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In-silico computational analysis of [6-(2, 3-Dichlorophenyl)-1, 2, 4-Triazine-3, 5-Diamine] metal complexes on voltage gated sodium channel and dihydrofolate reductase enzyme

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Abstract: Epilepsy is the disease associated with seizures and convulsions. Various antiepileptic drugs have been used widely to treat these disorders. Lamotrigine [6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine] shows certain adverse effects at small doses, to evaluate its efficacy lamotrigine schiff based metal complexes were screened in-silico at voltage gated sodium channel for antiepileptic effect and dihydrofolate reductase enzyme for anticancer activity. Post docking analysis revealed that lamotrigine shows greater antiepileptic effect with its Schiff base complex of tin, with greater binding affinities on voltage gated sodium channel. However, anticancer effect of lamotrigine with its Schiff base silver complex shows highest binding affinity on dihydrofolate reductase enzyme. Study concluded that Schiff base derivative and its metal complexes express significant binding interactions with voltage gated sodium channel and dihydrofolate reductase enzyme.

Keywords: Schiff base, antiepileptic, dihydrofolate reductase, anticancer.

INTRODUCTION

Schiff bases are compounds with imines functional group. These are the condensation product of carbonyl compound with primary amines (Cimernan *et al.,* 2000). Schiff bases of antiepileptic drugs protect against seizures through variety of cellular targets, like synaptic vesicle protein, neurotransmitter metabolic enzyme, neurotransmitter transporter and ion channels (Robert, 2010). Schiff bases of aldehyde are prepared as acetaldehyde accumulates after ethanol consumption within the body which is oxidized by aldehyde dehydrogenase enzyme (Kopakka *et al.,* 2012). They possess a versatile core structure due to their broad spectrum of biological activities and various applications in biological, inorganic chemistry and analytical field (Kajal *et al.,* 2013). With the passage of time it has been observed that dehydrogenase enzyme have correlations with various human cancers (Januchowski *et al.,* 2013; Imran *et al.,* 2017). This enzyme is responsible for maintaining cellular homeostasis and used in anticancer studies at molecular level (Nene *et al.,* 2017; Nadeem *et al.,* 2018).

Docking of metal based schiff base ligand is a new approach in computational studies. In one of the studies scientists use crystallographic data of macromolecule from protein data bank and dock against new ligands (Berman *et al.,* 2006). Docking is a method used to predict the preferred orientation of ligand to targeted protein and also predict the binding affinity of small molecules with receptor that results in new complex with overall minimum energy (Sankar and Varun, 2012; Mahmoud *et al.,* 2020). Heterocyclic compounds containing pyridine, pyrazine and related molecules are good ligand due to the presence of at least one ring nitrogen atom. The successful application of heterocyclic compounds has led to the formation of series of novel compounds with broad spectrum of reactivity and stability (Diab *et al.,* 2016). Our interest is focused on the investigation of coordination chemistry of transition metal complexes through molecular docking.

MATERIALS AND METHODS

Target protein preparation

Docking studies was carried out using Auto Dock Tools 1.5.6 (Garrette *et al.,* 1998). The crystal structures of receptor proteins were picked from RCSB Protein Data Bank (PDB) with PDB ID: 5KAV (https://www.rcsb.org/structure/5KAV) for protein binding to voltage gated sodium channel as antiepileptic drug; and the crystal structure of dihydrofolate reductase enzyme was obtained from RCSB Protein Data Bank having PDB ID-3GHW (https://www.rcsb.org/structure/3GHW) for anticancer activity (Gangjee *et al.,* 2009). Macromolecules were read by AutoDock 1.5.6; impurities were removed, whereas, partial charges and hydrogen atom were put onto the protein. Macromolecules were saved in their respective PDBQT format for ligand interaction (Yang *et al.,* 2013). Major

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aim of molecular docking was to achieve an optimized conformation for both drug and protein to minimize free energy of overall system with absolute orientation between them (EI-Bindary *et al.*, 2015). Binding site of target was selected based on amino acid residues involved in binding to enzymes; which would be considered as the best active region (Ebrahimipoura *et al.*, 2015). Docking was a well-established computational technique; used to predict interactions between enzyme receptor with newly designed compounds (Mahmoud *et al.*, 2020).

Ligand preparation

The coordination sphere of lamotrigine; as standard anticonvulsant agent; and its ligand-metal complexes were generated and optimized from ACD/Chem Sketch software as MDL file. Open Babel GUI 3.0 software converts MDL files to PDB format; which later on used for automated docking by AutoDock software (Morris *et al.*, 2009; Ebrahimipoura *et al.*, 2015). Molecular docking was used between schiff base ligands and receptor of voltage gated sodium channel (PDB: 5kav); and the receptor of breast cancer hormone (PDB: 3GHW), in order to compute the binding affinities of schiff base ligands (Diab *et al.*, 2016). Some properties of lamotrigine and its ligand-metal complexes with their structures and smile notation were presented in table 1.

Molecular docking and scoring

Schiff base ligand-metal complexes were analyzed for binding affinity at voltage gated sodium channel and dihydrofolate reductase enzymes for binding energy calculations and lead optimization (Elzaher *et al.*, 2016). AutoDock Tools 1.5.6 software was used to simulate the binding conformations of ligand on voltage gated sodium channel for antiepileptic effect and dihydrofolate reductase enzyme for anticancer action and explores the binding sites of docked molecule which utilizes Lamarckian Genetic Algorithm (LGA). The grid box was centered at -14.797, 10.381 and -0.196 Å, with grid spacing of 0.375 Å. The grid box was set at 126x126x126 Å along X, Y and Z axis with the auto dock parameters used were:

1. Genetic algorithm with population size = 150
2. Maximum number of energy evaluations = 2,500,000
3. Genetic algorithm cross-over mode = 2 points

All prepared compounds were docked into the same binding site of the native co-crystallized ligand. Ten docked conformations (poses) were obtained after protein-ligand docking at antiepileptic and anticancer receptors. Cluster analysis of protein binding sites with lowest binding energy was further explored using discovery studio visualizer (Poureshghi *et al.*, 2017).

Validation of docking protocol

The reliability of docking program was validated by using re-docking method. In both cases the co-crystallize ligands were redocked in the active site of enzyme. Both RMSD as well as native ligand interaction with in the crystal structure of voltage gated sodium channel and dihydrofolate reductase receptor were used as standard docked models (Elzaher *et al*., 2016). Root mean square deviation (RMSD) was then calculated and in all cases RMSD value of <2.0 Å was considered accurate in predicting binding orientation of ligand (Jabeen *et al.*, 2018).

RESULTS

On the basis of docking energies and good interaction with active site residue the docked ligand molecules were selected (Colovos and Yeates, 1993). 2D structures and smile notation for docking of schiff base ligands were presented in table-1; along with their molecular formula, formula weight and melting points. Docking of schiff based metal complexes of lamotrigine at voltage gated sodium channel and dihydrofolate reductase enzymes were carried out to compute different models with different binding energies of protein-ligand interaction. Some parameters of Auto Dock were estimated and presented in tables 3 and 5; whereas their interacting residues were presented in tables 2 and 4. Binding free energy, inhibition constant (Ki) and interacting surface area reveal the most favorable binding (Diab *et al.*, 2019). The model with least binding energy was selected, as the more negative value of estimated binding free energy represents more efficient binding. The docking score and binding interactions of docked ligands were associated with their capability to inhibit the activity of voltage gated sodium channel and dihydrofolate reductase enzyme and show a potential for its anticonvulsant and anticancer activities. LAC4 shows most significant binding with voltage gated sodium channel as shown in fig. 1 and 2; it also show significant activity at dihydrofolate reductase enzyme for anticancer activity as shown in fig. 3 and 4. Docking describes the binding mode or mechanism of chemical moieties in the pocket of enzymes (Mahmoud *et al.*, 2020).

DISCUSSION

The present work comprises of in-silico simulation studies of macromolecule and small molecule (Althchul *et al.*, 1990). Molecular docking studies were used to estimate the enzyme ligand interacting geometries for selected compounds (Elzaher *et al.*, 2016). The docking score and amino acid residues involved in binding at voltage gated sodium channel for antiepileptic activity of schiff base ligand of lamotrigine and its metal complexes was demonstrated in table 2.

The amino acid residues show hydrogen bonding, hydrophobic and ionic interactions with ligand at receptor site. It was discovered that the potential of LA, LAC1, LAC2, LAC3 and LAC4 against voltage gated sodium

channel was linked with binding energies and number of hydrogen bonds, hydrophobic bonds and ionic bonds formed at the catalytic site. The standard drug for voltage gated sodium channel was lamotrigine with amino acid residues showing hydrogen bonds were SER93, and SER94, whereas, ALA92, VAL128, ALA131, VAL155, and GLY157 shows hydrophobic interaction at the binding site, while, LYS95 and LYS156 shows ionic interaction. As we can see from table 2 the least binding energy was shown by LAC4 at voltage gated sodium channel and amongst the interacting amino acids THR90 shows hydrogen bonding, ILE62, ILE83, ALA84, TYR86, VAL88, ILE89, and PRO132 shows hydrophobic interactions, whereas, LYS17, and ASP87 shows ionic interactions. table 3 demonstrates the protein binding

Table 1: Properties and 2D structures of [6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine] metal complexes.

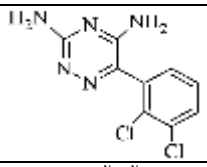
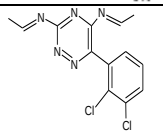
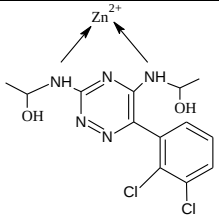
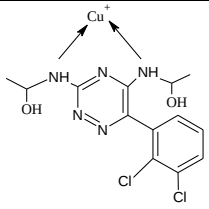
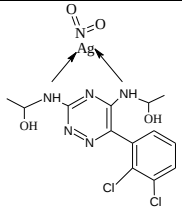
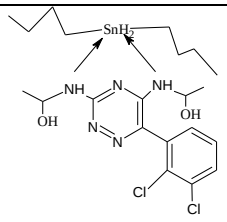
Compound identity codes	Molecular formula	Formula weight (g/mol)	2D structures with smile notation	Melting point (°C)
LMT (standard)	$C_9H_7Cl_2N_5$	256		200
LA	$C_{13}H_{15}Cl_2N_5O_2$	344	 CC(O)Nc1nc(NC(C)O)c(nn1)c2cccc(Cl)c2Cl (1*E*,1'*E*)-*N*,*N*'-[6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diyl]di(ethan-1-imine)	205
LAC1	$C_{13}H_{13}Cl_2N_5ZnO$	407	 CC(O)Nc1nc(NC(C)O)c(nn1)c2cccc(Cl)c2Cl	105
LAC2	$C_{13}H_{13}Cl_2CuN_5O_2$	405	 CC(O)Nc1nc(NC(C)O)c(nn1)c2cccc(Cl)c2Cl	78
LAC3	$C_{13}H_{11}AgCl_2N_5$	416	 CC(O)Nc1nc(NC(C)O)c(nn1)c2cccc(Cl)c2Cl	230
LAC4	$C_{21}H_{29}Cl_2N_5Sn$	541	 CC(O)Nc1nc(NC(C)O)c(nn1)c2cccc(Cl)c2Cl	Decompose above 200

Table 2: Lowest binding energy of lamotrigine analogues with amino acids residues involved in drug binding at voltage gated sodium channel (5kav).

No	Drug with voltage gated sodium channel	Lowest binding energy	Amino acid residues involved in binding with 5kav
1.	LA	-6.75	LEU8, GLY52, ASP53, LEU54, ALA86, GLN136, LEU138, ILE148, GLU150, TRP152
2.	LAC1	-6.83	GLU2, GLN4, GLN6, VAL96, PHE99, GLU100, GLU150
3.	LAC2	-6.19	GLU100, ALA101, GLN102, ALA108, GLN111, LYS112
4.	LAC3	-6.41	GLU100, ALA101, GLN102, ALA108, GLN111, LYS112, GLU115
5.	LAC4	-10.02	LYS17, ILE62, ILE83, ALA84, TYR86, ASP87, VAL88, ILE89, THR90, PRO132
6.	LMT std.	-6.13	ALA92, SER93, SER94, LYS95, VAL128, ALA131, VAL155, LYS156, GLY157

Table 3: Protein binding (5kav) and kinetic properties of schiff base derivative of lamotrigine and its metal complexes.

Property/Name	LMT(std.)	LA	LAC1	LAC2	LAC3	LAC4
Molecular mass (g)	256.091	344.196	407.589	405.727	416.033	541.103
No of rotatable bonds	1	1	1	1	1	2
Lowest binding energy (Å)	-6.13	-6.75	-6.83	-6.19	-6.41	-10.02
Cauchy alpha	0.0	0.0	0.0	0.0	0.0	0.0
Cauchy beta	1.0	1.0	1.0	1.0	1.0	1.0
Active torsions	1	1	1	1	1	2
Inhibition constant (ki)	32.24	11.36	9.81	28.87	19.99	45.12
Total intermolecular energy (kcal/mol)	-6.43	-7.04	-7.13	-0.61	-0.62	-10.62
Total internal energy (kcal/mol)	-0.23	-0.11	-0.61	-0.61	-0.62	-1.30
Torsional energy (kcal/mol)	+0.30	+0.30	+0.30	+0.30	+0.30	+0.60
Temperature (K)	298.15	298.15	298.15	298.15	298.15	298.15

Table 4: Lowest binding energy of lamotrigine analogues with amino acids residues involved in drug binding at dihydrofolate reductase (DHF) enzyme.

No	Drug with DHF enzyme	Lowest binding energy	Amino acid residues involved in binding with 3GHW
1.	LA	-6.99	GLY20, ASP21, LEU22, LYS55, THR56, SER59, GLY117, THR146
2.	LAC1	-6.61	LYS18, ASN19, ASP21, LEU22, PRO23, TRP24, PRO26, PHE31
3.	LAC2	-8.99	ALA9, ILE16, GLY17, GLY20, LEU22, LYS55, THR56, SER59, GLY117, SER118, TYR121, GHW187
4.	LAC3	-9.08	ALA9, ILE16, GLY17, GLY20, LEU22, LYS55, THR56, SER59, SER116, GLY117, TYR121, GHW187
5.	LAC4	-7.58	VAL1, LEU4, LEU97, LYS98, GLU101, VAL112, HIS127, PRO128, GLY129, HIS130
6.	LMT std.	-6.65	ILE16, GLY20, LEU22, LYS55, THR56, GLY116, GLY117, SER118, SER119, GHW187

Table 5: Protein binding (3GHW) and kinetic properties of schiff base derivative of lamotrigine and its metal complexes.

Property/Name	LMT(std.)	LA	LAC1	LAC2	LAC3	LAC4
Molecular mass (g)	256.091	344.196	407.589	405.727	416.033	541.103
No of rotatable bonds	1	1	1	2	2	2
Lowest binding energy (Å)	-6.65	-6.99	-6.61	-8.99	-9.08	-7.58
Cauchy alpha	0.0	0.0	0.0	0.0	0.0	0.0
Cauchy beta	1.0	1.0	1.0	1.0	1.0	1.0
Active torsions	1	1	1	2	2	2
Inhibition constant (ki)	13.33	7.47	14.29	255.42	221.72	27.61
Total intermolecular energy (kcal/mol)	-6.95	-7.29	-6.91	-9.29	-9.38	-8.78
Total internal energy (kcal/mol)	-0.23	-0.10	-0.61	-0.61	-0.61	-1.27
Torsional energy (kcal/mol)	+0.30	+0.30	+0.30	+0.30	+0.30	+1.19
Temperature (K)	298.15	298.15	298.15	298.15	298.15	298.15

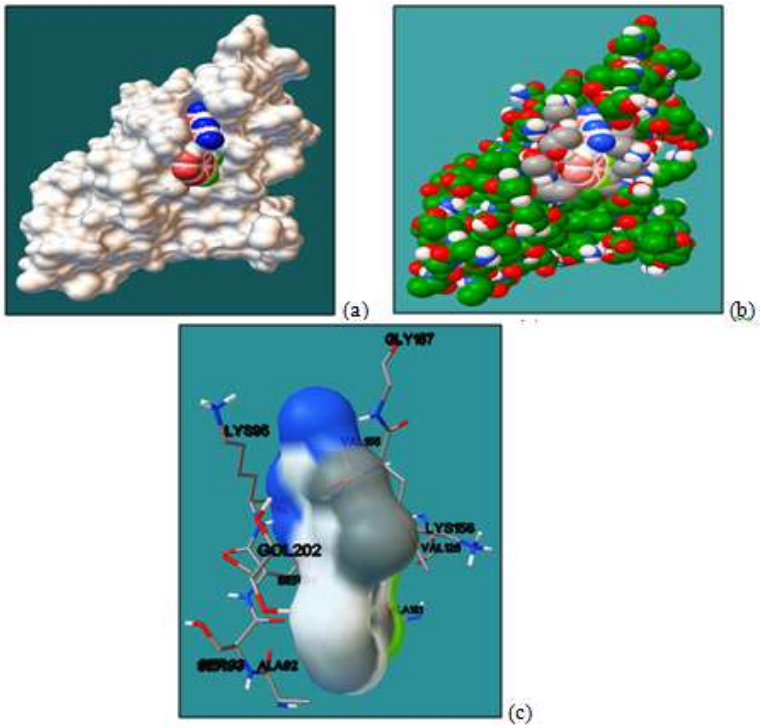


Fig. 1: (a) Ligand (pink) present on the molecular surface of protein (white); (b) The atomic surface of protein shown in green with the drug bound in pink color at catalytic site; (c) Amino acid residues involved in the binding of LMT at voltage gated sodium channel (5kav) with least binding energy.

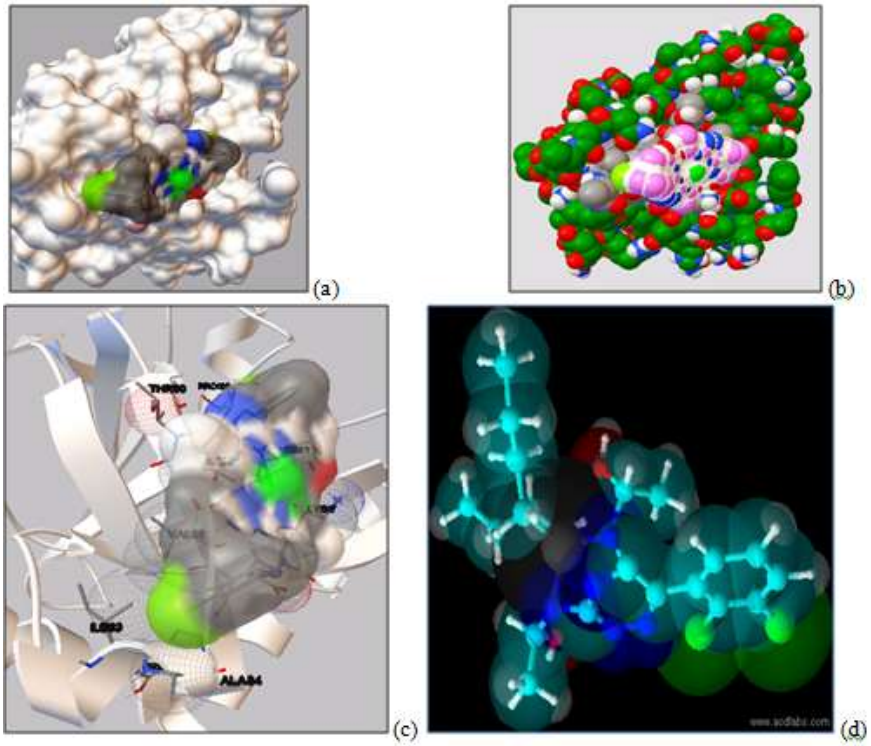


Fig. 2: (a) Molecular surface of protein (white) in interaction with ligand (grey) at catalytic site; (b) Atomic surface of protein 5kav (green) with interacting ligand (pink); (c) Secondary structure of protein with interacting amino acid residues at the catalytic site of voltage gated sodium channel for antiepileptic effect; (d) 3D structure of LAC4, metal is shown as black ball with surrounding amines (dark blue) and methyl (light blue) functional groups, whereas green balls represent chloride.

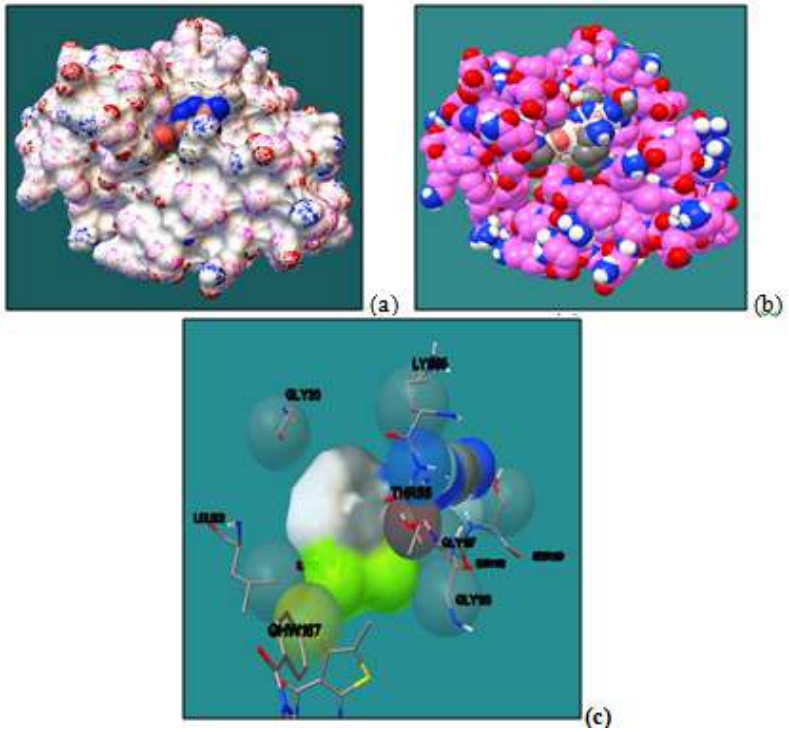


Fig. 3: (a) Molecular atomic surface of protein with interacting ligand (pink); (b) Atomic surface of protein (green) in contact with ligand (pink); (c) Amino acid residues of dihydrofolate reductase enzyme at the catalytic site bound with ligand.

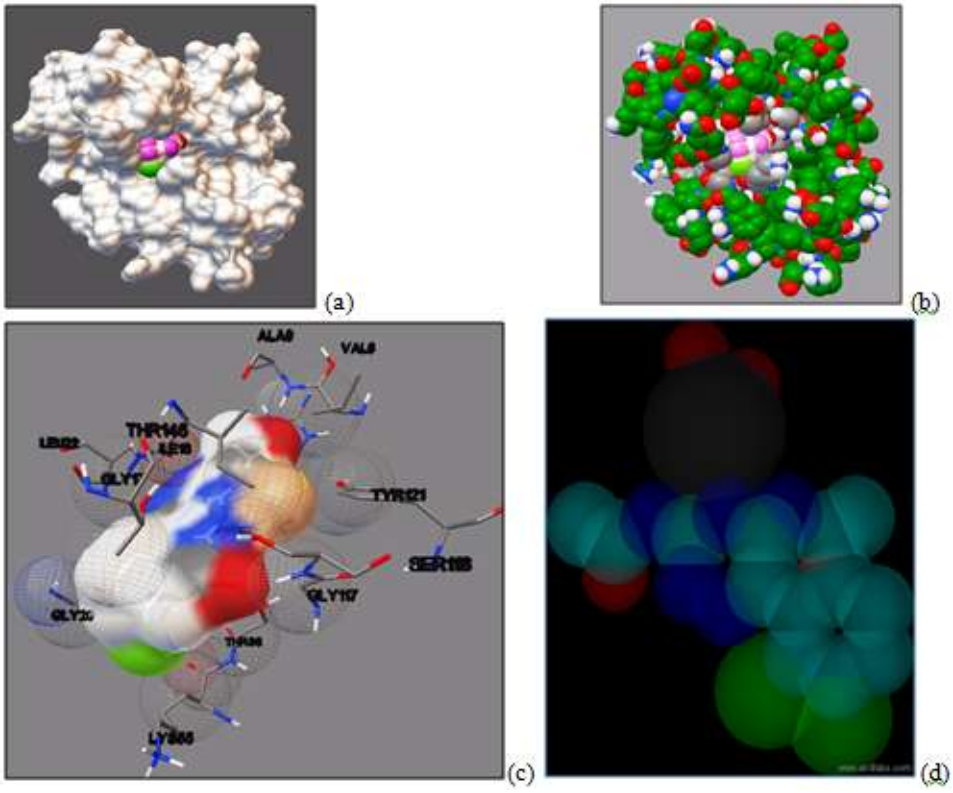


Fig. 4: (a) Ligand (pink) in interaction with the molecular surface (white) of protein; (b) Atomic surface (green) of dihydrofolate enzyme with interacting ligand (pink); (c) Amino acid residues in the binding pocket of protein (3GHW) in close interaction with ligand; (d) 3D molecular structure of LAC4, silver metal is shown as black ball with surrounding methyl (light blue) and amine (dark blue) functional groups, whereas green balls represent chloride.

properties at voltage gated sodium channel. LMT being standard shows binding energy of -6.13 Å, with 1 active torsion and 1 rotatable bond at 298.15K. LA, LAC1, LAC2, LAC3, and LAC4 have binding energies of -6.75Å, -6.83 Å, -6.19 Å, -6.41 Å and -10.02 Å respectively. All of them show 1 active torsion and 1 rotatable bond at 298.15 K on voltage gated sodium channel.

The docking score of LA, LAC1, LAC2, LAC3 and LAC4 with dihydrofolate reductase enzyme and interacting amino acid residues were demonstrated in table 4. It was discovered that LAC3 have the greatest potential against dihydrofolate reductase enzyme than others and was strongly related with the binding energies and number of hydrophobic interactions, hydrogen bonds and ionic bonds formed at the catalytic site of enzyme.

The drug used to standardize the results was lamotrigine. The amino acids involved in hydrogen bonding with lamotrigine at dihydrofolate reductase binding site were THR56, SER118 and SER 119, while, the amino acids involved in hydrophobic interaction were ILE16, GLY20, LEU22, GLY 116, and GLY117, whereas, amino acid involved in ionic interaction was found to be LYS55 and GHW187. We compare the binding energies of schiff base ligand and its metal complexes and LAC3 was found to be the most active dihydrofolate reductase inhibitor. The amino acid residues involved in hydrogen bonding of LAC3 at the binding site of dihydrofolate enzyme were THR56, SER59 and SER116, whereas, ALA9, ILE16, GLY17, GLY20, LEU22 and GLY117 were found to show hydrophobic interactions, while, LYS55, TYR121 and GHW187 were involved in ionic interactions. The protein binding properties of LA-series at dihydrofolate receptor was shown in table 5. The drug used to standardize the results was LMT, having binding energy of -6.65 Å with 1 active torsion and 1 rota table bond at 298.15 K.

LA, LAC1, LAC2, LAC3, and LAC4 have binding energies of -6.99 Å, -6.61 Å, -8.99 Å, -9.08 Å and -7.58 Å respectively. LA and LAC1 have 1 rotatable bond, while, LAC2, LAC3 and LAC4 have 1 rotatable bond. LA, LAC1, LAC2, and LAC3 shows only 1 active torsion, whereas, LAC4 shows 4 active torsions at anticancer binding site at 298.15 K. The binding energy of LMT (standard) was -6.13 Å and the residues involved in its binding are ALA92, SER93, SER94, LYS95, VAL128, ALA131, VAL155, LYS156 and GLY157 as shown in fig. 1c.

The binding energy of LAC4 was lowest as compared to other schiff base complexes at voltage gated sodium channel, hence LAC4 show greater affinity with voltage gated sodium channel. The molecular surface and atomic surface of voltage gated sodium channel is cleared from fig. 2 (a) and (b) The binding energy of LAC4 was found to be -10.02 Å; with the residues involved are LYS17, ILE62, ILE83, ALA84, TYR86, ASP87, VAL88, ILE89, THR90 and PRO132 in the binding pocket of protein as shown in fig. 2c. 3D structure of LAC4 was shown in fig. 2d along with interacting functional groups

The surface bound drug is shown in fig. 3 (a) and (b) with the binding energy of -6.65 Å. The amino acid residues involved in its binding at the binding site are ILE16, GLY20, LEU22, LYS55, THR56, GLY116, GLY117, SER118, SER119 and GHW187 as shown in fig. 3c. The binding energy of LAC3 (-9.08 Å) was found to be lowest as compared to other schiff based complex at dihydrofolate reductase binding site, hence show greater binding affinity at anticancer receptor.

The binding of drug molecule on surface of enzyme is shown in fig. 4 (a) and (b). The residues involved in binding of LAC3 at dihydrofolate reductase site involve ALA9, ILE16, GLY17, GLY20, LEU22, LYS55, THR56, SER59, SER116, GLY117, TYR121, and GHW187 as shown in fig. 4c. Three dimensional structure of LAC3 was shown in fig. 4d

These in-silico docking results of voltage gated sodium channel and dihydrofolate enzyme were in good agreement with the *in-vitro* experimental data for anticonvulsant and anticancer activities respectively. In particular, this study is the first report of antiepileptic effect of schiff base and its metal complexes on voltage gated sodium channel and anticancer activity on dihydrofolate reductase enzyme via molecular docking simulation technique. By molecular docking studies we can also predict the kinetic properties of drug and its complexes on its protein receptors. These are the molecular basis of our in-vitro studies. We synthesized the chemical compounds in laboratory and then perform in-vivo anticonvulsant and anticancer studies based on these results.

CONCLUSION

In conclusion, [6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine] metal derivatives were evaluated for their in-silico voltage gated sodium channel and dihydrofolate reductase enzymes inhibitory potentials. As a result of in-silico evaluation of compounds; LAC4 express significant binding affinity with voltage gated sodium channel at 5kav and show excellent antiepileptic property. Regarding in-silico studies of dihydrofolate reductase inhibitor, compound LAC3 shows strong interaction with active site residues of 3GHW enzyme. In short, the antiepileptic and anticancer potential of [6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine] metal derivatives was established for lab synthesis of compounds and their implementation on cellular level.

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