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SYNTHESIS, MOLECULAR DOCKING & EVALUATION OF ANTHELMINTIC ACTIVITY OF 2-(2-AMINO ETHYL)-1H-BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

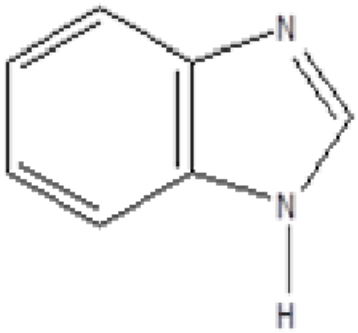
A novel series of 2 (2-aminoethyl)-1H-Benzimidazole was synthesized. The molecular docking study of these compound was performed at the active site of the β tubulin receptor. The synthesized compounds were characterized by spectral analysis (IR, ^{1}H NMR). The screening of synthesized compound for in-vitro anthelmintic activity was studied using albendazole as reference drug. All compounds have shown binding ability at the active site of receptor and posses anthelmintic activity comparable to albendazole.

KEYWORDS: 2(2-aminoethyl)-1H-Benzimidazole, β tubulin receptor, anthelmintic activity.

INTRODUCTION

Benzimidazole are important group of biological active heterocyclic compounds hence having significant importance in medicinal chemistry.[1]

Benzimdazole is a bicyclic compound in which imidazole ring is fused with at adjacent position benzene ring. The Chemistry and pharmacology of benzimidazole is of great interest to medicinal chemistry as its derivatives possessed various biological activities like Antioxidant, Antimicrobial, Anthelmintic, Anticancer, Antihypertensive, Antineoplastic, Anti-inflammatory, Analgesic, Antifungal and Antiviral Activity Variety of Benzimidazoles as anthelmintic agents are Albendazole, Mebendazole, thiabendazole, flubendazole are most popular Benzimidazoles utilized as anthelmintic agents.

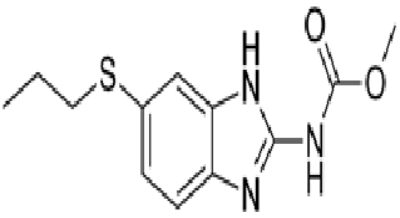


Benzimidazole.

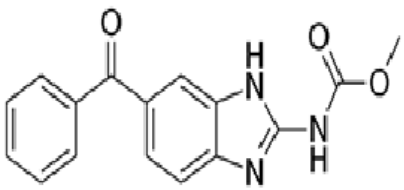
Among the benzimidazole derivatives, 2-substituted ones are found to be pharmacologically more potent and hence the design and synthesis of 2- substituted benzimidazoles is the potential area of research.[2]

Biological Profile

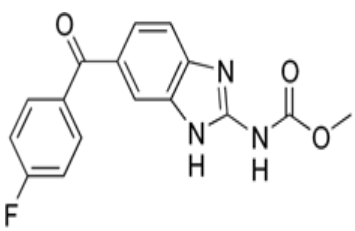
Benzimidazole possesses both acidic and basic characteristics. The cyclic –NH- group contributes for relatively strongly acidic and also weakly basic property. They possess affinity towards enzyme and protein receptor hence categorised as privileged 'sub-structure' for drug dosing. Broad-spectrum anthelmintics Albendazole, mebendazole, flubendazole, cyclobendazole, fenbendazole, oxfendazole, oxibendazole, ricobendazole, and luxabendazole as derivatives of carbabendazim i.e benzimidazole act by inhibiting the microtubule formation. Albendazole, fenbendazole and oxfendazole are the benzimidazole to used for the treatment of gastro-intestinal nematodes.[3,4]



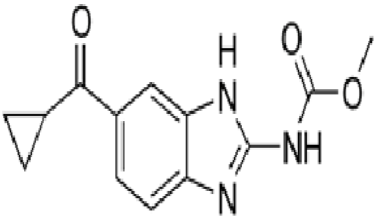
Albendazole



Mebendazole



Flubendazole



Cyclobendazole

Fig. 1: Some Benzimidazole derivatives used as anthelmintic agent.

MATERIAL AND METHOD

All reagent and solvent were purchased AR grade from Bansal sales corporation and Morden science apparatus Nasik. Solvents were dried by standard procedures. The purity of compound was checked by TLC using precoated Silica Gel G plates. Melting point were determined in capillary tubes on Remi apparatus and were presented uncorrected. Infrared (IR) Spectra were recorded using FTIR Spectrophotometer Model: IR affinity 1-S.

A Nuclear magnetic resonance spectra were recorded on Bruker Ultra Shield Model DPX 500 MHz spectrometer in $CDCl_3$ Solvent and TMS as internal standard. In silico modelling of the molecule was performed using Chem Draw were used for drawing, 2D molecule and calculating various physiochemical properties of the proposed molecules. The Molecular docking was carried out using by Vlife MDS 4.6 Software.

Synthesis of 2-(2-amino ethyl) -1H-Benzimidazole

Synthesis of Ortho phenylene diamine Dihydrochloride Salt

Ortho Phenylene Diamine(0.024mol) was dissolved in conc HCl to it stannous Chloride (0.024 mol) was added the reaction mixture was warmed & filtered allowed to cool in ice bath. Crystals of Dihydrochloride were collected and dried. M.P 120º C % Yield 54%.

Synthesis of 2-(2-amino ethyl) -1H-Benzimidazole

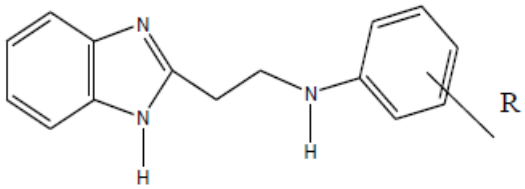
O-Phenylene diamine dihydrochloride (0.024mol), 3-Chloropropionic acid (0.024mol) and 5N HCl Hydrochloric acid were placed in an RBF and refluxed. The reaction was monitored by TLC. After Completion of the reaction the reaction mixture was poured in ice cold water. It was then basified. The solid precipitated was obtained which was filtered and dried. M.P 218º C % Yield 60%.

Synthesis of N-(2-(1H-benzimidazole-2-yl)-2-chlorobenzenamine (A1)

2-(2-chloro ethyl)-1H-Benzimidazole (0.005mol) o-Chloro aniline (0.005mol) were Separately dissolved in dioxone and placed in RBF, trimethylamine (0.005mol) was added to it and the reaction mixture was refluxed. The reaction was monitored by TLC. After completion of the reaction reaction mixture was dumped in ice cold water. Obtained oily liquid then converted to HCl salt.

Other derivatives were prepared using substituted anilines with same procedure.[5]

Table No. 1: Physical Characterization data of synthesized derivatives.



Compound	R	Melting point (°C)	Yield (%)	*Rf
A1	-o-Cl	218	60%	0.63
A2	-m- Cl	129	58%	0.54
A3	-p- Cl	138	64%	0.51
A4	-H	110	61%	0.59
A5	-o-CH_3	178	65%	0.60
A5	-p- CH_3	160	64%	0.53
A7	-p-OH	175	59%	0.45

Mobile phase for TLC: Ethyl acetate: Toluene (7:3)

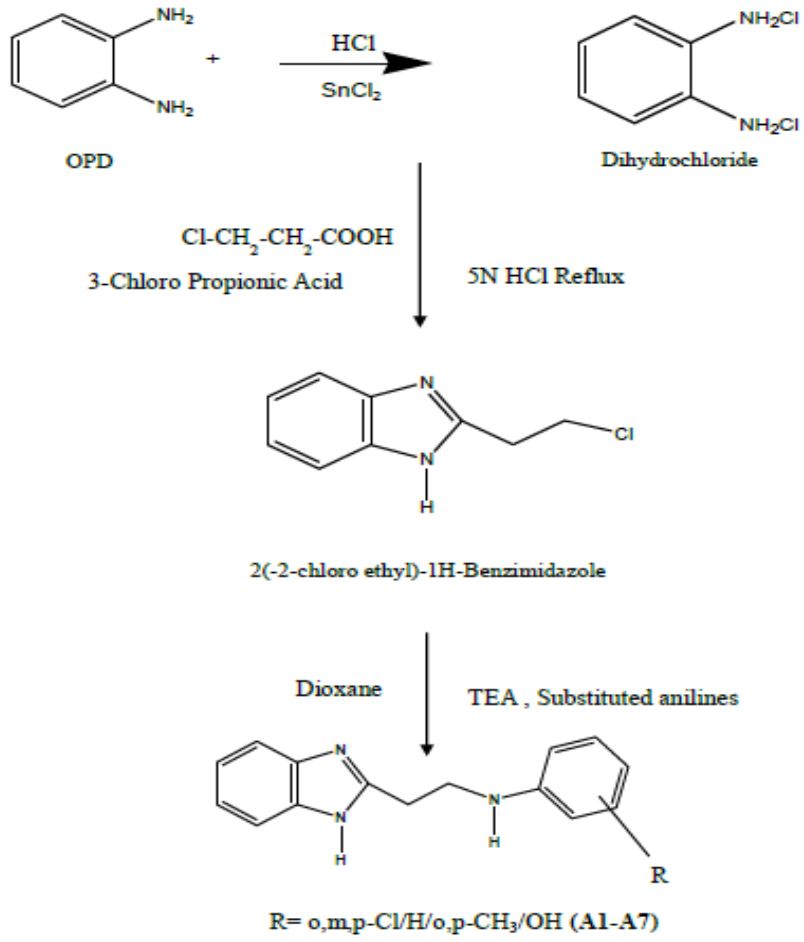


Fig. 2: Scheme for the synthesis route of 2-(2-amino ethyl)-1H-Benzimidazole derivatives.

2(-2-aminoethyl)-1H-Benzimidazole

FTIR 3450 (-NH str), 2900 (Ar-H str), 2365 (Ali-H str), 750.67 (Cl str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.16-3.19 (Ali H) 4H, 6.18-7.68 (Ar-H) 4H Benzimidazole, 6.61-7.41 (Ar-H) 4H Phenyl M.P 110º C % yield 59%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-2-chlorobenzenamine (A1)

FTIR FTIR 3050 (-NH str), 2611 (Ar-H str), 2341 (Ali-H str) 780 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.13-3.23 (Ali H) 4H 6.34-7.24 (Ar-4H) Benzimidazole, 6.76-6.85 (Ar-4H) Phenyl M.P 218º C % yield 60%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-4-chlorobenzenamine (A2)

FTIR 3052 ((-NH str), 2225 (Ar-H str), 2460 (Ali-H str) 754 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.12-3.17 (Ali H) 4H, 6.35-7.36 (Ar-H) 4H Benzimidazole, 6.41-7.55 (Ar-H) 4H Phenyl M.P 178º C % yield 65%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-4-chlorobenzenamine (A3)

FTIR 3122 ((-NH str), 2650 (Ar-H str), 2340 (Ali-H str) 745 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.11-3.22 (Ali H) 4H, 6.45-7.65 (Ar-H) 4H Benzimidazole, 6.22-7.45 (Ar-H) 4H Phenyl M.P 138º C % yield 58%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-Benzenamine (A4)

FTIR 3234 ((-NH str), 2475 (Ar-H str), 2470 (Ali-H str) 765 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.8-3.19 (Ali H) 4H, 6.15-7.55 (Ar-H) 4H Benzimidazole,6.45-7.44 (Ar-H) 4H Phenyl M.P 110º C % yield 61%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-2-methyl Benzenamine (A5)

FTIR 3456 ((-NH str), 2800 (Ar-H str), 2422 (Ali-H str) 743 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.19-3.25 (Ali H) 4H, 6.35-7.72 (Ar-H) 4H Benzimidazole,6.65-7.45 (Ar-H) 4H Phenyl M.P 178º C % yield 65%.

N-(2-(1H-benzimidazole-2-yl) ethyl)-4-methyl Benzenamine (A6)

FTIR 3350 ((-NH str), 2812 (Ar-H str), 2355 (Ali-H str) 742.60 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.12-3.21 (Ali H) 4H, 6.14-7.52 (Ar-H) 4H Benzimidazole, 6.50-7.43 (Ar-H) 4H Phenyl M.P 160º C % yield 64%.

4-(2-1H-benzo[di] imidazole-2yl) ethyl amino)-2-aminophenol (A7)

FTIR 3342 ((-NH str), 2972 (Ar-H str), 2500 (Ali-H str) 731.12 (Cl-str) ^{1}HNMR ($CDCl_3$ δ ppm) 3.9-3.27 (Ali H) 4H, 6.15-7.59 (Ar-H) 4H Benzimidazole, 6.68-7.41 (Ar-H) 4H Phenyl M.P 175º C % yield 59%.

MOLECULAR DOCKING

Receptor βTubulin is anticancer and anthelmintic drug target, investigation of β tubulin inhibitors may lead to the development of anthelmintic drug, Inhibitors bind selectively of β Tubulin of nematodes, cestodes and fluke, a protein unit of microtubule and thereby disrupting microtubule structure and function. The receptor β Tubulin (TUBULIN-COLCHICINE: STATHMIN-LIKE DOMAIN COMPLEX) (pdb code 1Sa0) was downloaded from RCBS pdb site and water molecules were removed from the protein. Receptor was loaded for docking in Biopredicta module of the software, virtual library of compound was prepared the Structure were drawn in **2D** in Vlife Engine Module of Software and converted to **3D.** then binding of compounds with the receptor was studied & score was noted and Compounds with lowest Score were studied for 2D & 3D interaction (hydrogen bonding, hydrophobic bonding & Vander wall forces, pi Stacking).[6]

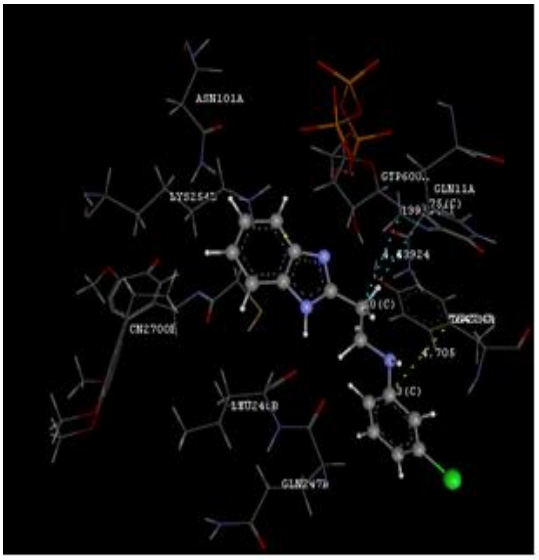


Fig. No: 3: Interaction of A1 with receptor.

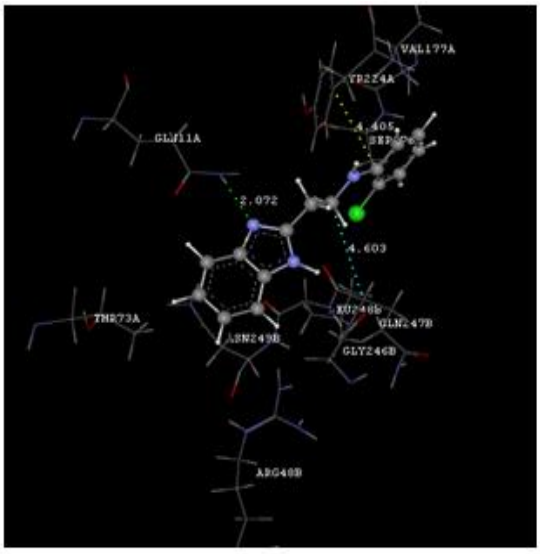


Fig No: 4 Interaction of A3 with receptor.

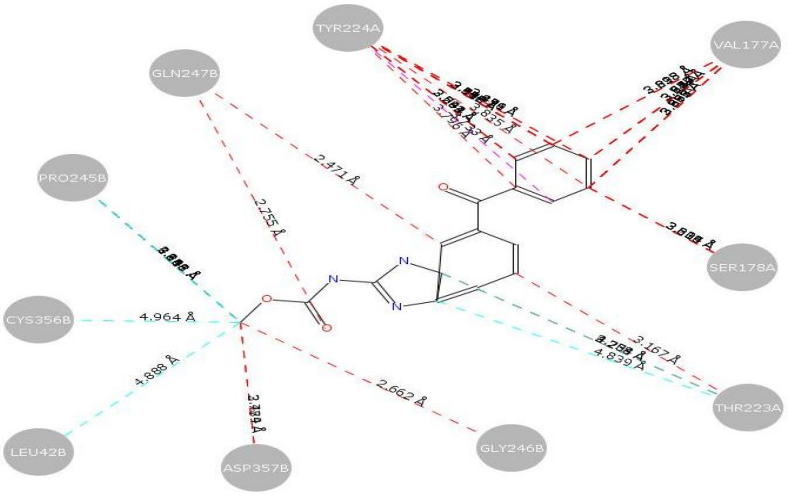


Figure No 5: 2D Interaction of Mebendazole with receptor.

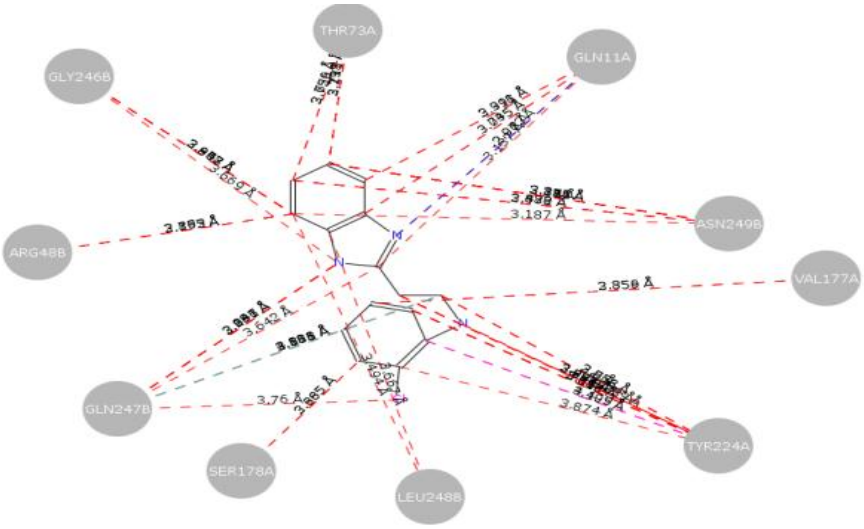


Figure No 6: 2D Interaction of A1 with receptor.

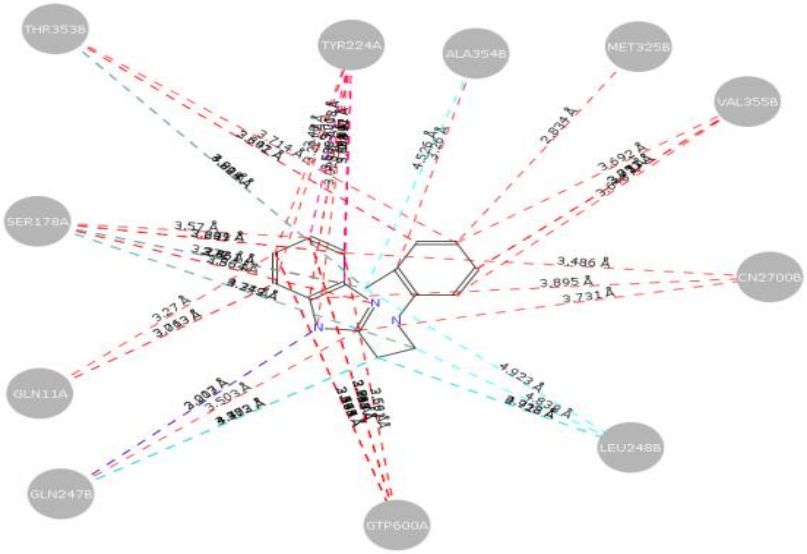


Figure No 7: 2D Interaction of A1 with receptor.

ANTHELMINTIC ACTIVITY

Anthelmintic screening was done on the synthesized novel 2(2-aminoethyl)-1H-benzimidazole derivative analogues. Albendazole (marketed preparation Bandy syrup) was used as standard. Adult earthworm *Eudrilus Eugenia* were washed with normal saline to remove all the faecal matter, were used the anthelmintic study. The earthworm of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites in human beings. Marketed preparation of Albendazole suspension was diluted with 40ml distilled water to obtain 0.4%w/v as standards and poured into petri dishes. All the test compounds were prepared in minimum quantity of DMSO and diluted to 40 ml distilled water to obtain 0.4% w/V solution which were then placed into petri dishes. Individual earthworm was placed in each petri dish at room temperature. The time taken for paralysis and death were recorded. The mean paralysis time and mean death time for each sample were recorded. Observation were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in distilled water. Death was concluded when the worms lost their motility followed with fading away of their body colors. The result was analyzed for statistical significant by one-way ANOVA. To ascertain death, each worm was frequently subjected to external stimuli that stimulate and induce movement in earthworm, if alive.[7,8,9]

Table No: 2: Dock score and anthelmintic activity.

Control Standard.(gm%)	Dock Score	Conc. (gm%)	Mean Time taken for Paralysis (Min)	Mean Time taken for death (Min)
Albendazole)	-37.62	0.4	19.64 ± 2.43	29.08 ± 2.166
A1	-51.70	0.4	4.64 ± 1.02	9.18 ± 0.79
A2	-38.99	0.4	9.16 ± 0.57	14.54 ± 0.82
A3	-51.70	0.4	9.75 ± 0.53	15.55 ± 0.85
A4	-38.67	0.4	10.24 ±0.79	19.77 ± 0.49
A5	-38.73	0.4	20.08 ± 0.82	25.12 ± 0.70
A6	-41.50	0.4	10.25 ± 0.70	20.58 ± 0.98
A7	-50.63	0.4	4.72 ± 0.99	9.26 ± 0.82

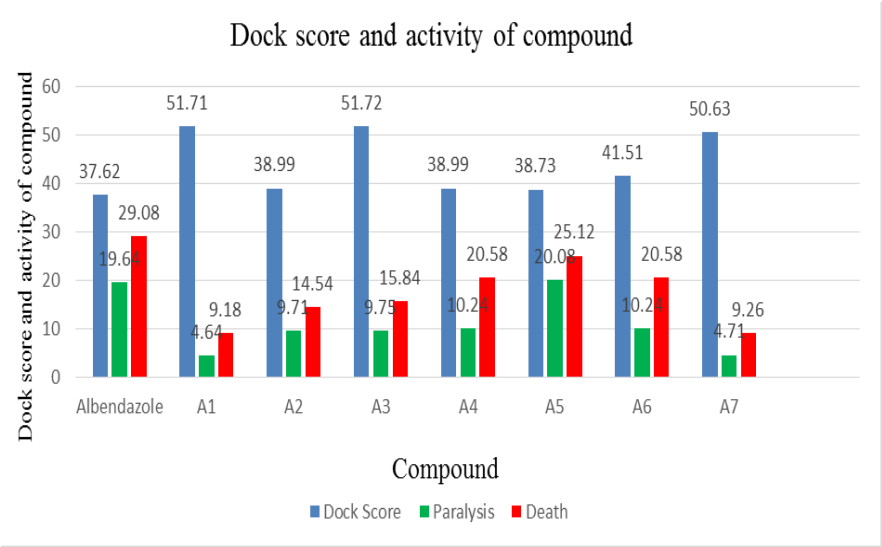


Figure No 8: Dock score and activity of Compound.

RESULT AND DISCUSSION

The 2-(2-amino ethyl)-1H-Benzimidazole derivatives were synthesized using appropriate synthesis route, further converted to HCL salt using conc HCL and checked the purity by thin layer chromatography. Characterization were done by R_f value, melting point, FTIR and ^{1}HNMR.

FTIR spectra of all synthesized compound compounds shows absorbance bonds at range 3050-3350cm^{-1} –NH associated with C-H stretching vibration and bands at 2800 cm^{-1} for Ar-H stretching.

The ^{1}HNMR spectrum of synthesized compounds exhibited peaks in the range of 6.18-7.68 ppm correspond to Ar-H (8H) and 3.16 – 3.19 Ali-H (4H) (2-(2-amino ethyl)-1H-Benzimidazole.

The Result of biological anthelmintic activity of test compound is given in Table No: 1.2 shows that A1 and A3 showed significant anthelmintic activity on comparison with standard Albendazole respectively.

CONCLUSION

Molecular modeling study of synthesized derivatives along with reference drugs Mebendazole and albendazole was performed with receptor β tubulin using V-Life MDS software. Albendazole **i**nteracts with receptor β tubulin at its active site by Pi stacking and Hydrogen bonding interaction. Amino acids at the active site Glycine 246B, Glutamine11A,

Proline 223A, Tyrosine 229A, Threonine 225A interacts by Pi stacking with Albnedazole while amino acid Valine 177A, Glutamine 247 interact by Hydrogen bonding with Albnedazole.

Synthesized compound A1-A7 also interacts with receptor β tubulin at its active site with Pi stacking and Hydrogen bonding interactions. Amino acids at active site Glycine 246B, Serine 178A, Tyrocine 224A, Valine 177A, Aspargine 249B interacts by Pi stacking with synthesized derivatives. While amino acids Arginine 48B, Glutamine 11A and Glutamine 22A interacts by Hydrogen bonding interaction with synthesized derivatives. All synthesized derivatives interact at active site of receptor in similar pattern that of reference drugs.

All synthesized derivatives along with refrence drug Albendazole were evaluated for invitro anthelmintic activity and it was found that all synthesized compounds posses anthelmintic activity comparable to that of reference drug that is Albendazole.

Compound A1 and A7 were found to have Biological activity more potent than Albendazole i.e time required for Paralysis (05min) and Death (10min) for A1 and A7 while for Albnedazole Paralysis (20min) and Death (30min).

Thus it can be concluded the nucleus 2(2-amino ethyl) benzimdazole posses high potential to exhibit anthelmintic activity.

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