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MOLECULAR DOCKING BASED DRUG DESIGN AND DISCOVERY: AN INSILICO APPROACH

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ABSTRACT

Computer aided drug design help to minimize the tedious drug discovery process over the traditional methods. Molecular docking based drug design and discovery is an In-Silico approach. Main objective of molecular docking to attain ligand-receptor complex with optimised conformation and with intention of possessing less binding free energy. The currently available drugs have limitations of toxicity, potential drug interaction with other drugs, insufficient pharmacokinetic properties and development of resistance. Thus, development of new drugs with less toxicity is urgently required. The present work is aimed to evaluate antibacterial, antitubercular, anticancer and antifungal activity of Benzimidazole, Benzotriazole;3,5-dimethyl pyrazole and 1,2,3,4-tetrahydro carbazole.

Keywords*: Molecular docking, Benzimidazole, Benzotriazole, pyrazole, 1,2,3,4-tetrahydro carbazole

INTRODUCTION

Discovery and development of a new drug is generally known as a very complex process which takes a lot of time and resources. So now a day's computer aided drug design approaches are used very widely to increase the efficiency of the drug discovery and development course. CADD consist use of any software program based process for establishing a standard to relate activity to structure.

Molecular docking is in-silico method which predicts placement of small molecules or ligands within the active site of their target protein (receptor). It is

mainly used to accurate estimation of most favorable binding modes and bio-affinities of ligands with their receptor **[1]**. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes **[2]**.

Chemsketch were used for drawing ligands. Ligand for docking were prepared using pubchem and online smile translator.Number of violations of Lipinski's rule were determined using Molinspiration server. Protein was prepared from RCSB Protein Data Bank and refined using Accelrys discovery studio viewer. Docking studies were carried out using Autodock4.2.

The goal of the anti-infective therapy is to rid the host of the pathogens, whereas the therapy of other diseases takes aim at direct the host Nitrogen containing heterocycles constitute an extra ordinary class of synthetic organic chemistry, natural products, drugs and food industry **[3]**. In this study we are conducting molecular docking studies of Azoles (Benzimidazole, Benzotriazole, 3,5-dimethyl pyrazole, 1,2,3,4-tetrahydrocarbazole)with targets of different disease *(Tuberculosis, E.coli infection,candida albicans* infection, and cancer). A bacterial infection is a proliferation of a harmful strain of bacteria on or inside the body. Glutamate racemase present in *escherichia coli* has been validated as promising target for antibacterial agent. Azoles can inhibit glutamate racemase which is essential to the bacterial cell wall biosynthesis pathway. Tuberculosis constitutes one of the most dangerous and serious health problems around the world. InhA was identified as an NADH-dependent enoyl-ACP (CoA) reductase specific for long-chain enoyl thioesters and is a member of the Type II fatty acid biosynthesis system, which elongates acyl fatty acid precursors of mycolic acids which are components of the mycobacterial cell wall **[4]**. In this study we carried out insilico molecular docking of azoles with Mycobacterium tuberculosis Enoyl acyl carrier protein reductase for antitubercular activity. Breast cancer is one of the most common types of malignancies in women worldwide. Binding energies of azoles with Maternal Embryonic Leucine Zipper Kinase was determined. Any disease caused by a fungus, that invades the tissue can cause a disease that's confined to the skin, spreads into tissue, bones and organs or affects the whole body. Currently, the main targets of the antifungal agents are the cytochrome P450 sterol 14a-demethylase (CYP51), **[5-8]**. Invasive Candida infections originate most frequently from

endogenous reservoirs in patients with lowered host defense but exogenous infections in hospitalized patients are frequently transmitted via the hands of health care workers **[9]**. The present work is aimed to evaluate antibacterial, antitubercular, anticancer and antifungal activity of azoles (Benzimidazole; Benzotriazole;3,5-dimethyl pyrazole;1,2,3,4-tetrahydro carbazole).

MATERIALS AND METHODS:

Drug discovery and development is an intense, interdisciplinary and endeavour, when a compound is under taken for discovery. It involves synthesis, characterisation, screening and evaluation for therapeutic efficiency. A number of researches have been performed to identify different azole compounds. Research report revealed that benzimidazole, benzotriazole, 3,5-dimethyl pyrazole and 1,2,3,4-tetrahydro carbazole are major among them. The aim of the present study was to evaluate the antifungal, antitubercular, anticancer and antibacterial activity by performing molecular docking studies of azoles. The important azoles and standard drugs are depicted in **Figure 1 and 2**.

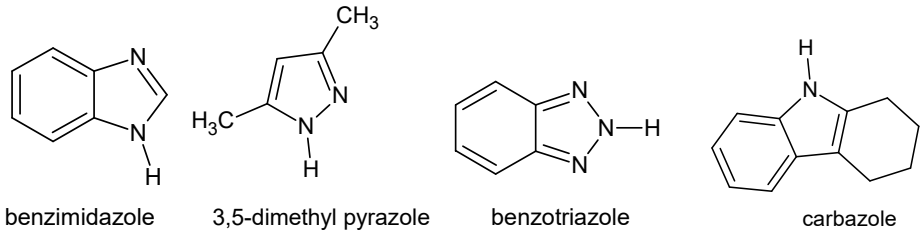


Figure 1: Chemical structure of Azoles

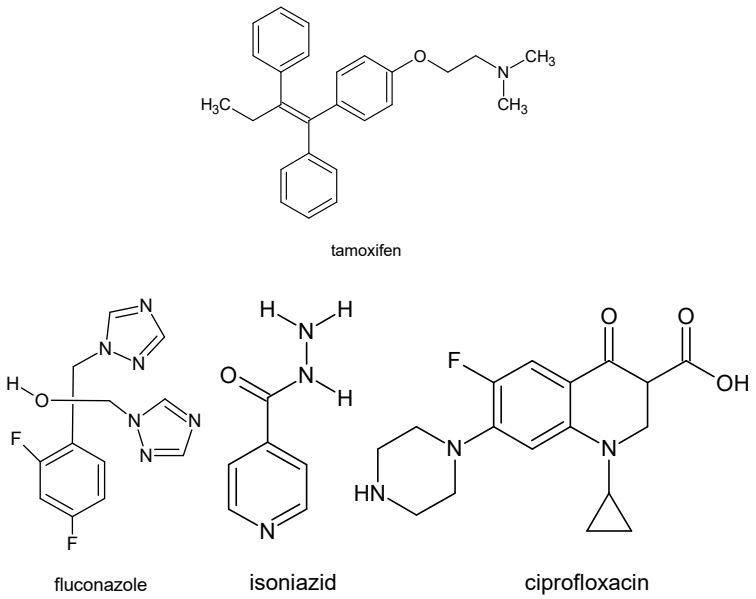


Figure 2: Chemical structure of Fluconazole(std), isoniazid (std), ciprofloxacin(std), tamoxifen(std)

Drug likeness and molinspiration

Drug likeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

The diversity of possible drug targets (of which each requires a different combination of matching molecular characteristics) is so enormous, that it is possible to find a common denominator for all of them and to express molecule drug-likeness by a single "magic number". Simple count criteria (like limits for molecular weight, logP, or number of hydrogen bond donors or acceptors) have also relatively limited applicability and are useful only to discard obvious non-drugs. At Molinspiration we believe that the strategy which leads to success is not a universal drug-likeness score, but focus on particular drug classes and development of specific activity score for each of these classes.

Lipinski Rules

Common routes of drug administration include - oral, nasal, sublingual, inhalation etc. However, when *patient convenience* is considered, *oral drug administration is the best way*. The below rules are principally from highly revealed work of Pfizer's Chris Lipinski.

A molecule has higher chances to be successful as oral drug (like tablets, capsules, emulsions, suspensions etc) if it satisfies Lipinski's 'rule of 5'. Remember that, there are only 4 rules - but go by the name *'rule of 5'* (not 5 rules). These four rules are physicochemical properties of that molecule **(Table 1)**.

Table 1: Drug likeness scores using molinspiration server

S. No.	Ligands	M logp	MW	No. of H acceptors	No. of H donors	No.of violations
01	Benzimidazole	1.43	118.14	2	1	0
02	Benzotriazole	1.29	119.13	3	1	0
03	3,5-dimethyl pyrazole	0.76	96.13	2	1	0
04	1,2,3,4-tetrahydro carbazole	3.33	171.24	1	1	0
05	Fluconazole(std)	-0.12	306.28	7	1	0
06	Ciprofloxacin	0.82	333.36	6	2	0
07	Isoniazid	-0.97	137.14	4	3	0
08	Tamoxifen	6.06	371.52	2	0	1

Drug likeness score

Molecular docking studies

In the field of molecular modelling, docking is a method which predicts the orientation of one molecule to a second when bound to each other to form a stable complex. Preferred orientation helps to predict the strength of association or binding affinity between two molecules. The associations with biological molecules such as proteins, nucleic acids, carbohydrates and lipids play an important role in signal transduction. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterisation of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.

In-silico methods

All the in-silico experiments are carried out at Sanjo College of Pharmaceutical Studies, vellapara, palakkad.

The leads selected were substituted with various substituents and they were optimized for the pharmacokinetic parameters by evaluating. The in-vivo absorption capabilities of the designed molecules were assessed by the means of Lipinski's rule of five using molinspiration server.

Docking studies for the lead molecules:

After the lead has been optimized, the protein was subjected to docking studies using AutoDock 4.2 **[10-12]** for evaluating the binding interactions.

Antibacterial activity

Azoles can inhibit glutamate racemase that present in the Escherichia coli which is an essential to the bacterial cell wall biosynthesis pathway. By considering the above observations, an attempt is made here to molecular docking studies of Azoles with glutamate racemase protein (PDB ID: 2JFN http://www.rcsb.pdb.org) by using in silico studies by molinspiration online tool and evaluated for their antibacterial activity.

Selection of Glutamate racemase from PDB [13-16]

Glutamate racemase of Escherichia coli PDB accession code is 2JFN Resolution is 1.90 Å Chain A, Sequence Length 285 **(Figure 4 and 5)**.

Anticancer activity of azoles

Azoles can inhibit Maternal Embryonic Leucine Zipper Kinase that present in the Homosapiens which is an essential for the. Proliferation of basal like breast cancer cells. By considering the above observations, an attempt is made here to molecular docking studies of Azoles with

Maternal Embryonic Leucine Zipper Kinase by using in silico studies by molinspiration online tool and evaluated for their anti breast cancer activity **(Figure 7, 8)**.

Selection of anticancer protein-5tx3From PDB.

Antifungal activities of azoles

Azoles can inhibit the E292Sglycosynthase variant of exo-1,3-beta-glucanase that present in C.albicans. By considering the above observations, an attempt is made here to molecular docking studies of azoles with *E292S glycosynthase variant of exo-1,3-beta-glucanase* (PDB ID: 4M82) by using in silico studies by autodock viamolinspiration online tool and evaluated for their antifungal activity.

E292S glycosynthase variant of exo-1,3-beta-glucanase from *C.albicans* PDB accession code is 4M82 Resolution is 1.59 A° .Chain A, Sequence length 399 **(Figure 9, 10)**.

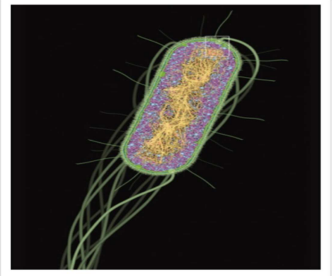


Figure 3: *Escherichia coli*

Figure 4: *Glutamate racemase*

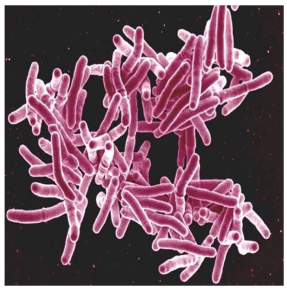
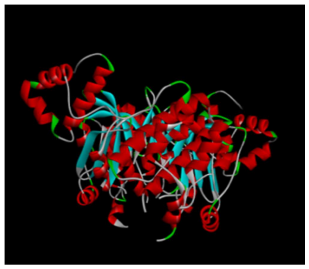
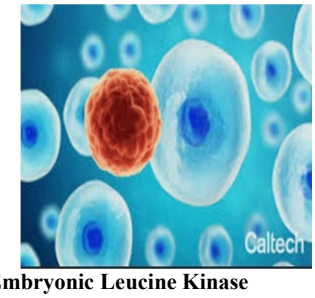


Figure 5: Mycobacterium tuberculosis.

Figure 6: Enoyl acyl carrier protein reductase





Figures 7 & 8: Cancer cell and Maternal Embryonic Leucine Kinase

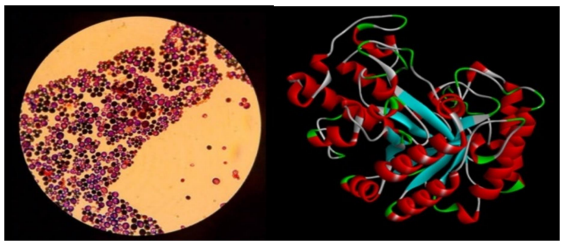


Figure 9 & 10: Candida albican and *E292S glycosynthase variant of exo-1,3-beta- glucanase*

RESULTS

Antibacterial activity

The results of docking of glutamate racemase with Azoles are reported below. The results are mentioned in the table **(Table 2)** followed by the snapshots **(Figure 11-15)**.

Antitubercular activity

The results of docking of InhA with Azoles are reported below. The results are mentioned in the table **(Table 3)** followed by the snapshots **(Figure 16-20)**.

Anticancer activity

The results of docking of Maternal Embryonic Leucine Zipper Kinase with different compounds are reported below. The results are mentioned in the table **(Table 4)** followed by the snapshots **(Figure 21-25)**.

Antifungal activity

The results of docking of E292S glycosynthase variant of exo-1,3-beta-glucanasewith azoles are reported below. The results are mentioned in the table **(Table 5)** followed by the snapshots **(Figure 26-30)**.

Table 2: Binding energies of Azoles with Glutamate racemase

S. No.	Ligand	Binding energy (ΔG =Kcal/mol)	No of hydrogen bonds
01	Benzimidazole	-5.79	1
02	Benzotriazole	-5.84	1
03	3,5-dimethyl pyrazole	-4.89	1
04	1,2,3,4-tetrahydrocarbazole	-7.93	0
05	Ciprofloxacin (std)	-5.67	0

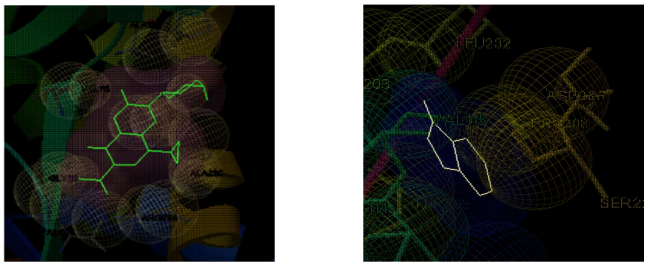


Figure 11 & 12: Snapshots and binding interactions of ciprofloxacin with Glutamate racemase and Benzimidazole respectively (Binding Energy=-5.67,-5.79.Kcal/mol respectively)

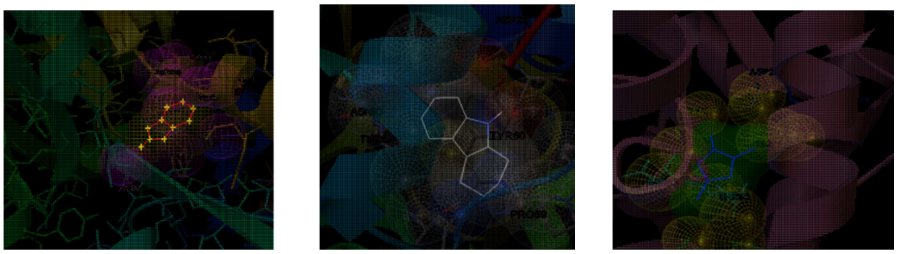


Figure 13, 14 & 15: Snapshots and binding interactions of Benzotriazole, 1,2,3,4-tetrahydrocarbazole and 3,5 -dimethyl pyrazole with Glutamate racemase (Binding Energy= -5.84,-7.93 and 4.89 Kcal/mol respectively)

Table 3: Binding energies of Azoles with InhA

Sl. No	Ligand	Binding energy (ΔG =Kcal/mol)	No of hydrogen bonds
01	Benzimidazole	-4.98	1
02	Benzotriazole	-5.01	1
03	3,5-dimethyl pyrazole	-4.31	1
04	1,2,3,4-tetrahydrocarbazole	-7.33	1
05	Isoniazid (Std)	-5.09	0

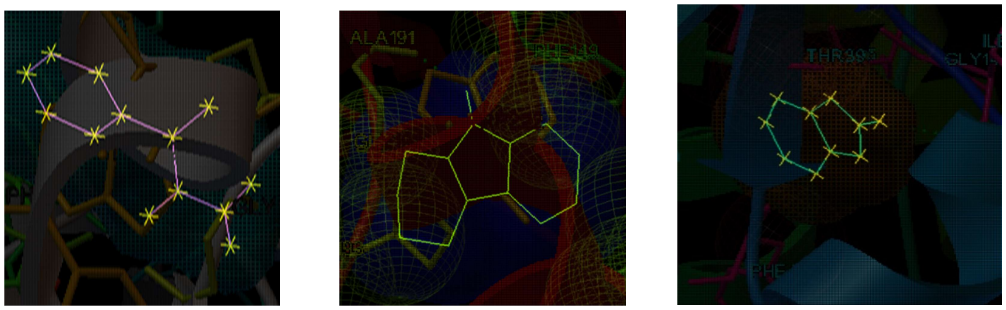


Figure 16, 17: Snapshots and binding interactions of Isoniazid, 1,2,3,4-tetrahydrocarbazole and Benzotriazole with Enoyl acyl carrier protein reductase Energy = 5.09, -7.33&-5.01 kcal/mol)

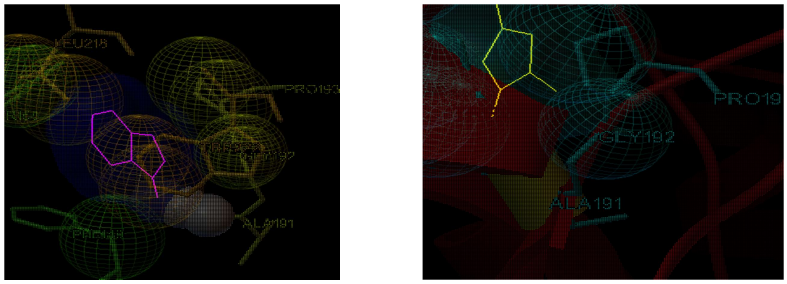


Figure 19 & 20: Snapshots and binding interactions of Benzimidazole and 3,5 -dimethyl pyrazole with Enoyl acyl carrier protein reductase (Binding Energy= -4.98&-4.31Kcal/mol)

Table 4: Binding energies of azoles with Maternal Embryonic Leucine Zipper Kinase

S. No.	Azoles for docking	Binding energy (ΔG =Kcal/mol)	No of hydrogen bonds
01	Benzimidazole	-4.39	1
02	Benzotriazole	-4.53	0
03	3,5-dimethyl pyrazole	-3.92	1
04	1,2,3,4,-tetrahydro carbazole.	-6.57	0
05	Tamoxifen	-8	1

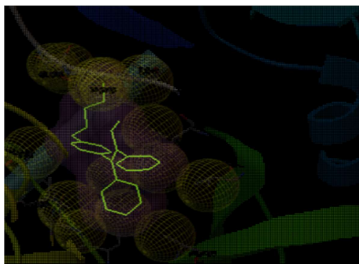
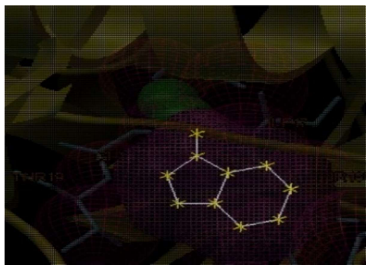



Figure 21 & 22: Snapshots and binding interactions of Tamoxifen and benzimidazole with Maternal Embryonic Leucine Zipper Kinase (Binding Energy= -8.69 & -4.39Kcal/mol)

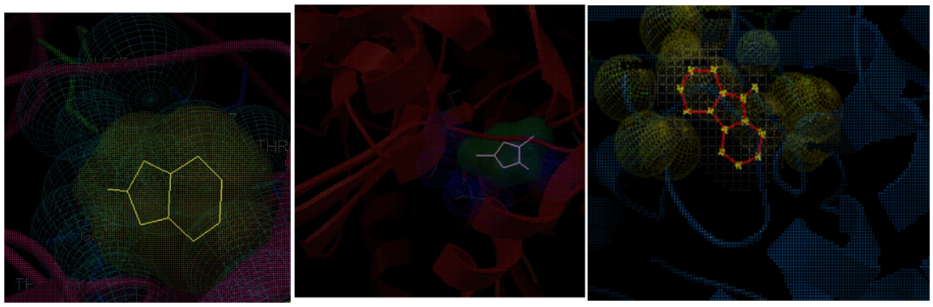


Figure 23, 24 & 25: Snapshots and binding interactions of Benzotriazole,3,5 dimethyl pyrazole &1,2,3,4,-tetrahydro carbazole with Maternal Embryonic Leucine Zipper Kinase (Binding Energy= -4.53,-3.92 &-6.57.Kcal/mol)

Table 5: Binding energies of azoles with E292S glycosynthase variant of exo-1,3-beta-glucanase

S. No.	LIGANDS	Binding energy (ΔG =Kcal/mol)	No of hydrogen bonds
01	Benzimidazole	-5.45	1
02	Benzotriazole	-5.41	1
03	3,5-dimethyl pyrazole	-4.77	1
04	1,2,3,4-tetrahydo carbazole	-7.65	1
05	Fluconazole (Std)	-6.47	1

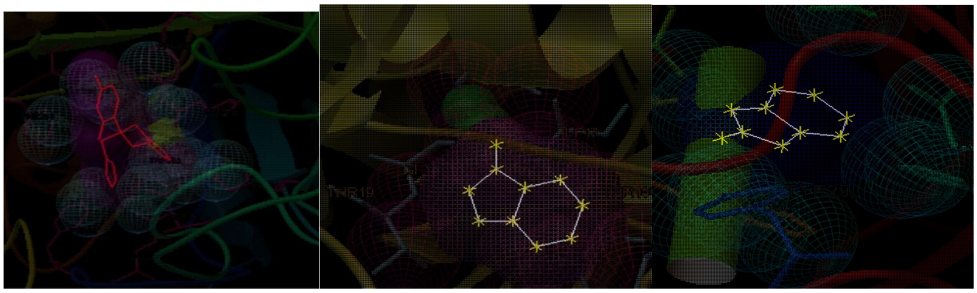


Figure 26, 27 & 28: Snapshots and binding interactions of Fluconazole (std) Benzimidazole& Benzotriazole with E292S glycosynthase variant of exo-1,3-beta- glucanase. (Binding Energy -6.47, -5.45, -5.41 Kcal/mol)

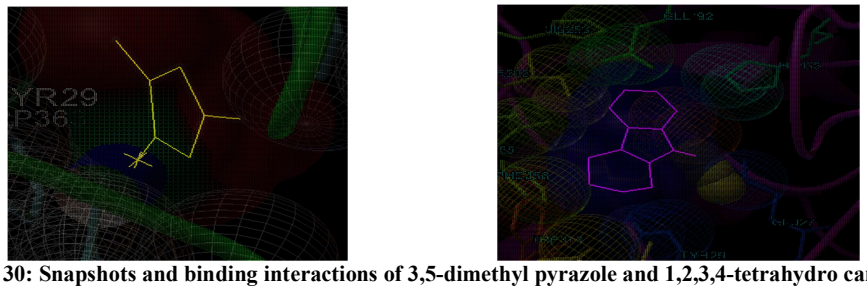


Figure 29 & 30: Snapshots and binding interactions of 3,5-dimethyl pyrazole and 1,2,3,4-tetrahydro carbazole with E292S glycosynthase variantofexo-1,3-beta-glucanase respectively. (Binding Energy= -4.77 &-7.65 kcal/mol respectively)

DISCUSSION

Antibacterial activity the results of docking of Glutamate racemase with Azoles are reported above. The binding sites were represented in the snap shots and the binding energy was compared with the standard ligand, ciprofloxacin(-5.67Kcal/mol). 1,2,3,4-tetrahydrocarbazole have highest binding energy.

Antitubercular activity

The results of docking of InhA with Azoles are reported above. The best docked structure should have the binding energy higher than that of the standard. The binding sites were represented in the snap shots and the binding energy was compared with the standard ligand, isoniazid (-5.09 Kcal/mol).1,2,3,4-tetrahydrocarbazole have highest binding energy - 7.33Kcal/mol. Other Azoles have binding energies nearer to the standard.

Anticancer activity

The results of docking of Maternal Embryonic Leucine Zipper Kinase with b Azoles are reported above. The best docked structure should have the binding energy higher than that of the standard. The binding sites were represented in the snap shots and the binding energy was compared with the standard ligand, Tamoxifen (-8.00 Kcal/mol).

Antifungal activity

The results of docking of E292S glycosynthase variant of exo-1,3-beta-glucanase with azoles are reported above. The best docked structure should have the binding energy higher than that of the standard. The binding sites were represented in the snap shots and the binding energy was compared with the standard ligand, Fluconazole (-6.47Kcal/mol). 3,5-dimethyl pyrazole have highest binding energy -4.77 Kcal/mol. Other azoles have binding energy near to standard.

CONCLUSION

Interaction of azoles with targets of different diseases were performed using docking studies. Binding energy obtained from docking studies confirm that the azoles inhibit enzymes E292S glycosynthase variant of exo -1,3-beta glucanase, InhA, glutamate racemase present in Candida albicans, mycobacterium tuberculosis, Escherichia coli respectively and also inhibit proliferation of breast cancer cell through inhibition of maternal embryonic leucine zipper kinase. Further studies of the structural activity relationship are required for the development of new derivaties with more pharmacological action.

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