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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF 2-SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Some 2- substituted benzimidazole derivatives were synthesized by condensation of o-phenylenediamine with carboxylic acid in presence of ring closing agents (polyphosphoric acid/Hcl).The Chemical structures of synthesized compounds were identified by spectral analysis. The synthesized compounds were screened for their in-vitro antibacterial activity against Standard strains by cup plate method.

Keywords: Benzimidazole, Antibacterial activity, Polyphosphoric acid.

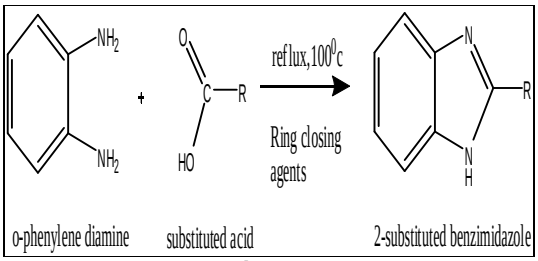
INTRODUCTION

Benzimidazole is a heterocyclic compound consists of benzene ring fused with imidazole ring. The chemistry and pharmacology of benzimidazoles have been of great interest to medicinal chemistry[1,2], because its derivatives possessed various biological activites such as anticancer[3], antihypertensive[4], anthelmenthic[5-7], anti-protozoal[8,9], antimicrobial[(10-15)], antioxidant[16,17],anti-inflammatory[18,19], analgesic[20] and anti-hepatitis-B-virus[21]. Moreover benzimidazoles are important intermediates in organic reaction.

A number of methods have been[22,23] reported for the synthesis of benzimidazoles and its derivatives. These methods include the coupling of o-phenylenediamine with carbonyl compounds in presence of various catalysts like zrcl$_4$, Sncl$_4$.5H$_2$o, BF3Et2o, polyethylene glycol, ceric ammonium nitrate[24]. In present study we reported the synthesis of 2-alkyl & aryl substituted benzimidazole derivatives in presence of ring closing agents and screened for anti bacterial activity.

MATERIAL AND METHODS

All the chemicals solvents used for this work were obtained from s d fine-chem limited (SDFCL), MUMBAI. Melting point of synthesized compounds were determined in open capillary tube using kshitij melting point apparatus, expressed in ^{0}c and were uncorrected silica gel chromatographic plates were used for TLC and solvent systems were ethylacetate : n-hexane (7:3) for all compounds. The purity of the compounds was checked by TLC and spots were visualised by iodine vapours [25]. IR spectra were recorded in KBr on bruker FT-IR spectrometer. The synthesis of compounds were carried according to scheme-1



Scheme-1

General Procedure for the Synthesis of 2-Substituted Benzimidazoles

Ortho Phenylenediamine (1mole) was made to condense with carboxylic acid derivatives (1mole) in presence of ring closing agents like hydrochloric acid or polyphosphoric acid. The mixture was kept for reflux and progress of the reaction was monitored by TLC. On completion of reaction, the reaction mixture was cooled and poured on to crushed ice. The cooled mixture was made basic by the gradual addition of concentrated ammonia solution. The precipitated product was then filtered and recrystallized from hot water. Decolourise with charcoal if necessary.

IR spectral data of synthesized compounds

1. 1H-benzo[d]imidazole(BZ): IR (KBr) cm^{-1}: 3413.67 (aromatic-NH streching), 1477.77 (-C=C streching), 1620 (-C=N streching), 3113.81 (=C-H streching), 1272.85 (-C-N streching), 1409.16 (aromatic –NH bending).
2. 2-Methyl-1H-benzo[d]imidazole(MBZ): IR (KBr) cm^{-1}: 3445.38 (aromatic-NH streching), 1462.98 (-C=C streching), 1556.17 (-C=N streching), 3176.26 (=C-H streching), 1270.86 (-C-N streching), 1477.09 (aromatic –NH bending), 2994.57 and 2874.95 (aliphatic- CH_3 Streching), 1386.23 (aliphatic $–CH_3$ bending).
3. 2-(Chloromethyl)-1H-benzo[d]imidazole(ClBZ): IR (KBr) cm^{-1}: 3390.67 (aromatic-NH streching), 1511.60 (-C=C streching), 1676.80(-C=N streching), 3055.76 (=C-H streching), 1271.04 (-C-N streching), 1430.42(aromatic –NH bending), 742.55 (-C-Cl).
4. 4-(1H-benzo[d]imidazol-2-yl)aniline(PABZ): IR (KBr) cm^{-1}: 3385.05 (aromatic-NH streching), 3470.12 (aromatic – NH_2 streching), 1500.59 (-C=C streching), 1605.21 (-C=N streching), 3143.35 (=C-H streching), 1274.01 (-C-N streching), 1444.98 (aromatic –NH bending).

Antibacterial Activity

The compounds were tested for their *in vitro* growth inhibitory activity against different bacteria.

The various organisms like *Staphylococcus aureus* ATCCBAA 1026, *Bacillus subtilis* ATCC 11774, *Staphylococcus werneri* ATCC 27836 (all Gram positive) and *Pseudomonas aeruginosa* ATCC 10662, *Proteus mirabilis* ATCC 14153 (all Gram negative) were procured from Microbes Speciality Lab, Danavaipeta, Rajahmundry, East Godavari District 533103, Andhra Pradesh, India. The inhibition zones of synthesized compounds were determined using cup plate method [26].

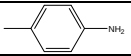
The sterilized medium (autoclaved at 121°C for 20min) was inoculated using 18hr slant cultures of the test organisms and transferred into sterile petri dishes and allowed to solidify the media. Cups of 8mm diameters were made on solidified media. Solutions of the synthesized compounds at a concentration of 50µg/ml and 100µg/ml were prepared in DMSO. 50µl of each solution was placed in cups by means of sterile pipette. In each plate one cup was used for standard and other two for test solutions. The plates thus prepared were left for 90min in a refrigerator for diffusion. The plates were incubated at 37°C for 24hrs and examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition was recorded. Gentamycin (50µg/ml) was used as standard.

RESULTS AND DISCUSSION

Chemistry

Different types of organic acids (aliphatic and aromatic) were used to condense with o-phenylenediamine to synthesise 2-sustituted benzimidazoles[27, 28]. The purity of synthesized compounds was checked by TLC and melting point. The physicochemical data of all synthesized compounds was represented in **table 1**. The synthesized compounds were analyzed by Infrared Spectroscopy.

Table 1

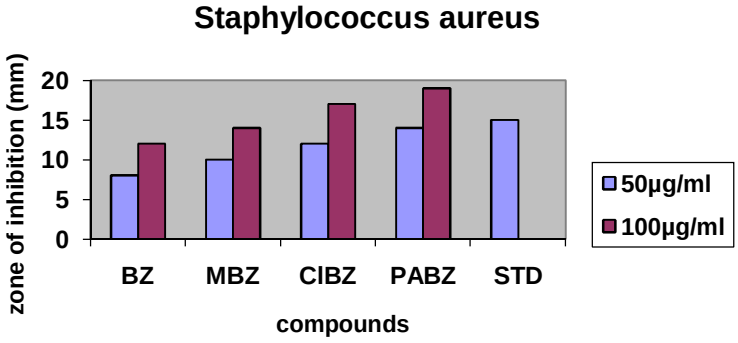
ENTRY	ACID	SUBSTITUTION	MOLECULAR FORMULA	MOLECULAR WEIGHT	MELTING POINT	TIME [MIN]	PERCENTAGE YIELD	RF VALUE
1.	HCOOH [FORMICACID]	-H	$C_7\ H_6\ N_2$	118gms	170^0c	3O	73.5	0.575
2.	CH_3COOH [ACETIC ACID]	$-CH_3$	$C_8H_8N_2$	132.16gms	180^0c	45	50	0.68
3.	$NH_2C_6H_4COOH$ [PABA]		$C_{12}H_{22}N_3$	209.25gms	310^0c	120	31.1	0.88
4.	$ClCH_2COOH$ [CHLOROACETIC ACID]	$-CH_2Cl$	$C_8H_7N_2Cl$	166.61gms	210^0c	45	54.6	0.565

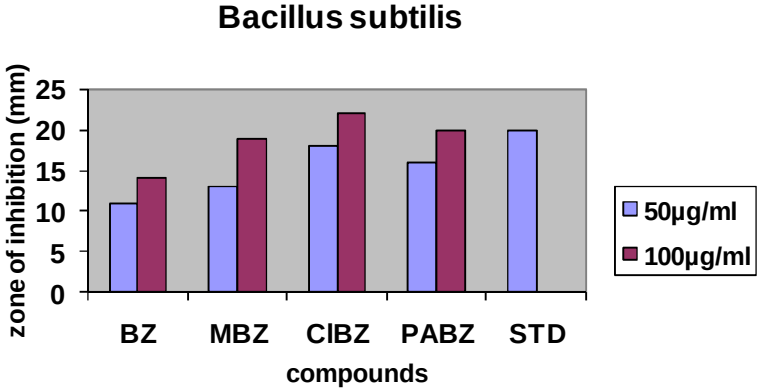
The *in vitro* antibacterial activity was performed using cup plate method with different strains of gram positive and gram negative bacteria. Gentamycin was used as standard. The results of final compounds for antibacterial activity were recorded in **table2** & **figure1,2.** The results revealed that synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the inhibitory activity against gram positive bacteria was higher than that of gram negative bacteria. Among the synthesized compounds, ClBZ showed potential antibacterial activity. The activity was due to the presence of halogen on substituent at C-2 of benzimidazole ring.

Table 2: Antibacterial Activity of Synthesized Compounds

Entry	Compound	ZONE OF INHIBITION (mm)									
		Gram positive bacteria						Gram negative bacteria			
		S. aureus		B. subtilis		S. werneri		P. mirabilis		P. aeruginosa	
		50 µg/ ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1	BZ	8	12	11	14	17	18	10	12	10	13
2	MBZ	10	14	13	19	16	19	11	15	16	19
3	ClBZ	12	17	18	22	24	27	14	19	17	22
4	PABZ	14	19	16	20	22	25	13	17	18	21
5	Control	-	-	-	-	-	-	-	-	-	-
6	STANDARD (Gentamycin 50 µg/ml)	15		20		24		18		20	

Control – DMSO, -- No activity





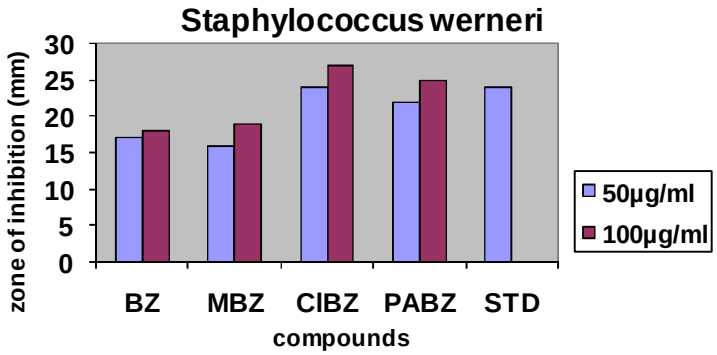
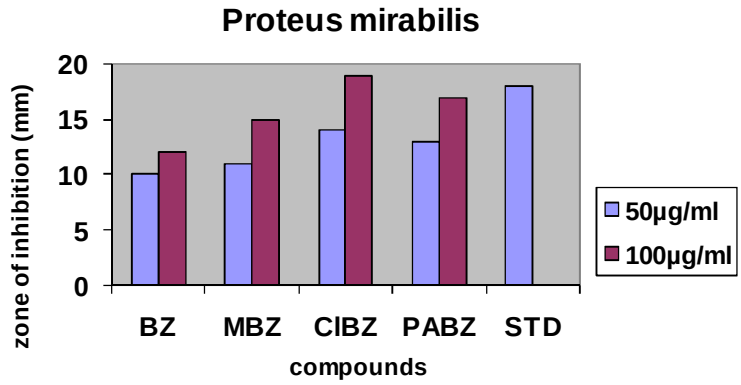
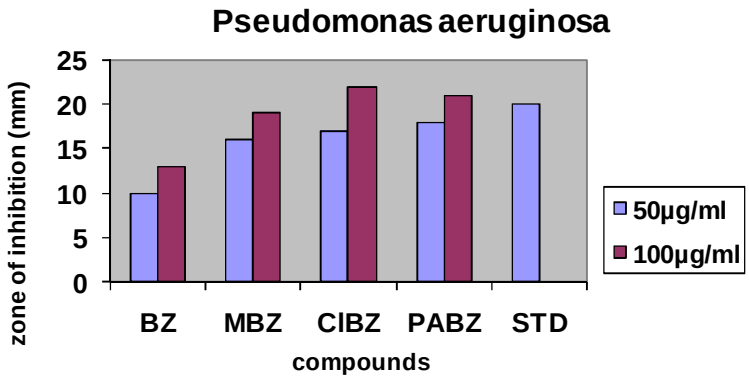


Fig. 1: Zone of Inhibition of Synthesized compounds against Gram Positive organisms





Note: Standard (STD) = GENTAMYCIN

Fig. 2: Zone of Inhibition of Synthesized compounds against Gram Negative organisms

CONCLUSION

A simple, convenient, synthetic method was developed for synthesis of 2-substituted benzimidazoles. Among the synthesized compounds, ClBZ exhibited prominent antibacterial activity. Further analysis of structure by NMR, Mass Spectroscopy is required to interpret the synthesized compounds. More extensive study is needed to confirm the mode of action studies to optimize the effectiveness of these compounds.

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