



MOLECULAR DOCKING STUDIES OF BISAZO DERIVATIVES OF 4-ACETYL RESORCINOL AGAINST LUNG CANCER PROTEIN EGFR

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Abstract

Lung cancer is one of the malignant diseases mostly caused by tobacco users. EGFR, proto oncogene plays an important role in many biological processes apoptosis, transcriptional regulation, cell differentiation, cell spreading and mobility. The mutation occurs in EGFR, the normal function of EGFR is over expressed and leads to develop lung cancer. In this present study, we have developed lung cancer drug from bisazo derivatives through molecular docking studies against EGFR using Glide. From the docking results the compound **I**, **IV** and **V** have a better life score when compared to other compounds. The ADME properties were carried out for five bis azo molecules. These compounds are under acceptable range with predicted ADME properties. From the *In-silico* docking studies, we conclude the compounds **I**, **IV** and **V** are good drug for lung cancer and inhibit over expression of EGFR in human.

Keywords: bis azo, Lung cancer, EGFR, Docking.

Introduction

Lung cancer is one of the most common malignant disease. In 2016, 224,390 (106,470 Female, 117,920 Male) new cases are diagnosed with lung cancer and 158, 080 (72,160 Female, 85, 960 Male) cases die from it [1]. The Epidermal Growth Factor Receptor (erbB1) one of the receptor tyrosine kinase family occupied in various essential cellular functions like cell proliferation, differentiation, metastasis and survival [2]. EGFR is expressed in all types of cell, activation in non-cancer cell types that are involved in cell growth and proliferation might lead to tumor progression [3]. The tyrosine kinase family consisting of four receptors structurally possesses an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain with tyrosine kinase activity. The binding of epidermal growth factor (EGF) leads to receptor-linked tyrosine kinase activation causing various effects like cell differentiation, apoptosis inhibition, maturation, metastatic, migration and angiogenesis [4, 5]. Mutation occurs in this EGFR that the normal function is over expressed, then the over expression of EGFR can lead to develop prostate, breast, lung [6]. In the present study we investigate antitumor activity of the compounds from bis azo derivatives for molecular docking studies against human lung cancer protein EGFR

and ADME properties were carried out for bis azo derivatives by using Schrodinger suite.

Materials and Methods

Synthesis of 2, 6-bis-(substituted phenyl) azo-4-acetylresorcinol

Substituted aniline (0.002mol) was dissolved in 3ml HCl and it was added 3ml of H₂O. The solution was cooled to 0-5°C in an ice bath and maintained this temperature. Sodium nitrite (0.004mol) in water (3ml) was then added drop wise. Stirring was continued for 20 minutes to produce diazonium salt at the same temperature. To this mixture, 2-acetylresorcinol (0.001mol, 0.152g) dissolved in 10% NaOH was added drop wise with stirring at 0-5°C. The mixture was stirred for 20 minutes. The precipitate was collected by filtration at vacuum and recrystallised from suitable solvent.

Detail of the target Protein Structure EGFR

The crystal structure of EGFR (PDB ID: 1M17), was retrieved from the Protein Data Bank (www.rcsb.org/pdb). The all unwanted water molecules were removed from the target protein structure and hydrogen atoms were added, Energy Minimizations were performed until the average root mean square deviations of non-hydrogen atoms reached 0.3Å [7].

Preparation of Ligand Structure

The five bis azo derivatives were drawn using chem draw in mol file format then the compounds and all these molecules were prepared for molecular docking studies using ligprep version 2.3 [8]. The tautomerism for each of these ligands were also generated and optimized. Partial atomic charges were computed using the OPLS-2005 force field.

Active Site Prediction Using Q-Site Finder

The target protein EGFR (PDB ID: 1M17) was retrieved from protein data bank further taken to Q-Site Finder for binding site prediction which revealed about ten different binding site. However the best binding residues given below PRO 770, LEU 694, LEU 768, LEU 820, ALA 719, MET 769, LEU 764 and VAL 702.

Docking Studies

The X-ray crystal structure of human protein EGFR (PDB ID: 1M17), was retrieved from the Protein Data Bank for molecular docking studies. So the active site has been predicted using Q-Site Finder. The predicted active site residues mainly involved in the receptor-ligand interaction. All the bis azo compounds were separately docked with binding site of the receptor by using glide XP mode from Schrodinger software [9] and also ADME properties were predicted using Qikprop[10, 11].

Results and discussion

Binding mode analysis of drug molecule I with EGFR

The binding conformation of **drug** molecule compound **I** within the active site of the EGFR has been analyzed. The glide score and glide energy values for drug molecule **I** were -4.981kcal/mol and -51.060kcal/mol respectively. Upon the examination of docking features between drug molecule **I** and EGFR, it was found only one hydrogen bond interaction. The side chain hydrogen atom of the hydrophobic residue of MET 769 was strappingly interacted with oxygen atom of the drug compound **I** with bond distance 3.75Å. Furthermore, the following residues ALA 719, LEU 694, MET 742 and PRO 770 are mainly involved in hydrophobic interactions with drug molecule **I**.

Binding mode analysis of drug molecule II with EGFR

The binding conformation of drug molecule **II** within the active site of the EGFR has been analyzed. The glide score and glide energy values for drug molecule **II** were -4.007 kcal/mol and -45.761kcal/mol, respectively. Upon the examination of docking features between drug molecule **II** and EGFR, it was found only three hydrogen bond interactions. The backbone oxygen atoms of the ASP 831

were strappingly interacted with hydrogen and oxygen atoms of C₃-OH of the drug molecule **II** respectively with bond distances 1.76Å and 2.21Å. The side chain hydrogen atom of the LYS 721 was strongly interacted with oxygen atom of C₃-OH of the drug molecule **II** with bond distance 2.10Å. Furthermore, that the following residues PRO770, LEU 768, ALA 719 and MET 769 are mainly involved in hydrophobic interactions with drug molecule **II**.

Binding mode analysis of drug molecule III with EGFR

The binding conformation of drug molecule **III** within the active site of the EGFR has been analyzed. The glide score and glide energy values for drug molecule **III** were -5.271 kcal/mol and -48.085kcal/mol, respectively. Upon the examination of docking features between drug molecule **III** and EGFR, it was found No hydrogen bond interactions. Furthermore that the following residues are mainly involved in hydrophobic interactions PRO 770, ALA 719, MET 769, LEU 764 and VAL 702.

Binding mode analysis of drug molecule IV with EGFR

The binding conformation of drug molecule **IV** within the active site of the EGFR has been analyzed. The glide score and glide energy

values for drug molecule **IV** were -3.954 kcal/mol and -55.032kcal/mol, respectively. Upon the examination of docking features between drug molecule **IV** and EGFR, it was found only two hydrogen bond interactions with drug molecule **IV**. The back bone hydrogen and oxygen atoms of met 769 interacted with C₁-OH oxygen and hydrogen respectively with bond length 2.33 and 1.77Å. Furthermore that the following residues are mainly involved in hydrophobic interactions PRO 770, LEU 694, LEU 768, LEU 820 and VAL 702.

Binding mode analysis of drug molecule V with EGFR

The binding conformation of drug molecule **V** within the active site of the EGFR has been analyzed. The glide score and glide energy values for drug molecule **V** were -3.620Kcal/mol and -61.705kcal/mol, respectively. Upon the examination of docking features between drug molecule **V** and EGFR, it was found only one hydrogen bond interactions with drug molecule **V**. The backbone hydrogen atom of the hydrophobic residue of CYS 773 was nicely interacted with oxygen atom of the drug molecule **V** with bond length 2.08Å. Furthermore that the following residues are mainly involved in hydrophobic interactions PRO 770, LEU 694, LEU 768 and MET 769.

ADME properties prediction of the ligand

The ADME properties of the ligands are predicted using the Qikprop. The predicted ADME properties such as Molecular weight, Hydrogen bond donor, Hydrogen bond acceptor, LogP (octanol/water), gut-blood barrier (QPPcaco2), LogIC50 value for blockage of K⁺ channels (QPlogHERG) permeability through MDCK cells (QPlogMDCK), determine which compound having certain biological activity. The chemical compounds are under acceptable range with predicted ADME (or) Pharmacokinetics properties were reported in Table 2. The first five properties are based on Lipinski rule of five, molecular weight (mol-MV) less than 500, partition coefficient between octanol and water (logPo/W) between 1.382 and 2.410, number of H bond donors between 0 and 1, number of H bond acceptors between 5.5 and 7.5 and aqueous solubility (logS) between -3.267 and -3.838, Brain/blood partition coefficient (logBB) between -1.377 and 2.701 indicated about the ability of drug pass through the blood-brain barrier which is mandatory for inhibition of EGFR kinase. All the synthesized compounds (I-V) obeyed Lipinski's rule of five and showed ADME properties in acceptable range.

Conclusion

EGFR is one of the important drug target for lung cancer. In this study the five bis azo

derivatives of 4-acetylresorcinol were docked into the active site of the EGFR. Computational tools such as molecular docking and insilico pharmacokinetics prediction are employed to know the significance of synthesized molecules. From the docking results that the compound **I**, **IV** and **V** having better glide score when compared to other compounds, predicted ADME properties of the compounds are under acceptable range, so compound **I**, **IV** and **V** will be a promising candidate and can further be validated in wet lab studies for its proper function.

Acknowledgement

The authors are thankful to the Principal and management committee, Jamal Mohamed College Trichy-20 for providing necessary facilities.

References

1. Cancer Facts & Figures. American Cancer Society 2016.
2. BO Ai-hua, HOU Jin-chao, LAN Yong-hao, TIAN Ya-ting, ZHANG Jun-yan (2007) Over-expression of EGFR in Breast Cancer. Chin J Cancer Res 20 (1): 69-72.
3. Y. Yarden (2001) The EGFR family and its ligands in human cancer: signaling mechanism and therapeutic Opportunities. European journal of Cancer 37: S3-S8.

4. Yarrom N, Jonker DJ (2011) The role of the epidermal growth factor receptor in the mechanism and treatment of Colorectal cancer. *Discov medicine* 57: 95-105.
5. Yiqing Tang, Li Zhu, Yafen Li, Jun Ji, Jianfang Li, Fei Yung, Dengbin Wang, Weiguo Chen, Ou Huang, Xiaosong Chen, Jiayi Wu, Kunwei Shen, Wings TY Loo, Louis WC Chow (2012) Overexpression of epidermal growth factor receptor (EGFR) predicts better response to neo-adjuvant chemotherapy in patients with triple-negative breast cancer. *Journal of Translational Medicine* 10: S1-S4.
6. B. Rude Voldborg, L. Damstrub, M. Spang-Thomsen & H. Skovgaard Poulsen (1997) Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. *Annals oncology* 8: 1197-1206.
7. protein preparation Wizard Maestro (2009) new York: Schrodinger LLC
8. Ligprep, Version 2.3, Schrodinger, LLC, New York, NY.
9. Glide, version 5.3, Schrodinger, LLC, New York, NY.
10. Qikprop, version 3.2, Schrodinger, LLC, New York, NY.
11. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development. *Advanced Drug Delivery Reviews* 23: 3-25.

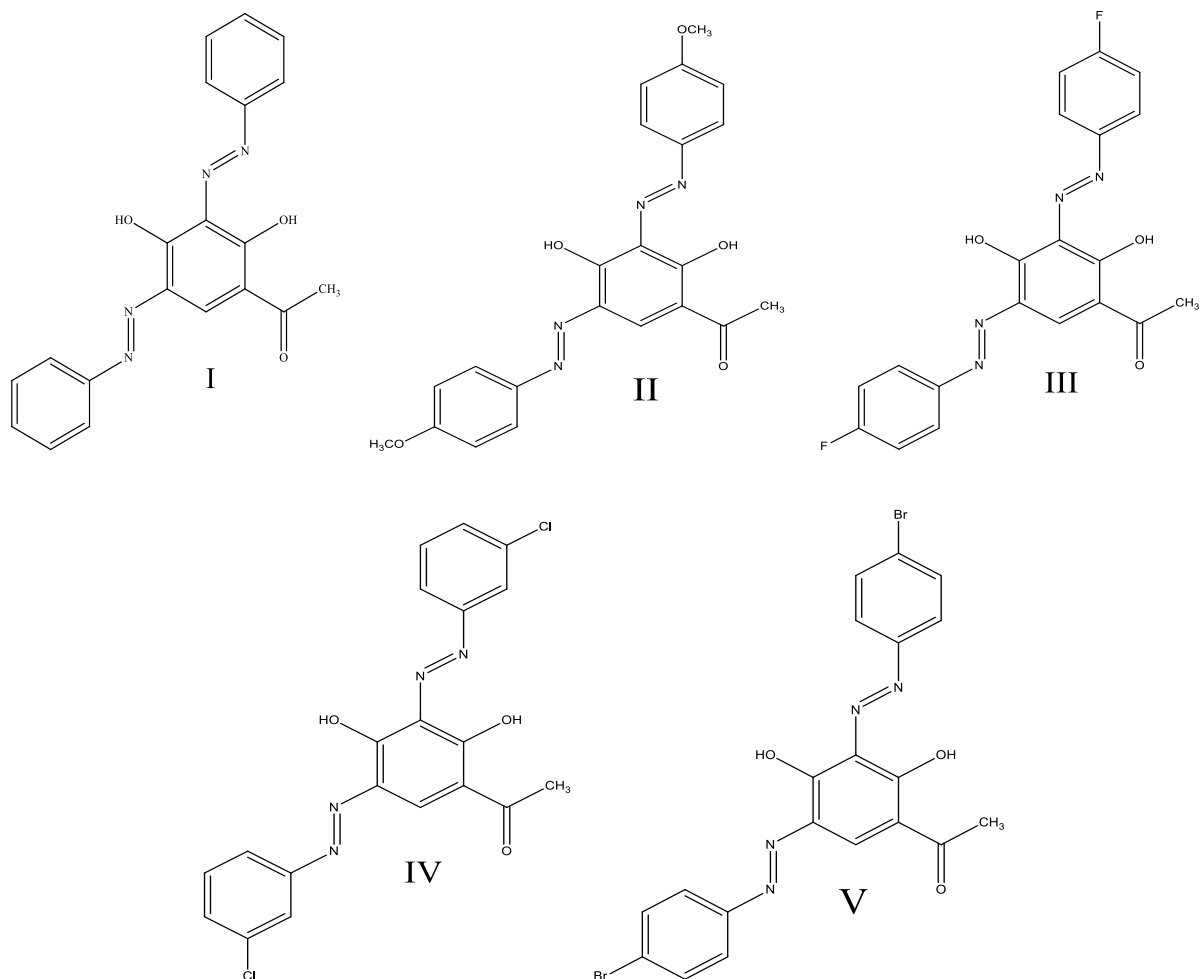


Figure 1 : Structure of the compounds I - V

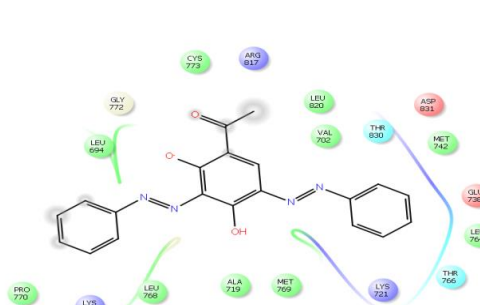
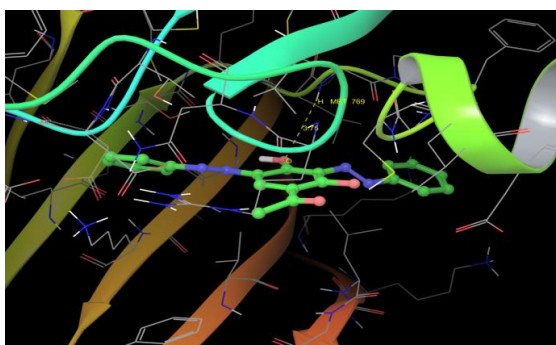


Figure 2: 3D and 2D docked structures of target protein EGFR with drug molecule - I

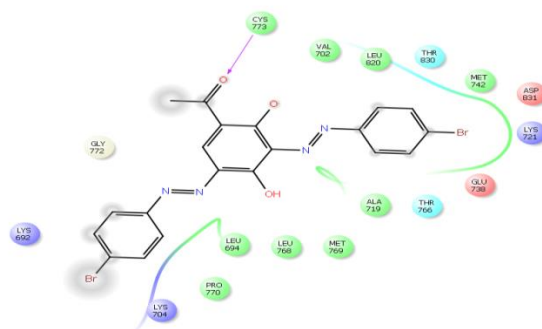
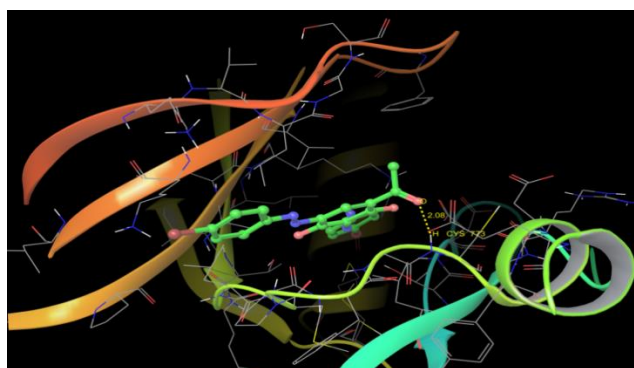


Figure6: 3D and 2D docked structures of target protein EGFR with drug molecule-V

Compound	Glide Score	Glide Energy	NO. of H bond interaction	Interaction Residues	Distance
I	-4.981	-51.060	1	MET 778	3.75
II	-4.00	-45.761	3	ASP 831 (2) LYS 721	2.21,1.76 2.10
III	-5.271	-48.085	-	-	-
IV	-3.954	-55.032	2	MET (2)	1.77,2.23
V	-3.620	-61.705	1	CYS 773	2.08

Table1: Docking result of EGFR with bis azo compound of 4-acetyl resorcinol (I-V).

Compound	Lipinski's rule of five (RO5)				QPLoS	Log BB
	Number of H Bond donors	Number of H bond acceptors	MW	Log P for octanol/Water		
I	0.00	5.500	360	2.070	-5.533	-1.473
II	1.00	8.00	420	3.613	-6.960	-1.28
III	0.00	5.500	396	2.310	-5.414	-1.374
IV	1.00	6.500	428	4.433	-7.003	-1.295
V	0.00	5.00	515	3.495	-5.702	-1.325

TABLE2 : ADME results based on rule five of bis azo compounds of 4-acetyl resorcinol (I-V)