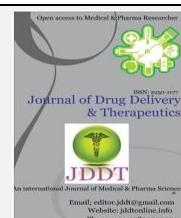


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Research Article

Synthesis, characterization of 2-substituted benzimidazole derivatives and evaluation of antimicrobial activity

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ABSTRACT

Benzimidazole has attracted a great deal of importance due to their interesting chemistry and wide utility. Benzimidazole has wide range of uses and applications and is produced in great quantities throughout the world. The current research work was aim to evaluate the in vitro antifungal activity of series of benzimidazole derivatives. A series of benzimidazole derivatives were synthesized by reacting the various amine derivatives of o phenylene diamine with carbon disulphide in presence of Potassium hydroxide. All the bis derivatives were characterized by IR, ¹H NMR and chromatography method (TLC). The antibacterial activity was evaluated by their MIC and zone of inhibition of synthesized compounds by taking ciprofloxacin as reference standard. The microbiological assay revealed that the compounds show promising antibacterial activity.

Keywords: Ciprofloxacin, Benzimidazole, antibacterial activity.

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INTRODUCTION

Benzimidazole

Historically, the first benzimidazole was prepared in 1872 by **Hoebrecker** who obtained 2, 5 or 2, 6-dimethylbenzimidazole by the reduction of 2-nitro-4-methyl-acetanilide. Several years later **Ladenburg** obtained the same compound by refluxing 3, 4-diaminotoluene with acetic acid. The benzimidazoles are also known as Benzimidazoles or Benzoglyoxalines.

Benzimidazoles are weakly basic, being somewhat less basic than the imidazoles. Benzimidazoles are also sufficient acidic to be generally soluble in aqueous alkali and form N-metallic compounds. The acidic properties of benzimidazoles, like those of the imidazoles seen to be due to stabilization of the ion by resonance. Those compounds possessing unsubstituted -NH grouping show molecular association through N-H-N bonds. The dipole moment of benzimidazoles has been determined to be 3.93D (in dioxane) and 4.08D. The benzimidazole ring possesses a high degree of

stability. Benzimidazole is neither affected by concentrated sulfuric acid when heated under pressure to 270°C nor by vigorous treatment with hot HCl or with alkalis. Oxidation cleaves the benzene ring of benzimidazole only under vigorous conditions. The benzimidazole ring is also quite resistant to reduction; however, tetra hydro and hexahydro benzimidazoles can be prepared by catalytic reduction only under certain conditions. Benzimidazole is non-toxic and has little effect on the blood pressure. It is reported to reduce skeletal muscle tone by acting on the central nervous system and it also inhibit the growth of several yeasts and bacteria.^{1,2}

Uses of Benzimidazole

A large number of patents describe benzimidazole derivatives of use in the textile industry as wetting, emulsifying, foaming agents or as dispersants for use in dying. In the main, these compounds are sulfonated benzimidazoles. Another use is in the treatment of fibers to improve whiteness of the undyed material or as

optical bleach. A number of 2-aminobenzimidazoles have been used in the preparation of fluorescent dyes for use in such preparation as inks for making clothes to the dry cleaner.

2-Mercaptobenzimidazole and several other benzimidazole derivatives have been found use in photographic industry. These compounds reduce photographic "Fog" and increase contrast and speed and hence have found use in photographic developing and fixing solutions. 2-mercaptobenzimidazoles has also found to be value as an antioxidant of rubber. Several benzimidazole derivatives have found use in the preparation of sun burn preventatives. These compounds protect the skin by absorbing ultraviolet rays.

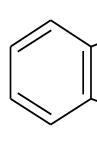
5-Methyl benzimidazole has been used as a camphor substitute. 2-methyl benzimidazole is said to be a value as a polymerization inhibitor and initiator in isoprene. 1-piperidinomethyl benzimidazole has been used as a booster compound with antioxidants in rubber. A number of salts of benzimidazole sulfonic acid are said to be value in the preparations for the care of the mouth and teeth.

Properties of Benzimidazole

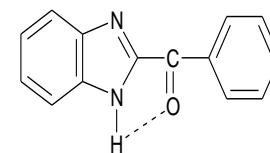
Benzimidazole with the imide nitrogen (i.e. hydrogen in the 1-position) is usually more soluble in polar solvents and less soluble in hot water but difficultly soluble in ether and insoluble in benzene. With the introduction of other non-polar substituent's in various positions of the benzimidazole ring, the solubility in non-polar solvent is increased i.e. 2-methylbenzimidazole is easily soluble in ether. The polar group into the molecule increases solubility in polar solvents i.e. 2-aminobenzimidazole is soluble in water.

- ❖ Benzimidazoles are weakly basic, being somewhat less basic then the imidazoles.
- ❖ They are general soluble in dilute acids.
- ❖ The more acidic benzimidazoles may be soluble in less basic solutions, such as potassium carbonate solution.
- ❖ 2(3H)-benzimidazolone is difficult to soluble in dilute sodium hydroxide solution. It is insoluble in dilute hydrochloric acid but is readily soluble in slightly warmed concentrated hydrochloric acid. 2-benzimidazolecarboxylic acids are easily soluble in dilute acids.
- ❖ The molecular weight of a number of benzimidazoles from freezing point data in naphthalene solution over a range of concentrations. Evidence was obtained indicating molecular association through N-H-N bonds in those compounds possessing an unsubstituted NH grouping. The strength of this bond is evidently enhanced by resonance of the benzimidazole nucleus. Those substances which are substituted in 1-position by an alkyl, aryl, acyl or amino group are not highly associated. Substances such as 2-benzoylbenzimidazole (1) occupy an intermediate position, being less highly associated then other benzimidazoles unsubstituted in the 1-position. It

would appear that majority of the molecules possess the internal chelate structure (2). Such internal chelation is possible in a number of 2-substituted benzimidazole.



(1)



(2)

- ❖ The dipole moment of benzimidazole have been determined, the values that have been obtaining 3.93D (in dioxane) and 4.08D.

- ❖ The melting point of a number of simple Benzimidazoles are:

Benzimidazole	- 170°C
1-methyl benzimidazole	- 61°C
2-methyl benzimidazole	-176°C
2-phenyl benzimidazole	-294°C
2(3H)-benzimidazolone	- 308°C

From this melting point it will be noted that the introduction of a substituent into the 1-position in general lowers the melting point. This appears to be due to the fact that benzimidazole containing hydrogen in the 1-position.

Mechanism of Action³

The primary action of the benzimidazole is associated with the ability of these drugs to bind to the protein tubulin and thus prevent tubulin polymerization to microtubules.

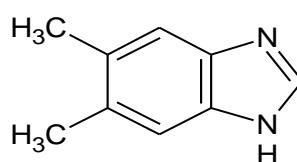
Metabolism⁴

The Benzimidazoles have been limited water solubility and as results are poorly absorbed from the GI tract (a fatty meal will increase absorption). Poor absorption may be beneficial since the drugs are used primarily to treat intestinal helminthes.

Natural Products Containing Benzimidazole Nucleus

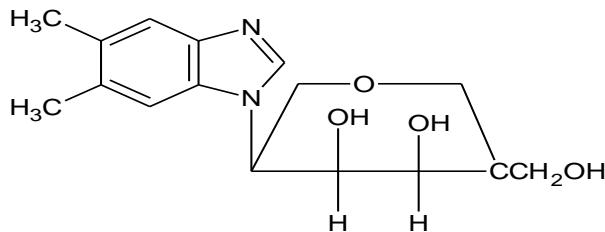
The Benzimidazole nucleus does not appear to occur very widespread in nature. However, very recently the 5, 6-dimethylbenzimidazole moiety has been shown to be part of the structure of vitamin B₁₂.

Vitamin B₁₂ on acid hydrolysis leads to the formation of three closely related substances designated compounds α , β & γ . Component γ is 5, 6-dimethylbenzimidazole (1).



(1)

Component β is 5, 6-dimethyl-benzimidazole-1- α -D-ribofuranoside (2).



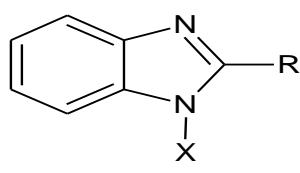
(2)

The α -component is probably a phosphorylated derivative of β -component. Since the α - component on acid hydrolysis yields the β -component and phosphate.

Biological Activities of Benzimidazole Derivatives

❖ Antimicrobial Activity^{5, 6}

Purathchikody et al., synthesized benzimidazole derivatives were shown potent antimicrobial activity.



X = CO-Ph etc.

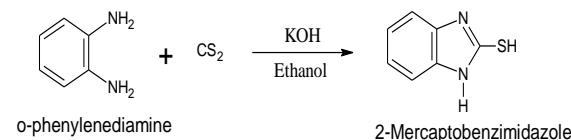
Guven et al., synthesized some novel phenyl and benzimidazole substituted benzyl ethers which show potent antimicrobial activity.

MATERIALS AND METHODS

All the chemicals used for synthetic work were purchased from Central drug house Pvt. Ltd (CDH), Hi-Media laboratories Pvt. Ltd. and MERCK. Melting points were determined by using Veego microprocessor based programmable melting point apparatus in open capillaries and are uncorrected. The completion of the reaction was checked by thin layer chromatography (TLC) by using 'silica gel G' procured from MERCK

and was coated on laboratory glass slides using Benzene:Ethylacetate (4:1) solvent system. TLC plates were visualized using iodine chamber or observed under UV light. IR spectra were recorded in cm^{-1} using KBr pellets on PERKIN ELMER spectrophotometer. ^1H NMR spectra (in δ ppm) on BRUKER AVANCE II 400 NMR spectrophotometer using CDCl_3 solvent and TMS as internal standard.

Step 1: Synthesis of 2-Mercaptobenzimidazole (BK1)



Procedure

Potassium hydroxide (1.9g, 0.03 moles) was dissolved in a mixture of ethanol (30 ml) and water (30 ml) in a 250ml round bottom flask. To this, CS_2 (2.7g, 0.03 moles) was added with stirring. This mixture was boiled and then o-phenylenediamine (3.24g, 0.03 moles) was added to it. After refluxing the reaction mixture for 4 hr at 100.110°C, cool the reaction mixture and was dissolved in water and the product was precipitated by the addition of dilute acetic acid (50%). The product was filtered and washed with water. It was recrystallized from ethanol.

Appearance: Light brown

M.F.: $\text{C}_7\text{H}_6\text{N}_2\text{S}$

Yield: 75%

$R_f(\text{CHCl}_3: \text{CH}_3\text{OH})$: 0.87

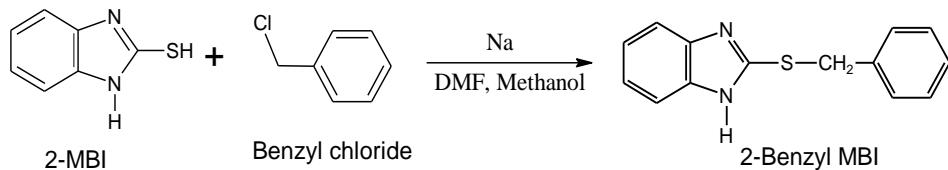
M.P: 301-303°C

I.R (KBr pellets): 3151.7 (Ar C-H), 1512.1 (C=C), 1355.8 (C-N), 709.2 (C-S).

NMR (DMSO): 7.06-7.16(m, 4H, Ar-H), 12.41(s, 1H, NH).

Step 2: Synthesis of 2-substituted mercaptobenzimidazole

(1) Synthesis of 2-benzyl mercaptobenzimidazole (BK2)



Procedure

In a 250ml beaker, 2-mercaptop benzimidazole (0.75g, 5mmol) in dry N, N-dimethylformamide (8ml) was added to a solution of sodium (0.12g, 5 m mol) in dry methanol (2.5ml). After 10 min of stirring at room

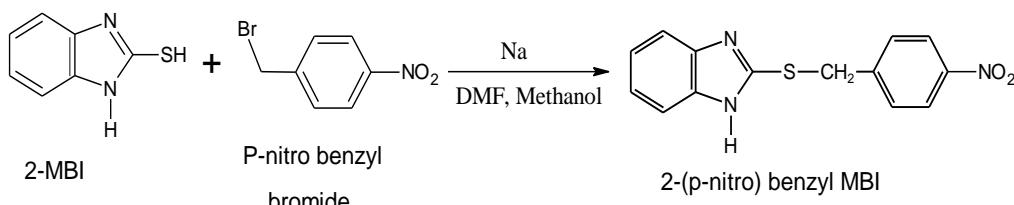
temperature, a benzyl chloride (0.63g, 5 m mol) was added in 2-3 portions, and the resultant suspension was stirred for 5h. The reaction mixture was then poured into an ice bath and left at overnight. The solid was filtered off, washed with cold water and air-dried. It was recrystallized from methanol.

Appearance:	White
M.F.:	C ₁₄ H ₁₂ N ₂ S
Yield:	70%
R _f (CHCl ₃ ; CH ₃ OH):	0.75
M.P.:	179-181°C

IR (KBr pellets): 3435.7(N-H), 3048.33(Ar C-H), 2963.21(C-H), 1511.88(C=C), 1350.05(C-N), 699.08(C-S).

NMR (DMSO): 4.57 (s, 2H, CH₂), 7.14-7.65 (m, 9H, Ar-H).

(1) Step 3: Synthesis of 2-(p-nitro) benzyl mercaptobenzimidazole (BK3)



Procedure

In a 250ml beaker, 2-mercaptop benzimidazole (0.75g, 5mmol) in dry N, N-dimethylformamide (8ml) was added to a solution of sodium (0.12g, 5 mmol) in dry methanol (2.5ml). After 10 min of stirring at room temperature, a p-nitro benzyl bromide (1.08g, 5 mmol) was added in 2-3 portions, and the resultant suspension was stirred for 6h. The reaction mixture was then poured into an ice bath and left at overnight. The solid was filtered off, washed with cold water and air-dried. It was recrystallized from methanol.

Appearance:	Light brown
M.F.:	C ₁₄ H ₁₁ N ₃ O ₂ S
Yield:	68%
R _f (CHCl ₃ ; CH ₃ OH):	0.70
M.P.:	183-186°C

IR (KBr pellets): 3401.23(N-H), 3085.15 (Ar C-H), 2959.12 (C-H), 1478.80 (C=C), 1361.22 (N=O), 1349.40 (C-N), 700.50 (C-S).

NMR (DMSO): 4.66(s, 2H, CH₂), 7.10-8.12(m, 8H, Ar-H).

Antibacterial Activity

The antibacterial activity of newly synthesized compounds was evaluated against Gram +ve bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*, Gram -ve bacteria i.e. *Escherichia coli*. Ciprofloxacin was used as a standard drug.

Preparation of Nutrient Agar Medium

1.	Peptic digest of animal tissue	5gm
2.	Sodium chloride	5gm
3.	Beef extract	1.50 gm
4.	Yeast extract	1.50 gm
5.	Agar	15 gm
6.	Distilled water	1000 ml

All the ingredients were dissolved in distilled water; Adjust the pH to 8.0-8.4 with 5M NaOH solution and boil for 10-15 min. filtered the solution. Adjust the pH

of the medium by 7.4±0.2 by the addition of dil. HCl. Sterilized the medium in autoclave for 15 min. at 121°C.

Preparation of Test Solution

The solution of the various benzimidazole derivatives in concentration of 100 µg was prepared in DMSO.

Preparation of Standard Solution

Weigh 10mg of standard drug and diluted to 10ml to form 1000 µg/ml of stock solution. From this stock solution, we took 1ml and diluted to 10ml to form 100 µg/ml of standard solution.

Procedure

Inoculate a previously liquefied medium appropriate to the assays, with requisite quantity of suspension of the microorganism; add the suspension to the medium at temp. 40-50°C and immediately pour the inoculated medium into Petri dishes to give a depth of 3-4 min. Ensures that the layer of the medium are uniform in thickness by placing the dishes on the level of surface. Made few cavities on the surface of medium. Poured the solution of known concentration of the standard preparation and test preparation to cavities by means of micropipette in a sterile condition. Leave the dishes standing for 1-4 hrs in refrigerator as appropriate as a period of pre incubation diffusion to minimize the effects of variation in time between the applications of different solution. Incubate them for about 24 hrs at the temp. indicated. Accurately measured the diameter of zone of inhibition.

Observation

Observe the zone of inhibition by visually.

RESULT AND DISCUSSION

The compounds were evaluated in vitro antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* using Ciprofloxacin as standard. The zone of inhibition of the synthesized compounds against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* is presented in table I.

Table I: Data of Antibacterial Activity of synthesized compounds

S.No.	Compounds	Zone of inhibition in mm		
		Staphylococcus aureus	Bacillus subtilis	Escherichia coli
1.	BK-1	05	12	08
2.	BK-2	13	11	0
3.	BK-3	14	19	17
4.	Ciprofloxacin	25	28	24

CONCLUSION

Three 2-substituted benzimidazole derivatives were synthesized and evaluated for their antibacterial activity. Derived compounds shows moderate antibacterial activity

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Conflict of Interest: The author does not have any conflict of interest.

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