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Synthesis and antitumor activity of aromatic camptothecin esters

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Abstract. Twenty-eight new aromatic esters of camptothecins **2-29** were prepared in yields of 5 to 96% by straight acylation of camptothecin **(1a)** and 9-nitrocamptothecin **(1b)** with various aromatic acids as acylating agents. All of these esters were tested against 14 different human cancer cell lines. The antitumor activity of these compounds was related to the nature of the substituting groups of their side aromatic chains. In general, esters with strong electron-withdrawing groups on their side aromatic chains were active; esters with halogen-substituted side aromatic chains were slightly active; and esters without any substituting groups on their side aromatic chains were practically inactive. The IC_{50} studies showed that the majority of these esters were not as potent as their parental compounds **1a** and **1b**; whereas, the potencies of esters **6** and **25** were exceptionally high, much higher than the commercial camptothecin analogues and comparable to (or slightly more potent than) their parental compounds.

Introduction

Camptothecin, a natural product originally found in China, was isolated and purified by Wall and his co-workers in 1966 (1). Since it demonstrated effective anticancer activity in animal models, camptothecin was quickly tested in human clinical trials. Unfortunately, no anticancer activity was found; instead, severe toxicities were observed in patients (2-6). Trials were accordingly discontinued. The reason for the failure of the early trials was later found to be the incorrect drug formulation selected. As it is insoluble in water, camptothecin was converted to its sodium salt form for intravenous administration. This form of the molecule, although water-soluble, is practically devoid of anticancer activity. A careful evaluation of these agents in animal models made by Wani *et al* revealed that the sodium salt has only one-tenth the potency of the parent compound (7). Important parameters for the anticancer activity of camptothecin derivatives have now been established (8), showing that the intact lactone form with an α-hydroxyl group at position 20 of the molecule with an *S*-configuration is required for antitumor activity.

In our laboratory, camptothecin and its semi-synthetic derivative 9-nitrocamptothecin, have shown significant activity against a wide spectrum of human tumors grown as xenografts in nude mice (9,10), but considerably less activity for these two compounds was observed in human clinical trials. This difference in antitumor activity between humans and mice has been associated with the finding that the opening of the lactone of the molecule to its carboxylate form is much more advanced in humans. For example, ~50% of 9-nitrocamptothecin is present as the lactone form in mice, but only 2-5% of the molecule can be found as the lactone form in humans (unpublished data). Further studies on the stability of camptothecin derivatives in human serum have been conducted by Burke (11).

Clearly, there is a need for a camptothecin derivative which is able to maintain the molecule as an intact lactone when circulating in the human body. A number of attempts at improving the lactone stability of camptothecins have been undertaken, of which acylation of 20-OH of the molecule has proven to be the most efficient. For example, Zhao and his co-workers reported that 20-acyl derivatives of camptothecin were substantially more stable in lactone form than their 20-OH parents and found that 20-*O*-acyl camptothecin derivatives remained unaffected even at pH 9.5 (12), whereas the lactone of parental camptothecin molecules underwent hydrolysis reaction to form carboxylate salt at physiological pH 7.4 (13,14). We previously reported the preparation of camptothecin esters by acylating the 20-OH group with organic anhydrides, which resulted in significant increases in the stability of ester compounds in the human body, and the presence of much higher concentrations of the lactone form of the molecules (15).

The SAR studies with our synthetic 20-acyl camptothecin derivatives showed that all alkyl esters had low toxicity, and that their antitumor activities varied depending on the property of their side ester chains (16,17). Only compounds with a straight C_2 or C_3 alkyl ester chain were found to be active; the remainder were either slightly active or not active

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at all. Alkyl camptothecin esters are not independently active. They are activated by a group of enzymes called esterases. Camptosar (CPT-11), a clinically useful anticancer agent, is a prodrug of 10-hydroxy-7-ethylcamptothecin (SN38). A number of studies have shown that, in various mammals and humans, camptosar is converted to its active metabolite SN-38 by liver carboxylesterase (18-21). Carboxylesterase enzymes exist in different organs and tissues. Many of these enzymes have been purified and characterized. For example, Mentlein *et al* and Meintlein and Heymann purified five carboxylesterases from rat liver microsomes, investigated their activity, and found that these enzymes had high carboxylesterase activity with simple aliphatic and aromatic esters (22,23). Our results are consistent with Mentlein's observations, that is, only those simple alkyl camptothecin esters with a C_2 or C_3 chain have significant antitumor activity. In order to obtain optimal camptothecin ester derivatives with high effectiveness and low toxicity for clinical use, we prepared many aryl camptothecin esters. We also assayed some of their biological properties and obtained some notable results, which we report in this paper.

Materials and methods

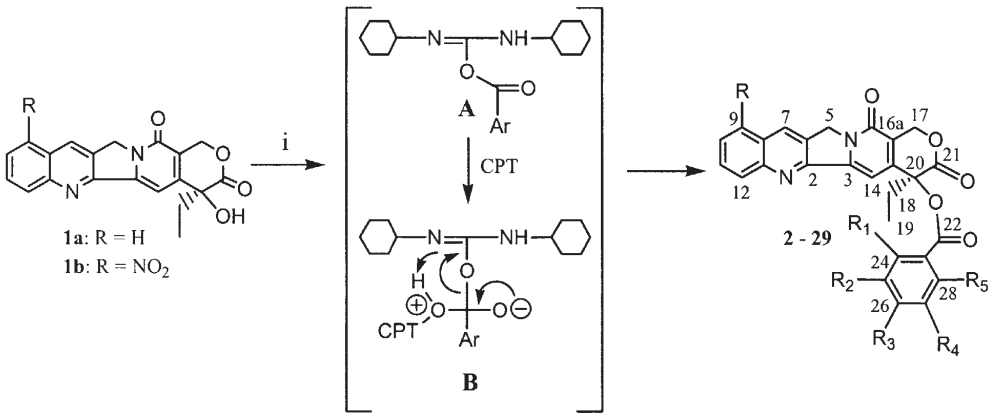
Dry nitrogen was routinely used as the reaction atmosphere in all reactions. All glassware was baked at 70±10˚C for a minimum of 2 h before being used. Melting points were obtained with a Mel-Temp melting point apparatus and were uncorrected. The ^{1}H NMR spectrum of approximately 10% (w/v) solution in $CDCl_3$ or DMSO was obtained at 270.05 MHz with a Jeol GX-270 WB NMR spectrometer. Chemical shifts are reported in parts per million (δ scale), employing tetramethylsilane as an internal standard. In reporting the NMR data, we used the following abbreviations: coupling constants in Hertz (J), singlet (s), doublet (d), triplet (t), broad singlet (bs) and multiplet (m). Mass spectra were recorded using a VG ZAB-SEQ mass spectrometer (VG Analytical Co., UK) with a resolution of 10,000. Routinely used solvents such as THF and methylene chloride were dried and freshly distilled. Silica gel (230-400 mesh, Aldrich) for column chromatography was used for all product separations. Eastman chromagram (silica gel with fluorescent indicator on polyethylene) sheets were employed in thin-layer chromatography (TLC) operations. The numbering system used in reporting NMR data is shown in Table I.

The dicyclohexylcarbodiimide(DCC)/dimethylaminopyridine (DMAP) reagent system is frequently used for the esterification reaction of carboxylic acids with alcohols. This procedure offers a convenient method for the acylation of alcohols and thiols with carboxylic acids under mild conditions (24-26). The success of the reaction depends on the high efficiency of dimethylaminopyridine as a nucleophilic catalyst in group transfer reactions (27). The acylation proceeds without the need of a pre-formed, activated carboxylic acid derivative, at room temperature, under nonacidic or mildly basic, conditions. A variety of aprotic solvents of comparable polarity such as methylene chloride, diethyl ether, tetrahydrofuran, and acetonitrile can be used. Two research groups used this method to successfully prepare water-soluble camptothecin glycinates (28,29). We also used this chemistry to synthesize our aromatic esters of camptothecins.

Camptothecin **(1a)** and 9-nitrocamptothecin **(1b)** were allowed to react with aromatic carboxylic acids and dicyclohexylcarbodiimide with DMAP as the catalyst to give the corresponding ester products **2-29** as depicted in Fig. 1. The reaction yields varied from 5 to 96% depending on the nature of the substituting group on the benzene ring of carboxylic acids. For example, the reaction of **1b** with 2-hydroxyl benzoic acid gave product **23** in only a 5% yield; while the reaction of **1a** with p-nitrobenzoic acid gave product **7** in a 96% yield. The starting aromatic acids react with dicyclohexyldiimide under room temperature with dimetylaminopyridine as the catalyst to form intermediate product **A**. The subsequent nucleophilic substitution of **A** with **1a** or **1b** gives ester products. When the Ar group has an electron-donating group on its benzene ring, the carbonyl carbon of **A** becomes less cationic, which makes the nucleophilic substitution by **1a** or **1b** more difficult. When the Ar group of **A** has an electron-withdrawing group on its benzene ring, the nucleophilic substitution by **1a** or **1b** becomes easier due to the highly cationic nature of the carbonyl carbon of **A**. A strong electron-donating group such as the N,N-dimethyl amino group can even prevent the reaction from occurring. For example, the reaction between camptothecin **1a** and 4-N,N-dimethylaminobenzoic acid under the same reaction conditions did not produce any ester product; **1a** was completely recovered. We only isolated one product for most reactions, but the reaction of 2-nitrobenzoic acid with 9-nitrocamptothecin **1b** gave two isomeric esters, **10** and **29**, in the same amount of yield (50/50) with a total yield of 9%. As depicted in Fig. 2, attacking both sides of the carbonyl group of intermediate **A** by **1b**, produces isomeric esters **10** and **29** equally. Esters **10** and **29** are not convertible into each other as rotation around the bond C_{20}-O-C_{22}-C_{23} is not possible due to the stereo hindrance between the C19-methyl group and the nitro group attaching on either C_{24} or C_{28}.

The starting camptothecin was purchased from P.R. China and was purified before being used. The starting 9-nitrocamptothecin was prepared following an established procedure (30). All other chemicals were from Aldrich Chemical Co. (Milwaukee, WI) and used as purchased. Preparation of the new aromatic esters of the camptothecins, and the corresponding structural data of these new compounds are described compound by compound as follows.

Camptothecin-20-O-benzoate [2]. Camptothecin (0.8 g, 0.0023 mol), benzoic acid (1.8 g, 0.014 mol), DCC (1.2 g, 0.0058 mol), and DMAP (0.3 g, 0.0025 mol) were added to 60 ml DMF in a 250-ml round-bottomed flask equipped with a mechanical stirrer. The mixture was stirred under N_2 at room temperature for 72 h. Dicyclohexyl urea formed during the reaction was removed by filtration. The filtrate was poured onto 600 ml ice water while stirring. The stirring was maintained for 30 min. After filtration the crude product was chromatographically separated with THF-CH_2Cl_2 (1:15) as eluent. Pure product **2** (0.4 g) was obtained as a white powder after precipitation from petroleum ether, yield 38%, purity 99% (HPLC). ^{1}H NMR in DMSO: δ 1.13 (3H, t,



i. Aromatic carboxylic acid, DCC, DMAP, DMF, and rt

2: R = R_1 = R_2 = R_3 = R_4 = R_5 = H
3: R = R_1 = R_2 = R_4 = R_5 = H, R_3 = CF_3
4: R = R_1 = R_3 = R_4 = R_5 = H, R_2 = CF_3
5 : R = R_2 = R_4 = R_5 = H, R_1 = R_3 = NO_2
6: R = R_1 = R_3 = R_5 = H, R_2 = R_4 = NO_2
7: R = R_1 = R_2 = R_4 = R_5 = H, R_3 = NO_2
8: R = R_1 = R_3 = R_4 = R_5 = H, R_2 = NO_2
9: R = NO_2, R_1 = R_2 = R_3 = R_4 = R_5 = H
10: R = R_1 = NO_2, R_2 = R_3 = R_4 = R_5 = H
11: R = R_2 = NO_2, R_1 = R_3 = R_4 = R_5 = H
12: R = R_3 = NO_2, R_4 = R_5 = R_1 = R_2 = H
13: R = NO_2, R_1 = R_3 = R_4 = R_5 = H, R_2 = CN
14: R = NO_2, R_1 = R_2 = R_4 = R_5 = H, R_3 = CN
15: R = NO_2, R_1 = F, R_2 = R_3 = R_4 = R_5 = H;
16: R = NO_2, R_1 = R_3 = R_4 = R_5 = H, R_2 = F
17: R = NO_2, R_1 = R_2 = R_4 = R_5 = H, R_3 = F
18: R = NO_2, R_1 = Cl, R_2 = R_3 = R_4 = R_5 = H
19: R = NO_2, R_1 = R_3 = R_4 = R_5 = H, R_2 = Cl
20: R = NO_2, R_1 = R_2 = R_4 = R_5 = H, R_3 = Cl
21: R = NO_2, R_1 = Br, R_2 = R_3 = R_4 = R_5 = H
22: R = NO_2, R_1 = R_3 = R_4 = R_5 = H, R_2 = Br
23: R = NO_2, R_1 = OH, R_2 = R_3 = R_4 = R_5 = H
24: R = R_1 = R_3 = NO_2, R_2 = R_4 = R_5 = H
25: R = R_2 = R_4 = NO_2, R_1 = R_3 = R_5 = H
26: R = R_2 = R_4 = NO_2, R_1 = R_5 = H, R_3 = CH_3
27: R = NO_2, R_1 = R_3 = R_4 = R_5 = H, R_2 = CF_3
28: R = NO_2, R_1 = R_2 = R_4 = R_5 = H, R_3 = CF_3
29: R = NO_2, R_1 = R_2 = R_3 = R_4 = H, R_5 = NO_2

Figure 1. Ester products **2-29** derived from camptothecin and 9-nitrocamptothecin.

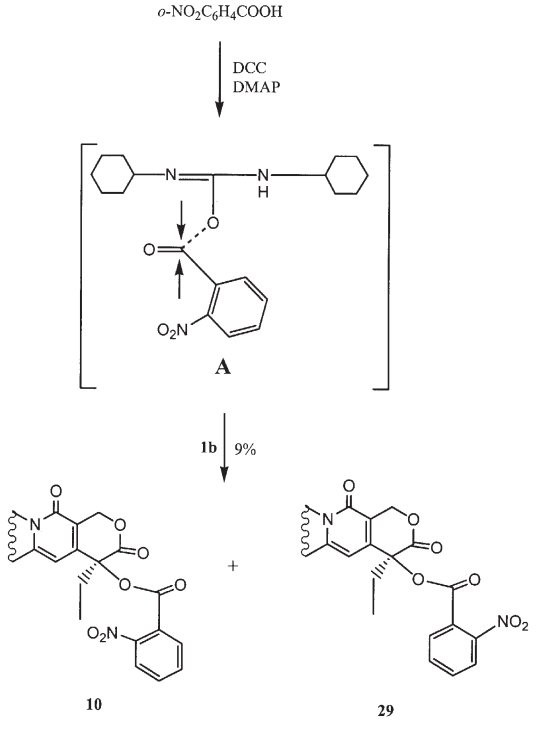


Figure 2. Isomeric esters **10** and **29** are produced in equal amounts by the reaction of 2-nitrobenzoic acid and 9-nitrocamptothecin.

J=7.04 Hz, C19-methyl protons), 2.20-2.40 (2H, m, C18-methylene protons), 5.28 (2H, s, C5-methylene protons), 5.56 (2H, s, C17-methylene protons), 7.05 (1H, s, C14-H), 7.50-8.20 (9H, m, C9-C12-Hs and C23-C28-Hs), 8.68 (1H, s, C7-H); ^{13}C NMR (DMSO): δ 8.0 (C19), 32.2 (C18), 49.0 (C5), 64.9 (C17), 74.8 (C20), 92.6 (C14), 117.5, 125.8, 126.0, 126.3, 126.8, 127.0, 127.2, 127.6, 128.0, 128.6, 130.1, 133.0, 144.1, 144.8, 146.3, 150.5, 155.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 162.8, 165.9 (C21, C22); mass m/e (relative intensity): 452 (m^+, 25), 330 (100), 315 (35), 302 (60), 287 (40), 169 (12), 122 (36), 105 (70), 77 (35), 69 (26); precise mass ($C_{27}H_{20}N_2O_5$): found, 452.137; required, 452.137.

*Camptothecin-20-O-p-triflouromethylbenzoate [**3**].* Using camptothecin (0.8 g, 0.0023 mol), trifluoro-p-toluic acid (1.6 g, 0.0084 mol), DCC (1.2 g, 0.0058 mol), DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **3** (1 g) was obtained as a white powder, yield 84%, purity 99% (HPLC). ^{1}H NMR (DMSO): δ 1.11 (3H, t, J=7.10 Hz, C19-methyl protons), 2.25-2.50 (2H, m, C18-methylene protons), 5.28 (2H, s, C5-methylene protons), 5.56 (2H, s, C17-methylene protons), 7.12 (1H, s, C14-H), 7.66 (1H, t, J=8.50 Hz, C10-H), 7.80 (1H, t, J=8.48 Hz, C11-H), 8.00 (2H, d, J=8.46 Hz, C9-H, C12-H), 8.12 (2H, d, J=8.38 Hz, C24-H, C28-H), 8.35

(2H, d, J=8.34 Hz, C25-H, C27-H), 8.68 (1H, s, C7-H); ^{13}C NMR (DMSO): δ 7.6 (C19), 30.5 (C18), 47.5 (C26-$\underline{C}F_3$), 50.4 (C5), 66.5 (C17), 77.1 (C20), 119.0, 126.0, 127.6, 127.8, 128.2, 128.8, 129.7, 130.1, 130.6, 131.4, 132.0, 145.0, 146.3, 147.3, 152.0, 156.5 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.0, 166.5 (C21, C22); mass m/e (relative intensity): 520 (m^+, 45), 330 (100), 315 (20), 302 (78), 173 (40), 147 (9), 56 (12); precise mass ($C_{28}H_{19}N_2O_5F_3$): found, 520.124; required, 520.125.

*Camptothecin-20-O-m-triflouromethylbenzoate [**4**]*. Using camptothecin (0.8 g, 0.0023 mol), trifluoro-m-toluic acid (1.7 g, 0.0089 mol), DCC (1.2 g, 0.0058 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **4** (1.07 g) was obtained as a white powder, yield 89%, purity 99% (HPLC). ^{1}H NMR (DMSO): δ 1.08 (3H, t, J=7.08 Hz, C19-methyl protons), 2.30-2.50 (2H, m, C18-methylene protons), 5.28 (2H, s, C5-methylene protons), 5.58 (2H, s, C17-methylene protons), 7.18 (1H, s, C14-H), 7.66 (1H, t, J=8.14 Hz, C10-H), 7.80 (1H, t, J=8.09 Hz, C11-H), 7.90 (1H, t, J=8.08 Hz, C25-H), 8.08 [2H, t (d+d), J=8.08 Hz, C9-H, C12-H], 8.16 (1H, d, J=8.07 Hz, C24-H), 8.36 (1H, s, C28-H), 8.44 (1H, d, J=8.08 Hz, C26-H), 8.68 (1H, s, C7-H); ^{13}C NMR (DMSO): δ 7.6 (C19), 33.0 (C18), 46.5 (C27-CF3), 47.2 (C5), 65.0 (C17), 76.0 (C20), 95.5 (C14), 117.0, 124.5, 126.0, 126.2, 127.0, 127.1, 127.5, 128.0, 128.5, 128.8, 129.0, 129.5, 132.2, 143.2, 144.5, 146.0, 150.8, 155.2 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 161.5, 165.5 (C21, C22); mass m/e (relative intensity): 520 (m^+, 50), 330 (100), 315 (40), 302 (97), 287 (38), 246 (6), 190 (10), 173 (56), 145 (38), 124 (3), 75 (3); precise mass ($C_{28}H_{19}N_5F_3$): found, 520.125; required, 520.125.

*Camptothecin-20-O-o,p-dinitrobenzoate [**5**]*. Using camptothecin (0.8 g, 0.0023 mol), 2,4-dinitrobenzoic acid (2 g, 0.0094 mol), DCC (1.3 g, 0.0063 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **5** (0.13 g) was obtained as a white powder, yield 10%, purity 99% (HPLC). ^{1}H NMR in $CDCl_3$: δ 1.10 (3H, t, J=7.06 Hz, C19-methyl protons), 2.20-2.45 (2H, m, C18-methylene protons), 5.32 (2H, s, C5-methylene protons), 5.40-5.85 (2H, dd, J=18.50, 18.52 Hz, C17-methylene protons), 7.53 (1H, s, C14-H), 7.66 (1H, t, J=8.05 Hz, C10-H), 7.83 (1H, t, J=8.07 Hz, C11-H), 7.96 (1H, d, J=8.08 Hz, C9-H), 8.18 (1H, d, J=8.07 Hz, C12-H), 8.24 (1H, d, J=8.05 Hz, C28-H), 8.42 (1H, s, C25-H), 8.60 (1H, d, J=8.06 Hz, C27-H), 8.85 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.8 (C19), 32.1 (C18), 50.3 (C5), 67.4 (C17), 78.4 (C20), 95.4 (C14), 120.2, 123,4, 128.3, 128.5, 129.4, 129.6, 130.6, 131.3, 132,4, 144.5, 146.6, 148.6, 152.1, 157.3 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.5, 166.7 (C21, C22); mass m/e (relative intensity): 542 (m^+, 10), 330 (100), 315 (38), 302 (78), 287 (42), 272 (12), 195 (10), 168 (40), 120 (20), 75 (18); precise mass ($C_{27}H_{18}N_4O_9$): found, 542.107; required, 542.107.

*Camptothecin-20-O-m,m-dinitrobenzoate [**6**]*. Using camptothecin (0.8 g, 0.0023 mol), 3, 5-dinitrobenzoic acid (2 g, 0.0094 mol), DCC (1.3 g, 0.0063 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **6** (1.2 g) was obtained as a white powder, yield 96%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.13 (3H, t, J=7.03 Hz, C19-methyl protons), 2.30-2.60 (2H, m, C18-methylene protons), 5.35 (2H, s, C5-methylene protons), 5.45-5.85 (2H, dd, J=17.90, 17.98 Hz, C17-methylene protons), 7.20 (1H, s, C14-H), 7.66 (1H, t, J=8.06 Hz, C10-H), 7.78 (1H, t, J=8.04 Hz, C11-H), 7.94 (1H, d, J=8.06 Hz, C9-H), 8.13 (1H, d, J=8.05 Hz, C12-H), 8.40 (1H, s, C26-H), 9.18 (2H, s, C24-H, C28-H), 9.26 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.5 (C5), 67.2 (C17), 78.3 (C20), 95.4 (C14), 120.2, 123.1, 128.1, 128.3, 129.5, 129.8, 130.9, 131.4, 132.6, 144.6, 146.8, 148.5, 152.0, 157.2 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 161.8, 166.5 (C21, C22); mass m/e (relative intensity): 542 (m^+, 4), 330 (44), 317 (20), 235 (10), 212 (100), 195 (15), 150 (35), 93 (25), 75 (23); precise mass ($C_{27}H_{18}N_4O_9$): found, 542.109; required, 542.107.

*Camptothecin-20-O-p-nitrobenzoate [**7**]*. Using camptothecin (0.8 g, 0.0023 mol), p-nitrobenzoic acid (2 g, 0.0120 mol), DCC (1.2 g, 0.0063 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **7** (1.1 g) was obtained as a white powder, yield 96%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.06 Hz, C19-methyl protons), 2.30-2.60 (2H, m, C18-methylene protons), 5.50 (2H, s, C5-methylene protons), 5.71-6.10 (2H, dd, J=18.01, 19.04 Hz, C17-methylene protons), 7.23 (1H, s, C14-H), 7.78 (1h, t, J=8.05 Hz, C10-H), 7.98 (1H, t, J=8.06 Hz, C11-H), 8.05 (1H, d, J=8.04 Hz, C9-H), 8.21 (1H, d, J=8.06 Hz, C12-H), 8.30-8.60 (4H, m, C24-H, C25-H, C27-H, C28-H), 9.10 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.2 (C5), 67.3 (C17), C20 buried in the area of solvent peaks, 95.8 (C14), 121.5, 123.8, 128.3, 128.5, 129.5, 131.0, 131.5, 134.0, 145.2, 146.5, 148.8, 151.1, 152.0, 156.2, 157.1 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.6, 167.0 (C21, C22); mass m/e (relative intensity): 497 (m^+, 35), 330 (100), 315 (30), 302 (86), 287 (38), 205 (8), 179 (12), 113 (16), 100 (35), 65 (20); precise mass ($C_{27}H_{19}N_3O_7$): found, 497.122, required, 497.122.

*Camptothecin-20-O-m-nitrobenzoate [**8**]*. Using camptothecin (0.8 g, 0.0023 mol), m-nitrobenzoic acid (2 g, 0.0120 mol), DCC (1.2 g, 0.0058 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **8** (1.1 g) was obtained as a white powder, yield 96%, purity 99%(HPLC). ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.08Hz, C19-methyl protons), 2.20-2.60 (2H, m, C18-methylene protons), 5.30 (2H, s, C5-methylene protons), 5.40-5.82 (2H, dd, J=17.53, 17.56 Hz, C17-methylene protons), 7.24 (1H, s, C14-H), 7.56-7.86 (3H, m, C10-H, C11-H, C25-H), 7.88-8.20 (2H, dd, J=8.05, 8.07 Hz, C9-H, C12-H), 8.3-8.55 (3H, m, C24-H, C26-H, C28-H), 8.95 (1H, s, C7-H). ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 31.8 (C18), 49.8 (C5), 66.7 (C17), C20 buried in solvent peaks, 95.5 (C14), 120.4, 1224.5, 124.6, 127.7, 127.9, 128.1, 129.5, 129.7, 129.8, 130.1, 130.5, 130.9, 135.7, 145.0, 146.4, 148.1, 148.5, 151.9, 159.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.0, 166.9 (C21, C22). Mass m/e (relative intensity): 497 (m^+, 6), 330 (28), 315 (12), 302 (18), 287 (15), 167 (l00), 121 (40), 100 (10), 65 (35); precise mass ($C_{27}H_{19}N_3O_7$): found, 497.122; required, 497.122.

*mptothecin-20-O-benzoate [**9**]*. Using 9-nitrocampto-5 g, 0.0038 mol), benzoic acid (1 g, 0.0082 mol), DCC (1.7 g, 0.0083 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **9** (0.2 g) was obtained as a yellow powder, yield 11%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3h, t, J=7.05 Hz, C19-methyl protons), 2.20-2.45 (2H, m, C18-methylene protons), 5.36 (2H, s, C5-methylene protons), 5.48-5.85 (2H, dd, J=18.03, 18.05 Hz, C17-methylene protons), 7.21 (1H, s, C14-H), 7.60-8.60 (8H, m, C10-C12-Hs, and C24-C28-Hs), 9.22 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.1 (C18), 50.6 (C5), 67.2 (C17), 78.3 (C20), 97,6 (C14), 121.0, 124.1, 126.3, 127.2, 128.1, 131.1, 131.5, 132.5, 133.0, 137.0, 145.2, 145.6, 149.1, 154.2, 157.3 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.8, 166.9 (C21, C22); mass m/e (relative intensity): 497 (m^+, 10), 392 (6), 375 (100), 360 (35), 347 (80), 332 (30), 319 (15), 302 (10), 286 (20), 274 (8), 258 (5), 216 (7); precise mass ($C_{27}H_{19}N_3O_7$): found, 497.123; required, 497.122.

*9-Nitrocamptothecin-20-O-o-nitrobenzoate [isomers **10** and **29**]*. Using 9-nitrocamptothecin (0.8 g, 0.0020 mol), 2-nitrobenzoic acid (0.8 g, 0.0048 mol), DCC (1 g, 0.0049 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, two isomers were obtained as a yellow powder, total yield 9%. Isomer **10**: ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, J=7.08 Hz, C19-methyl protons), 2.25-2.42 (2H, m, C18-methylene protons), 5.44 (2H, s, C5-methylene protons), 5.45-5.82 (2H, dd, J=17.51, 17.58 Hz, C17-methylene protons), 7.24 (1H, s, C14-H), 7.60-8.58 (7H, m, C10-C12-Hs, and C24-C27-Hs), 9.26 (1H, s, C7-H); ^{13}CNMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.8 (C5), 67.5 (C17), 78.0 (C20), 97.9 (C14), 121.1, 124.1, 126.0, 127.4, 128.7, 131.0, 131.4, 132.7, 133.5, 137.1, 145.5, 145.8, 149.0, 154.0, 157.5 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 167.0 (C21, C22); mass m/e (relative intensity): 542 (m^+, 2), 389 (38), 375 (85), 347 (100), 361 (35), 332 (80), 306 (55), 286 (36), 272 (25), 260 (16), 230 (18), 203 (12); precise mass ($C_{27}H_{18}N_4O_9$): found, 542.108; required, 542.107. Isomer **29**: ^{1}H NMR ($CDCl_3$): δ 1.14 (3H, t, J=7.06 Hz, C19-methyl protons), 2.28-2.46 (2H, m, C18-methylene protons), 5.40 (2H, s, C5-methylene protons), 5.45-5.84 (2H, dd, J=17.50, 17.55 Hz, C17-methylene protons), 7.25 (1H, s, C14-H), 7.60-8.58 (7H, m, C10-Cl2-Hs, and C25-C28-Hs), 9.35 (1H, s, C7-4); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.1 (C18), 50.8 (C5), 67.5 (C17), 78.0 (C20), 96.6 (C14), 121.4, 124.0, 126.0, 127.5, 128.8, 131.1, 131.4, 132.7, 133.3, 137.1, 145.4, 145.8, 148.9, 149.2, 157.6 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 167.0 (C21,C22); mass m/e (relative intensity): 542 (m^+, 2), 389 (38), 375 (85), 347 (100), 36l (35), 332 (80), 306 (55), 286 (36), 272 (25), 260 (16), 230 (18), 203 (12); precise mass ($C_{27}H_{18}N_4O_9$): found, 542.108; required, 542.107.

*9-Nitrocamptothecin-20-O-m-nitrobenzoate [**11**]*. Using 9-nitrocamptothecin (0.6 g, 0.0015 mol), 3-nitrobenzoic acid (0.8 g, 0.0048 mol), DCC (1 g, 0.0049 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **11** (0.3 g) was obtained as a yellow powder, yield 37%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.13 (3H, t, J=7.0 Hz, C19-methyl protons), 2.30-2.60 (2H, m, C18-methylene protons), 5.40 (2H, s, C5-methylene protons), 5.46-5.85 (2H, dd, J=17.50, 17.55 Hz, C17-methylene protons), 7.25 (1H, s, C14-H), 7.72 (1H, t, J=8.02 Hz, C25-H), 7.88 (1H, t, J=8.01 Hz, C11-H), 8.37-8.52 (4H, m, C10-H, C12-H, C24-H, C26-H), 8.95 (1H, s, C28-H), 9.28 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.8 (C5), 67.5 (C17), C20 buried by $CHCl_3$ peaks, 96.7 (C14), 121.0, 121.8, 125.0, 126.1, 127.7, 128.5, 128.8, 130.1, 130.8, 131.6, 135.9, 136.5, 145.5, 146.0, 148.2, 148.5, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.4, 166.9 (C21,C22); mass m/e (relative intensity): 542 (m^+, 3), 389 (20), 375 (100), 360 (38), 347 (78), 332 (58), 306 (30), 286 (261), 272 (15), 258 (10), 229 (8); precise mass ($C_{27}H_{18}N_4O_9$): found 542.107; required, 542.107.

*9-Nitrocamptothecin-20-O-p-nitrobenzoate [**12**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 4-nitrobenzoic acid (0.5 g, 0.0030 mol), DCC (0.8 g, 0.0039 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **12** (0.18 g) was obtained as a yellow powder, yield 26%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, J=7.05 Hz, C19-methyl protons), 2.20-2.53 (2H, m, C18-methylene protons), 5.39 (2H, s, C5-methylene-protons), 5.40-5.83 (2H, dd, J=17.50, 17.54 Hz, C17-methylene protons), 7.22 (1H, s, C14-H), 7.86 [1H, t (d+d), J=8.03 Hz, C11-H], 8.20-8.60 (6H, m, C10-H, C12-H, C24-H, C25-H, C27-H, and C28-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.1 (C5), 67.3 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 96.6 (C14), 121.0, 121.8, 123.9, 126.0, 127.8, 128.8, 130.3, 131.4, 131.5, 133.9, 136.5, 137.0, 145.1, 146.0, 148.5, 151.4, 153.5, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.6, 166.6 (C21, C22); mass m/e (relative intensity): 542 (m^+, 8), 375 (95), 347 (100), 333 (14), 304 (8), 258 (6), 203 (4); Precise mass ($C_{27}H_{18}N_4O_9$): found, 542.109; required, 542.107.

*9-Nitrocamptothecin-20-O-m-cyanobenzoate [**13**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 3-cyanobenzoic acid (1 g, 0.0068 mol), DCC (1.5 g, 0.0073 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **13** (0.26 g) was obtained as a yellow powder, yield 38%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.14 (3H, bs, C19-methyl protons), 2.25-2.26 (2H, m, C18-methylene protons), 5.38 (2H, s, C5-methylene protons), 5.40-5.78 (2H, dd J=17.50, 17.54 Hz, C17-methylene protons), 7.21 (1H, s, C14-H), 7.60-7.70 (1H, m, C25-H), 7.80-7.96 (2H, m, C11-H, C24-H), 8.25-8.55 (4H, m, C10-H, C12-H, C26-H, C28-H), 9.28 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): 7.9 (C19), 32.5 (C18), 50.8 (C5), 67.5 (C17), C20 buried by $CHCl_3$ peaks in the area of 76.0-78.0 ppm, 96.8 (C14), 113.2, 117.8, 121.0, 121.8, 126.1, 127.8, 128.8, 129.9, 130.1, 131.6, 133.9, 134.1, 136.7, 137.0, 145.4, 146.0, 148.4, 153.1, 156.1, 156.5 (C2, C3, C6-C13, C15, C216, C16a, C23-C28, C27-cyano carbon), 163.1, 166.5 (C21, C22); mass m/e (relative intensity): 522 (m^+, 3), 389 (18), 375 (100), 360 (40), 347 (80), 332 (61), 306 (56), 286 (35), 272 (15), 216 (10); precise mass ($C_{28}H_{18}N_4O_7$): found, 522.117; required, 522.118.

*9-Nitrocamptothecin-20-O-p-cyanobenzoate [**14**]*. Using 9-nitrocamptothecin (0.58 g, 0.0015 mol), 4-cyanobenzoic

acid (1 g, 0.0068 mol), DCC (1.5 g, 0.0073 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **14** (0.3 g) was obtained as a yellow powder, yield 38%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.15 (3H, t, J=7.05 Hz, C19-methyl protons), 2.30-2.60 (2H, m, C18-methylene protons), 5.44 (2H, s, C14-H), 5.55-5.85 (2H, dd, J=17.50, 17.53 Hz, C17-methylene protons), 7.30 (1H, s, C14-H), 7.85 (2H, d, J=8.08 Hz, C24-H, C28-H, 7.94 (1H, t, J=8.02 Hz, C11-H), 8.26 (2H, d, J=8.09 Hz, C25-H, C27-H), 8.45-8.55 (2H, m, C10-H, C12-H), 9.33 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 8.0 (C19), 32.0 (C18), 50.6 (C5), 67.5 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 96.6 (C14), 117.2, 117.3, 121.0, 121.4, 126.0, 127.5, 128.5, 130.6, 131.6, 132.7, 136.7, 145.2, 145.9, 149.0, 153.9, 157.4, (C2, C3, C6-C13, C15, C16, C16a, C23-C28, C26-cyano carbon), 163.6, 166.8 (C21, C22); mass m/e (relative intensity): 522 (m^+, 2), 389 (4), 375 (100), 360 (35), 347 (85), 332 (55), 306 (20), 286 (25), 272 (8), 229 (5), 203 (2); precise mass ($C_{28}H_{18}N_4O_7$): found, 522.118; required, 522.118.

*9-Nitrocamptothecin-20-O-o-fluorobenzoate [**15**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 2-fluorobenzoic acid (1 g, 0.0071 mol), DCC (1.5 g, 0.0073 mol), and DMAP (0.2 g, 0.0016 mol) as starting reaction materials, pure product **15** (0.12 g) was obtained as a yellow powder, yield 18%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.08 Hz, C19-methyl protons), 2.20-2.50 (2H, m, C18-methylene protons), 5.39 (2H, s, C5-methylene protons), 5.45-5.84 (2H, dd, J=17.51, 17.58 Hz, C17-methylene protons), 7.24 (1H, s, C14-H), 7.16-7.40 (2H, m, C24-H, C27-H), 7.52-7.70 (1H, m, C25-H), 7.89 (1H, t, J=8.09 Hz, C11-H), 8.05 (1H, t, J=6.8HZ, C26-H), 8.36-8.60 (2H, m, C10-H, C12-H), 9.28 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.6 (C5), 67.4 (C17), C20 buried by $CHCl_3$ peaks, 97.1 (Cl4), 117.0, 117.6, 121.0, 121.6, 124.4, 125.9, 127.4, 128.7, 131.4, 122.8, 135.8, 136.7, 145.0, 146.0, 148.6, 157.2, 160.8 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.5, 167.2 (C22, C22); mass m/e (relative intensity): 515 (m^+, 2), 375 (22), 347 (18), 332 (8), 286(3), 140 (63), 123 (100), 45 (36), 75 (16); precise mass ($C_{27}H_{18}N_3O_7F$), found, 515.113; required, 515.113.

*9-Nitrocamptothecin-20-O-m-fluorobenzoate [**16**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 3-fluorobenzoic acid (1 g, 0.0071 mol), DCC (1.5 g, 0.0073 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **16** (0.6 g) was obtained as a yellow powder, yield 90%, purity 99%. ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.08 Hz, C19-methyl protons), 2.23-2.55 (2H, m, C18-methyl protons), 5.33 (2H, s, C5-methylene protons), 5.42-5.82 (2H, dd, J=17.50, 17.54 Hz, C 17-methylene protons), 5.42-5.82 (2H, dd, J=17.50, 17.541 Hz, C17-methylene protons), 7.25 (1H, s, C14-H), 7.30-7.52 (2H, m, C24-H, C26-H), 7.75-7.93 (3H, m, C14-H, C25-H, C28-4), 8.40-8.50 (2H, d, J=8.08 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.8 (C5), 67.5 (C17), C20 buried by solvent peaks, 97.0 (C14), 118.0, 118.2, 121.0, 121.4, 122.0, 126.2, 127.4, 128.6, 131.0, 131.2, 131.9, 137.0, 145.0, 145.5, 145.7, 148.5, 153.9, 157.5, 161.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.3, 167.0 (C21, C22); mass m/e (relative intensity): 5l5 (m^+, 7), 375 (38), 347 (32), 332 (10), 286 (3), 140 (60), 123 (l00), 95 (50), 175 (15); precise mass ($C_{27}H_{18}N_3O_7F$): found, 515.133, required, 515.133.

*9-Nitrocamptothecin-20-O-p-fluorobenzoate [**17**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 4-fluorobenzoic acid (1 g, 0.0071 mol), DCC (1.5 g, 0.0073 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **17** (0.15 g) was obtained as a yellow powder, yield 22%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.04 Hz, C19-methyl protons), 2.20-2.50 (2H, m, C18-methylene protons), 5.38 (2H, s, C5-methylene protons), 5.4l-5.82 (2H, dd, J=17.42, 17.49 Hz, C17-methylene protons), 7.18 [2H, t (d+d), J=8.05 Hz, C25-H,C27-H], 7.27 (1H, s, C14-H), 7.85 (1H, t, J=8.04 Hz, C11-H), 8.10-8.20 (2H, d+d, J=8.06, 8.05 Hz, C24-H, C28-H), 8.45 (2H, d, J=8.08 Hz, C10-H, C12-H), 9.26 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.2 (C18), 50.6 (C5), 67.6 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 97.3 (C14), 116.0, 116.3, 121.0, 121.6, 125.0, 126.0, 127.5, 128.0, 131.6, 133.2, 136.3, 145.0, 146.0, 148.6, 153.4, 156.8, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.2, 168.1 (C21,C22); mass m/e (relative intensity): 515 (m^+, 8), 375 (60), 360 (14), 347 (50), 332 (10), 155 (6), 140 (18), 123 (100), 95(30); precise mass ($C_{27}H_{18}N_3O_7F$): found, 515.113; required, 515.113.

*9-Nitrocamptothecin-20-O-o-chlorobenzoate [**18**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 2-chlorobenzoic acid (1 g, 0.0063 mol), DCC (1.5 g, 0.0073 mol), and AMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **18** (0.25 g) was obtained as a yellow powder, yield 36%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.10 Hz, C19-methyl protons), 2.20-2.50 (2H, m, C18-methylene protons), 5.38 (2H, s, C5-methlene protons), 5.42-5.85 (2H, dd, J=17.51, 17.56 Hz, C17-methylene protons), 7.38 (1H, s, C14-H), 7.35-7.50 (3H, m, C25-H, C26-H, C27-H), 7.87 (1H, t, J=8.08 Hz, C11-H), 8.05 (lH, d, J=8.06 Hz, C24-H), 8.44-8.54 (2H, d+d, J=8.05, 8.08 Hz, C10-H, C12-H), 9.26 (1H, s, C7-H); ^{13}C NMR: δ 7.9 (C19), 32.3 (C18), 50.8 (C5), 67.7 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 97.3 (C14), 121.0, 121.2, 125.9, 126.9, 127.4, 128.7, 131.3, 131.6, 132.2, 133.7, 134.8, 136.8, 145.0, 146.0, 148.8, 153.7, 156.6, 158.9 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 166.8 (C21, C22); mass m/e (relative intensity): 53l (m^+, weak), 375 (100), 360 (20), 347 (85), 335 (80), 285 (38), 235 (38), 185 (8), 147 (75), 139 (85), 111 (18), 97 (25), 77 (15); precise mass ($C_{27}H_{18}N_3O_7Cl$): found, 531.083; required, 531.083.

*9-Nitrocamptothecin-20-O-m-chlorobenzoate [**19**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 3-chlorobenzoic acid (0.5 g, 0.0032 mol), DCC (0.8 g, 0.0039 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **19** (0.06 g) was obtained as a yellow powder, yield 9%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.04 Hz, C19-methyl protons), 2.20-2.54 (2H, m, C18-methyl protons), 5.38 (2H, s, C5-methylene protons), 5.40-5.83 (2H, dd, J=17.52, 17.55 Hz, C17-methylene protons),

s, C14-H), 7.44 [1H, t (d+d), J=8.06 Hz, C25-H], d, J=8.08 Hz, C26-H), 7.86 [1H, t (d+d), J=8.08 Hz, C11-H], 7.98 (1H, d, J=8.04 Hz, C24-H), 8.10 (1H, s, C28-H), 8.45 (2H, d, J=8.06 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (48), 50.4 (C5), 67.0 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 96.9 (C14), 120.8, 121.5, 125.8, 127.4, 128.3, 128.6, 129.7, 129.8, 129.9, 131.2, 134.0, 134.9, 136.6, 144.9, 145.5, 145.8, 148.8, 157.2 (C2, C3, C6-Cl3, C15, C16, Cl6a, C23-C28), 164.2, 167.1 (C21, C22); mass m/e (relative intensity): 531 (m^+, 1), 375 (35), 360 (6), 347 (25), 243 (15), 231 (15), 156 (100), 139 (90), 119 (82), 111 (35), 100 (28), 75 (8); precise mass ($C_{27}H_{18}N_3O_7Cl$): found, 531.084; required, 531.083.

*9-Nitrocamptothecin-20-O-p-chlorobenzoate [**20**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 4-chlorobenzoic acid (0.5 g, 0.0032 mol), DCC (1 g, 0.0049 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **20** (0.05 g) was obtained as a yellow powder, yield 7%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, J=7.06 Hz, C19-methyl protons), 2.20-2.50 (2H, m, C18-methylene protons), 5.35 (2H, s, C5-methylene protons), 5.40-5.82 (2H, dd, J=17.51, 17.55 Hz, C17-methylene protons), 7.23 (1H, s, C14-H), 7.47 (2H, d, J=8.09 Hz, C25-H, C27-H), 7.86 (1H, t, J=8.0 Hz, C11-H), 8.04 (2H, d, J=8.07 Hz, C24-H, C28-H), 8.43 (2H, d, J=8.03 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 50.5 (C5), 67.2 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 96.8 (C14), 120.9, 121.5, 125.9, 127.4, 128.8, 129.1, 131.5, 136.5, 140.4, 145.0, 145.8, 145.9, 148.7, 153.6, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.5, 167.0 (C21, C22); mass m/e (relative intensity): 531 (m^+, weak), 375 (95), 360 (35), 347 (70), 332 (38), l56 (43), 139 (100), 111 (35), 75 (10). Precise mass ($C_{27}H_{18}N_3O_7Cl$): found, 531.083; required, 531.083.

*9-Nitrocamptothecin-20-O-o-bromobenzoate [**21**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 2-bromobenzoic acid (1 g, 0.0050 mol), DCC (90.75 g, 0.0036 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **21** (0.14 g) was obtained as a yellow powder, yield 19%, purity 99% (HPLC). ^{1}H NMR (DMSO): δ 1.00 (3H, t, J=7.06 Hz, C19-methyl protons), 2.20-2.40 (2H, m, C18-methylene protons), 5.35 (2H, s, C25-methylene protons), 5.58 (2H, s, C17-methylene protons), 7.26 (1H, s, C14-H), 7.50-8.60 (7H, m, C10-C12-Hs, C25-C28-Hs), 9.15 (1H, s, C7-H); ^{13}C NMR (DMSO): δ 7.6 (C19), 30.6 (C18), 50.8 (C5), 66.1 (C17), 77.3 (C20), 95.5 (C14), 119.6, 119.9, 121.2, 125.1, 127.3, 128.6, 129.0, 131.8, 132.6, 134.0, 134.5, 136.0, 144.5, 144.8, 146.2, 147.5, 156.6 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 163.6, 166.4 (C21, C22); mass m/e (relative intensity): 577 (M+2, 5), 575 (m^+, 5), 375 (58), 347 (38), 332 (12), 286 (4), 202 (26), 183 (36), 84 (100); precise mass ($C_{27}H_{18}N_3O_7Br$): found, 575.032; required 575.033.

*9-Nitrcamptothecin-20-O-m-bromobenzoate [**22**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 3-bromobenzoic acid (0.5 g, 0.0025 mol), DCC (0.75 g, 0.0036 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **22** (0.06 g) was obtained as a yellow powder, yield 8%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.04 Hz, C19-methyl protons), 2.20-2.52 (2H, m, C18-methylene protons), 5.40 (2H, s, C5-methylene protons), 5.41-5.85 (2H, dd, J=17.50, 17.55 Hz, C17-methylene protons), 7.24 (1H, s, C14-H), 7.36 (1H, t, J=8.03 Hz, C25-H), 7.76 (1H, d, J=8.04 Hz, C26-H), 7.88 (1H, t, J=8.05 Hz, C11-H), 8.05 (1H, d, J=8.06 Hz, C24-H), 8.26 (1H, s, C28-H), 8.48 (2H, d, J=8.05 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 50.4 (C5), 67.5 (C17), C20 buried by $CHCl_3$, 96.9 (C14), 121.0, 121.8, 122.5, 125.9, 127.6, 128.7, 128.9, 130.2, 130.5, 131.3, 133.1, 136.6, 137.0, 145.1, 145.7, 145.9, 148.5, 153.5, 157.0, 157.6 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 167.0 (C21, C22); mass m/e (relative intensity): 575 (m^+, 5), 389 (10), 375 (100), 360 (35), 347 (74), 332 (48), 318 (8), 286 (16), 258 (8), 224 (8). Precise mass ($C_{27}H_{18}N_3O_7Br$): found, 575.032; required, 575.034.

*9-Nitrocamptothecin-20-O-o-hydroxylbenzoate [**23**]*. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 2-hydroxylbenzoic acid (0.5 g, 0.0036 mol), DCC (0.75 g, 0.0036 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **23** (0.03 g) was obtained as a yellow powder, yield 5%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, J=7.05 Hz, C19-methyl protons), 2.20-2.52 (2H, m, C18-methylene protons), 5.40 (2H, s, C5-methylene protons), 5.41-5.85 (2H, dd, J=17.51, 17.55 Hz, C17-methylene protons), 6.80-7.06 (2H, m, C25-H, C27-H), 7.24 (1H, s, C14-H), 7.55 [1H, t (d+d), J=8.02 Hz, C26-H], 7.90 [1H, t (d+d), J=8.05 Hz, C11-H], 8.10 (1H, d, J=8.03 Hz, C24-H), 8.45 (2H, d, J=8.04 Hz, Cl0-H, C12-H), 9.25 (1H, s, C7-H), 10.0 (1H, s, C28-phinolic proton); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.3 (C5), 67.5 (C17), C20 buried by solvent peaks in the area of 76.0-78.0 ppm, 96.9 (C14), 111.0, 118.1, 119.7, 121.0, 121.6, 126.0, 127.5, 128.8, 130.2, 131.3, 137.0, 137.2, 145.0, 145.2, 145.5, 148.5, 153.5, 157.3 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 162.5, 168.6 (C21, C22); mass m/e (relative intensity): 513 (m^+, 1), 375 (25), 347 (12), 138 (62), 120 (100), 92 (56), 64 (10). Precise mass ($C_{27}H_{19}N_3O8$): found, 513.116; required, 513.117.

*9-Nitrocamptothecin-20-O-o,p-dinitrobenzoate [**24**]*. Using 9-nitrocamptothecin (0.8 g, 0.0020 mol), 2,4-dinitrobenzoic acid (2 g, 0.0094 mol), DCC (1 g, 0.0049 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **24** (0.1 g) was obtained as a yellow powder, yield 9%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t, J=7.56 Hz, C19-methyl protons), 2.l5-2.40 (2H, m, C-18-methylene protons), 5.40 (2H, s, C5-methylene protons), 5.41-5.85 (2H, dd, J=17.50, 17.58 Hz, C17-methylene protons), 7.58 (1H, s, C14-H), 7.94 (1H, t, J=8.08 Hz, C11-H), 8.15 (1H, d, J=8.06 Hz, C10-H), 8.45-8.70 (3H, m, C12-H, C27-H, C28-H), 8.86 (1H, s, C25-H), 9.28 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 31.6 (C18), 51.0 (C5), 67.5 (C17), 79.0 (C20), 97.5 (C14), 119.8, 121.4, 126.0, 127.4, 128.0, 128.6, 131.6, 131.8, 132.2, 137.0, 145.5, 145.8, 146.0, 147.5, 149.2, 149.4, 153.5,

157.4 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 167.8 (C21, C22); mass m/e (relative intensity): 587 (m^+, weak), 389 (4), 377 (6), 347 (6), 306 (5), 212 (75), 168 (100), 120 (85), 75 (70). Precise mass ($C_{27}H_{18}N_5O_{11}$): found, 587.092; required, 587.092.

9-Nitrocamptothecin-20-O-m,m-dinitrobenzoate [25]. Using 9-nitrocamptothecin (0.8 g, 0.0020 mol), 3,5-dinitrobenzoic acid (1.5 g, 0.0071 mol), DCC (1.3 g, 0.0063 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **25** (0.9 g) was obtained as a yellow powder, yield 77%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.50 Hz, C19-methyl protons), 2.30-2.60 (2H, m, C18-methylene protons), 5.37 (2H, s, C2-methylene protons), 5.40-5.84 (2H, dd, J=17.50, 17.55 Hz, C17-methylene protons), 7.20 (1H, s, C14-H), 7.88 (1H, t, J=8.15 Hz, C11-H), 8.35-8.50 [2H, t (dd), J=8.09 Hz, C10-H, C12-H], 9.18 (2H, strong s, C24-H, C28-H), 9.30 (2H, s, C7-H, C26-H); ^{13}C NMR ($CDCl_3$): δ 17.8 (C19), 32.0 (C18), 50.6 (C5), 67.6 (C17), 78.8 (C20), 96.5 (C14), 121.0, 122.1, 123.5, 126.4, 128.0, 129.1, 130.0, 131.6, 132.3, 136.4, 144.5, 145.1, 145.8, 149.0, 153.2, 157.4 (C2, C3, C6-C13, C15-C16, C16a, C23-C28), 161.5, 166.4 (C21, C22), mass m/e (relative intensity): 587 (m^+, weak), 389 (1), 377 (3), 306 (2), 212 (100), 166 (26), 120 (20), 75 (40). Precise mass ($C_{27}H_{17}N_5O_{11}$): found, 587.092; required, 587.092.

9-Nitrocamptothecin-20-O-p-methyl-m,m-dinitrobenzoate [26]. Using 9-nitrocamptothecin (0.8 g, 0.0020 mol), 4-methyl-3,5-dinitrobenzoic acid (2 g, 0.0088 mol), DCC (1.3 g, 0.0063 mol), and DMAP (0.3 g, 0.0025 mol) as the starting materials, pure product **26** (0.15 g) was obtained as a yellow powder, yield 12%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.12 (3H, t, J=7.08 Hz, C19-methyl protons), 2.20-2.60 (2H, m, C18-methylene protons), 2.68 (3H, s, C26-methyl protons), 5.40 (2H, s, C5-methylene protons), 5.41-5.84 (2H, dd, J=17.52, 17.56 Hz, C17-methylene protons), 7.20 (1H, s, C14-H), 7.90 (1H, t, J=8.08 Hz, C11-H), 8.45 (2H, d, J=8.06 Hz, C10-H, C12-H), 8.64 (2H, s, C24-H, C28-H), 9.27 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 15.0 (C26-methyl carbon), 32.4 (C18), 50.5 (C5), 67.4 (C17), 78.0 (C20), 96.8 (C14), 121.0, 121.8, 125.4, 126.0, 127.6, 128.2, 128.3, 129.0, 129.2, 131.5, 132.8, 136.6, 144.5, 145.9, 146.1, 148.9, 152.1, 153.6, 157.2 (C2, C3, C6-C13, C15, C16, C16a, C23-C28, 161.6, 166.5 (C21, C22); mass m/e (relative intensity): 602 (M+1, 45), 449 (100), 376 (40), 347 (12), 332 (15), 136 (15), 72 (46). Precise mass ($C_{28}H_{20}N_5O_{11}$): found, 602.116; required, 602.116.

9-Nitrocamptothecin-20-O-m-trifluoromethylbenzoate [27]. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 3-trifluoromethylbenzoic acid (1 g, 0.0053 mol), DCC (0.8 g, 0.0039 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **27** (0.45 g) was obtained as a yellow powder, yield 61%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.08 Hz, C19-methyl protons), 2.20-2.50 (2H, m, C18-methylene protons), 5.38 (2H, s, C5-methylene protons), 5.40-5.85 (2H, dd, J=17.51, 17.58 Hz, C17-methyl protons), 7.24 (1H, s, C14-H), 7.65 (1H, t, J=8.08 Hz, C25-H), 7.82-7.95 (2H, m, C11-H, C26-H), 8.28 (1H, d, J=8.07 Hz, C24-H), 8.38 (1H, s, C28-H), 8.47 (2H, d, J=8.06 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.5 (C5), 67.6 (C17), 76.2 (C27-trifluoromethyl carbon), 77.2 (C20), 96.9 (C14), 121.0, 121.4, 126.0, 127.2, 127.5, 128.7, 129.4, 129.8, 130.7, 131.4, 133.3, 136.4, 145.2, 145.3, 148.8, 153.5, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.0, 167.1 (C21, C22); mass m/e (relative intensity): 565 (m^+, 3), 375 (18), 360 (5), 34 (18), 332 (4), 190 (68), 173 (100), 145 (85), 95 (8). Precise mass ($C_{28}H_{18}N_3O_7F_3$): found, 565.110; required, 565.110.

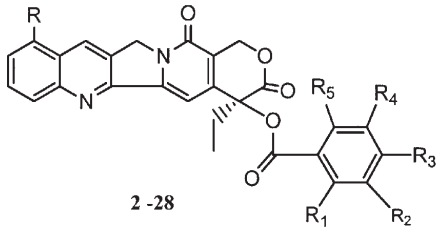
9-Nitrocamptothecin-20-O-p-trifluoromethylbenzoate [28]. Using 9-nitrocamptothecin (0.5 g, 0.0013 mol), 4-trifluoromethylbenzoic acid (1 g, 0.0053 mol), DCC (0.8 g, 0.0039 mol), and DMAP (0.2 g, 0.0016 mol) as the starting materials, pure product **28** (0.35 g) was obtained as a yellow powder, yield 48%, purity 99% (HPLC). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, J=7.05 Hz, C19-methyl protons), 2.20-2.52 (2H, m, C18-methylene protons), 5.39 (2H, s, C5-methylene protons), 5.40-5.82 (2H, dd, J=17.50, 17.55 Hz, C17-methylene protons), 7.22 (1H, s, C14-H), 7.78 (2H, d, J=8.03 Hz, C25-H, C27-H), 7.86 (1H, t, J=8.04 Hz, C11-H), 8.24 (2H, d, J=8.06 Hz, C24-H, C28-H), 8.45 (2H, d, J=8.06 Hz, C10-H, C12-H), 9.25 (1H, s, C7-H); ^{13}C NMR ($CDCl_3$): δ 7.9 (C19), 32.0 (C18), 50.4 (C5), 67.5 (C17), C20 buried by $CHCl_3$ peaks, 96.8 (C14), 121.0, 121.6, 125.8, 126.0, 127.5, 128.7, 130.6, 131.5, 131.9, 136.5, 145.0, 145.2, 145.8, 148.5, 153.5, 157.0 (C2, C3, C6-C13, C15, C16, C16a, C23-C28), 164.7, 166.8 (C21, C22); mass m/e (relative intensity): 565 (m^+, 4), 375 (50), 360 (20), 347 (48), 332 (15), 302 (6), 190 (45), 173 (100), 145 (60), 95 (4). Precise mass ($C_{28}H_{18}N_3O_7F_3$): found, 565.109; required, 565.110.

Determination of drug-induced antiproliferative activity and toxicity in cultured cells. To assess the antiproliferative activity of the various ester drugs, identical cell cultures were treated with equimolar concentrations of these esters and the cell number per ml was counted. Stocks consisted of a fine suspension of esters in polyethylene glycol (PEG-400; Aldrich). Control cultures received only the carrier. The cell number was counted at 24, 72 and 120 h of treatment. Control compounds included the parental compounds **1a** and **1b** for positive control. The targeted cells included the following 14 human cancer cell lines: breast (CLO and KIE), colon (McCN and HT-29), lung (SPA and DOY), melanoma (BRO and SB1B), ovarian (EFO-27 and 2774), pancreatic (MIA and Panc-1) and prostate (PC-3 and DU-145). The average response of ester compounds to 14 human cancer cell lines is shown in Tables I and II.

HPLC procedure for purity analysis of ester products 2-29

Instrumentation. The HPLC system consisted of a Beckman 421 controller with two 110A pumps and a 2-ml injection loop. The UV detector was a SPD-110AV model (Shimadzu, Kyoto, Japan). The HPLC detector was set to monitor the UV absorbance at 220 nm. The integrating software used for the analyses was EZChrome (Shimadzu,

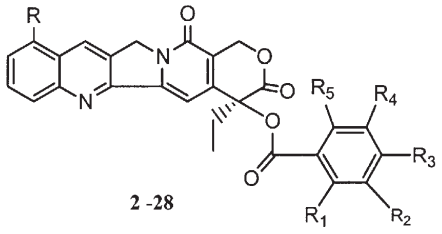
Average response of 14 malignant cell lines to f esters **2-28**.



2 -28

Compounds	Time (Day) 3	5	7
Control	4.00	4.00	4.00
2	4.00 (0%)	4.00 (0%)	4.00 (0%)
3	3.93 (2%)	3.93 (2%)	3.86 (3%)
4	3.86 (3%)	3.64 (9%)	3.07 (23%)
5	3.71 (7%)	3.07 (23%)	2.43 (39%)
6	1.93 (52%)	1.21 (70%)	0.93 (77%)
7	3.57 (11%)	2.93 (27%)	2.29 (23%)
8	2.79 (30%)	2.07 (48%)	1.36 (66%)
9	4.00 (0%)	4.00 (0%)	4.00 (0%)
10	4.00 (0%)	3.93 (2%)	3.79 (5%)
11	2.36 (41%)	1.50 (63%)	1.00 (75%)
12	2.71 (32%)	2.00 (50%)	1.00 (75%)
13	3.20 (20%)	2.60 (35%)	1.90 (53%)
14	2.80 (30%)	2.00 (50%)	1.30 (68%)
15	3.60 (10%)	3.30 (18%)	3.10 (23%)
16	3.90 (2%)	3.90 (2%)	3.90 (2%)
17	4.00 (0%)	3.90 (2%)	3.90 (2%)
18	4.00 (0%)	4.00 (0%)	4.00 (0%)
19	4.00 (0%)	3.86 (3%)	4.00 (0%)
20	4.00 (0%)	3.93 (2%)	3.93 (2%)
21	4.00 (0%)	4.00 (0%)	4.00 (0%)
22	4.00 (0%)	3.97 (1%)	3.50 (13%)
23	3.14 (21%)	1.93 (52%)	1.43 (64%)
24	2.64 (34%)	1.86 (54%)	1.43 (64%)
25	1.86 (53%)	1.21 (70%)	0.86 (79%)
26	1.71 (57%)	1.29 68%)	0.93 (77%)
27	3.86 (3%)	3.14 (21%)	2.14 (46%)
28	3.64 (9%)	2.79 (30%)	2.07 (48%)

Table II. Average response of 14 malignant cell lines to 800 nM of esters **2-28**.



2 -28

Compounds	Time (Day) 3	5	7
Control	4.00	4.00	4.00
2	3.71 (7%)	3.86 (3%)	3.93 (2%)
3	3.86 (3%)	3.14 (21%)	2.36 (41%)
4	3.14 (21%)	2.14 (46%)	1.57 (61%)
5	2.50 (37%)	1.86 (53%)	1.21 (70%)
6	1.14 (71%)	0.79 (80%)	0.50 (88%)
7	2.93 (27%)	1.79 (55%)	1.21 (70%)
8	2.07 (48%)	1.21 (70%)	0.71 (82%)
9	4.00 (0%)	4.00 (0%)	4.00 (0%)
10	3.21 (17%)	2.50 (37%)	1.93 (52%)
11	1.86 (53%)	0.86 (78%)	0.43 (89%)
12	1.79 (55%)	0.79 (80%)	0.50 (88%)
13	2.30 (42%)	1.40 (65%)	0.90 (78%)
14	2.00 (50%)	1.10 (72%)	0.50 (88%)
15	2.60 (35%)	1.60 (60%)	1.11 (72%)
16	3.70 (7%)	2.90 (27%)	2.40 (40%)
17	3.90 (2%)	3.90 (2%)	3.90 (2%)
18	4.00 (0%)	3.93 (2%)	3.86 (3%)
19	3.71 (7%)	2.64 (34%)	1.93 (52%)
20	3.79 (5%)	3.57 (11%)	3.14 (22%)
21	4.00 (0%)	4.00 (0%)	3.93 (2%)
22	3.36 (16%)	2.50 (37%)	1.57 (61%)
23	2.00 (50%)	1.00 (75%)	0.79 (80%)
24	1.79 (55%)	1.29 (68%)	1.00 (75%)
25	1.21 (70%)	0.71 (82%)	0.64 (84%)
26	1.36 (66%)	0.71 (82%)	0.64 (84%)
27	2.57 (36%)	1.64 (59%)	1.21 (70%)
28	2.64 (34%)	1.86 (53%)	1.21 (70%)

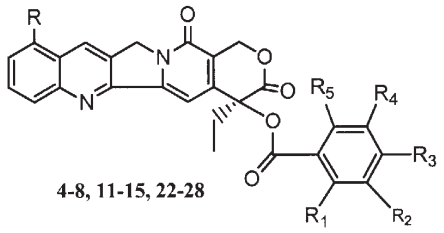
Japan) and Flo-One\beta (Radiomatic Instruments, Meridian, CT). A C-8 Microsorb was from Rainin Instruments (Woburn, MA).

HPLC analysis. Reverse-phase HPLC analysis of the samples was performed using an acetonitrile-acetic acid-water mobile phase system. Analyses were carried out at room temperature with a flow rate of 1 ml/min. The solution with a concentration of approximately 0.1 mg/ml of sample in acetonitrile was prepared by dissolving it in the solvent. A 300-μl portion of this solution was taken and added to a 700-μl solution of 0.1% acetic acid in water. After shaking for ~10 sec, 100 μl of this solution was injected through a 2-ml loop onto a column and chromatographed with 70% water with 0.1% acetic acid and 30% acetonitrile as the mobile phase for the first 5 min, and then the gradient of the mobile phase was programmatically increased to 100% acetonitrile over a period of 4 min. A complete HPLC spectrum was obtained at 15 min. The purity of the sample was determined by measuring the UV peak areas at 254 nm and calculating the percentage associated with the sample peak.

Results and discussion

The *in vitro* anticancer activity of esters **2-28** was evaluated with the following 14 different human cancer cell lines: breast (CLO and KIE), colon (McCN and HT-29), lung (SPA and DOY), melanoma (BRO and SB1B), ovarian (EFO-27 and 2774), pancreatic (MIA and Panc-1), and prostate (PC-3 and DU-145). Two concentrations were tested. The data are expressed in growth levels relative to the untreated (control) group. The growth level of the untreated group is defined as level 4, the full growth. Levels 3, 2 and 1 indicate the 75%, 50%, and 25% growths relative to the corresponding control groups, respectively. Table I shows the average response of the 14 malignant cell lines to 200 nM of compounds **2-28**. Table II shows the average response of the same cell lines to 800 nM of the same compounds. The data in parentheses in

Table III. Potency of ester products **4-8**, **11-15** and **22-28** against 8 different cancer cell lines.



4-8, 11-15, 22-28

Compounds	IC50s (nM)								
	DOY	Du145	Ht29	Kielty	McCN	MPC2	Schc11	T47D	**Ave.**
CPT	65±10	50±7	46±25	63±14	39±6	41±4	47±3	36±12	**48±10**
9NC	18±0	15±1	23±8	22±4	36±13	23±5	18±3	11±3	**19±5**
Irinotecan	583±29	572±20	1099±54	622±239	696±158	589±87	833±13	387±164	**673±96**
Topotecan	164±2	158±43	234±72	134±9	125±3	119±37	133±9	96±24	**145±24**
4	262±21	316±44	256±68	627±214	810±215	344±20	452±71	445±80	**439±92**
5	275±11	302±25	235±60	280±82	1093±193	284±21	369±80	446±157	**410±78**
6	13±1	20±4	15±0	16±0	44±1	15±2	17±1	20±3	**20±2**
7	464±88	626±112	508±186	597±200	405±81	659±47	715±96	743±241	**590±113**
8	113±39	94±23	110±25	216±86	395±62	112±20	157±32	208±79/	**176±46**
11	40±5	92±8	91±6	154±12	/	80±11	76±12	/	**89±9**
12	67±6	75±5	128±18	152±18	380±25	125±9	102±23	56±8	**136±14**
13	87±5	466±54	966±98	258±34	428±45	242±24	674±65	603±54	**466±47**
14	25±3	133±16	578±76	149±25	146±32	58±7	272±25	430±46	**224±7**
15	399±19	289±32	816±65	467±45	500±67	1065±98	467±54	120±12	**515±49**
22	100±9	449±65	624±76	1840±96	556±76	358±34	1538±98	781±76	**781±66**
23	203±11	152±13	312±45	324±46	750±87	386±54	266±43	306±32	**337±41**
24	95±8	/	109±12	98±10	572±96	160±8	94±16	36±6	**166±22**
25	10±3	16±7	11±9	16±8	31±5	12±8	17±10	9±3	**15±7**
26	16±5	27±4	/	41±5	92±9	19±6	32±7	/	**38±6**
27	73±10	60±7	/	214±23	/	92±8	14±6	/	**91±11**
28	922±237	254±40	290±59	431±131	466±44	296±51	345±85	291±95	**412±93**

Tables I and II show the corresponding percentage inhibitions, another expression of the anticancer activity of these compounds. The results showed that, at the concentration of 200 nM, compounds **2-4**, **9-10**, and **15-22** were basically inactive, showing no significant inhibitory effects on the growth of cells. When the concentration was elevated to 800 nM, compounds **3**, **4**, **15**, **16**, **19**, and **22** became active. Further analysis of the data in Tables I and II also indicated that the antitumor activity of these esters correlated with the nature of their side aromatic chains. Strong electron-withdrawing groups such as NO_2, CN, and CF_3 are beneficial for antitumor activity. For example, esters **6**, **8**, **11**, **12**, **13**, **14**, **24**, **25**, **26**, **27**, and **28** were all active against the 14 cell lines tested (Table I). The position where the nitro group attaches did not have any significant effects on antitumor activity. For example, the antitumor activity of esters **11** and **12** was almost identical, although the nitro groups attached to their side aromatic chains were positioned differently. The antitumor activity of halogen-substituted esters, on the other hand, was clearly dependent on where the halogen atom was attached. For example, as shown in Table II, the *o*-F-substituted ester **15** was active, and *m*-F-substituted **16** was slightly active, while *p*-F-substituted **17** was basically inactive; the *m*-Cl-substituted **19** was slightly active while the *o*-Cl- and *p*-Cl-substituted esters **18** and **20** were inactive. Compounds **2** and **9** were completely inactive against the tested cell lines. The side aromatic chains of these two compounds only contain a plain benzene ring with no substituting groups attached at all. The OH-substituted ester, **23**, was also active.

As also shown in Tables I and II, esters **4-8**, **11-15**, and **22-28** were active against the cancer cells tested. To compare the potency of these active ester compounds with their parental compounds and with commercially available camptothecin analogues, we measured the IC_{50} values of these esters and that of the control compounds **1a**, **1b**, irinotecan, and topotecan in a parallel manner against 8 different human cancer cell lines. The results are shown in Table III. Overall, the potency of the most active ester compounds was lower than that of their parental compounds **1a** and **1b** and higher than the commercial CPT derivatives irinotecan and topotecan. Of all these active compounds, esters **6** and **25** showed remarkable potencies against these 8 cancer cell lines. On average, ester **6** was twice as potent as the parental **1a**, and 7-33 times more potent than the commercial CPT analogues. Ester **25** was also more potent than the parental **1b** and many times (9-45) more potent than its commercial CPT analogues. Clearly, ester derivatives **6** and **25** were stronger in activity against these 8 human cancer cell lines than their parental compounds and commercial

[illegible]. One explanation for this observation is that these [illegible]pounds may themselves be active with no need of the enzymatic cleavage of the ester bond, and if this is the case, these compounds may have different action mechanisms from their parental compounds. Another is that the uptake of these two compounds by the testing cell lines may be easier than that of their parental compounds, and thus the local concentrations of the active metabolites for these two compounds in cells are actually higher than their parental compounds. We think the latter scenario is the more probable. Both of these hypotheses need to be further investigated experimentally, and our institution is undertaking related studies to this end.

In conclusion, the chemistry employed in the preparation of these new aromatic esters is straightforward. The reaction yields vary depending on the nature of the substituting groups on the aromatic side chains. Generally, the reactions with the electron-withdrawing group substituted acids such as nitrobenzoic acids and cyanobenzoic acids as acylating agents, gave good yields. Many ester compounds showed good anticancer activity against the human cancer lines tested. Compounds **6** and **25** are particularly impressive with regard to antitumor activity and potency, and thus have high potential for use as effective agents in cancer treatment. Further biological and preclinical studies such as *in vivo* antitumor activity and toxicity, pharmacokinetics, and the action mechanism for these two significant camptothecin derivatives are currently being undertaken in our laboratory and the results will be reported.

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