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Synthesis and Anticancer Evaluation of Novel 10-Methoxycamptothecin Derivatives

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As part of our continuing search for potential anticancer drug candidates, we have synthesized four 10-methoxycamptothecin derivatives. The compounds were synthesized by facile procedures and characterized by ^{1}H NMR, ^{13}C NMR and mass spectra study. The synthesized compounds were examined for their cytotoxic effect on a panel of five human cancer cell lines. Three out of five compounds were found to exhibit moderate anticarcinogenic activities in all cell lines. The result of the present investigation encourage us to develop more other related compounds and to screen them for a wide range of biological activity.

Keywords: 10-Methoxycamptothecin derivative, Anticancer, Cytotoxic.

INTRODUCTION

Camptothecin (CPT), a naturally occurring pentacyclic indole alkaloid, was isolated from the oriental tree *Camptotheca acuminata*[1], a native tree of China. It appeared antitumor activity and inhibiting effect against solid tumors and leukemia[2,3]. Camptothecin can make cells die by inhibiting the replication of DNA[4], which makes it become one of unique anticancer drugs. The E-ring lactone structure of camptothecin undergo a rapid, reversible and non-enzymatic hydrolysis to the water-soluble carboxylate form at basic pH. The water-soluble carboxylate form of camptothecin, which was used in early clinical trials due to the poor water-solublity of camptothecin, is easy to conjugate with serum protein and makes it lose anticancer activity[5]. Even worse, it showed severe side effect on the urinary and digestive systems[6] implying that the intact E-ring lactone structure is the critical structural feature with respect to anticancer activity[7]. In recent years, a number of scientists have engaged in modifing the structure of camptothecin to enhance its activity and reduce its toxicity[8-13]. In order to stablize the lactone ring, a esterification with the hydroxyl group at C-20 position can derive prodrugs which can be converted back to camptothecin by carboxylesterase *in vivo*[14]. A series of novel CPT-bile acids and some simple amino acid prodrugs were synthesized as well[15,16]. 10-Methoxycamptothecin (MCPT), a native derivative of camptothecin, few exist in *C. acuminata*, possessing better activities against several cell lines *in vitro* and showed higher cytotoxicity than 10-hydroxycamptothecin[17]. However 10-methoxycamptothecin shares the same E-ring lactone structure, its *in vivo* anticancer activity would decrease when the open-ring carboxylate formed. In this article, esterified prodrugs of 10-methoxycamptothecin were synthesized with different amino acid derivatives at C-20 position and their cytotoxic effect were examined on a panel of five human cancer cell lines. The result showed that three of the derivatives exhibit moderate anticarcinogenic activities against all cell lines. It brings new intrest to the potential use of 10-methoxycamptothecin in the development of novel anticancer drugs.

EXPERIMENTAL

Melting points of the synthesized compounds are uncorrected and performed by melting point apparatus. Mass spectra were taken on Micromass Quattro-Micro TM. The NMR spectra were recorded using the Varian Unity Inova 500 NB.

The purity of each compound was detected by a Waters HPLC system equipped with a 1525 binary pump, a 717 plus autosampler and a 2996 PDA detector (Milford, MA, USA). A Thermo ODS C18 column (4.6 × 250 mm i.d., 5 μm) was used.

Preparation of 10-methoxy-camptothecin: A mixture of different anhydrous potassium carbonate (1 g, 0.007 mol) and methyl iodide (1 mL) were added into acetone (15 mL) with 10-hydroxycamptothecin (0.5 g, 0.0014 mol). To this solution methyl iodide (1 mL) was added in at room temperature when it was stirred for 0.5 h. Stirring was continued for an additional 12 h. The solution was dissolved in dichloromethane (200 mL), filtered, washed with water and dried with anhydrous sodium sulfate, recrystallized with anhydrous ethanol after removing the dichloromethane to obtain 10-methoxycamptothecine (MCPT).

General procedure for preparation of 10-methoxycamptothecin N-t-boc-amino acid derivatives (C1-C4): N-t-boc-amino acid (0.003 mol) was dissolved in 20 mL of anhydrous dimethyl formamide in a round-bottom flask at room temperature. To this solution a mixture of 10-methoxycamptothecin (0.2 g, 0.0006 mol), dicyclohexylcarbodiimide (DCC, 0.52 g, 0.0025 mol) and 4-dimethylaminopyridine (DMAP, 0.06 g, 0.0005 mol) was added drop wise at 0 °C. The solution was allowed back to room temperature after stirring at 0 °C for 1 h. Filtered the resulted dicyclohexylurea followed by a dilution with 100 mL distilled water to form precipitate. The precipitate was filtered, washed with water and dried, purified by column chromatography (dichloromethane/ethyl alcohol =1:100) to obtain 10-methoxycamptothecin N-t-boc-amino acid derivatives (C1- C4).

Cytotoxicity assay: The cell lines MIA PaCa-2, HT-29, DU-145, NCI-H520 and 2774 were included in this study. Each of the cell lines was grown in RPMI 1640 medium containing 100 IU/mL G-penicillin, 100 µg/mL streptomycin and supplemented with 10 % fetal calf serum. Cells were maintained at 37 °C in a humidified atmosphere with 5 % of CO_2. Cells were subcultured every 4-5 days by total replacement using 0.25 % (w/v) trypsin.

Cells were seeded in 96-well culture plates in 0.2 mL of growth medium per well and allowed to attach for 24 h. Then the culture medium was replaced with each compound at different concentrations in four replicates followed by 72 h of incubation. Each concentration Blank wells only filled with fresh medium but not cells. camptothecin was used as positive control. After incubation, 30 µL of MTT solution at a concentration of 3 mg/mL was added to each well followed by 4 h of incubation. MTT solution was then aspirated and 150 µL of DMSO was added to each well to dissolve the dark blue crystals thoroughly. The absorbance was measured at 490 nm using a microplate reader. The relative growth rate (%) was calculated as (mean absorbance of the sample/mean absorbance of the control) × 100 %, considering the optical density of the control[18,19] as 100 %.

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± sem.

Characterization of the synthesized compounds

10-Methoxylcamptothecin-20-O-2-(*tert*-butoxycarbonylamino) acetic acid ester (C1): Pale yellowish powder; m.p. 139-141 °C, MS: *m/z* 533.3 $(M + H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.94 (3H, t, *J* = 7.2 Hz, H-18), 1.32 (1H, s, t-boc), 1.39 (8H, s, t-boc), 2.13 (2H, m, H-19), 3.81 (1H, dd, *J* = 24 Hz, CH_2), 3.90 (3H, s , OCH_3), 3.98 (1H, dd, *J* = 24 Hz, CH_2), 5.17 (2H, s, H-5), 5.48 (2H, s, H-17), 7.13 (1H, s, H-14), 7.43 (1H, d, *J* = 2.8 Hz, H-11), 7.44 (1H, t, *J* = 2.8 Hz, NH), 7.47 (1H, d, *J* = 2.8Hz, H-9), 7.97 (1H, d, *J* = 9.2 Hz, H-12), 8.44 (1H, s, H-7); ^{13}C NMR (400 MHz, DMSO-d_6): δ 7.99, 19, 28.27, 28.62, 30.77, 42.55, 50.56, 56.11, 56.50, 66.66, 76.69, 78.95, 95.29, 106.56, 118.50, 123.41, 129.77, 130.28, 130.42, 130.71, 144.38, 145.82, 146.60, 150.26, 156.34, 156.97, 158.55, 167.58, 169.99.

10-Methoxylcamptothecin-20-O-2-(*tert*-butoxycarbonylamino) propanoic acid ester (C2): Pale yellowish powder; m.p. 129-130 °C, MS: *m/z* 548.4 $(M + H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96 (3H, t, *J* = 7.2 Hz, H-18), 1.33 (3H, t, *J* = 7.2 Hz, CH_3), 1.44 (9H, s, t-boc), 2.09 (2H, m, H-19), 3.90 (3H, s, OCH_3), 4.09 (1H, t, *J* = 7.2 Hz, CH), 5.17 (2H, q, *J* = 7.2 Hz, H-5), 5.48 (2H, s, H-17), 7.18 (1H, s, H-14), 7.39 (1H, d, *J* = 2.8 Hz, H-11), 7.46 (1H, d, *J* = 6.4 Hz, NH), 7.61 (1H, d, *J* = 6.4 Hz, H-9), 7.90 (1H, d, *J* = 5.2 Hz, H-12), 8.42 (1H, s, H-7); ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.02, 16.93, 19, 28.85, 30.56, 50.55, 56.08, 56.50, 66.52, 76.50, 79.10, 95.66, 106.54, 118.28, 123.37, 129.72, 130.18, 130.40, 130.63, 144.40, 146.11, 146.48, 150.31, 156.10, 157.01, 158.51, 167.63, 172.46.

10-Methoxylcamptothecin-20-O-2-(*tert*-butoxycarbonylamino)-3-henylpropanoic acid ester (C3): Pale yellowish powder; m.p. 134-136 °C, MS: *m/z* 624.4 $(M + H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.89 (3H, t, *J* = 7.2 Hz, H-18), 1.89 (3H, t, *J* = 7.2 Hz, CH_3), 1.87 (2H, m, H-19), 2.68 (2H, q, H-CH_2), 5.25 (2H, s, H-5), 5.42 (2H, s, H-17), 6.54 (1H, s, 20-OH), 7.32 (1H, s, H-14), 7.64 (1H, d, H-11), 7.87 (1H, d, *J* = 9.2 Hz, H-9), 8.16 (1H, d, H-12), 8.63 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.24, 9.28, 27.43, 30.75, 50.69, 55.38, 65.71, 72.84, 97.16, 119.59, 119.68, 126.55, 128.79, 130.86, 131.64, 145.81, 146.33, 149.53, 150.47, 152.99, 157.26, 172.91, 173.10.

10-Methoxylcamptothecin-20-O-2-(*tert*-butoxycarbonylamino)-3-methyl-butanoic acid ester (C4): Pale yellowish powder; m.p. 141-143 °C, MS: *m/z* 576.4 $(M + H)^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.96 (3H, t, *J* = 7.2 Hz, H-18), 1.33 (3H, t, *J* = 7.2 Hz, CH_3), 1.44 (9H, s, t-boc), 2.09 (2H, m, H-19), 3.90 (3H, s, OCH_3), 4.09 (1H, t, *J* = 7.2 Hz, CH), 5.17 (2H, q, *J* = 7.2 Hz, H-5), 5.48 (2H, s, H-17), 7.18 (1H, s, H-14), 7.39 (1H, d, *J* = 2.8 Hz, H-11), 7.46 (1H, d, *J* = 6.4 Hz, NH), 7.61 (1H, d, *J* = 6.4 Hz, H-9), 7.90 (1H, d, *J* = 5.2 Hz, H-12), 8.42 (1H, s, H-7); ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.02, 16.93, 19, 28.85, 30.56, 50.55, 56.08, 56.50, 66.52, 76.50, 79.10, 95.66, 106.54, 118.28, 123.37, 129.72, 130.18, 130.40, 130.63, 144.40, 146.11, 146.48, 150.31, 156.10, 157.01, 158.51, 167.63, 172.46.

RESULTS AND DISCUSSION

The synthesis of 10-methoxycamptothecin bearing a methoxy side chain at C-10 was realized starting from 10-hydroxycamptothecin. The reaction with iodomethane in the presence of acetone proceeded smoothly. N-t-boc-amino acids were dissolved in DMF and an acyl born at C-terminal. DMAP was added as a catalyzer for acylation. Dicyclohexylcarbodiimide was used to connect the acyl to the C-20 of 10-methoxycamptothecin (Fig. 1).

The compouds were assayed for their cytotoxic activity against five cancer cell lines consisting of MIA PaCa-2 (human pancreatic cancer cell line), HT-29 (human colon cancer cell), DU-145 (human prostate cancer cell), NCI-H520 (human lung cancer cell), 2774 (human ovarian cancer cell) using the thiazolyl blue tetrazolium bromide (MTT) method. Medium was used as the solvent and blank. As shown in Table-1, compound **C2** was more effective against all the cell lines. Compound **C4** has poor cytotoxicity against all 5 cell lines. Other examined compounds exhibited a moderate inhibitory effect, depending

TABLE-1
in vitro ANTITUMOR ACTIVITY OF THE DERIVATES OF 10-METHOXYCAMPTOTHECIN

Sample	IC_{50}(nM)				
	HT29	MPC2	DU145	NCI-H520	2774
Camptothecin	83.67 ± 3.05	90.64 ± 4.93	33.45 ± 1.54	25.01 ± 1.24	12.09 ± 1.20
10-Methoxycamptothecin	76.80 ± 6.41	35.34 ± 1.37	16.22 ± 0.59	13.05 ± 1.26	8.72 ± 0.73
C1	884.00 ± 94.11	540.42 ± 69.95	306.59 ± 27.48	151.47 ± 1.17	105.66 ± 2.12
C2	408.67 ± 16.04	229.90 ± 14.81	76.06 ± 3.64	79.62 ± 5.60	39.02 ± 0.40
C3	541.33 ± 39.58	371.59 ± 11.08	165.80 ± 9.63	104.37 ± 9.16	53.34 ± 2.81
C4	> 1000	> 1000	> 1000	> 1000	> 1000

MPC-2: human pancreatic cancer cell line; HT-29: human colon cancer cell; DU-145: human prostate cancer cell; NCI-H520: human lung cancer cell; 2774: human ovarian cancer cell.

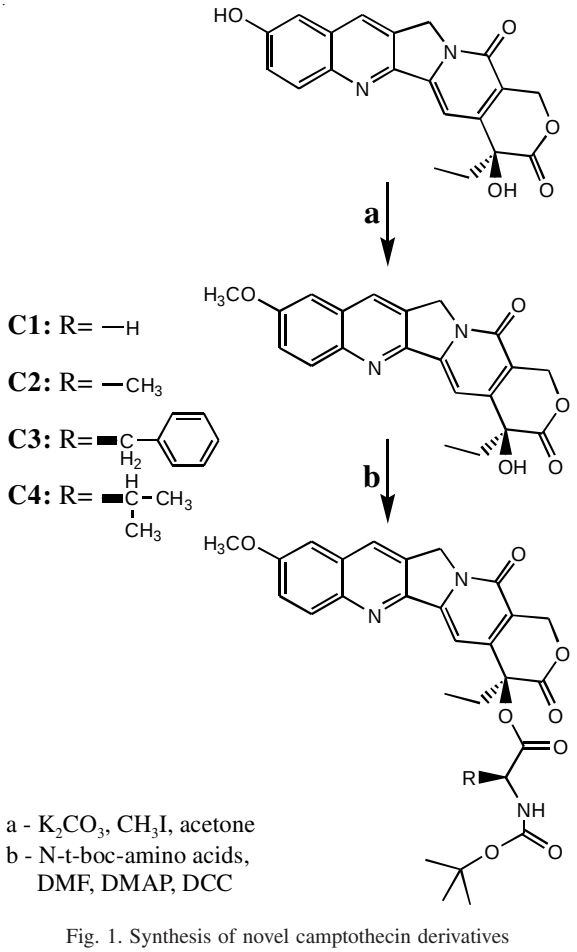

Fig. 1. Synthesis of novel camptothecin derivatives

on the type of the cell line (Table-1). In the four compounds **C2** showed the lowest half inhibitory concentration (IC_{50}), compound **C4** showed least activity and compounds **C1** and **C3** were moderate. The result elucidated that the prodrugs could cleave by carboxylesterase and released the active 10-methoxycamptothecin and the less *in vitro* antitumor activity than 10-methoxycamptothecin was happened because the prodrugs need to be activated by hydrolysis to 10-methoxy-camptothecin.

Conclusion

The anticancer screening of synthesized compounds (**C1** to **C4**) were evaluated against cancer cell lines and the synthesized compounds were found to exhibit mild to moderate anticancer in all cell lines.

In conclusion, CPT > MCPT> C2 > C3 > C1> C4. In our study, all of the 10-methoxycamptothecin derivatives were formed prodrugs by esterification at C-20 hydroxyl group. The IC_{50} values of compounds **C1** to **C3**, range of 79.62-884 nM, are higher than that of 10-methoxycamptothecin, which implies that the prodrugs could cleave by carboxylesterase and released the active 10-methoxycamptothecin and they have great potential for further study.

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