CHAPTER 3

3. MATERIALS AND METHODS

3.1 Chemicals

The chemicals like 2-amino-5-fluorobenzonitrile benzoaldehyde, 4-Chloro benzoaldehyde, 4-Flurobenzaldehyde, 4-Methylbenzaldehyde, 4-Methoxy benzoaldehyde, Zinc Chloride, Thiglycolic acid, Chloro acetyl chloride and solvents like Ethanol, 1,4 Dioxan, Triethyl amine, Dimethy formamide, Dimethyl sulphoxide were supplied from Merck, Fischer and Himedia Scientific they were used as received.

3.2 Melting point determination

Melting points of the synthesized compounds were determined in open-glass capillaries on a Stuart-SMP10 melting point apparatus and recorded in °C without correction. It is widely used physical constant in the characterization of an organic compound.

3.3 Ultrasonic Cleaning Bath

Sonication was performed in Shanghai Branson-CQX ultrasonic cleaner (with a frequency of 40 kHz and a nominal power 250 W): (Shanghai Kudos Ultrasonic Instrument Co., Ltd.). The reaction flask was located in the cleaner, where the surface of reactants is slightly lower than the level of the water. The reaction temperature was controlled by addition or removal of water from ultrasonic bath.
3.4 Purification

The synthesized compounds have been purified by recrystallization methods. The purity has been ascertained by TLC.

3.5 Characterization

The synthesized compounds have been characterized by melting point and study of spectral data.

3.5.1 IR Spectroscopy

Information about the structure of a molecule could frequently obtain from its absorption spectrum. The atomic and electronic configuration of a molecule is responsible for the position of absorption bands.

The peaks in the IR spectra give an idea about the probable structure of the compound. IR region ranges 4000-400 cm\(^{-1}\). The IR spectra were recorded on SHIMADZU FT-IR spectrophotometer (Thermo Nicolet), the sample was mixed with KBr and the pellet technique was adopted to record the spectra. Various peaks obtained for different functional groups of individual compounds. The characteristic peaks confirm synthesized derivatives.

3.5.2 NMR Spectroscopy

a) Proton NMR:

The proton NMR spectrum enables us to know how many different kinds of environment are there in the molecules and also which atoms are present in neighbouring groups. The proton NMR was recorded at room
temperature using TMS an internal reference. The spectra were recorded on BRUKER AMX 400 spectrometer. Samples were prepared by dissolving about 10mg of compounds in 0.5mole of CDCl₃ and DMSO. ¹H-NMR chemical shifts were measured in ppm and the following notations are used for multiplicity; s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet.

b) ¹³C-NMR Spectra

¹³C NMR spectra were recorded on BRUKER AMX-400 spectrometer in proton decoupled mode samples were prepared by dissolving 50mg of the compound in 0.5ml of CDCl₃ and DMSO. All the spectral values from NMR spectra confirm the structure of synthesized derivatives.

3.6 Antimicrobial Assay

a) Assay of Antibacterial Activity

This will be carried out on both Gram positive and Gram negative organisms like Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Streptococcus pyogenes using sterile Media like Mueller-Hinton Agar etc by Disc Diffusion Method. Zone of inhibition of the compounds synthesized will be noted and compared with that of standard drugs like Streptomycine
b) **Assay of Antifungal Activity**

This will be carried out on organisms like *Aspergillus flavus*, *Penicillium chryogenum*, *Tricoderma viride*, *Fusarium oxysporum* and *Aspergillus niger* using Media like Potato Dextrose Agar (PDA). Zone of inhibition of the synthesized compounds will be compared with that of standard drugs like *Amphotericin-B*.

**3.7 Acoustical Studies**

The ultrasonic velocity in the mixture was measured using a variable path fixed frequency ultrasonic interferometer working at 2 MHz frequency (Mittal enterprises, New Delhi). The accuracy of sound velocity was ±0.1 ms\(^{-1}\). The density was determined at the experimental temperature using 10ml capacity specific gravity bottle immersed in a thermostatic bath (accuracy +0.01°C). The volume of the bottle at the experimental temperatures, viz. 303.15K was ascertained using doubly distilled water. The densities of water at these temperatures were obtained from literature. The viscosity of pure liquids and liquid mixtures at 303.15K were determined using an Ostwald viscometer.