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Synthesis and biological evaluation of bis and monocarbonate prodrugs of 10-hydroxycamptothecins

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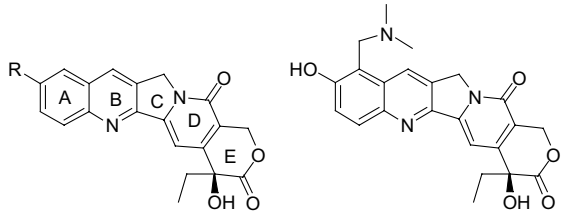
Abstract—In an effort to improve the stability of labile lactone ring of camptothecins, the bis and mono-alkyl carbonate prodrugs of 10-hydroxycamptothecins were synthesized and their chemical and enzymatical stability as well as antitumor activity were studied. The in vitro evaluation of the stability of these carbonates indicates that the 10,20-biscarbonates are firstly hydrolyzed to afford the stable 20-monocarbonates. And the 10-carbonates are not stable in human plasma, mouse plasma and pH 7.4 phosphate buffer, while the 20-carbonates are relatively stable in the three media and can be readily cleaved by porcine liver esterase. The overall toxicity of the tested carbonate against mice bearing S180 sarcoma is much lower when compared with the parent compound, and the antitumor activity is maintained.

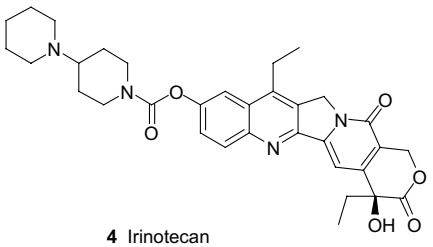
1. Introduction

The pentacyclic natural alkaloid 20(*S*)-camptothecin (CPT, **1**), acting by inhibiting DNA topoisomerase I,[1] was first discovered in 1966[2] from *Camptotheca acuminata* and showed excellent in vitro antitumor activity. Compound **1** was administered as the water-soluble sodium salt of the ring-opened carboxylate form in initial clinical trial to overcome the poor solubility of the lactone form. However, severe side effect led to the termination of clinical trial.[3] The 10-hydroxy analogue (**2**) was less toxic and was used in clinic in China.[4] The effort to find water-soluble derivatives led to the identification and development of topotecan (**3**)[6] and irinotecan (**4**)[5] in 1990s. Compounds **2**, **3**, and **4** are the only three agents of topoisomerase I inhibitors that enter clinical use. More than 10 camptothecin analogues are in their various stages of development[6] and are expected to enter clinical use in following years.

SAR studies have showed that the intact E lactone ring is the most important structural feature. The administration of camptothecins with closed lactone ring is far superior to the injection of water-soluble carboxylate salt in inhibiting growth of human cancer xenografts in mice.[7,8] However, the common problem confronting camptothecins is the instability of lactone ring in plasma. The instability results mainly from the intramolecular hydrogen bond formation of 20-hydroxy with the



1 R=H Camptothecin
2 R=OH 10-hydroxycamptothecin
3 Topotecan



4 Irinotecan

Keywords: Camptothecin; 10-Hydroxycamptothecin; Carbonate; Antitumor.
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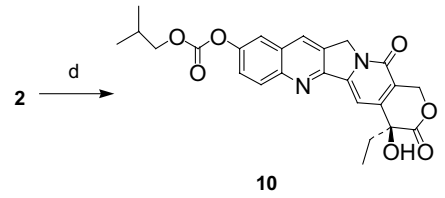
carbonyl moiety of the lactone ring, which accelerates the hydrolysis of the otherwise stable lactone.[9] Masking the 20-hydroxy group by transforming it to the corresponding water-insoluble alkyl esters increased the biological life span of lactone in human and mouse plasma compared with their parent compounds. And meanwhile the overall toxicity of tested esters against nude mice was much lower and their antitumor activity against human tumor xenografts in nude mice was maintained.[10] Some ester prodrugs including afeletecan (BAY38-3441), prothecan, and camptothecin polyglutamate, have entered clinical trial.[6]

Almost all the ester prodrugs are the esters of camptothecin, which release the toxic camptothecin in vivo. However, the structural feature in common of the three drugs or the active metabolite used in clinic is that they all contain a hydroxyl group at 10 position,[6] which are less toxic. In this paper, synthesis and stability study of alkyl carbonate prodrugs **5**–**10** of 10-hydroxycamptothecins as well as in vivo antitumor activity are described.

2. Chemistry

The biscarbonates **5**–**7** were prepared in relatively high yield by the straightforward reaction of corresponding chloroformate with **2** (Scheme 1). Biscarbonate **5** was obtained in 89.2% yield by the reaction of **2** with excessive ethyl chloroformate in dichloromethane with pyridine as acid removal agent at room temperature. In the same way, biscarbonate **6** and **7** were obtained in yields of 81% and 80%, respectively. The ^{1}H NMR spectra obtained from $CDCl_3$ showed corresponding characteristic proton peaks for the respective alkyl side chains of **5**, **6**, and **7**. The 10-carbonate **10** was obtained in 55% yield in a similar manner using pyridine as solvent instead (Scheme 2). No 20-carbonate product formed as a result of hydrogen bond formation of 20-hydroxyl group with the lactone carbonyl group in polar solvent. While the 20-monocarbonate **8** could only be synthesized by selectively hydrolyzing biscarbonate **7** in 71% yield with dilute sodium carbonate in methanol at low temperature (Scheme 1). And the topotecan analogue **9** was prepared by Mannich reaction of **8** with bis-(dimethylamino)methane in a way similar to topotecan[11] (Scheme 1). The 10- and 20-monocarbonates differ in that, the methylene protons of the 20-ethyl group of camptothecins in ^{1}H NMR of 20-carbonate from



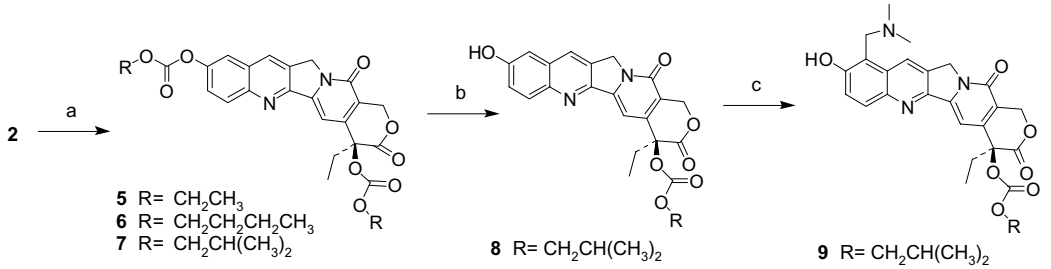
Scheme 2. Reagents and conditions: (d) isobutyl chloroformate, pyridine, rt.

DMSO-d_6 show two multiplets at δ 1.88 and 2.16 ppm, a phenomenon similar to the biscarbonates **6** and **7**, while only one multiplet at δ 1.87 ppm for **10** appears. In addition, the fluorescence of **10** under UV lamp on TLC plate was similar to camptothecin and 10-carboxylates while that of **8** was the same as **2**. And the ^{13}C NMR and high-resolution mass spectra of all these carbonates are consistent with the structure.

3. Results and discussion

The result of in vitro determination, based on literature method, [12] of lactone levels in human and mouse plasma as well as pH 7.4 phosphate buffer for **7** are shown in Figure 1. As shown in Figure 1, almost 8% of **7** was remained in human plasma after 4 h, while **7** was not detectable in mouse plasma after half an hour. And the percent lactone of **7** in pH 7.4 phosphate buffer is about 48% even after 24 h. In other words, the cleavage of **7** in mouse plasma is much faster than in human plasma, and the cleavage of **7** is the result of the combination of chemical and enzymatic effect in both mouse and human plasma. Compound **7** was cleaved to afford mainly the 20-carbonate **8** and a very small amount of **10** in human plasma (Fig. 2). It was also the case in mouse plasma (Fig. 3) and pH 7.4 phosphate buffer (data not shown).

The in vitro determination of lactone levels in human and mouse plasma as well as pH 7.4 phosphate buffer for **8** and **9** was also carried out independently in the same way as **7**. The result was shown in Figures 4 and 5. As indicated in Figure 4, the lactone of **8** in human plasma is stable in human and mouse plasma. For example, 45% of **8** is still detected as the closed lactone form even after 8 h, whereas there is only 25% of its mother compound **2** detected as closed lactone form after 4 h (data not shown). And there is still over 50% of **9** detected as the closed lactone form even after 8 h (Fig. 5).



Scheme 1. Reagents and conditions: (a) ROCOCl, cat. pyridine, CH_2Cl_2, rt; (b) 3% Na_2CO_3, CH_3OH, 0–5 °C; (c) $[(CH_3)_2N]_2CH_2$, *n*-PrOH, CH_2Cl_2.

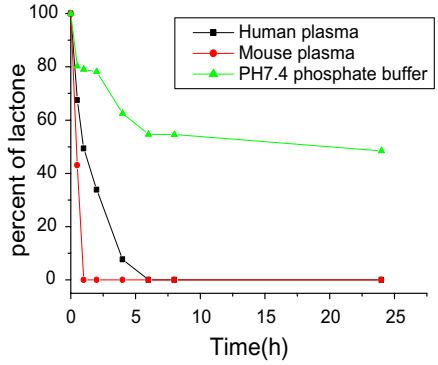


Figure 1. Cleavage of **7** in human plasma, mouse plasma, and pH 7.4 phosphate buffer.

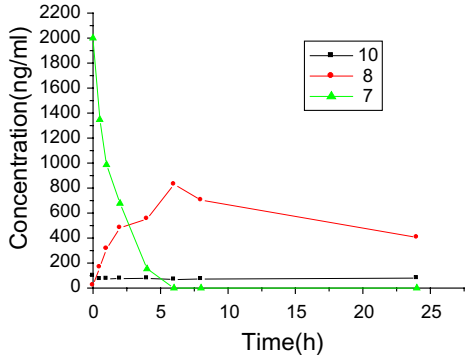


Figure 2. Cleavage of **7** to **8** and **10** in human plasma.

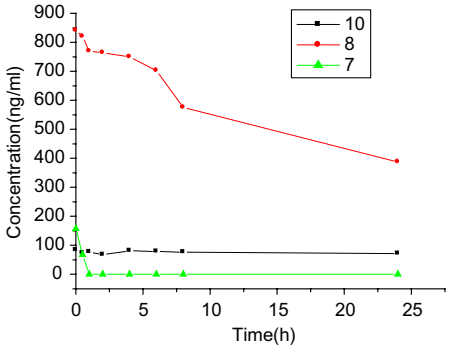


Figure 3. Cleavage of **7** in mouse plasma to **8** and **10**.

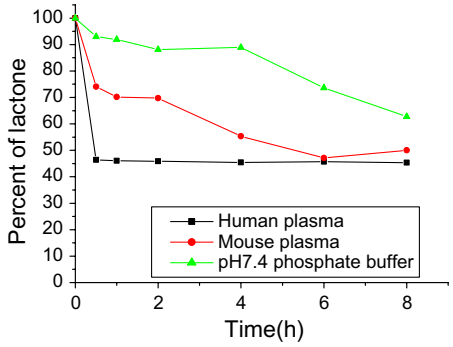


Figure 4. Cleavage of **8** in human plasma, mouse plasma and pH 7.4 phosphate buffer.

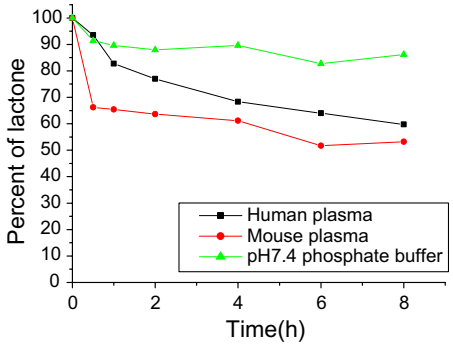


Figure 5. Cleavage of **9** in human plasma, mouse plasma and pH 7.4 phosphate buffer.

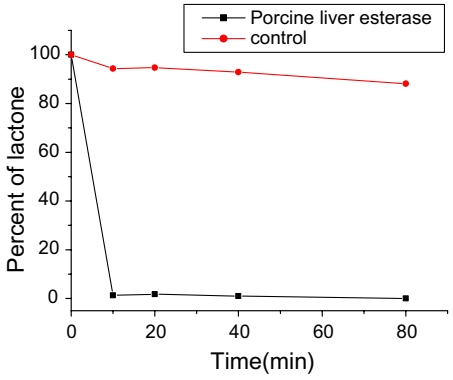


Figure 6. Cleavage of **8** in porcine liver esterase.

Hydrolysis of carbonates in the presence of porcine esterase was also investigated in vitro according to literature method using the same volume of 0.9% physiological saline instead of esterase as control parallelly.[13] It was found that both the bis and monocarbonates could be hydrolyzed by enzyme present in porcine liver, liberating **2**. For example, the monocarbonate **8** was hydrolyzed completely in 10 min (Fig. 6) and **7** was cleaved very quickly and completely if the esterase doubled (data not shown).

As a result, the 20-carbonates are stable enough at physiological pH and can therefore enhance the stability of the lactone ring. And at the same time, the 20-carbonates can be hydrolyzed by esterase to release the active parent compounds.

All the carbonates were tested in vitro against mouse leukemia (P388) based on SRB assay[14] and human lung cancer (A549) cell line based on MTT assay.[15] The result indicated that the antitumor activity of bis and monocarbonates of 10-hydroxycamptothecins were much reduced (Table 1), which was almost the common feature of prodrugs. The cytotoxicity of monocarbonates were higher than that of the biscarbonates.

The in vivo antitumor activity of biscarbonate **7** was evaluated with mice bearing S180 sarcoma as the animal

Table 1. In vitro cytotoxicity activity of the prodrugs

Samples	IC_{50} (μM)	
	A549	P388
5	1.27	0.15
6	3.85	3.66
7	3.45	4.28
8	0.62	0.42
9	0.70	0.68
10	1.21	1.65
HCPT[a]	0.10	0.023
Topotecan	0.038	0.24

[a] HCPT: 10-hydroxycamptothecin.

model in comparison with the mother compound **2**. Overall toxicity can be judged using various criteria. For example, loss of body weight can be considered as one sign of toxicity. In addition, loss of mobility and activity and signs of diarrhea or cystitis in a subject can also be evidence of toxicity. The in vivo toxicity study showed that **7** had little toxicity in mice at doses of 9.3 mg/kg (i.p.*7qd) (Table 2) and 18.6 mg/kg (i.g.*7qd) (Table 3), and the antitumor activity was maintained, while **2** showed obvious toxicity at 6 mg/kg (i.p.*7qd) and 12 mg/kg (i.g.*7qd).

4. Conclusion

We have described the synthesis of bis and mono-alkyl carbonates prodrugs of 10-hydroxy-camptothecins. Some compounds were selected for the in vitro evaluation of the stability. The result indicates the 10,20-bis-carbonates are firstly hydrolyzed to mainly afford 20-monocarbonates. And the 10-carbonates are not stable in human plasma, mouse plasma and pH 7.4 phosphate buffer, while the 20-carbonates is stable in the three media and can be readily cleaved by porcine esterase. The overall toxicity of the tested carbonate against mice bearing S180 sarcoma was much lower when compared with the parent compound. Meanwhile, the antitumor activity is maintained.

5. Experimental

Melting point was measured on Buch 510 without correction. ^{1}H NMR was recorded on Varian Mercury 400 and ^{13}C NMR on Varian Mercury 300. HRESI-MS spectra were obtained with LCQDECA and HREI-MS spectra with Finnigan/MAT95. Flash chromatography was performed on silica gel H (200–300 mesh).

5.1. 10-Hydroxycamptothecin 10,20-diethyl carbonate 5

To the suspension of **2** (200 mg) in dichloroethane (15 mL) and pyridine (0.8 mL) cooled with ice bath was added ethyl chloroformate (1.0 mL) dropwise. Afterwards, the reaction mixture was stirred at room temperature overnight. Dichloroethane (40 mL) was added. The resulting solution was washed with water, dilute HCl, then water, and saturated Saline, respectively, dried over anhydrous sodium sulfate. After filtration and recovery of solvent, the residue was purified by column chromatography to afford slight yellow solid (250 mg) with a yield of 89.2%, mp 150–152 °C. NMR (ppm, $CDCl_3$) δ_H: 0.99 (t, 3H), 1.28 (t, 3H), 1.43 (t, 3H), 1.96 (q, 2H), 4.16 (q, 2H), 4.39 (q, 2H), 5.28 (s, 2H), 5.41 (d, 1H, $J = 17$ Hz), 5.68 (d, 1H, $J = 17$ Hz), 7.32 (s, 1H), 7.67 (dd, 1H, $J = 9.2$ and 2.6 Hz), 7.78 (d, 1H, $J = 2.6$ Hz), 8.23 (d, 1H, $J = 9.2$ Hz), 8.35 (s, 1H); δ_C: 7.79, 14.24, 14.38, 32.10, 50.09, 65.32, 65.55, 67.21, 77.75, 96.27, 118.40, 120.64, 125.65, 128.63, 129.32, 131.06, 131.38, 145.97, 146.29, 146.96, 150.10, 152.62, 153.41, 153.86, 157.41, 167.62; HREI-MS: calcd for $C_{26}H_{24}N_2O_9$ 508.1482. Found 508.1482.

5.2. 10-Hydroxycamptothecin 10,20-dibutyl carbonate 6

Using *n*-butyl chloroformate (1.1 mL), pyridine (0.85 mL), and starting material **2** (200 mg), product **6** was obtained in the same manner as in preparing **5**, gray-white powder, yield 81%, mp 147–150 °C. NMR (ppm, $CDCl_3$) δ_H: 0.90 (t, 3H), 1.00 (m, 6H), 1.25–1.80

Table 2. The in vivo antitumor activity (i.p.*7qd) and body weights of mice before and at the end of treatment with prodrug **7** and its parent compound **2**

Sample	Dose (mg/kg)	Animal no beginning/end	Body weight beginning/end	Tumor weight[a]	Percent of tumor inhibitor (%)
7	9.3	10/10	20.4/23.0	1.31 ± 0.40	52.19
2	6	10/9	20.4/20.1	0.59 ± 0.15	78.47
Control	Solvent[b]	20/20	20.4/24.6	2.7 ± 0.34	—

[a] $P < 0.01$ compared with control.
[b] Solvent refers to solvent used to dissolve drugs.

Table 3. The in vivo antitumor activity (i.g.*7qd) and body weights of mice before and at the end of treatment with prodrug **7** and its parent compound **2**

Sample	Dose (mg/kg)	Animal no beginning/end	Body weight beginning/end	Tumor weight[a]	Percent of tumor inhibitor (%)
7	18.6	10/10	19.7/24.5	1.71 ± 0.22	40.83
2	12	10/9	19.4/22.5	0.69 ± 0.12	76.12
Control	Solvent[b]	20/20	20.4/24.6	2.7 ± 0.34	—

[a] $P < 0.01$ compared with control.
[b] Solvent refers to solvent used to dissolve drugs.

(m, 8H), 1.93–1.97 (m, 1H), 2.13–2.15 (m, 1H), 4.14 (q, 2H), 4.33 (q, 2H), 5.30 (s, 2H), 5.38 (d, 1H, $J = 17$ Hz), 5.68 (d, 1H, $J = 17$ Hz), 7.32 (s, 1H), 7.68 (dd, 1H, $J = 9.3$ and 2.6 Hz), 7.79 (d, 1H, $J = 2.6$ Hz), 8.24 (d, 1H, $J = 9.3$ Hz), 8.36 (s, 1H); δ_C: 7.83, 13.81, 13.86, 19.00, 19.12, 30.69, 30.77, 32.12, 50.12, 67.23, 69.20, 69.43, 77.78, 96.28, 118.42, 120.67, 125.69, 128.68, 129.34, 131.06, 131.43, 146.08, 146.33, 147.03, 150.18, 152.66, 153.58, 154.03, 157.47, 167.63. HREI-MS: calcd for $C_{30}H_{32}N_2O_9$ 564.2108. Found 564.2113.

5.3. 10-Hydroxycamptothecin 10,20-diisobutyl carbonate 7

Using isobutyl chloroformate (3.3 mL), pyridine (2.6 mL), and starting material **2** (600 mg), product **7** was obtained in the same manner as in preparing **5**, gray-white powder, yield 80%, mp 144–146 °C. NMR (ppm, $CDCl_3$) δ_H: 0.91–1.05 (m, 15H), 1.95–1.98 (m, 1H), 2.12–2.14 (m, 2H), 2.26–2.29 (m, 1H), 3.84–3.87 (m, 2H), 3.95 (d, 2H), 5.29 (s, 2H), 5.42 (d, 1H, $J = 17.4$ Hz), 5.68 (d, 1H, $J = 17.4$ Hz), 7.26 (s, 1H), 7.67 (dd, 1H, $J = 9.0$ and 2.7 Hz), 7.78 (d, 1H, $J = 2.6$ Hz), 8.24 (d, 1H, $J = 9.0$ Hz), 8.36 (s, 1H); δ_C: 7.81, 18.90, 18.95, 19.05, 19.09, 27.90, 27.97, 32.06, 50.08, 67.19, 75.16, 75.43, 77.76, 96.19, 119.39, 130.63, 125.65, 128.63, 129.31, 131.03, 131.39, 146.00, 146.28, 146.98, 150.13, 152.61, 153.57, 154.04, 157.40, 167.59. HREI-MS: calcd for $C_{30}H_{32}N_2O_9$ 564.2108. Found 564.2109.

5.4. 10-Hydroxycamptothecin 20-isobutyl carbonate 8

In a 150 mL round bottom flask, prodrug **7** (200 mg) was dissolved in the mixture of methanol (12 mL) and chloroform (5 mL). After being cooled to 0 °C, ice-cooled 1 N aqueous sodium hydroxide (20 mL) was slowly added. After 2 h, the reaction solution was neutralized with dilute HCl and solid precipitated. The product was collected by filtration. After purifying by column chromatography, **8** was obtained as slight yellow powder, yield 71%, mp 248 °C (dec.). NMR(ppm, DMSO-d_6) δ_H: 0.84 (t, 9H), 1.86–1.89 (m, 1H), 2.14–2.18 (m, 2H), 3.88 (d, 2H), 5.25 (s, 2H), 5.49 (s, 2H), 6.95 (s, 1H), 7.29 (d, 1H, $J = 2.7$ Hz), 7.42 (dd, 1H, $J = 9.2$ and 2.7 Hz), 8.01 (d, 1H, $J = 9.2$ Hz), 8.46 (1H, s); δ_C: 7.58, 18.43, 18.47, 27.31, 30.24, 50.16, 66.37, 74.19, 77.67, 93.29, 108.77, 118.19, 123.12, 129.23, 129.74, 129.80, 130.57, 143.16, 145.04, 146.61, 148.97, 153.06, 156.51, 156.77, 167.24. HREI-MS: calcd for $C_{25}H_{24}N_2O_7$ 464.1584. Found 464.1584.

5.5. Topotecan 20-isobutyl carbonate 9

To the solution of **8** (100 mg) in dichloromethane (10 mL) and *n*-propanol (10 mL) was added bis(dimethyl-amino)methane (0.08 mL). The reaction mixture was stirred at room temperature overnight. Dichloroethane (40 mL) was added. The resulting solution was washed with water, dilute HCl, then water, and saturated Saline, respectively, dried over anhydrous sodium sulfate. After filtration and recovery of solvent, the residue was purified by column chromatography to afford yellow powder (60 mg), yield 53.6%, mp 166–168 °C. NMR (ppm, DMSO-d_6) δ_H: 0.80–0.92 (m, 9H), 1.85–2.88 (m, 1H), 2.12–2.14 (m, 2H), 2.31–2.33 (m, 2H), 2.49 (s, 6H), 3.86 (d, 2H), 5.26 (s, 2H), 5.47 (s, 2H), 6.94 (s, 1H), 7.50 (d, 1H, $J = 9.2$ Hz), 7.95 (d, 1H, $J = 9.2$ Hz), 8.71 (s, 1H); δ_C: 7.58, 18.43, 18.47, 27.31, 29.58, 30.24, 44.34, 50.38, 54.49, 66.38, 74.19, 77.67, 93.27, 113.88, 118.16, 122.89, 126.17, 128.61, 129.54, 129.92, 143.35, 145.05, 146.63, 148.35, 153.06, 156.53, 156.59, 167.24. HRESI-MS (M+1): calcd for $C_{28}H_{32}N_3O_7$ 522.2240. Found 522.2240.

5.6. 10-Hydroxycamptothecin 10-isobutyl carbonate 10

The suspension of **2** (200 mg) in pyridine (10 mL) was cooled with ice bath and isobutyl chloroformate (1.0 mL) was added dropwise. Afterwards, the reaction mixture was stirred at room temperature overnight. After removal of pyridine, dichloroethane (40 mL) was added. The resulting solution was washed with water, dilute HCl, then water, and saturated Saline, respectively, dried over anhydrous sodium sulfate. After filtration and recovery of solvent, the residue was purified by column chromatography to afford slight yellow solid (140 mg) with a yield of 55%, mp 245 °C (dec.). NMR (ppm, DMSO-d_6) δ_H: 0.86 (t, 3H), 0.96 (d, 6H), 1.84–1.88 (m, 2H), 2.00–2.03 (m, 1H), 4.06 (d, 2H), 5.29 (s, 2H), 5.41 (s, 2H), 7.35 (s, 1H), 7.76 (dd, 1H, $J = 9.1$ and 2.7 Hz), 8.02 (d, 1H, $J = 2.7$ Hz), 8.21 (d, 1H, $J = 9.1$ Hz), 8.67 (s, 1H); δ_C: 7.78, 18.65, 18.66, 27.28, 30.30, 50.24, 65.26, 72.38, 74.53, 96.78, 118.82, 119.21, 125.41, 128.24, 130.51, 130.62, 131.31, 145.27, 145.92, 149.11, 149.99, 152.75, 152.96, 156.78, 172.45. HREI-MS: calcd for $C_{25}H_{24}N_2O_7$ 464.1584. Found 464.1583.

5.7. In vitro determination of lactone levels in human, mouse and pH 7.4 phosphate buffer for 7, 8, 9, and 10

To 0.8 mL pre-incubated human plasma, mouse plasma and pH 7.4 phosphate buffer was added, respectively, test compounds (0.2 mL, 100 μg/mL) in acetonitrile. The mixture was incubated at 37 °C, and 100 μL aliquots were taken at 0.5, 1, 2, 4, 6, 8, and 24 h. To precipitate plasma protein, 400 μL acetonitrile (−20 °C) was added, vortexed for 20 s, and centrifuged at 10,000 rpm for 5 min. Supernatant was transferred to a glass vial and stored at −20 °C immediately until HPLC analysis. HPLC (HP1100) analysis: 20 μL of solution obtained above was injected onto a C-18 column (Zobax SB, 4.6 × 150 mm) and chromatographed with methanol/water/0.1% acetic acid as mobile phase. Compounds **7** and **10** were detected (detector: DAB) at 254 nm, while **8** at 268 nm, and **9** at 267 nm. The percent of lactone was determined by the ratio of lactone levels measured at different time points to the lactone levels measured at starting point ($t = 0$ h).

5.8. In vitro determination of carbonates cleavage by porcine liver esterase

Dispersion of porcine liver esterase (1 mL, 142 U/mg protein), purchased from Flucka Co., in 3.2 M ammonium sulfate, was desalted by dialysis using 0.01 M Tris–HCl (pH 8.0) at 4 °C. The resulting eaterase solution (40 μL), test compounds solution (0.75 mL) in DMSO, Tween 80 and water, 0.01 M Tris–HCl (0.74 mL, pH 8.0) were mixed after preincubation at 30 °C. The mixture was incubated at 30 °C and 150 μL aliquots were taken at 10, 20, 40, and 80 min and analyzed by HPLC. HPLC (HP1100) analysis: 20 μL of solution obtained above was injected onto a C-18 column (Zobax SB, 4.6×150 mm) and chromatographed with methanol/water/0.1% acetic acid as mobile phase. Compounds **7** and **10** were detected (detector: DAB) at 254 nm, while **8** at 268 nm and **9** at 267 nm. The percent of lactone was determined by the ratio of lactone levels measured at different time points to the lactone levels of test compounds diluted with the same volume of physiological saline.

5.9. Cytotoxicity assays

Cytotoxicity assays are performed on human lung cancer (A549) and mouse leukemia (P388) cell lines. Cells (6000–10,000) in 100 μL culture medium per well were seeded into 96-well microtest plates (Falcon, CA). Cells were treated in triplicate with gradient concentration of test prodrugs and incubated at 37 °C for 72 h. For leukemia cell lines, the microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT; Sigma, St. Louis, MO] assay was performed to measure the cytotoxic effects. The growth inhibitory effect on human solid tumor cell lines (A549) was measured by the Sulforhodamine B (SRB; Sigma, St. Louis, MO) assay. The drug concentration required for 50% growth inhibition (IC_{50}) of tumor cells was determined from the dose-response curve.

5.10. Evaluation of in vivo antitumor activity of 7

Kunming strain female mice (Grade II, Certificate No 005, weighing $20\,g \pm 2\,g$) were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences. Sarcoma 180 cell suspensions were implanted subcutaneously into the right axilla region of mice. Daily treatment with drugs or normal saline commenced 1 day after implantation of cells. Mice were administered by i.p. or i.g. injection with vehicle or **7** once daily for consecutive days. All the mice were euthanized and the tumors were weighed on day 8 after implantation of cells. The rate of inhibition of tumor growth in vivo was calculated using the following formula:

$$\text{Growth inhibition} = (\text{Average tumor weight of control group} - \text{Average tumor weight of test group})/\text{Average tumor weight of control group} \times 100\%.$$

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