Polym Int 51:569–576 (2002)
DOI: 10.1002/pi.801

Synthesis and biological activity of phthalimide-based polymers containing 5-fluorouracil

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Abstract: The attachment of anticancer agents to polymers is a promising approach towards reducing the toxic side-effects and retaining the potent antitumour activity of these agents. A new tetrahydrophthalimido monomer containing 5-fluorouracil (ETPFU) and its homopolymer and copolymers with acrylic acid (AA) and with vinyl acetate (VAc) have been synthesized and spectroscopically characterized. The ETPFU contents in poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) obtained by elemental analysis were 21 mol% and 20 mol%, respectively. The average molecular weights of the polymers determined by gel permeation chromatography were as follows: $M_n = 8900\ g\ mol^{-1}$, $M_w = 13\,300\ g\ mol^{-1}$, $M_w/M_n = 1.5$ for poly(ETPFU); $M_n = 13\,500\ g\ mol^{-1}$, $M_w = 16\,600\ g\ mol^{-1}$, $M_w/M_n = 1.2$ for poly(ETPFU-*co*-AA); $M_n = 8300\ g\ mol^{-1}$, $M_w = 11\,600\ g\ mol^{-1}$, $M_w/M_n = 1.4$ poly(ETPFU-*co*-VAc). The *in vitro* cytotoxicity of the compounds against FM3A and U937 cancer cell lines increased in the following order: ETPFU > 5-FU > poly(ETPFU) > poly(ETPFU-*co*-AA) > poly(ETPFU-*co*-VAc). The *in vivo* antitumour activities of all the polymers in Balb/C mice bearing the sarcoma 180 tumour cell line were greater than those of 5-FU and monomer at the highest dose (800 mg kg^{-1}).

Keywords: 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidopentanoyl-5-fluorouracil (ETPFU); poly(ETPFU-*co*-AA); poly(ETPFU-*co*-VAc); photopolymerization; *in vitro* cytotoxicity; *in vivo* antitumour activity

INTRODUCTION

The severity of side-effects associated with conventional cancer chemotherapy has prompted the development of a variety of drug delivery approaches, with the ultimate goal of improving the therapeutic index of the drug *via* either site-specific delivery or site-specific drug activation. One promising approach for targetable drug delivery is the use of polymers,[1–3] and several polymer–drug conjugates are currently in advanced clinical trials.[4–7]

The non-specific toxicity-derived limitations of all anticancer drugs are also evident in the case of 5-fluorouracil (5-FU), a pyrimidine analogue with a broad spectrum of anticancer activity. Over the past 30 years, this compound has been used for the treatment of solid tumours, including advanced breast cancer and adenocarcinomas of the gastrointestinal tract.[8–10] However, despite its many beneficial effects, administration of 5-FU is accompanied by disorders of the bone marrow and the epithelium of the gastrointestinal tract, which seriously limit its therapeutic effect.[11,12] Moreover, 5-FU has a very short half-life in plasma as a result of its fast metabolism in liver, and a sustained intravenous infusion is often required to maintain an adequate drug concentration in the blood.

In an attempt to minimize the toxic side-effects and overcome the delivery problems, several polymeric routes to controlled administration of 5-FU have been reported.[13–20] These strategies include encapsulation of 5-FU in synthetic or natural hydrogels followed by diffusive release,[18] encapsulation in degradable polymer devices from which release occurs as the polymer degrades[19] and conjugation to soluble peptides.[20] In parallel, research from our laboratories has been focused to develop new bioactive polymers containing 5-FU.[21–23] A distinct feature of our strategy is to introduce 5-FU to suitably functionalized monomers which are subsequently polymerized in a well defined manner. With this goal in mind, we have reported the synthesis and polymerization of 1,2,3,6-tetrahydrophthaloyl or itaconyl monomers containing 5-FU and discussed their reduced toxicity and improved anti-

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(*Received 17 May 2001; revised version received 2 August 2001; accepted 20 August 2001*)

tumour activity.[24–32] Among them, the tetrahydrophthalic-acid-based polymers showed strong antitumour activity against cancer cell lines, while they displayed low cytotoxicity against normal cell lines.[30–32]

In continuation of our studies, we report herein the synthesis and biological evaluation of new antitumour active polymers based on the tetrahydrophthalimide template. The monomeric unit was built by conjugating 5-FU to tetrahydrophthalic anhydride using 5-aminopentanoic acid. Its homopolymer and copolymers with acrylic acid (AA) or vinyl acetate (VAc) were prepared by photopolymerization. The obtained monomeric unit and its polymers were identified by FTIR, ^{1}H NMR and ^{13}C NMR spectroscopies, and elemental analysis. The average molecular weights of the polymers were measured by gel permeation chromatography (GPC). The *in vitro* cytotoxicities were evaluated with mouse mammary carcinoma (FM3A) and human histiocytic lymphoma (U937) as cancer cell lines and mouse liver cells (AC2F) as a normal cell line. The *in vivo* antitumour activities of the synthesized polymers against mice bearing the sarcoma 180 tumour cell line were evaluated. Our data demonstrate that the synthesized polymers exhibit higher antitumour activities than the monomers and 5-FU. In addition, they display low cytotoxicity against normal cell lines, suggesting that they can be used for pharmacological and clinical studies.

EXPERIMENTAL

Materials

Acrylic acid (AA, Junsei Chemicals, Tokyo, Japan) was dried with NaCl and distilled under vacuum (7 mmHg, 45 °C). Dimethoxyethane (DME, Fluka Chemicals, Wisconsin, USA), triethylamine (TEA, Junsei Chemicals, Tokyo, Japan) and other chemicals were used without further purification. Vinyl acetate (VAc, Junsei Co, Tokyo, Japan) was purified by distillation with benzoyl peroxide under nitrogen. 3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalic anhydride (MPA; Fluka Co, Tokyo, Japan), 5-fluorouracil (5-FU), 2,2-dimethoxy-2-phenylacetophenone (DMP) and 5-aminovaleric acid were used as received from Aldrich Chemicals (Wisconsin, USA) without further purification.

For *in vitro* tests, FM3A and U937 were used as cancer cell lines and AC2F was used as a normal cell line. For *in vivo* tests, Balb/C mice and cells of the sarcoma 180 line were purchased from the Center of Genetic Engineering (Korea Institute of Science and Technology).

We received the approval from University Ethics Committee of Kosin University, in order to conduct this experiment. The mice were kept under pathogen free environment (4 mice per cage) and were allowed to consume experimental diet and tap water freely during the experimental period. The experimental conditions were as follows: The relative humidity was maintained within a range of 55%. Temperature was regulated at 22 °C by having thermostatic control. We used 15–17 air changes per hour for ventilation. Ammonia gas was kept below 20 ppm. Light at the cage level was 250–300 lux. Noise was below 60 dB. Water was treated to eliminate contamination. Bedding was used in amounts sufficient to keep animals dry between cage changes. We changed bedding twice a week. At the end of the experiment, mice inhaled CO_2 for euthanasia. This guideline was based on: Guide for Care and Use of Laboratory Animals from NIH. (http://cacu.od.nih.gov/regs/guide/guidex.htm)

Instruments

^{1}H and ^{13}C NMR spectra were recorded on a FT-300 MHz Varian Gemini (Paolo Alto, USA) 2000 spectrophotometer. Tetramethylsilane (TMS) and DMSO-d_6 were used as an internal standard and the solvent, respectively. Elemental analyses were carried out with an elemental analyser (Carlo Erba, Peapack, USA, model EA 180). The number and weight average molecular weights were measured by gel permeation chromatography (GPC; Waters, Massachusetts, USA, 410 differential refractometer).

Synthesis of monomer

The ETPFU monomer was synthesized according to Scheme 1.

3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalimidopentanoic acid (ETPA)

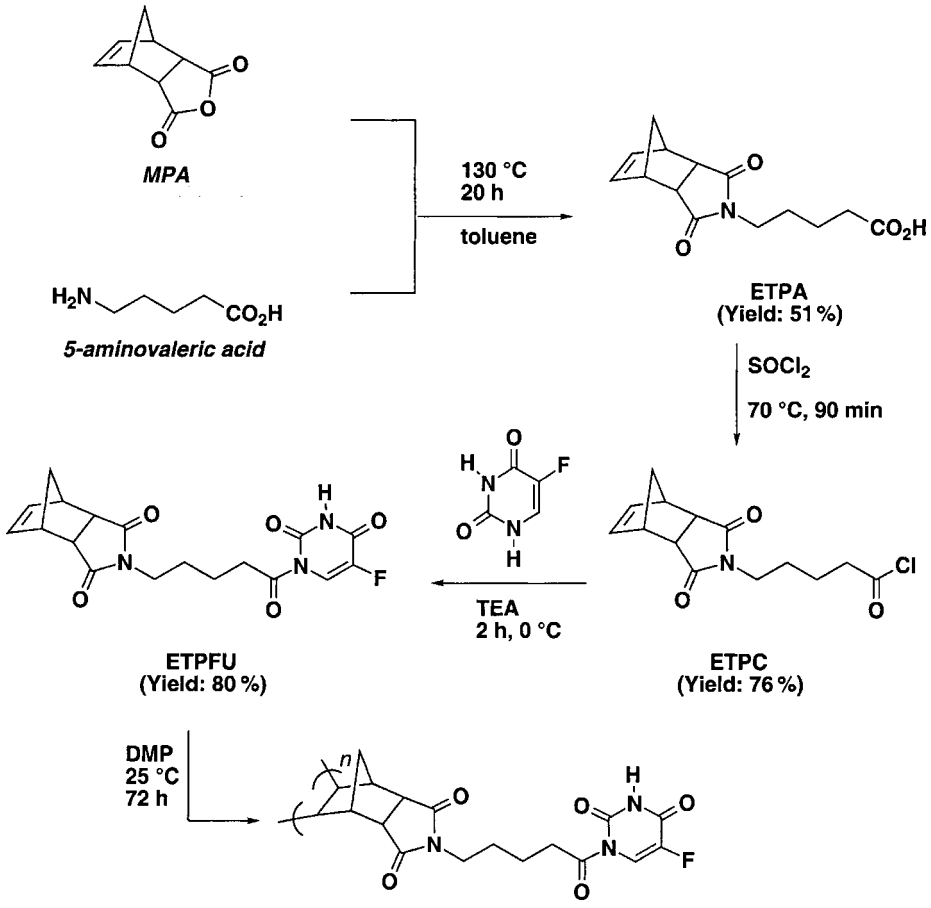
MPA (10.81 g, 65.8×10^{-3} mol), 5-aminovaleric acid (7.76 g, 65.8×10^{-3} mol) and TEA (0.62 g, 6.17×10^{-3} mol) were refluxed in toluene (100 ml) for 20 h with azeotropic removal of water *via* a Dean–Stark apparatus. After evaporation of the solvent, the residue was titrated with 0.1 N HCl (60 ml) and dissolved in saturated aqueous $NaHCO_3$ (40 ml). The aqueous solution was washed with ethyl acetate (2×25 ml) to remove any organic soluble materials. The aqueous layer was acidified to pH 2 by adding concentrated HCl and extracted twice with $CHCl_3$ (2×25 ml). The combined $CHCl_3$ layers were dried over anhydrous $MgSO_4$ and evaporated to obtain pure ETPA in 51% yield. The melting point was 104–108 °C.

3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalimidopentanoyl chloride (ETPC)

A solution of ETPA (2.5 g, 9.50×10^{-3} mol) and thionyl chloride (3.5 ml, 47.5×10^{-3} mol) was stirred at 70 °C for 90 min. The excess thionyl chloride was evaporated to obtain the crude product, which was recrystallized from petroleum ether to give pure ETPC in 76% yield. ETPC was stored under nitrogen atmosphere before use.

3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalimidopentanoyl-5-fluorouracil (ETPFU)

A solution of 5-FU (0.5 g, 3.8×10^{-3} mol) and TEA (0.5 ml) in DME (70 ml) was stirred at 70 °C for 2 h



Scheme 1. Synthesis of monomer ETPFU and poly(ETPFU).

and then cooled to 0 °C. To this solution, ETPC (1.07 g, 3.8×10^{-3} mol) in DME (25 ml) was added dropwise with vigorous stirring under nitrogen at 0 °C for 2 h. The formed TEA hydrochloride salt was removed by filtration. The filtrate was allowed to stand for 1 day and was subsequently concentrated to 20% of the original volume and poured into n-hexane (300 ml). The resulting precipitate was dried under reduced pressure to obtain pure ETPFU in 80% yield. ^{1}H NMR (DMSO-d_6): δ (ppm) = 11.5 (s, 1H, NH of 5-FU), 8.3 (s, 1H, CH=CF of 5-FU), 6.1 (d, 2H, CH=CH), 3.4 (t, 2H, —N—CH_2CH_2), 3.2–3.3 (m, 4H, —COCHCH—CH_2—CHCH—CO—), 2.2 (t, 2H, —CH_2CH_2CON), 1.7 (d, 2H, —$CHCH_2$CH—), 1.3–1.5 (m, 4H, —$NCH_2CH_2CH_2CH_2$CON), ^{13}C NMR (DMSO-d_6): δ (ppm) = 178 (CONCO), 172 (CH_2CON), 157 and 158 (CH=CFCONH of 5-FU), 150 (NCONH of 5-FU), 142 and 140 (CH=CF of 5-FU), 134 (CH=CH), 126 (CH=CF of 5-FU), 52 (CHCONCOCH), 45 (CHCH=CHCH), 44 ($CHCH_2$CH). HRMS for $C_{18}H_{18}FN_3O_5$: calculated $[M+H]^+ = 376.1308$; found 376.1332. Elemental analysis: calculated (%) for $C_{18}H_{18}FN_3O_5$, C 59.6, H 4.7, N 11.2; found (%), C 56.2, H 4.9, N 10.6.

Synthesis of poly(ETPFU)

A solution of ETPFU (0.5 g, 1.3×10^{-3} mol) and DMP (0.02 g, 0.065 mmol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was flushed twice with N_2 gas, sealed and placed in a photochemical chamber where it was irradiated at 313 nm (115 V, 60 Hz power supply) at 25 °C for 72 h. The obtained polymer solution was slowly dropped into n-hexane (300 ml) to precipitate the polymer. The precipitated polymer was collected by filtration and washed several times with acetone. The obtained homopolymer was dried under reduced pressure to constant weight. The conversion was 74%.

Syntheses of poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc)

A solution of ETPFU (0.5 g, 1.3×10^{-3} mol) and AA (0.1 ml, 1.3 mmol) with DMP (0.04 g, 0.13 mmol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was sealed after flushing twice with bubbling purified N_2 gas. The preparation procedure for poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) was the same as that described for the homopolymerization of ETPFU except for the

monomer pairs. The copolymerization conversion of ETPFU with AA and VAc was 84% and 77%, respectively.

Measurements of average molecular weight and composition

To compare the average molecular weights of the synthesized polymers, we determined their apparent molecular weights by GPC using a micro-Styragel column and low polydispersity polystyrene as a standard at 40 °C. Dimethylformamide was used as an eluent. The contents of ETPFU moity in the copolymers were calculated from C, H and N data obtained by elemental analysis.

Biological activity tests

In vitro *cytotoxicities of ETPFU and its polymers*

The cytotoxic effects of ETPFU and its polymers against cancer cells *in vitro* were tested in a standard MTT assay.[33] The MTT assay is based on the reduction of soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. All the cell lines used for the cytotoxicity assay were maintained in RPMI-1640 medium containing 10% heat inactivated foetal calf serum. The synthesized polymers were dissolved in a minimum quantity of dimethylsulfoxide (DMSO) and diluted with phosphate-buffered saline just before use. The solution was added to the cells (2×10^4) in 96-well microtiter plates and cultured for 3 days at 37 °C. The cultured cells were mixed with 20 µl of MTT solution and incubated for 4 h at 37 °C. The supernatant was carefully removed from each well and 100 µl of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance was measured at 570 nm using a microplate reader. All measurements were done in triplicate. The cytotoxicity is represented as the IC_{50}, which denotes the concentration of 50% growth inhibition. There was a good reproducibility between replicate wells with standard errors below ±10%.

In vivo *antitumour activity test*

For the antitumour activity test sarcoma 180 cells (2×10^5) isolated from ascitic fluid of tumour-bearing mice were injected intraperitoneally into Balb/C mice (6 weeks, 25 g). Every day for four consecutive days starting from 24 h after the cell injection, the synthesized polymers in physiological saline were administered intraperitoneally to each group. The different doses tested were as follows: 0.8, 80 and 800 mg kg^{-1}. Each sample-treated group for each dose consisted of ten mice. Mice were observed until the time of death. For comparison, the antitumour activity of 5-FU was also tested by the same method. The control group was divided into two groups: one subgroup was treated with sarcoma 180 cells together with neat saline by replacing the sample solution; the other subgroup was treated with sarcoma 180 cells alone. The comparative antitumour effect of various dosages on the median survival time in days for treated *versus* control groups was expressed as *T/C*%.

RESULTS AND DISCUSSION

Identification of monomer and polymers

The structures of the synthesized monomer and polymers were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopies. The FTIR characteristic absorption peaks for ETPFU appeared at 3500–3000 cm^{-1} (NH stretching), 1720 and 1705 cm^{-1} (C=O stretching), and 1670 cm^{-1} (C=C stretching). The ^{1}H NMR and ^{13}C NMR spectra of ETPFU are shown in Fig 1. The peaks of vinyl protons in ETPFU appeared at 6.1 ppm, and the olefin proton (CH=CF) and N—H proton of the 5-FU moiety in ETPFU appeared at 8.3 ppm and at 11.5 ppm, respectively.

Poly(ETPFU) was synthesized *via* radical polymerization through the carbon–carbon double bond of the norbornene moiety. The FTIR spectrum of poly(ETPFU) showed the characteristic absorption peaks at 1720 and 1705 cm^{-1} with the disappearance of vinyl absorptions at 1670 cm^{-1} which appeared in ETPFU monomer. The characteristic peak for the methine protons of the polymer backbone of poly(ETPFU) was observed at 0.9 ppm in the ^{1}H NMR spectrum; the peak for the vinyl protons of monomeric ETPFU at 6.1 ppm was not observed. The peaks for the olefinic and N—H protons in the 5-FU moiety appeared at 8.3 and 11.5 ppm, respectively.

For the copolymer, poly(ETPFU-*co*-AA), the characteristic peaks for the methine proton on the ETPFU moiety were observed at 0.9 ppm in the ^{1}H NMR spectrum. The peak for the vinyl protons of monomeric ETPFU at 6.1 ppm was not observed. The peaks for the olefinic and N—H protons in the 5-FU moiety appeared at 8.3 ppm and 11.5 ppm, respectively. The proton peaks for the carboxylic acid, methine, and methylene protons of the AA moiety in poly(ETPFU-*co*-AA) were observed at 12.3 ppm, 1.0 ppm and 1.2 ppm, respectively.

For the copolymer, poly(ETPFU-*co*-VAc), the proton peaks of the ETPFU moiety were assigned to the same as those of poly(ETPFU-*co*-AA). The proton peaks on the methyl, methine and methylene protons of the VAc moiety in poly(ETPFU-*co*-VAc) were observed at 1.5 ppm, 1.1 ppm and 2.1 ppm, respectively. The peaks due to vinyl protons in ETPFU and VAc were not observed.

Solubility of ETPFU and its polymers

The solubilities of ETPFU and its polymers are shown in Table 1. ETPFU is soluble in acetone, 2-butanone, DMF, DMSO and THF. Its polymers are insoluble or poorly soluble in diethyl ether, n-hexane, toluene and water.

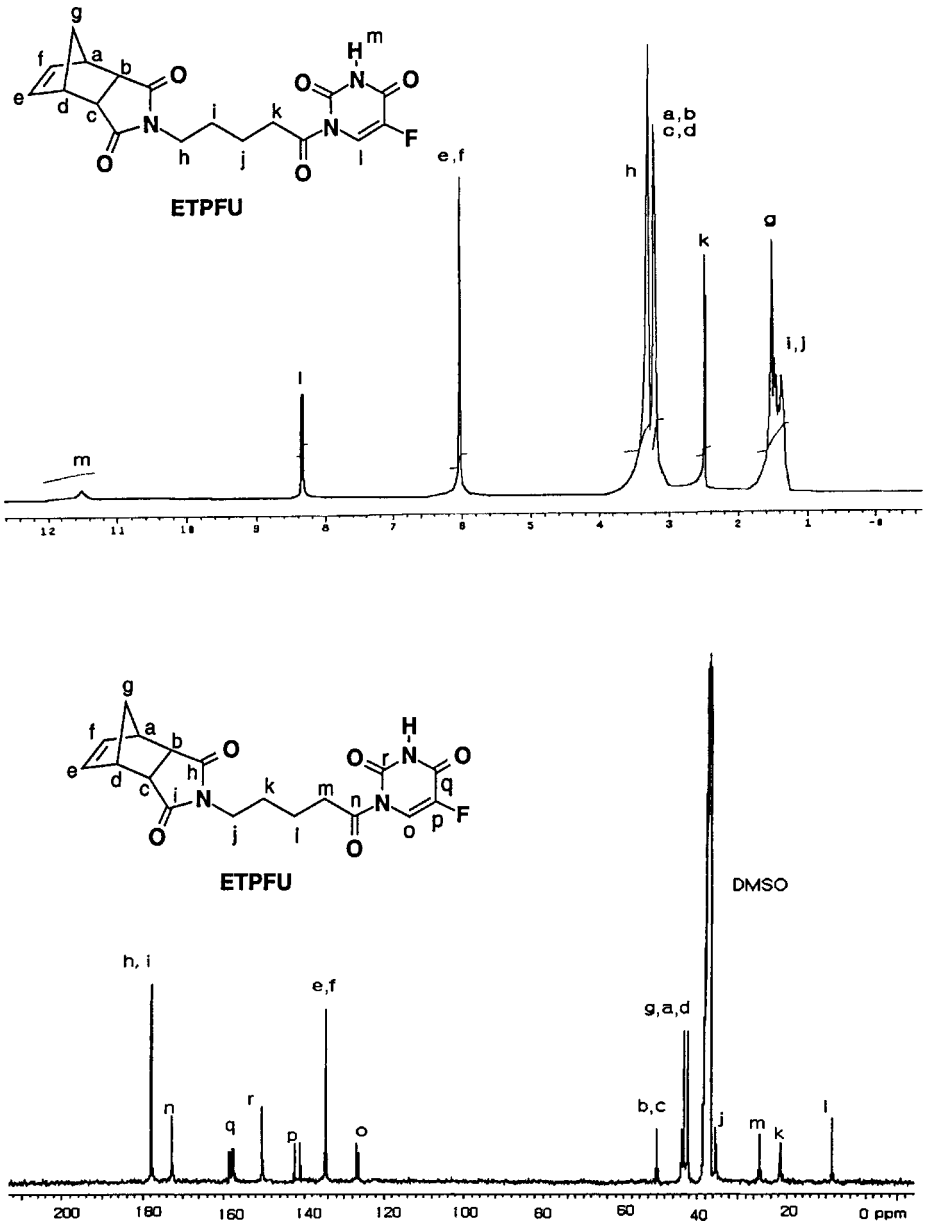


Figure 1. ^{1}H NMR and ^{13}C NMR spectra of ETPFU.

Average molecular weights and compositions of polymers

The average molecular weights and polydispersity indices of the polymers are listed in Table 2. The number average molecular weights (M_n) of the polymers ranged from 8300 to 13 500 mg mol^{-1} and molecular weight distributions (M_w/M_n) were 1.2–1.5. Ottenbrite[34] reported that polymers having molecular

	Sample			
Solvent	*ETPFU*	*Poly(ETPFU)*	*Poly(ETPFU-co-AA)*	*Poly(ETPFU-co-VAc)*
Water	PS	PS	PS	PS
DMSO	S	S	S	S
DMF	S	S	S	S
Acetone	S	S	S	S
2-Butanone	S	S	S	S
THF	S	S	S	S
Diethyl ether	IS	IS	IS	PS
Toluene	IS	IS	IS	IS
n-Hexane	IS	IS	IS	IS

Table 1. Solubility of ETPFU and its polymers[a]

[a] S, soluble; PS, poorly soluble; IS, insoluble.

Table 2. Average molecular weights and polydispersity of the polymers

Polymer	M_n[a] *(g mol^{-1})*	M_w[a] *(g mol^{-1})*	M_w/M_n
Poly(ETPFU)	8900	13300	1.5
Poly(ETPFU-*co*-AA)	13500	16600	1.2
Poly(ETPFU-*co*-VAc)	8300	11600	1.4

[a] Determined by GPC in DMF.

Table 3. *In vitro* cytotoxicity of ETPFU and its polymers against cell lines

	IC_{50} (μg ml^{-1}) for cell lines[a]		
	Cancer cells		*Normal cells*
Sample	*FM3A*[b]	*U937*[c]	*AC2F*[d]
5-FU	0.02	0.01	0.03
ETPFU	0.04	0.03	0.05
Poly(ETPFU)	0.16	0.05	0.28
Poly(ETPFU-*co*-AA)	0.35	0.25	0.10
Poly(ETPFU-*co*-VAc)	1.20	1.35	0.95

[a] 50% growth inhibition.
[b] Mouse mammary carcinoma cell.
[c] Human histiocytic lymphoma cell.
[d] Mouse liver cell.

weight of 10000–30000 g mol^{-1} exhibit optimal biological activity. The polymer that we synthesized using a previously reported method,[25–32] met the minimum chain length requirement to exhibit antitumour activity based on Ottenbrite's findings.

The elemental analysis values (in %) of the copolymers are as follows. Poly(ETPFU-*co*-AA): C 45.2, H 6.12, N 6.4. Poly(ETPFU-*co*-VAc): C 38.2, H 5.6, N 5.8. The ETPFU compositions in poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) were determined by the elemental analysis and were 21 mol% and 20 mol%, respectively.

In vitro cytotoxicity of ETPFU and its polymers

The *in vitro* cytotoxicity of ETPFU and its polymers was evaluated against two cancer cell lines and one normal cell line. As shown in Table 3, the monomeric unit (ETPFU) is more effective against all cell lines than its polymers and has an activity similar to that of 5-FU. The cytotoxicity of 5-FU, ETPFU and the synthesized polymers against cancer cell lines decreased in the following order: 5-FU > ETPFU > poly(ETPFU) > poly(ETPFU-*co*-AA) > poly(ETPFU-*co*-VAc). The values of 50% cytotoxicity (IC_{50}) for ETPFU and its polymers were in the range of 0.03–1.35 μg ml^{-1} against cancer cell lines. In the normal cell line (AC2F), the cytotoxicity of the synthesized ETPFU and its polymers was much lower than that of 5-FU.

In vivo antitumour activity of ETPFU and its polymers

The *in vivo* antitumor activity of ETPFU and its polymers against mice bearing sarcoma 180 is listed in Table 4 together with that of 5-FU for comparison. Mortality was recorded and mean survival time was calculated for each compound. The activity of polymers was expressed as a survival effect (*T*/*C*), where *T* is the mean survival time of mice treated with the

Entry	*Sample*	*Dosage (mg kg^{-1})*	*Mean survival time (days)*[a]	*T/C (%)*[b]	*S/E*[c]
1	Control	–	14.7±2.3	100	0/10
2		Saline	15.7±0.5	100	0/10
3	5-FU	800.0	5.9±0.3	39	0/10
4		80.0	21.3±2.8	140	0/10
5		0.8	20.3±1.8	134	0/10
6	ETPFU	800.0	14.0±0.0	92	0/10
7		80.0	108.8±8.4	715	7/10
8		0.8	104.6±10.5	688	5/10
9	Poly(ETPFU)	800.0	82.8±23.9	544	0/10
10		80.0	80.8±26.8	531	4/10
11		0.8	88.25±26.1	581	5/10
12	Poly(ETPFU	800.0	112.0±0	737	10/10
13	-*co*-AA)[d]	80.0	101.1±13.5	665	4/10
14		0.8	108.6±8.4	715	7/10
15	Poly(ETPFU	800.0	85.6±29.4	563	5/10
16	-*co*-VAc)[e]	80.0	93.0±7.8	612	0/10
17		0.8	88.4±24.6	581	4/10

[a] Mean survival time of animals dying within the experimental period of 112 days.
[b] *T*/*C* (%) represents the ratio of the survival time of the mice treated with a sample (*T*) to the control (*C*) mice × 100.
[c] *S*/*E* denotes the ratio of the number of surviving mice (*S*) to that of experimental mice (*E*) after the experimental period of 112 days.
[d] For poly(ETPFU-*co*-AA), the ETPFU composition is 21%, which means that the drug composition is 7.1%.
[e] For poly(ETPFU-*co*-VAc), the ETPFU composition is 20%, which means that the drug composition is 6.8%.

Table 4. *In vivo* antitumor activity of 5-FU, ETPFU and its polymers

sample and *C* is the survival time of mice in a control group.

As shown in entries 3–5 of Table 4, low dosage of 5-FU (0.8 mg kg^{-1}) led to good antitumour activity (140% of that of the control group). However, increasing the drug dosage to 800 mg kg^{-1} led to a sharp decrease of the mean survival time (to 40% of that of the control group). This is attributed to the inherent toxicity of 5-FU.

The anticancer activity of the monomeric unit (ETPFU) at different dosages is shown in entries 6–8 of Table 4. At low and medium drug dosages (0.8 and 80 mg kg^{-1}) we observed a significant increase (to about 700%) of the mean survival time *versus* that of the control group (100%). However, a high dose of ETPFU (800 mg kg^{-1}) resulted in a notable decrease of the survival time. This suggests that at high concentration the monomer displays significant toxicity which overrides its potential therapeutic value. It was, nonetheless, encouraging to observe that a low dose of ETPFU led to a statistically significant prolongation of the life of mice beyond the experimental period (112 days). This result indicates that conjugation of 5-FU to the phthalimide framework increases the therapeutic potential of the drug.

Evaluation of poly(ETPFU) at different dosages is presented in entries 9–11 of Table 4. Independently of the dose administered, we recorded a notable increase of mean survival time (about 550%) *versus* that of the control group. Similarly to ETPFU, no mice survived the entire experimental time when poly(ETPFU) was administered at high dose (800 mg kg^{-1}). However, at low or medium dosages about 50% of the mice lived beyond 112 days.

As indicated in entries 12–14 of Table 4, the, best results were obtained using the copolymer of ETPFU with acrylic acid [poly(ETPFU-*co*-AA)]. Treatment of mice with this copolymer resulted in a substantial increase of the mean survival time (to about 700% of that of the control group). It was very rewarding to observe that administration of this polymer at high dosage (800 mg kg^{-1}) resulted in 100% survival of mice beyond the experimental period of 112 days, while lower concentrations led to at least 40% survival. These results demonstrate that this polymer has a very low toxicity even at high dosage, and can therefore overcome the inherent toxicity associated with high doses of 5-FU.

Increase of mean survival time (to about 600% of that of the control) was also recorded upon administration of the copolymer of ETPFU with vinyl acetate [poly(ETPFU-*co*-VAc)] (entries 15–17 of Table 4). Nonetheless, the survival of mice after 112 days was at the best case 50%, and overall the data were less impressive than those reported for poly(ETPFU-*co*-AA).

Previous studies from our laboratory have addressed the synthesis and biological examination of several phthalimide-based polymers containing 5-FU.[30–32] The polymers presented herein, in which 5-FU is attached to the phthalimide framework using a four carbon methylene linker (5-aminovaleric acid), have shown lower toxicity together with a dramatic improvement in survival time. This effect could probably be attributed to the structures of the new polymers that allow a slower release of 5-FU from the polymeric matrix *via* hydrolysis of the amide or imide bonds.

It has been shown that the polymers containing 5-FU exhibit longer half-lives than the corresponding monomers in plasma, which enhances their likelihood of being taken up by the tumour cells.[35] Moreover, it is known that such polymers enter the tumour cell membrane by endocytosis, where they release 5-FU due to degradation by endogenous lysosomes.[36–38] The antitumour effect of the drug is then initiated by preventing DNA synthesis in the tumour cells.[39,40] To this end, the enhanced stability of the polymers containing 5-FU in blood plasma provides an efficient way of sustaining an effective concentration of the drug in tumour cells over a long period of time.

CONCLUSIONS

A new monomer, 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidopentanoyl-5-fluorouracil (ETPFU), was synthesized from 5-fluorouracil (5-FU) and 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidopentanoyl chloride (ETPC). Its homopolymer and copolymers with acrylic acid (AA) or vinyl acetate (VAc) were prepared by photopolymerization. ETPFU, poly(ETPFU), poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) were identified by ^{1}H NMR and ^{13}C NMR spectroscopies. The polydispersity indices of all synthesized polymers ranged from 1.2 to 1.5. The contents of ETPFU in poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) were found to be 21 mol% and 20 mol%, respectively.

The range of IC_{50} values obtained from the *in vitro* test for ETPFU, poly(ETPFU), poly(ETPFU-*co*-AA) and poly(ETPFU-*co*-VAc) were 0.03–1.35 μg ml^{-1} against cancer cell lines, which were much lower than those of 5-FU. In a normal cell line, the cytotoxicities of ETPFU and its polymers were much lower than that of 5-FU. From estimation in mice bearing the sarcoma 180 tumour cell line, the *in vivo* antitumour activities of the synthesized polymers were 3–19 times superior to that of 5-FU at corresponding dosages. The highest *T/C* value was 737% for poly(ETPFU-*co*-AA) at 800 mg kg^{-1}. This polymer exhibited much lower toxicity than previously reported polymers containing 5-FU, which opens the way for further clinical studies.

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