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Benzimidazole compounds: As antimicrobial agents

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ABSTRACT

Heterocyclic compounds exhibited remarkable pharmacological activities. Literature indicates that compounds of pyrimidine, pyridine, benzimidazole nucleus have wide range of therapeutic uses such as antitubercular, anticancer, antihelmintic, antioxidant and antimicrobial activities. It is also believed that the presence of >N-C=S linkage is responsible for the amoebicidal, anticonvulsant, fungicidal and antiviral activities. In this direction, the work is being pursued to investigate the antimicrobial activity of some heterocyclic compounds prepared in our laboratory. Various benzimidazole derivatives of o-phenylene diamine , 4,5-dimethyl-1,2-phenylene diamine , 4-chloro-1,2-phenylenediamine **(IVa,b,c respectively)**, S-methylated o-phenylene diamine , S-methylated 4,5-dimethyl-1,2-phenylene diamine , S-methylated 4-chloro-1,2-phenylenediamine **(Va,b,c respectively)** have been synthesized. All the synthesized derivatives have been screened with various bacterial and fungal strains viz. *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonaie., Penicillium chrysogenum, Aspergillus niger, Aspergillus japanicus, Microsporum gypseum.* After the antimicrobial studies, it were found that Compound **(IVb, Vb, Vc)** showed excellent activity against the becterial strain of *Pseudomonas aeruginosa* and compound **(IVa, Va)** showed very good activity against the fungal strain of *Aspergillus niger* and compound **(Vb)** also showed excellent activity against the fungal strain of *Aspergillus japanicus* as these derivatives showed more inhibition zone than the standard drug Amoxycillin and Ketoconazole respectively. Thus these compounds can be used as a standard drug having less side effects.

Keywords: Benzimidazole compounds, antibacterial activity, antifungal activity.

1. INTRODUCTION

A wide variety of benzimidazole derivatives have been described for their chemotherapeutic importance. The similarity in ring structure between the benzimidazole nucleus and natural purines, hypoxanthine or guanines, made it one of the most important nuclei that has been reported to be associated with microbial potency. [1] Moreover, an appreciable antimicrobial activity was found to be associated with many banzimidazole derivatives carrying different heterocyclic ring systems at their 2-position. The introduction of a thiazolidinone nucleus to enhance the antimicrobial activity of benzimidazoles was demonstrated recently in several studies from the laboratories. A wide application of benzimidazoles in agriculture and veterinary medicine as fungicides and anthelminthic drugs, and their experimental use in cancer chemotherapy, has led to intensive research to elucidate their mode of action in detail.[2] Various benzimidazole derivatives are known to possess a broad spectrum of biological activity such as antitrichomonad [3], antiviral [4], anticoagulant[5], anti-inflammatory [6,], analgesic [7], antitumor [8], antimicrobial activities. [9,10,11,12,13,14,15,] In continuation of our efforts in search of potential anti-inflammatory, analgesic and antiamoebic activities [16],we have studied the reactions of various derivatives of o-phenylenediamine, 4,5-dimethyl-1,2-phenylenediamine and 4-chloro-1,2-phenylenediamine with 4-isothiocyanato-4-methylpentan-2-one (MOIC)and evaluated them for their antibacterial and antifungal activities.

2 MATERIALS AND METHODS:

Step 1: Synthesis of 4-isothiocyanato-4-methylpentan-2-one (MOIC):
4-Isothiocynanato-4-methylpentan-2-one was prepared by adding sulphuric acid (27 ml; 0.25 mole) diluted with 25 ml. distilled water to mesityl oxide (49 ml; 0.5 mole) over a period of 25 minutes at 15°C. Ammonium thiocyanate (38 g; 0.5 mole) dissolve in 50 ml. distilled water was added to the above prepared mixture at 21°C. After stirring of 15 minutes, the upper oily layer was separated and washed with aqueous sodium carbonate and finally with water to free it from acid. The contents were left over fused calcium chloride for 24 hrs. and subjected to fractionation. [16] The pure product was collected, the yield being 30.2 ml. (38.47%). **(Scheme-1).**

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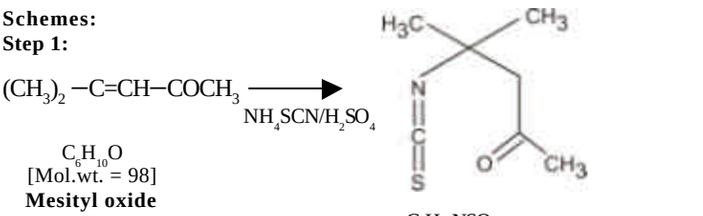
Schemes:
Step 1:



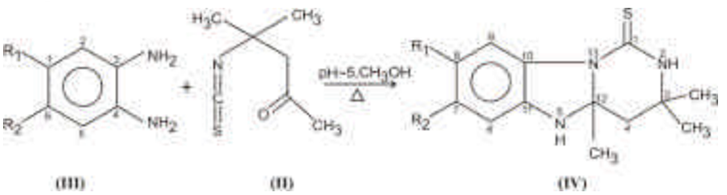
$C_6H_{10}O$
[Mol.wt. = 98]
Mesityl oxide
(I)

$C_7H_{11}NSO$
[Mol.wt. = 157]
4-Isothiocyanato-4-methylpentan-2-one [MOIC]
(II)

Scheme-1 Synthesis of 4-Isothiocyanato-4-methylpentan-2-one (MOIC).

Step 2: General procedure for the condensation of different substituted phenylene diamine with 4-Isothiocyanato-4-methylpentan -2-one:
4-Isothiocyanato-4-methylpentan-2-one (0.8ml; 5 mmole) is added to a solution of different substituted phenylene diamine (1.0g) in methanol (10-20 ml.). The pH of the reaction medium was adjusted to about 5 by adding a few drops of 10% sulphuric acid (10% sulphuric acid in methanol). The reaction mixture was heated under reflux for 8 hrs. After about 20 minutes, solid product started to separate out. After cooling, the solid was collected and washed with chilled methanol to give compound. The remaining compound in reaction solution is separated by the column chromatography and yield were different for different compounds. **(Scheme-2)**

Step 2:



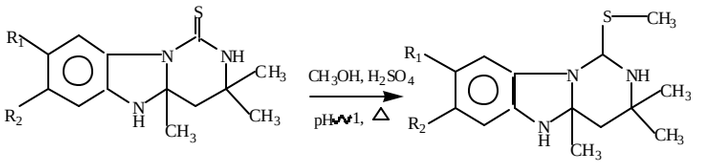
(III) (II) (IV)

Where,	Mol. Formula	Mol. Wt.
(a) R_1 = H, R_2 = H,	$C_{13}H_{17}N_3S$	247
(b) R_1 = CH_3, R_2 = CH_3,	$C_{15}H_{20}N_3S$	274
(c) R_1 = H, R_2 = Cl,	$C_{13}H_{16}N_3SCl$	281.5

Scheme-2 Condensation of different substituted phenylene diamine with 4-Isothiocyanato-4-methylpentan-2-one (MOIC).

Step 3: General procedure for S-methylation of different substituted phenylene diamine derivatives :
Different substituted phenylene diamine derivatives (1 g) was dissolve in a minimum amount of CH_3OH and to it was added concentrated sulphuric acid (1ml). Reaction contents having pH~1 was heated under reflux for 8 hrs. and then solvent was removed under reduced pressure. The residue left behind was basified with 50% aqueous sodium carbonate solution. Solid product separated out was filtered, washed with water and air dried to give crude product. The crude product was purified by column chromatography over silica-gel. **(Scheme-3)**

Step 3:



IV **V**

Where,		**Mol. Formula**	**Mol. Wt.**
(a) R_1 = H,	R_2 = H,	$C_{13}H_{20}N_3S$	250
(b) R_1 = CH_3,	R_2 = CH_3,	$C_{15}H_{24}N_3S$	278
(c) R_1 = H,	R_2 = Cl,	$C_{13}H_{19}N_3SCl$	284.5

Scheme-3 S-methylation of different substituted phenylene diamine derivatives.

Step 4: Antimicrobial Assay:
The *invitro* antibacterial and antifungal effect of benzimidazole derivatives were determined by Disc and Hole method. The bacterial strains were sub-cultured in Muller-Hinton broth and incubated at 37°C for 24 hrs. Turbidity of the suspension was adjusted to the Mac Farland Standard (0.5) and 100 µl of suspension plated on Muller-Hinton agar, wells were made with the help of (6 mm) borer. Prepare the solution of each compounds and standard drug in 200 mg/ml concentration and 100 µl of each solution of compounds loaded in each well against the control (solvent) and standard drug amoxicillin. Plates were incubated at 37^{0}C for 24 hrs and recorded the zone of inhibition or sensitivity against *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonaie* and compared with the standard drug amoxicillin.

For antifungal test, the fungal cultures were grown in Sabourauds dextrose agar for 96 hrs adopting the above procedure, made suspension of sub-cultured organisms. Plates were incubated at 26°C for 72 hrs and recorded the zone of inhibition or sensitivity against *Penicillium chrysogenum, Aspergillus niger, Aspergillus japanicus, Microsporum gypseum* and comparaed with the standard drug Ketoconazole.

3 RESULTS AND DISCUSSION
The physical properties and spectral data of various prepared Benzimidazole derivatives **(IVa, IVb, IVc, and Va, Vb, Vc)** are given in **Table-1**.

Antibacterial activity indicated that, o-phenylenediamine derivative **(IVa)** was mild active against *Escherichia coli, Klebsiella pneumonaie, Pseudomonas areuginosa* and showed comparable activity against *Proteus mirabilis.* 4,5-dimethyl-1,2-phenylenediamine derivative **(IVb)** was inactive against *E.coli* and very mild active against *Klebsiella pneumonaie* and *Proteus mirabilis.*. This derivative showed excellent activity against *Pseudomonas areuginosa.* 4-chloro-1,2-phenylenediamine derivative **(IVc)** was inactive against *Proteus mirabilis* and very mild active against *Escherichia coli* and *Klebsiella pneumonaie.* This derivative showed comparable activity against *Pseudomonas aeruginosa.* S-methylated o-phenylenediamine derivatives **(Va)** was mild active against *Pseudomonas areuginosa, Proteus mirabilis, Escherichia coli* . This derivative showed good activity against *Klebsiella pneumonaie.* S-methylated-4,5-dimethyl-1,2-phenylenediamine derivative **(Vb)** was inactive against *Escherichia coli* and *Proteus mirabilis* and very mild active against *Klebsiella pneumonaie*. This derivative showed excellent activity against *Pseudomonas areuginosa*. S-methylated 4-chloro-1,2-phenylenediamine derivative **(Vc)** was inactive against *Proteus mirabilis* and very mild active against *Escherichia coli* and *Klebsiella pneumonaie.* This derivative also showed excellent activity against *Pseudomonas areuginosa.* Thus the derivatives **(IVb, Vb and Vc)** showed more potent activity against *Pseudomonas areuginosa* which was more than standard drug Amoxycillin. **(Table-2)** Antifungal activity indicated that, o-phenylenediamine derivative **(IVa)** was mild active against *Penicillium chrysogenum, Aspergillus japanicus, Microsporum gypseum*. This derivative showed excellent activity against *Aspergillus niger*. 4,5-dimethyl-1,2-phenylenediamine derivative **(IVb)** was mild active against *Aspergillus niger Penicillium chrysogenum, Aspergillus japanicus and Microsporum gypseum.* 4-chloro-1,2-phenylenediamine derivative **(IVc)** was inactive against *Aspergillus japanicus* and showed mild activity against *As-*

Table 1: The various prepared Benzimidazole derivatives (IVa, IVb, IVc, IVd and V) having following physical properties and spectral data.

Properties	**o-phenylene diamins derivative (IVa)**	**4,5-dimethyl-1,2-phenylene diamine derivative (IV-b)**	**4chloro-1,2-phenylene diamine derivative (IVc)**	**S-methylated o-phenylene diamins Derivative (Va)**	**S-methylated 4,5-dimethyl-1,2-phenylene diamine derivative (Vb)**	**S-methylated 4-chloro-1,2-phenylene diamine derivative (Vc)**
Yield (gm)	1.614	1.90	1.606	0.665	0.258	0.125
% Yield	74.3	94.05	81.32	86	24.80	25.2
m.p. (°C)	217	213	202	218	180	190
Solubility	$CHCl_3$, Dimethyl Sulfoxide	Dimethyl Sulfoxide	Dimethyl Sulfoxide, Tetra hydrofuran	Dimethyl formamide, Tetra hydrofuran	$CHCl_3$, Dimethyl formamide	$CHCl_3$, Dimethyl formamide
Element detection	N&S are present and Halogen are absent	N&S are present and Halogen are absent	N&S and Halogen are present	N&S are present and Halogen are absent.	N&S are present and Halogen are absent	N&S are present and Halogen are absent
Elution	Pet. Ether : $CHCl_3$ (5:5) $CHCl_3$ (Pure) $CHCl_3$:Ethyl acetate (9:1)	$CHCl_3$: Ethyl acetate (8:2)	$CHCl_3$ (Pure) $CHCl_3$: Ethyl acetate (9:1)	$CHCl_3$: Ethyl acetate (5:5)	$CHCl_3$: Ethyl acetate (5:5)	Pure $CHCl_3$
Solvent of Crystallization	MeOH	MeOH	MeOH	MeOH	MeOH	Me
IR (KBr) cm^{-1}	3215.26 (NH) 1603.5 (C=C) (Ar) 1177.32 (C=S) 890.35 (Substitution on Aromatic ring)	3198.05 (NH) 2966.39 (CH st) 1179.56 (C=S) 882.18 (Substitution on Aromatic ring)	3172.76 (NH) 1601.62 (C=C) 1178.75 (C=S) 898.95 (Substitution on Aromatic ring) 801.52 (C-Cl)	3224.81 (NH) 2972.14 (C-H,st) 1597 (C=N,str) 1378.26 (gem dimethyl bending in CH_3) 621.98(C=S)	3250.29 (NH) 1589.98 (C=O) 1496.16 (C=C) 1291.21 (CH def. gem dimethyl) 628.23 (C-S)	3230.09 (NH), 2970.03 (C-H,) 1616.85 (C==Nstr) 746.49 (C-Cl)
^{1}HNMR (DMSO) δ,J (H_2)	NMR was also done and reported our published paper .[16,18]					

Table 2: Antibacterial Activity of various prepared Benzimidazole derivatives and standard drug Amoxycillin.

Test Organisms	**O-phenylene diamine derivative (IVa)**	**4,5-dimethyle 1,2-phenylene diamine derivative (IVb)**	**4-Chloro -1,2-phenyle diamine derivative (IVc)**	**S-methylated o-phenylene diamins Derivative(Va)**	**S-methylated 4,5-dimethyl-1,2-phenylene diamine derivative (Vb)**	**S-methylated 4-chloro-1,2-phenylene diamine derivative (Vc)**	**Standard Drug Amoxycillin**
Escherichia coli	14mm	(-)	10mm	16mm	(-)	5mm	22mm
Pseudomonas areuginosa	12mm	**30mm**	20mm	10mm	**32mm**	**31mm**	26mm
Klebsiella pneumonaie	9mm	7mm	8mm	18mm	10mm	7mm	21mm
Proteus mirabilis	18mm	7mm	(-)	15mm	(-)	(-)	22mm

Table 3: Antifungal Activity of various prepared Benzimidazole derivatives and standard drug Ketoconazole:

Test Organisms	o-phenylene diamine derivative (IVa)	4,5-dimethyle 1,2-phenylene diamine derivative (IVb)	4-chloro- 1,2-phenyle diamine derivative (IVc)	S-methylated o-phenylene diamins Derivative(Va)	S-methylated 4,5-dimethy l-1,2-phenylene diamine derivative (Vb)	S-methylated4-chloro -1,2- phenylene diamine derivative (Vc)	Standard Drug Ketoconazole
Aspergillus niger	**50mm**	15mm	20mm	**50mm**	18mm	(-)	30mm
Penicillium chrysogenum	16mm	12mm	7mm	17mm	8mm	(-)	28mm
Aspergillus japanicus	17mm	25mm	(-)	10mm	**28mm**	5mm	31mm
Microsporum gypseum	18mm	10mm	15mm	20mm	8mm	10mm	30mm

pergillus niger Penicillium chrysogenum and *Microsporum gypseum.* S-methylated o-phenylenediamine derivatives **(Va)** was mild active against *Aspergillus japanicus, Penicillium chrysogenum* and *Microsporum gypseum*. This derivative showed excellent activity against *Aspergillus niger*. S-methylated-4,5-dimethyl-1,2-phenylenediamine derivative **(Vb)** was mild active against *Aspergillus niger Penicillium chrysogenum*, and *Microsporum gypseum* and showed excellent activity against *Aspergillus japanicus.* S-methylated 4-chloro-1,2-phenylenediamine derivative **(Vc)** was inactive against *Aspergillus niger, Penicillium chrysogenum* and mild active aganist *Aspergillus japanicus*, and *Microsporum gypseum*. Thus the derivative **(IVa, Va and Vb)** showed excellent activity against *Aspergillus niger* and *Aspergillus japanicus respectively* which was more than standard drug Ketoconazole. **(Table-3)** .

CONCLUSIONS

4,5-dimethyl-1,2-phenylenediamine derivative **(IVb)**, S-methylated-4,5-dimethyl-1,2-phenylenediamine derivative **(Vb),** and S-methylated 4-chloro-1,2-phenylenediamine derivative **(Vc)** acts as a standard drug against bacterial strain *Pseudomonas areuginosa*. The o-phenylenediamine derivative **(IVa),** S-methylated o-phenylenediamine derivatives **(Va)** act as a standard drug against the fungal strains *Aspergillus niger,* and S-methylated-4,5-dimethyl-1,2-phenylenediamine derivative **(Vb)** act as a standard drug against the fungal strains *Aspergillus japanicus* because it showed more inhibition zone than the standard drug Amoxycillin for bacterial strain and Ketoconazole for fungal strain.

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