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Discovery of Novel Alanine Racemase Inhibitors Through Cheminformatics and Biophysical Approaches

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Abstract

Antibiotic resistance in bacteria is rapidly increasing across the globe which warrants the development of new drugs to tackle this issue. Since alanine racemase is essential to bacterial survival, novel antibiotics could have efficacy targeting it, particularly in light of the growing prevalence of antibiotic resistance. This work employs cheminformatics and biophysics approaches to find and optimize new inhibitors of alanine racemase. The objective of the research is to effectively find strong inhibitors and advance the development of novel drugs to fight resistant bacterial infections by integrating virtual screening with molecular dynamics simulations. Herein, using clerodane furanolactones as a parent structure, we identified several derivatives of the compound to show the best binding with bacterial alanine racemase enzyme and block the biosynthesis of bacterial cell walls. Three compounds; Top-1, Top-2, and Top-3 with a binding affinity (in kcal/mol) of -8.7, -8.6, and -8.5, respectively were identified. The compounds were classified as good drug-like molecules as they clear parameters of Lipinski, Ghose Muegge, Vber, Egan, and MDDR drug-like rules. According to molecular dynamics simulation findings, the enzyme remained in good stable dynamics in the presence of compounds throughout the length of simulation time with average RMSD within the 3 Å range. Further observation noticed the enzyme residues in good overall stable behavior, particularly the active site residues. The intermolecular strength of interactions between the enzyme and compound was additionally cross-validated by binding free energy analysis. The net binding free energy (in kcal/mol) of Top-1, Top-2, and Top-3 is -22, -25.21, and -17, respectively in the MM-GBSA method and -32.14 (Top-1),-29.45 (Top-2) and -32.87 (Top-3) in WaterSwap. Together this study indicated the screened compounds as promising antibacterial and might be investigated in the experimental analysis for biological activity.

Keywords: Alanine Racemase; Molecular docking; Auto-dock Vina; Molecular Dynamic Simulation; Water Swap

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1. INTRODUCTION

The antibiotic resistance crisis is increasing to a very high level across the globe both at the hospital and community level [1]. Bacterial pathogens have acquired new resistant genetic determinants not reported previously and are spreading fast [2]. This results in difficult treatment, makes the hospital stay longer, and is associated with a high mortality rate [3]. Statistically, approximately 100,000 people have died annually due to antibiotic-resistant bacterial infections within the last 35 years is recorded [4]. the US Centers for Disease Control and Prevention (CDC) estimated 2.8 million cases of antibiotic-resistant bacterial infections and 35,000 deaths in the United States. This roughly equals one infection and death after every 11 seconds and 15 minutes, respectively [5]. Considering this worrisome situation with the inappropriate use of antibiotics and alarming evaluation of antibiotic resistance, new therapeutic approaches are urgently needed to overcome this global burden of antibiotic resistance and reduce antibiotic resistance-associated mortality and morbidity [2,6].

Clerodane furanolactones is a heterocyclic chemical compound that basically contains lactone and furan rings in its chemical composition, this compound is basically isolated from different plants including Sphenocentrum jollyanum Pierre and Jateorhiza calumba Miers. Recently notable biological activity has been reported, as well as this compound can act as a chemical defense against phytophagous animals or diseases. Furthermore, several other biological potency which are mainly include piscicidal trypanocidal and antimicrobial properties, however, the antifeedant properties of the said compound have been extensively reported. Heretofore 5 clerodane diterpenoids have been isolated from Pulicaria wightiana which represents highly antibacterial activity against Bacillus subtilis and several other bacterial species like staphylococcus aureus and gram-negative Klebsiella aerogenes, also antibacterial activity against Chromobacterium violaceum was reported. The gram-negative bacterial pathogens in particular are more problematic have higher pathogenic potential and are reported to be resistant to most of the clinically used antibiotics. In a study carried out from 2009 to 2012, this group of bacterial pathogens revealed a high degree of resistance to ceftazidime and imipenem, in U.S hospitals both in ICU and non-ICU units. For example, Escherichia coli resistance is recorded to show 11% resistance to ceftazidime and 0.3% to imipenem in ICU units while in the non-ICU units, 6.9% were to ceftazidime and 0.1% to imipenem. The Klebsiella pneumonia resistance in ICU units is 26.8% to ceftazidime, and 11.5% to imipenem while in non-ICU, 14.5% were resistant to ceftazidime and 5.8% to imipenem. The superbug Acinetobacter baumanni was resistant to ceftazidime (60.1%), imipenem (52%) in ICU and ceftazidime (35%) and imipenem (28%) in non-ICU while Pseudomonas aeruginosa resistant is 18.6% to ceftazidime and 23.2% to imipenem in ICU and 7.3% ceftazidime and 8.4% to imipenem in non-ICU [10].

Alanine racemase is a PLP-dependent enzyme which supervise the conversion of I-alanine to d-alanine during bacterial peptidoglycan biosynthesis. As there is known homolog present in humans, the enzyme is considered as an excellent antimicrobial target. For the development of new drugs computational studies play a major role in the designing and discovery of drugs in order to reduce the time, computational studies include molecular docking, de-novo design as well as virtual screening [11]. The computational studies of drug design give us information about the structure of the target and ligand in the discovery of new drugs [12]. The screening technique is used to minimize the time and price for the discovery/development of potent drugs. The impotence via structure-base

and ligand-based drug design suggests their compulsory use. More ever there integration with experimental routines, so have a good impact on rational drug design (Macalino, Gosu, Hong, & Choi, 2015).

2. MATERIALS AND METHODS

The current study was designed for the identification of potential antibacterial agents by targeting alanine racemase enzyme using a multi-stage structure-based virtual screening approach as the flow diagram is presented in Figure 1.
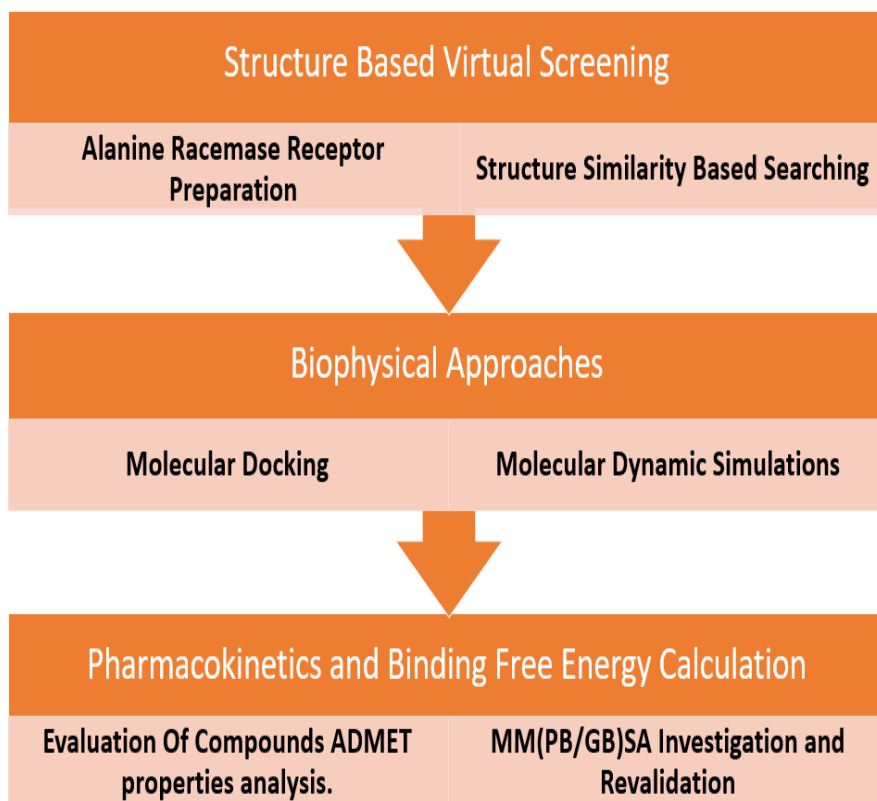
Structure Based Virtual Screening

Alanine Racemase Receptor Preparation | Structure Similarity Based Searching

Biophysical Approaches

Molecular Docking | Molecular Dynamic Simulations

Pharmacokinetics and Binding Free Energy Calculation

Evaluation Of Compounds ADMET properties analysis. | MM(PB/GB)SA Investigation and Revalidation

Figure 1. The study was started using Clerodane furanolactones as a parent structure to search for novel derivatives. The identified compounds were used in virtual screening against the alanine racemase enzyme. The study was further supported by molecular dynamics simulation and binding free energies.

2.1. Alanine Racemase Receptor Preparation

The 3D crystal complex structure of alanine racemase (PDB I.D; 1BD0) was downloaded from the protein data bank (PDB). The energy minimization of the receptor was done via UCSF Chimera software 1.15 to make it ready for docking calculations. The energy minimization consists of 1000 steps of the conjugate and steepest descent gradient algorithms. The protein was then saved to be employed in docking studies.

2.2. Structure Similarity-based Searching

The energy minimization of the target protein was performed to remove any unwanted residues or water molecules and prepare it for the molecular docking step. The structure of Clerodane furanolactones was drawn using ChemDraw ultra 12.0 and energy minimized in chem3D ultra 10.0 and saved as a PDB. The PDB file of the compound was used in an online smile translator and compound SMILE was generated. The Clerodane furanolactones was then used in the Chembl database; this database is used for the identification of the same structure compounds based on the percentage similarity ratio. The 100% similarity ratio means that the database will find the 100% same structure compound present in the database and the 60% similarity ratio means that the database will find the 60% matching structure compound. So 106 ligands were identified based on a 60% similarity ratio S-Table 1. To utilize these inhibitors in the docking procedure, the 2D structure of the compounds was drawn in ChemDraw keeping the exact bound angles and lengths [18]. Again the compounds were used in Chem3D ultra for the energy minimization process.

2.3. Molecular Interactions Analysis

Molecular docking method is a computational-based study to predict intermolecular conformation and interactions between a receptor and a ligand by using docking software PyRx 0.8. The docking was completed using Autodock-Vina Software 4.2.6 [20]. First of all, the docking of the 105 inhibitors including the Clerodane furanolactones with the targeted alanine racemase enzyme was executed. The grid box dimensions were set with search area coordinates at the center along the X, Y, and Z axis. The X=18.6609 Å, Y=19.3931 Å, and Z=71.3607 Å, and the size of the box was set to 25 Å. The binding site was defined to cover all possible binding sites of the receptor. Before docking the inhibitors, the docking protocol was validated by re-docking the co-crystallized ligand (IN5) against the identified active pocket of the enzyme. The produced RMSD value (less than 2 Å) indicated the validity of the process (1). The binding score of the best-generated pose was used as a threshold for screening the inhibitors. The energy is measured in kcal/mol and the lowest binding energy is considered the best docking complex. The best docked soluations were analyzed through UCSF Chimera 1.15 [20], VMD v1.93 [21] and Discovery Studio (DS) Visualizer v2021 [22].

2.4. Evaluation of Compounds Pharmacokinetics

Computational pharmacokinetics of selected best-hit compounds was done through preADMET [23] and SwissADMETto find out the absorption, distribution, metabolism, excretion, and toxicity profile of the hit compounds in the body. [24].

2.5. Unveiling Complexes Dynamics

This study was carried out after docking analysis. The best-docked complexes were subject to molecular dynamic simulations through the AMBER software v20 to check the interaction of ligand and protein in dynamic environment. (Weiner & Kollman, 198c1). The preprocessing of complexes was done using antechamber program. The force field used for the protein is FF14Sb and the compound is GAFF. The energy minimization of complexes was done using the steepest descent and conjugate gradient algorithms. The complexes were then heated gradually to 310 K, followed by the equilibration phase. The production run was performed for 100 ns where the temperature was kept

constant using the Langevin algorithm. The trajectories were evaluated using the CPPTRAJ algorithm while plots were made using XMGRACE.

2.6. MM (PB/GB) SA Investigation and Revalidation

The trajectories of simulations were used further in MM (PB/GB) SA of AMBER20 [30]. The methods describe the difference between complex and protein and ligand alone. The frames were picked after each 0.2 ns from simulation trajectories and subjected to MMPBSA.py module [31]. The MMPBSA and MMGBSA binding free energies were revalidated by another sophisticated method such as WaterSwap. This method works on reaction coordinates where a bounded ligand is swapped with a cluster of water molecules of equal size and shape present in the active pocket. Further, the entropy energy calculation was done on selected simulation trajectory frames using AMBER normal mode analysis**.**

3. RESULTS AND DISCUSSIONS

3.1. Binding Mode and Interactions Analysis

A molecular docking study is an in silico study to decipher biomolecule dynamics. The study herein aims to determine the best docked intermolecular conformation of used compounds with alanine racemase. Re-docking of the co-crystallized ligand was also done to assess docking accuracy, and an RMSD of 1.829 Å was obtained, as well as a docking score of -6.6 kcal/mol for reference; the top three complexes showed binding affinity higher than the reference. The ligands of the top three complexes are tabulated in Table 1. The Top-1 binding energy (in kcal/mol) was -8.7, Top-2 was -8.6 and Top-3 was -8.5. The binding conformation of the compounds with the alanine racemase is illustrated in Figure 2.
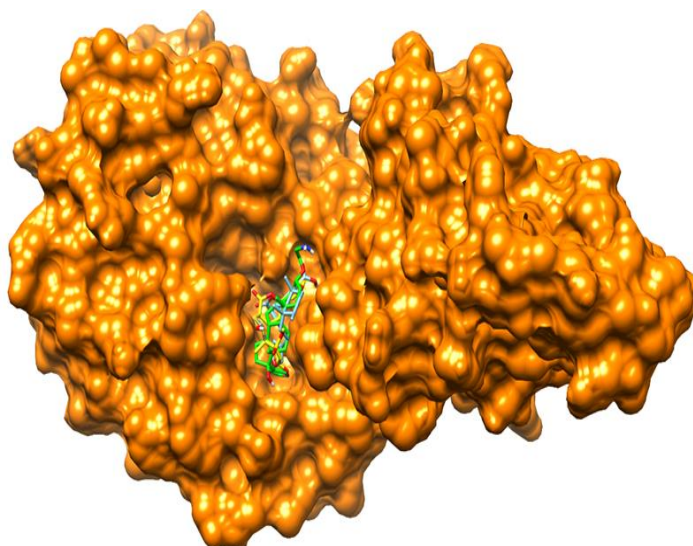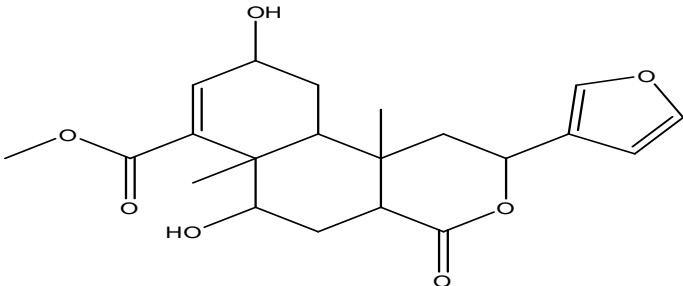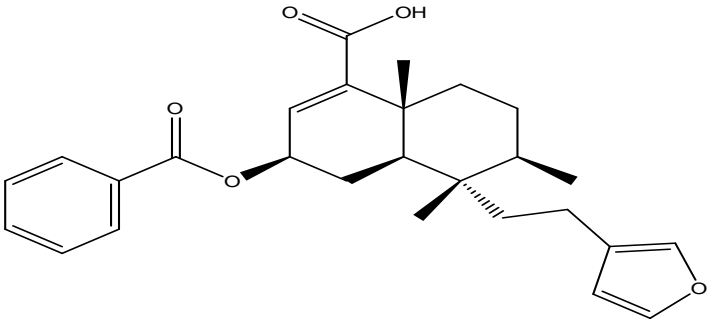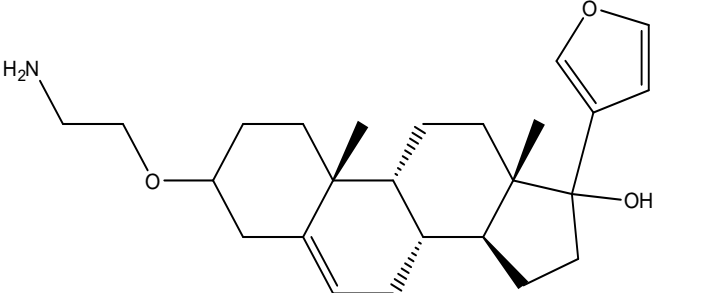

Figure 2. Binding conformation of compounds with the alanine racemase enzyme. The compounds are in sticks while alanine racemase is on an orange surface.

The binding interactions of complexes were determined using Discovery Studio Visualizer 2021 software. The Top-1 involves the van der Waals, conventional hydrogen bonds, pi-Sulphur, and Pi-Pi T-shaped interactions shown in Virtual Screening software. The active residues such as Tyr43 are

attached to furan with the help of Pi-Pi T-shaped interaction at a distance of 5.88 Å, while the adjacent carbon of furan interacts with the active residue of Cys358 is attached with the help of pi-Sulpher interaction at a distance of 8.44 Å. The tetrahydro-2H-pyran-2-one showed interaction with the active residue of Lys39 via position 4 at a distance of 5.67 Å attached with oxygen with the help of a conventional hydrogen bond. The Top-1 from position 5 interacts with the active residue of Arg136 at a distance of 6.26 Å with the help of a conventional hydrogen bond. Moreover, the active residue associated with cyclohexane-2 enol is Tyr354, Asn203, Asp171, Pro229, and Ile352S with the help of conventional hydrogen bonds. The active residue that is found in front of the cyclohexane is His A: 166 Met A: 134 and Ala A: 168. Are connected with the help of conventional hydrogen bonds Figure 3. The Top-2 compound involves the Van der Waals, Pi-Pi stacked conventional hydrogen bond and Pi-Alkyl interaction. The cyclohexane-1-carboxylic acid ring contains the active residue of Cys358 at a distance of 5.38 Å and Lys39 at a distance of 4.35 Å via conventional hydrogen bond interaction. The furan ring attaches the active residue of Tyr354 at a distance of 4.94 Å by Pi-pi stacked. The methyl benzoate ring attached to an active residue of His166 at a distance of 5.44 Å and Lys39 at a distance of 5.44 Å through the interaction of conventional hydrogen bond. The other active residue of an enzyme formed interaction with residues such as Tyr43, Ile222, Arg219, Gly221, Ser204, Asn203, Asp171, and Arg136 by the van der Waals interactions. The 1, 1, 2 trimethylcyclohexane ring of the compound interacts with Ala 40 and Ile352 through van der Waals and hydrogen interactions Figure 3. The Top-3 interaction involves the van der Waals, pi-alkyl and pi-anion interacting with furan moiety through Asp171 at a distance of 5.14 Å by pi-anion and Ala205, Pro229, Asn203, Ala168, Phe167, Met134 attached via van der Waals interaction and 3-methyl-Cyclohex-1-ene ring interact with Lys39 Cys358, Arg136, His166, and Ile352 by van der Waals force and the 2-(cyclohexyl oxy) ethanamie interact with Tyr354 at a distance of 4.81 Å by pi-alkyl bond. The interaction of the compound with Ala40, Lys39, and Cys358 by Van der Waals bond is shown in Figure 3.

Table 1. Selected best binders to alanine racemase enzyme. All values in kcal/mol.

S.no	Compound	Structure	Energy value
1	Top-1 SCHEMBL12544489		-8.7 kcal/mol

2	Top-2 SCHEMBL12655234		-8.6 kcal/mol
3	Top-3 SCHEMBL12655360		-8.5 kcal/mol

A

Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Positive-Positive
Pi-Pi Stacked

B

Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Sulfur
Pi-Pi T-shaped

C

Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Pi Stacked
Pi-Alkyl
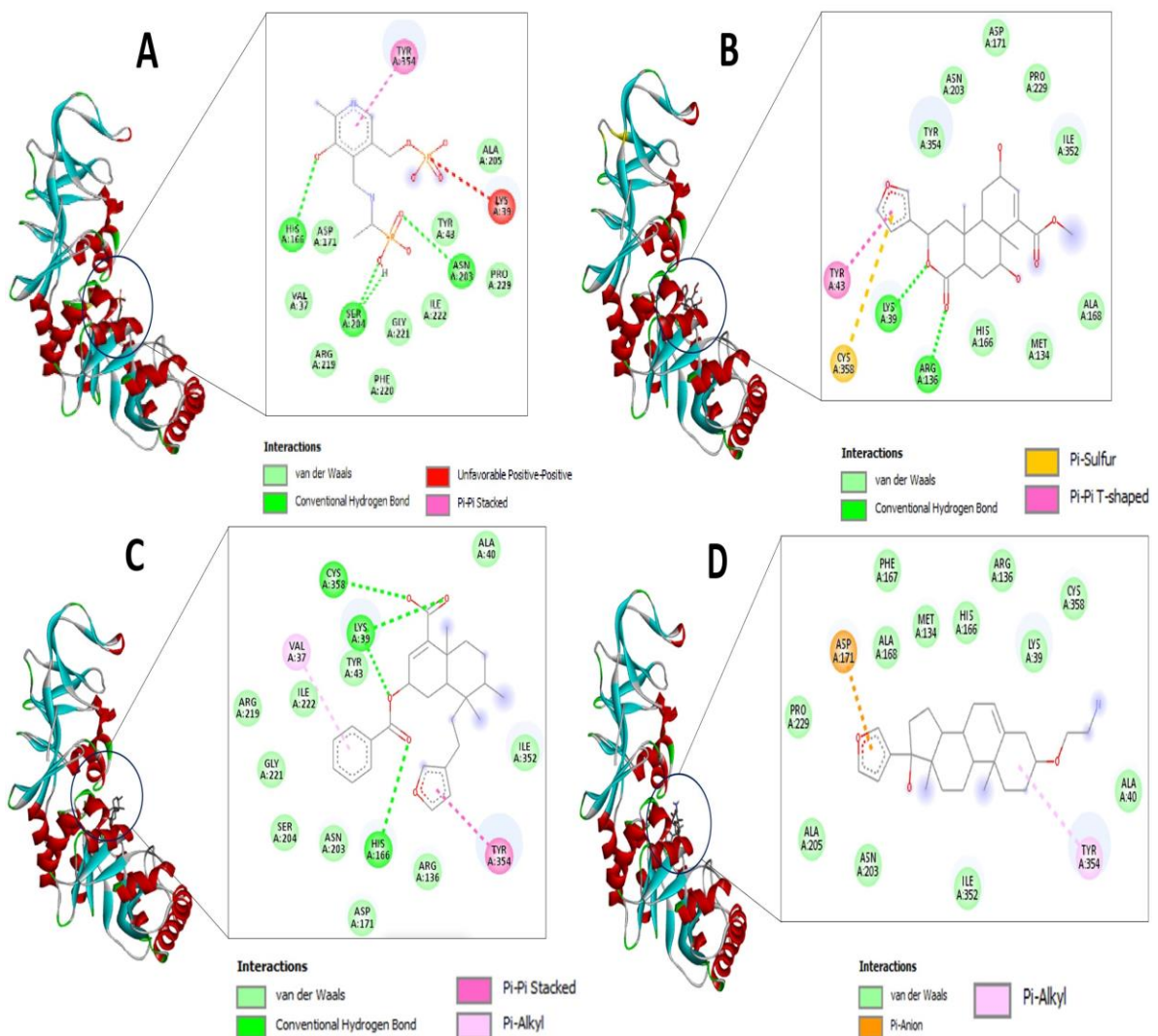
D

Interactions
van der Waals
Pi-Anion
Pi-Alkyl

Figure 3. Docked complexes of alanine racemase with different ligands (Alanine racemase shown probable interaction with different compounds, the highest initiative amino acids residues are presented with different colors. (A) Re-docking of the co-crystallized ligand. (B) Top-1 complex. (C) Top-2 complex (D) Top-3 complex.

3.2. Prediction of Compounds Pharmacokinetic Properties

The development of drugs often suffers from unsuccessful clinical testings which can waste time and the assioacted cost. Therefore, the prediction of pharmacokinetic properties is vital in preclinical drug development to minimize drug failure [32]. The prediction of compounds pharmacokinetics properties has facilitated the selection of appropriate drugs for lead optimization. All the priortized compounds in the study were classified as a druglike and follow druglike rules scuh as Muegge, Vber, Egan, and MDDR rules. The gastrointestinal absorption (GI) of all compoudns was high thus demonstrating the good amount of the drugs can be obtained at the target site for biolgoical action. The compounds were found not to able to cross the blood-brain barrier (BBB). The ability of drugs to be retained in the blood stream for an extended for a good tieme period is an important property for its delivery to the target sites [33]. The bioavailability is recorded as Top-1 (0.55), Top-2 (0.85) and Top-3 (0.55). The bioavailability of compounds depends on their predominant charge at biological pH. This property of the compound can be used to evaluate its binding efficacy for the plasma protein binding (PPB). The logk value was in the following order; Top-1 (-8.40cm/s), Top-2 (-5.11cm/s), and Top-3 (-7.20cm/s). The elevated percent of PPB and small logk indicates that the compounds may be retained within the bloodstream for an excessive period of time as a consequence maximizing its availability to the target sites. In addition, the absorption capability of the compound in the skin and the intestinal is measured via Sklog P and log D values [35]. The excessive Sklog P and log D values further reveal that the compounds may be correctly absorbed through the skin pores and from the intestine. Moreover, the action of the ligand on CNS (Central Nervous System) and the activeness of the compound to pass the CNS barrier changed into computed on the idea of the molecular weight, the molecular weight of Top-1 (392.44 g/mol), Top-2 (438.56 g/mol) and Top-3(401.58 g/mol). The TPSA of Top-1 is 392.44 g/mol), Top-2 is 438.56 g/mol) and Top-3 is 401.58 g/mol. It is believed that lower molecular weight, topological polar surface area (TPSA), and NRB values are related to extended CNS penetration. In this analysis, it was found that the compounds are appropriate candidates for designing derivatives with more improved physiochemical, pharmacokinetics, and ADMET features.

3.3. Molecular dynamic simulations

The dynamic behavior of docked complexes was investigated using molecular dynamics simulation to gain a detailed analysis of the structural adjustment adopted by the enzyem in the presence of inhibitors during simulation time. The extent that a protein or other macromolecule deviates from its initial form throughout the course of a simulation has been tracked using Root mean square deviation (RMSD). First of all, RMSD analysis was conducted to measure the average distance among superimposed complexes snapshots picked from different time frames of simulation trajectories. The docked intermoelcular conformation was used as a reference strucutre. To study complexes RMSD is vital to decipher the structure stability of receptor enzyme in the presence of ligand molecules. The Top-1 complex revealed the lowest RMSD compared to the other complexes. The RMSD plot of the Top-1 complex showed fluctuations within the range of 1.3–3.16 Å and finally reached a stable state after 95 ns of simulation. The mean RMSD value for the Top-1 complex is 1.98 Å. The RMSD of the Top-2 complex increased from the beginning of the simulation until 65 ns, reaching a value of 3.82 Å. From that point until the end of the simulation, the RMSD value decreased slightly. The mean RMSD value for the Top-2 complex is 2.6 Å. The RMSD plot of the Top-3 complex increased from the beginning of the simulation until 18 ns, then ranged between 2 and 3.2 until 94 ns and subsequently decreased. The Top-3 mean RMSD value is 2.37 Å. These RMSD values demonstrate that the receptor enzyme remained structurally intact and no major global or local deviations were noticed.The root mean square fluctuation (RMSF) plot provides crucial insights regarding the flexibility of the complexes. High fluctuations in the plot suggest increased flexibility and unstable bonds, while lower values or less fluctuations indicate well-structured sections in the complexes. The mean RMSF values of

Top-1, Top-2, and Top-3 complexes were 1.03 Å, 1.01 Å, and 0.95 Å, respectively. The RMSF analysis revealed that the enzyme residues experienced good stable energy except for flexible loop deviation located at the N-terminal and C-terminal of the enzyme. The loops are naturally flexible and behave more dynamically when ligands bind. However, the loops deviations were not found to destabilize the ligands binding and conformation. As shown in Figure 4, all of the complexes showed almost similar patterns. The Beta Factor plot in Figure 4 is in accordance with RMSF trends, whereby the mean value of the Beta Factor of the Top-1 complex is 34.43 Å, Top-2 complex is 31.05 Å, and Top-3 complex is 28.18 Å. The radius of gyration (Rg) tells about enzyme compact nature. The Rg of the Top-1 complex found to be almost stable with low amount of Rg flucutations seen. The mean Rg of Top-1 complex was 25.24 Å. The Top-2 complex from 20 to 100 ns has the highest Rg value when compared to other complexes, indicating less regid secondary structure elements compared to other complexes. The mean Rg value of Top-2 complex was 25.50 Å. The Top-3 complex exhibited minimum Rg value between 38 and 48 ns; the mean Rg value of the Top-3 complex is 25.3 Å. These values illustrate the stable structure dynamics of the selected complexes and witness the good compact nature during simulation. The simulation-based plots are given in Figure 4.
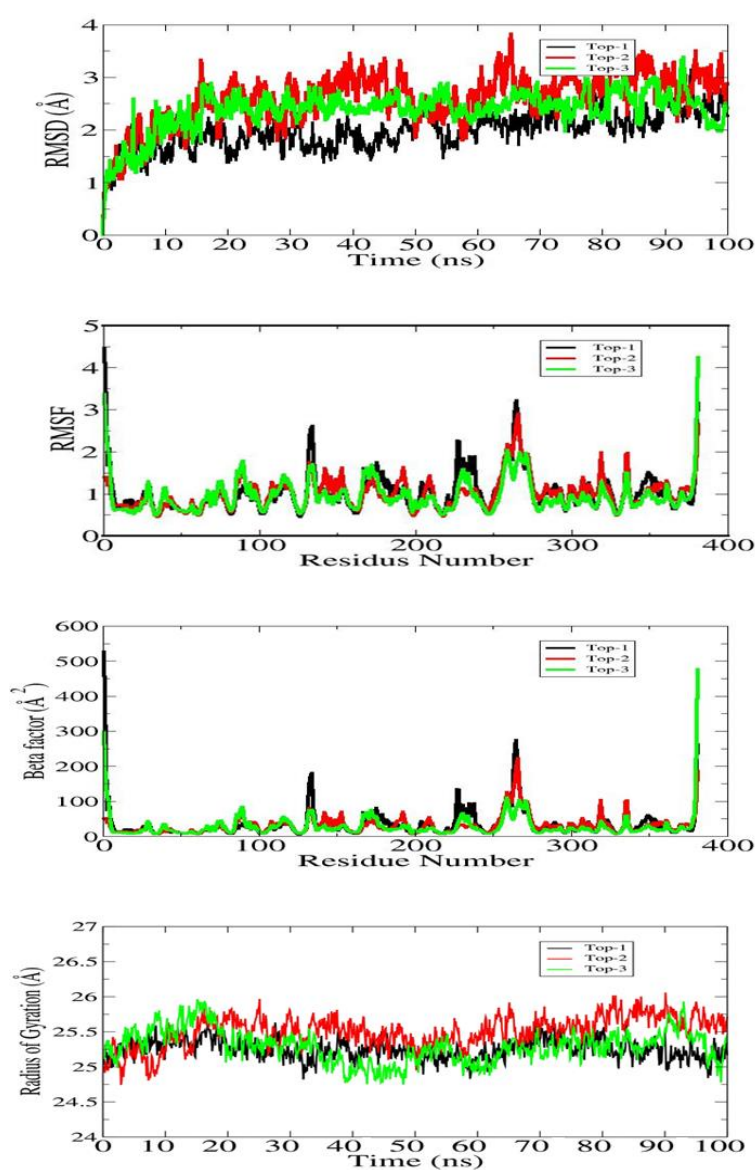

Figure 4. Molecular dynamics simulation analysis. The simulation analysis was conducted for 100 ns. All the analyses were done based on carbon alpha atoms.

3.4. Estimating Binding Free Energies

The binding free energy calculation is considered more reliable than docking and often more comparable to experimental studies. The binding free energy value (in kcal/mol) of 5000 frames for the Top-1, Top-2, and Top-3 complex in MMGBSA was -33.45, -30.87, and -31.65, respectively. In MMPBSA, the corresponding free energy values for the Top-1, Top-2, and Top-3 complexes was -34.18, -31.05, and -32.36, respectively. The van der Waals and electrostatic energies played a significant role in complexes stabilization while solvation energy is non-favorable. Based on the calculated values, the contributions of Van der Waals energy appear to be significant in both methods. The data reveal that the Top-1 complex has the most negative binding free energy in both MMGBSA and MMPBSA, indicating a stronger binding of the Top-1 compound to alanine racemase; the Top-3 and Top-2 compounds also showed a favorable binding free energy with alanine racemase. Table 2 shows the contributions of various energy components to the net binding free energy of complexes.

Table 2. Calculated binding free energies of complexes. All of the energy values are in kcal/mol.

Energy Parameter	Top-1	Top-2	Top-3
MMGBSA			
Van der Waals	-29.00	-26.11	-28.00
Electrostatic	-10.00	-11.06	-10.00
Delta G gas	-39	-37.17	-38
Delta G solv	5.55	6.30	6.35
Delta Total	-33.45	-30.87	-31.65
MMPBSA			
Van der Waals	-29.00	-26.11	-28.00
Electrostatic	-10.00	-11.06	-10.00
Delta G gas	-39	-37.17	-38
Delta G solv	4.82	6.12	5.64
Delta Total	-34.18	-31.05	-32.36

3.5. AMBER Entropy Analysis

The AMBER normal mode entropy calculation was done to estimate the entropy energy contribution to each system's intermolecular docked stability. As the process is computationally very expensive, only 5 frames were processed. The net entropy energy value (in kcal/mol) of Top-1, Top-2, and Top-3 was -1.52, -1.89, and 0.24, respectively. The values show that the systems have no or very little freedom energy due to which the intermolecular docked complexes are very stable in terms of conformational energy.

3.6. Water Swap Binding Energies

The Water Swap calculations were done for default 1000 iterations and the results seem well converged. The Water Swap absolute binding energies for the complexes are given in Figure 5. All three complexes reported stable binding energy scores. The difference in energy among the Water Swap algorithm was found small, indicating well convergence of the systems. The Top-3 was found as the most stable with a binding energy (in kcal/mol) of -22.85, -22.08, and -21.39 for Bennett's, thermodynamic integration (TI), and free energy perturbation (FEP) algorithms, respectively.
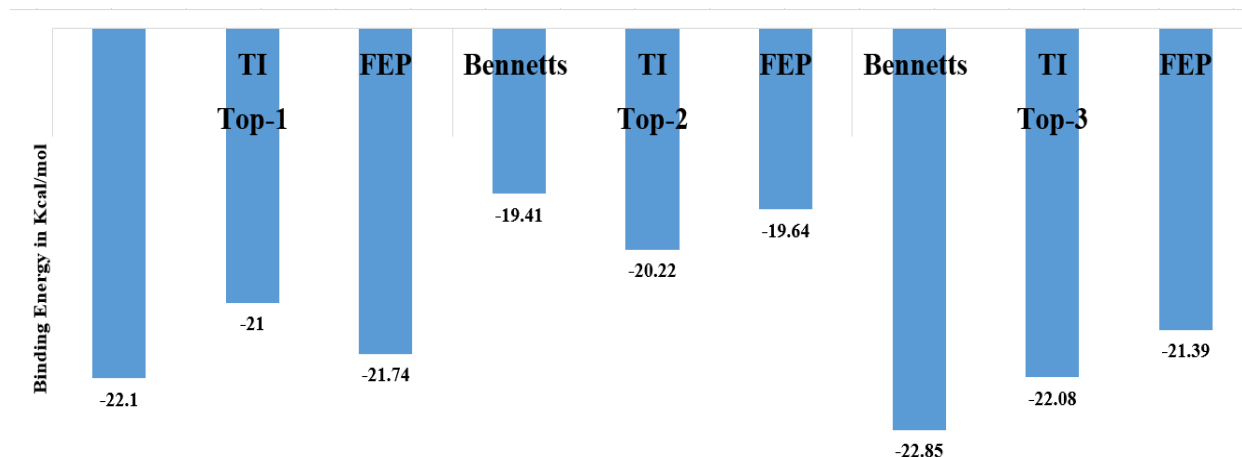

Figure 5. WaterSwap energy calculation for selected top complexes.

4. CONCLUSIONS

One interesting pharmacological target for fighting bacterial infections is the enzyme alanine racemase. The potential of several clerodane furanolactone derivatives to inhibit alanine racemase was assessed in this work. Based on their stable docking inside the active site of the enzyme and significant binding affinity, several top candidates were identified. These compounds met essential requirements for possible drug development by exhibiting positive pharmacokinetic characteristics. The docking studies were confirmed by the use of molecular dynamics simulations, which confirmed the stability of the ligand-enzyme interactions. Even though the results are encouraging, further study such as experimental validation, structure activity-relationship analysis, extended pharmacokinetics profile studies and exploration of derivatives librarries is necessary to confirm the findings and develop the compounds as possible medicinal agents.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Tacconelli, E.; Magrini, N.; Kahlmeter, G.; Singh, N. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Heal. Organ. 2017, 27.

2. Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; et al. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. Lancet Infect. Dis. 2018, 18, 318–327.

3. Myers, A.G.; Clark, R.B. Discovery of Macrolide Antibiotics Effective against Multi-Drug Resistant Gram-Negative Pathogens. Acc. Chem. Res. 2021, 54, 1635–1645.

4. Mendelson, M.; Matsoso, M.P. The World Health Organization Global Action Plan for Antimicrobial Resistance. SAMJ South African Med. J. 2015, 105, 325.

5. Morehead, M.S.; Scarbrough, C. Emergence of Global Antibiotic Resistance. Prim Care 2018, 45, 467–484.

6. Rather, I.A.; Kim, B.-C.; Bajpai, V.K.; Park, Y.-H. Self-Medication and Antibiotic Resistance: Crisis, Current Challenges, and Prevention. Saudi J. Biol. Sci. 2017, 24, 808–812.

7. Gould, I.M. The Epidemiology of Antibiotic Resistance. Int. J. Antimicrob. Agents 2008, 32, S2--S9.

8. Ventola, C.L. The Antibiotic Resistance Crisis: Part 1: Causes and Threats. Pharm. Ther. 2015, 40, 277.

9. Otto, M. MRSA Virulence and Spread. Cell. Microbiol. 2012, 14, 1513–1521.

10. Shlaes, D.M.; Sahm, D.; Opiela, C.; Spellberg, B. The FDA Reboot of Antibiotic Development. Antimicrob. Agents Chemother. 2013, 57, 4605–4607.

11. Ram\'\irez, D. Computational Methods Applied to Rational Drug Design. Open Med. Chem. J. 2016, 10, 7.

12. Macalino, S.J.Y.; Gosu, V.; Hong, S.; Choi, S. Role of Computer-Aided Drug Design in Modern Drug Discovery. Arch. Pharm. Res. 2015, 38, 1686–1701.

13. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera—a Visualization System for Exploratory Research and Analysis. J. Comput. Chem. 2004, 25, 1605–1612.

14. Naas, T.; Oueslati, S.; Bonnin, R.A.; Dabos, M.L.; Zavala, A.; Dortet, L.; Retailleau, P.; Iorga, B.I. Beta-Lactamase Database (BLDB)--Structure and Function. J. Enzyme Inhib. Med. Chem. 2017, 32, 917–919.

15. Zhang, H.; Forman, H.J. Redox Regulation of γ-Glutamyl Transpeptidase. Am. J. Respir. Cell Mol. Biol. 2009, 41, 509–515.

16. Azam, M.A.; Jayaram, U. Inhibitors of Alanine Racemase Enzyme: A Review. J. Enzyme Inhib. Med. Chem. 2016, 31, 517–526.

17. de Melo, E.B.; da Silveira Gomes, A.; Carvalho, I. α-and β-Glucosidase Inhibitors: Chemical Structure and Biological Activity. Tetrahedron 2006, 62, 10277–10302.

18. Kerwin, S.M. ChemBioOffice Ultra 2010 Suite 2010.

19. Morris, G.M.; Lim-Wilby, M. Molecular Docking. In Molecular modeling of proteins; Springer, 2008; pp. 365–382.

20. Korb, O.; Stützle, T.; Exner, T.E. An Ant Colony Optimization Approach to Flexible Protein--Ligand Docking. Swarm Intell. 2007, 1, 115–134.

21. Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual Molecular Dynamics. J. Mol. Graph. 1996, 14, 33–38.

22. BIOvIA, D.S. Discovery Studio Modeling Environment 4, Dassault Syst. Release, San Diego 2015.

23. Lee, S.K.; Chang, G.S.; Lee, I.H.; Chung, J.E.; Sung, K.Y.; No, K.T.; others The PreADME: Pc-Based Program for Batch Prediction of Adme Properties. EuroQSAR 2004, 9, 5–10.

24. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. Sci. Rep. 2017, 7, 1–13, doi:10.1038/srep42717.

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25. Weiner, P.K.; Kollman, P.A. AMBER: Assisted Model Building with Energy Refinement. A General Program for Modeling Molecules and Their Interactions. J. Comput. Chem. 1981, 2, 287–303.

26. Özp\inar, G.A.; Peukert, W.; Clark, T. An Improved Generalized AMBER Force Field (GAFF) for Urea. J. Mol. Model. 2010, 16, 1427–1440.

27. Maier, J.A.; Martinez, C.; Kasavajhala, K.; Wickstrom, L.; Hauser, K.E.; Simmerling, C. Ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from Ff99SB. J. Chem. Theory Comput. 2015, 11, 3696–3713.

28. Berendsen, H.J.C.; Postma, J.P.M. van; van Gunsteren, W.F.; DiNola, A.; Haak, J.R. Molecular Dynamics with Coupling to an External Bath. J. Chem. Phys. 1984, 81, 3684–3690.

29. Vaught, A. Graphing with Gnuplot and Xmgr: Two Graphing Packages Available under Linux. Linux J. 1996, 1996, 7--es.

30. Massova, I.; Kollman, P.A. Combined Molecular Mechanical and Continuum Solvent Approach (MM-PBSA/GBSA) to Predict Ligand Binding. Perspect. drug Discov. Des. 2000, 18, 113–135.

31. Miller, B.R.; McGee, T.D.; Swails, J.M.; Homeyer, N.; Gohlke, H.; Roitberg, A.E. MMPBSA.Py: An Efficient Program for End-State Free Energy Calculations. J. Chem. Theory Comput. 2012, 8, 3314–3321, doi: 10.1021/ct300418h.

32. Wadood, A.; Ghufran, M.; Hassan, S.F.; Khan, H.; Azam, S.S.; Rashid, U. In Silico Identification of Promiscuous Scaffolds as Potential Inhibitors of 1-Deoxy-d-Xylulose 5-Phosphate Reductoisomerase for Treatment of Falciparum Malaria. Pharm. Biol. 2017, 55, 19–

33. Yang, Y.; Engkvist, O.; Llinàs, A.; Chen, H. Beyond Size, Ionization State, and Lipophilicity: Influence of Molecular Topology on Absorption, Distribution, Metabolism, Excretion, and Toxicity for Druglike Compounds. J. Med. Chem. 2012, 55, 3667–3677.