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Synthesis, Characterization, *In vitro* and *In silico* Studies of a Novel Hydrazone Compound

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The geometric increase of drug-resistant bacteria pathogens has made urgent the research, development and production of new antibacterial and antifungal compounds, hence the synthesis of this novel compound as a potential antibacterial drug. 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 aerogene.* The compound was found to be active against *Escherichia coli* as the test result recorded ++ indicating 'very active' unlike the other three microbes which either had + or none. 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, rifamycin, rifaximin and sulfamethoxazole were subjected to same type of silico studies. The result of the docking indicated

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that the novel hydrazone compound is more efficient and effective than five of the *E. coli* inhibitory drugs, as the novel compound has lower binding energy of-6.6 after the docking, while ciproxafloxacin drug is -6.0, doxycycline drug, -6.2, rifamycin drug, -5.2, rifaximin drug, -5.7 and sulfamethoxazole drug, -6.6. The synthesized compound has also better interaction with the active site of the microbe concerned as shown in the images for the interactions as follows:- The newly synthesized compound has 5Hb & 9vdW = 14bonds, ciproxafloxacin has 3Hb & 9vdW = 12bonds, doxycycline has 2Hb & 4vdW = 06bonds, rifamycin has 2Hb & 4vdW = 06bonds, rifaximin has 0Hb & 4vdW = 04bonds, sulfamethoxazole has 4Hb & 6vdW = 10bonds. Compounds with lower binding energy and good interactions with the active site of the enzyme prove to be better drugs in pharmacy. Sulfamethoxazole drug which has same binding energy with the novel compound has lower interaction with the active site of the bacterium. Therefore, Salicylaldehyde -4- thiazoleacetic acid hydrazone could be a potential and better drug for the cure of *E. coli* infection.

Keywords: Escherichia coli; antimicrobial screening; molecular docking; binding affinity; salicylaldehyde -4- thiazoleacetic acid hydrazone.

1. 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 [1]. Hydrazides are the acylated derivatives of hydrazine [2].

A great attention is given to hydrazones because of their biological and physiological activities [3]. They generally exhibit very strong antibacterial and antifungal activities.

A good number of researchers are therefore 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 [4,3]. Examples of such hydrazones 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 [5].

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 [6].

A compound known as acetone [4-(5-nitro-2-furyl)-2-thiazolyl]hydrazone is found to have anti cancer potentials [7].

Escherichia coli occur as single and straight rods. This bacteria is mostly responsible for acute 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 infection grows as a biofilm, it is often present in the environment [8]

Aminopeptidase N 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 [9]. It belongs to the aminopeptidase class with active region similar to the active region of thermolysin. They are zinc dependent enzymes. They are secreted precisely from the acinar cells of the pancreas. This enzyme is one of the known and attractive targets of *E coli* associated disease [10].

The *In vitro* 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. The Salicylaldehyde -4-thiazoleacetic acid hydrazone was tested for sensitivity using *staphylococcus aureus, Escherichia coli, streptococcus and klebsialla aerogenes* microbes and the novel compound docked on aminopeptidase N enzyme from *E coli* [11,12]. The binding energy and mechanism of action was compared with those of six known

drugs for the treatment of *E coli* bacteria in Nigeria.

1.1 Aim of this research

The aim of this work is to synthesize and produce from handy chemicals a very less resistant and more efficacious drug for the cure of *E coli*, an infection which is rampart in Nigeria and which is very much a drug-resistant bacteria. The new drug being synthesized from handy materials in Nigeria promises to be cost effective.

2. 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 standard 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.

The Infrared spectra of the synthesized compound in Nujol were taken on FTIR-8400S Fourier Transformation Infrared Spectrophotometer and the proton NMR done using dimethyl sulfoxide DMSO-d_6 and recorded on a Bruker Avance 400 NMR spectrometer at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

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.

2.1 Preparation of 2-Amino-4-Thiazoleacetic Acid Hydrazide) (ATAH)

Standard method was used to prepare the hydrazides. 1mole of ethyl-2-amino-4-thiazoleacetate was reacted with 1 mole of the hydrazine hydrate to give the required hydrazide (Fig. 1a,1b).

2.50 ml (2.40g; 0.04 moles) 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, dried over silica gel in a vacuum desiccators and weighed (Yield, 4.61g; 65.86%). The crystals obtained were light brown in colour.

2.2 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%).

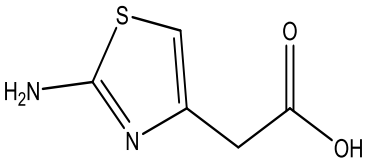

Fig. 1a. Structure of the intermediate compound

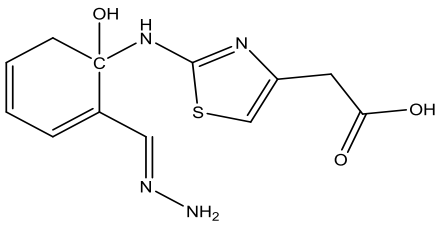

Fig. 1b. Structure of salicyladehyde-2-amino-4-thiazoleacetic acid hydrazone

Characterization and structure determination of the synthesized compound was done using the FTIR obtained using Nujol disc and proton NMR. Other physical analyses were also carried out to confirm the structure.

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

Disks were improvised using filter papers. The papers were cut into approximately equal sizes. Then, they were sterilized in the oven at a temperature of 60^{o}C for one hour. Next, the papers were allowed to cool and then they were soaked accordingly into the solution of the new compound obtained (in 0.005g/ml ethanol). Drying followed, sterilization in the oven at a temperature of 37°C for 24 hours.

The Petri- dishes containing already gelled nutrient agar were innoculated with some micro-organisms-,*staphylococcus aureus, Escherichia coli, streptococcus, klebsialla aerogenes.*

These Petri-dishes were impregnated with the disks containing the novel compound. They were arranged radially from the centre of the dishes and incubation was done for 24 hours. The result obtained is shown in Table 3.

2.3 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 studies [13] 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 [14].

3. RESULTS AND DISCUSSION

The physical analysis; solubility test, conductivity, melting point, colour determination are reported in Table 1.

Table 1. Results of physical analysis

Compound	Formular	FM WT	Colour	MPT/DEC	%Yeild	Conductivity Ω-1 cm3 mol-1
SAFTAH	C12H12N40S	171	Yellow	210°	81	-0.289
			Solubility test			
Compounds	**Water**	**Acetone**	**Ethanol**	**Methanol**		
SAFTAH	IS	S	S	S		

Key: IS- Insoluble; S-Soluble

1 Infrared spectra

The frequency of absorption obtained from infra red spectra is shown in Table 2. 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) $\Delta\upsilon$(C=O) υ(C=N) $\Delta\upsilon$(C=N) $\upsilon(SO_4^{2-1})$ υ(M-N)

SAFTAH 3788 1750 1589

H-NMR Spectrum of the novel compound : SAFTAH

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

2. The *E. coli* enzyme and the various interractions with its key active regions

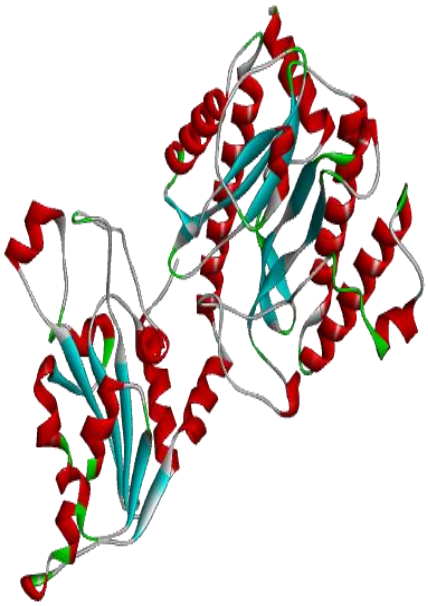

Fig. 2. Aminopeptidase N enzyme from *E coli*

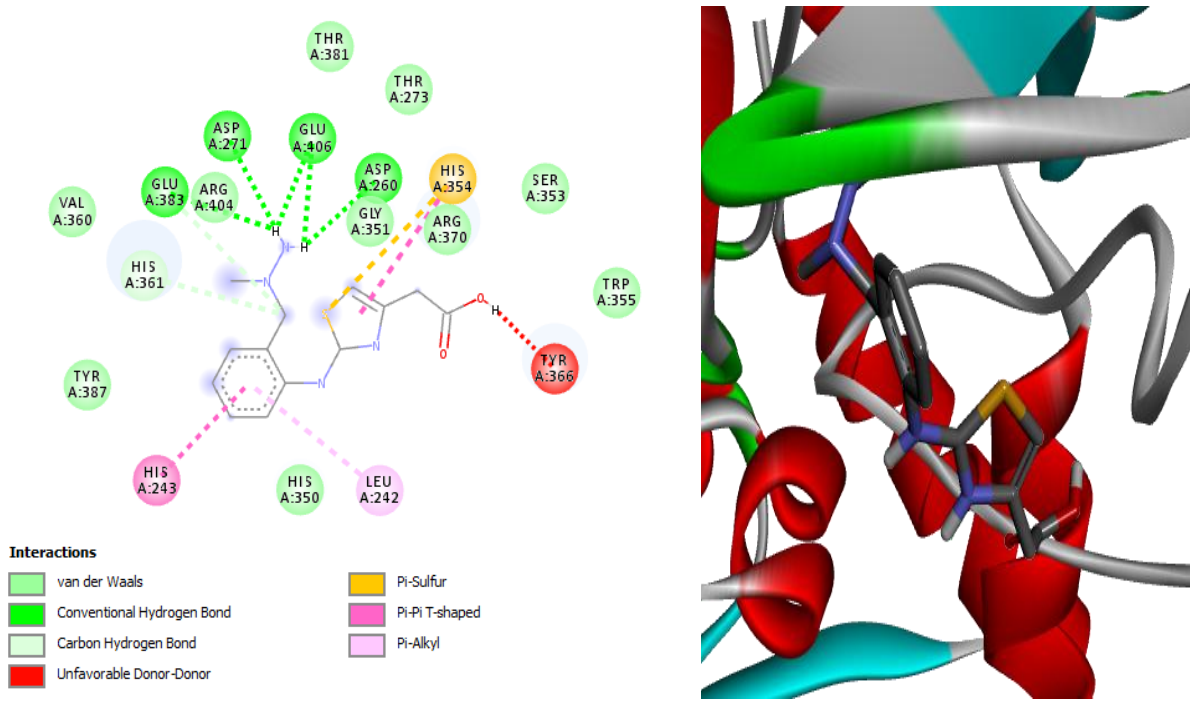

Fig. 3. The 2D interaction of SAFTAH with the active sites of the enzyme from *E. coli*

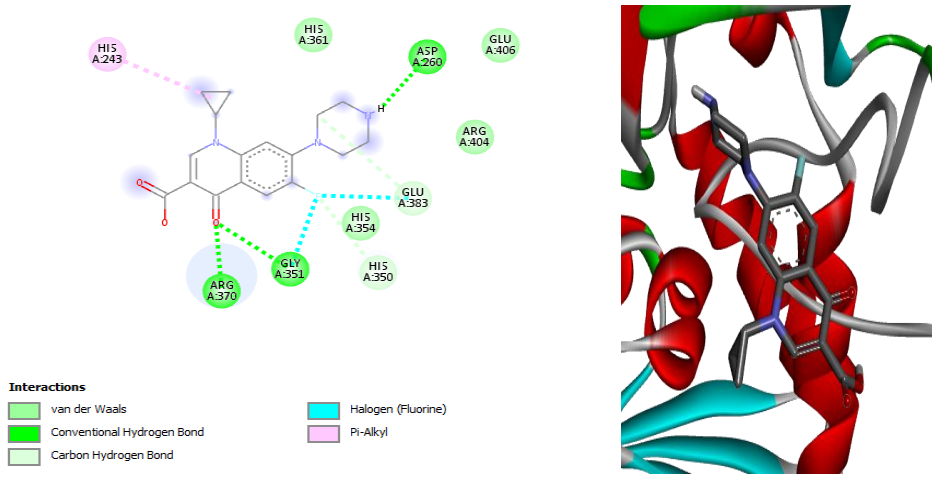

Fig. 4. The 2D interaction of ciprofloxacin drug with the enzyme from *E coli*

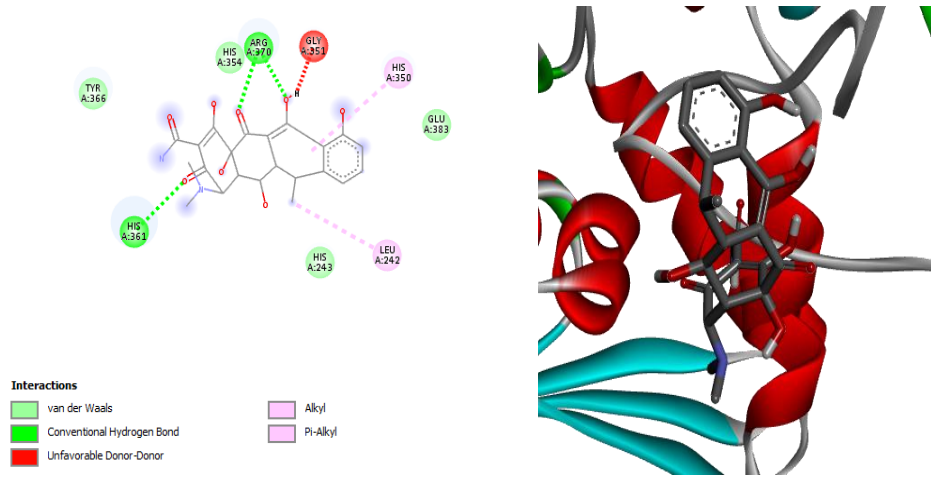

Fig. 5. The 2D interaction of Doxycycline drug with the enzyme from *E coli*

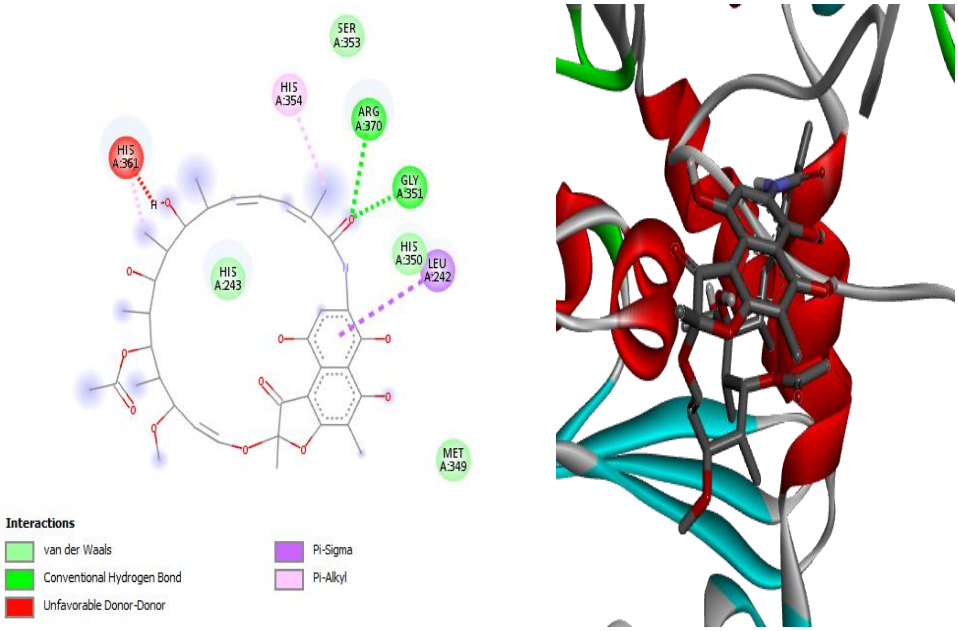

Fig. 6. The 2D interaction of Rifamycin drug with the enzyme from *E coli*

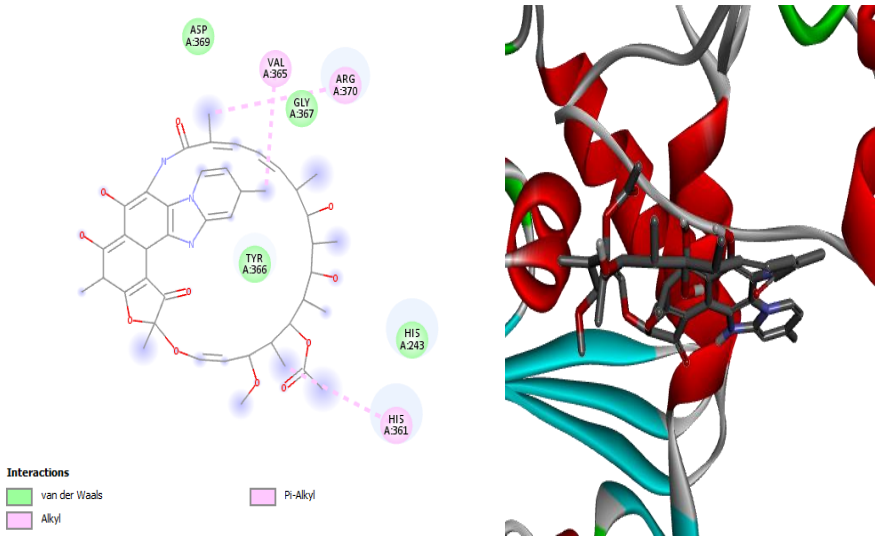

Fig. 7. The 2D interaction of Rifaximin drug with the enzyme from *E coli*

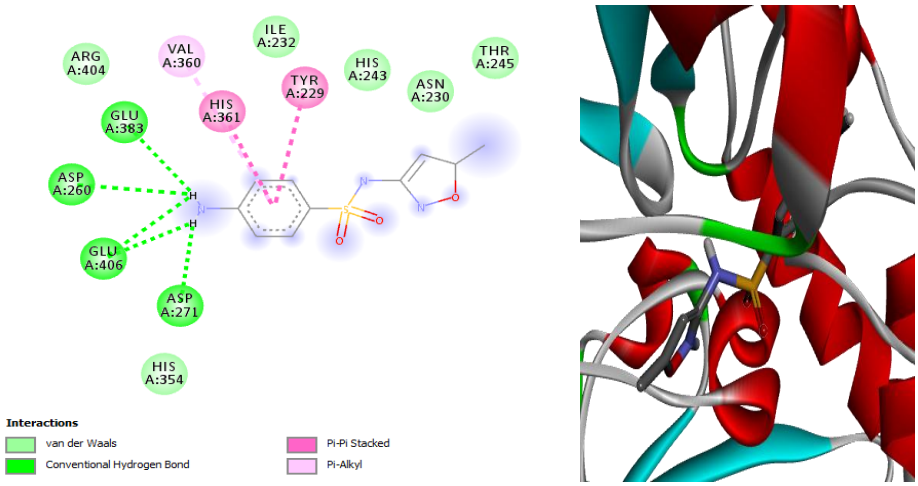

Fig. 8. The 2D interaction of Sulfamethoxazole drug with the enzyme from *E coli*

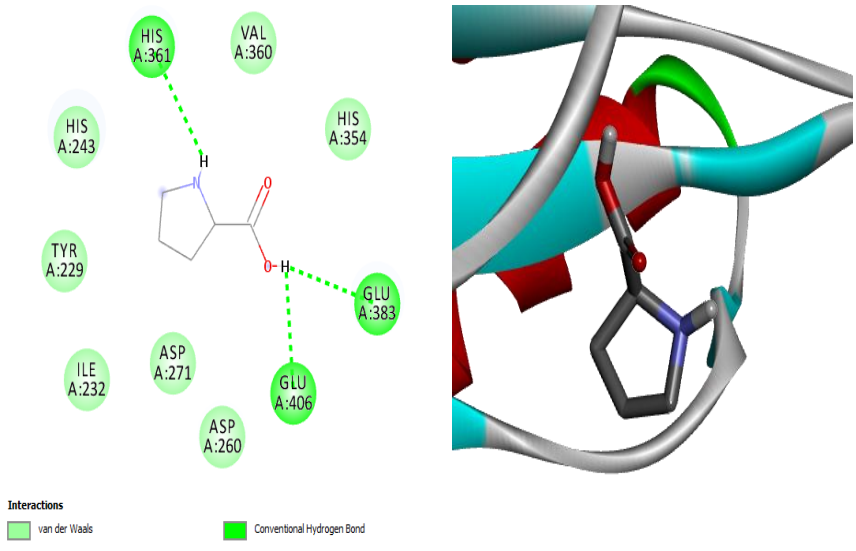

Fig. 9. The 2D interaction of Cocrystallized ligand with the enzyme from *E coli*

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 is very positive. Therefore, the activity profile of the novel compound indicates the existence of a very significant correlation between the screening and the computational data studies. Moreover, in its interaction with the bacteria, 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. Interaction with the active site of the *E coli* is as follows; the new compound is 5Hb & 9vdW = 14bonds, ciproxafloxacin is 3Hb & 9vdW = 12bonds, doxycycline is 2Hb & 4vdW = 06bonds, rifamycin is 2Hb & 4vdW = 06bonds, rifaximin is 0Hb & 4vdW = 06bonds, sulfamethoxazole is 4Hb & 6vdW = 10bonds. This proves that the synthesized compound interacted better with the active site of the *E coli (*Figs. 2-9).

It follows then that the newly synthesized compound thiazole compound Salicylaldehyde-2-amino-4-thiazoleacetic acid hydrazone performs better than these known drugs. Even the sulfamethoxazole drug which has same binding energy with the novel compound, fell below it considering the interaction ability with the *E coli*. with 4Hb & 6vdW = 10bonds as against 5Hb & 9vdW = 14bonds of the novel compound. When the interaction is high, the inhibition of the enzyme increases and therefore have better curative effect.

4. CONCLUSION

Considering the success recorded - both *in vitro and in silico* analyses of this novel compound synthesized, salicylaldehyde-2-amino-4-thiazoleacetic acid hydrazone (SAFTAH) emerges as an excellent potential and more effective drug for the cure of *Escherichia coli* infection in Nigeria, given its in vitro 'very active' inhibitory property with the *E coli* enzyme of double plus (++), its lower binding energy in silico, of -6.6 and better interaction with the active site of the enzyme of 5Hb & 9vdW = 14bonds, when compared with other known drugs for the treatment of the disease 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 therefore promises to be more stable and permanent other than what obtains currently, considering this low binding energy of interaction and the attack on the active site of the disease.

5. RECOMMENDATION

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 posseses more e coli treatment ability than other hydrazides.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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