Leu932 (2), Arg980
20	0.303	2	-8.86	-52.44	2	Leu932 (2)
21	0.132	3	-9.36	-53.64	4	Leu932 (2), Arg980 (2)
22	0.01	2	-5.79	-49.76	2	Gln853, Arg980
23	0.03	1	-7.79	-60.44	3	Leu932 (2), Asp994
24	0.549	2	-9.24	-56.15	2	Leu932 (2)
25	0.298	1	-8.93	-57.44	2	Leu932 (2)
26	0.374	1	-9.25	-53.66	4	Leu932 (2), Ser936, Asp994
27	0.166	1	-9.57	-61.95	4	Leu932 (2), Tyr934, Asp994
28	0.163	1	-6.9	-54.41	2	Gly993, Asn981
29	0.116	2	-8.52	-59.11	3	Leu932 (2), Asp994
30	0.113	1	-9.12	-56.7	2	Leu932 (2)
31	0.069	2	-9.55	-56.79	3	Leu932 (2), Asp994
32	0.047	2	-10.01	-57.09	3	Leu932 (2), Asp994
33	0.0077	1	-10.03	-52.38	3	Leu932 (2), Asp994
34	0.0016	3	-10.25	-53.13	4	Leu932 (2), Asp994, Asp939
35	0.0037	3	-10.81	-66.64	3	Leu932 (2), Glu1015
36	0.0365	1	-10.36	-54.41	3	Leu932 (2), Arg980
37	0.2193	2	-9.48	-59.99	2	Leu932 (2)
38	0.0651	3	-10.18	-60.44	2	Leu932, Lys857
39	0.0025	1	-11.15	-57.43	4	Leu932 (2), Asp939, Ser936
40	0.036	3	-8.61	-53.64	5	Glu930, Leu932 (2), Leu855, Arg980
41	0.011	3	-8.07	-46.68	3	Leu932 (2), Glu930
42	0.048	3	-8.61	-54.91	2	Asp939, Glu930
43	0.108	3	-9.1	-48.94	2	Leu932 (2)
44	0.004	4	-8.34	-46.15	5	Leu932 (2), Glu930, Leu855, Arg980
45	0.01	6	-10.21	-43.39	4	Leu932 (2), Glu930, Leu855
46	0.488	5	-8.09	-40.39	2	Leu932, Glu930
47	0.815	6	-8.9	-49.41	2	Leu932 (2)
48	0.009	3	-9.83	-44.17	3	Leu932 (2), Glu930
49	0.03	3	-8.47	-47.82	3	Leu932 (2), Asp939
50	0.008	3	-8.58	-52.52	2	Leu855, Ser936
51	0.09	3	-6	-50.26	2	Leu932, Arg980
52	0.037	1	-7.67	-51.23	3	Leu932 (2), Asp939
53	0.022	2	-7.44	-46.48	3	Leu932 (2), Asp939
54	0.015	1	-7.69	-52.67	3	Leu932 (2), Asp939
55	0.017	4	-8.02	-54.42	3	Leu932 (2), Asp939
R1*	0.00026	6	-8.09	-45.62	2	Leu932 (2)
R2*	0.003	3	-10.22	-60.67	3	Leu932 (2), Arg980
R3*	0.00048	2	-8.82	-64.27	1	Leu855

R1*=AZD-1480 compound; R2*=TG101348 compound; R3*=NVP-BSK805 (3KRR bound native ligand); [a]No. of Poses generated; [b]Glide score (XP); [c]Glide energy (Kcal/mol); [d]No. of H-bonds.

The docking results of Phenylaminopyrimidine and Pyrazolylaminopyrimidine molecules has shown a good binding affinity to the target protein JAK2, as indicated by the presence of two-five hydrogen bonding interactions between the ligands and the catalytic site amino acids Leu932, Asp 939, Asp994, Glu 930 and Leu 855, etc. (Figure 2). The docking analysis of the compounds shows following interactions. The Pyrimido amino group of compound seven (7) shows hinge hydrogen bond interactions with Leu932 (2) with G. Score = -10.07 (Figure 2a); The binding mode of compound 17 describes that, the Pyrimido amino group interacts with hinge region Leu932 (2), and the Piperazin group have hydrophobic contact with Gln853, G. Score = -10.09(Figure2b); Compound 24 describes that, the Pyrimido amino group interacts with kinase domain amino acid residue Leu932 (2), G. Score = -9.24 (Figure 2c); Compound 32 describes that, the Pyrimido amino group interacts with kinase domain Leu932 (2), and the amide group with Asp994 G. Score = -10.01 (Figure 2d); Compound 34 describes that, the Pyrimido amino group interacts with the kinase domain amino acid residue Leu932 (2), and the pyrrolidine group interacts with Asp939 and a methanol group with Asp994, G. Score = -10.25 (Figure2e);

Compound 35 describes that, the Pyrimido amino group interacts with the kinase domain amino acid residue Leu932 (2), and the ethanol group with Glu1015, G. Score = -10.07 (Figure2f) [40-45]. The above interaction of ligands with JAK2 protein explains the importance of Pyrimido amino group in chemical structure regarding the binding affinity against the JAK2 protein. More negative Glide score value indicates the better interaction of the ligand with the target protein. A positive correlation was observed between the binding affinity and their experimental inhibitory activity of ligands against the JAK2 protein.

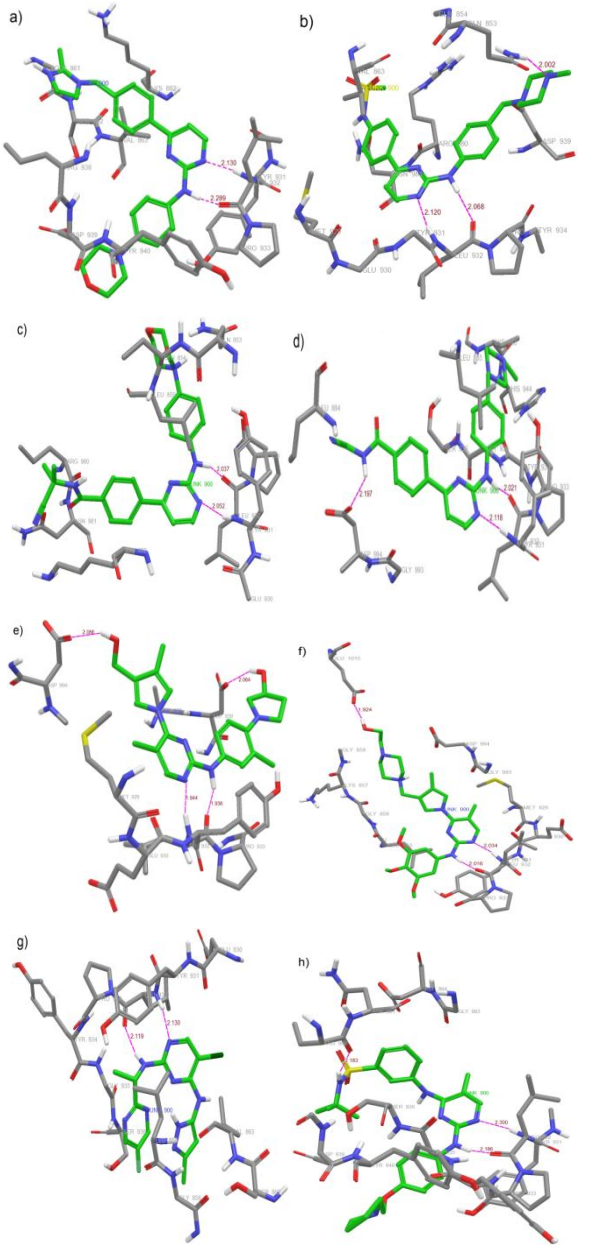


Fig. 2: Binding mode of ligands with JAK2 kinase active site. a). Crystal structure of Compound seven; b). Compound 17; c). Compound 24; d). Compound 32; e). Compound 34; f). Compound 35; g). Compound AZD-1480; h). Compound TG101348 bound to JAK2 protein respectively.

The binding mode of reference compound AZD-1480 is shown in Figure2g, showing two hydrogen bond interactions with hinge region residue Leu932 and also having a Glide score of -8.09. Figure2h, the compound TG101348 showing interactions with hinge region amino acids Leu932 (2) and hydrophobic cavity Arg980 with a Glide score of -10.22.

According to the binding interactions of the known ligands and reference compounds, it is indicated that the hinge and a hydrophobic region amino acids are the most intractable residues present in the kinase domain. It is also noted that most of the compounds are showing two hydrogen bond interactions with hinge region residue Leu932. The docking results illustrate that, the Pyrimido amino group is the most interactive group of the molecules having good binding interactions with the hinge and hydrophobic region residues of the kinase domain of JAK2.

CONCLUSION

In this study, molecular docking studies were carried out in a series of Phenylaminopyrimidine and Pyrazolylaminopyrimidine derivatives using Glide software by flexible docking method to describe the binding mechanism of ligands to the target JAK2 protein. These docking results precisely indicate that the Phenylaminopyrimidine and Pyrazolylaminopyrimidine derivatives have good hinge hydrogen bond interactions with Leu932, Glu930 and hydrophobic contacts with Asp939, Arg980, Leu855 and Asp994 residues present at the catalytic site of the JAK2 kinase protein. The generated molecular docking model has given scope for the development of novel chemical entities with potent JAK2 inhibitory activity.

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Authors' Competing Interests

The authors declare no conflict of interest.

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