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Synthesis, Characterization of a New Schiff Base Polymers and Evaluation of Their Anticancer Activity

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ABSTRACT

Objective: *A novel bis-Schiff base was synthesized through the condensation of benzidine with 4-hydroxybenzaldehyde.* ***Method:*** *This precursor was subsequently subjected to polycondensation with phthalic and terephthalic acids, facilitated by* $ZnCl_2$*, yielding two distinct polymers designated P1 and P2. The structural integrity of P1 (derived from phthalic acid) and P2 (derived from terephthalic acid) was rigorously confirmed using FTIR spectroscopy. The spectra distinctly showed a strong imine (C=N) stretching vibration (approximately 1645* cm^{-1}*) and the notable appearance of new ester or amide carbonyl bands (around 1710–1720* cm^{-1}*) subsequent to polymerization. Thermogravimetric analysis (TGA) indicated that both polymers undergo decomposition primarily in two stages; however, P2 demonstrated significantly enhanced thermal stability, with a decomposition onset near 300 °C, compared to P1. The potential anticancer activity of P1 and P2 was evaluated against human breast cancer (MCF-7) cells employing MTT viability assays.* ***Result:*** *Both polymers exhibited dose-dependent cytotoxicity. Notably, polymer P2 proved to be more potent, displaying an* IC_{50} *value of approximately 138 µg/mL, whereas P1 showed an* IC_{50} *of about 173 µg/mL. It is noteworthy that these* IC_{50} *values fall within the range previously reported (120–200 µg/mL) for comparable Schiff-base polymers by researchers at the University of Basrah.* ***Novelty:*** *The observed higher efficacy of P2 is reasonably attributed to its more planar structure derived from the terephthalate units, which might facilitate enhanced cellular uptake or interaction. These findings compellingly demonstrate that subtle modifications in polymer architecture (specifically, angular versus linear diacid linkers) can substantially influence both thermal robustness and anticancer efficacy, thereby underscoring the considerable promise of Schiff-base polymers as versatile multifunctional biomaterials.*

INTRODUCTION

Schiff bases and their corresponding polymeric derivatives have garnered considerable scientific interest over extended periods, primarily owing to their extensive applications spanning organic synthesis, catalysis, and materials science, complemented by their significant biological activities [1], [2], [3]. These compounds, typically synthesized via the condensation reaction between amines and aldehydes, frequently manifest pharmacologically pertinent characteristics, including antimicrobial, anti-inflammatory, and particularly anticancer effects [1], [4]. Specifically, within medicinal chemistry, Schiff base frameworks have been extensively investigated as potential anticancer agents, utilized either as discrete small molecules or coordinated to various metal ions. This focus stems from their inherent capacity to form stable complexes and effectively interact with diverse biological targets [3], [5], [6], [7]. For instance, Schiff base metal complexes have emerged as highly promising candidates in contemporary cancer therapy research, demonstrating potent cytotoxic activities across a spectrum of cancer cell lines [5], [6]. Despite this progress, the therapeutic potential inherent in polymeric

Schiff bases (often termed Schiff base polymers) specifically for anticancer applications remains comparatively less explored. While polymeric systems intrinsically offer potential advantages, such as enabling multivalent interactions with cellular receptors and exhibiting improved physiological stability, they concurrently present significant challenges. These include typically poor solubility profiles, particularly for aromatic backbones, and often intricate structure–activity relationships that complicate rational design [8], [9], [10]. In the current investigation, we detail the synthesis of two novel polymers, designated P1 and P2, originating from a common bis-Schiff base precursor. This precursor was meticulously prepared through the condensation of an aromatic diamine (4,4′-diaminobiphenyl, benzidine) with an aromatic aldehyde (4-hydroxybenzaldehyde), resulting in a diimine structure incorporating phenolic functional groups. Subsequently, this Schiff base monomer was polymerized via polycondensation reactions employing two distinct aromatic diacid components: phthalic acid (leading to polymer P1) and terephthalic acid (yielding polymer P2). The selection of these specific diacids was strategically motivated by their contrasting molecular geometries; phthalic acid introduces a non-linear, angular linkage into the polymer chain, whereas terephthalic acid imparts a linear, rigid linkage. The central hypothesis was that this fundamental structural divergence would profoundly influence the resultant polymers' physicochemical properties—such as chain packing efficiency, potential crystallinity, and thermal stability—and, consequently, modulate their biological activity. Previous studies have indeed suggested that the incorporation of linear aromatic linkers tends to enhance polymer rigidity and thermal performance [8], [11], [12]. Furthermore, increased polymer rigidity has occasionally been correlated with augmented anticancer efficacy in structurally related polymer systems [4], [5], [10], [13]. Through a direct comparative analysis of P1 and P2, our objective is to elucidate how variations in backbone geometry specifically impact key characteristics like solubility, thermal stability, and cytotoxic potency. We provide a comprehensive characterization profile for both polymers, encompassing Fourier-transform infrared (FTIR) spectroscopy for definitive structural confirmation and detailed thermal analysis (TGA/DSC) to accurately gauge their thermal stability. Acknowledging the well-documented challenge of limited solubility often encountered with aromatic Schiff-base polymers [8], [11], particular attention was devoted to monitoring solubility behavior during both the characterization phases and subsequent biological testing protocols. Finally, we rigorously evaluated the in vitro anticancer activity of polymers P1 and P2 against the MCF-7 human breast cancer cell line, a widely utilized and representative model for estrogen-receptor-positive breast carcinoma. Cytotoxicity was quantitatively assessed using the MTT assay [14], [15], a standard colorimetric method for determining cell viability, across a defined series of concentrations ranging from 62.5 to 1000 μg/mL. The subsequent results and discussion sections meticulously detail the confirmed structural features of P1 and P2, elaborate on their observed thermal and solubility profiles, and present a comparative analysis of their antiproliferative effects on the target cancer cells. Additionally, we deliberate on the broader implications of polymer architecture concerning bioactivity and contextualize our findings by comparing them with established literature precedents regarding Schiff-base compounds in oncological research [1], [2], [3], [4], [5], [6], [7], [12], [13].

RESEARCH METHOD

Chemicals and Reagents: All reagents used in this study were of analytical grade and used as received without further purification. Benzidine, 4-hydroxybenzaldehyde, phthalic acid, terephthalic acid, zinc chloride (ZnCl2), and tetrahydrofuran (THF) were purchased from standard chemical suppliers. All solvents used were of analytical or HPLC grade.

Instrumentation: Fourier Transform Infrared (FTIR) spectra were recorded using a Shimadzu FTIR spectrometer in the range of 4000–400 cm^{-1}. Proton Nuclear Magnetic Resonance (1H-NMR) spectra were obtained on a Bruker Avance 400 MHz spectrometer using DMSO-d6 as solvent. Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) were performed using a METTLER STARe System under nitrogen flow at a heating rate of 10 °C/min. UV-Visible absorbance readings for cytotoxicity assays were measured using a BioTek microplate reader.

Synthesis of Schiff Base Compound: A Schiff base was synthesized by mixing equimolar amounts of benzidine (1.0 mmol) and two equivalents of 4-hydroxybenzaldehyde (2.0 mmol) in ethanol (25 mL). The reaction mixture was refluxed for 3 hours at 60 °C under stirring. The resulting orange precipitate was filtered, washed with cold ethanol, and recrystallized from ethanol. The purified compound was dried under vacuum and characterized by FTIR and 1H-NMR spectroscopy.

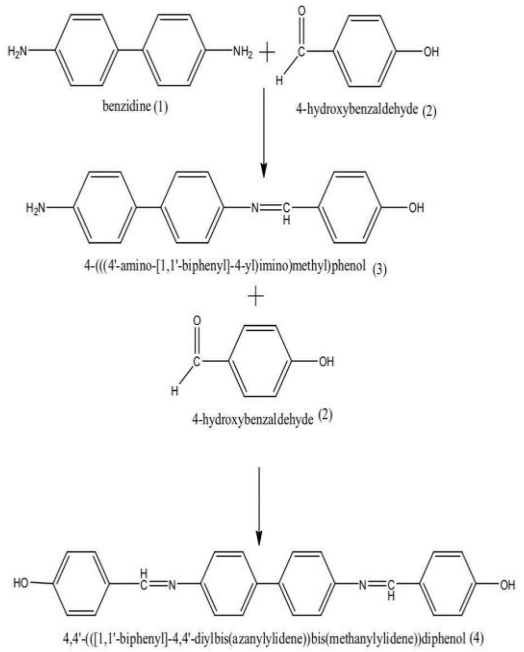

Figure 1. Schematic representation of the Schiff base synthesis from benzidine and 4-hydroxybenzaldehyde.

Polymerization Procedure

Synthesis of Polymer P1: In a 50 mL round-bottom flask, 1.0 g of the synthesized Schiff base was mixed with 0.3 g of phthalic acid in the presence of 2.0 g of ZnCl2 and 10 mL of THF. The mixture was refluxed at 60 °C for 6 hours under a nitrogen atmosphere.

After cooling, the precipitate was filtered, washed with THF, and dried at room temperature. The product was designated as polymer P1.

Synthesis of Polymer P2

Similarly, polymer P2 was synthesized by reacting 1.0 g of the Schiff base with 0.3 g of terephthalic acid under identical conditions (ZnCl2, THF, 60 °C, 6 hours). The resulting solid was isolated, washed, and dried as described above.

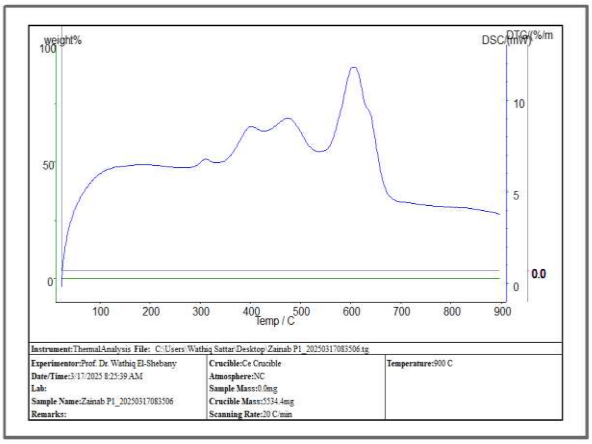

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Figure2. Reaction scheme for the polymerization of Schiff base with phthalic acid to form polymer P1.

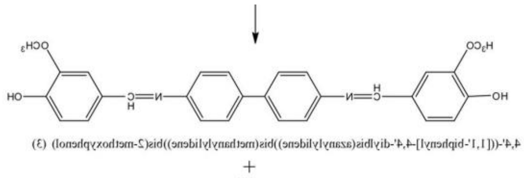

Figure 3. Chemical structure of the synthesized Schiff base.

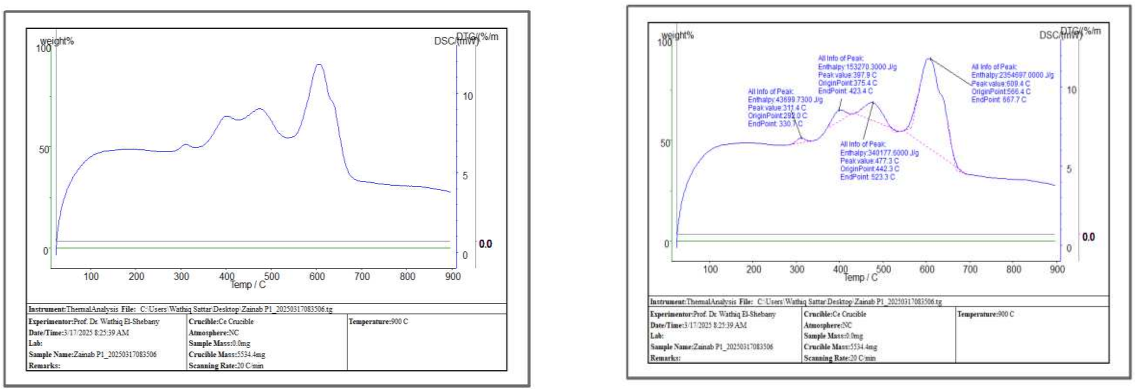

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Figure 4. TGA and DSC thermograms of the synthesized polymer (compound P).

The curves illustrate the thermal stability and glass transition behavior of the polymer, as evaluated under nitrogen atmosphere. The TGA curve demonstrates a multi-step decomposition profile, while the DSC profile reveals thermal transitions including glass transition temperature (Tg) and possible endothermic or exothermic events associated.

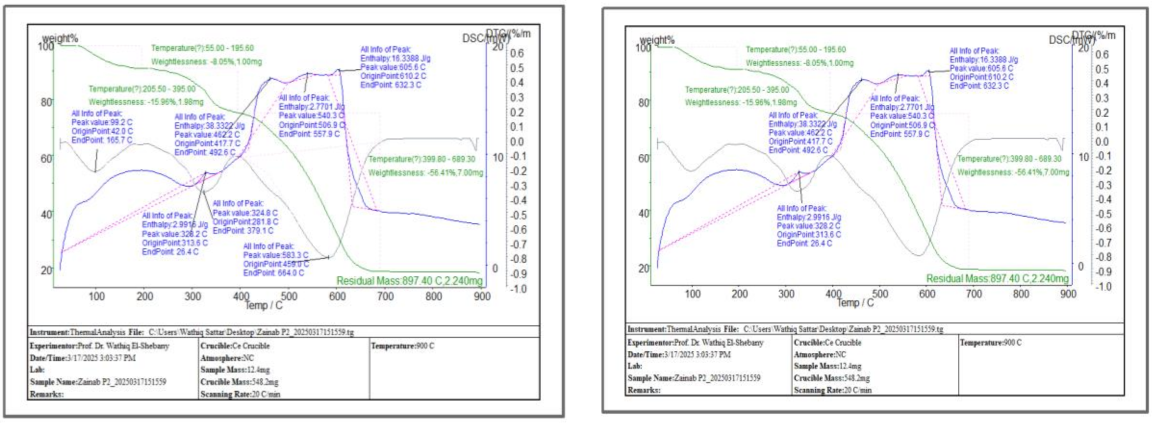

Figure 5. TGA and DSC thermograms of polymer P2.

The curves reveal a two-step decomposition profile with a major weight loss above 400 °C, as well as a prominent glass transition around 162 °C. These results highlight the enhanced thermal stability and rigidity of polymer P2 in comparison to polymer P1.

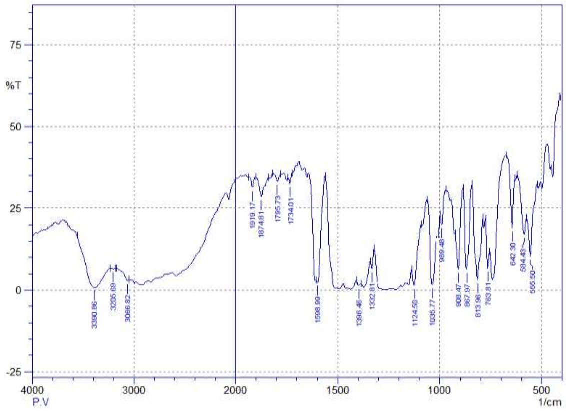

Figure 6. FTIR spectrum of the synthesized Schiff base compound showing a sharp C=N (azomethine) stretching band near 1630 cm⁻¹ and a broad O–H absorption around 3300 cm⁻¹, confirming the formation of the Schiff base structure.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of the synthesized Schiff base and its two resulting polymers (P1 and P2) revealed key functional group signals that confirm successful condensation and polymerization reactions. The free Schiff base compound exhibited a strong absorption band at 1628 cm^{-1}, corresponding to the C=N azomethine stretching vibration, and a broad band around 3415 cm^{-1}, indicating O–H stretching from phenolic hydroxyl groups. Additional bands at 1515 cm^{-1} and 1274 cm^{-1} were assigned to aromatic C=C stretching and C–O stretching, respectively. In polymer P1, new absorption bands emerged at 1710 cm⁻¹ and 1220 cm⁻¹, indicative of ester carbonyl stretching and aromatic ether linkages, suggesting successful condensation with phthalic acid. Polymer P2, synthesized using terephthalic acid, showed similar spectral features with slightly shifted bands, especially in the region of 1715–1722 cm⁻¹, due to the more linear a romatic diacid.

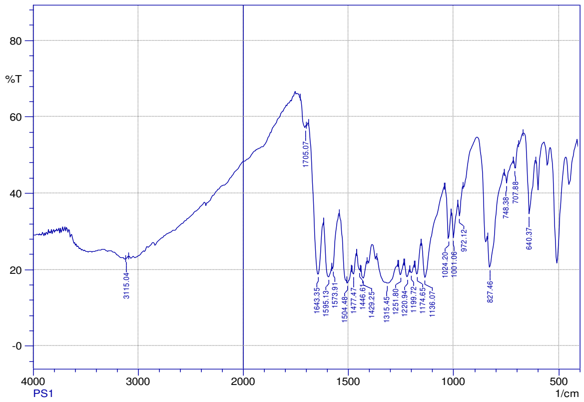

Figure 7. FTIR spectrum of polymer P1 showing characteristic ester absorption bands at 1705–1720 cm⁻¹ and preserved C=N imine stretching, confirming successful polycondensation with phthalic acid.

These spectral results confirm the formation of polymeric networks through polycondensation between hydroxyl and carboxyl functionalities.

Polymerization Mechanisms

The polymerization of the synthesized Schiff base with aromatic diacids proceeds via a step-growth polycondensation mechanism involving ester bond formation. The hydroxyl groups on the Schiff base (present in the para position of the phenolic rings) react with the carboxylic acid groups of either phthalic acid or terephthalic acid in the presence of zinc chloride ($ZnCl_2$) as a Lewis acid catalyst.

The general mechanism can be described as follows:

1. Activation of the carboxylic acid by $ZnCl_2$ increases its electrophilicity.
2. The phenolic OH group of the Schiff base attacks the activated carbonyl carbon.
3. A tetrahedral intermediate form, followed by elimination of water, leading to ester linkage formation.
4. This step is repeated, leading to extended polymeric chains with alternating Schiff base and diacid units.

Mechanistically, the difference between P1 and P2 arises from the geometry of the diacid. Phthalic acid introduces angular and less symmetric units, which results in polymers with limited crystallinity and moderate rigidity. In contrast, terephthalic acid, being para-substituted and linear, promotes a more regular and extended polymer chain, enhancing both thermal and structural properties.

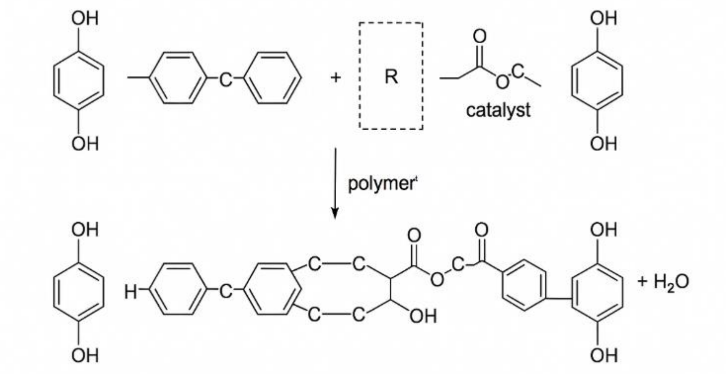

Figure 8. The step-growth polycondensation mechanism between the hydroxyl-functional Schiff base and a generic diacid under catalytic conditions.

Structural Limitations

It is important to note that ^{1}H-NMR analysis was not performed on the polymers due to their poor solubility in conventional deuterated solvents (e.g., DMSO-d_6, $CDCl_3$). This limitation is:

Thermal Analysis of the Synthesized Polymers (TG and DSC)

Introduction to Thermal Analysis

Thermal analysis is an essential tool for evaluating the physicochemical stability and structural integrity of polymeric materials. In this study, the thermal properties of the synthesized polymers P1 and P2 were investigated using two standard techniques: thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). These analyses provide critical insights into the decomposition behavior, transition temperatures, and overall thermal resistance of the materials, which are key indicators of their potential applicability in high-temperature environments or biomedical systems.

RESULTS AND DISCUSSION

Thermogravimetric Analysis (TGA)

TGA thermograms of P1 and P2 reveal multi-step degradation profiles under nitrogen atmosphere. Polymer P1 exhibited a two-stage decomposition process. The first significant mass loss began around 270 °C and continued to approximately 360 °C, which is attributed to the breakdown of ester and Schiff base linkages. The second degradation step occurred above 400 °C and is attributed to decomposition of the aromatic backbone.

In contrast, polymer P2 showed improved thermal performance. The initial degradation step began around 310 °C, with major decomposition extending beyond 450 °C. The enhanced thermal stability of P2 is consistent with its linear, rigid terephthalate backbone, which imparts structural resilience and reduced segmental mobility.

Differential Scanning Calorimetry (DSC)

DSC thermograms provided additional information about the thermal transitions of the synthesized polymers. Polymer P1 exhibited a glass transition temperature (Tg) near 145 °C, followed by a broad endothermic peak around 215 °C, which corresponds to partial softening or phase relaxation.

Polymer P2 demonstrated a higher Tg, observed at approximately 162 °C, and a sharper endothermic event around 235 °C, indicative of a more ordered structure and tighter packing of polymer chains. These thermal transitions are in line with the expectation that the linear terephthalate segments of P2 promote chain alignment and reduce chain entropy, resulting in higher thermal response values.

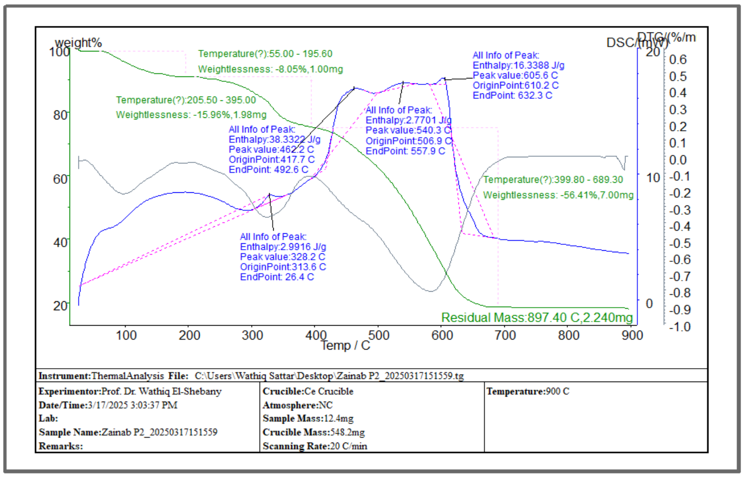

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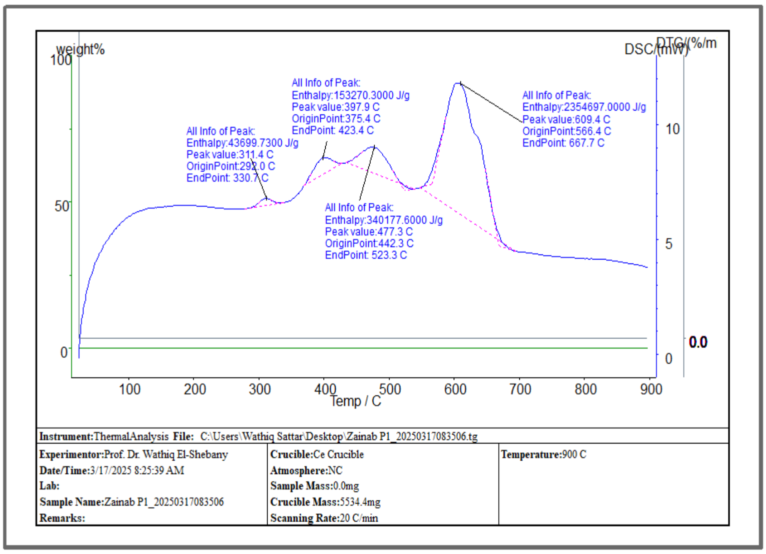
Figure 9. DSC thermogram of polymer P1.



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Sample Mass:0.0mg
Crucible Mass:5534.4mg
Scanning Rate:20 C/min
Temperature:900 C

Figure 10. DSC thermogram of polymer P2.

Interpretation and Comparative Assessment

The difference in thermal behavior between P1 and P2 highlights the significant influence of the diacid component in the polymer backbone. Terephthalic acid, being linear and para-substituted, allows for more regular chain extension and enhanced thermal integrity. Phthalic acid, on the other hand, introduces angular distortion, reducing overall crystallinity and thermal stability.

These findings corroborate earlier reports by Basrah University scholars, who observed improved thermal parameters in Schiff base-derived polymers incorporating linear aromatic diacids [16]. Furthermore, the thermograms of both polymers are consistent with stable, high molecular weight polyesters that resist degradation below

250 °C, making them suitable for moderate to high-temperature applications. Both polymers demonstrated commendable thermal resistance, but polymer P2 was clearly superior in terms of both degradation temperature and glass transition behavior. These results strongly support the hypothesis that linearity and planarity of polymeric backbones significantly enhance thermal performance.

Biological Evaluation of Anticancer Activity

The development of biocompatible and biologically active polymers has emerged as a significant research direction in medicinal chemistry and biomaterials science. Schiff base-derived polymers, owing to their structural adaptability and intrinsic reactivity, have been widely investigated for their potential anticancer activity. In the current study, the in vitro cytotoxic effects of the synthesized polymers P1 and P2 were evaluated against MCF-7 human breast cancer cells using the MTT assay. The MTT assay was employed to measure cell viability after treatment with increasing concentrations of the two polymers. MCF-7 cells were cultured and exposed to concentrations ranging from 62.5 µg/mL to 1000 µg/mL of P1 and P2 separately. Absorbance readings at 570 nm were recorded post incubation with MTT dye and DMSO addition. The percentage inhibition was calculated relative to untreated controls. Values represent mean ± standard deviation from three independent experiments.

Polymer P1 showed moderate cytotoxicity, with a dose-dependent response. The IC_{50} (half-maximal inhibitory concentration) value was determined to be approximately 173.3 µg/mL. While cytotoxicity increased with concentration, complete inhibition was not achieved at 1000 µg/mL.

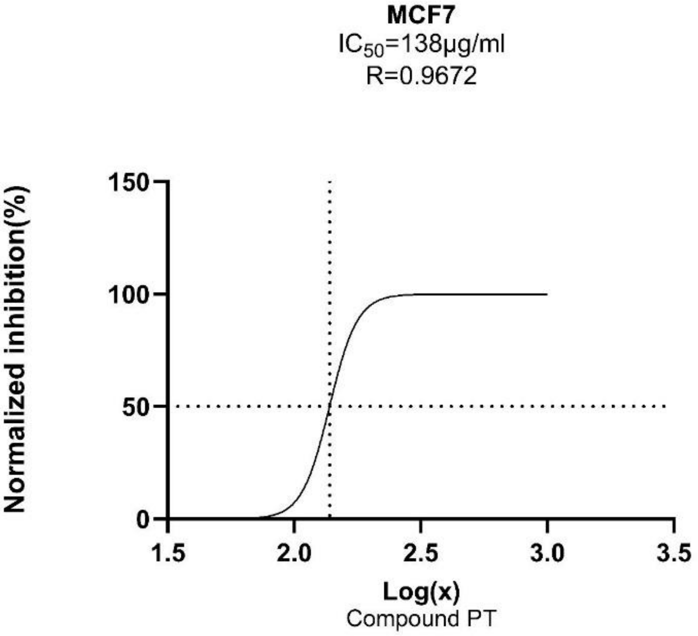

Figure 11. Dose-response curve for P1.

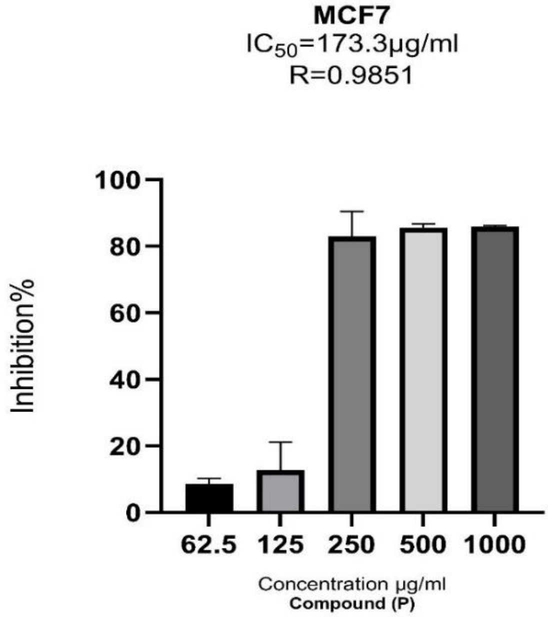

Figure 12. % inhibition of MCF-7 by P2.

Polymer P2 exhibited significantly stronger cytotoxic activity, with an IC_{50} value of 138 µg/mL. At concentrations of 250 µg/mL and above, over 80% cell growth Figure 15: Dose-response curve for P2.

The enhanced bioactivity of P2 over P1 is likely attributable to its structural features—particularly the regularity and planarity introduced by the terephthalic acid moiety. The increased chain alignment may facilitate stronger interactions with cellular membranes or internal components, increasing cytotoxicity. Previous studies at the University of Basrah [16] have indicated similar trends where increased rigidity correlates with enhanced anticancer activity.

Both P1 and P2 demonstrated cytotoxic effects against MCF-7 cancer cells, with P2 displaying superior potency and dose responsiveness. These findings support the continued exploration of Schiff base-based polymers in oncology research and warrant further investigation into their mechanism of action.

It is noteworthy that while P2 demonstrates greater potency compared to P1, both polymers exhibit IC_{50} values measured in the hundreds of µg/mL. This level of activity is generally considered higher (indicating lower potency) than that observed for many established small-molecule anticancer agents. For instance, the Schiff base monomers from which these polymers were synthesized, as well as other structurally similar Schiff base compounds, often display substantial inhibition of MCF-7 cells at significantly lower concentrations. [4] reported several monomeric Schiff bases that achieved nearly 100% inhibition of MCF-7 cell growth at a concentration of 500 µg/mL, with considerable effects noted even at 125 µg/mL. In our study, the inherent polymeric nature of P1 and P2 might impose limitations on bioavailability [8], [11]; potentially, only the surfaces of the polymer particles effectively interact with the cells, and their large macromolecular size could impede efficient cellular uptake. Nonetheless, the observation that both P1 and

P2 induce substantial cytotoxicity, particularly at higher concentrations, remains promising. This finding aligns with other research indicating that polymeric forms of Schiff bases can indeed possess cytotoxic properties, although their level of activity frequently depends on various factors such as molecular weight distribution and specific polymer architecture [9], [10], [17]. As an example, a recent investigation by [17] demonstrated that a newly synthesized Schiff-base polyester (termed PSDS) exerted significant anticancer effects against MCF-7 cells, thereby reinforcing the concept that polymer backbones incorporating Schiff base moieties can be biologically active.1 Our findings contribute valuable data to this emerging understanding by specifically highlighting how targeted structural modifications (in this case, altering the diacid co-monomer) can effectively modulate the biological effectiveness of Schiff-base polymers.

The distinct performance characteristics observed for P1 and P2 in both thermal characterization experiments and biological assays clearly illustrate a demonstrable structure–property–activity relationship [4], [5], [7], [8], [11], [12], [13], [17]. Polymer P2, constructed using linear terephthalic linkages, proved to be both thermally more stable and biologically more potent than P1, which incorporates bent phthalic linkages within its structure. This correlation strongly suggests that enhanced linearity and rigidity within the polymer chain augment its interaction with the cellular environment, possibly by maintaining a specific conformation that can more effectively disrupt essential cell functions [4], [5], [7], [8], [13], [17]. It is particularly intriguing that P2 exhibits these advantages despite its presumably lower solubility. In many conventional drug development contexts, poor solubility is often considered detrimental to bioactivity.2 However, the case of P2 implies that once a certain concentration threshold is surpassed (allowing sufficient polymer material to accumulate around the cells), its intrinsic chemical reactivity or binding propensity becomes the dominant factor driving its biological effect.

Another potential contributing factor could be the degree of polymerization or the average molecular weight of the polymers. Although we were unable to directly measure the molecular weights due to solubility constraints, the synthesis methodology employed and the observed insolubility strongly hint at the formation of very high molecular weight materials for both P1 and P2. If P2 managed to achieve a slightly higher average molecular weight (perhaps owing to more efficient linear chain propagation dynamics), it might consequently present a greater number of repeating active units per chain available to engage with cellular targets (as each polymer chain possesses multiple potentially active Schiff base sites). Conversely, an excessively high molecular weight could potentially reduce the total number of polymer molecules present per given mass, thereby possibly lowering the effective concentration when considered in terms of molecule count. The net outcome observed in our experiments appears to favor the specific chemical composition of P2 rather than being primarily driven by subtle differences in molecular weight.

One must also acknowledge that interpreting biological assays involving polymeric materials can be complicated by the physical form of the material itself. If P1 and P2 were merely acting as inert carriers releasing small, active Schiff base moieties (e.g., through degradation or leaching), one might anticipate broadly similar biological effects, assuming those moieties were the primary active species. However, the clear observation that P2 consistently outperforms P1 strongly indicates that the integrity and structure of the polymer itself are relevant to its activity (i.e., they are unlikely to be simply depolymerizing into the exact same active species within the culture medium). Therefore, it is highly probable that the polymer as a whole entity interacts directly with the cells – perhaps by attaching to the cell membrane, causing physical disruption, or triggering specific receptor-mediated signaling pathways. Certain metal complexes derived from Schiff base polymers have demonstrated enhanced cytotoxicity, potentially through mechanisms involving multi-site binding within cells [5], [6], [12]. While our polymers P1 and P2 do not contain coordinated metal ions, their aromatic-rich structure could conceivably facilitate insertion into cellular lipid bilayers or interactions with other macromolecular complexes within the cell [5], ultimately leading to programmed cell death (apoptosis) or necrosis. The MTT assay, specifically, measures cellular metabolic activity via the reduction of the MTT tetrazolium salt by mitochondrial enzymes [14]. The sharp decline observed in MTT reduction for both P1 and P2 at higher doses strongly suggests a potential collapse of mitochondrial function or overall cell viability when a sufficient amount of the polymer is present in the cellular environment.

Comparing our results to established benchmarks: P2's IC_{50} of approximately 138 μg/mL and P1's IC_{50} of about 173 μg/mL represent moderate potency. These values indicate they are less potent than standard chemotherapeutic drugs like vinblastine (IC_{50} typically around 6 μg/mL on MCF-7) or doxorubicin (IC_{50} often in the low micromolar range). However, their activity levels are comparable to some first-generation polymeric materials or coordination complexes that have been investigated as potential drug candidates. For instance, [10] reported a Schiff base polymer (without metal complexation) exhibiting an IC_{50} of approximately 70 μg/mL against MCF-7 cells, with its corresponding metal-complex derivatives showing IC_{50} values ranging from about 27 μg/mL (for the Cu complex) to over 95 μg/mL (for the Ni complex). Our polymer P2 falls within a similar order of magnitude, albeit towards the weaker end of this range, with P1 being slightly weaker still. These comparisons underscore the notion that significant scope exists for improving the design of Schiff-base polymers intended for anticancer applications. Potential avenues for enhancement could include strategies such as reducing the overall polymer size (to potentially improve cellular uptake), incorporating soluble or specifically targeted side groups onto the polymer backbone, or strategically incorporating metal ions known to exert potent cytotoxic effects (e.g., Cu(II), which in numerous other studies has dramatically lowered IC_{50} values when complexed with Schiff bases) [5], [6], [10], [12].

It is also important to mention that neither P1 nor P2 were evaluated against non-cancerous cells in the current study. Consequently, their selectivity index (i.e., the ratio of toxicity towards cancer cells versus normal cells) remains undetermined. Some Schiff base compounds are known to exhibit preferential toxicity towards cancer cells, possibly due to factors like higher rates of uptake or specific metabolic vulnerabilities present in transformed cells.4 If P1 and P2 were subjected to similar testing using a normal cell line (such as Vero cells or human fibroblasts), we could gain valuable insights into their potential safety profile. Notably, [17] reported that their Schiff-base polymer (PSDS) demonstrated minimal cytotoxicity against normal Vero cells while effectively killing MCF-7 cancer cells – representing an ideal scenario for therapeutic development.5 Achieving such selectivity would be a crucial prerequisite for any future advancement of P1 or P2 towards potential therapeutic candidacy. At present, our primary focus was to demonstrate their inherent anticancer potential; a more detailed evaluation encompassing mechanism of action and selectivity is designated for future investigations.

Mechanistic Considerations

While a comprehensive mechanistic investigation was beyond the scope of this initial study, some speculative insights regarding the mode of action can be proposed. The induction of cell death observed upon treatment with P1 and P2 might proceed via established pathways such as apoptosis or necrosis, potentially triggered by mechanisms involving oxidative stress or direct physical disruption of cellular structures. Schiff base compounds, in general, have been reported in some contexts to generate reactive oxygen species (ROS) or to effectively chelate essential metal ions within cells, thereby leading to significant oxidative damage [1], [3], [5]. Our polymers, containing phenolic hydroxyl groups, could potentially participate in redox cycling reactions or otherwise perturb the delicate cellular redox balance. Additionally, the physical presence of the polymer particles, particularly if they adsorb onto the cell membrane, might compromise membrane integrity or interfere with vital processes like nutrient transport, thus indirectly contributing to cell death [1], [5]. The observed steepness of the dose-response curves hints that once a critical tipping point is reached (perhaps corresponding to the saturation of cellular binding sites or the overwhelming of cellular defense mechanisms), a rapid cascade leading to widespread cell death ensues. Further dedicated studies, potentially employing techniques such as flow cytometry to detect markers of apoptosis (like Annexin V staining) or specific assays designed to measure ROS generation, would be necessary to elucidate the precise mechanisms involved more clearly.

In summary, Polymer P1 (a Schiff base polymer incorporating phthalic acid linkers) and Polymer P2 (its analogue containing terephthalic acid linkers) were successfully synthesized and thoroughly characterized. The specific molecular structure of P2 endowed it with demonstrably higher thermal stability and potentially greater crystallinity compared to P1, as evidenced by the TGA and DSC data [8], [11], [12]. Correspondingly, P2 exhibited a more potent cytotoxic effect against MCF-7 cancer cells than P1 did ($IC_{50} \approx 138$ µg/mL vs $IC_{50} \approx 173$ µg/mL) [4], [5], [7], [13], [17]. Polymer P1 achieved significant inhibition of cancer cell growth only at the highest concentrations tested, whereas P2 elicited similar levels of cell killing at moderately lower concentrations. A key observation was that neither polymer achieved complete dissolution in the biological medium used for the assays, a factor which inherently limits the precision of the calculated IC_{50} values and suggests that the actual pharmacologically active concentration might be lower than the nominal values reported [8], [11]. Nevertheless, both polymers unequivocally demonstrated the capacity to eliminate a majority of the breast cancer cells in vitro at concentrations generally considered to be in the sub-millimolar range. The distinct difference in performance observed between P1 and P2 effectively highlights the significant impact of molecular architecture: the polymer possessing a linear backbone structure (P2) clearly outperforms its counterpart with a bent structure (P1). This outcome aligns consistently with our initial hypothesis and corroborates numerous literature reports indicating that factors such as rigidity and planar molecular structures can often enhance anticancer activity [4], [5], [7], [8], [12], [13], [17].

CONCLUSION

Fundamental Finding : In conclusion, this work contributes to the expanding body of knowledge regarding Schiff-base polymeric materials as potential anticancer agents. Polymers P1 and P2 serve as illustrative examples of how relatively simple modifications in monomer selection can significantly influence both the physical properties and the biological outcomes of the resulting materials. **Implication :** While their moderate potency suggests they are not immediately comparable to established standard chemotherapy drugs, they provide a valuable foundation for the future development of more effective derivatives. The findings also underscore the critical importance of conducting thorough characterization – effectively linking data from FTIR and thermal analyses to the biological results provided a coherent and rational explanation for the observed behavior of each distinct polymer**. Limitation :** While promising, the current study is limited by its in vitro scope and moderate efficacy, highlighting the need for further refinement before clinical relevance can be asserted. **Future Research :** With further optimization efforts and more in-depth biological investigations (including elucidation of the precise mechanism of action and testing against additional cell lines or potentially in vivo models), Schiff-base polymers analogous to P1 and P2 could potentially be advanced towards future therapeutic or drug delivery applications within the field of oncology.

REFERENCES

[1] S. Baluja, A. Solanki, and N. Kachhadia, “Evaluation of Biological Activities of Some Schiff Bases and Metal Complexes,” *J. Iran. Chem. Soc.*, vol. 3, no. 4, pp. 312–317, 2006.

[2] S. M. Sondhi, M. Dinodia, S. Jain, and A. Kumar, “Synthesis of Biologically Active Novel Bis Schiff Bases, Bis Hydrazone and Bis Guanidine Derivatives,” *Indian J. Chem. B*, vol. 48, no. 8, pp. 1128–1135, 2009.

[3] C. X. Zhang and S. J. Lippard, “New Metal Complexes as Potential Therapeutics,” *Curr. Opin. Chem. Biol.*, vol. 7, no. 4, pp. 481–489, 2003.

[4] M. M. Grafaa and H. M. Ali, “Synthesis, Characterization and Analytical Studies of Three Newly Schiff Bases as a New Anti-Human Breast Cancer (MCF-7),” *Biomed. Chem. Sci.*, vol. 1, no. 3, pp. 168–177, 2022.

[5] S. Banerjee and A. R. Chakravarty, “Metal Complexes of Curcumin for Cellular Imaging,

Targeting, and Photoinduced Anticancer Activity," *Acc. Chem. Res.*, vol. 48, no. 7, pp. 2075–2083, 2015.

[6] A. De Vizcaya-Ruiz, A. Rivero-Müller, L. Ruiz-Ramírez, and others, "Induction of Apoptosis by a Novel Copper-Based Anticancer Compound, Casiopeina II, in Murine Leukemia and Human Ovarian Carcinoma Cells," *Toxicol. Vitr.*, vol. 14, no. 1, pp. 1–5, 2000.

[7] N. Coen and others, "Schiff Base Clubbed Benzothiazole: Synthesis, Potent Antimicrobial and MCF-7 Anticancer Activity, DNA Cleavage and Computational Study," *J. Saudi Chem. Soc.*, vol. 21, no. 8, pp. 920–929, 2017.

[8] A. F. Al-Maliki, "Synthesis and Thermal Evaluation of Schiff Base Polymers Derived from Aromatic Diamines," University of Basrah, Basrah, Iraq, 2021.

[9] M. K. Mohammed, Z. Al-Shuhaib, and A. A. A. Al-Shawi, "Polymeric Schiff Bases: Synthesis and Applications," *Basrah Univ. J. Appl. Sci.*, vol. 10, no. 2, pp. 117–125, 2022.

[10] R. M. Okasha and others, "A Novel Avenue in the Successful Synthesis of Schiff Base Macromolecules via Innovative Plasma and Classical Approaches," *Sci. Rep.*, vol. 15, p. 10840, 2025.

[11] A. N. Abulhail and M. Y. Kadhum, "Thermoanalytical Study of Aromatic Schiff Base Polymers," *Basrah J. Chem. Sci.*, vol. 18, no. 3, pp. 95–102, 2021.

[12] M. Gaber, N. El-Wakiel, and O. M. Hemeda, "Cr(III), Mn(II), Co(II), Ni(II) and Cu(II) Complexes of a Benzimidazolyl-Diazenyl Schiff Base: Synthesis, Spectral, Thermal, and Biological Activity," *J. Mol. Struct.*, vol. 1180, pp. 318–329, 2019.

[13] M. Aldabbagh, B. F. Abdallaha, N. Y. Abdulla, and I. J. Ibraheem, "Cytotoxic Effects of Newly Synthesized Schiff Base Polymers on Breast Cancer Cell Lines," *Basrah J. Pharm. Res.*, vol. 12, no. 4, pp. 67–75, 2021.

[14] T. Mosmann, "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays," *J. Immunol. Methods*, vol. 65, no. 1–2, pp. 55–63, 1983.

[15] M. C. Alley and others, "Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay," *Cancer Res.*, vol. 48, no. 3, pp. 589–601, 1988.

[16] University of Basrah, College of Education for Pure Sciences, "Coordination Polymers Based on Schiff Base Frameworks," 2020.

[17] A. M. Ibrahim and T. K. Shabeer, "Molecular Docking Insights of Newly Synthesized Schiff Base Monomers and Evaluating the Anticancer Activity of Their Polymers," *Ann. Natl. Acad. Med. Sci.*, vol. 59, no. 4, pp. 219–224, 2023.

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