Research Article

# Docking Studies on Eupalitin-3-o-β-D-galactopyranoside as Potential Inhibitor of Cyprinus *Carpio* (Koi Carp) Mapk/Erk Kinase 2 and Mitogen-activated Protein Kinase (p38)

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**ABSTRACT**

In the current study molecular docking was used as a tool to evaluate the immunosuppressive activity of Eupalitin-3-o-β-D-galactopyranoside towards two specific kinases, p38 mapkinase and MEK2. In this work, a homology model for (koi carp) *Cyprinus carpio* MEK2, p38 MAPKinase was performed and possible inhibitory effect of Eupalitin-3-o-β-D-galactopyranoside on p38 Mapkinase and MEK2 was investigated by docking study. The docking results of Eupalitin-3-o-β-D-galactopyranoside with p38 Mapkinase and MEK2 emphasize Eupalitin-3-o-β-D-galactopyranoside as a potential TNF-α inhibitor.

**Keywords:** *Cyprinus carpio,* Eupalitin-3-o-β-D-galactopyranoside, MEK2, p38 Mapkinase, TNF-α inhibitor.

## INTRODUCTION

Flavonoids have been found to inhibit the upstream signaling molecules that are involved in TNF-α expression. Drugs derived from natural compounds might provide an alternative approach for the treatment of inflammatory diseases via modulation of the TNF-α signaling pathway. Eupalitin-3-O-β-D-galactoside a bioflavonoid isolated from *Boerhaavia diffusa* has been reported to possess immunosuppressive activity.[1] In the present docking study with Eupalitin-3-o-β-D-galactopyranoside involvement of MEK2, and p38 MAPKinase in TNF-α signalling was evaluated. MEK2 is a protein kinase that participates in the RAS-RAF-MEK-ERK signal transduction cascade in the regulation of a large variety of processes including apoptosis, cell cycle progression, cell migration, differentiation, metabolism and proliferation.[2] Mitogen-activated protein kinases (MAPK) are intracellular signaling molecules involved in cytokine synthesis. The role of MAPK in inflammation makes them attractive targets for advanced therapies and recent research is progressing to identify more selective inhibitors for inflammatory diseases. p38 alpha is a key MAPK involved in tumor necrosis factor alpha and other cytokine production. Increased activity of MAPK, in particular p38 MAPK, and their involvement in the regulation of the synthesis of inflammation mediators at the level of transcription and translation, make them potential targets for anti-inflammatory therapeutics. MAPK inhibitors appears to be an attractive strategy as they are capable of inhibiting both the synthesis of pro-inflammatory cytokines and their signaling.[3]

Homology models of proteins are of great interest for designing and evaluating biological experiments when no experimental structures are available. Homology modeling was done in this study as there is no existing data on the three dimensional structures for (koi carp) *Cyprinus carpio* MEK2 and p38 MAP Kinase. In this work, a homology model for (koi carp) *Cyprinus carpio* MEK2, p38 MAPKinase was performed for the first time. The 3D models of MEK2, p38 MAPKinase were constructed using the protein structure homology model building program MODELLER 9.11 with energy minimization parameters.

## MATERIALS AND METHODS

### Ligand structure

2D and 3D structure of Eupalitin-3-o-β-D-galactopyranoside (Ligand) was sketched using ACD Chemsketch Tool in this study.

### Retrieval of Target Protein sequence

The protein sequence of *Cyprinus carpio* (koi carp) was obtained from protein sequenced database of Uniprot.

MEK2 MAPKINASE (Accession No: Q90321.1) (http://www.uniprot.org/uniprot/Q90321) and p38 MAPK (Accession No: BAA11881.1)

(http://www.uniprot.org/uniprot/Q90336).

### Template identification

The BLAST tool was used to identify the best template against PDB for modeling the 3Dstructure of MEKK2 (koi carp) (*Cyprinus carpio*). The results yielded by p BLAST against the PDB database revealed that chain C ksr-2-mek1dimer from *orytolagus cuniculus* (Rabbit) *with* (PDB ID: 2Y4I) a resolution 3.46 Å as a suitable template. The template and the target have 78% of *identical* residues.

The Template Chain A-crystal structure of *Salmo salar* (Salmon) p38 alpha with (PDBID: 3OHT) and 2.7 Å resolution aligned with the target sequence with 93% identity.

### Model generation and Evaluation

### Homology modeling

The three dimensional structure of MEK2 and p38 MAPKinase has been predicted using MODELLER 9.11.A rough 3D model (visualized using RASMOL 2.7.5.2) was then obtained and the backbone conformation of the rough model was evaluated using the Ramachandran plot in the SPDBV 3.7. The results of Ramachandran plot indicates that the rough model generated had more residues in the allowed region.

### Domain analysis

The functional of analysis were predicted using Pfam Database (http://pfam.sanger.ac.uk/).

### Active site Prediction

The possible binding sites of MEK2 and p38 MAPKinase were searched using Q-Site Finder after obtaining the final model. (http://www.modelling.leeds.ac.uk /q site finder/). Ten binding sites were obtained.

### Docking

MEK2 and p38 MAPKinase were docked using the Lamarckian Genetic Algorithm (LGA) provided by the AutoDockProgram version 4.0. The best ligand-receptor structure from the docked structures was chosen based on lowest energy and minimal solvent accessibility of the ligand.

## RESULTS AND DISCUSSION

### Eupalitin-3-o-beta-D-galactopyranoside

2D and 3D structures (Figure-1) of Ligand are drawn using ACD Chemsketch Tool

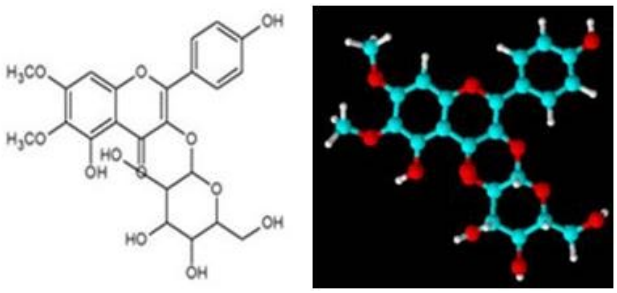


**Figure 1:** 2D and 3D Structure of Eupalitin-3-O-Beta-D-Galactopyranoside

### Homology Modelling for MAPK/ERK kinase 2(EC-2.7.12.2) and Mitogen-Activated Protein Kinase (p38) (EC-2.7.11.24) in *Cyprinus carpio* (koi carp)

The absence of three dimensional structure of MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38) in *Cyprinus carpio* (koi carp) in PDB interested us to construct the 3D model. The three dimensional structure provides valuable insight into molecular functions and also enables the analysis of its interactions with suitable inhibitors. The best Structure for MAPK/ERK kinase 2 (Figure 2) and Mitogen-Activated Protein Kinase (p38) (Figure 3) was visualized using Rasmol tool.

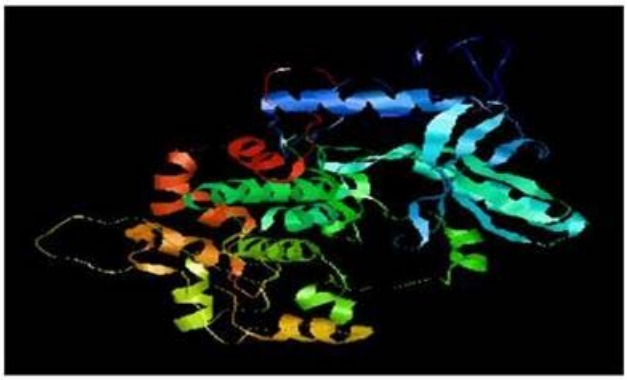

**Figure 2:** Visualization of modeled structure for MAPK/ERK kinase 2 using Rasmol (version 2.7.5.2) Tool

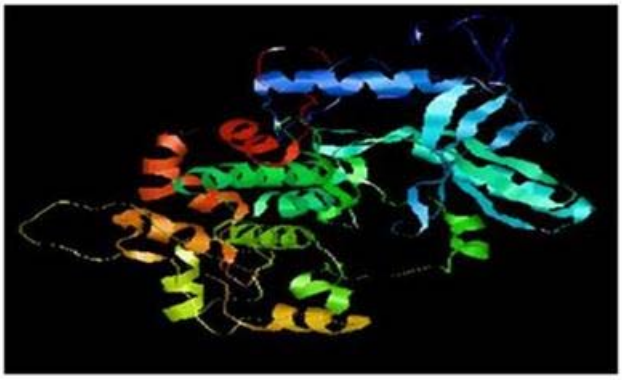

**Figure 3:** Visualization of modeled structure for Mitogen-Activated Protein Kinase (p38) using Rasmol (version 2.7.5.2) Tool

### Active site identification for MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38)

Among the ten binding sites obtained from Q-SiteFinder, site 1 is highly conserved. The residues at site 1 for the enzyme MAPK/ERK kinase 2 are: LYS98, LEU116, LEU119, CYS122, ASN123, ILE127, VAL128, GLY129, PHE130, ILE142, CYS143, MET144, LEU207, CYS208, ASP209, PHE210, GLY211, VAL212, SER213, LEU216, ILE217, MET220. The residues at site 1 for the enzyme Mitogen - Activated Protein Kinase (p38) are: ILE142, HIS143, ALA145, ASP146, ILE147, ILE148, HIS149, ARG150, ASP151, LEU152, LYS153, PRO154, MET195, LEU196, TRP198, MET199, ASN202, MET203, ASP206, THR204. Thus, site 1 has been chosen in this study as the most favorable site for docking. The residues forming the binding pocket for MAPK/ERK kinase 2 are shown in Figure 4 and for Mitogen-Activated Protein Kinase (p38) in Figure 5.

### Domain analysis

The functional regions of the *Cyprinus carpio* (koi carp) MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38) was predicted using pfam and found to have single domain region such as pkinase domain 70 - 366 for

MAPK/ERK kinase 2 and pkinase domain of 25 - 309 for Mitogen-Activated Protein Kinase (p38).

**Docking of MAPK/ERK kinase 2 and Mitogen -Activated Protein Kinase (p38) with Eupalitin-3-O-Beta-D-Galactopyranoside.**

Docking of MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38) was performed with Eupalitin-3-O-Beta-D-Galactopyranoside.The docked conformations were evaluated based on Docking Score and the number of hydrogen bonds formed between active site and inhibitor as represented in Table-1. Docked Complex of Eupalitin-3-O-Beta-D-Galactopyranoside with MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38) using Pymol Viewer (1.3) is depicted in Figure 6 and Figure 7.

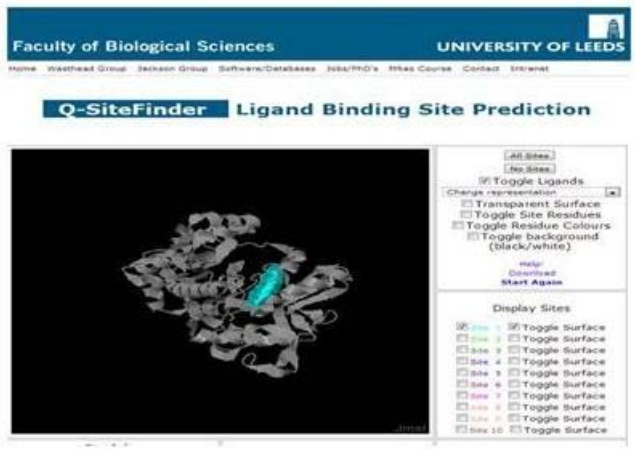


**Figure 4:** Active site Prediction for MAPK/ERK kinase 2 using Q-Site Finder

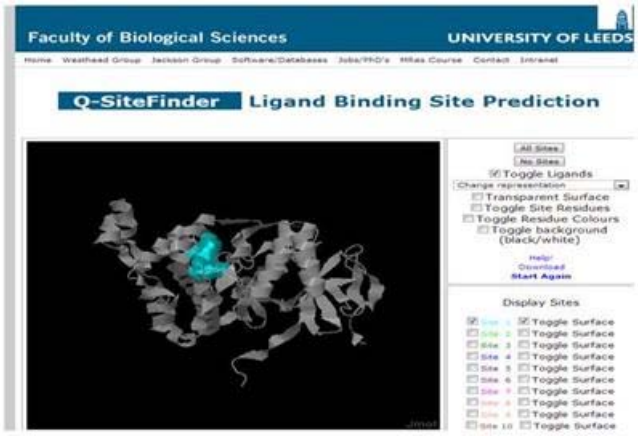


**Figure 5:** Active site Prediction for Mitogen-Activated Protein Kinase (p38) using Q-Site Finder

## DISCUSSION

Molecular docking is a computational tool that predicts the binding site location and conformation of a compound when bound to a protein.[4-6] The process by which molecular docking is used to rank compounds with in a library based on a predicted binding affinity is known as virtual screening.[7,8] The potential benefit to drug discovery has inspired the development and evaluation of numerous virtual screening methodologies. All docking algorithms make use of a scoring function in combination with a method of search. AutoDock 4.0 was used to calculate an affinity constant for ligand-protein configuration in the present study. The best ligand-protein structure from the docked structures was chosen based on lowest energy and minimal solvent accessibility of the ligand.

Flavonoids have been identified as invitro enzyme inhibitors and ligands of receptors involved in signal transductions. Intrinsically, the phenolic nucleus is a structural unit that is favourable to molecular interactions such as Vanderwaals interactions and Electrostatic interactions with proteins.[9,10]

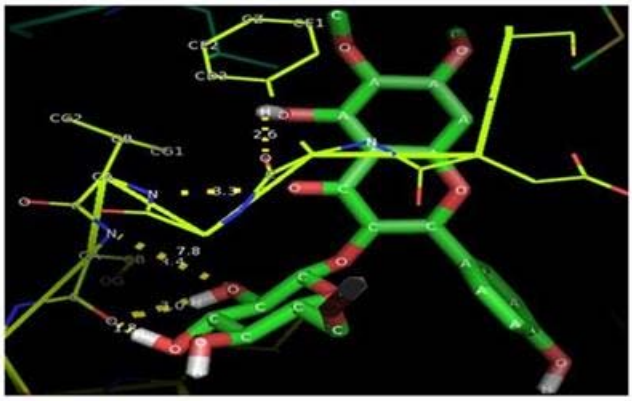

**Figure 6:** Visualization of Docked Complex of Eupalitin-3-O-Beta-D-Galactopyranoside with MAPK/ERK kinase 2using Pymol Viewer (1.3).

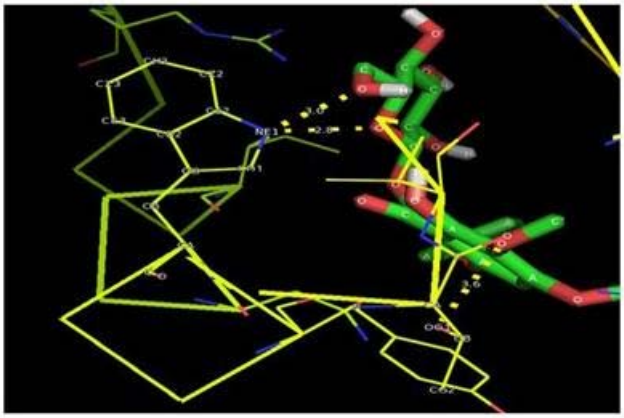

**Figure 7:** Visualization of Docked Complex of Eupalitin-3-O-Beta-D-Galactopyranoside with Mitogen-Activated Protein Kinase (p38) using Pymol Viewer (1.3).

Flavonoids, as integral constituents of the diet, have been proposed to exert beneficial effects in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders.[11,12] Protein kinases are recognized as potential molecular targets for chemoprevention by flavonoids as they regulate signal pathways by post-translational modification mechanism.[13] Modulation of the activity of MAPKs by flavonoids is suggested as a possible mechanism for their potential anti neurodegenerative action[14,15] and

protection against autoimmune, allergic and cardiovascular diseases.[16] Quercetin-3-O-glucuronide,a Quercetin metabolite, is more specific than Quercetin in inhibiting the activity of MAPKinases in vascular smooth cells.[16] Several studies have reported the inhibitory activity of flavonoids against protein kinases, using different descriptors and methods of modeling.[17-19]

Lee etal[20] have reported Quercetin could be docked to the pocket separate from, but adjacent to the ATP-binding site of MEK1. The C-ring of Quercetin interacts with the residues in the activation loop of the inactive MEK1. Val211 and Leu215 form van der Waals interactions with the C-ring of Quercetin. The hydroxyl group at the 3′ position of the C-ring can make a critical hydrogen bond with the backbone amide group of Ser212. These interactions of Quercetin with the activation loop would lock MEK1 into a catalytically inactive species by stabilizing the inactive conformation of the activation loop. Inhibition of TPA-induced the phosphorylation of ERK and p90RSK, and the activation of AP-1 and NF-κB by Quercetin inhibited TPA-induced cell transformation.[20]

**Table 1:** Binding of the Eupalitin-3-O-Beta-D-Galactopyranoside, showing best docking energy with the active site of MAPK/ERK kinase 2 and Mitogen-Activated Protein Kinase (p38)

| MAPK/ERK kinase 2 | | Eupalitin-3-O-Beta-D-Galactopyranoside | Distance (Å) | Docking Score (Kcal/Mol) |
|---|---|---|---|---|
| **Residue** | **Atom** | | | |
| SER213 | O | O | 3.0 | -10.4 |
| SER213 | N | O | 3.4 | |
| SER213 | O | H | 1.8 | |
| PHE210 | O | H | 2.6 | |
| VAL212 | N | O | 3.3 | |
| **Mitogen-Activated Protein Kinase (p38)** | | **Eupalitin-3-O-Beta-D-Galactopyranoside** | **Distance (Å)** | **Docking Score (Kcal/Mol)** |
| **Residue** | **Atom** | | | |
| TRP198 | NE1 | O | 2.6 | -10.1 |
| TRP198 | NE1 | O | 3.0 | |
| THR204 | OG1 | O | 3.6 | |

MEKK2 is a 70 kDa member of the MEKK group of MAP3Ks that has been shown to regulate the JNK and ERK5 pathways.[2, 21] MEKK2 is the first MAP3K shown to be required for mast cell tyrosine kinase receptor signaling controlling cytokine gene expression. MEKK2 is involved in the signaling of antigen receptors in both lymphocytes and mast cells.[22] In the present investigation the inhibitory effect of Eupalitin-3-o-β-D-Galactoside on MEKK2 was evaluated to study the involvement of MEKK2 in TNF-α signalling.

The mitogen-activated protein (MAP) kinases are essential signaling molecules that mediate many cellular effects of growth factors, cytokines, and stress stimuli. Mitogen-activated protein kinases (MAPKs)[23] play a pivotal role in controlling numerous cellular processes, including differentiation, mitogenesis, oncogenesis and apoptosis.[24-27]

Like many protein kinases, the activity of MAPKs is regulated by phosphorylation in an activation loop located near their active sites.[28] The hallmark of the MAPKs is their unique requirement for dual phosphorylation at a conserved threonine and tyrosine residue belonging to the consensus sequence TXY for catalytic activation (where *X* is Glu in ERKs, Pro in JNKs and Gly in p38 kinases).[29-31]

The p38 mitogen-activated protein (MAP) kinase plays an important role in the inflammatory diseases. The p38mapkinase belong to serine/threonine protein kinases family and are involved in cellular responses to external stress signals.[32] As the p38 MAP kinase plays crucial role in the cascade for the regulation of proinflammatory cytokine production, [33] it can be a good potential target for TNF-α inhibition. In current study, an approach of molecular docking was used to identify the potential of Eupalitin-3-o-β-D-Galactoside as inhibitor of this kinase by measuring their binding affinities.

Specificity of MAPK signaling is maintained primarily through structural mechanisms that limit protein interactions. p38-contain a specific sequence in their activation loop (TEY, TPY and TGY, respectively) that is recognized by the MAPKK of the pathway. In turn, MAPKs only efficiently phosphorylate the consensus motif S/TP in their target proteins. In addition to specific phosphorylation motifs in both MAPKs and their substrates, another level of specificity is ensured by conserved docking domains. These domains form a binding site for the kinase and are required for phosphorylation of the substrate.[34] It has been shown that docking interactions between MAPK and their substrates are necessary for signaling and that docking site structures can influence pathway-specific input and output.[35,36]

Docking results indicated that Eupalitin-3-o-β-D-Galactoside interacted with MAPK/ERK kinase 2 at highly conserved active site 1 with residues SER213, PHE210 and VAL212 with binding energy value of -10.4kcal/mol whereas Eupalitin-3-o-β-D-Galactoside interacted with p38 Mapkinase at highly conserved active site1 with residues TRP198 and THR204 with binding energy value of -10.1 kcal/mol. RMSD values for ligand docking obtained for MEK2 and p38 MAPKinase were 42.5660Å and 17.8460 Å respectively.

The binding score value and the interaction of Eupalitin-3-o-β-D-Galactoside with the ATP binding sites of MAPK/ERK kinase 2 and p38 Mapkinase emphazise the role of Eupalitin-3-o-β-D-Galactoside in regulation of TNF-α signaling through inhibition of MAPK/ERK kinase 2 and p38 Mapkinase involved in TNF-α signaling pathway.

## CONCLUSION

The results in this study suggests the potential role of Eupalitin-3-o-β-D-Galactoside in TNF-α signaling through MEK2 and p38 MAPKinase. In this study, it is reported that Eupalitin-3-o-β-D-galactoside act as an inhibitory agent for Cyprinus carpio(koi carp)MAPK/ERK kinase 2 and p38 Mapkinase and thus from the interesting results, obtained in this study, indicate that human studies are needed to evaluate Eupalitin-3-o-β-D-galactoside for their immunosuppressive studies. The use of Eupalitin-3-o-β-D-galactoside as an efficient immunosuppressor may be possible in future.

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