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Synthesis, characterisation and antimicrobial activity of azo compounds of benzimidazole

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ABSTRACT

In this study, azo compounds are synthesized in excellent yields via the diazotization of five different aromatic amines followed by coupling with benzimidazole. These compounds are characterized by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectral techniques. The synthesized compounds have been tested *in vitro* against a number of microorganisms in order to assess their antimicrobial properties using disk diffusion method. All compounds exhibit comparable antimicrobial activity.

Key words Benzimidazole, Azo compounds, Antimicrobial, Disc diffusion

1. INTRODUCTION

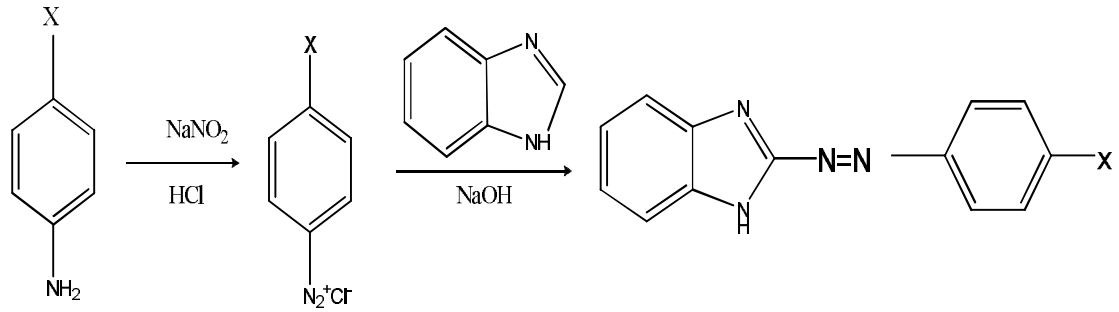
In the family of heterocyclic compounds nitrogen containing heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes [1].The ring system in which a benzene ring is fused to the 4,5-positions of imidazole is designated as benzimidazole [2]. Benzimidazole derivatives are very useful intermediates or subunits for the development of pharmaceutical or biological interest [3]. Benzimidazole derivatives are an important class of bioactive molecules in the field of drugs and pharmaceuticals [4]. A compound containing benzimidazole and benzene rings have been used extensively for pharmaceutical purpose since 1960. 1-H-Benzimidazole rings, which exhibit remarkable basic characteristics due to their nitrogen content, comprise the active substances for several drugs. A number of biological activities have been attributed to these compounds [5]. Biological importance of azo compounds is well known for their use as antibacterial, antifungal anti neoplastics, antidiabetics, antiseptics, anticancer, anti-inflammatory, and other useful chemotherapeutic agents [6-9]. Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation [10, 11]. Evans blue and Congo Red are being studied as HIV inhibitors of viral replications. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of this virus [12]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticidal activity [13, 14]. Since compounds with azo moiety and benzimidazole moiety have been extensively used as dyes, but biological activity is less reported. In the present work, we have synthesized and characterized five azo compounds namely 2-[phenylazo]-benzimidazole (PAB), 2-[4-methylphenylazo]-benzimidazole(MPAB), 2-[4-nitro phenylazo]-benzimidazole (NPAB), 2-[4-chloro phenylazo]-benzimidazole (CPAB) and 2-[4-methoxy phenylazo]-benzimidazole (MyPAB). The antimicrobial activities of the synthesized azo compounds were reported *in vitro* using disc diffusion method.

2. EXPERIMENTAL

All chemicals were of analytical grade and were obtained from Merck, Nice and CDH. Melting points were determined in open capillary tubes and are uncorrected. The purity of the synthesized compounds was checked by TLC using silica gel G. For TLC, Merck silica gel 60 G plate was used. In the present investigation the IR spectra of azo compounds were recorded on Schimadzu FTIR spectrophotometer model 8400 S in KBr wafer and the NMR spectra were obtained on 400 MHz FT NMR spectrometer using $CDCl_3$ as solvent and reported relative to TMS as internal standard.

2.1. Synthesis of azo compounds

Azo compounds were synthesized according to the method reported in literature [15]. There are two steps in the synthesis of azo compounds:



(I) X = H, (II) X = CH_3, (III) X = NO_2, (IV) X = Cl, (V)X = OCH_3

Scheme 1: Preparative route of substituted azo compounds

Table 1: Physical and Analytical data of substituted azo compounds of Benzimidazol

Compounds	Molecular Formula	Molecular Weight (Calculated)	Colour	% Yield	Melting Point
I(PAB)	$C_{13}H_{10}N_4$	222	Reddish brown	72	148 °C
II(MPAP)	$C_{14}H_{12}N_4$	236	Dark brown	64	64°C
III(NPAB)	$C_{13}H_9N_5O_2$	267	Orange red	67	119°C
IV(CPAB)	$C_{13}H_9N_4Cl$	256	Orange	64	140°C
V(MyPAB)	$C_{14}H_{12}N_4O$	252	Dark red	71	73°C

Table 2: Antimicrobial screening data (zone of inhibition in mm) of the synthesized azo compound

Compds	*Klebsiellapneumoniae (-)*	*E.coli (-bacilli)*	*Staphylococcus aureus(+cocci)*	*Streptococcus (+cocci)*	*Candida albicans*	*Candida glabrata*
I	12	12	11	11	8	9
II	10	15	10	12	7	10
III	10	10	8	9	9	9
IV	8	13	12	15	6	9
V	10	12	10	9	7	9
Std	20	22	23	20	16	15

Table 3: Infrared spectral data of substituted azo compounds of benzimidazole

Compounds	X	νN-H (cm-1)	νN=N (cm-1)	νC=N (cm-1)	$\nu_{CH=CH}$ (cm-1)
I	H	3434	1456	1301	1670
II	CH_3	3502	1444	1346	1668
III	NO_2	3477	1458	1301	1670
IV	Cl	3502	1456	1340	1670
V	OCH_3	3502	1444	1302	1668

2.2. Diazotisation of amines

A solution aromatic amine (10 mmol) and 8 mL of 3 M HCl was heated gently, then water (10 mL) was added in order to dissolve the solid. The mixture was cooled to 0° C in an ice bath with stirring. This solution was cooled to 0–5 ºC, and a freshly prepared solution of 1 M sodium nitrite (10 mL) was then added drop wise, maintaining the temperature below 5 º C. The solution was kept in an ice bath and used immediately in the next step.

2.3. Coupling with Benzimidazole

Benzimidazole (10 mmol) was dissolved in 10 mL of 2 M sodium hydroxide, and cooled to 0–5 ºC in an ice bath. This solution was then gradually added to the cooled benzene (or substituted) diazonium chloride solution. The resulting mixture was stirred at 0–5°C for at least 15 minutes until the crystallization is complete (giving a coloured solid). The pH of the solution was adjusted with dilute HCl or NaOHsolutions (0.1 M) in order to induce precipitation. The resulting coloured precipitate was filtered, washed several times with cold water and was recrystallized from hot chloroform to yield azo compound.Azo compounds were synthesized according to following scheme 1. The physical and analytical data obtained for these compounds are shown in Table 1.

2.4. Antimicrobial activity

The synthesized azo compounds were screened for the presence of antibacterial constituents against six strains of bacteria *i.e.Staphylococcus aureus, Klebsiellapneumoniae, E.Coli, Streptococcus*, and two species of fungi *i.e.,* against *Candida albicans and Candida glabrata*by disc diffusion method [16, 17, 18]. Nutrient agar was used as culture medium for bacterial growth while fungi were subcultured in potato dextrose agar medium. Measured quantities of the test compounds were dissolved in DMF to get final concentrations of 500 ppm and soaked in filter paper discs of 6 mm diameter. These discs were placed on the previously seeded plates and incubated at 35°C. All compounds were dissolved in DMF. Amikasin (5 mcg/disc for bacteria) and Fluconozole (100 units/disc for fungi) was used as reference antibiotic and DMF as control. The zones of inhibition were determined at the end of an incubation period of 24 hr at 37◦ C. During this period, the test solution diffused and the growth of inoculated microorganism was affected. The bacterial inhibition zone values are summarized in table 2. All the azo compounds showed remarkable activity against used microbes and results were compared with standard drugs.

3. RESULTS AND DISCUSSION

In this study five azo compounds were synthesized by coupling of different aromatic amines with benzimidazole. They were characterized by IR spectrum. Table 3 shows important IR peaks values of newly synthesized azo compounds.

3.1. Spectroscopic characterization of synthesized compounds

The glance at the structure of azo compounds, one may expect the absorption bands due to N=N, -N-H, C-H=C-H and C-N vibrations in IR region. All the synthesized compounds shows absorption bands for different types of vibrations which were shown by azo compounds. This confirms the success of the synthesis.

3.2. Antimicrobial activity

The synthesized azocompounds of benzimidazole shows bactericidal and fungal activity. All the compounds were found to exhibit moderate to good antifungal activity against both the test bacteria and fungi.

3.3. Mode of action

Although the exact mechanism is not understood biochemically, mode of action of antimicrobials may involve various targets in microorganisms [19].

(i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered (or) they may disorganize the lipoprotein leading to the cell death.

(ii) Deactivate various cellular enzymes, which play a vital role in different metabolic pathways of these microorganisms.

(iii) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired.

(iv) Formation of a hydrogen bond through the azo group with the active centre of cell constituents, resulting in interference with the normal cell process.

From the antimicrobial screening it was observed that all the compounds exhibited activity against all the organisms employed. As we consider all results obtained from antibacterial and antifungal tests together we can say that entire compounds tested were active towards bacteria and fungi.

4. CONCLUSION

Novel azo compounds are prepared and are characterized on the basis ofanalytical and spectral data. Screening of these compounds against pathogenic microorganism reveals that

these compounds have the capacity of inhibiting metabolic growth of some microorganisms to different extent. The antimicrobial activity of the compounds depends on the nature of substituent present on the aromatic ring. More extensive study is needed to confirm the preliminary results and mode of action to optimize the effectiveness of this series of compounds.

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