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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

TQ-B3203, a potent proliferation inhibitor derived from camptothecin

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Abstract To develop topoisomerase I targeted drug candidates with sophisticated liposolubility, a series of novel camptothecin derivatives were synthesized through structure-based molecular hybridization and prodrug design approach. The compounds were used as compositions in micellar emulsion preparations, and the antiproliferative efficacy of these preparations were evaluated in two cancer cell lines (A2780s and A549) in vitro. The designed molecules were afterwards optimized for better potency by modifications at the aliphatic chain, the linker and the camptothecin-yl group to reach the optimal structure **7c** (TQ-B3203), an SN-38 (camptothecin derivative, 7-ethyl-camptothecin-10-yl) containing compound. **7c** showed excellent capacity of inhibiting cell proliferation with IC_{50} value at nanomolar level, and the potency was further confirmed in other human cancer cell lines (HT-29 and HePG2) superior to the positive reference irinotecan. **7c** can be a promising candidate as antitumor drug. Its micellar emulsion preparation has succeeded in the preclinical studies and is in process for investigational new drug(IND) application.

Keywords Camptothecin · Trolox · SN-38 · Antiproliferative activity · Liposoluble

Introduction

Although molecular targeted small-molecule antitumor drugs is developing rapidly, in modern clinical practice, classical chemotherapeutics still plays an important role because of their well-studied pharmacological mechanism and their economic advantage. Targeting property as well as safety of classical chemotherapeutics are improvable with the support of preparations using bio-material constituted carriers, regaining them with competitiveness (Barenholz 2012; Gabizon et al. 1994; Gabizon et al. 2003; Koudelka and Turanek 2012; Lee and Low 1995; Noble et al. 2006; Zhang et al. 2004; Zhang et al. 2013). Among the most common classical chemotherapeutics, camptothecin and its derivatives have good antiproliferation activity, representing a typical treatment for wide range of carcinomas including gastric cancer, colorectal cancer, ovarian cancer, leukemia and liver cancer (Li et al. 2006; Lorence and Nessler 2004; Oberlies and Kroll 2004; Thomas et al. 2004). Camptothecin is a topoisomerase I (topo 1) inhibitor (Redinbo et al. 1998) identified from Traditional Chinese Medicine prescription (Wall et al. 1966). Camptothecin binds to the topo I and DNA complex to form a ternary complex that stabilizes the structure, thereby prevents DNA re-ligation and causes DNA damage, which eventually results in cell apoptosis (Fig. 1) (Sukhanova et al. 2003).

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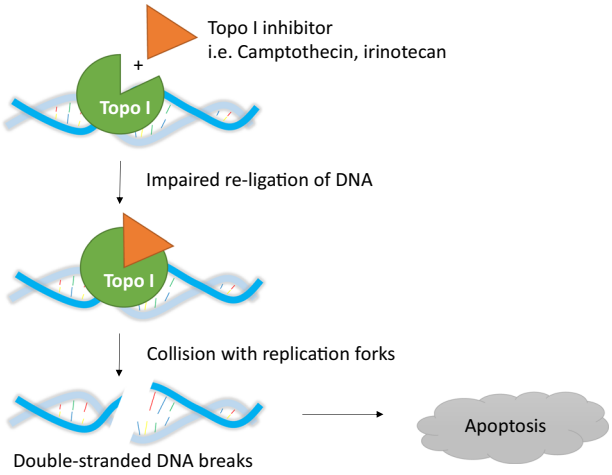


Fig. 1 Diagram of antitumor mechanism for topoisomerase I (topo I) inhibitors

Because of fairly pool solubility in either water or lipid, meaning low bioavailability and adverse drug reaction, camptothecin itself has limitations in clinical use. It is preferable to obtain solubility-improved camptothecin by chemical modification. Topotecan and irinotecan are most representative drugs among the chemical modified camptothecin derivatives (Flowers et al. 2003). Topotecan is the first marketed topo I inhibitor for oral use, while irinotecan, a medicine for injection use, is approved by FDA much earlier and readily on the WHO Model List of Essential Medicines, and comparatively, irinotecan is of greater utilization potential in clinical. The antitumor activity of irinotecan relies on its hydrolysis product in-vivo, SN-38 (Fig. 2), a camptothecin-derived active metabolite playing a direct inhibition role against topo I, leading to suppression of both DNA replication and transcription (Chazin et al. 2014; Kawato et al. 1991; Liu et al. 2015; Rivory et al. 1996). It suggests that to obtain a better therapeutic based on camptothecin, the idea that improving bioavalability of the natural product (better solubility in irinotecan case) by molecular editing at the same time modifying the compound into a prodrug structure that releases an active metabolite after administration can be very practical and inspirational.

To obtain modified camptothecin or camptothecin-derived prodrug with better solubility, hydrophilicity and lipophicity should be balanced firstly before designing of the aimed compounds. The lactone ring in camptothecin core-structure is highly susceptible to hydrolysis, especially under alkaline conditions (Adams et al. 2006b), indicating that modifications to camptothecin derivatives with less hydrophilicity should be preferred. Also, cellular uptake and intracellular accumulation of camptothecin derivatives favors lipophilicity. Lipophilicity makes these compounds more stable because of improved lactone partitioning into red blood cells and consequently less hydrolysis of the lactone. Since camptothecin has affinity for human serum albumin (HSA), reduced drug-HSA interactions could result in improved activity (Adams et al. 2006a; Zunino et al. 2002). Using lipid carriers for delivery in circulation may protect camptothecin derivatives with oil phase surroundings which avoid the direct exposing of the chemical to water soluble hydrophilic HSA in the plasma.

It is reported that tocopherols are commonly used as covalent conjugates to improve the lipophilicity of drugs that are poor in liposolubility (Duhem et al. 2014; Nishina et al. 2015). In fact, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, can be a good option as modification tool to contribute the chemical building blocks (Wu et al. 1992). From this perspective, the camptothecin derivatives consisting of an alkyl trolox-2-carboxylate group as lipophilic moiety, a linker and a camptothecin/SN38 were designed, synthesized and evaluated in this work. The first studied compounds were camptothecin-4-yl ester derivatives **6a–6d**. These compound showed only weak antiproliferation activity comparing with irinotecan. Further modification using SN-38 (7-ethyl-camptothecin-10-yl) as pharmacophore was implemented, giving 7-ethyl-camptothecin-10-yl ester derivatives **7a–7f**. In the cell viability tests using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, compound **7c** among these SN-38 analogs that consists of an (*R*)-2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl moiety and a succinate linker showed the strongest potency against A2780s and A549 cancer cell lines. The high antiproliferation efficacy of **7c** against HT-29 and HePG2 was later confirmed, appeared to be more potent comparing with irinotecan as reference preparation.

Experimental methods

The reagents were purchased from Sigma–aldrich, Sinopharm and ENERGY, China and used without further purification. All yields refer to isolated products after purification. Compounds were characterized by spectroscopic data (mass spectrometry, MS and nuclear magnetic resonance, NMR). The NMR were measured in $CDCl_3$ relative to tetramethylsilane (TMS, 0.00 ppm), and recorded on a Bruker-400 MHz NMR spectrometer. MS were obtained from Finnigan MAT-95 Spectrometry Services.

The synthesis of alkyl 6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylate/carboxamide (2)

A solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (**1**, 3 mmol) in 10 mL N,N'-dimethylformamide (DMF) was added slowly to the stirring solution of

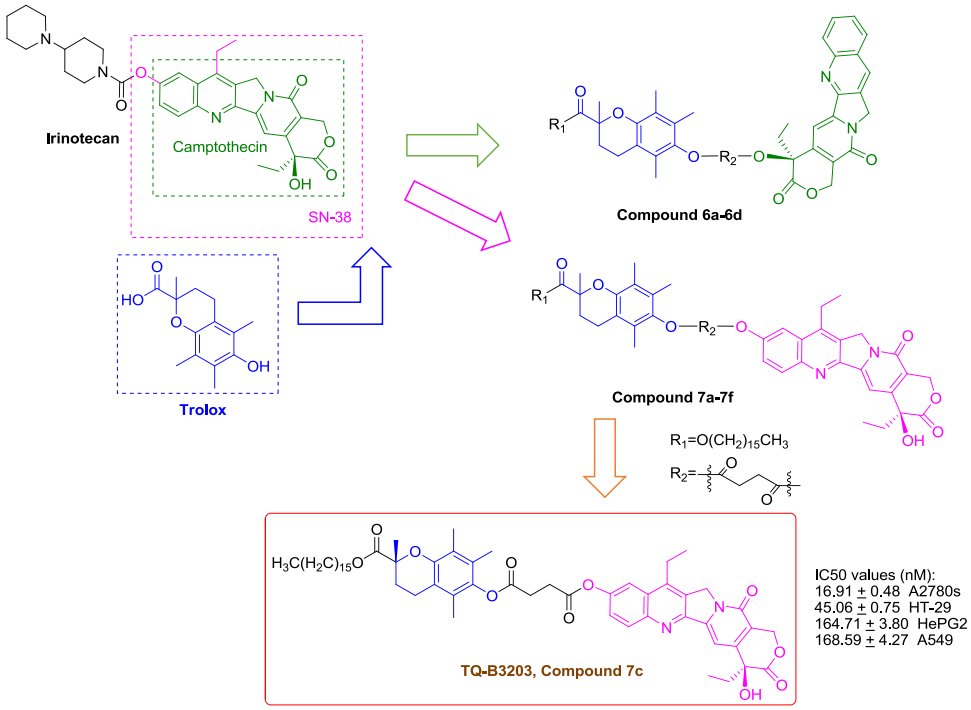


Fig. 2 Two series of compounds derived from camptothecin/SN-38 was designed by incorporation into a trolox group with the purpose of obtaining liposoluble antiproliferative drug candidates. After a brief screening, one compound (**7c**) showed very good lipophicity, and displayed significant antiproliferative potential on all four tested neoplasms cell lines

appropriate aliphatic alcohol/ammine (3 mmol), dimethylaminopyridine (DMAP, 6 mmol) and 2-Chloro-1-methylpyridinium iodide (CMPI, 3 mmol) in DMF (20 mL) in a 50 ml flask. The stirring was continued at room temperature under the atmosphere of nitrogen for 12 h and the completion of reaction was monitored by thin layer chromatography (TLC). The reaction on completion was evaporated under reduced pressure, and mixed with diethyl ether (50 mL). After 2 h of stirring, the mixture was filtered, and the filtrate was separated by chromatography (silica gel, 230–400 mesh) eluted by hexane/ethyl acetate. The physical data for the characteristic compounds is shown below.

Hexadecyl 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate

Yield: 65.4%; MS (Positive ESI): m/z = 475.3 $(M+H)^+$, 497.3 $(M+Na)^+$,971.5 $(2M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.167 (s, 1H,ArOH), 4.077–3.974 (m, 2H, O=$COCH_2$), 2.647–2.383 (m, 3H, CH_2, $ArCH_2CH_2$), 2.160 (s, 3H, CH_3), 2.135 (s, 3H, CH_3), 2.037 (s, 3H, CH_3), 1.878–1.801 (m, 1H, CH_2, $ArCH_2CH_2$), 1.577 (s, 3H, CH_3), 1.535–1.471 (m, 2H, CH_2), 1.282–1.174 (m, 26H, CH_2), 0.879–0.844 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.3 (C=O), 148.7 (C_{Ar}), 140.1 (C_{Ar}), 126.5 (C_{Ar}), 124.9 (C_{Ar}), 121.7 (C_{Ar}), 117.0 (C_{Ar}), 76.9 (C, O-C-COO), 64.5 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.0 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 22.0 (CH_2), 20.2 (CH_2), 13.8 (CH_3), 12.6 (CH_3), 11.6 (CH_3), 11.5 (CH_3).

(R)-hexadecyl 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate

Yield 72.0%; MS (Positive ESI): m/z = 475.3 $(M+H)^+$, 497.3 $(M+Na)^+$, 971.6 $(2M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.173 (s, 1H, ArOH), 4.079–3.690 (m, 2H, O=$COCH_2$), 2.648–2.384 (m, 3H, CH_2, $ArCH_2CH_2$), 2.161 (s, 3H, CH_3), 2.136 (s, 3H, CH_3), 2.038 (s, 3H, CH_3), 1.879–1.802 (m, 1H, CH_2, $ArCH_2CH_2$), 1.577 (s, 3H, CH_3), 1.538–1.475 (m, 2H, CH_2), 1.302–1.176 (m, 26H, CH_2), 0.880–0.846 (t, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.9 (C=O), 148.5 (C_{Ar}), 140.7 (C_{Ar}), 126.6 (C_{Ar}), 125.0 (C_{Ar}), 121.7 (C_{Ar}), 117.0 (C_{Ar}), 76.9 (C, O-C-COO), 64.5 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.0 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 22.1 (CH_2), 20.2 (CH_2), 13.8 (CH_3), 12.5 (CH_3), 11.7 (CH_3), 11.5 (CH_3).

(R)-hexyl 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate

Yield: 85.0%; MS (Positive ESI): m/z = 335.3 $(M+H)^+$, 357.2 $(M+Na)^+$, 691.5 $(2M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.081–3.959 (m, 2H, O=$COCH_2$), 2.674–2.392 (m, 3H, CH_2, $ArCH_2CH_2$), 2.159 (s, 3H, CH_3), 2.134 (s, 3H, CH_3), 1.991 (s, 3H, CH_3), 1.877–1.737 (m, 1H, CH_2,

$ArCH_2CH_2$), 1.578 (s, 3H, CH_3), 1.534–1.471 (m, 2H, CH_2), 1.294–1.165 (m, 6H, CH_2), 0.876–0.839 (t, $J = 7.4$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.0 (C=O), 148.8 (C_{Ar}), 140.9 (C_{Ar}), 126.6 (C_{Ar}), 124.9 (C_{Ar}), 121.7 (C_{Ar}), 117.0 (C_{Ar}), 76.9 (C, O-C-COO), 64.5 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 28.9 (CH_2), 28.5 (CH_2), 28.1 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 22.1 (CH_2), 14.1 (CH_3), 12.6 (CH_3), 11.6 (CH_3), 11.5 (CH_3).

(R)-dodecyl l 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate

Yield: 43.6%; MS (Positive ESI): m/z = 419.4 $(M+H)^+$, 441.4 $(M+Na)^+$, 859.7 $(2M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 4.183 (s, 1H, ArOH), 4.079–3.961 (m, 2H, O=$COCH_2$), 2.655–2.383 (m, 3H, CH_2, $ArCH_2CH_2$), 2.161 (s, 3H, CH_3), 2.135 (s, 3H, CH_3), 2.037 (s, 3H, CH_3), 1.879–1.803 (m, 1H, CH_2, $ArCH_2CH_2$), 1.578 (s, 3H, CH_3), 1.525–1.459 (m, 2H, CH_2), 1.304–1.178 (m, 18H, CH_2), 0.884–0.849 (t, $J = 7.0$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.9 (C=O), 148.9 (C_{Ar}), 140.8 (C_{Ar}), 126.5 (C_{Ar}), 124.9 (C_{Ar}), 121.8 (C_{Ar}), 117.1 (C_{Ar}), 76.9 (C, O-C-COO), 64.6 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.9 (CH_2), 25.0 (CH_3, CH_3-C-C=O), 21.9 (CH_2), 20.1 (CH_2), 13.9 (CH_3), 12.6 (CH_3), 11.7 (CH_3), 11.6 (CH_3).

The synthesis of 2-(alkyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy acid derivatives (3)

A solution of alkyl 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate/carboxamide (**2**, 2 mmol), appropriate acid chloride, acid anhydride or halogen ester (3 mmol), cesium carbonate (2.5 mmol) in 20 mL DMF was stirring at room temperature under the atmosphere of nitrogen for 12 h and the completion of reaction was monitored by TLC. The reaction on completion was mixed with ethyl acetate (100 mL), and washed 3 times with water (50 mL each). After drying and evaporating, the residue of the organic phase was separated by chromatography (silica gel, 230–400 mesh) eluted by hexane/acetone. To obtain the free acid from the product of halogen ester, an aqueous solution of LiOH (2 mmol in 5 mL) was added slowly to the stirring solution of the ester in a 50 mL flask. The stirring was continued for 2 h and the completion of reaction was monitored by TLC. The methanol in the reaction on completion was evaporated and the pH value of the rest solution was adjusted to 3~4 by adding HCl (0.1 N) dropwise. After lyophilization, the product was obtained after recrystallized from ethanol. The physical data for the characteristic compounds is shown below.

4-(2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy)-4-oxobutanoic acid

Yield: 87.2%; MS (Positive ESI): m/z = 597.3 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 4.066–3.999 (m, 2H, O=$COCH_2$), 2.916–2.778 (m, 4H, CH_2, CH_2-C=O), 2.625–2.363 (m, 3H, CH_2), 2.136 (s, 3H, CH_3), 1.994 (s, 3H, CH_3), 1.903 (s, 3H, CH_3), 1.866–1.790 (m, 1H, CH_2), 1.579 (s, 3H, CH_3), 1.512–1.496 (m, 2H, CH_2), 1.298–1.193 (m, 26H, CH_2), 0.876–0.842 (t, $J = 6.8$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 174.2 (C=O), 173.5 (C=O), 170.8 (C=O), 149.0 (C_{Ar}), 141.4 (C_{Ar}), 126.8 (C_{Ar}), 124.7 (C_{Ar}), 122.7 (C_{Ar}), 117.8 (C_{Ar}), 77.8 (C, O-C-COO), 65.8 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 27.9 (CH_2), 24.9 (CH_3, CH_3-C-C=O), 22.4 (CH_2), 20.0 (CH_2), 14.1 (CH_3), 12.2 (CH_3), 11.9 (CH_3), 11.6 (CH_3).

(R)-4-(2-(hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy)-4-oxobutanoic acid

Yield 79.3%; MS (Positive ESI): m/z = 597.5 $(M+Na)^+$, 1194.0 $(2M+2Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 4.084–4.002 (m, 2H, O=$COCH_2$), 2.911–2.776 (m, 4H, CH_2, CH_2-C=O), 2.633–2.363 (m, 3H, CH_2), 2.137 (s, 3H, CH_3), 1.995 (s, 3H, CH_3), 1.903 (s, 3H, CH_3), 1.867–1.791 (m, 1H, CH_2), 1.580 (s, 3H, CH_3), 1.514–1.499 (m, 2H, CH_2), 1.330–1.240 (m, 26H, CH_2), 0.878–0.844 (t, $J = 6.8$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 173.2 (C=O), 172.6 (C=O), 170.7 (C=O), 148.7 (C_{Ar}), 140.9 (C_{Ar}), 126.6 (C_{Ar}), 124.9 (C_{Ar}), 121.7 (C_{Ar}), 117.0 (C_{Ar}), 76.9 (C, O-C-COO), 64.5 (CH_2, OCH_2), 31.2 (CH_2), 29.8 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.0 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 22.0 (CH_2), 20.2 (CH_2), 13.8 (CH_3), 12.6 (CH_3), 11.6 (CH_3), 11.5 (CH_3).

(R)-4-(2-(hexyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy)-4-oxobutanoic acid

Yield: 79.0%; MS (Positive ESI): m/z = 457.3 $(M+Na)^+$, 891.6 $(2M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 4.086–3.974 (m, 2H), 2.915–2.883 (t, $J = 6.4$ Hz, 2H), 2.809–2.777 (t, $J = 6.4$ Hz, 2H), 2.667–2.373 (m, 3H), 2.135 (s, 3H), 1.992 (s, 3H), 1.901 (s, 3H), 1.864–1.788 (m, 1H), 1.580 (s, 3H), 1.491 (s, 2H), 1.281–1.189 (m, 6H), 0.857–0.822 (t, $J = 7$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 174.9 (C=O), 171.2 (C=O), 170.3 (C=O), 149.3 (C_{Ar}), 141.8 (C_{Ar}), 127.2 (C_{Ar}), 127.0 (C_{Ar}), 123.8 (C_{Ar}), 117.2 (C_{Ar}), 79.5 (C, O-C-COO), 65.9 (CH_2, OCH_2), 30.6 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 28.2

(CH_2), 25.6 (CH_3, CH_3-C-C=O), 22.1 (CH_2), 13.4 (CH_3), 11.9 (CH_3), 11.6 (CH_3), 11.2 (CH_3).

(R)-4-(2-(dodecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy)-4-oxobutanoic acid

Yield: 86.1%;MS (Positive ESI): m/z = 541.4 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 4.089–3.999 (m, 2H), 2.911–2.879 (t, *J* = 6.4 Hz, 2H), 2.807–2.774 (t, *J* = 6.6 Hz, 2H), 2.664–2.363 (m, 3H), 2.136 (s, 3H), 1.994 (s, 3H), 1.902 (s, 3H), 1.866–1.790 (m, 1H), 1.579 (s, 3H), 1.496 (m, 2H), 1.299–1.192 (m, 18H), 0.878–0.844 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 174.7 (C=O), 171.1 (C=O), 170.9 (C=O), 149.2 (C_{Ar}), 141.0 (C_{Ar}), 127.8 (C_{Ar}), 126.9 (C_{Ar}), 123.3 (C_{Ar}), 116.9 (C_{Ar}), 79.4 (C, O-C-COO), 65.7 (CH_2, OCH_2), 31.2 (CH_2), 29.6 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 27.8 (CH_2), 24.9 (CH_2), 24.7 (CH_3, CH_3-C-C=O), 22.7 (CH_2), 14.0 (CH_3), 12.0 (CH_3), 11.9 (CH_3), 11.7 (CH_3).

The synthesis of 2-(alkyloxycarbonyl)-tocopherol-6-yl camptothecin-4-yl ester derivatives (6)

A solution of appropriate 2-(alkyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy acid derivative (**3**, 0.5 mmol), camptothecin (**4**, 0.5 mmol), DMAP (1.2 mmol) and CMPI (0.6 mmol), in 20 mL DMF was stirring at room temperature under the atmosphere of nitrogen for 4 h and the completion of reaction was monitored by TLC. The reaction on completion was poured onto ethyl acetate (100 mL) and filtered. The filtrate was evaporated and the residue was separated by chromatography (silica gel, 230–400 mesh) eluted by hexane/acetone. The physical data for the synthesized compounds is shown below.

*2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl camptothecin-4-yl succinate (**6a**)*

Yield: 69.4%; MS (Positive ESI): m/z = 906.3 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.071–7.980 (m, 2H, Ar-H), 7.846 (s, 1H, Ar-H), 7.780–7.584 (m, 2H, Ar-H), 6.744 (s, 1H, Ar-H), 4.762–4.738 (m, 2H, CH_2, OCH_2), 4.224 (s, 2H, NCH_2), 4.121–4.050 (m, 2H, OCH_2), 2.905–2.715 (m, 4H, CH_2), 2.559–2.425 (m, 2H, CH_2), 2.270–2.199 (m, 2H, CH_2), 2.079 (s, 9H, CH_3), 2.009–1.879 (m, 2H, CH_2), 1.638–1.606 (m, 5H, CH_3&CH_2), 1.498–1.249 (m, 26H, CH_2), 1.004–0.875 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.3 (C=O), 171.5 (C=O), 170.4 (C=O), 168.3 (C=O), 156.4 (C=O), 153.8 (C_{Ar}), 149.6 (C_{Ar}), 149.4 (C_{Ar}), 146.7 (C_{Ar}), 145.2 (C_{Ar}), 140.1 (C_{Ar}), 131.3 (C_{Ar}), 130.8 (C_{Ar}), 129.2 (C_{Ar}), 128.6 (C_{Ar}), 127.4 (C_{Ar}), 127.0 (C_{Ar}), 126.9 (C_{Ar}), 126.7 (C_{Ar}), 126.0 (C_{Ar}), 119.7 (C_{Ar}), 115.8 (C_{Ar}), 97.0 (C_{Ar}), 73.9 (C, O-C-COO), 76.8 (C, O-C-COO), 66.4 (CH_2, OCH_2), 66.3 (CH_2, OCH_2), 54.5 (CH_2, NCH_2), 34.9 (CH_2), 32.2 (CH_2), 31.8 (CH_2), 30.3 (CH_2), 30.0 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 25.2 (CH_3, CH_3-C-C=O), 22.2 (CH_2), 20.7 (CH_2), 16.4 (CH_3), 13.9 (CH_3), 12.2 (CH_3), 11.8 (CH_3), 7.7 (CH_3).

*Hexadecyl 6-(2-(camptothecin-4-yloxy)-2-oxoethoxy)-2,5,7,8-tetramethylchroman-2-carboxylate (**6b**)*

Yield: 73.0%; MS (Positive ESI): m/z = 864.5 $(M+H)^+$; ^1H NMR($CDCl_3$, 400 MHz): δ = 8.094–7.977 (m, 2H, Ar-H), 7.850 (s, 1H, Ar-H), 7.779–7.719 (m, 1H, Ar-H), 7.619–7.574 (m, 1H, Ar-H), 6.748 (s, 1H, Ar-H), 4.901 (s, 2H, OCH_2), 4.764–4.744 (m, 2H, OCH_2), 4.230 (s, 2H, NCH_2), 4.119–4.043 (m, 2H, OCH_2), 2.858–2.753 (m, 2H, CH_2), 2.490–2.225 (m, 2H, CH_2), 2.084 (s, 9H, CH_3), 1.995–1.934 (m, 2H, CH_2), 1.630–1.619 (m, 5H, CH_3&CH_2), 1.461–1.401 (m, 2H, CH_2), 1.340–1.258 (m, 24H, CH_2), 0.927–0.863 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.5 (C=O), 168.6 (C=O), 168.3 (C=O), 157.6 (C=O), 152.0 (C_{Ar}), 149.2 (C_{Ar}), 148.1 (C_{Ar}), 146.6 (C_{Ar}), 145.4 (C_{Ar}), 145.0 (C_{Ar}), 132.8 (C_{Ar}), 130.5 (C_{Ar}), 129.3 (C_{Ar}), 128.6 (C_{Ar}), 127.9 (C_{Ar}), 127.1 (C_{Ar}), 127.0 (C_{Ar}), 126.7 (C_{Ar}), 119.8 (C_{Ar}), 119.6 (C_{Ar}), 117.3 (C_{Ar}), 96.9 (C_{Ar}), 77.0 (C, O-C-COO), 73.9 (C, O-C-COO), 67.3 (CH_2, OCH_2), 65.3 (CH_2, OCH_2), 65.0 (CH_2, OCH_2), 53.1 (CH_2, NCH_2), 34.4 (CH_2), 31.7 (CH_2), 30.9 (CH_2), 29.7 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 26.2 (CH_3, CH_3-C-C=O), 23.1 (CH_2), 20.6 (CH_2), 15.9 (CH_3), 15.6 (CH_3), 14.4 (CH_3), 12.9 (CH_3), 7.6 (CH_3).

*Hexadecyl 6-((camptothecin-4-yl)phosphoryloxy)-2,5,7,8-tetramethylchroman-2-carboxylate (**6c**)*

Yield: 82.9%; MS (Positive ESI): m/z = 824.4 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.012–7.963 (m, 2H, Ar-H), 7.842 (s, 1H, Ar-H), 7.776–7.737 (m, 1H, Ar-H), 7.609–7.553 (m, 1H, Ar-H), 6.740 (s, 1H, Ar-H), 4.765–4.736 (m, 2H, OCH_2), 4.223 (s, 2H, NCH_2), 4.141–4.055 (m, 2H, OCH_2), 2.862–2.740 (m, 2H, CH_2), 2.487–2.226 (m, 2H, CH_2), 2.112–2.013 (m, 11H, CH_3&CH_2), 1.695–1.603 (m, 5H, CH_3&CH_2), 1.511–1.406 (m, 5H, CH_3&CH_2), 1.338–1.240 (m, 24H, CH_2), 0.941–0.839 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.2 (C=O), 171.5 (C=O), 156.6 (C=O), 152.5 (C_{Ar}), 150.2 (C_{Ar}), 149.7 (C_{Ar}), 146.0 (C_{Ar}), 145.5 (C_{Ar}), 143.1 (C_{Ar}), 132.1 (C_{Ar}), 129.4 (C_{Ar}), 128.6 (C_{Ar}), 128.6 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 127.3 (C_{Ar}), 127.0 (C_{Ar}), 123.1 (C_{Ar}), 121.8 (C_{Ar}), 119.6 (C_{Ar}), 117.6 (C_{Ar}), 98.1 (C_{Ar}), 74.8 (C, O-C-COO), 73.7 (C, O-C-COO), 65.7 (CH_2, OCH_2), 65.6 (CH_2, OCH_2), 53.4 (CH_2, NCH_2), 34.1 (CH_2), 32.3 (CH_2), 30.1 (CH_2), 29.5 (CH_2), 28.8 (CH_2), 28.8

(CH_2), 28.4 (CH_2), 25.2 (CH_3, CH_3-C-C=O), 21.7 (CH_2), 21.6 (CH_2), 14.7 (CH_3), 14.2 (CH_3), 13.7 (CH_3), 12.2 (CH_3), 11.0 (CH_3), 8.4 (CH_3).

*(R)-2-(hexadecylcarbamoyl)-2,5,7,8-tetramethylchroman-6-yl camptothecin-4-yl succinate (**6d**)*

Yield: 70.2%; MS (Positive ESI): m/z = 905.5 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.105–7.964 (m, 3H, Ar-H), 7.844 (s, 1H, Ar-H), 7.780–7.706 (m, 1H, Ar-H), 7.632–7.559 (m, 1H, Ar-H), 6.746 (s, 1H, Ar-H), 4.766–4.740 (m, 2H, OCH_2), 4.223 (s, 2H, NCH_2), 3.259–3.155 (m, 2H, NCH_2), 2.850–2.752 (m, 4H, CH_2), 2.495–2.551 (m, 2H, CH_2), 2.374–2.122 (m, 2H, CH_2), 2.084 (s, 9H), 1.995–1.926 (m, 2H, CH_2), 1.637 (s, 3H, CH_3), 1.548–1.508 (m, 2H, CH_2), 1.324–1.259 (m, 26H, CH_2), 0.923–0.809 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.1 (C=O), 173.3 (C=O), 170.8 (C=O), 167.3 (C=O), 157.1 (C=O), 152.0 (C_{Ar}), 149.2 (C_{Ar}), 148.1 (C_{Ar}), 145.8 (C_{Ar}), 144.9 (C_{Ar}), 140.7 (C_{Ar}), 131.9 (C_{Ar}), 130.0 (C_{Ar}), 128.7 (C_{Ar}), 127.9 (C_{Ar}), 127.8 (C_{Ar}), 127.8 (C_{Ar}), 127.0 (C_{Ar}), 126.9 (C_{Ar}), 120.3 (C_{Ar}), 115.5 (C_{Ar}), 96.8 (C_{Ar}), 76.4 (C, O-C-COO), 75.1 (C, O-C-COO), 65.7 (CH_2, OCH_2), 54.1 (CH_2, NCH_2), 40.8 (CH_2, NCH_2), 33.6 (CH_2), 32.6 (CH_2), 31.2 (CH_2), 30.4 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.3 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 26.8 (CH_2), 23.1 (CH_3, CH_3-C-C=O), 22.7 (CH_2), 15.4 (CH_3), 14.4 (CH_3), 12.1 (CH_3), 11.6 (CH_3), 7.8 (CH_3).

The synthesis of 2-(alkyloxycarbonyl)-tocopherol-6-yl 7-ethyl-camptothecin-10-yl ester derivatives (7)

A solution of appropriate 2-(alkyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yloxy acid derivative (**3**, 1 mmol), thionyl chloride (2 mmol), 10 μL DMF in 20 mL toluene was stirring at room temperature under the atmosphere of nitrogen for 4 h and the completion of reaction was monitored by TLC. After evaporation, 10 mL chloroform was added to the reaction, and the resulted solution was added slowly to the stirring solution of SN-38 (**5**, 0.5 mmol) and triethylamine (0.6 mmol) in 20 mL DMF in a 100 mL flask. The stirring was continued at room temperature under the atmosphere of nitrogen for 4 h and the completion of reaction was monitored by TLC. The reaction on completion was poured onto ethyl acetate (100 mL), and washed 3 times with water (50 mL each). The organic phase was evaporated and the residue was separated by chromatography (silica gel, 230–400 mesh) eluted by hexane/acetone. The physical data for the synthesized compounds is shown below.

*2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7a**)*

Yield: 50.0%; MS (Positive ESI): m/z = 949.4 $(M+H)^+$, 1898.8 $(2M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 8.238–8.215 (d, J = 4.6 Hz, 1H, Ar-H), 7.798 (s, 1H, Ar-H), 7.645 (s, 1H, Ar-H), 7.645–7.529 (m, 1H, Ar-H), 5.758–5.718 (d, J = 8 Hz, 1H, OCH_2), 5.313–5.272 (d, J = 8 Hz, 1H, OCH_2), 5.243 (s, 2H, NCH_2), 4.075–4.017 (m, 2H, OCH_2), 3.709 (s, 1H, OH), 3.144–3.082 (m, 6H, CH_2), 2.597–2.382 (m, 3H, CH_2), 2.145 (s, 3H, CH_3), 2.020 (s, 3H, CH_3), 1.928 (s, 3H, CH_3), 1.902–1.801 (m, 1H, CH_2), 1.598–1.507 (m, 5H, CH_3&CH_2), 1.374–1.336 (t, J = 7.6 Hz, 3H, CH_3), 1.289–1.187(m, 26H, CH_2), 1.024–1.005 (t, J = 7.4 Hz, 3H, CH_3), 0.869–0.835 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.6 (C=O), 172.4 (C=O), 171.0 (C=O), 170.6 (C=O), 156.7 (C=O), 151.9 (C_{Ar}), 150.0 (C_{Ar}), 149.1 (C_{Ar}), 146.5 (C_{Ar}), 145.8 (C_{Ar}), 145.1 (C_{Ar}), 140.8 (C_{Ar}), 131.3 (C_{Ar}), 128.5 (C_{Ar}), 127.0 (C_{Ar}), 126.5 (C_{Ar}), 125.3 (C_{Ar}), 124.9 (C_{Ar}), 121.8 (C_{Ar}), 119.0 (C_{Ar}), 117.1 (C_{Ar}), 115.0 (C_{Ar}), 96.6 (C_{Ar}), 77.0 (C, O-C-COO), 72.3 (C, O-C-COO), 65.2 (CH_2, OCH_2), 64.6 (CH_2, OCH_2), 49.5 (CH_2, NCH_2), 31.2 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 28.3 (CH_2), 27.9 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 25.0 (CH_2), 22.2 (CH_2), 22.0 (CH_2), 20.2 (CH_2), 13.8 (CH_3), 13.7 (CH_3), 12.6 (CH_3), 11.7 (CH_3), 11.6 (CH_3), 7.7 (CH_3).

*Hexadecyl 6-(2-(7-ethyl-camptothecin-10-yloxy)-2-oxoethoxy)-2,5,7,8-tetramethylchroman-2-carboxylate (**7b**)*

Yield: 57.0%; MS (Positive ESI): m/z = 908.4 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.869–7.844 (d, J = 10.2 Hz, 1H, Ar-H), 7.750 (s, 1H, Ar-H), 7.515–7.492 (d, J = 9.2 Hz, 1H, Ar-H), 6.747 (s, 1H, Ar-H), 5.154 (s, 2H, OCH_2), 4.761–4.739 (t, J = 8.8 Hz, 2H, OCH_2), 4.226 (s, 2H, NCH_2), 4.119–4.057 (m, 2H, OCH_2), 3.659 (s, 1H, OH), 2.858–2.754 (m, 2H, CH_2), 2.663–2.553 (m, 2H, CH_2), 2.486–2.231 (m, 2H, CH_2), 2.090 (s, 9H, CH_3, $ArCH_3$), 1.894–1.851 (q, 2H, CH_2), 1.699–1.586 (m, 5H, CH_3&CH_2), 1.482–1.415 (m, 2H, CH_2), 1.396–1.251 (m, 27H, CH_2), 0.925–0.854 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 180.2 (C=O), 171.5 (C=O), 171.3 (C=O), 157.3 (C=O), 153.3 (C_{Ar}), 150.3 (C_{Ar}), 148.6 (C_{Ar}), 145.8 (C_{Ar}), 146.2 (C_{Ar}), 142.7 (C_{Ar}), 139.9 (C_{Ar}), 129.1 (C_{Ar}), 128.0 (C_{Ar}), 127.5 (C_{Ar}), 127.6 (C_{Ar}), 125.9 (C_{Ar}), 124.9 (C_{Ar}), 120.4 (C_{Ar}), 118.8 (C_{Ar}), 116.8 (C_{Ar}), 113.5 (C_{Ar}), 97.4 (C_{Ar}), 79.6 (C, O-C-COO), 75.4 (C, O-C-COO), 66.8 (CH_2, OCH_2), 65.2 (CH_2, OCH_2), 65.1 (CH_2, OCH_2), 50.9 (CH_2, NCH_2), 35.7 (CH_2), 32.7 (CH_2), 30.8 (CH_2), 30.0 (CH_2), 29.3 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 25.5 (CH_3, CH_3-C-C=O), 23.5 (CH_2), 22.4 (CH_2), 20.1

(CH_2), 16.5 (CH_3), 15.9 (CH_3), 15.1 (CH_3), 14.6 (CH_3), 12.5 (CH_3), 8.1 (CH_3).

*(R)-2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7c**)*

Yield 76.2%; MS (Positive ESI): m/z = 949.7$(M+H)^+$, 971.7$(M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.223–8.200 (d, J = 9.2 Hz, 1H, Ar-H), 7.796 (s, 1H, Ar-H), 7.624 (s, 1H, Ar-H), 7.554–7.532 (d, J = 8.8 Hz, 1H, Ar-H), 5.757–5.717 (m, 1H, OCH_2), 5.312–5.271 (m, 1H, OCH_2), 5.240 (s, 2H, NCH_2), 4.092–4.021 (m, 2H, OCH_2), 3.714 (s, 1H, OH), 3.142–3.081 (m, 6H, CH_2), 2.632–2.389 (m, 3H, CH_2), 2.146 (s, 3H, $ArCH_3$), 2.020 (s, 3H, $ArCH_3$), 1.929 (s, 3H, $ArCH_3$), 1.903–1.802 (m, 1H, CH_2), 1.589–1.545 (m, 7H, CH_3&CH_2), 1.374–1.335 (t, J = 7.8 Hz, 3H, CH_3), 1.229 (m, 26H, CH_2), 1.042–1.005 (t, J = 7.4 Hz, 3H, CH_3), 0.869–0.836 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.5 (C=O), 171.0 (C=O), 170.6 (C=O), 156.7 (C=O), 151.9 (C_{Ar}), 150.0 (C_{Ar}), 149.0 (C_{Ar}), 148.9 (C_{Ar}), 146.5 (C_{Ar}), 145.8 (C_{Ar}), 145.1 (C_{Ar}), 140.8 (C_{Ar}), 131.3 (C_{Ar}), 128.4 (C_{Ar}), 127.0 (C_{Ar}), 126.5 (C_{Ar}), 125.3 (C_{Ar}), 124.9 (C_{Ar}), 121.8 (C_{Ar}), 119.0 (C_{Ar}), 117.1 (C_{Ar}), 114.9 (C_{Ar}), 96.6 (C_{Ar}), 76.9 (C, O-C-COO), 72.3 (C, O-C-COO), 65.2 (CH_2, OCH_2), 64.6 (CH_2, OCH_2), 49.4 (CH_2, NCH_2), 31.2 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 28.3 (CH_2), 27.9 (CH_2), 25.0 (CH_3, CH_3-C-C=O), 22.2 (CH_2), 22.0 (CH_2), 20.2 (CH_2), 13.8 (CH_3), 13.7 (CH_3), 12.6 (CH_3), 11.7 (CH_3), 11.6 (CH_3), 7.7 (CH_3).

*(R)-2-(hexyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7d**)*

Yield: 89.8%; MS (Positive ESI): m/z = 809.5 $(M+H)^+$, 831.5 $(M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.224–8.202 (d, J = 9.2 Hz, 1H, Ar-H), 7.795 (s, 1H, Ar-H), 7.625 (s, 1H, Ar-H), 7.555–7.532 (d, J = 9.2 Hz, 1H, Ar-H), 5.759–5.718 (m, 1H, OCH_2), 5.313–5.273 (m, 1H, OCH_2), 5.241 (s, 2H, NCH_2), 4.066–4.020 (m, 2H, OCH_2), 3.705 (s, 1H, OH), 3.142–3.083 (6H, CH_2), 2.632–2.382 (m, 3H, CH_2), 2.144 (s, 3H, $ArCH_3$), 2.018 (s, 3H, $ArCH_3$), 1.927 (s, 3H, $ArCH_3$), 1.901–1.799 (m, 1H, CH_2), 1.590–1.512 (m, 7H, CH_3&CH_2), 1.372–1.334 (t, J = 7.6 Hz, 3H, CH_3), 1.232–1.170 (m, 6H, CH_2), 1.041–1.005 (t, J = 7.2 Hz, 3H, CH_3), 0.857–0.822 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.7 (C=O), 171.3 (C=O), 171.6 (C=O), 156.1 (C=O), 153.5 (C_{Ar}), 150.5 (C_{Ar}), 149.2 (C_{Ar}), 148.0 (C_{Ar}), 145.2 (C_{Ar}), 142.9 (C_{Ar}), 140.2 (C_{Ar}), 140.9 (C_{Ar}), 130.1 (C_{Ar}), 127.9 (C_{Ar}), 127.3 (C_{Ar}), 126.4 (C_{Ar}), 127.8 (C_{Ar}), 126.0 (C_{Ar}), 124.8 (C_{Ar}), 119.1 (C_{Ar}), 116.1 (C_{Ar}), 112.7 (C_{Ar}), 98.3 (C_{Ar}), 77.0 (C, O-C-COO), 75.0 (C, O-C-COO), 65.9 (CH_2, OCH_2), 64.8 (CH_2, OCH_2), 51.6 (CH_2, NCH_2), 34.5 (CH_2), 32.3 (CH_2), 29.7 (CH_2), 30.2 (CH_2), 29.7 (CH_2), 28.5 (CH_2), 25.4 (CH_2), 25.1 (CH_3, CH_3-C-C=O), 22.1 (CH_2), 21.0 (CH_2), 15.9 (CH_3), 15.8 (CH_3), 15.1 (CH_3), 12.1 (CH_3), 11.9 (CH_3), 7.6 (CH_3).

*(R)-2-(dodecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7e**)*

Yield: 76.2%; MS (Positive ESI): m/z = 893.7 $(M+H)^+$, 915.7 $(M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.220–8.197 (d, J = 9.2 Hz, 1H, Ar-H), 7.794 (s, 1H, Ar-H), 7.623 (s, 1H, Ar-H), 7.552–7.529 (d, J = 9.2 Hz, 1H, Ar-H), 5.755–5.714 (m, 1H, OCH_2), 5.310–5.269 (m, 1H, OCH_2), 5.238 (s, 2H, NCH_2), 4.075–4.108 (m, 2H, OCH_2), 3.739 (s, 1H, OH), 3.140–3.081 (m, 6H, CH_2), 2.633–2.397 (m, 3H, CH_2), 2.145 (s, 3H, $ArCH_3$), 2.020 (s, 3H, $ArCH_3$), 1.929 (s, 3H, $ArCH_3$), 1.902–1.801 (m, 1H, CH_2), 1.589–1.506 (s, 7H, CH_3&CH_2), 1.373–1.335 (t, J = 7.6 Hz, 3H, CH_3), 1.272–1.223 (m, 18H, CH_2), 1.041–1.004 (t, J = 7.4 Hz, 3H, CH_3), 0.869–0.835 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.3 (C=O), 171.8 (C=O), 170.2 (C=O), 157.4 (C=O), 152.5 (C_{Ar}), 151.0 (C_{Ar}), 150.0 (C_{Ar}), 148.3 (C_{Ar}), 144.7 (C_{Ar}), 141.3 (C_{Ar}), 140.7 (C_{Ar}), 139.4 (C_{Ar}), 129.6 (C_{Ar}), 129.0 (C_{Ar}), 127.9 (C_{Ar}), 127.2 (C_{Ar}), 127.2 (C_{Ar}), 126.6 (C_{Ar}), 125.2 (C_{Ar}), 119.5 (C_{Ar}), 116.1 (C_{Ar}), 113.0 (C_{Ar}), 97.5 (C_{Ar}), 77.5 (C, O-C-COO), 75.0 (C, O-C-COO), 66.6 (CH_2, OCH_2), 65.4 (CH_2, OCH_2), 51.5 (CH_2, NCH_2), 35.4 (CH_2), 32.6 (CH_2), 30.7 (CH_2), 30.3 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 25.9 (CH_2), 24.3 (CH_3, CH_3-C-C=O), 22.2 (CH_2), 21.8 (CH_2), 15.9 (CH_3), 15.7 (CH_3), 14.4 (CH_3), 12.6 (CH_3), 11.8 (CH_3), 7.5 (CH_3).

*(R)-2-(hexadecylcarbamoyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7f**)*

Yield: 68.0%; MS (Positive ESI): m/z = 949.5 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.035 (s, 1H, NH), 7.854 (m, 1H, Ar-H), 7.755 (m, 1H, Ar-H), 7.468–7.575 (m, 1H, Ar-H), 6.748 (s, 1H, Ar-H), 4.763–4.739 (t, J = 9.6 Hz, 2H, OCH_2), 4.226 (s, 2H, NCH_2), 3.654 (s, 1H, OH), 3.234–3.176 (m, 2H, NCH_2), 2.851–2.755 (m, 2H, CH_2), 2.712 (s, 4H, CH_2), 2.668–2.542 (m, 2H, CH_2), 2.372–2.122 (m, 2H, CH_2), 2.083 (s, 9H, $ArCH_3$), 1.930–1.813 (m, 2H, CH_2), 1.633 (s, 3H, CH_3), 1.562–1.477 (m, 2H, CH_2), 1.381–1.196 (m, 29H, CH_2), 0.930–0.879 (m, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 175.6 (C=O), 172.8 (C=O), 171.9 (C=O), 171.4 (C=O), 156.4 (C=O), 152.7 (C_{Ar}), 149.9 (C_{Ar}), 149.4 (C_{Ar}), 149.3 (C_{Ar}), 146.3 (C_{Ar}), 142.5 (C_{Ar}), 141.8 (C_{Ar}), 139.3 (C_{Ar}), 129.0 (C_{Ar}), 128.3 (C_{Ar}), 127.3 (C_{Ar}), 127.1 (C_{Ar}), 126.8 (C_{Ar}), 126.4 (C_{Ar}), 125.4 (C_{Ar}), 119.0 (C_{Ar}),

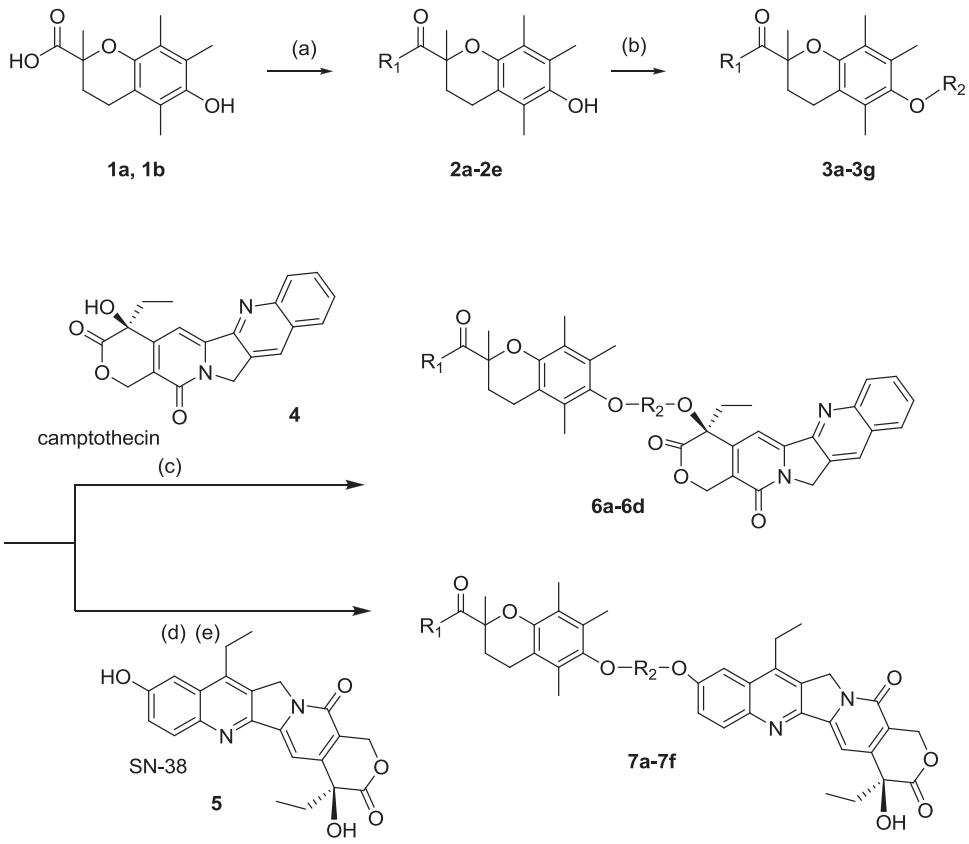


Fig. 3 General synthetic route to camptothecin derivatives **6a–6d** and **7a–7f**. Reagents and conditions: **a** $CH_3(CH_2)_nOH$, DMAP, CMPI, DMF, r.t. ($n = 5$, 11, 15); or $CH_3(CH_2)_{15}NH_2$, DCC, DMF, r.t.; **b** succinic anhydride or ethyl 2-bromoacetate, or methylphosphonic dichloride, Cs_2CO_3/ET_3N, DMF, r.t.; for $R_2 = OCH_2COOC_2H_5$, a further esterolysis gives the free carboxylic acid; **c** DMAP, CMPI, DMF, r.t. **d** $SOCl_2$, DMF, toluene, r.t.; **e** Et_3N, DMF, r.t

115.9 (C_{Ar}), 113.2 (C_{Ar}), 98.4 (C_{Ar}), 77.7 (C, O-C-COO), 74.6 (C, O-C-COO), 65.0 (CH_2, OCH_2), 51.5 (CH_2, NCH_2), 40.3 (CH_2, NCH_2), 35.2 (CH_2), 31.5 (CH_2), 31.2 (CH_2), 30.5 (CH_2), 29.8 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 27.4 (CH_2), 24.5 (CH_3, CH_3-C-C=O), 22.2 (CH_2), 22.0 (CH_2), 15.5 (CH_3), 15.0 (CH_3), 13.9 (CH_3), 12.3 (CH_3), 11.0 (CH_3), 7.4 (CH_3).

Preparation of the micellar emulsions

Solutions of designed compounds (0.5 mmol) in the mixture of Tween-80 (0.5 mL), ethanol (0.5 mL) and polyethyleneglycol 200 (PEG200, 0.5 mL) were mixed with deionized water (8.5 mL) by vigorous vibration. Concentration of each resulted micellar emulsion was estimated to be 50 mM, and diluted to indicated concentration with RPMI 1640 (HyClone) plus 10% Bovine Calf Serum (Gibco) for the activity assay.

Antiproliferation activity

The antiproliferation activity of compounds was established using MTT method, against human ovarian carcinoma cell line A2780s and human lung carcinoma cell line A549, and further confirmed against human colon adenocarcinoma cell line HT-29 and human liver carcinoma cell line HePG2. These cells were provided by Chia-tai Tianqing Pharmaceutical, Jiangsu, China., In brief, cells were seeded in 96-well plates at a density of 1×10^4 cells/well and then cultured at 37 °C 18 h. The micellar emulsion of compounds **6a–6d** and **7a–7f** were added to each well (final concentrations: 2, 5, 10, 20, 40, 70 and 100 μM). After 72 h treatment, 20 μL of MTT solution in phosphate buffered saline (PBS, 5 mg/mL) was added to each well, and the cells were incubated for another 4 h at 37 °C. After the culture medium removed, 100 μL of dimethylsulfoxide (DMSO) was added to dissolve formazan crystal; the percentage of cell viability was determined using a microplate reader (ELx808, BioTek). The IC50 values were defined as the drug concentrations resulting in 50% cell viability compared to the controls. The antiproliferation activity of compounds was determined in triplicate, in comparison with Irinotecan.

Result and discussion

Chemistry

The general synthetic procedures for the target compounds **6a–6d** and **7a–7f** are outlined in Fig. 3. The commercially available starting material 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) **1** was treated

with aliphatic alcohol/ammine in the presence of DMAP and CMPI in DMF. This reaction and the reactions there after should be carried out under the atmosphere of nitrogen to prevent byproducts from the oxidation of tocopherol group. To achieve adequate coupling efficiency, the amount of DMAP was increased from catalytic amount (0.5 Eq, yield 20 ~ 50%) to 2 Eq (yield 40 ~ 85%). Purified compound **2** was reacted at room temperature with succinic anhydride, ethyl 2-bromoacetate, or methylphosphonic dichloride to obtain **3** with the linker conjugation, so that thecarboxyl esterificated trolox can connect covalently to camptothecin (compound **4**) or SN-38 (compound **5**) to give **6a–6d** or **7a–7f**. Coupling of **3** and **4** were readily realized using DMAP (2.4 Eq) in presence of CMPI (1.2 Eq). However, coupling of **3** and **5** did not efficiently initiate under the same condition. Another strategy to obtain **7a–7f** includes two steps, in which firstly the compound **3** was activated with chlorinated reagents such as $SOCl_2$, and the activated product was then reacted with **5** (0.5 Eq). The crudes were purified using column chromatography on silica gel. The chemical structures of novel compounds were confirmed through spectroscopic techniques including MS and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The results are presented in the Experimental section.

Antiproliferation activity

The in vitro antiproliferative activities were evaluated against A2780s cells and A549 cells. Irinotecan was used as positive reference. As shown in Table 1, compound **6a** showed only weak antiproliferative activity against both cell lines. For similar camptothecin-4-yl esters with linkers other than succinate (**6b** and **6c**), no activity improvement comparing to **6a** was observed. Moreover, using hexadecylamine in substitute of hexadecanol did not improve the camptothecin-4-yl ester (**6d**) activity, either. The viability of the cells after **7a** treatment, on the other hand, displayed significant decrease at compound comcentration ≥ 2 μM, far exceeded the potency of **6a** and even surpassed that of irinotecan, the positive control. Comparing **6a** and **7a** for activities, SN-38 was considered a preferred pharmacophore than camptothecin. Another linker for the 2-(alkyloxycarbonyl)-tocopherol-6-yl 7-ethyl-camptothecin-10-yl ester derivatives similar to **7a**, -CH_2CO-, was also tested (**7b**). However **7b** showed to compromised antiproliferation potency, so the succinate linker in **7a** is considered preferable. Based on the above results, the 7-ethyl-camptothecin-10-yl pharmacophore and succinate linker were more promising and selected for further structural modification.

Since compound **7a** is racemic, we examined the potential of its chiral isomer. The *R*-form (**7c**) was tested against A2780s cells and A549 cells, and showed improved potency than **7a** on both cell lines, suggesting *R*-form is a preferred chiral isomer. Moreover, considering the carbon chain on the trolox-2-carboxylate affects significantly the liposolubility of target compound, we also tested other aliphatic alcohol substitution instead of hexadecanol. Compound **7d** and **7e**, containing a hexyl and a dodecyl group, respectively, displayed decreased antiproliferation potency with the alkyl chain length decreasing. To confirm the 2-amide form of the trolox moiety is less preferable compared to 2-ester form, hexadecylamine derivative **7f** was tested, and the activity is weaker comparing with **7c**, as expected. In summary, compound (*R*)-2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate (**7c**) displayed excellent antiproliferative activities against the two cell lines compared with the positive reference drug. The IC_{50} values (concentration required to achieve 50% inhibition of the tumor cell proliferation) of the tested compounds for each cell line are presented in Table 1. Compound **7c** was selected as potential antitumor drug candidate and designated as TQ-B3203 for further evaluation.

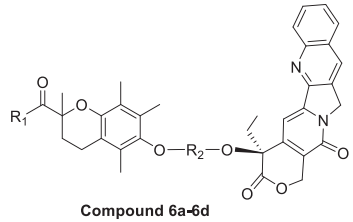
Another antiproliferative activity evaluation using HT-29 cells and HePG2 cells was continued for TQ-B3203 (**7c**) in comparing with irinotecan. As shown in Fig. 4, in both cell lines TQ-B3203 showed antiproliferative activity more than 10-folds stronger than the positive control irinotecan, indicating the candidate TQ-B3203 is very valuable for antitumor drug developing.

Structure activity relationship

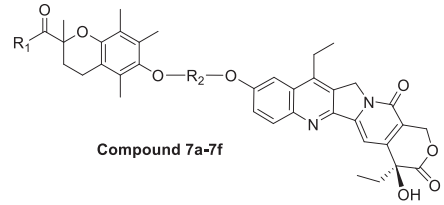
The activity of compound **7c**, **7e** and **7d** decreased with their aliphatic side chain (from long chain aliphatic alcohol to short chain aliphatic alcohol), suggesting that certain length of the carbon chain plays important role in the pharmacological function of 2-(hexyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinates. This moiety may provide the compound with appropriate lipophilic ability to better disperse in the micellar emulsion, and it could thereby facilitate the molecule to undergo more efficient uptake by the host cells.

It's also worth mentioning that succinic acid and succinate participate in the citric acid cycle, an energy-yielding process in all living organisms. Similarly, trolox is a water-soluble analog of vitamin E used in biological or biochemical applications to reduce oxidative stress or damage. And the fatty alcohols are very common in daily diet. All three above chemical building blocks of **7c** have no or very limited activities against neoplasms. For this reason we assume that the antiproliferative activity of TQ-B3203 (**7c**) is from the 7-ethyl-camptothecin-10-yloxy moiety which could process hydrolysis in cells and therefore release

Table 1 Antiproliferative activities of the target compounds (**6a–6d** and **7a–7f**) against A2780s and A549 cells in vitro



Compound 6a-6d



Compound 7a-7f

Compd.	Stereo-chemical[a]	R_1	R_2	IC_{50} (μM)[b,c]	
				A2780s	A549
6a	*R, S*	$O(CH_2)_{15}CH_3$	$CO(CH_2)_2OC$	35.45 ± 0.84	72.11 ± 4.05
6b	*R, S*	$O(CH_2)_{15}CH_3$	CH_2OC	93.54 ± 3.90	>100
6c	*R, S*	$O(CH_2)_{15}CH_3$	$POCH_3$	>100	>100
6d	*R*	$NH(CH_2)_{15}CH_3$	$CO(CH_2)_2OC$	50.23 ± 3.73	45.39 ± 3.62
7a	*R, S*	$O(CH_2)_{15}CH_3$	$CO(CH_2)_2OC$	1.87 ± 0.27	0.96 ± 0.14
7b	*R, S*	$O(CH_2)_{15}CH_3$	CH_2OC	>100	70.69 ± 4.21
7c	*R*	$O(CH_2)_{15}CH_3$	$CO(CH_2)_2OC$	0.0169 ± 0.0048	0.1687 ± 0.023
7d	*R*	$O(CH_2)_5CH_3$	$CO(CH_2)_2OC$	0.0409 ± 0.0105	4.611 ± 0.33
7e	*R*	$O(CH_2)_{11}CH_3$	$CO(CH_2)_2OC$	0.0178 ± 0.0090	2.102 ± 0.39
7f	*R*	$NH(CH_2)_{15}CH_3$	$CO(CH_2)_2OC$	5.72 ± 2.16	11.34 ± 1.21
Irinotecan	–	–	–	19.30 ± 1.62	58.07 ± 3.51

[a] Stereochemistry of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)

[b] Results are expressed as means ± SD (standard deviation) of three independent experiments

[c] Compounds with IC_{50} values > 100 μM are considered to be inactive

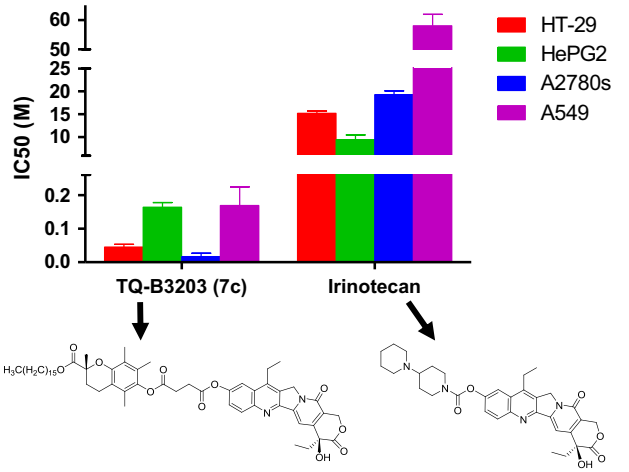


Fig. 4 Antiproliferative activities of compound **7c** against HT-29 and HePG2 cells in vitro. Comparing to irinotecan, TQ-B3203 has a stronger activity in inhibiting the cell viability. The results were in consistence with the Antiproliferative activity results of TQ-B3203 and irinotecan against A2780s and A549 cells

SN-38. Based on this hypothesis, the trolox moiety with the linker on the compound is preferred to be easily cleaved in tissue. TQ-B3203 could be considered a prodrug of SN-38 from this perspective, similar to irinotecan in pharmacological mechanism (Rivory et al. 1996). Our data showed that succinate as the linker provided better potential for designed compounds than other testedl inkers, indicating that succinate was efficient in covalently connect the pharmacophore with cooperating groups at the same time performing better in the release of the active hydrolysis product SN-38.

Conclusion

A series of novel camptothecin derivatives were successfully synthesized and tested for antiproliferative activities against two cancer cell lines (A2780s and A549). Each designed camptothecin-yl compound contain an aliphatic chain, a water soluble vitamin E moiety and a linker, with the purpose of being liposoluble for micellar emulsion preparations. This study led to a potent series of 7-ethyl-camptothecin-10-yl succinates, which were further optimized for higher potency. Among these compounds, TQ-B3203 (**7c**, (*R*)-2-(Hexadecyloxycarbonyl)-2,5,7,8-tetramethylchroman-6-yl 7-ethyl-camptothecin-10-yl succinate) showed promising inhibition activity in antiproliferation tests using two additional tumor cell lines (HT-29 and HePG2), and proved to be the optimal molecule even stronger than the positive reference drug irinotecan. TQ-B3203 can be a preferable candidate for antitumor drug development. Its micellar emulsion preparation has gained success in the preclinical studies and it is now in process as investigational new drug(IND) for clinical assessment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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