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APPLICATION OF COMPUTATIONAL STRATEGIES FOR OPTIMIZATION OF ASPARGINYL t-RNA SYNTHETASE DOCKING STUDY OF QUINOXALINES

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ABSTRACT

The potential use of asparginyl t-RNA synthetase inhibitors as antifilarial agents for the treatment of filariasis. In our present work we have synthesized series of diphenyl quinoxalines derivatives. Molecular docking studies of these compounds were undertaken to evaluate the binding affinities of the compounds with enzyme asparginyl t-RNA synthetase (PDB: 2kqr). The current result shows fine correlation in enzyme inhibitory activity with docked ligand.

KEYWORDS: asparginyl t-RNA synthetase, quinoxaline, docking.

INTRODUCTION

Quinoxalines are becoming the attractive target for extensive research due to its inherent diverse biological properties.[1] Various potential activities of the quinoxalines have been explored recently like antimicrobial agents, cytotoxic agents, anti-tubercular, anxiolytic, anti-HIV, anti-inflammatory, antioxidant, antileishmanial etc. In the recent year, 2,3-disubstituted quinoxalines reported to posse's significant potential against parasites, bacteria, fungi, and mycobacterium.[2] Design of quinoxaline antibiotics have undertaken by several workers,[3] but they posses limited application due to their toxic effect. We undertaken the molecular docking studies of these compounds to evaluate the binding affinities of the synthesized compounds with enzyme asparginyl t-RNA synthetase (PDB: 2kqr).[4]

MATERIALS AND METHODS

Given quinoxaline derivatives docked with titled enzyme and observed the all parameters and binding interactions with various sites on receptor. From various conformations only most

stable conformer get selected and docked with ligands. For docking purpose download the PDB file from online (PDB: 2kqr) and purify proteins by removal of binded ligand, removal of water molecule and addition of hydrogen atom and make it ready for docking. Second step was drawn ligand in Chembiodraw 2D structures and get converted into 3D form. (.mol) Later on minimized the energy of selected molecules and optimized molecules for stability. Energy minimization was done by semi empirical method by MMFF (Merck Molecular Force Field)[5] and molecules became ready for docking purpose. Later on Grip study was done. Now picked up the molecules and open the grid option in software and set the receptor and chosen the compound protein structure which is in .mol file. Afterwards; specify the protein cavity and its number. Docking was supposed to be performed by using reference ligand around which grid could be generated. Now started the docking and observed various results. For best ligand it has to compare the molecules and done proper ligand merging by using ligand merge option. In given study we also optimized the complex by minimizing its energy and made it more stable conformer. All the interactions given by compound R6 were depicted in Table 1. Given table provides idea about which residue atom binds with which ligand atom and state the distance between them.

Table 1: Binding interactions of compound R6.

Sr. no.	Residue Atoms	Ligand Atoms	Distance	Interaction Type
1	TYR26A 2220C	2743C	4.56	HYDROPHOBIC_INTERACTION
2	ARG 2223C	2743C	3.621	HYDROPHOBIC_INTERACTION
3	ASP 2224C	2743C	4.454	HYDROPHOBIC_INTERACTION
4	ASP 2229C	2743C	4.452	HYDROPHOBIC_INTERACTION
5	TYR26A 2234C	2743C	4.599	HYDROPHOBIC_INTERACTION
6	MET26A 2236C	2743C	4.549	HYDROPHOBIC_INTERACTION
7	TYR26A 2241C	2743C	4.802	HYDROPHOBIC_INTERACTION
8	TYR26A 2244C	2743C	3.969	HYDROPHOBIC_INTERACTION

Molecular docking analysis

Molecular docking simulations were employed to predict the best conformation of screened molecules into the active site of protein (PDB: 2kqr), using software's V-Life MDS 4.5. The docked ligands were analyzed manually to explore their binding mode with receptor 2kqr. The binding energy score, hydrogen bond interactions with active site residues along with the corresponding distance notation were analyzed. Various steps used in the methodology of molecular docking studies are discussed as under.[6]

Target preparation

The 3D structure of 2kqr was retrieved from the Protein Data Bank (www.rcsb.org). Finally, the 3D structures of protein 2kqr were imported into the workspace of V-Life MDS suit with the removal of all water molecules having more than 5Å specific distance. The standard V-Life algorithm was employed for rendering the missing charges, protonation states and assigning of polar hydrogen to the receptor.

Preparation of ligand molecules

The ligands were built in chemSketch all hydrogens in the structure were added, 2D molecules were cleaned into 3D and the conformational energy of molecules was minimized using MMFF94 force field. The resulting structures were saved in Chem. Sketch as MDL molfile (*.mol). After that ligands were imported in V-Life which helps in assigning the missing bond orders, charges, bonds and hybridization states of the imported ligands.

Docking studies on V-Life MDS 4.5

V-Life was used for docking studies, which has been considered more accurate than other docking tools. Comparatively V-Life was used to calculate the interaction energies between ligands and macromolecular systems from the 3D structures of the protein and ligands. The candidates with the best conformation and energy scores were selected. The algorithm used to be the MolDock Score, which is an adaptation of Differential Evolution (DE) algorithm. Finally docking of ligands and protein was performed in docking wizard with the score function dock score. The parameters for docking were set as default, which includes number of runs 10, maximum iterations 1500, maximum population size 50, but maximum number of poses was increased to 10.

Docking energy calculations

The Dock score energy, Escore was calculated with the help of Eq. (1), where Einter represent the ligand-protein inter action energy, whereas Eintra was the internal energy of the ligand. Einter was calculated by Eq. (2) whereas calculation of Eintra was made with the help of Eq. (3)

$$E_{\text{score}} = E_{\text{inter}} + E_{\text{intra}} \quad (1)$$

$$E_{inter} = \sum_i ligand \sum_j protein \left[\frac{\text{EPLP(rij)} + 332.0\text{qiq}}{4\text{r2ij}}\right] \quad (2)$$

$$E_{inter} = \sum_i ligand \sum_j protein \lceil \text{EPLP(rij)} \rceil + \sum_{felxiblebonds} A \lceil 1 - \cos(\text{M}\theta - \theta 0 \rceil E_{clash} \quad (3)$$

Represents the term "piecewise linear potential" which comprised two different parameters, one of the potential energies of hydrogen words and second for the Vander wall interactions between atoms. Second term in the Eq. (2) was used for the calculation of electrostatic interactions between charged atoms. The second term in the Eq. (3) was for the calculation of tensional energy where the tensional angle is. The last term Eclash in the Eq. (3).

AutoDock 4.2 uses a semi-empirical free energy force field to evaluate conformations during docking simulations. The force field was parameterized using a large number of protein-inhibitor complexes for which both structure and inhibition constants, and Ki, are known.

Table 2: The calculated Docking score of the substitution at different confirmation of the moiety.

Placement	Score
R6_5_3D_opt_C68_P1	**-66.61**
R__5_2_3D_opt_C48_P5	**-60.509**
R7_6_3D_opt_C45_P4	**-56.708**
R7_6_3D_opt_C75_P29	**-55.604**
R4_4_3D_opt_C63_P28	**-55.429**
R7_6_3D_opt_C71_P30	**-55.408**
R-LEAD_1_3D_opt_C74_P24	**-55.124**
R6_5_3D_opt_C92_P21	**-55.101**
R4_4_3D_opt_C11_P9	**-54.804**
R7_6_3D_opt_C49_P6	**-54.499**
R7_6_3D_opt_C68_P7	**-54.158**
R-LEAD_1_3D_opt_C38_P13	**-53.886**
R__5_2_3D_opt_C14_P16	**-53.619**
R__5_2_3D_opt_C14_P25	**-53.349**
R7_6_3D_opt_C16_P10	**-53.311**
R__5_2_3D_opt_C69_P3	**-52.442**
R-LEAD_1_3D_opt_C94_P12	**-52.227**
R7_6_3D_opt_C29_P26	**-51.997**
R-LEAD_1_3D_opt_C13_P19	**-51.658**
R__5_2_3D_opt_C57_P8	**-51.558**
R4_4_3D_opt_C46_P15	**-51.298**
R_9_3_3D_opt_C29_P17	**-51.127**
R-LEAD_1_3D_opt_C57_P23	**-51.124**
R4_4_3D_opt_C70_P2	**-51.061**
R-LEAD_1_3D_opt_C6_P18	**-50.92**
R_9_3_3D_opt_C7_P27	**-50.558**
R4_4_3D_opt_C63_P14	**-50.531**
R-LEAD_1_3D_opt_C11_P22	**-50.466**
R-LEAD_1_3D_opt_C86_P11	**-50.276**
R-LEAD_1_3D_opt_C42_P20	**-50.14**

Docking studies on Autodock 4.0

The force field appraise binding in two stepladders. The ligand and protein start off in an liberated conformation. In the first step, the intramolecular energetic are approximate for the evolution from these unbound states to the conformation of the ligand and protein in the spring state. The second step then evaluates the intermolecular energetics of uniting the ligand and protein in their bound conformation.

The force field includes six pair-wise evaluations (V) and an approximation of the conformational entropy lost upon binding (ΔSconf):

ΔG = (Vbound L-L +V unbound L-L)+(Vbound P -P +V unbound P-P)+(Vbound P-L +Vunbound P-L +ΔSconf)

Where, L refers to the "ligand" and P refers to the "protein" in a ligand-protein docking calculation. Every of the pair-wise energetic terms includes evaluations for dispersion/repulsion, hydrogen bonding, electrostatics, and desolvation. The weighting constants we have been optimized to calibrate the empirical free energy based on a set of experimentally determined binding constants. The first term is a typical 6/12 potential for dispersion/repulsion interactions. The parameters are based on the Amber force field. The second term is a directional H-bond term based on a 10/12 potential. The parameters C and D are assigned to give a maximal well intensity of 5 kcal/mol at 1.9Å for hydrogen bonds with oxygen and nitrogen, and a well depth of 1 kcal/mol at 2.5Å for hydrogen bonds with sulfur. The function E(t) provides directionality based on the angle t from ideal H-bonding geometry. The third term is a screened Coulomb potential for electrostatics. The final term is a desolvation potential based on the volume of atoms (V) that surround a given atom and shelter it from solvent, weighted by a solvation parameter (S) and an exponential term with distance-weighting factor σ=3.5Å. For a detailed presentation of these functions.

Prediction of ADME descriptors and toxicity

The major reason for the failure of most of the drug candidates during clinical trial are poor ADME and high toxicity profile. Thus an important aspect of drug discovery is to evaluate critical physiochemical as well as toxicity profile in initial stages of drug discovery. Hence, to select a potential drug candidate, all hypothetical ligands were screened on the basis of ADME and toxicity filter using preadmet from PreMetabo software and was also employed to filter non-toxic hits on the basis of carcinogenicity and mutagenicity (rat model) of the hypothetical designed ligands. input. Output showed a number of principal descriptors and

ADME properties as shown in Table 3 Lipinski rule of five was applied on the ligands and hits which were having more than one violation were rejected. Various other physicochemical properties were also calculated, represented by different descriptors such as molecular weight (MW), number of rotatable bonds (NRB), lipophilicity parameter [log P(o/w)], number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), total polar surface area (TPSA), solubility (log s), solvent-accessible surface area (SASA), skin permeability (log Kp), binding to human serum albumin (log Khsa), blood-brain partition coefficient (logBB), apparent MDCK cell permeability (affyPMDCK), apparent Caco-2 cell permeability (affyPCaco), percentage human oral absorption. Ideal ranges of various descriptors calculated with the reference to 95% of are presented in Table 3.

RESULTS AND DISCUSSION

Pharmacophore and synthesis of test series

The substituted sulfonamido quinoxalines were synthesized[8–14] using 2, 3-diphenylquinoxaline-6-sulfonylchloride as an important intermediate which was synthesized by reported method in which was achieved by treating of 2, 3-diphenyl quinoxaline was refluxed with excess chlorosulfonic acid[15] on water bath for 5 hrs. The resultant mixture was poured into water to give sulfonylchloride derivative. Various different organic amines were treated at different reaction conditions to give the series of 6-sulfonamido derivatives of 2, 3-diphenylquinoxaline.

Now, docked the synthesized molecules on parasite *Brugia malayi* asparginyl t-RNA synthetase enzyme[16] which we got downloaded from protein data bank (PDB) site (PDB: 2kqr).

Here use of V-Life MDS 4.5 Software to explore the binding mode of ligands. All ligands exhibited negative docking scores and analyzed for various types of interactions like hydrophobic bonding, aromatic bonding, hydrogen bonding and Vanderwaal's interactions.[17–21]

Docked selected synthesized quinoxaline molecules with parasite *Brugia malayi* asparginyl t-RNA synthetase ligase enzyme. The results obtained from activity were in excellent correlation with docking outcomes of title compounds. Molecular docking was carried out to study the interaction of quinoxaline derivatives with *Brugia malayi* parasite. The most active compound of dataset was found to have dock score -66.609758 for R6 compound. In case of

parasitic enzyme compound R6 shows most promising dock score (Table 2) and binding interactions and affinity towards receptor.

It was observed that *in silico* and *in vitro* results of synthetic compounds was well correlated as the top scoring compounds obtained after docking displayed most promising activity than others.

Final screening on the basis of ADME and toxicity analysis

All 20 hits hypothetical compounds were screened for drug likeliness and "non-toxic" profile on the basis of various parameters of the software preadmet and metabo respectively. ADME filter lead to five drugs like hits. The ADME prediction studies data of five hits The range of various important parameters were predicted, like molecular weight lied in range between 400 and 482, the value of *in vitro* Caco2 cell permeability (Human colorectal carcinoma), estimated *in vitro* CYP 2C19 inhibition *in vitro* plasma protein binding ,(%)Water solubility in pure water (SK atomic types, mg/L), *in vitro* skin permeability (logKp, cm/hour), K logD in pH 7.4 (SK atomic types), *in vivo* blood-brain barrier penetration (C. brain/C. blood), *in vitro* MDCK cell permeability (Mandin Darby Canine Kidney) were studied shown in Table.3.

Table 3: Toxicity prediction data of best hits using PreMetabo; ADME-T descriptors of best hits calculated from Preadmet.

ID	Value	ID	Value
Caco2	19.3724	Carcino_Mouse	negative
CYP_2C19_inhibition	Inhibitor	Carcino_Rat	negative
CYP_2C9_inhibition	Inhibitor	daphnia_at	0.00421
CYP_2D6_inhibition	Non	hERG_inhibition	low_risk
CYP_2D6_substrate	Non	medaka_at	6.18253
CYP_3A4_inhibition	Inhibitor	minnow_at	9.16303
CYP_3A4_substrate	Weakly	TA100_10RLI	negative
HIA	97.6465	TA100_NA	negative
MDCK	0.07375	TA1535_10RLI	negative
Pgp_inhibition	Inhibitor	TA1535_NA	negative
Plasma_Protein_Binding	95.9338		
Pure_water_solubility_mg_L	0.00306		
Skin_Permeability	-1.51462*		
SKlogD_value	5.42433		
SKlogP_value	5.42433		
SKlogS_buffer	-6.3055		
SKlogS_pure	-8.1985		

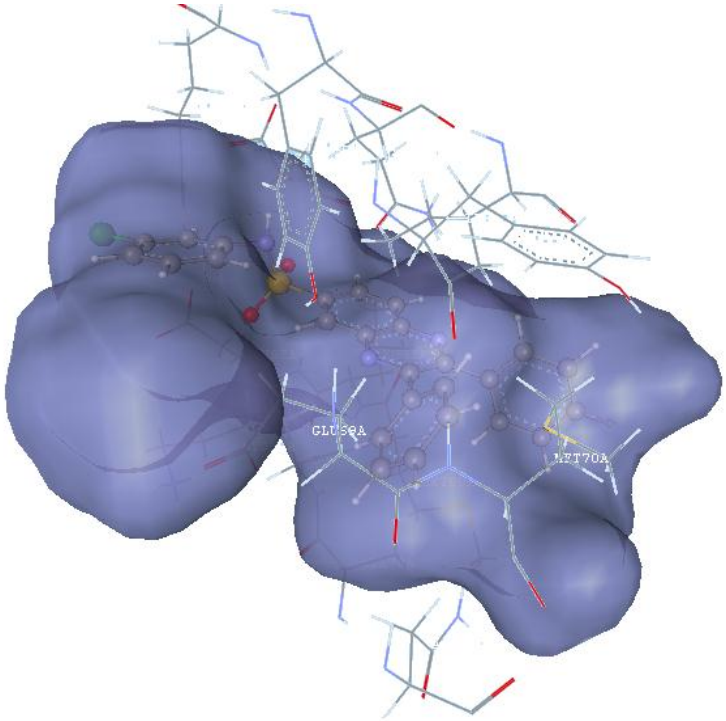

Figure 1: The crystal structure of asparginyl t-RNA synthetase in complex with a R6 (pdb: 2kqr). There is an intramolecular VDW interaction (length = 3.528Å) between the oxygen atom O of ASP12A 183C residue and the oxygen atom O of ASP12A residue in 2kqr protein.

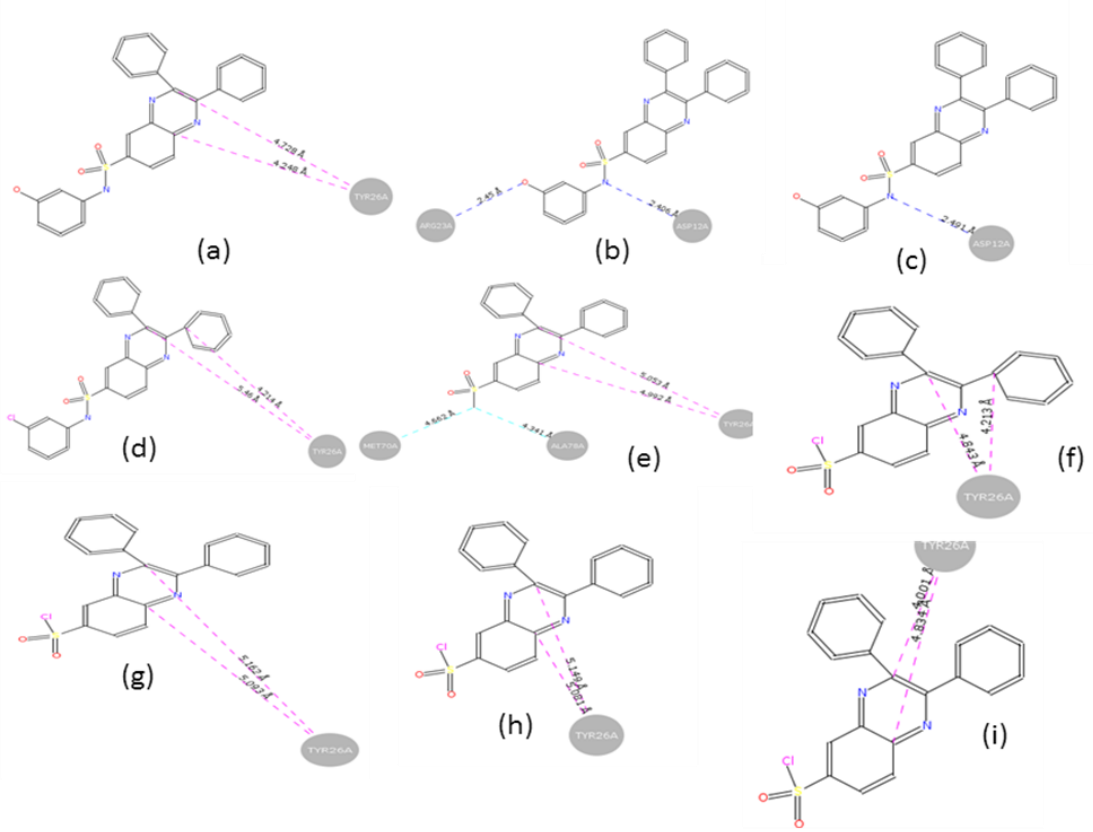

Figure 2: Confirmation and amino acid interactions.

a) Conformation for R7 compound (R_7 C_75 P_29) b) Conformation for R7 compound (R7 C_71 P_30).

c) Conformation for R7 compound (R7 C_45 P_4) d) Conformation for R7 compound (R7 C_92 P_21).

e) Conformation for R4 compound (R4 C_63 P_28) f) Conformation for parent compound (Lead C_94 LP_3).

g) Conformation for parent compound (Lead C_38 P_13) h) Conformation for parent compound (Lead 13_LP_4) i) Conformation for parent compound (Lead C_15 LP_2).

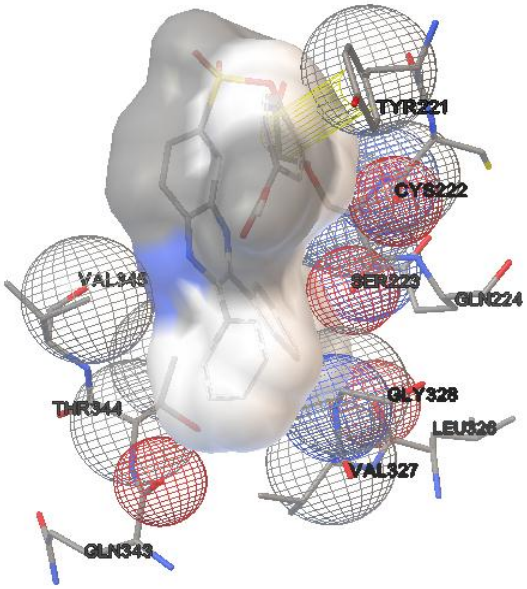

Figure 3: Superimposition of R6_3D from the X-Ray crystal structure from the docking result.

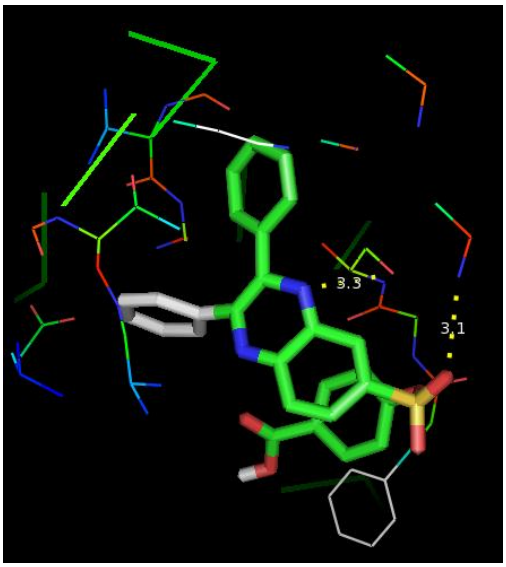

Figure 4: Model of interaction of R6_3D and asparginyl t-RNA synthetase.

CONCLUSION

Docking of quinoxaline derivatives with *Brugia malayi* enzyme out of which derivative R6 gives best docking score as it good *in vitro* antifilarial study also. On the basis of dock score it was concluded that compound having good asparginyl t-RNA synthetase receptor binding capacity. ADME-T was performed successfully. Reported results revealed that almost all 44 analogues of the series, having a good binding affinity and exhibited strong hydrogen bond interactions with the receptor. Final screening was based on the ADME-T filter. The results indicated that Autodock algorithms were valid, as docking the native ligand to binding site of asparginyl t-RNA synthetase showed a RSMD value less than 4 A 3.3. The docking result revealed sulfonamido quinoxalines exhibited good binding interaction to catalytic site of parasite asparginyl t-RNA synthetase.

Finally best hits were found on the basis of molecular docking and ADME-T studies. These most promising analogues can be explored further in designing of drugs against *Brugia malayi* also provide a way to synthesize the optimized lead compounds in the laboratory.

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