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Synthesis, Molecular Docking and Biological Evaluation of Schiff Base Derived from 2-Aminobenzothiazole and 2-Aminopyrimidine as Anthelmintic and Anti-Bacterial Agents

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Keywords: Schiff base,2-aminobenzothiazole, 2-aminopyimidine, antibacterial activity, anthelmintic activity.

ABSTRACT

Schiff bases are compounds containing imine or azomethine functional group that exhibits wide range of pharmacological activities. A new series of Schiff bases were synthesized by condensation of 2-Aminobenzothiazole and 2-Aminopyimidine with substituted benzaldehydes. The synthesized compounds were screened for antibacterial activity against different bacterial strains viz Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) and some were found good. The compounds were screened for anthelmintic activity against Indian earthworm (*Pheretima posthuma*). The compounds were tested for their onset of paralysis time followed by time of death of worms. The tested compounds were found not only paralyze (vermifuge) but also to kill the earthworms (vermicidal). Molecular docking studies were carried out on investigated compounds using PyRx software to understand their binding interaction towards target proteins with PDB IDs 4URM and 3VRA.

INTRODUCTION

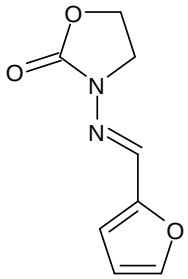
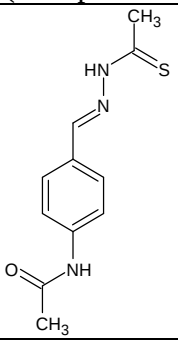
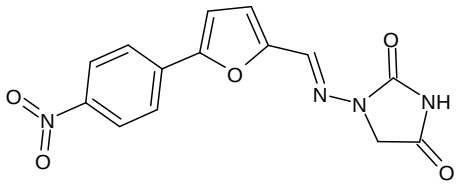
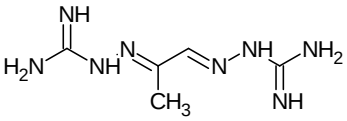
Schiff bases are the compounds carrying imine or azomethine (–C=N–) functional group and are found to be a versatile pharmacophore for design and development of various bioactive lead compounds [2]. They were first reported in 1864 by German Chemist, Hugo Schiff and hence they are named so. Schiff bases are condensation product of primary amine and active carbonyl compound (aldehydes/ketones) formed by nucleophilic addition reaction.

Schiff bases and their metal complexes are utilized as pharmaceutically and medicinally important scaffolds because of their versatile biological profile [11]. Schiff bases possess wide range of pharmacological activities like anticancer, antibacterial, antioxidant, antitumor, anti-inflammatory, antiviral, antimalarial, muscle relaxant, anthelmintic and various other biological activities [3,4,7,8,9,10]. Examples of drugs with Schiff base that are available in the market for the treatment of human ailments are given below **(Table. 1).**

In this study a series of novel Schiff bases were synthesized from 2-aminopyrimidine and 2-aminobenzothiazole with benzaldehyde, p-chlorobenzaldehyde and 2-chloro-5-nitrobenzaldehyde. Compounds were screened for their antibacterial activity using different strains of bacteria viz Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) and compared with standard Gentamicin by disc diffusion method. The compounds were also screened for anthelmintic activity against Indian earthworm (*Pheretimaposthuma*) and compared with standard Albendazole.

Molecular docking was carried out on the newly synthesized Schiff base compounds to analyse their binding interactions with the target proteins. For antibacterial screening the target protein is 4URM (Crystal Structure of Staph GyraseB 24kDa in complex with Kibdelomycin) and for anthelmintic screening the target protein is 3VRA (Mitochondrial rhodoquinol-fumarate reductase from the parasitic nematode Ascaris suum with the specific inhibitor Atpenin A5). The binding affinity and docked poses were identified and compared with standard drug.

Table 1: Marketed drugs with Schiff bases

Structure (Compound Name & Use)	Structure (Compound Name & Use)
	
Furoxone (Antibacterial activity)  Dantrolene (Muscle relaxant)	Thiacetazone(Antitubercular agent)  Mitoguazone (Anticancer agent)

1. MATERIALS AND METHODS

Experimental:

Melting point were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellet on SHIMADZU FT-IR. ^{1}H NMR and ^{13}C NMR were recorded with TMS as internal standard on a Jeol (JNM-ECZ400S) spectrometer at 400MHz. Purity of the compounds was checked by TLC on silica gel plates and spots were visualized by iodine chamber using n-Hexane: Ethyl acetate (8:2).

2-Aminobenzothiazole,2-Aminopyrimidine, ethanol, benzaldehyde, 4-Chlorobenzaldehyde, 2-Chloro-5-nitrobenzaldehyde, glacial acetic acid. The synthesis was carried out in the Department of Pharmaceutical chemistry, year 2024 in Grace college of Pharmacy, Palakkad.

All the synthesized test compounds were tested for their antimicrobial activity against various bacterial strains like gram-positive bacteria; *Bacillus subtilis* (ATCC-6633), gram-negative bacteria; *Escherichia coli* (NCIM 2809). All the strains used for bacteria were collected from Department of Biotechnology, Grace College of Pharmacy, Palakkad,

678004. All the strains used were pure cultures purified and preserved by the method of Raistrick and Hetherington as stab slant cultures at a temperature of 4c. DMSO was used as solvent.

Adult Indian earthworms, *Pheretemaposthuma* (Family: Lumbricida) were used to study the anthelmintic activity. The earth worms (*Pheretemaposthuma*) 6 cm in length and 0.1–0.2 cm in width weighing 0.8–3.04 g was used for all experiment protocols. Other materials used are normal saline, dextrose(control) etc.

Software's and databases used for molecular docking studies include; ACD/ ChemSketch (ligand preparation), Biovia discovery studio (For viewing, sharing and analysing protein and small molecule data), PyRx(To estimate the free energy of binding of ligands to receptors), RCSB PDB(Database for the three-dimensional structural data of proteins and nucleic acids).

Chemical synthesis:

Add 1g of primary amine (2-Aminobenzothiazole/2-Aminopyimidine) in 10 ml of ethanol to a beaker. Add 1.5 ml of benzaldehyde derivative in 10 ml of ethanol in another beaker. Mix both in a 50 ml RB flask. Add few drops of Glacial Acetic Acid in to it. Reflux it in a condenser for 5 hours. Add cold water to the RB flask. The product is then filtered, dried, and recrystallized from ethanol.

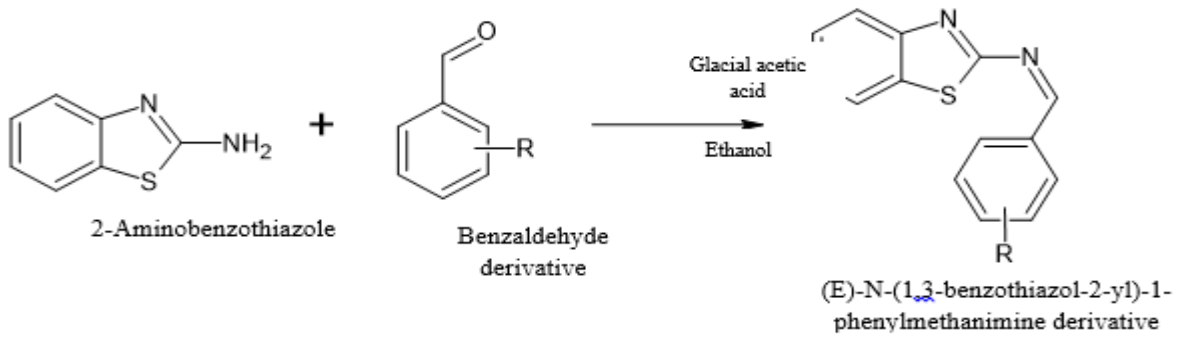


Figure 1:- Synthesis of Schiff base from 2-Aminobenzothiazole

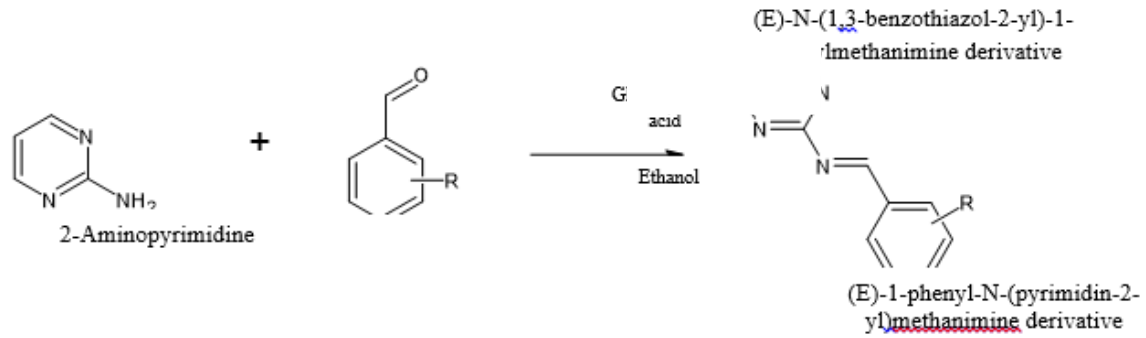


Figure 2:- Synthesis of Schiff base from 2-Aminopyrimidine

2. ANTIMICROBIAL ACTIVITY

Preparation of standard and test stock solution

Test compounds and standard drug Gentamycin were dissolved in Dimethyl sulfoxide (DMSO) to obtain a concentration of 100 and 50 /ml.

Determination of Zones of Inhibition by Disc Diffusion Technique [6]

The antimicrobial activity of all the newly synthesized compounds was determined by disc diffusion method. In this method, pure Gentamycin was taken as the standard antibiotic for the comparison of the results. Stock solutions (each of 1 mg/mL, concentration) of both compounds (**ABT_1, ABT_2, ABT_3, AMP_1, AMP_2& AMP_3**) and that of the standard antibiotic were prepared. From these stock solutions, 5 dilutions (50,100,150 µg/ml) of compounds (**ABT_1, ABT_2, ABT_3, AMP_1, AMP_2& AMP_3**) in DMSO and Gentamycin (100 µg/ml in sterile distilled water) were prepared in sterile McCartney bottles. Antibacterial activity was determined by disc diffusion assay method employing 24 h cultures of test organisms. Sterile nutrient agar plates were prepared and incubated 35-37°C for 24h to check gram-positive bacteria; *Bacillus subtilis*, gram-negative bacteria; *Escherichia coli* for contamination if any. Each sterile nutrient agar plate was then flooded with the corresponding peptone culture of the test organism, dried for 30 minutes and after drying of the flooded plate, wells were made using a cork borer (size-3) on the solidified medium. Prepared wells were filled with respective dilutions (100 µg/mL) of the test and standard compounds and marked as quadrants at the back of the plates. The same technique was repeated in the case of the remaining test organisms for both the compounds and the standard antibiotic. All the flooded

plates were incubated at 35-37°C for 24h. The diameters of the zones of inhibition were measured in mm and their means were compared accordingly.

3. ANTHELMINTIC ACTIVITY

The Indian earthworm, *Pheretema posthuma* 4-5cm length and 0.1-0.2 cm in width weighing 0.8–3.04g were used for the in vitro Anthelmintic bioassay of all newly synthesised compounds. The earthworms were washed with normal saline to remove all the fecal matter and waste surrounding their body. The worms were divided into the respective groups containing 2 earthworms in each group. All the compounds were dissolved in minimum quantity of 2%v/v Tween 80 and the volume was adjusted to 10ml with normal saline for making the concentration. All the compounds and the standard drug were freshly prepared before the commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follow, vehicle (2%v/v Tween 80 in normal saline), Albendazole and compounds. The Anthelmintic activity was observed. 2 earthworms of about same size petri dish were used. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.

Statistical Analysis

Results were expressed as mean± S.E.M. Statistical significance was determined by anthelmintic activity. one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

4. MOLECULAR DOCKING STUDIES

The 2D structures of the 6 Schiff base ligands were sketched using ChemSketch software. By using online SMILES translator, SMILE strings are converted into 3D structure and were saved in PDB format.

Crystal structures for target proteins 4URM and 3VRA were downloaded from RCSB PDB. All the proteins were docked with the 3D structures of the Schiff base ligands using PyRx

software. The protein and ligand input files were given in PDB format and grid box was set to maximum to get the best orientation with the lowest binding affinity.[8]

Molecular docking interactions and docked conformations of the proteins with Schiff base ligands were visualized using biovia discovery studio**(fig.3-5,7-9)**. The docking interactions were then compared with standard drugs **(Table.7-8) (fig.6,10)**.

5. RESULTS AND DISCUSSION

Evaluation of antimicrobial activity:

The result of antimicrobial activity indicated that all the compounds **ABT_1, ABT_2, ABT_3, AMP_1, AMP_2 and AMP_3** were found to be significant active against all bacterial strains used in this invitro bioassay at the concentration range of 100 µg/ml. The test compound **ABT_3** and **AMP_2** exhibited a significant activity against *Bacillus subtilis* (Gram-positive) and *Escherichia Coli* (Gram-negative), the prototype compounds shown a significant activity against the both gram positive and gram-negative strains but mild or less activity against the same strains at 10µg/ml concentration. In fact, these compounds exhibited equipotent activity at 100 µg/ml with that of standard Gentamycin at 10µg/ml. Therefore, it has clearly shown the title compounds 2-aminopyrimidine and 2-aminobenzothiazole derivatives incorporating Schiff base having a good antibacterial activity against the all the bacterial strains what we have been used in this research. Rather the test compounds **ABT_1** and **AMP_1** showed comparatively lesser activity than the other compounds **(Table.3-5)**.

Table 2:- List of designed compounds

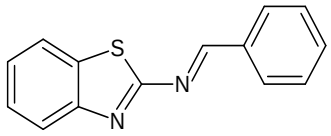
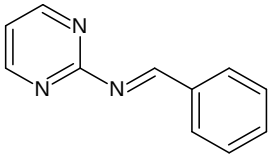
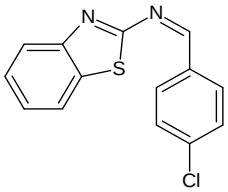
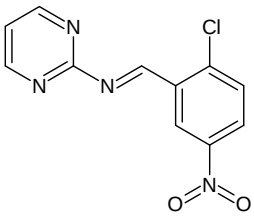
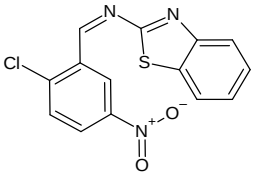
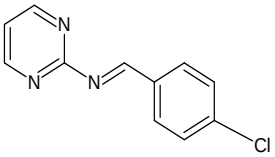
Compound Code	Structures	Compound Code	Structures
ABT_1		**AMP_1**	
ABT_2		**AMP_2**	
ABT_3		**AMP_3**	

Table 3:- Antibacterial activity of the test compound ABT_3 against bacterial strain *Bacillus subtilis*

Compound Code	Concentration (µg/ml)	Zone of inhibition
		Bacillus subtilis **(Gram-positive)**
ABT_3	25	3
	50	6
	75	10
	100	13
STANDARD (GENTAMYCIN)	25	16
CONTROL (DMSO)	0	0

Table 4:- Antibacterial activity of the test compound AMP_2 against bacterial strain *Bacillus subtilis*

Compound Code	Concentration (µg/ml)	Zone of inhibition
		Bacillus subtilis (Gram-positive)
AMP_2	25	2
	50	5
	75	9
	100	12
STANDARD (GENTAMYCIN)	25	16
CONTROL (DMSO)	0	0

Table 5:- Antibacterial activity of the test compound AMP_3 against bacterial strain *E.Coli*

Compound Code	Concentration (µg/ml)	Zone of inhibition
		E. Coli (Gram-negative)
AMP_3	25	4
	50	5
	75	7
	100	12
STANDARD (GENTAMYCIN)	25	14
CONTROL (DMSO)	0	0

Evaluation of anthelmintic activity:

Earthworm, each of average length of 6 cm, were placed in petri dishes containing 2 ml of various drug concentrations, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml of solutions. Albendazole solution was used as reference standard drug and D-glucose as a control. The worms were observed for the motility after incubating at 37˚C. This was done after pouring the petri dishes content in the wash basin and allowing the worms to move freely. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were alive showed motility and those dead were non-motile. The motile worms were returned to the respective petri dishes containing drug solutions and the incubation process was carried out again. In the control, the worms were viable for at least twelve days, which is similar to the findings reported earlier. The time taken for paralysis, motility activity of any sort and death time of worms were observed and recorded after ascertaining that the worms did not move neither when shaken vigorously nor when dipped in warm water(50˚C) **(Table.6)**.

Table 6:- Anthelmintic activity of 2-aminobenzothiazole and 2-aminopyrimidine containing Schiff base

Sl.no	Compound Code	Time taken for paralysis(minutes)	Time taken for death (minutes)
1	**ABT_2**	5 ± 0.5	26 ± 0.63
2	**ABT_3**	9 ± 0.34	40 ± 0.24
3	**AMP_2**	6 ± 0.2	15± 0.87
4	**AMP_3**	7 ±0.54	36 ± 0.72
5	**STANDARD (Albendazole)**	4 ± 0.45	51±0.59
6	**CONTROL (D-Glucose)**	0	0

6.1 MOLECULAR DOCKING RESULTS

PyRx RESULTS OF ANTIMICROBIAL ACTIVITY:

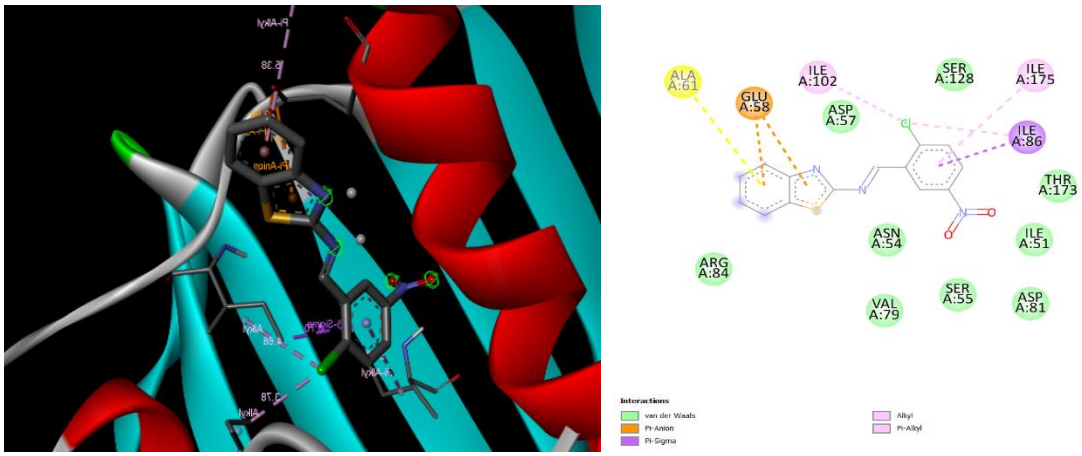


Figure3:- Docking of ABT$_3$ with 2D representation against 4URM

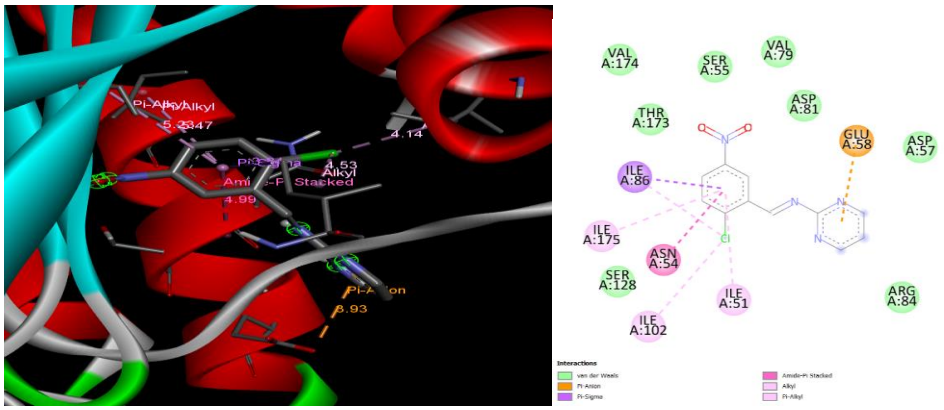


Figure 4:- Docking of AMP$_2$ with 2D representation against 4URM

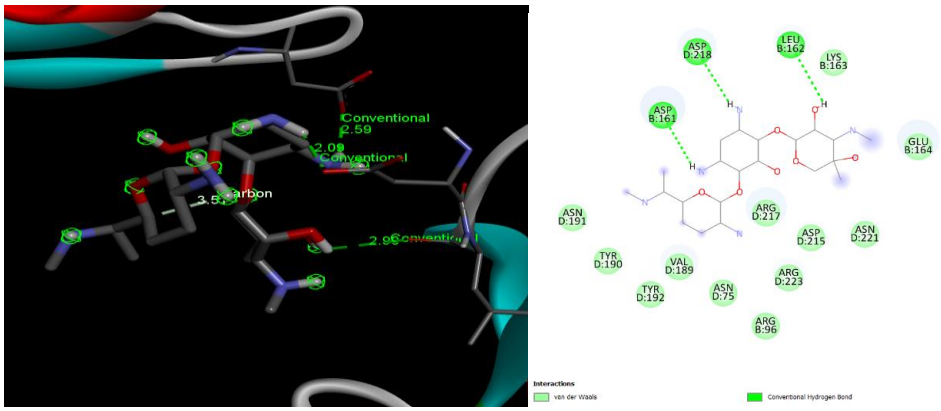


Figure 5:- Docking of Gentamycin with 2D representation against 4URM

Table 7:- Docking results of designed derivatives towards PDB ID: 4URM by using PyRx software.

Sl.no	Compound Code	Binding energy(kcal/mol)
1	ABT_1	-6.1
2	ABT_2	-6.8
3	ABT_3	-7.7
4	AMP_1	-6.9
5	AMP_2	-7.6
6	AMP_3	-7.1
7	GENTAMYCIN	-6.6

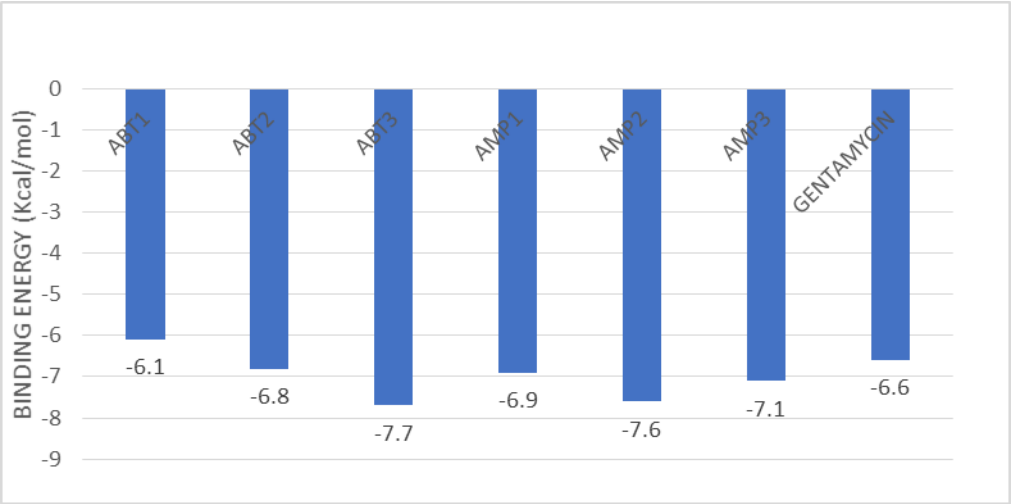


Figure 6:- Histogram of Antimicrobial activity of the compounds based on docking.

The PyRx results between the interaction of 4URM inhibitor with the designed compounds have given an account of -7.7 Kcal/mol energy for **ABT_3**.

PyRx RESULTS OF ANTHELMINTIC ACTIVITY:

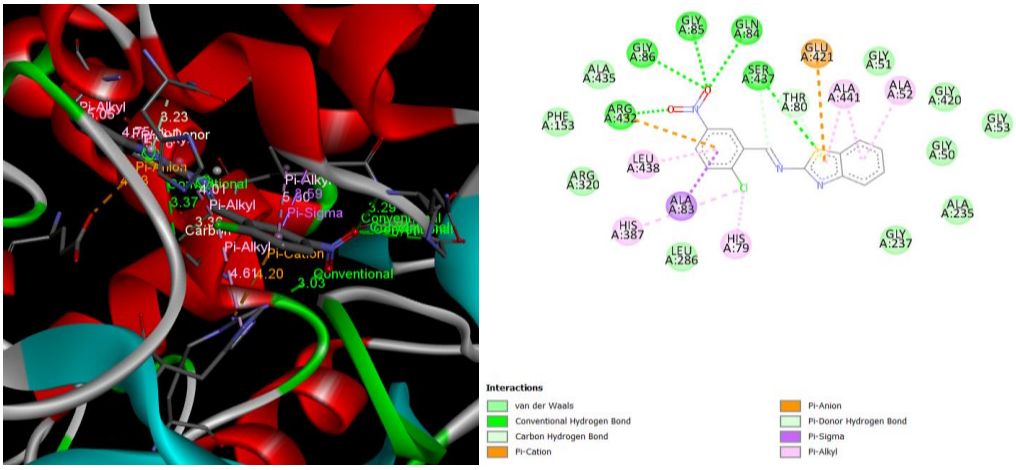


Figure 7:- Docking of ABT$_3$ with 2D representation against 3VRA.

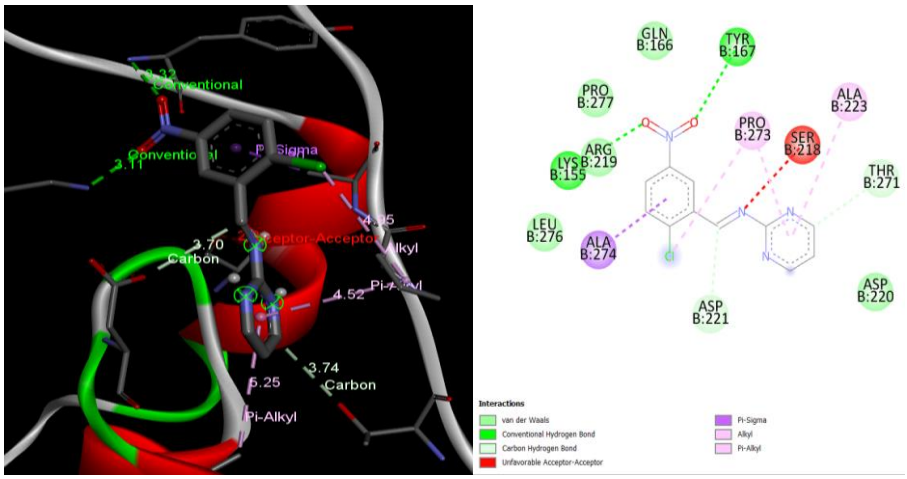


Figure 8:- Docking of AMP$_2$with 2D representation against 3VRA

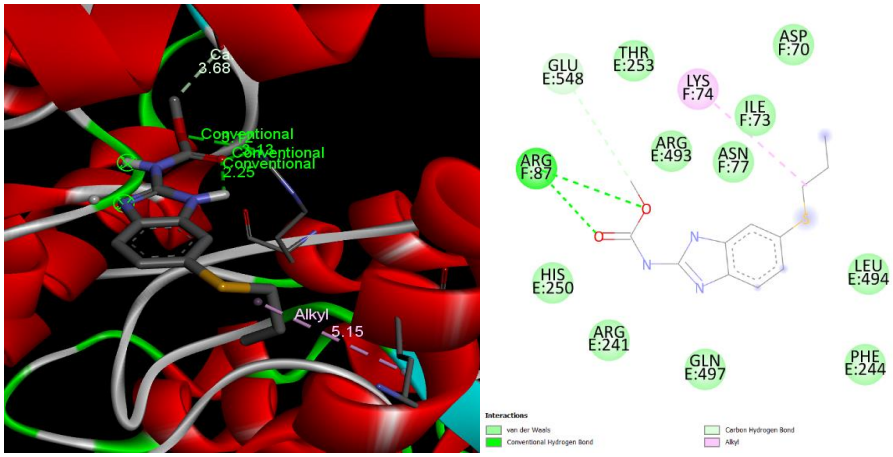


Figure 9:- Docking of Albendazole with 2D representation against 3VRA

Table 8:- Docking results of designed derivatives towards PDB ID: 3VRA by using PyRx software.

Sl.no	Compound Code	Binding energy(kcal/mol)
1	ABT_1	-6.4
2	ABT_2	-7.5
3	ABT_3	-10.1
4	AMP_1	-6.6
5	AMP_2	-9.1
6	AMP_3	-6.9
7	ALBENDAZOLE	-6.2

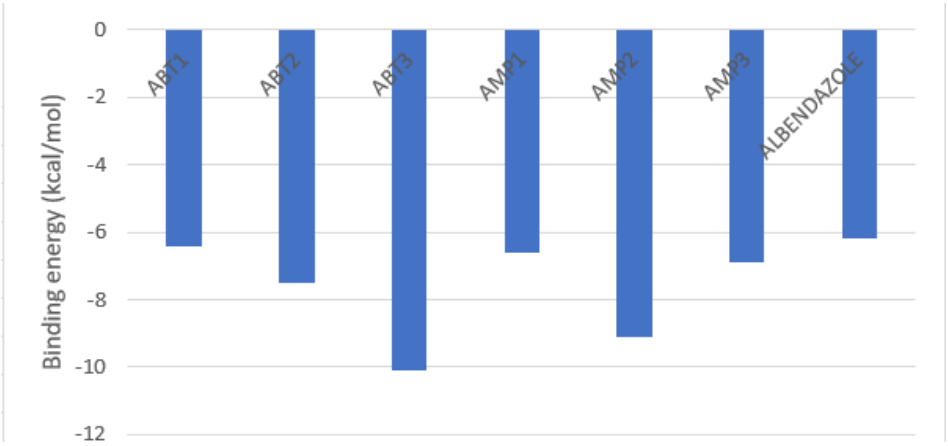


Figure 10:- Histogram of anthelmintic activity of the compounds based on docking.

The PyRx results between the interaction of 3VRA inhibitor with the designed compounds have given an account of 10.1 Kcal/mol energy for **ABT_3**.

6. CONCLUSION

In this study a series of novel Schiff bases from 2-Aminobenzothiazole and 2-Aminopyimidine with substituted benzaldehydes **(ABT_1, ABT_2, ABT_3, AMP_1, AMP_2 andAMP_3)** were synthesized **(Table.2)**. Their chemical structures were confirmed using melting point, TLC, and spectral studies including UV, FTIR, ^{1}H NMR, ^{13}CNMR).The compounds were screened for antibacterial activity against different bacterial strains viz Gram-positive bacteria (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*).All the compounds **ABT_1, ABT_2, ABT_3, AMP_1, AMP_2 and AMP_3** were found to be significant active against all bacterial strains used in this invitro bioassay.The compounds were screened for anthelmintic activity against Indian earthworm (*Pheretima posthuma*). The compounds were tested for their onset of paralysis time followed by time of death of worms.

The tested compounds were found not only paralyze (vermifuge) but also to kill the earthworms (vermicidal). All the compounds possess significant anthelmintic activity. Molecular docking studies indicates significant binding interactions for all the compounds with the target proteins for antimicrobial and anthelmintic activity.

7. ACKNOWLEDGEMENT

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