Design, Synthesis, Characterization of Chalcone Derivatives PTSH and PITI and Their Potential Anticancer Properties against MDA-MB-231 Cell Lines

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ABSTRACT

Chalcones (1,3-diaryl-2-propane-1-ones), biogenetic precursor of open chain flavonoids, which are highly multifunctional proven to have antitumor activity either in-vitro or in-vivo and their target cover all of the actions of tumour cells. Chalcone derivatives exhibit antitumor activity by anti-initiation, apoptosis induction, antiproliferation, antiangiogenesis and so forth. We synthesized six copolyesters and were characterized by UV, FT-IR, ¹H NMR, ¹³C NMR. Their thermal stability was studied by DSC and TGA. Cytotoxicity activity of these copolyesters was evaluated by studying their responses to MDA-MB-231 Cell Line (human Breast Cancer) by MTT Assay. UV, FT-IR, ¹H NMR, ¹³C NMR confirmed the insertion of correct functional groups. DSC and TGA showed that the synthesized compounds were of good thermoregulatory we. The drug, PTSH was effective at 10 µg/mL and 51 % inhibition was observed at 100 µg/mL. Most importantly, the drug, PITI was more significant at 0.1 µg/mL concentration itself and 58 % inhibition was noted at 1 µg/mL.

Keywords: Chalcone, MDA-MB-231 Cell Line, Breast Cancer, MTT Assay.

1. INTRODUCTION

Breast cancer is one of the major malignant tumor found in humans especially in women and they partially treated by radiotherapy, hormone therapy surgery and chemotherapy¹.
These therapeutic effects with current drugs were more effective \(^2\). The Taxane is showed a better treatment. Clinically, Taxol drug causes cell cycle arrest i.e G2/M phase and cease the mitosis \(^3\). This drug possesses the activity against head, breast, ovarian, nonsmall cell lung cancer\(^4\). Although taxol suggested being an act against these pathophysiology conditions it is coming under a category of multidrug resistance (MDR) immediately after its administration\(^5,6\).

The naturally occurring flavonoids and its isoforms are present in the edible plants such as fruits, vegetables, spices, tea and soy and derived an important product is a chalcone which is chemically an open chain flavanoid contains two aromatic rings joined by three-carbon \(\alpha,\beta\)-unsaturated carbonyl linkages having valuable pharmacological property \(^7\). Chalcones have been reported to have multifunctional biological activity against bacteria, fungus, virus, malarial parasites, inflammation and which result in cancer \(^8\)–\(^10\) all these living endanger species and pathogenesis kept under control by this small molecule. Substitutions of the allylic group in chalcones exhibit antioxidant and antimicrobial activity \(^11,12\). Additionally, these chalcones containing ring B substitute with hydroxyl and methoxy groups showed inhibition of proliferative activity in human colon HT-29 cancer \(^13\). This study is focused to synthesize six copolyesters by incorporating the Chalcone derivatives in the copolyester the main chain and then analyze for anticancer activity against human breast cancer cell lines.

2. MATERIALS AND METHODS

2.1. Chemistry

All chemicals were purchased from Merck and Fluka chemical companies. Melting points were taken in open capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer V IR spectrophotometer. \(^1\)H NMR and \(^13\)C NMR spectra were recorded CDCl\(_3\) on Bruker NMR spectrometers at 400 and 500, 100 and 125 MHz, respectively. All reactions were conducted open to the atmosphere and the yields refer to the isolated products. Aldrich samples of 4-hydroxy acetophenone and 3-ethoxy, 4-hydroxy benzaldehyde, 3-methoxy, 4-hydroxy acetophenone and were used as received. Ethanol (Merck) was used as non-solvent for the copolyesters and as a solvent for the preparation of the two chalcone diols. Aldrich samples of succinyl chloride, oxalyl chloride, glutaryl and is phthaloyl chloride were used as such in the synthesis of the five copolyesters. Spectral grade DMSO-d6 was used as internal standard for recording NMR spectra.

2.2. Synthesis of chalcone diols

The monomer diols namely (2E)-1-(4-hydroxyphenyl)-3(4-hydroxy, 3-ethoxy phenyl) prop-en-1-one and (2E)-1-(4-hydroxy-3-methoxy phenyl)-3(4-hydroxy, 3-ethoxy phenyl) prop-en-1-one were synthesized by the process reported \(^14\).

2.3. Synthesis of Copolyesters

The procedure for the synthesis of typical aliphatic diacid chloride-based copolyester is given here. The diol HHEP (1mmol) was dissolved in 10 mL of dry DMF and taken in a
100 mL round-bottomed flask. To this 0.2mL of succinyl chloride and 0.2 mL of oxalyl chloride were added with constant stirring and the temperature was maintained at 120ºC with continuous stirring for 3 hours. At the end, the reaction mixture was cooled to room temperature and poured into 50 mL of n-hexane and the copolyesters were precipitated. It was filtered reprecipitated with methanol and dried in vacuum. The other four copolyesters were also prepared by the similar method. The diols and the diacid chlorides used and the copolyester code of the four copolyesters are presented (Table 1).

2.4. Viscosity Studies

The η_{inh} value of all the five copolyesters was determined in DMAc solution at 30ºC using Ubbelohde viscometer. 25mg of each of pure dry copolyester sample was dissolved in 25mL of DMAc, kept aside for some time with occasional shaking. Then they were left undisturbed for 24 hours. The η_{inh} was calculated from the flow time measurements \(^\text{14}\). The inherent viscosity values were found to be in the range of 0.73–1.10dL/g and are presented in Table 1. The data shows that these copolyesters are reasonable of high molecular weight.

2.5. Solubility Studies

The copolyesters stated here are found to be soluble in highly polar solvents such as DMAc and dimethyl formamide, partially soluble in moderately polar solvents like tetrahydrofuran and acetone but thoroughly insoluble in the least polar solvents like benzene and hexane. Copolyesters with methoxy substituent in the benzene ring of the chalcone moiety had better solubility which may be attributed to their competence to disrupt the macromolecular chain.

2.6. Spectral Studies

The FT-IR spectrum of the six copolyesters was recorded using Shimadzu FT-IR instrument. The FT-IR spectrum of all the six copolyesters revealed characteristic absorption in the range of 1742–1764cm\(^{-1}\) due to ester C=O stretching frequency. The NMR spectra were recorded with BRUKER AV III 500 MHz NMR instrument in a DMSO-d6 solvent to categorize the repeating structural units present in the copolyester chain.

2.7. Thermal Studies (DSC and TGA)

The influence of polymer structure on the thermal properties of copolyester PSGH, PITI was investigated by thermogravimetric analysis (TGA) at a heating rate of 10ºC min\(^{-1}\) in a nitrogen atmosphere and the TGA curve. The glass transition temperature \(T_g\) and the melting temperature \(T_m\) was determined by DSC at a heating rate of 10ºC min\(^{-1}\) under nitrogen atmosphere and the DSC thermogram.

2.8. Cell culture conditions

The human breast cancer cell line, MDA-MB-231S were purchased from the National Centre for Cell Science (NCCS) Pune, India. The cells were cultured in high-glucose
Dulbecco’s modified Eagle’s medium (DME)

2.9. Cytotoxicity assay

The anti-proliferative effect of the drugs was tested for *in vitro* cytotoxicity analysis, using MDA-MB-231 cells by 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay\[^{[5]}\]. The cultured MDA-MB-231 cells were harvested by trypsinization. Then, the cells were plated at a density of 1×10^5 cells/mL (50 µL) into 96-well microtitre plates. Briefly, 180 µL of media (DME-High Glucose) was added into each of the 96-well plates and 20 µL of diluted synthetic drugs with different concentration ranging from 0.1, 1, 10 and 100 µg/mL were added in the wells with positive and negative controls for comparison of results with test samples.

Each sample was triplicated and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 48 h. After the incubation period, MTT (50 µL of 5 mg/mL) was added to each well and the cells incubated for 4 h until purple precipitates were clearly visible. The medium was removed completely and the formazan crystals were dissolved in DMSO. The optical density was measured using an ELISA reader (Molecular Devices, Spectra Max 190, USA) at 570 nm and the percentage cell viability was calculated manually using the following formula:

\[
\text{% Cell Viability} = \frac{(A_{570 \text{ sample}} - A_{570 \text{ blank}})}{(A_{570 \text{ control}} - A_{570 \text{ blank}})} \times 100.
\]

The calculation of concentrations that inhibits 50% of the (IC\(_{50}\)) MDA-MB-231 cells was formulated in the graph.

2.10. Evaluation of cell morphological changes

The morphological changes in the cells were observed under the inverted fluorescent microscope (Nikon Te200, USA).

2.11. Statistical analysis

Each experiment was done in triplicates and the values are mean of three independent experiments. Mean ± S.D. p < 0.05 was considered to indicate a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Chalcone diols

Chalcone diol BIMP was synthesized by acid catalyzed Claisen-Schmidt reaction are shown in Schema 1. The bright brown colored solid got precipitated and the yield was 76%. The melting point was determined the open capillary method and was corrected. The melting
point was about 239° C. The IR peak at 1642 cm\(^{-1}\) indicates the presence of C=O (stretching) of ketoethylinic group. The \(^1\)H NMR spectrum of BIMP shows at δ 6.9 and 7.1 was due to protons of -CH=CH- of ketoethylinic group and δ 7 to 7.2 ppm is due to an aromatic proton, δ 4 ppm is due to the proton of –OCH\(_3\) and δ 9.9 ppm was due to proton –OH group. The spectral study was in agreement with the structural orientation of chalcone diol BIMP.

Schema 1. (A) synthesis of (2E)-1-(benzyl)-3-(3-hydroxy-4-methoxy phenyl)- prop-2-ene-1-one.[BIMP] (B)(C) Synthesis of Copolyesters PTSH and PITI.
3.2. Viscosity Studies

The increase in the value of Inherent Viscosity will increase the molecular weight of the copolyesters. Among the six copolyesters, PITI was found to be having highest inherent viscosity because of the rigidity of the aromatic in the polymer backbone as both the acid chlorides used were aromatic in nature. This data supports that these copolyesters are reasonable high molecular weight are shown in Tab.1.

Table 1. Monomers diols used for six copolyesters synthesis with code and the percentage of yield and inherent viscosities ($\eta_{inh}$).

<table>
<thead>
<tr>
<th>Diol</th>
<th>Diacid chloride-I</th>
<th>Diacid chloride-II</th>
<th>Copolyester Code</th>
<th>Percentage Yield</th>
<th>Inherent Viscosity (dL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHEP</td>
<td>Succinyl Chloride</td>
<td>Glutaryl Chloride</td>
<td>PSGH</td>
<td>81</td>
<td>1.1800</td>
</tr>
<tr>
<td>HIMP</td>
<td>Terephthaloyl Chloride</td>
<td>Oxalyl Chloride</td>
<td>PTOI</td>
<td>79</td>
<td>1.0179</td>
</tr>
<tr>
<td>MHEP</td>
<td>Isophthaloyl Chloride</td>
<td>Oxalyl Chloride</td>
<td>PIOM</td>
<td>87</td>
<td>1.1728</td>
</tr>
<tr>
<td>HHEP</td>
<td>Isophthaloyl Chloride</td>
<td>Glutaryl Chloride</td>
<td>PIGH</td>
<td>79</td>
<td>1.5602</td>
</tr>
<tr>
<td>HHEP</td>
<td>Terephthaloyl Chloride</td>
<td>Succinyl Chloride</td>
<td>PTSH</td>
<td>86</td>
<td>1.2321</td>
</tr>
<tr>
<td>HIMP</td>
<td>Isophthaloyl Chloride</td>
<td>Terephthaloyl Chloride</td>
<td>PITI</td>
<td>80</td>
<td>1.9081</td>
</tr>
</tbody>
</table>

3.3. Solubility Studies

The solubility of the copolyesters was tested qualitatively by dissolving 5 mg of the copolyesters in 1 mL of the solvent. The copolyesters are easily soluble in a polar aprotic solvent such as DMSO, DMAc, and DMF and they are insoluble in nonpolar solvents benzene, n-hexane, and toluene are shown in Table 2. The copolyesters are soluble in polar solvents like methanol upon warming may be due to intermolecular interaction for solvents with ester linkage of the polymer.

Table 2. Determination of Solubility of six copolyesters with different solvent system

<table>
<thead>
<tr>
<th>S.No</th>
<th>Polymer Code</th>
<th>DMAc</th>
<th>DMF</th>
<th>DMSO</th>
<th>CH$_3$OH</th>
<th>C$_6$H$_6$</th>
<th>n-hexane</th>
<th>H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PSGH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>PTOI</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3.</td>
<td>PIOM</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>PIGH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>5.</td>
<td>PTSH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6.</td>
<td>PITI</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

++ = Soluble        ++ = Soluble on warming       -- = Insoluble
Fig. 1. UV-Visible spectrum for (A) PTSH and (B) PITI

Fig. 2. FT-IR spectrum (A) PTSH and (B) PITI complex
3.4 Spectral Studies
3.4.1. UV – Vis Spectrum

The absorption peak at wavelength range 230-390 nm of PTSH and PITI showed the chalcone is incorporated in a polymer backbone. This is due to \( n \rightarrow \pi^* \), \( \pi \rightarrow \pi^* \) transition and also due to the C=O group of the molecule and also due to the excitation of the C=O group of the molecule shown in Fig.1.

3.4.2. FT-IR Spectrum

The FT-IR Spectrum of the six copolyesters namely PIGH, PIOM, PTOI, PSGH, PTSH, PITI were recorded by using Shimadzu FT-IR instrument. The IR Spectra of the copolyesters of PTSH and PITI were characterised by strong absorption band 1709-1759 cm\(^{-1}\) and 1583-1598 cm\(^{-1}\) corresponding to ester and ketone carbonyl (C=O) stretching respectively. The strong absorptions at 1679-1695 cm\(^{-1}\) were due to olefinic group (CH=CH) of chalcone unit are shown in Fig.2 A & B.

3.4.3 NMR Spectrum

The \(^1\)H and \(^{13}\)C NMR spectra of PTSH signals in the range of 170–205ppm and 165–175ppm in the \(^{13}\)C NMR spectra of the co-polyesters are due to the carbonyl carbon of the \( \alpha, \beta \)-unsaturated ketone and ester groups respectively, which indicates the formation of the copolyester as shown in Fig.3,4.

\(^1\)H and \(^{13}\)C NMR spectra of (DMSO-d6 solutions) of PITI are displayed in Fig. 5,6. The aromatic protons of PITI are observed in the range of 7.2–8.1 ppm. The vinylic protons attached to the carbonyl carbon are observed in the range of 6.7–6.9 ppm. The methoxy protons in the chalcone moiety are represented in the range of 3.1–3.4 ppm. The methylene protons are observed in the range of 1.3–3.3 ppm with an expected integral value.

![Fig. 3. \(^1\)H NMR spectra (DMSO-d6 solutions) of PTSH with the assignment of the detected signals (see Figure for the atom-numbering scheme).](image-url)
Fig. 4. $^{13}$C NMR spectra (DMSO-d6 solutions) of PTSH with the assignment of the detected signals (see Figure for the atom-numbering scheme).

Fig. 5. $^1$H NMR spectra (DMSO-d6 solutions) of PITI with the assignment of the detected signals (see Figure for the atom-numbering scheme).
3.5. Thermal Stability

3.5.1. Differential Scanning Calorimetry (DSC)

The endothermic peak at 99°C, 103.57°C for PTSH and PITI respectively. They indicate good thermal stability required for their biomedical application. Table 3. Fig. 7 (A),(B).
Table. 3. Both copolyestes PTSH and PITI having transitional temperature with melting temperature confirming their stability.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Copolyesters code</th>
<th>Glass transition temperature $T_g$</th>
<th>Melting temperature $T_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PTSH</td>
<td>52°C</td>
<td>138.19</td>
</tr>
<tr>
<td>2.</td>
<td>PITI</td>
<td>50°C</td>
<td>103.57</td>
</tr>
</tbody>
</table>

3.5.2. Thermo Gravimetric Analysis (TGA)

The temperature for the 50% weight loss was found to be 325°C, 320°C for PTSH and PITI respectively in Fig. 8.A & B. This shows that the copolyesters were of high thermal stability.

![Fig. 8. The TGA (A) PTSH and (B) PITI](image)

3.6. Cytotoxicity effect of PTSH and PITI co-polymers

The effect of the synthetic drugs on cellular morphology was investigated and the phenomenal changes were displayed in Fig. 9. Among the various drugs tested, PIOM, PIGH, PTSH, and PITI exhibited marked changes in the cellular morphology, indicating its cytotoxic effect on the cancerous cells. Significant changes in the nuclear fragmentation, chromatin condensation, and apoptosis were noted in the PITI treated cells compared to the rest of the drugs.
At higher concentration (100 µg/mL) of the drug PITI, the breast cancer cells were completely disintegrated (figure 1). PIGH treated cells showed a vacuolation in the cell cytoplasm, which might have resulted in autophagy. Interestingly, PITI cells got shrunk, thus denoting cell death. The MTT data substantiates the findings of the morphological studies, as shown in Fig. 10,11.

![Morphological changes of MDA-MB-231 cells after the exposure of synthetic drugs PSGH, PTOI, PIOM, PIGH, PTSH and PITI.](image)

![MTT Assay in MDA-MB-231 cells treated with synthetic compounds](image)

**Incubation time**: 48 hours at 37°C

ELISA plate with MDA-MB-231S cells organized in different wells along with non treated cells, and 0.1% DMSO (solvent control) treated cancer cells with different concentration 0.1 to 100 µg/mL incubated 48 hours at 37°C.
The breast cancer cell inhibition is shown in figure 14. The cytotoxicity effect of the synthetic compounds was tested against MDA-MA-231 cells by MTT assay. After 48 hours of synthetic compound treatment, the IC$_{50}$ dose levels were calculated. Each line graph showed the percentage of cell inhibition depending on the dose and the inhibition of 50% of the cells is indicated as IC$_{50}$. The drugs, PSGH, PTOI, PIOM, and PIGH showed about 33% and 43% inhibition of the cells even at 100 µg/mL. While, the drug, PTSH was effective at 10 µg/mL and 51% inhibition was observed at 100 µg/mL. Most importantly, the drug, PITI was more significant at 0.1 µg/mL concentration itself and 58% inhibition was noted at 1 µg/mL, thus signifying the potential of this drug as a promising anti-proliferative agent.

The above synthetic drugs PTSH and PITI primarily involved in the killing mechanism by targeting the Bax proteins, which was stimulated in the late 24-48 h stage leading to apoptosis. This evidence suggested that the induction of other protein, p21 which paves the way to apoptosis through a p53-dependent pathway. Nevertheless, this mechanism needed to be further clarified by immunoblot analysis.

4. CONCLUSIONS

In conclusion, a novel series of copolymers was synthesized especially six chalcone based copolyesters, characterize them and their physical properties were studied by using spectroscopic techniques (IR, and NMR) and checked for anticancer activity against one cell lines (Human-MDA-MB-231). From our study results revealed that among the six compounds synthesized PTSH and PITI was found to be more sensitive to MDA cell lines. Many synthetic chalcones derived compounds with higher concentration was studied and it is difficult to treat, hence, we synthesized compounds by substitution of functional groups studied compounds against the cancer cell line invitro. We found that copolyesters PTSH and PITI having good cytotoxic property at very low concentration (1µg/ml) compared to other studies.
ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

S.Kothai and D.Lakshmi Devi, designed the research work and performed the research, analyzed the data and wrote the paper. S.Venkatesh and S.Kothai corrected this manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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