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Research Article

Cytotoxic Anti-Cancer Activity of Certain Thermotropic Liquid Crystalline Poly (Ester-Amides) Containing 2, 6-Bis (Benzylidene)Cyclohexanone Moiety in the Main Chain

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ABSTRACT

Five thermotropic liquid crystalline poly(ester-amides) were synthesized by polycondensation method. The poly(ester-amides) were synthesized from varying dicarboxylic acids with a common diamine namely 4,4'-diaminobenzene and a common diol namely 2,6-bis(4-hydroxybenzylidene)cyclohexanone. For qualitative characterization, viscosity measurements and solubility data were used for these synthesized poly(ester-amides). The spectroscopic techniques such as FT-IR, ¹H NMR, ¹³C NMR were performed to investigate the microstructural features of these synthesized poly(ester-amides). The thermal phase transition behavior of these poly(ester-amides) were studied by Differential Scanning Calorimetry (DSC) and Hot-stage Optical Polarized Microscopy (HOPM). The degree of crystallinity was assessed by X-ray diffraction (XRD) patterns. Scanning Electron Microscopic (SEM) technique was used to illustrate the morphology of these poly(ester-amides). The copolymer synthesized was subjected into *in vitro* anti-cancer activity studies against human breast cancer (MCF-7) cell line.

Keywords: Bisbenzylidenecyclohexanone; poly(ester-amides); polycondensation; thermotropic liquid crystalline properties; cytotoxicity, anticancer.

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1. INTRODUCTION

The thermotropic liquid crystalline poly(ester-amides) (TLCPs) incorporating flexible methylene groups, aromatic groups, and arylidene-keto moiety were reported.¹⁻³ The bisbenzylidene cyclohexanone has both mesogenic and photo-active properties. They were found to be a potential mesogen that imparted thermotropic liquid crystalline property to the polymeric materials.⁴ Phase transitions of liquid crystals were reported by Marin et al.⁵ by using Hot-stage Optical Polarized Microscopy (HOPM), Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD) studies. The poly(ester-amides) containing 2,5-bis(benzylidene) cyclopentanone moiety were reported by Kannappan et al.⁶ Suhas Thatte and co workers,⁷ discussed the new and improved drug delivery methodology in the several polymer-drug systems. The first drug delivery application is reported using hydrogel in 1960.⁸ They found that these compounds were cytotoxic to a number of human

tumours *in vitro*, particularly towards colon cancer and leukemic cells.

Keeping in mind all these facts, we thought it is interesting to synthesize a series of five thermotropic liquid crystalline poly(ester-amides) containing bisbenzylidenecyclohexanone moiety in the polymer backbone. In the current work, poly(ester-amides) are a category of polymeric materials which contains both ester and amide linkages and are synthesized by the copolymerization of a diacid with that of a diamine and a diol.

2. EXPERIMENTAL

2.1 Chemicals

Aldrich samples of diphenyl chlorophosphate, terephthalic acid, isophthalic acid, phthalic acid, adipic acid, azelaic acid, and 4,4'-diaminobenzene were used as received. Lithium chloride (SD Fine), vanillin (CDH), and cyclohexanone (Fluka) were used as received. Merck sample of pyridine, Merck LR sample of methanol, SD-Fine AR samples of N,N-

dimethyl acetamide (DMAc) and N,N-dimethyl formamide (DMF), and Aldrich spectral grade DMSO-d₆ were used.

2.2 Preparation of monomer

2.2.1 Synthesis of arylidene-keto diol: The arylidene-keto diol namely 2,6-bis(4-hydroxybenzylidene)cyclohexanone (CHBB) was synthesized by the method reported in literature.⁷

Preparation of 2,6-bis(4-hydroxybenzylidene)cyclohexanone (CHBB): In a 250 mL conical flask, a

solution containing p-hydroxy benzaldehyde (0.2 mol) in 100 mL of dry methanol was taken. To this solution, cyclohexanone (0.1 mol) was added drop wise and the mixture was shaken well. Then with external cooling in an ice bath, fuming sulphuric acid (5 mL) was added drop wise so that an exothermic reaction took place. The reaction mixture turned bright yellow initially, then dark green and finally pink. The precipitated diol was filtered and washed with aqueous methanol. It was filtered and recrystallised from aqueous methanol. Yield: 95% (m.p.: 195 °C). It is represented in Scheme 1.

Scheme 1. Synthesis of 2,6-bis(4-hydroxybenzylidene)cyclohexanone (CHBB).

2.2.2 Synthesis of poly(ester-amides): Two methods were employed to synthesize the poly(ester-amides) which depends upon the type of dicarboxylic acid monomer (aromatic or aliphatic).

Method 1: The procedure for the synthesis of a typical poly(ester amide) derived from aromatic dicarboxylic acid is represented here.⁹ Three poly(ester-amides) were prepared by the direct polycondensation method using diphenyl chlorophosphate (DPCP) as the condensation agent in the mole ratio 1:1:2 of a diamine, a diol and a diacid in pyridine solution. This method avoids the tedious preparation of acid chloride and hence, yield of the polymer is high.

In a 100 mL round-bottomed flask, a solution containing aromatic dicarboxylic acid (5 mmol), DPCP (12 mmol), and LiCl (10 mmol) in pyridine was continuously stirred at room temperature for 30 minutes. Then the temperature was raised to 115°C and 4,4'-diaminodiphenyl (2.5 mmol) and the diol (2.5 mmol) were added and the stirring was continued for 3 hours. The reaction mixture was cooled and poured into methanol. The poly(ester-amide) was precipitated, filtered and washed with methanol and dried in vacuum. It is represented in Scheme 2.

Scheme 2. Synthesis of poly(ester-amides) PBBI (I), PBBI (II) and PBBI (III) derived from aromatic dicarboxylic acid.

Method 2: The procedure for the synthesis of a typical aliphatic diacid-based polymer is given here. Two aliphatic diacid-based poly(ester-amides) were prepared by the

polycondensation method in the mole ratio 1:1:2 of a diamine, a diol and a diacid in DMF solution.¹⁰ It is represented in Scheme 3.

Scheme 3. Synthesis of poly(ester-amides) PBBA (IV) and PBBAz (V) derived from aliphatic diacid chloride

In a 100 mL round-bottomed flask, a solution containing 4,4'-diaminodiphenyl (2.5 mmol), diol (2.5 mmol) and DMF (30 ml) were taken. Then, diacid chloride (5 mmol) was added dropwise at 100°C. At nitrogen atmosphere, the reaction mixture was heated at 115°C for 8 hours with constant stirring. The contents were cooled and poured into methanol for precipitation and kept in a refrigerator

overnight and then filtered. The poly(ester-amide) was dissolved in acetone, filtered and poured into water for precipitation and was dried in vacuum over phosphorus pentoxide. It is represented in Scheme 3.

In Table 1, the various poly(ester-amides) prepared by these methods were listed along with the polymer code, percentage of yield and inherent viscosities.

Table 1. List of monomers used, polymer code of poly(ester-amides) with percentage of yield and inherent viscosities (η_{inh})

S. No.	Common diamine: 4,4'-diamino benzene		Polymer Code	Yield (%)	η_{inh} (dL/g)
	Diol	Diacid / Diacid chloride			
1.	CHBB	Terephthalic acid	PBBT - I	73	1.05
2.	CHBB	Isophthalic acid	PBBI - II	70	0.98
3.	CHBB	Phthalic acid	PBBP - III	69	0.95
4.	CHBB	Adipoyl dichloride	PBBA - IV	55	0.45
5.	CHBB	Azelaoyl dichloride	PBBAz - V	58	0.53

CHBB: 2,6-(bis(4-hydroxybenzylidene))cyclohexanone

2.3 Characterization methods

2.3.1 Solubility: In a small stoppered test tube, about 50 mg of the polymer containing 1 mL of the solvent was taken and kept for 24 hours with occasional shaking. In various solvents, the solubility was tested qualitatively.

2.3.2 Viscosity: The inherent viscosity (η_{inh}) was determined in DMAc solution by using Ubbelohde viscometer in which the pure solvent had a flow rate of 470 seconds at 30°C. In each case, dry poly(ester-amide) sample (25 mg) was dissolved in 25 mL of DMAc and kept aside for 12 hours with occasional shaking. From the flow time measurement at 30°C, the η_{inh} was calculated.

2.3.3 Fourier Transform Infrared (FT-IR) spectroscopy: Shimadzu FT-IR instrument was used to record the FT-IR spectra. The sample was taken in the form of potassium bromide pellet.

2.3.4 ¹H and ¹³C NMR Spectra: Bruker AVANCE III 500 MHz instrument was used to record the ¹H and ¹³C NMR spectra. DMSO-d₆ was used as solvent with TMS as internal reference.

2.3.5 Differential Scanning Calorimetry (DSC): NETZSCH DSC 200F3 differential scanning calorimeter using 5mg samples under nitrogen atmosphere was used for thermal analysis of these poly(ester-amides) at a heating rate of 10°C/min.

2.3.6 Hot-stage Optical Polarized Microscopy (HOPM): The polarized optical microscopic technique was used to identify the mesophases using Olympus BX51P model with a source of 100W halogen lamp housing by using Linksys 32 software for Hot Stage temperature controller.

2.3.7 X-Ray Diffraction (XRD): GE-Inspection Technology Diffractometer System XRD 3003 TT model made in Germany with a source of copper target operating voltage 40 Kv 300 mA° current rate was used to record X-ray diffraction

Figure 2(a,b). ^1H NMR spectrum of random poly(ester-amides) (a) PBBT and (b) PBBA.

In the ^{13}C NMR spectra of the poly(ester-amides), the signal in the range of $\square$ 188–200 ppm is due to the carbonyl carbon of the $\square\square\square$ -unsaturated ketone. Due to the carbonyl carbon of the amide and ester groups, the signals in the range of $\square$ 171–

185 ppm and $\square$ 160–170 ppm were appeared which indicates the formation of poly(ester-amide).⁶ They are represented in Figures 3(a) and 3(b) for PBBT and PBDA, respectively.

Figure 3(a,b). ^{13}C NMR spectrum of random poly(ester-amides) (a) PBBT and (b) PBBA.

3.4 Thermal analysis and phase behavior

By using Differential Scanning Calorimetry (DSC) and Hot-stage Optical Polarized Microscopy (HOPM), the thermal and phase behaviors of the five poly(ester-amides) were investigated.

3.4.1 Differential Scanning Calorimetry (DSC): In Table 3, the glass transition temperature (T_g), crystal to crystal transition ($T_{K1 \rightarrow K2}$), melting temperature (T_m), isotropization temperature (T_i), and liquid crystalline range (ΔT) obtained from DSC are tabulated.

Table 3. Differential Scanning Calorimetry (DSC) data of poly(ester-amides)

S.No.	Polymers	Differential Scanning Calorimetry (DSC)				
		T_g ($^{\circ}\text{C}$)	$T_{K \rightarrow K}$ ($^{\circ}\text{C}$)	T_m ($^{\circ}\text{C}$)	T_i ($^{\circ}\text{C}$)	ΔT
1	PBBT	59.31	189.34	369.44	536	166.56
2	PBBI	52.73	188.49	360.78	521	160.22
3	PBBP	49.34	187.98	351	509	158
4	PBBA	45.45	179.87	340.21	493.21	153
5	PBBAz	37.868	180.898	333.868	430.86	96.992

In Figure 4, the DSC thermograms of all poly(ester-amides) are shown. By analyzing the DSC data, we could interpret that the poly(ester-amides) II and III synthesized from aliphatic dicarboxylic acids have lower glass transition temperature (T_g) values than those synthesized from aromatic diacid chloride monomers. In these polymers, an exothermic peak appears before melting transition, which seems to be related to crystal to crystal transition resulted from different crystalline polymorphs.¹⁴ The liquid crystalline range of the poly(ester-amide) derived from terephthalic acid monomer is broader than compared to the poly(ester-amide) derived from other monomers. This may be due to effective molecular packing by coplanar geometry. The other monomers molecular packing may be due to the rigidity of o-phenylene or m-phenylene moiety in the polymer chain.

3.4.2 Hot-stage Optical Polarized Microscopy (HOPM): Mesophase identification of poly(ester-amides) have been achieved by Hot-stage Polarized Optical Microscopy (HOPM). From Figures 5 to 9, the different textures of poly(ester-amides) have been identified at different temperatures. The poly(ester-amides) exhibited high birefringence when it analyzed with a HOPM.¹⁵ In Figure 5(a), crystalline phases of the mesogens has been shown. By increasing the temperature, crystal to crystal transition was observed. They are shown in Figure 5(b). This on further heating, a crystalline to nematic transition of the mesogens takes place. They are shown in Figure 5(c). On further heating of these poly(ester-amides), the nematic phase lost its birefringence and transformed to an isotropic phase where there is no texture identification i.e. it appears as black domain.

Figure 4. DSC thermograms of random poly(ester-amides).

Figure 5(a-c). Hot-stage optical polarized micrographs for (a) PBBA at 25°C, crystalline phase of the mesogens (b) PBBA at 135°C, crystal to crystal transition, and (c) PBBA at 267°C, crystalline to nematic transition.

3.5 X-Ray Diffraction studies

In Figures 10(a) and 10(b), the X-Ray diffraction pattern of poly(ester-amides) PBDT and PBDA has been shown that indicates the semi-crystallinity of these polymers with an

amorphous background which may be due to the presence of carbonyl and C=C groups. In the region $2\theta = 10 - 50^\circ$, these poly(ester-amides) showed few reflection peaks that confirms the presence of semi-crystallinity.^{9,10}

Figure 10(a,b). X-ray diffraction pattern of random poly(ester-amides) (a) PBDT and (b) PBDA.

3.6. Morphological study by SEM

In Figure 11, the SEM images of the poly(ester-amides) have been shown that illustrates the morphology of these poly(ester-amides).

Figure 11(a-e). Scanning electron micrographs of random poly(ester-amides) (a) PBBT, (b) PBBI, (c) PBBP, (d) PBBA, and (e) PBBAz.

3.7 Cytotoxic Anticancer Evaluation of Synthesized Polymer

Viable cells were determined by the absorbance. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The anti cancer activity and anti bacterial activity have been studied by Chitra and Roop Singh.¹⁶ The absorbance was measured with a UV-Spectrophotometer using wells without sample containing

cells as blanks. The effect of the polymer on the proliferation of MCF-7 was expressed as the % cell viability. In Figure 12, a graphical representation of the polymer effect on cancer cells by % cell viability is shown. The affected MCF-7¹⁷ cell line at different concentration was shown in Figure 13. IC of the polymer was determined and was shown in Table 4. The study on anti-cancer activity containing arylidene ketone moiety was studied by Rajam and Roop Singh.¹⁸⁻²⁰

Table 4. MCF7 Cell line for PBBP

S.No	Concentration $\mu\text{g/ml}$	Absorbance 540nm	% cell Viability
1	100	0.00	0.0
2	50	0.00	0.0
3	25	0.00	0.0
4	12.5	0.17	15.0
5	6.25	0.30	26.5
6	3.12	0.57	50.4
7	1.56	0.81	71.6
8	Control Cells	1.13	100

Figure 12. A graphical representation of PBBP polymer effect on cancer cells by % cell viability

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Figure 13. The affected MCF-7 cell line PBBP at different concentration

Table 5. MCF7 Cell line for PBBA

S.No	Concentration $\mu\text{g/ml}$	Absorbance 540nm	% cell Viability
1	100	0.00	0.0
2	50	0.00	0.0
3	25	0.00	0.0
4	12.5	0.09	7.9
5	6.25	0.20	17.6
6	3.12	0.43	38.0
7	1.56	0.79	69.9
8	Control Cells	1.13	100

Figure 14. A graphical representation of PBBA polymer effect on cancer cells by % cell viability

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Figure 15. The affected MCF-7 cell line at PBBA different concentration

Table 5. MCF7 Cell line for PBBI

S.No	Concentration $\mu\text{g/ml}$	Absorbance 540nm	% cell Viability
1	100	0.00	0.0
2	50	0.00	0.0
3	25	0.00	0.0
4	12.5	0.09	7.9
5	6.25	0.21	18.5
6	3.12	0.40	35.3
7	1.56	0.78	69.0
8	Control Cells	1.13	100

Figure 16. A graphical representation of PBBI polymer effect on cancer cells by % cell viability

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Figure 17. The affected MCF-7 cell line at PBBI different concentration
IC₅₀ Value, MCF7

PBBP= 3.12µg; PBBA=2.496 µg; PBBI= 2.496 µg

By comparing MCF7 IC₅₀ values, PBBP has the highest MCF7 IC₅₀ values

4. CONCLUSION

A series of five poly(ester-amides) have been synthesized successfully by direct polycondensation method. Solubility studies reveal that these poly(ester-amides) are highly soluble in polar organic solvents and the viscosity studies reveal that these are of high molecular weight. These are characterized by FT-IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ spectral studies that supports the structural assignments. From the thermal studies such as DSC and HOPM, the phase transition and texture behavior of these poly(ester-amides) were identified. The degree of crystallinity were confirmed by X-ray diffractograms. The morphology of these five poly(ester-amides) are illustrated by SEM. Because of their high thermal stability as thermotropic liquid crystals, they might be utilized for flame retardant applications and optical device technology. Some of the synthesized poly(ester-amides) tested for anti-cancer activity against human breast cancer MTC7 cells using MTT assay showed an excellent anti-cancer activity against human breast cancer cells.

REFERENCES

1. Ling A-L, Hsu K-Y, Li C-H and Chang T-C, Studies on thermotropic liquid crystalline polymers: Synthesis and properties of poly(ether ester amide)s, *Polymer*, 1992; 33(10):2217-2221.
2. Aharoni SH, Hydrogen-bonded highly regular strictly alternating aliphatic-aromatic liquid crystalline poly(ester amides), *Macromolecules*, 1988; 21(7):1941-1961.
3. Chen W-H, Chang T-C and Higashi F, Liquid-crystalline copoly(ester amide)s prepared from hydroxybenzoic and 4-aminobenzoic acids, *Journal of Polymer Science Part A: Polymer Chemistry*, 1988; 26(12):3269-3274.
4. Murali M and Samui AB, Bisbenzylidene cycloalkanone: a versatile molecule as a polymer building block, *Journal of Materials Chemistry*, 2010; 20(14):2714-2737.
5. Marin L, Damaceanu MD and Timpu D, New thermotropic liquid crystalline polyazomethines containing luminescent mesogens, *Soft Materials*, 2009; 7(1):1-20.
6. Kannapan V and Reuben Jonathan D, A study on the synthesis and bactericidal efficacy of certain poly(ester-amides) containing 2,5-bis(benzylidene)cyclopentanone moiety in the main chain, *Journal of Chemical and Pharmaceutical Research*, 2013; 5(4): 382-386.
7. Thatte S, Datar K and Ottenbrite RM, Perspectives on: polymeric drugs and drug delivery systems, *Journal of Bioactive and Compatible Polymers*, 2005; 20(6): 585-601.
8. Wichterle O. and Lim D, Hydrophilic gels for biological use, *Nature*, 1960; 185: 117-118
9. Kannapan V, Arumugasamy E, Ravichandran E and Baskar B, Synthesis and characterization of certain thermotropic liquid crystalline random copolyesters, *Journal of Polymer. Materials* 2000; 17: 4-9.
10. Arul Moli J, Vasanthi S, Prakash N and Roop Singh D, Synthesis and characterization of certain photocrosslinkable thermotropic liquid crystalline random copolyesters containing arylidene moiety, *High Performance Polymers*, 2012; 24: 507-520.
11. Rajakumar P, Ganesan K, Jayavelu S and Murugesan K, Synthesis and bactericidal efficacy of novel dendrimers, *Synthetic Letters*, 2005; 7: 1121-1124.
12. Perundevi TS, Reuben Jonathan D and Kothai S, Synthesis and studies on non-linear optical property of new chalcone based polymers, *Chemical Science Transactions*, 2017; 6(2): 199-208.
13. Khairou KS, Abdullah MA, Aly KI, Nahas NM and Al-Bonian AM, Synthesis and characterization of new poly(ester-amide)s containing diarylidenecyclohexanone in the main chain. Part: II *Arabian Journal of Chemistry*, 2009; 2: 65-71.
14. Mulani K, Momin M, Ganjave N and Chavan N, Thermotropic liquid crystalline polyesters derived from bis-(4-hydroxybenzoyloxy)-2-methyl-1,4-benzene and aliphatic dicarboxylic acid chlorides, *Bulletin of Materials Science*, 2015; 38(5): 1301-1308.
15. Qaddoura M A and Belfield K D, Synthesis, characterization and texture observations of calamitic liquid crystalline compounds, *International Journal of Molecular Science*, 2009; 10(11) 4772-4788.
16. Rajam C, Roop Singh D, Synthesis, characterization and studies on anticancer activity of certain polymers containing arylidene moiety in their main chain, *World Wide Journal of Multidisciplinary Research and Development*, 2017; 3(7):252-258.
17. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journals of Immunological Methods*, 1983; 65: 55-63.
18. Edlund U, Albertsson AC, Polyesters based on diacid monomers, *Advanced Drug Delivery Reviews*, 2003; 55(4):585-609.
19. Murase SK, Puiggali J, Poly(Ester Amide)s: Recent Developments on Synthesis and Applications, *Natural and Synthetic Biomedical Polymers* 2014; 145-166.
20. Chitra V and Roop Singh D, Anti-bacterial activity, anti-cancer activity and nanofiber formation of certain poly (ester amides) from 2,5-pyridine dicarboxylic acid, *Journal of Drug Delivery and Therapeutics*, 2018; 8(6-s):166-173.