Bioorganic & Medicinal Chemistry Letters 26 (2016) 864–874

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# 3D-QSAR, molecular dynamics simulations and molecular docking studies of benzoxazepine moiety as mTOR inhibitor for the treatment of lung cancer

Udit Chaube, Dhara Chhatbar, Hardik Bhatt *

*Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University, S.G. Highway, Chharodi, Ahmedabad 382 481, India*

ARTICLE INFO

*Article history:*
Received 15 September 2015
Revised 7 December 2015
Accepted 22 December 2015
Available online 23 December 2015

*Keywords:*
Mammalian target of rapamycin (mTOR)
Lung cancer
CoMFA
CoMSIA
Molecular dynamics simulations
Molecular docking

ABSTRACT

According to WHO statistics, lung cancer is one of the leading causes of death among all other types of cancer. Many genes get mutated in lung cancer but involvement of EGFR and KRAS are more common. Unavailability of drugs or resistance to the available drugs is the major problem in the treatment of lung cancer. In the present research, mTOR was selected as an alternative target for the treatment of lung cancer which involves PI3K/AKT/mTOR pathway. 28 synthetic mTOR inhibitors were selected from the literature. Ligand based approach (CoMFA and CoMSIA) and structure based approach (molecular dynamics simulations assisted molecular docking study) were applied for the identification of important features of benzoxazepine moiety, responsible for mTOR inhibition. Three different alignments were tried to obtain best QSAR model, of which, distil was found to be the best method, as it gave good statistical results. In CoMFA, Leave One Out (LOO) cross validated coefficients ($q^2$), conventional coefficient ($r^2$) and predicted correlation coefficient ($r^2_{pred}$) values were found to be 0.615, 0.990 and 0.930, respectively. Similarly in CoMSIA, $q^2$, $r^2_{ncv}$ and $r^2_{pred}$ values were found to be 0.748, 0.986 and 0.933, respectively. Molecular dynamics and simulations study revealed that B-chain of mTOR protein was stable at and above 500 FS with respect to temperature (at and above 298 K), Potential energy (at and above 7669.72 kJ/mol) and kinetic energy (at and above 4009.77 kJ/mol). Molecular docking study was performed on simulated protein of mTOR which helped to correlate interactions of amino acids surrounded to the ligand with contour maps generated by QSAR method. Important features of benzoxazepine were identified by contour maps and molecular docking study which would be useful to design novel molecules as mTOR inhibitors for the treatment of lung cancer.



Cancer is the second most common disease after cardiovascular diseases resulting into deaths. According to fact sheets of WHO, updated on November 2014, cancer is accounted for 8.2 million deaths in 2012.[1] The most commonly found cancers are Lung, Liver, Stomach, Colorectal and Breast.[1] Lung cancer is one of the leading causes of death among all types of cancer.[1] It is mainly classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC); among which, cases of NSCLC (85%) were found to be more as compared to SCLC.[2] NSCLC is further classified as Adenocarcinoma (AC), Squamous Cell Carcinoma (SCC), and Large Cell Carcinoma (LCC).[2] Many genetic mutations take place in lung cancer, but mainly KRAS, EGFR, PIK3CA and PTEN are more common in lung cancer.[3–5] KRAS and EGFR mutations are found in AC,[2,3] while PIK3CA[2] and PTEN mutations occurred in the case of SCC.[6] No drugs are available in the market which directly acts on KRAS[2] and this problem can be solved by targeting the pathway associated with KRAS mutant in NSCLC viz. RAS/RAF/MEK pathway or PI3K/AKT/mTOR pathway. Selumetinib and BEZ-235 (as shown in Fig. 1A and B, respectively) are the drugs available in the market for targeting the RAS/RAF/MEK and PI3K/AKT/mTOR pathway, respectively. Similarly in the case of EGFR-mutant AC, drugs available in market are Erlotinib and Gefitinib (as shown in Fig. 1C and D, respectively).[3] Major problem associated with these types of drugs is resistance[4] which resulted into decrease in their therapeutic efficacy. In case of SCC, genes mutated are PTEN and PIK3CA, thus the inhibitors targeting PI3K/AKT/mTOR pathway might be effective within SCC as there are involvement of PTEN and PIK3CA in PI3K/AKT/mTOR pathway.[7] As per the available literature, it is well understood that PI3K/AKT/mTOR pathway is altered in lung cancer[8] and discovery of new inhibitors against any target on this pathway might be helpful for the treatment of lung cancer.

* Corresponding author. Tel.: +91 79 30642727; fax: +91 2717 241916.
*E-mail address:* hardikbhatt23@hotmail.com (H. Bhatt).

http://dx.doi.org/10.1016/j.bmcl.2015.12.075
0960-894X/

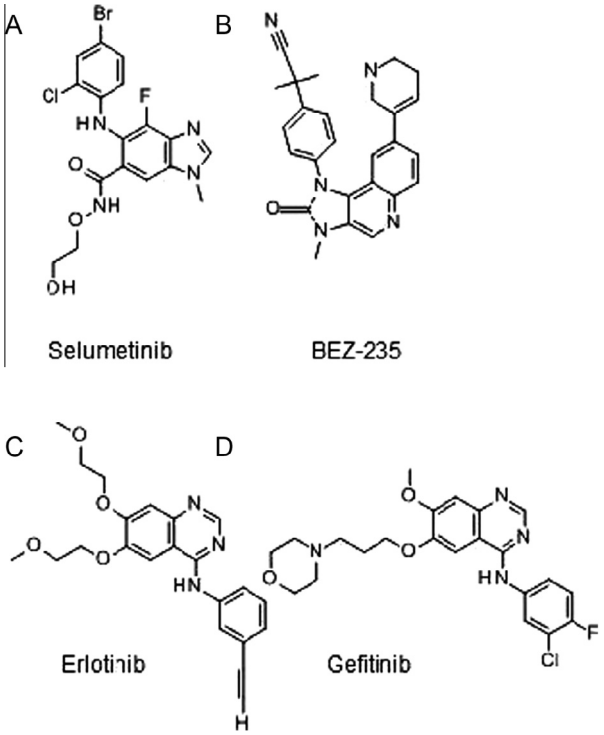


**Figure 1.** Inhibitors of various pathways (A) RAS/RAF/MEK pathway (Selumetinib); (B) PI3K/AKT/mTOR pathway (BEZ-235); (C) EGFR (Erlotinib and Gefitinib).

In the study of CoMFA (Comparative Molecular Field Analysis); steric and electrostatic properties of structures are correlated with their activity, while in the case of Comparative Molecular Similarity Indices Analysis (CoMSIA); hydrophobic field, hydrogen bond donor/hydrogen bond acceptor and steric/electrostatic fields are correlated with their activity. CoMFA and CoMSIA are an efficient tools in ligand based drug design, which were used for contour map generation and identification of favourable and unfavourable regions in a moiety. Further, these contour maps were validated by Molecular dynamics and simulations (MD/MS) assisted molecular docking study on the protein structure of mTOR (PDB ID: 4JT6).[9] Molecular dynamics and simulations study generally performed for understanding the activity and behaviour of biomolecules. In the present research, MD/MS study was performed on protein structure to obtain the stable conformation of it. Molecular docking is a structure based approach where interactions of amino acids surrounding to the ligand are correlated with contour maps generated by QSAR studies. B-chain of mTOR protein was simulated before docking on crystal structure, which resulted into the increase in the docking interactions with improvement in docking score.

Here in the present research, synthetic molecules bearing benzoxazepine moiety, reported as mTOR inhibitors,[10] were selected from the literature to carry out the CoMFA and CoMSIA study for the identification of important features responsible for anticancer activity. Further contour maps generated through the CoMFA and CoMSIA models were validated by MD/MS assisted molecular docking study.

28 synthetic molecules bearing benzoxazepine moiety, reported as mTOR inhibitors, were identified from literature as shown in Table 1. $IC_{50}$ values of all these molecules were converted into $pIC_{50}$. These $pIC_{50}$ values were utilised as dependent variable, while CoMFA and CoMSIA were utilised as independent variable. SKETCH function of Sybyl X was utilised for drawing the structure and charges were calculated by Gasteiger-Huckel method.

Tripose force field was utilised for energy minimisation of these molecules. These 28 molecules were divided in the ratio of 75:25, in such a way that both dataset consisted of balanced active and less active molecules.[11–13] Molecular alignment is one of the important parameter for the generation of QSAR model. Statistical values of CoMFA and CoMSIA depends on molecular alignment techniques. Hence in the present study, three alignments methods viz. distil based, docking based and pharmacophore based alignment methods were carried out to find out the best alignment method for the generation of 3D-QSAR model.[14–17] Alignment-1 (distil based alignment), shown in Figure 2A, was carried out by distil method. In this method, common core moiety was identified among all 28 molecules and all these molecules were aligned over each other. Compound **12** was selected as a template molecule, as it was the most active in the series,[18,19] and all others molecules were aligned over this template molecule. Alignment-II (docking based alignment) is shown in Figure 2B was carried out by SurFlex module of Sybyl X, in which protein of mTOR (PDB ID: 4JT6) was selected from protein data bank. Binding pocket was generated by the protomol generation technique of SurFlex and all molecules were docked in this binding pocket. Alignment-III (pharmacophore based alignment) is shown in Figure 2C where conformers of each molecule were generated and all conformers were aligned over each other to get a pharmacophore. This alignment technique was carried out by DiscoTECH module of Sybyl X.

Among all the alignment techniques, statistical results of distil were found to be good and hence it was proved as a good alignment technique which was used for further studies.

In case of CoMFA, steric and electrostatic properties of aligned molecules were calculated. These properties were responsible for variations in biological activity ($pIC_{50}$) of molecules. Principle of Lenard Jones potential was utilised for the calculation of electrostatic property while columbic potential was utilised for the calculation of steric property.[20] CoMSIA consisted of grid box, which included five CoMSIA fields viz. steric, electrostatic, hydrophobic, hydrogen bond donor atom and hydrogen bond acceptor atom. These fields were calculated at each lattice point of grid box. This grid box consisted of probe atom with radius of 1 Å at each lattice point with charge of +1, hydrophobicity of +1 and hydrogen bond donor and acceptor property of +1 which were utilised to calculate steric, electrostatic, hydrophobic, hydrogen bond donor atom and hydrogen bond acceptor atom fields.[21]

PLS method correlated CoMFA and CoMSIA fields with the activity value.[22] This method is generally utilised in QSAR study to determine the different statistical values like Leave One Out (LOO), cross validated coefficients ($q^2$), conventional coefficient ($r^2$), predicted correlation coefficient ($r^2_{pred}$), Standard Error of Estimation (SEE) and optimal number of components. Among these statistical values, $q^2$ is the most important to determine the reliability of the model. QSAR model is said to be good when $q^2$ value is more than 0.5.[23] During this analysis SAMPLES remain turned off and column filtering set to 2.0 kcal·mol$^{-1}$ to speed up the analysis.[24] Leave One Out (LOO) method includes hauling out one of the compound from the training set and then predicts the activity of each compound hauled out to ensure that the results of CoMFA and CoMSIA models are predictive for compounds that are not in the training set.[25–28] After several different trials, best CoMFA and CoMSIA models were decided based upon the highest $q^2$ value, lower Standard Error of Estimation (SEE) and optimal number of components.[29,30] Predictive capability ($q^2$) of model was found by Leave One Out (LOO) method. Optimum number of components, 7 in CoMFA and 4 in CoMSIA were selected which gave good $q^2$ value. Statistical confidence of derived model was done by 10-cycle bootstrap analysis. Conventional correlation coefficient ($r^2$), SEE and *F*-values were used to assess the non-cross validated models.