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SYNTHESIS, ANTIMICROBIAL AND DOCKING STUDIES OF NOVEL BENZOTRIAZOLE DERIVATIVES

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ABSTRACT

In view of the biological and clinical importance of Benzotriazole compounds, some new derivatives were synthesized and characterized on the basis of analytical and spectral data. All the new compounds were evaluated at 500µg/ml, 250µg/ml and 100µg/ml for the antibacterial and antifungal activity. Some of the compounds showed moderate to good antimicrobial activity against gram positive and gram negative bacteria. Compound BT1 was found to be of potent activity against *Candida albicans* at a concentration of 500µg/ml. Docking studies of the synthesized compounds were carried out with the help of VLife MDS 4.2 software using GRIP batch docking method to validate the results obtained in *in-vitro* studies.

KEYWORDS: **Antibacterial, Antifungal, Docking, Benzotriazole, *Candida albicans*.**

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INTRODUCTION

Today, the majority of patients seeking medical advice are those suffering from some sort of infectious diseases and more than half a total deaths in the world are associated with the microbial infection. Millions of infants die of bacterial, viral and protozoal infection and antimicrobial drug resistance. Hence new drugs and vaccines are needed for emerging diseases. Moreover the development of resistance for current antibacterial therapy continues to drive the search for new and more effective agents[1]. In the recent decade a number of Benzotriazoles have been reported for its antibacterial, antifungal, anti-inflammatory, antihypertensive and diuretic activity[2, 3]. Thus synthesis of benzotriazole is of current interest because of their medical importance. The Schiff bases, Thiadiazole[4] and Triazole thione[5] synthesised in this study are also reported to exhibit various biological activities.Various heterocyclic compounds such as 1,3,4 oxadiazoles, 1,2,4 triazole[6], 1,3,4 thiadiazoles[7] and thiosemicarbazide derivatives have occupied an important place in synthetic organic chemistry because of their usefulness as drugs and are known to possess interesting pharmacological properties like antitubercular, antifungal, bacteriostatic, hypoglycemic, diuretic, antiviral, anthelmentic and anti-inflammatory activities[8].There are many compounds which are basic in nature and having antimicrobial activity such as Pyrimidine, INH, Streptomycin and other well-known antibiotics. As we know benzotriazole itself has antimicrobial activity, we thought of synthesizing Schiff bases of benzotriazole with various substituted aromatic amines.

MATERIALS AND METHODS

All the chemicals used for the synthesis were obtained from Sigma Aldrich, Ranbaxy Chemical, Nice Chemicals, Merck andCDH Pvt. Ltd, India. Equipments such asShimadzu FTIR-8310 spectrophotometer, Gemini-200MHz spectrometer, Autospec Mass spectrometer, Shimadzu, UV-1601 spectrometer were used.

SYNTHESIS

Preparation of benzotriazole (BT1): Dissolve 0.1 mol of o-phenylene diamine in a mixture of 0.2mol of glacial acetic acid and 30ml of water. Cooled the clear solution to 15^0C, stir and then added a solution of sodium nitrite (0.1mol) in 15ml of water. Collected the solid recrystalized with benzene.

Preparation of ester: Benzotriazole (1.1g) was dissolved in 10ml of DMF and added ethylchloroacetate (0.01mol). Anhydrous potassium carbonate (2.3g) was added and stirred for 24hrs. Mixture was poured into ice-cold water and extracted with ether and was evaporated to obtain ester.

Preparation of hydrazide (BT2) Benzotriazole ester (0.01mol) and Hydrazine hydrate (0.96ml) was dissolved in 10ml of alcohol and refluxed for 12-16 hrs. Mixture was poured into cold water and pricipitate was filtered.

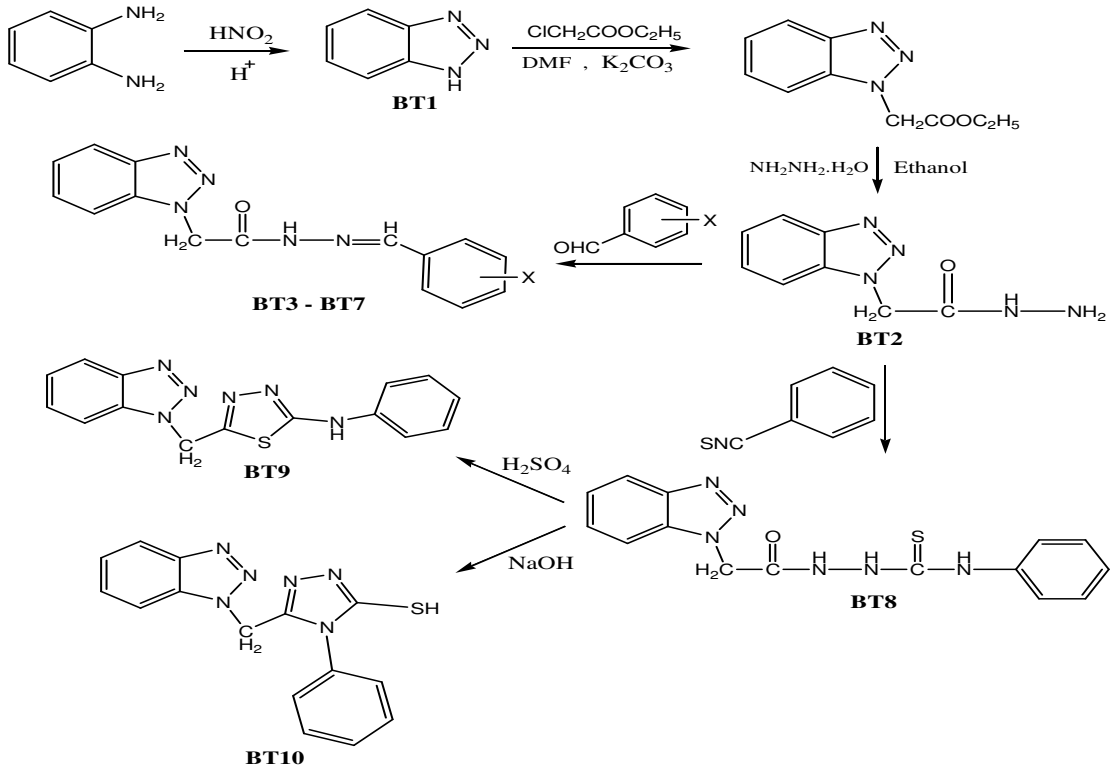
Preparation of Schiff bases (BT3-BT7) Hydrazide derivative of benzotriazole (0.01mol) was refluxed with different substituted aldehyde in 10ml of alcohol for 10hrs. The product was washed with sodium bisulphate solution and filtered.

Preparation of thiosemicarbazide (BT8) Mixture of hydrazide derivative of benzotriazole (0.01mol) and phenylisothiocyanate (0.01mol) in ethanol (80ml) was refluxed for 6 hrs.Solid product was filtered.

Preparation of 1, 3, 4-thiadiazole derivative (BT9): Sulphuric acid (10ml) was added to thiosemicarbazide (0.01mol) and the mixture was kept at room temperature. Then poured into crushed ice and solid was separated.

Preparation of mercapto triazole (BT10) Thiosemicarbazide (0.01mol) was refluxed with 4% sodium hydroxide solution (60ml) for 3-4 hrs. Then reaction mixture was cooled and filtered.

Scheme for the synthesis of Benzotriazole derivatives



Compound	X
BT3	H
BT4	2-NO_2
BT5	4-CH_3
BT6	2-OCH_3
BT7	4-Cl

Table 1
Physical data of the Benzotriazole derivatives

Compound	Mol. Formula	% yield	Melting point^{0}C	Rf value
BT1	$C_6H_5N_3$	90	96-99	0.77
BT2	$C_8H_9N_5O$	86	191-194	0.63
BT3	$C_{15}H_{13}N_5O$	75	162-164	0.60
BT4	$C_{15}H_{12}N_6O_3$	63	193-196	0.47
BT5	$C_{16}H_{15}N_5O_2$	82	213-216	0.45
BT6	$C_{16}H_{15}N_5O_2$	78	203-207	0.68
BT7	$C_{15}H_{12}N_5O_2Cl$	66	212-215	0.58
BT8	$C_{15}H_{14}N_6OS$	80	182-186	0.75
BT9	$C_{15}H_{12}N_6S$	60	188-191	0.63
BT10	$C_{15}H_{12}N_6S$	72	278-280	0.50

SPECTRAL DATA

1H-benzo[d][1,2,3]triazole (BT1): IR (KBr): 3037(C-H), 1267.1(C-N), 3236 (N-H), cm^{-1}. GC-MS: 119 (M+).

2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide(BT2): IR(KBR): 2980.26(C-H), 1283.01(C-N), 3230(N-H), 1720.77(C=O), cm^{-1}. GC-MS: 191(M+).

2-(1H-benzo[d][1,2,3]triazol-1-yl)-N-benzylideneacetohydrazide (BT3): IR (KBr): 3029(C-H), 1727.70(C=O), 1658.54(C=N), 1373.31(C-N), cm^{-1}. GC-MS: 279(M+).

N-(2-nitrobenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (BT4): IR (KBr): 2982.40(C-H), 1729.50(C=O), 1698.44(C=N), 1383.81(C-N), cm^{-1}. GC-MS: 324(M+).

N-(4-methylbenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (BT5): IR (KBr): 2792.25(C-H), 1715.34(C=O), 1630.17(C=N), 1358.13(C-N), cm^{-1}. GC-MS: 293(M+).
N-(2-methoxybenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (BT6): IR (KBr): 3012.37(C-H), 1722.70(C=O), 1664.56(C=N), 1323.20(C-N), cm^{-1}. GC-MS: 309(M+).
N-(4-chlorobenzylidene)-2-(1H-benzo[d][1,2,3]triazol-1-yl)acetohydrazide (BT7): IR (KBr): 2929.11(C-H), 1715.17(C=O), 1675.42(C=N), 1392.51(C-N), cm^{-1}. GC-MS: 314(M+).
1-(2-(1H-benzo[d][1,2,3]triazol-1-yl)acetyl)-4-phenylthiosemicarbazide (BT8): IR (KBr): 3008.69(C-H), 1722.60(C=O), 1618.14(C=N), 1377.36(C-N), 1120.62(C=S), cm^{-1}. GC-MS: 326(M+).
5*-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine (BT9):* IR (KBr): 2972.40(C-H), 1716.70(C=O), 1658.55(C=N), 1383.33(C-N), 769.62(C-S), cm^{-1}. GC-MS: 308(M+).
5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol (BT10): IR (KBr): 3045.43(C-H), 1740.77(C=O), 1666.32(C=N), 1363.26(C-N), 2585.51(H-S), cm^{-1}.GC-MS: 308(M+).

BIOLOGICAL EVALUATION

Antibacterial activity

The synthesized compounds were dissolved separately in DMSO. The entire compounds were tested at varying level of concentrations of 500µg/ml, 250µg/ml and 100µg/ml. The dehydrated medium was obtained from Himedia Laboratories Pvt. Ltd., India. The antibacterial activity of the test compound was determined by using *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Klebsilla pneumoniae and Salmonella typhi*, isolated from clinical specimen.The plates were inoculated by dipping a sterile swab into the microbial growth. Excess of inoculate was removed by passing and rotating the swab firmly against the side of the tube, above the level of the liquid.0.1 ml of the test drug was added to each of the plate and standard drug and control solution was added. Plates were kept at room temperature for 24hrs for diffusion. All the plates were incubated for 24hrs at 37^0C. After incubation, the diameter of each zone was measured with the help of a ruler and the reading was recorded in mm (Table 2 and 3).

Antifungal activity

Sabouraud's Dextrose Agar Broth medium was poured in 20ml quantities to petridishes. The inoculums were spread evenly over the whole plate with a sterile cotton swaband dried in an incubator..The different concentrations of the drug solution was poured into the cups bored in the plate. It was transferred quickly to the incubator for 24 hrs at 37^0C, at the end diameter of zone of inhibition produced were measured.The fungi selected for the study was *Candida albicans*. The fungi was subcultured in S.D.A broth and incubated at room temperature. After getting growth, fungal suspension was standardized spectrophotometrically to an absorbance at 0.600 at 450nm. Fungal susceptibility of synthesized benzotriazoles were done using cup plate method for different concentration of 500µg/ml, 250µg/ml and 100µg/ml using Amphoterecin B as standard. (Table 4).

Table 2
Gram Negative Bacteria

Compound	Zone of inhibition in mm														
	S.typhi			*E.coli*			*P.aeruginosa*			*P. vulgaris*			*K. pneumoniae*		
µg/ml	100	250	500	100	250	500	100	250	500	100	250	500	100	250	500
BT1	18	18	18	-	-	12	10	16	16	11	15	15	11	12	15
BT2	10	10	10	-	-	-	-	-	10	-	-	-	-	-	-
BT3	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-
BT4	-	-	10	-	-	-	-	-	-	-	-	10	-	-	-
BT5	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-
BT6	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-
BT7	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BT8	11	-	-	-	-	-	-	-	9	-	-	-	-	-	-
BT9	11	-	-	-	-	-	-	-	11	-	-	-	-	-	-
BT10	10	10	10	-	-	-	-	-	10	-	-	-	-	-	-
Streptomycin	21														

Table 3
Gram Positive Bacteria

Compound	Zone of inhibition in mm					
	B.subtilis			*S.aureus*		
µg/ml	100	250	500	100	250	500
BT1	11	15	15	10	11	12
BT2	-	-	10	-	-	-
BT3	-	-	-	-	-	-
BT4	-	-	-	-	-	-
BT5	-	-	-	-	--	-
BT6	-	-	-	-	-	-
BT7	-	-	-	-	-	-
BT8	-	-	-	-	-	10
BT9	-	-	-	-	-	-
BT10	-	-	-	10	-	-
Penicillin	23			30		

Table 4
Fungal Organism

Compound	Zone of inhibition		
	Candida albicans		
µg/ml	100	250	500
BT1	-	-	13
BT2	-	-	-
BT3	-	-	-
BT4	-	-	-
BT5	-	-	-
BT6	-	-	-
BT7	-	-	-
BT8	-	-	-
BT9	-	-	-
BT10	-	-	-
Amphotericin B	17		

DOCKING STUDIES

Docking was done by GRIP batch docking method with the help of Vlife MDS 4.2 software. The crystal structure of *E. coli* 24KDa domain (PDB Code: 1KZN) for antibacterial docking studies and cytochrome P450 14 alpha – sterol demethylase (PDB Code: 1EA1) for antifungal docking studies was obtained from the protein data bank[9]. The parameter fixed for docking simulation was like, number of placements: 30, rotation angle: 15º, ligand flexible, exhaustive method, scoring function: dock score. The ligand forming most stable drug-receptor complex is the one which is having minimum dock score. After docking simulation, the best docked conformer of each ligand was checked for various interactions with receptor like hydrogen bonding, hydrophobic bonding and Van der waal's interaction.Compound BT9 was possessing highest negative dock score with both the receptors (Table 5), meaning that it forms most stable drug-receptor complex amongst all the compounds. It was found to form hydrogen bonding, hydrophobic bonding and Van der waal's interaction. The residue of 1KZN involved in hydrogen bonding was Arg76 (Fig 1), the residues involved in hydrophobic bonding are Glu50, Gly77, Ile78 and Thr165 (Fig 2). It exhibited a large number of Van der waal's bonding with a wide range of residues. Some of the residues involved in this type of interaction are Arg76, Glu50, Gly77, Thr165, Val120, and Val143 etc. (Fig. 3). The residue of 1EA1 involved in hydrogen bonding was Val395 (Fig. 4), the residues involved in hydrophobic bonding are Ala256 and Gly396 (Fig 5). It also exhibited more number of Van der waal's bonding, some of the residues involved in this type of interaction are Arg96, Cys394, Val395, Gly396 and Ala400 (Fig 6).

Table 5
Docking scores of the synthesized compounds

Compound Code	Dock score with 1KZN 1KZN with	Dock score with 1EA1 with 1EA1
BT1	-39.05	-39.31
BT2	-57.72	-53.20
BT3	-72.61	-66.16
BT4	-58.25	-69.18
BT5	-67.14	-69.68
BT6	-60.71	-67.09
BT7	-67.29	-72.44
BT8	-59.96	-74.69
BT9	-79.66	-77.22
BT10	-70.52	-67.41

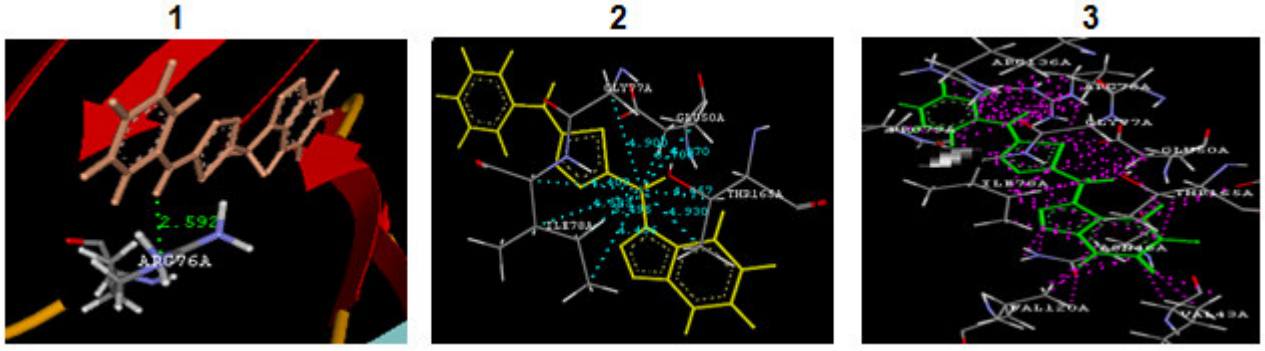


Figure 1 Hydrogen interaction (green dotted line)
Figure 2 Hydrophobic interaction (light blue dotted line)
Figure 3 Van der wall's interaction (pink dotted line) of BT9 with receptor (PDB: 1KZN)

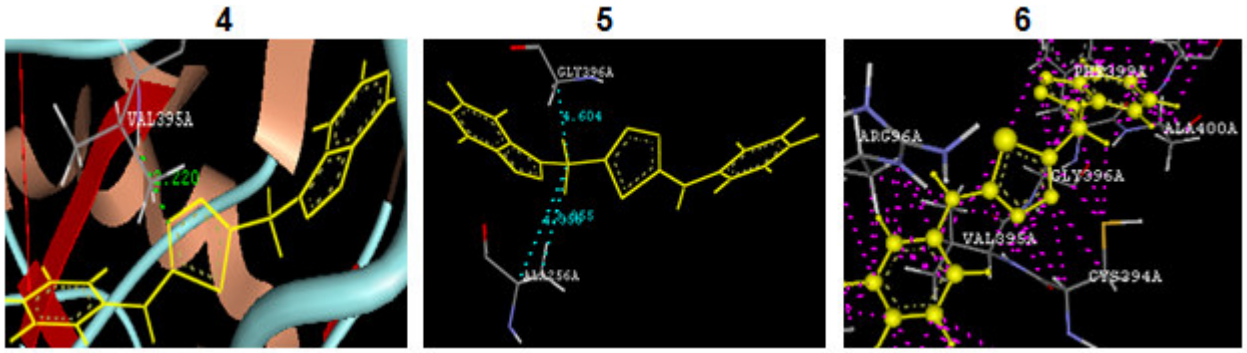


Figure 4 Hydrogen interaction (green dotted line)
Figure 5 Hydrophobic interaction (light blue dotted line)
Figure 6 Van der wall's interaction of BT9 with receptor (PDB: 1EA1)

RESULTS AND DISCUSSION

From the antimicrobial studies, we can infer that BT1 showed good activity against both the Gram positive and Gram negative organism in comparison with the control Penicillin and Streptomycin. BT1 showed potent activity at 500µg/ml where as BT2 showed moderate activity against *Bacillus subtilis*. Similarly in case of *Staphylococcus aureu*s BT1 and BT8 showed moderate activity at 500µg/ml. In case of *Salmonella typhi*, BT2, BT3, BT4, BT5, BT6, BT10 showed moderate activity at concentration of 500µg/ml and BT7, BT8 and BT9 shows moderate activity at 100µg/ml concentration. BT1 showed potent activity against *E.coli* at only 500µg/ml concentration. BT1 also showed moderate activity against *Pseudomonas aerugenosa* compared to other derivatives at the mentioned concentration. BT1 showed potent activity against *Proteus vulgaris* at 500µg/ml concentration, while BT4 showed moderate activity in comparison with BT1 at the similar concentration. Moreover BT1 showed potent activity against *Klebsilla pneumonia* whereas this organism was found to be resistant to other derivatives.BT1 was found to be of potent activity against *Candida albicans* at a concentration of 500µg/ml in comparison with the control Amphoterecin B. *Candida albicans* was found resistant to other derivatives.Docking studies was carried out by taking the crystal structure of *E.coli* 24KDa domain (PDB Code: 1KZN) for antibacterial studies and cytochrome P450 14 alpha – sterol demethylase (PDB Code: 1EA1) for antifungal studies. The compound BT9 was found to have highest negative dock score with both the receptor (-79.66 with 1KZN and -77.22 with 1EA1). It means that it can fit well in the receptor cavity forming energetically most stable drug receptor complex.

CONCLUSION

The compounds synthesized during the present work were found to be not that promising though the moieties such as Schiff bases, Thiasemicarbazide, Triazole thione and Thiadiazole are of antibacterial and antifungal nature. This was because benzotriazole nucleus itself is more active than its derivatives synthesised. Hence the hypothesis of improving the activity was not successful. Therefore the logical conclusion for this study is blocking the secondary -NH- group in first position is not useful in improving the antimicrobial activity and further substitutions on phenyl ring should be tried for improving antimicrobial activity.

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