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SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF NOVEL BENZIMIDAZOLE DERIVATIVES

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Abstract:

Benzimidazole is a heterocyclic aromatic organic compound. Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Looking at the importance of benzimidazole and oxadiazole nucleus, it was thought that it would be worthwhile to design and synthesize new benzimidazole derivatives. In recent years, attention has increasingly been given to the synthesis of benzimidazole derivatives. Hence, there will always be a vital need to discover new chemotherapeutic agents to overcome the emergence of resistance and ideally shorten the duration of therapy. Due to the structural similarity to purine, antibacterial ability of benzimidazoles is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins. Thus, from the present study it can be concluded that cyclohexyl derivatives of benzimidazole ring have significant antibacterial activity.

Keywords: *Benzimidazoles, anti-malarial, anti-fungal, anti-bacterial, anti-viral, anti biotics.*

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INTRODUCTION:

Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B_{12} [1]. This ring system is present in numerous antioxidant [2,3], antiparasitic [4,5], antihelmintics [6], antiproliferative [7], anti- HIV [8], anti-inflammatory [9-10] and antineoplastic [11,12] activities. Our research efforts are directed to find new chemical classes of antimycobacterially active agents. A lot of studies were carried out on heterocyclic systems bearing an alkylsulfanyl group as pharmacophore. Varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities. This ring system is present in numerous antiparasitic, fungicidal, anithelemintic and anti-inflammatory drugs. The earliest report of their antibacterial activity appeared in 1964 [13], and more recently we have found two groups of substituted benzimidazoles, namely the 5, 6-dinitro and 2- trifluoromethyl derivatives, to be promising candidates for antimicrobial drugs [14]. In this paper author present new data on the antimicrobial activities of derivatives of benzimidazole ring. Various functional groups, such as, chloro, fluoro, nitro, methoxy, and so on, have an important significance in medicinal chemistry. Especially the dihalogen-like dichloro, as in antifungal azoles, provides a potent antifungal activity for drugs such as oxiconazole, ketoconazole, terconazole, itraconazole and so on.

MATERIAL AND METHODS:

Chemistry: General procedures followed during synthesis are as follow:

Reaction Monitoring:

Synthetic procedures employed were monitored by Thin Layer Chromatography (TLC) employing 6'' x 2" plates coated with 0.25 mm thick layer of silica gel G. Solvent systems of chloroform: methanol mixtures (5:1) were used to monitor the reactions. The dried plates after development were visualized in an iodine chamber.

Product separation:

Desired products were extracted with suitable solvent (ether). Crushed ice was used instead of cold water wherever required in very hot days. Solvents were dried using anhydrous magnesium sulphate, fused calcium chloride, or specifically reported material.

Purification procedures:

Recrystallization was carried out using dry solvents. Charcoal treatment was given to purify compounds. Column Chromatography was carried out using silica gel (60-120 mesh) using Chloroform as solvent.

Spectral characterization:

^{1}H-NMR and ^{13}C-NMR Spectra were recorded on a 300 MHz FT-NMR and 75.45 MHz respectively. In ^{1}H-NMR chemical shifts were reported in δ values using tetramethylsilane as internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd-double doublet) and coupling constants (J) in Hz (Hertz) in the solvent indicated.IR spectra were recorded as KBr pellets on Shimadzu 8400 S
FT-IR Spectrophotometer and intensities mentioned as **s**-strong, **m**-medium and **w**-weak.

Material

All the chemicals used for preparing reagents were procured from Hi-media and were of analytical grade. All the glassware's like test tubes, beaker and Erlenmeyer's flask were of Borosil grade.

Test Organism

Microbial strains used were procured from Institute of Microbial Technology, Chandigarh, India. The stock cultures were maintained on nutrient agar slants.

Preparation of Test Substance: The tested compounds and standard were dissolved in dimethyl sulfoxide (DMSO) using 1000µg/ml concentration. DMSO was used as negative control and ampicillin was used as positive control.

Preparation of Mc Farland Standard: 0.5 Mc equivalent turbidity was prepared by using standard method.

Antibacterial Activity Assay: The cup plate or cylindrical plate method was applied to determine the growth inhibition of bacteria by compounds to be tested. In this method, petri plates were prepared by pouring about 25ml of Muller Hinton Agar in each plate for *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus and Bacillus subtilis* respectively. One plate each for standards DMSO and ampicillin were prepared. Then, 100µl of standardized inoculum suspension was poured, spreaded uniformly on respective agar plates and dried for 5 minutes. 1µl test substance was poured in the uniform wells created in the centre of the petriplates and then the plates were kept undisturbed for 5-10 minutes followed by incubation at 37^{0}C for 24hrs. After incubation the diameter of circular area around the well known as Zone of Inhibition (ZOI)

was measured which is directly proportional to the sensitivity of test organisms to the test substance.

Minimum inhibitory concentration (MIC): MIC is the concentration required to inhibit bacterial cell proliferation by 50% after exposure of cells to the test compounds. MIC values (ug/ml) were determined using turbidimetry method. In this method, test and standards both were serially diluted with different concentration (500, 250, 125 67 ug/ml) in Muller Hinton Broth (MHA). After inoculation, the tubes were incubated at 37°C for 24 hrs. Cell growth was determined by visible turbidity, in order to evaluate the results. The lowest compound concentration, showing no visible growths after incubation were expressed as Minimum inhibitory concentration (MIC) in µg/ml.

Biological activity

Bacteria and media

In vitro antibacterial studies of various synthesized compounds were carried out on gram negative and gram positive bacterial strains. The gram negative strains are *Escherichia. Coli* (MTCC 1678, aerobic), *Salmonella typhi* (MTCC 733, aerobic) and gram positive strains are *Staphylococcus aureus* (MTCC 3160, aerobic), *Bacillus subtillis* (MTCC 441, p aerobic), procured from Microbial Type Culture Collection and gene bank (MTCC), IMTECH Chandigarh.

Bacteria were cultivated at 37°C in Muller Hinton Broth (MHB) and Muller Hinton agar (MHA). The tested compounds and standard were dissolved in dimethyl sulfoxide (DMSO) using 1000 µg /ml concentration. DMSO was used as negative control and ampicillin (standard) was used as positive control.

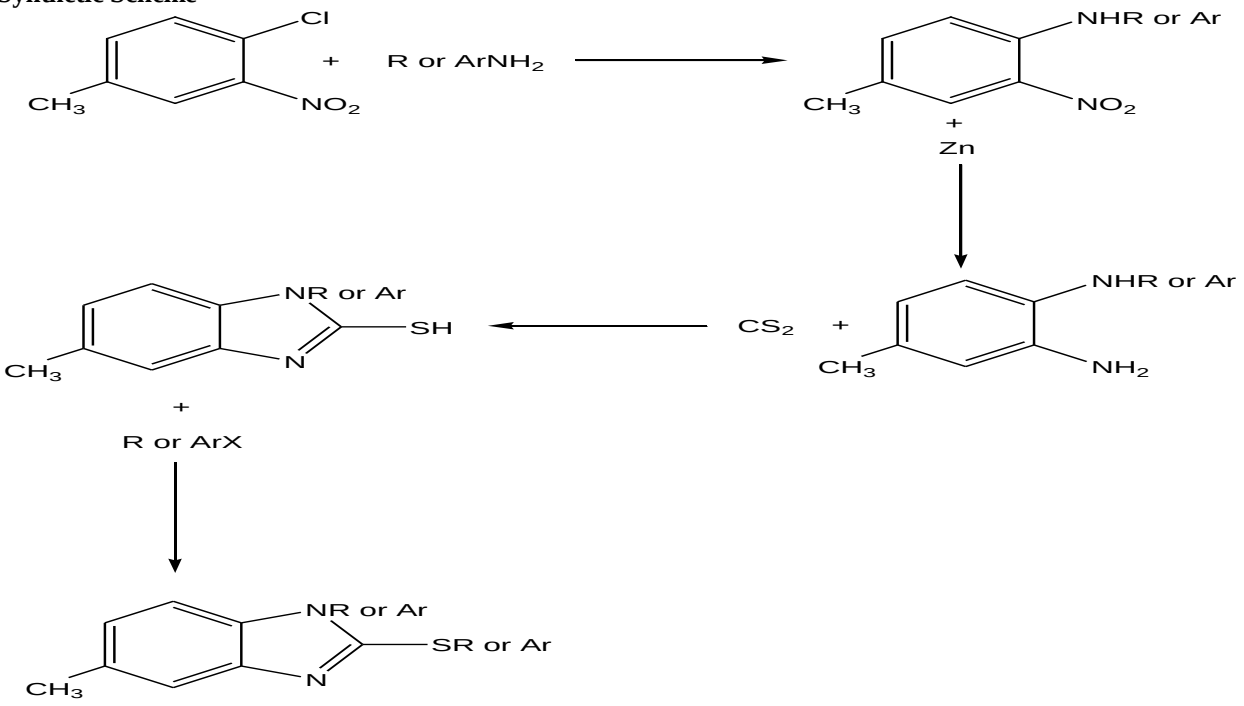
Antibacterial activity

The cup plate method was applied to determine the growth inhibition of bacteria by compounds to be tested. This method depends upon the diffusion of test substance from vertical cavity through solidified agar layer on petriplate. The growth of test microorganism is inhibited entirely around the circular area or the cavity containing test substance which is measured after the incubation for 24hrs at 37°C.

Determination of MIC

For minimum inhibitory concentration, tested and standard compounds were serially diluted with different concentration in Muller Hinton broth (MHB) at pH 7.4. The dilutions were inoculated with a suspension of bacterial culture in Muller Hinton Agar (MHA). After inoculation, tubes were incubated at 37°C for 24 hrs. Cell growth was determined by visible turbidity, in order to evaluate the results. The lowest compounds concentration, showing no visible growths after incubation were expressed as minimum inhibitory concentration (MIC) in µg/ml.

Synthetic Scheme



Spectral analysis of compounds

1] $C_{16}H_{16}N_2S$,Yield M.P 82-83°C Solubility: - Chloroform and Ethanol

Spectral analysis: - **IR Specturm** (KBr):1450cm^{-1} (C−N), 790 cm^{-1}(C-S)

NMR Specturm **^{1}H NMR** (300 MHz, $CDCl_3$): δ_H2.46 (s,-3H,-CH_3) δ_H3.45(s,-3H,-CH_3) δ_H3.54(s,-3H,-CH_3) δ_H4.56(s,-2H,-CH_2) δ_H7.05(q,-1H,-Ph) δ_H7.33(m,-5H,-Ph) 7.38(dd,-1H,-Ph) : δ_H 7.50 (s,-1H,-Ph)).^{13}C NMR (75.45 MHz, $CDCl_3$):24.34, 31.26, 112.56, 115.46, 125.82, 127.23,127.85,128.82,131.32,132.71, 138.83,139.77, 152.61 M^+268

[2] $C_{13}H_{18}N_2S$:- **IR Specturm:** δ max(KBr): 2850-2960 cm^{-1} (C- Hstr aliohatic) ,1487 (C−N), 788 cm^{-1}(C-S)

NMR Specturm: **^{1}H NMR** (300 MHz, $CDCl_3$): δ_H 7.4 (s,-1H,-Ph) δ_H 7.0 (d,-1H,-Ph) δ_H 6.9 (d,-1H,-Ph) δ_H 3.6(s,-3H,-CH_3) δ_H 3.3(t,-2H,-CH_2) δ_H 2.4(s,-3H,-CH_3) δ_H 1.7(m,-2H,

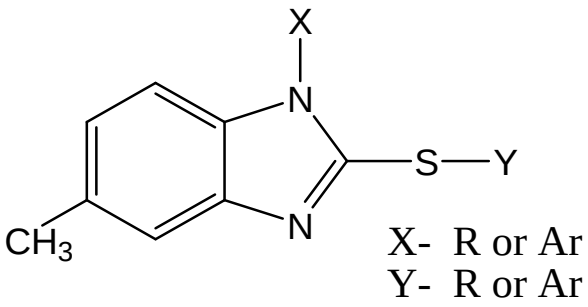
CH_2) δ_H 1.4 (m,-2H,-CH_2) δ_H 0.9 (t,-3H,-CH_3)

^{13}CNMR(75.45MHz,$CDCl_3$):13.5,21.4,21.7,29.9,31.3 ,32.3,107.8,117.9,123.1,131.4,134.5,143.1,152.0 and M^+234

BIOLOGICAL EVALUATION

The synthesized compounds were evaluated for antibacterial and antimycobacterial activity. Ampicillin was used as standard for antibacterial activity.

Serial No.	Code	X	Y
1.	HA-1	CH_3	CH_2-C_6H_5
2.	HA-2	CH_3	C_4H_9
3.	HA-3	C_2H_5	CH_2-C_6H_5
4.	HA-4	C_2H_5	C_4H_9
5.	HA-5	C_3H_7	CH_2-C_6H_5
6.	HA-6	C_3H_7	C_4H_9
7.	HA-7	C_4H_9	CH_2-C_6H_5
8.	HA-8	C_4H_9	C_4H_9
9.	HA-9	CH_2-C_6H_5	CH_2-C_6H_5
10.	HA-10	CH_2-C_6H_5	C_4H_9
11.	HA-11	C_6H_{11}	CH_2-C_6H_5
12.	HA-12	C_6H_{11}	C_4H_9



ANTIBACTERIAL ACTIVITY

The compounds were screened for their antibacterial activity against different bacterial strains (*E.coli*, *S.tyhpi*, *B.subtilis*, *S.aureus*) using concentration of 1000µg/ml in Dimethyl sulfoxide solution and evaluated by cup-plate or cylindrical plate method. The compounds were assigned codes by HA-1-12. The results obtained are shown in Table 1. It has been found that good activity was exhibited by HA-8 and HA-12 against all bacterial strains.

Table 1: *In vitro* zone of inhibition of various bacterial strains by benzimidazole (HA1-HA12) derivatives

Compound code	Growth inhibition zone diameter (mm)			
	E.coli (1678)	S.tyhpi (733)	B.subtilis (441)	S.aureus (3160)
HA-1	-	-	3	6
HA-2	-	-	5	6
HA-3	-	-	4	5
HA-4	-	-	4	7
HA-5	-	-	3	6
HA-6	-	-	-	4
HA-7	-	3	-	5
HA-8	5	8	5	8
HA-9	-	4	-	-
HA-10	-	-	-	-
HA-11	-	3	-	-
HA-12	6	7	4	9
Ampicillin	11	14	10	12

RESULTS AND DISCUSSION:

The *in vitro* evaluation of the synthesized compound against four different bacterial strains i.e *E.coli, S.typhi, B.subtilis, S.aureus* have shown moderate to significant activity. Compound HA-1,2,3,4 and 5 shows less zone of inhibition ranges between 3mm to 7mm against gram positive strains i.e. *B.subtilis* and *S.aureus* as compared to Ampicillin (standard) but no activity against gram negative strains i.e. *E.coli* and *S.typhi*. Compound HA-6 shows mild activity against *S.aureus* but no activity against all other strains. Compound HA-7 shows zone of inhibition against one gram negative bacteria i.e. *S.typhi* and one gram positive bacteria i.e. *S.aureus* and no activity against *E.coli* and *B.subtilis*. Compound HA-8 and HA-12 shows good zone of inhibition against all strains [15]. Compound HA-10 shows no inhibition against all strains. Compound HA-9 and HA-11 having aromatic group at 1-position and benzyl at 2-position shows inhibition against only one gram negative strain i.e *S.typhi* and no inhibition against all other strains.

Further MIC values of these two compounds lies within the range of less than 125µg/ml and greater than 500µg/ml. Thus, it can be found that mostly compounds having aliphatic group at 1-position of benzimidazole ring shows activity against gram positive strains i.e. *B.subtilis* and *S.aureus*. Compound HA-12 shows MIC value less than 125µg/ml against *E.coli* strain and less than 250 µg/ml against *S.aureus* and less than 500µg/ml against *S.typhi* [16]. Compound HA-8 shows moderate MIC value greater than 500µg/ml against all strains (Table 2).

Table 2: In vitro antibacterial activity of compound HA-8 and HA-12 against E.coli, S.typhi, B.subtilis, S.aureus (MIC expressed in µg/ml)

Concentration	67 µg/ml			125 µg/ml			250 µg/ml			500 µg/ml		
Strains	HA-8	HA-12	Ampicillin	HA-8	HA-12	Ampicillin	HA-8	HA-12	Ampicillin	HA-8	HA-12	Ampicillin
E.coli	-	-	>67	-	>125	-	-	+	-	<500	+	-
S.typhi	-	-	>67	-	-	-	-	-	-	<500	>500	-
B.subtilis	-	-	>67	-	-	-	-	-	-	<500	<500	-
S.aureus	-	-	>67	-	-	-	-	>250	-	<500	+	-

CONCLUSION:

The synthesized compounds have been evaluated for their in- vitro activity against four bacterial strains. Out of twelve compounds selected for antibacterial evaluation, two compounds show good zone of inhibition and MIC values against all strains. The good MIC value less than 125µg/ml against *E.coli* is shown by compound 12 having cyclohexyl at 1-position with butyl substitution at 2- position on thiol group. Thus, from the present study it can be concluded that cyclohexyl derivatives of benzimidazole ring have significant antibacterial activity.

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