Design And Synthesis of Potential Anti- Inflammatory Agents

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Abstract- Inflammation is a complex biological response involved in the pathogenesis of various acute and chronic disorders. The limitations associated with currently available anti-inflammatory drugs, including adverse effects and reduced efficacy upon prolonged use, necessitate the development of safer and more effective therapeutic agents. In the present study, a series of novel compounds were rationally designed and synthesized as potential anti-inflammatory agents based on structure–activity relationship considerations.

The synthesized compounds were obtained using conventional organic synthetic methodologies and characterized by spectroscopic techniques such as FT-IR, ^1H NMR, ^13C NMR, and mass spectrometry. The anti-inflammatory activity of the prepared compounds was evaluated using standard in vivo and/or in vitro models, and the results were compared with those of standard reference drugs. Several compounds demonstrated significant anti-inflammatory activity, indicating that appropriate structural modifications play a crucial role in enhancing biological efficacy. The findings suggest that these newly designed molecules may serve as promising lead candidates for further optimization in the development of novel anti-inflammatory drugs.

Keywords- Anti-inflammatory agents; Drug design; Synthesis; Structure–activity relationship; Spectral characterization; Biological evaluation

I. INTRODUCTION

Inflammation is a vital defense mechanism of the human body that protects against pathogens, toxins, tissue injury, and other harmful stimuli. Acute inflammation is a rapid and regulated immune response that helps eliminate injurious agents and initiate tissue repair. However, if unresolved, acute inflammation may progress into chronic inflammation, which is associated with several life-threatening diseases such as cardiovascular disorders, cancer, diabetes, chronic respiratory diseases, and autoimmune conditions. According to the World Health Organization, chronic inflammatory diseases represent one of the major global health challenges.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for the management of inflammation, pain, and fever. These agents exert their effects primarily by inhibiting cyclooxygenase (COX) enzymes, which catalyze the conversion of arachidonic acid into pro-inflammatory eicosanoids. Non-selective NSAIDs inhibit both COX-1 and COX-2 enzymes, often resulting in gastrointestinal, renal, and hepatic adverse effects.

p38α MAP Kinase as a Therapeutic Target

The p38 mitogen-activated protein (MAP) kinase pathway plays a central role in regulating inflammatory responses by controlling the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-8. Activation of p38 MAP kinase also induces the expression of inflammatory enzymes including COX-2 and inducible nitric oxide synthase. Experimental studies with selective p38 inhibitors, such as SB203580, have demonstrated significant therapeutic benefits in inflammatory disease models. Among the p38 isoforms, p38α is the most extensively studied and is considered a promising molecular target for the treatment of chronic inflammatory diseases including rheumatoid arthritis, asthma, psoriasis, and COPD.

Quinoxaline Scaffold in Anti-Inflammatory Drug Design

Heterocyclic compounds play a crucial role in medicinal chemistry due to their structural diversity

and biological relevance. Quinoxaline, a bicyclic nitrogen-containing heterocycle formed by the fusion of benzene and pyrazine rings, is a bioisostere of quinoline and naphthalene. Although naturally occurring quinoxaline derivatives are rare, synthetic quinoxalines have demonstrated a wide range of pharmacological activities, including antimicrobial, anticancer, antiviral, and anti-inflammatory properties. Several marketed drugs and clinical candidates contain the quinoxaline nucleus, highlighting its therapeutic significance. Importantly, quinoxaline derivatives have been reported as potent p38α MAP kinase inhibitors.

II. OBJECTIVES OF THE STUDY

Based on the above considerations, the present study aims to design and synthesize novel quinoxaline derivatives bearing hydrazone and 1,3,4-oxadiazole moieties as potential anti-inflammatory agents. The specific objectives include:

- Synthesis of new quinoxaline analogues
- Structural characterization using IR, ^{1}H NMR, ^{13}C NMR, and HRMS
- Evaluation of anti-inflammatory activity through in-vitro and in-vivo assays
- Assessment of ulcerogenicity and lipid peroxidation
- Establishment of structure–activity relationships (SAR)
- Molecular docking and molecular dynamics studies targeting p38α MAP kinase
- ADME profiling of synthesized compounds
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III. MATERIALS AND METHODS

All chemicals, reagents, and solvents used in the present study were of analytical or synthetic grade and were procured from standard commercial suppliers. Starting materials required for the synthesis of target compounds were used without further purification unless otherwise stated. Solvents were dried and distilled according to standard laboratory procedures when necessary.

Carrageenan, bovine serum albumin (BSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, indomethacin, diclofenac sodium, and other reagents required for biological assays were obtained from certified suppliers. Experimental animals were procured from the institutional animal facility and maintained under standard laboratory conditions

Instrumentation

Melting points were determined using an open capillary method and are uncorrected.

Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates, and spots were visualized under UV light (254 nm).

Infrared (IR) spectra were recorded using FT-IR spectrophotometer with KBr pellets.

^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz NMR spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.

High-resolution mass spectra (HRMS) were obtained using ESI-MS technique.

Procedure for the Synthesis of Target Compounds

The target anti-inflammatory agents were designed based on rational drug design principles incorporating heterocyclic pharmacophores known for anti-inflammatory activity. The synthetic pathway involved the preparation of key intermediates followed by functionalization to obtain the final compounds.

Typically, the appropriate substituted heterocyclic precursor was reacted with the corresponding hydrazine derivative or carboxylic acid derivative under suitable reaction conditions. The reaction mixture was refluxed for the specified time, and the progress of the reaction was monitored by TLC. After completion, the mixture was cooled to room temperature and poured into ice-cold water. The precipitated solid was filtered, washed with water, dried, and purified by recrystallization or column

chromatography using silica gel and suitable solvent systems.

IV. IN-VITRO ANTI-INFLAMMATORY ACTIVITY

DPPH Radical Scavenging Assay

Antioxidant activity was assessed using the DPPH free radical scavenging method. Various concentrations of the test compounds were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid served as the standard. Percentage scavenging activity was calculated using standard formula.

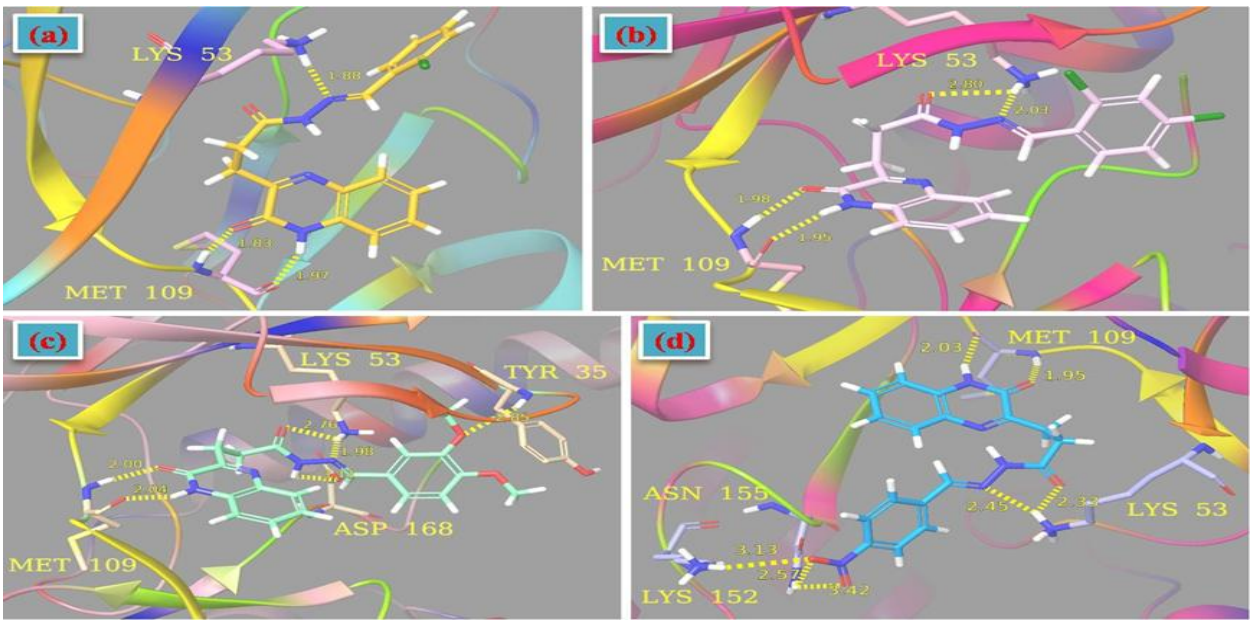
p38α MAP Kinase Inhibition Assay

The inhibitory activity of synthesized compounds against p38α MAP kinase was evaluated using an in-vitro enzyme inhibition assay kit, following the manufacturer's protocol. Test compounds were incubated with recombinant p38α enzyme and substrate in assay buffer. The enzyme activity was measured spectrophotometrically, and IC_{50} values were determined by plotting percentage inhibition versus concentration.

V. RESULTS AND DISCUSSION

Table 1. Docking scores and hydrogen bonding interactions of synthesized derivatives (4a-q and 5a-m), co-crystal and standard inhibitor (SB203580).

Compounds	Docking scores	Glideenergy	Hydrogenbond/π-π/π-citation interactions
4a	-4.909	-44.625	MET109, ASP 112
4b	-8.485	-45.01	MET109,LYS 53
4c	-6.979	-43.695	MET109,LYS 53
4d	-6.992	-39.030	MET 109
4e	-8.249	-48.55	MET109,LYS 53
4f	-8.018	-42.48	MET109,LYS53,ASP168
4g	-5.164	-48.856	LYS53,ASP 112



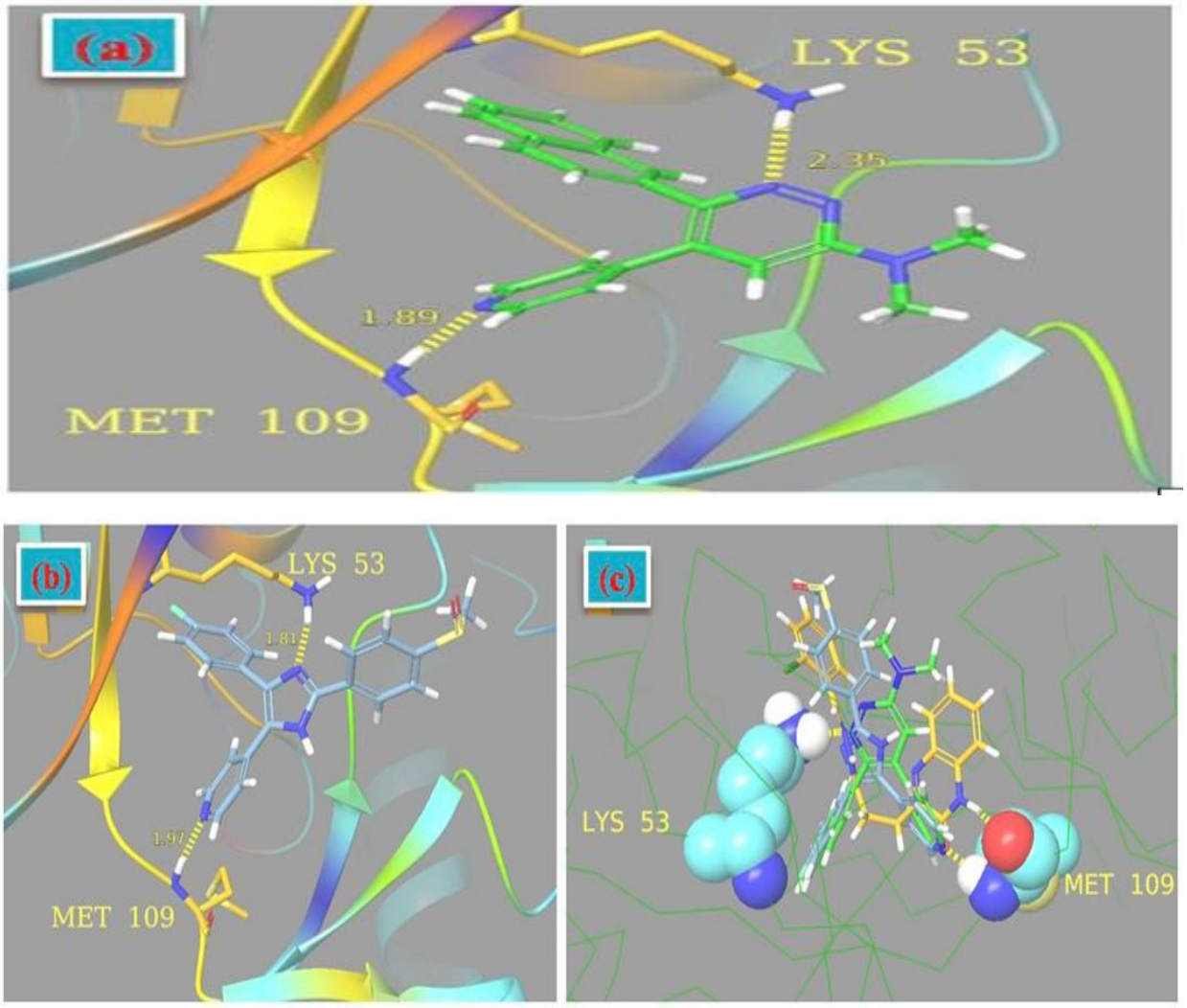


Figure 1. Docked image of (a) co-crystal ligand (green colour stick model) (b) SB203580 (sky blue colour stick model) (c) superimposed compound 4b, co-crystal ligand and SB203580 at the binding site of p38α MAP kinase. The yellow colour dashed line showed hydrogen bonding interaction.

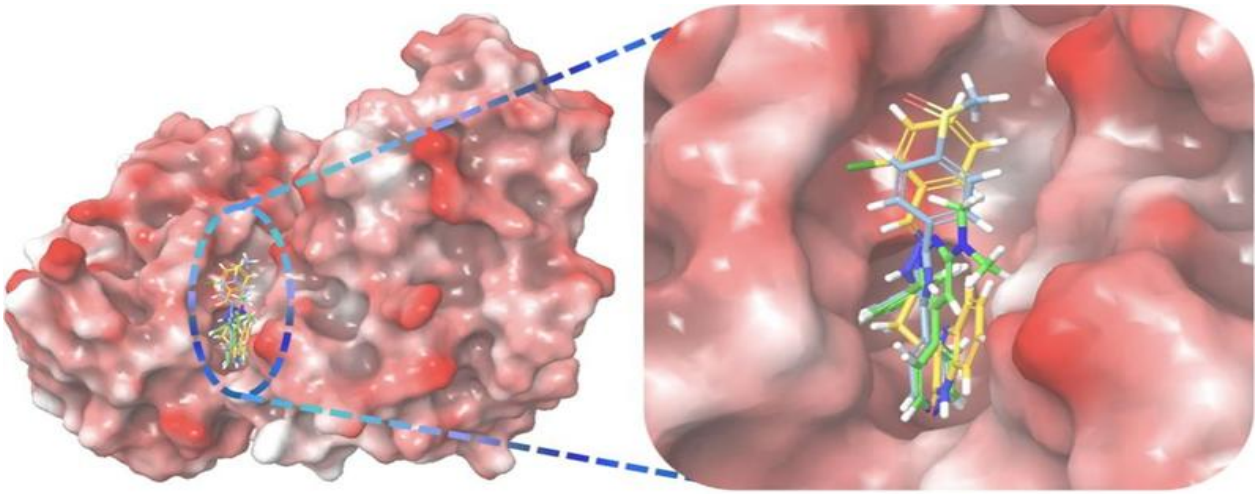


Figure 2. Molecular surface view of compound 4b (yellow stick model), co-crystal ligand (green stick model) and SB203580 (sky blue stick model) complexes withp38α MAP kinase.

Moleculardynamic (MD)simulation

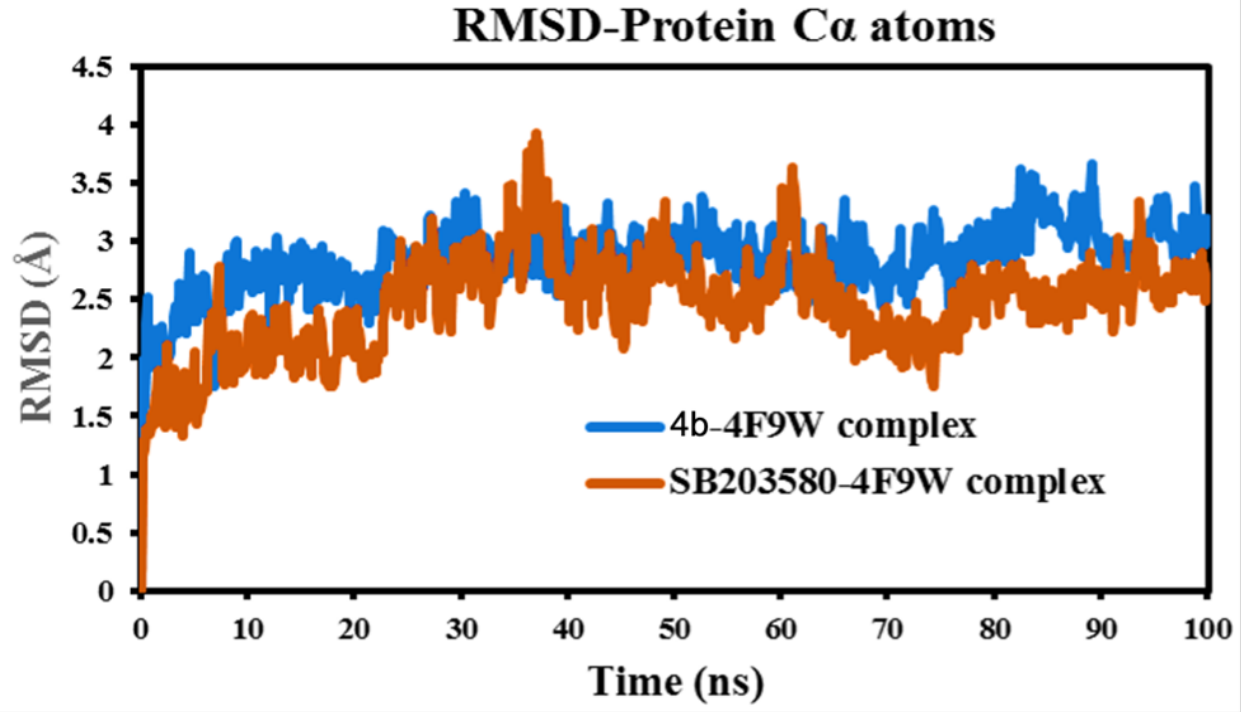


Figure 3. RMSD plot 4F9W bound with compounds 4b and SB203580 vs. time of the simulation

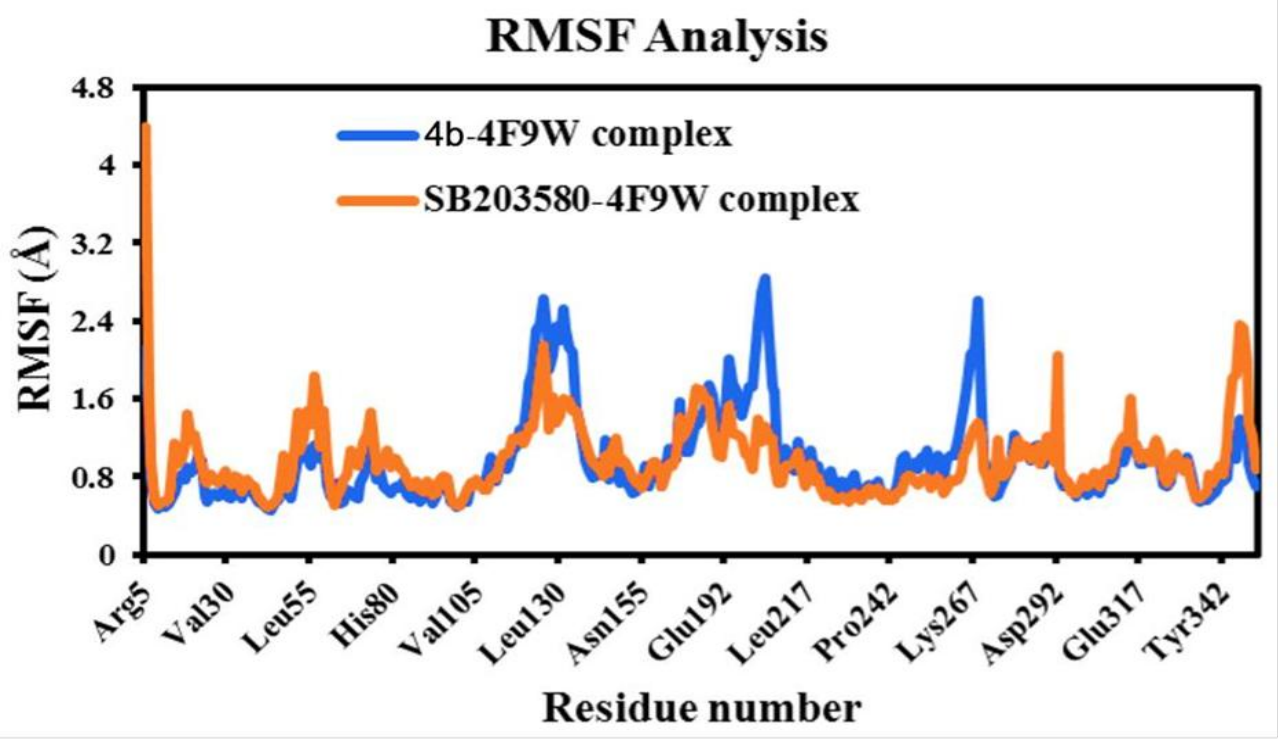


Figure 4 .RMSF plot of each amino acid residue of 4F9W bound with compounds 4b And SB203580 calculated from MD simulation trajectories.

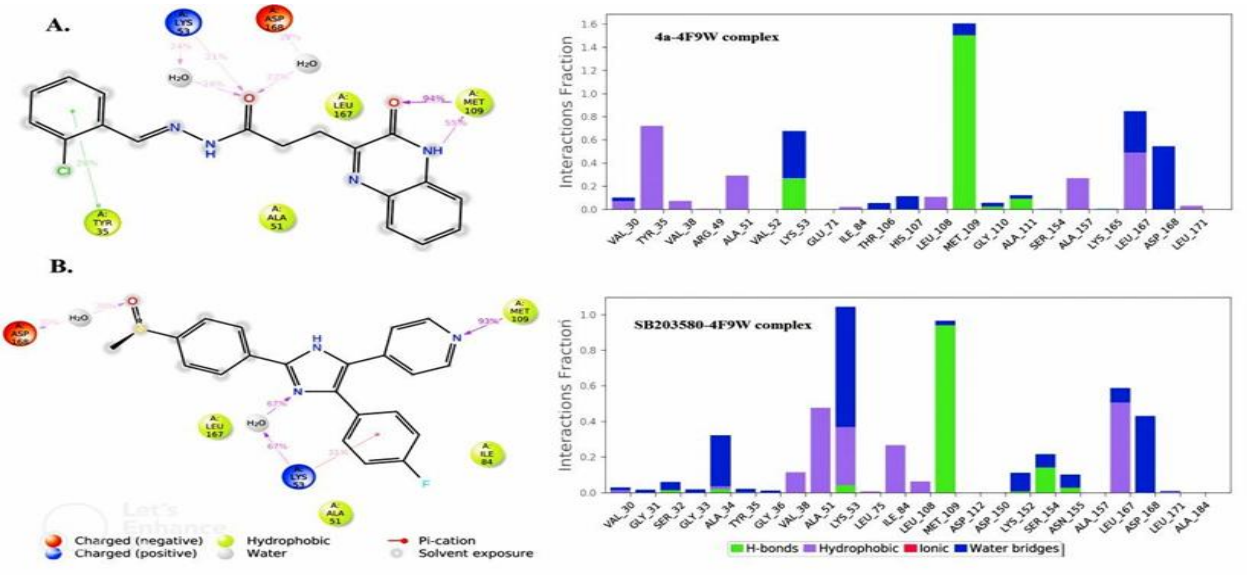


Figure 5 . Ligand 2 Dinteraction (Leftside) and protein ligand contact (Rightside) of 4b-4F9W complex (a) and SB203580-4F9W complex (b) during 100n ssimulation time

VI. CONCLUSION

Somenewquinoxalinecongenerspossessinghydrazone moietyweresynthesizedso as to develop potent p38α

MAP kinase inhibitors with improved anti-inflammatory activity and ulcerogenicsafety profile. All the compounds were synthesized by multistep reaction protocol and evaluated for their *in-vitro* activity. M,respectively). Both these compound s also exhibited excellent carrageenan induced rat paw edema inhibition (83.61 and 82.92% respectively). Furthermore, 4b and 4h showed reduced ulcerogenicity (0.416 0.154 and 0.583 0.239 respectively) and better lipid per oxidation profile (4.20 0.142 and 5.69 0.079 respectively) as compared to standard drug diclofenac sodium (SI1.750 0.112and lipid peroxidation 6.76 0.059). SAR analysis of all the synthesized compounds demonstrated that the presence of electron withdrawing group on phenyl ring is crucial for anti-inflammatory activities. In molecular docking studies, it was revealed that 4b and 4h formed strong hydrogen bonding interaction with amino acid residues MET 109 and LYS 53 in the active site of p38α MAP kinase. The ADME prediction of these compounds also demonstrated that all the compounds (4a-qand5a-m) fall in the range of drug-likeness properties and there is no deviation from Lipinki's rule of five. Furthermore, the results obtained from 100 ns MD simulation studies also confirmed the findings from molecular docking studies by keeping stable hydrogen bonding interactions with MET 109 and LYS 53 in addition to few more interactions with GLY 110, ALA 111, LYS 53, THR106, HIS107 and LEU167. The consistency of hydrogen bond inginteraction between 4b and MET 109 suggested that this compound has the capacity to inhibit p38α MAP kinase. Therefore, based on above findings it is concluded that these compounds could provide a framework to further develop novel p38α MAP kinase inhibitors.

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