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In-silico designing of an inhibitor against mTOR FRB domain: Therapeutic implications against breast cancer

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ABSTRACT

Worldwide breast cancer causes significant fatalities in women. The effective therapeutic solution for treating the disease is using new and probable antagonistic biologically available ligands as anticancer drugs. To identify a successful therapeutic approach, the scientific community is now interested in creating novel ligands that in the future may be used as anticancer drugs. The mechanistic target of rapamycin (mTOR) is a protein kinase connected to several processes governing immunity, metabolism, cell development, and survival. The proliferation and metastasis of tumors have both been linked to the activation of the mTOR pathway. Female breast cancer represents about 15.3% of all new cancer cases in the U.S. alone and is frequently diagnosed among women aged 55 to 69 years. Given that the P13K/AKT/mTOR pathway is one of the most often activated in cancer, much attention has been paid to its resistance as a novel oncological treatment approach. mTOR/FRB Domain's recruitment cleft as, well as substrate recruitment mechanism, was targeted using a structural-based approach. A series of selective inhibitory small molecules have been designed and screened for the best inhibiting target binding triad of the FRB Domain with better ADME and no detectable toxic effects.

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1 Introduction

The cancer cells transfuse and are transported via the blood to other parts of the body, causing Breast cancer. One in eight women will develop breast cancer during their lifetime. The past several years have seen dramatic changes in anticancer drug development. More specifically, improvements have been seen in the production of anticancer drugs during the last few years. Even though various inhibitors have been discovered in recent times, and quite a few have been efficaciously developed to cure breast cancer, there has been more emphasis on the discovery of value-added anticancer drugs (Sharma et al. 2022a). The FRB domain with its rapamycin-binding site functions as a sentinel. By inhibiting substrate recruitment and further restricting active-site access, rapamycin-FKBP12 inhibits the kinase. By directly preventing substrate recruitment and further restricting active-site access, rapamycin-FKBP12 inhibits the kinase. PI3K/AKT/mTOR pathway is the most promising target for cancer treatment, although clinical trials are still being conducted (Sharma et al., 2017). PI3K/Akt/mTOR pathway being a complex intracellular pathway is known to result in cell growth and tumor proliferation, and thus it could act as a striking target for anticancer drug development (Sharma, 2020, 2022). Growth factors binding to the receptors activate PI3K, resulting in phosphorylation of the PI3-K-dependent kinases, which trigger AKT (Sharma et al. 2021b). AKT further triggers mTOR by phosphorylation and inhibition of TSC2, which negatively regulates mTOR. Further, p70S6 kinase upon phosphorylation by mTORC1 phosphorylates the 4E-BP1 and S6 (a ribosomal protein) which leads to protein translation. mTORC1 and mTORC2 are the two main complexes of mTOR. The components of mTOR Complex 1 comprise MTOR, RAPTOR, MLST8, PRAS40 , and DEPTOR (Kim and Guan 2019), while the Complexes of mTORC2 consist of MTOR, RICTOR, MLST8, and mSIN1. At the C-terminus, MTOR has four different domains for catalytic activity; FAT, FKBP12-rapamycin-binding domain (FRB), the kinase domain, and the FATC domain. At the N-terminus, MTOR contains 20 HEAT (Huntingtin elongation factor 3) repeats (Singh et al. 2022). The cleft is shaped like a deep V, thanks to the FRB or FKBP12-rapamycin-binding domain, which also limits access to the substrate-binding region (Ram et al. 2020). When rapamycin-FKBP12 binds to the FRB (Raghav et al. 2022), it effectively blocks all access to the substrate-binding site (Sharma et al. 2010). Besides aiding in a restriction to the active site, FRB also facilitates S6K1 (a ribosomal protein kinase) access to the active site, yielding increased kinase activity (Raghav et al. 2020). mTOR binds to the FK506-binding protein, FKBP-12, which upon binding, performs mTOR reticence (Sharma et al. 2020). mTOR upon inhibition further reduces phosphorylation of both the downstream targets, 4E-BP1 and S6K(Sharma et al. 2019a), inhibiting protein synthesis (Sun et al. 2020; Wakchaure and Ganguly 2021). Current investigation has proposed de-novo generated new lead molecule targeting FRB domain of mTOR, one of the key molecules in PI3K/AKT/mTOR pathway using the structure-based designing approach (Sehrawat et al. 2020). The current study has proposed a de-novo generated new lead molecule targeting the mTOR FRB domain, using a structure-based designing approach in the PI3K/AKT/mTOR pathway (Sharma et al. 2019b).

2 Materials and Methods

The energy-refined models of mTOR were engendered through iterative threading assembly refinement (I-TASSER) (Yang et al. 2015). PROCHECK was used for cross-checking the predicted models for energy distribution and stereochemistry. The substrate recruiting triad was then picked as the site of investigation, and in this complete study, we questioned the complementation of the same site. The substrate recruitment triad was then chosen as the site of inquiry, and we addressed the complementation of the same site in our complete analysis. Natural ligand search, as a seed molecule of 4JSP, revealed Benzoxazepine. Benzoxazepine was found in 4JSP's natural ligand quest. Its natural tendency to bind with class AKT (Sharma et al. 2021a) kinase and be a small molecular structure (molecular weight ~145.16) fits over accepted rules of the SBDD approach (Sharma and Sharma 2021). Thus Benzoxazepine as a whole was picked as a starting point for the de-novo generation of ligands. Benzoxazepine clean 3D structure PDB file was generated using ACD Labs freeware Chemsketch (Samanta et al. 2010). It was then used in the Schrodinger Maestro suite as an input file. Using "Maestro" and site-directed approach, we then placed seed molecule Benzoxazepine in available search space close to the binding triad. The seed molecule was placed such that it would interact well with the location (Figure 1). Utilizing an internal library of organic fragments, the growth strategy of Ligbuilder (v1.2genetic) algorithm was employed to populate more than 500000 molecules. Binding affinities were calculated by an empirical scoring function (Wang et al. 2000). Chemical stability, synthesis feasibility, and toxicity were also considered by defining "forbidden structure" libraries. It is also reasonable to consider chemical stability, synthesis feasibility, and toxicity by identifying libraries of "forbidden structure". Lipinski's parameters were used for screening and processing the population of produced compounds, and a total of 20 different fragment combinations were ultimately chosen for research (Sjöholm and Sandler 2019).

Then, using MGL and Autodock tools, the binding energies of generated ligands and mTOR were examined. The binding energy of developed ligands was further assessed by MGL and Autodock tools (Huey et al. 2012). The docking experiment was completed using the Lamarckian Genetic algorithm and the default Local Search parameters. With a decreased RMSD from the initial

conformation, Gibbs free energy (G) demonstrated strong interaction between produced ligands and mTOR (Table 1). The examined ligands' activity concentration (IC50) was also acceptable within the micro-molar range (Table 2). Biosafety and Bioavailability studies were then carried out through different web tools, and a comparison of candidates for the arrest of the mTOR FRB domain-mediated substrate recruitment mechanism revealed the best-fit candidate (Sharma et al. 2022b). To better understand the conformational dynamics of docked complexes, GROMACS was used to conduct molecular dynamics simulations (Berendsen 1995). Newton's equation of motion was solved by using the all-atoms simulation technique. Protein topology was created using GROMACS pdb2gmx modules. In addition, the topology of the ligand was created by PRODRG 2.5, an online tool (Adams et al. 2010). The complex was positioned in a dodecahedron box with a 10 Å distance from the edges using the edit conf module. The system was solvated using the specific point charges water model to keep equilibrium{SPC216}. The solvated system was then minimized with a cut-off of 50,000 steps at a maximum force of 1000.0 KJ/mol/nm. The system was also approaching equilibration at a temperature of 300K for the ensemble of the system's number of atoms, volume, and temperature (NVT) (Sharma et al. 2021a). For 100 ps, NVT and NPT was calcuted to keep the system in equilibrium. The system was put through a 1ns molecular dynamics simulation to test the complex stability. The structural analyses (Rg, RMSF andRMSD) was done and graphs were produced using the Graphing, Advanced Computation and Exploration application Xmgrace (Bansal et al. 2022).

3 Results and Discussion

Computer-aided drug design (CADD) methodologies are potentially promising and are playing an ever-increasing role in the drug discovery process, which is a cost-effective and suitable path provider for wet lab experiments. Moreover, CADD has been widely employed for the identification of promising drug candidates. This study designed a novel lead molecule, which has the best possible characteristic targeting properties, especially targeting the FRB domain. The same has exhibited significant overall goodness (G factor) and substantial core residual value (80.3%) of the modelled structures. Upon structure modelling, Model-1 has proven to be the best model. The results generated are in agreement with other studies reported in the literature, which prove the geometry and stereochemistry checks also along with the energy distribution using PROCHECK (Sharma et al. 2021b). The values obtained using the tool are following the studies reported in the literature.

Model 1, with 82.3 percent core residues, was found to be the most stable structure after projected models were reevaluated for energy distribution and stereochemistry using PROCHECK (Aruleba et al. 2018). Cross-validation of the shown models was done using a

Table 1 Models evaluated in terms of c-Score, Z-score, and Goodness factor values

Model	C-score	Core Value	G-factors	Z-Score
Model 1	-5.00	82.3% core	-0.39	-11.42
Model 2	-2.66	76.3% core	-0.52	-10.48
Model 3	-3.00	78.2% core	-0.49	-9.34
Model 4	-5.00	77.8% core	-0.32	-10.4
Model 5	-5.00	76.8% core	-0.40	-12

Table 2 Analysis of Various ligands based on their best conformations attained, ΔG (kcal/mol) value, RMSD values, and inhibition constant values.

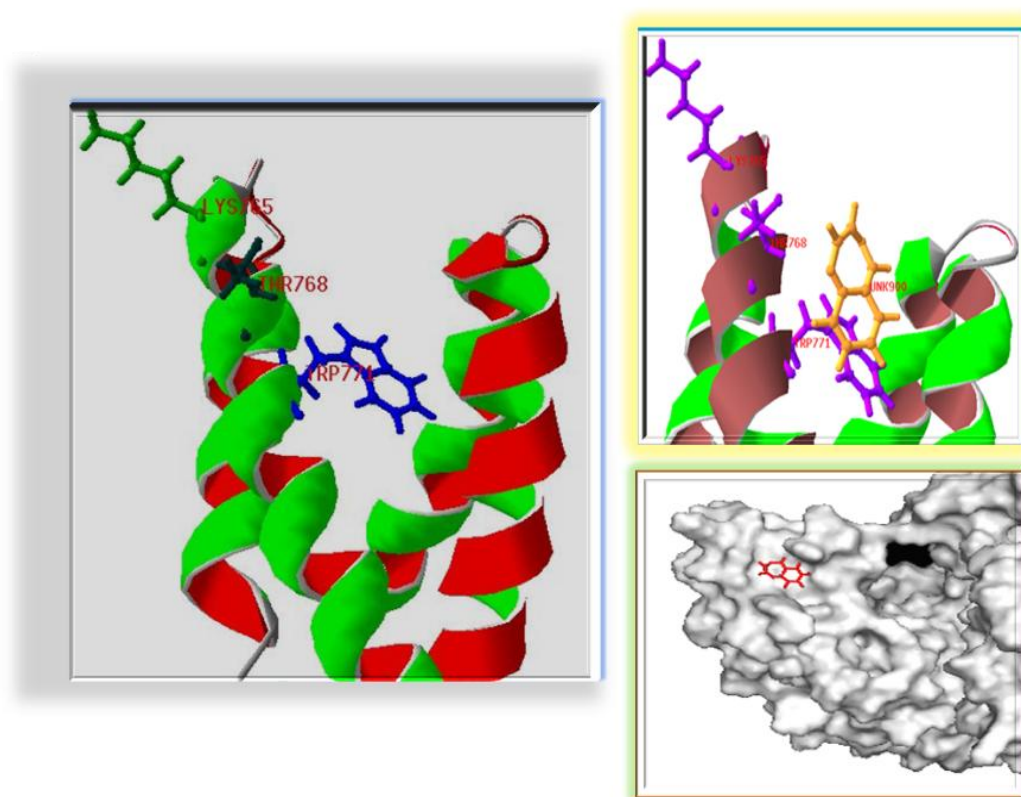| Ligand Name	Best Conformation	ΔG (kcal/mol)	Ref RMSD	Inhibition Constant
Ligvar 2	2	-3.53	2.6	3.8 mM (millimolar)
Ligvar 21	3	-3.24	2.83	4.15 mM (millimolar)
Ligvar 22	5	-3.25	3.05	4.76 mM (millimolar)
Ligvar 23	4	-5.86	4.26	6.17 uM (micromolar)
Ligvar 26	7	-4.17	1.2	3.79 mM (millimolar)
Ligvar 37	2	-2.48	1.2	3.80 mM (millimolar)
Ligvar 38	8	-4.03	2.65	5.15 mM (millimolar)
Ligvar 39	9	-3.25	2.26	4.32 mM (millimolar)

Figure 1 Benzozapine and its best bound position A. Ribbon view B. Surface view

comprehensive examination of model quality (Z-Score), and the findings showed that model 1 was the best fit (Table 1). FRB domain analysis and literature search showed that the active triad (Lysine (K) 765, Threonine (T) 768, and Tryptophan (W) 768) (Figure 1) plays a crucial role in substrate recruitment mechanism. The Ligsitemeta server also predicted enough active space around these three residues. Which have been reported in the literature as well.

Three amino acids viz. K-765, T-768, and W-771 at the specified positions were the primary focus of our study. Benzoxazepine was considerably used for the growth strategy, with which a population of binding fragments was generated, with pKd values ranging from 5-7 molars (Chaube et al. 2016). The designed ligands were subjected to molecular docking analysis to determine their binding energies with the protein of interest, which revealed that amongst all the designed ligands, Ligvar23 suggested positive affinity i.e ΔG -5.86 Kcal/mole around the catalytic triad position. Previous studies have convincingly shown that the active triad of K-765, T-768, and W-771 is capable enough in the fulfillment of substrate recruitment mechanism, which blocks the disease-causing path in the FRB domain. After performing docking studies, Ligand 23 displayed nominal bonding with Threonine-768. For MD simulations,

water was added into the periodic box along with three chlorine ions by using the genion module of GROMACS. The system's volume was found 632.22 nm^3 while the density of the system was observed at 1003.04 (g/l). A total of 19285 solvent (water) molecules were added (Lemkul 2018). The potential energy graphical representation displayed a sharp drop in the system's PE at the beginning, after which it was stabilized. The system's PE was accomplished at 884 EM steps (Figure 2). Protein was in a dynamic state at 300K and constant pressure after NVT and NPT ensemble (Figures 3 and 4). Throughout the course, density fluctuated from 1017 to 1022 kg/m^3, with an average value close to 1020 kg/m^3 (Figure 5). MD was then run for a total of 1ns. In addition, the RMSD value was observed over time. Higher values suggest protein misbehavior during simulation, while changes in the range of 1-3 Å are acceptable (Lemkul 2018).

While the complex was under simulation, it was perceived that it passes through minute changes, with a modest rise in RMSD (Cabeza de Vaca et al. 2018). Red denotes the equilibrated protein backbone, which remains in the acceptable range compared to the black line (crystal structure) (Figure 6). RMSF was computed to demonstrate the undulation of amino acid residues of the complex during the MD run. The peak depicts the residues that fluctuate the most during the simulation (Figure 7).
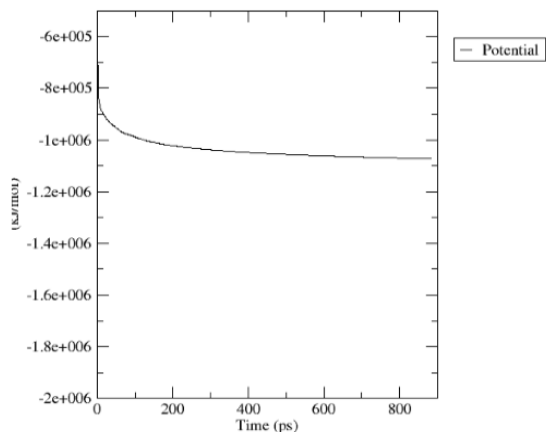
GROMACS Energies

Figure 2 System PE minimization was accomplished
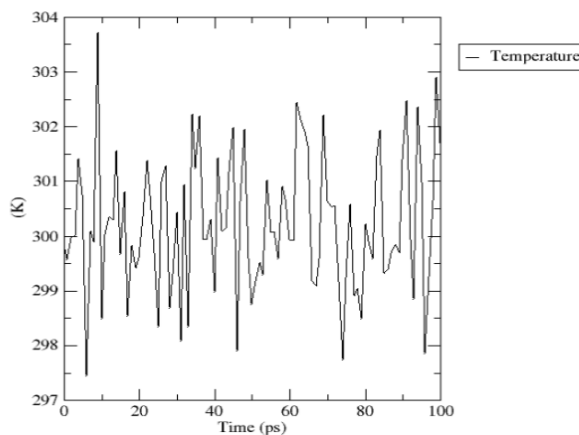after 884 EM steps

GROMACS Energies

Figure 3 NVT of the system for 100 picoseconds
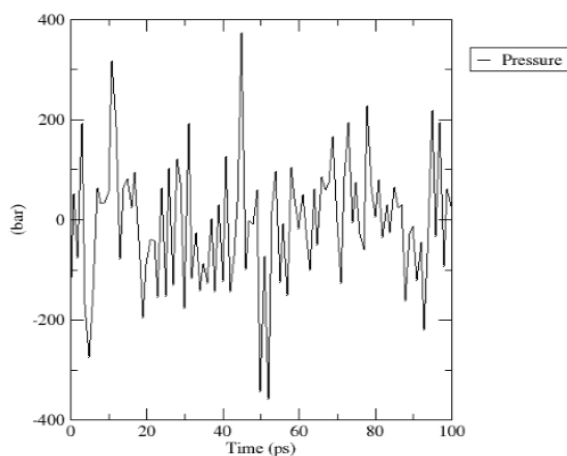
GROMACS Energies

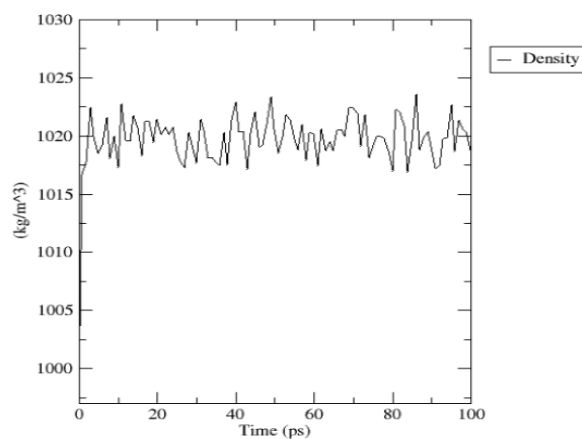Figure 4 NPT of the system for 100 picoseconds

GROMACS Energies

Figure 5 System density observed during the study
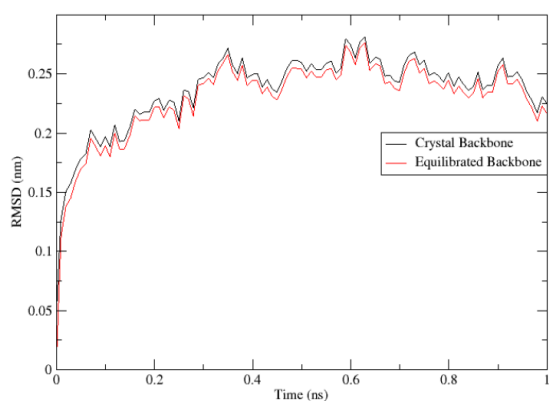which fluctuates around 1020 kg/m^3

Comparative RMSD

Figure 6 Comparative RMSD of the system. Crystal backbone
vs. equilibrated backbone
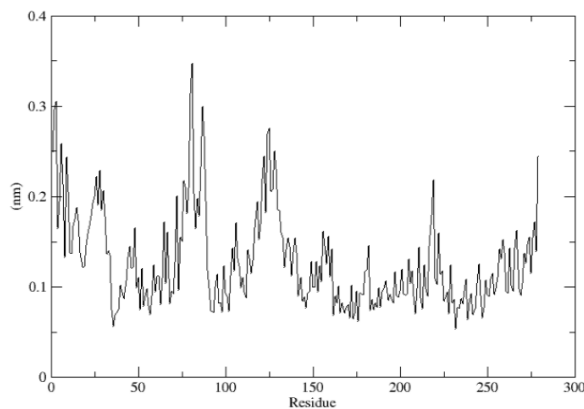
RMS fluctuation

Figure 7 System RMSF shows fluctuation over all residues
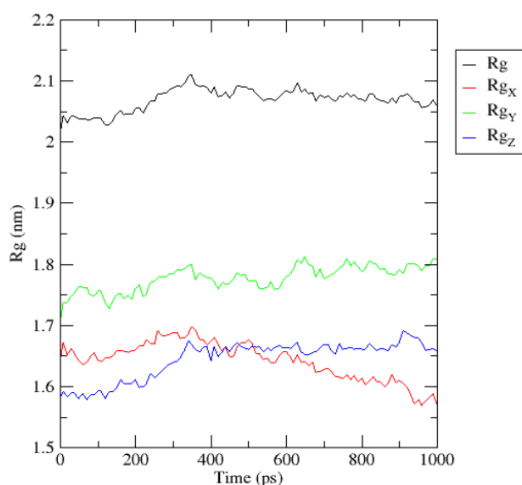of the protein

Radius of gyration (total and around axes)

Figure 8 Radius of gyration of the system for 1000 Ps

Figure 9 Heat map of RMSD over 101 frames. Encircled area showing highest RMSD

A minute change in radius of gyration having a value of 0.05nm was observed over 1000ps, indicating that protein remains stable during MD simulations (Figures 8 and 9). In terms of bioavailability and biosafety parameters, ligand Ligvar 23 displayed enzyme inhibition properties and could also act as the best candidate due to its non-toxic fragments. The present MD study based on Equilibrated NVT-NPT, PEM, RMSD, RMSF, and Radius of Gyration revealed that the complex remains stable throughout the observation period which was validated previously as well (Valdés-Tresanco et al. 2021). The findings are certainly significant from a therapeutic perspective against breast cancer especially targeting the mTOR/FRB domain.

Conclusions

The FRB domain of mTOR protein was targeted in the present study for which a library of designed molecules was constructed. After performing an extensive screening based on binding affinities, binding energy, bioavailability, toxicity, and biosafety parametric calculations, Ligvar 23 was found to be the most promising with the best binding affinity along with the best-fitted biosafety parameters, having the capability to act as potent drug molecules. The MD study based on Equilibrated NVT-NPT, PEM, RMSD, RMSF, and Radius of Gyration revealed that the complex remained stable throughout the study, and the complex was proven to be a bioavailable and the most suitable candidate.

Acknowledgments

Conflict of Interest

Regarding the publication of this paper, there are no conflicts of interest among the authors.

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