Chapter-VI

Evaluation of anti-microbial activity of
Synthesized compounds
Chapter –VI

Chapter at a Glance

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Abstract

A few selected synthesized compounds from II III IV V respectively (viz. oxidaizolo, imidazolo, benzmidazolo, thiadiazole, isoxazole, pyrazolo, pyrimidine, 1,5-benzodiazipino, 1,5-benzothiazepino, 1,5-benzoaxazepino) incorporated analogues of face ‘c’ cyclohexano annulated 1,5-benzodiazipine appended on to it through the oxyphenyl or aminophenyl bridge) were screened for their in vitro antimicrobial activities against bacterial species such as (E. coli and B. subtilis) and fungal species such as (A. niger and F. soloni) by disc diffusion method against the standard drugs (Ciproflaxacin for bacteria and Fluconazole for fungi) and results which have emanated from these studies have been discussed in this chapter.
6.1 General Introduction

Condensed heterocyclic systems containing oxadiazoles, imidazoles, benzimidazoles, thiadiazoles, pyrazoles, isoxazoles, pyrimidines and azepines (1,5-benzodiazepine, benzothiazepines and benzoxazepines) have attracted the attention of chemists owing to these nuclei having been recognized in the literature as the most active pharmacophores in the drug design and synthesis. It has been observed that incorporation of certain bioactive pharmacophores in the existing drug molecules sometimes exert a profound influence on the biological profiles of that molecule. Based on these observations, it was thought of interest in the present work to incorporate these bioactive pharmacophores into a single molecular framework, on face ‘c’ cyclohexano annulated analogue of [1,5]-benzodiazepines to study their impact on the biological activity in the newer materials, produced by the additive or cumulative effects excercised by the presence of each of these moieties.

To test this hypothesis, a series of novel compounds 6.01-6.06 (fig-6.1) were selected for their biological screening.

In chapter-III, IV and V the synthesis of these materials has been described. The present chapter describes the result obtained on in vitro screening of 6 representative members selected from each class for study of their antibacterial activity against Esherichia coli (MTCC 119) and Bacillus subtilis (MTCC 619) and antifungal activity against Aspergillus niger (MTCC 282) and Fusarium solani (MTCC 350). The zone of inhibition was determined in comparison to the standard drugs, Ciprofloxacin” for bacterial strains and “Fluconazole” for the fungal strains (Fig-6.1). The outcome of this study is presented in tubular form in table-6.1.

6.2 Antibacterial Studies1-4

6.2.1 Bacterial strain:

Pure culture of pathogenic bacteria for anti-bacterial activity, were procured from the Microbiology Research Laboratory, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan. These were sub-cultured and characterized by standard methods of identification5. E. coli (MTCC 119) and
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*B. subtilis* (MTCC 619) strain of various bacteria were used for anti-bacterial screening.

6.2.2 Different controls:

In the second phase, three types of controls were ensured to allow the organism to grow in petridishes. This was done as follows:

**Organism control:**

This included sterilized medium and organism. Organism was placed in the medium. If no growth was observed, it was concluded that the media was not suitable for the organism.

**Media control:**

Fully sterilized medium was chosen to check the presence of organism. If, some unwanted growth was observed, it was concluded that the media were not fully sterilized and hence, the results were not valid.

**Solvent control:**

The necessary solvents and solutions (except test compounds) were properly sealed by good quality cotton and were subjected to very high pressure and temperature. All work of pouring and preparing the media were done between two burners in a Laminar Air Flow (LAF) to repeal unwanted organism from coming in contact to the agar media taken in petri dish.

For media control to be assured, a mild solvent (DMF) was taken which was found to favour the organism to grow. Transfer of test compounds solutions was done by sterilized micropipette in between two burners in LAF.

6.2.3 Anti-bacterial activity of the compounds:

The newly synthesized benzimidazo 6.01, thiadiazolo 6.02, isoxazolo 6.03, pyrimido 6.04, azepino 6.05 and 6.06 condensed analogues of face ‘c’ cyclohexano annulated analogue of 1,5-benzodiazepine were screened for their antibacterial activity against randomly chosen two different bacterial strains containing both gram positive and gram negative bacteria. The different strains chosen were *E.coli*
(MTCC 119) and *B. subtilis* (MTCC 619) and the activity of the synthetic materials of these strains were studied by disc diffusion method.

To study the anti-bacterial activity, the compounds were first dissolved in the solvent DMF so as to make necessary dilution at 400, 200 and 100 µg/ml to form the stock solutions of the compounds to be tested.

### 6.3 Methodology\(^6\)\(^-\)\(^9\)

#### Growth medium (Disc diffusion method):

**Preparation of media:**

The media, which was used for testing of anti-bacterial activity, had the following composition for one liter solution and nutrient agar was developed for the microbes to grow the organisms by settling over Petri dishes in the compositions given below:

- **Peptone**: 5 g
- **NaCl**: 5 g
- **Meat extract**: 3 g
- **Distilled water**: 1000 mL

The media, which was used for testing of anti-fungal activity, had the following compositions for one liter solutions. The composition of potato dextrose (procured from HI-Media) was developed for the microbes to grow the organisms by settling over petridishes.

- **Glucose**: 20 g
- **Peptone**: 10 g
- **Agar-agar**: 15 g
- **Distilled water**: 1000 mL

In this method, all the above components were mixed and dissolved in one liter of distilled water by heating the mixture. The pH of the media was adjusted to 5.0.
The media was then sterilized in the autoclave along with the glasswares, petri dishes, pipettes etc. at 15 lb pressure and 120°C for half an hour. The sterilized nutrient agar solution was taken out by micro-pipettes and a large number of Petri dishes were prepared by pouring agar solution to it between two burners in laminar air flow (LAF). The solution gradually solidified to a thickness of around 0.30.5-cm. The plates were then tightly tapped and kept at room temperature.

Under the aseptic conditions, the sterilized filter paper discs were dipped in the diluted test solutions with different concentrations in DMF placed in the petri dishes. This was again done in laminar air flow. All the agar plates with different organisms and disc of test solutions were accordingly utilized. Then properly cupped plates were placed in incubator at 37°C for 12 hours and 28±2°C at 48 hours for bacterial fungal growth respectively.

Analysis and Interpretation of data:

After 24 hours of incubation, the plates were analyzed and the diameter of the zones of inhibition was measured to the nearest whole in millimeter with a sliding calipers. Standard drugs used for anti-bacterial and anti-fungal activity were ciproflaxin for bacteria and Fluconazole for fungi.

6.4 Anti-Fungal Studies

6.4.1 Fungal strains:

Pure culture of fungi used for anti-fungal activity, were procured from the Microbiology Research Laboratory, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan. These were sub-cultured and characterized by standard methods of identification. A. Niger (MTCC 282) and F. Solani strains were used for anti-fungal screening.

6.4.2 Anti-fungal activity of compounds:

The newly synthesized substituted benzimidazo 6.01, thiadiazole 6.02, isoxazole 6.03, pyrimido 6.04, azepino 6.05 and 6.06 condensed derivatives of face ‘c’ cyclohexano annulated analogue of 1,5-benzodiazepine were screened for their antifungal activity against randomly chosen two different fungal strains A.niger
(MTCC 282) and F. solani (MTCC 350) The activity was studied by disc diffusion method and standard used Fluconazole for fungi.

The stock solutions of standard and test compounds were prepared in DMF and subsequent dilutions i.e. 400, 200 and 100 µg/mL were made with the same solvent. In the second phase, three types of controls were assured to allow the organism to grow in the petri dishes. The preparation of media was done as described in anti-bacterial studies.

6.5 Result and Discussion
6.5.1 Anti-bacterial activity at minimum inhibitory concentration (MIC, 400-100 µg/ml)

The antibacterial screening against E.coli and B. subtilis showed the following order of activity among the compounds 6.01-6.06, compared to the standard drug ciprofloxin.

On comparing the antibacterial activity of the synthesized compounds (Shown in tabular form in table-6.1 and in graphical from in graph-6.1 and 6.2), the following conclusions were drawn.

- The result clearly indicated that increase in the concentration of compounds, increased the antibacterial activity. A regular fall in the activity was recorded when the concentration of the compounds were reduced.
- The results of the antibacterial activity revealed that all the compounds showed the following order of antibacterial activity with E. coli and B. Subtilis.
- Following decreasing order of antibacterial activity was shown by compounds 6.01-6.06 against E.coli. 6.06 (F) > 6.02 (B) > 6.03 (C) > 6.01 (A)> 6.04 (D) >6.05 (E)
- Following decreasing order of antibacterial activity was shown by compounds 6.01-6.06 against B.subtilis. 6.06 (F) > 6.03 (C) > 6.02 (B) > 6.05 (E) > 6.04 (D) > 6.01(A)
The benzodiazepine derivatives 6.06 showed the maximum activity and pyrimido derivatives 6.05 showed the minimum activity against *E. coli* for *B. subtilis* the benzodiazepine derivatives 6.06 showed the maximum activity and benzimidazo derivatives 6.01 showed the minimum activity.

### 6.5.2 Anti-fungal activity at minimum inhibitory concentration (MIC400-100 µg/ml):

The antibacterial screening against *A.niger* and *F. solani* showed the following order of activity among the compounds 6.01-6.06, compared to the standard drug Fluconazole.

On comparing the antibacterial activity of the synthesized compounds (Shown in tabular form in table-6.1 and in graphical from in graph-6.3 and 6.4), the following conclusions were drawn.

- The result clearly indicated that increase in the concentration of compounds, increased the antifungal activity. A regular fall in the activity was recorded when the concentration of the compounds were reduced.
- The results of the antifungal activity revealed that all the compounds showed the following order of antifungal activity with *A.niger* and *F. Solani*.
- Following decreasing order of antifungal activity was shown by compounds 6.01-6.08 against *A.niger*.
  - 6.04(D) > 6.03(C) > 6.02(B) > 6.05(E) > 6.06(F) > 6.01(A)
- Following decreasing order of antifungal activity was shown by compounds 6.01-6.08 against *F. Solani*.
  - 6.05(E) > 6.04(D) > 6.02(B) > 6.01(A) > 6.06(F) > 6.03(C)

The pyrimido derivatives 6.04 showed the maximum activity and benzimidazo derivatives 6.01 showed the minimum activity against *A.niger* for *F. solani* the benzothiazepino derivatives 6.05 showed the maximum activity and isoxazolo derivative 6.03 showed the minimum activity.
Compounds (6.01-6.06) whose anti-bacterial and anti-fungal activities have been studied in this chapter:

6.01(A)
6.02 (B)
6.03 (C)
6.04(D)
6.05(E)
6.06 (F)

Fig-6.1
Figure-6.1  Anti-bacterial activity of synthesized compounds 6.01-6.06 (A-F) against *E. coli*:

6.01(A)                        6.02(B)
6.03(C)                        6.04(D)
6.05(E)                        6.06(F)
Figure-6.2 Anti bacterial activity of synthesized compounds 6.01-6.06 (A-F) against *B. subtilis*:
Figure-6.3  Anti fungal activity of synthesized compounds 6.01-6.06 (A-F) against A.Niger:
Figure-6.4 Anti fungal activity of synthesized compounds 6.01-6.06 (A-F) against *F. solnoni*:
### Table: 6.1 Antimicrobial activities of the synthesized compounds (6.1-6.1)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Structure of compounds</th>
<th>Conc. (µg/ml)</th>
<th>E. coli</th>
<th>% activity compared to the standard</th>
<th>Zone of inhibition (mm)</th>
<th>B. subtilis</th>
<th>% activity compared to the standard</th>
<th>Zone of inhibition (mm)</th>
<th>A.niger</th>
<th>% activity compared to the standard</th>
<th>Zone of Inhibition (mm)</th>
<th>F. solani</th>
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<td>68.96</td>
<td>24.0</td>
<td>6.05(E)</td>
<td>8.1</td>
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<td>72.77</td>
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<td><em>Std. Anti bact.</em></td>
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*Std. Anti bact.: Ciproflaxacin
*Std. Antifungal: Fluconazole
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Graph 6.1-Anti bacterial activity of synthesized compounds (A-F) against *E.coli*

Graph 6.2-Anti bacterial activity of synthesized compounds (A-F) against *B. substilis*
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Graph 6.3-Anti fungal activity of synthesized compounds (A-F) against A. niger

Graph 6.4-Anti fungal activity of synthesized compounds (A-F) against F. solani