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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activity of pyrano[3,2-*i*]-fused camptothecin derivatives

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Abstract

A novel series of A-ring modified hexacyclic camptothecin derivatives containing a pyranone ring fused at the 9-positions and 10-positions were designed and synthesized. These new derivatives were screened against four human tumor cell lines and the results showed most of the compounds possessed comparable cytotoxicity to 10-hydroxycamptothecin (HCPT) in vitro. The pyranono-fused derivatives, except for the one with methoxyformyl group at 4′-position (**12**), displayed similar inhibitory efficiency to saturated dihydropyrano-fused camptothecins (**6** and **7**) and superior cytotoxic activity compared with topotecan (TPT). These results indicated that the saturated and unsaturated fused-pyran rings would not disrupt the base-stacking between camptothecins and the DNA base pair, as well as the interactions between the compounds and Topoisomerase I.

Keywords Hexacyclic camptothecins · Pyranono-fused camptothecins · Cytotoxicity · Structure–activity relationship

Introduction

Camptothecin (CPT, **1**, Fig. 1) is a cytotoxic pentacyclic ring alkaloid, which was first isolated from the Chinese tree *Camptotheca acumunata* by Wall and co-workers in 1966 (Wall et al. 1966, 1993). CPT has attracted worldwide attention because of its novel structure and its mechanism of action as an inhibitor of topoisomerase I (Topo I), which is an essential enzyme that relaxes DNA supercoiling and relieves torsional strain during DNA processing, including transcription and replication (Wang 1996). Up to now, four camptothecin analogs (Gao et al. 2005; Martino et al. 2017), including 10-hydroxycamptothecin (HCPT, **2**), topotecan (TPT, **3**), irinotecan and belotecan have been launched in several countries for the treatment of ovarian cancer, lung cancer, colorectal cancer and the other cancers. However, their significant advances in clinical application are strongly limited due to the underlying side effects and inherently low water-solubility. Therefore, many efforts were made to overcome these defects and several other camptothecins, such as exatecan, gimatecan, CKD-602, diflomotecan and silatecan are in various stages of preclinical or clinical development (Thomas et al. 2004; Venditto and Simanek 2010; Hu et al. 2015).

The SAR studies showed that substitutions at the 7-position, 9-position, or 10-position of camptothecin could enhance their antitumor activity (Hsiang et al. 1985; Sheng et al. 2008). For instance, some small lipophilic groups, such as ethyl, oxyiminoalkyl, alkylsilyl and alkylsilylalkyl at the 7-position of camptothecin can promote not only the stability of the E-lactone ring but also their antitumor activities (Martino et al. 2017). When an additional ring is combined with ring-A and -B, such as in lurtotecan (**4**) and exatecan (**5**), the derivatives demonstrate potent antitumor activity, superior to that of the original pentacyclic camptothecins, possibly because of the increased planarity exerted by an additional ring (Kingsbury et al. 1991).

In our previous studies, topotecan hydrochloride was found that could react with a series of alcohols and thiols to produce the corresponding 9-alkanyloxy-camptothecins and

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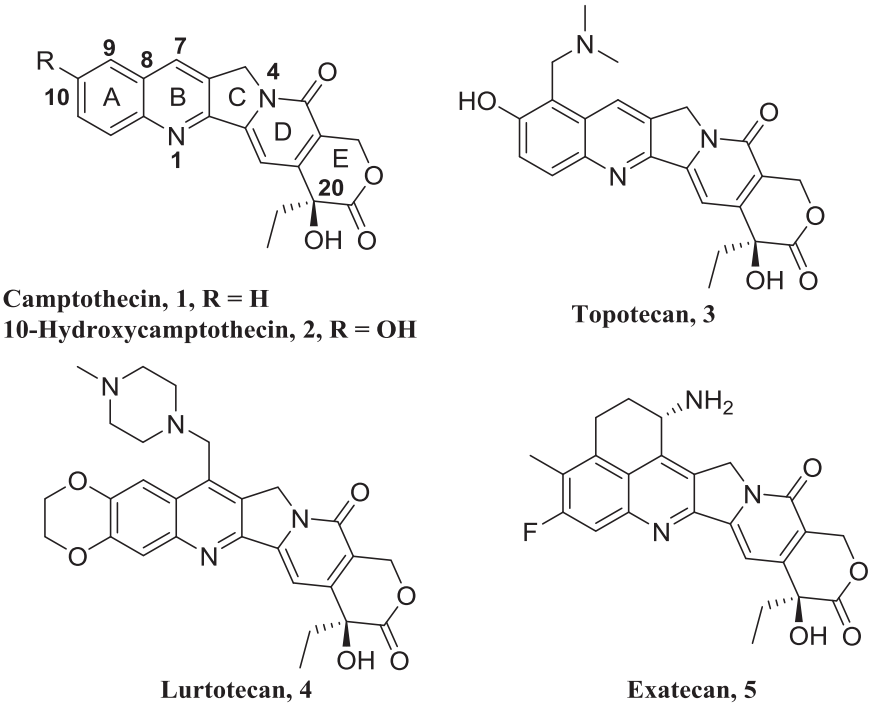


Fig. 1 Structures of camptothecin (**1**) and its derivatives

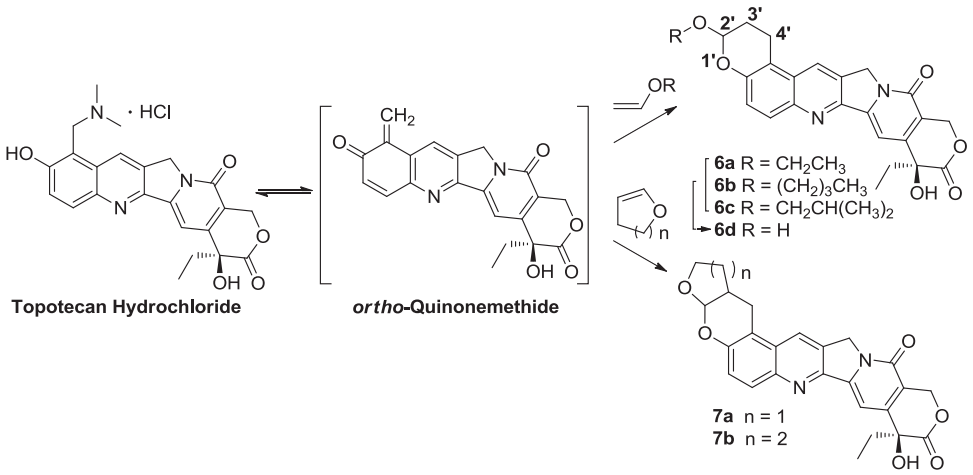


Fig. 2 The formation of 2′-alkoxydihydropyrano-fused camptothecins

9-alkanylthio-camptothecins by an *ortho*-quinone methide (*o*-QM) mechanism (Li et al. 2011). Subsequently, a series of dihydropyrano-fused camptothecins (**6a**–**d** and **7a**–**b**) were prepared, as shown in Fig. 2, through [4 + 2] cycloaddition of the same *o*-QM intermediate from topotecan hydrochloride with various alkyl vinyl ethers (Tan et al. 2015). All the dihydropyrano-fused compounds demonstrated comparative anti-proliferative activity to HCPT and superior activity to TPT, against cancer cell lines, such as HepG2, KB, HCT-8, and SGC7901 (Tan et al. 2015). Hence, it is reasonable to suppose that the presence of bulky hydrophobic side chains at the 9-position and 10-position of camptothecin is beneficial to their bioactivity.

Herein, the synthesis and antitumor activity of a series of novel unsaturated pyrano-fused camptothecins, fused at the 9-position and 10-position are reported to further explore the impact of a different ring type on the antitumor activity. Theoretically, unsaturated fused rings should increase the planarity more than saturated rings. We postulated that the structural characteristics of these new hexacyclic camptothecins should increase the stability of the E-lactone ring and improve the antitumor activity.

Materials and methods

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), proton nuclear magnetic resonance (^{1}H-NMR, 400 MHz), electrospray ionization mass spectrometry (ESI-MS), melting

point, and high resolution mass spectrum (HR-MS). Silica gel thin layer chromatography (TLC) was performed on precoated plates (Qingdao Haiyang Chemical Ltd, China). ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were determined with Bruker-DPX 400 and 600 MHz spectrometer respectively, using TMS as an internal standard and DMSO-d_6 as solvent. Chemical shifts are given in parts per million (ppm) (δ). ESI-MS was performed using an Agilent G1946D. Melting points were determined on a SGWX-4 microscopic melting point apparatus. HR-MS was performed on a Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS. Column chromatography separations were performed on silica gel (300–400 mesh, Qingdao Huanghai Chemical Ltd., China). All commercially available solvents and reagents were used without further purification.

9-Formoxyl-10-hydroxycamptothecin (8)

Compound **2** (300 mg, 0.824 mmol) was dissolved in TFA (6 ml). A solution of hexamethylenetetramine (HMTA) (2.4 g) in 40 ml TFA was added at 0 °C. The solution was stirred at 85 °C for 20 h under N_2. The residue obtained after eliminating TFA through vacuum distillation was poured into ice-cold water to give an orange solid. The crude product was purified by flash chromatography (CH_2Cl_2: MeOH = 100: 1, v/v) to give **8** as a yellow solid (60 mg, 18.6%). m.p.: 247–249 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.85 (t, J = 7.24 Hz, 3H, 18-CH_3), 1.84 (m, 2H, 19-CH_2), 5.28 (s, 2H, 5-CH_2), 5.40 (s, 2H, 17-CH_2), 6.52 (s, 1H, 20-OH), 7.25 (s, 1H, 14-CH), 7.58 (d, J = 9.39 Hz, 1H, 11-CH), 8.30 (d, J = 9.39 Hz, 1H, 12-CH), 9.71 (s, 1H, 7-CH), 10.74 (s, 1H, 9-CHO); ESI-MS *m/z* 393.2 $[M+H]^+$.

General method for the preparation of compounds 9 and 10

A mixture of 9-formoxyl-10-hydrocamptothecin (**8**) (100 mg, 0.255 mmol) and ylide reagent $Ph_3P{=}C(R_1)$-$COOR_2$ (0.306 mmol) was stirred in a mixed solvent composed of xylene (8 ml) and DMSO (1 ml) at 80 °C for 4 h. The resulting mixture was poured into ice-cold water and filtered. The filtrate was extracted twice with ethyl acetate and was dried over $MgSO_4$. The organic residue and the filtered solid were combined and purified by chromatography on silica gel (CH_2Cl_2: MeOH = 80: 1, v/v) to afford yellow solids.

2′-Oxo-2H-pyrano[3,2-*i*]camptothecin (9a)

Yield 7.1%. m.p.: 293–295 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.87 (t, 3H, 18-CH_3), 1.85 (m, 2H, 19-CH_2), 5.34 (s, 2H, 5-CH_2), 5.42 (s, 2H, 17-CH_2), 6.54 (s, 1H, 20-OH), 6.77 (d, 1H, J = 9.78 Hz, 5′-CH), 7.34 (s, 1H, 14-CH), 7.92 (d, 1H, J = 9.39 Hz, 11-CH), 8.37 (d, J = 9.39 Hz, 1H, 12-CH), 8.95 (d, 1H, J = 8.60 Hz, 4′-CH), 9.33 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.2, 159.4, 156.6, 153.4, 152.1, 149.9, 145.0, 139.6, 133.6, 131.5, 126.5, 124.3, 121.2, 119.1, 116.3, 113.2, 113.1, 96.6, 72.2, 65.1, 50.3, 30.2, 7.6; **ESI-MS** *m/z* 417.1 $[M+H]^+$; HRMS calcd. for $C_{23}H_{15}N_2O_6$ $[M-H]^-$ 415.0936, found 415.0934.

3′-Allyl-2′-oxo-2H-pyrano[3,2-*i*]camptothecin (9c)

Yield 4.1%. m.p.: 317–319 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.87 (t, 3H, 18-CH_3), 1.87 (m, 2H, 19-CH_2), 3.37 (d, 2H, J = 6.26 Hz, 5′-CH_2-), 5.22 (m, 2H, 5′-C–C=CH_2), 5.34 (s, 2H, 5-CH_2), 5.403 (s, 2H, 17-CH_2), 6.08 (m, 1H, J = 6.26 Hz, 5′-C–CH), 6.55 (s, 1H, 20-OH), 7.34 (s, 1H, 14-CH), 7.92 (d, 1H, J = 9.00 Hz, 11-CH), 8.33 (d, 1H, J = 9.00 Hz, 12-CH), 8.76 (s, 1H, 4′-CH), 9.33 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.3, 159.4, 156.6, 153.4, 152.2, 149.9, 145.0, 142.7, 139.6, 138.3, 133.6, 131.4, 128.6, 126.5, 121.2, 119.1, 116.3, 113.2, 96.6, 95.9, 72.2, 65.1, 50.4, 50.2, 30.2, 7.6; **ESI-MS** *m/z*: 455.0 $[M-H]^-$; **HRMS** calcd. for $C_{26}H_{21}N_2O_6$ $[M+H]^+$ 457.1394, found 457.1386.

3′-Methyl-2′-oxo-2H-pyrano[3,2-*i*]camptothecin (9d)

Yield 4.9%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.87 (t, 3H, 18-CH_3), 1.85 (m, 2H, 19-CH_2), 2.24 (s, 3H, 5′-CH_3), 5.35 (s, 2H, 5-CH_2), 5.42 (s, 2H, 17-CH_2), 6.56 (s, 1H, 20-OH), 7.34 (s, 1H, 14-CH), 7.91 (d, 1H, J = 9.78 Hz, 11-CH), 8.30 (d, 1H, J = 8.61 Hz, 12-CH), 8.88 (s, 1H, 4′-CH). 9.33 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.2, 160.7, 156.6, 152.0, 149.9, 145.0, 135.1, 132.2, 131.2, 128.6, 126.4, 125.4, 123.9, 120.9, 119.1, 115.5, 113.7, 96.6, 72.2, 65.1, 50.2, 30.2, 16.9, 7.6; **ESI-MS** *m/z*: 429.0 $[M-H]^-$; **HRMS** calcd. for $C_{24}H_{17}N_2O_6$ $[M-H]^-$ 429.1092, found 429.1108.

3′-Methoxy-2′-oxo-2H-pyrano[3,2-*i*]camptothecin (9e)

Yield 7.8%. m.p.: 301–303 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.87 (t, 3H, 18-CH_3), 1.86 (m, 2H, 19-CH_2), 3.94 (s, 3H, 5′-OCH_3), 5.35 (s, 2H, 5-CH_2), 5.42 (s, 2H, 17-CH_2), 6.54 (s, 1H, 20-OH), 7.34 (s, 1H, 14-CH), 8.24 (s, 2H, 11-CH, 12-CH), 8.61 (s, 1H, 4′-CH), 9.20 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.2, 158.7, 156.6, 153.0, 151.5, 149.9, 145.8, 145.3, 131.5, 130.8, 129.9, 127.6, 122.9, 122,5, 118.8, 116.9, 113.7, 96.3, 72.2, 65.1, 52.3, 50.2, 30.2, 7.6; **ESI-MS** *m/z* 447.1 $[M+H]^+$; **HRMS** calcd. for $C_{24}H_{19}N_2O_7$ $[M+H]^+$ 447.1186, found 447.1179.

3′-Bromo-2′-oxo-2H-pyrano[3,2-*i*]camptothecin (9f)

Yield 15.3%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.87 (t, 3H, 18-CH_3), 1.86 (m, 2H, 19-CH_2), 5.33 (s, 2H, 5-CH_2), 5.43 (s, 2H, 17-CH_2), 6.55 (s, 1H, 20-OH), 7.34 (s, 1H, 14-CH), 7.93 (d, 1H, $J = 9.39$ Hz, 11-CH), 8.39 (d, 1H, $J = 9.39$ Hz, 12-CH), 9.40 (s, 1H, 4′-CH), 9.48 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.3, 156.6, 156.2, 152.6, 149.9, 144.9, 140.9, 133.8, 131.6, 126.8, 123.7, 121.0, 119.2, 114.0, 111.9, 96.8, 72.2, 65.1, 50.2, 45.6, 30.2, 8.5, 7.6; **ESI-MS** *m/z*: 493.0 $[M-H]^-$; **HRMS** calcd. for $C_{23}H_{16}N_2O_6Br$ $[M+H]^+$ 495.0186, found 495.0168.

(*E*)-9-Methoxycarbonylvinyl-10-hydroxycamptothecin (10a)

Yield 13.9%. m.p.: 297–299 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.86 (t, 3H, 18-CH_3), 1.83 (m, 2H, 19-CH_2), 3.77 (s, 3H, –$COOCH_3$), 5.20 (s, 2H, 5-CH_2), 5.25 (s, 2H, 17-CH_2), 6.50 (s, 1H, 20-OH), 6.92 (d, 1H, $J = 15.65$ Hz, 9-β-CH), 7.25 (s, 1H, 14-CH), 7.58 (d, 1H, $J = 9.39$ Hz, 11-CH), 8.08 (d, 1H, $J = 9.39$ Hz, 12-CH), 8.24 (d, 1H, $J =$ 16.43 Hz, 9-CH), 8.89 (s, 1H, 7-CH), 11.39 (s, 1H, 10-OH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.3, 167.2, 156.7, 149.9, 149.4, 145.4, 143.3, 136.2, 132.5, 130.8, 127.9, 126.1, 122.6, 121.9, 118.3, 112.4, 95.9, 72.2, 65.1, 51.4, 50.4, 30.1, 7.6; **ESI-MS** *m/z*: 447.0 $[M-H]^-$; **HRMS** calcd. for $C_{24}H_{19}N_2O_7$ $[M-H]^-$ 447.1198, found 447.1209.

(*E*)-9-Ethoxycarbonylvinyl-10-hydroxycamptothecin (10b)

Yield 10.5%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.86 (t, 3H, 18-CH_3), 1.28 (t, 3H, $COOCH_2CH_3$), 1.84 (m, 2H, 19-CH_2), 4.23 (m, 2H, –$COOCH_2$–C), 5.21 (s, 2H, 5-CH_2), 5.25 (s, 2H, 17-CH_2), 6.50 (s, 1H, 20-OH), 6.92 (d, 1H, $J = 16.04$ Hz, 9-β-CH), 7.25 (s, 1H, 14-CH), 7.58 (d, 1H, $J = 9.39$ Hz, 11-CH), 8.08 (d, 1H, $J = 9.39$ Hz, 12-CH), 8.24 (d, 1H, $J = 15.65$ Hz, 9-CH), 8.89 (s, 1H, 7-CH), 11.39 (s, 1H, 10-OH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.3, 166.8, 156.9, 156.7, 149.9, 149.5, 145.4, 143.3, 136.0, 132.4, 130.8, 127.9, 126.1, 122.5, 122.4,118.3, 112.5, 95.9, 72.2, 65.1, 59.9,50.4, 30.1, 14.1, 7.6; **ESI-MS** *m/z*: 463.0 $[M+H]^+$; **HR-MS** calcd. for $C_{25}H_{22}N_2NaO_7$ $[M+Na]^+$ 485.1319, found 485.1330.

(*E*)-2′-Allyl-9-methoxycarbonylvinyl-10-hydroxycamptothecin (10c)

Yield 26.6%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.85 (t, 3H, 18-CH_3), 1.84 (m, 2H, 19-CH_2), 2.88 (d, 2H, $J = 6.26$ Hz, 9-β-CH_2-), 3.79(s, 3H, –$COOCH_3$), 4.78 (m, 2H, –CH_2CH=CH_2), 5.22 (s, 2H, 5-CH_2), 5.28 (s, 2H, 17-CH_2), 5.66 (m, 1H, $J = 6.26$ Hz, –CH_2CH=), 6.50 (s, 1H, 20-OH), 7.25 (s, 1H, 14-CH), 7.54 (d, 1H, $J = 9.00$ Hz, 11-CH), 7.73 (s, 1H, 9-CH), 8.04 (d, 1H, $J = 9.00$ Hz, 12-CH), 8.29 (s, 1H, 7-CH), 10.74 (s, 1H, 10-OH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.4, 166.8, 156.7, 153.3, 149.8, 149.3, 145.6, 143.0, 134.6, 134.4, 133.6, 130.7, 130.1, 127.2, 127.0, 122.4, 118.2, 115.9, 114.4, 95.8, 72.2, 65.1, 51.8, 50.2, 32.5, 30.1, 7.6; **ESI-MS** *m/z*: 487.0 $[M-H]^-$; **HRMS** calcd. for $C_{27}H_{23}N_2O_7$ $[M-H]^-$ 487.1511, found 487.1527.

(*E*)-2′-Methyl-9-methoxycarbonylvinyl-10-hydroxycamptothecin (10d)

Yield 26.6%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.85 (t, 3H, 18-CH_3), 1.68 (s, 3H, 2′-CH_3), 1.84 (m, 2H, 19-CH_2), 3.80 (s, 3H, –$COOCH_3$), 5.34 (s, 2H, 5-CH_2), 5.39 (s, 2H, 17-CH_2), 6.49 (s, 1H, 20-OH), 7.25 (s, 1H, 14-CH), 7.54 (d, 1H, $J = 9.00$ Hz, 11-CH), 7.75 (s, 1H,9-CH), 8.05 (d, 1H, $J = 9.39$ Hz, 12-CH), 8.29 (s, 1H, 7-CH). 10.55 (s, 1H, 10-OH). **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.4, 167.3, 156.7, 153.5, 149.8, 149.3, 145.6, 143.0, 132.8, 131.8, 130.7, 130.2, 127.2, 127.1, 122.4, 118.2, 114.6, 95.8, 72.2, 65.1, 51.8, 50.3, 30.1, 14.8, 7.6; **ESI-MS** *m/z*: 463.0 $[M-H]^-$; **HRMS** calcd. for $C_{25}H_{22}N_2NaO_7$ $[M+Na]^+$ 485.1319, found 485.1333.

1′-Methoxycarbonyl-9-methoxycarbonylvinyl-10-hydroxycamptothecin (11)

A mixture of 10-hydrocamptothecin (**2**) (500 mg, 1.37 mmol) and Ph_3P (540 mg, 2.06 mmol) was stirred in a mixed solvent composed of xylene (25 ml) and DMSO (2 ml) at room temperature. A solution of dimethylacetylene-dicarboxylate (DMAD) in xylene (0.31 ml DMAD in 10 ml xylene) was added at 0 °C. The solution was stirred at 100 °C for 10 h. The residue obtained after eliminating most of the solvent through vacuum distillation was poured into ice-cold water to give an orange solid. The filtrate was extracted twice with DCM and was dried over $MgSO_4$. The organic layer and solid were combined, purified by chromatography on silica gel (CH_2Cl_2: MeOH = 100:1, v/v) to afford compound **12** as a mixture of *cis* and *trans* isomers (*cis*:*trans* 1:2). Yield: 51.1%. *Cis* isomer: **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.86 (t, $J = 7.04$ Hz, 3H, 18-CH_3), 1.83 (m, 2H, 19-CH_2), 3.68 (s, 3H, 9-C–$COOCH_3$), 3.77 (s, 3H, 9-C=CH–COO**CH_3**), 5.16 (s, 2H, 5-CH_2), 5.38 (s, 1H, 9-C=CH–), 5.39 (s, 2H, 17-CH_2), 6.50 (s, 1H, 20-OH), 7.25 (s, 1H, 14-CH), 7.48 (d, 1H, $J = 9.78$, 11-CH), 8.05 (d, $J = 9.00$ Hz, 1H, 12-CH), 8.49 (s, 1H, 7-CH). 10.77 (s, 1H, 10-OH). *Trans* isomer: **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.86 (t, $J = 7.04$ Hz, 3H, 18-CH_3), 1.83 (m, 2H, 19-CH_2), 3.32 (s, 3H, 9-C–$COOCH_3$), 3.44 (s, 3H, 9-C=CH–$COOCH_3$), 5.16 (s, 2H, 5-CH_2), 5.23 (s, 1H, 9-C=CH–),

5.39 (s, 2H, 17-CH_2), 6.50 (s, 1H, 20-OH), 7.25 (s, 1H, 14-CH), 7.48 (d, 1H, $J = 9.78$,11-CH), 8.05 (d, $J = 9.00$ Hz, 1H, 12-CH), 8.15 (s, 1H, 7-CH), 10.44 (s, 1H, 10-OH). **ESI-MS** *m/z* 507.4 $[M+H]^+$; **HRMS** calcd. for $C_{26}H_{22}N_2NaO_9$ $[M+Na]^+$ 529.1218, found 529.1207.

4′-Methoxycarbonyl-6-pyrano[3,2-*i*]camptothecin (12)

Compound **11** was dissolved in a mixed solvent composed of xylene (10 ml) and DMSO (1 ml) and stirred at 160 °C for 14 h. The residue obtained after eliminating most of the solvent through vacuum distillation was poured into ice-cold water to give an orange solid. The filtrate was extracted twice with DCM and was dried over $MgSO_4$. The organic layer and solid were combined, purified by chromatography on silica gel (CH_2Cl_2:MeOH = 100:1, v/v) to afford compound **12** as a yellow solid. Yield: 46.0%. m.p.: >330 °C. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ: 0.86 (t, $J = 7.04$ Hz, 3H, 18-CH_3), 1.85 (m, 2H, 19-CH_2), 4.08 (s, 3H, 4′-$COOCH_3$), 5.32 (s, 2H, 5-CH_2), 5.42 (s, 2H, 17-CH_2), 6.50 (s, 1H, 20-OH), 7.00 (s, 1H, 5′-CH), 7.32 (s, 1H, 14-CH), 7.96 (d, 1H, $J = 8.99$ Hz, 11-CH), 8.43 (d, $J = 9.00$ Hz, 1H, 12-CH), 8.40 (s, 1H, 7-CH); **^{13}C NMR** (DMSO-d_6, 150 MHz) δ: 172.3, 166.3, 158.3, 156.6, 154.5, 152.2, 149.8, 145.4, 144.7, 144.1, 135.0, 131.5, 126.8, 122.9, 121.7, 119.3, 117.1, 109.7, 96.8, 72.2, 65.1, 54.0, 50.8, 30.1, 7.6; **ESI-MS** *m/z* 475.3 $[M+H]^+$; **HR-MS** calcd. for $C_{25}H_{218}N_2NaO_8$ $[M+Na]^+$ 497.0955, found 497.0974.

Cell culture

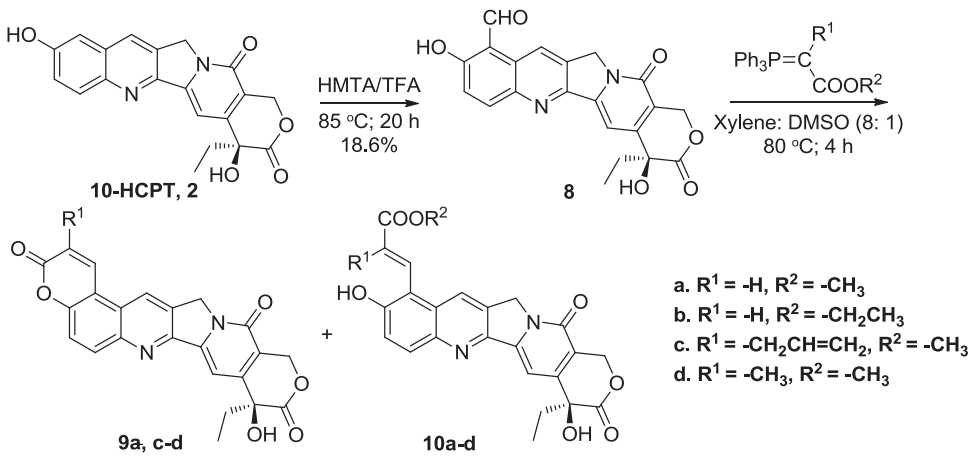
The cell lines HepG2, KB, HCT-8, and SGC7901 were maintained as a monolayer culture in DMEM or RPMI1640, supplemented with 10% FBS, in a humidified atmosphere (5% CO_2) at 37 °C.

Cell proliferation assay

Cell proliferation was determined using CCK8 dye (Wuhan Boster) according to manufacturer′s instructions. Cells ($3–5 \times 10^3$ per well) were seeded in a 96-well plate and grown at 37 °C for 12 h. Subsequently, cells were treated with compounds at increasing concentrations (0.5, 5, 50, 500, 5000, and 50,000 μmol/l) in the presence of 10% FBS for 48 h. After 10 μl CCK8 dye was added to each well, cells were incubated at 37 °C for 1–2 h. The absorbance (OD) of the plates was read using a Spectra Max M5 microplate reader (Molecular Devices) at 450 nm. The percentage inhibition was calculated by comparison of the IC_{50} values of compounds with DMSO-treated control wells. All the experiments were performed three times.

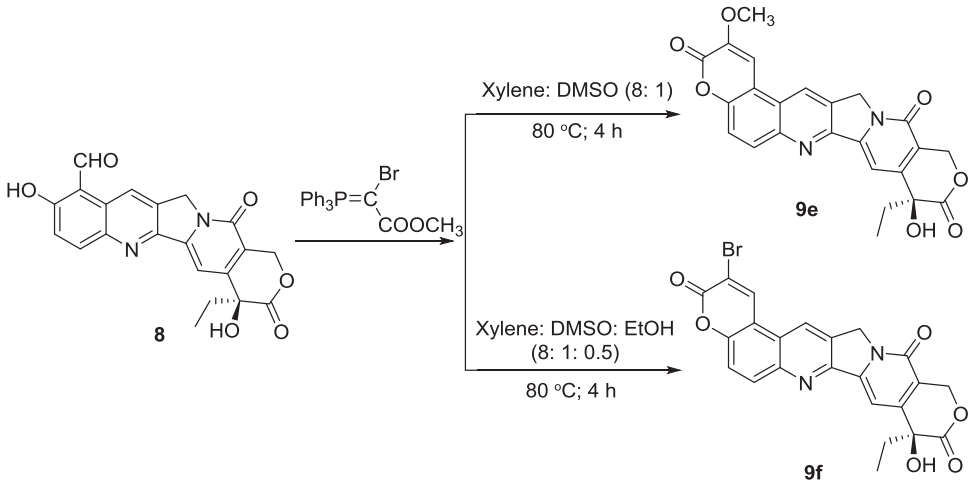
Results and discussion

All the 2′-oxopyrano-fused camptothecins were synthesized as shown in Schemes 1 and 2. In earlier work, various synthetic methods for constructing pyranones were conducted in an attempt to synthesize the desired compounds, such as the Perkin reaction (Bahekar and Shinde 2004), Pechmann condensation (Bose et al. 2003), and Knoevenagel reaction (Song et al. 2003) using 10-hydroxycamptothecin (10-HCPT, **2**) as substrate. But none of them found success, which can be ascribed, at some level, to the low reactivity of A-ring of 10-HCPT. When 10-HCPT was heated with trifluoroactic acid (TFA) and hexamethylenetetramine (HMTA) at 85 °C, the Duff reaction afforded 9-formoxyl-10-hydrocamptothecin (**8**) according to the literature method (Dallavalle et al. 2008; Rodriguez-Berna et al. 2013) with a yield of only 18.6% after separation by column chromatography. The key intermediate **8** was then reacted with different phosphorus ylides, $Ph_3P{=}C\text{-}(R_1)\text{-}COOR_2$, to

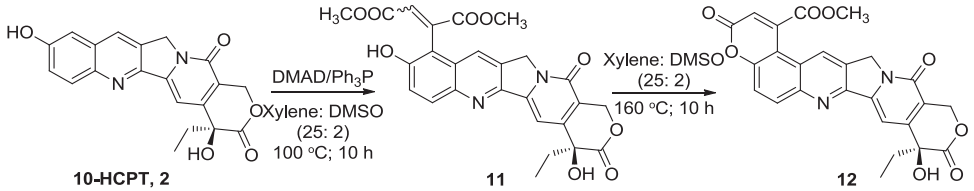


Scheme 1 Synthesis of 3′-substituted-2′-oxopyrano[3,2-*i*] camptothecins **9a, c–d**

Scheme 2 Synthesis of 3′-substituted-pyrano[3,2-*i*] camptothecins **9e** and **9f**



Scheme 3 Synthesis of 4′-methoxycarbonylpyrano[3,2-*i*] camptothecin **12**



give the desired 2′-oxopyrano-fused camptothecins (**9**) and corresponding ring-opened compounds (**10**). The configuration of **10** was confirmed to be (*E*) based on the ^{1}H NMR spectrum of compound **10a** and **10b** (R^1=H), in which the *J*-values of the olefin hydrogens are approximately 16–20 Hz. It can be presumed that the products of the Wittig reaction are a mixture of *E* and *Z* isomers, and the latter configuration undergoes cyclization to form a pyranone ring (compound **9**). The ratio of the yield of compound **10** to compound **9** is approximately 2–5:1, indicating that the *E*-configuration is the main product in the reaction. The cyclization of *Z* isomers of **10a** and **10b** all resulted in the same product **9a**.

When the ylide reagent was methyl 2-bromo-2-(triphenylphosphanylidene)acetate as shown in Scheme 2, the reaction gave the unexpected product **9e** under stirring in xylene: DMSO (8:1) at 80 °C. The reason for the formation of this product can be attributed to intramolecular nucleophilic substitution by CH_3O^-, which formed as the cyclization proceeded. When ethanol was added into the reaction to block the CH_3O^-, the product was the expected 3′-bromo-2′-oxopyrano-fused camptothecin (**9f**). It is worth noting that when the R^1 group of the ylide reagent included any electron-withdrawing groups, such as –$COOCH_3$ or –CN, unfortunately, the Wittig reaction was unable to occur to provide the desired compounds.

In the interest of synthesizing derivatives with substituent groups at the 4-position of the pyrone ring, dimethyl acetylene dicarboxylate (DMAD) and Ph_3P were used as the source of zwitterionic species (Galariniotou et al. 2007) in xylene/DMSO (25:2) under 100 °C to give compound **11** as a *Z*/*E*-mixture in 34.9% yield. Without separation, the mixture was transformed to **12**, also in xylene and DMSO under reflux, with a yield of 46.1% (Scheme 3). Notably, this method provides direct access to a carbon-carbon bond and is a useful way to construct a pyranone ring in poorly reactive natural products.

All the synthesized derivatives were screened against HCT-8 (colonic carcinoma cell), SGC7901 (gastric carcinoma cell), HepG2 (human hepatoma cell), and KB (human oral epidermoid carcinoma cell) cell lines to evaluate their antiproliferative activity, using 10-hydroxycamptothecin (**2**) and topotecan hydrochloride (**3**) as reference compounds. As shown in Table 1, the data depicted that camptothecins **9a, 9c–f**, and **10a–d**, exhibited considerable cytotoxic activity with IC_{50} values in the range of 0.030–4.553 μM in all the four cell lines, and showed superior cytotoxic activity to TPT, and comparable cytotoxicity with HCPT. In particular, compound **10d** was approximately 6-fold and 60-fold more potent than HCPT and TPT, respectively, toward HCT-8 cell lines. However, compounds **11** and **12** showed lower anti-proliferative activity than the other compounds in the series and the reference compounds, which indicated that the ester group at the 4′-position in the pyranone ring has a negative impact on the activity.

The X-ray co-structure (PDB code: 1K4T) indicated that topotecan binds to the covalent complex of Topo I and DNA mimicing a DNA base pair and binds at the site of

Table 1 In vitro anti-proliferative activity of synthesized compounds against four tumor cell lines

Compd	IC_{50}/(μmol l^{-1})			
	HCT-8	SGC7901	HepG2	KB
9a	0.205	1.132	0.404	0.531
9c	0.156	1.318	0.518	1.669
9d	0.186	2.642	0.207	0.335
9e	0.534	1.426	0.742	1.589
9f	0.554	1.720	1.706	2.058
10a	2.229	3.792	2.811	2.060
10b	0.120	0.858	0.972	4.553
10c	0.197	1.279	0.549	0.940
10d	0.030	0.262	0.176	0.730
11	2.508	7.485	21.75	7.703
12	6.036	39.24	5.232	8.202
6d	0.567	0.536	0.184	0.777
HCPT, 2	0.181	0.835	0.499	0.128
TPT, 3	1.875	2.292	4.208	2.282

DNA cleavage by base-stacking (Staker et al. 2002; Fan et al. 1998). Although 2′-oxopyrano-fused camptothecins **9a** and **9c–f** are more rigid partly compared with the dihydropyrano-fused camptothecins **6** and **7**, shown in Fig. 2, all these compounds still possess similar activity. This fact suggests that neither saturated nor unsaturated pyran rings affect the base-stacking between camptothecins and the DNA base pair. Furthermore, the binding mode of dihydropyrano-fused and pyrano-fused camptothecin derivatives with the Topo I-DNA complex was investigated via molecular docking simulation of 2′-hydroxyldihydropyrano [3,2-*i*]camptothecin (**6d**) and 2′-oxo-2*H*-pyrano[3,2-*i*] camptothecin (**9a**) in the complex of 1K4T. The result suggested that both compounds were intercalated at the site of DNA cleavage and interacted with Topoisomerase I through H-bonds formed between the groups at E ring and the fused pyran-ring and the surrounding residues (see Supplementary Material).

It is worth noting that the substituents at the 3′-position did not have an obvious influence on the activity except for compound **9f** with bromine group, which went against the trend for improvement in activity compared with the reference compounds. This subtle change in activity was possibly related to the electron-withdrawing of the bromine group, which changes the electronic properties of the whole ring system (Tan et al. 2015). In addition, the ring-opening products **10b–d** but **10a**, demonstrated cytotoxicity similarly as compound **9** did, which confirmed our speculation to some extent regarding the equivalency of the new ring and alkyl groups at 9-position or/and 10-position.

Conclusion

In summary, six A-ring modified hexacyclic camptothecin derivatives containing a pyranone ring fused at 9-positions and 10-positions and five corresponding ring-opened products were designed and synthesized. Preliminary in vitro antiproliferative activity demonstrated that all of the new derivatives, except compounds **11** and **12**, exhibited very potent biological activity comparable to the clinically used drug 10-hydroxycamptothecin (**2**) and topotecan (**3**). In particular, compound **10d** showed more potent in vitro antiproliferative activity than HCPT. Furthermore, the similar anti-proliferative of dihydropyrano-fused and pyrano-fused camptothecin derivatives indicate that the saturated and unsaturated pyran rings do not affect the base-stacking between camptothecins and the DNA base pair, as well as the interactions between the compounds and Topoisomerase I.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Bahekar SS, Shinde DB (2004) Samarium (III)-catalyzed one-pot construction of coumarins. Tetrahedron Lett 45:7999–8001

Bose DS, Rudradas AP, Babu MH (2003) The indium (III) chloride catalyzed von Pechmann reaction: a simple and effective procedure for the synthesis of 4-substituted coumarins. Cheminform 34:9195–9197

Dallavalle S, Rocchetta DG, Musso L, Merlini L, Morini G, Penco S, Tinelli S, Beretta GL, Zunino F (2008) Synthesis and cytotoxic activity of new 9-substituted camptothecins. Bioorg Med Chem Lett 18:2781–2787

Fan Y, Weinstein JN, Kohn KW, Pommier Y (1998) Molecular modeling studies of the DNA-topoisomerase I ternary cleavable complex with camptothecin. J Med Chem 41:2216–2226

Galariniotou E, Fragos V, Makri A, Litinas KE, Nicolaides DN (2007) Synthesis of novel pyridocoumarins and benzofused 6-azacoumarins. Tetrahedron 63:8298–8304

Gao H, Zhang X, Chen Y, Shen H, Sun J, Huang M, Ding J, Li C, Lu W (2005) Synthesis and antitumor activity of 7-ethyl-9-alkyl derivatives of camptothecin. Bioorg Med Chem Lett 15:2003–2006

Hsiang YH, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA Topoisomerase I. J Biol Chem 260:14873–14878

Hu G, Zekria D, Cai X, Ni X (2015) Current status of CPT and its analogues in the treatment of malignancies. Phytochem Rev 14:429–441

Kingsbury WD, Boehm JC, Jakas DR, Holden KG, Hecht SM, Gallagher G, Caranfa MJ, Mccabe FL, Faucette LF, Johnson RK (1991) Synthesis of water-soluble (aminoalkyl)camptothecin analogues: inhibition of topoisomerase I and antitumor activity. J Med Chem 34:98–107

Li J, Wang G, Dong M, Zhang Q (2011) The solvolysis of topotecan in alcohols and acetic anhydride. Bioorg Med Chem Lett 21:2324–2326

Martino E, Della VS, Terribile E, Benetti E, Sakaj M, Centamore A, Sala A, Collina S (2017) The long story of camptothecin: from traditional medicine to drugs. Bioorg Med Chem Lett 27:701–707

Rodriguez-Berna G, Cabanas MJD, Mangas-Sanjuan V, Gonzalez-Alvarez M, Gonzalez-Alvarez I, Abasolo I, Schwartz S, Bermejo M, Corma A (2013) Semisynthesis, cytotoxic activity, and oral availability of new lipophilic 9-substituted camptothecin derivatives. ACS Med Chem Lett 4:651–655

Sheng W, Li Y, Liu Y, Lu A, You Q (2008) Novel hexacyclic camptothecin derivatives. Part 1: synthesis and cytotoxicity of camptothecins with an A-ring fused 1,3-oxazine ring. Bioorg Med Chem Lett 18:4095–4097

Song A, Wang X, Lam KS (2003) A convenient synthesis of coumarin-3-carboxylic acids via Knoevenagel condensation of Meldrum's acid with ortho-hydroxyaryl aldehydes or ketones. Tetrahedron Lett 44:1755–1758

Staker BL, Hjerrild K, Feese MD, Behnke CA, Burgin Jr AB, Stewart L (2002) The mechanism of topoisomerase I poisoning by a camptothecin analog. PNAS 99:15387–15392

Tan H, Wang G, Li J, Meng G, Liu Z, Dong M, Li Y, Ju D, Zhang Q (2015) Synthesis of novel 10-hydroxycamptothecin derivatives utilizing topotecan hydrochloride as ortho-quinonemethide precursor. Bioorg Med Chem 23:118–125

Thomas CJ, Rahier NJ, Hecht SM (2004) Camptothecin: current perspectives. Bioorg Med Chem 12:1585–1604

Venditto VJ, Simanek EE (2010) Cancer therapies utilizing the camptothecins: a review of the in vivo literature. Mol Pharm 7:307–349

Wall ME, Wani MC, Cook CE, Palmer KH, Mcphail AT, Sim GA (1966) Plant antitumor agents I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminate. J Am Chem Soc 88: 3888–3890

Wall ME, Wani MC, Nicholas WA, Manikumar G, Tele C, Moore L, Truesdale A, Leitner P, Besterman JM (1993) Plant antitumor agents. 30. Synthesis and structure activity of novel camptothecin analogs. J Med Chem 36:2689–2700

Wang JC (1996) DNA topoisomerases. Annu Rev Biochem 65:635–692