Original Research Article

Synthesis, Characterization And Antimicrobial Screening - In Vitro And In Silico Of A Novel Hydrazone Compound Exhibiting Strong Bacterial Escherichia Coli Inhibitory Potentials.

ABSTRACT

The geometric increase of drug-resistant bacteria pathogens has made urgent the research, development and production of new antibacterial and antifungal compounds. As hydrazones enhanced generally with thiazoles which are 5-membered ring compound containing active nitrogen and sulphur molecules (C_3H_3NS) have been proven to exhibit strong antibacterial and antifungal activities, in this study, salicylaldehyde -4- thiazoleacetic acid hydrazone(SAFTAH) compound was synthesized. The novel compound was characterized and subjected to anti-microbial screening. The microbes employed were *staphylococcus aureus, Escherichia Coli, streptococcus and klebsialla aero genes.* The compound was found to be active against *Escherichia coli*. To validate this inhibition of *Escherichia coli* by the novel compound and to detect the active site of their interactions, in silico molecular docking analysis of the novel compound against aminopeptidase N from *E. coli* which is known to promote virulence to the microbe in question was carried out. Six drugs commonly used for the treatment of E. coli vis-a-viz, ciprofloxacin, levofloxacin, doxycycline, trimethoprim, rifamycin, rifaximin and sulfamethoxazole were subjected to same type of silico studies. The result of the docking indicated that the novel hydrazone compound was more efficient and effective than five of these *E. coli* inhibitory drugs.The success recorded from in vitro and in silico analyses stems from the efficacy of the novel compound not clear. Salicylaldehyde -4- thiazoleacetic acid hydrazone could therefore be a potential drug for the cure of *E. coli* infection.

Keywords

Escherichia coli, Antimicrobial screening, Molecular docking, Binding affinity, Salicylaldehyde -4-thiazoleacetic acid hydrazone

INTRODUCTION

Generally, acyl hydrazone are compounds that contain the -CNN- group, synthesized generally by eliminating water molecule between hydrazine and any of aldehyde or ketone (carbonyl compound)
It could be by the condensation of hydrazide with carbonyl compounds [K. K. Bedia et al; 2006]
Hydrazides are the acylated derivatives of hydrazine [J. Pisk, I. Dilovic et al; 2020]..

A great attention is given to hydrazones because of their biological and physiological activities [Mohamma Asif and Asif Husan 2013].They generally exhibit very strong antibacterial and antifungal activities.

A good number of researchers are interested in synthesizing variety of acyl hydrazones which are known to associate with antimalarial, antitumour, analgesic, antibacterial, antifungal, antitubercular antihelmintic, anticonvulsant, antimicrobial, antidiabetic and/or anti-inflammatory activities [M. E. Shakdofa et al, 2017, M. Asif and A. Husain; 2013]. Examples of such hydrazone include; N- (4-tert-Butylbenzoyl)-2-hydraxynapthalaldehyde (BBNH) a potent inhibitor of the ribonuclease H (R Nase H) activity of human immunodeficiency virus (HIV)-1 reverse transcriptase (RT). This BBNH binds to the HIV- 1 RT R Nase H active site via coordination to the metal and to amino acid. HIV- 1 RT R Nase H can reasonably be inhibited by N- acylhydrazone and phenylhydrazones.[Y. Jin, Z. Tan et al; 2010, K. Nagano, H. Kinoshita and A. Hirakawa; 1964, R.C. Aggarwal, N.K Singh]
A Research programme carried out on a series of these acyl hydrazones discovered that they are potentially tridentate ligands.
In addition, hydrazones of **thiazoleacetic acid?** are so enhanced that they are used as monoamine oxidase inhibitors and could be applied for curing psychotic illnesses[J.N. Nwabueze ; 1997].
These thiazolehydrazones are of the following structure

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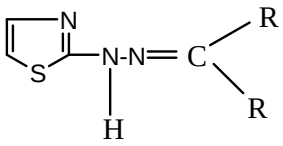

Fig. 1. Chemical structure of acetone[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazone

Another compound known as acetone[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazone is found to have anti cancer potentials[. S. Emily; 2011**]**

Escherichia coli (E coli) belongs to a family of microbes known as Enterobacteriaceae. These are gram-negative anaerobic rod. Generally, E coli are about 2.6μm long and 1.1 -1.5 μm wide which occur as single and straight rods. This bacteria is mostly responsible foracute urinary tract infection and tract sepsia in human. It causes acute enteritis together with haemorrhagic colitis. Its presence can lead to neonatal meningitis. As this infections grows as a biofilm, it is often present in the environment.[C.A Batt(2014) in Encyclopedia of food Microbiology 2nd edition].

Aminopeptidase N from Escherichia coli is a major metalloprotease which can be located in the small intestine and it participates in the controlled hydrolysis of peptides. That is hydrolysis of proteins, [https://www.ncbi.nim.nih.gov] It is a M1 class aminopeptidase with active region similar to the active region of thermolysin. They are zinc dependent enzymes. They are secreted by, precisely from the acinar cells of the pancreas. This enzyme is one of the known and attractive targets of e? coli associated desease (Sonia George et al 2014)

The in vivo microbial screening and in silico analysis of the novel compound; salicylaldehyde -4-thiazoleacetic acid hydrazone was performed in this study The docking was on Aminopeptidase N from Escherichia coli. The Salicylaldehyde -4- thiazoleacetic acid hydrazone was tested for sensitivity using *staphylococcus aureus, Escherichia Coli, streptococcus and klebsialla aero genes* microbes, the novel compound was docked on aminopeptidase N enzyme from e coli. The binding energy and mechanism of action was compared with those of seven known drugs for the treatment of e coli bacteria in Nigeria.

Materials and Methods

Ethyl-2-amino-4-thiazoleacetate was obtained from Sigma – Aldrich Chemical Company Ltd and used without further purification, while other reagents which include the carbonyl compound-salicylaldehyde together with the solvents used which were ethanol and methanol were from the BDH Chemical Ltd, Pools England.Known drugs for treatment of *E. coli* in Nigeria which include Ciprofloxacin, levofloxacin, doxycycline, trimethoprim, rifamycin, rifaximin and sulfamethoxazole were bought from Orchad Pharmacy Owerri and Pax Pharmacy Onitsha in Nigeria.

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The infrared spectra of the synthesized compound in Nujol were taken on FTIR-8400S Fourier Transformation Infrared Spectrophotometer and the proton NMR using dimethyl sulfoxide DMSO-d6 and recorded on a Bruker Avance 400 NMR spectrometer were both carried out at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

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The antimicrobial screening of the novel compound was carried out at University of Abuja Teaching Hospital and Peak Medical Laboratory, Gwagwalada using nutrient Agar on bacteria *staphylococcus aureus, Escherichia Coli, Streptococcus and klebsialla aerogenes* obtained from the Teaching hospital.

Preparation of 2-amino-4-thiazoleacetic acid hydrazide) (ATAH)

Standard method was used to prepare the hydrazides [120]?. 1mole of ethyl-2-amino-4-thiazoleacetate was reacted with 1 mole of the hydrazine hydrate to give the require hydrazide.

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2.50ml ((2.40g; 0.04moles) of hydrazine hydrate was added to 7.0g (0.040 moles) of ethyl-2-amino-4-thiazoleacetate in 50ml of absolute ethanol. Antibumping granules were added and the mixture was refluxed, on a water bath for six hours in a 100ml round-bottom flask. The mixture was poured into a beaker, left for three days to crystallize, and the crystals formed were filtered and recrystallized from ethanol. The resulting crystals were filtered and dried over silica gel in a vacuum desiccators and weighed (Yield, 4.61g; 65.86%). The crystals obtained were light brown in colour.

Preparation of Salicylaldehyde-2-amino-4-thiazoleacetic acid hydrazone (SAFTAH)

0.86g, (0.005 mole) of 2-amino-4-thiazoleacetic acid hydrazide (ATAH) was mixed with 053ml(0.601g,(0.005 moles) of salicylaldehyde in 50ml ethanol and refluxed for 4 hours in a 150ml round bottom flask on water bath. The solution was left for one day to crystallize. The crystals obtained were filtered and were recrystallized from ethanol. The yellow crystals were then dried in a desiccator over silica gel.(Yield,0.97g; 80.78%)

The structure and characterization of the synthesized compound was elucidated using the FTIR infrared spectra using Nujol disc and proton NMR spectrometer and some physical analyses.

Invitro susceptibility testing of the synthesized compound was carried out on four types of micro-organisms viz *taphylococcus aureus, Escherichia coli, Streptococcus, Klebsialla aerogenes*.

Docking procedure

The energy minimized compounds, (synthesized and the known existing drugs) were subjected to docking analyses on aminopeptidase N from E coli so as to predict their various interactions with the main binding sites on this enzyme. The Autodock Vina in Pyrx virtual screening software 20 (version 0.8) was employed for the docking analysis. [Duru et al; 2020] The grid box sizes were x centre: 19.19, y centre: 17.59, z centre: 20.88.

Biovia Discovery studio (Biovia 2020) was used to visualize the molecule-ligand interaction (enzyme-drugs) at the end of the docking process and to understudy the simulations

The binding affinities of the different compounds (drugs) docked on the protein target were obtained and the result organized on an excel spreadsheet [Chioma Nnenna Nwofor et al: 2022]

Results and Discussion

The physical analysis carried out has the following result

Table 1. Results of physical analysis

Compound	Formular	FM WT	Colour	MPT/DEC	% Yeild	Conductivity Ω^{-1} cm^3 mol^{-1}
SAFTAH	$C_{12}H_{12}N_40S$	171	Yellow	210°	81	-0.289

Solubility Test

Compounds	Water	Acetone	Ethanol	Methanol
SAFTAH	IS	S	S	S

Key

IS Insoluble

S Soluble

Infrared spectra.

The infrared spectra is seen in Table 4.3 .The band of interest include the v(OH) v(NH) v(C=N), v(C=O), v(C=N),

Compound	υ(OH)	υ(N-H)	υ(C=O)	Δ υ(C=O)	υ(C=N)	Δ υ(C=N)	υ(SO_4^{2-1})	υ(CH_3COO^-)$_2$	υ(M-O)	υ(M-N)	(
SAFTAH	3788			1750		1589				423	

H-NMR Spectrum of the novel compound - SAFTAH

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Table 2. Chemical Shift and multiplicity

s/n	Chemical Shift	Multiplicity
5	11.15	Singlet
6	3.76	Singlet
8	10.06	Singlet
10	8.38	Singlet
12	8.23	Singlet
13	7.61	Multiple
14	7,26	Multiple
15	7.50	Multiple
16	6.89	Multiple
17	3.39	Singlet

Table 3. Antimicrobial test result for the compound.

Compound	***S.aureus***	***E.Coli-***	***S.Spp***	***K.genes***
SAFTAH	-	+ +	+	-

KEY
S.aureas= Staphylococcus aureas
E.Coli= Escherichia coli
S.spp= Streptoccus species
K.aerogenes= Klebsialla aero gene

The Ecoli Enzyme and the various Interractions with its Key Active Regions

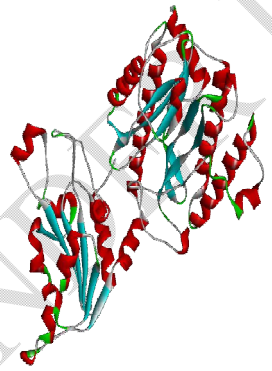

Fig. 2. Aminopeptidase N enzyme from E coli

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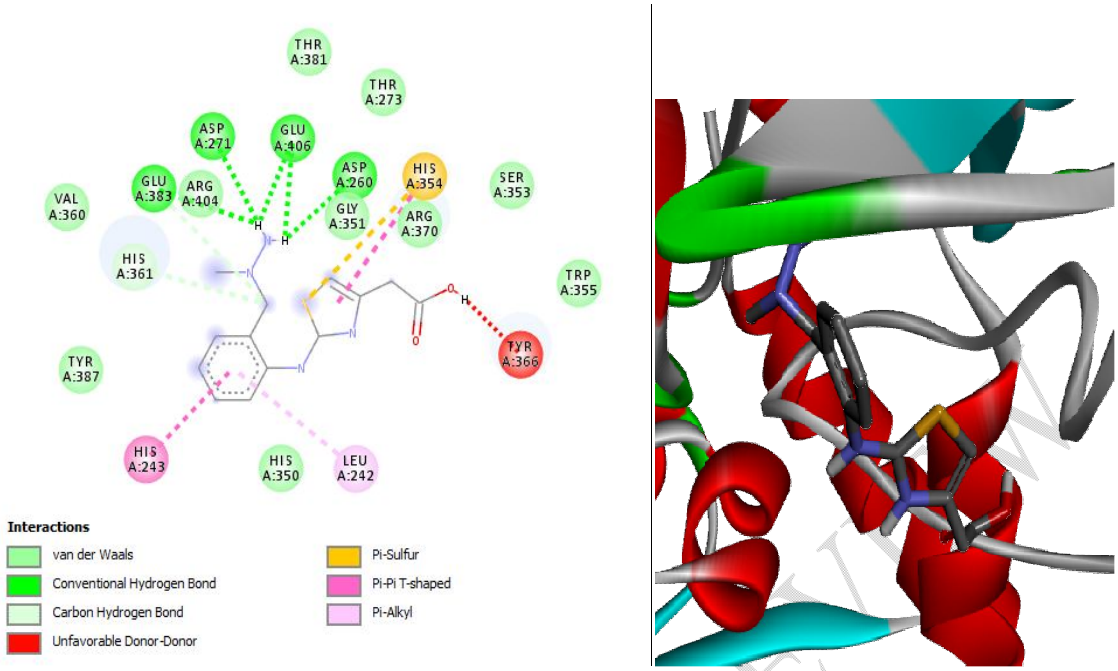

Fig. 3. The 2D interaction of SAFTAH with the active sites of the enzyme from E coli

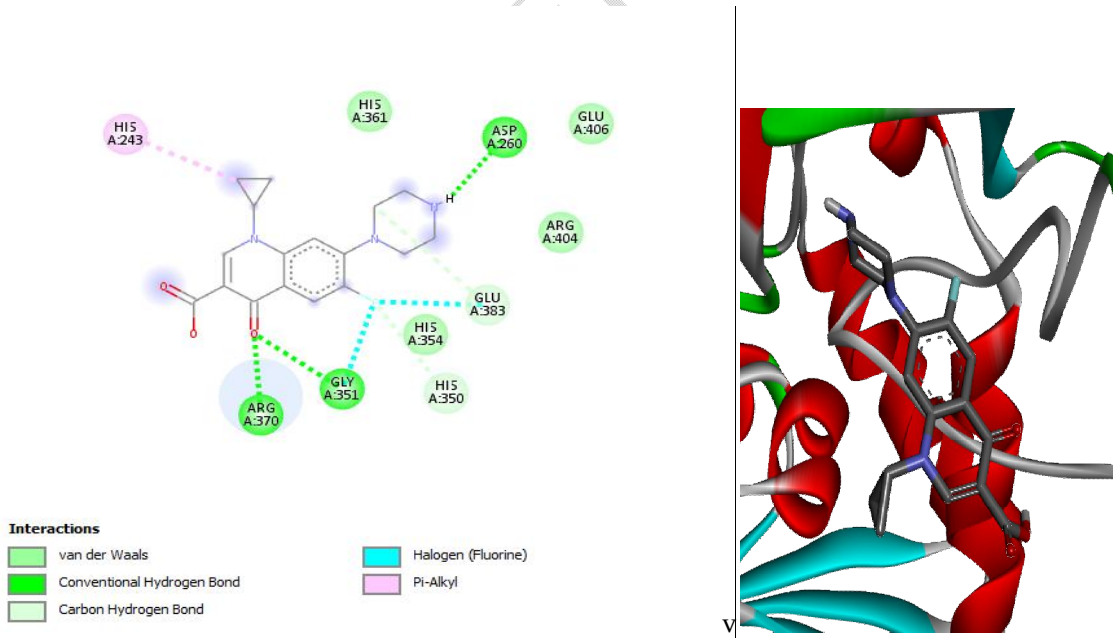

Fig. 4 The 2D interaction of Ciprofloxacin drug with the enzyme from E coli

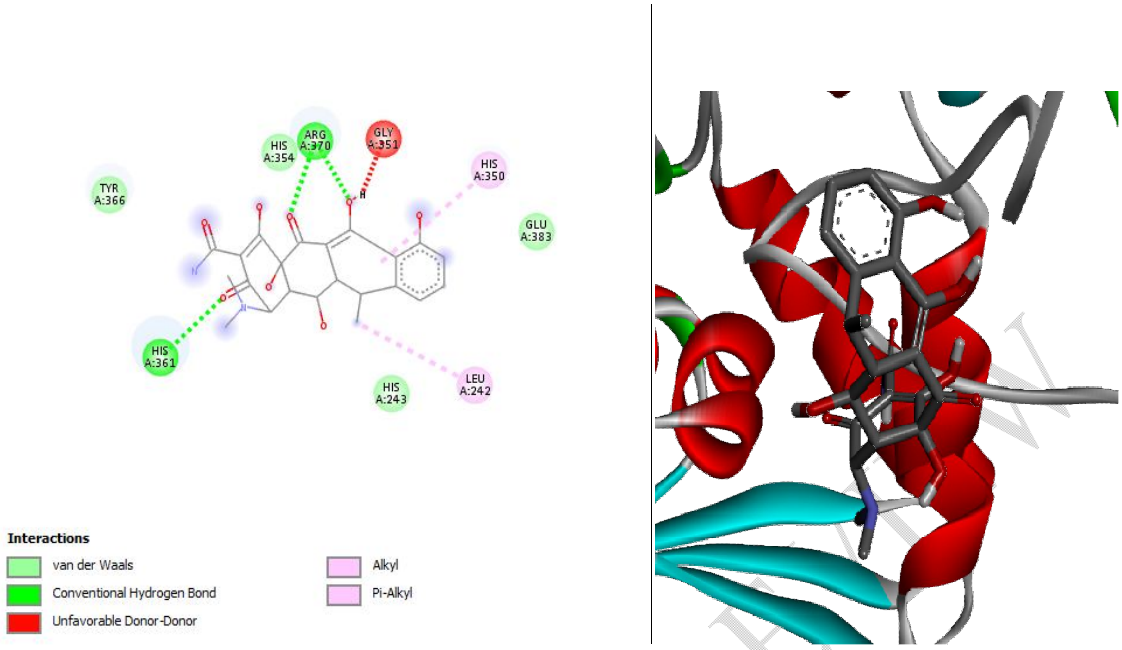

Fig. 5. The 2D interaction of Doxycycline drug with the enzyme from E coli

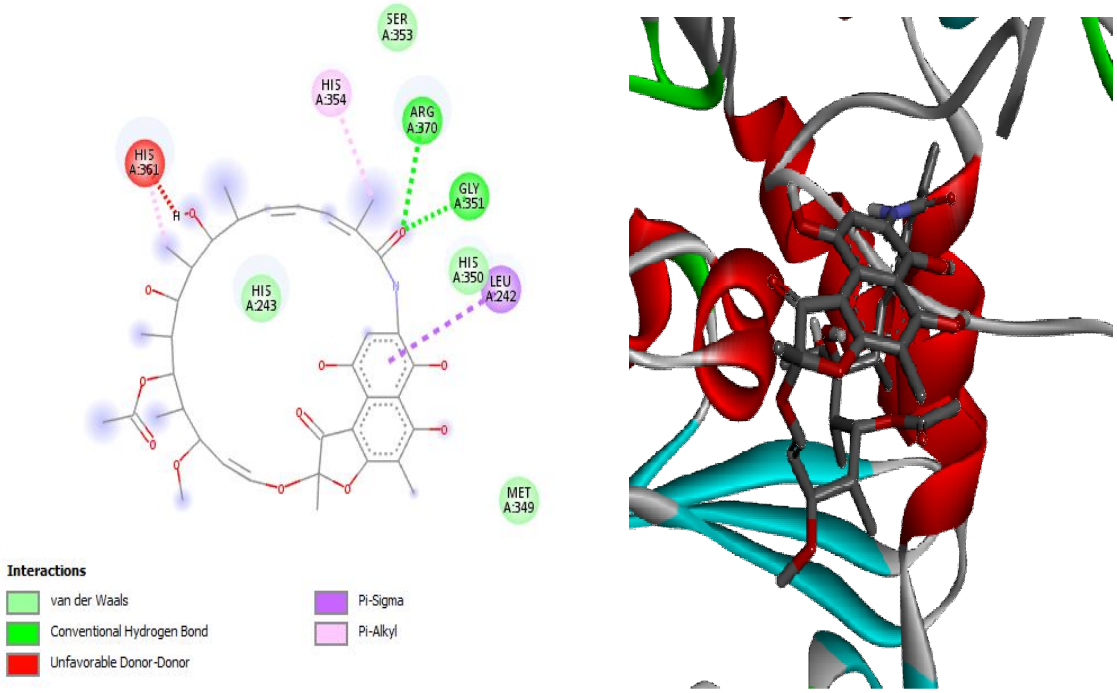

Fig. 6. The 2D interaction of Rifamycin drug with the enzyme from E coli

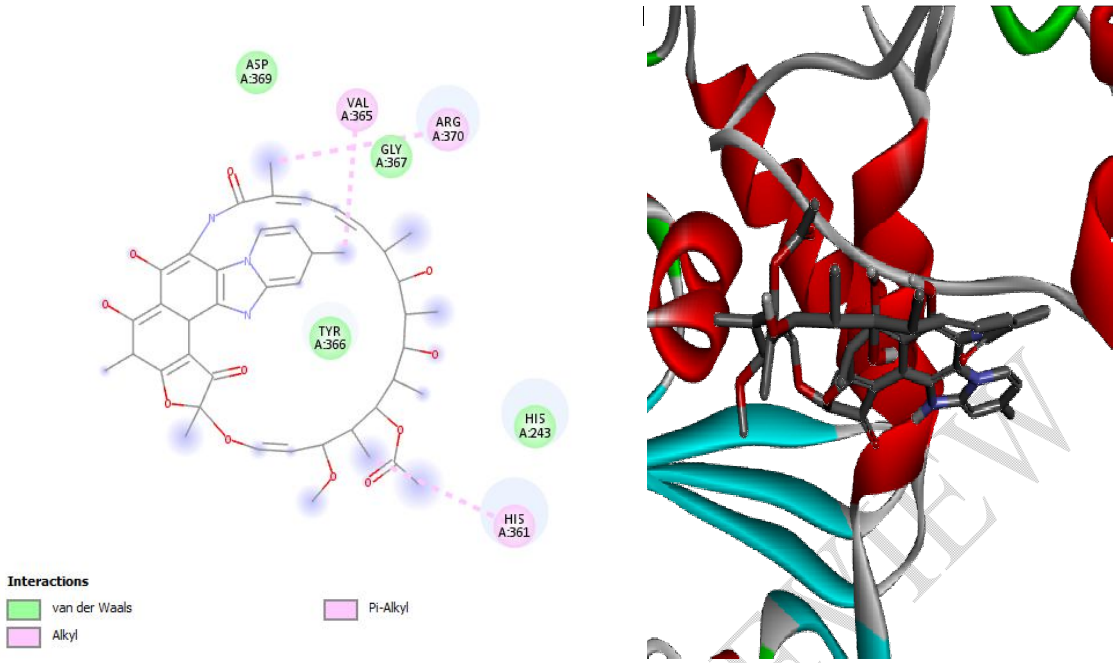

Fig. 7. The 2D interaction of Rifaximin drug with the enzyme from E coli

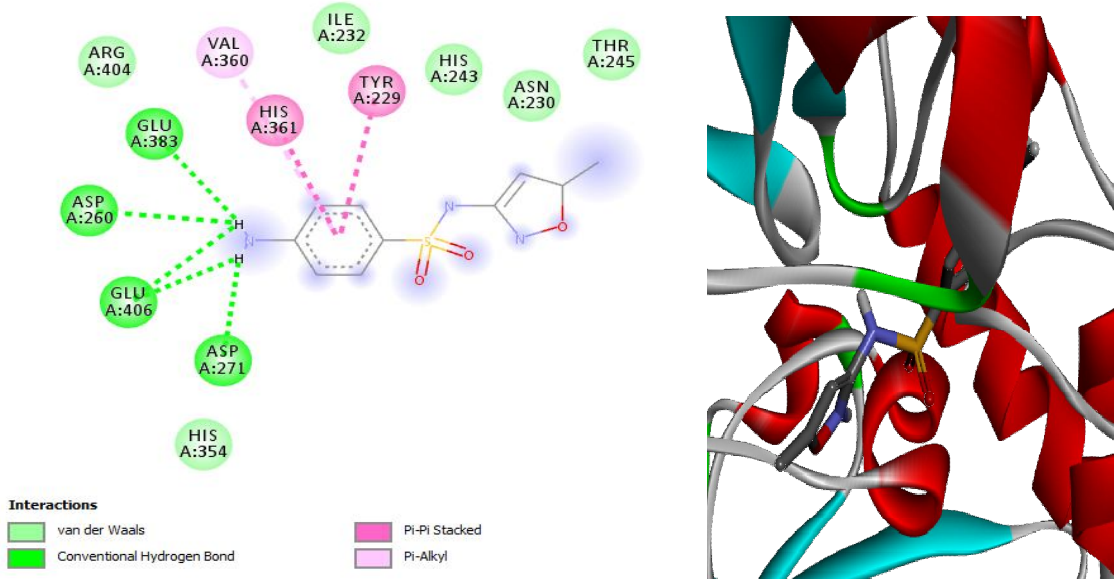

Fig.8. The 2D interaction of Sulfamethoxazole drug with the enzyme from E coli

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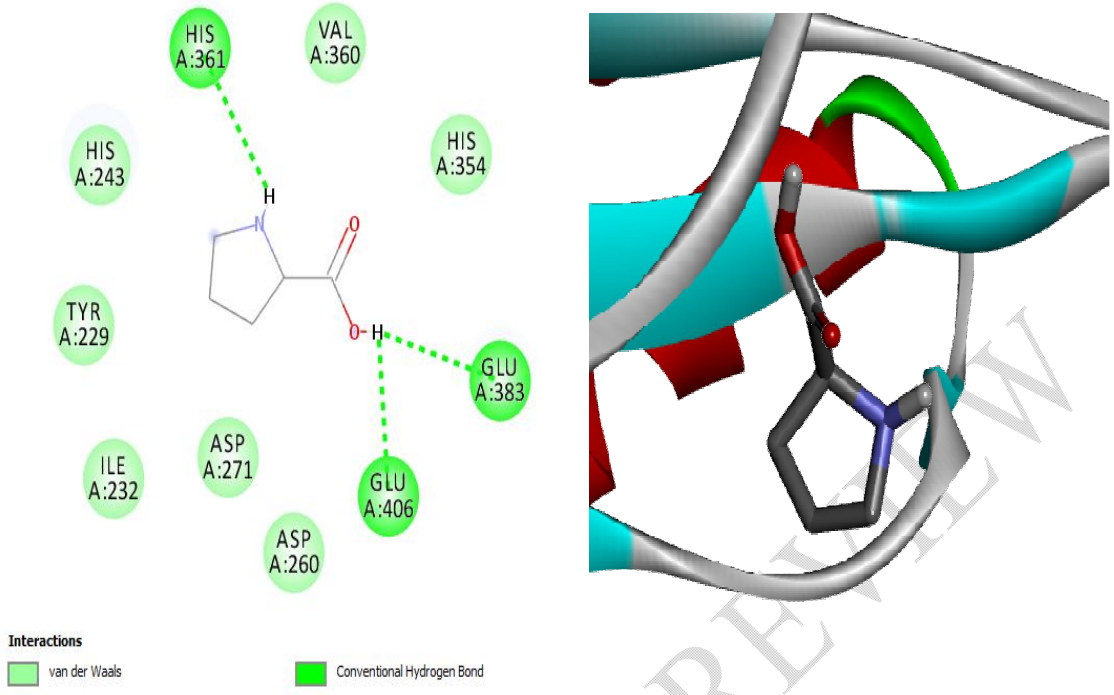

Fig. 9. The 2D interaction of Cocrystallized ligand with the enzyme from E coli

In vitro, the compound obtained was active against *E. coli* used for the screening and in silico, the interaction of the novel compound with the aminopeptidase N enzyme from E coli is very positive. Therefore, the activity profile of the novel compound indicates the existence of a very significant correlation between the in vitro screening and the computational data from the in silico studies. Moreover, in its interaction with the E coli enzyme, It competes and surpasses five (5) of the commonest known drugs for the treatment of patients suffering from E coli in Nigeria..This is so because the novel compound binds better than these drugs at the active sites of the protein, The binding energy of the novel compound is appreciably lower than those of the known drugs as follows; Binding energy of the novel compound -6.6, while ciproxafloxacin drug is -6.0, doxycycline drug is -6.2, rifamycin drug is -5.2, rifaximin drug is -5.7 and sulfamethoxazole drug is -6.6. It follows then that the thiazole compound which has been proven to exhibit strong antibacterial and antifungal activities was highly enhanced and strengthened by the salicylaldehyde compound for the **2-amino-4-thiazoleacetic acid hydrazide** alone was subjected to same screening - in vitro and in silico and the result obtained fell below. Salicylaldehyde on its own does not have a history of drug potentiality when used alone but does exhibit the ability of adequate enhancement for compounds with drug potentials. Salicylaldehyde certainly complemented the known efficacy of **2-amino-4-thiazoleacetic acid hydrazide** making this novel compound a potential E coli drug.
Reacting this novel hydrazide compound with some other carbonyl compounds and subjecting salicyladehyde into reactions with different hydrazides, all for comparative studies would make a good research and offer proof of whether or not the salicyladehyde is more efficacious than other carbonyl compounds and whether the novel hydrazide compound posses more e coli treatment ability than other hydrazides..

Conclusion

The success recorded - both in vitro and in silico analyses of the novel compound synthesized, certainly stems from its efficacy. Therefore, **Salicylaldehyde-2-amino-4-thiazoleacetic acid**

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hydrazone (SAFTAH) emerges as an excellent potential and effective drug for the cure of Escherichia coli infection in Nigeria. Synthesis of the compound is not difficult and so can be very handy for the treatment of the E coli infection which appears to be incessant in Nigeria. The cure promises to be more stable than what obtains currently considering the low binding energy of interaction..

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