

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF PHENOTHIAZINE-BASED HETEROCYCLIC POLYAMIDES

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ABSTRACT

Current research has focused a lot of curiosity on heterocyclic polymers because of their unique structural features, diverse physical properties, and many uses. In this work, diacid derivatives of phenothiazine and furan were refluxed with specific amines and citramide in dimethylformamide as the reaction medium to form heterocyclic polyamides. The colour, solubility traits, yield percentage, and thin-layer chromatographic features of the resultant polymers were evaluated. Nuclear magnetic resonance spectroscopy, infrared spectroscopy, thermal studies, and fluorescence studies were used to authenticate the structural characteristics. Using the agar disc diffusion method, the antibacterial qualities of the produced polymers were assessed against certain Gram-positive and Gram-negative bacterial strains, showing significant inhibitory effects. The results obtained show that heterocyclic polyamides based on phenothiazine may be useful contenders for the creation of new antibacterial materials. By providing insightful information about structure–activity relationships, this work advances the field of bioactive polymers and may help in the development of better medicinal and functional systems in subsequent research. Additionally, these polymers have the capacity for use in the pharmaceutical and biomedical industries, especially in undertaking issues related to microbial resistance.

Keywords: Phenothiazine Derivatives, Heterocyclic Polymer, Furan, Spectroscopy, Biological Activity.

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INTRODUCTION

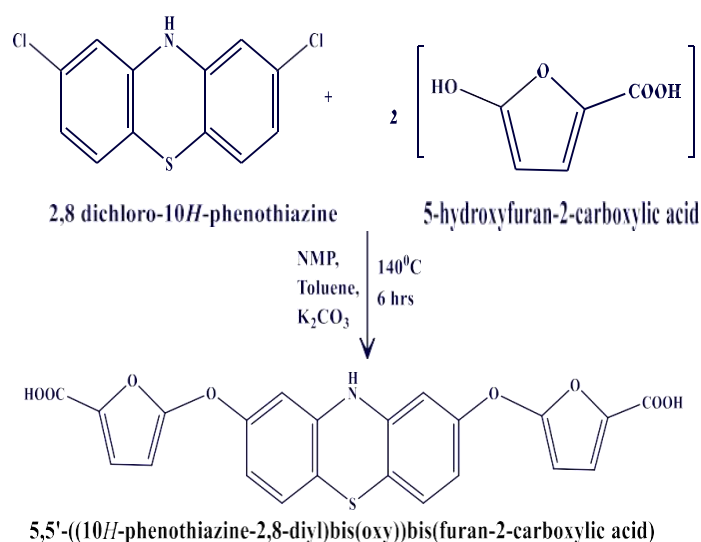
Because of their usefulness, strength, and wide range of functional capabilities, polymeric materials have become important in today's world.^{1,2} Because of their substantial biological efficiency and structural diversity, heterocyclic polyamides containing nitrogen and oxygen atoms have drawn ongoing attention, particularly in agrochemical and medicinal research. For almost an era, phenothiazines, a significant class of tricyclic heterocyclic molecules with nitrogen and sulfur atoms, have been the focus of much research.³⁻⁵ Many phenothiazine-based derivatives have been developed as a result of their distinct molecular structure, which supports a broad range of chemical reactivity and biological activity.^{6,7} Modifications at the tenth position of the phenothiazine ring are known to significantly impact its pharmacological properties, especially when dialkylamino groups are introduced. Such structural changes have been connected with enhanced activities, including neuroleptic, antiemetic, antihistaminic, antipruritic, analgesic, and anthelmintic effects.⁸⁻¹⁰ Because of their wide range of properties, phenothiazine derivatives have been used in several medicinal formulations. Studies have shown that these compounds exhibit various biological activities such as anticancer, antibacterial, antiplasmid, and multidrug resistance reversal effects. Furthermore, they have also been discovered for their potential role in treating neurodegenerative disorders like Alzheimer's disease and Creutzfeldt–Jakob disease.^{11,12} Phenothiazines are attractive candidates for material and biomedical research because of their availability, economic viability, favourable tolerance, and low toxicity.¹³ The biological and physicochemical behaviour of phenothiazine-based systems can be finely tuned through suitable substitution patterns and controlled modification of the tricyclic core.¹⁴⁻¹⁸ In the present study, a series of heterocyclic polyamides designated A1-A6 were synthesised via a condensation polymerisation route. Phenothiazine- and furan-

derived diacids were reacted with selected aromatic and aliphatic diamines to obtain the target polymers. The synthesised materials were characterised using infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, fluorescence spectroscopy, differential scanning calorimetry, and thermogravimetric analysis to study their structure, thermal behaviour, and optical properties. Although phenothiazine-based compounds and heterocyclic polymers have been extensively examined, the development of phenothiazine-containing heterocyclic polyamides with tunable structures and improved antimicrobial properties has not been explored in detail. In addition, studies that clearly relate their structural features to biological activity are still inadequate. In this context, the present work emphasises the synthesis of a series of phenothiazine-based heterocyclic polyamides and the evaluation of their physicochemical and antimicrobial properties, to understand their structure–activity relationship. The present study also supports Sustainable Development Goal 3 (Good Health and Well-being) of the United Nations, as it contributes toward the development of antimicrobial materials that may help in addressing issues related to microbial resistance and improving healthcare applications.

EXPERIMENTAL

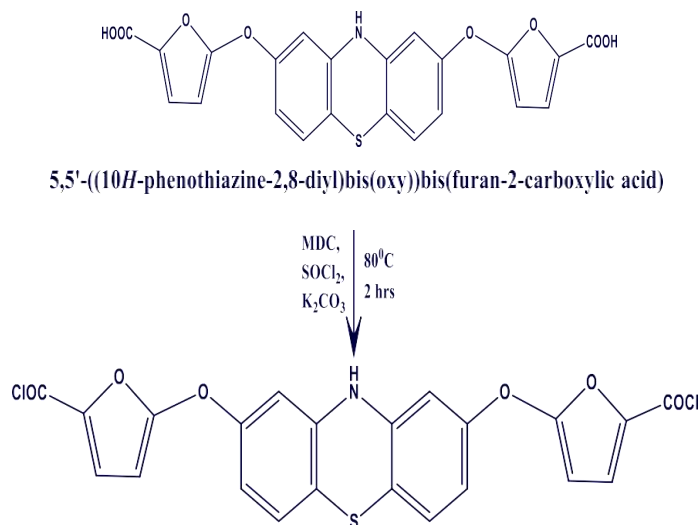
Chemicals and Reagents

All chemicals and solvents used in this study were acquired from standard commercial suppliers and were used as received, unless otherwise specified. Magnesium sulfate, potassium carbonate, and sodium chloride were used as received. 2,8-dichloro-10H-phenothiazine, 5-hydroxy furan 2-carboxylic acid, and dimethylformamide were obtained from JSK Chemicals Private Limited. N methyl pyrrolidone and dimethyl sulfoxide were purchased from CDH, while chlorobenzene and chloroform were acquired from SRL Private Limited. Various diamines, such as ortho-phenylenediamine, meta-phenylenediamine, para-phenylenediamine, 4,4'-diaminodiphenyl ether, 1,3-diaminopropane, and 3,4-diaminobenzophenone, were acquired from Sigma-Aldrich and Loba Chemie and used without further purification. The melting points of the synthesized compounds were determined using the open capillary method and are uncorrected. Proton nuclear magnetic resonance spectra were recorded on a Bruker Avance 400 MHz spectrometer using dimethyl sulfoxide as the solvent, and the chemical shifts are expressed in parts per million (ppm). Infrared spectra were obtained using potassium bromide pellets on a Shimadzu Fourier transform infrared spectrometer. Fluorescence measurements were carried out using a Fluoromax instrument with dimethyl sulfoxide solutions. The progress of the reactions was observed using thin-layer chromatography. Structural characterization of the synthesized diacid and polyamides was confirmed using spectroscopic techniques.



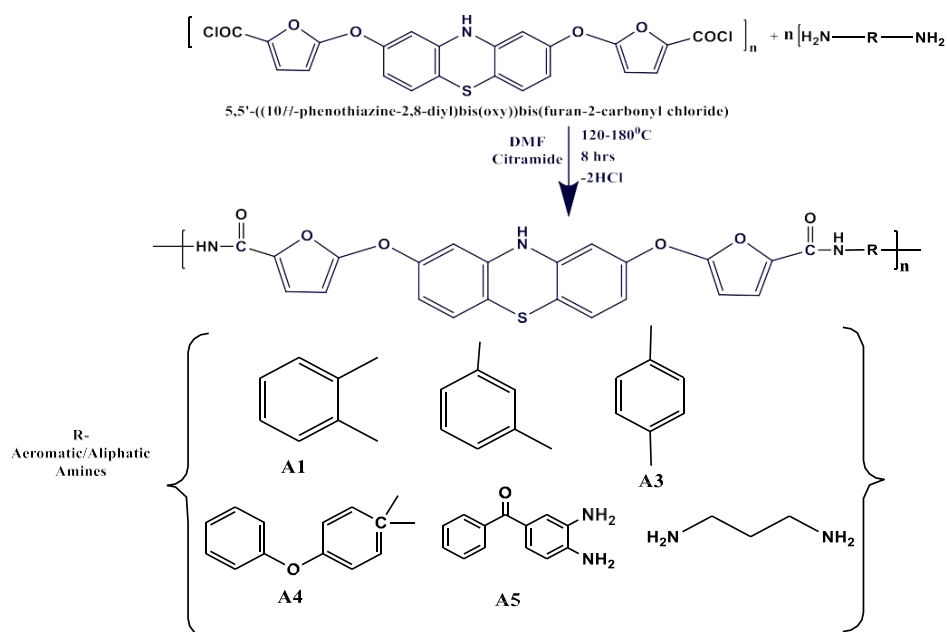
Scheme-1: Synthesis of Diacid

A three-necked round-bottom flask was filled with 2,8-dichloro-10H-phenothiazine, 5-hydroxy furan 2-carboxylic acid, dry N-methyl pyrrolidone, and dry toluene in an atmosphere of nitrogen. Potassium carbonate was then added to the reaction mixture, which was heated to 140 °C and stirred continuously for six hours (Scheme-1). Water formed during the reaction was removed azeotropically. The temperature was then increased to one hundred sixty-five degrees Celsius and maintained for twenty hours. The progress of the reaction was tracked using thin-layer chromatography. Upon completion, the reaction mixture was allowed to cool and then poured into water. The obtained product was washed with a 3% sodium hydroxide solution to isolate the final compound in good yield.^{19,20}



Scheme-2: Synthesis of Monomer

Thionyl chloride (8 mL) and diacid (1.58 g) were placed in a 100 mL round-bottom flask. The reaction mixture was refluxed for two hours, and the evolved HCl gas was trapped in water (Scheme-2). A brown solid was produced after the mixture was distilled to eliminate any leftover thionyl chloride. The diacid chloride was washed several times with dry n-hexane before recrystallisation from a toluene and n-hexane mixture.^{21,22} The yield (M.P. 200–230 °C) was 90%.

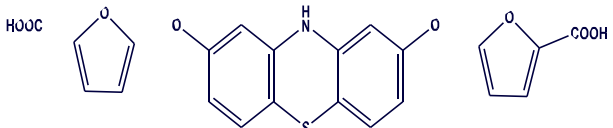


Scheme-3: Synthesis of Polyamide

In a two-neck round-bottom flask, 5,5'-((10H-phenothiazine-2,8-diyl)bis(oxy))bis(furan-2-carbonyl chloride) (0.1 M) was dissolved in a minimum amount of DMF (≈ 10 mL). Cetramide (0.25 g) was introduced as an initiator, and the reaction was heated to 150 °C. Diamines (0.2 M) were then added, and the temperature was maintained between 160 and 180 °C for 8 hours. After completion, the reaction was allowed to cool and then slowly poured into ice-cold water with continuous stirring (Scheme-3). The precipitated substance was filtered, rinsed with hot water, and then dried.^{23,24} Polyamides A1-A6 were synthesized using different diamines using the same approach, yielding around 85% and melting temperatures below 240 °C.

RESULTS AND DISCUSSION

The synthesized polyamides exhibited a range of colours varying from brown to black, with most of the samples appearing black, while compounds A2 and A4 showed a brown appearance. The polymers were obtained through polycondensation, giving yields in the range of 58.78% to 81.45%. Among the series, A2 showed the highest yield (81.45%), whereas A6 showed the lowest (58.78%), with an average yield between 60–70%. The reaction time varied from 1.2 to 1.5 hours, and it was observed that a longer reaction time generally favoured a higher polymer yield. All synthesized polyamides exhibited melting points below 250 °C, indicating moderate thermal stability suitable for material applications. The FT-IR spectra confirmed the formation of polyamide structures, showing characteristic absorption bands corresponding to N–H stretching vibrations in the range of 3500–3150 cm^{-1} and C=O stretching vibrations between 1700–1630 cm^{-1} . The solubility behavior of the synthesized polyamides at 3 wt% concentration is presented in Table 2. The polymers showed good solubility in polar Aprotic solvents like N-Methyl-2-pyrrolidone (NMP), Dimethyl Sulfoxide (DMSO), and Dimethylformamide (DMF) at room temperature. Limited solubility was observed in solvents such as ethyl acetate, 1,4-dioxane, diethyl ether, carbon tetrachloride, finsolv, ligroine, and tetrahydrofuran (THF), while they were found to be insoluble in acetonitrile, hexane, 1-propanol, acetone, toluene, and methanol. Polyamides containing the phenothiazine moiety also showed insolubility in chlorinated solvents. This solubility behavior suggests good processability of the polymers in suitable solvents, which is advantageous for their potential applications. Thermal properties of the synthesized polyamides were evaluated using thermogravimetric analysis (TGA) under a nitrogen atmosphere at a heating rate of 10 °C/min. The TGA curves (Fig.-1 and 2) showed that the decomposition temperatures corresponding to 10% weight loss (T10) ranged between 250 and 500 °C, while the initial decomposition temperatures (IDT) were observed between 250 and 310 °C. These results indicate that the polymers possess good thermal stability, which is an essential characteristic for high-performance materials. The TGA profiles of polyamides A2 and A6 revealed an initial weight loss around 100 °C, which can be attributed to the removal of absorbed moisture or volatile impurities. The major degradation of A2 occurred between 200 and 300 °C, whereas A6 showed slightly higher thermal stability, with degradation occurring between 250 and 310 °C. Both polymers exhibited gradual decomposition at higher temperatures and retained significant char residues of 27.9% (A2) and 21.0% (A6) at 800 °C, confirming their strong thermal resistance. Differential Scanning Calorimetry (DSC) was carried out to investigate the thermal transitions of the synthesized polyamides. A nitrogen atmosphere was provided for the analysis at a heating rate of 10 °C/min. The polymers exhibited high glass transition temperatures (T_g) in the range of 260–350 °C, indicating rigid polymer structures. Among the samples, A2 showed a T_g value of 264.69 °C, while A6 exhibited T_g values of 236.93 °C and 284.94 °C (Fig.-3 and 4). The high T_g values can be attributed to the presence of aromatic and bulky groups in the polymer backbone, which restrict chain mobility and enhance thermal stability. These findings further support the suitability of these materials for applications requiring high thermal resistance.

Compound: Di-acid	
Melting point (°C):	>250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	6.1, 6.2, 6.9, 7.4, 13.7
IR cm^{-1} (KBr)	3344, 3065, 2956, 2652, 2258, 2036, 1688, 1599, 1268, 995, 508.

Compound: A1	
Melting point (°C):	<250 °C
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	2.8, 6.1, 6.2, 6.7-6.9, 7.5, 7.7, 9.6
IR cm ⁻¹ (KBr):	3232, 3028, 2954, 2600, 2021, 1748, 1635, 1564, 1286, 919, 512
Compound: A2	
Melting point (°C):	<250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	6.1, 6.2, 6.7, 7.0, 7.2, 7.5, 10.15
IR cm ⁻¹ (KBr):	3328, 3034, 2962, 2515, 2222, 1754, 1622, 1526, 1290, 900, 562
Compound: A3	
Melting point (°C):	<250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	2.6, 2.8, 6.1, 6.2, 6.6, 6.7, 6.9, 7.4, 9.9
IR cm ⁻¹ (KBr):	3287, 3114, 2900, 2589, 2188, 1794, 1673, 1542, 1116, 894, 516
Compound: A4	
Melting point (°C):	<250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	2.8, 6.1, 6.3, 6.7, 6.9, 7.2, 7.7, 9.8
IR cm ⁻¹ (KBr):	3421, 3099, 2942, 2584, 2336, 1842, 1679, 1266, 909, 426
Compound: A5	
Melting point (°C):	<250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	2.5, 3.2, 5.8, 6.1, 6.7, 6.9, 7.5, 7.7, 9.4
IR cm ⁻¹ (KBr):	3389, 3192, 2912, 2857, 2542, 2024, 1896, 1644, 1579, 1242, 1006, 694
Compound: A6	
Melting point (°C):	<250
¹ H NMR (400 MHz, CDCl ₃), δ (ppm):	1.7, 2.5, 2.8, 3.3, 6.1, 6.2, 6.9
IR cm ⁻¹ (KBr):	3411, 3049, 2924, 2896, 2545, 2234, 1749, 1614, 1486, 1314, 1256, 890, 343

Biological Activity

The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the synthesised compounds in order to assess their in vitro antibacterial activity. The study was carried out against four bacterial strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, along with three fungal strains, including *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. The Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology in Chandigarh, India, provided all of the microbial strains. The

inoculum density was adjusted using the turbidimetric method to approximately 10^8 CFU/mL. Mueller–Hinton broth and Sabouraud dextrose broth were used as nutrient media for bacterial and fungal strains, respectively. Standard antibacterial agents included ampicillin and chloramphenicol, while the usual antifungal medications were griseofulvin and nystatin. DMSO was used as the solvent for preparing both test and standard solutions.

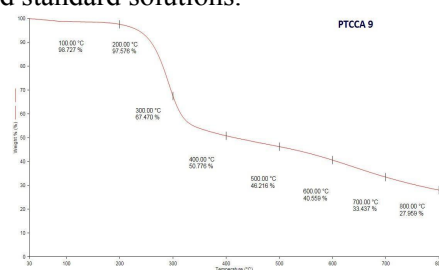


Fig.-1: TGA Curve of Polymer A2

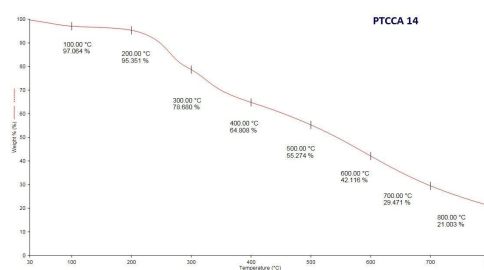


Fig.-2: TGA Curve of Polymer A6

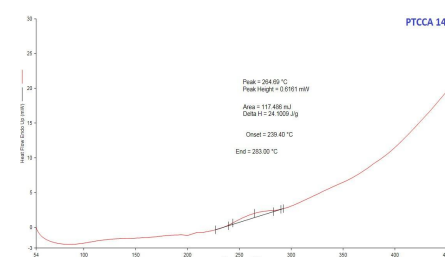


Fig.-3: DSC Curve of Polymer A2

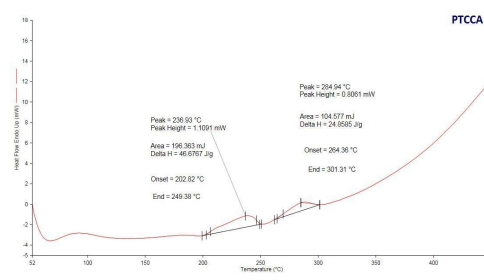


Fig.-4: DSC Curve of Polymer A6

The antimicrobial activity of the synthesized compounds is summarized in Tables-1 and 2. In antibacterial studies, most compounds showed moderate activity against the tested strains. Compounds A2 and A5 exhibited relatively better activity against *Staphylococcus aureus* with MIC values of 200 μ g/mL, while other compounds showed activity comparable to the standard drug ampicillin. In the case of *Escherichia coli* and *Pseudomonas aeruginosa*, the compounds generally showed moderate to low activity when compared to standard drugs. In antifungal studies, the synthesized compounds showed limited activity against *Aspergillus niger* and *Aspergillus clavatus*. However, compounds A1 and A6 demonstrated comparatively better activity against *Candida albicans*, with MIC values of 100 μ g/mL and 250 μ g/mL, respectively, compared to griseofulvin (500 μ g/mL).

Table-1: Antibacterial Activity of the Compounds

Compound	<i>E.Coli</i> MIC (μ g/mL)	<i>P. Aeruginosa</i> MIC (μ g/mL)	<i>S. Aureus</i> MIC (μ g/mL)	<i>S. Pyogenes</i> MIC (μ g/mL)
A1	200	500	250	200
A2	250	500	200	500
A3	200	500	250	250
A4	250	1000	250	500
A5	250	500	500	500
A6	250	500	250	250
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50

Table-2: Antifungal Activity of the Compounds

Compound	<i>C. Albicans</i> MIC(μ g/mL)	<i>A.Niger</i> MIC(μ g/mL)	<i>A.Clavatus</i> MIC(μ g/mL)
A1	100	500	1000
A2	500	500	500
A3	1000	500	500
A4	500	1000	1000

A5	500	500	1000
A6	250	200	200
Nystatin	100	100	100
Griseofulvin	500	100	100

The antimicrobial activity observed for the synthesized compounds suggests that these materials possess promising biological potential. It can be inferred that the presence of heterocyclic units in the polymer backbone contributes to their activity, possibly due to enhanced interaction with microbial systems. These findings provide useful insight into the structure–activity relationship of heterocyclic polymers and indicate their potential for further development in antimicrobial applications. Overall, the combined antimicrobial performance and thermal stability of the synthesized polyamides highlight their relevance in advanced functional and biomedical materials, thereby supporting Sustainable Development Goal 3 (Good Health and Well-being) by contributing to efforts aimed at combating microbial resistance.

CONCLUSION

In summary, synthesizing, defining, and evaluating the biological activity of phenothiazine-based heterocyclic polyamides opens up fascinating research directions with a wide range of potential in both science and industry. We created a variety of functionalized polyamides (A1–A6) via polycondensation polymerization. Their structures and stability were confirmed by several spectroscopic and thermal techniques. The compounds exhibited moderate to strong antibacterial activity when evaluated against a range of bacterial strains. These findings make phenothiazine-based heterocyclic polyamides attractive candidates for advancements in materials science and biomedicine. These results indicate that the presence of heterocyclic and phenothiazine units plays a significant role in improving the biological performance of the polymers, providing useful insight into their structure–activity relationship. The combination of good thermal stability and antimicrobial activity suggests that these materials may be further explored for applications in advanced functional materials and biomedical fields. Overall, the outcomes of this study are aligned with Sustainable Development Goal 3 (Good Health and Well-being), as they support the development of new antimicrobial materials that may contribute to addressing challenges related to microbial resistance.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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