Cup plate method | cultivation of microorganism

An Adduct formed by stirring (0. 01 mole) of aromatic aldehyde with the 40% of NaHSO3. O-phenylenediamine (0. 01 mole) was dissolved in 50 ml of warm Ethanol 80. The NaHSO3 adduct of the aldehyde is added slowly with constant stirring in the warm solution of O-phenylenediamine stirred for 20-30 min still solid product obtained, then added 100 ml of Distilled water and filtered . Now the product was recrystallised by using Ethanol.

Step 2: Nicotinoyl Cloride

0. 1 mole of Nicotinic Acid was refluxed for 6 hrs with the 20 ml of Thionyl Chloride. After this the excess of Thionyl Chloride was distilled off and separated from the product and dried it.

Step 3:

0. 01 mole of 2-phenylbenzimidazole solution in 100 ml Pyridine stirred for 8 hrs constantly with the 0. 01 mole of Nicotinoyl Chloride , then the water added 50 ml to get a solid product. The product was filtered, dried and recrystallised using Ethanol.

Scheme:

AIM AND OBJECTIVES

Molecular modification of a promising lead compound is still a major line of approach for the discovery of new drug. Molecular modification involves substituting, elimination, or adding new moieties to a parent lead compound, there by making gradual changes in the physico-chemical properties of the parent compound and thus biological activity of the compound.

It is clear from the literature review that a number of Benzimidazole derivatives are known for the, antibacterial, antifungal and ant-inflammatory activities properties.

The present studies were performed with the following objectives:

- Synthesis of new series of 1, 2-substituted benzimidazole derivatives.
- Characterization of newly synthesized compounds by spectra methods viz. infrared spectra (IR spectra), Nuclear magnetic resonance spectra (^{1}H NMR spectra) and (Mass spectra).
- Screening of the antibacterial and Antifungal of the newly synthesized compounds using various strains of bacteria and fungi by determining their MIC.
- Screening of anti-inflammatory action of Benzimidazole derivatives.

Scope and Plan of work:

Literature survey revealed that Benzimidazole nucleus is a part numerous class of reported molecules exhibiting diverse range of biological activities like antibacterial, antifungal, antiviral, anticancer, analgesic , anti-inflammatory activity, antihyperlipidemic, antihistaminic, antiulcer, anti-arrhythmic , HIV-RT inhibitor. Considering the reported data about Benzimidazole nucleus we have tried to synthesize some Nicotinoyl derivatives of Benzimidazole. The Benzimidazole derivatives of all above mentioned activities are mostly of 2-substituted type . The synthesis of 2-(substituted phenyl)-benzimidazolyl-1-pyridinyl-3-methanone was carried out and screened for antibacterial, antifungal, and anti-inflammatory activity.

The present work was divided in to three sections:

Synthesis of 1, 2-substituted derivatives of Benzimidazole .

Chemical Characterisation of the synthesized compounds.

Biological evaluation of synthesized compounds.

Pharmacological screening of the synthesized compounds.

ANTIMICROBIAL SCREENING

An antibiotic is a chemical compound that in high dilution hinders the growth and the survival of one or more species of microorganism. A drug is considered to have bacteriostatic or fungistatic activity when it inhibits the growth of bacteria or fungi respectively and bactericidal or fungicidal activity when it kills the bacteria or fungi. In vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infection to determine which of the available drug might be useful therapeutically.

Important factors for antimicrobial activity are size of the inoculums, metabolic state of microorganism, pH, temperature, and duration of interaction, concentration of the inhibitor and presence of interfering substance.

ANTIBACTERIAL ACTIVITY STUDIES

Literature survey reveals that the synthesis and evaluation of antibacterial activity of various 2-substituted benzimidazole derivatives. The development of resistant among various pathogenic microorganisms towards the antibiotics has increased the impetus for investigating new antimicrobial agent. When a compound are synthesized in the hope that one of them

would be more effective than the existing one. The antimicrobial effectiveness of a compound can be evaluated by serial dilution method and cup plate method. Dilution susceptibility tests are used to determine the Minimum Inhibitory Concentration (MIC).

MIC is the lowest concentration of a drug that inhibits the growth of a particular organism under specific condition. The sensitivity of a compound against a particular organism can be studied by cup plate method. Initially the zone of inhibition method was carried out to evaluate the sensitivity of the organism were selected for determination of MIC.

CUP PLATE METHOD:

Cultivation of Microorganism:

The following microorganisms were used to study the antibacterial activity.

Bacillus subtilis – Gram positive bacteria

Staphylococcus aureous – Gram positive bacteria

Escherichia coli – Gram negative bacteria

Salmonella typhi – Gram negative bacteria

Standard: Streptomycin (1000mcg)

Solvent: DMF

All the test compounds were tested at 250 µg, 500 µg , and 1000 µg.

Preparation of the medium:

Composition of nutrient agar medium

Beef extract……. 10g

Peptone…….. 10g

Sodium chloride…….. 5g

Agar…….. 20g

Purified water……..1000ml

pH 7. 2± 0. 2

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water by heating on a water bath and were dispensed in 100 ml volume conical flasks. The conical flasks were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes.

The contents of the conical flasks were poured aseptically into sterile Petridishes are allowed to solidify. These sterilized Medias were used to subculture the bacterial culture.

PROCEDURE:

Each Petridish was filled to a depth of 4-5 mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify. The petridish were specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness. The petridishes were sterilized at 160-170°C in hot air oven for 30 mins before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal

diameter of approximately 6-8 mm and made of aluminium (or) stainless steel. Each plate was divided in to four equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. Three cavities for test compounds and one cavity for the standard. The petridishes were incubated at 37°C for 18 hours. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained by the test sample was compared with that produced by standard Streptomycin.

CUP PLATE METHOD:

Cultivation of Microorganism

The following fungal strains were used to study the antibacterial activity.

1. C. raphigera

2. A. polytricha

Standard: Ketocanazole (1000mcg)

Solvent: DMF

All the test compounds were tested at 250 µg, 500 µg , and 1000 µg.

Preparation of the medium:

Composition of nutrient agar medium

Sabraoud Dextrose broth..... 64gm

Distilled water..... 1000ml

pH..... 7. 2± 0. 2

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water by heating on a water bath and were dispensed in 100 ml volume conical flasks. The conical flasks were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes.

The contents of the conical flasks were poured aseptically into sterile Petridishes are allowed to solidify. These sterilized medias were used to subculture the fungal culture.

ROCEDURE:

Each Petridish was filled to a depth of 4-5 mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify. The petridish were specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness. The petridishes were sterilized at 160-170°C in hot air oven for 30 mins before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of aluminium (or) stainless steel. Each plate was divided in to four equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. Three cavities for test compounds and one cavity for the standard. The petridishes were incubated at 37°C for 18 hours. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained by the test sample was compared with that produced by standard Ketocanazole.