Research Article

In silico docking study of azo compounds of 4,6-dipropanoylresorcinol as EGFR antagonists

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ABSTRACT

A new series of azo compounds of 4,6-dipropanoylresorcinol were synthesized and molecular docking studies were performed on newly synthesized six compounds on the active site of EGFR to study the binding mode of these analogs. ADME properties of all the newly synthesized six compounds were calculated by Qik Prop v3. 7. All the designed compounds were found to exhibit lead like properties from the calculated ADME properties. We worked with successful insilico approaches like molecular docking and pharmacokinetic properties. As a result, we found all the six azo derivatives are exceedingly capable of acting as antagonist for EGFR.

Key words: In Silico, Azo, Dipropanoylresorcinol, EGFR, ADME.

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INTRODUCTION

Cancer is defined as a group of diseases characterized by uncontrolled growth and the spread of abnormal cells which if left untreated may lead to death [1]. Cancer continues to be a major health problem worldwide and more than ten million new cancer cases occur annually, roughly half of which is prevalent in the developed countries, and the disease causes over six million deaths a year [2]. About 13 percent of all the death worldwide is due to cancer, surpassing cardiovascular disease and taking number one place[3,4]. Chemotherapy of cancer is associated with various adverse effects *viz*., bone marrow depression, alopecia, drug induced caner, etc., and is often associated with cytotoxicity, genotoxicity to normal cells together with the development of resistance [5]. Medicinal chemists have great perseverance in research and development (R & D) for the search of newer and safer anticancer agents. The uncontrolled proliferation of tumor cells is a hallmark of cancer. EGFR family of Tyrosine Kinases (TK) play a vital role in cancer proliferation. Dysregulation of tyrosine kinase activity in cancer patients results in enhanced proliferation of cancer cells and it is suggested that any agent which would inhibit the TK activity may have substantial role in the cancer treatment [6].

The mutations of the EGFR kinase domain have been emphasized in majority of cancers including breast, lung, ovarian, and anal cancers, and head/neck and brain tumors. Owing to their dominant role in cancer, therefore, extensive research has been focused on the inhibition of EGFR for anticancer drug design. So EGFR family of TK has been selected and explored the binding mode of the newly synthesized compounds **I-VI** to EGFR tyrosine kinase active site.

Lipinski's rule of five also known as the Pfizer's rule of five or simply the Rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). Many drugs often fail to enter the market as a result of poor pharmacokinetic profiles Thus, it has become imperative nowadays to design lead compounds which can be easily orally absorbed, easily transported to their desired site of action, not easily metabolized into toxic metabolic products before reaching the targeted site

of action and easily eliminated from the body before accumulating in sufficient amounts that may produce adverse side effects. Nearly 40% of drug candidates fail in clinical trials due to poor ADME properties. These late-stage failures contribute significantly to the skyrocketing cost of new drug development. The ability to detect problematic candidates early will dramatically reduce the amount of wasted time and resources, and streamline the overall development process. In order to determine ADME property of the synthesized compounds, Lipinski's rule of five is also applied.

EXPERIMENTAL SECTION

Synthesis of 2-(substituted phenyl)azo-4,6-dipropanoylresorcinol
Substituted aniline (0.001mol) was dissolved in 2ml HCl and to it was added 1ml of H_2O. The solution was cooled to 0-5°C in an ice bath and maintained this temperature. Sodium nitrite (0.002mol) in water (2ml) was then added drop wise. Stirring was continued for 20minutes to produce diazoniun salt at the same temperature. To this mixture, 4,6-dipropanoylresorcinol (0.001mol, 0.222g) dissolved in 10% NaOH was added drop wise with stirring at 0-5°C. The mixture was stirred for 15minutes. The precipitated crude azo product was collected by filtration at vacuum and recrystallized from appropriate solvent [7].
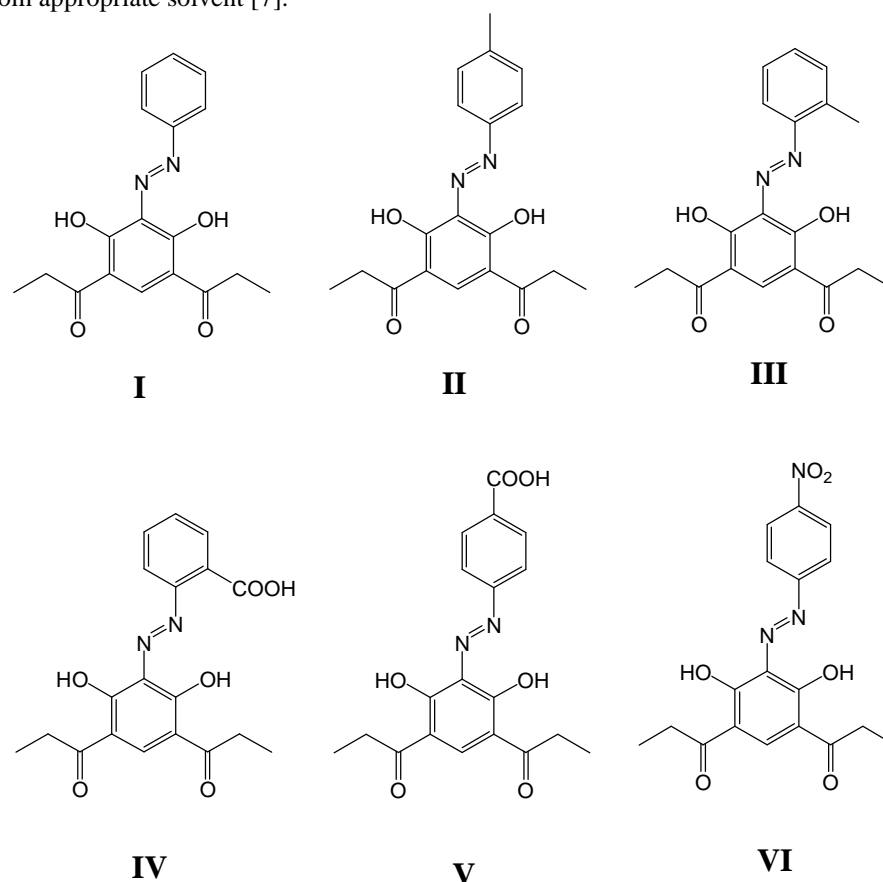

Figure 1: Structures of compounds I-VI

Molecular docking studies
Protein structure preparation
The X-ray crystallographic structure of the Epidermal growth factor receptor (PDB ID: 2ITO) was obtained from Protein Data Bank.[8] As a rule, the protein was prepared using the Protein Preparation Wizard. Preprocessed bond orders were assigned, hydrogens were added, metals were treated, water molecules were deleted and co- crystal ligand was removed from the crystal structure. Protein energy minimization was carried out until the average RMSD 0.30Å [9].

Ligand structure preparation
The six synthesized azo compounds were drawn using Chemdraw [10]. Then these compounds were further imported into ligprep for ligand preparation. LigPrep involves series of steps that can produce a number of structures from each input structure with various ionization states, tautomers, stereo chemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional

groups present. The default options in the LigPrep panel remove unwanted molecules, add hydrogens, and minimize the ligand structure [11].

Docking and scoring function Docking simplifies the amount of time spent for the library screening at the same time, efficient method which operates with algorithm includes several quantum mechanical parameters. In this present study, a top notch docking performer, glide version 5.5 was employed for molecular docking studies. Protein – ligand is a step by step methodology, which starts from protein and ligand molecule preparation, grid generation and actual docking process. Since the crystal structure of target protein has a co-crystal ligand, Grid generation can be restricted to that region alone for further assessment of unknown ligands. Hence, six synthesized ligand molecules were docked into active site of the receptor using glide extra precision method (XP) [12]. Docking score, hydrogen bond interactions, hydrophobic interactions were analyzed by the use of glide XP visualizer.

ADME prediction
The key criteria which determine the successful transmission of just a lead molecule to drug like molecule is when they possess necessary absorption, distribution, metabolism and excretion properties to trigger a biological act. ADME properties were predicted using QikProp v3.7. It predicts both physicochemically significant descriptors and pharmacokinetically relevant properties. All the analogs were neutralized before being used by QikProp [13]. The predicted properties were reported in Table 2.

RESULTS AND DISCUSSION

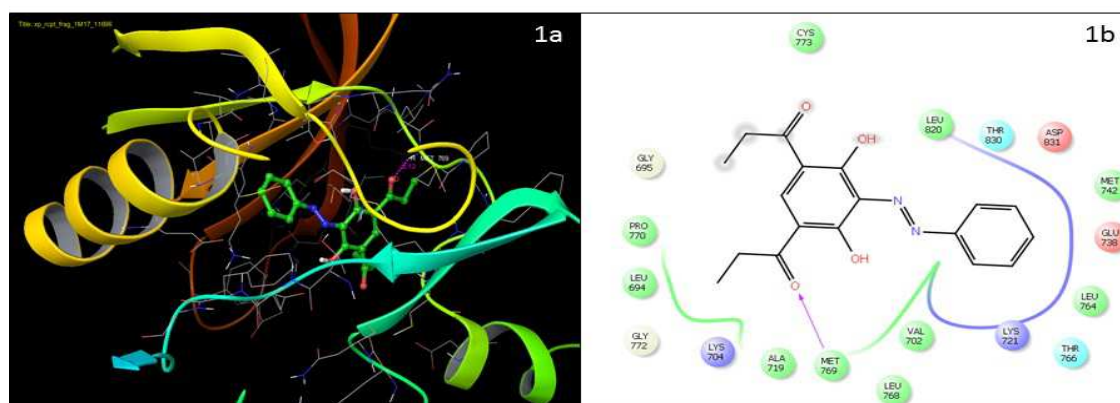**Binding mode analysis of compound I with EGFR**

Figure 2. (a) 3D docked structure of compound I and EGFR, (b) 2D docked structure of compound I and EGFR

The binding conformation of **compound I** within the active site of the EGFR has been clearly shown in 3D docked structure.   Upon the examination of docking features between **compound I** and EGFR it is found that there is only one hydrogen bond interaction. The side chain hydrogen atom of the hydrophobic MET 769 is interacted with oxygen atom of propanoyl group of the **compound I** with bond length 2.12Å. Further, pro 770, leu 694, ala 719, leu 768, val 702, leu 764, met 742, cys 773 and leu 820 residues are mainly involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound **I** shows that the **compound I** is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR.

The glide score and glide energy values for **compound I** were **-7.847**kcal/mol and -60.297kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -3.894 mol dm^{-3}, log BB for brain / blood is -1.406. These two values lie in the recommended range. Hence molecular docking study result shows that the **compound I** has minimum binding energy, good affinity towards the active pocket and blood-brain barrier crossing ability.   Thus, it may be considered as a good inhibitor of EGFR. The 91%   human oral absorption in gastro intestine shows that it is a high qualitative model for oral absorption. Number of hydrogen bond donors in **compound I** is zero,   number of hydrogen bond acceptors is 5.5, molecular mass is 326 and octanol-water partition coefficient log P is 2.916. These data show that it obeys **Lipinski's rule of five** (RO5). So it may have drug-like physicochemical properties with low attrition rate during clinical trials and increased chance of reaching market.
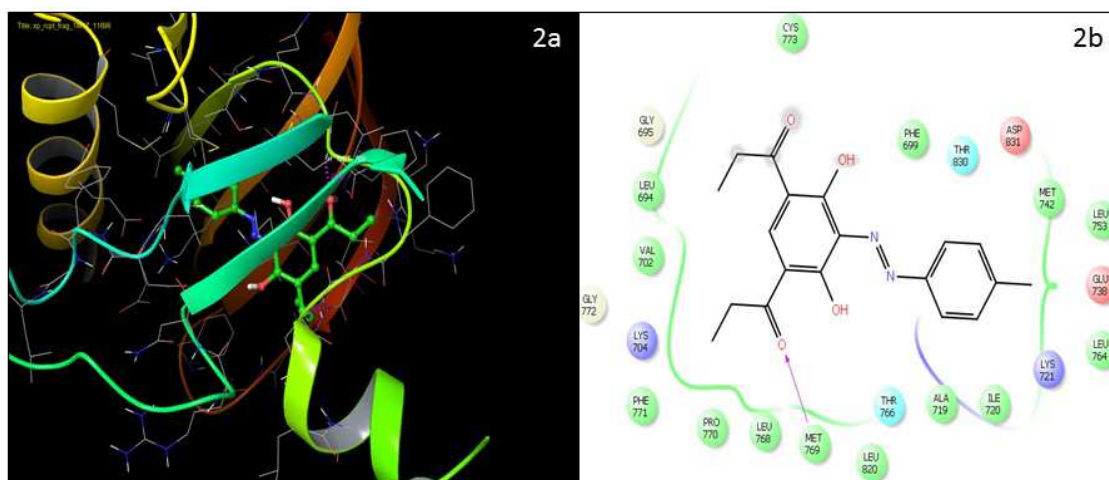
Figure 3. (a) 3D docked structure of compound II and EG FR, (b) 2D docked structure of compound II and EGFR

Upon the examination of docking features between **compound II** and EGFR it is found that there is only one hydrogen bond interaction. The side hydrogen atom of the polar MET 769 is interacted with hydrogen atom of the **compound II** with bond length 2.24Å. Further, leu 694, val 702, phe 771, pro 770, leu 768, leu 820, ala 719, ile 720, leu 764, leu 753, met 742, phe 699 and cys 773 residues are involved in hydrophobic interactions. The glide score and glide energy values were **-**6.848kcal/mol and -55.722kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -4.505 mol dm^{-3}, log BB for brain / blood is -1.448. These two values lie in the recommended range.
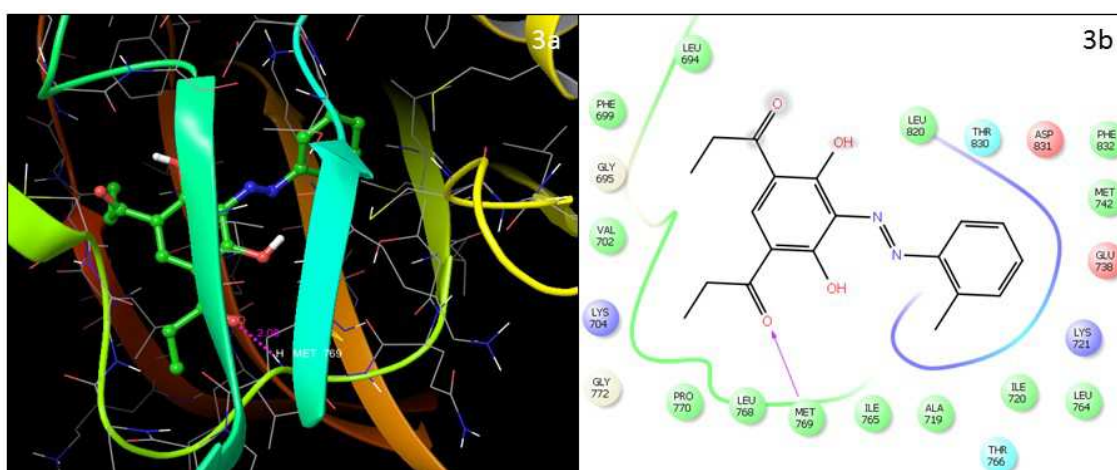

Figure 4. (a) 3D docked structure of compound III and EGFR, (b) 2D docked structure of compound III and EGFR

Upon the examination of docking features between **compound III** and EGFR it is found that there is only one hydrogen bond interaction. The backbone hydrogen atom of the hydrophobic MET 769 is interacted with oxygen atom of propanoyl group of the **compound III** with bond length 2.08Å. Further, leu 694, phe 699, val 702, pro 770, leu 768, ile 765, ala 719, ile 720, leu 764, met 742, phe 832 and leu 820 residues are involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound **III** shows that the **compound III** is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR.   The glide score and glide energy values for **compound III** were **-**7.927kcal/mol and -60.919kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -3.909 mol dm^{-3}, log BB for brain / blood is -1.235. These two values lie in the recommended range.
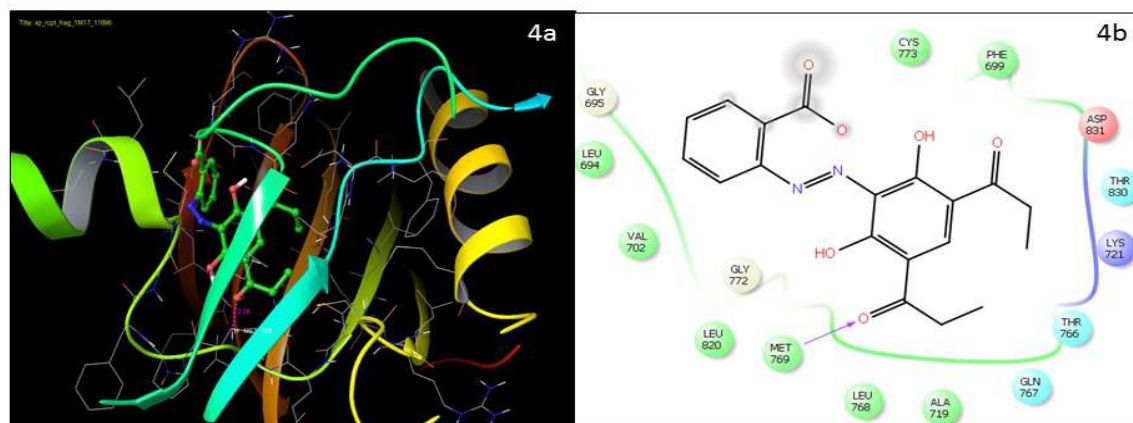
Figure 5. (a) 3D docked structure of compound IV and EGFR, (b) 2D docked structure of compound IV and EGFR

Upon the examination of docking features between **compound IV** and EGFR it is found that there is only one hydrogen bond interaction. The backbone hydrogen atom of the hydrophobic MET 769 is interacted with oxygen atom of propanoyl group of the **compound IV** with bond length 2.08Å. Further, leu 694, val 702, leu 820, leu 768, ala 719, phe 699 and cys 773 residues are mainly involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound **IV** shows that the compound **IV** is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR. The glide score and glide energy values for compound **IV** are **-**6.878kcal/mol and -45.482kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -3.123 mol dm^{-3}, log BB for brain/blood is -2.074. These two values lie in the recommended range.
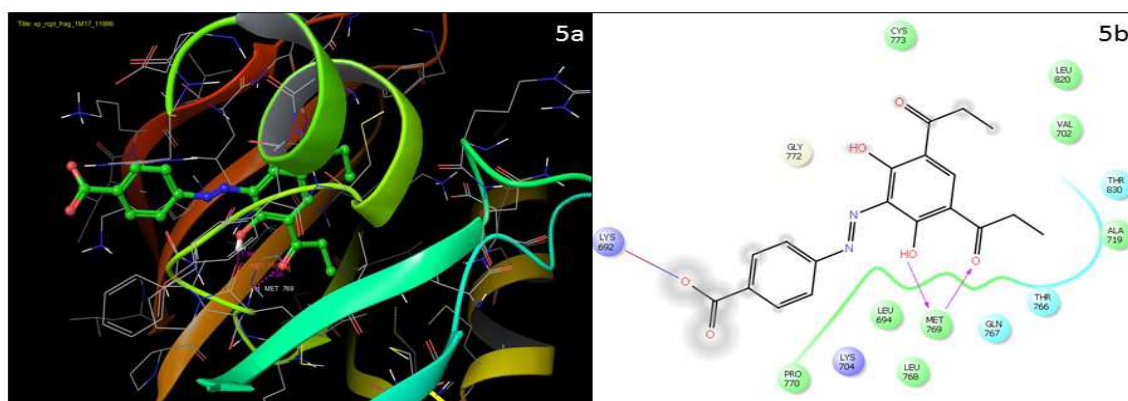

Figure 6. (a) 3D docked structure of compound V and EGFR, (b) 2D docked structure of compound V and EGFR

Upon the examination of docking features between compound **V** and EGFR it is found that there are two hydrogen bond interactions. One is in between the back bone oxygen atom of met 769 and hydrogen of –OH group with bond length 1.84Å and another hydrogen bond is in between the back bone hydrogen atom of the same met 769 and oxygen atom of propanoyl group with bond length 2.00Å. Further, pro 770, leu 694, leu 768, ala 719, val 702, leu 820 and cys 773 residues are involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound **V** shows that the compound **V** is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR.

The glide score and glide energy values for compound **V** were -7.003kcal/mol and -55.272kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -3.909 mol dm^{-3}, log BB for brain / blood is -1.235. These two values lie in the recommended range.
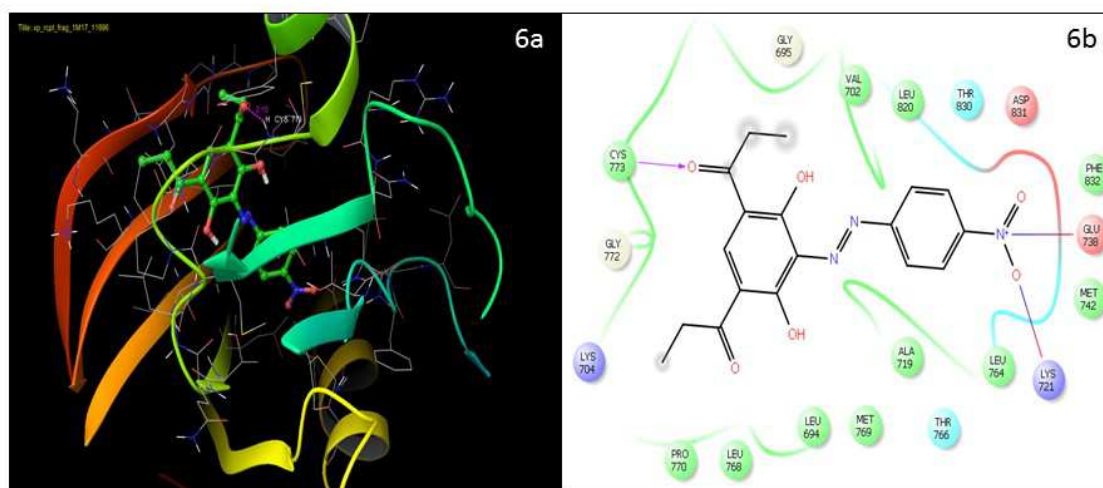
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Figure 7. (a) 3D docked structure of compound VI and EGFR, (b) 2D docked structure of compound VI and EGFR

Upon the examination of docking features between compound **VI** and EGFR it is found that there is only one hydrogen bond interaction. The backbone hydrogen atom of hydrophobic cys 778 is interacted with oxygen atom of propanoyl group of the compound **VI** with bond length 2.10Å. Further, val 702, leu 820, phe 832, met 742, leu 764, ala 719, met 769, leu 694, leu 768 and pro 770 residues are involved in hydrophobic interactions. The 2D and 3D Docked structures of the target protein EGFR between compound **VI** shows that the compound **VI** is well interacted with EGFR and has good affinity towards the active pocket, thus, it may be considered as a good antagonist of EGFR. The glide score and glide energy values for compound **VI** are -5.310kcal/mol and -60.929kcal/mol, respectively. These two low negative values indicate that it is a stable system and thus a likely interaction. The log S for aqueous solubility is -3.901mol dm^{-3}, log BB for brain / blood is -2.640. These two values lie in the recommended range.

Table -1. Docking results of EGFR with azo compounds of 4,6-dipropanoylresorcinol (I-VI)

Compd	Glide Score kcal/mol	Glide Energy kcal/mol	Number of H bond interactions	Interacting Residues	Distance (Å)	log S for aqueous solubility* (mol dm^{-3})	Log BB for brain/ blood†	% Human oral absorption in GI#
I	-7.847	-60.297	1	MET 769	2.12	-3.894	-1.406	91
II	-6.848	-55.722	1	MET 769	2.24	-4.505	-1.448	92
III	-7.927	-60.919	1	MET 769	2.08	-3.909	-1.235	94
IV	-6.878	-45.482	1	MET 769	2.08	-3.123	-2.074	62
V	-7.003	-55.272	2	MET 769 (2)	1.84, 2.00	-4.209	-2.615	59
VI	-5.310	-60.929	1	CYS 778	2.10	-3.901	-2.640	67

: range= -6.5 to 0.5,   † : range = -3 to 1.2 , # : < 25%=poor, > 80% = high

Table -2. ADME results based on RO5 of azo compounds of 4,6-dipropanoylresorcinol (I-VI)

Compd	Lipinski's rule of five (RO5)				Number of violations
	Number of H bond donors	Number of H bond acceptors	MW	log P for octanol/water	
I	0.0	5.5	326	2.916	0
II	0.0	5.5	340	3.242	0
III	0.0	5.5	340	3.178	0
IV	1.0	7.5	370	2.199	0
V	1.0	7.5	370	2.387	0
VI	0.0	6.5	371	2.060	0

ADME and Lipinski's rule of five
We have analyzed a new series of azo compounds of 4,6-dipropanoylresorcinol (**I-VI**) using Qikprop v3. 7 tool of Schrodinger software.

All the compounds docked showed significant values for the properties analyzed (Table 1 and 2) and exhibited drug-like characteristics based on Lipinski's rule of 5. The ADME values of newly synthesized compounds (I-VI) are given in Table 2. The first five properties are based on Lipinski rule of five, molecular weight (mol_MW) less than 500, partition coefficient between octanol and water (logPo/w) between 1.38 and 2.41, number of hydrogen bond donors between 0 and 1, number of hydrogen bond acceptors between 5.5 and 7.5 and aqueous solubility (logS)

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between -3.26 and -3.83, Brain/blood partition coefficient (logBB) between -1.377 and -2.701 indicated about the ability of the drug to pass through the blood–brain barrier which is mandatory for inhibition of EGFR kinase. Percent human oral absorption is between 49 and 85 %. All synthesized compounds (**I-VI**)   obeyed Lipinski's rule of five and showed ADME properties in acceptable range.

CONCLUSION

All the synthesized azo compounds of 4,6-dipropanoylresorcinol (I-VI) have minimum binding energy, good affinity towards the active pocket and blood-brain barrier crossing ability and obey Lipinski's rule of five. In future research work, the ADME/T properties of these compounds can be tested in wet lab and research can be extended for clinical trials to test its effectiveness and for social benefit thus reducing the time and cost in drug discovery process.

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