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Study of Some Benzimidazole Compounds as Antibacterial and Antifungal Agents

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Abstract

There is a clinical need for new treatment option as a result of continued increase in the expression of resistance among bacterial and fungal pathogens. A number of compounds currently in development show promise. However, in some cases, there is concern that resistance may develop quickly to new compounds that are based on existing antimicrobial agents. So it is therefore desirable to develop new potent antifungal, antibacterial drugs which produces minimum or no side effect and low cost. In continuation of this studies, some benzimidazole derivatives have been prepared and screened their antimicrobial activities with various strains.

Various benzimidazole derivative of o-phenylene diamine, 4,5-dimethyl-1,2-phenylene diamine, 4-chloro-1,2-phenylenediamine, 3,4-diaminobenzophenone and S-methylated 3,4-diaminobenzophenone have been prepared by the reaction of 4-isothiocyanato-4-methyl pentan-2-one. All synthesized derivatives have been screened with the various bacterial and fungal strains viz. *Escherichia coli, Bacillus pumilus, Micrococcus luteus, Bacillus cereus, Klebsiella pneumonaie, Aspergillus niger, Aspergillus flavus, Trichosporum flavurusclem and Microsporum gypseum.*

After the antimicrobial studies, it were found that the S-methylated 3,4-diamino benzophenone derivative (V) acts as a standard drug against bacterial strain *Klebsiella pneumonaie* and all tested fungal strains, because it showed more inhibition zone than the standard drug Amoxycillin and Ketoconazole respectively.

Keywords: Antibacterail activity, Antifungal activity, Benzimidazole derivatives, 4-Isothiocyanato-4-methylpentan-2-one (MOIC).

INTRODUCTION

Benzimidazoles are an important class of heterocyclic compounds, several derivatives of which have been found to possess diverse types of biological activities. The compounds bearing a thiazolyl, pyridyl and indolyl moieties possess a wide spectrum of biological activities which is related to their capacity to transfer electrons, to scavenge reactive oxygen species and presence of >N-CH=S linkage. It is believed that these properties are responsible for the amoebicidal, anticonvulsant, fungicidal, antibacterial and antiviral activities. Defining and redefining the use of antimicrobial therapy is significant on both an individual and a global level because the emergence of resistant organisms is a public health concern. The development of new and different antimicrobial agents has been a very important step. [1] Much of the research programme efforts are directed toward the design of new and available drugs because of the unsatisfactory status of present drugs, side effects and the acquisition of resistance by the infecting organisms [2-4]. Various review has been written on the recent development in chemical and biological profiles of heterocyclic systems such as antitumor [5], anti-inflammatory [6], analgesic [7] and antimicrobial activities [8-14]. In continuation of our efforts in search of potential anti-inflammatory and analgesic agents [15], we have studied the reactions of 4-isothiocyanato-4-methylpentane-2-one with o-phenylenediamine and their derivatives and evaluated the resulting benzimidazole compounds for their antimicrobial activity, which we wish to report in this paper.

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 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)**

Step 2: General procedure for the condensation of different substituted phenylene diamine with 4-Isothiocyanato-4-methylpentan -2-one:

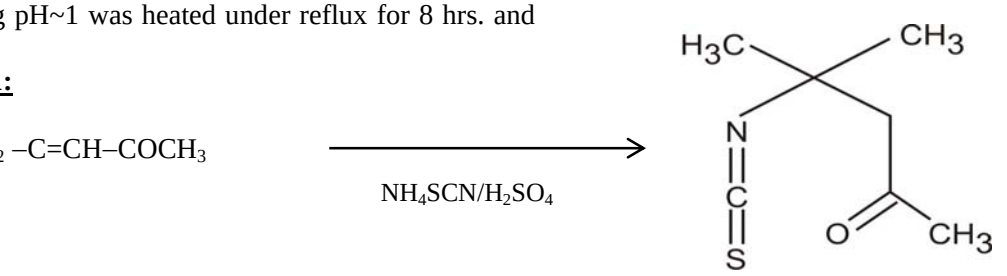
4-Isothiocyanato-4-methylpentan-2-one (0.8ml; 5 mmole) is added to a solution of different substituted phenylene diamine (1.0g) in methanol (10-20 ml.). The pH of the reaction medium was adjusted to about 5 by adding a few drops of 10% sulphuric acid (10% sulphuric acid in methanol). The reaction mixture 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 3: General procedure for S-methylation of 3,4-diamino benzophenone derivative (IVd):

3,4-diaminobenzophenone (IVd) (1 g; 3 mmole) was dissolve in CH_3OH (20ml) 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% aq. 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 1:

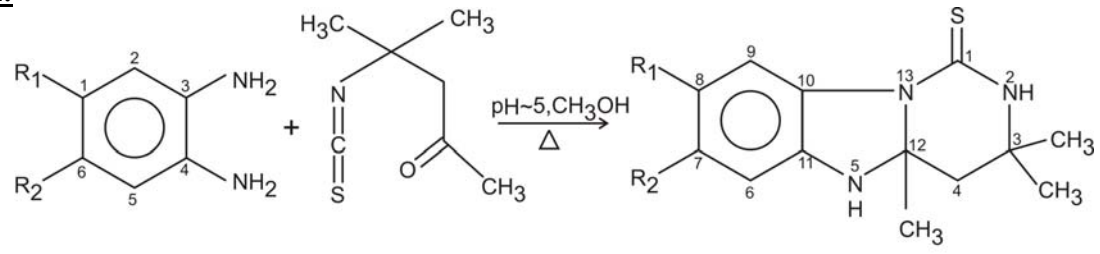


$C_6H_{10}O$
[Mol.wt. = 98]
Mesityl oxide
(I)

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

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

Step 2:

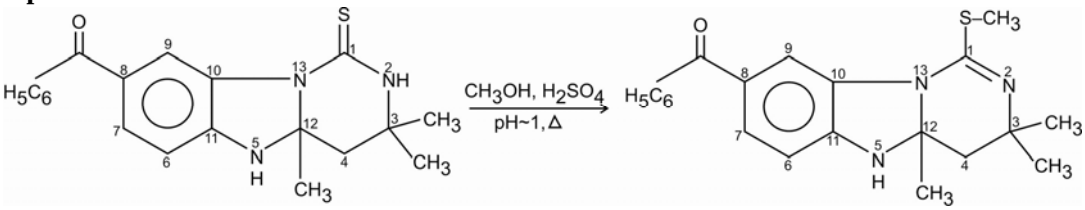


(III) **(II)** **(IV)**

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,	$C_{15}H_{21}N_3S$	275
(c)	R_1 = H, R_2 = Cl,	$C_{13}H_{16}N_3SCl$	281.5
(d)	R_1 = C_6H_5CO, R_2 = H,	$C_{20}H_{21}N_3SO$	335

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

Step 3:



$C_{20}H_{21}N_3SO$
[Mol.wt. = 335]
3,4-diaminobenzophenone derivative
(IVd)

$C_{21}H_{23}N_3SO$
[Mol.wt. = 349]
S-methylated 3,4-diaminobenzophenone derivative
(IVd)

Scheme-3 S-methylation of 3,4-diaminobenzophenone derivative (IVd).

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 37^0C for 24 hrs and recorded the zone of inhibition or sensitivity against *Escherichia coli, Bacillus cereus, Micrococcus luteus, Bacillus pumilus, Klebsiella pneumonaie.*

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 *Aspergillus niger, Aspergillus flavus, Microsporum gypseum, and Trichosporum flavurusclem* comparable with Ketoconazole..

RESULTS AND DISCUSSION

The physical properties and spectral data of various prepared Benzimidazole derivatives (IVa, IVb, IVc, IVd and V) are given in Table-1.Antibacterial activity indicated that, o-phenylenediamine derivative (IVa) was inactive against *E.coli, B. pumilus, M. luteus* and *Klebsiella pneumonaie* and mild active against *B. cereus*. 4,5-dimethyl-1,2-phenylenediamine derivative (IVb) was inactive against *E.coli, M. luteus* and very mild active against *Klebsiella pneumonaie.* This derivative showed significant activity against *B. cereus* and *B. pumilius*. 4-chloro-1,2-phenylene diamine derivative (IVc) was inactive against *M. luteus* and *B. cereus* and very mild active against *E.coli.* This derivative showed significant activity against *B. pumilus* and *Klebsiella pneumonaie.* 3,4- diamino benzophenone derivative (IVd) was inactive against *E.coli* and *B. pumilus* and very mild active against *M. luteus.* This derivative showed significant activity against *B. cereus* and *Klebsiella pneumonaie.* S-methylated 3,4-diaminobenzophenone derivative (V) was inactive against *E.coli* and very mild active against *B. pumilus* and *M. luteus*.

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)***	***4-chloro-1,2-phenylene diamine derivative (IVc)***	***3,4-diamino banzophenone derivative (IVd)***	***5-methylated 3,4-diamino benzophenone derivative (V)***
Yield (gm)	1.614	1.90	1.606	1.299	0.258
% Yield	74.3	94.05	81.32	82.21	24.80
m.p. (°C)	217	208	204	223	180
Solubility	CCl_4, Dimethyl Sulfoxide	Dimethyl Sulfoxide	Dimethyl Sulfoxide,Tetra hydrofuran	Dimethyl formamide, Tetra hydrofuran	$CHCl_3$, 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
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 (8:2)	$CHCl_3$: Ethyl acetate (5:5)
Solvent of Crystallization	MeOH	MeOH	MeOH	MeOH	MeOH
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)	3242.81 (NH) 1641.80 (C=O) 1498.71 (C=C) 1291.18 (CH def. gem dimethyl) 1200.85 (C=S)	3250.29 (NH) 1589.98 (C=O) 1496.16 (C=C) 1291.21 (CH def. gem dimethyl) 628.23 (C-S)
^{1}HNMR (DMSO) δ,J (H_2)	NMR was also done and reported in our published paper [15].				

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)***	***3,4-diamino benzophenone derivative (IVd)***	***S-methylated 3,4-diamino benzophenone derivative (V)***	***Standard Drug Amoxycillin***
Escherichia coli	(-)	(-)	4mm	(-)	(-)	37mm
Bacillus pumilus	(-)	20mm	15mm	(-)	3mm	40mm
Micrococcus luteus	(-)	(-)	(-)	2mm	10mm	53mm
Bacillus cereus	18mm	18mm	(-)	22mm	28mm	30mm
Klebsiella pneumonaie	(-)	12mm	15mm	18mm	32mm	27mm

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)***	***3,4-diamino benzophenon e derivative (IVd)***	***S-methylated 3,4-diamino benzophenon e derivative (V)***	***Standard Drug Ketoconazole***
Aspergillus niger	(-)	(-)	10mm	(-)	15mm	16mm
Aspergillus flavus	11mm	(-)	19mm	(-)	35mm	34mm
Trichosporum flavurusclem	(-)	8mm	12mm	10mm	22mm	22mm
Microsporum gypseum	18mm	14mm	19mm	20mm	28mm	22mm

This derivative showed good activity against *B. cereus*, which was more closely to the standard drug Amoxycillin. This derivative showed more potent activity against *Klebsiella pneumonaie* which was more than standard drug Amoxycillin. (Table-2) Antifungal activity indicated that, o-phenylenediamine derivative (IVa) was inactive against *A. niger, T. flavurusclem* and mild active against *A.flavus.* This derivative showed significant activity against *M. gypseum* . 4,5-dimethyl-1,2-phenylene diamine derivative (IVb) was inactive against *A. niger, A. flavus* and mild active against *T. flavurusclem, M. gypseum.* 4-chloro-1,2-phenylene diamine derivative (IVc) showed mild activity against *A. flavus* and *T. flavurusclem*. This derivative showed significant activity against *A. niger* and *M. gypseum.* 3,4-diamino benzophenons derivative (IVd) was inactive against *A. niger, A. flavus* and mild active against *T. flavurusclem.* This derivative showed significant activity against *M. gypseum* . S-methylated 3,4-diaminobenzophenone derivative (V) showed good activity against *A. niger, A. flavus, T. flavurusclem* which were more closely to the standard drug. This derivative showed more potent activity against *M. gypseum* because the compound (V) had more inhibition zone than the standard drug Ketoconazole.

CONCLUSIONS

S-methylated 3,4-diaminobenzophenone derivative (V) acts as a standard drug against bacterial strain *Klebsiella pneumonaie* and against all fungal strains *A. niger, A. flavus, T. flavurusclem and M. gypseum* because it showed more inhibition zone than the standard drug Amoxycillin and Ketoconazole respectively.

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