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***RESEARCH ARTICLE***

# Anti-microbial Studies of Potential Cyclopentanone derived Spiropyrroidines

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## ABSTRACT:
A Series of Various cyclopentanone derived spiro-pyrrolidines were synthesized using 1,3-dipolar cycloaddition methodology . The compounds found to have very good antimicrobial properties and some of the compounds shown properties greater than the reference compounds. The attachment of cyclopentanone moiety to spiropyrrolidines enhanced the antimicrobial properties and it is shown in the results.

**KEYWORDS:** Nutrient agar, bacterial culture, spiropyrrolidines, well diffusion method.

## INTRODUCTION:
Great attention has been paid for curing diseases caused by microorganisms. Many infectous of pathogenic microorganisms develop resistance against the prevailing drugs, and this situation has necessitated a search for new source of compounds. Heterocyclic compounds, particularly five- and six-membered ring compounds have occupied a prominent place among the organic compounds in view of their diverse biological activities. The spiro-pyrrolidine and oxindole ring systems have acquired a prominent place among various heterocyclic compounds owing to their specific structural motif in many pharmacologically relevant alkaloids, as typyfied by rhyncoohylline, cornoxeine, mitraphylline, horsifline and spirotryprostatins.[1,2] Cycloalkanone derivatives are also important synthetic precursors for biologically active materials. [3]

Cycloalkanone based compounds turn out to be nontoxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall[4,.5]

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Cycloalkanone find widespread application as anti-inflammatory, diuretic, anabolic, contraceptive, antiandrogenic, progestational and anticancer agents.[6]

Many of the cycloalkanone heterocycles have been found to exhibit potent biological activities, such as anti-inflammatory, anabolic, anti-cancer and anti-microbial activities.[7-15] Recently, heterocyclic cycloalkanone derivatives have been found to exhibit potential antibacterial, antifungal and antiproliferative activities.[16]

With this perspective we have carried out bioactivity studies on some of the spiro-pyrrolidines and the results are discussed

## MATERIAL AND METHODS:
### Clinical Bacterial isolates:
All the pathogenic bacterial and fungal isolates were obtained from Department of Clinical Medical Microbiology, Apollo Hospital at Chennai-06, India. All the clinical isolates were identified by standard methods. From this departmental culture collection unit, clinically important bacterial isolates were obtained namely *Staphylcoccus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* strains were used. Screening of antimicrobial activity was performed by following the sequence as given below:

1) Preparation of Nutrient agar,
2) Preparation of McFerland standards,

3) Inoculums preparation and
4) *In vitro* Antimicrobial Sensitivity Determination by Agar well diffusion method.

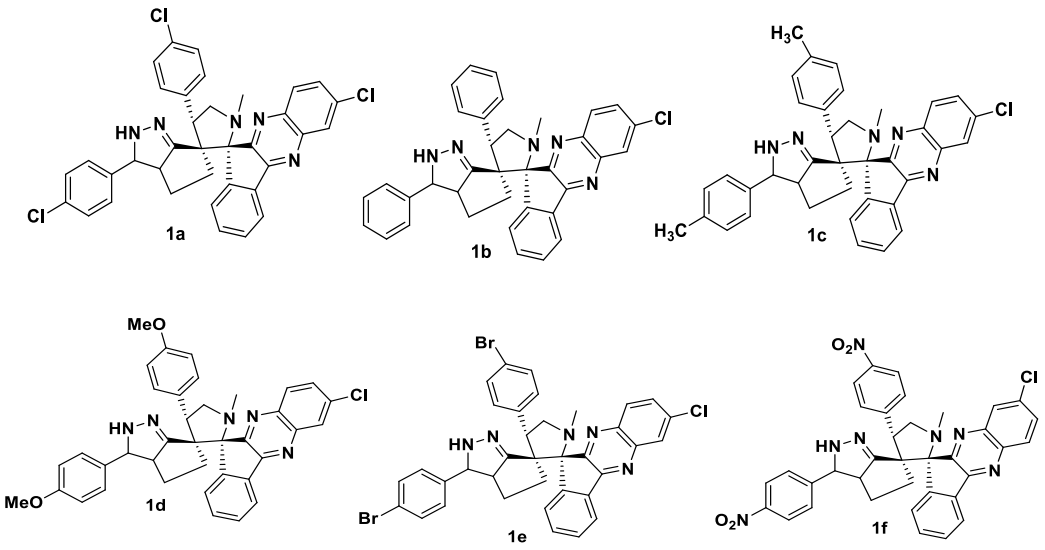
**Preparation of nutrient agar:**
The nutrient agar was prepared by dissolving beef extract (1.5g), peptone (0.5g), yeast extract (1.5g), sodium chloride (0.5g) and agar (1.5g) in 100mL of distilled water. The pH was adjusted to 7.2 followed by sterilization in an autoclave at 121oC/15 Lb for 15minutes. The sterile molten agar media was then cooled to 50oC. About 15ml of the media was poured on a sterile petriplate and allowed to cool at room temperature.

**Preparation of 0.5 Mcferland standards:**
0.5 mL of solution A (1.175g of barium chloride in 100mL of distilled water) was added to 99.5 mL of solution B (1mL of 0.36 N sulfuric acid in 100mL of distilled water) and mixed well with magnetic stirrer, then disturbed in test tubes with a screw cap of the same size as those containing the bacterial culture, the turbidity of which must be evaluated.

**Preparation of bacterial inoculums:**
The cooled sterile broth medium was poured into sterile petri-plates having a uniform depth of 4 mm; this is equivalent to approximately 25mL in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. The turbidity of the culture was adjusted with sterile cotton swab was immersed in the bacterial/fungal suspension and then rotated and compressed against the wall of the test tube so as to remove the excess fluid.

## RESULTS AND DISCUSSION:

**Invitro Antimicrobial Sensitivity Determination Test by Well Diffusion Method:**
The invitro antimicrobial sensitivity of the antibiotics and the test compounds synthesized were determined by well diffusion method[9] as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[10] The well diffusion test was performed using medium, as per the procedure described by Magaldi et al., 2004. A sterilized 10 mm cork borer was used to make agar wells on the sterile cotton swab.




**Spiropyyrolidines derived cyclopentanone**

**Table 1**

| Compounds | Escherichia coli | | Pseudomonas Aeruginosa | | Bacillus substills | | Staphylococcus Auerus | |
|---|---|---|---|---|---|---|---|---|
| | 50 µg/ml | 100 µg/ml | 50 µg/ml | 100 µg/ml | 50 µg/ml | 100 µg/ml | 50 µg/ml | 100 µg/ml |
| **1a** | 13.47 | 17.73 | 13.34 | 15.47 | · | ·- | · | ·- |
| **1b** | 19.89 | 21.11 | 18.89 | 21.11 | · | 13.33 | · | ·14.22 |
| **1c** | ·- | 14.33 | ·- | ·- | ·- | · | ·- | ·- |
| **1d** | 13.33 | 17.77 | 13.33 | 17.78 | · | ·- | 13.33 | 15.45 |
| **1e** | · | 12.24 | · | ·- | · | ·- | · | ·- |

**Table 2: Antibacterial activity of Kanamycin (10 µg/ml) against human pathogens**

| Organisms | Zone of inhibition (mm) (µg/ml) | Percentage of inhibition (%) |
|---|---|---|
| *Escherichia coli* MTCC 733 | 25.00±2.13 | 29.88 |
| *Pseudomonas aeruginosa MTCC1688* | 37.98±2.1 | 35.33 |
| *Bacillus subtilis* MTCC 41 | 28.25±1.81 | 31.38 |
| *Staphylococcus aureus* MTCC 96 | 31.25±2.05 | 31.50 |

Given values are mean values of triplicate and standard deviations (Mean ± SD)

**Table 3: Minimum Inhibitory concentration (MIC) of compounds against human pathogens**

| COMPOUNDS | Minimum inhibitory concentration (µg/ml) | | | |
|---|---|---|---|---|
| | Gram negative | | Gram positive | |
| | *Escherichia coli* | *Pseudomonas aeruginosa* | *Bacillus subtilis* | *Staphylococcus aureus* |
| **27a** | 50 | 100 | ND | ND |
| **27b** | 50 | 50 | 50 | 50 |
| **27c** | 100 | ND | ND | ND |
| **27d** | 50 | 100 | 100 | ND |
| **27e** | 100 | ND | ND | 100 |

**ND:** Not Determined

Compounds were dissolved in DMSO solvent separately and poured in the wells with varying concentrations ranging from 50µL and 100 µL using a micropipette. Antibiotic kanamycin (10mg/ml) was used as a standard to compare the results. The percentage of inhibition was calculated by the formula.

## CONCLUSIONS:

Five synthetic compounds were tested for antibacterial activity against four pathogens *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus.* All the compounds showed moderate activity against the tested pathogens. The bioactivity was tested at two different concentrations 50 to 100 **µg/ml** with reference compound Kanamycin. For the compounds 1**a, 1b and 1d** MIC was found to be 50 **µg/ml** against *E.coli*. and 1**b** exhibited MIC 50 **µg/ml** against *P.aeruginosa*. *1***b** showed same MIC against *B.subtilis* and *S.aureus.* The compounds 1**a** and **1d** exhibited MIC at 100 **µg/ml** against *P.aeruginosa* while the same MIC was showed by **1d** against *Bacillus subtilis* and 1**e** against *S.aureus. The* remaining compounds did not show any antibacterial activity against the tested pathogens (Table 3).

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