Abstract

Present study describes antifungal and antibacterial screening of the previously synthesized novel substituted benzyl 4-ketohexanoates (1-17) against two fungi and two bacteria. All of the substituted benzyl 4-ketohexanoates exhibited antimicrobial activities in terms of inhibition zones and Minimum Inhibitory Concentrations (MICs). Halogens substituted benzyl 4-ketohexanoates in general and Iodinated analogues showed maximum activity in terms of inhibitions zones as well as MICs.

Keywords: Antibacterial, Antifungal, Benzyl 4-ketohexanoates, MICs, Zones of Inhibition

1. Introduction

To combat against illnesses nature provides us with curing medicines in the form of natural products. However, the natural medicines are mostly present in very low % age and exploration for new alternative synthetic therapeu tic agents is the main area of the current research. There is an increasing trend that many of synthesized compounds are being continually tested for their antimicrobial activities. Such compounds in future could be used as therapies for various diseases in human being as well as animals. Compounds derived from gamma ketoesters and 2-aminopyridine have shown bioactivity against microbes. Author in their studies have investigated that halo-benzyl substituted compounds have been shown to possess anti-fungal and anti-bacterial activity. Present study deals with the already prepared substituted benzyl 4-ketohexanoates (1-17). In the present study the substituted benzyl 4-ketohexanoates were screened against two fungal and two bacterial strains. These studies revealed that all of the substituted benzyl 4-ketohexanoates possessed antimicrobial activities.

2. Material and Methods

2.1 Preparation of Substituted Benzyl 4-Ketohexanoates

All of substituted benzyl 4-ketohexanoates (1-17) employed in this study were previously synthesized and characterized by various spectroscopic techniques. Briefly, compounds 1-17 were prepared by adding corresponding 4-benzyloxy-4-ketobutanoyl chloride (5 mmol) to the freshly prepared solution of diethyl cadmium. The reaction mixture was refluxed and allowed to stand overnight. The material was then poured into a beaker containing ice and aq. H₂SO₄. The organic layer was extracted with diethyl ether, washed with an aq. NaHCO₃ solution (20 mL), and dried over anhydrous Na₂SO₄. After removal of the solvent, the material thus obtained was purified by chromatography on silica gel and characterized by their physical characteristics and spectroscopic data (Figure 1).
Antifungal and Antibacterial Activities of Substituted Benzyl 4-Ketohexanoates

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Briefly, microbial inoculum matching with McFarland standard (0.5) was applied to the Mueller-Hinton agar petri plates (100 mm) with the help of a sterile cotton swab. Filter papers disks (6.5 mm) impregnated in the solution of antimicrobial agents each at concentration 50 μg/mL were then placed with the help of a pre-sterilized tuning fork on the inoculated petri plates. The petri plates were allowed to dry at room temperature for 20 minutes. Zones of inhibition were measured with the help of a Vernier caliper after 24 h incubation at 34 °C for bacteria and 28 °C for fungi.

Ketoconazole and Imipenem both at a concentration of 50 μg/mL were employed as standards for antifungal and antibacterial activities while DMSO was used as a solvent as well as a control. The zones of inhibition of the antimicrobial agents are given in Table 1.

2.4 Minimum Inhibitory Concentrations (MICs) of Benzyl 4-ketohexanoates

Minimum Inhibitory Concentrations (MICs) of 4-ketoesters (1-17) against microbes was determined using Broth dilution method. Briefly, for MICs determinations an overnight culture of Bacteria/fungi was diluted with liquid broth to get inoculum suspension such that its turbidity matched McFarland standard (0.5). To 32 wells (labelled 1-32) of micro titer 96 wells plate 50μL of the liquid broth was introduced. All 4-ketoesters (1-17) were dissolved in DMSO (1000 μg/mL) to obtain a stock solution. The stock solution was serially 1:2 diluted to get the solutions of working concentrations of 1, 2, 3, 4, 5...30 μg/mL. These concentrations of the antimicrobial agents were placed in the wells labelled 1-30 of micro titer 96 wells plate. Inoculum suspension was serially 1:10 diluted and employing multi-channel pipette, 5μL of it was added to 30 wells. In well 31 DMSO was added without antimicrobial agent while into well 32, 50 μL of the standard drug was added. The micro titer plates were incubated for twenty four hours. This process was repeated twice for each of 4-ketoesters. Minimum inhibitory concentration which was the lowest concentration (μg/mL) of 4-ketoesters that completely inhibited the growth of the microbes in the wells of micro titer 96 wells plate was noted. The results are presented in Table 2.

Figure 1. Structure of substituted benzyl 4-ketohexanoates.

2.2 Microorganism

For the purpose of zones of inhibition/Minimum Inhibitory Concentrations (MICs), all of the compounds 1-17 were screened against two bacteria; Bacillus cereus (B. cereus) and Micrococcus luteus (M. luteus) and two fungal strains; Aspergillus niger (A. niger) and Candida albicans (C. albicans).

2.3 Zones of Inhibition (Mm) of Benzyl 4-Ketohexanoates

Zones of inhibition of 4-ketohexanoates (1-17) against bacteria and fungi were determined following the method of...
Table 1. Antimicrobial activity in terms of zones of inhibition (mm)

| Compound | B. cereus | M. luteus | A. niger | C. albicans |
|----------|-----------|-----------|----------|-------------|
| Ctrl (DMSO) | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 | 15.2±0.3 | 16.4±0.5 | 16.7±0.1 | 15.6±0.4 |
| 2 | 15.4±0.8 | 16.6±0.3 | 16.4±0.1 | 16.2±0.7 |
| 3 | 16.1±0.4 | 16.5±0.6 | 14.1±0.2 | 15.9±0.1 |
| 4 | 15.4±0.6 | 15.1±0.7 | 13.3±0.3 | 15.2±0.2 |
| 5 | 15.1±0.2 | 15.2±0.5 | 14.2±0.5 | 15.3±0.3 |
| 6 | 15.8±0.1 | 15.7±0.3 | 12.7±0.6 | 13.5±0.4 |
| 7 | 13.3±0.2 | 13.6±0.6 | 11.6±0.4 | 12.2±0.5 |
| 8 | 13.4±0.4 | 13.5±0.7 | 11.2±0.3 | 12.1±0.8 |
| 9 | 17.3±0.3 | 17.4±0.3 | 12.5±0.2 | 14.3±0.4 |
| 10 | 17.6±0.7 | 17.6±0.5 | 14.9±0.3 | 15.5±0.3 |
| 11 | 17.1±0.8 | 17.2±0.3 | 14.3±0.6 | 16.2±0.4 |
| 12 | 18.±0.3 | 18.4±0.6 | 16.7±0.3 | 17.0±0.3 |
| 13 | 18.4±0.6 | 18.8±0.2 | 15.9±0.1 | 17.1±0.7 |
| 14 | 17.7±0.7 | 17.8±0.2 | 16.4±0.3 | 17.2±0.7 |
| 15 | 20.5±0.6 | 20.5±0.4 | 18.3±0.8 | 19.4±0.3 |
| 16 | 20.4±0.3 | 20.6±0.9 | 18.5±0.4 | 19.5±0.5 |
| 17 | 22.6±0.2 | 22.5±0.5 | 18.3±0.4 | 21.1±0.3 |
| Imipenem | 36.3±0.3 | 38.6±0.5 | 30.4±0.3 | 31.5±0.3 |

Table 2. Antimicrobial activity in terms of MICs (μg/mL)

| Compound | B. cereus | M. luteus | A. niger | C. albicans |
|----------|-----------|-----------|----------|-------------|
| 1 | 30 | 30 | 26 | 27 |
| 2 | 28 | 28 | 26 | 27 |
| 3 | 28 | 28 | 26 | 27 |
| 4 | 26 | 26 | 24 | 25 |
| 5 | 25 | 25 | 26 | 17 |
| 6 | 23 | 24 | 24 | 23 |
| 7 | 23 | 24 | 24 | 24 |
| 8 | 26 | 26 | 23 | 25 |
| 9 | 25 | 25 | 22 | 23 |
| 10 | 25 | 25 | 22 | 24 |
| 11 | 24 | 24 | 24 | 23 |
| 12 | 23 | 23 | 22 | 23 |
| 13 | 23 | 23 | 21 | 23 |
| 14 | 25 | 25 | 20 | 22 |
| 15 | 23 | 23 | 20 | 21 |
| 16 | 21 | 21 | 19 | 20 |
| 17 | 21 | 21 | 19 | 20 |
| Imipenem | 10 | 9 |
| Ketoconazole | 8 | 7 |

3. Results and Discussion

The biological activities of the benzyl 4-ketohexanoates are not known because all of the compounds are being subjected to antimicrobial activity for the first time. Antimicrobial activity of all compounds (1-17) was investigated against B. cereus, M. luteus, A. niger and C. albicans. All compounds (1-17) showed good response for microbes both in terms of zones of inhibition and MICs. Methoxysubstitutedbenzyl 4-ketohexanoates (1-8) showed smaller values zones of inhibition than halogen substitutedbenzyl 4-ketohexanoates (9-17). In general the difference in values Zones of inhibition was not large. The lowest zones of inhibition were displayed by compounds 7 and 8 against fungus A. niger. The largest zones of inhibition of 22 mm were shown by compound 17 against both bacteria. Standard drugs imipenem and ketoconazole showed zones of inhibitions of 36.3±0.3, 38.6±0.5,
30.4±0.3 and 31.5±0.3 mm while solvent DMSO displayed zero vale of zone of inhibition. Results are displayed in Table 1. Zones of inhibition are also displayed in the form of a graph (graph 1).

Zones of inhibition of the compounds (1-17) were reflected by their MICs values which were reverse of inhibition zones. Smallest MIC of 19 μg/mL was displayed by compounds 16 and 17 against fungi. The largest MIC value of 30 μg/mL was shown by 1 against bacteria. All other compounds exhibited MICs values between 19 and 30 μg/mL. Standard drugs imipenem displayed the MICs values of 10 and 9 μg/mL whereas ketoconazole showed MICs of 8 and 7 μg/mL. The results are displayed in Table 2. MICs results are also displayed in the form of a Graph 2.

4. References

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