Available online on 15.10.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Food constituents for inhibition of BabA of *Helicobacter pylori*

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ABSTRACT

Helicobacter pylori causes several gastric pathogeneses to human, nowadays the bacterium developed incredible drug and antibiotic resistance. The bacterium starts its activities by attachment to gastric epithelia via BabA as the main player in the \is process. The study was carried out to find/discover food constituents as inhibitors. Five molecules were obtained from the screening process, 2_3_4_5_6_Penta_O_acetyl_D_glucose, N2_N2_Dimethylguanosine, 5__Methylthioadenosine, Glyceryl_5_hydroxydecanoate, Monoisopropyl_citrate, in addition to two drugs Rivoglitazone and Tiapirinol not used for *Helicobacter pylori* before. The molecules were docked with considerable binding affinities with different types of interactions. The molecules were checked for the safety of different aspects, they are of good synthetic accessibility and in agreement with the Lipinski rule of 5 which is essential for Helicobacter therapy.

Keywords: *Helicobacter pylori*, food constituents, BabA inhibition, SBDD

Article Info: Received 13 Aug 2020; Review Completed 22 Sep 2020; Accepted 06 Oct 2020; Available online 15 Oct 2020

Cite this article as:

Al-Khafaji ZM, Mahmood AB, Mahmood MB, Food constituents for inhibition of BabA of *Helicobacter pyloris*, Journal of Drug Delivery and Therapeutics. 2020; 10(5-s):152-163 http://dx.doi.org/10.22270/jddt.v10i5-s.4358

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INTRODUCTION

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium colonizes the human stomach; it is involved in chronic gastritis and peptic ulcers. Colonization of *H. pylori* can lead to mucosa-associated lymphoid tissue lymphoma and gastric cancer [1,2]. It has been characterized as a class I Carcinogen by the world health organization (WHO) [1,3,4] . About 80% of the middle-aged adults may be infected with this bacterium, and gastric cancer is one of the most common cancer and is the third leading cause of cancer-related death worldwide [4,5]

The adhesion process is quite important to the bacterium, since any *H. pylori* cell that does not adhere to an epithelial layer would be quickly removed, so the adhesion represents a method of protection [6,7]. This bacterium is equipped with an extraordinary large set of virulence factors, among them a set of outer membrane proteins (OMPs), about 4% of the bacterial genome responsible for coding these proteins [8,9] , some of them were identified as adhesins or adherence-associated proteins[10]. In addition, the bacterium got high plasticity of its genome caused by high mutation rate and extensive exchange of genetic materials [11,12], leading to continuous changes as in the appearance of antibiotic resistance in an incredible manner [13,14,15].

The recognition, adhesion, and persistence of bacterium in the stomach is mainly mediated by several OMPs (hop) group including blood group antigen-binding adhesion, one of the most important is BabA which is also known as HopS [6,16,17] with molecular weight 78kDa.This protein is known to be important in the initial colonization of *H. pylori*, it has been reported on the oral cavity and stomach [7,18] , it has binding affinities(Ka~10^{7}-10^{12}/M) with order magnitude greater than most carbohydrates binding protein [19,20]. This protein mediates binding to fucosylated Lewis b (Leb) blood group antigens and facilitates the injection of some virulence factors such as CagA and VacA [17,21,22]. In addition, BabA binding contributes to gene mutations through the formation of double-stranded DNA breaks in host cell lines [23]. This might lead to the suggestion that BabA and other binding proteins such as SabA are involved in carcinogenesis [24]. This protein was reported to be necessary for *H. pylori* replication and nutrient acquisition [25,26], it has been estimated in population with the highest incidence of gastric cancer worldwide as in East Asia [22].

On the other side, foods have been used for a long time as anti-Helicobacter. Food constituents work in different mechanisms to inhibit *H. pylori* infection, therefore, they can be considered as alternatives to prevent and manage *H. pylori* infection [27,28]. To explore that computational aid drug design (CADD) can play an important in drug design and discovery using different approaches, among these Structure-Based Drug Design (SBDD), since a lot of 3D structures are now available. SBDD is the most powerful and efficient process for accelerating drug discovery as it is specific and cost-effective [29,30], so it has emerged as a promising tool for the drug industry to design and optimizes ligands/drugs and become as an integral part of most drug discovery [31].

This study aims to use BabA crystal structure (pdb ID 4zh0) to screen food database and drug database using SBDD approach in an attempt to find out new inhibitors or drugs from food constituents to inhibit the attachment of *H. pylori*.

MATERIALS AND METHODS

Different databases and software were used, for different purposes:

Databases

NCBI: https://www.ncbi.nlm.nih.gov/

Used for retrieve sequences and BLASTing

pdb database: https://www.rcsb.org/

Used to find out the pdb structure of BabA (4zh0) protein .

Uniprot database: https://www.uniprot.org/

To find out some information about the target (BabA).

Zinc database: http://zinc.docking.org/

Used to download different chemical formats, and information about compounds. **SOFTWARE**

MarvinSketch : https://chemaxon.com/products/marvin

Used for chemical format manipulation, and finding some molecule descriptors.

Online SMILES Translator and Structure File Generator https://cactus.nci.nih.gov/translate/

Used to get SMILES format of some molecules.

Swiss ADME: http://www.swissadme.ch/

Used for finding pharmacokinetic characters of molecules.

T.E.S.T. software: https://www.epa.gov/

Toxicity-Estimation-Software tool-Test , to find out the safety of molecules.

PyRx software v.8: https://pyrx.sourceforge.io/

Used for molecular docking.

PyMOL software https://pymol.org/2/.

Used for docking vitalization.

Discovery Studio Visualizer Used for docking vitalization

RESULTS AND DISCUSSION

Target importance and characterization:

The choice of a drug target is primarily made on a biological and biochemical basis, it should have well-defined binding pockets and the goal is total inhibition leading to death and eradication of the pathogens. The target chosen in this study BabA (pdb ID 4zh0) is druggable and got the most criteria to be a good drug target, it has been characterized in a previous study [32] . It has been used in the SBDD process which had been carried out in multistep relies on the knowledge of the 3D of the protein. The steps involve protein preparation, binding site identification, ligand library preparation, docking, and scoring function, so current SBDD methods consider the key features of binding cavities of a therapeutic target to design efficient ligands [33,34,35]. Many tools can perform such tasks, among them MTiOpenScreen which use AutoDock4.2 and automated virtual screening with AutoDock Vina and provides valuable starting collections using pdb structure to screen several databases [36], it has been used to find and identifying ligands for human acetylcholinesterase (Alzheimer's disease) [37].

In this study, hundreds of molecules resulted in a screening food database and a drug database. Once small molecules have been identifying as potentially binding to the target, it must be evaluated before proceeding to further steps, since even molecule with high scoring could fail in some aspects of characterizations or in vivo and in vitro assays. In the case of H. pylori, the rule of 5 should be considered [33,38].

The resulted molecules were subjected to different filtration steps. first of all, molecules with rotatable bonds more than 7 were omitted since such molecules could bind to off-targets [38,39]. Then their mutagenicity, teratogenicity, and carcinogenicity were estimated. Such a survey resulted in two molecules from the drug database and 5 molecules from the food database. Table 1 shows the molecules

Table 1: Molecules found from drug database screening food database

Molecule #	Molecule ZINC766	Comments
Rivoglitazone	Drug database	ZINC4214702
Tiapirinol	Drug database	ZINC766
Food database		
81	2_3_4_5_6_Penta_O_acetyl_D_glucose	ZINC000003861047
86	N2_N2_Dimethylguanosine	ZINC000005115341
91	5__Methylthioadenosine	ZINC000005929300
97	Glyceryl_5_hydroxydecanoate	ZINC000002557905
101	Monoisopropyl_citrate	ZINC000002528012

Other properties such as bioavailability and ADME (Absorption, Distribution, Metabolism, Excretion) i.e. Pharmacokinetics and Druglikeness and Medicinal Chemistry characters and hERG activity were estimated in addition to synthetic accessibility, as shown in Table 2

Table 2: Some characters of suggested molecules

Molecule #	RTs	GI absorption	BBB permeant	P-gp substrate	Druglikeness/ Lipinski	PAINS	Synthetic accessibility	Bioavailability
Rivoglitazone	6	High	NO	NO	0 violation	NO	3.54	0.55
Tiapirinol	3	High	NO	NO	0 violation	NO	3.45	0.55
81	6	High	NO	NO	1 violation: NorO>10	NO	4.79	0.55
86	3	High	NO	NO	0 violation	NO	4.10	0.55
91	3	Low	NO	NO	0 violation	NO	4.12	0.55
97	5	High	NO	NO	0 violation	NO	3.61	0.55
101	7	High	NO	NO	0 violation	NO	3.04	0.56

From the results it is obvious that chemical synthesis is possible, however, optimization of these molecules, if required, can be done using the scaffold hopping approach. It has been suggested that the inhibition could be active site as in enzymes, but inhibitor could affect the assembly sites with other macromolecules or communication sites or proteins with other function as for BabA, so the potential drug targets are not necessarily disease-causing but could sharing in disease development [16,40]. Therefore, the anti-adhesion would be able to clear *H. pylori* out of the stomach wall through dislodging off the bacterium, since that BabA is unique to *H. pylori* without affecting the other good bacteria of normal flora [6].

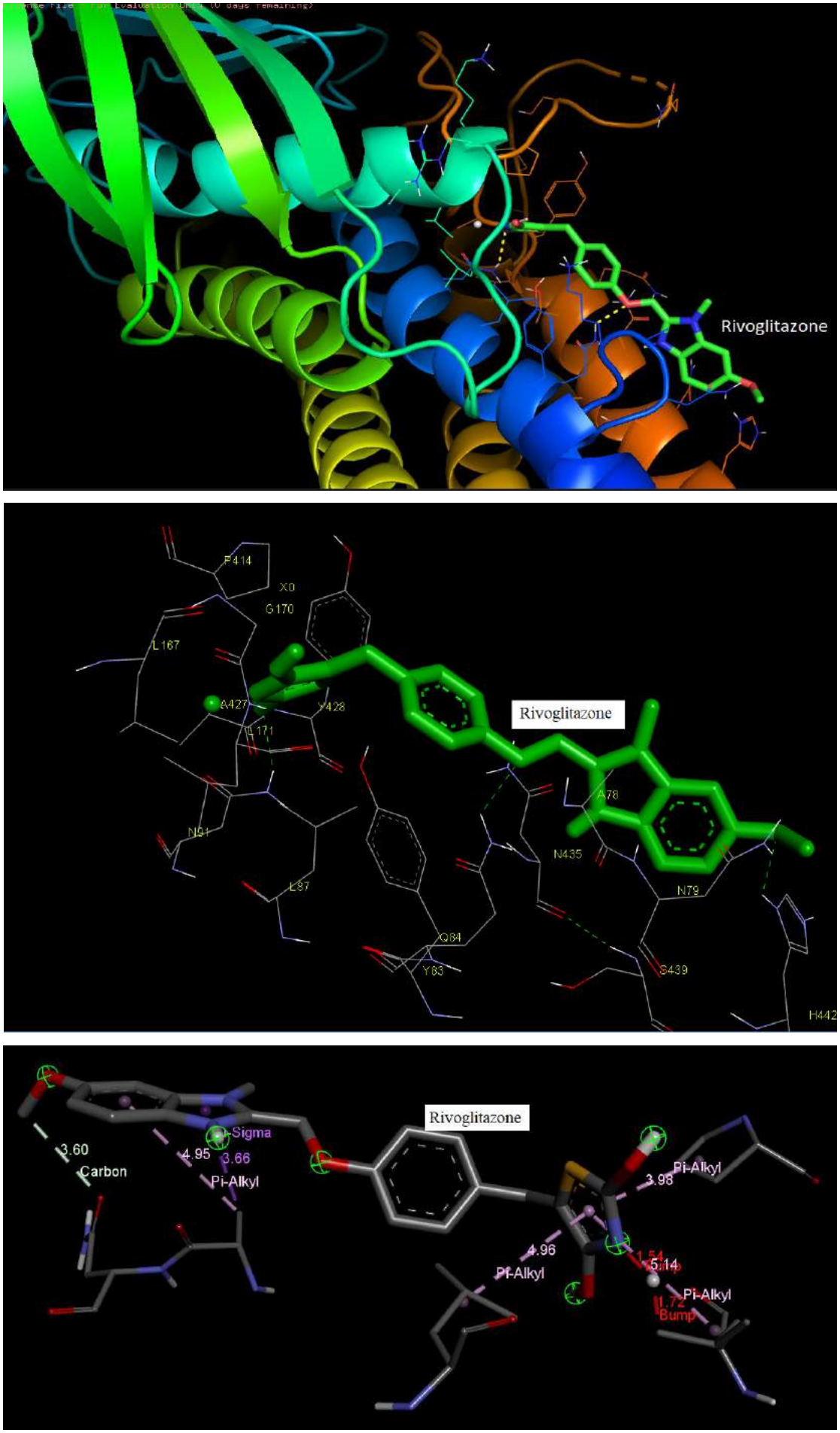
Docking studies

Docking is the critical step in SBDD, which helps in visualizing the interaction patterns and binding energy of protein/receptor-ligand complexes [33], and gives insight into the interactions at the atomic level, offering the opportunity to fully characterize the binding site of each molecule. The obtained molecules were docked with BabA using PyRx package v.8, the top affinities results with RMSD value of zero are shown in Table 3

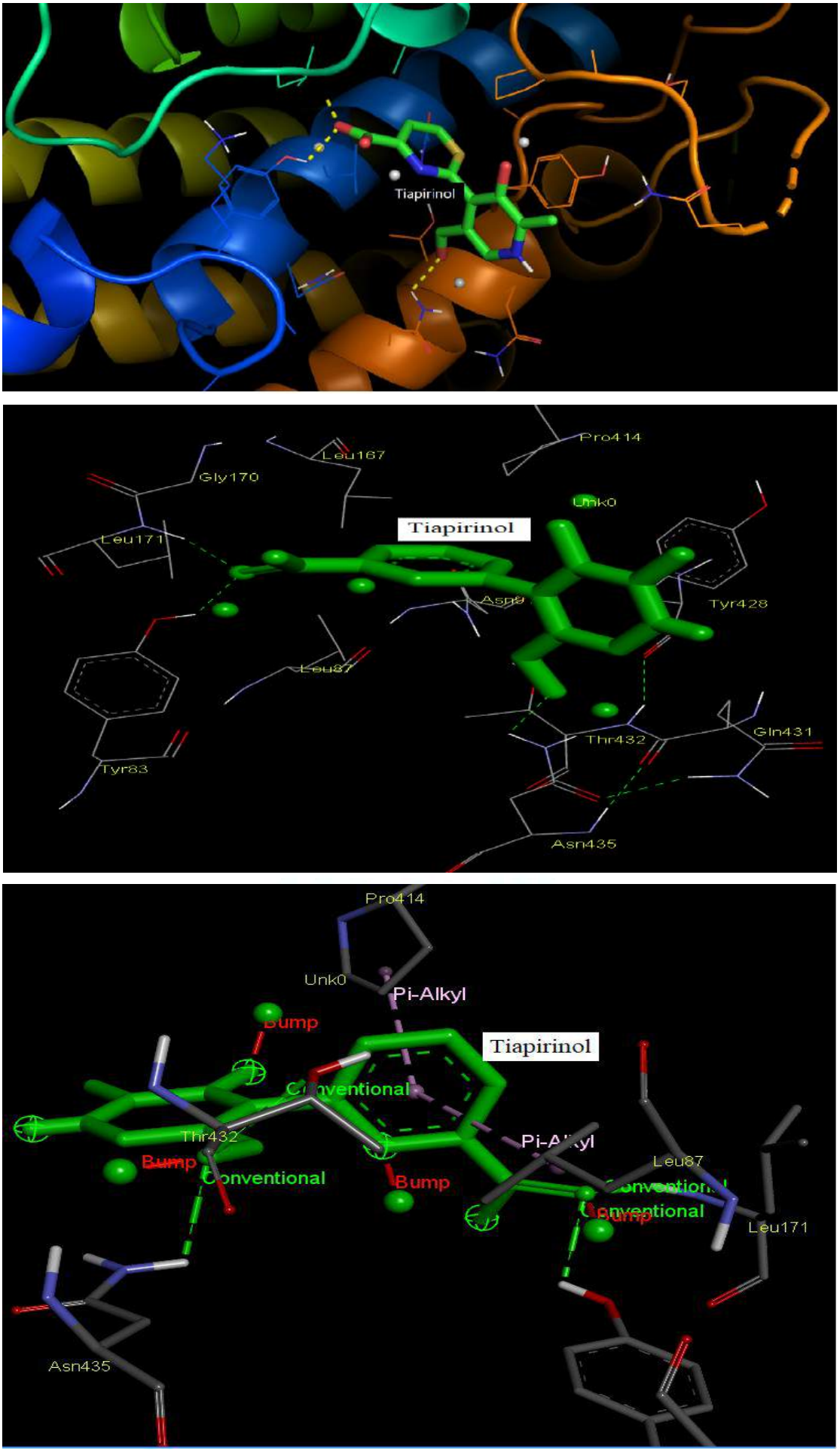
Table 3: Binding affinity of docked molecules

Molecule #	Comments	Binding affinity kcal/mol
Rivoglitazone	Drug databse	-6.9
Tiapirinol	Drug databse	-5.9
81	ZINC000003861047	-6.8
86	ZINC000005115341	-5.7
91	ZINC000005929300	-6.0
97	ZINC000002557905	-5.2
101	ZINC000002528012	-5.2

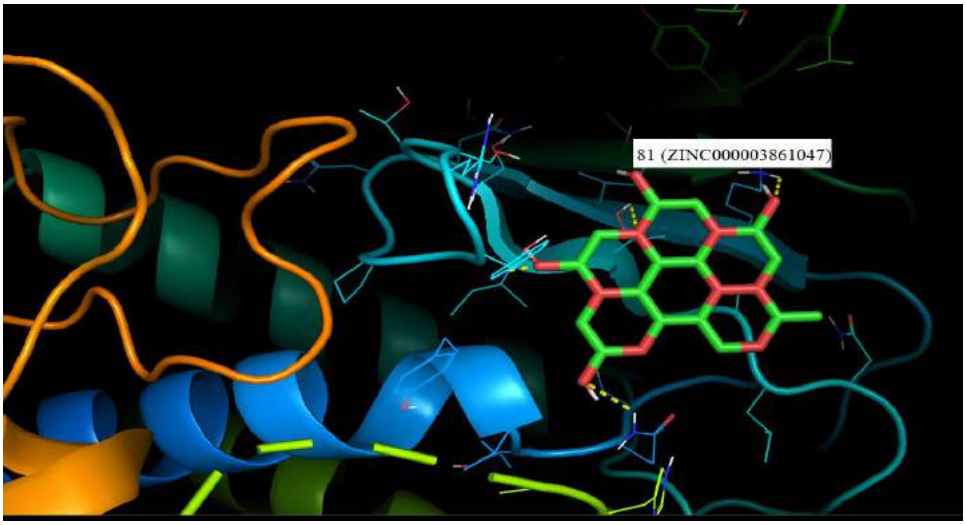
It is known that docking accuracy can be evaluated by values of RMSD with a threshold of 1.0-3.0 A^{O} between the docked ligand and X-ray pose which is generally considered to be successful [41,42,43]. However, this could depend on the resolution of the X-ray structure of the protein (26). The docking depends on a variety of interactions such as electrostatics, potential hydrogen bond donors and acceptors, hydrophobic patches, van der Waals and also effected by neighboring patches near the ligand-binding sites [29,40,44]. Different types of interactions were observed between the selected molecules and BabA protein as shown in the following figure (Figure 1)

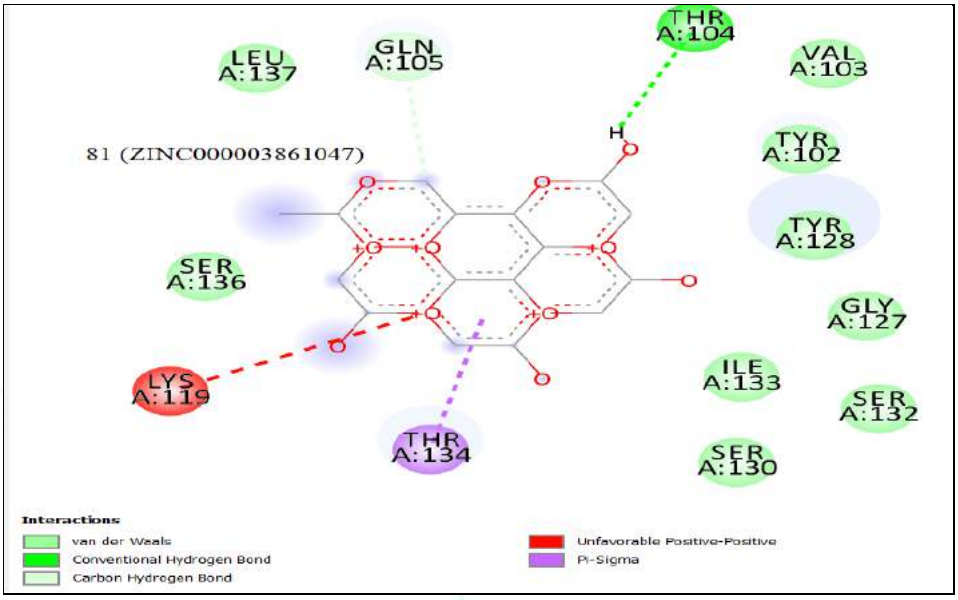


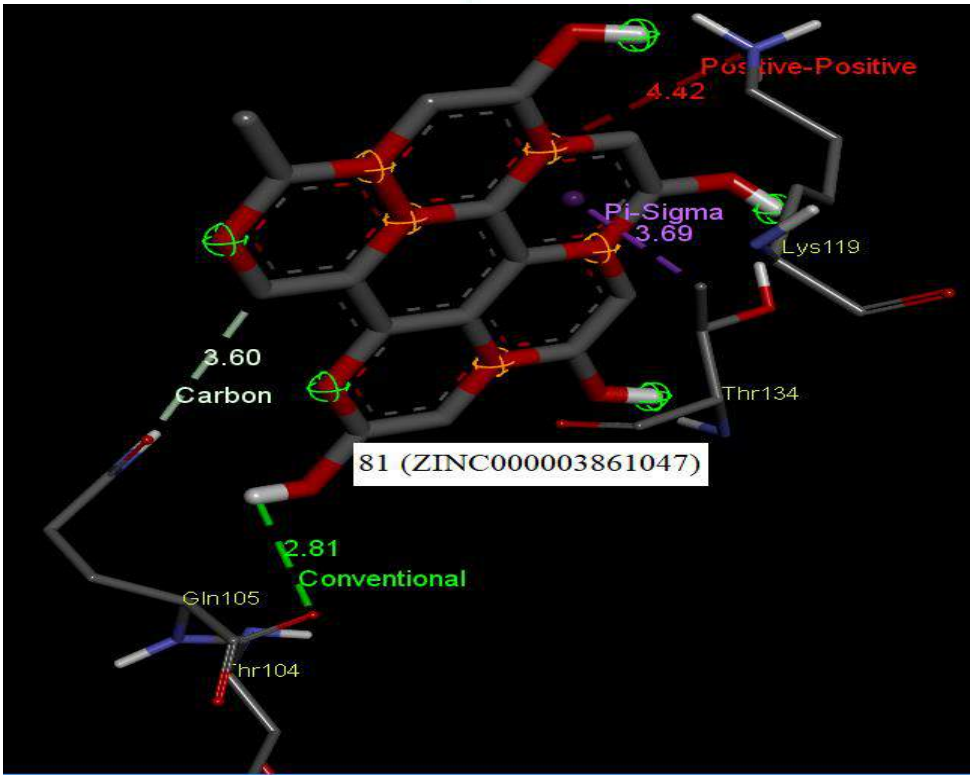
Rivoglitazone


Tiapirinol
Pro414
Leu167
Gly170
Unk0
Tiapirinol
Leu171
Asp9
Tyr428
Leu87
Thr432
Gln431
Tyr83
Asn435
Pro414
Unk0
Pi-Alkyl
Bump
Tiapirinol
Conventional
Thr432
Pi-Alkyl
Leu87
Bump
Conventional
Bump
Conventional
Bump
Leu171
Asn435

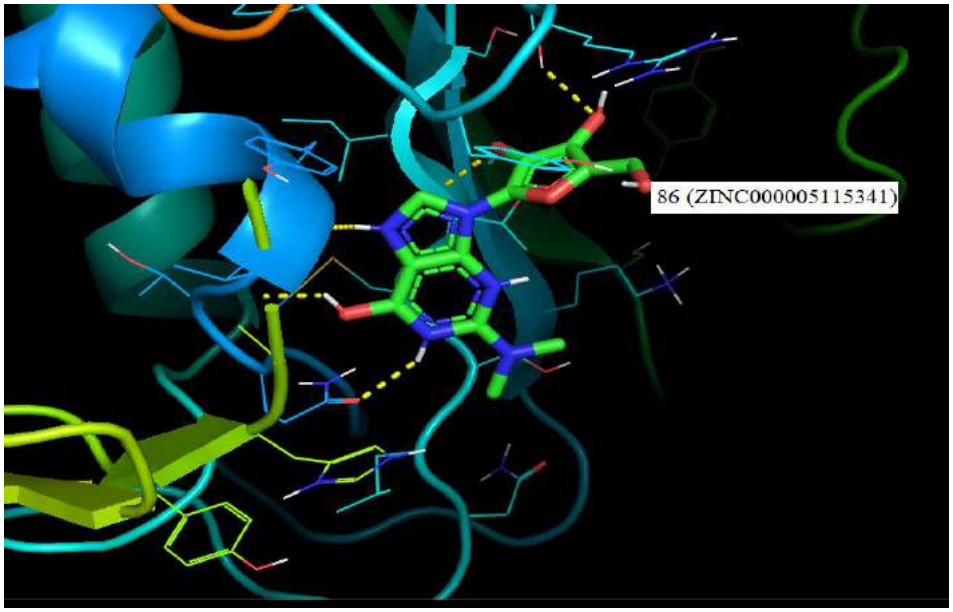
Tiapirinol

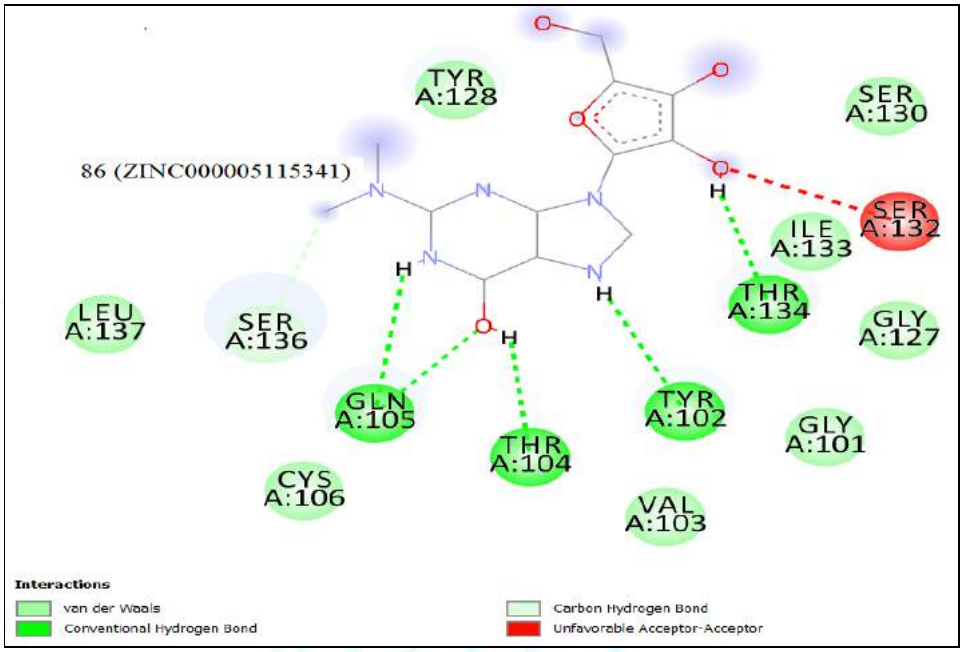


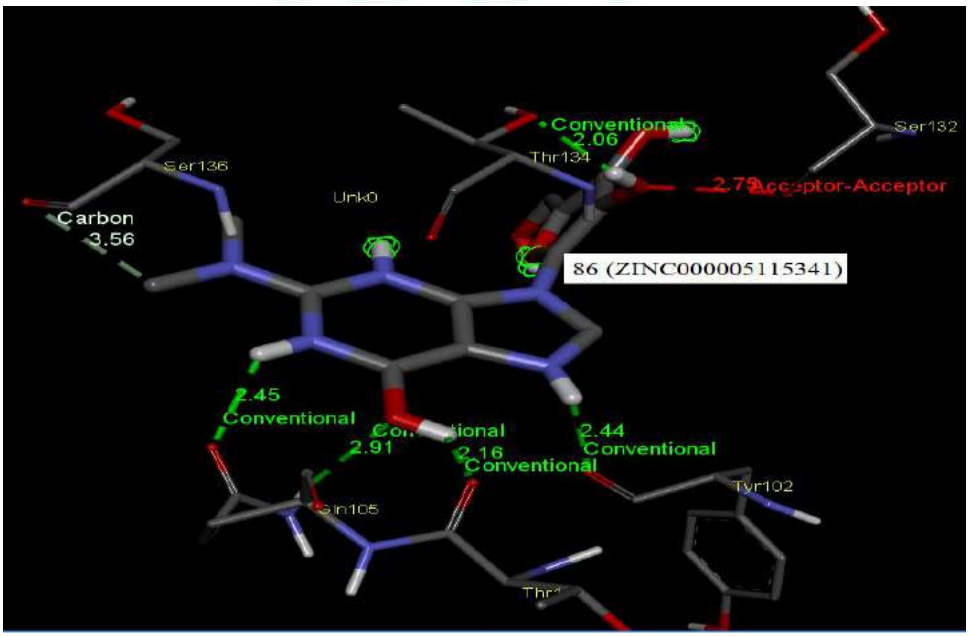




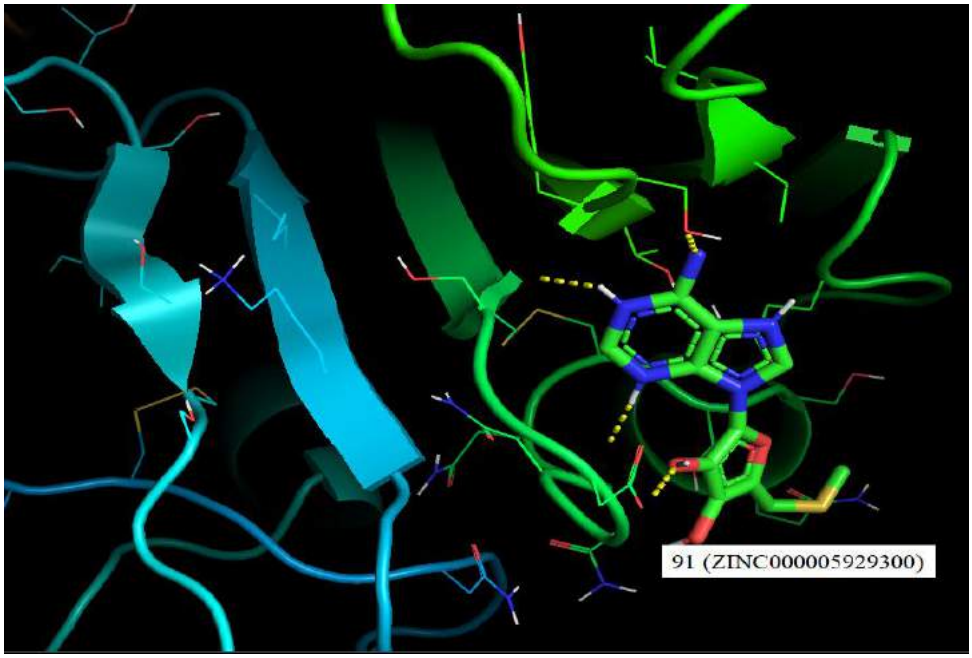
81 (ZINC000003861047)

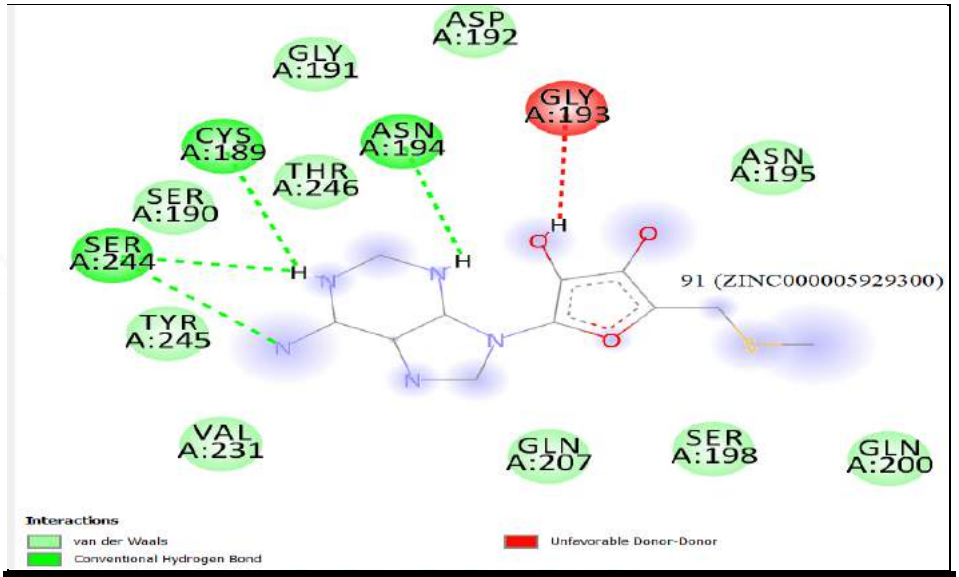


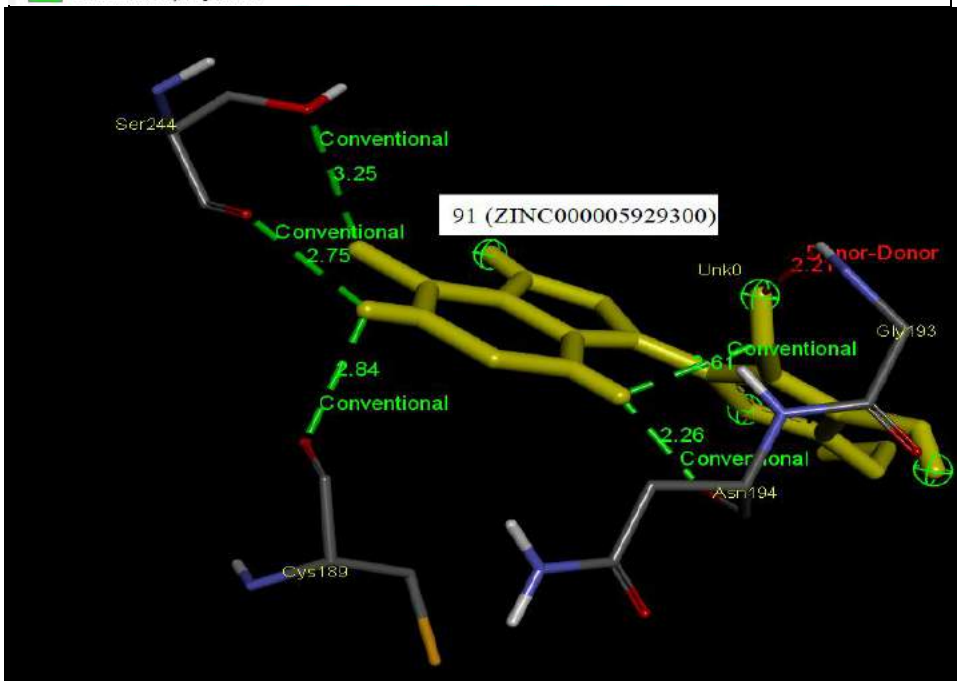




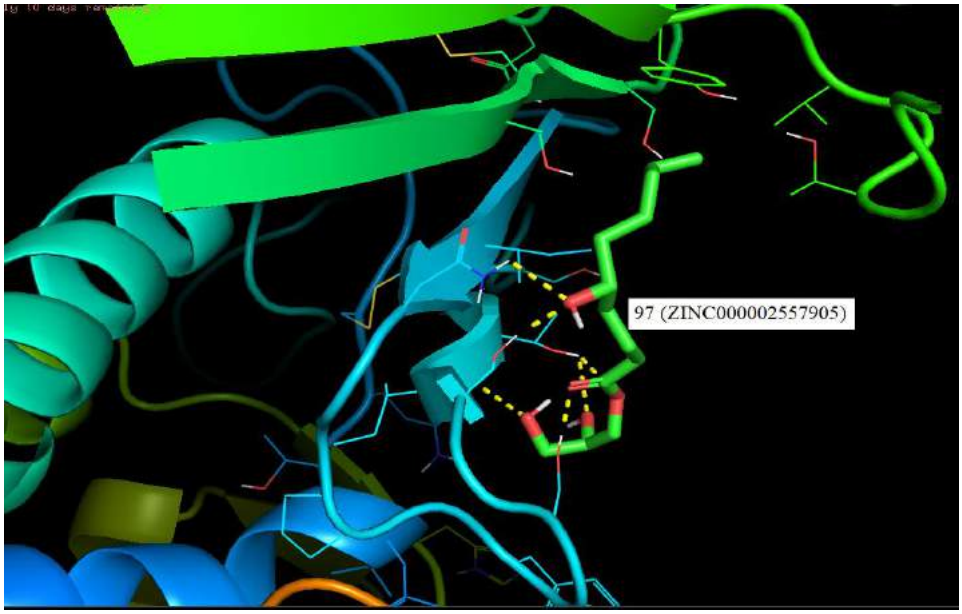
86 (ZINC000005115341)

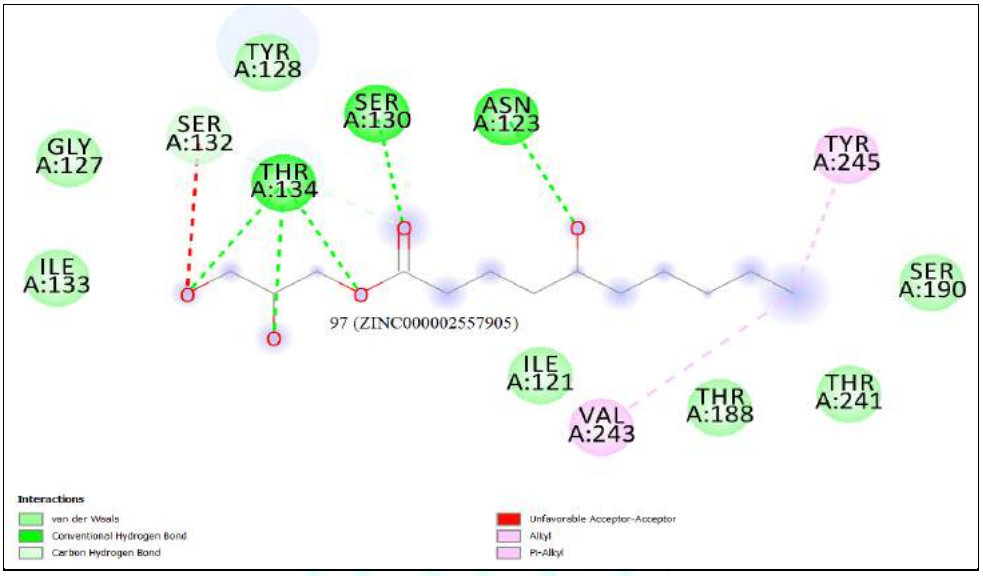


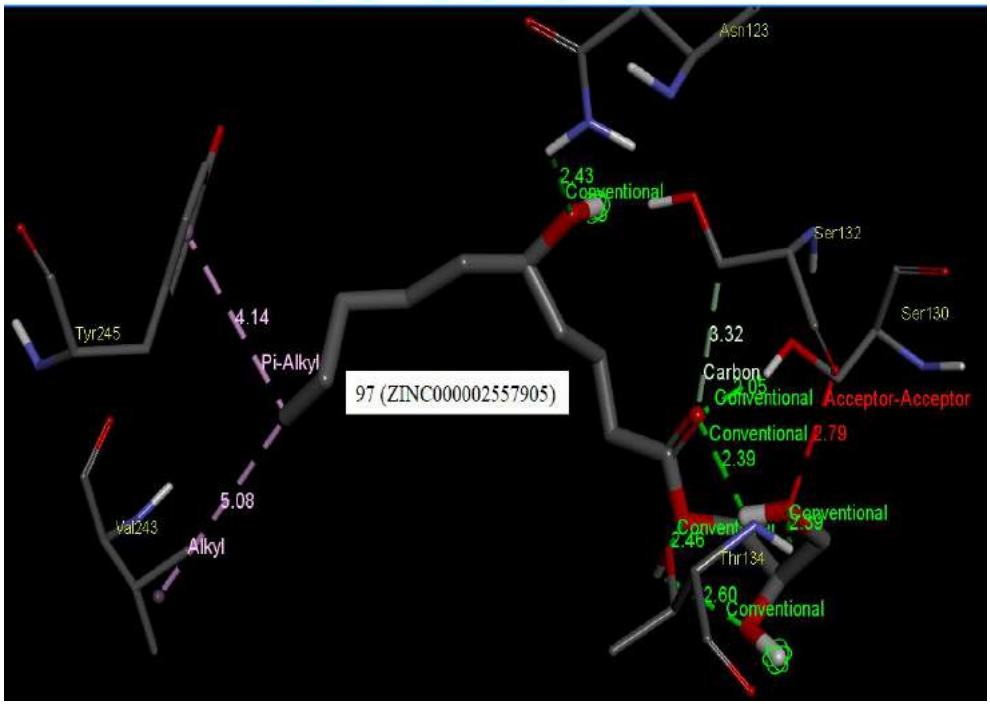




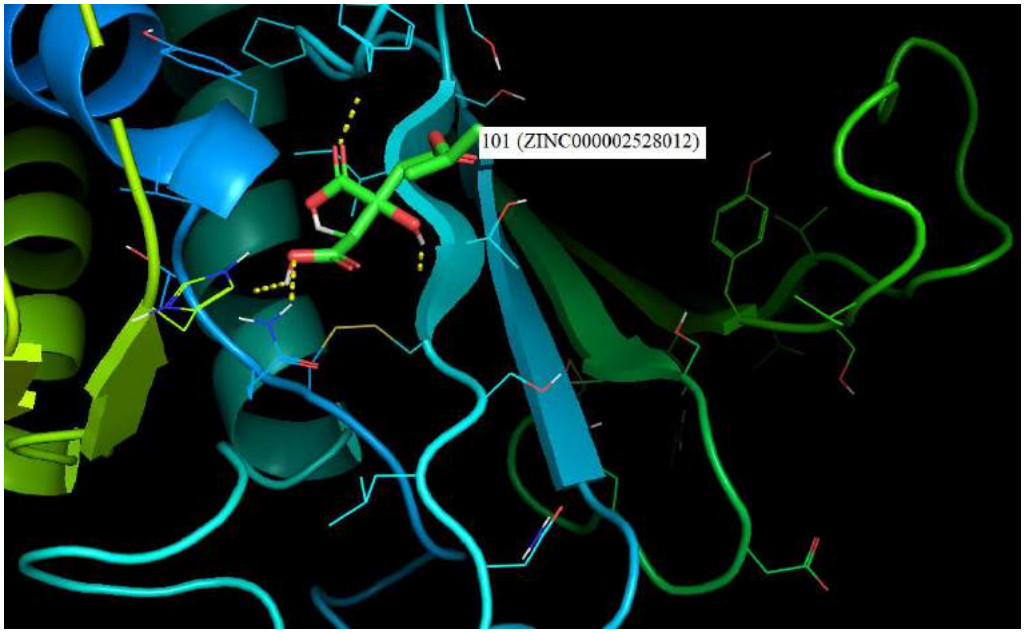
91 (ZINC000005929300)

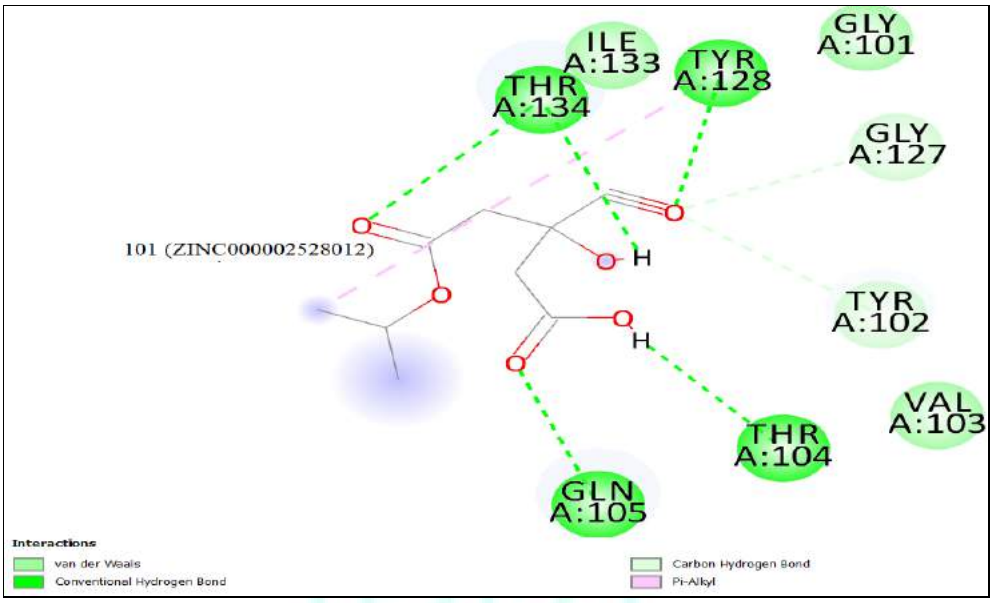


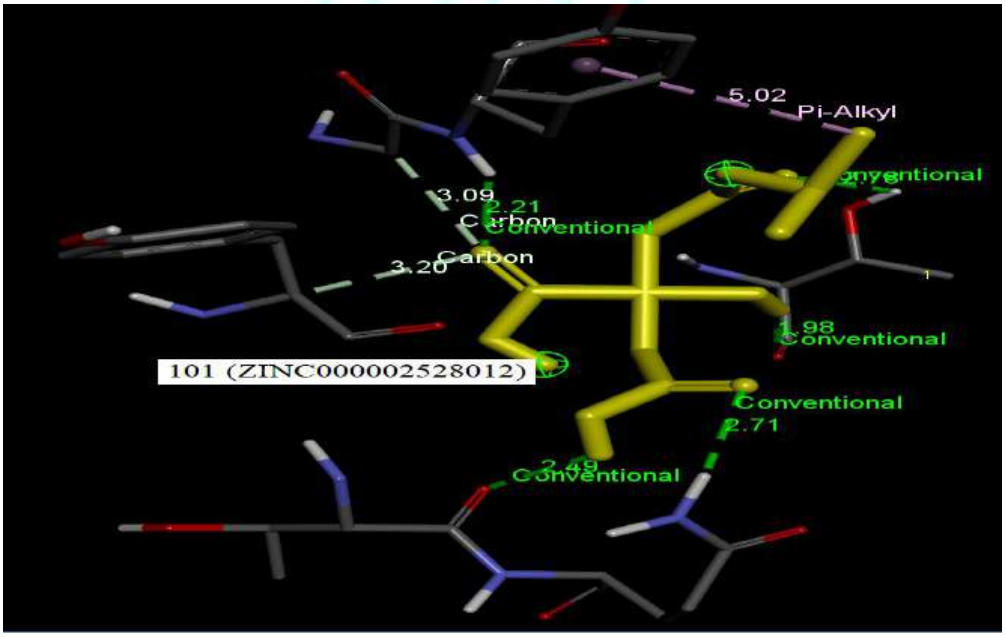




97 (ZINC000002557905)







101 (ZINC000002528012)

Figure 1: Attachments and types of molecular interaction with BabA (4zh0)

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From the results above, it can be noticed that most of the molecules have an aromatic ring(s) (5 out of 7) which is in agreement with the fact that 75% of marketed drugs contain one or more aromatic rings [45,46]. van der Waals forces are the most frequent interactions, these are formed between atoms, molecules, and ions when they are sufficiently close to each other, although they are weak but they are abundant and have additive action with no directional characteristics [47]. The docking interactions involve H-bonds for some molecules which are stronger than van der Waals and are mainly electrostatic forces between an electronegative atom and partial positive hydrogen atom, they are long-lived and strong forces. The other interactions are of π types, π-sigma considered as the strongest interaction are found in molecule 81.

The anti-adhesion molecule could be direct or indirect as found for the extract of Okra fruits, it acts by changing the binding capacity of the bacterial adhesion to Leb by interaction with surface structures in the vicinity of BabA[48] , this supported by Kaelin [49] that the loss of function in one molecule often correlated with a gain of function in another.

Generally, it has been reported of antibiotic resistance of *H. pylori* is increasing worldwide [50] which led to a low eradication rate and redistricted the application of triple therapy, this means that the novel diet-based therapeutics should be used when conventional antibiotic therapies failed and this received considerable attention [27,51].

CONCLUSION

BabA is one of the significant protein involving in many inflammatory processes in addition to its role in the attachment for persistent colonization [7], *H. pylori* eradication might be impossible on these days due to antibiotic resistance, and alternatives must be used, foods and natural products offer such alternative, especially those work early at the begging of infection processes as for the activity of BabA and other adhesins. The anti-adhesion molecules are safe and representing interesting tools for future medicinal developments since they interact with surface proteins of pathogens.

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