Short Communication

Molecular docking analysis of 5,6,7,8-tetrahydropyrido[3,4-d] pyrimidine derivatives as ERK2 inhibitors

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ABSTRACT

ERK1 and ERK2 are one of the important targets, involved in various types of cancers such as breast, lung, prostate, and ovarian cancer. ERK1 and ERK2 belong to the mitogen-activated family, thus also known as mitogen-activated protein kinases (MAPKs) and both possess 85% similarity in their amino acid sequence. In silico techniques like molecular docking are continuously utilized in the identification of new molecules as lead for the development of bioactive compounds. Molecular docking predicts the binding orientation of small molecule drug candidates to their protein targets. In this study, we performed the molecular docking studies of small library of heterocycles against ERK2 (PDB ID: 2OJJ) using docking software MOE 2008.10. Binding energy score of each designed molecules was calculated and results were compared with standard ligand 82A. The results displayed that most of the compounds were occupied the same binding cavity of protein and also showed the similar interactions when compared to the standard ligand 82 A. Among all ligand 11 exhibits the highest binding energy score of −11.6464 kcal/mol comparable to the 82A with binding energy score of −9.2447kcal/mol and showed interactions with residue Met-106.

Keywords: ERK2, *In silico*, Molecular docking analysis, MOE

INTRODUCTION

ERK1 and ERK2 are one of the promising targets, which seek the attention of many researchers. ERK1 and ERK2 belong to the mitogen-activated family, so also called as mitogen-activated protein kinases (MAPKs).[1] Both ERK1 and ERK2 contain same amino acid sequence up to 85% similarity. These kinases are part of RAS–RAF–MEK–ERK signaling cascade due to their active involvement in the cell functions such as cell growth, cell differentiation, and proliferation. Mutation leads to dysregulation and increased kinases activity and thus increased phosphorylation.[2] Due to this, ERKs or MAPKs have been considered as one of the target in various types of cancers such as breast, lung, prostate, and ovarian cancer.[3] About 33% of the human cancers are over expressed with RAS proteins.

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At present, various *in silico* approaches like molecular docking have been used for the discovery of new molecules as lead for the development of pharmacologically active compounds. Molecular docking is an important tool in computer-assisted drug design which determines the binding mode of a ligand with a target protein.[4] In docking methods, two parameters, i.e., search algorithm and scoring function are used. The docking score of molecules is calculated by scoring function and rank the molecules according to docking score. Docking studies also utilized in performing virtual screening on large libraries of compounds. Through this method, a basic idea about the mechanistic action of ligands toward the target can be suggested, which is useful in lead optimization. It is a technique which identifies the preferred orientation of molecule in the target protein through forming a stable complex.[5] This information of the preferred orientation was then utilized to predict the strength of association or binding affinity between two molecules. For docking-based study, the known structure of the protein of interest is required. The structure of protein was usually determined by various biophysical techniques such as X-ray crystallography, and NMR

spectroscopy.[6] Many derivatives containing different heterocyclic moieties have been reported as MAPK/ERK inhibitors.[7] Some of the reported molecules as MAPK/ERKs inhibitors are shown in Figure 1.

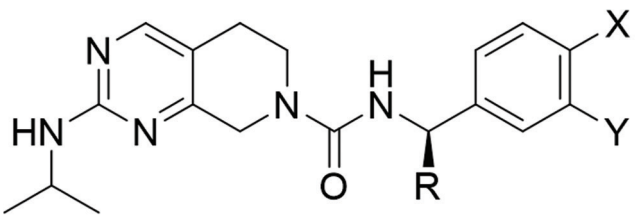
Amin *et al.* had reported the synthesis and molecular docking study of new benzofuran and furo[3,2–g]chromone-based cytotoxic agents against breast cancer and *p38*α MAP kinase inhibitors.[8] Hamed *et al.* had reported most potent MAPK inhibitors and are great substituents for trametinib to be used and evaluated in clinical trials as alternative cancer drugs.[9] Kohno *et al.* have discussed the possibility that combination of ERK pathway inhibitors and conventional anticancer drugs provides an excellent basis for the development of new chemotherapeutic strategies against cancer.[10] PD184352[11] and AZD6244[12] as MEK1/2 inhibitors have reached the clinical trial stage.

Therefore, in the current study, we performed the molecular docking analysis of a library of 5,6,7,8-tetrahydropyrido[3,4-*d*] pyrimidine derivatives[13] against ERK2 to evaluate their binding mode and analyze their mechanistic behavior. Further the binding conformation of the library of molecules was compared with the standard molecule co-crystallized within the catalytic domain of ERK2. Furthermore, the binding affinity was evaluated to understand the variation in the inhibitory potential of the molecules of library.

METHODOLOGY

Library generation

First, a library of derivatives of 5,6,7,8-tetrahydropyrido[3,4-d] pyrimidine [Table 1], inhibitors for ERK were designed and prepared using MOE 2008.10.[14]



Selection and preparation of protein

The ERK2 protein is selected for the docking studies, as it is found over-expressed in the MDA-MB-231 cancer cell line. Protein target was downloaded from database Protein Data Bank (PDB). The crystal structure of ERK 2 protein (PDB ID: 2OJJ) having resolution 2.4 Å was obtained from the Protein Data Bank.

The protein was prepared by the structure preparation process in MOE. First, hydrogen positions have been added in the protein structure. Then, water molecules were removed. Finally, partial charges were calculated, and the active site of the protein has been defined in collection of residues within 10.0 Å of the bound inhibitor.

Selection and Preparation of the Ligand

All the compounds were built using the builder tool of the MOE program. All the compounds are then convert into mol file. The parameters such as hydrogen atoms were added, correct bond types were defined, charges were assigned to each atom, and finally the energy minimization of structures was done using force field MMFF94x.

Molecular Docking Analysis

First, the docking of standard ligand, i.e., 82A was carried out by dock module in MOE program in the active site of protein. Then individual designed compounds were docked and the results were compared with the 82A. The free energy of ligand binding was calculated using scoring function for each compound. The ligand-enzyme complex with lowest *S*_score was selected and was used to rank the docked compounds.

Interaction Studies

The goal of ligand-protein docking is to predict the predominant binding mode of a ligand with a protein of known three-dimensional

Table 1: Different derivatives of 5,6,7,8-tetrahydropyrido[3,4-*d*] pyrimidine

Ligand No.	R	X	Y
1.	H	H	H
2.	H	F	H
3.	H	Cl	H
4.	H	H	Cl
5.	H	H	F
6.	H	F	Cl
7.	CH_2OH	F	Cl
8.	$CH_2NHSO_2CH_3$	F	Cl
9.	$CH_2NHSO_2CH_3$	Cl	F
10.	$CH_2NH_2CH_3$	F	Cl
11.	$CH_2NHCH_2CH_2OH$	F	Cl
12.	CH_2NH_2	F	Cl
13.	$CH_2OCH_2CH_2OCH_3$	F	Cl
14.	CH_2OCH_3	F	Cl
15.	$CH_2(S\text{-}OH)CH_2CH_3$	F	Cl

Table 2: Binding energy scores of the library of 5,6,7,8-tetrahydropyrido[3,4-*d*] pyrimidine derivatives

Ligand No.	R	X	Y	Binding Score (kcal/mol)
1.	H	H	H	−9.1998
2.	H	F	H	−8.6700
3.	H	Cl	H	−9.8154
4.	H	H	Cl	−10.2481
5.	H	H	F	−9.2451
6.	H	F	Cl	−9.0622
7.	CH_2OH	F	Cl	−10.3884
8.	$CH_2NHSO_2CH_3$	F	Cl	−10.8964
9.	$CH_2NHSO_2CH_3$	Cl	F	−8.7241
10.	$CH_2NH_2CH_3$	F	Cl	−8.3927
11.	$CH_2NHCH_2CH_2OH$	F	Cl	−11.6464
12.	CH_2NH_2	F	Cl	−10.7067
13.	$CH_2OCH_2CH_2OCH_3$	F	Cl	−11.0955
14.	CH_2OCH_3	F	Cl	−8.2792
15.	$CH_2(S\text{-}OH)CH_2CH_3$	F	Cl	−9.3192

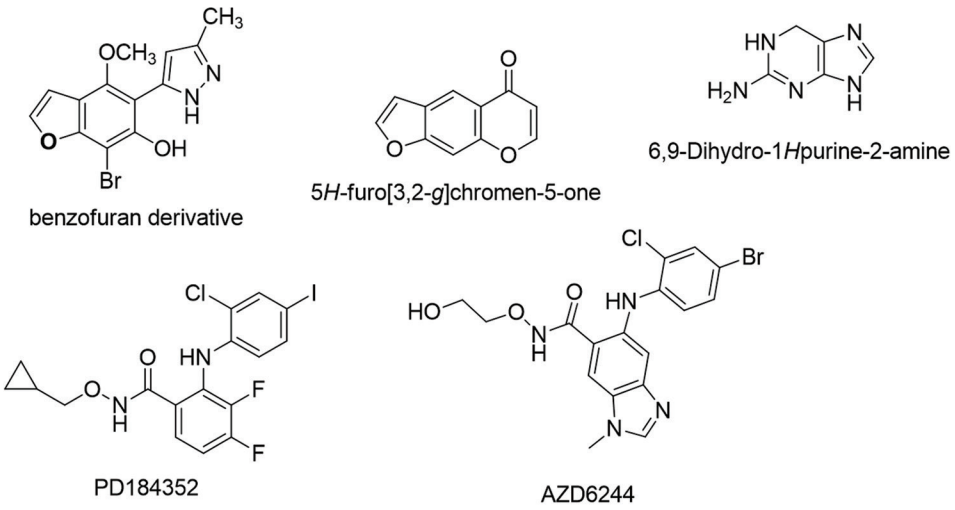


Figure 1: Some of the reported molecules as MAPK/ERK inhibitors

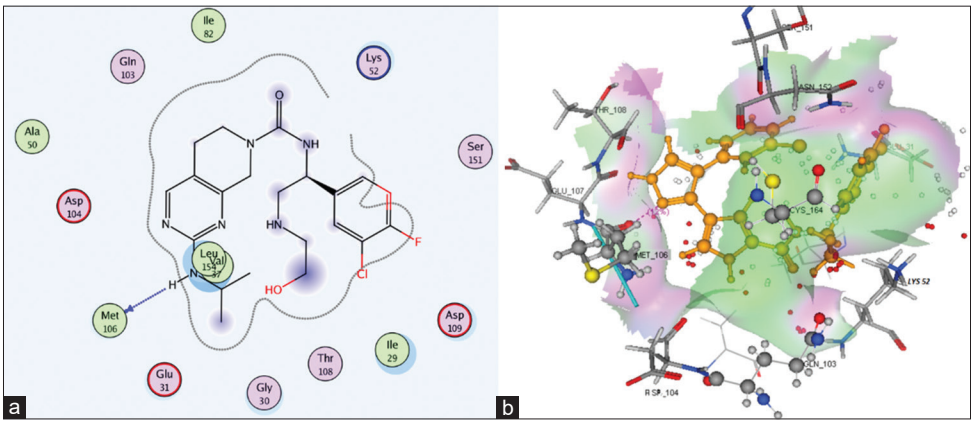


Figure 2: (a) 2D diagram of ligand 11 showing interactions with residues Met 106. (b) 3D diagram of ligand 11 showing same interactions in the active site of protein showed as graphical molecular surface

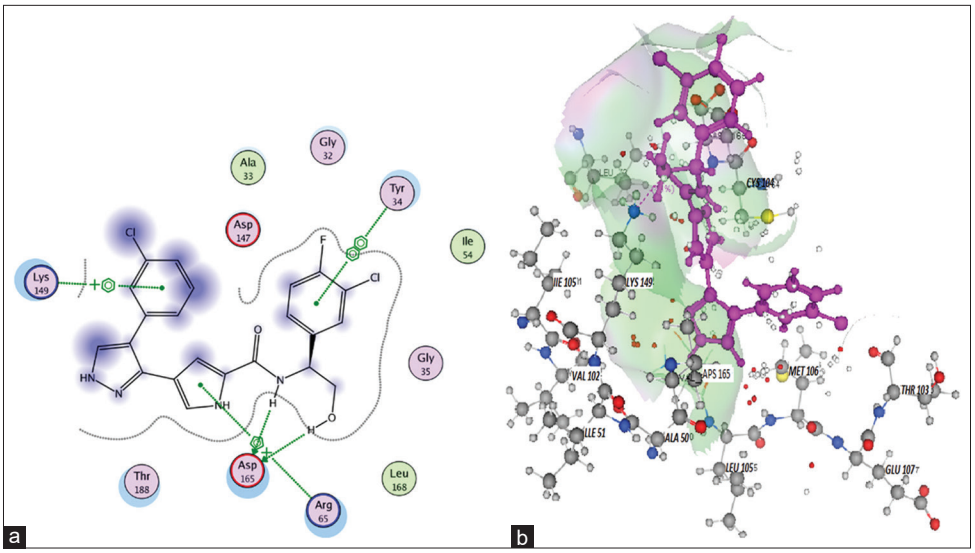


Figure 3: (a) 2D diagram of ligand 82 A showing interactions with residues Aps165 and Lys149. (b) 3D diagram of ligand 82 A showing same interactions in the active site of protein showed in graphical molecular surface

structures. To study the binding modes of bioactive compounds in the binding site of human ERK2 protein, intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the ERK2-inhibitor complexes. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein.

RESULTS AND DISCUSSION

Molecular docking analysis of compounds against ERK 2 protein (PDB ID: 2OJJ) showed that most of the compounds were occupied the same binding cavity of protein and also showed the similar interactions when compared to the standard ligand 82A. Ligand 82A showed interactions with residues Aps165 and Lys149 [Figure 2]. For each compound 10, binding conformations were generated. The conformation with the highest docking score was selected as the optimum binding pose (best fit conformation).

Among them, compound 11 exhibits highest binding energy score of −11.6464 kcal/mol, which is comparable to the 82A with binding energy score of −9.2447kcal/mol. The docking energy score of all compounds is depicted in Table 2. Similarly, in ligand 11 the ring showed interactions with Met-106 [Figure 3]. Other docked compounds also retained at least one of the interactions in their structures.

CONCLUSION

In the current work, reported anticancer compounds were evaluated for their binding mode in the catalytic domain of ERK2 receptor. On molecular docking, the compounds have showed commendable binding affinity in comparison to the reference compound. The compounds occupied the same binding cavity as the reference compound maintaining the interactions with conserved amino acid residues essential for significant inhibitory potential, especially for compound 11. This analysis provided a mechanistic justification for the anticancer potential of the compounds under study. Further structural modifications of these compounds can be done for the generation of potent analogs.

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