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In vitro Antifungal and Antibacterial Activity of Carbazoles

GHAZALA YAQUB[1,*], ABDUL HANNAN[2], MUHAMMAD USMAN[2], ERUM AKBAR[3] and BUSHRA MATEEN[3]

[1]Department of Environmental Sciences, Kinnaird College for Women, Lahore-54000, Pakistan
[2]Department of Microbiology, University of Health Sciences, Lahore-54000, Pakistan
[3]Department of Chemistry, Lahore College for Women University, Lahore, Pakistan

*Corresponding author: Tel: +92 331 4063466; E-mail: ghazala_yaqub@yahoo.com

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Carbazoles of synthetic origin were screened for their *in vitro* antibacterial and antifungal activities against human pathogens *i.e.*, *Salmonella typhi*, methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* by agar dilution assay. Targeted compounds were found to possess moderate to excellent activities against these human pathogens and their MIC values ranged from 18 µg/mL to > 512 µg/mL.

Key Words: Carbazole, Antibacterial activities, Antifungal activities, Agar dilution assay.

INTRODUCTION

Bacterial and fungal infections cause many global health problems. Antibiotics[1] and antifungal medicines are used for the treatment of clinically important infections. In treatment of these infections a hurdle comes when these microbes acquire resistance against existing antifungal agents and antibacterials[2,3] *e.g. Enterococcus* spp has developed resistance against the existing antibiotics such as oxacillin, nafcillin and vancomycin[4]. Clinical isolates of MRSA are also found to be resistant to vancomycin now[5] and promising new antibacterial drug linezolid (zyvox)[6]. Thus the need for the isolation and synthesis of new compounds never comes to an end. There is a pressing need of new antimicrobial agents which could be effective against drug resistant bacteria. Luckily, carbazoles are one of the predominant antimicrobial agents. Carbazoles, both synthesized and isolated, are among those N-containing heterocyclic compounds which acquired their place as emerging antibiotics[7] and are the part of many medicines *i.e.*, vetprofen (carprofen)[8], carvedilol (used in chronic heart failure)[9], ondansetron[10] *etc*., due to their remarkable antibacterial[11], antifungal, antitumor[12], anti cancer, anti HIV, anti-inflammatory, antioxidant[13] activities *etc*. Carbazoles are the subject of intensive investigation by chemists and biochemists because they serve as important leads among the novel biologically active motifs.

In our previous studies[14,15] we have reported the protocols for the synthesis of some known and novel derivatives of carbazole alkaloids (Fig. 1). Here in this study we will explore their role as antimicrobial agent.

EXPERIMENTAL

Antibacterial and antifungal activities of all synthesized compounds were determined in terms of minimum inhibitory concentration (MIC). All MIC experiments were performed in triplicate to be sure about reproducibility of results. Agar dilution assay was performed according the Clinical laboratory Standards Institute guidelines (CLSI 2011).

Procedure for Antimicrobial Studies: Antimicrobial activity of all synthesized carbazoles was determined in terms of MIC to check the lowest concentration of the compounds at which bacterial growth was completely inhibited. Initially twelve compounds were screened for antimicrobial activity using agar well diffusion assay. Among the screened compounds, those having good activity were chosen for MIC determination. MIC was determined against thirty four clinical isolates of *Salmonella typhi*, methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* (fungi) including the ATCC reference strains.

Preparation of Muller Hinton agar plates incorporated with compounds: Muller-Hinton agar (38 g) was dissolved in 1 L of distilled water. Thirty Three flasks (100 mL volume) for each compound were taken. 20 mL Mueller Hinton agar was poured in each flask and autoclaved at 121 ºC for 15 min. The medium was allowed to cool down to 50 ºC before the addition of compound. Stock solution of each compound was prepared in dimethyl sulfoxide. Eleven different working concentrations to obtain 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048 µg/mL of the medium were prepared from the stock solution. The respective volume for each concentration

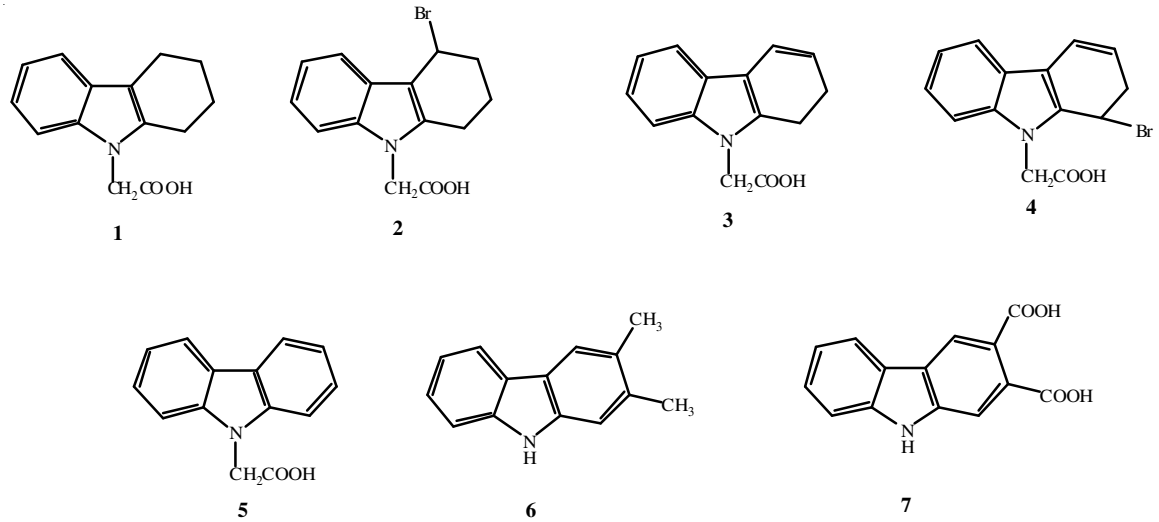

Fig. 1. Antimicrobial carbazoles

was determined using (M1V1=M2V2) and incorporated in 20 ml of Mulller-Hinton agar to achieve the desired concentration. The flasks were well shaken to mix the compound thoroughly and poured into 90 mm petri dishes. The plates were allowed to solidify at room temperature and then stored in refrigerator. The plates were taken out of the refrigerator and placed in hot oven at 55 ºC for about 5 min to permit the evaporation of surface moisture if present.

Procedure for MIC: The isolates stored at -70 ºC were refreshed on Blood agar plates. Bacterial suspensions with turbidity equivalent to 0.5 McFarland standard were made for each isolate in tryptic soya broth. The suspensions were further diluted 1 in 10 with normal saline. 600 µL of each suspension was added to their respective labelled wells of the Multi-inoculator grid.

Compound incorporated plates were taken out of the refrigerator, dried in hot air oven (55 ºC) for about 5 to 10 min and inoculated with multipoint multi-inoculator (Mast Diagnostic, UK). Two control plates were also set: one with Muller-Hinton agar without any compound incorporated at the start of inoculation with all strains to confirm the viability, growth and purity of the isolates. Second control plate was included without any inoculation to check the sterility of the medium. Plates were then incubated at 37 ºC for 18-24 h and were observed for the growth inhibition by unaided eye. Potato dextrose agar was used for antifungal screening rather than Muller Hinton agar.

RESULTS AND DISCUSSION

Literature reveals that the synthesis of novel carbazoles and their amazing antimicrobial assay[16] especially against multi resistant microbes[17] is pillaring up their role as an antibiotic. In near future they will definitely emerged as novel class of antibiotics against drug resistant microbes.

Here in this report, we have presented amazing results of antimicrobial screening of the carbazole alkaloids **1-7** which we synthesized previously in terms of MIC (minimum inhibitory concentration) against Methicillin-resistant *Staphylococcus aureus*, a gram positive bacterium, which becomes resistant to many antibiotics and is responsible for severe infections *i.e*., shortness of breath, fever, chills, infections on the skin causing pimples or boils, serious skin[18], urinary tract and surgical wound infections, *Salmonella enterica serovar typhi*, a gram negative bacterium, is the most important microorganism responsible for foodborne illness and typhoid fever causing nearly 600,000 deaths annually[19] and the fungi *Candida albicans*[20], causes a range of clinical infections including irritating infections of the oral mucosa (thrush) to life-threatening systemic disease in immune-compromised patients and have an effect on numerous organ systems such as the eyes, lungs, kidneys, heart and central nervous system.

Seventeen different isolates of the MRSA, *S. typhi* and *C. albicans* including reference ATCC isolates were used for this purpose.

Among the tested compounds **2** and **4** having bromo-substitution overall showed excellent activity against all three microbes predominantly against MRSA and *C. albicans*. Compound **6** having carboxylic functionality was also effective against tested microbes. Antimicrobial activity of remaining carbazoles was moderately good against different strains of all three tested microbes (Table-1).

TABLE-1
RESULTS OF ANTIMICROBIAL SCREENING OF COMPOUND 1-7 IN TERMS OF MIC (µg/mL)

Tested compounds	MRSA	*S. typhi*	*C. albicans*
1	512	>512	512
2	18	128	128
3	>512	>512	512
4	32	64	64
5	256	>512	>512
6	128	512	64
7	512	>512	512

DMSO: Negative control.

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