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Virtual screening of novel pyridine derivatives as effective inhibitors of DNA gyrase (GyrA) of *salmonella typhi*

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C H R O N I C L E

Article history:
Received April 23, 2022
Received in revised form
June 25, 2022
Accepted October 10, 2022
Available online
October 10, 2022

Keywords:
Pyridine
DNA gyrase
Salmonella typhi
Pharmacokinetics
Drug-likeness

A B S T R A C T

In a bid to discovering novel antibiotics to combat growing trend of multi-drug resistance strains of *Salmonella typhi*, 48 new pyridine derivatives with significant inhibitory activities against the aforementioned bacterium were subjected to molecular docking against DNA gyrase protease of the bacterium, drug likeness evaluation and pharmacokinetics profiling. All the 48 leads displayed better binding affinity values when compared with Amoxicillin, Ciprofloxacin, Ceftriaxone, Ampicillin, and chloramphenicol, the standard antibiotics used herein for quality assurance. Furthermore, the majority of the compounds were, however, screened out due to their poor pharmacokinetics profiles and drug-likeness. Only five compounds emerged as the most promising leads and they include C4 with binding affinity of -8.0 kcal/mol, C8 (-8.6 kcal/mol), C9 (-8.1 kcal/mol), C26 (-8.3 kcal/mol), and C27 (-8.0 kcal/mol). These compounds not only displayed better binding affinity when compared with the reference antibiotics but also exhibit different modes of interactions with the target protease of the bacterium making them more potent and drug like. Toxicity evaluation of the leads also revealed that the compounds are neither tumorigenic nor mutagenic. In view of the excellent binding affinity, high pharmacokinetics profile and positive drug-likeness of the novel ligands, we recommend these promising compounds for in vitro and in vivo studies in order to discover novel antibiotics that could curb the dangerous trend of multiple drug resistance by *Salmonella typhi*.

1. Introduction

Salmonella typhi is a bacterium responsible for typhoid fever, a contagious disease characterized by persistent high temperature that increases gradually each day, headache, general aches, fatigue, cough and constipation.[1] World Health Organization estimated 11–21 million cases of typhoid fever and approximately 128 000–161 000 deaths annually across the globe.[2]

This poor hygiene-associated infectious disease is prevalent in developing countries such as South and South East Asia as well as sub-saharan Africa.[2,3] Lack of proper sanitation and access to safe drinking water are triggers to the spread of this disease.[4,5]

Although typhoid fever is treated with antibiotics but the emergence of multi-drug resistant strains of *Salmonella typhi* is constituting a serious threat to public health and may pose serious global health burden, thus a need for discovery of newer antibiotics in the drug development pipeline has become necessary.[6-12]

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doi: 10.5267/j.ccl.2022.10.002

Among the recent computational studies geared towards the discovery of novel inhibitors of DNA gyrase is the work of Anebi *et al.* [13] wherein the authors carried out molecular docking study, drug-likeness and pharmacokinetic properties (ADMET) prediction of some novel thiophene derivatives as *Salmonella typhi* inhibitors. Also, Abhishek *et al.* [14] performed Virtual Screening, Molecular Docking, and ADME/T Analysis of Natural Product Library against Cell Invasion Protein SipB of *Salmonella enterica serotype typhi.* Likewise, Arunkumar *et al.* [15] evaluated seaweed sulfated polysaccharides as natural antagonists targeting *Salmonella typhi* OmpF using virtual screening, molecular docking and ADMET analysis.

Organic compounds, especially organic compounds containing pyridine moiety, are very important and have a lot of biological and industrial applications.[16-19] Pyridine based compounds has shown significant inhibitory activity against *Salmonella typhi*.[20-25] Theoretical investigation of the binding modes of these bioactive compounds to GyrA, an enzyme that plays essential role in the supercoiling and replication of *Salmonella typhi* and assessment of their drug likeness would provide scientific roadmaps towards the discovery and development of novel drugs for the treatment of typhoid fever.

In this research work, we investigated the inhibitory activities of some pyridine derivatives against *Salmonella typhi* using virtual screening techniques to filter out most promising leads that could be used to design novel antibiotics against this pathogenic bacterium. This has become necessary considering the rising cases of multi-drug resistance threats posed by this microbe.

2. Materials and Methods

2.1 Materials

The computer hardware and software used for this insilico research include Dell computer system, with processor properties of Intel ® Core i3-6100U CPU Dual @ 2.30 GHz, 12 GB (RAM) size on Microsoft windows 8.1 Ultimate Operating System, Spartan 14 V.1.1.0, ChemDraw Ultra 12, Spartan 14 V 1.1.2 developed by Wavefunction Inc., AutoDock tools in AutoDock 4.3 program, Discovery Studio Visualizer V.16.1.0, EasyDockVina v2.2, DataWarrior v.4.2.2., and SwissADME online tools.

2.2 Methods

2.2.1 Lead identification and optimization

Lead in this context is a compound that demonstrates a desired biological activity against *Salmonella typhi* on a validated molecular target. In this work, a total of 48 leads were obtained from recent literatures.[26-27] The inhibitory activities of the leads were measured using the same experimental procedures and a wide range of inhibitory activities were covered. In order to get the minimum energy geometry of the leads and the standard antibiotics, they were optimized using the Density Functional Theorem (6-311G* basis set) technique embedded in Spartan'14 software (www. wavef un. com). The optimized structures were saved in protein data bank (PDB) and sdf file format for further analysis. The structures of the leads and their anti- *Salmonella typhi* inhibitory activities are presented in Table 1.

2.2.2 Molecular Docking

Molecular docking, a technique that allows accurate prediction of the strength of association or binding affinity between the leads (ligands) and the target protein (GyrA) was used to screen the novel ligands for their binding affinity to the target protein. The optimized ligands were prepared using the AutoDock Vina interface and saved as pdbqt files. The 3-D crystal structure of the target protein was retrieved from RCSB (www. rcsb. org/pdb) protein data bank (PDB Code: 5ztj). The protein was prepared on the Discovery Studio interface where water molecules and heteroatoms were removed. The prepared protein was further exported into the AutoDock Vina interface where missing atoms were checked and repaired, partial atomic charges were assigned and all necessary valency tests and H-atom additions were performed. The results of the docking simulation were validated with the aid of AutoDock Vina (EasyDock Vina software) with the following binding location; center_x = 26.438; center_y = 22.971; center_z = 22.2 ; Dimension-x = 40; Dimension-y = 40; Dimension-z = 40. The Discovery Studio Visualizer v16.1.0.15350 was deployed to picture the kind of interactions in the stable protein-ligand complex formed. **Fig. 1** shows the 3D structure of the protein target. As a quality control measure, structures of five common antibiotics currently in use for the treatment of *Salmonella typhi* infections were retrieved from PubChem drug data bank (https://pubchem.ncbi.nlm.nih.gov/compound) and use as standards for comparison. These ligands were optimized, treated and docked with GyrA target to examine their binding affinities using the aforementioned procedures for the lead molecules. The standard antibiotics used are Amoxicillin, Ciprofloxacin, Ceftriaxone, Ampicillin, and chloramphenicol.

2.2.3 Pharmacokinetics and Toxicity Evaluation

Drug candidates must possess low toxicities and high pharmacokinetic profile. Thus, pharmacokinetic profiling and toxicity evaluation was performed on all the novel ligands with the aid of DataWarrior v.4.2.2 software and SwissADME online tools (http://www.swissadme.ch). Evaluation of passive gastrointestinal absorption (HIA) and brain penetration

(BBB) of the leads were performed using the BOILED-Egg method while drug-likeness of the leads were assessed using the Bioavailability Radar models.[28]

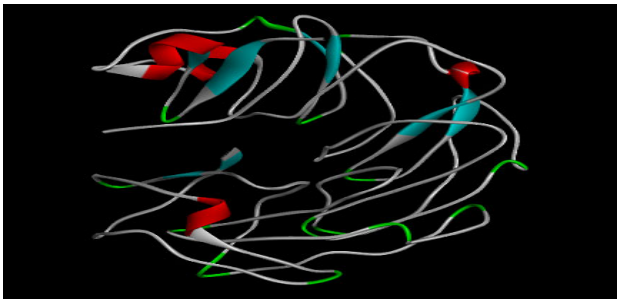
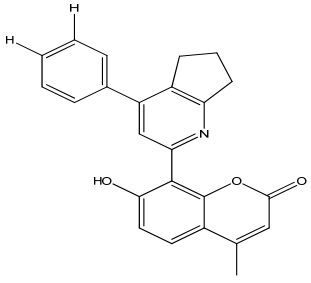
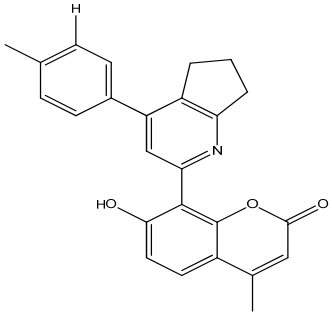
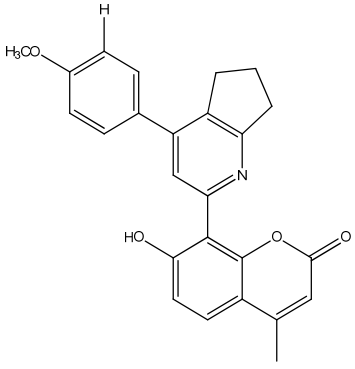
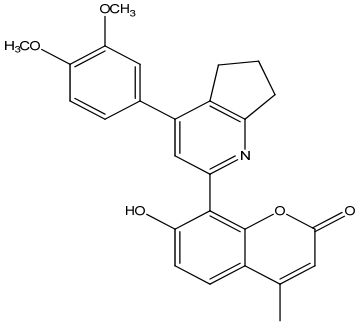
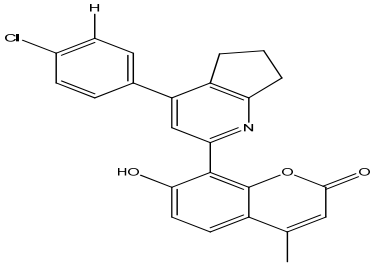
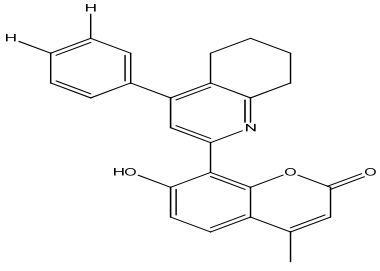
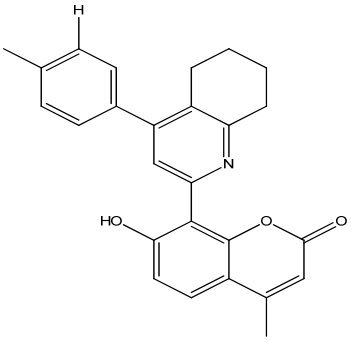
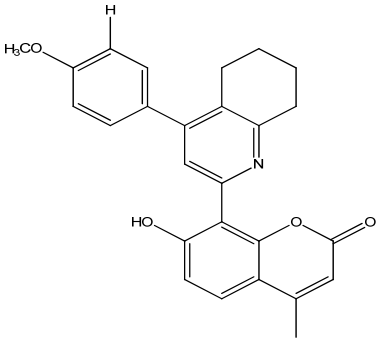
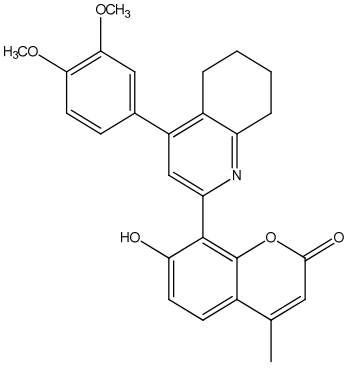
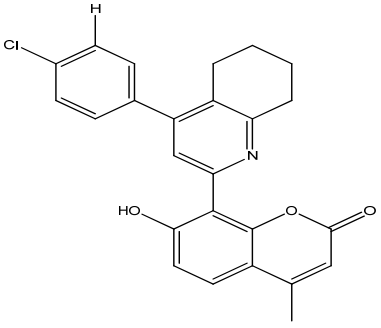
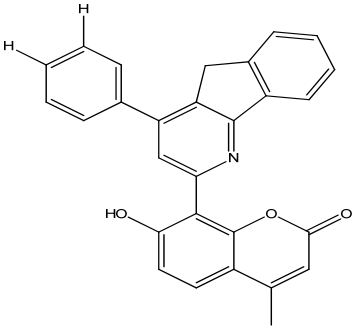
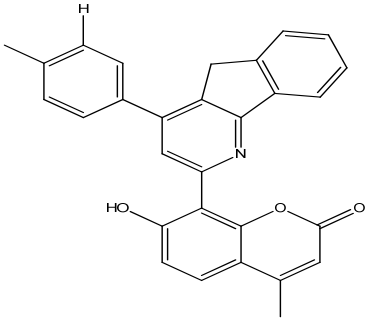
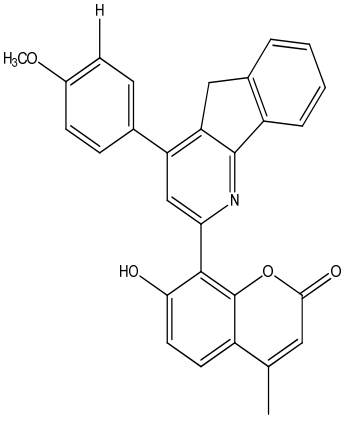
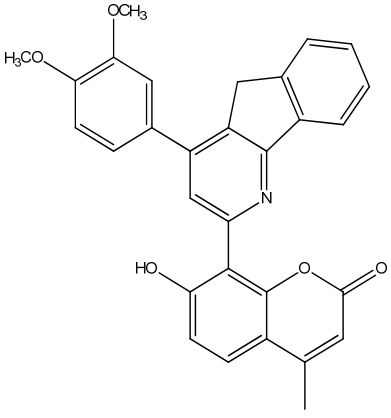


Fig. 1. 3D structure of the protein target (gyrA)

Table 1. Molecular structures and experimentally determined Minimum inhibitory concentration of the leads

S/n	Structure	MIC (µg/mL)	S/n	Structure	MIC (µg/mL)
C1.		200	C2.		500
C3.		250	C4.		200
C5.		500	C6.		200
C7.		63	C8.		500

					
C9.		100	C10.		500
C11.		200	C12.		250
C13.		100	C14.		250
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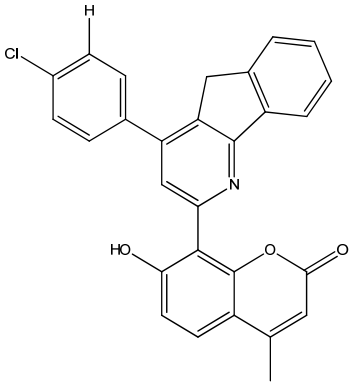
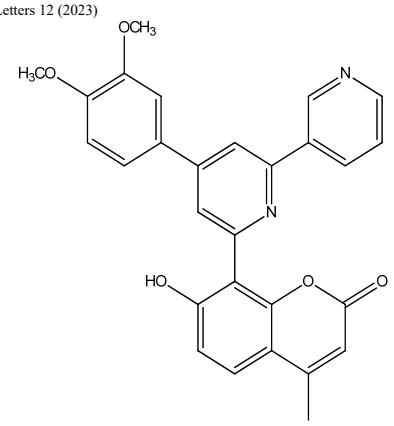
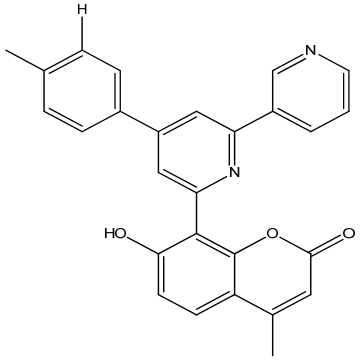
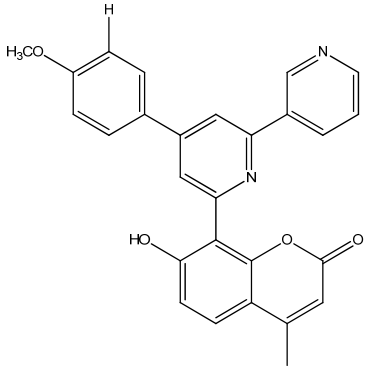
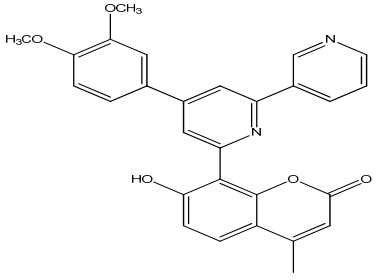
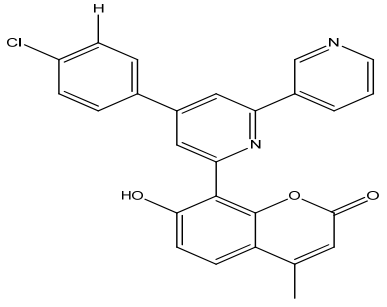
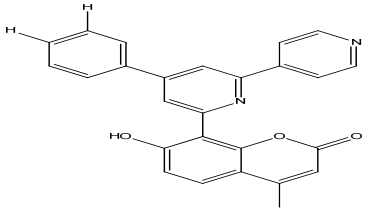
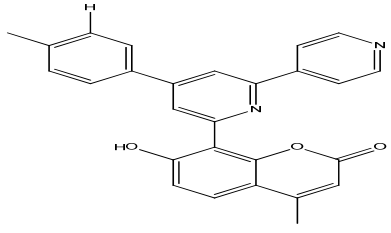
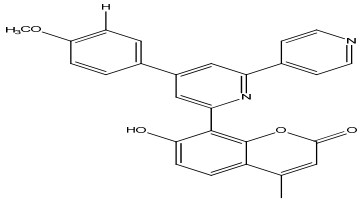
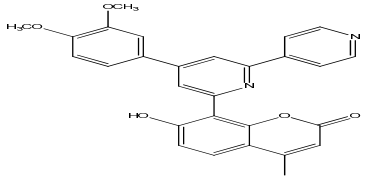
					
C17.		250	C18.		50
C19.		100	C20.		200
C21.		200	C22.		250
C23.		500	C24.		100
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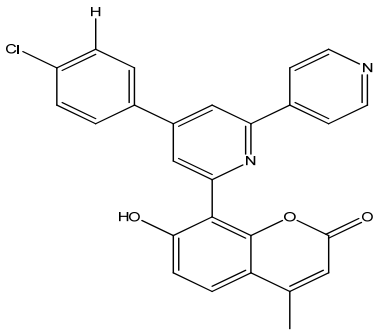
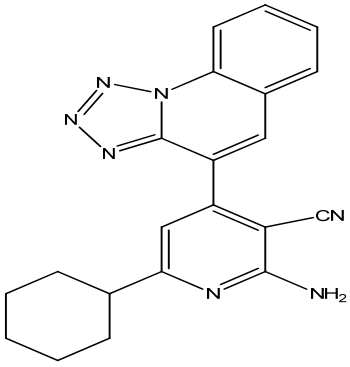
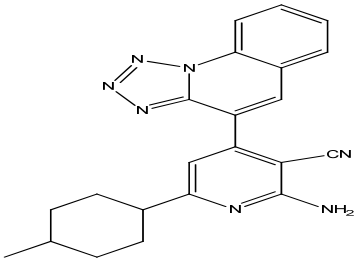
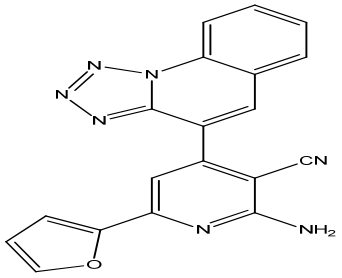
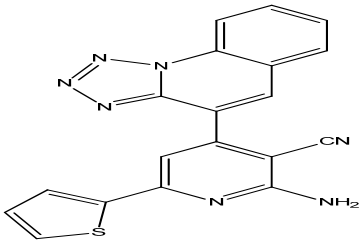
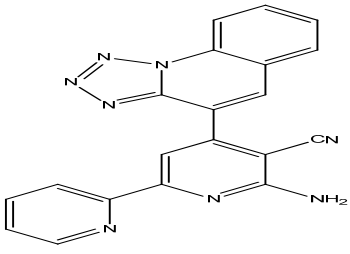
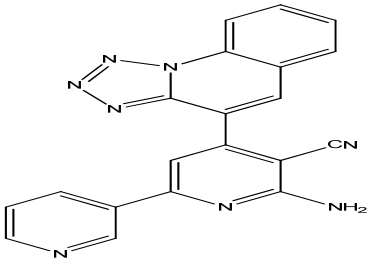
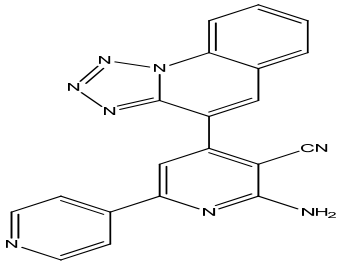
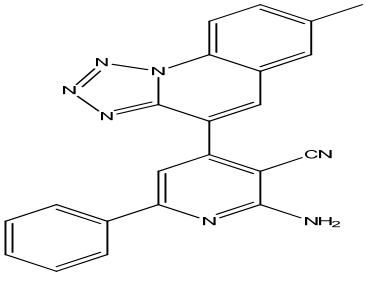
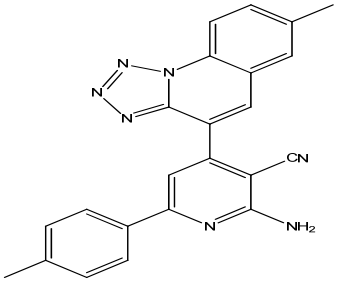
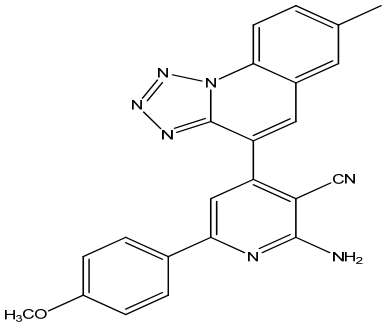
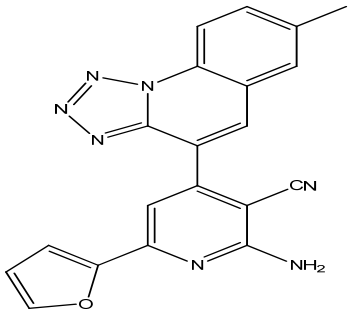
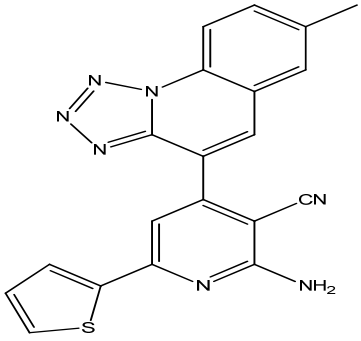
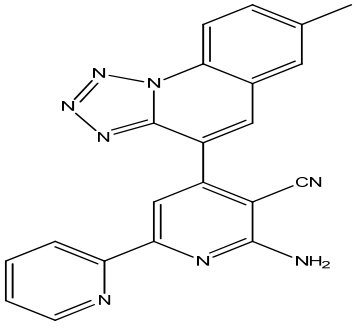
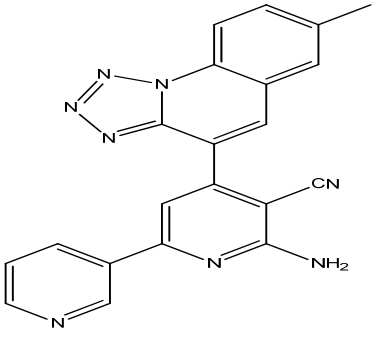
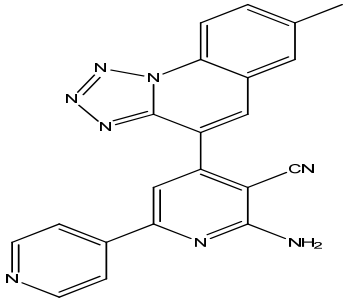
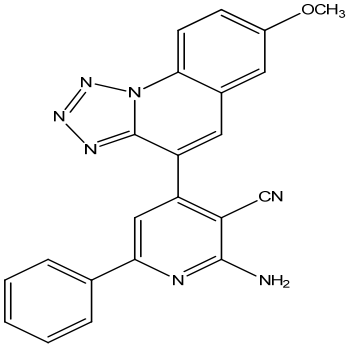
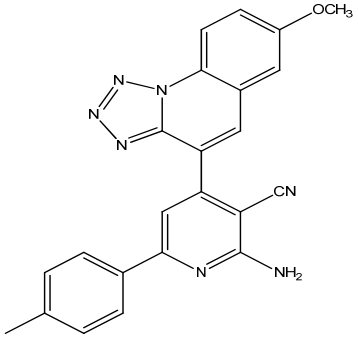
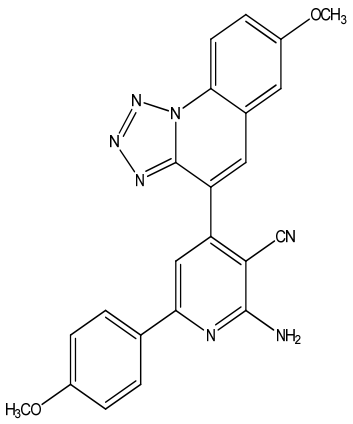
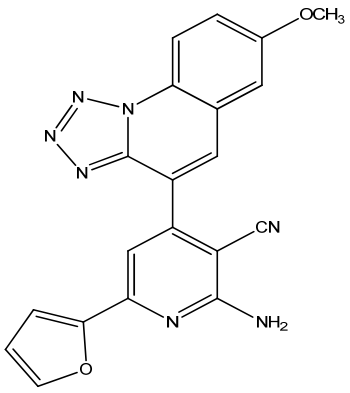
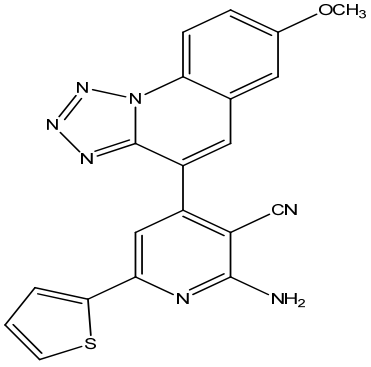
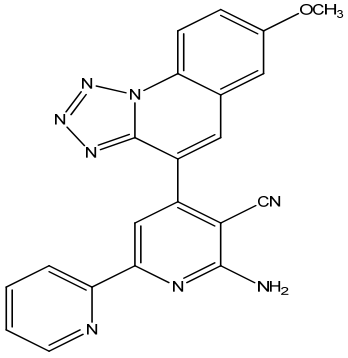
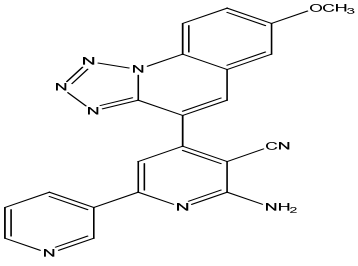
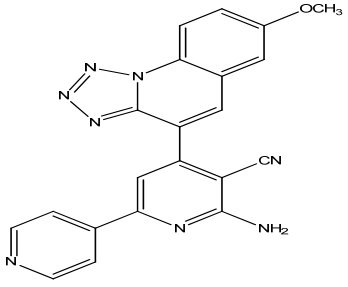
					
C27.		500	C28.		500
C29		630	C30.		200
C31.		500	C32.		500
C33.		200	C34.		200

C35.		500	C36.		150
C37.		100	C38.		200
C39.		125	C40.		150
C41.		125	C42.		1000
C43.		500	C44.		100





C45.		200	C46.		500
C47.		50	C48.		125

3. Results and Discussions

3.1 Molecular docking on most promising hit compounds

The results of molecular docking for the leads and standards are presented in **Tables 2** and **3,** respectively. The best result was selected on the basis of the conformation with the least distance from the root mean square (rmsd) upper bound (u.b) and lower bound (l.b). The molecular docking studies revealed that the 48 novel pyridine-based GyrA inhibitors showed better docking scores compared to the standard antibiotics. Majority of the ligands, despite their excellent binding affinity to the target protein were screened out due to poor pharmacokinetic profiles, poor water solubility and poor oral bioavailability, except compounds C4, C8, C9, C26, and C27. Thus, these compounds were selected as the most promising leads and their binding affinity values to the target protein are presented in **Table 2**.

Among the promising leads, C8 has the best dock score with a binding affinity of -8.6 kcal/mol. The 3D and 2D diagrams of interactions with the amino acid side chains of the target protein is shown in **Figure 2**. It displayed two pi-cation interactions with ARG612; two pi-sigma interactions with VAL838 and ILE578; five pi-alkyl interactions with VAL839, LEU581 and ILE578; an alkyl interaction with MET796; a carbon-hydrogen interaction with ARG615. There was however, an unfavorable acceptor-acceptor interaction with ARG806.

Also, **Fig. 3** gives the 3D and 2D diagram of interaction of C4 with the target. The binding affinity of this ligand to the protease is -8.0 kcal/mol and it displays the following modes of interaction: two pi-cation interactions with ARG612; five

pi-alkyl interactions with VAL839, LEU581 and ILE578; an alkyl interaction with MET796; a pi-sigma interaction with VAL839, a water-hydrogen bond at HOH1003; two carbon-hydrogen bond at ALA614 and ARG615; unfavorable acceptor-acceptor interaction with ARG806.

An excellent binding score of -8.1kcal/mol was recorded for C9. The 3D and 2D diagram of its complex with the protease (**Fig. 4**) reveals that it forms five pi-alkyl interactions with VAL839, LEU581 and ILE578; an alkyl interaction with MET796; two pi-cation interactions with ARG612; a pi-sigma interaction with VAL839; two carbon-hydrogen bond at ALA614 and ARG615; and unfavorable acceptor-acceptor interaction with ARG806.

Furthermore, the binding score for the interaction of C26 with the target is -8.3 kcal/mol. This ligand exhibits five binding interactions in all (**Fig. 5**): two alkyl interactions with ILE578 and VAL839; two pi-alkyl interaction with ILE578 and MET796; and an unfavorable bump interaction with ARG806.

C27 with a binding score of -8.0 kcal/mol appears to have the best interactions as no unfavorable bump was found. This ligand exhibits a total of five interactions as shown in **Fig. 6**. It displayed a water hydrogen bond at HOH983; two conventional hydrogen bond interactions with ILE578 and ARG806; a pi-cation interaction with ARG612; and an alkyl interaction with ARG612.

The interaction of the three reference antibiotic ligands with the active sites of the target protein are presented in **Fig. 7**. **Fig. 7a** gives the complex of amoxicillin ligands within the active sites of GyrA protein target. This standard drug displayed the following interactions with GyrA target; three alkyl interactions with MET796 and LEU804; three conventional hydrogen bond interactions with ARG612, ASP576, and ILE578; a pi-cation interaction with LYS550; and water hydrogen bond interaction at HOH918.

Also, the diagram of interaction of ceftriaxone with protease target is shown in **Fig. 7b** and their binding affinity values in **Table 3**. A sum total of four interactions were observed; two alkyl interactions with LEU687 and PRO636; a conventional hydrogen bond interaction with LEU581; and an unfavorable donor-donor interaction with GLY837.

Furthermore, the ciprofloxacin- GyrA interaction is presented in **Fig. 7c**. A total of nine interactions abound. Three water hydrogen bond interactions with HOH957, HOH947, and HOH979; two pi-pi stacking interactions with HIS545 and ARG630; a conventional hydrogen bond interaction with ARG580; one pi-alkyl interaction with HIS545; and a van der waals interaction with VAL834.

As a way of comparison, the novel pyridine based leads displayed interactions with the amino acids side chains of GyrA similar to amoxicillin, ceftriaxone, and ciprofloxacin.

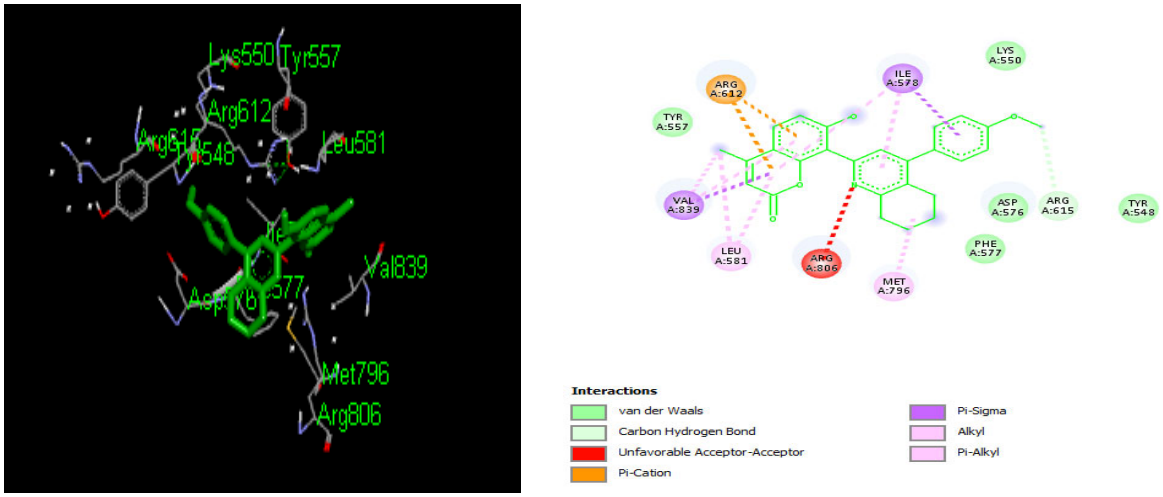
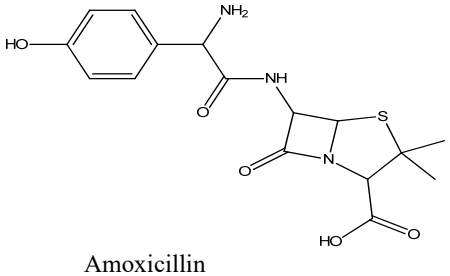
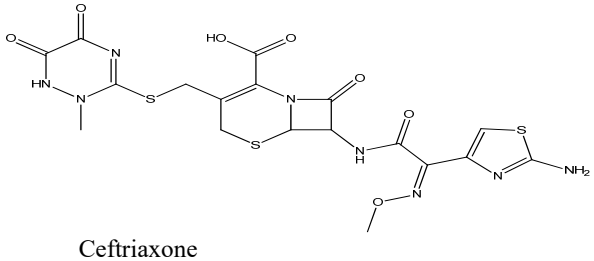
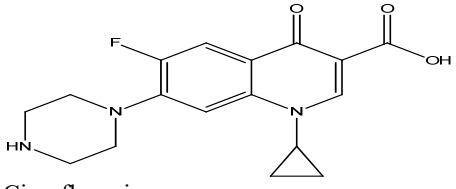


Fig. 2. 3D and 2D diagram of interaction of C8 with the target

Table 2. Molecular Docking Result for the Leads

Compound	Affinity (kcal/mol)	Compound	Affinity (kcal/mol)	Compound	Affinity (kcal/mol)
C1	-8.3	C17	-8.9	C33	-8.6
C2	-8.5	C18	-8.4	C34	-9.0
C3	-8.4	C19	-8.4	C35	-8.5
C4	-8	C20	-8.6	C36	-8.1
C5	-8.4	C21	-8.5	C37	-8.1
C6	-8.3	C22	-8.9	C38	-8.5
C7	-8.8	C23	-8.5	C39	-8.4
C8	-8.6	C24	-8.4	C40	-8.4
C9	-8.1	C25	-8.7	C41	-8.5
C10	-8.7	C26	-8.3	C42	-8.7
C11	-9.3	C27	-8	C43	-8.2
C12	-9.7	C28	-7.9	C44	-8.1
C13	-9.6	C29	-7.7	C45	-8.1
C14	-9	C30	-8.3	C46	-8.3
C15	-9.6	C31	-8.1	C47	-8.2
C16	-8.4	C32	-8.1	C48	-8.2

Table 3. Molecular Docking Result for the reference antibiotics

S/n.	Antibiotic structure/name	Pubchem CID	Affinity (kcal/mol)
1	 Amoxicillin	33613	-6.7
2	 Ceftriaxone	5479530	-7.4
3	 Ciprofloxacin	2764	-6.6

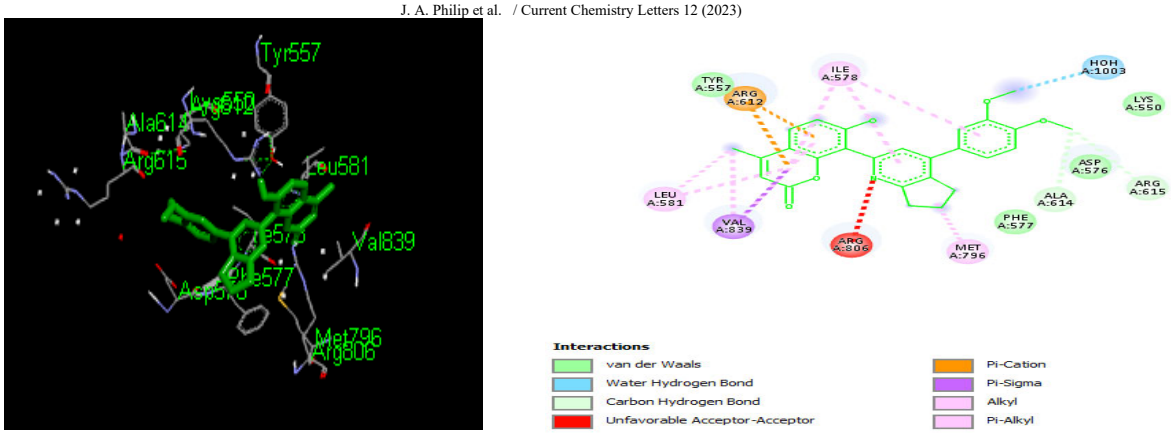


Fig. 3. 3D and 2D diagram of interaction of C4 with the target

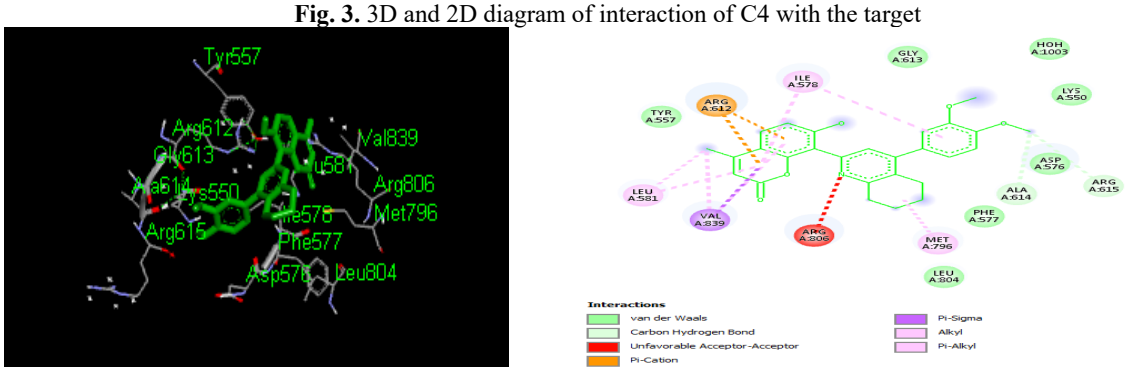


Fig. 4. 3D and 2D diagram of interaction of C9 with the target

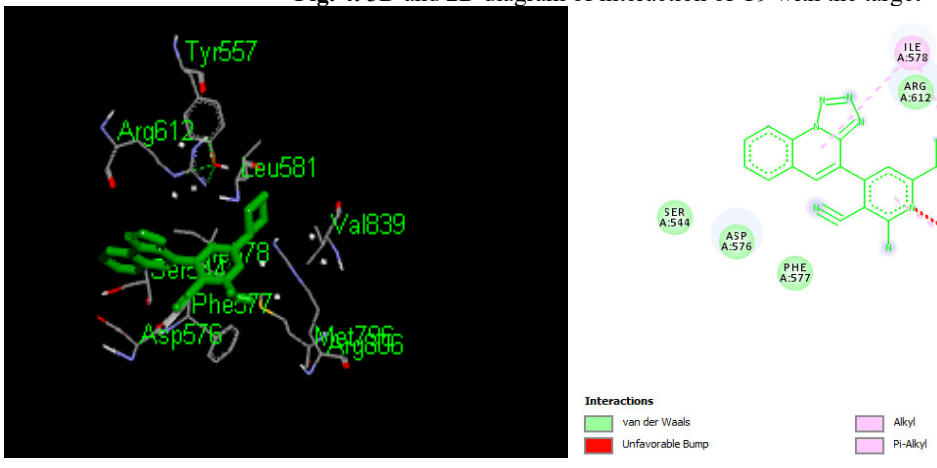


Fig. 5. 3D and 2D diagram of interaction of C26 with the target

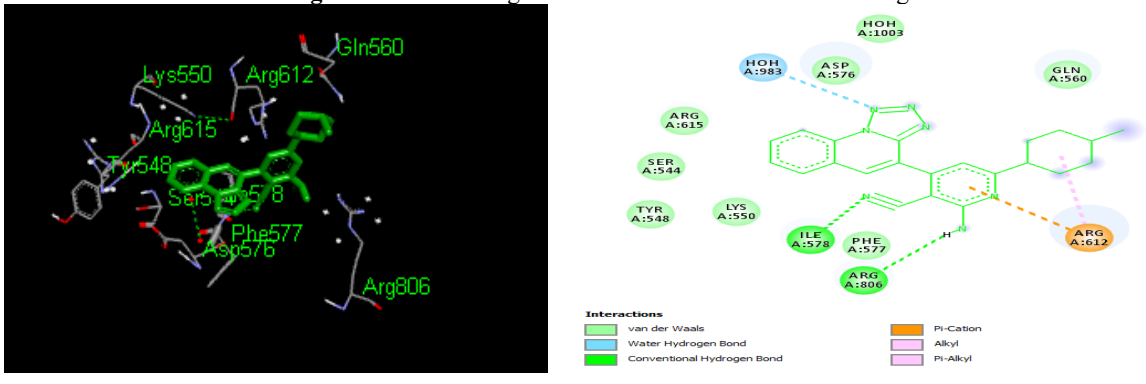
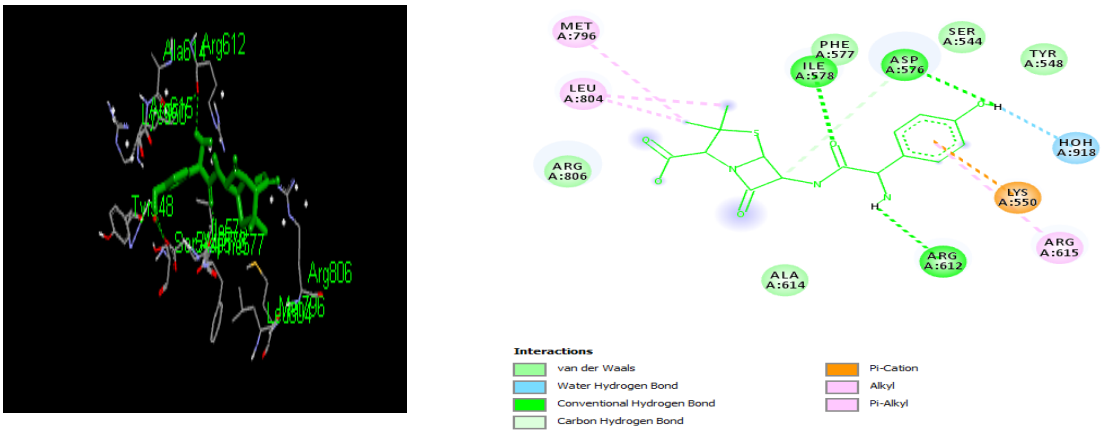
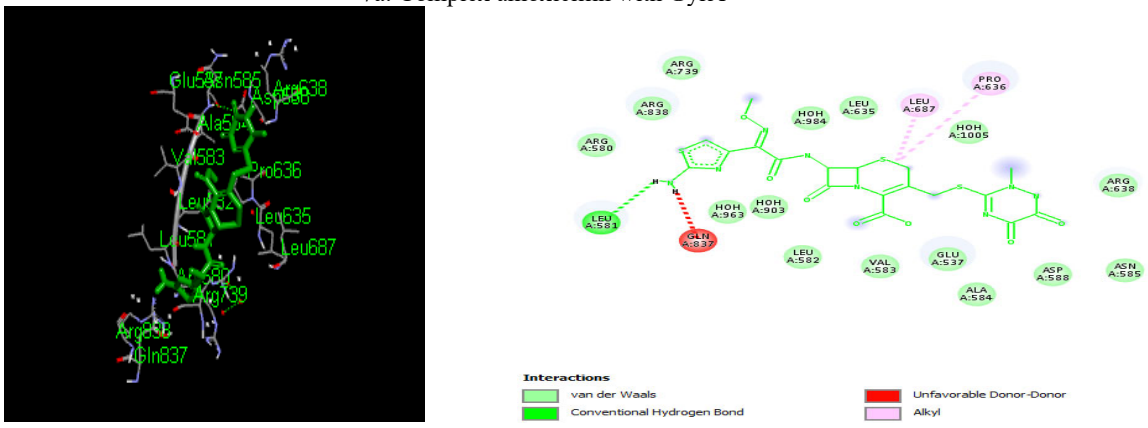


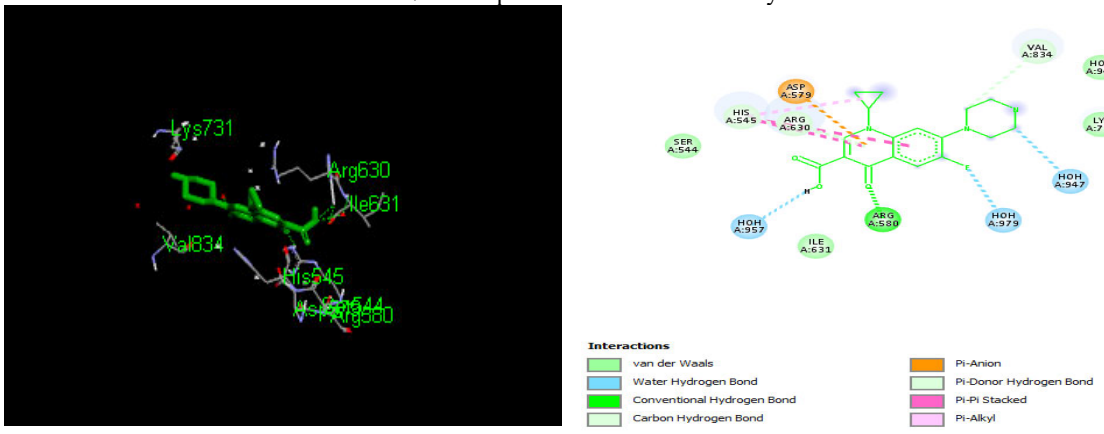
Fig. 6. 3D and 2D diagram of interaction of C27 with the target



7a: Complex amoxicillin with GyrA



7b: Complex of Ceftriazone with GyrA



7c: Ciprofloxacin-GyrA complex

Fig. 7. 3D and 2D diagram interaction of the standard antibiotics with the target protein

3.2 Pharmacokinetics and Drug-likeness Analysis

The physicochemical properties and Pharmacokinetics profiles of the most promising leads are displayed in **Tables 4** and **5**, respectively. Our result as it concerns the drug-like features showed that all the most promising leads respected the Lipinski's and the Veber's rules (**Table 4**). Lipinski's rule of five (RO5) states that a compound with a molecular mass below 500 Dalton (MW), an octanol-water partition coefficient ($LogP_{o/w}$) less than 5, no more than five hydrogen bond donors (HBD) and no more than 10 hydrogen bond acceptors (HBA) could be a good drug candidate. Veber's rule on the other hand states that a compound with 10 or fewer rotatable bonds (RTB) and a topological polar surface area (TPSA) not greater than 140 $Å^2$ should be a good orally bioavailable drug.

The pharmacokinetics data (**Table 5**) reveals that all the compounds possess high gastrointestinal absorption and no brain barrier penetration potential. Toxicological data shows that none of the compounds was found to be tumorigenic or mutagenic.

Graphically, drug-likeness of the leads assessed using the Bioavailability Radar models are presented in **Fig. 8 (a-e).** This radar enables a first glance at the drug-likeness of the molecules. The pink area represents the optimal range for each property. As seen from the diagram, all the leads exhibited positive drug-likeness as all the indices; lipophilicity (LIPO), size, solubility (INSOLU), Polarity, saturation (INSATU), flexibility (FLEX), fell within the optimum range of the radar.

In order to complement pharmacokinetics information in Table 5 graphically, intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) was performed on the leads using the BOILED-Egg model [28] in **Fig. 9**. The result shows that all the ligands except C27 fell within the white region, an indication that all the compounds possess high gastrointestinal absorption.

P-glycoprotein (P-gp) is one of the proteins expressed naturally on the plasmatic membranes of endothelial cells at the blood-brain barrier (BBB) that protects the brain from harmful substances by excluding them from entering into the parenchyma from blood circulation. It plays important role for the blood brain barrier as a defense against penetration of toxins and drugs into the central nervous system.[29] It was found that all the most promising ligands with the exception of C27 are substrate of P-glycoprotein (P-gp^{+}) and as such could be actively effluxed by P-gp.

Table 4. Physicochemical Properties of the leads

Compound Rule	C4	C8	C9	C26	C27
Lipinski's	yes	yes	yes	yes	yes
HBA	1	1	1	1	1
HBD	6	5	6	5	5
MW ($gmol^{-1}$)	393.41	413.47	443.49	369.42	383.45
$LogP_{(o/w)}$	4.49	4.81	4.77	3.46	3.67
Veber's	yes	yes	yes	yes	yes
RTB	4	3	4	2	2
TPSA ($Å^2$)	81.79	72.56	81.79	105.78	105.78

Table 5. Pharmacokinetics profiles of the most promising leads

Compound	GI Absorption	BB permeant	P-gp substrate	Tumorigenic	mutagenic
C4	High	No	Yes	No	No
C8	high	No	yes	No	No
C9	High	No	Yes	No	No
C26	High	No	Yes	No	No
C27	High	No	No	No	No

GI = gastro intestinal absorption, BBB=blood brain barrier, P-gp = P-glycoprotein

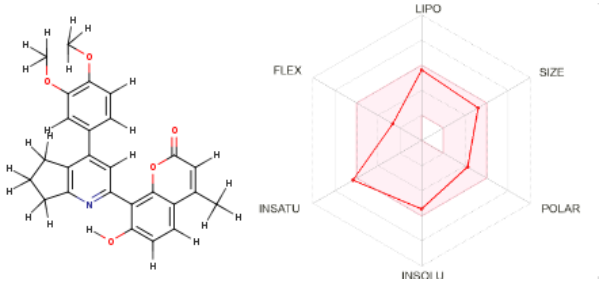


Fig. 8a. Bioavailability Radar for C4

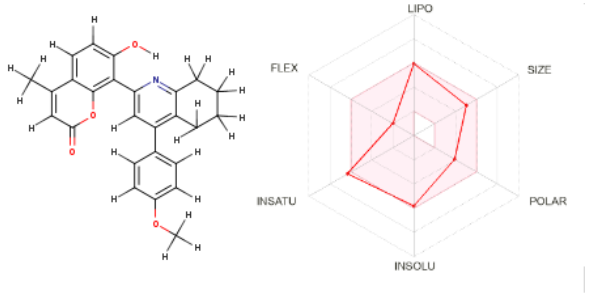


Fig. 8b. Bioavailability Radar for C8

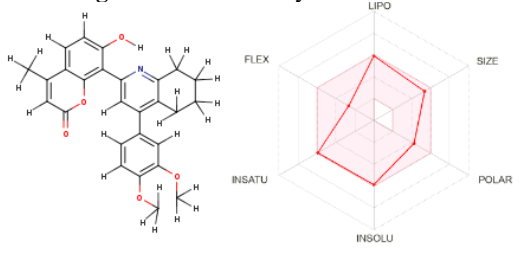


Fig. 8c. Bioavailability Radar for C9

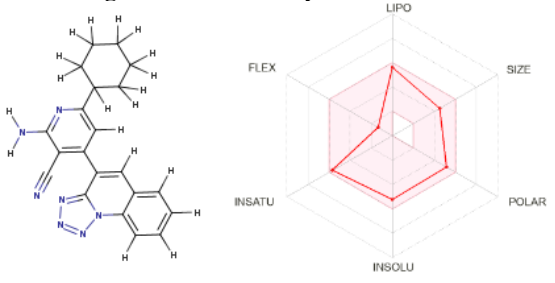


Fig. 8d. Bioavailability Radar for C26

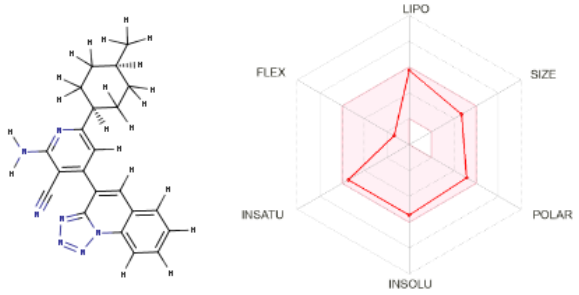
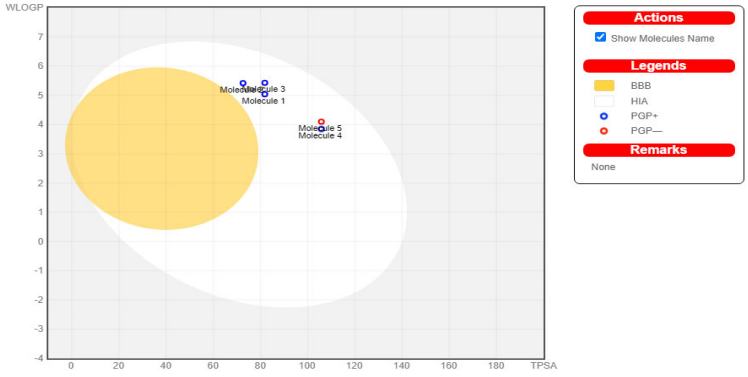


Fig. 8e. the Bioavailability Radar for C27



C4 = molecule 1; C8 =molecule 2; C9 = molecule 3; C26 = molecule 4; C27 = molecule 5

Fig. **9**. The BOILED-Egg Diagram for the most promising leads

4. Conclusion

DNA gyrase (GyrA) plays essential role in the supercoiling and replication of *Salmonella typhi*, making this macromolecule a major target of antibiotics. In this research, 48 series of pyridine based heterocycles with potent anti-*Salmonella typhi* activities were subjected to virtual screening using molecular docking, drug likeness assessment and pharmacokinetics analysis techniques. As a quality control measure, three standard antibiotics used for routine treatment of typhoid fever were also docked with the GyrA protein target to ascertain their binding affinities. The result showed that all the ligands possessed better binding affinity to the protein target in comparison to the antibiotics. Pharmacokinetic and drug-likeness evaluation revealed that only five of the ligands displayed positive pharmacokinetics profile and drug-likeness. We therefore recommend these promising compounds for lead optimization with the view of discovering novel antibiotics that could curb the dangerous trend of multiple drug resistance by *Salmonella typhi*.

Abbreviations

gyrA: DNA gyrase sub-unit A; GI: gastro intestinal absorption; BBB: blood brain barrier; P-gp: P-glycoprotein; LIPO: lipophilicity; INSOLU: solubility; INSATU: saturation; FLEX: flexibility; RO5: rule of five; $LogP_{o/w}$: octanol-water partition coefficient; HBA:hydrogen bond acceptors; HBD:hydrogen bond donors; RTB:rotatable bonds; TPSA:total polar surface area; HIA:passive gastrointestinal absorption

Acknowledgements

We are thankful to the members of staff in the physical chemistry unit, chemistry department of Ahmadu Bello University, for providing the essential facilities to carry out this research work

Authors' contribution

AU outlined and designed the research work. GAS and SU analyzed and supervised the study. JPA handled the computational chemistry software and drafted the manuscript. In addition, all authors read and approved the manuscript

Conflicts of interest

The authors have no conflicts of interest to declare

References

1. Fauci A.S., Kasper D.L., Longo D.L., Braunwald E., Hauser S.L., Jameson J.L., Loscalzo J. (**2008**) Harrison´s Principles of Internal Medicine, 17th edition. *Internal Medicine Journal*, 38 (12) 932-932, https://doi.org/10.1111/j.1445-5994.2008.01837.x
2. World Health Organization (**2018**) *Typhoid*. Retrieved from http://www.who.int/news-room/fact-sheets/detail/typhoid on September 5, 2022
3. Gasem M.H., Dolmans W.M., Keuter M.M., Djokomo eljanto R.R. (**2001**) Poor food hygiene and housing as risk factors for typhoid fever in Semarang, Indonesia. *Trop Med Int Health*, 6(6) 484-490, https://doi: 10.1046/j.1365-3156.2001.00734. x.
4. Dewan A.M., Corner R., Hashizume M., Ongee E.T. (**2013**) Typhoid Fever and its association with environmental factors in the Dhaka Metropolitan Area of Bangladesh: a spatial and time-series approach. *PLOS Neglected Tropical Disease*. 7(1): p.e1998. https://doi: 10.1371/journal.pntd.0001998 PMID: 23359825
5. Khan M.I., Ochiai R.L., Soofi L.V., Khan M.J., Sahito S.M., Habib M.A., Puri J.K., You Y.A. (**2012**) Risk factors associated with typhoid fever in children aged 2–16 years in Karachi, Pakistan. *Epidemiol Infect.,* 140(4) 665–72. doi: 10.1017/S0950268811000938 PMID: 21676350
6. Klemm E.J., Shakoor S., Page A.J., Qamar F.N., Judge K., Saeed D.K., Wong V.K., Dallman T.J., Nair S., Baker S., Shaheen G., Qureshi S., Yousafzai M.T., Saleem M.K., Hasan Z., Dougan G., Hasan R. (**2018**) Emergence of an extensively drugresistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio 9:e00105-18. https://doi.org/10.1128/mBio .00105-18.
7. Peirano G., Van der B.I.J., Freeman K.A., Poirel L.J., Nordmann L., Costello P., et al. (**2014**) Characteristics of Escherichia coli sequence type 131 isolates that produce extended-spectrum _-lactamases: Global distribution of the H 30-Rx sublineage. Antimicrob. Agents Chemother, 58 3762–3767.
8. Smith S.M., Palumbo P.E., Edelson P.J. (**1984**) Salmonella strains resistant to multiple antibiotics: Therapeutic implications. Pediatric Infect. Dis., 3 455–460.
9. Chen S., Cui S., McDermott P.F., Zhao S., White D.G., Paulsen I., Meng J. (**2007**) Contribution of target gene mutations and efflux to decreased susceptibility of Salmonella enterica serovar Typhimurium to fluoroquinolones and other antimicrobials. Antimicrob. Agents Chemother, 51 535–542.
10. Gaind R., Paglietti B., Murgia M., Dawar R., Uzzau S., Cappuccinelli P., Deb M., Aggarwal P., Rubino S. (**2006**) Molecular characterization of ciprofloxacin-resistant Salmonella enterica serovar Typhi and Paratyphi A causing enteric fever in India. J. Antimicrob. Chemother, 58 1139–1144.
11. Hirose K., Hashimoto A., Tamura K., Kawamura Y., Ezaki T., Sagara H., Watanabe H. (**2002**) DNA sequence analysis of DNA gyrase and DNA topoisomerase IV quinolone resistance-determining regions of Salmonella enterica serovar Typhi and serovar Paratyphi A. Antimicrob. Agents Chemother, 46 3249–3252.
12. Menezes G.A., Harish B.N., Khan M.A., Goessens W., Hays J. (**2016**) Antimicrobial resistance trends in blood culture positive Salmonella Paratyphi A isolates from Pondicherry, India. Indian J. Med. Microbiol, 34 222–227.
13. Anebi E.I., Shallangwa M.T., Isyaku G.A., Abdulsalam S.S., Danmallam A.M. (**2020**) Molecular docking study, drug-likeness and pharmacokinetic properties (ADMET) prediction of some novel thiophene derivatives as salmonella typhi inhibitors, *Bayero Journal of Pure and Applied Sciences*, 14 (2). doi: 10.4314/bajopas.v14i2.29
14. Abhishek K.V., Aminu I.D., Avinash K., Binta S.S., Umar A.H., Najib L.Y., Usman R.B., Zaharaddeen U.N., Abubakar D.D. (**2020**) Virtual Screening, Molecular Docking, and ADME/T Analysis of Natural Product Library against Cell Invasion Protein SipB from *Salmonella enterica serotype typhi: In Silico* Analysis". *Acta Scientific Pharmaceutical Sciences* 4 (8) 20-30. DOI:10.31080/ASPS.2020.04.0563
15. Arunkumar M., Murugan M., Vairamuthu A., Manikka K.A., Sathaiah G., Verma M., Balasubramaniem A., Perumal V. (**2022**) Evaluation of seaweed sulfated polysaccharides as natural antagonists targeting *Salmonella typhi* OmpF: molecular docking and pharmacokinetic profiling. *Beni-Suef Univ J Basic Appl Sci*, 11(8)
16. Mamaghani M., Tabatabaeian K., Bayat M., Nia R.H., Rassa M. (**2013**) Regioselective Synthesis and Antibacterial Evaluation of a New Class of Substituted Pyrazolo[3,4-b] Pyridines. J. Chem. Res., 37 494–498.
17. Anderson D.R., Hegde S., Reinhard E., Gomez L., Vernier W.F., Lee L., Liu S., Sambandam A., Snider P.A., Masih L. (**2005**) Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2). Bioorganic Med. Chem. Lett., 15 1587–1590.
18. Zhang X., Qiu Y., Li X., Bhattacharjee S., Woods M., Kraft P., Lundeen S.G., Sui Z. (**2009**) Discovery and structure–activity relationships of a novel series of benzopyran-based KATP openers for urge urinary incontinence. Bioorganic Med. Chem., 17, 855–866.
19. Samadi A., Marco-Contelles J., Soriano E., Álvarez-Pérez M., Chioua M., Romero A., González-Lafuente L., et al., (**2010**) Multipotent drugs with cholinergic and neuroprotective properties for the treatment of Alzheimer and neuronal vascular diseases. I. Synthesis, biological assessment, and molecular modeling of simple and readily available 2-aminopyridine-, and 2-chloropyridine-3,5-dicarbonitriles. Bioorganic Med. Chem., 18, 5861–5872
20. Hamada Y (**2018**). Role of Pyridines in Medicinal Chemistry and Design of BACE1 Inhibitors Possessing a Pyridine Scaffold. In Pyridine, Pandey, P.P., Ed.; IntechOpen: London, UK, 10–29
21. Ling Y., Hao Z.Y., Liang D., Zhang C.L., Liu Y.F., Wang Y. (**2021**) The Expanding Role of Pyridine and Dihydropyridine Scaffolds in Drug Design. Drug Des. Devel., 15 4289–4338.

22. Altaf A.A., Shahza A., Gul Z., Rasool N., Badshah A., Lal B., Khan E. (**2015**) A Review on the Medicinal Importance of Pyridine Derivatives. *J. Drug Des. Med. Chem*., 1; 1–11.
23. Zalaru C., Dumitrascu F., Draghici C., Tarcomnicu I., Tatia R., Moldovan L., Chifiriuc M.C., Lazar V., Marinescu M., Nitulescu M.G., et al. (**2018**) Synthesis, spectroscopic characterization, DFT study and antimicrobial activity of novel alkylaminopyrazole derivatives. J. Mol. Struct., 1156 12–21.
24. Marinescu M., Cinteza L.O., Marton G.I., Chifiriuc M.C., Popa M., Stanculescu I., Zalaru C.M., Stavarache C.E. (**2020**) Synthesis,density functional theory study and in vitro antimicrobial evaluation of new benzimidazole Mannich bases. BMC Chem., 14, 45.
25. Marinescu M. (**2021**) Synthesis of Antimicrobial Benzimidazole–Pyrazole Compounds and Their Biological Activities. Antibiotics, 10 1002.
26. Lad B.H., Giri R.R., Chovatiya L.Y., Brahmbhatt I.D. (**2015**) Synthesis of modified pyridine and bipyridine substituted coumarins as potent antimicrobial agents. *J. Serb. Chem. Soc, 80* (6) 739–747, https://doi: 10.2298/JSC140804004L
27. Mungra C.D., Patel P.M., Patel G.R. (**2009**) An efficient one-pot synthesis and *in vitro* antimicrobial activity of new pyridine derivatives bearing the tetrazoloquinoline nucleus. ARKIVOC, 14 64-74.
28. Daina A., Michielin O., Zoete V. (**2017**) SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. SCientifiC REpOrtS, 7 42717 DOI: 10.1038/srep42717
29. Konig J., Muller F., (**2013**) Transporters and drug-drug interractions: Important determinants of drug disposition and effects. Phamacol Rev, 65 944-966