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RESEARCH ARTICLE

Insights into the Identification of p38-alpha Mitogen activated Protein Kinase against Pyridazinopyridinone Derivatives in the Treatment of Rheumatoid Arthritis

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ABSTRACT:
Rheumatoid arthritis (RA) is a long-term disease that leads to inflammation of the joints and surrounding tissues. It also affects other organs and considered as an autoimmune disease. The body's immune system fights off foreign substances, like viruses by producing cytokines. But in an autoimmune disease, the immune system confuses healthy tissue for foreign substances and the body attacks its own tissues. The p38 Mitogen Activated Protein Kinase (MAPK) plays an important role in the production of proinflammatory cytokines, making it an attractive target for the treatment of Rheumatoid Arthritis and various inflammatory diseases. Currently Methotrexate, Leflunomide, and anti-inflammatory drugs like Aspirin, Ibuprofen are given to patients affected with Rheumatoid Arthritis, but these drugs have serious side effects. Recently, pyridazinopyridinone and its derivatives were found to be effective in inhibiting the p38 alpha MAP kinase and thereby help in treating inflammatory diseases. Docking analysis was performed using Schrödinger's GLIDE and potent ligands were selected from the literature. The structures of the ligands were determined and energy was minimized using OPLS force field. These energy minimized ligand structures were then subjected to High throughput virtual screening against the target protein p38-α. The ligands which showed best energy and score were selected for Induced Fit Docking (IFD). The interactions between the ligands and the protein were observed in different poses and the final docking results were observed. The ligand31 has a good docking score of -11.20 and the Glide energy -72.00 with hydrogen bonding between ASP 168 and GLU 71 in the active site region. Hence, pyridazinopyridinone derivatives were found to be potent inhibitors of the target p38 Mitogen Activated Protein.

KEYWORDS: Rheumatoid Arthritis, p38 MAPK, pyridazinopyridinone, induced fit Docking, GLIDE.

INTRODUCTION:
Rheumatoid Arthritis (RA) is an autoimmune disease of unknown aetiology that primarily targets synovial tissues, cartilage and bone, and is the most common form of immune-mediated arthritis[1]. RA is an inflammatory disease that affects nearly 1% of the world's adults. It is characterized by symmetric polyarticular inflammation of the synovium, typically of the small joints of the hands (MCP and PIP), wrists and feet. This inflammation results in pain and stiffness, and can lead to progressive joint damage resulting in deformities and loss of function. The treatment of RA has been revolutionized by advances in the understanding of its pathologic mechanisms and the development of drugs which target them[2]. Anaemia is a characteristic of rheumatoid arthritis whereas by contrast, iron is elevated in the synovial fluid of arthritic joints. This suggests a significant derangement of iron metabolism in RA as well, and a mechanism in which elevated superoxide liberates free iron from ferritin in synovial fluid, thereby

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catalysing further the damaging production of hydroxyl radicals[3]. Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that play essential roles in eukaryotic cells by transducing environmental stress signals into altered gene expression[4-7]. MAP kinases are activated in response to a diverse array of extracellular stimuli ranging from growth factors and cytokines to environmental stress factors[8-11]. The p38 MAPK family of serine/threonine protein kinases was explicitly implicated in the regulation of key inflammatory responses in mammals, contributing to a large body of evidence that eventually established it as a therapeutic target for a range of diseases that have inflammation as a common disease progression mechanism. p38α MAPK is an established drug discovery target for peripheral inflammatory diseases, including rheumatoid arthritis, where increased levels of proinflammatory cytokines coincide with disease progression[12-15]. Initial research concentrated on defining the components and organization of MAPK signalling cascades, but recent studies have begun to shed light on the physiological functions of these cascades in the control of gene expression, cell proliferation and programmed cell death[16,17].

MATERIALS AND METHODS:

Molecular Docking:

Docking is the technique which envisages the preferred orientation of one molecule to a second when bound to each other to form a stable complex in three dimensional spaces. Docking tools are based on the search, algorithm and the scoring function. A search algorithm finds the best docking pose measured by the scoring function. A scoring function differentiates correct docking poses from incorrect ones[18]. Therefore, structure based drug design has gained importance which is facilitated through computational docking studies employing programs such as GLIDE docking[19,20].

Ligplot:

The LIGPLOT program automatically generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. The output is a colour, or black-and-white, PostScript file giving a simple and informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities. The program is completely general for any ligand and can also be used to show other types of interaction in proteins and nucleic acids. It was designed to facilitate the rapid inspection of many enzyme complexes, but has found many other applications[21].

Protein Preparation:

The three dimensional structure of p38 alpha Mitogen Activated Protein was retrieved from the RCSB Protein data bank (PDB ID: 3LHJ). All the water molecules and ligands were removed from the PDB file prior to docking[22].

Ligand Preparation:

The Schrödinger ligand preparation is designed to prepare high quality, all-atom 3D structures for large numbers of druglike molecules. GLIDE (Grid-based Ligand Docking with Energetics) is a ligand binding program provided by Schrödinger that searches for favourable interactions between one or more ligand molecules and a receptor molecule, usually a protein. It provides a complete solution for ligand-receptor docking. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. Finally, the minimized poses are re-scored to generate the Score or Glide score is the sum total of various figures generated for each ligand during the docking process. The best G Score is obtained as the most negative value and the most active ligands in terms of G Score are enlisted in descending order[23].

Table 1: IC_{50} values of various pyridazinopyridinone derivatives

LIGANDS	MOLECULAR FORMULA	IC_{50} μM
Ligand 17	$C_{20}H_{11}F_3N_4O$	2.2 ± 0.2
Ligand 18	$C_{20}H_{12}F_4N_4O$	0.9 ± 0.3
Ligand 19	$C_{19}H_{12}F_3N_5O$	328 ± 200
Ligand 20	$C_{19}H_{11}F_4N_5O$	111 ± 24
Ligand 21	$C_{21}H_{15}F_3N_4O$	299 ± 22
Ligand 22	$C_{20}H_{12}F_3N_3O_2$	78 ± 68
Ligand 23	$C_{20}H_{11}F_4N_3O_2$	8.4 ± 1.9
Ligand 24	$C_{21}H_{14}F_3N_3O$	55.8 ± 3.2
Ligand 25	$C_{21}H_{13}F_4N_3O$	6.1 ± 0.2
Ligand 26	$C_{22}H_{15}F_2N_3O_3$	> 1000[b]
Ligand 30	$C_{22}H_{16}F_2N_4O_2$	2.7 ± 0.8
Ligand 28	$C_{23}H_{18}F_2N_4O_2$	2.7 ± 1.0
Ligand 31	$C_{25}H_{20}F_2N_4O_2$	2.7 ± 0.8
Ligand 32	$C_{26}H_{22}F_2N_4O_2$	3.1 ± 0.5
Ligand 33	$C_{25}H_{19}F_3N_4O_2$	1.4 ± 0.5
Ligand 29	$C_{24}H_{17}ClF_2N_4O_2$	2.6 ± 0.4
Ligand 34	$C_{24}H_{19}F_2N_5O_2$	14.5 ± 3.4
Ligand 31	$C_{25}H_{20}F_2N_4O_2$	2.7 ± 0.8
Ligand 35	$C_{26}H_{24}N_4O_2$	2.7 ± 1.0
Ligand 36	$C_{24}H_{19}ClN_4O_2$	2.1 ± 0.5
Ligand 37	$C_{26}H_{21}F_3N_4O_2$	3.9 ± 0.9
Ligand 38	$C_{26}H_{23}FN_4O_2$	3.1 ±1.2
Ligand 39	$C_{26}H_{20}F_4N_4O_2$	2.9 ± 0.7
Ligand 40	$C_{25}H_{20}ClFN_4O_2$	1.6 ± 0.6

GLIDE:

Extra precision (XP) mode of Glide program was used for all docking calculations. The binding site is defined in terms of two concentric cubes such as bounding box and enclosing box. The former box includes the center of any acceptable ligand pose, and the latter one encloses all ligand atoms of an acceptable pose. Various energy grids for binding site were calculated and stored. During the initial phase of docking calculation, the maximum poses generated from the variables were fixed to 5000 and the best variable which set the number of poses per ligand that enters the energy minimization was set to 1000. The dielectric constant of 4.0 and 1000 steps of conjugate gradient was applied in the energy minimization protocol. At the ending of each docking calculation, utmost 100 poses per ligand were generated. Using Glide scores (G-score) function, the best docked structure was chosen. E-model is also one of the scoring functions used by Glide, which is inferred from the combination of the G-score, van der Waals and Coulombic interactions, and the strain energy of the ligand[24]. At the end of each docking run, interactions are shown in the form of "poses" with the energy values given in kcal/mol for each pose[25].

RESULTS AND DISCUSSION:

The ligands were chosen by high throughput virtual screening method in Maestro, out of which four ligands were selected for induced fit docking method including the native ligand based on the scores and the lowest energy. The interaction between the native ligand and the protein could be seen in ligplot. Ligplot generates 2D representation of the protein-ligand complex. The interaction of the native ligand with the protein is shown in Fig. 1.

The aminoacid residue Asp 168 has formed a protein – ligand complex with an interaction of 2.67Å. The interaction has been shown in green colour. Pyridazinopyridinone derivatives were chosen as ligands. Its molecular formula and values of IC_{50} are tabulated in Table 1.

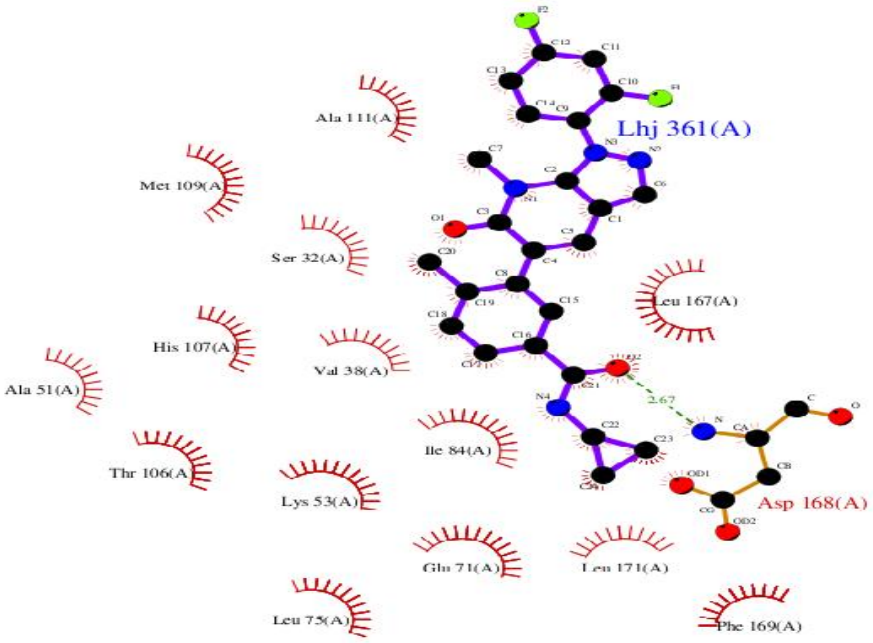


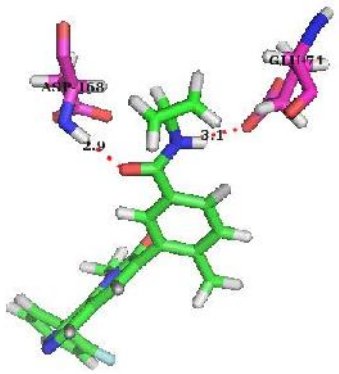
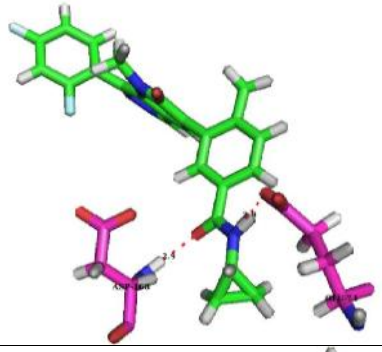
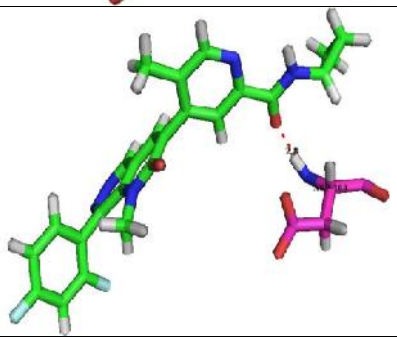
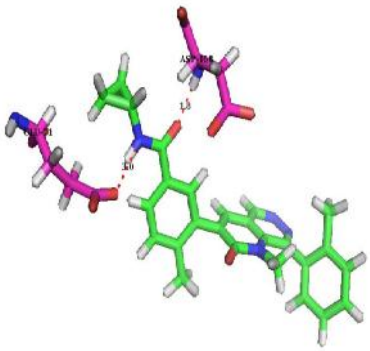
Fig. 1: Interaction of the native ligand with the protein

Out of performing the high throughput screening, finally four ligands were chosen as the best ones. The ligands chosen were ligand 31, 33,34 and 35. The ligand 31 showed a dock score of -11.20 and Glide Energy -72.00 with an interaction of 2.974 Å at ASP 168 and 3.131 Å at GLU 71. The ligand 33 showed a dock score of -11.36and Glide Energy -71.19 with an interaction of 2.854 Å at ASP 168 and 2.897 Å at GLU 71.

The ligand 34 showed a dock score of -10.50 and Glide Energy -69.14 with an interaction of 2.773 Å at ASP 168. The ligand 35 showed a dock score of -10.52 and Glide Energy --69.86 with an interaction of 2.863 Å at ASP 168 and 3.035 Å at GLU 71.

The ligand showing best Glide Energy among the four ligands was ligand 31. Therefore, ligand 31 of the pyridazinopyridinone derivatives could emerge as a potent inhibitor of the p38 mitogen actigen protein kinase which could play a vital role in the treatment of Rheumatoid Arthritis.

Table 2: Interaction of Ligands 31, 33, 34, 35 showing Dock score, Glide Energy and Distance

LIGAND	DOCK SCORE/ GLIDE ENERGY Kcal/mol	D-H…A	DISTANCE Å	INTERACTIONS
Ligand 31	11.20 / -72.00	(ASP 168) N-H…O N-H…O(GLU 71)	2.974 3.131	
Ligand 33	-11.36/ -71.19	(ASP 168) N-H…O N-H…O(GLU 71)	2.854 2.897	
Ligand 34	-10.50/ -69.14	(ASP 168) N-H…O	2.773	
Ligand 35	-10.52/ -69.86	(ASP 168) N-H…O N-H…O(GLU 71)	2.863 3.035	

CONCLUSION:

Rheumatoid arthritis is an autoimmune disease caused by the activation of pro inflammatory cytokines against the own host cells by p38 alpha MAPK protein. Pyridazinopyridinone compound derivatives were found to be effective in blocking the p38 protein which causes rheumatoid arthritis. The pyridazinopyridinone compound derivatives were designed targeting the ATP binding site of the protein p38 alpha MAPK and were tested for their ability to inhibit the protein p38 alpha MAPK. The ligand pyrazolopyridinone interacted mainly with the residues ASP 168 and with the hydrophobic residue GLU71. Induced fit docking studies showed that the ligands 31, 33, 35 and 34 had low energy and best glide score. The first pose of these ligands were analyzed to be the best poses of these ligands. Docking studies showed that the ligand 31 has a good docking score of -11.20 and the Glide energy -72.00 when compared with other pyridazinopyridinone compounds. It also has strong hydrogen bonding interaction with ASP 168 and GLU 71 in the active site region. Hence, ligand 31 has emerged to be a new potent drug candidate for inflammatory diseases especially rheumatoid arthritis.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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REFERENCES:

1. Josef S, Edward C. Keystone. Rheumatoid arthritis: where are we now? Rheumatology (Oxford) 2012; 51: 5: v1-v2.
2. J. Michelle and David AF. Advances in the Medical Treatment of Rheumatoid Arthritis. Hand Clin. 2011; 27(1): 11–20.
3. Douglas BK. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. BMC Med Genomics. 2009 8; 2:2.
4. Deborah B, Nobuyuki T, Anja J, Juan-Jose V, Nyaya K, Yoshinori T, *et al.,* Mechanism of p38 MAP kinase activation in vivo. Genes Dev. 2003; 17: 1969-1978.
5. Dérijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ *et al.*. Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. Science. 1995; 3; 267(5198):682-5.
6. Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr Opin Cell Biol. 1999; 11(2):211-8.
7. Ono K, Han J. The p38 signal transduction pathway: activation and function. Cell Signal. 2000; 12(1): 1-13.
8. Chung-I C, Bing-e X, Radha A, Melanie HC and Elizabeth JG. Crystal Structures of MAP Kinase p38 Complexed to the Docking Sites on Its Nuclear Substrate MEF2A and Activator MKK3b. Molecular Cell. 2000; 9: 1241–1249.
9. Ge B, Gram H, Di PF, Huang B, New L, Ulevitch RJ *et al*., MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha. Science. 2002; 15; 295(5558):1291-4.
10. Han J, Lee JD, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science. 1994. 5; 265(5173): 808-11.
11. Joël R, Shashi G, Jeffrey SR, Martin D, Jiahuai H, Richard JU *et al.,* Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem. 1995. 31; 270(13):7420-6.
12. Aaron SB, Lucia de A, Linda JV and Martin W. The p38α mitogen-activated protein kinase as a central nervous system drug discovery target. BMC Neuroscience. BMC Neuroscience 2008, 9(2): S12.
13. Baoxue G, Xinsheng X, Qing J, Jennifer LM, Angela F, Dafang B *et al.,* TAB1β (Transforming Growth Factor-β-activated Protein Kinase 1-binding Protein 1β), a Novel Splicing Variant of TAB1 That Interacts with p38α but Not TAK1. The Journal of Biological Chemistry. 2003; 278: 2286-2293.
14. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science. 2002 6; 298(5600): 1911-2.
15. Angel RN, Almudena P. p38 MAP kinases: beyond the stress response. Trends in Biochemical Sciences. 2000; 25 (6): p257–260.
16. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001; 1-410(6824):37-40.
17. Pearson G, Robinson F, Beers G T, Xu B, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001; 22(2): 153-83.
18. Nandhini S, Radha R, Vadivu R. Docking of Hematoporphyrin On Various Anticancer Drugs Targeting Enzymes. Asian J. Pharm. Res. 2016; 6: 3.
19. Hemalatha K, Girija. K. Evaluation of Drug Candidature of Some Benzimidazole Derivatives as Biotin Carboxylase Inhibitors: Molecular Docking And Insilico Studies. Asian J. Res. Pharm. Sci. 2016; 6: 1.
20. Shruthy VS, Shakkeela Y. In Silico Design, Docking, Synthesis And Evaluation of Thiazole Schiff Bases. Int J Pharm Pharm Sci. 6; 3: 271-275.
21. Wallace AC, Laskowski RA and Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. 1995; 8(2): 127-34.
22. Murugesan A, Shanmugam R. In Silico Molecular Docking Studies on the Phytoconstituents of Cadaba Fruticosa (L.) Druce for Its Fertility Activity. Asian J Pharm Clin Res. 2016. 9 (2): 48-50.
23. Sathyan SL, Ponnusamy RD and Arumugam M. In Silico Docking Studies of RP2 (X-Linked Retinitis Pigmentosa) Protein Using Anthocyanins As Potential Inhibitors. Bangladesh J Pharmacol. 2013; 8: 292-299
24. Kalirajan R, Sankar S, Jubie S, Gowramma B. Molecular Docking Studies And In-Silico ADMET Screening of Some Novel Oxazine Substituted 9-Anilinoacridines As Topoisomerase II Inhibitors. Indian J of Pharmaceutical Education and Research. 2017; 51(1):110-115.
25. I. E. Otuokere, Amaku FJ and Alisa CO. In Silico Geometry Optimization, Excited – State Properties of (2E)-N-Hydroxy-3-[3-(Phenylsulfamoyl) Phenyl] Prop-2-Enamide (Belinostat) and Its Molecular Docking Studies With Ebola Virus Glycoprotein. Asian J. Pharm. Res. 2015; 5(3)3: 131-137.