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In silico docking of phytochemicals from *Indigofera aspalathoides* to identify potent inhibitors of MAP kinase

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ABSTRACT
In the present article, around 40 compounds from *Indigofera aspalathoides* were screened for inhibition of MAP Kinase, as mitogen-activated protein Kinase plays a vital role in many signaling pathways for transducing the extracellular signals. Screening of MAPK inhibitors is essential for direct inhibition of other effector proteins downstream in MAPK pathway. For this purpose *In silico* molecular modeling and molecular docking were performed to predict the variation in binding efficiency of a diverse set of compounds. The bonding energy and docking score were utilized to determine the binding efficiency of tested compounds. Results showed that the three compounds were found to be a potent inhibitors of MAPK. It was concluded that **t**hese compounds might be explored for inhibiting MAPK and other proteins involved in cell proliferation and cell division in diseases related to neuronal dysfunction and tumorigenesis.
Keywords: MAP kinase, *Indigofera aspalathoides*, phytochemical, anticancer activity, *Insilico*

1. INTRODUCTION

Protein kinases have been explored as the primary drug target for developing novel lead molecules in combating several human diseases and associated complications. Protein phosphorylation is involved in most biochemical and cellular processes such as differentiation, secretion, proliferation, and apoptosis. In regulating these processes, protein kinases and phosphatases play vital roles and thus influence the cellular reaction to several extracellular signaling molecules [1]. Changes in the stage, subcellular position, and activity of kinases and phosphatases affect normal cell function and cell homeostasis maintenance [1,2]. Either directly or indirectly, more than 400 diseases have been linked to protein kinases [3]. MAP Kinase belongs to the family of protein tyrosine kinases. It plays a vital role in regulating basic biological processes by phosphorylating specific serine and threonine residues belonging to a target protein. MAPKs are the critical regulators of various cellular processes such as proliferation, differentiation gene expression, and programmed cell death [4-7].

MAPKs are among the oldest signal transduction pathway. They are widely involved during the evolution of many physiological procedures [8]. The wide range of functions regulated by MAPKs is mediated by the phosphorylation of several substrates, including members of a family of protein kinases called MAPK-activated protein kinases [9,10]. Once the MAP kinases are engaged, they can phosphorylate various transcription factors and other proteins that regulate gene transcription, mRNA stability, and gene translation. The subcellular distribution of downstream effectors is also regulated by MAPKs, thus influencing the signaling properties of these proteins. The functional studies proved that MAP kinases are expressed at the sites of much of human disease. Many of the anti-inflammatory and anti-cancer drugs function by inhibiting the MAP kinase cascade of signaling events. Thus, MAP kinase down regulation can influence the secretion of cytokines and other mediators known to be involved in pathogenic processes. The potent inhibition of MAP kinase can thus increase the efficacy of drugs.

The development of biological databases and *In silico* tools lead to novel drug target identification against many human diseases. Hence the primary aim of this study involves the *In silico* docking approach to identify a binding mode of MAPK with lead molecules from *Indigofera aspalathoides* using molecular docking. The lead molecule with maximum binding energy is determined to be a potent MAPK inhibitor.

2. MATERIALS & METHODS

2.1 Protein sequence retrieval

The protein sequence in raw form was obtained from the UNIPROT database [11]. http://www.uniprot.org,

2.2 Preparation of ligands

The optimized 3D structure of all the ligands was downloaded in SD format from the Pubchem database available at NCBI (www.ncbi.nlm.nih).

2.3 Secondary structure prediction using SOPMA

The possible probability with which the amino acid residues in a protein sequence can form a secondary structure has been predicted by SOPMA [12].

2.4 Molecular modeling

Homology modeling is used to model the 3D structure of the target protein. The target protein was subjected to an all-against-PDB in BLAST search to identify the template sequence that is highly homolog to the target sequence [13]. The template was selected based on a high degree of similarity, and the 3D structure was modeled using a modeler [14].

2.5 Molecular docking

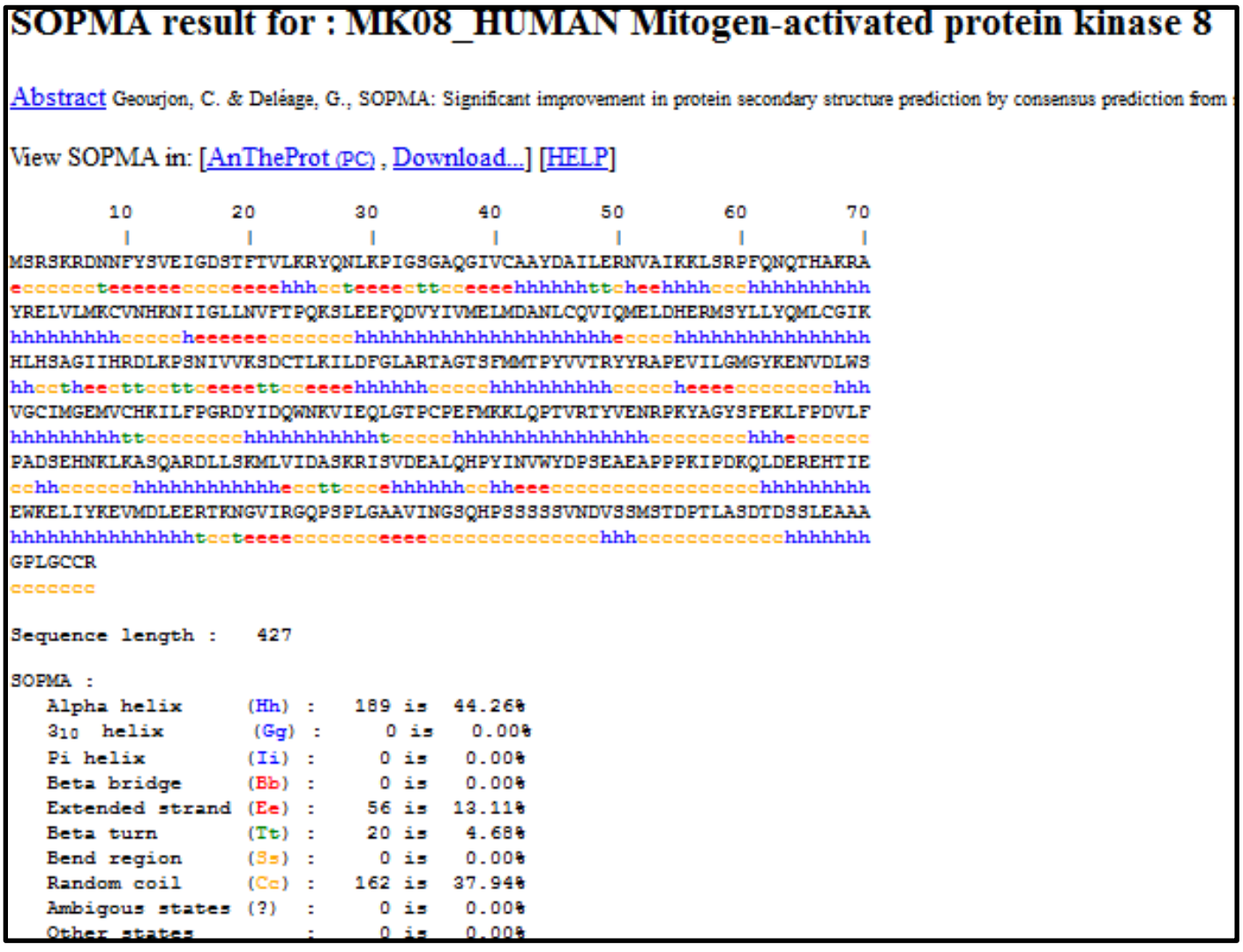
The ligands were docked the predicted structure of the target MAP Kinase to determine the mode of interaction of target protein with the selected lead molecules. Molecular docking was carried out using PyRX, a standalone tool used to visually screen bioassays [15]. Virtual screening has become an effective tool in drug discovery as it filters the molecule as it scores these compounds.

3. RESULTS

The protein sequence of Mitogen-activated protein kinase 8 was retrieved from the Primary protein sequence database, UNIPROT with ID- P45983. It consists of 472 amino acids.

>sp|P45983|MK08_HUMAN Mitogen-activated protein kinase 8 OS=Homo sapiens GN=MAPK8 PE=1 SV=2
MSRSKRDNNFYSVEIGDSTFTVLKRYQNLKPIGSGAQGIVCAAYDAILERNVAIKKLSRP
FQNQTHAKRAYRELVLMKCVNHKNIIGLLNVFTPQKSLEEFQDVYIVMELMDANLCQVIQ
MELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVKSDCTLKILDFGLARTAGTSF
MMTPYVVTRYYRAPEVILGMGYKENVDLWSVGCIMGEMVCHKILFPGRDYIDQWNKVIEQ
LGTPCPEFMKKLQPTVRTYVENRPKYAGYSFEKLFPDVLFPADSEHNKLKASQARDLLSK
MLVIDASKRISVDEALQHPYINVWYDPSEAEAPPPKIPDKQLDEREHTIEEWKELIYKEV
MDLEERTKNGVIRGQPSPLGAAVINGSQHPSSSSSVNDVSSMSTDPTLASDTDSSLEAAA
GPLGCCR

The protein sequence was subjected to secondary structure prediction using SOPMA with default parameters set within the tool. The output revealed the residues of protein to have a 44.26% probability of forming an alpha helix. The probability of forming a random coil within the structure predominated compared to the Beta strand (Figure 1).



SOPMA result for : MK08_HUMAN Mitogen-activated protein kinase 8

Abstract Geourjon, C. & Deléage, G., SOPMA: Significant improvement in protein secondary structure prediction by consensus prediction from

View SOPMA in: [AnTheProt (PC) , Download...] [HELP]

```
        10         20         30         40         50         60         70
         |          |          |          |          |          |          |
MSRSKRDNNFYSVEIGDSTFTVLKRYQNLKPIGSGAQGIVCAAYDAILERNVAIKKLSRPFQNQTHAKRA
eccccccteeeeeecccceeeehhhcctceeeecttccceeeehhhhhhttchheehhhhcccchhhhhhhhhh
YRELVLMKCVNHKNIIGLLNVFTPQKSLEEFQDVYIVMELMDANLCQVIQMELDHERMSYLLYQMLCGIK
hhhhhhhhhccccchheeeeeeccccccchhhhhhhhhhhhhhhhhhhhhhecccchhhhhhhhhhhhhhhh
HLHSAGIIHRDLKPSNIVVKSDCTLKILDFGLARTAGTSFMMTPYVVTRYYRAPEVILGMGYKENVDLWS
hhcchheecttcctttceeeettcceeeehhhhhhccccchhhhhhhhhhcccccheeeeccccccccchhh
VGCIMGEMVCHKILFPGRDYIDQWNKVIEQLGTPCPEFMKKLQPTVRTYVENRPKYAGYSFEKLFPDVLF
hhhhhhhhhhttccccccccchhhhhhhhhhhhtccccchhhhhhhhhhhhhhhhhcccccccchhheccccccc
PADSEHNKLKASQARDLLSKMLVIDASKRISVDEALQHPYINVWYDPSEAEAPPPKIPDKQLDEREHTIE
ccchhccccccchhhhhhhhhhhhheccttcccehhhhhhccchheeeccccccccccccccccchhhhhhhhh
EWKELIYKEVMDLEERTKNGVIRGQPSPLGAAVINGSQHPSSSSSVNDVSSMSTDPTLASDTDSSLEAAA
hhhhhhhhhhhhhhhhtcctceeeeccccccceeeecccccccccccccchhhccccccccccchhhhhhh
GPLGCCR
ccccccc

Sequence length :   427

SOPMA :
   Alpha helix      (Hh) :   189 is  44.26%
   3₁₀ helix        (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :    56 is  13.11%
   Beta turn        (Tt) :    20 is   4.68%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :   162 is  37.94%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

Figure 1:SOPMA results of MAP kinase 8

3.1 Molecular modeling of MAPK

Using the BLAST instrument issued by NCBI, this sequence was subjected to BLAST against the Protein Data Bank. Later, the templates were chosen based on structural hits and their alignment pattern against the query sequence. The selected templates were chain A of 1OHV, chain A of 2CIN, and chain P belonging to 2JJF. The corresponding structures were retrieved from Protein Data Bank. Templates and their identity with the Mitogen-activated protein kinase 8 sequences were given in Table 1.

Table 1: Templates used in molecular modeling

S. No.	Template(PDB)	Chain	Length	Identity score with cox-2 seq
1	1JNK	A	423	83.00%
2	2XS0	A	386	95.00%
3	3TTJ	A	464	90.00%

Molecular modeling using an advanced modeling package provided 5 modeled structures. The optimized structure was chosen with the aid of a DOPE (Discrete optimized protein energy) score. -47885.152344 was the DOPE score for the best model structure. With the PROCHECK structural validation method, the stereo-chemistry properties of the structures were validated. PROCHECK results indicated the higher fidelity of modeled Mitogen-activated protein kinase 8 structure. Further, 89.7% of residues were determined to fall in the allowed regions of the Ramachandran plot, with only 0.8% residues in the disallowed region. This strongly proves the stability and absence of steric hindrance in the modeled 3D structure (Figure 2).

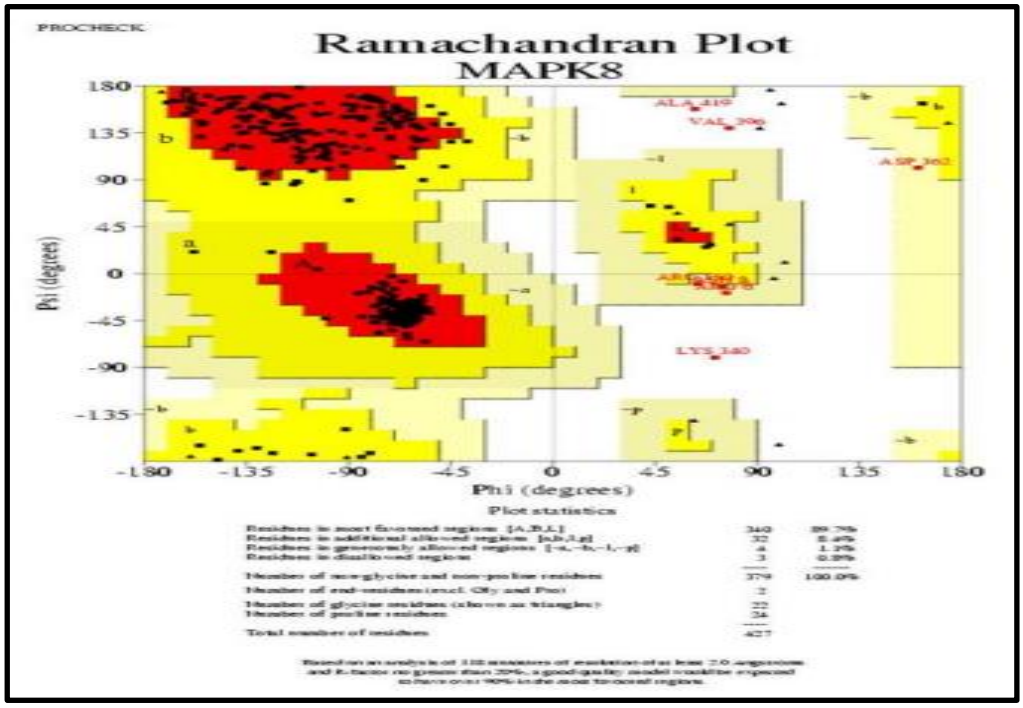


Fig. 2: Ramachandran plot for MAPkinase 8

Modeled structure of Mitogen-activated protein kinase 8 obtained using chimera. The structure was given with the combination of cartoon and surface model with 60% transparency.

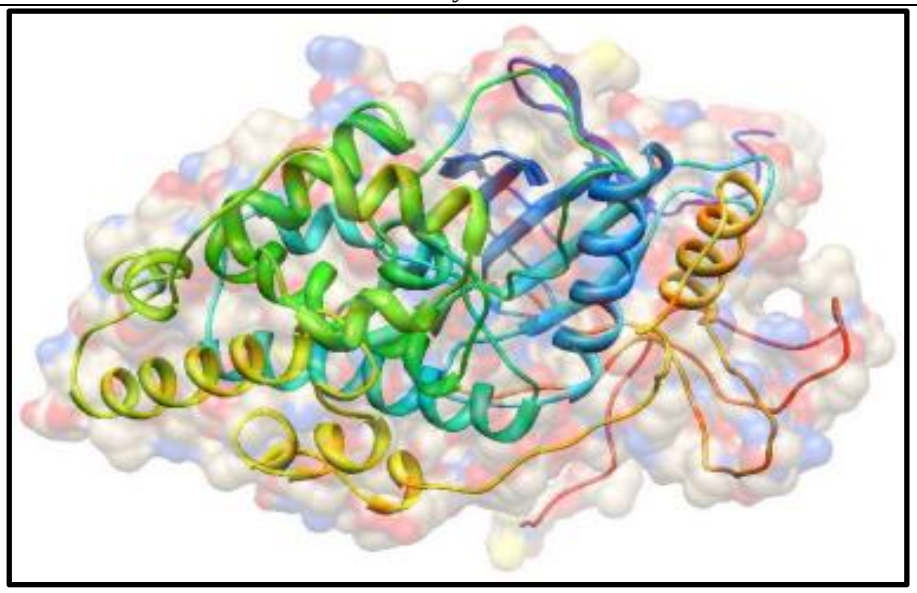


Fig. 3: Modelled structure of MAP kinase 8 with ligand molecules

3.2 Molecular docking

Table 2: Compounds that showed best binding potential

S. No.	Name of the compound	Pubchem ID	Protein	Binding energy	Ligand efficiency	Inhibitory constant	Ref. RMS	Hydrogen bond
1	Carotal	4631	MPK8	-4.13	-0.22	9.78	35.17	SER 155 ASN 114
2	Spathulenol	522266	MPK8	-7.13	-0.45	5.9	36.18	MET 111 MET 111
3	Tau.- Cadinol	6429185	MPK8	-7.25	0.46	4.61	34.93	MET 111

About 40 different compounds were chosen as lead molecules. Their binding efficiency with MAPK was determined using Vina Wizard available in PyRX-0.8 software. The compounds that showed the best binding potential are given in Table 2. The mode of interaction between MAPK and ligand molecules is shown in Figure 3.

4. DISCUSSION

Pharmacological inhibitors of MAPK pathway components are also a feasible alternative or a complementary method for understanding the functional requirements of the pathway. It has also been reported that the p38 activation causes the activation of inflammatory cytokines such TNFα, IL-1β, cyclooxygenase (COX)-2, IL6, IL-12, and IFN-γ, which play essential roles in autoimmune, neurodegenerative, and cardiovascular diseases [16-18]. The ATP binding pocket common to all protein kinases is connected to several inhibitors. Allosteric inhibitors are becoming increasingly valuable for achieving greater precision. The specificity of the interaction outside the ATP pocket has continued to stimulate the search for inhibitors of several kinases on kinase surfaces that bind to other pockets [19]. Some inhibitors that block significant protein-protein interactions are currently being developed.

Similarly, in the present study, the primary raw sequence of MAP Kinase was retrieved from UniProt, and the 3D structure was modeled to obtain a stable, optimized molecular structure. The helical structures in the optimized structure can witness the high probability of helix formation as predicted by SOPMA. The presence of phi and psi angle values of most amino acids in the allowed regions of the Ramachandran plot revealed the stable conformation of modeled structure. Among 40 different lead molecules, 3 ligand molecules were found to have better binding efficiency within the active site of MAPK. The same set of ligand molecules were also screened for binding potential with Hemopexin and reported. Molecular docking studies of lupeol with MAPK and its effector proteins were reported by Bhatt *et al*., [20].

5. CONCLUSION:

It was concluded that the protein-ligand interaction plays a crucial role in structure drug discovery. The screening of lead molecules for MAPK inhibition will pave to identify of new MAPK inhibitors, thereby indirectly increasing the efficacy of drug molecules.

DATA AVAILABILITY: The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request

REFERENCES

1. T. Hunter. *Signaling—2000 and beyond Cell* 100:113-127. **(2000).**

2. T. Hunter. *The Croonian Lecture 1997. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* 353:583-605. **(1998).**
3. I. Melnikova, & J. Golden. *Nat Rev Drug Discov*, 3: 993-4. **(2004).**
4. C. Dong, R. J. Davis, and R. A. Flavell. *Annual review of immunology*, 20: 55-72. **(2002).**
5. J. M. Kyriakis, & J. Avruch. *Physiological reviews*, 81(2), 807-869. **(2001).**
6. H. J. Schaeffer & M. J. Weber. *Molecular and cellular biology*, 19: 2435-2444. **(1999).**
7. T. Wada and J. M. Penninger. *Oncogene*, 23: 2838-2849. **(2004).**
8. C. Widmann, S. Gibson, M. B. Jarpe & G. L. Johnson. *Physiological reviews*, 79: 143-180. **(1999).**
9. M. Gaestel. *Front Biosci*, 13, 6050-6059. **(2008).**
10. P. P. Roux and J. Blenis. *Microbiology and molecular biology reviews*, 68: 320-344. **(2004).**
11. M. Magrane. *Database*, 2011. **(2011).**
12. J. A. Cuff, and G. J. Barton. *Proteins: Struc. Func. and Bioinfor*. 40: 502-511. **(2000).**
13. R. Ghosh, A. D. Upadhayay and A. K. Roy. J. *applied Biotechnl. and bioengin*., 3, 55. **(2017).**
14. Z. Li, S. Zhang and Q. Liu. PLoS One, 3: e1940. **(2008).**
15. G. Deleage, C. Blanchet, C. Geourjon. *Biochimie*, 79: 681-686. **(1997).**
16. C. Ding. *Curr. Opin. in investing. drugs*, 7: 1020-1025. **(2006)**.
17. F. Dodeller, and H. Schulze-Koops. *Arthr. res. & ther.* 8: 1-11. **(2006)**.
18. M. A. Palladino, F. R. Bahjat, E. A. Theodorakis, and L. L. Moldawer. *Nature revi. Drug disco.*, 2: 736-746. **(2003).**
19. M. F. Favata, K. Y. Horiuchi, E. J. Manos, A. J. Daulerio, D. A. Stradley, W. S. Feeser, and J. M. Trzaskos, *J. Bio. Chem.*, 273: 18623-18632. **(1998).**
20. M. H. Bhatt, C. K. Prajapati, M. N. Reddy. *J. exp. Therap. & oncol.*12: **(2017)**.

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