MOLECULAR DOCKING STUDY OF SIX PYRIMIDINE DERIVATIVES AS EGFR (EPIDERMAL GROWTH FACTOR RECEPTOR) AND CA IX (CARBONIC ANHYDRASE IX) INHIBITOR

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ABSTRACT

Objective: The present study was carried out to discover whether these pyrimidine derivatives have the potential to be used as epidermal growth factor receptor (EGFR) and carbonic anhydrase (CA) IX inhibitors through structure-based in silico study.

Methods: Docking was performed on 6 pyrimidine analogs; cetuximab and curcumin were taken as reference drug. The structure of the target protein retrieved from the RCSB Protein databank and the protein-ligand docking was performed using Pyrx AutoDock wizard with MGL tools 1.5.6 by using Lamarckian algorithm.

Results: All the compounds have shown lower binding energy and inhibition constant (Ki) value than reference drug cetuximab and curcumin. Out of the 6 inhibitors analyzed vkh has shown minimum binding energy against the target protein EGFR and CA IX respectively. Smaller Ki value shows stronger interaction. The scoring value of the interaction of vkh is -9.93 Kcal/mol and Ki 53.04µM against the target protein EGFR and CA IX respectively while the reference drug cetuximab has shown binding energy-6.09 Kcal/mol with Ki value 34.44 µM and curcumin in has shown binding energy-6.02 kcal/mol with Ki value 38.60 µM.

Conclusion: It can be concluded that the molecule vkh could have potential to be used as an EGFR inhibitor and CA IX inhibitor.

Keywords: Docking, Pyrimidine, Pyrx, Molecule

INTRODUCTION

Pyrimidines are the most vital heterocyclic aromatic organic compound containing two nitrogen atoms at positions 1 and 3 of the six-membered ring which shows the wide range of biological activities. These are the essential constituent of all cells and thus of all living matter. DNA and RNA is the main component of the chromosome carrying genetic information contain pyrimidine base in cytosine, uracil, and thymine. These occur in nature in two forms glycosylated pyrimidines and unglycosylated pyrimidines. Unglycosylated pyrimidines such as amino acids (ecotine), vitamins (B1), antibiotics (Becimethrin, Bleomycin) and quinazoline alkaloids while glycosylated as DNA and RNA [1]. Pyrimidine derivatives have been reported as antitubercular [2], antifungal [7], and anti-tumor [8] agents. Anti-breast cancer activity of some novel pyrimidine derivatives has been also reported [9]. Various activities displayed by these nitrogen-containing heterocyclic rings such as pyrimidine made it promising structural moiety for future drug design.

Cancer is the most dreadful disease affecting the human population these days. It is characterized by abnormal growth of the cells. Different mechanisms account for the cytotoxic effect of pyrimidines, where they had been reported to act as glycogen synthase kinase (GSK) inhibitors [10], cyclin-dependent kinase (CDK) inhibitors [11], dual src/Ab1 kinase inhibitors, epidermal growth factor receptor (EGFR) inhibitors [12] and carbonic anhydrase (CA) [13]. In the present study, docking is performed against two macromolecules i.e. EGFR and CA IX. FDA approved EGFR as the successful target in colorectal cancer and various other types of cancers (as shown in table 1). The CA IX is reported to be associated with tumorigenesis being highly overexpressed in hypoxic tumors and restrictedly expressed in normal tissues. CA IX monoclonal antibody is already in Phase III clinical trials (as shown in table 1) and several small molecule inhibitors are in advanced preclinical evaluation with its overexpression in many cancer tissues and not in their normal counterparts [14]. This study includes the molecular docking study of the pyrimidine derivatives with EGFR and CA IX. All of the compounds were auto docked for the inhibition of the EGFR and CA IX. Cetuximab was taken as the reference drug in case of EGFR and curcumin for CA IX. This paper aims to elucidate the anti-cancer molecular mechanism of pyrimidines and provide the reference for its clinical application and further drug development.

Table 1: TTD ID of targeted receptor

| TTD ID     | Target name                     | Target type                  | Disease          | Drugs        |
|------------|---------------------------------|------------------------------|------------------|--------------|
| TTDS00355  | Epidermal growth factor receptor| Successful target            | Colorectal cancer, Cancers | Cetuximab    |
| TTDR00211  | Carbonic anhydrase IX           | Clinical trial target        | Cancer, Discovery agent | Curcumin     |

MATERIALS AND METHODS

Procurement of sample

The samples were procured from Saurashtra University and code name was given to each of the molecule vkb, vkc, vkd, vke, vkg, and vkh respectively. The “IUPAC” name of samples was obtained by using ChemDraw online while the molecular properties were generated by using ACD chemsketch (freeware) 2015 2.5 (as shown in table 2 and 3).

Preparation of protein

Three-dimensional structure of the protein should be retrieved from the RCSB Protein data bank (PDB); afterward the retrieved structure
should be pre-processed for removal of heteroatoms then energy minimization was performed by using Argus lab and visualization was done by using UCSF Chimera 1.11.2. Ramachandran plot was generated by using Discovery studio 3.5.

Table 2: Chemical structure, code name and IUPAC name of compounds

| Structure | Code | IUPAC Name |
|-----------|------|------------|
| ![Structure](image1) | vkb | "4-(3,4-dimethoxyphenyl)-6-methyl-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide", |
| ![Structure](image2) | vkc | "4-(3-bromophenyl)-6-methyl-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide" |
| ![Structure](image3) | vkd | "6-methyl-4-(3-nitrophenyl)-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide" |
| ![Structure](image4) | vke | "4-(1H-indol-3-yl)-6-methyl-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide", |
| ![Structure](image5) | vkg | "4-(4-hydroxyphenyl)-6-methyl-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide" |
| ![Structure](image6) | vkh | "4-(anthracen-9-yl)-6-methyl-N-{4,5,6,7-tetrahydro-3al5,7al5-benzo[d]thiazol-2-yl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide" |

Preparation of ligand

Ligands can be retrieved from several databases such as ZINC, PubChem or can be sketched by applying the Chemsketch tool. Ligand 2D structures were drawn using ACDchemsketch (freeware) 2015 2.5. Chem 3D viewer was used to convert the 2D structure into 3D. The drug molecules of cetuximab and curcumin were collected in 3D SDF format from the PubChem database. The compounds were added hydrogens and energy minimized with UFF force field using conjugate gradient algorithm by open babel in pyrx. All structures were saved as the pdb file format for input to pyrx 0.8. All the ligand structures were then saved in Pdbqt file format, for input into AutoDock version. Later, all lead molecules were converted into Auto Dock Pdbqt format.

While picking out the ligand, the LIPINSKY'S RULE OF 5 should be utilized. Lipinski rule of 5 assists in discerning amongst non-drug like and drug-like candidates. It promises the high chance of success or failure due to drug-likeness for molecules abiding by with 2 or more than of the complying rules. For the choice of a ligand allowing to the LIPINSKY'S RULE: (1) Less than five hydrogen bond donors (2) Less than ten hydrogen bond acceptors (3) Molecular mass of less than 500 Da (4) High lipophilicity (not over 5) and (5) Molar refractivity should be between 40-130. The rule is important in the drug discovery process to ensure the selectivity of the compound or determine if a chemical compound has physical or chemical properties that would make it likely orally active.

Docking

Ligand was docked against the protein and the interactions were analyzed by using by pyrx 0.8. For the docking of ligands into protein active site and to estimate the binding affinities of docked compounds, an advanced molecular docking program AutoDock Vina (4) was used in this study. All computational studies were carried out using pyrx AutoDock wizard with MGL tools 1.5.6 installed in a Pentium ®Dual-Core CPU T4200 machine running on a 2.0 GHz Intel core processor with 2GB RAM by using the lamarckian algorithm. The scoring function gives the score on the basis of best-docked ligand complex is picked out.

RESULTS

EGFR and CA IX is a clinically validated target for the treatment of various types of cancer and tumors has received considerable interest from the scientist in the design and development of newer anticancer drugs. The EGFR family plays an essential role in normal organ development by mediating morphogenesis and differentiation through effects on cell proliferation, differentiation, apoptosis, invasion, and angiogenesis [15, 16]. Whereas the CA family from a family of enzymes that catalyze the interconversion between carbon dioxide and water and the dissociated ions of carbonic acid (i.e. bicarbonate and protons) and leads to regulation of tumor microenvironment. This interconversion is a reversible reaction and the enzyme catalyzes both reactions, forward and reverse. The active site of most carbonic anhydrases contains a zinc ion; they are therefore classified as metalloenzymes [17]. This study is primarily concerned with the in silico molecular docking of six pyrimidine derivatives with EGFR and CA IX proposing their role as an inhibitor in cancer studies. These compounds are tested in silico for drug-likeness and anticancer activity by docking with the protein via pyrx docking software.
The Protein-Ligand interaction plays a significant role in structural based designing. All the compounds have shown hydrogen bond donors and hydrogen bond acceptors, molecular mass ≥500 Da, highly lipophilic in nature and molar refractivity between 40-130 (except for vkh which has shown molar refractivity of 138.71) as shown in Table 4. The rule describes molecular properties imperative for drug’s pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion.

Docking studies of pyrimidine derivatives with both the receptors revealed that all the compounds have lower binding energy and inhibition constant (K_i) value than reference drugs. 3-D structure of EGFR, Ramachandran plot of EGFR and various docked conformations

### Table 3: Molecular properties of the compounds

| Compound | vkb | vkc | vkd | vke | vkh |
|----------|-----|-----|-----|-----|-----|
| Molecular formula | C_3H_3N_4 | C_3H_3Br | C_3H_3N_5 | C_3H_3N_4 | C_3H_3N_4 |
| Composition | C(56.73%) | C(49.24%) | C(55.13%) | C(59.55%) | C(56.98%) |
| Molar mass (m+H) | 119.95 ± 114.94 ± 113.26 ± 118.70 ± 108.75 ± 142.34 ± |
| Molar refractivity | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 |
| Polarisability | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 |
| Parachor | 927.9 ± m^2 | 861.7 ± m^2 | 867.7 ± m^2 | 876.9 ± m^2 | 825.9 ± m^2 |
| Molecular properties | Vkh | Vkg | Vkf | Vkd | Vkc |
|-------------------|-----|-----|-----|-----|-----|
| Vkh | 119.95 ± 114.94 ± 113.26 ± 118.70 ± 108.75 ± 142.34 ± |
| Vkg | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 |
| Vkf | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 |
| Vkd | 927.9 ± m^2 | 861.7 ± m^2 | 867.7 ± m^2 | 876.9 ± m^2 | 825.9 ± m^2 |
| Vkc | 119.95 ± 114.94 ± 113.26 ± 118.70 ± 108.75 ± 142.34 ± |
| Vkb | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 | 0.4 cm^3 |
| Vkc | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 | 3.0 cm^6 |
| Vkd | 927.9 ± m^2 | 861.7 ± m^2 | 867.7 ± m^2 | 876.9 ± m^2 | 825.9 ± m^2 |

### Table 4: Results of the lipinski rule calculator

| Compound | Mass (Da) | H bond donor | H bond acceptors | Log P | Molar refactivity |
|----------|-----------|--------------|------------------|-------|------------------|
| Vkb      | 444       | 1            | 6                | 4.7463 | 124,68478 |
| Vkc      | 420       | 1            | 4                | 5.548411 | 117,3252 |
| Vkd      | 421       | 1            | 6                | 4.56402 | 114,10259 |
| Vkf      | 420       | 3            | 1                | 0.854811 | 115,55609 |
| Vkg      | 394       | 2            | 5                | 2.0183  | 110,24445 |
| Vkh      | 482       | 2            | 4                | 4.73961 | 138,71896 |

### Table 5: Output of molecular docking score of ligand-EGFR with respect to minimum binding energy and inhibition constant

| S. No. | Name | Run | Minimum binding energy (Kcal/mol) | Inhibition constant |
|--------|------|-----|----------------------------------|---------------------|
| 1      | vkb  | 6   | -6.63                            | 13.71 µM            |
| 2      | vkc  | 9   | -8.03                            | 1.29 µM             |
| 3      | vkd  | 5   | -7.77                            | 2.03 µM             |
| 4      | vkf  | 8   | -8.7                             | 4.5955 mM           |
| 5      | vkg  | 5   | -7.7                             | 2.26 µM             |
| 6      | vkh  | 5   | -10.75                           | 13.17 mM            |
| 7      | Cetuximab | 4 | -6.09                           | 34.44 µM            |
and active site interactions with EGFR have been shown in fig. 1 and 3-D structure of CA IX, Ramachandran plot of CA IX and various docked conformations and active site interactions with CA IX have been shown in fig. 2. Further details of important interactions in terms of energies between ligands and the proteins with EGFR and CA IX receptor with predicted Ki values are shown in the table is given in table 5 and 6.

Table 6: Output of molecular docking score of ligand-CA IX with respect to minimum binding energy and inhibition constant

| S. no | Name   | Run | Minimum binding energy (Kcal/mol) | Inhibition constant |
|-------|--------|-----|-----------------------------------|--------------------|
| 1     | vkb    | 7   | -7.83                             | 1.82 µM            |
| 2     | vkc    | 9   | -9.47                             | 114.57 nM          |
| 3     | vkd    | 9   | -7.17                             | 5.55 µM            |
| 4     | vkf    | 5   | -8.4                              | 690.47 nM          |
| 5     | vkg    | 5   | -9.39                             | 131.71 nM          |
| 6     | vkh    | 9   | -9.93                             | 53.04 nM           |
| 7     | Curcumin | 4   | -6.02                             | 38.60 µM           |

Fig. 1: (a) 3D structure of EGFR; (b) Ramachandran plot of EGFR; (c) Docking of EGFR Protein receptor with Cetuximab; (d) Docking of EGFR Protein receptor with vkb; (e) Docking of EGFR Protein receptor with vkc; (f) Docking of EGFR Protein receptor with vkd; (g) Docking of EGFR Protein receptor with vkf; (h) Docking of EGFR Protein receptor with vkg; (i) Docking of EGFR Protein receptor with vkh
DISCUSSION

The EGF is the prototype of a large family of peptide ligands that bind to cell membrane receptors and activate a myriad of intracellular signaling pathways to control tumor cell growth, proliferation, survival, metastasis, and angiogenesis [18]. Whereas CA IX enzymes catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissue/organs, biosynthetic reactions, bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes [19]. In this work, totally 6 compounds which are pyrimidine derivatives were examined for ligand-based docking. The ligands are screened for their ability to dock within the active site of the inhibitor protein. All the compounds have shown 5 hydrogen bond donors, ≥10 hydrogen bond acceptors, molecular mass ≥500 Da, log P value ≥5 and molar refractivity between 40-130 (except for vkh has shown molar refractivity of 138.71) which means compounds have good oral bioavailability. The Ramachandran plot generated displays the dihedral angles φ and ψ for each residue in the protein that is displayed in the workspace. The plot area displays a plot of protein dihedrals for all residues in the protein. The area "green region" corresponds to "core" region representing the most favorable combinations. Ideally, 90% of the residues should be in this "core" region. After analyzing the different docking interactions of ligands, out of the 6 inhibitors analyzed vkh has shown the binding energy of -10.74 and -9.93 Kcal/mol and Ki 13.17 nM and 53.04 nM against the target protein EGFR and CA IX respectively. The best drug was selected, depending upon the binding energy and Ki. Smaller Ki value shows the stronger interaction [20, 21]. Out of the 6 derivatives compound, vkh shows the highest affinity towards the protein compared with the standard drug cetuximab and curcumin. Thus compound vkh may act as a better and efficient anticancer drug.
This work is carried out by Shikha Sharma under the valuable guidance of Dr. V. J. Shukla sir. He guided me and helped me throughout my studies.

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AUTHORS CONTRIBUTIONS

This work is carried out by Shikha Sharma under the valuable guidance of Dr. V. J. Shukla sir. He guided me and helped me throughout the study.

CONFLICT OF INTERESTS

All the authors declared that there is no conflict of interest

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Fig. 2: (a) 3D structure of CA IX; (b) Ramachandran plot of CA IX; (c) Docking of CA IX Protein receptor with Cetuximab; (d) Docking of CA IX Protein receptor with vkb; (e) Docking of CA IX Protein receptor with vkc; (f) Docking of CA IX Protein receptor with vkd; (g) Docking of CA IX Protein receptor with vke; (h) Docking of CA IX Protein receptor with vkf; (i) Docking of CA IX Protein receptor with vkg; (j) Docking of CA IX Protein receptor with vkh