Design, Synthesis and Evaluation of Phthalimide and Its Derivatives

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***Abstract*—The synthesis, structural characterisation, and antimicrobial assessment of phthalimide and its major derivatives potassium phthalimide, N-benzyl phthalimide, N-phenyl phthalimide, and N-anthraniloyl phthalimide are the main objectives of this study. Under carefully monitored circumstances, nucleophilic substitution and condensation reactions were used to create these derivatives. Thin Layer Chromatography (TLC), melting point determination, and spectroscopic methods including infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy were used to characterise the compounds and verify their structures and purity. Disc diffusion and minimum inhibitory concentration (MIC) techniques were used to evaluate antimicrobial activity against specific bacterial strains. N-benzyl and N-anthraniloyl phthalimide showed encouraging antibacterial activity, suggesting that they could be used as lead molecules in the creation of novel therapeutic agents.**

***Keywords*—Phthalimide; Potassium Phthalimide; N-Benzyl Phthalimide; N-Phenyl Phthalimide; N-Anthraniloyl Phthalimide; Antimicrobial Activity; Synthesis; Characterisation; TLC; IR Spectroscopy; NMR Spectroscopy; Drug Discovery; Nitrogen-Containing Heterocycles**

I. Introduction

A structurally significant scaffold frequently employed in synthetic organic chemistry and pharmaceutical research is phthalimide, a classical nitrogen-containing heterocyclic compound. Phthalimide, a thermally stable, planar molecule with a fused isoindoline-1,3-dione ring, is usually made by condensing phthalic anhydride with urea or ammonia. The imide functional group, surrounded by two electron-withdrawing carbonyls, gives the nitrogen proton acidity while promoting nucleophilic substitution reactions, making phthalimide a crucial precursor for a variety of N-substituted derivatives.

Derivatives of phthalimide have a wide range of pharmacological applications, including immunomodulatory, antibacterial, anti-inflammatory, anticancer, and anticonvulsant properties. Notable examples include thalidomide and its analogs, lenalidomide and pomalidomide, which have become effective treatments for autoimmune diseases and cancer. N-alkylation, N-arylation, or acylation can be used to modify the structure of phthalimide derivatives during synthesis, allowing for systematic exploration of structure-activity relationships (SAR).

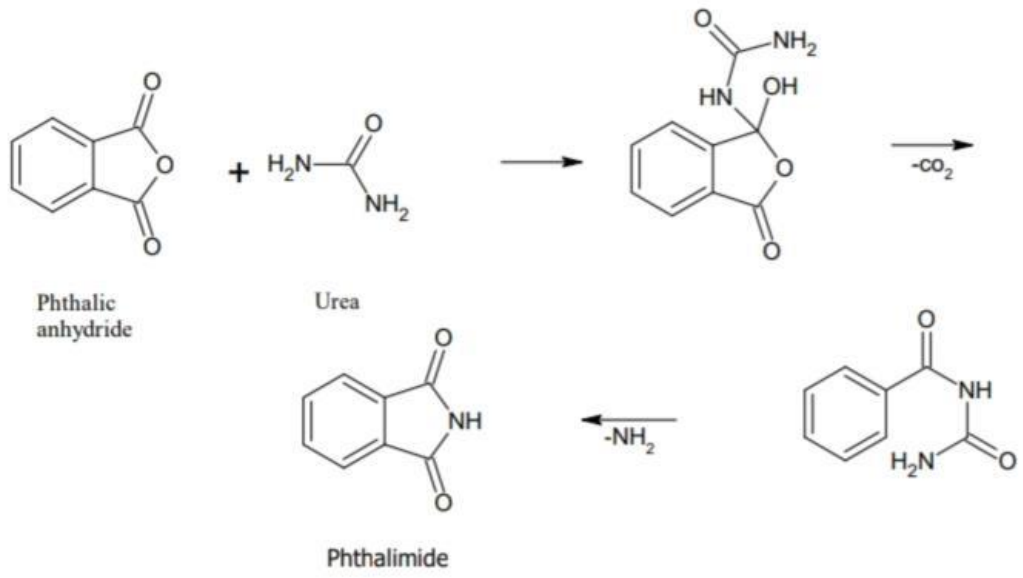
The purpose of this project was to investigate the structural properties, antibacterial potential, and feasibility of synthesising phthalimide and a few of its derivatives: potassium phthalimide, N-benzyl phthalimide, N-phenyl phthalimide, and N-anthraniloyl phthalimide. Potassium phthalimide is a crucial step in the Gabriel synthesis of primary amines. N-benzyl and N-phenyl phthalimides demonstrate N-substitution, providing pathways to bioactive compounds with enhanced lipophilicity and membrane permeability via SN2 and condensation reactions respectively. N-anthraniloyl phthalimide is a hybrid compound combining the pharmacophores of phthalimide and anthranilic acid, potentially exhibiting dual biological activities.

To verify the identity and purity of the products, analytical methods including NMR spectroscopy, IR spectroscopy, and TLC were used. Agar diffusion and MIC assays were used in an in vitro antimicrobial evaluation to assess biological activity. This investigation not only enhances understanding of imide chemistry but also contributes to the ongoing search for novel scaffolds to combat microbial resistance.

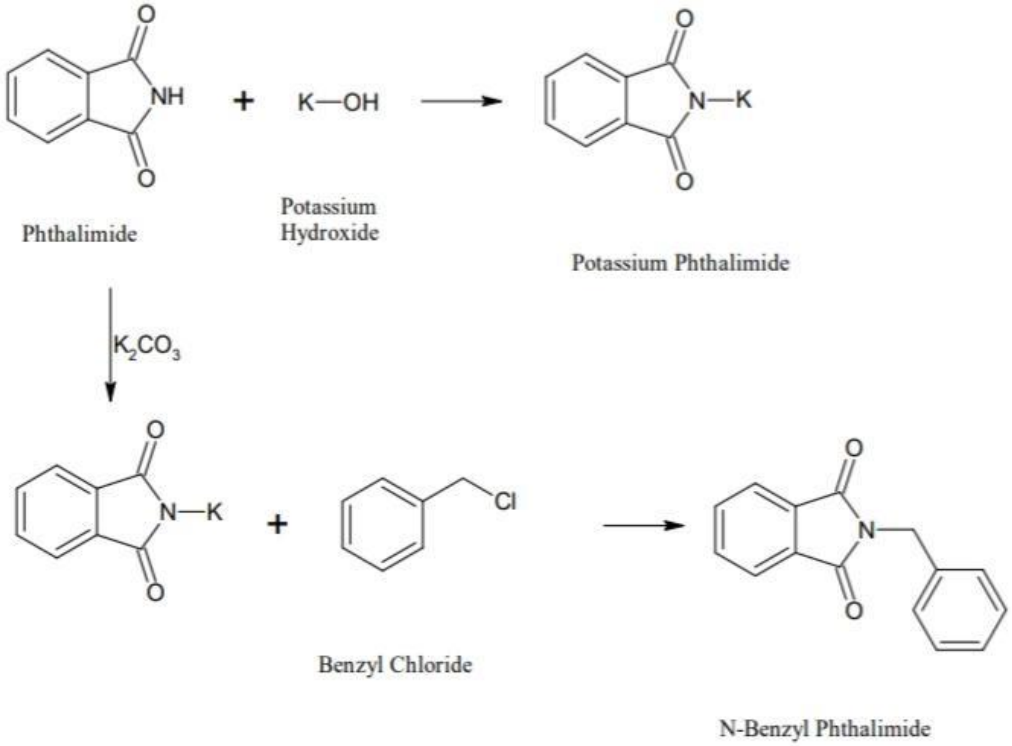
II. Materials and Methods

The starting materials for this work included analytical grade phthalic anhydride, urea, phthalimide, KOH, benzyl chloride, aniline, o-toluidine, anthranilic acid, and glacial acetic acid. Phthalimide was synthesised by heating phthalic anhydride with urea followed by hydrolysis and recrystallisation. Reaction of phthalimide with ethanolic KOH afforded the potassium salt of phthalimide. N-benzyl phthalimide was synthesised by alkylating phthalimide with benzyl chloride in the presence of potassium carbonate in DMF. N-phenyl and N-(o-tolyl) phthalimides were obtained by condensation reactions with aniline or o-toluidine in glacial acetic acid. N-anthraniloyl phthalimide was synthesised from anthranilic acid and phthalic anhydride. Products were purified by recrystallisation and characterised by melting point, TLC, IR, and ^{1}H-NMR. Antibacterial activity was tested by agar well diffusion and MIC methods.

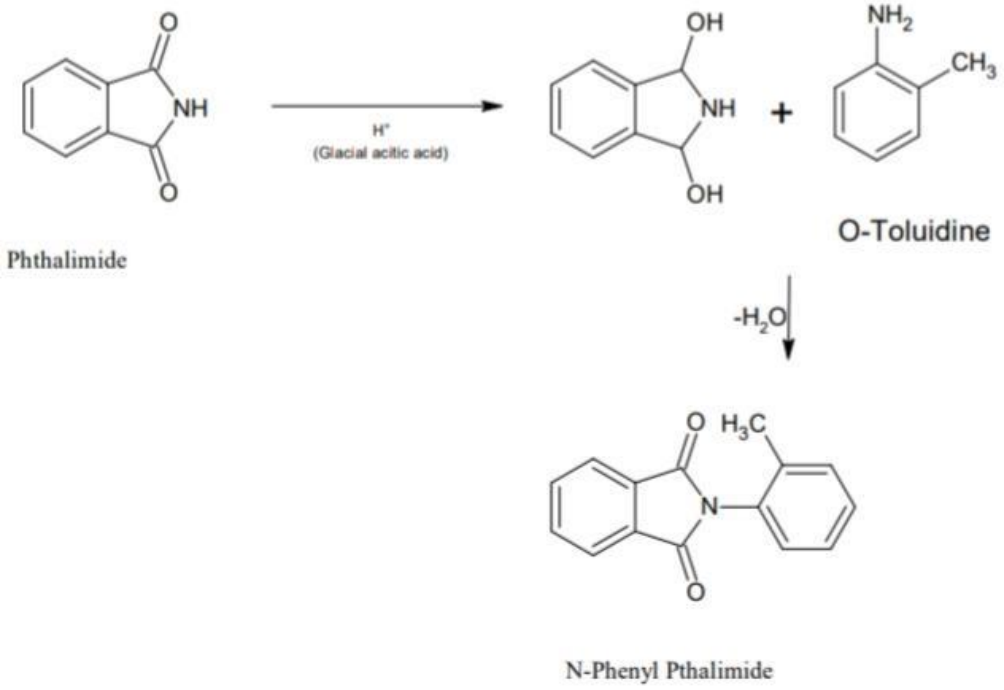
III. Reaction Schemes



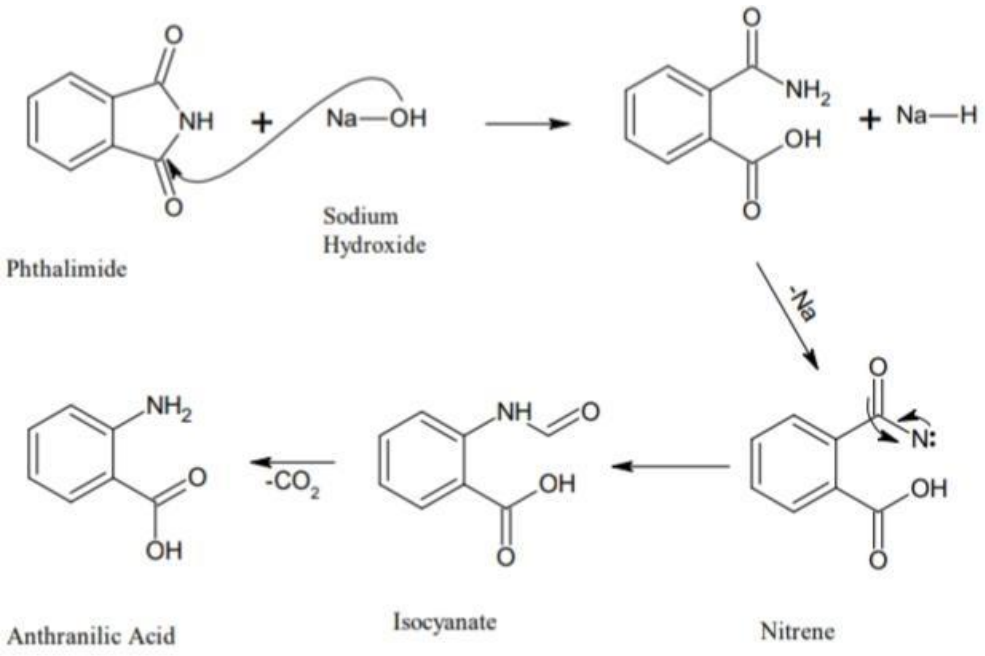
Scheme 1. Synthesis of Phthalimide (Isoindoline-1,3-dione) from phthalic anhydride and urea.



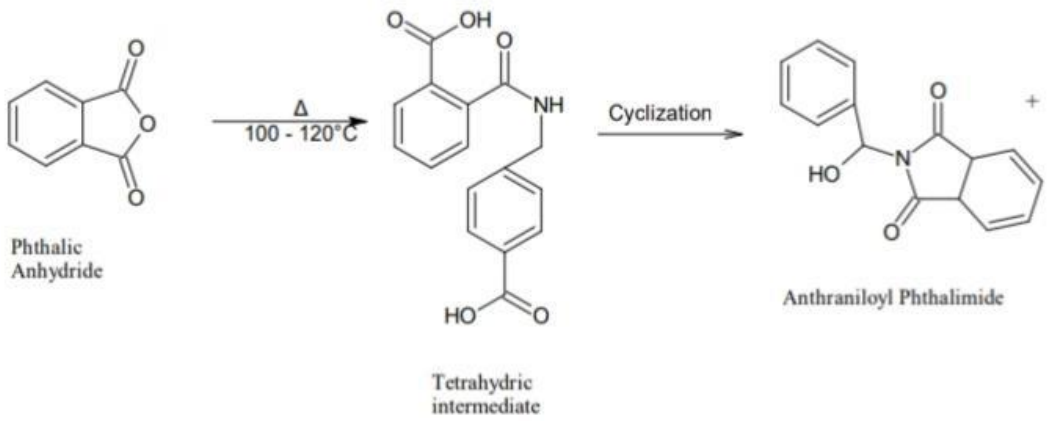
Scheme 2. Synthesis of Potassium Phthalimide from phthalimide and KOH.



Scheme 3. Synthesis of N-Benzyl Phthalimide via SN2 alkylation with benzyl chloride.



Scheme 4. Synthesis of N-Anthraniloyl Phthalimide from anthranilic acid and phthalic anhydride.



Scheme 5. Synthesis of N-Phenyl Phthalimide via condensation with aniline/o-toluidine.

IV. Procedure

4.1 Preparation of Isoindoline-1,3-dione (Phthalimide)

In the synthesis of phthalimide, 15 g of phthalic anhydride and 3 g of urea were carefully weighed and charged into a clean, dry 250 mL round-bottom flask. The flask was set on a heating mantle and the mixture was gradually heated in the range of 75–90°C. As the components melted, a condensation reaction set in, with gaseous by-products (carbon dioxide and ammonia) being released, noticed by foaming and a considerable increase in the volume of the molten mass. Heating was continued until the reaction mass became homogeneous, with subsidence of foaming indicating completion of the reaction.

The mixture was allowed to cool naturally to room temperature. On cooling, 30–40 mL of distilled water was added to dissolve unreacted urea and other water-soluble impurities. The suspension was vacuum filtered through a Büchner funnel, and the crude phthalimide was washed several times with cold distilled water. For purification, the crude phthalimide was dissolved in hot ethanol and slowly cooled to room temperature, then placed in an ice bath for crystallisation. The purified crystals were collected by vacuum filtration and dried at 50–60°C.

4.2 Preparation of Potassium 1,3-Dioxoisoindolin-2-ide (Potassium Phthalimide)

All glassware was sterilised by ethanol and dried to ensure a contaminant-free reaction environment. Four grams of potassium hydroxide were dissolved in 50 mL of ethanol under continuous stirring. Ten

grams of finely powdered phthalimide were gradually added to the solution with constant stirring to avoid clumping, and the mixture was gently heated at 50–60°C for approximately 30 minutes to promote the reaction. After heating, the mixture was cooled to room temperature and further cooled in an ice bath to promote crystallisation of potassium phthalimide. The white precipitate was collected by vacuum filtration, washed thoroughly with cold ethanol, and dried at 60°C until constant weight was obtained.

4.3 Preparation of 2-Benzylisoindoline-1,3-dione (N-Benzyl Phthalimide)

N-benzyl phthalimide was synthesised by dissolving one equivalent of phthalimide in anhydrous DMF in a 100 mL round-bottom flask. Two equivalents of K_2CO_3 were added and the mixture was stirred and heated at 60–70°C for 30 minutes to form the nucleophilic intermediate. Thereafter, 1.1 equivalents of benzyl chloride were added dropwise to the stirred mixture and allowed to react at 70°C for 2–3 hours. The reaction was monitored by TLC using a hexane:ethyl acetate (7:3) solvent system until the phthalimide spot was consumed. After cooling, the mixture was poured into crushed ice to precipitate the crude product, which was collected by vacuum filtration, washed with cold distilled water, and recrystallised from hot ethanol–water mixture.

4.4 Preparation of 2-(1,3-Dioxoisoindolin-2-yl)benzamide (N-Anthraniloyl Phthalimide)

N-anthraniloyl phthalimide was synthesised in two steps. Step 1: Anthranilic acid was prepared via alkaline hydrolysis of phthalimide. Fifteen grams of NaOH were dissolved in 100 mL of water in a 250 mL round-bottom flask, and 10 g of phthalimide was added. The mixture was refluxed at 100°C for 1.5–2 hours. After cooling, concentrated HCl was used to acidify the solution to pH 1–2, triggering precipitation of anthranilic acid. The product was filtered, washed with cold water, and dried under reduced pressure. Step 2: 1.5 g of anthranilic acid and 1.5 g of phthalic anhydride were dissolved in 20 mL of glacial acetic acid and heated at 90–100°C for 2–3 hours. The crude product was collected after pouring into ice-cold water, then recrystallised from hot ethanol.

4.5 Preparation of 2-Phenylisoindoline-1,3-dione (N-Phenyl Phthalimide)

In a 100 mL round-bottom flask, 20 g of phthalimide and 14 mL of o-toluidine were mixed with 140 mL of glacial acetic acid. The mixture was stirred thoroughly and refluxed at 110–120°C for approximately 2 hours to promote the condensation reaction. After cooling, the reaction mixture was poured slowly into ice-cold water, facilitating precipitation of crude N-(o-tolyl) phthalimide. The product was collected by vacuum filtration, washed with cold distilled water to remove acetic acid and other soluble impurities, purified by recrystallisation from hot ethanol, and dried at 60°C in a desiccator for further characterisation.

V. Evaluation of Antimicrobial Activity

Antimicrobial activities were determined by the cup plate agar diffusion method. Sterilised, melted agar medium was inoculated with Staphylococcus aureus and Streptococcus pyogenes, poured into Petri dishes, and allowed to solidify. A hole was aseptically punched in the agar medium using a sterile borer, and various samples and standard solutions were added. All glassware was sterilised by autoclaving at 121°C, 15 psi for 15 minutes. Bacterial specimens were transferred into sterile nutrient broth (1 mL) and incubated for 18–24 hours at 37°C.

Test compounds (10 mg) were weighed in a sterile glass vial, and 1 mL of water for injection was added to prepare stock solutions (800 µg/mL), followed by serial dilutions to 400, 200, 100, 50, and 25 µg/mL. An aliquot of 0.1 mL was removed from each dilution and added into each agar well. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured and their diameters were compared to determine antimicrobial activities.

VI. Results and Discussion

6.1 Percentage Yield

Table 1. Percentage yield of synthesised phthalimide derivatives.

S.No.	Name of Compound	Theoretical Yield (g)	Practical Yield (g)	Percentage Yield
1	Phthalimide	14.89	13.70	92%
2	Potassium Phthalimide	12.59	11.35	90.1%

3	N-Benzyl Phthalimide	6.35	5.40	85%
4	N-Anthraniloyl Phthalimide	3.42	2.90	84.79%
5	N-Phenyl Phthalimide	7.58	5.49	72.4%

6.2 Thin Layer Chromatography (TLC)

Table 2. TLC data for phthalimide derivatives.

Compound	Stationary Phase	Mobile Phase (Solvent System)	Solvent Distance (cm)	Spot Distance (cm)	Rf Value
Phthalimide	Silica gel (SiO_2)	Ethyl acetate : Hexane (4:1)	5	4	0.80
Potassium Phthalimide	Silica gel (SiO_2)	Toluene : Ethyl acetate (7:3)	5	3.5	0.70
N-Benzyl Phthalimide	Silica gel (SiO_2)	Chloroform : Methanol (9:1)	5	2.8	0.58
N-Anthraniloyl Phthalimide	Silica gel (SiO_2)	Ethyl acetate : Hexane (7:3)	5	0.9	0.18
N-Phenyl Phthalimide	Silica gel (SiO_2)	Ethyl acetate : Hexane (7:3)	5	3.8	0.76

6.3 FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the structural identity of all synthesised compounds. The characteristic absorption bands observed were consistent with the expected functional groups: the symmetric and asymmetric C=O stretching of the imide ring at approximately 1770 and 1700 cm^{-1} respectively, N–C=O stretching, and aromatic C–H stretches. The FTIR spectra confirmed successful synthesis and the presence of the phthalimide core in all derivatives, with substituent-specific bands validating the respective N-substitution patterns.

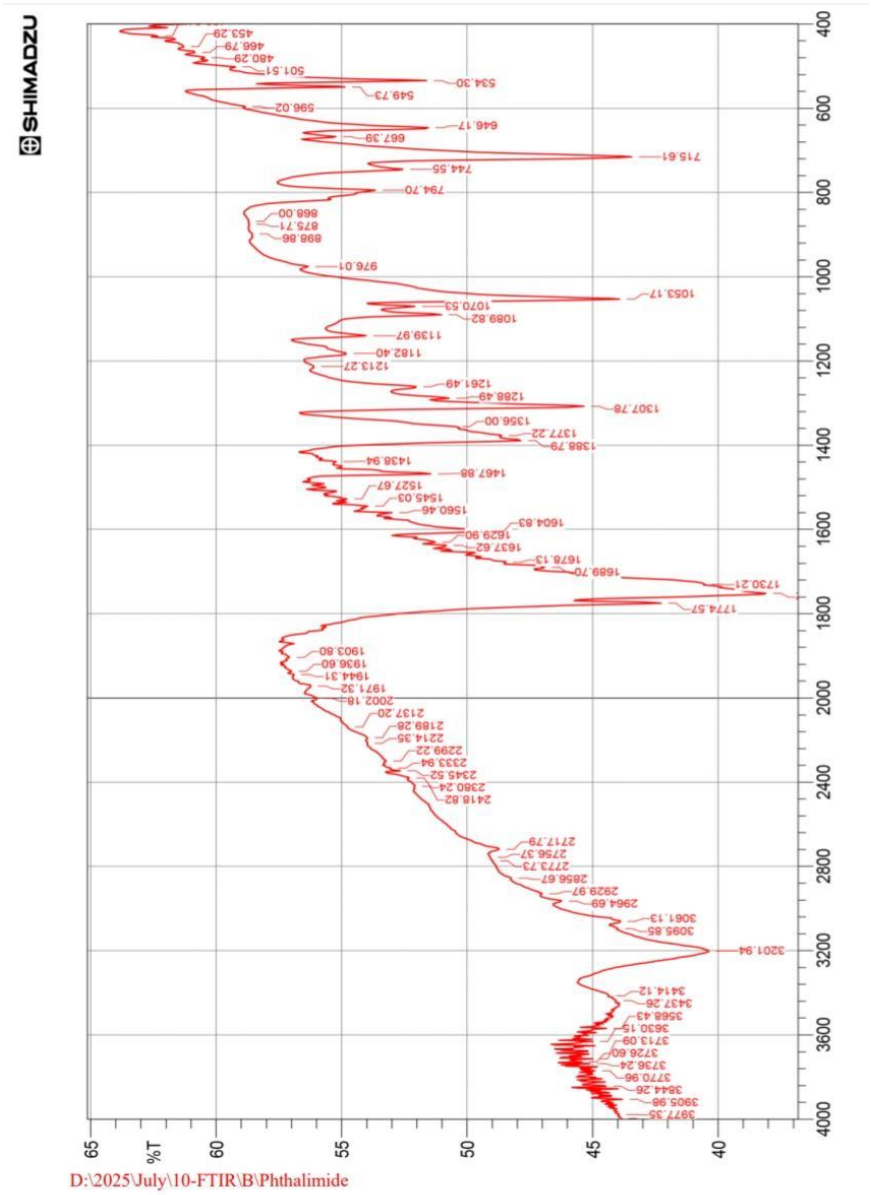


Fig. 1. FTIR spectra of synthesised phthalimide and its derivatives.

Antimicrobial screening results demonstrated that all synthesised derivatives exhibited varying degrees of antibacterial activity against Staphylococcus aureus. Among the derivatives, N-benzyl phthalimide and N-anthraniloyl phthalimide showed the most significant zones of inhibition, indicating enhanced antibacterial potency relative to the parent phthalimide scaffold. The enhanced activity of N-benzyl phthalimide is likely attributable to the increased lipophilicity conferred by the benzyl group, facilitating better membrane penetration. The dual-pharmacophore nature of N-anthraniloyl phthalimide, combining phthalimide and anthranilic acid moieties, is consistent with its improved antibacterial profile. These findings highlight the role of N-substitution in modulating the antimicrobial activity of phthalimide derivatives.

VII. Conclusion

This paper highlights the design, synthesis, and assessment of phthalimide and its analogs in respect to their physico-chemical and antimicrobial characteristics. Phthalimide, N-benzyl phthalimide, N-anthraniloyl phthalimide, and N-phenyl phthalimide were prepared using established methods, yielding moderate to high yields in the range of 72.4–92%. The structure and purity of all compounds were verified by TLC and FTIR analysis, with drug-likeness properties assessed within Lipinski's Rule of Five showing acceptable bioavailability profiles.

Results from antimicrobial screening against Staphylococcus aureus using the agar cup plate diffusion method demonstrated significant antibacterial activity. Among the derivatives, N-benzyl phthalimide and N-anthraniloyl phthalimide showed increased zones of inhibition, emphasising the critical role of N-substitution in modulating antimicrobial activity. In summary, these findings reinforce the relevance of the phthalimide core to pharmacology and warrant further structural modification for its potential in developing novel antimicrobial agents.