4 methyl benzyl amine substituted benzopyran, antimicrobial activity

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Abstract
Benzo pyran is one of the privileged medicinal pharmacophore, which appears as an important structural component in natural compounds and generated great attention because of their interesting biological activity. The derivatives of benzo pyran moiety can be capable of interacting with a variety of cellular targets which leads to their wide ranging biological activities such as antitumor, anti-hepatotoxic, antioxidant, anti-inflammatory, diuretic, anticoagulant, antispasmodic, estrogenic, antiviral - helminthic, hypothermal, vasodilatory, anti-HIV, antitubercular, herbicidal, anticonvulsant and analgesic activity.

Keywords: Methyl benzyl, amine substituted benzopyran, antimicrobial

Introduction
Medicinal chemistry is the branch of science, which has remarkable value for synthesis of novel drugs with intense therapeutic activity. It concerns with discovery, development, identification and interpretation of mode of action of biologically active compounds at molecular level. These developments have provided new challenges and opportunities for drug research in general and drug design in particular. Pure organic compounds, natural or synthetic products are the chief source of agents for the cure, the mitigation or the prevention of disease today. The major objectives of the medicinal chemists are transformation of patho biochemical and physiological data into a chemical language with the aim of designing molecules interacting specifically with the derailed or degenerating processes in the diseased organisms.

Antimicrobial Study
An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, viruses. Antimicrobial drugs either kill microbes (Microbicidal) or prevent the growth of microbes (Microbiostatic).

The history of antimicrobials begins with the observation of pasteur and joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that one of the bacteria was producing an antibiotic which inhibits the growth of the other microorganism. Technically, antibiotics are only those substances that are produced by microorganisms that kill or prevent the growth of another microorganism. Of course, in today’s common usage, the term antibiotic is used to refer to almost any drug that cause a bacterial infection. Antimicrobials include not just antibiotic but synthetically formed compound as well.

Review of Literature

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Synthesis of Derivatives
To a 50 ml of RBF 250mg (1.066 mmol) of compound 7 was taken in 30ml of ethanol. After that 0.271ml (2.136 mmol, 2 equivalents) of 4-methyl-benzylamine was added and the reaction was refluxed at 80°C for 4hours. After completion of reaction the solvent was evaporated.

M+1 Peak: 313

The anti-bacterial screening was carried out in the pharmaceutical biotechnology laboratory, Nehru College of Pharmacy, Pampady, Thrissur.

Media used in the study
Nutrient agar
Nutrient agar gelled by the addition of 2% agar (bacteriological grade)

Ingredients
Peptic digest of animal tissue : 5g/Ltr
Sodium chloride : 5g/Ltr
Beef extract : 1.5g/Ltr
Yeast extract : 1.5g/Ltr
Agar : 50g/Ltr
Final PH (at 25°C) : 7.4

Preparation
The ingredients were dissolved in distilled water with the aid of heat. PH was adjusted to 7.2-7.6 using alkali diluted acid.

Sterilization
15-20ml of Nutrient agar was transferred to test tubes and scaled with non-absorbent cotton. It was then autoclaved at a pressure of 15 psi (121 °C) for 15 min.

Organisms used
S. aureus MTCC 405, Pseudomonas aeruginosa, were collected from Institute of Microbial Technology, Chandigarh and stored in the Pharmaceutical Biotechnology Laboratory, Nehru College of Pharmacy, Pampady, Kerala. The strain was confirmed for their purity and identity by Gram’s staining method and characteristic biochemical reactions. The selected strains were preserved by sub-culturing them periodically on other slants and storing them under frozen conditions. For the study, fresh 24 hrs broth cultures were used after standardization of the culture.

Working conditions
The entire work was done using horizontal laminar flow hood so as to provide aseptic conditions. Before commencement of the work air sampling was carried using a sterile nutrient agar plate and exposing it to the environment inside the hood. On incubation it was checked for the growth of microorganism and absence of growth confirmed by aseptic working condition.
Preparations of inoculums
The inoculums for the experiments were prepared fresh in Nutrient agar from preserved frozen slant culture. It as kept incubated at 37° c for 24 hrs.

Drug used: t1 (1000mcg/100ml)
Standard Used: Levofloxacin (5mcg/disc)
Vehicle Used: Ethanol

Antibacterial Screening
Two Nutrient agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37° c before inoculation. The organisms were inoculated in the plates prepared by spread plate method. i.e, using a micropipette, the culture place randomly on the agar plate and it is spread by using L-shaped glass rod where it is just touch the surface of the agar and rotating it to and fro direction.

The organisms used were Gram positive S. aureus and Gram negative Pseudomonas aeruginosa

The standard and test drugs were introduced in two agar plates by using cup plate method.

- By using the tips of borer, the four agar wells were made at each quadrant and central well for control.
- Add three different dilution of test drug which has been prepared from previously prepared stock solution of 1g test drug per 100mL ethanol.
- The different dilutions are prepared by taking 1 mL stock solution and dilute with 4 mL solvent (ethanol) similarly two more dilutions were prepared in the ratio 2:3 and 3:2.

Also add the standard drug to one well, which has prepared in the ratio 1:4 and ethanol was added as control at the centre.
- It were kept in the refrigerator for one hour to facilitate uniform diffusion of drugs.
- Two plates prepared were then incubated for 18-24 hrs.
- Observations were made for zone of inhibition around the drug and compared with of standard.
- The compound synthesized was tested for antibacterial activity against gram positive and gram negative bacteria.

<table>
<thead>
<tr>
<th>Name of organism</th>
<th>Compounds</th>
<th>Dilutions (compound: Solvent)</th>
<th>Total Diametre (T) (cm)</th>
<th>Well Diametre (W)</th>
<th>Zone of Inhibition (T-D)*10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Stdard</td>
<td>1:4</td>
<td>4</td>
<td>0.6</td>
<td>34</td>
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<tr>
<td></td>
<td>Solvent</td>
<td></td>
<td>0.6</td>
<td>0</td>
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<td></td>
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<td>1.5</td>
<td>0.6</td>
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<td>11</td>
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<td></td>
<td>Sample</td>
<td>3:2</td>
<td>2</td>
<td>0.6</td>
<td>13</td>
</tr>
</tbody>
</table>

Zone of inhibition of the compound against Gram negative Pseudomonas aeruginosa
Drug used: Test sample (1000mcg/100ml) of different dilution in the ratio 1:4, 2:3 & 3:2
Standard: Levofloxacin (1mL/4ml)
Solvent: Ethanol

<table>
<thead>
<tr>
<th>Name of organism</th>
<th>Compounds</th>
<th>Dilutions (compound : Solvent)</th>
<th>Total Diametre (T) (cm)</th>
<th>Well Diametre (W)</th>
<th>Zone of Inhibition (T-D)*10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
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<td>4.1</td>
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<td></td>
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<td>0.6</td>
<td>21</td>
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<tr>
<td></td>
<td>Sample</td>
<td>3:2</td>
<td>3.1</td>
<td>0.6</td>
<td>24</td>
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</tbody>
</table>
Result and Discussion
The antibacterial activity of newly synthesized compound was evaluated by using both gram positive and gram negative organism by agar diffusion method. 
Gram positive organism - *S. aureus*
Gram negative organism - *Pseudomonas aeruginosa*
Various dilution of 1000mcg/100ml was used for the test compound and results were compared with the standard drug Levofloxacin 1mL/4mL concentration and ethanol as vehicle. The results were interpreted as the KB method (Kirby-Bauer method). The test organism *Pseudomonas aeruginosa* was found to be moderately sensitive at given concentration of test compound.
The test organism *S. aureus* was found to be highly sensitive at given concentration of test compound. Therefore the drug is more effective against Gram positive organism.

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