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Molecular Docking Studies of Substituted Pyrazolone Derivatives as Cytokine Synthesis Inhibitors

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ABSTRACT

In this continuing efforts toward the development of disease modifying treatments for inflammatory diseases, we are targeting the development of novel p38 Mitogen-activated Protein (MAP) kinase inhibitors. In present study, we reported molecular docking studies of substituted pyrazolone derivatives with cytokine synthesis inhibitors.

Key words: Cytokine synthesis inhibitors, p38 kinase, docking, anti-inflammatory

INTRODUCTION

Numerous efforts in anti-inflammation research have been focused on the development of small molecule inhibitors of cytokine release. The over expression of cytokines, such as TNF-α and IL 1β, has been implicated in a number of serious inflammatory disorders (Golebiowski *et al*., 2005). Consequently, agents that inhibit the production of Tumor Necrosis Factor-alpha (TNF-α) can decrease levels of these proinflammatory cytokines (Palladino *et al*., 2003; Baugh and Bucala, 2001) and thereby reduce inflammation and prevent further tissue destruction in diseases such as Rheumatoid Arthritis (RA) (Smolen and Seiner, 2003; Brennan and Feldmann, 1996), Osteoarthritis (OA) (Camussi and Lupia, 1998) and Crohn's disease. Researcher reported docking studies of PI-3 kinase with substituted pyrazolo ligands, so there is also scope for pyrazolo which exhibit as kinase inhibitor (Sujatha and Silja, 2011).

Activated microglia release TNF-α as shown in *in vivo* and *in vitro* studies (Krady *et al*., 2005). TNF-α is an important pro-inflammatory factor in retinal and Central Nervous System (CNS) neurodegenerative diseases, such as Diabetic Retina (DR), Parkinson's disease (Sriram *et al*., 2002). TNF-α has the potential to induce apoptosis, fibroblast proliferation, NF-kβ activation and cell adhesion molecule activation (Hemalatha *et al*., 2012). There is convincing evidence to suggest that neuropathy is a feature of DR as ganglion cell death was observed in the diabetic retina (Barber, 2003; Wang *et al*., 2007).

Tumour necrosis factor-alpha (TNF-α) is a multi-functional cytokine that can regulate many cellular and biological processes such as immune function, cell differentiation, proliferation, apoptosis and energy metabolism. It is synthesized as a 26-k Da transmembrane monomer

(mTNF-α) (Gupta and Kumaran, 2006) that undergoes proteolytic cleavage by the TNF-α Converting Enzyme (TACE) to yield a 17-kDa soluble TNF-α molecule (sTNFα) (Black *et al.*, 1997). Both sTNF-α and mTNF-α can effect biological and metabolic responses (Perez *et al.*, 1990; Xu *et al.*, 1999) suggesting that mTNF-α may mediate paracrine and autocrine signals, leaving sTNF-α to mediate endocrine effects (Cawthorn and Sethi, 2008).

In the previous study, we reported the synthesis of a novel class of substituted pyrazolone derivatives with anti-inflammatory activity (Antre *et al.*, 2011). The two definitely known isomers of COX, named COX-1 and COX-2 shows distinct expression patterns and distinct biological activities. COX-1 is constituently expressed proteins that are responsible for the physiological production of prostaglandins. COX-2 is rapidly regulated at inflammatory sites and appeared responsible for the formation of prostaglandins. In inflammatory processes COX-2 is over expressed (Levita *et al.*, 2010). As PDB code 1YWR is related with compounds were tested for the inhibition of TNF-α production using Lipopolysaccharide (LPS) stimulated human monocytic cells (THP-1) (Golebiowski *et al.*, 2005). We collected protein from RCSB data bank, docked with our synthesized ligand molecule and compared MolDOCK score with calculated pIC_{50} from our literature.

MATERIALS AND METHODS

Experimental methods

Data preparation: The Mitogen-activated protein kinase 14 (1YWR) with monocyclic pyrazolone inhibitor were collected from the literature. The biological activity data as anti-inflammatory activity collected from literature (Antre *et al.*, 2011). The percent inhibition after 3 h in anti-inflammatory activity were collected and converted to (pIC_{50}) by using reported formula (Raparti *et al.*, 2009). The dataset for molecular docking study consisted of 1YWR with 10 ligand molecules.

Model building: The current study was performed using the following programs: Molegro Virtual Docker (MVD 4.3.0)-[Win32] (Trial version) which performance flexible ligand docking. The structure of substituted pyrazolone derivatives were drawn in Chem Sketch ACDFREE10 and the molecule was saved as MolDock format. All the programs were installed on an Intel based windows workstation with Intel Dual-Core processor (2.0 GB RAM) integrated with Nvedia graphical display. Molegro module works in five steps:

Step 1: Start with crystal coordinates of target receptor
Step 2: Generate molecular surface for receptor
Step 3: Generate spheres to fill the active site of the receptor: The spheres become potential locations for ligand atoms
Step 4: **Matching:** Sphere centers are then matched to the ligand atoms, to determine possible orientations for the ligand
Step 5: **Scoring:** Find the top scoring orientation

RESULTS AND DISCUSSION

Focused compound screening libraries are commonly used to improve the efficiency and productivity of early drug discovery efforts. Traditionally each compound in a focused library is selected based upon structural and physical properties that will increase its probability of having activity for one specific target. In this study we have extended this approach in order to identify potential multi-ligands as inhibitors. Following are the active residues involved in docking:

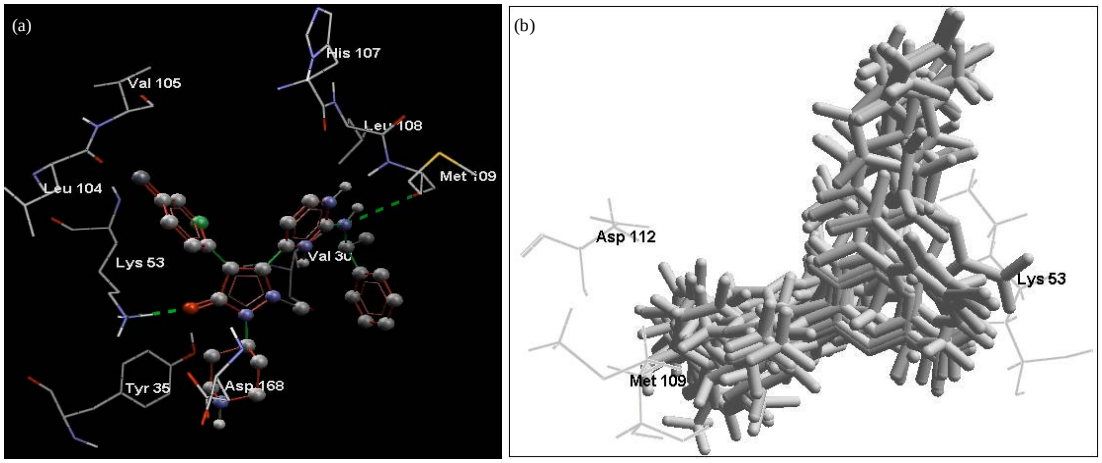


Fig. 1(a-b): (a) Active site residues involved in docking (PDB: 1YWR) and (b) Binding mode of ligand molecules (5a-5j) wih 1YWR. The side-chain residue mainly involved in docking Asp112, Met109, Lys53 are shown

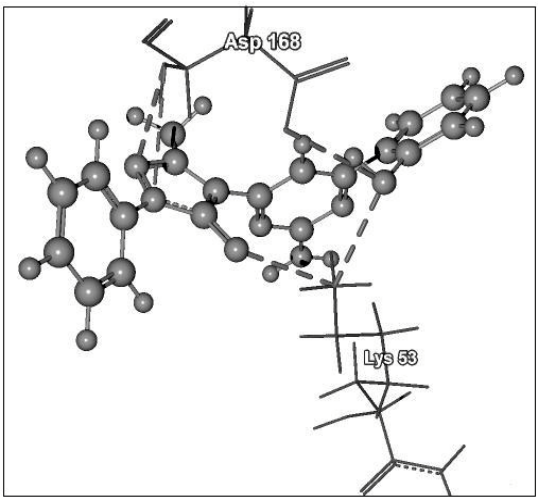


Fig. 2: The broken line represents hydrogen bonding between ligand molecule 5d and active site residues Asp168, Lys53

Met-109, Leu-108, His-107, Val-105, Leu-104, Lys-53, Asp-186, Tyr-35, Val-30 (Hydrogen bond interaction between active site residues and ligand highlighted with dashed line) (Fig. 1a, b). The broken line represents hydrogen bonding between ligand molecule 5d and active site chain residues Asp168, Lys53 reported in Fig. 2. Ligand (5a-5j) binds into active site of p38 kinase with reference ligand are reported in Fig. 3a and b. Correlation between intermolecular energies (ΔEs, kcal moL^{-1}) and experimental bioactivities (pIC_{50}) reported in Fig. 4.

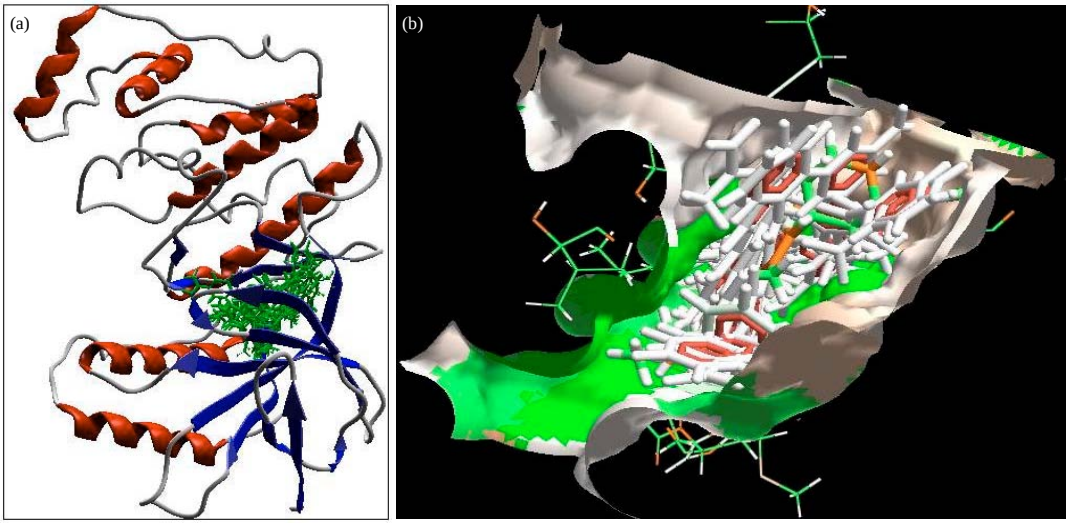


Fig. 3(a-b): (a) Binding mode of ligands 5a-5j in ribbon p38 kinase and (b) Binding mode of ligands (5a-5j) into cavity of P38 kinase (PDB: 1YWR)

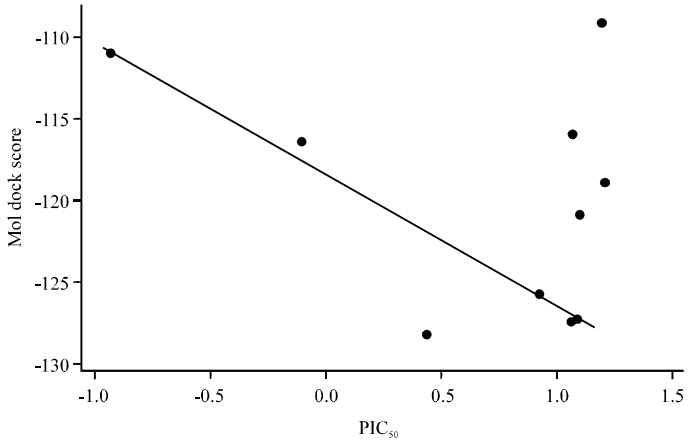


Fig. 4: Correlation between intermolecular energies (ΔEs, kcal mol^{-1}) and experimental bioactivities (pIC_{50})

Log p-value (Table 1) of ligands 5b, 5g, 5i showed very less as compared with other ligands, because 5b, 5g and 5i contains NO_2 substitution on pyrimidine ring attached to 4th position of pyrazolone ring. Although, the structures of substituted pyrazolone elucidated, molecular docking and ligand-displacement experiments indicate that ligand binds at active site residue only as predicted by Molegro Virtual Docker, is shown in Fig. 2. The pyrazolone rings of ligands are within Grid resolution: 0.30A°. p38 kinase docking analysis and comparison with the observed activity showed p38 docking analysis both with and without water molecules which involves the substrate-enzyme interactions had shown no significant differences between the docking and observed position of the inhibitor.

Table 1: Interaction energy values (kcal mol^{-1}) between p38 kinase and ligands, Rerank Score, HBond energy, log p and pIC_{50} for ligands (5a-5j)

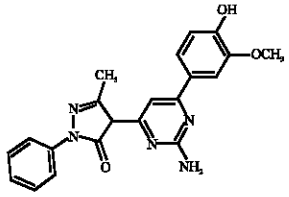
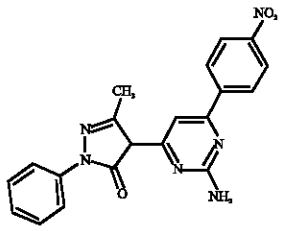
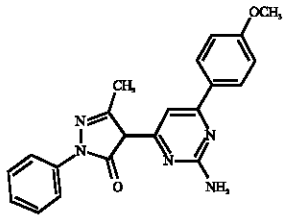
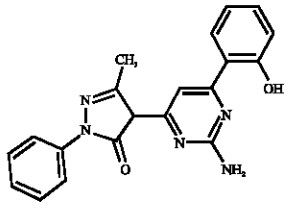
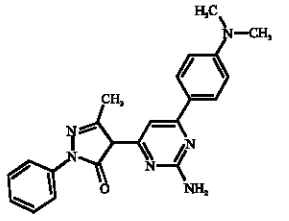
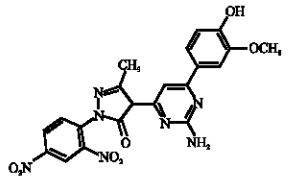
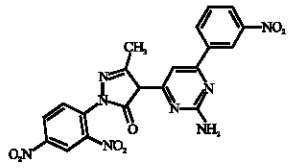
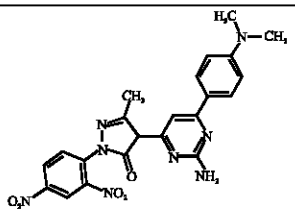
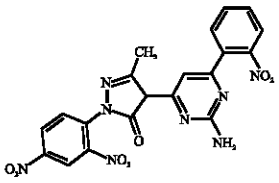
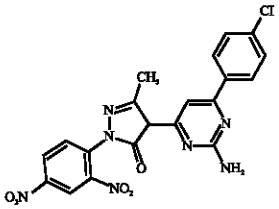
Ligand	Structure	MolDock score	Rerank score	HBond energy	log p	pIC_{50}
5a		-127.252	-98.3936	-5.06159	2.69	1.0898
5b		-128.191	-92.1346	-5.40921	-0.48	0.4345
5c		-127.305	-94.2764	-4.95564	3.08	1.0678
5d		-115.965	-58.3012	-9.18528	2.82	1.0662
5e		-120.882	-73.0481	-4.48282	3.49	1.1002
5f		-118.909	-92.0024	-6.90915	-0.77	1.2037
5g		-125.78	-70.4493	-3.38049	-0.56	0.92281

Table 1: Continued

Ligand	Structure	MolDock score	Rerank score	HBond energy	log p	pIC_{50}
5h		-109.192	-78.7819	-1.2792	0.32	1.1927
5i		-116.464	-86.8039	-5.34182	-0.56	-0.10841
5j		-111.019	-83.4219	-2.5	0.1	-0.9338

CONCLUSION

To our knowledge, this is the first report correlating anti-inflammatory activity and cytokine synthesis inhibitors protein and describing the binding site of protein. We reported that, Asp112, Met109, Lys53 residues involved in molecular docking. Furthermore, we showed the linear Correlation between intermolecular energies (ΔEs, kcal mol^{-1}) and experimental bioactivities (pIC_{50}). All these evidences lead to the conclusion that the anti-inflammatory activity of pyrazolone derivatives is due to basic moiety. In conclusion, we have also reported a novel series of pyrazolone scaffold that maintains the important binding sites in the cytokine synthesis inhibitors. Three of the pyrazolone derivatives 5a, 5b, 5c showed good mol dock score against 1YWR.

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REFERENCES

Antre, R.V., A. Cendilkumar, D. Goli, G.S. Andhale and R.J. Oswal, 2011. Microwave assisted synthesis of novel pyrazolone derivatives attached to a pyrimidine moiety and evaluation of their anti-inflammatory, analgesic and antipyretic activities. Saudi Pharm. J., 19: 233-243.

Barber, A.J., 2003. A new view of diabetic retinopathy: A neurodegenerative disease of the eye. Prog. Neuropsychopharmacol. Biol. Psychiatry, 27: 283-290.

Baugh, J.A. and R. Bucala, 2001. Mechanisms for modulating TNF α in immune and inflammatory disease. Curr. Opin. Drug Discov. Devel., 4: 635-650.

Black, R.A., C.T. Rauch, C.J. Kozlosky, J.J. Peschon and J.L. Slack *et al.*, 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature, 385: 729-733.

Brennan, F.M. and M. Feldmann, 1996. Cytokines in autoimmunity. Curr. Opin. Immunol., 8: 872-877.

Camussi, G. and E. Lupia, 1998. The future role of anti-tumour necrosis factor (TNF) products in the treatment of rheumatoid arthritis. Drugs, 55: 613-620.

Cawthorn, W.P. and J.K. Sethi, 2008. TNF-α and adipocyte biology. FEBS Lett., 582: 117-131.

Golebiowski, A., J.A. Townes, M.J. Laufersweiler, T.A. Brugel and M.P. Clark *et al.*, 2005. The development of monocyclic pyrazolone based cytokine synthesis inhibitors. Bioorg. Med. Chem. Lett., 15: 2285-2289.

Gupta, S.P. and S. Kumaran, 2006. Quantitative structure-activity relationship studies on benzodiazepine hydroxamic acid inhibitors of matrix metalloproteinases and tumor necrosis factor-α converting enzyme. Asian J. Biochem., 1: 47-56.

Hemalatha, R., M. Karthik, K.N. Babu and B.D. Kumar, 2012. Immunomodulatory activity of triticum aestivum and its effects on Th1/Th2 cytokines and NFêB P 65 response. Am. J. Biochem. Mol. Biol., 2: 19-29.

Krady, J.K., A. Basu, C.M. Allen, Y. Xu, K.F. Lanoue, T.W. Gardner and S.W. Levison, 2005. Minocycline reduces proinflammatory cytokine expression, microglial activation and caspase-3 activation in a rodent model of diabetic retinopathy. Diabetes, 54: 1559-1565.

Levita, J., A. Nawawi, A. Mutholib and S. Ibrahim, 2010. Andrographolide inhibits COX-2 expression in human fibroblast cells due to its interaction with arginine and histidine in cyclooxygenase site. J. Applied Sci., 10: 1481-1484.

Palladino, M.A., F.R. Bahjat, E.A. Theodorakis and L.L. Moldawer, 2003. Anti-TNF-alpha therapies: the next generation. Nat. Rev. Drug. Discov., 2: 736-746.

Perez, C., I. Albert, K. Defay, N. Zachariades, L. Gooding and M. Kriegler, 1990. A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell, 63: 251-258.

Raparti, V., T. Chitre, K. Bothara, V. Kumar and S. Dangre *et al.*, 2009. Novel 4-(morpholin-4-yl)-N0-(arylidene)benzohydrazides: Synthesis, antimycobacterial activity and QSAR investigations. Eur. J. Med. Chem., 44: 3954-3960.

Smolen, J.S. and G. Seiner, 2003. Therapeutic strategies for rheumatoid arthritis. Nat. Rev. Drug Discov., 2: 473-488.

Sriram, K., J.M. Matheson, S.A. Benkovic, D.B. Miller, M.I. Luster and J.P. Ocallaghan, 2002. Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: Implications for Parkinson's disease. FASEB J., 16: 1474-1476.

Sujatha, S. and S. Silja, 2011. Finding the potential inhibitors of phosphoinositide 3-kinase as a anti-cancerous drug target. Asian J. Biotechnol., 3: 177-185.

Wang, A.L., A.C. Yu, Q.H. He, X. Zhu and M.O. Tso, 2007. AGEs mediated expression and secretion of TNF alpha in rat retinal microglia. Exp. Eye Res., 84: 905-913.

Xu, H., J.K. Sethi and G.S. Hotamisligil, 1999. Transmembrane Tumor Necrosis Factor (TNF)-alpha inhibits adipocyte differentiation by selectively activating TNF receptor 1. J. Biol. Chem., 274: 26287-26295.