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Investigation of salicylidene acylhydrazides derivatives: Molecular Docking, ADMET, and Molecular Dynamic Simulations were used in conjunction towards the design of new Yersinia pseudotuberculosis inhibitors

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Abstract: LysR-type transcription factor RovM is an important target of Yersinia pseudotuberculosis drug discovery and the discovery of antibacterial is considered one of the greatest medical achievements of all time. In this research work, a combination of three docking tools with different algorithms was applied in Salicylidene acylhydrazides derivatives intended toward gram-negative bacterium Yersinia pseudotuberculosis to evaluate their binding interactions. The analysis of the molecular docking results obtained from the 3-docking software system succeeded in screening twelve fascinating compounds with higher restrictive concentrations having a decent affinity to LysR-type transcription factor RovM macromolecule. Then the Lipinski's and Veber's rule properties were calculated to spot the drug-likeness properties of the investigated candidate compounds. To anticipate the toxicity of the predicted candidate chemicals, in-silico toxicity tests were conducted. Furthermore, golden triangle and drug scores were performed, the investigated compounds which fall within the golden triangle indicate that these compounds would not have clearance problems. 5 of the 12 hits drugs pass the golden triangle screening step. These selected drugs undergo a drug score test which only compound 17 passed. To validate the stability, 1 ns molecular dynamic simulations were done on the highest-ranking drug score compound 17 / 3onm complexes. These findings point to interesting avenues for the development of new compounds that are more effective against *Yersinia pseudotuberculosis*.

Keywords: *Yersinia pseudotuberculosis, Salicylidene acylhydrazides, Docking, ADMET, golden triangle, and MD simulations*

1. Introduction

Yersinia pseudotuberculosis (YP) could be a gram-negative foodborne microorganism that causes a spread of enteral and extraintestinal syndromes (yersiniosis), together with self-limiting inflammation, diarrhea, peritoneum inflammation, and response disorders [1]. Once within the viscus, the microorganism ought to penetrate through the animal tissue cells to succeed in the underlying humor tissues [2]. Multidrug-resistant gram-negative organisms have emerged as a serious threat to hospitalized patients and are related to mortality rates starting from 30 to 70% [3]. They are classes of approved antibiotics drugs to treat or prevent certain bacterial infections. However, as a result of side effects, development of resistance, and challenges with patient compliance there's an unbroken demand for brand spanking new generations of antibiotics medicine [4]. Ciprofloxacin (webmd.com/drugs/2/drug-7748/ciprofloxacin-oral/details), a family of quinolone and one of the most widely used antibiotics has severe side effects such as boxed warnings, tendonitis and tendon rupture, nervous-system effect includes insomnia, restlessness, seizure, convulsion, psychosis, serious tears in the aorta, hypertension, certain genetic conditions such as Marfan Syndrome, and Ehlers-Danlos Syndrome [5]. Other medicine area units Ampicillin, Chlortetracycline, and Oxytetracycline will harm

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calcium-rich organs, like teeth and bones [5]. They can additionally result in the epithelial duct and sensitive allergies. Currently, the increasing number of multidrug-resistant microbes is a huge burden on the world's health and economy [6]. Serious infections and deaths are caused by the resistance of bacteria to antibiotics [7]. The emergence of drug-resistant bacterial variants and also the facet effects of those compounds (i.e., Ciprofloxacin Ampicillin, Chlortetracycline, and Oxytetracycline) entail research into new drugs that concentrate on completely different stages of the bacterium [8]. Transcription factor of the LysR type One of the host proteins that could be exploited as a target is RovM. Transcription factor of the LysR type RovM from Yersinia pseudotuberculosis belongs to one of the largest families of prokaryotic transcriptional regulators of genes that code for proteins with a variety of functions, including aromatic compound degradation, amino acid biosynthesis, virulence factor synthesis, CO2-fixation, N2-fixation, antibiotic resistance, cell division, quorum sensing, and oxidative stress responses [9]. LysR-type transcriptional regulators typically consist of ~300 amino acids and bind their target promoters as homo-tetramers. Transcription factors of the LysR type RovM regulators are a subset of the MarR-like family of transcriptional regulators that regulate several physiological processes in bacterial pathogens, including stress adaption and pathogenicity in response to environmental and host-associated stress [10]. Therefore, due to the essential nature of LysR-type transcription, RovM has been an important drug target for Yersinia pseudotuberculosis inhibitors [9]. Conventional drug findings and the need to develop new and more efficient drugs still represent an unsatisfied challenge [11]. The production of any drug for human use currently involves intensive trials and expensive methods, so that it takes about 10- 15 years on average before it can reach the market [12]. Over the past decade, much attention has been placed on the study of phytochemicals for their antibacterial activity, especially against multidrug-resistant Gram-negative and Gram-positive bacteria [13]. In recent years, computer-aided drug design (CADD) studies have greatly impacted the field of drug development, especially as a fast tool to evaluate and screen only molecules that are likely to be active, to indicate which of them are worth to be synthesized and experimentally tested [15]. Present research investigation deals with the combination of (1) molecular docking simulations; a frequently used method for evaluating the complex formation of small ligands with large biomolecules [15], (2) ADMET; in silico prediction of the ADMET properties has a significant impact in the antibiotic drug discovery process. Nowadays ADME (absorption, distribution, metabolism, and elimination) is applied at an early phase of the drug development process, to remove molecules with poor ADME properties from the drug development pipeline and leads to significant savings in research and development costs [16]. (3) Golden triangle and drug score; is a visualization tool that allows to screen out metabolically stable and permeable drug candidates [17, 18], lastly (4) Molecular dynamics (MD) simulations; are used to predict the stability more reliably for the receptor-lead complex. The MD simulations enable flexibility for both the lead and the receptor, allowing for the induced fit of the receptor active region around the inserted lead [19]. We expected the outcome from this study could provide an insight into a novel antibacterial treatment for Y. pseudotuberculosis infection.

2. Computational Method

2.1 Ligands Selection and Preparation

The chemical structure and anti-bacterial activity (IC50) of 58 salicylidene acylhydrazides derivatives were obtained from PubChem accession number AID 473049 (https://pubchem.ncbi.nlm.nih.gov/bioassay/473049) (Table S1). The 58 salicylidene acylhydrazides derivatives were optimized using the Spartan'14 v1.1.4 program and the semi-empirical (PM3) approach (www.wavefun.com). Because docking software accepts .pdb format as an input file, the optimized ligands were stored in .pdb format for further research.

2.2 Target selection and preparation

The reported three-dimensional (3D) structure of the effector binding domain of LysR-Type transcription factor RovM from Y. pseudotuberculosis (PDB ID: 3ONM) [20] was retrieved from RCSB PDB (3onm). The water molecules, as well as co-crystallized ligands, were deleted from the protein PDB file. Hydrogen and charge were added and save in the .pdb format.

2.3 Docking simulations and ADMET

To study the molecular interaction between salicylidene acylhydrazides derivatives and effector binding domain of LysR-Type transcription factor RovM from Y. pseudotuberculosis (PDB ID: 3ONM) [20], a molecular docking simulation-based in silico approach was applied. The molecular docking simulations were done by three powerful docking tools with a different algorithm to select the best conformations. Molecular docking studies were performed using Molegro virtual docker (MVD) [21], iGemDock [22], and the AutoDock-vina [23] with PyRx software. The prepared ligand and protein files were uploaded in ".pdb" format and the parametric quantity was set to the default setting. After the completion of docking, a ranked list of predicted complexes can be downloaded. The docking output results were rendered with excel and Discovery Studio visualizer programs, respectively. The complexes that have a docking score better than the reference drug were taken for pharmacokinetic screening. The pharmacokinetic properties of the complexes screened through docking were calculated using Data-Warrior version 5.5.0 of Idorsia Pharmaceuticals Ltd – engineering by Thomas Sander, to assess the compounds' oral bioavailability and drug-likeness. The software (OSIRIS) was also utilized to forecast the values of the compounds' molecular mutagenic, tumorigenic, irritating, and reproductive effective qualities.

2.4 Golden Triangle and Molecular Dynamics (MD) Simulations

After successful ADMET predictions, the selected compounds were subjected to the Golden Triangle for permeability, bioavailability, and clearance. Johnson and co-workers reported that the distribution coefficient at pH 7.4 (LogD) and the molecular weight (MW), have a crucial effect on the permeability, bioavailability, and clearance behavior of active compounds [17, 24]. Based on this information, the Golden Triangle was developed. The Golden Triangle [17, 18] is a visualization tool for identifying metabolically stable and permeable medication candidates. It was developed based on experimental results about the permeability and clearance of existing drugs [25]. It is characterized by a baseline of calculated clogP = −2 to 5 at MW = 200 and a peek at calculated clogP = 1 to 2 and MW = 450 [25]. The triangle displays more perspective compounds that are metabolically unstable as they are located outside it. The compound that falls within the Golden Triangle were taken for further simulation. The molecular dynamics simulations (MDS) were carried out using the nanoscale molecular dynamics (NAMD) software version 2.13 [26], and the visual molecular dynamics (VMD) software version 1.9.3 [27] was used to visually display, analyzed, and animate trajectories [27]. The CHARMM-GUI web server was used for the complex parameterization of the lead compound to mimic the physiological condition of the complex. To establish charge neutrality, the salt (KCl) concentration was fixed to 0.15M, then the net and counter ions (potassium and chloride) were added. The CHARMM36m force field was used in the calculations. To optimize the complex, several cycles of the steepest descent process were run. The system was gradually heated to 303.15K before being equilibrated as a canonical ensemble (NVT) at that temperature. For complexes, the usual CHARMM-GUI equilibration approach was used. For the production dynamics, one nanosecond (1 ns) unconstrained isothermal isobar ensemble (NPT) experiments were carried out at 303.15K, utilizing 1 atm and 2 fs time increments.

3. Results and discussion

Authentic prediction of binding affinity to targets with inhibitors can give what is desired or needed, especially support, or guide for rational drug design. The binding mechanism of a compound in the active site is best understood by the analysis of bonding interactions. Four drugs were selected as the control as anti-Yersinia pseudotuberculosis namely: Ampicillin, Oxytetracycline, Chlortetracycline, and Ceftriaxone (Azithromycin). The active site for the receptor was predicted using Molegro virtual docker (MVD), which yielded various cavities in the receptor, cavity 1: volume = 34. 816, surface = 101.12; cavity 2: volume = 20.992, surface = 72.96; cavity 3: volume = 17.408, surface = 72.96; cavity 4: volume = 12.288, surface = 56.32, and cavity 5: volume = 11.264, surface = 47.36 and the largest cavity "cavity 1" was presumed to be the active site (Fig. 1). The docking scores of the 4 standards were displayed in Table 1 and their interactions with the amino acid residues are presented in Fig. 2.

Based on the results, Ceftriaxone (Azithromycin) was found to possess the lowest MolDock score (-176.051) and forms series of interactions with the amino acid residues, among which are hydrogen bonds were found interacting with Tyr198, Arg136, Asp108, Arg238, and Ser137 (Figure 2). All the reference drugs except Ampicillin are unable to form stability with the protein by unfavorable bumps and unfavorable donor-donor interaction (Fig. 2). Out of the whole compound library of 58

salicylidene acylhydrazides derivatives, compounds 25, 31, 35, and 41 showed the strongest interaction (Figure 3) towards the receptor with the MolDock score of -137.051, -132.013, -143.983, and -137,71 (Table 1) compares with the standards except Ceftriaxone (Azithromycin). The least MolDock score (compound 35 with PubChem-CID: 136167972) binds effectively with the receptor active site by interacting through the amino acids including Ser137, Arg136, Pro138, Pro195, Ser196, etc. (Figure 3).

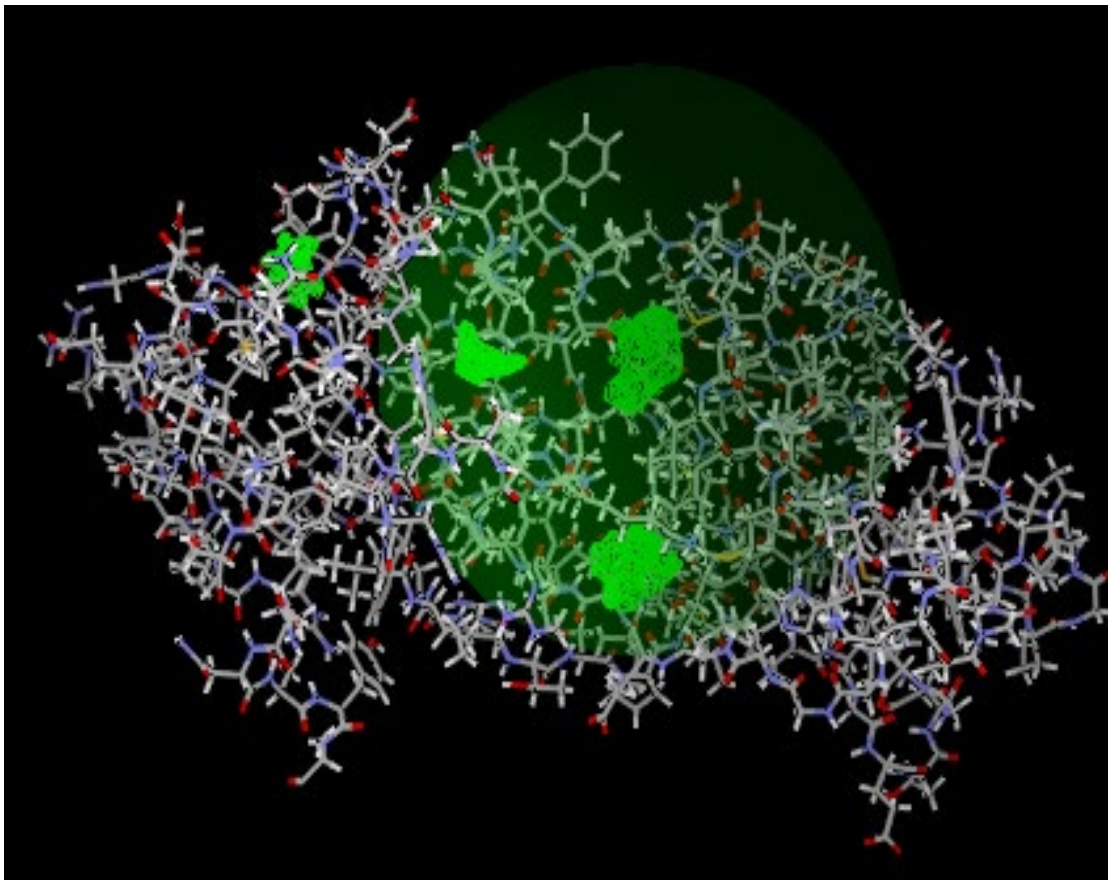


Figure 1. The binding pocket of LysR-Type transcription factor RovM

Table 1. Docking results of the reference drugs using MVD software

Name	MolDock score (kcal/mol)	Rerank score (kcal/mol)	HBond (kcal/mol)
Ampicillin	-120.545	-87.452	-7.478
Oxytetracycline	-61.033	85.056	-20.014
Chlortetracycline	-61.279	86.389	-20.002
Ceftriaxone(Azithromycin)	-176.051	-68.607	-9.612

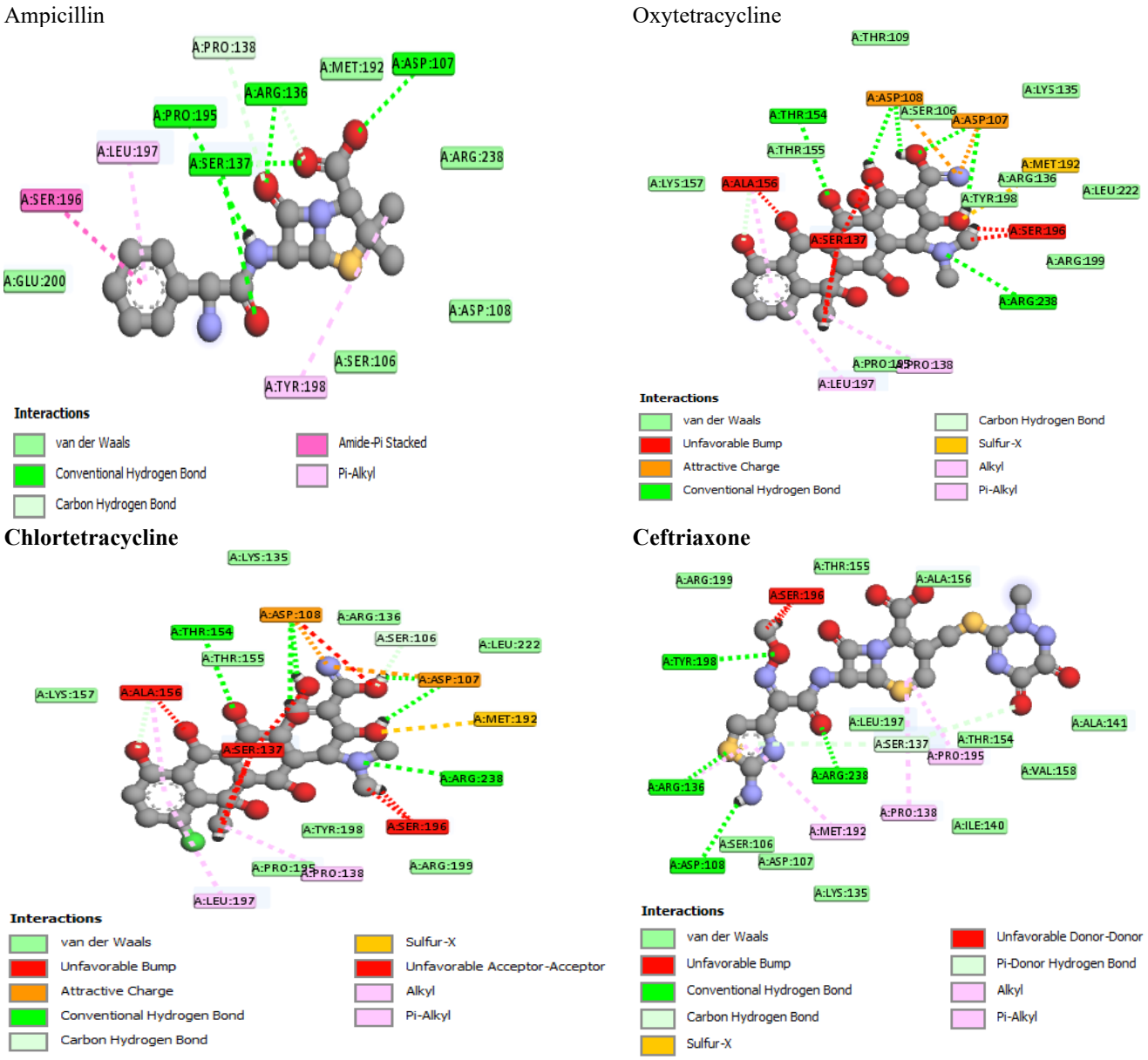


Figure 2. Docking interactions of the four reference drugs using Molrgro Virtual Docker.

Table 2. Results for the molecular docking Scores Using MVD and AutoDock-vina with PyRx

	Molegro Virtual Docker			**AutoDock Vina**	
Compounds	MolDock Score (kcal/mol)	Rerank Score (kcal/mol)	HBond (kcal/mol)	Ligand	Binding Affinity (kcal/mol)
3onm-1	-113.139	-85.521	-8.512	3onm-20	-7.9
3onm-2	-97.644	-85.013	-9.627	3onm-44	-7.9
3onm-3	-117.820	-91.477	-4.832	3onm-49	-7.6
3onm-4	-101.465	-91.156	-6.783	3onm-36	-7.5
3onm-5	-113.020	-91.924	-7.118	3onm-17	-7.4
3onm-6	-100.026	-86.294	-9.472	3onm-18	-7.4
3onm-7	-112.163	-94.877	-6.288	3onm-4	-7.1
3onm-8	-102.146	-87.422	-6.617	3onm-57	-7.1
3onm-9	-128.815	-111.029	-6.442	3onm-1	-7

3onm-10	-117.022	-87.931	-8.101	3onm-26	-7
3onm-11	-123.083	-86.298	-6.970	3onm-29	-7
3onm-12	-108.707	-89.709	-2.500	3onm-21	-6.9
3onm-13	-115.614	-48.123	-8.655	3onm-32	-6.9
3onm-14	-116.137	-94.125	-10.014	3onm-39	-6.9
3onm-15	-130.710	-103.720	-12.163	3onm-42	-6.9
3onm-16	-112.049	-90.801	-12.952	3onm-54	-6.9
3onm-17	-99.690	-87.810	-16.942	3onm-22	-6.8
3onm-18	-112.574	-91.048	-1.666	3onm-30	-6.8
3onm-19	-123.422	-93.225	-9.444	3onm-37	-6.8
3onm-20	-113.054	-82.948	-5.286	3onm-5	-6.8
3onm-21	-107.379	-91.157	-9.355	3onm-51	-6.7
3onm-22	-107.930	-85.990	-7.882	3onm-53	-6.7
3onm-23	-118.603	-98.819	-7.508	3onm-6	-6.7
3onm-24	-115.085	-88.490	-4.054	3onm-11	-6.6
3onm-25	-137.051	-117.814	-5.834	3onm-23	-6.6
3onm-26	-114.011	-93.575	-9.068	3onm-25	-6.6
3onm-27	-122.522	-101.203	-7.278	3onm-28	-6.6
3onm-28	-114.350	-92.768	-8.305	3onm-34	-6.6
3onm-29	-127.125	-91.180	-9.485	3onm-43	-6.6
3onm-30	-119.090	-97.775	-9.928	3onm-56	-6.6
3onm-31	-132.013	-104.800	-15.295	3onm-15	-6.5
3onm-32	-117.748	-93.499	-7.882	3onm-3	-6.5
3onm-33	-103.402	-68.938	-11.244	3onm-40	-6.5
3onm-34	-118.421	28.210	-2.500	3onm-41	-6.5
3onm-35	-143.983	-67.464	-6.226	3onm-24	-6.4
3onm-36	-112.550	-6.473	-4.963	3onm-27	-6.4
3onm-37	-117.768	-94.858	-10.080	3onm-31	-6.4
3onm-38	-113.792	-96.986	-7.844	3onm-58	-6.4
3onm-39	-124.920	-101.885	-4.022	3onm-10	-6.3
3onm-40	-109.901	-82.258	-9.703	3onm-16	-6.3
3onm-41	-137.710	-119.990	-8.234	3onm-48	-6.3
3onm-42	-120.924	-99.738	-10.712	3onm-13	-6.2
3onm-43	-119.313	-42.618	-6.910	3onm-38	-6.2
3onm-44	-115.463	-93.567	-6.249	3onm-14	-6.1
3onm-45	-121.293	-101.498	-8.584	3onm-2	-6.1
3onm-46	-112.352	-79.709	-7.232	3onm-50	-6.1
3onm-47	-129.780	-94.593	-9.985	3onm-55	-6.1
3onm-48	-124.221	-96.941	-10.962	3onm-7	-6.1
3onm-49	-101.331	-85.736	-10.184	3onm-46	-6
3onm-50	-112.676	-93.517	-8.249	3onm-9	-6
3onm-51	-114.827	-83.010	-7.930	3onm-12	-5.9
3onm-52	-98.3169	-86.222	-9.777	3onm-33	-5.9
3onm-53	-102.233	-83.405	-7.911	3onm-52	-5.8

3onm-54	-108.752	-94.109	-5.910	3onm-19	-5.7
3onm-55	-118.876	-94.393	-9.111	3onm-35	-5.7
3onm-56	------	------	------	3onm-47	-5.7
3onm-57	------	------	------	3onm-45	-5.3
3onm-58	------	------	------	3onm-8	-5.3

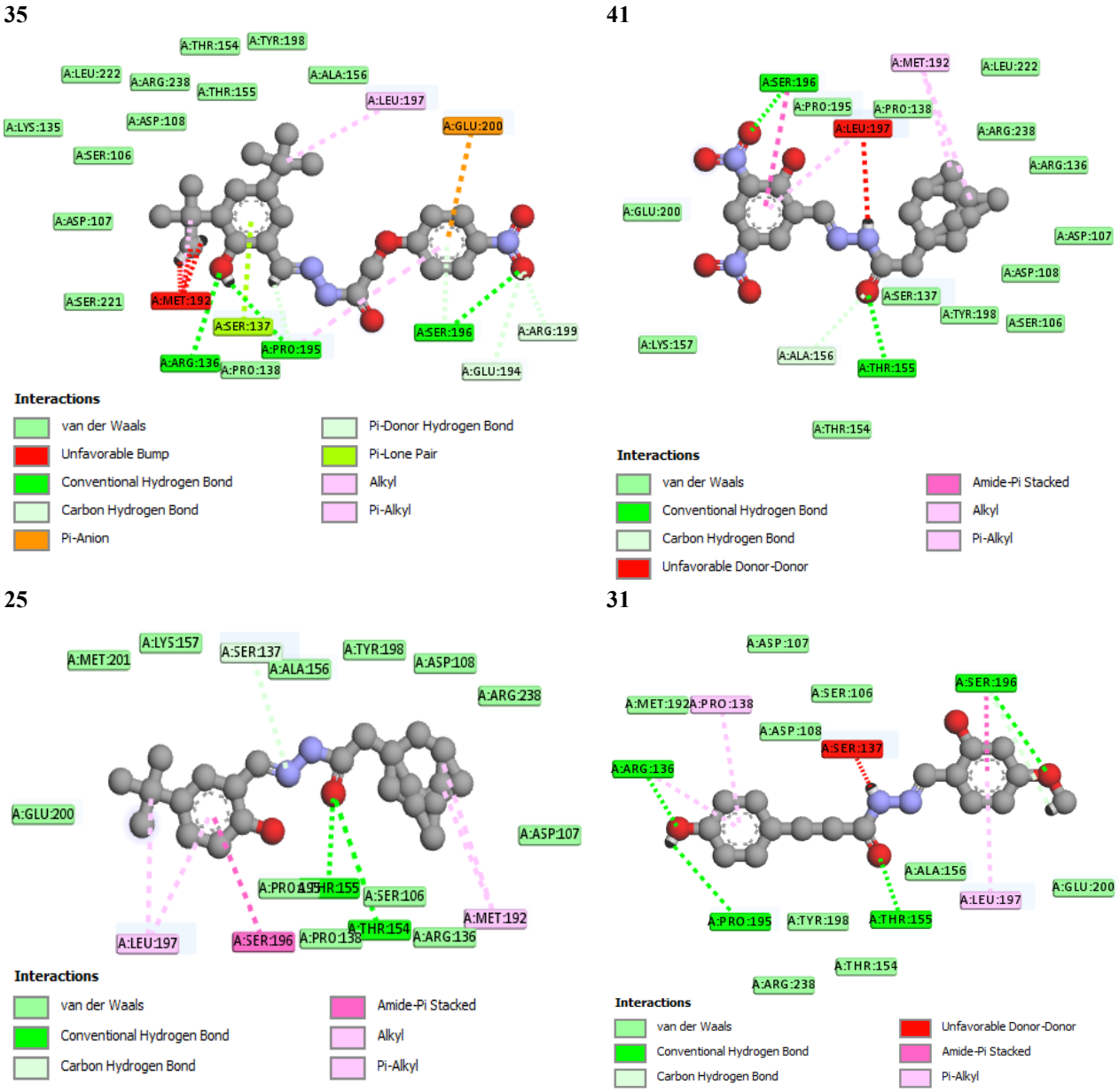


Figure 3. Binding interaction between compounds 25, 31, 35, and 41 and the active site of the protein using Melogro virtual docker.

The whole compound library of 58 salicylidene acylhydrazides derivatives with the standards undergoes another docking screening with iGemDock v2.1 software. The following parameters for docking were used: Population size = 200; Number of generations = 70; and Number of solutions = 3. The findings of iGemDock analysis revealed that compound 41 is the topmost in the rank with a total energy of -106.975 kcal/mol which was shared with van der Waals forces, hydrogen

bond, and electrostatic interaction (-60.0817, -48.1473, and 1.25433 Kcal/mol), respectively, followed by compound 49 with the overall total energy of -105.987 Kcal/mol which was shared with van der Waals and hydrogen bond interaction (-71.1538 and -34.8333 Kcal/mol). In the docking analysis for compound 49, no electrostatic interactions were recorded as shown in Table 3.

From the docking poses, compound 41 formed van der Waals, hydrogen bonding and hydrophobic interactions with Val134, Arg136, Thr155, Ser137, Asp107, Asp108, Ser106, Lys135, Met192, Thr154, Leu197, Arg199, Tyr198, Ser196, and Arg238 amino acid residues present in C-terminal and N-terminal regions of the binding pocket played a crucial role in ligand binding (Figure 4). Table 4 showed the results of the reference drugs in which Chlortetracycline is the topmost in the rank with -105.022 kcal/mol which was shared among van der Waals (-71.1492 kcal/mol) and hydrogen bond (-33.8726 kcal/mol). The binding interactions of the reference drugs and compounds 41 and 49 were analyzed and the results obtained were compared. From the docking poses, the reference drugs Ampicillin, Oxytetracycline, and Ceftriaxone (Figure 5) have unfavorable dumps in their interactions which may lead to a side effect of unfavorable interactions with the protein. It was observed that compounds 41 and 49 acted better than the reference drugs. Their interactions show that hydrophobic interaction enhances the activity of the compounds and helped their biological activity than the reference drugs.

Table 3. Summary of the molecular docking score using iGemDock software.

Ligand	TotalEnergy (kcal/mol)	VDW	HBond (kcal/mol)	Elec	AverConPair
3onm-41-2.pdb	-106.975	-60.082	-48.147	1.254	32.250
3onm-49-0.pdb	-105.987	-71.154	-34.833	0	25.304
3onm-39-2.pdb	-102.751	-74.133	-28.618	0	23.923
3onm-51-0.pdb	-102.539	-60.901	-41.638	0	25.296
3onm-19-0.pdb	-100.699	-53.968	-46.731	0	31.471
3onm-17-2.pdb	-100.564	-67.700	-32.864	0	23.044
3onm-47-0.pdb	-99.933	-75.539	-24.393	0	21.560
3onm-40-2.pdb	-99.632	-67.104	-33.445	0.918	24.826
3onm-18-0.pdb	-99.435	-73.529	-25.906	0	23.958
3onm-42-2.pdb	-98.985	-81.786	-16.702	-0.497	23.482
3onm-45-1.pdb	-96.407	-67.528	-28.879	0	24.889
3onm-56-1.pdb	-95.486	-72.508	-22.978	0	27.950
3onm-35-0.pdb	-95.074	-63.803	-31.271	0	28.412
3onm-21-0.pdb	-95.063	-76.647	-16.949	-1.467	23.000
3onm-22-0.pdb	-94.292	-75.071	-19.221	0	24.191
3onm-6-1.pdb	-93.444	-63.128	-30.317	0	24.238
3onm-1-1.pdb	-93.217	-68.710	-24.507	0	21.870
3onm-10-1.pdb	-92.997	-70.320	-22.677	0	24.050
3onm-44-1.pdb	-92.634	-47.925	-40.794	-3.915	14.172
3onm-14-0.pdb	-92.472	-70.359	-22.113	0	26.727
3onm-52-2.pdb	-91.963	-61.878	-30.086	0	34.222
3onm-34-0.pdb	-91.935	-63.765	-28.169	0	23.333
3onm-13-1.pdb	-91.817	-76.460	-15.356	0	22.217
3onm-55-0.pdb	-91.756	-64.416	-27.339	0	23.526
3onm-16-2.pdb	-91.205	-68.379	-22.826	0	24.565

3onm-29-1.pdb	-91.144	-71.813	-19.332	0	26.348
3onm-11-2.pdb	-91.063	-81.755	-9.3078	0	23.542
3onm-7-1.pdb	-91.008	-69.981	-21.027	0	24.714
3onm-3-2.pdb	-90.820	-67.572	-23.248	0	24.000
3onm-50-1.pdb	-90.493	-63.642	-26.851	0	27.375
3onm-20-0.pdb	-89.543	-66.283	-23.261	0	20.609
3onm-31-0.pdb	-89.271	-78.291	-10.980	0	23.462
3onm-58-2.pdb	-89.168	-73.192	-15.976	0	22.773
3onm-28-1.pdb	-89.054	-73.671	-15.383	0	23.875
3onm-23-2.pdb	-89.054	-60.332	-28.722	0	24.579
3onm-2-0.pdb	-88.401	-68.216	-20.185	0	23.250
3onm-12-0.pdb	-87.369	-62.715	-24.654	0	24.625
3onm-26-2.pdb	-87.112	-76.516	-10.596	0	31.913
3onm-48-0.pdb	-86.970	-64.731	-22.239	0	19.923
3onm-54-0.pdb	-86.553	-61.691	-24.863	0	17.200
3onm-4-2.pdb	-86.401	-67.955	-18.446	0	25.174
3onm-30-0.pdb	-86.171	-65.363	-20.808	0	22.773
3onm-43-2.pdb	-86.064	-45.895	-39.587	-0.581	16.520
3onm-53-1.pdb	-85.742	-73.632	-12.110	0	25.417
3onm-9-2.pdb	-85.557	-66.268	-19.289	0	26.773
3onm-32-0.pdb	-85.519	-54.524	-29.763	-1.232	21.087
3onm-25-1.pdb	-85.046	-60.066	-24.980	0	21.360
3onm-5-0.pdb	-84.337	-62.802	-21.536	0	22.682
3onm-37-0.pdb	-83.978	-69.009	-14.969	0	26.516
3onm-57-2.pdb	-83.499	-70.994	-12.505	0	24.476
3onm-8-2.pdb	-83.314	-58.716	-24.596	0	23.889
3onm-46-2.pdb	-82.949	-66.593	-16.355	0	18.19
3onm-36-2.pdb	-82.848	-59.914	-22.933	0	23.033
3onm-24-0.pdb	-81.667	-58.929	-22.738	0	27.313
3onm-15-2.pdb	-81.190	-64.690	-16.500	0	17.769
3onm-27-0.pdb	-80.910	-58.950	-21.960	0	16.444
3onm-33-2.pdb	-80.633	-51.460	-29.173	0	22.087
3onm-38-2.pdb	-78.629	-65.853	-12.776	0	17.821

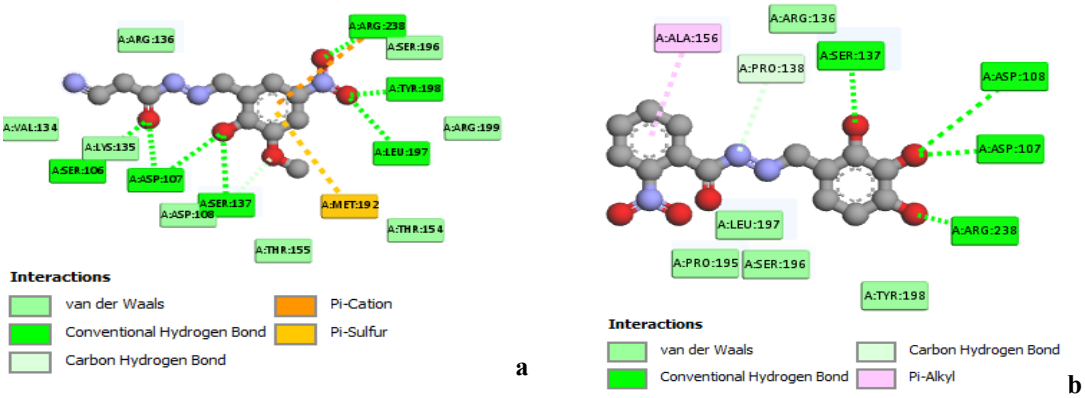


Figure 4. Docking interaction (a) compound 41 (b) compound 49 with the protein.

Table 4. Results of the reference drugs using iGemDock software

Ligand	TotalEnergy (kcal/mol)	VDW	HBond (kcal/mol)	Elec	AverConPair
Ampicillin	-82.362	-62.462	-17.235	-2.665	18.25
Oxytetracycline	-90.635	-52.316	-38.320	0	18.061
Chlortetracycline	-105.022	-71.149	-33.873	0	16.353
Ceftriaxone	-91.721	-84.510	-8.7608	1.550	15.5278

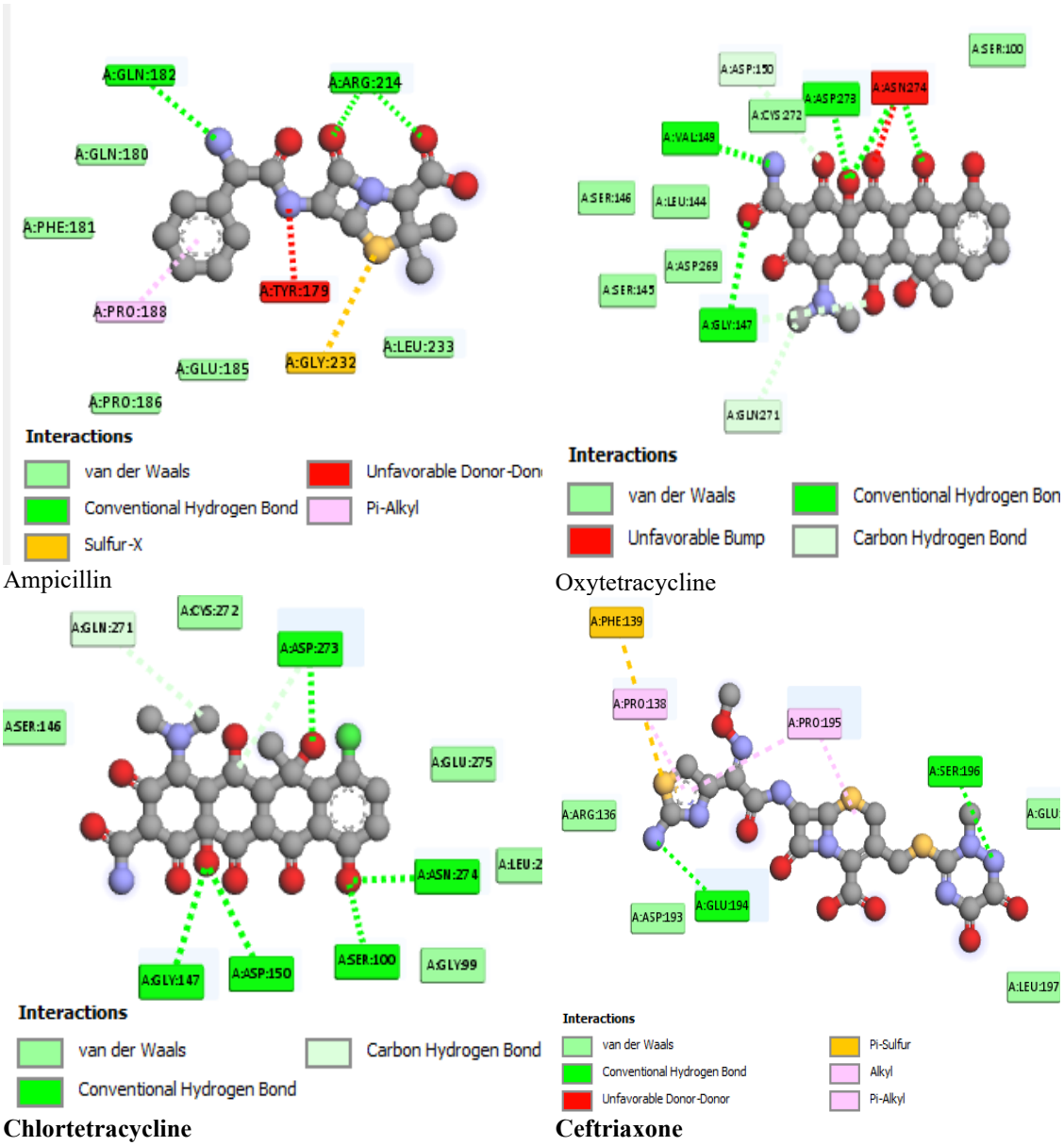


Figure 5. Summary of the docking interactions of the reference drugs with the amino acid residuals.

The structure of the four reference (standard) drugs and the 58 salicylidene acylhydrazides derivatives were considered for another virtual screening using AutoDock Vina with PyRx. The AutoDock Vina uses a 3D grid broadly encompassing the active site of the protein and allowing free rotation of the ligand in the active site. In this case, the center of this box is determined by the coordinates X = -24. 8918, Y = 8.7616, and Z = -6.8212 with size X = 33.7623, Y = 61.2411, and Z = 46.0013. The box covered almost the protein structure and its dimension are proportional to the size of the

ligands. After the docking simulation, the binding affinity of the reference drugs Ampicillin, Ceftriaxone, Chlortetracycline, and Oxytetracycline was observed: -6.1, -7.0, -6.7, and -6.2 kcal/mol, respectively (Table 5). The molecular docking interactions of the reference drugs with the protein are shown in Figure 6. The important residues have direct interaction with the inhibitors. The docking results indicate that the best score is -7.0 kcal/mol for the protein-ligand interaction of "Ceftriaxone" incorporating the sum of the van der Waals, conventional-carbon hydrogen bond, electrostatic, and hydrophobic interactions. Ceftriaxone docked well into the active pocket of the protein and represents the most stable binding conformation.

Table 5. Results of the reference drugs using AutoDock-vina with PyRx

Ligand	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
Ampicillin	-6.1	0	0
Ceftriaxone	-7	0	0
Chlortetracycline	-6.7	0	0
Oxytetracycline	-6.2	0	0

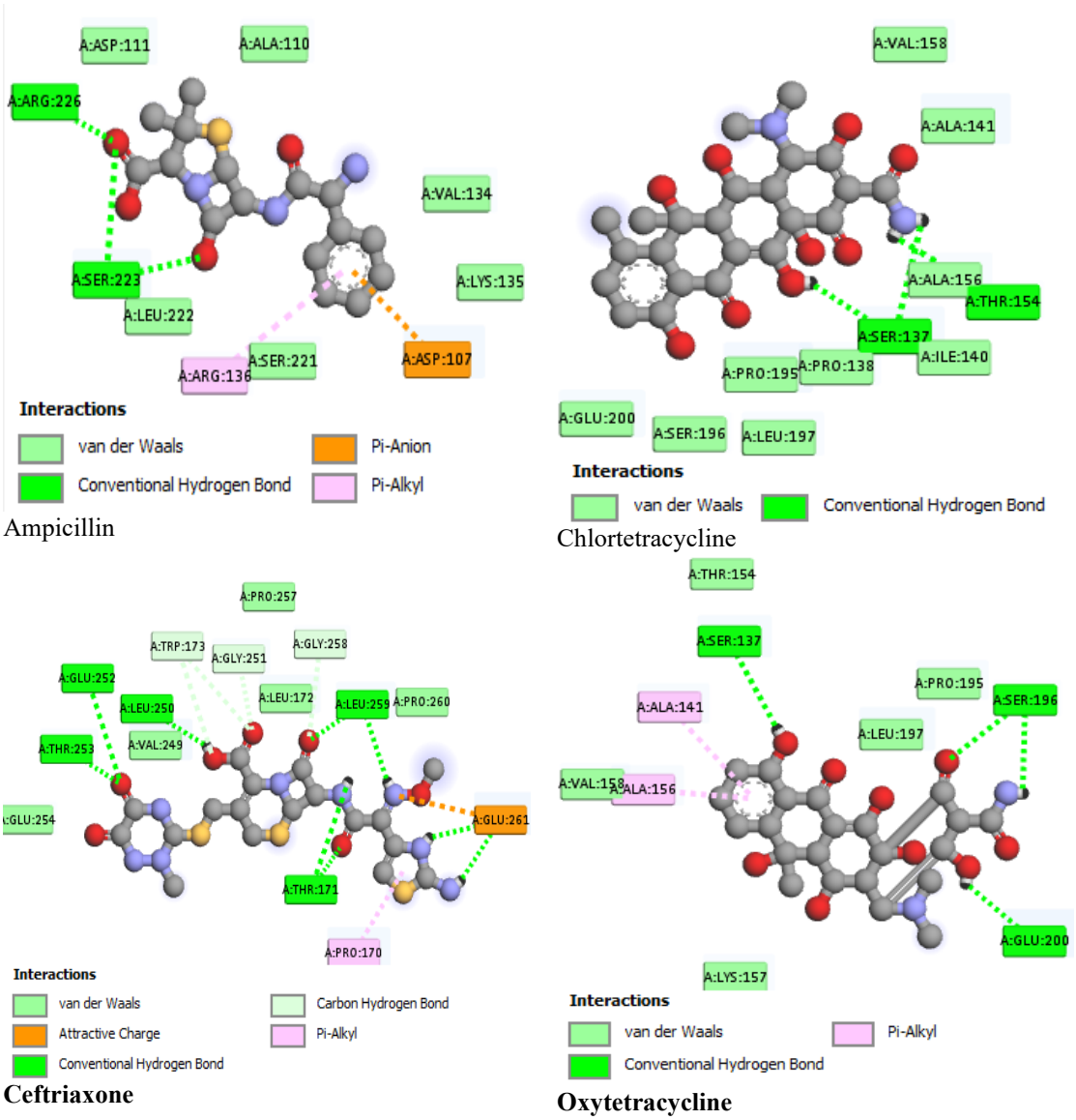


Figure 6. Interaction of the reference drugs with the protein crystal structure

The -C=O group of Ceftriaxona forms four hydrogen bonds with Glu252, Thr171, Thr253, and Leu259. The -OH group to form one Hydrogen bond with Leu250, while -NH group form four hydrogen bonds with Thr171, Leu259, and Glu261, respectively. To get insight into the 58 salicylidene acylhydrazides derivatives basis of the detailed interactions between the top four lead compounds with the protein, binding affinities, and molecular interaction analysis was carried out. Among all the docked compounds with the protein, compound 4, 17, 18, 20, 36, 44, 49, and 57 shows highest binding affinity toward the protein more than the reference drugs with a score greater than -7.0 kcal/mol. Compound 20 and 44 have the highest docking score with the protein having a score of -7.9 kcal/mol, respectively. Compound 20 bound to the protein structure has shown two conventional hydrogen bonds and one carbon-hydrogen bond with Ser137, His161, Ser137 amino acid residues, respectively. It showed binding energy (binding affinities) of -7.9 kcal/mol. Apart from these interactions, compound 20 was further stabilized with halogen interaction with Pro195. It also makes hydrophobic interactions (Pi-sigma, Amide-Pi Stacked, Alkyl, and Pi-alkyl). Compound 44 also has a binding affinity of -7.9 kcal/mol and is involved in hydrogen bonding with Thr154, Thr155, and Ala156. Furthermore, it is stabilized with hydrophobic interaction (Alkyl and Pi-alkyl) with Met192 and Ala141 and van der Waals interactions with Pro138, 135, Ser106, 137,196, Leu197, Asp107, 108, Arg136, 238, Tyr198, and Lys157, respectively. Compound 49 binds with the protein structure with a binding affinity score of -7.6 kcal/mol and forms four hydrogen bonds, one carbon-hydrogen bond, five van der Waals interactions, and hydrophobic contacts. Compound 49 is unable to form stability with the protein by unfavorable donor-donor interaction with Arg136, Ser137, and Arg238, respectively. Compound 36 with a binding affinity of -7.5 kcal/mol with the protein form one conventional hydrogen bond with His161. It also forms halogen interaction with Ala141. The compound is also involved in hydrophobic interactions with Leu144, Cys267, Ala156, Leu197, Val158, Pro195 (Figure 7).

According to the results obtained from the molecular docking interactions (1) with Molegro Visual Docker, compounds 25, 31, 35, and 41 have the least energies interactions with the protein crystal structure than the reference drugs except for Ceftriaxone (Azithromycin). (2) with iGemDock, compounds 41 and 49 have the least total energy values than the reference drugs. (3) lastly using AutoDock Vina, compound 4, 17, 18, 20, 36, 44, 49, and 57 shows the best binding energy with the protein structure than the reference drugs.

3.1 ADMET Properties

Lipinski's rule of 5 [28] and Veber's rule of 2 [29] propose that molecular weight (MW), hydrogen bond acceptors (HA), hydrogen bond donors (HD), rotatable bond (R-bond), polar surface area (PSA) are crucial to influencing the oral bioavailability, good absorption or permeation, if MW < 500 Da, The HD < 5 (counting the sum of all NH and OH groups), partition coefficient octanol/water cLog P < 5, The HA < 10 (counting all N and O atoms). The other two Veber's parameters: Number of Rotatable bonds (R-bond) < 10 and Polar surface area (PSA) < 140 Å2. The molecular weight (MW) of all the selected compounds is less than 450 Da, unlike the reference drugs that have more than 450 Da except Ampicillin with 349.41 Da. Increasing MW reduces the compound concentration at the surface of the intestinal epithelium, therefore reducing absorption. Increasing size also blocks passive diffusion through the tightly packed aliphatic side chains of the bilayer membrane [30]. The 12 selected compounds are likely soluble and easily pass-through cell membranes. Compound 35 and the reference drugs have negative cLogP indicates that the compounds are too hydrophilic, therefore, it has good aqueous-solubility, better gastric tolerance, and efficient elimination through the kidneys. Since their clogP is less than zero, the drug has difficulty penetrating the lipid membranes. The rest selected compounds have a positive cLogP value indicates that the compound is too lipophilic (Table 6). So, it has a good permeability through the biological membrane, a better binding to plasma proteins, elimination by metabolism but poor solubility, and gastric tolerance [10]. From the Table, compound 17, 41, 44, and 49 has their cLogP greater than zero and less than three (3) shows that it has better oral bioavailability. An analysis of drug-like molecules (cLogS > -4) suggests that for better absorption and good solubility. Typically, a

high solubility goes along with good absorption. Therefore, compound 4, 18, 20, 25, 31, 36, 44, and 57 have their cLogS calculations greater than -4, while all the reference drugs are less than -4 shows low solubility with bad absorption. Molecules showing good absorption or permeation are likely to have hydrogen bond donors (HD) not more than 5 and hydrogen bond acceptors (HA) not more than 10 to enhance the probability of good intestinal permeability. The selected compounds are within the required range, while all the reference drugs except Ampicillin have their HA and HD in more than the required range (Table 6).

The rotatable bond (R-bond) of all the compounds is less than 10, show that the compounds are flexible and more adaptable for efficient interaction with the protein binding pocket. A successful CNS drug has its R-bond less than 8 [31]. All the selected compounds as Yersinia pseudotuberculosis inhibitors have an R-bond less than 8 (Table 6). The polar surface area (PSA) is formed by the polar atoms of a molecule. Compounds with PSA less than 140Å2 show a good correlation with passive molecular transport through membranes, and so allows estimation of transport properties of drugs.

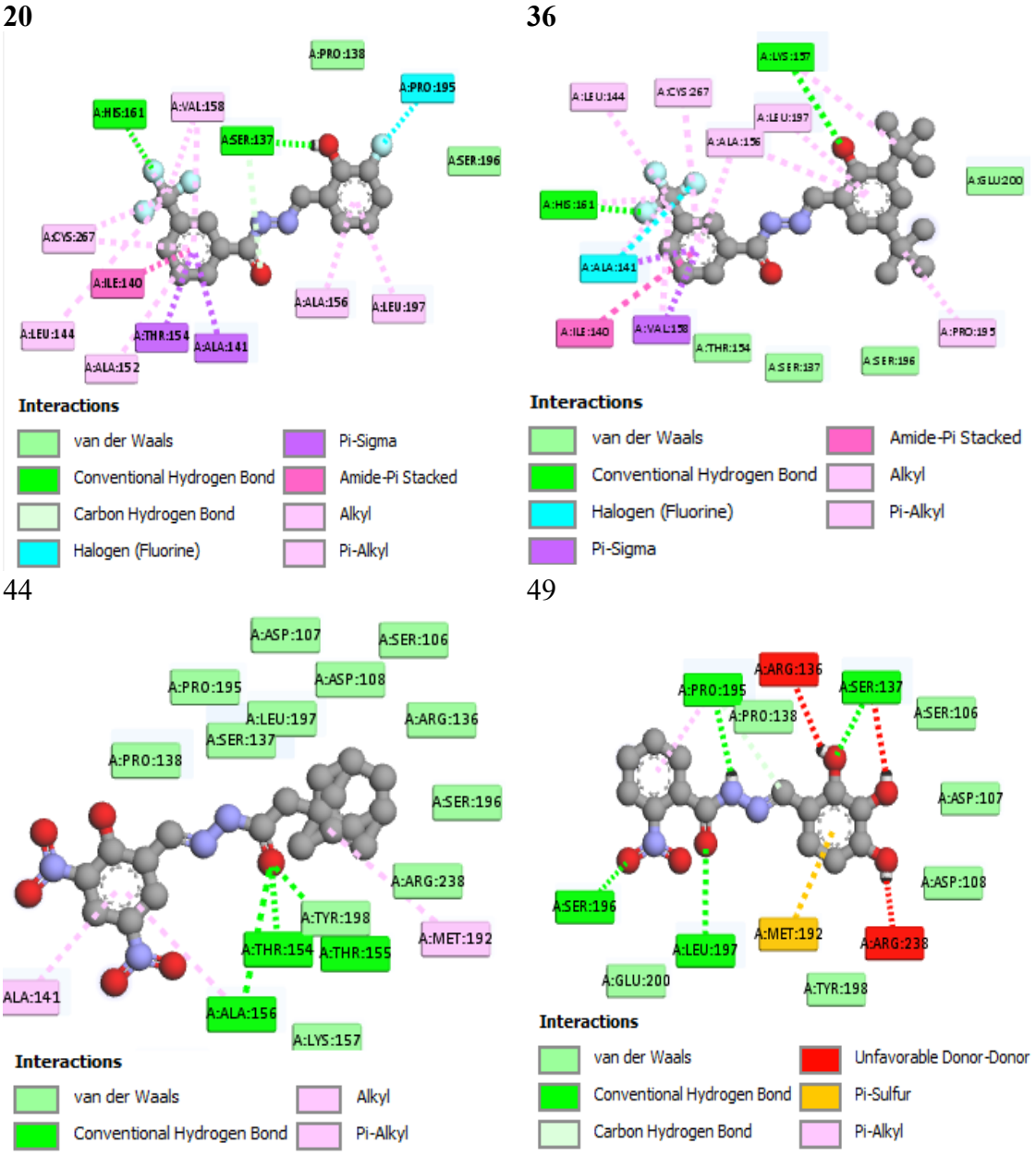


Figure 7. Interaction of the selected compounds with the protein crystal structure

Ceftriaxone, Chlortetracycline, and Oxytetracycline have very high values of PSA results except for Ampicillin, worsening of the absorption of a drug. Compounds 41, 44, and 49 with PSA values between 140 and 155 Å2 belong to the compounds with reduced absorption (Table 6).

3.2 Drug-likeness properties and lipophilicity indices

Since passing Lipinski's and Veber's rules is not a guarantee that a compound is drug-like, other parameters were carried out called the lipophilicity indices as shown in Table 7. The values of drug-likeness, ligand efficiency (LE), Lipophilic ligand efficiency (LLE), ligand efficiency lipophilic price (LELP), mutagenic, tumorigenic, and reproductive effectiveness were compared. The drug-likeness may be defined as a complex balance of various molecular properties and structural features that determine whether a particular molecule is similar to the known drugs [32].

Table 6. Absorption, permeability, and bioavailability properties of the selected compounds

Compound No.	MW (g/mol)	cLogP	cLogS	HA	HD	TSA (Å^2)	R-bond	PSA (Å^2)
4	310.168	4.782	-4.929	4	2	252.79	4	61.69
17	316.316	2.271	-2.772	7	5	239.91	5	122.38
18	362.792	3.692	-4.918	6	4	252.00	3	130.39
20	326.248	3.806	-4.517	4	2	229.15	4	61.69
25	405.291	5.093	-5.437	5	2	281.42	5	70.92
31	399.229	3.858	-4.951	7	2	290.96	6	89.38
35	237.214	-0.332	-1.824	7	3	182.11	4	114.01
36	420.473	6.868	-6.523	4	2	317.00	6	61.69
41	278.223	0.121	-3.151	9	2	214.98	5	140.53
44	402.406	1.920	-5.6	10	2	284.65	6	153.33
49	317.256	1.243	-3.293	9	4	229.71	4	147.97
57	349.183	3.511	-4.277	5	2	234.23	4	70.92
Ampicillin	349.410	-1.657	-1.565	7	3	238.56	4	138.03
Ceftriaxona	554.588	-3.011	-2.953	15	4	365.48	8	287.82
Chlortetracycline	494.882	-1.578	-2.166	11	7	311.79	2	201.85
Oxytetracycline	460.438	-2.183	-1.43	11	7	296.37	2	201.85

MW = Molecular weight; HA = hydrogen bond acceptor; HD = hydrogen bond donor; TSA = total surface area; PSA = polar surface area

In drug-likeness property, a positive value for the chemicals states that the molecule contains predominantly fragments that are frequently present in commercial drugs [33]. Compounds 20, 25, 36, 41, 44, and 49 showed negative values for the drug-likeness properties (i.e., they do not contain fragments that are frequently present in market drugs). The toxicity risk values were predicted using the software Data warrior (OSIRIS) are shown as none, low, and high for its mutagenic, tumorigenic, irritant, reproductive effective properties. The high risks of undesired effects like mutagenicity tumorigenic, and reproductive effective properties are shown in Table 7. The none value indicates the absence of risk alerts that a particular substance would be completely free of any toxic effect, the low and high values show the level of toxicity [34]. Compounds 18, 20, and 35 showed high mutagenic toxicity risk, compound 31 shown high tumorigenic toxicity risk, while compounds 31 and 35 shows high reproductive effective properties, respectively (Table 7).

The ligand efficiency (LE) is free binding energy in kcal/mol per nonhydrogen atom or heavy atom calculated from IC50 [35]. A truly good hit or lead compound has LE greater than 0.3 and the results show that all the selected compounds have their LE greater than 0.3 [11, 36]. This indicates that the compounds have a desirable LE potency at the right molecular weight. The lipophilic ligand efficiency (LLE) is used to identify low potency target compounds that are small in size and have low lipophilicity, it evaluates how well compounds improve potency while asserting low lipophilicity. The score ranges from 5 to 7 or more. The results

showed that compounds 17, 44, and 49 displayed a significantly high value of LLE. Ligand efficiency lipophilic price (LELP) becomes a useful function to follow during hit-to-lead optimization. The LELP could distinguish between marketed drugs over drug candidates. The ideal LELP values have been stated to be between -10 and 10 for acceptable leads [37]. The closer the LELP is to zero in the positive range, the better, and the desirable range is between 0 to 7.5. Compound 17, 41, 44, 49, and 57 have their LELP value within the stipulated range and they have the chance of success in the Yersinia Pseudotuberculosis inhibitors drug development process.

These selected compounds were also tested for irritants, the nasty function (Nasty F), shape index, molecular flexibility, molecular complexity, and the electronegative atoms were also computed as shown in Table 8. All the selected compounds and the reference drugs are free from the irritant, while compound number 31 was associated with a high risk of irritant. The shape index (Shap-I) works with the two-dimensional non-hydrogen atoms and bond graph of the molecule. It ranges from 0 to 1.

Table 7. Lipophilicity and toxicity properties of the selected compounds

Compound No.	Drug-likeness	LE	LLE	LELP	Mutagenic	Tumorigenic	R-E
4	1.0753	0.5009	3.6162	9.546	None	None	None
17	3.1799	0.4634	5.4983	4.9011	None	None	None
18	5.5897	0.4427	4.053	8.339	High	None	None
20	-2.7696	0.4592	3.894	8.2858	High	None	None
25	-0.7374	0.4172	2.5091	12.209	None	None	None
31	4.3397	0.3962	3.6507	9.7375	None	High	High
35	2.1895	0.6017	7.7879	-0.552	High	None	High
36	-6.1147	0.3404	0.5757	20.177	None	None	None
41	-8.0604	0.5067	7.2667	0.2378	None	None	None
44	-0.4717	0.3480	5.4361	5.5182	None	None	None
49	-07802	0.4360	6.0669	2.8506	None	None	None
57	2.5245	0.4732	3.733	7.4193	None	None	None
Ampicillin	9.3648	--------	------	-------	None	None	None
Ceftriaxona	16.694	-------	------	------	None	None	None
Chlortetracycline	5.2162	-------	--------	------	None	None	None
Oxytetracycline	5.1656	------	--------	------	None	None	None

LE = ligand efficiency; LLE = lipophilic ligand efficiency; LELP = ligand efficiency lipophilic price; R-E = reproductive effective

The value of 1 represents perfect chains and the smaller the value the more rings and bridges in the compounds. All the compounds are closed to linearity (straight-chain) with a shape index greater than 0.5 except compound compounds 36 and 44. The molecular flexibility (Mol Fle) of a ligand has a substantial influence on the affinity and specificity when binding to a protein. Molecular flexibility relates to the ease by which the molecule transverse the membrane [38]. The molecular flexibility ranges from 0 (rigid) to 1 (completely flexible). All the selected compounds are a bit rigid since their molecular flexibility is less than 0.5. Reduced molecular flexibility (measured by the number of rotatable bonds) and low polar surface areas are found to be important predictors of good oral bioavailability [39]. The molecular complexity also plays important role in molecular properties predictions. The reference drugs have their molecular complexity value higher than the selected compounds; this suggests that the selected compounds can easily be reproduced or synthesized.

Globularity values play an important part in molecular property prediction. The globularity of a compound is a value that describes how well the molecule's 3D shape resembles a sphere. From Table 9, the number of heavy atoms (non-hydrogen atoms) of the reference drugs is higher than the selected drugs except Ampicillin with 24. So, the ligand efficiency decreases with the increase of the number of heavy atoms. Decrease of heavy atoms increases the lipophilicity (ligand efficiency) which leads to

low aqueous solubility and difficulties in penetrating the lipid bilayers of cell membranes [11, 18, 40].

The non-carbon/hydrogen atoms (non-C/H) are all greater than one. A lower carbon to heteroatom balance may be required to achieve acceptable aqueous solubility and lipophilicity for uptake and movement in mammals [41]. Globularity using single value decomposition of 3D-atom coordinate (SVD) values ranges from close to 1.0 for spherical molecules to 0.0 for perfectly flat or linear molecules. The SVD values reference drugs and some of the selected compounds are less than 0.5; which suggested that they are flat or linear in shape except compounds 25, 44, and 49 that have their SVD values greater than 0.5 (Table 9). The globularity from molecular volume and surface (GMVS) values ranges from close to 1.0 for spherical molecules to about 0.6 for linear with irregular shapes. All the compounds are non-spherical with compound 44 with a greater irregular shape.

Table 8. Comparison of the molecular shape of the selected compounds with the standards

Compound No.	Irritant	Nasty F.	Shap-I	Mol Fle	Mol Com	Electro.
4	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.6087	0.3767	0.6863	4
17	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.6957	0.4675	0.6392	7
18	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.5833	0.3585	0.7938	8
20	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.5652	0.4483	0.7090	8
25	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.56	0.4006	0.7590	6
31	High	Acyl-hydrazone; imine/hydrazone of aldehyde	0.5384	0.3707	0.7787	9
35	None	Acyl-hydrazone; imine/hydrazone of aldehyde; Limit! Oxal-diamide	0.7059	0.3182	0.6007	7
36	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.4356	0.4355	0.7982	7
41	None	Aromatic nitro; Acyl-hydrazone; imine/hydrazone of aldehyde	0.6	0.3489	0.7426	9
44	None	Aromatic nitro; Acyl-hydrazone; imine/hydrazone of aldehyde	0.4828	0.4400	0.8364	10
49	None	Aromatic nitro; Acyl-hydrazone; imine/hydrazone of aldehyde	0.5652	0.3873	0.7618	9
57	None	Acyl-hydrazone; imine/hydrazone of aldehyde	0.6191	0.3563	0.6886	6
Ampicillin	None	----------	0.5417	0.3818	0.8929	8
Ceftriaxone	None	Acyl-hydrazone; imine/hydrazone of aldehyde; Limit! Oxal-diamide	0.5278	0.3882	0.9593	18
Chlortetracycline	None	Twice activated DB	0.3529	0.3261	1.1057	12
Oxytetracycline	None	Twice activated DB	0.3636	0.3283	1.0879	11

Nasty-F = nasty function; Shap-I = shape index; Mol Fle = molecular flexibility; Mol Com = molecular complexity; Electro = electronegativity.

Table 9. Globularity and Size evaluation for the selected compounds and standard drugs

Compound No.	Non-H Atom	Non-C/H	SVD	GMVS	V-Surf.	VDW-Vol.	SP^3
4	23	4	0.4816	0.7288	326.96	345.79	6
17	23	7	0.2789	0.7068	310	305.26	6
18	24	8	0.3605	0.7161	313.71	316.54	3
20	23	8	0.3509	0.7178	286.26	276.90	2
25	25	6	0.6305	0.7301	344.01	374.15	7
31	26	9	0.3195	0.6701	363.72	357.69	7
35	17	7	0.1278	0.7388	237.78	218.88	3
36	30	7	0.3504	0.6727	403.64	421.04	10
41	20	9	0.2701	0.7178	266.89	249.28	5
44	29	10	0.6908	0.83163	296.24	363.52	14
49	23	9	0.6095	0.7299	287.37	285.57	4
57	2	6	0.2832	0.6983	299.81	284.77	3
Ampicillin	24	8	0.2640	0.7519	313.98	340.99	10
Ceftriaxona	36	18	0.2812	0.6429	451.43	464.78	10

Chlortetracycline	34	12	0.388	0.7416	366.68	421.52	16
Oxytetracycline	33	11	0.4653	0.7558	351.19	406.49	16

Other properties calculated are the solvent excluded surface area (Van der Waals surface) using VMD-radii and 1.4A probe (V-Surf), the molecular volume inside solvent exclude surfacing using VMD-radii, and 1.4A probe (VMD-Vol) as shown in Table 9.

3.3 Golden Triangle and Drug Score test

As indicated in Fig. (8), around 42 percent of the examined chemicals lie within the golden triangle, indicating that they will not have clearance issues, while 52 percent fall outside the golden triangle [12, 42]. Out of the 12 candidate drugs, compound 17, 35, 41, 44, and 49 passes the golden triangle screening. These selected drugs undergo a drug score test. The drug score combines all other predictions into one total [32]. The drug score is used to assess the drug candidate's potential [43]. The chemical has a better possibility of becoming a drug candidate when the drug score is higher. The drug score values such as 1.0, 0.8, 0.6 are associated with no risk, medium risk, and high risk, respectively. Compounds 17, 35, 41, 44, and 49 drug score are 0.87, 0.14, 0.46, 0.38, and 0.45, respectively. This shows that compound 17 possesses the value of medium risk and may be used as a drug molecule.

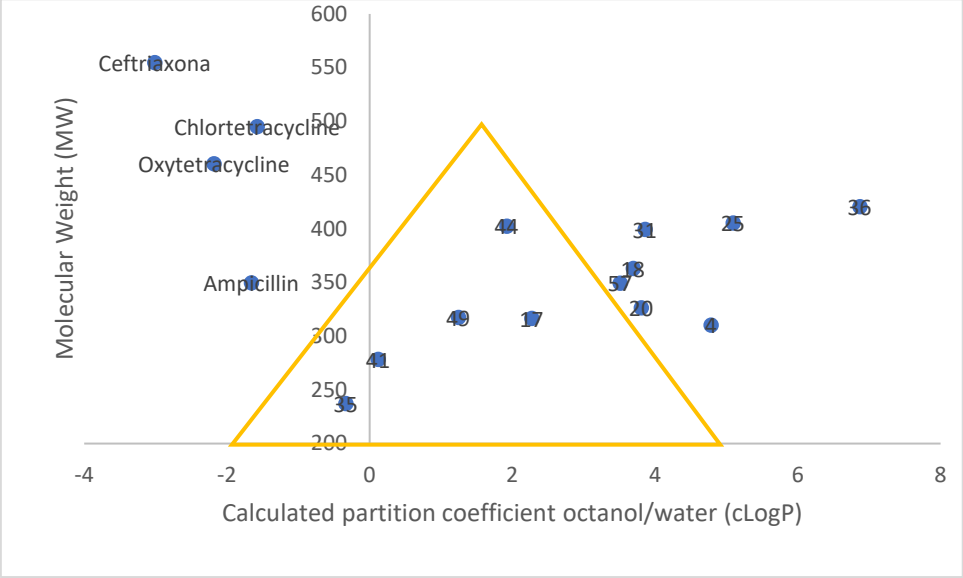


Figure 8. Golden triangle plot of molecular weight (MW) vs calculated LogP

3.4 Molecular Dynamics Simulations

Finally, compounds 17 with approved drug-likeness (Table 7) and drug score undergo 1 ns molecular dynamics simulations from the docked complexes (Fig. 9), since docking was not considered conclusive because *in vivo* binding of the inhibitor to a protein is a dynamic process.

The comparison between the docked conformation and 1 ns MD simulated stable conformation of compound 17 is shown in Figs. 9 and 10. The conformations of the docked and the simulated were well aligned with slight differences. The root means square deviation (RMSD), kinetic, total, and potential energy was monitored during the simulations to ensure the stability of the simulated system, and plots are shown in Fig. 11. By comparing docking complex (Fig. 9) and the 1 ns MD simulations complex (Fig. 10), compound 17 retained the hydrogen bonds with Ser137, Ser106, Leu197, electrostatic interaction with Asp108, and the van der Waals interaction with Pro195, respectively. The docking complex and the MD simulation complex form an equal number of hydrogen bonds. Furthermore, compound 17 formed six additional van der Waals interactions and one additional hydrophobic interaction during the 1 ns MD simulations. The binding interaction pattern during MD simulation was almost consistent with the docking results. The simulation result reveals that the RMSD tends to be steady and wavered approximately at 1.1 Å (Fig. 11a). The kinetic, total, and potential energy as a function of time was assessed to check the complexes during the 1ns molecular dynamic simulations (Fig. 11b-d). The

average kinetic, total and potential energy of the complexes was set at about 32,752.47, -175963.42 and -143210.95 Kcal/mol at a temperature of 301.99 K, respectively. The shape of the curve shows that the kinetic, total and potential energy of the systems is stable, no abnormal fluctuation was noticed during the whole simulation.

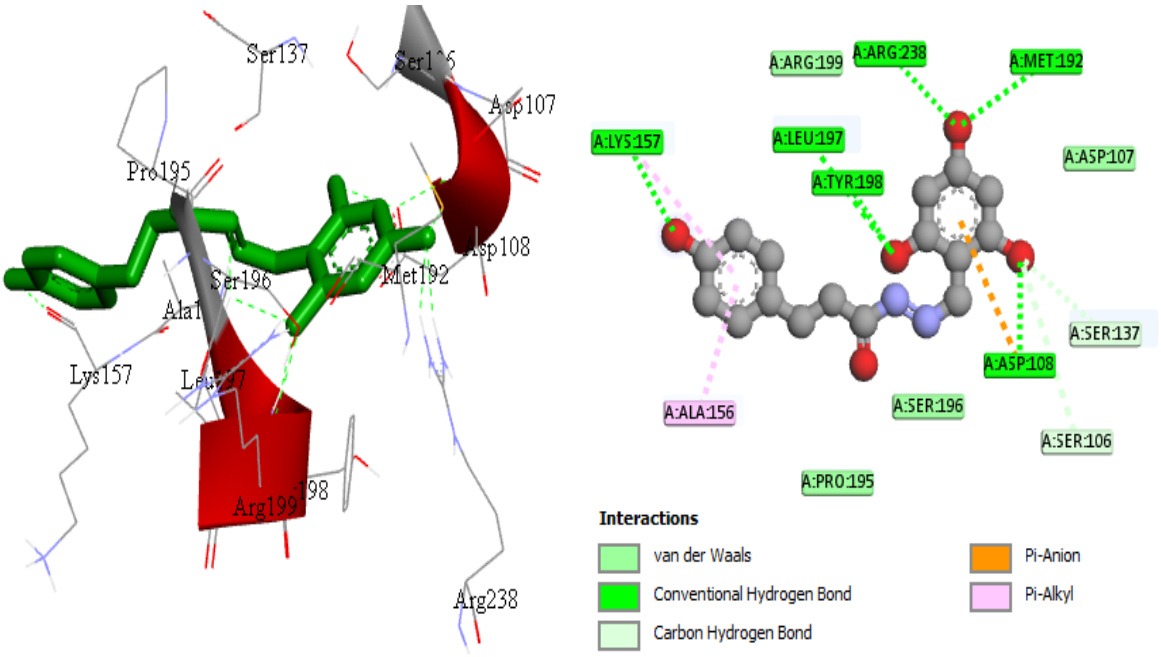


Figure 9. Interaction of compound 17 with the protein crystal structure before MDs simulations.

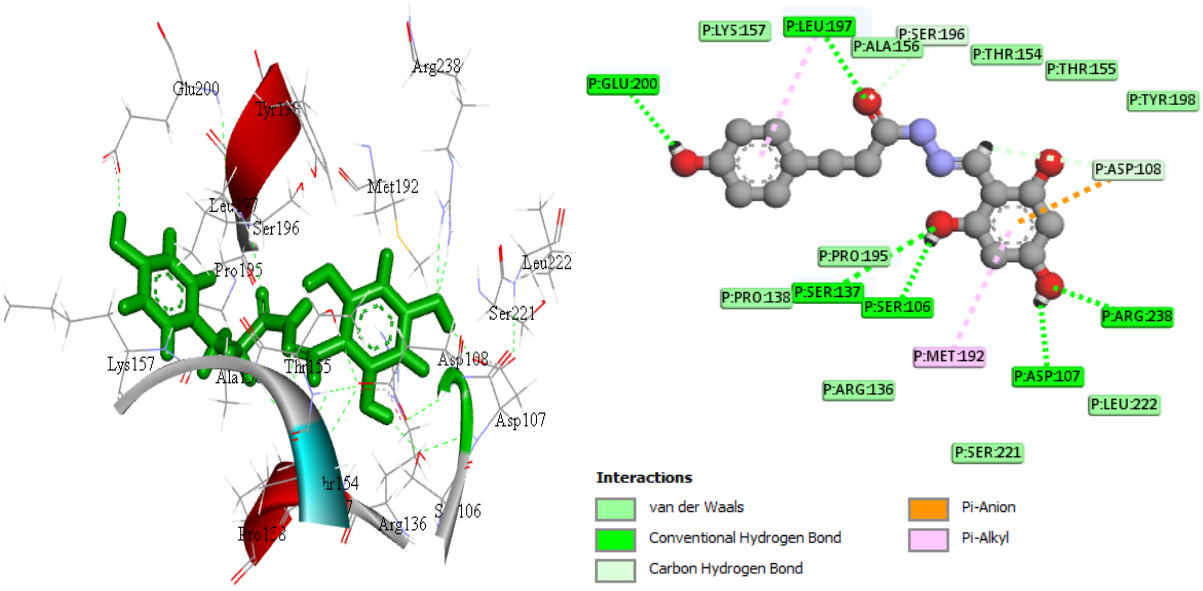
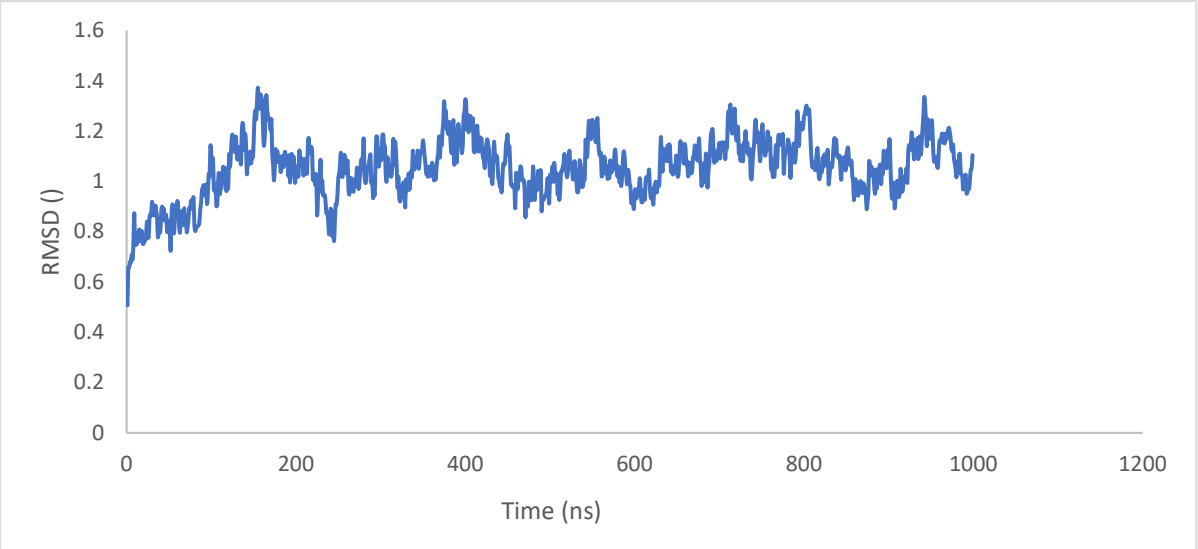


Figure. 10. Interaction of compound 17 with the protein crystal structure after molecular dynamics simulations

The results obtained in this study are promising and could aid in the development of new anti-Y. pseudotuberculosis drugs. It would be interesting to test the inhibitory activity of the predicted salicylidene acylhydrazides derivatives against the effector binding domain of the LysR-Type transcription factor RovM from Y. pseudotuberculosis.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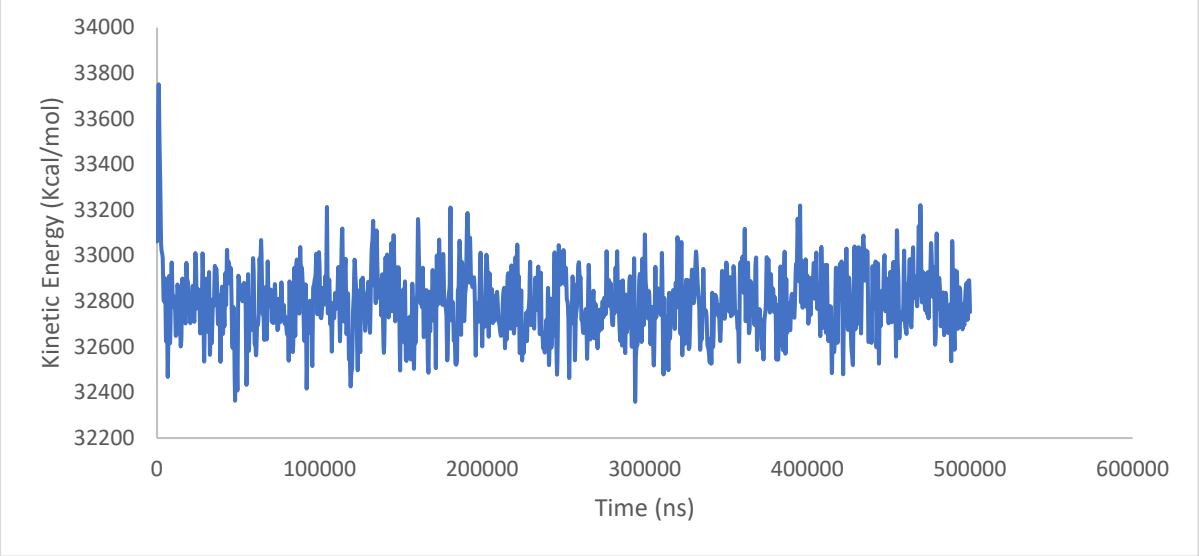
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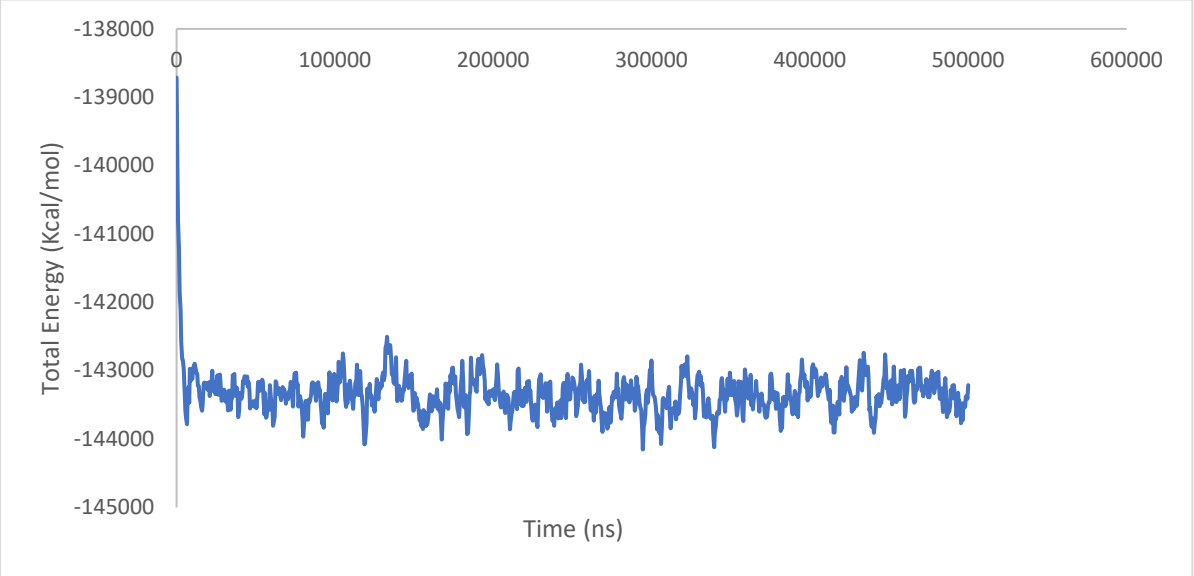
600000
Time (ns)


Total Energy (Kcal/mol)
-138000
-139000
-140000
-141000
-142000
-143000
-144000
-145000
0
100000
200000
300000
400000
500000
600000
Time (ns)

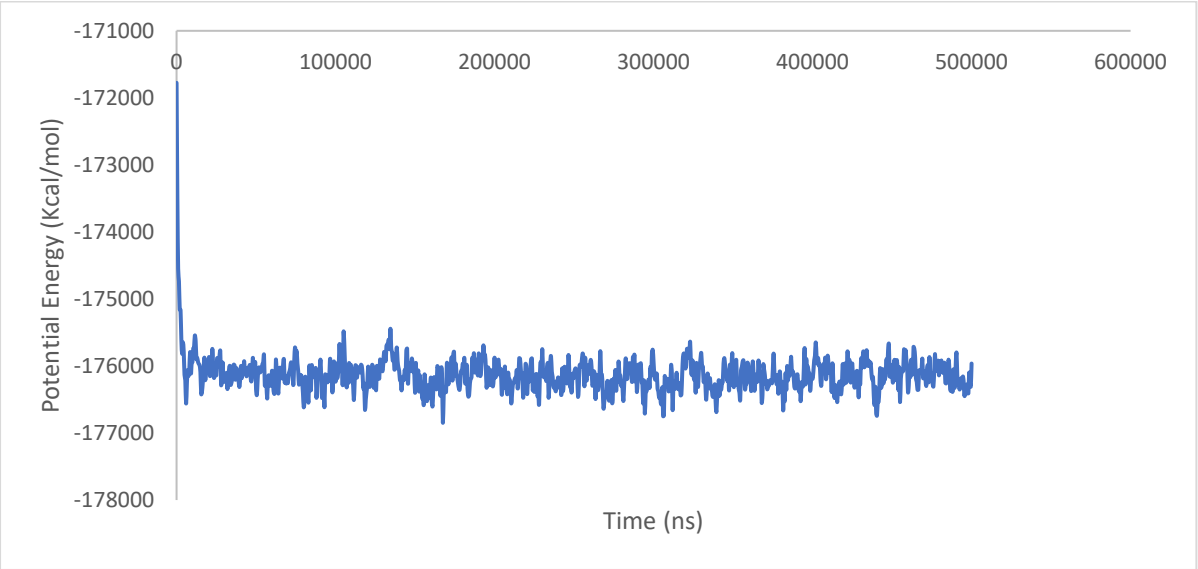


Figure 11. The plot of (a) RMSD, (b) kinetic energy, (c) total energy, and (d) potential energy per time (ns) of the protein-ligand complex.

4. Conclusions

Drug targets derived from infections' specific pathways are of particular relevance in the development of medications to combat the bacterium Y. pseudotuberculosis. This is a lethal infection brought about by microorganisms of the class mycobacterium which influences people. Because of the unavailability of preventive vaccines this disease is becoming endemic in many countries. The computational mechanisms have improved the identification of vaccines by reverse vaccinology. To find the best-screened compounds, we performed molecular docking simulations from three separate software packages (MVD, iGemDock, and AutoDock-vina), ADMET, golden triangle visualizer, drug scores, and molecular dynamics simulations. Computational toxicity and drug-likeness tests also showed good results. The analysis of the golden triangle showed that compounds 17, 35, 41, 44, and 49 wouldn't have clearance and cytomembrane permeableness issues except for all the references medication, compounds four, 18, 20, 25, 31, 36, and 57, respectively. Based on all the simulations that have been done, compounds 17 and 44 were the potential candidate to inhibit Y. pseudotuberculosis. We conclude these compounds are also potential ligands to be developed as a drug. Therefore, in vitro and in vivo tests are mandatory to establish these facts

References

[1] A.K. Heroven, P. Dersch, RovM, a novel LysR-type regulator of the virulence activator gene rovA, controls cell invasion, virulence, and motility of Yersinia pseudotuberculosis. Molecular Microbiology 62(5) (2006)1469-1483. doi: 10.1111/j.1365-2958.2006.05458.x.

[2] A.K. Heroven, A.M. Nuss, P. Dersch "RNA-based mechanisms of virulence control in Enterobacteriaceae." RNA Biology 14.5 (2017) 471-487.

[3] P.D. Tamma, S.E. Cosgrove, L.L. Maragakis, Combination Therapy for Treatment of Infections with Gram-Negative Bacteria. Clinical Microbiology Reviews 25(3) (2012) 450–470. doi:10.1128/CMR.05041-11.

[4] B. Malgija, H.M. Rajendran, S. Darvin, J. Priyakumari, In silico exploration of HIV entry co-receptor antagonists: a combination of molecular modeling and molecular dynamics simulations. Medicinal Research Reviews 42(3) (2019), 249-253.

[5] E.I. Edache, A. Uzairu, P.A. Mamza, G.A. Shallangwa, Molecular Docking Study of Chlamydia Trachomatis Using Salicylidene Acylhydrazides as Inhibitors. Biomedical Journal of Scientific & Technical Research 36(4) (2021) 26472-26489. DOI: 10.26717/BJSTR.2021.36.005895.

[6] Z. Hafidi, M.O. El Achouri, F.F. Sousa, L. Pérez, Antifungal activity of amino-alcohols based cationic surfactants and in silico, homology modeling, docking, and molecular dynamics studies against lanosterol 14-α-demethylase enzyme. Journal of Biomolecular Structure and Dynamics (2021) 1-17.

https://doi.org/10.1080/07391102.2021.1902396.

[7] Q. Zhang, G. Lambert, D. Liao, H. Kim, K. Robin, C.K. Tung, N. Pourmand, R.H. Austin, Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. Science (New York, NY), 333 (2011) 1764–1767. https://doi.org/10.1126/science.1208747

[8] J. Davies, D. Davies, Origins and Evolution of Antibiotic Resistance. Microbiology and Molecular Biology Reviews 74(3) (2010) 417-433. doi:10.1128/MMBR.00016-10.

[9] M.P. Koentjoro, N. Ogawa, Structural studies of transcriptional regulation by LysR-TYPE transcriptional Regulators in bacteria. Reviews in Agricultural Science 6 (2018) 105-118. http://dx.doi.org/10.7831/ras.6.105.

[10] N. Quade, M. Dieckmann, M. Haffke, A.K. Heroven, P. Dersch, D.W. Heinz, Structure of the effector-binding domain of the LysR-type transcription factor RovM from Yersinia pseudotuberculosis. Acta Crystallographica Section D 67 (2011) 81–90. doi:10.1107/S0907444910049681.

[11] E.I. Edache, A. Uzairu, P.A. Mamza, G.A. Shallangwa, Docking Simulations, and Virtual Screening to find Novel Ligands for T3S in Yersinia pseudotuberculosis YPIII, A drug target for type III secretion (T3S) in the Gram-negative pathogen Yersinia pseudotuberculosis. Chemical Review and Letters 4 (2021) 130-144. doi: 10.22034/CRL.2021.254804.1088.

[12] W. Sobhi, A. Attoui, T. Lemaoui, A. Erto, Y. Benguerba, In silico drug discovery of Acetylcholinesterase and Butyrylcholinesterase enzymes inhibitors based on Quantitative Structure-Activity Relationship (QSAR) and drug-likeness evaluation. Journal of Molecular Structure 1229 (2021) 129845.

[13] R. Shivakumar, K. Venkatarangaiah, S. Shastri, R.B. Nagaraja, A. Sheshagiri, Antibacterial Property and Molecular Docking Studies of Leaf Calli Phytochemicals of Bridelia scandens Wild. Pharmacognosy Journal 10(6) (2018) 1221-1229. DOI: 10.5530/pj.2018.6.209

[14] M.H. Baig, K. Ahmad, G. Rabbani, M. Danishuddin, I. Choi, Computer-Aided Drug Design and its Application to the Development of Potential Drugs for Neurodegenerative Disorders. Current Neuropharmacology 16 (2018) 740-748. DOI: 10.2174/1570159X15666171016163510.

[15] M. Mahdavia, V. Moreau, In silico designing breast cancer peptide vaccine for binding to MHC class I and II: A molecular docking study. Computational Biology and Chemistry - Journals 65 (2016) 110–116. http://dx.doi.org/10.1016/j.compbiolchem.2016.10.007.

[16] R. Ganesh, I. Kannan, Molecular Docking Study of Certain Plant Alkaloid Derivatives as Inhibitors of Various Drug Targets of Alzheimer's Disease. Biomedical and Pharmacology Journal 10(3) (2017) 1489-1494. http://dx.doi.org/10.13005/bpj/1257.

[17] T.W. Johnson, K.R. Dress, M. Edwards, Using the Golden Triangle to optimize clearance and oral absorption. Bioorganic & Medicinal Chemistry Letters 19 (2009) 5560–5564, doi: 10.1016/j.bmcl.2009.08.045.

[18] E.I. Edache, A. Uzairu, P.A. Mamza, G.A. Shallangwa, Prediction of HemO Inhibitors Based on Iminoguanidine using QSAR, 3DQSAR Study, Molecular Docking, Molecular Dynamic Simulation, and ADMET. Journal of Drug Design and Discovery Research 1(2) (2020) 36-52.

[19] P. Munikumar, P. Natarajan, U. Amineni, R.K.V. Krishna, Discovery of potential lumazine synthase antagonists for pathogens involved in bacterial meningitis: In silico study. Informatics in Medicine Unlocked 15 (2019) 100187. https://doi.org/10.1016/j.imu.2019.100187.

[20] N. Quade, M. Dieckmann, M. Haffke, A.K. Heroven, P. Dersch, D.W. Heinz, Structure of the effector-binding domain of the LysR-type transcription factor RovM from Yersinia pseudotuberculosis. Acta Crystallographica Section D 67(Pt2) (2011) 81-90. doi: 10.1107/S0907444910049681.

[21] R. Thomsen, M.H. Christensen, MolDock: a new technique for high-accuracy molecular docking. Journal of Medicinal Chemistry 49(11) (2006) 3315-21. doi: 10.1021/jm051197e.

[22] K.C. Hsu, Y.F Chen, S.R. Lin, J.M. Yang, iGemDock: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis, BMC Bioinformatics 2(1) (2011) 1-11.

[23] O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry 31 (2009) 455–461. https://doi.org/10.1002/jcc.21334.

[24] M.J. Waring, Defining optimum lipophilicity and molecular weight ranges for drug candidates-Molecular weight dependent lower log D limits based on permeability, Bioorganic & Medicinal Chemistry Letters 19 (2009) 2844–2851. doi: 10.1016/j.bmcl.2009.03.109.

[25] A. Zerroug, S. Belaidi, I. Benbrahim, L. Sinha, S. Chtita, Virtual screening in drug-likeness and structure/activity relationship of pyridazine derivatives as Anti-Alzheimer drugs, Journal of King Saud University-Science 31 (2019) 595–601. doi: 10.1016/j.jksus.2018.03.024.

[26] J.C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kale, K. Schulten, Scalable molecular dynamics with NAMD. Journal of Computational Chemistry 26 (2005) 1781–1802.

[27] W. Humphrey, A. Dalke, K. Schulten, "VMD – Visual Molecular Dynamics", Journal of Molecular Graphics 14 (1996) 33-38.

[28] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Advanced Drug Delivery Reviews 46(1-3) (2001) 3-26.

[29] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, Journal of Medicinal Chemistry 45(12) (2002) 2615-2623.

[30] T.S. Maliehe, P.H. Tsilo, J.S. Shandu, Computational Evaluation of ADMET Properties and Bioactive Score of Compounds from Encephalartos ferox. Pharmacognosy Journal 12(6) (2020) 1357-62. DOI: 10.5530/pj.2020.12.187.

[31] H. Pajouhesh, G.R. Lenz, Medicinal chemical properties of successful central nervous system drugs. Neuro Rx. 2 (2005) 541–553. DOI: 10.1602/neurorx.2.4.541.

[32] T.V.A. Kumar, S. Kabilan, V. Parthasarathy, Screening and Toxicity Risk Assessment of Selected Compounds to Target Cancer using QSAR and Pharmacophore Modelling. International Journal of PharmTech Research 10(4) (2017) 219-224. http://dx.doi.org/10.20902/IJPTR.2017.10428.

[33] O. Ursu, A. Rayan, A. Goldblum, T.I. Oprea, Understanding drug-likeness. WIREs Computational Molecular Science 1 (2011) 760-781. doi:10.1002/wcms.52.

[34] M. von Korff, T. Sander, Toxicity-indicating structural patterns. Journal of Chemical Information and Modeling 46 (2) (2006) 536-544.

[35] S.A. Egieyeh, J. Syce, S.F. Malan, A. Christoffels, Prioritization of anti-malarial hits from nature: chemo-informatic profiling of natural products with in vitro antiplasmodial activities and currently registered anti-malarial drugs. Malaria Journal 15(50) (2016) 1-23. DOI 10.1186/s12936-016-1087-y.

[36] M. Ouassaf, S. Belaidi, I. benbrahim, H. Belaidi, S. Chtita, Quantitative Structure-Activity Relationships of 1.2.3 Triazole Derivatives as Aromatase Inhibition Activity. Turkish Computational and Theoretical Chemistry 4(1) (2020) 1-11. doi.org/10.33435/tcandtc.545369.

[37] G.M. Keseru, G.M. Makara, The influence of lead discovery strategies on the properties of drug candidates. Nature Reviews Drug Discovery 3 (2009) 203-212. doi: 10.1038/nrd2796.

[38] C. Forrey, J.F. Douglasb, M.K. Gilson, The fundamental role of flexibility on the strength of molecular binding. Soft Matter 8 (2012) 6385-6392. DOI: 10.1039/C2SM25160D.

[39] F.Z. Fadel, N. Tchouar, S. Belaidi, V. Soualmia, O. Oukil, K. Ouadah, Computational Screening and QSAR Study on a Series of Theophylline Derivatives as Aldh1a1 Inhibitors. Journal of Fundamental and Applied Sciences 13(2) (2021) 942-964. doi: http://dx.doi.org/10.4314/jfas.v13i2.17.

[40] E.H. Kerns, D. Li, Drug-Like Properties: Concepts, Structure Design and Methods from ADME to Toxicity Optimization. Elsevier, United States (2008) 86-98.

[41] D.E. Clarke, J.S. Delaney, Physical and molecular properties of agrochemicals: An analysis of screen inputs, hits, leads, and products, CHIMIA 57(11) (2003) 731-734.

[42] E.I. Edache, A. Uzairu, P.A. Mamza, G.A. Shallangwa, Molecular docking, molecular dynamics simulations, and ADME study to identify inhibitors of Crimean-Congo Hemorrhagic Fever (CCHF) viral ovarian tumor domain protease (vOTU). Chemistry Research Journal 5(5) (2020) 16-30.

[43] H. Alonso, A.A. Bliznyuk, J.E. Gready, Combining Docking and Molecular Dynamic Simulations in Drug Design. Medicinal Research Reviews 26 (2006) 531-568. doi:10.1002/med.20067.