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Research Article

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In vitro anti-microbial and DNA cleavage studies of benzimidazole derivatives

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ABSTRACT

*A series of reported benzimidazoles derivatives **L(1-11)** were screened for in vitro anti-microbial and DNA cleavage by taking Ciprofloxcin & Fluconazole and Escherichia coli as standards for studies respectively. These compounds showed promising anti-microbial and DNA cleavage activities.*

Keywords: benzimidazole derivatives, *in vitro*, anti-bacterial, anti-fungal, DNA cleavage

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INTRODUCTION

The increase in bacterial resistance has attracted considerable interest in the discovery and development of new classes of antibacterial agents [1]. The new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents.

Benzimidazoles are remarkably effective compounds both with respect to their bacteria inhibitory activity and their favorable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms [2, 3]. Some benzimidazole compounds inhibit the biosynthesis of ergosterol, required in the cell membrane of fungi. They have antibacterial, antifungal, and antiviral activity [4-7]. This ring system is present in numerous antiparasitic and antitumoral drugs [8, 9]. The benzimidazole structure is part of the nucleotide portion of vitamine B_{12} and the nucleus of some drugs, such as proton pump inhibitors and anthelmintic agents. Proton pump inhibitors (PPIs) are substituted benzimidazole derivatives that selectively and irreversibly inhibit the gastric hydrogen–potassium adenosine triphosphatase (H^+K^+-ATPase) pump mechanism. The antimicrobial activity of this class of compounds was investigated against Helicobacter pylori[10] and against oral streptococci[11]. Also, benzimidazole and its derivatives are of considerable importance because of their antihistaminic, cytostatic, local analgesic, hypotensive and anti-inflammatory activity [12]. Benzimidazole was confirmed to have moderate *in vitro* anti-HIV effect [13, 14].

The aforesaid numerous pharmacological activities of benzimidazoles and in continuation of our ongoing research project on benzimidazole derivatives[15] prompted us to study the *in vitro* anti-microbial and DNA cleavage of reported benzimidazole derivatives **L(1-11)** [16] **Fig.1** from our research group.

EXPERIMENTAL SECTION

2. Biological assay

2.1. Anti-microbial activity screening

All the compounds prepared herein were screened for *in vitro* anti-microbial activities and the compounds were screened against bacterial and fungal strain by the Agar-Well Diffusion Method[17-19]. The results were based on

the comparison of inhibition of growth of bacteria and fungi by measured concentration of anti-microbial substance to be examined with that produced unknown concentration with standard (Ciprofloxcin & Fluconazole) antibiotic[20,21].

2.2. DNA Cleavage screening

In DNA cleavage experiments were done by Agarose gel electrophoresis method [22]. The difference was observed in the bands of compounds **L(1-11)** compared to the control DNA of *E. coli*.

RESULTS AND DISCUSSION

3.1. Anti-microbial activity

The reported compounds **L(1-11)** were screened *in vitro* anti-bacterial activity against two Gram-positive (*S. aureus* & *B. subtilis*) and two Gram-negative (*S.typhi & E.coli)* bacterial strains and *A. Niger* and *C.albicans* fungal strains by the agar well diffusion method. The results were compared with Ciprofloxacin and Fluconazole drug as a reference standard for anti-bacterial and anti-fungal activities. The MIC values of the compounds are tabulated and graphically showed in **Table-1** and **Fig. 2** and **3**.

The results indicate that all the synthesized compounds of benzimidazole series are good to moderate activity against a panel of microorganisms. In anti-bacterial, the compound which contains chloro group at ortho & meta position to benzene ring, bromo to both benzene and benzimidazole rings and the compound contains bromo at benzene and nitro to benzimidazole moiety shows remarkable anti-bacterial activity against panel of strains. Remained derivatives also show promising activity at 20 µg/mL concentration. In anti-fungal activities of benzimidazole derivatives, the results indicate that unsubstituted derivative and chloro, bromo substituted derivatives shows potent activity against A. niger and C. albicans at 20 µg/µL concentration. Remaining compounds also promising the activity of 30 µg/µL against fungal strains. The results are graphically showed in **Fig. 2** & **3**.

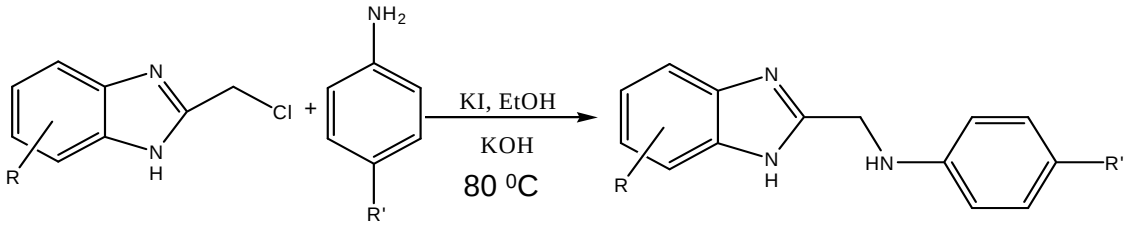
3.2. Electrophoretic analysis

The DNA cleavage of benzimidazole derivatives **L(1-11)** were studied by agarose gel electrophoresis method and are presented in **Fig. 4**. The gel after electrophoresis clearly revealed that, the intensity of **L(4, 5, 6, 8, 9)** and **L10** treated DNA samples has diminished because of the partial cleavage of the DNA. The DNA band in **L11** lane has disappeared, indicating the complete cleavage of the DNA. The presence of nitro group in benzimidazole ring results the complete cleavage. The difference was observed in the bands of compounds **L(1-11)** compared to the control DNA of *E.coli*. This shows that the control DNA alone does not show any apparent cleavage as the compounds did. However, the nature of reactive intermediates involved in the DNA cleavage by the compounds has not been clear. The results indicate the importance of benzimidazole derivatives in these isolated DNA cleavage reactions. As the compound **L11** observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

TABLE 1: Minimum Inhibitory Concentration (MIC) of synthesized compounds

	MIC (µg/µL)					
Compound	***S.aureus***	***B.subtilis***	***S.typhi***	***E.coli***	***A.niger***	***C.albicans***
10-50 (µg)	**Antibacterial strains**				**Antifungal Strains**	
1	30	30	30	30	**20**	**20**
2	30	30	**20**	30	30	**20**
3	30	30	40	30	40	**20**
4	30	30	40	30	30	40
5	40	30	30	30	40	30
6	30	30	30	40	40	**20**
7	30	**20**	30	30	40	30
8	**20**	**20**	30	30	30	30
9	30	30	30	30	30	30
10	30	30	30	30	30	30
11	30	30	**20**	**20**	30	30
Control	0	0	0	0	0	0

Standard drug : Bacteria (Ciprofloxcin), Fungal (Fluconazole) (40 µg in100µl)
Control : DMSO (DI-methyl sulphoxide)



Where R = H, Br, NO_2
R'= H, Cl, Br, CH_3, OCH_3

Fig. 1. Schematic representation of synthesis of benzimidazole derivatives

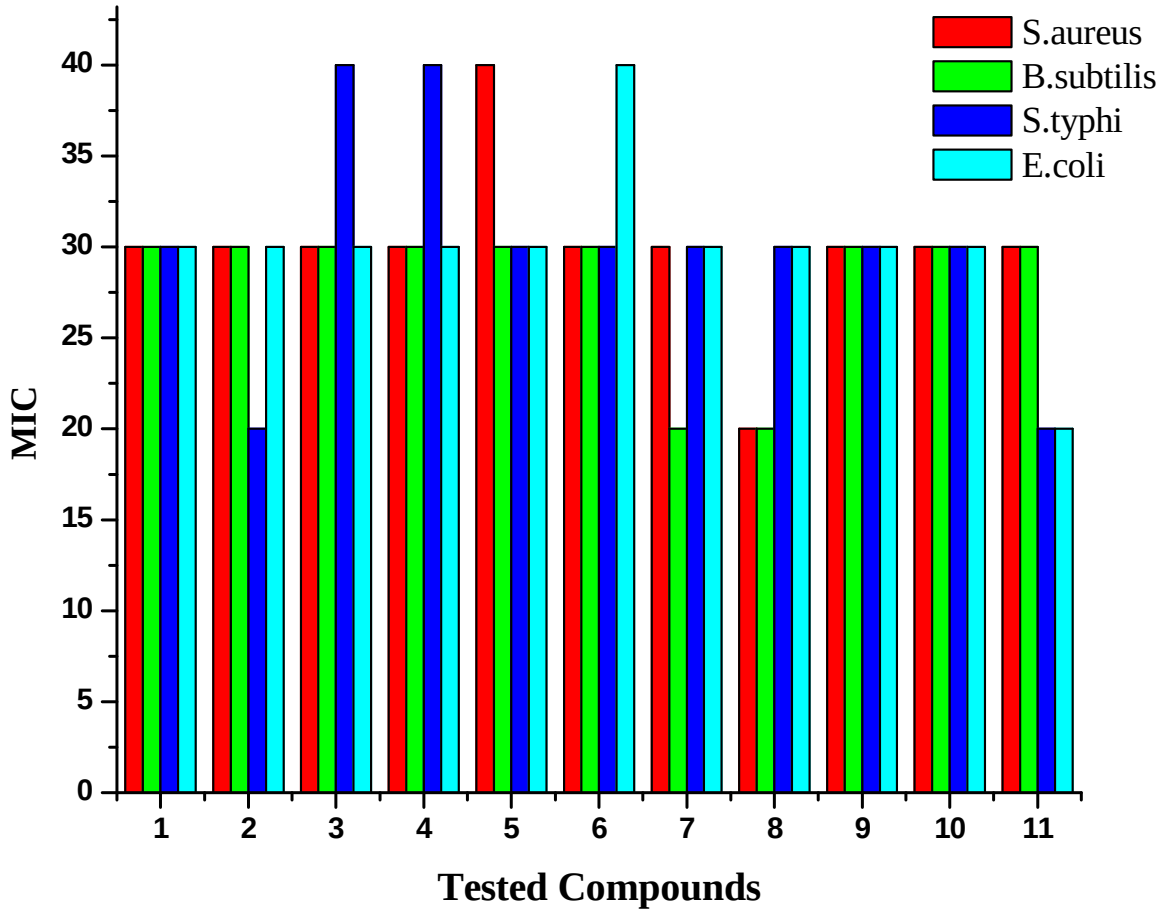


Fig. 2. Anti-bacterial activity of tested compounds and standard drug Ciprofloxcin (40 µg in100µl)

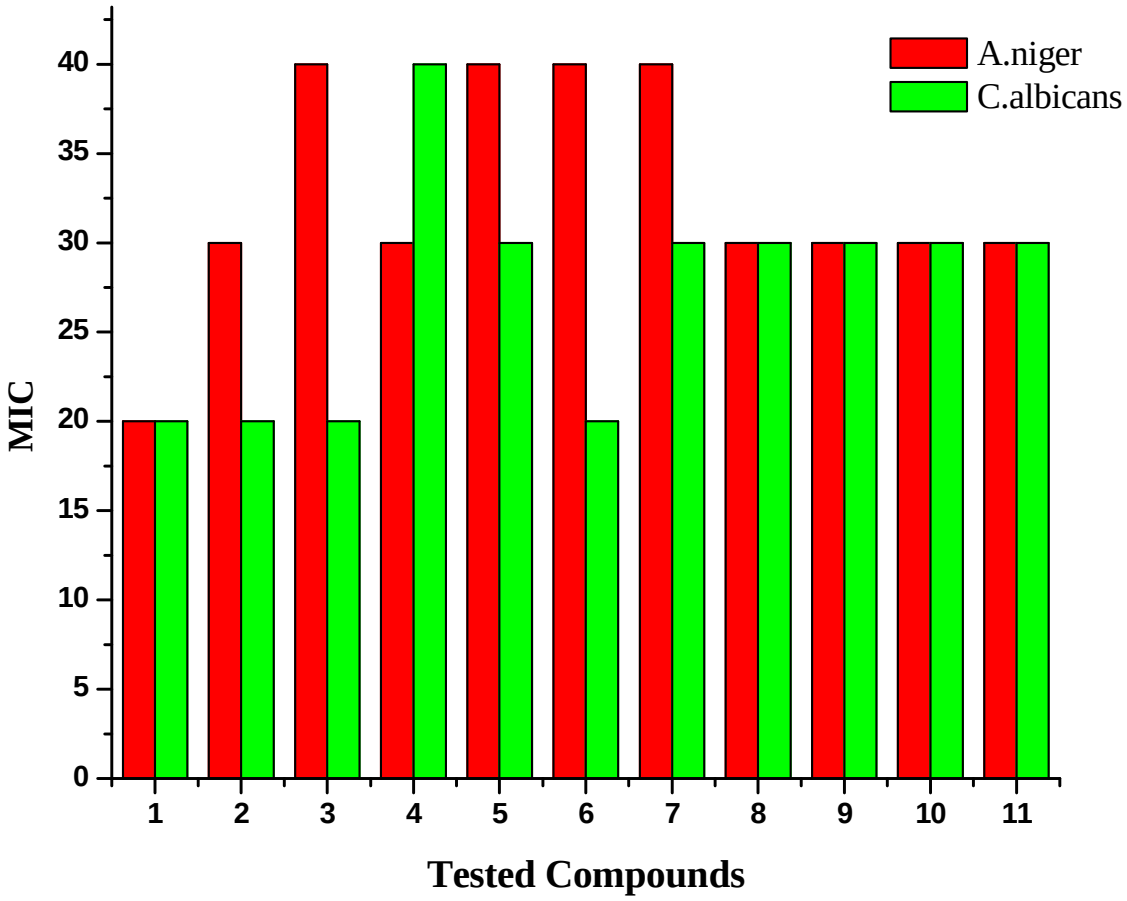
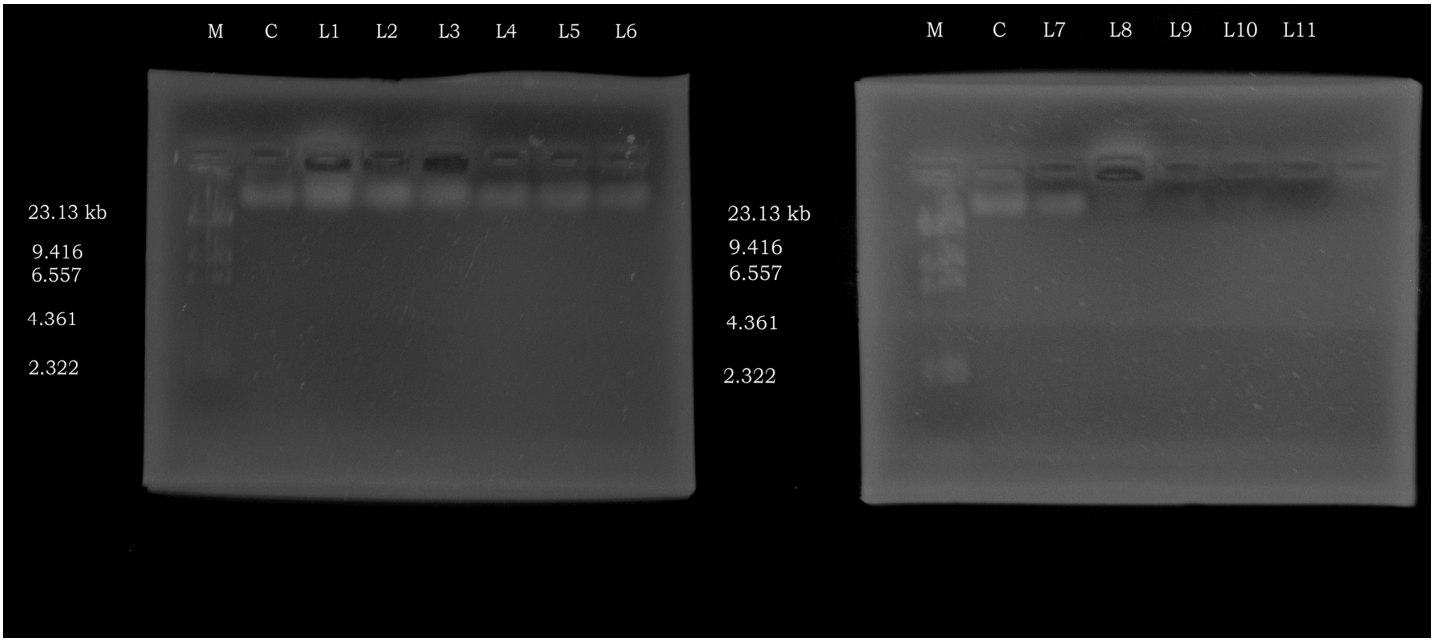


Fig. 3. Anti-fungal activity of tested compounds and standard drug Fluconazole (40 µg in100µl)



M- Standard DNA molecular weight marker (λ DNA HindIII digest, Banglore Genei, Bangalore)
C- Control DNA (untreated sample)

Fig. 4. DNA Cleavage photos of tested compounds

CONCLUSION

In conclusion, we have screened for *in vitro* anti-bacterial and anti-fungal activities against the several microorganisms. Some of the synthesized compounds which contain chloro, bromo derivatives showed excellent activity against the bacterial strains and other compounds showed moderate activity comparable with Ciprofloxcin standard drug. In anti-fungal activity, compounds are screened by taking two fungii starins. Some of the compounds like **L1, 2, 3** and **L6** showed excellent anti-fungal activity against fungii strains and other compounds showed good to moderate activity comparable with Fluconazole standard drug. The DNA cleavage studies revealed that the compound **L11** showed complete cleavage. While the other samples showed partial cleavages.

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