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Studies on Antimicrobial Activity of Novel Naphthyridine Derivatives

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Abstract: *Naphthyridine compounds, due to their unique cellular action, Stability under extreme conditions and broad spectrum antimicrobial activity, can be potential therapeutic agents against various microbial infections. In this present investigation, 10 novel naphthyridine derivatives were examined for their antimicrobial activity. Out of all the compounds tested, 8,10-Dibromo-6-phenyl-6a,7,10,12-tetrahydro-1,7,12a-triaza-benzo[a]anthracen-12-one and 8,10-Dibromo-6-(4-methoxy-phenyl)-1,7,12a-triaza-benzo[a]anthracen-12-one compounds were found to possess potent antimicrobial and antifungal properties respectively. In addition, we observed that antimicrobial activity is highly influenced by the type of halogen atom and position of its substitution in the molecules.*

Key Words: Naphthyridine, antimicrobial activity, halogen substitution, antifungal activity,

1. INTRODUCTION

Pathogenic microbes can cause serious and devastating diseases in humans, animals and crops. Until now, by using chemotherapeutics and antibiotics, pathogenic microbes were kept under control. However, development of antibiotic resistance among pathogenic microbes has become a major concern and serious problem for researchers and clinicians [1,7]. Owing to the short life-cycle of bacteria, ability to undergo rapid genetic changes and adapt to a constantly changing ecosystem, it is presumed that antibiotic resistance process can be prevented or reversed by using antibiotic cycling [17] . However, theoretical models and experimental studies showed that antibiotic cycling will not bring back the antibiotic susceptibility, instead leave a mark with every probability to surface back when exposed to the antibiotic [1,20]. Therefore, novel types of molecules are needed, which can replace antibiotics in the long run. Currently there is a dearth of new anti-infective agents that are active against resistant pathogenic species as no new antibiotics have added to the arsenal in last 50 years [25].

Given this many researchers around the globe have focused their research efforts on discovering alternatives for antibiotics. Naphthyridine compounds are hot area of research due to their wide spectrum of antimicrobial activity [8,10] . Synthetically formulated naphthyridine compounds have become popular antimicrobial agents owing to their stability under extreme conditions and effective antimicrobial and apoptotic activity [2]. Molecules belonging to this group are widely used as chemotherapeutics for infectious diseases [11,13,19,24] , antitumor, anti-inflammatory, antiplatelet, antiallergic, anticancerous drugs [21]. Moreover, some naphthyridine derivatives have been recently patented as new generation plant growth regulators, fungicides [3,12] , bactericides[14] herbicides, insecticides and nematocides [22]. Given this input, we decided to investigate the antimicrobial activity of 10 novel naphthyridine derivatives.

I.1 MATERIAL & CHEMICALS: All the chemicals used in this work were of analytical grade and were obtained from Himedia, India unless stated. Ten novel naphthyridine derivatives (NND) used in the study (Table 1) were obtained from Department of chemistry, Kakatiya University, India [18] . Three bacteria (*Escherichia coli, Bacillus subtilis, Enterococcus faecalis)* and four fungi (*Aspergillus niger, Pencillum chrysogenum, Fusarium oxysporum*, and *Drechslera sp)* were obtained from the National Chemical Laboratory, India. All the fungal strains were grown on potato dextrose agar (PDA) and bacterial strains were grown on nutrient agar medium (NAM). For the resuspension and dilution of bacteria and fungi, sterile saline was used. Streptomycin and amphotericin B at two concentrations (250 and 500 μg/ml) were used as positive control for comparison with NND. Acetone, water, saline were used as negative controls.

1.2 ANTI-MICROBIAL ACTIVITY: In order to make the disc containing the test compound, discs of size 0.5mm in diameter were made from Whatman filter paper 3 by using cork borer and sterilized by dry heat at 140 ⍰ for 1 h. Stock solution of eac h NND was made in acetone at the concentration of 1 mg/ml. Final working solution (250 or 500 μl) was transferred into fresh tube and a single disc was transferred followed by incubation in water bath preset to 55 ⍰ until the entire acetone

evaporated. Positive as well as negative controls were also made in the above said procedure. For negative control, discs were first socked in water and processed as stated above.

For antibacterial activity disc diffusion method was employed. Briefly, a loop full of bacteria was inoculated in 10 ml nutrient medium (NM) and incubated for 12-16 hours at 37 ± 1°C. Later, 200 µL of the overnight culture was transferred into fresh 10 ml NM and grown up to mid logarithium phase (0.4 - 0.5_{620}). The microorganisms were then washed twice with sterile saline pH 7.4 and resuspened in 1 ml of saline. Subsequently, bacterial load (1-2 X 10^7 CFU) was added to 5 ml agar NM and spread in a petriplate. Then sterile disc previously soaked in a known concentration of the NND were placed on top and plates were incubated for 24 h at 37 ± 1°C in inverted position. The inhibition zones around each disc was measured by using Hiantibiotic zone scale™ (Himedia, India) and compared with the controls after 24 h zone of incubation. All the experiments were carried out in triplets and best representing experiment is presented here. Streptomycin was used as a standard drug.

For antifungal screening, fungal isolates were grown on PDA at 30 ± 1°C for 7 days. Ten agar discs containing the mycelia were made by using a 0.5mm diameter sterile cork borer which was transferred aseptically into 10 ml of sterile saline and vortexed for 10 min to disintegrate the agar. Inoculum (0.1ml) were applied on the surface of the PDA plate and spread by using sterile glass spreader. Sterile disc with known concentration of the NND or negative controls were placed on top and incubated for 48-64 h at 30±1°C. The inhibition zones were measured and compared with the control amphotericin B.

In this present study, ten novel naphthyridine derivatives were analyzed for antimicrobial activity by disc diffusion assay method. For all the experiments two additional controls were maintained one with microorganism and other without microorganism for a better clarity in results and avoid ambiguity in results. This strategy was followed to check whether there is any contamination in working area and to confirm the culture purity all along the work. It has been shown compounds dissolved in organic solvents showed a higher antimicrobial activity over the compounds dissolved in water [15,16] , hence NND were dissolved in acetone over water. This strategy was employed in order to prevent false negative results by compounds with lower activity and also removing the organic solvent without destroying the compound is easy than in water by heating. The results of these investigations (Table –II) showed that most of the compounds tested have more antifungal activity rather than antibacterial. Among all the compounds tested NND-VII has the most antifungal activity and rest of them have a moderate or lesser activity to that of positive controls.

The antimicrobial assay results reveal that majority of the NND showed a variable inhibition activities against the tested microbes. Molecular structural analysis of compounds reveals that the molecule, which ever possessed the halogen atom (e.g NND-II, VII) were found to have both antibacterial and antifungal activity. Recently it has been clear that presence of halogen atoms in molecules increase the target binding ability and also stabilises ligand-target complex [9]. Interestingly, molecules (NND-V, VIII) without any halogen group were showing only antibacterial activity especially against gram negative bacteria [4] . The difference in the activity could be attributed to the difference in the membranes architectures of bacteria and fungi.

It widely known that the molecules biophysical properties influence the spectrum of action of various biothereaputic molecules. Interestingly, we observed that even the position and number of halogen atoms in the molecule are having some effect on the activity and host spectrum. Molecules with a halogen at both 8 and/or 10 (NND- II, VII/VI) positions were found to have more antimicrobial activity when compared to molecules having a halogen at some other place (NND-IX). Noteworthy, presence of halogen at 8^{th} position is more important than at 10^{th} position (NND-VI) for higher antimicrobial activity.

Shirinzadeh et.al, reported that compounds with monohalogen are less antimicrobial in comparison to dihalogenated ones [23] . It is suggested that ionization of the N-H bond might be an important step in the difference in molecular mode of action for the antimicrobial activity [5,6]. Our results also confirm this observation, compounds containing two bromine atoms (NND II, VII, X) are more antimicrobial than those with one (NND VI). The unique observation in this study is that not only the number and position of the halogen even the type of halogen atom have some effect on the spectrum of antimicrobial activity. NND-III with iodine atom at C-10 position was showing activity, even though less than control, against all the bacteria and one fungal species that are studied, where, as molecule NND-IV with Br was observed to posses lesser spectrum of antimicrobial activity [4]. Our results show that the presence of more number of halogen atoms at right position is crucial in deciding the spectrum and specificity of antimicrobial activity. As the antimicrobial activity varied from low to moderate, future investigations are necessary to elucidate the structure activity relationship. Even though the synthesised compounds did not exhibit high levels of antibacterial activity in comparison to that of streptomycin, we believe that the data presented here is of immense value for the people working in the antimicrobial activity as the data clearly demonstrate the significance of position and halogen atom present. In this paper, we report superior antifungal activity by some of the naphthyridine derivatives.

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Table 1: **Nomenclature of Naphthyridine compounds used in the present study as per IUPAC**

Code*	COMPOUND
NND I	3-Bromo-10-(4-methoxy-phenyl)-2,3,9,10-tetrahydro-1,4a,5-triaza-phenanthren-4-one
NND II	8,10-Dibromo-6-phenyl-6a,7,10,12-tetrahydro-1,7,12a-triaza-benzo[a]anthracen-12-one
NND III	10-Iodo-6-phenyl-1-7-12a-triaza-benzo[a]anthracen-12-one
NND IV	10-Bromo-6-phenyl-1-7-12a-triaza-benzo[a]anthracen-12-one
NND V	6-phenyl-5,6-dihyro-1,7,12a-triaza-benzo[a]anthracen-12-one
NND VI	10-Bromo-6-(4-chloro-phenyl)-1,7,12a-triaza-benzo[a]anthracen-12-one
NND VII	8,10-Dibromo-6-(4-methoxy-phenyl)-1,7,12a-triaza-benzo[a]anthracen-12-one
NND VIII	6-(4-Methoxy-phenyl)-5,6-dihydro-1,7,12a-triaza-benzo[a]anthracen-12-one
NND IX	6-(4-chloro-phenyl)-1,7,12a-triaza-benzo[a]anthracen-12-one
NND X	8,10-Dibromo-6-(4-chloro-phenyl)-10,12-dihydro1,7,12a-triaza-benzo[a]anthracen-12-one

* For this work

Table II : Zones of inhibition (mm) observed for the microorganism when incubated with NND.

MO	µg / disc	Streptomycin	Amphotericin B	I	II	III	IV	V	VI	VII	VIII	IX	*X*
E.coli	250	3.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.5	1.0	0.0	1.0
	500	5.0	0.0	3.0	3.0	1.0	1.0	10.0	0.0	1.5	2.0	0.0	2.0
B.subtilis	250	2.0	0.0	*	1.0	0.5	0.0	3.0	0.0	1.0	0.0	0.0	1.0
	500	6.0	0.0	1.0	2.0	1.0	0.0	7.0	0.0	3.0	1.0	0.0	2.0
E. faecalis	250	3.0	0.0	0.0	2.0	1.5	1.0	0.0	0.0	2.0	0.0	0.0	0.0
	500	7.0	0.0	0.0	3.0	2.5	1.5	0.0	0.0	2.5	0.0	0.0	0.0
A.niger	250	0.0	3.0	3.0	6.0	4.0	0.0	0.0	0.0	3.0	0.0	0.0	3.0
	500	0.0	4.0	4.0	1.0	5.0	1.0	3.0	0.0	9.0	0.0	0.0	7.0
P. chrysogenum	250	0.0	2.0	0.0	2.5	*	*	0.0	0.0	*	0.0	0.0	0.0
	500	0.0	3.0	0.0	3.5	*	*	0.0	0.0	*	0.0	0.0	0.0
F. oxysporum	250	0.0	1.0	0.0	7.0	0.0	0.0	0.0	0.0	7.0	0.0	0.0	0.0
	500	0.0	3.0	0.0	9.0	0.0	0.0	0.0	7.0	8.0	0.0	0.0	9.0
Drechslera sp	250	0.0	2.0	0.0	0.0	2.0	0.0	0.0	2.0	4.0	0.0	0.0	2.0
	500	0.0	4.0	5.0	0.0	5.0	0.0	0.0	5.0	5.0	0.0	0.0	4.0

* trace inhibition

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