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Research Article

DESIGN, SYNTHESIS, MOLECULAR DOCKING AND BIOLOGICAL EVALUATION OF NITROGEN CONTAINING ARYL SCAFFOLDS AS ANTHELMINTIC AGENTS

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ABSTRACT
Compounds that possess an imine or azomethine functional group that exhibit a range of pharmacological actions are referred to as Schiff bases. By condensing 2-Aminobenzothiazole, 2-Aminopyimidine, Indole-3-carboxaldehyde and Aniline with substituted benzaldehydes, a novel sequence of Schiff bases was produced. Some of the synthetically produced compounds demonstrated good anthelmintic action when tested against various Indian earthworm (*Pheretima posthuma*). The compounds were investigated regarding whether the worms collapsed and when they would become immobilized. It was discovered that the tested compounds were both vermicide and vermifuge. Molecular docking investigations were carried out on the drugs under research to see how they might interact with the target protein 3VRA using PyRx Software and Discovery studio for visualization.

Keywords: Schiff base, 2-aminobenzothiazole, 2-aminopyimidine, Indole-3-carboxaldehyde, Aniline, Anthelmintic activity.

1. INTRODUCTION

Schiff bases provide a flexible pharmacophore allowing the production of multiple bioactive lead compounds [1]. They obtained the title as German chemist Hugo Schiff reported their existence for the first time in 1864. Schiff bases are the condensation outcome of an aldehyde or ketone which is an active carbonyl molecule generated through a nucleophilic addition reaction involving a primary amine. In addition to their broad biological outline, Schiff bases and their metal complexes are employed as major scaffolds in the pharmaceutical and medicinal industries [2]. Extensive biological actions including anthelmintic, anticancer, antibacterial, antioxidant and antiviral properties are possessed by Schiff bases [3,4,5,6,7].

In this research, 2-aminopyrimidine, 2-aminobenzothiazole, indole-3-carboxaldehyde and aniline were combined with various benzaldehyde to produce an array of novel Schiff bases. Using *Pheretima posthuma* or Indian earthworms, the compounds were evaluated for their anthelmintic activity.

Chemical synthesis: Take a beaker, add 10 ml of ethanol and 1g of primary amine (2-Aminobenzothiazole/ 2-Aminopyimidine/ indole-3-Carboxaldehyde/Aniline). In another beaker, mix 1.5 ml of the benzaldehyde derivative with 10 ml of ethanol. In a 50 ml RB flask, blend both. Stir

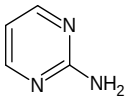
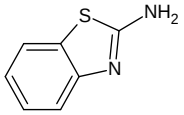
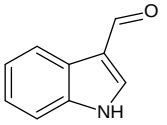
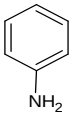
The results were compared with the standard drug albendazole. The newly synthesized Schiff base compounds were subjected to molecular docking for the purpose to investigate their binding interactions with the target protein. The target protein for anthelmintic screening is 3VRA (Mitochondrial rhodoquinol-fumarate reductase from the parasitic nematode Ascaris suum with the specific inhibitor Atpenin A5). The docked poses and binding affinity have been assessed and compared with the standard drug Albendazole.

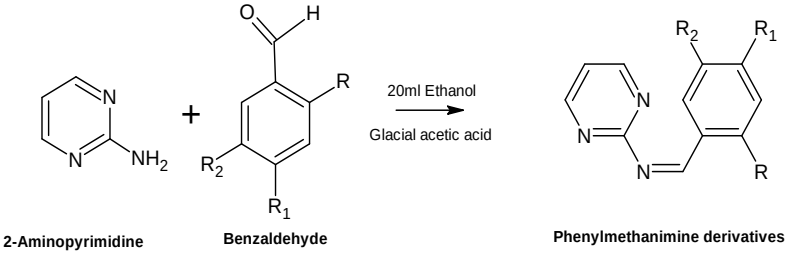
2. EXPERIMENTAL

a) Materials and Methods

2-aminopyrimidine, 2-aminobenzothiazole, indole-3-Carboxaldehyde and aniline were acquired from Sigma -Aldrich USA. Melting points of all the generated were estimated by open capillary tube methods and values were uncorrected. The 2-aminopyrimidine derivatives [AB_1-AB_3] (Fig.1), 2-aminobenzothiazole derivatives [BT_1-BT_3] (Fig.2), Indole-3-carboxaldehyde derivatives [IC_1-IC_3] (Fig.3) and Aniline derivatives [AN_1-AN_3] (Fig.4), are synthesized.

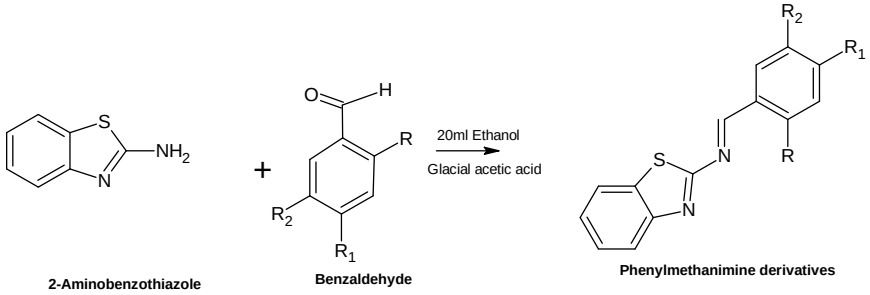
in just a little of glacial acetic acid. Reflux it for five hours in a condenser. Pour cold water into the flask of RB. Upon ethanol extraction, the product is dried, filtered, and recrystallized.

			
2-Aminopyrimidine	2-Aminobenzothiazole	Indole-3-carboxaldehyde	Aniline



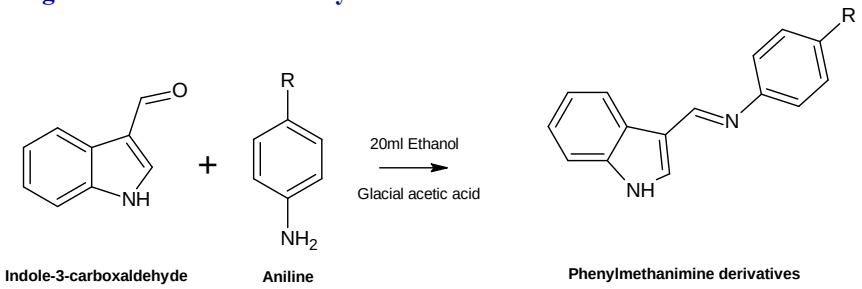
Compound Code	R	R_1	R_2
AB_1	H	Cl	H
AB_2	Cl	H	NO_2
AB_3	H	NO_2	H

Figure 1: - Scheme for the Synthesis of 2-aminopyrimidine derivatives



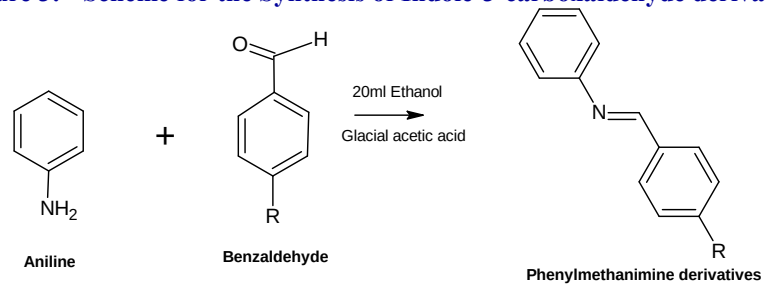
Compound Code	R	R_1	R_2
BT_1	H	Cl	H
BT_2	Cl	H	NO_2
BT_3	H	NO_2	H

Figure 2: - Scheme for the Synthesis of 2-aminobenzothiazole derivatives



Compound Code	R
IC_1	Cl
IC_2	NO_2
IC_3	H

Figure 3: - Scheme for the Synthesis of Indole-3-carboxaldehyde derivatives



Compound Code	R
AN_1	Cl
AN_2	NO_2
AN_3	H

Figure 4: - Scheme for the Synthesis of Aniline derivatives

Melting point evaluations were conducted in uncorrected open capillary tubes; IR spectra were captured in KBr pellet on SHIMADZU FT-IR; 1H NMR and 13C NMR were reported on a Jeol (JNM-ECZ400S) spectrometer at 400MHz using TMS as internal standard; TLC was employed to confirm the compounds' purity on silica gel plates; spots were displayed using an iodine chamber using n-Hexane: Ethyl acetate (8:2).

3. MOLECULAR DOCKING

In this study, PyRx Software was used to perform a ligand-based computer modeling program for forecasting binding affinity of the selected compound [8]. The structures of all synthesized test compounds are produced using Chemsketch software (http://www.acdlabs.com/resources/freeware/). Chem3D pro 8.0 was employed to optimize the structures and to reduce the energy. Molecular docking was performed out using the optimized compounds. The synthesis was carried out in the Department of Pharmaceutical chemistry, 2024 in Grace college of Pharmacy, Palakkad.

a) Preparation of grid and docking parameters

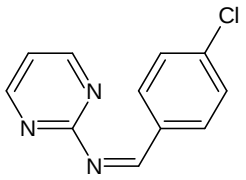
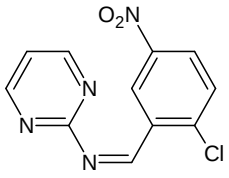
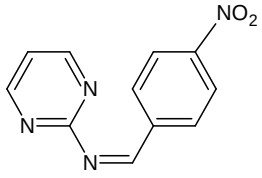
The 3D structure of the molecular target was taken from Protein Data Bank (PDB) (www.rcsb.org). Loading the molecules in to PyRx workspace. Converting the pdb file to pdbqt files. Select the protein and ligand by simply clicking and run vina. Select vina search space. Enclose the labels within grid box. The active sites were selected using grid boxes around the bound cocrystal ligands, which was like this: number of grid points (60×60×60), center (xyz coordinates) and the grid point spacing was 0.375 Å. In order to correlate the test compounds for their respective activity and to examine their *in-silico* interaction, they were docked into the active site. Click forward button in order to start vina calculations. Once the calculations are done, results will be populated by giving binding affinity (Kcal/mol) values. Docking study was performed using PyRx Software and Discovery Studio is employed for the visualization.

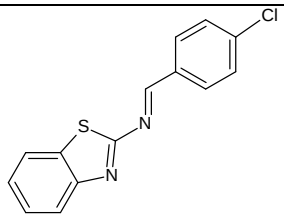
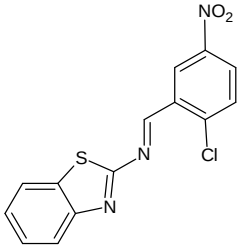
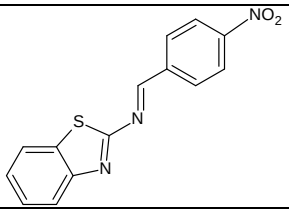
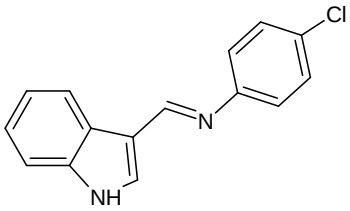
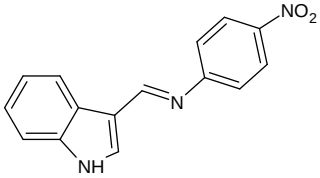
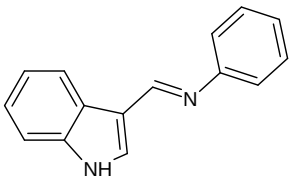
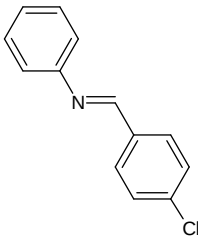
4. ANTHELMINTIC ACTIVITY

Pheretema Posthuma, the Indian adult earthworm was used to determine the anthelmintic activity due to its anatomical resemblance with the intestinal roundworm parasites of human beings. All recently synthesized compounds (AB_1-AB_3, BT_1-BT_3, IC_1-IC_3, AN_1-AN_3) were subjected to an *in vitro* anthelmintic bioassay, with dimensions of 4-5 cm in length and 0.1-0.2 cm in width, weighing 0.8-3.04g earth worms. To get rid of all the trash and feces around their bodies, the earthworms were washed with normal saline solution. Two earthworms per group were formed when the worms were split into their separate groups. To make the concentration, all of the compounds were dissolved in a minimum of 2% v/v Tween 80, and the volume was adjusted to 10 ml using regular saline. Before the trials started, every compound and the reference medication were made from the beginning. In advance of being discharged into 10 ml of the appropriate formulation including the following ingredients: vehicle (2% v/v Tween 80 in normal saline), compounds, and albendazole, all earthworms were cleaned in normal saline solution. There is evidence of anthelmintic action. Two petri dishes with almost equal sizes of earthworms were employed. Their evoked responses and spontaneous movement were detected. The amount of time it took for each worm to become paralyzed, and death was noted. When the worms fail to resurrect in regular saline, it was claimed that paralysis had occurred. The worms' loss of motility and subsequent loss of body color signaled the end of their existence.

5. RESULTS AND DISCUSSION

a) List of Synthesized Drugs

SI No.	COMPOUND CODE	STRUCTURE	NAME
1.	AB_1		(*Z*)-1-(4-chlorophenyl)-*N*-(pyrimidin-2-yl)methanimine
2.	AB_2		(*Z*)-1-(2-chloro-5-nitrophenyl)-*N*-(pyrimidin-2-yl)methanimine
3.	AB_3		(*Z*)-1-(4-nitrophenyl)-*N*-(pyrimidin-2-yl)methanimine

4.	BT_1		(*E*)-*N*-(1,3-benzothiazol-2-yl)-1-(4-chlorophenyl)methanimine
5.	BT_2		(*E*)-*N*-(1,3-benzothiazol-2-yl)-1-(2-chloro-5-nitrophenyl)methanimine
6.	BT_3		(*E*)-*N*-(1,3-benzothiazol-2-yl)-1-(4-nitrophenyl)methanimine
7.	IC_1		(*E*)-*N*-(4-chlorophenyl)-1-(1*H*-indol-3-yl)methanimine
8.	IC_2		(*E*)-1-(1*H*-indol-3-yl)-*N*-(4-nitrophenyl)methanimine
9.	IC_3		(*E*)-1-(1*H*-indol-3-yl)-*N*-phenyl methanimine
10.	AN_1		(*E*)-1-(4-chlorophenyl) -*N*-phenylmethanimine

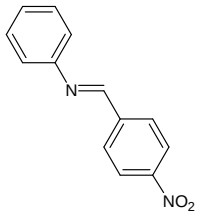
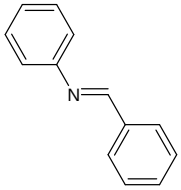
11.	AN_2		(*E*)-1-(4-nitrophenyl) -*N*-phenylmethanimine
12.	AN_3		(*E*)-*N*,1- diphenylmethanimine

Table 1: Synthesized Derivatives of Test compounds

b) Evaluation of Anthelmintic Action

Earthworms with an average length of 6 cm were placed in petri dishes with 2 ml of various drug concentrations: 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml. D-glucose served as the control and albendazole solution as the reference standard drug. The worms were incubated at 37°C before their motility was assessed. After transferring the contents of the petri dishes into the sink and letting the worms roam around freely, this was done. The living worms displayed motility when the end of each worm was tapped with the index finger and slightly compressed, however the dead worms did not exhibit any movement. The process of incubation was repeated when the motile worms were placed back into their corresponding petri dishes that contained drug solutions. The worms in the control group remained alive for a minimum of twelve days, which is consistent with previous research findings. After confirming that the worms failed to move when shaken violently or when submerged in warm water (50°C), the duration of paralysis, the amount of motility activity, and the time until death were examined and recorded.

SI NO	Compound Code	Time taken for paralysis (minutes)	Time taken for death (minutes)
1	BT_2	7 ± 0.5	26 ± 0.63
2	BT_3	9 ± 0.34	40 ± 0.24
3	AB_2	6 ± 0.2	15± 0.87
4	AB_3	8 ±0.54	36 ± 0.72
5	STANDARD (Albendazole)	4 ± 0.45	51±0.59
6	CONTROL (D-Glucose)	0	0

Table 2: Anthelmintic activity of test compounds

Figure 1: Anthelmintic activity results of AB_2

Figure 2: Anthelmintic activity results of AB_3

Figure 3: Anthelmintic activity results of BT_2

Figure 4: Anthelmintic activity results of BT_3

Figure 5: Anthelmintic activity results of Albendazole

Figure 6: Anthelmintic activity results of Dextrose

The earth worm (*Pheretema Posthuma*) died most frequently in compound AB_2 and survived more time in compound BT_3. It indicates that Compound AB_2 have more Anthelmintic activity while comparing with all other test compounds.

c) Molecular Docking Results

SI NO.	COMPOUND CODE	BINDING ENERGY (kcal/mol)
1.	AB_1	-6.6
2.	AB_2	**-9.1**
3.	AB_3	-6.9
4.	BT_1	-6.4
5.	BT_2	-7.5
6.	BT_3	**-10.1**

7.	IC_1	-6.1
8.	IC_2	-6.7
9.	IC_3	-7.2
10.	AN_1	-6.0
11.	AN_2	-5.8
12.	AN_3	-6.7
13.	Albendazole	-6.2

Table 3: Docking Results of test compounds against anthelmintic protein (PDB ID: 3VRA)

The results obtained from these experiments shows the strong interactions of Potential drug molecules towards the PDB ID: 3VRA.

Figure 7: The best and stable conformation of the synthesized compounds along with the standard drug against the protein (PDB ID: 3VRA)

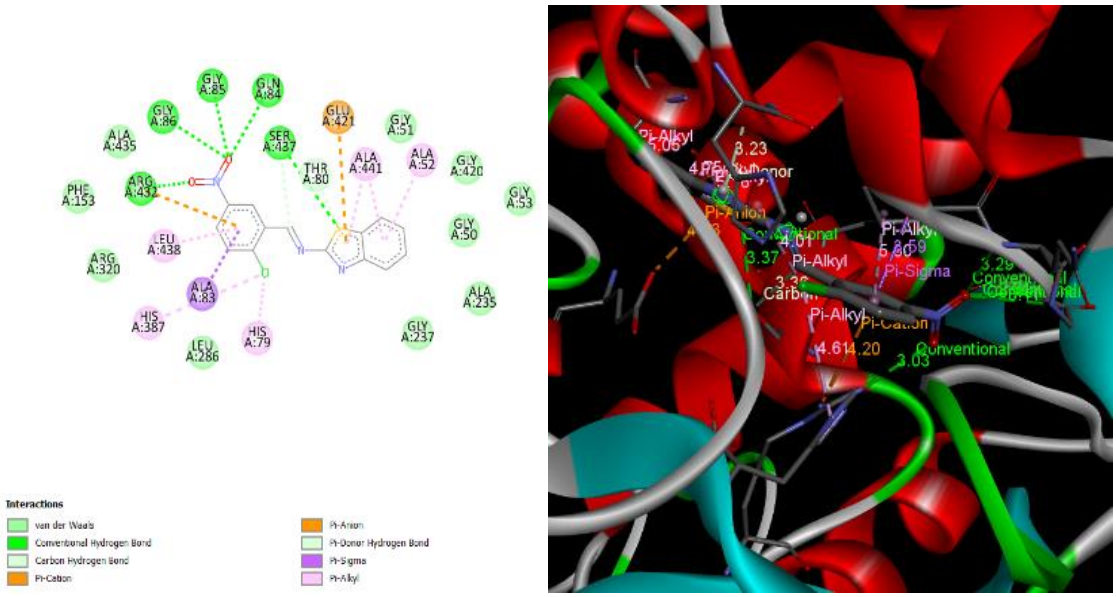


Figure 7a: Docking of BT3 with 2D representation against 3VRA

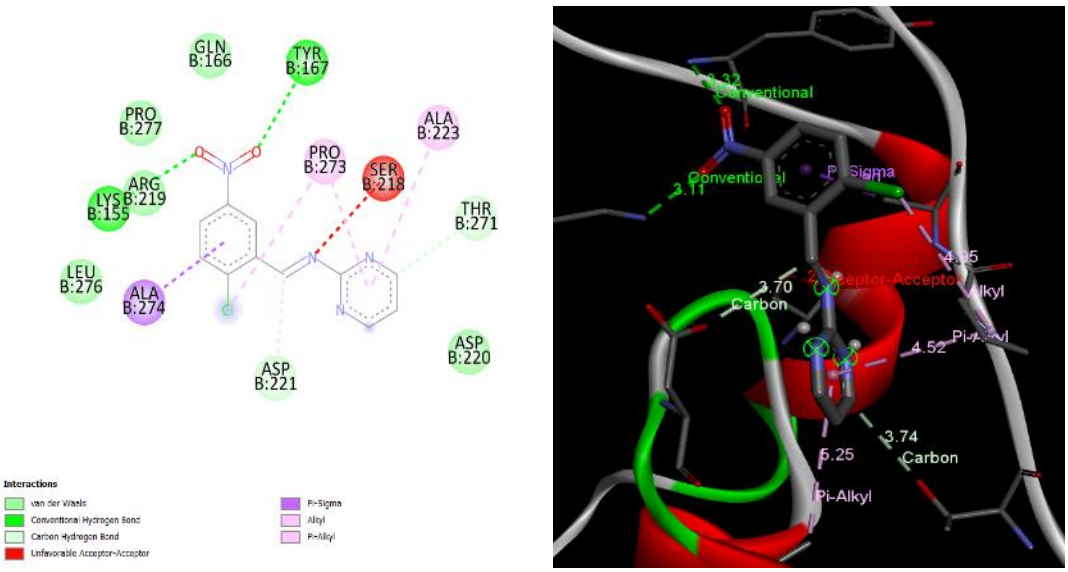


Figure 7b: Docking of AB2 with 2D representation against 3VRA

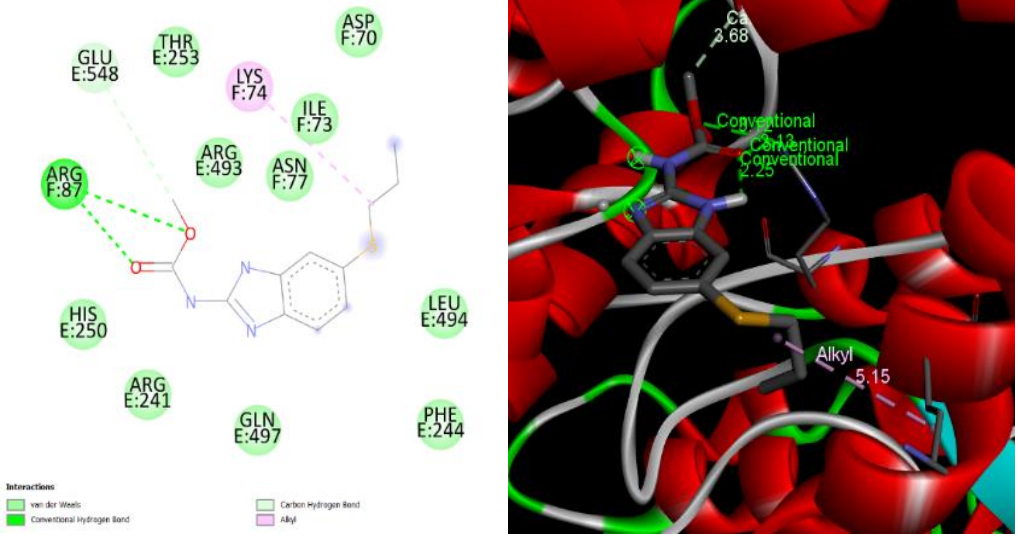


Figure 7c: Docking of Albendazole with 2D representation against 3VRA

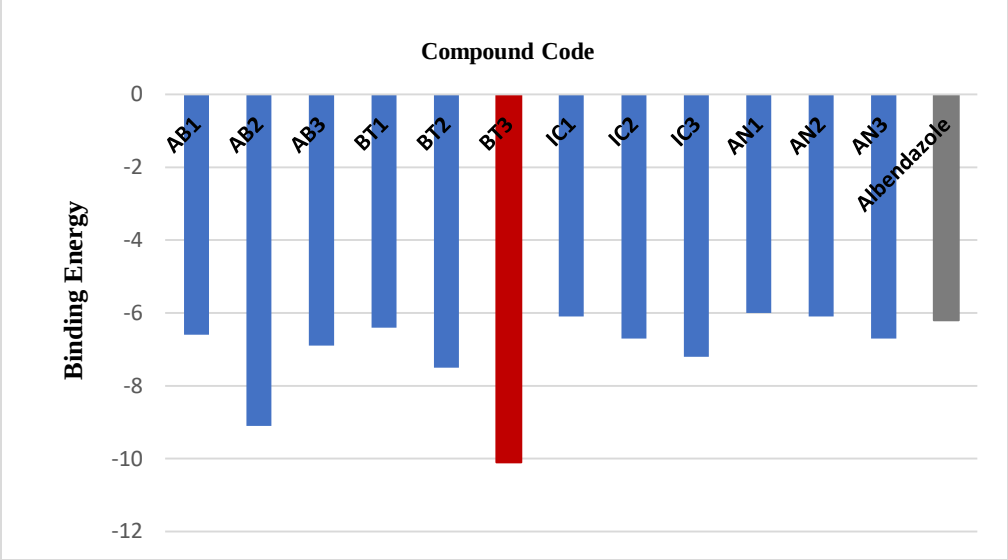


Figure 8: Histogram of Anthelmintic activity of the test compounds based on docking.

Docking of all the ligands towards the target protein has done effectively. From this synthesized nitrogen containing aryl derivatives (AB, BT, IC, AN) in Table 3. For this investigation 12 compounds (AB1-AB3, BT1-BT3, IC1-IC3, AN1-AN3) were designed and synthesized, each ligand docked towards the target protein. All the derivatives exhibited their anthelmintic activity. According to this, the compounds were analyzed and complexed with Albendazole, which was used as a standard drug for this study. The compound BT3 (figure: 7a) showing high binding energy among these 12 derivatives, if compared to the standard drug of Albendazole (figure: 7c).

So various benzaldehyde derivatives with 2-aminobenzothiazole is more effective than other three set of compounds. However, we can consider the 2-aminobenzothiazole derivatives are one of the lead molecules against the anthelmintic infections. Then the compound AN2 shows minimal activity while comparing with the standard drug. As for as our study concerned, we found that the 2-aminobenzothiazole with different benzaldehyde derivatives have an important role against the anthelmintic activity.

6. CONCLUSION

To be precise, we believe that the nitrogen containing aryl scaffold is significant in the context of the present scenario. We are aware that as an outcome of advancement, both our medical problems and the number of new developments or changes experienced by the younger generation have increased. Because the inner nucleic acids comprise the nitrogen containing aryl scaffold base unit, it is imperative that they be changed for the purpose to improve potent, resistant medicines for our new generation and to help people maintain good health, especially against emerging diseases.

A more effective lead compound as an anthelmintic agent can be identified by employing structure-based drug designing. The majority of compounds exhibit enhanced effectiveness.

Using structure-based drug designing enables the study to accomplish its purpose. It provides the analogue with the potential to be a powerful agent against the anthelmintic activity. The recognition of twelve superior analogues, one of which is superior as an anthelmintic agent, demonstrated the value of the current investigation. The lead compounds were identified, and the analogs are developed to increase the therapies' effectiveness.

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