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Cytotoxic efficacy of aliphatic aromatic copolyesters containing chalcone moiety against MCF7 breast cancer cell lines

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Abstract

One of the most obstinate diseases in human beings, with uncontrollable growth and spreading of abnormal cells is cancer. Cancers like breast cancer, blood cancer, liver cancer, lung cancer, brain cancer, colon cancer, prostate cancer, cervical cancer and ovarian cancer etc. play a vital role in the mortality rate of humans. According to WHO, due to cancer, worldwide 9.6 million people were died in the year 2018 and it is expected to affect 22 million by 2030. American Cancer Society survey of 2015 reports that, breast cancer ranks second in the death of women. Despite treatments like surgery, radiation, chemotherapy, hormone therapy, immune therapy, targeted therapy, have reduced the risks of lower lifespan, number of deaths in the world population are always on the rise due to serious side effects and multidrug resisting cancer cells . Therefore, a greater need of anticancer agents with low toxicity and targeted therapies are necessary. Apart from cancer, contagious diseases like SARS and COVID 19 have consumed more lives in short time world over. It is also feared that the humans along with pets are going to encounter epidemics and pandemics in not so distant future.

Introduction

A very big challenge, always placed before the scientist, is to invent new drugs that are free of undesirable side effects and simultaneously at the affordable rate. Besides producing new drugs, scientists are keenly interested in mimicking privileged class of organic compounds found in natural products like fruits, vegetables, tea, spices and so on. One of the gifts from God is chalcone which occurs naturally, ubiquitously in edible plants, vegetables, fruits, spices tea and natural foodstuff as an open chain flavonoid compound. Chalcones and their derivatives have demonstrated their efficacy in broad range of pharmacological activities like antimalarial, anticancer, antiprotozoal, anti-inflammatory, antibacterial, antifilarial, antifungal, antimicrobial, larvicidal, anticonvulsant, antioxidant, anti-diabetic, anti-neoplastic , antihypertensive, anti-retroviral, anti-histaminic, anti-ulcer activities [1-4]. Chalcones also have the ability to inhibit the enzymes like mammalian alpha-amylase, cyclo-oxygenase (COX) and monoamine oxidase (MAO) and so on.

In recent years, studies have revealed that, biomaterials such as chalconate complexes of Ruthenium (II), chalcone based co-ordination compounds, dihydroartemisinyl chalcone esters, the adhesive coating on cotton fabric using ZnO nanorods and chalcone were found to be an excellent antimicrobial agent. Furthermore, it was noted interestingly, that when chalcone moiety linked with ether or ester group are found to be more cytotoxicity than the chalcone monomer unit. On the other hand, whenever a drug is given orally or injected into the affected tissues it should sustain in the human body for a certain period of time by releasing in a controlled manner. However, this controlled release of drug can be achieved efficiently by using polymeric materials. Felix et al reveals that polymers can bind to the surfaces without losing their biological activity which results in prolonged activity and less toxicity [5]. For example, aliphatic aromatic copolyesters are widely used as biomaterials for a variety of biomedical applications, which highlight the significance of polymers as therapeutic agents. In the present work, we investigated the cytotoxic efficacy of eight synthesized aliphatic aromatic copolyesters containing chalcone moiety.

Materials and Methods

All the chemical reagents, solvents used in the synthesis and characterization of copolyesters are listed as follows: Sigma-Aldrich chemicals of 4-hydroxy-3-methoxybenzaldehyde (99%), 4-hydroxyacetophenone (99%), 4-hydroxy 3-methoxyacetophenone (99%), terephthaloyl chloride (99%), succinyl chloride (95%), adipoyl chloride (98%), were used as such without any purification. Merck sample of sulphuric acid (98%) was also used as purchased. Merck sample of methanol was used as a non-solvent to precipitate all the copolyesters. It was refluxed over quick lime for 6 h followed by distillation. Dimethyl sulphoxide (DMSO) was dried over anhydrous calcium sulphate and distilled under reduced pressure. N, N-Dimethyl acetamide (DMAc) was dried with calcium hydride for 24 h and distilled under vacuum [6]. N, N-Dimethyl formamide (DMF) was dried with potassium carbonate for 12 h under reduced pressure, followed by distillation. Tetrahydrofuran (THF) was kept over sodium hydroxide pellets and distilled. Chloroform, acetone, were used as such for the qualitative test of solubility of the copolyesters. Merck sample of spectral grade DMSO-d6 (Aldrich) containing TMS as internal standard was used for recording NMR Spectra. Cell lines were obtained from National Centre for Cell Sciences Pune (NCCS), India. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO2 at 37 °C. The Reagents MEM, Fetal bovine serum (FBS), Trypsin, Methyl Thiazolyl Diphenyl Tetrazolium Bromide (MTT), Dimethyl sulfoxide (DMSO) were purchased from Hi media, Sigma Aldrich Mumbai [7].

Synthesis of Chalcone diols

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A series of four chalcone diols used as monomers in this work were synthesized by conventional acid catalyzed Claisen-Schmidt condensation method as mentioned in the literature. The chalcone diols thus prepared are 1,3-bis-(4-hydroxyphenyl) prop-2-en-1-one (BHPP), 1-(4-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (HMPP), 1-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one, (HMHP), and 1,3-bis-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (BHMP). The typical method of synthesis of BHPP is as follows [8].

Synthesis of 1, 3-bis-(4-hydroxyphenyl) prop-2-en-1-one (BHPP)

In a 250 mL double necked round bottomed flask provided with a magnetic stirrer and a gas inlet, 4-hydroxy benzaldehyde 5 g (0.04 mole) and 4-hydroxy acetophenone 5.57 g (0.04 mole) were dissolved in 100 mL of dry ethanol. Anhydrous dry HCl gas was passedinto the reaction mixture at room temperature and it was stirred continuously for 1 h. The reaction mixture was thoroughly mixed with 300 mL ice cold water. That resulted in the precipitation of yellow coloured BHPP. It was filtered, washed with double distilled water, dried and re-crystallized from hot methanol [9].

1, 3-bis-(4-hydroxyphenyl) prop-2-en-1-one (BHPP)

Yield: 90%; m.p.:183.2 °C. IR(KBr): 3301 (b, O–H), 1648(s, –C=O) cm-1, 1605 (s, –CH=CH); 1H NMR (500MHz, DMSO-d6, ppm) 9.9 (s, 2H, –OH), 7.4–8.4 (m, 8H, aromatic), 6.7–6.9 (dd, 2H, –CH=CH–) and MS (EI) m/z 240 [M]+.

1-(4-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (HMPP)

Yield: 85%; m. p. 202 °C; IR (KBr): 3320 (b, O–H), 1658(s, –C=O) cm-1; 1H NMR (500MHz, DMSO-d6, ppm) 10.3 (s, H, –OH), 9.6 (s, H, –OH),7.4–8.0 (m, 7H, aromatic), 6.8–7.2 (dd, 2H, –CH=CH–), 3.87 (m, –OCH3) and MS (EI) m/z 270 [M]+

1-(4-hydroxy-3-methoxyphenyl) -3-(4-hydroxyphenyl) prop-2-en-1-one (HMHP)

Yield: 83% m. p.110 °C; IR (KBr): 3320 (b, O–H), 1658(s, C=O) cm-1; 1H NMR (500MHz, DMSO-d6, ppm) 9.9-10.0 (s, 2H, –OH), 7.6–8.0 (m, 6H, aromatic), 6.8–6.9 (dd, 2H, –CH=CH–), 3.7 (m, –OCH3) and MS (EI) m/z 270 [M]+.

1, 3-bis-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (BHMP):

Yield: 86% m. p.182 °C; FT-IR (KBr): 3320 (b, O–H), 1658(s, –C=O) cm-1; 1H NMR (500MHz, DMSO-d6, ppm) 10.1 (s, H, –OH), 9.8 (s, H, –OH), 7.3–7.9 (m, 7H, aromatic), 6.7–6.9 (dd, 2H, –CH=CH–) 3.5-3.7 (m, –OCH3) and MS (EI) m/z 300 [M]+.

Synthesis of Copolyesters

The copolyesters were prepared by polycondensation technique shown in the Scheme.2. In a 250 mL double necked round bottom flask equipped with a magnetic stirrer and a double surface condenser, the monomer (0.8 g) and triethylamine (1 mL) were dissolved in 10 mL of DMF. The reaction mixture was stirred well for about 15 minutes. Then 0.2 mL of terephthaloyl chloride and 0.2 mL of adipoyl chloride were added to the reaction mixture with constant stirring while the temperature was raised to 120 ° C. The reaction mixture was stirred vigorously for a span of 3 h. The mixture was poured into 100 mL of methanol after cooling it to room temperature[10].

Characterization of copolyesters

The synthesized copolyesters were purified and recrystallized from methanol. The structures of all the synthesized copolyesters were elucidated by FT-IR, 1H NMR and 13C NMR Spectral analysis. FT-IR spectra were obtained by JASCO Model P-4600 instrument using KBr pellets. 1H NMR Spectra were obtained with Bruker 500 MHz Avance III and 13C NMR Spectra were obtained with Bruker 125 MHz Avance III Instrument at room temperature. Viscosity measurements were made by using Ubbelohde Viscometer at 30 °C using DMAc at the concentration of 0.1 dL g-1.The cytotoxicity of copolyesters on MCF-7 was determined by the MTT assay method. The concentration required for a 50 % of viability (IC50) was determined by making use of MTT Cell Proliferation Assay Instruction Guide.

Copolyester PATS

Brown solid, yield: 75%.

IR (KBr, cm-1): 2936-2361 cm-1 (C–H Str. Aliphatic), 1755 cm-1 (C=O Str. of ester), 1679 cm-1 (C=O Str. of chalcone), 1599 cm-1 (C=C Str. of aromatic), 1419 cm-1 (C=C Str. of ethylenic double bond), 1211-1133 cm-1 (C–O–C Str. of alkoxy), 1018 cm-1 (C–O Str. of ester), 880 and 781 cm-1 (C–H out of plane bending), 680 cm-1 (C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 1.39-2.73 (m, –CH2 of acid chlorides), 6.85 (m, –CH=CH– of chalcone moiety), 7.15 (d, olefinic proton), 8.2 (m, Ar-H), 10.2 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 24.4-31.5 (–CH2 of succinyl moiety), 110.2-144.6 (–CH=CH of chalcone), 149.1-160 (Ar-C), 165 (C=O of ester), 175 (–C=O of chalcone moiety).

Copolyester PBTS

Brown solid, yield: 80%.

IR (KBr, cm-1): 2936 cm-1 (C–H Str. Aliphatic), 1750 cm-1 (C=O Str. of ester), 1640 cm-1 (C = O Str. of chalcone), 1426 cm-1 (C=C Str. of aromatic), 1200-1111 cm-1 (C–O–C Str. of alkoxy), 1064 cm-1 (C–O Str. of ester), 810 and 723 cm-1 (C–H out of plane bending), 695 cm-1 (C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 2.16-3.17 (m, –CH2 of acid chlorides), 3.4 (m, –OCH3 of chalcone unit), 6.4 (m, –CH=CH of chalcone moiety), 7.6 (d, olefinic proton), 8.23 (m, Ar–H), 10.5 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 29.2-35 (–CH2 of succinyl moiety), 55.5 (–OCH3), 112.84-135 (–CH=CH of chalcone), 152.3 (Ar–C), 167.7 (–C=O ester), 174 (–C = O chalcone)

Copolyester PCTS

Brown solid, yield: 75%. IR (KBr, cm-1): 2534-3062 cm-1 (–CH Str. of aliphatic), 1754 cm-1 (C=O Str. of ester), 1677 cm-1 (C=C Str.of chalcone), 1573-1411 cm-1 (C=C Str. of aromatic), 1259-1161 cm-1 (C–O–C Str. of alkoxy), 1110 cm-1 (C–O Str. of ester), 978 and 726 cm-1 (C–H out of plane bending), 688 cm-1 (–C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 2.4-2.89 (m, –CH2 of acid chlorides), 3.8 (m, –OCH3 of chalcone unit), 6.8 (m, –CH=CH of chalcone moiety), 7.7 (d, olefinic proton), 8.3 (m, Ar-H), 10.1 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 29.0-30.2 (–CH2 of succinyl moiety), 56.5 (–OCH3), 112-125 (–CH=CH of chalcone), 152 (Ar–C), 160.2-167.1 (–C=O ester), 174.0 (–C=O chalcone)

Copolyester PDTS

Brown solid, yield: 77%. IR (KBr, cm-1): 2362-2938 cm-1 (C–H Str. Of aliphatic), 1755 cm-1 (C–O Str. of ester), 1677 cm-1 (C=C Str. of chalcone),

1504-1421 cm-1 (C=C Str. of aromatic), 1258-1022 cm-1 (C–O–C Str. of alkoxy), 1109 cm-1 (C–O Str. of ester), 911, 798, 628 cm-1 (C–H out of plane bending), 538 cm -1(C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 2.2-2.8 (m, –CH2 of aliphatic acid chloride), 3.7 (m, –OCH3 of chalcone unit), 6.26 (m, –CH=CH of chalcone moiety), 8.4 (m, Ar–H), 7.2 (d, olefinic proton), 10.3 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 25.2 -31.5 (–CH2 of succinyl moiety), 58.5 (–OCH3), 115.1- 135 (–CH=CH of chalcone), 153.5 (Ar–C), 162-166.9 (–C=O ester), 175.2 (–C=O chalcone).

Copolyester PATA

Brown solid, yield: 70%. IR (KBr, cm-1): 2942-2348 cm-1 (C–H Str. Aliphatic), 1729 cm-1 (C=O Str. of ester), 1640 cm-1 (C=O Str. of chalcone), 1596 cm-1 (C=C Str. of aromatic), 1427 cm-1 (C=C Str. of ethylenic double bond), 1208-1110 cm-1 (C–O–C Str. of alkoxy), 1036 cm-1 (C–O Str. of ester), 817 and 749 cm-1 (C–H out of plane bending), 672 cm-1 (C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 1.49-2.93 (m, –CH2 of aliphatic acid chloride), 6.86 (m, –CH=CH of chalcone moiety), 8.17 (m, Ar–H), 7.58 (d, olefinic proton), 8.1 (m, Ar–H), 10.8 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 24.7-37.1 (–CH2 of adipoyl moiety), 116.2-143.6 (–CH=CH of chalcone), 147.1-160.2 (Ar–C), 165.9 (–C=O of ester), 172.2 (–C=O of chalcone)

Copolyester PBTA

Brown solid, yield: 70%. IR (KBr, cm-1): 2942-2348 cm-1 (C–H Str. Aliphatic), 1729 cm-1 (C=O Str. of ester), 1628 cm-1 (C = O Str. of chalcone), 1468 cm-1 (C=C Str. of aromatic), 1200-1106 cm-1 (C–O–C Str. of alkoxy), 1025 cm-1 (C–O Str. of ester), 817 and 749 cm-1 (C–H out of plane bending),672, 495 cm-1 (C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 1.26-2.89 (m, –CH2 of acid chloride), 3.3 (m, –OCH3 of chalcone unit), 6.87 (m, –CH=CH of chalcone moiety), 8.23(m, Ar–H), 7.47 (d, olefinic proton), 10.45 (s, end –OH).

13C NMR: (500MHZ, DMSO-d6, ppm): 24.4-38.2 (–CH2 of adipoyl moiety), 56.5 (–OCH3), 115.84-151.6 (Ar–C), 162.7-165.7 (C=O ester), 174.7 (C = O chalcone).

Copolyester PCTA

Brown solid, yield: 70%. IR (KBr, cm-1): 2947 cm-1 (C–H Str. of aliphatic), 1717 cm-1 (C=O Str. of ester), 1646 cm-1 (C=C Str.of chalcone), 1598-1415 cm-1 (C=C Str. of aromatic),1256-1025 cm-1 (C–O–C Str. of alkoxy), 1113 cm-1 (C–O Str. of ester), 975and 727 cm-1 (C–H out of plane bending), 524 (C=C in plane bending).

1H NMR (500MHz, DMSO-d6, ppm): 1.5-2.2 (m, –CH2 of acid chloride), 3.9 (t, –OCH3), 6.8 (m, –CH=CH of chalcone moiety), 8.0 (m, Ar–H), 7.47 (d, olefinic proton), 10.3(s, end –OH).

13C NMR (500MHZ, DMSO-d6, ppm): 24.48- 33.7 (–CH2 of adipoyl moiety), 56.1 (–OCH3), 116.2-152.5 (Ar–C), 160.2-167.1(C=O ester), 174.7 (C=O chalcone).

Copolyester PDTA

Brown solid, yield: 70%. IR (KBr, cm-1): 2658-3058 cm-1 (C–H Str. Of aliphatic), 1717 cm-1 (C–O Str. of ester), 1686 cm-1 (C=C Str. of chalcone), 1508-1418 cm-1 (C=C Str. of aromatic),1286–1019 cm-1 (C-O–C Str. of alkoxy), 1124 cm-1 (C–O Str. of ester), 939,783,657 cm-1 (C–H out of plane bending),538(C=C in plane bending).

1H NMR (500 MHz, DMSO-d6, ppm): 2.19 (m, –CH2), 2.9 (t, –CH2), 6.56 (m, –CH=CH of chalcone moiety), 8.1 (m. Ar–H), 7.51(d, olefinic proton), 10.8 cm-1 (s, end -OH).

13C NMR (500MHZ, DMSO-d6, ppm): 24.7-32.5 (–CH2 of adipoyl unit), 56.8 (–OCH3), 116.2-147.1 (Ar-C), 160.2-165.9 (C=O ester), 172.2 (C=O chalcone).

In vitro cytotoxicity study

All the synthesized eight copolyesters were evaluated for their cytotoxic activity in human breast cancer cell lines by the MTT assay method. The cancer cells (1 × 105/well) were plated in in 0.2 mL of medium/well in 96-well plates and it was incubated for 72 h at 5 % CO2 condition. Then, various concentrations of copolyesters were added to the sample plates and to the control plates in 0.1% DMSO and incubated for 24 h at 5 % CO2 condition. The sample solution was washed with phosphate-buffered saline (pH 7.4) and 20 µL /well (5 mg mL-1) of 0.5 % 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added, and then the cultures were incubated for 4 h. After 4 h of incubation, the insoluble purple formazan product formed, was dissolved with 1 mL of DMSO. Viable cells were determined by measuring the absorbance of this coloured solution at 540 nm. The concentration required for a 50% inhibition of viability (IC50) was determined from the graph.

Results and Discussion

Solubility

Solubility of copolyesters in various solvents is presented in [Table 2]. The copolyesters synthesized were found to be soluble in highly polar solvents like DMAc, DMF, DMSO, THF and they are insoluble in hydrocarbons like benzene, toluene, xylene and hexane. It was insoluble in hydroxyl group containing solvents like methanol, ethanol, 2 - propanol and in water. Copolyesters with methoxy substituent in the benzene ring of chalcone moiety had greater solubility than those without methoxy substituents. The electron releasing methoxy group caused disruption in the macromolecular chain and makes the easy penetration of solvent molecule into the polymer chain which enhances its solubility.

Table 2: Comparison Between Succinyl and Adipoyl Based Copolyesters Cytotoxicity

Copolyesters concentration µg/ml	% cell viabilityin PDTS	% cell viability in PDTA
100	6.5	0
50	10.2	0
25	14.7	2.9
12.5	12.5	5.9
6.25	40.7	12.8
3.12	51.1	26.7
1.56	65.3	55.4

Viscosity measurements

Berry and Fox certifies that the inherent viscosity is directly related with the molecular weight as well as the rigidity of the polymers. Inherent viscosity η inh of the random copolyesters were measured from the flow time of the pure solvent and copolyester solution by using Ubbelohde viscometer in DMAc at 30 °C. About 25 mg of pure dry copolyester was dissolved in 25 mL of DMAc. The inherent viscosity values were found to be in the range

of 0.27 - 0.45 dL g-1and is presented in [Table 1]. These data show that the copolyesters are of lower to moderate molecular weight. The copolyesters having one or two methoxy substituent in the aromatic ring exhibit higher viscosity than those of the copolyesters without methoxy substituent. This trend might be due to the interlocking effect obtained by the methoxy groups in the polymer chain. Whereas copolyester PATA has very low viscosity than the other one, this might be due to the flexible methylene spacer in the polymer backbone. It is inferred that these copolyesters with low molecular weight is best suitable for the biological applications.

Table 1: Synthesized Copolyesters Inherent Viscosity

Copolyester code	Chalcone diol used	ηinh(dL/g)
PATS	BHPP	0.38
PBTS	HMPP	0.37
PCTS	HMHP	0.44
PDTS	BMMP	0.45
PATA	BHPP	0.27
PBTA	HMPP	0.38
PCTA	HMHP	0.38
PDTA	BMMP	0.45

Spectral studies

The FT-IR spectrum of PCTA copolyester is shown in [Figure1]. FT-IR spectra of all eight copolyesters were also obtained. All the eight copolyesters have shown strong absorption bands due to carbonyl stretching vibration of ester between 1716 cm-1 and 1755 cm-1. The ketonic carbonyl carbon stretching vibration is observed between 1600 cm-1 and 1679 cm-1 due to the presence of chalcone moiety. The peaks at 1411–1599 cm-1 are assigned to the C=C stretching vibrations of the ethylene double bond and aromatic moiety. The absorption band in the region 1018-1110 cm-1 is assigned to –C–O–C– group. All the eight copolyesters have shown absorption peak around 2348–3058 cm-1 due to the presence of the –CH2 stretching of aliphatic group in the polymer chain. The peak at 628-978 cm-1 is attributed to aromatic C-H out-of-plane bending vibrations.

1H–NMR spectrum of the copolyester PCTA is represented in [Figure 2]. 1H–NMR spectra were obtained for all other copolyesters also. The multiple signals observed between 7.15-8.2 ppm corresponding to the aromatic protons of chalcone, terephthaloyl ring resonated in the upfield region. This can be ascribed to the delocalization of lone pair of electrons of the ester oxygen with the benzene ring which shield the protons and consequently absorps at the upfield region. The multiple signals recorded between 6.26-7.0 ppm correspond to deshielded vinylic protons of chalcone moiety. The sharp singlet peak of methoxy protons appeared at 3.9 ppm. The methylene protons of the succinyl and adipoyl moiety were observed at 1.5 - 2.2ppm. The signal at 3.4 ppm is due to the methyl protons of deuterated solvent DMSO-d6, whereas the signal resonate at 2.5 ppm is due to protons of water, present in the DMSO solvent.

The proton decoupled 13C NMR spectroscopy is employed by many workers to provide information about the backbone of copolyesters. 13C–NMR spectrum of the copolyester PCTA is represented in [Figure 3]. 13C–NMR spectra were obtained for all other polymers also. The presence of α, β-unsaturated ketone carbonyl carbon of chalcone moiety resonate signals at 172-175 ppm. The ester carbonyl carbons shows signal in the region of 160.2–166.9 ppm. The two resonance signals at 147 ppm and 132 ppm are of aromatic carbons of chalcone and terephthaloyl parts. The olefinic carbons of chalcone moieties resonate in the region of 116 ppm. The methoxy carbon occurs at 55.5-58.5 ppm. The signals between 24.4-29.2 ppm and 31.2-41.9 ppm are methylene carbon of aliphatic acid chlorides in the polymer chain.

Cytotoxicity screening

The entire adipoyl unit based copolyesters namely PATA, PBTA, PCTA and PDTA and the entire succinyl unit based copolyesters namely PATS, PBTS, PCTS and PDTS were investigated for their cytotoxic activity in human breast cancer cell lines by the MTT assay method. Fig. 9 represents the image of the vero cell line. The affected MCF7 cell at different concentrations and the IC50 value of all the copolyesters are shown in the Table 3. By this method, the tetrazolium salt (5 mg of MTT was dissolved in 10 ml of serum free DMEM medium), when added to the solubilizing solution (50 % DMSO), it gets reduced to formazan dye by the mitochondrial dehydrogenase enzyme of the viable cells. This formazan dye is insoluble in water, but forms a purple coloured complex with viable cells and the absorbance is measured at 540 nm.

In vitro cytotoxicity of all the copolyesters is presented in [Table 4] [Figure 4-7]. From the observed values, it is explicit that all the aliphatic aromatic copolyesters exhibit significant cytotoxicity effect on the cancer cell line. The copolyesters induced greater cytotoxicity due to the conjugation of chalcone unit through ester linkage which increases the cell permeability and restricts the growth of cancer cells. Interestingly, IC50 profile of copolyesters possessing succinyl moiety, reports that PDTS has 51.1% cell viability at 3.12 µg mL-1, PCTS has 57.6% cell viability at 3.12 µg mL-1, PBTS has 52.5% cell viability at 3.12 µg mL-1, PATS has 54.7% cell viability at 12.5 µg mL-1 respectively. These values certify that copolyesters induced greater cytotoxicity at lowest concentration itself (IC50 = 3.12 µg mL-1).

Copolyesters containing adipoyl moiety

Fig. 8-11 represents the images of the cytotoxicity of copolyesters containing adipoyl moiety on breast cancer cell line at various concentrations. The IC50 profile of the copolyesters containing adipoyl moiety, reveals that all the aliphatic aromatic copolyesters have significant cytotoxicity effect on the cancer cell line. The copolyesters induced greater cytotoxicity at lowest concentration. (IC50 = 1.56 µg mL-1). PDTA has 55.4% cell viability at 1.56 µg mL-1, PCTA has 64% cell viability at 1.56 µg mL-1, PBTA has 63.3% cell viability at 1.56 µg mL-1, PATA 55.9% cell viability at 6.25 µg mL-1 respectively. From the IC50 values shown in the Table 3, it is evident that cytotoxicity increases with increasing polymer concentration. Interestingly, it was noted that, the adipoyl moiety based copolyesters have shown enhanced cytotoxicity than the succinyl moiety based copolyesters. This might be attributed to the increasing flexible spacer length in the polymer backbone. Moreover, the copolyesters have shown excellent cytotoxicity when compared with the aliphatic aromatic copolyester bearing biscoumarin unit synthesized by Narendran et al. Fig. 13 represents the cytotoxicity of all the copolyesters at different concentrations.

Comparison between cytotoxicity of the succinyl and adipoyl based copolyesters is shown in the fig. 14 It is inferred from the [Table 2]. PDTS has 51.1% cell viability at 3.12 µg mL-1 whereas PDTA has 55.4% cell viability at 1.56 µg mL-1. The percentage of cell viability is found to be lower with PDTA copolyester than the PDTS copolyester. Among the succinyl and adipoyl moiety based copolyesters, adipoyl based copolyesters exhibit good cytotoxicity in comparison with succinyl based copolyesters.

Figure 1: FT-IR spectrum of PCTA copolyester

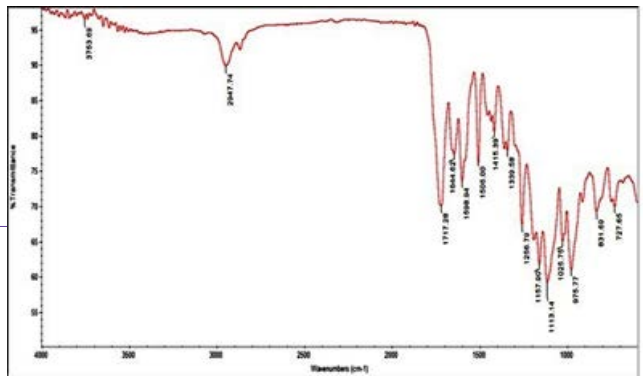

Figure 2: 1H–NMR spectrum of the copolyester PCTA

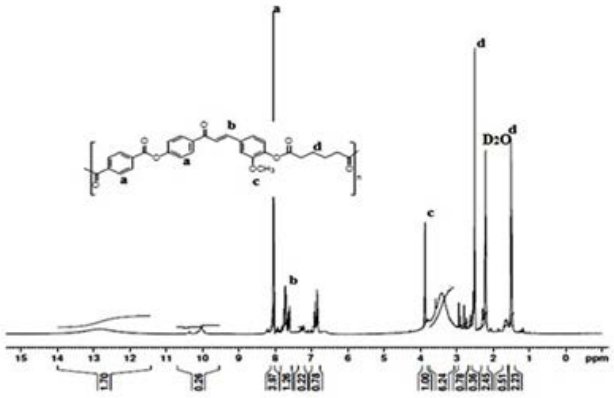

Figure 3: C–NMR spectrum of the copolyester PCTA

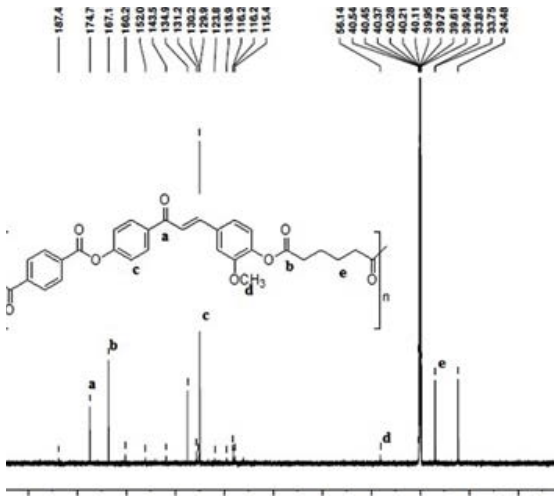

Figure 4:MCF-7 Cell line; Cytotoxicity of PATS (b) at 100 µg (c) at 50 µg (d) at 12.5 µg

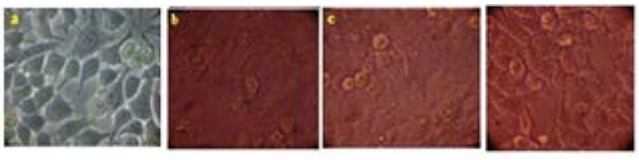

Figure. 5:MCF-7 Cell line; Cytotoxicity of PBTS (b) at 100 µg (c) at 50 µg (d) at 12.5 µg

Conclusion

This might be attributed to the longer alkyl chain flexible spacer, which enhances the easy penetration of the copolyester into the cancer cell lines to prevent its growth. And also, it was evident that, greater lipophilicity group, lowers the percentage of cell viability. Thus, the cytotoxic activity of copolyesters increases, as the alkyl chain length increases which was reported by the previous workers. The increasing order of in vitro cytotoxic activity of copolyesters is as follows: PATS > PATA > PBTS = PCTS = PCTA = PDTS > PBTA > PDTA. Among all the copolyesters, PDTA and PDTS were found to be the most potent compound with IC50 values 1.56 µg mL-1and 3.12 µg mL-1. This study has widened the scope of using chalcone based aliphatic aromatic copolyesters as a promising candidate in chemotherapeutic treatment

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Conflicts of Interest

Authors do not have any conflicts of interest in this manuscript.

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