Research Article

Synthesis and *In-Vitro* Evaluation of Novel Bischalcone Polymers as Potential Anticancer Agents

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ABSTRACT

Cancer is a group of diseases involving abnormal cell growth with the potential to invade other parts of the body and a threat to human life. As part of the ongoing efforts to purge cancer we were interested in evaluating the cytotoxic prospective of a series of polymers bearing bis-chalcone moiety. In this study a series of polymers have been synthesized using two varying aliphatic diacid chlorides, namely succinyl and glutaryl chloride, one aromatic diacid chloride, and two varying diols 3,3'-(1,4-phenylene) bis (1-(4-hydroxyphenyl)prop-2-en-1-one) (THAP) and 3,3'-(1,4-phenylene) bis (1-(4-hydroxy-3-methoxy phenyl)prop-2-en-1-one) (TMAP) by solution polycondensation. Acid catalyzed Claisen-Schmidt reaction was employed to synthesize the two varying diols. The copolyesters were characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectra. Thermal transitions were recorded from DSC thermograms. The synthesized copolyesters were evaluated for in vitro cytotoxic activity by method using MCF-7 cancer cell lines. *In-vitro* observations indicated that the polymers are potent anticancer agents. IC_{50} values of one of the copolyester was too low which depicts this study as a preliminary step towards understanding the cytotoxicity activity of polymers with bischalcone moiety in their backbone.

Keywords: Bischalcones, copolyesters, Cytotoxicity, MTT assay.

INTRODUCTION

Cancer has become one of the most difficult health challenges of current time, accounting for millions of death annually[1]. Cancer chemotherapy is not always successful. Difficulties in drug delivery to the tumor, drug toxicity to normal tissues and drug stability in the body add to this problem. The existing situation highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal antitumor potency.

Chalcones constitute one of the important classes of anticancer agents that have shown promising therapeutic efficacy in the management of human cancer[2]. Chalcone and its derivatives have attracted increasing attention due to numerous pharmacological applications[3]. The extremely simple skeleton of chalcone compared to the complex structures of most other anticancer drug candidates makes this scaffold very attractive to chemists for alteration and structure activity relationship (SAR) modifications[4]. Chalcones have displayed a wide array of pharmacological activities, among which antimalarial[5-8], anticancer[9-13], antiprotozoal [antileishmanial and antitrypanosonal][14], anti-inflammatory[15,16], antibacterial[17,18], antifilarial[19], antifungal[20,21], antimicrobial[22], larvicidal[23], anticonvulsant[24], antioxidant[25-27], activities have been reported.

Chalcones display chemopreventive and antitumor effects. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a naturally occurring anticancer agent that induces apoptosis in cancer cells and is nontoxic to normal cells. Szliszka[12], examined the cytotoxic and apoptotic effect of five chalcones in combination with TRAIL and prostrated cancer cells and evaluated the cytotoxicity by MTT and lactate dehydrogenase (LDH) assays. They showed for the first time that chalcones sensitize prostrate cancer cells to TRAIL–induced apoptosis. Studies have indicated that cytotoxicity of chalcones is related linearly to their ability to form phenoxy radicals by oxidation, as well as to their hydrophilicity[28].

Recent studies have thrown light on some aromatic chalcones which exhibit antitumor activity[29]. Recently Jayashree[30] explored the anticancer activity of newer synthetic chalcones. Furthermore Mielcke[31] have also studied the activity of chalcone derivatives on glioma cell proliferation.

Polymers have been extensively thought out in the improvement of localized delivery systems. Sustained drug delivery by biodegradable polymer devices can increase the therapeutic efficacy of drugs by producing high local tissue concentrations over extended periods of time[32]. Polymeric materials afford an alternate means for delivering chemotherapeutic agents.

When anticancer drugs are encapsulated in polymer they can be protected from degradation. Implanted polymeric pellets or injected microspheres localize therapy to specific anatomic sites, providing a continuous sustained release of anticancer drugs while minimizing systemic exposure. In certain cases polymeric microspheres delivered intravascularly can be targeted to specific organs or tumors[33]. Of late Gowsika[34] have studied cytotoxicity of biodegradable copolyesters.

In the present study it is proposed to synthesize polyester using bischalcone moiety which itself has good anticancer activity and drug release system.

MATERIALS AND METHODS

Materials

Terepthalaldehyde, 4-hydroxy acetophenone, 3-methoxy-4-hydroxy acetophenone, succinyl and glutaryl chloride were purchased from Sigma Aldrich.

Dimethyl acetamide (DMAc) used for evaluating the inherent viscosity of the copolyester in solution was purchased from SD-Fine (AR Grade). DMSO (d_6) of spectral grade was used as internal standard for recording NMR Spectra. Cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin 20µl (100U), and streptomycin (1000µg/ml) and amphotericin B (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C.

Instruments

Shimadzu FT-IR spectrometer was used to record the FTIR spectrum of the copolyesters in the range of 4000-400 cm^{-1} using KBr pellets. Bruker AV III 500MHz instrument was used to record ^{1}H-NMR and ^{13}C-NMR spectra. Inherent viscosity of the copolyester was established using Ubbelhode Viscometer and dimethyl acetamide was employed as the solvent. DSC thermograms were recorded on a DSC 200 F3 Maia instrument. Anticancer activity was picturized using Lobomed Inverted Microscope of 40x magnification.

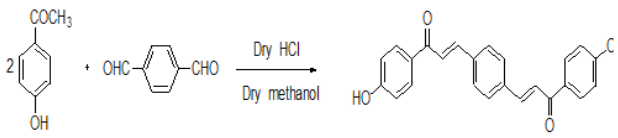
Synthesis of Chalcone Diol

The monomers 3,3'-(1,4-phenylene) bis(1-(4-hydroxy phenyl)prop-2-en-1-one) (THAP) and 3,3'-(1,4-phenylene) bis(1-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one) (TMAP) were synthesized by the process reported by Chitra and coworkers[35].

Preparation of THAP

A well cooled and stirred solution containing 4-hydroxyacetophenone (60mmol) and terephthaldehyde (30 mmol) in 50ml of dry methanol was exposed to dry HCl gas. Yellow crystals of THAP were collected. It was washed with double-distilled water and re-crystallized from hot methanol.

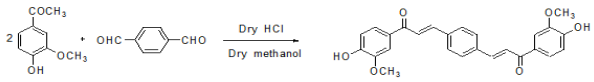
Yield: 90% m.p.: 262–264°C; FTIR(KBr) 3597 (b, O–H), 1652(s, C=O) cm-1; ^{1}H-NMR (DMSO-d6) δ 9.1 (s, 2H, –OH), δ 7.5–8.2 (m, 12H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH) and MS (EI) m/z 370 [M]+.



Preparation of TMAP

A well cooled and stirred solution containing 3-methoxy, 4-hydroxyacetophenone (60mmol) and terephthaldehyde (30mmol) in 50ml of dry methanol was exposed to dry HCl gas. Yellow crystals of THAP were collected. It was washed with double-distilled water and re-crystallized from hot methanol.

Yield: 85% m.p.: 239°C; FTIR(KBr) 3508 (b, O–H), 1642(s, C=O) cm^{-1}; ^{1}H NMR (DMSO-d6) δ 9.8 (s, 2H, –OH), δ 7.2–8.3 (m, 7H, aromatic), δ 6.7–6.9 (dd, 2H, –CH=CH–), δ 3.5 (s, 6H, $–OCH_3$) and MS (EI) m/z 430 [M]+.



Synthesis of Copolyesters

The procedure[36] for the synthesis of a typical diacid-chloride, diol based copolyester is given here.

The monomer THAP (1mmol.) and the diacid chlorides, isophthaloyl chloride and either succinyl or glutaryl chloride were dissolved in 15ml of DMF in a 100mL round-bottomed flask. After 5 minutes 1mL of triethylamine was added and stirred. The mixture was allowed to stir at room temperature for 15 minutes in inert atmosphere. Then the temperature was raised to 100°C and maintained at this temperature with continuous stirring for a span of 3 hours. At last the reaction mixture was poured into 100ml of methanol when the copolyester was precipitated. It was filtered, washed with dry methanol and dried in vacuum.

The diacid chlorides I, II and diol used and the copolyester code of the four copolyesters are represented in Table 1.

Anticancer Evaluation

The anticancer activity of samples on **VERO cells** were determined by the MTT assay[37]. Cells (1 × 10^5/well) were plated in 0.2ml of medium/well in 96-well plates. Incubate at 5% CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 48hrs at 5 % CO_2 incubator.

After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate-buffered saline solution was added.

After 4 hours incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm.

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically.

RESULTS

Solubility of all the copolyesters was determined in various solvents qualitatively and they were found to be readily soluble in aprotic polar solvents. The inherent viscosity (ηinh) of the polyesters was determined in DMAc solution using Ubbelohde viscometer at 30°C. The values ranged from 0.51 to 1.02 which indicates the polymers were of high molecular weight.

FT-IR spectra of the entire random copolyesters were recorded using Shimadzu FT-IR instrument. The ^{1}H and ^{13}C-NMR spectra were recorded with BRUKER AV III 500 MHz NMR instrument in DMSO-d6 solvent.

The cytotoxicity of the synthesized copolyesters were screened using the MTT assay in human Breast cancer cell line (MCF-7). All the four copolyesters exhibited potent anticancer activity. PGIM showed high cytoxicity in cell lines with IC50 concentrations lines.

DISCUSSION

Table 1: Monomer diols used and the copolyester code of the four copolyesters together with Percentage of yield and inherent viscosities (η_{inh})

Diacid chloride I: Isophthaloyl chloride		Copolyester code	Yield (%)	η_{inh} (dL/g)
Diol	Diacid chloride II			
THAP	Succinyl chloride	PSIH	78.2	1.0274
TMAP	Succinyl chloride	PSIM	74.7	0.6682
THAP	Glutaryl chloride	PGIH	80.3	0.5449
TMAP	Glutaryl chloride	PGIM	76.43	0.5102

Solubility

The copolyesters synthesized were subjected to solubility tests and the results indicated that they were readily soluble in high polar solvents like DMAc and dimethyl formamide, partially soluble in moderately polar solvents like tetrahydrofuran and acetone but thoroughly insoluble in least polar solvents like benzene and hexane.

Copolyesters with methoxy group in the benzene ring of the bischalcone moiety was found to be more soluble endorsing their ability to disrupt the macromolecular chain[38].

Viscosity Measurements

The inherent viscosity (η_{inh}) of the four copolyesters was estimated using an Ubbelohde viscometer in DMAc as solvent at room temperature.

The inherent viscosity values were found to be in the range of 0.51 to 1.072 gdL^{-1} and are presented in Table 1.

The data ensures that the copolyesters are rationally of high molecular weight.

Spectral Studies

FT-IR spectrum of the synthesized copolyesters are presented in Figure 1.

The copolyesters exhibited distinctive absorptions in the range of 1705cm^{-1} to 1742cm^{-1} due to ester C=O stretching frequency. Peaks observed at 1581cm^{-1} to1595cm^{-1} are assigned to trans olefinic double bonds.

The NMR spectra were recorded to recognize the structural units present in the copolyester chain.

The peak in the range of 7.3ppm to 8.6ppm is due to aromatic protons, while peaks in the range of 6.91ppm to 6.95ppm were due to vinyllic protons attached to the carbonyl carbon. The methoxy protons in the bischalcone moiety is indicated by a peak at 3.38ppm. Methylene protons were in the range of 2.4ppm - 2.6ppm.

Signals in the range of 192ppm and 142ppm in ^{13}C-NMR is due to the carbonyl carbon of the α,β-unsaturated ketone and ester groups, respectively, which indicates the formation of copolyester.

Anti-cancer evaluation of synthesized polymers

The cytotoxicity of the synthesized polymers were screened using the MTT assay in human Breast cancer cell line (MCF-7). The results are depicted in Table 2.

All the polymers showed cytotoxic activity. PGIM showed high cytotoxicity in cell lines with IC50 concentrations lines.

$$\% \text{ cell viability} = \frac{\text{A540 of treated cells}}{\text{A540 of control cells}} \times 100\%$$

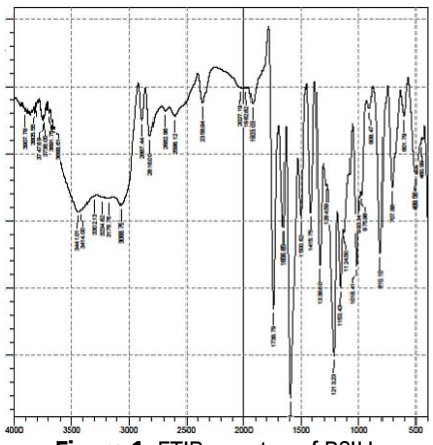


Figure 1: FTIR spectra of PSIH

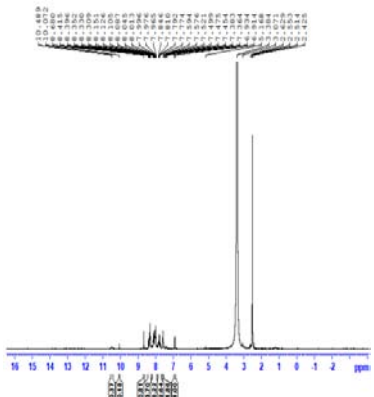


Figure 2: ^{1}H-NMR spectra of PSIH

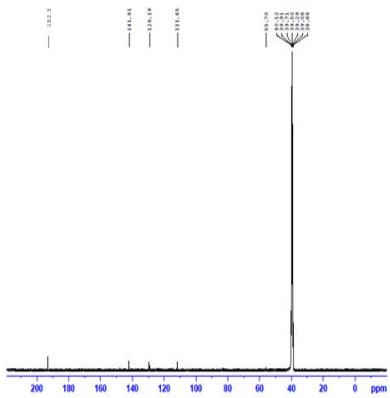


Figure 3: ^{13}C-NMR spectrum of PGIM

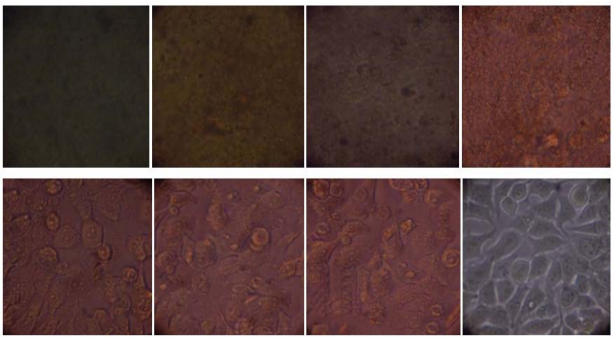


Figure 4: Representative images of MCF7 cells treated with PGIH at various concentrations (a)

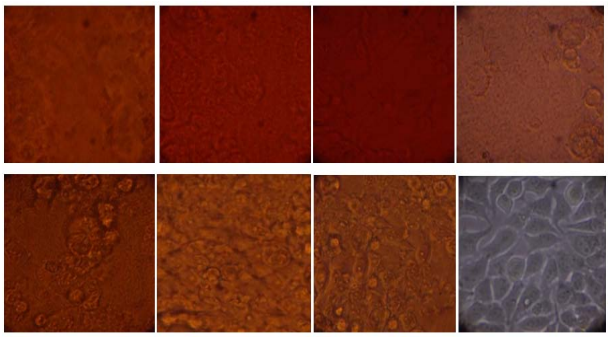


Figure 5: Representative images of MCF7 cells treated with PGIM at various concentrations

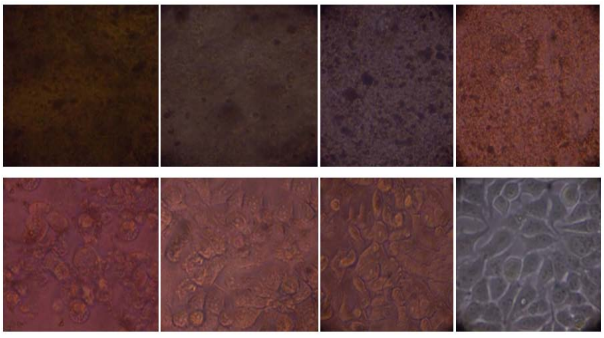


Figure 6: Representative images of MCF7 cells treated with PSIH at various concentrations

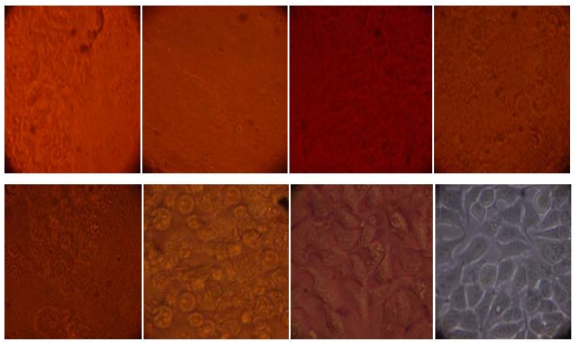


Figure 7: Representative images of MCF7 cells treated with PSIM at various concentrations

CONCLUSION

The study comprises of synthesis of four copolyesters using two varying diols THAP, TMAP, two aliphatic diacid chlorides namely succinyl and glutaryl chloride and one common aromatic diol isopthaloyl chloride by polycondensation method. The random copolyesters PSIH, PSIM, PGIH and PGIM were assayed for anticancer activity in human breast cancer cells. From the results it is clear that, the copolyester PGIM exhibits higher anticancer activity than the other compounds. The IC_{50} concentration of the polymer PGIM was also very low compared with other polymers, hence it can be used as a potent anticancer agent.

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