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MOLECULAR DOCKING STUDY, DRUG-LIKENESS AND PHARMACOKINETIC PROPERTIES (ADMET) PREDICTION OF SOME NOVEL THIOPHENE DERIVATIVES AS *Salmonella typhi* INHIBITORS

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ABSTRACT

Typhoid fever is a gram- negative bacterial infection caused by the bacterium called Salmonella Typhi, a rod-like shaped pathogen. Salmonella typhi is both food-born and water-born pathogen. This disease is affecting people in both developed and underdeveloped countries. In this research, geometry optimization was carried out using density functional theory (DFT) at B3LYP/6-31G* level of theory in finding the most stable structures of all the studied molecules. Molecular docking studies was carried out on novel thiophene derivatives (anti-typhoid agents) against S. Typhi target (DNA gyrase B) with the pdb ID: 1TM2 to virtually screened the studied molecules. The drug-likeness and pharmacokinetic properties of the investigated molecules were also evaluated using SWISSADME and pkCSM online web tools, respectively. The molecular docking virtual screening carried out on the anti-typhoid agents has explored their theoretical binding affinities (which ranges between -7.1 to 7.8 kcal/mol) and poses with the active sites of the S. Typhi target (DNA gyrase B). Compound A11 with the highest binding affinity of -7.8 Kcal/mol interacted with the following amino acid residues LYS35, THR247, ASP166, VAL164, TRP170, ALA222, PHE41, TR266, ASP116 and PHE41 in the active site of the receptor via conventional hydrogen bond, carbon hydrogen bond, hydrophobic and electrostatic interactions, respectively. The drug-likeness of the studied compounds showed good response to the Lipinski's rule of five filtering criterion by not violating more than one of the filtering criterion thereby confirming their drug-likeness properties and oral bioavailability, respectively. Their ADMET properties showed that they were all absorbed in the intestinal at a rate above 80% and well distributed in the brain and the central nervous system. They were all found to be both substrate and inhibitors of 3A4 which indicated their metabolic properties. And based on their predicted total clearance values, they can be excreted out. All the reported compounds had a negative AMES toxicity which mean they were all non-toxic. With reference been made to an approved already existing anti-typhoid agent ciprofloxacin with a binding affinity of -7.3 Kcal/mol to the investigated molecules (most especially compound A11), All the reported molecules have higher binding affinities than the ciprofloxacin, having good pharmacokinetic properties therefore proves the tendency of their effectiveness when been administered as drug.

Keywords: Molecular docking; Drug-likeness; Pharmacokinetic; Thiophene.

INTRODUCTION

Typhoid is a bacterial infection, caused by the bacterium Salmonella Typhi (S. Typhi), a Gram-negative bacterium, that can lead to a high fever, diarrhea, and vomiting which can be fatal (Hurley et al., 2014; Orish et al., 2014).The infection is often transmitted through contaminated food and drinking water, and it is more prevalent in places where hand-washing is less frequent. It can also be passed on by carriers who do not know they carry the bacteria. A growing body of research in immunology and neuro-physiology has led to the recent understanding that fever is generally an adaptive physiological response to some threat (Abioye et al., 2017; Bhargava and Chandra, 2016). It is difficult to estimate the real burden of typhoid fever in the world because the clinical picture is confused with many other febrile infections and the disease is underestimated because of lack of laboratory resources in most areas in developing countries (Wibisono et al., 2020). Annually, there are around 5,700 cases in the United States, and 75 percent of these start while traveling internationally. Globally, around 21.5 million people contract typhoid annually

(Mulu et al., 2021). If typhoid is caught early, it can be successfully treated with antibiotics, if it is not treated the bacterium can live in the intestines and bloodstream of humans. It spreads between individuals by direct contact with the feces of an infected person (Mulu et al., 2021).

The Salmonella typhi DNA Gyrase B is a subunit in the Salmonella receptor, due to the receptor been a multi-domain protein. The inhibitory action of the drugs is through the inhibition of DNA gyrase as a potential mechanism of action. The DNA gyrase, is essential for chain elongation during replication of the chromosome in the bacterial cells (Kourlaba et al., 2016).

Due to the growing resistance of the disease to several anti-biotics such as quinolones, penicillin, cephalosporin macrolides and others, drew attention of medicinal chemist into brewing of new novel inhibitors with improved bioactivities (Glomb and Świątek, 2021). Therefore, there urgent need for more effective and less toxic anti-salmonella typhi inhibitors that can overcome the resistance developed by the bacteria.

The concept of computational chemistry like computer-aided drug design (CADD) might save the time of discovering or designing new compounds with better potency, and also reduce the cost and time spent in synthesizing new compounds. Molecular docking is one of the most frequently used methods in structure-based drug design (SBDD) because of its ability to predict with a substantial degree of accuracy, the conformation of small-molecule ligands within the appropriate target binding site. Molecular docking contributes to the virtual screening of a library of compounds at the pre-clinical stage of drug development. This model predicts how a protein (enzyme) interact with small molecules (ligand) when bind togethers (Ibrahim et al., 2020a).

Drug-likeness properties give the conditions or criteria for drug potency of a particular chemical compound using set of filtering criteria such as of Lipinski's rule of five to predict the drug-likeness of the selected drugs, which states that if any chemical violates more than two of these criteria (Molecular weight ≤ 500g/mol, Number of hydrogen bond donor ≤ 5, Number of hydrogen bond acceptors ≤ 10, calculated logP ≤ 5), the molecule is said to be impermeable or badly absorbed (Lei et al., 2019). The Absorption, Distribution, Metabolism Excretion and Toxicity (ADMET) properties also known as pharmacokinetic properties describe the fate of a small molecule (ligand) in the body of a living organism when administered into the body (Chandrasekaran et al., 2018).

The aim of this work is to carry out molecular docking virtual screening on thiophene derivatives (as anti-Salmonella typhi inhibitors) to identify best hit compounds that can be used as template for designing new anti-salmonella typhi inhibitors with the prediction of their drug-likeness and ADMET properties.

MATERIALS AND METHODS

Materials

A logiq model M76T computer system, with the following specifications: Genuine Intel(R) CPU 585 @ 2.16GHz, 2.16 GHz, 4.00GB (RAM) with a system type of 64-bit operating system, x64-based processor and a computer name of Desktop-Stall13 was used to explore the nature of interactions between the active site of receptor target (Salmonella typhi) and the ligand (drug) with the help of spartan'14 version 1.1.4, auto-dock vina version 4.2, chimera version 1.10.2, and discovery studio visualizer version 16.1.0.15350, respectively (Ibrahim et al., 2019).

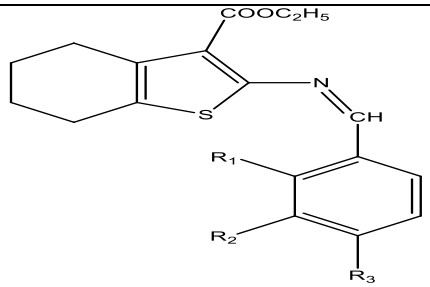
Molecular Docking Method

Molecular docking simulation predicts the binding affinities an orientation when two molecules bind with each other to form a stable complex (Ibrahim et al., 2020a).

Dataset Collection

The dataset which comprises eighteen (18) set of thiophene derivatives as anti-typhoid agents used in this work were collected from the literature (Sahu et al., 2008). The 2D structures of the dataset are shown in Table 1, respectively.

Table 1**:** The thiophene derivatives



S/N	R_1	R_2	R_3
A_1	Cl	H	Cl
A_2	H	Br	H
A_3	H	NO_2	H
A_4	OCH_3	H	H
A_5	H	H	$N(C_2H_5)_2$
A_6	H	OCH_3	OH
A_7	H	OCH_3	OCH_3
A_8	H	H	Br
A_9	Cl	H	H
A_{10}	NO_2	H	H
A_{11}	H	H	NO_2
A_{12}	H	H	H
A_{13}	H	H	$N(CH_3)_2$
A_{14}	H	H	OH
A_{15}	OH	H	H
A_{16}	H	OH	H
A_{17}	H	H	OCH_3
A_{18}	OCH_3	H	OCH_3

Drawing of Structures, Geometry Optimization and Ligand Preparation

In this research after data collection, the next is drawing of the 2D structures of the dataset. The 2D structures of the dataset were drawn using chemdraw version 12.0 software. After the 2D generation of the studied molecules, the 2D structures were automatically converted to 3D by Spartan 14 software prior to energy minimization. Energy minimization was carried out to reduce constraint in the structures before finding the most stable geometry of the studied molecules using the same software. Density Functional theory (DFT) at B3LYP/6-31G* level of theory was used in finding the most stable structures of all the studied molecules. Before the docking analysis, ligands were prepared from the optimized structures of the investigated compounds (Ibrahim et al., 2020b).

Receptor Retrieval and Preparation

The 3D structure of the S. typhi was downloaded from the protein data bank (pdb ID: 1TM2) the enzyme was prepared with the help of discovery studio visualizer for the docking analysis. In the course of the preparation of the receptor, polar hydrogen was added, water molecule was eliminated from the crystalized 3D structure of the receptor and saved in pdb file using discovery studio visualizer (Ibrahim et al., 2020c).

Docking Analysis

The docking of the ligands to the active site of S. typhi receptor (DNA Gyrase B) was achieved with the help of Auto dock vina version 4.2 by following successful docking protocols (Abdullahi et al., 2020).

Recoupling of Docked Ligand, Receptor and Visualization of Complex Interaction

Re-coupling of the docked ligands and receptor for further investigation was performed using chimera version 1.10.2 software. Discovery studio visualizer version 16.1.0.15350 was used to investigate the interaction of the complexes. The 2D and the 3D structures of the interactions were saved using snipping tool (Ibrahim et al., 2020d).

Drug-likeness and Pharmacokinetic

The pharmacokinetics properties or the ADMET properties and the drug likeness predictions were determined using SwissADME (http://www.swissadme.ch/index.php) and pkCSM (http:// structure.bioc.cam.ac.uk/pkCSM) free web tools used in evaluating ADMET properties and drug-likeness of small molecules (Daina et al., 2017).

RESULTS AND DISCUSSION

Molecular Docking

The binding mode of thiophene derivatives in the active site of salmonella typhi (receptor) was explored via molecular docking. Table 2 shows the binding affinities and mode of interaction of five the most potent thiophene anti-typhoid agents. The binding affinities of the studied molecules range from -7.1 to -7.8 Kcal/mol, respectively. The results of the best five (Compound A11, A9, A1, A2 and A16) among the investigated ones are reported and shown on Table 2. From Table 2, molecule A11 was identified to have the highest binding affinity of -7.8 Kcal/mol among the other the investigated compounds followed by molecule A9 with a binding affinity of -7.7 Kcal/mol, molecule A1 with a binding affinity of -7.6 Kcal/mol, molecule A2 with a binding affinity of -7.5 Kcal/mol and molecule A16 having -7.5 Kcal/mol as its binding affinity.

Table 2: Binding affinities and mode of interactions of the best five (5) selected ligands

Entry	Binding affinity (Kcal/mol)	Hydrogen bond interactions	Carbon hydrogen bond interactions	Hydrophobic and other interactions
A11	-7.8	LYS35 (2.44Å) and THR247 (2.47 Å)	ASP166 and VAL164	TRP170, ALA222, PHE41, TRP266 and ASP116
A9	-7.7	LYS35 (2.15 Å)	TRP170	LYS35, PHE42, ALA222, PHE41, ASP116 and LEU265
A1	-7.6	LYS35 (8.1 Å)	VAL164	TRP170, and TRP266 and VAL39, ALA222, PHE42, ASP116 and PHE41
A2	-7.5	ASP116(2.01 Å)	LEU265 TRP266 and TRP170	ALA222, PHE41, LYS35 and ASP116
A16	-7.5	LYS35 (2.90 Å), GLN167 (2.33 Å) and PRO220 (2.43 Å)	ALA222	PHE42, PHE41, TRP170 and ASP116

Compound A11 with the highest binding affinity of -7.8kcal/mol formed a conventional hydrogen bond with LYS35 (2.44Å) and THR247 (2.47Å) amino acid residue in the binding pose of the receptor. It also formed a carbon hydrogen bond with ASP166 and VAL164 amino acid residue with the back bone of the receptor. In addition to that, it also formed hydrophobic interactions with TRP170, ALA222, PHE41 and TR266 amino acid residue, although no halogen bond interaction was established but electrostatic interactions were observed with ASP116 and PHE41 amino acid residues. It's high binding affinity toward the target receptor may be as a result of it interactions with the mentioned amino acid residues in the active site of the receptor. Figure 1 shows the 2D structure of molecule A11 in complex with DNA gyrase B receptor.

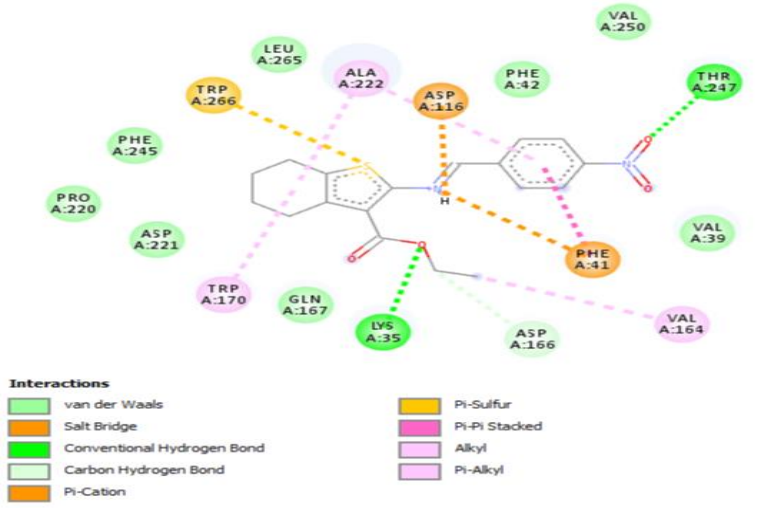


Figure 1: 2D structure of molecule A11 in complex with DNA gyrase B receptor.

The second ligand with the higher binding affinity was A9 (-7.7 Kcal/mol). It formed a conventional hydrogen bond with LYS247 (2.15 Å), a carbon hydrogen bond with TRP170 amino acid residues, respectively. It also formed hydrophobic interactions with LYS35, PHE42, ALA222 and LEU265 amino acid residues respectively. Halogen interactions were formed with ALA222 and LEU265 amino acid residues. Electrostatic interactions were also formed with PHE41 and ASP116 amino acid residues respectively. Figure 2 shows the 2D structure of molecule A9 in complex with DNA gyrase B receptor.

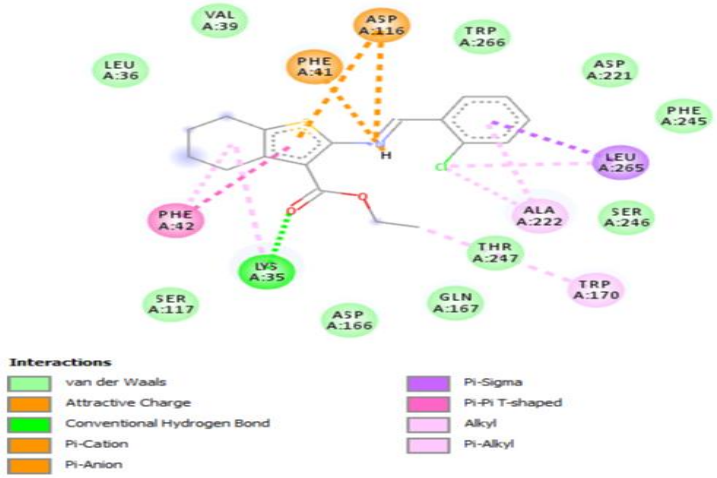


Figure 2: 2D structure of molecule A9 in complex with DNA gyrase B receptor

A1 is the third in terms of binding affinity (-7.6kcal/mol) which formed a conventional hydrogen bond interaction with LYS35 (8.1 Å), also a carbon hydrogen bond was observed with VAL164. Hydrophobic interactions with TRY170, ALA222, PHE41 and TRP266 amino acid residue were also observed with the receptor. Halogen bonds were formed with LYS35, VAL39, ALA222 and PHE42 amino acid residues. Electrostatic interactions were also formed with ASP116 and PHE41 amino acid residues respectively. Figure 3 shows the 2D structure of molecule A1 in complex with DNA gyrase B receptor.

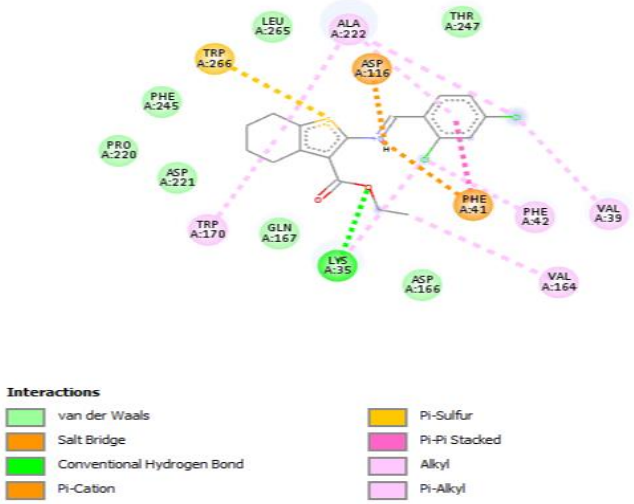


Figure 3: 2D structure of molecule A1 in complex with DNA gyrase B receptor

Compound A2 and A16 are seen to have the same binding affinities of -7.5kcal/mol. A2 had a conventional hydrogen bond interaction with ASP116 (2.01 Å). Carbon hydrogen bonds were formed with LEU265, TRP266 and TRP170 amino acid residues respectively. Hydrophobic interactions were formed with ALA222 and PHE41 residues. LYS35 amino acid residue formed electrostatic interaction with A2. Figure 4 shows the 2D structure of molecule A2 in complex with DNA gyrase B receptor.

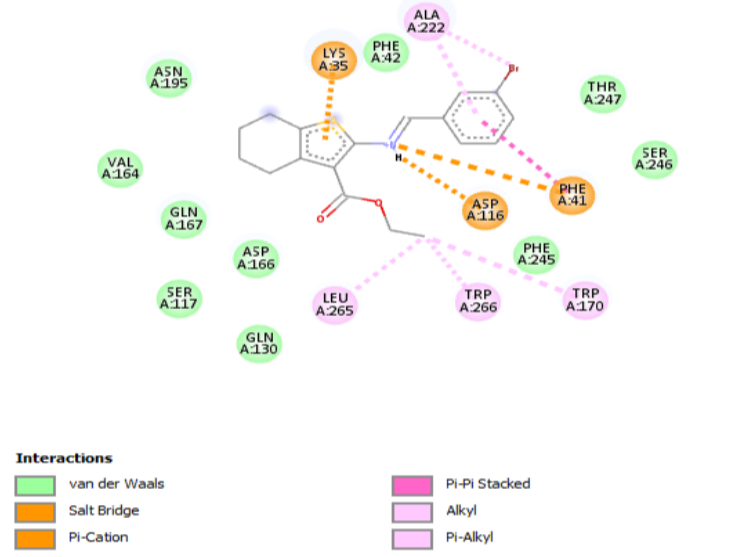


Figure 4: 2D and 3D structure of molecule A2 in complex with DNA gyrase B receptor

A16 (-7.5kcal/mol) had a conventional hydrogen bond interaction with LYS35 (2.9Å), GLN167 (2.33Å) and PRO220 (2.43Å) amino acid residue respectively. A carbon hydrogen bond was observed with ALA22 and A16 ligand. Hydrophobic interactions with PHE42, PHE41 and TRP170 with amino acid residues were observed. No halogen interaction was observed but electrostatic interaction was observed with ASP166 and ASP116 amino acid residues. Figure 5 shows the 2D structure of molecule A16 in complex with DNA gyrase B receptor.

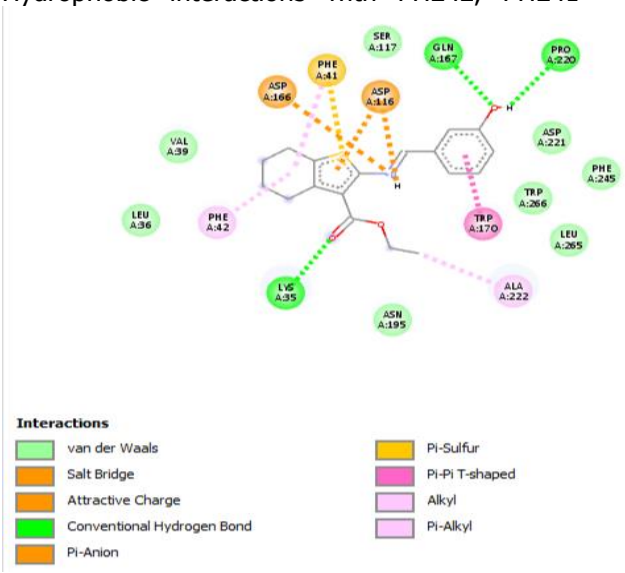


Figure 5: 2D structure of molecule A16 in complex with DNA gyrase B receptor

The Drug-likeness Properties

The drug-likeness of the thiophene derivatives (Compound A11, A9, A1, A2 and A16) were predicted following the Lipinski's rule of five which states that if any small molecule violates more than two of these criteria (Molecular weight ≤ 500g/mol, Number of hydrogen bond donor ≤ 5, Number of hydrogen bond acceptors ≤ 10, calculated logP ≤ 5), the molecule is said to be impermeable or badly absorbed (Table 3). All the selected and reported thiophene derivatives (Compound A11, A9, A1, A2 and A16) violated neither of the Lipinski rule of five except molecule A1 which have one (1) violation (WLOG P was >5) respectively. Their Molecular Weight was <500, the number of hydrogen bond donors and acceptors for all were less than 5 and 10, respectively. Since none of the molecules violated more than one of Lipinski's rule of five, in that regard the compounds are predicted to be drug-like in nature, orally bioavailable and active. The Bioavailability Radar gives an over view of the drug-likeness of a molecule (Figure 6). The region painted pink indicates the range for each properties. The bioavailability radar of all the reported compounds is shown in Figure 6.

Table 3: Drug-likeness of the thiophene derivatives

S/N	M. F	M. W (g/mol)	WLOGP	H. Bond donors	H. Bond acceptors	Lipinski violation
A11	$C_{18}H_{18}N_2O_4S$	358.41	4.46	0	5	0
A9	$C_{18}H_{18}ClNO_2S$	347.86	5.21	0	3	0
A1	$C_{18}H_{17}Cl_2NO_2S$	382.3	5.86	0	3	1
A2	$C_{18}H_{18}BrNO_2S$	392.31	5.32	0	3	0
A16	$C_{18}H_{19}NO_3S$	329.41	4.26	1	4	0

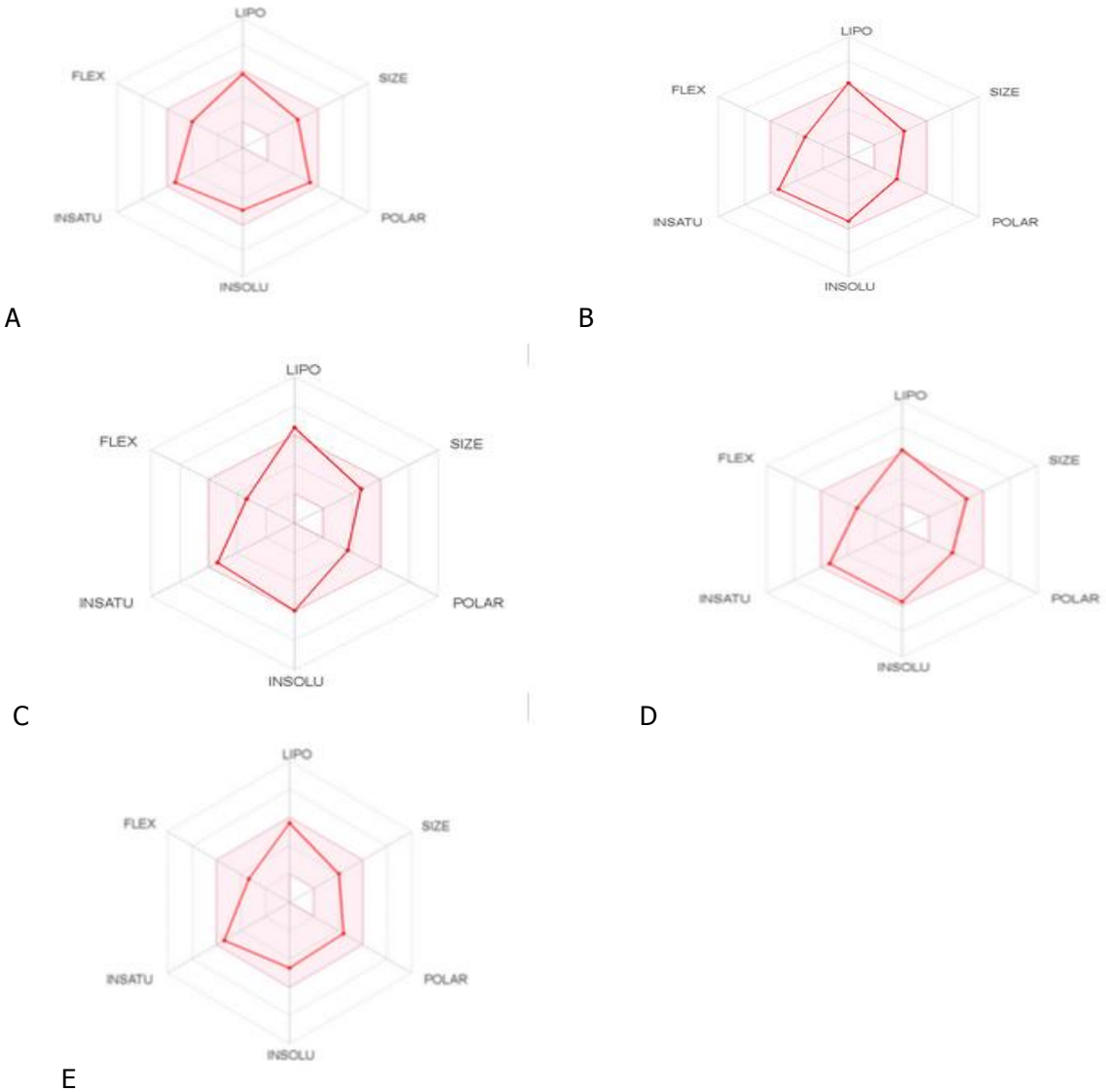


Figure 6: The Bioavailability Radar of (A) molecule (A11), (B) molecule A9, (C) molecule A1, (D) molecule A2 and (E) molecule A16

The ADMET/ Pharmacokinetics Properties

The ADMET/Pharmacokinetics properties predicted for the five selected compounds are presented in Table 4. The intestinal absorption values for these compounds were all above 90% but less than 100%. Their intestinal absorption values have passed the threshold value of 30%, which clearly shows that these reported compounds have high human intestinal absorption properties. The BBB permeability (log BB) values for some of the selected ligands were < -1, which signifies those compounds are poorly distributed through the brain. The CNS permeability (log PS) values of some were > -2 which are considered to penetrate the central nervous system. The reported compounds (most especially A11 with the highest binding affinity) were found to be both substrates and inhibitors of CYP3A4, there by affirming their metabolic properties.

In addition to that, their total clearance value was within the permissive limits. All the reported compounds were found to be non-toxic. With respect to the predicted parameters the compounds are said to have high absorption value, low toxicity level and good permeability to the cell membrane. All the reported compounds were predicted to have good pharmacokinetic profiles.

The Boiled- egg plot between WLOGP and TPSA to predict gastrointestinal absorption and brain penetration of the selected molecule was shown in (Figure 7). It can be seen that none of the molecule possess the BBB permeant but are within the GI absorption region.

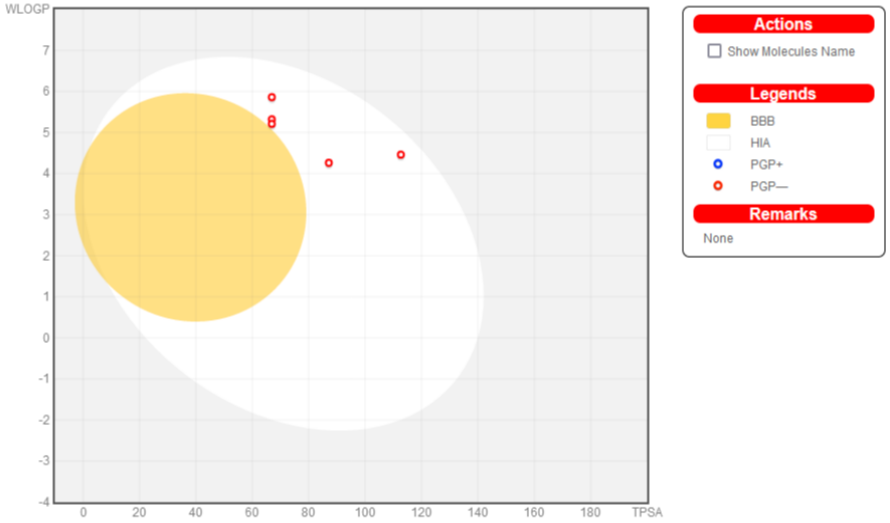


Figure 7: The boiled egg plot of thiophene derivatives

Table 4**:** The ADMET properties of the five selected thiophene derivatives with the highest binding affinity

S/N	Absorption Intestinal absorption	Distribution Permeability BBB Log BB	CNS Log PS	Metabolism							Excretion Total Clearance	Toxicity AMES toxicity
				CYP Substrate		CYP Inhibitors						
				2D6	3A4		1A2	2C19	2C9	2D6 3A4		
A11	91.997	-0.633	-1.913	No	Yes	Yes	Yes	Yes	No	Yes	0.001	No
A9	93.181	0.238	-1.37	No	Yes	Yes	Yes	Yes	No	Yes	0.009	No
A1	90.983	0.25	-1.325	No	Yes	Yes	Yes	Yes	No	Yes	0.082	No
A2	92.199	0.223	-1.358	No	Yes	Yes	Yes	Yes	No	Yes	-0.049	No
A16	91.206	-0.141	-1.856	No	Yes	Yes	Yes	Yes	No	Yes	-0.013	No

BBB=Blood brain barrier, CNS=Central nervous system, CYP=Cytochrome

Comparison Between Compound A11 (the best hit) and Ciprofloxacin (approved drug)

From Table 5, it is observed that compound A11 have higher binding affinity than ciprofloxacin. It can be seen that all the two compounds have not violated the Lipinski's rule of five, therefore are said to be orally bioavailable with good bioavailability scores. Their gastrointestinal absorption value was found to be all greater than 30% which indicate that they can be absorbed within the intestine. They were also found to be well distributed within the brain and the central nervous system. A11 was seen to be both substrate and inhibitors of CYP3A4 but ciprofloxacin was found to be only substrate of CYP3A4 and not an inhibitor of CYP3A4. A11 was seen to be non-toxic and ciprofloxacin gave a positive result to the AMES toxicity test (is toxic).

Table 5: Comparison between compound A11 and ciprofloxacin (approved drug)

Parameters	A11	Ciprofloxacin
Binding affinity (kcal/mol)	-7.8	-7.3
M.W	358.41	331.34
WLOGP	4.46	1.18
H.B donors	0	2
H.B acceptors	5	5
Lipinski violation	0	0
Bioavailability score	0.55	0.55
Absorption		
Intestinal absorption (%)	91.997	95.274
Distribution		
BBB (log BB)	-0.633	-0.724
CNS (log PS)	-1.913	-3.024
Metabolism		
CYP Substrates		
2D6	No	No
3A4	Yes	Yes
CYP Inhibitors		
1A2	Yes	No
2C19	Yes	No
2C9	Yes	No
2D6	No	No
3A4	Yes	No
Excretion (Total clearance)	0.001	0.502
Toxicity (AMES toxicity)	No	Yes

CONCLUSION

The molecular docking virtual screening carried out on the studied compounds in the active site of Salmonella typhi DNA Gyrase B (receptor) has identified compound A11 with the highest binding affinity of -7.8 Kcal/mol to be the best hit among the investigated compounds. It interacted with the following amino acid residues LYS35, THR247, ASP166, VAL164, TRP170, ALA222, PHE41, TR266, ASP116 and PHE41 in the active site of the receptor via conventional hydrogen bond, carbon hydrogen bond, hydrophobic and electrostatic interactions, respectively. The ADMET and drug-likeness of the compounds which predicted via pkCSM and SwissADME were found to be pharmacologically active, non-toxic, orally bioavailable and permeable (most especially compound A11). None of these molecules violate more than one Lipinski's rule of five which is the minimum criteria for drug-likeness of a small molecule. With reference been made to an approved already existing anti-typhoid agent ciprofloxacin with a binding affinity of -7.3 Kcal/mol to the investigated molecules (most especially compound A11), All the reported molecules have higher binding affinities than the ciprofloxacin, having good pharmacokinetic properties therefore proves the tendency of their effectiveness when been administered as drug.

Author's contribution:

Ibrahim M. T. and Anebi E: Conducted the research and wrote the manuscript

Shallangwa G. A., Abdulsalam S. and Danmallam A. M.: Provide the materials for the research and read the manuscript

Conflict of interest:

Authors declare no conflict of interest.

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