CHAPTER 4

BIOLOGICAL ACTIVITIES
INTRODUCTION

Biological activity spectrum of a compound represents the pharmacological effects, physiological and biochemical mechanisms of action, specific toxicity that can be revealed in compound’s interaction with biological system. Further, it describes the intrinsic properties of the compound, which depends on its structure.

The tetrahydropyrimidine derivatives are also known to have wide spectrum of therapeutic activities such as calcium antagonist\textsuperscript{1-3}, anti-inflammatory\textsuperscript{4-6}, analgesic\textsuperscript{7,8}, antitumor\textsuperscript{9,10}, antidepressant\textsuperscript{11}, antibacterial and antifungal effects\textsuperscript{12-14}. These compounds act as muscarinic agonist in the rat central nervous system\textsuperscript{15,16}. Upshall\textsuperscript{17} has reported the nicotinic activity of these compounds.

Dihydropyrazole are always an attraction point for researchers because of its efficiency towards various pharmacological usages. Dihydropyrazole posses possess valuable bioactivities like, antiinflammatory\textsuperscript{18,19}, antitumor\textsuperscript{20,21}, analgesic\textsuperscript{22,23}, bactericidal\textsuperscript{24}, fungicidal\textsuperscript{25}, anticancer\textsuperscript{26}, anticonvulsant\textsuperscript{27}, pesticidal\textsuperscript{29}, antiamoebic\textsuperscript{30}, antidepressant\textsuperscript{31}, insecticidal\textsuperscript{32}, antineoplastic\textsuperscript{33,34} etc.

1,3,4-Oxadiazoles are a class of heterocycles, which have attracted significant interest in medicinal chemistry\textsuperscript{35}. These heterocyclic compounds have been subjected to a large variety of structural modifications in order to synthesize derivatives with different biological properties. Their various derivatives have been reported to possess antibacterial\textsuperscript{36}, antiinflammatory\textsuperscript{37}, analgesic\textsuperscript{38}, anticancer\textsuperscript{39}, antihypertensive\textsuperscript{40}, anticonvulsant\textsuperscript{41,42}, antifungal\textsuperscript{43}, cardiovascular\textsuperscript{44}, hypoglycemic\textsuperscript{45}, Mao inhibitor\textsuperscript{46,47}, antituberculosis\textsuperscript{48}, anti-tumor\textsuperscript{49}, anthelmintic\textsuperscript{50}, antioxidant\textsuperscript{51} etc.

Thiazolidinone derivatives are also known to possess interesting biological activities such as anticancer\textsuperscript{52}, anti-HIV\textsuperscript{53}, antimalarial\textsuperscript{54}, tuberculostatic\textsuperscript{55}, antihistaminic\textsuperscript{56}, anticonvulsant\textsuperscript{57,58}, antibacterial\textsuperscript{59}, antiarrythmic\textsuperscript{60}, antiproliferative\textsuperscript{61,62}, antiinflammatory\textsuperscript{63}, cox I inhibition\textsuperscript{64}, anti tumor\textsuperscript{65}, analgesic\textsuperscript{66}, and antidiabatic\textsuperscript{67,68} etc.

In the present chapter, antibacterial and antifungal activities of some synthesized compounds have been screened against some Gram positive and Gram negative bacteria as well as some fungal strains.
EXPERIMENTAL

The antibacterial and antifungal activities of all synthesized compounds were studied in DMSO. All the synthesized compounds were recrystallized prior to use.

The solvent DMSO was also purified before use by standard method\(^9\). For all the compounds, agar well diffusion method was used.

**Test Microorganisms:**

The synthesized compounds were tested for its antibacterial activity against three Gram positive bacteria *Staphylococcus aureus* ATCC29737, *Bacillus cereus* ATCC11778 and *Micrococcus flavus* ATCC10240 and three Gram negative bacteria *Escherichia coli* NCIM2931, *Salmonella typhimurium* ATCC23564 and *Proteus mirabilis* NCIM2241 and two antifungal strains *Candida glabrata* NCIM3448 and *Candida neoformans* NCIM3542. The microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India and were maintained at 4°C on nutrient agar slants.

**Preparation of test compounds:**

The solutions were prepared at a concentration of 1 mg/μl for all the compounds.

**Preparation of the plates and microbiological assay:**

The test organism was activated by inoculating a loop full of the strain in 25 ml of Nutrient broth/ Sabouraud Dextrose Broth and kept overnight on a rotary shaker. Mueller Hinton agar and Sabouraud Dextrose Agar medium were used for antibacterial and antifungal activity respectively. The assay was performed by agar well diffusion method\(^{70,71}\). 200 μl inoculums (1 x 10\(^8\) cfu/ml) was introduced into molten Muller Hinton agar/ Sabouraud Dextrose agar and poured into Petri dishes when temperature was reached to 40 – 42°C. The media was solidified and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). 100 μl of the test compound (20 mg/ml DMSO) was introduced into the well and the plates were incubated at 37/28°C for 24/48 h for bacteria and fungi, respectively. Dimethyl sulfoxide (DMSO) was taken as a negative control. All the tests were performed in triplicate under strict aseptic conditions. The microbial growth was determined by measuring the diameter of the zone of inhibition in mm.
RESULTS AND DISCUSSION

Tetrahydropyrimidine derivatives (PAB 101-PAB-110):

Figure 4.1 shows the antibacterial activity of tetrahydropyrimidine derivatives (PAB 101-PAB-110) against Gram positive and Gram negative bacteria as well as fungal strains.

It is evident from Figure 4.1[A] that PAB-103 exhibited maximum inhibition against *B. Cereus*. Against both *M. flavus* and *S. aureus*, PAB-108 showed maximum inhibition. PAB-105, PAB-106 showed no inhibition at all against *M. flavus* whereas against *S. aureus*, PAB-105 and PAB-106 exhibited minimum inhibition.

Figure 4.1[B] shows inhibition against Gram negative bacteria. It is observed that against *E. Coli*, PAB-109 and PAB-110 showed maximum inhibition whereas PAB-106 showed minimum inhibition whereas against *P. Mirabilis*, PAB-103 exhibited maximum inhibition and PAB-105 showed minimum inhibition. Against *S. Tuplemurium*, again PAB-110 exhibited maximum inhibition.

Against both fungal strains, *C. Glabrata*, *C. Neoformans*, PAB-109 showed maximum inhibition, as evident from Figure 4.1[C].

The inhibition depends on the solvent, compound structure and bacterial strain. In the present study, solvent is same throughout so this parameter is not considered in this study. All the compounds of tetrahydropyrimidine derivatives contain same central moiety but different substitution. Figure 4.2 shows the general structure of tetrahydropyrimidine derivatives along with different substitutions. These substitutions are aryl ring with different functional groups. Thus, different substitutions affect different strains differently. PAB-103 contains m-chloro group whereas in PAB-108 m-methoxy and p-hydroxy groups are present. In PAB-109, it is furan whereas in PAB-110, hydroxy group is present at para position.

Thus, m-chloro group is more effective against *B. Cereus* and *P. Mirabilis* whereas m-methoxy and p-hydroxy groups are effective for *M. flavus* and *S. aureus* strains. Against *E. Coli* and *S. Tuplemurium*, hydroxy group is highly effective at para position.
Figure: 4.1: Antibacterial activity of tetrahydropyrimidine compounds against [A] Gram positive bacteria [B] Gram negative bacteria and [C] fungal strains.

[A]

[B]

[C]
Figure 4.2: General structure of tetrahydropyrimidine derivatives (PAB-101 – PAB-110)

![General structure of tetrahydropyrimidine derivatives](image)

R=Substituted aldehyde

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Dihydropyrazole derivatives (PAB 301-PAB-320):

Figure 4.3 shows the inhibition of dihydropyrazole derivatives (PAB 301-PAB-320) against Gram positive bacteria. It is observed from Fig. 4.3 that against *B. Cereus*, PAB-314 showed maximum inhibition whereas PAB-305 showed minimum inhibition. For *M. flavus*, only few compounds exhibited inhibition and PAB-311 showed maximum inhibition. Against *M. flavus*, PAB-317 showed minimum inhibition whereas against *S. aureus*, PAB-308 exhibited maximum inhibition, which is followed by PAB-312.

All these dihydropyrazole derivatives contain the same moiety but different side chains as shown in Figure 4.4 where general structure of these derivatives are given along with different R and R$_1$ groups. PAB-314 containing p-chloro and p-nitro group groups at R$_1$ and R positions respectively affects *B. Cereus* to maximum extent. When one of these groups is present with some other functional group as in PAB-304, PAB-307, PAB-308, PAB-311, PAB-317 and PAB-318), the inhibition is considerably reduced. However, against *M. flavus*, PAB-311 containing p-chloro and p-methyl group is found to be most effective whereas against *S. aureus*, PAB-308 containing p-chloro and o-nitro groups at R$_1$ and R positions respectively is more effective. Thus, it is concluded that against the studied Gram positive bacteria, mostly compounds containing p-chloro groups at R$_1$ position are found to be more effective.

Figure 4.5 shows the inhibition of dihydropyrazole derivatives (PAB 301-PAB-320) against Gram negative bacteria. It an be noticed that against *E. Coli*, PAB-305 exhibited maximum inhibition whereas minimum is observed for PAB-307. PAB-314 could inhibit *P. Mirabilis* to maximum extent in comparison to other compounds whereas PAB-306 showed maximum inhibition against *S. Tuphimurrium*. Some of the compounds had no effect on *S. Tuphimurrium*.

PAB-305 contains p-nitro and p-fluoro groups at R$_1$ and R positions respectively, which is found to be more effective against *E. Coli*. PAB-314 containing p-chloro and p-nitro group groups at R$_1$ and R positions respectively affects *P. Mirabilis* to maximum extent whereas o-nitro and p-nitro groups at R$_1$ and R positions (as in PAB-306) could affect *S. Tuphimurrium* more effectively. Thus, against the studied Gram negative bacteria, compounds containing p-nitro groups are more effective.
Figure: 4.3: Antibacterial activity of dihydropyrazole compounds against Gram positive bacteria in DMSO
Figure 4.4: General structure of dihydropyrazole derivatives (PAB-301-PAB-320)

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<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
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Figure 4.5: Antibacterial activity of dihydropyrazole compounds against Gram negative bacteria in DMSO
Against fungal strains, it is observed from Figure 4.6 that all the studied compounds could not inhibit *C. Glabrata*. However, against *C. Neoformans*, some compounds exhibited inhibition and PAB-307 and PAB-318 showed maximum inhibition.

PAB-307 contains p-chloro and p-aryl groups at R<sub>1</sub> and R positions respectively whereas PAB-318 contains p-chloro and p-methoxy groups at R<sub>1</sub> and R positions respectively. Thus, for fungal strain *C. Neoformans*, again compounds with p-chloro groups are effective with p-aryl and p-methoxy groups in combination.

Thus, in case of studied dihydroxypyrazole derivatives in DMSO solutions, *M. flavus* is most resistant among three Gram positive bacteria, *S. Tuphimurrium* is most resistant among three Gram negative bacteria and *C. Glabrata* is most resistant among the two fungal strains.

**Oxadiazole derivatives (PAB-401-PAB-420):**

Figure 4.7 shows the zone of inhibition of oxadiazole derivatives (PAB-401-PAB-420) against Gram positive bacteria. It is observed from this figure that against *B. Cereus*, PAB-402 exhibited maximum inhibition and PAB-405 showed minimum inhibition. For *M. flavus*, PAB-416 exhibited maximum inhibition whereas PAB-413 and PAB-414 showed minimum inhibition. For *S. aureus*, maximum and minimum inhibitions were exhibited by PAB-411 and PAB-413 respectively.

As evident from Figure 4.8, where general structure of these derivatives are given along with the substitution, that PAB-402 contains m-bromo benzyl group as side chain whereas in PAB-405, no side chain is in the benzene ring. Thus, it is concluded that when R is benzene ring with side chain, inhibition is higher and it is maximum when side chain is bromo group at para position. Without side chain, compound is not very effective as in case of PAB-405. When methyl group is present at meta position, the compound exhibited maximum inhibition against *M. flavus*. Against *S. aureus*, m-chloro group is found to be most effective (as in PAB-411).

Figure 4.9 shows the zone of inhibition of oxadiazole derivatives (PAB-401-PAB-420) against Gram negative bacteria. It is observed that against *E. Coli*, *P. Mirabilis* and *S. Tuphimurrium*, PAB-406, PAB-403, PAB-412 exhibited maximum inhibition. Against *E. Coli*, PAB-402 and PAB-415 showed minimum inhibition whereas PAB-420 had no effect at all.
Figure 4.6: Antifungal activity of dihydropyrazole compounds in DMSO
Figure 4.7: Antibacterial activity of oxadiazole compounds against Gram positive bacteria in DMSO
Figure 4.8: General structure of oxadiazole derivatives (PAB-401 – PAB 420)

R = Substituted Acid

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Figure 4.9: Antibacterial activity of oxadiazole compounds against Gram negative bacteria in DMSO
Against *P. Mirabilis*, PAB-407 and PAB-413 exhibited minimum inhibition whereas PAB-403 showed minimum inhibition against *S. Tuphimurrium*. However, few compounds had no inhibition at all against *S. Tuphimurrium*.

Thus, 2,4-dichloro groups (at ortho and para positions) in PAB-406, p-chloro group as in PAB-403 and m-nitro group (as in PAB-412) are most effective in inhibiting Gram negative bacteria.

Against fungal strains *C. Glabrata* and *C. Neoformans*, most of oxadiazole derivatives had no effect at all as shown in Figure 4.10. However, PAB-403 containing p-chloro group showed maximum inhibition against both fungal strains. Thus, p-chloro group alone as side chain in benzene ring is most effective for selected fungal strains. When other groups are present along with p-chloro group, inhibition is decreased.

Thus, in case of studied oxadiazole derivatives in DMSO solutions, *S. aureus* is most resistant among three Gram positive bacteria, *S. Tuphimurrium* is most resistant among three Gram negative bacteria and *C. Glabrata* is most resistant among the two fungal strains.

**Thiazolidinone derivatives (PAB-501-PAB-510):**

Figure 4.11 [A] shows inhibition against Gram positive bacteria for thiazolidinone derivatives (PAB-501-PAB-510). It is observed that against all the three selected Gram positive bacteria, viz. *B. Cereus*, *M. flavus*, *S. aureus*, PAB-501 exhibited maximum inhibition. Against *B. Cereus*, PAB-506 and PAB-507 exhibited minimum inhibition. Some compounds such as PAB-503, PAB-506, PAB-507, PAB-508 and PAB-510 could not inhibit *M. flavus* at all. However, against *S. aureus*, only PAB-508 showed no inhibition.

The general structure of thiazolidinone derivatives along with different substitution groups are given in Figure 4.12. Again, in all these compounds central moiety is same but side chains are different. Thus, p-chloro group, which is present in PAB-501, is found to be most effective against all the three studied Gram positive bacteria.

In Figure 4.11 [B], zone of inhibition against Gram negative bacteria for thiazolidinone derivatives (PAB-501-PAB-510) is shown. Against *E. Coli* and *S. Tuphimurrium*, PAB-504 exhibited maximum inhibition whereas against *P. Mirabilis*, both PAB-502 and PAB-503 showed maximum inhibition. Against *S. Tuphimurrium*, PAB-501, PAB-505, PAB-506 and PAB-508 had no effect at all.
Figure 4.10: Antifungal activity of oxadiazole compounds in DMSO
Figure 4.11: Antibacterial activity of thiazolidinone compounds against [A] Gram positive bacteria, [B] Gram negative bacteria and [C] fungal strains.

[A]
Figure 4.12: General structure of thiazolidinone derivatives (PAB-501 – PAB-510)

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As given in Figure 4.12, PAB-504 contains p-bromo group, which is found to be most effective against *E. Coli* and *S. Tuphimurrium*. Whereas against *P. Mirabilis*, 2,5-dimethoxy group (present in PAB-502) is also found to be equally effective as p-nitro group (as in PAB-503).

Figure 4.11 [C] shows inhibition zones against fungal strains *C. Glabrata* and *C. Neoformans*. It is found that for these two fungal strains some of the compound had no effect at all. However, PAB-510 exhibited maximum inhibition against *C. Glabrata* whereas PAB-508 showed maximum inhibition for *C. Neoformans*.

Thus, in case of studied thiazolidinone derivatives in DMSO solutions, *M. flavus* is most resistant among three Gram positive bacteria, *S. Tuphimurrium* is most resistant among three Gram negative bacteria and *C. Neoformans* is most resistant among the two fungal strains.

Comparison of antimicrobial activity of all the above mentioned four series shows that tetrahydropyrimidine compounds are most effective. Except oxadiazole, in all other series, side chains are almost same. Thus, the central moiety of tetrahydropyrimidine compounds along with selected side chains is most effective in inhibiting studied bacteria and fungal strains than other moieties.
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