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Original article

Synthesis, cytotoxicity, metabolic stability and pharmacokinetic evaluation of fluorinated docetaxel analogs

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ABSTRACT

Three novel fluorinated docetaxel analogs, along with six previous reported, were evaluated for their cytotoxicity against five tumor cell lines. The results indicated that these analogs maintained similar/more potent activity than docetaxel against these tumor cell lines. They were also evaluated for their metabolic stability and pharmacokinetics, which demonstrated that these analogs showed better profiles of metabolic stability and pharmacokinetics than that of docetaxel.

1. Introduction

The naturally occurring paclitaxel (Taxol) is a diterpenoid originally isolated from the bark of *Taxus brevifolia* [1], which is also prepared by semisynthesis and microorganisms now. Paclitaxel (**1**) and its semi-synthetic derivative docetaxel (**2**) (Fig. 1), are currently considered to be the most important and promising anticancer agents in the treatment of refractory breast and ovarian cancers due to their unique mechanism of action by binding tubulin and stabilizing microtubule formation, which ultimately disrupts mitosis and causes cell death [2]. However, some drawbacks of paclitaxel hampered its clinical usefulness, such as poor aqueous solubility, multi-drug resistance (MDR), and low activity for oral administration [3]. To develop new anticancer agents, many approaches have been applied in preparing of analogs with better activity, conjugates or prodrugs with better bioavailability and specificity, and new formulations with improved physical properties [4].

Extensive studies on the structure–activity relationships of paclitaxel and docetaxel were mainly focused on the modifications on C-13 side chain, B and C ring [5]. The importance of C-13 substituted phenylisoserine side chain to the bioactivity of paclitaxel has been acknowledged for a long time. Ojima's group [6] reported a series of taxoids in which 3′-Ph was replaced by alkyl or alkenyl substitutions, in combination with the changes of the substitutions on the C-2 and C-10. The 3′-trifluoromethyl-10-acetyl analogs of docetaxel exhibited better cytotoxicity than paclitaxel and possessed more than one order of magnitude higher potency than paclitaxel and docetaxel against a drug-resistant human breast cancer cell line. Further systematically studies [7–9] demonstrated that incorporating fluorine at the metabolism site in the paclitaxel molecules did indeed block some of the metabolic pathways by enzymes of P450 (CYP) family and increased their stability, in which no hydroxylation was observed for 2-(3-fluorobenzoyl) paclitaxel both at the phenyl ring of 2-(3-fluorobenzoyl) and at the 3′-phenyl ring. This remarkable effect may be contributed by the direct influence from the outlying fluorine moiety upon enzyme–substrate recognition and action. These findings lay the solid foundation for this course of work that the fluorine replacement on the taxoid should be a viable tool to enhance the potency, perhaps against MDR cancer cell lines.

Along with the pursuing of more potent docetaxel analogs as anticancer agents, we also pay our attention to these analogs' metabolic stability and pharmacokinetic profiles. It is believed that only the optimized combination can lead to the success to find desired clinical candidates. As our continuous work to design and synthesis of fluorine-containing docetaxel analogs [10], three novel docetaxel analogs (**3a**–**c**) were synthesized here (Fig. 2). Along with the previous six analogs (**3d**–**i**), these nine analogs will be evaluated for their cytotoxicity against five tumor cell lines. Also

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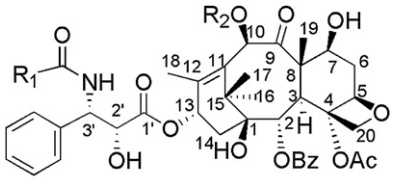


paclitaxel (**1**): R_1 = Ph, R_2 = Ac
docetaxel (**2**) : R_1 = *t*-BuO, R_2 = H

Fig. 1. Structures of paclitaxel and docetaxel.

preliminary metabolic stability and pharmacokinetic study will be executed. Based on the observation from Ojima group, it was anticipated that fluorination(s) on docetaxel should have impact on their metabolic stability and pharmacokinetics profiles. Herein, we reported the syntheses, cytotoxic activity, metabolic stability and pharmacokinetics of a series of fluorinated docetaxel analogs.

2. Results and discussion

2.1. Chemistry

The synthesis of side chain [11–14] commenced from 4-fluorobenzaldehyde (**4**), a commercially available starting material, as illustrated in Scheme 1. 4-Fluorobenzaldehyde was first transformed into 4-fluoro-(*E*)-cinnamic acid (**6**) via the reaction of ethyl bromoacetate with triphenylphosphine in the presence of catalytic amount of titanium tetrachloride, followed by hydrolysis and crystallization. The obtained 4-fluoro-(E)-cinnamic acid (**6**) was then converted to isopropyl cinnamate (**7**) by using isopropanol and thionyl chloride at 0 °C, and then stirred at room temperature overnight in 78% yield. Compound **7** was subjected to a Sharpless asymmetric aminohydroxylation by using $(DHQ)_2PHAL$ as the ligand, freshly prepared N-bromoacetamide as the nitrogen source, and potassium osmate as the oxidant to give the desired amino alcohol (**8**) as a single isomer in 83% yield and with >99% ee. Compound **8** was subjected to hydrolysis in aqueous 10% HCl under reflux to furnish the free amine which was concomitantly transferred to be the methyl ester (**10**). Subsequently, the amine group was transformed into the Boc protected amino alcohol **11** by using Boc_2O in the presence of triethylamine. Cyclic protection using methoxypropene in the presence of catalytic amount of pyridinium para-toluenesulfonate (PPTS) followed by saponification of formed intermediate **12** afforded acid **13** in 98.6% yield.

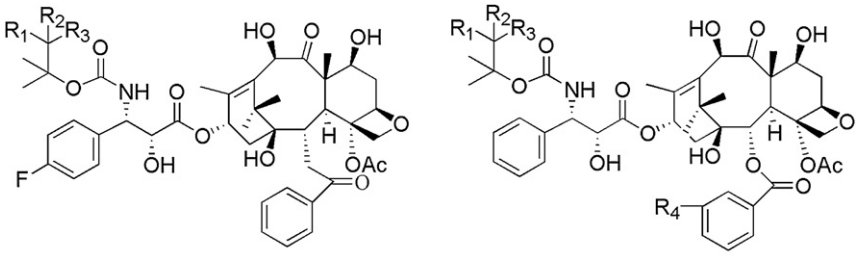
With intermediate **13** in hand, the previous optimized synthetic route was adopted for the synthesis of target compounds **3a–c** (Scheme 2) [10]. First, hydroxyl groups at the C7 and C10 of the natural product 10-deacetylbaccatin III (10-DAB) were protected with 2,2,2-trichloroethyl chloroformate (TrocCl) by using pyridine as the base to obtain 7,10-diTroc-10-deacetylbaccatin (**15**). Then, **15** was coupled with **13** to provide the corresponding intermediate **16** in 86% yield in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). After removing the 3′-N-Boc and acetonide-protecting group of **16** with 98% formic acid at room temperature, the amino alcohol **17** was obtained in 55% yield. After that, **19a–c** were prepared by acylation of amino group of **17** with freshly prepared fluorine containing acyl chlorides (**18a–c**) [15,16], removing the 7,10-Troc protecting groups on **19a–c** with zinc in acetic acid, three corresponding fluorinated docetaxel derivatives (**3a–c**) were synthesized in desirable yields.

2.2. In vitro cytotoxic activity

The *in vitro* cytotoxicity of fluorinated docetaxel derivatives (**3a–i**) were evaluated against two human cancer cell lines [oral epidermoid carcinoma (KB-0528) and human promyelocytic leukemia cells (HL60-0607-2)], and three multiple drug resistance (MDR) cancer cell lines (KBR-0530, KBR-0602, and HL60OR-0604-2) (Table 1). Compared with docetaxel, except **3c** which had 18-fold more potent than docetaxel against KB-0528, all other analogs (**3a–b** and **3d–i**) showed similar or slight better potency against both human cancer cell lines (KB-0528 and HL60-0607-2). Interestingly, analog **3c** also exhibited 20- and 10-fold more potent than docetaxel against HL60-0607-2 and HL60OR-0604-2, while others had similar potency as docetaxel.

2.3. In vitro metabolic stability

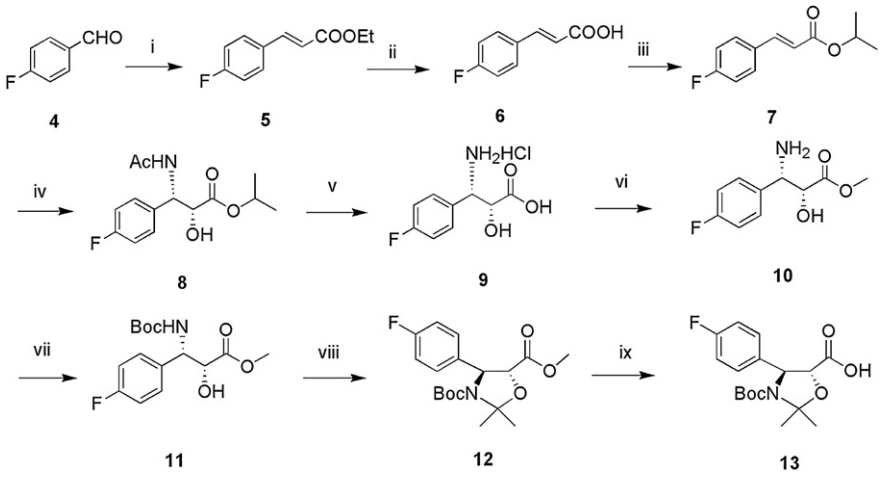
The metabolic study results for fluorinated docetaxel analogs upon incubation with pooled human liver microsome are shown in Table 2 [17–19]. Compared with Docetaxel (22.6% remaining after 60 min), all analogs (**3a–i**) exhibited much better metabolic stability profile. Among them, **3a** and **3e** were two most metabolic stable analogs in this condition with 88.5% and 89.5% intact after 60 min. This result demonstrated that incorporating fluorine(s) at the metabolism site(s) in the paclitaxel molecules did indeed block



3a R_1=F, R_2=H, R_3=H
3b R_1=F, R_2=F, R_3=H
3c R_1=F, R_2=F, R_3=F

3d R_1=F, R_2=H, R_3=H, R4=H
3e R_1=F, R_2=F, R_3=H, R4=H
3f R_1=F, R_2=F, R_3=F, R4=H
3g R_1=F, R_2=H, R_3=H, R4=F
3h R_1=F, R_2=F, R_3=H, R4=F
3i R_1=F, R_2=F, R_3=F, R4=F

Fig. 2. Fluorinated docetaxel analogs.



Scheme 1. Synthesis of key intermediate **13**. Reagents and conditions: (i) $Ph_3P{=}CHCOOEt$, toluene, reflux, 3 h; rt, overnight; (ii) KOH/EtOH, rt, 3 h; (iii) i-PrOH/$SOCl_2$, 0 °C - rt, overnight; (iv) LiOH–H_2O/$K_2OsO_2(OH)_4$, *t*-BuOH–H_2O/$(DHQ)_2PHAL$, AcNHBr, 0 °C, 10 h; (v) 10% HCl aqueous, reflux, 4 h; (vi) MeOH/$SOCl_2$, 0 °C - rt, overnight; (vii) Boc_2O/Et_3N, CH_2Cl_2, 0 °C - rt, 48 h; (viii) $CH_2{=}C(OCH_3)CH_3$, PPTs, toluene, 90 °C, 3 h; (ix) LiOH, THF/MeOH/H_2O, rt, 2 h.

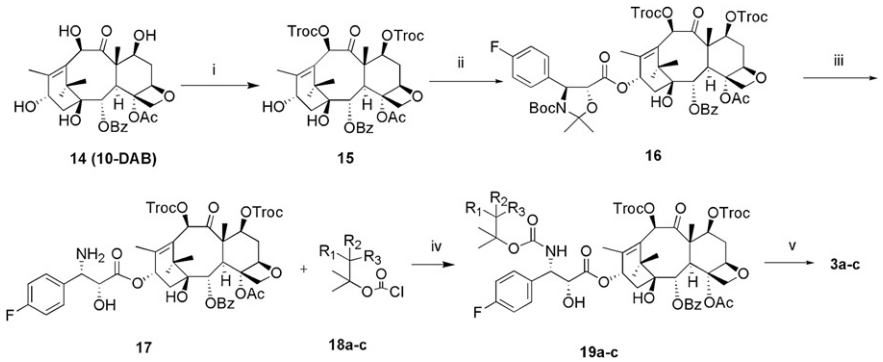
some of the metabolic pathways by enzymes of P450 (CYP) family, which resulted in the significant increase of their metabolic stability. In the inactive pooled human liver microsome, the unchanged drugs of **3a**–**i** at 60 min were 109.2%, 96.9%, 85.2%, 73.2%, 86.9%, 95.0%, 54.6%, 92.9% and 82.5%, respectively, in which the concentration of both **3d** and **3g** decreased along with the prolongation of the incubation time may due to the adsorption of protein or self-degradation. The results of CL_{int} (Table 2) indicated that **3c** was a low intrinsic clearance drug and had a better metabolic stability, while the others (**3a**–**b** and **3d**–**i**) were medium intrinsic clearance drugs, compared with docetaxel. In conclusion, mono-fluorinated tert-butyl docetaxel analogs (eg. **3a**, **3d** and **3g**) had lower metabolic stabilities than those with di- or tri-fluorinated tert-butyl docetaxel analogs (eg. **3b**–**c**, **3e**–**f**, and **3h**–**i**).

2.4. In vivo pharmacokinetics

The pharmacokinetics of fluorinated docetaxel analogs were defined after i.v. administration of 5 mg/kg in rat. The plasma was sampled between 5 min and 24 h after dosing. Liquid–liquid extraction followed by isocratic high-performance liquid chromatography (HPLC) with MS–MS was used to determine drug concentrations in plasma. Compared with docetaxel, AUC_{0-t} and $AUC_{0-\infty}$ of fluorinated docetaxel analogs increased significantly, especially the AUC of **3c**, **3f**, and **3i** increased 6-fold. Meanwhile, the total plasma clearance (CLz) and apparent volume of distribution (Vz) decreased significantly. The AUC of tri-fluorinated tert-butyl docetaxel analogues (**3c**, **3f**, and **3i**) was higher than those of mono, or di-fluorinated tert-butyl docetaxel analogs, while CLz and Vz of those (**3c**, **3f**, and **3i**) were lower. This indicated that fluorine substitution on the side chain of tert-butyl could improve the AUC_{0-t} and $AUC_{0-\infty}$, however, 2-(3-fluorobenzoyl) substitution and at the 3′-(4-fluorophenyl) substitution didn't influence the AUC_{0-t} and $AUC_{0-\infty}$. Except **3a** and **3g**, the other fluorinated docetaxel analogs had a longer elimination half-life time than docetaxel. All fluorinated derivatives had a lower total plasma clearance than docetaxel. Interestingly, the AUC_{0-t} and $AUC_{0-\infty}$ of **3h** were lower than those of **3g**, and the result was confirmed by repeated experiments. Further investigation is on the way (Table 3).

3. Conclusions

Fluorinated docetaxel analogs were synthesized and evaluated for their cytotoxicity, metabolic stability and pharmacokinetics. It was found that some analogs showed potent cytotoxicity, good metabolic stability, and improved pharmacokinetics. Among them, **3c** was shown up as one of the most promising analogs. It was also found that fluorinated substituted on the side chain tert-butyl significantly improved the AUC_{0-t} and $AUC_{0-\infty}$, while 2-(3-fluorobenzoyl) substitution and at the 3′-(4-fluorophenyl) substitution



Scheme 2. Synthesis of new fluorinated docetaxel derivatives (**3a**–**c**). Reagents and conditions: (i) TrocCl, Pyridine, rt, 30 m; (ii) **13**, DCC, DMAP, toluene; (iii) HCOOH, rt, 2 h; (iv) Et_3N, CH_2Cl_2; (v) Zn/HOAc, MeOH, 50 °C.

Table 1
In vitro cytotoxicities (IC_{50} μM) of fluorinated docetaxel analogs.

	KB-0528	KBR-0530	KBR-0602	HL60-0607-2	HL60OR-0604-2
3a	9.146×10^{-4}	2.318×10^{-4}	1.945×10^{-4}	1.399×10^{-4}	5.100×10^{-4}
3b	3.969×10^{-4}	3.207×10^{-4}	8.854×10^{-4}	1.681×10^{-5}	2.085×10^{-3}
3c	2.589×10^{-5}	3.318×10^{-4}	4.323×10^{-4}	3.583×10^{-7}	1.545×10^{-5}
3d	5.057×10^{-4}	0.322×10^{-4}	7.917×10^{-4}	1.564×10^{-4}	9.583×10^{-4}
3e	2.820×10^{-4}	3.321×10^{-4}	3.693×10^{-4}	1.762×10^{-4}	5.539×10^{-4}
3f	8.342×10^{-5}	6.941×10^{-4}	6.260×10^{-4}	2.625×10^{-4}	4.099×10^{-4}
3g	2.476×10^{-4}	6.695×10^{-5}	2.049×10^{-4}	1.110×10^{-6}	2.973×10^{-3}
3h	7.076×10^{-5}	1.609×10^{-4}	1.817×10^{-4}	1.834×10^{-4}	5.840×10^{-4}
3i	2.101×10^{-4}	1.076×10^{-4}	1.492×10^{-4}	5.468×10^{-5}	1.909×10^{-4}
Docetaxel	4.536×10^{-4}	1.317×10^{-4}	2.341×10^{-4}	1.149×10^{-5}	1.140×10^{-4}

didn't significantly influence the AUC_{0-t} and $AUC_{0-\infty}$. Overall, trifluorinated tert-butyl docetaxel analogs exhibited the best metabolic stability and the lowest total plasma clearance.

4. Experimental section

4.1. Material and physical measurements

Reagents were purchased from Aldrich and TCI Chemical companies. All solvents are purified and dried in accordance with standard procedures, unless otherwise indicated. Ether, tetrahydrofuran and toluene ware dried over natrium/benzophenone; dichloromethane and pyridine ware distilled from calcium hydride; and DMF was distilled from calcium hydride by reduced pressure. Oxygen-free and water-free operations were carried out under argon atmosphere in dried glassware unless otherwise noted. All reactions were monitored by TLC with precoated silica gel plates GF_{254}, 10–40 μm (Yantai, China). The purity of the samples was by column chromatography with the silica gel H 300–400 mesh (Yantai, China). Melting points (mp) were determined using an X-4 microscope melting point apparatus and were uncorrected. All ^1H NMR spectra were recorded on Varian Mercury 300 (300 MHz), or Bruker DRX-400 (400 MHz) spectrometer at room temperature, and chemical shifts are reported in ppm (δ) relative to TMS (0.00) as internal standard; expressed in peak shape, the number of hydrogen, and coupling constant in Hertz. ^{13}C NMR spectra data were recorded on Bruker DRX-400 (100 MHz), or Bruker DPX-300 (75 MHz) spectrometer at room temperature, and chemical shifts are reported in ppm (δ) relative to d-substituted solvents (d-chloroform at 77.2 ppm, d6-acetone at 30.6 ppm and 206.7 ppm) as internal standard. Mass spectra ware performed at the testing and analysis center of Shanghai Institute of Organic Chemistry: Low-resolution mass spectra (ESI) were performed on Shimadzu LCMS-2010EV; High-resolution mass spectra on IonSpec 4.7 T FTMS (MALDI) or Bruker Daltonics, Inc. APEXIII7.0 TESLA FMS (ESI).

4.2. Chemistry

4.2.1. Iso-propyl (2E)-3-(4-fluorophenyl) prop-2-enoic acid (**7**)

Compound **5** (16.08 g, 82.86 mmol) was treated with KOH (16 g) in ethanol (220 mL) at room temperature for 3 h. Then the ethanol was removed under reduced pressure. The residue was diluted with water and extracted with CH_2Cl_2 twice to remove organic impurities. The obtained aqueous layer was then acidified with dilute HCl, extracted with ether (400 mL × 2). The combined ether was dried over anhydrous Na_2SO_4 and evaporated to afford the crude acid. The crude acid was dissolved in i-PrOH (230 mL), and $SOCl_2$ (9.05 mL, 124.3 mmol) was added dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 85 °C for 4 h, and then quenched with saturated $NaHCO_3$ to pH = 8. The solvent was evaporated under reduced pressure, and diluted with H_2O. The water phase was extracted with EtOAc for three times. The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to give the product **7** (13.42 g, 77.9%); ^1H NMR (300 MHz, $CDCl_3$): δ 1.31 (d, J = 6.3 Hz, 6H), 5.14 (m, 1H), 6.35 (d, J = 15.9 Hz, 1H), 7.10 (*t*, J = 8.7 Hz, 2H), 7.51 (dd, J = 8.7, 5.4 Hz, 2H) 7.63 (d, J = 15.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.1, 68.1, 116.1, 116.3, 118.8, 130.0, 131.0, 143.2, 166.6; EIMS *m/z* 208, 166, 149; HRMS (EI) *m/z* calcd for $C_{12}H_{13}O_2F$: 208.0906, found 208.0900.

4.2.2. Isopropyl (2R,3S)-3-acetylamino-2-hydroxy-3-(4-fluorophenyl) propionate (***8***)

To a flash (100 mL) charged with aqueous solution (25 mL) of LiOH-H_2O (0.222 g, 5.27 mmol) was added $K_2OsO_2(OH)_4$ (0.045 g, 0.122 mmol, 2.4 mmol%). The mixture was stirred for 30 min and then *t*-BuOH (50 mL) and ligand $(DHQ)_2PHAL$ (1.58 mmol%, 0.062 g, 0.0789 mmol) were added. The resulting mixture was stirred for 1 h at room temperature to give a clear solution. Water (25 mL) was subsequently added and the reaction mixture was cooled to 0 °C, **7** (1.04 g, 5.0 mmol) was added followed by the addition of fresh

Table 2
$T_{1/2}$ in liver microsomes and predicted CL_{int} *in vivo* of fluorinated docetaxel analogs.

	Unchanged drug at 60 min (%)	$T_{1/2}$ (min)	CL_{int} (mL/min/kg)
3a	53.8	144.4	8.62
3b	72.4	364.8	3.41
3c	88.5	3465.7	0.36
3d	47.2	121.6	10.24
3e	89.5	385.1	3.23
3f	58.1	239.0	5.21
3g	46.8	123.8	10.06
3h	71.0	266.6	4.67
3i	63.6	216.6	5.75
Docetaxel	22.6	70.0	17.78

Table 3
Pharmacokinetics parameters *in vivo* of fluorinated docetaxel analogs.

	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	MRT (h)	$t_{1/2}$ (h)	CLz (L/h/kg)	Vz (L/kg)
3a	1640	1659	2.13	5.06	3.02	22.0
3b	3147	3361	4.28	13.4	1.51	27.4
3c	3874	3979	2.78	8.50	1.28	15.8
3d	914	994	5.62	12.2	5.09	89.7
3e	2857	2932	2.76	7.69	1.79	20.2
3f	3859	3960	2.32	9.91	1.26	18.1
3g	2192	2238	2.30	6.80	2.26	22.7
3h	1932	1989	2.40	10.15	2.55	36.2
3i	3867	3944	2.13	7.62	1.28	13.7
Docetaxel	646	667	3.34	7.37	7.51	78.8

AcNHBr (0.897 g, 6.5 mmol) in one portion. The reaction mixture was stirred at 0 °C and monitored by TLC. After completion, the reaction mixture was treated with Na_2SO_3 (2 g) and stirred for 30 min at room temperature, after which EtOAc was added. The organic layer was separated and the water layer was extracted with EtOAc for three times. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography using petroleum ether/EtOAc (3/2) to afford **8** (1.176 g, 83.0%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (d, J = 6.6 Hz, 3H), 1.32 (d, J = 6.3 Hz, 3H), 2.00 (s, 3H), 3.23 (br s, 1H), 4.44 (d, J = 2.1 Hz, 1H), 5.11 (m, 1H), 5.53 (dd, J = 9.0, 1.5 Hz, 1H), 6.41 (d, J = 9.3 Hz, 1H), 7.03 (*t*, J = 8.7 Hz, 2H), 7.38 (dd, J = 8.7, 5.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.7, 21.7, 23.3, 58.6, 69.9, 73.7, 115.5, 115.7, 128.9, 135.1, 163.7, 172.5; ESIMS *m/z* 306.0 $[M + Na]^+$; HRMS (ESI) *m/z* calcd for $C_{14}H_{19}NO_4F^+$: 284.1301 $[M + H]^+$, found 284.12926.

*4.2.3. General procedure for the preparation of **19a–c***

To a stirred solution of **17** (500 mg, 0.46 mmol) in anhydrous THF (10 mL) was dropwise added excessive **18** at 0 °C, followed by a solution of triethylamine (0.13 mL, 0.93 mmol) in anhydrous THF (5 mL). The resulting mixture was stirred at room temperature for 1 h, and then extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by silica gel flash chromatography column (petroleum ether/ethyl acetate: 2/1) to afford **19a–c** as white solid.

*4.2.3.1. N-De-t-butoxycarbonyl-N-[2-(1-fluoro-2-methyl)propyloxycarbonyl]-7,10-di-(2,2,2-trichloroethyloxycarbonyl)-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**19a**).* Yield 55.8%; mp 156–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 3H), 1.27 (s, 3H), 1.35 (s, 6H), 1.87 (s, 3H), 1.96 (s, 3H), 2.32 (m, 2H), 2.39 (s, 3H), 2.07 and 2.63 (2m, 2H), 3.91 (d, J = 6.9 Hz, 1H), 4.19 and 4.34 (2d, J = 8.1 Hz, 2H), 4.32 (m, 2H), 4.61 and 4.91 (2d, J = 11.7 Hz, 2H), 4.62 (m, 1H), 4.78 (s, 2H), 4.96 (d, J = 8.4 Hz, 1H), 5.27 (m, J = 9.6 Hz, 1H), 5.53 (d, J = 9.9 Hz, 1H), 5.54 (m, 1H), 5.70 (d, J = 6.9 Hz, 1H), 6.24 (s, 1H), 6.24 (*t*, J = 8.1 Hz, 1H), 7.10 (*t*, J = 8.7 Hz, 2H), 7.38 (dd, J = 8.7, 5.1 Hz, 2H), 7.51 (*t*, J = 7.5 Hz, 2H), 7.63 (*t*, J = 7.5 Hz, 1H), 8.11 (d, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.7, 14.7, 20.9, 22.2, 22.5, 26.3, 33.3, 35.3, 43.2, 46.9, 53.4, 56.3, 72.3, 73.3, 74.1, 76.4, 77.1, 78.6, 79.1, 79.9, 80.9, 83.6, 86.8, 94.2, 115.8, 126.7, 128.6, 128.7, 128.8, 129.0, 130.2, 132.1, 133.8, 138.0, 142.3, 153.2, 153.2, 154.8, 161.1, 166.8, 170.4, 172.4, 200.7; MALDIMS *m/z* 1192.0 $[M + H]^+$; HRMS (ESI) *m/z* calcd for $C_{49}H_{53}NO_{18}F_2Cl_6Na^+$: 1214.1267 $[M + Na]^+$, found 1214.1258.

*4.2.3.2. N-De-t-butoxycarbonyl-N-[2-(1,1-difluoro-2-methyl)propyloxycarbonyl]-7,10-di(2,2,2-trichloroethyloxycarbonyl)-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**19b**).* Yield 58.1%; mp 172–174 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 3H), 1.27 (s, 3H), 1.39 (s, 6H), 1.86 (s, 3H), 1.94 (s, 3H), 2.31 (d, J = 8.7 Hz, 2H), 2.37 (s, 3H), 2.07 and 2.63 (2m, 2H), 3.90 (d, J = 6.6 Hz, 1H), 4.18 and 4.34 (2d, J = 8.7 Hz, 2H), 4.60 and 4.92 (2d, J = 12.0 Hz, 2H), 4.62 (m, 1H), 4.78 (s, 2H), 4.95 (d, J = 11.4 Hz, 1H), 5.25 (d, J = 8.7 Hz, 1H), 5.54 (dd, J = 10.8, 7.2 Hz, 1H), 5.63 (d, J = 9.9 Hz, 1H), 5.69 (d, J = 6.6 Hz, 1H), 6.00 (*t*, J = 5.7 Hz, 1H), 6.24 (s, 1H), 6.24 (*t*, J = 7.5 Hz, 1H), 7.11 (*t*, J = 8.7 Hz, 2H), 7.38 (dd, J = 8.7, 5.1 Hz, 2H), 7.51 (*t*, J = 7.5 Hz, 2H), 7.64 (*t*, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.7, 14.7, 19.8, 20.8, 22.5, 26.3, 33.3, 35.2, 43.1, 46.9, 55.5, 56.3, 72.3, 73.2, 74.1, 76.4, 77.1, 77.4, 78.6, 79.1, 79.9, 80.9, 83.6, 94.1, 114.8, 115.7, 115.9, 128.5, 128.6, 128.7, 128.9, 129.8, 130.1, 132.2, 133.9, 142.1, 153.2, 153.2, 154.2, 161.3, 163.8, 166.8, 170.4, 172.2, 200.6; MALDIMS *m/z* 1232.2 $[M + Na]^+$; HRMS (ESI) *m/z* calcd for $C_{49}H_{52}NO_{18}F_3Cl_6Na^+$: 1232.1173 $[M + Na]^+$, found 1232.1168.

*4.2.3.3. N-De-t-butoxycarbonyl-N-[2-(1,1,1-trifluoro-2-methyl)propyloxycarbonyl]-7,10-di(2,2,2-trichloroethyloxycarbonyl)-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**19c**).* Yield 50.0%; mp 170–172 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 3H), 1.27 (s, 3H), 1.54 and 1.58 (2s, 6H), 1.86 (s, 3H), 1.93 (s, 3H), 2.31 (m, 2H), 2.36 (s, 3H), 2.07 and 2.63 (2m, 2H), 3.90 (d, J = 6.6 Hz, 1H), 4.18 and 4.34 (2d, J = 8.7 Hz, 2H), 4.60 and 4.92 (2d, J = 12.0 Hz, 2H), 4.62 (s, 1H), 4.78 (s, 2H), 4.96 (d, J = 10.2 Hz, 1H), 5.25 (br d, J = 9.0 Hz, 1H), 5.53 (dd, J = 10.8, 7.5 Hz, 1H), 5.68 (m, 1H), 5.69 (d, J = 6.3 Hz, 1H), 6.24 (s, 1H), 6.24 (*t*, J = 9.3 Hz, 1H), 7.11 (*t*, J = 8.7 Hz, 2H), 7.40 (dd, J = 8.4, 5.4 Hz, 2H), 7.51 (*t*, J = 7.5 Hz, 2H), 7.64 (*t*, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.7, 14.7, 19.4, 19.6, 20.7, 22.5, 26.4, 33.3, 35.2, 43.1, 46.9, 55.6, 56.3, 72.3, 73.2, 74.0, 76.4, 77.1, 77.4, 78.6, 79.1, 81.0, 83.6, 94.1, 115.8, 128.6, 128.7, 128.9, 130.1, 132.3, 133.9, 142.0, 153.2, 153.3, 154.8, 163.7, 166.9, 170.4, 172.1, 200.6; MALDIMS *m/z* 1266.2 $[M + K]^+$; HRMS (ESI) *m/z* calcd for $C_{49}H_{51}NO_{18}F_4Cl_6Na^+$: 1250.1078 $[M + Na]^+$, found 1250.1125.

*4.2.4. General procedure for the preparation of **3a–c***

To a solution of **19a–c** (0.19 mmol) in methanol (10 mL) were added glacial acetic acid (4.60 mL) and zinc powder (0.46 g, 7.08 mmol). The resulting mixture was stirred at 50 °C for 1 h. The reaction mixture was filtered to remove the zinc and solid formed. Removal of the solvent by distillation gave a white solid. The obtained solid was then dissolved in ethyl acetate (60 mL), which was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The obtained residue was purified by silica gel flash chromatography column (petroleum ether/Acetone: 2/1) to give **3a–c** as a white solid.

*4.2.4.1. N-De-t-butoxycarbonyl-N-[2-(1-fluoro-2-methyl)propyloxycarbonyl]-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**3a**).* Yield 57.5%; mp 168–170 °C; [α]20D −9.8° (*c* 0.50 MeOH); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 1.16 (s, 3H), 1.21 (s, 3H), 1.36 (s, 6H), 1.73 (s, 3H), 1.91 (s, 3H), 2.31 (m, 2H), 2.42 (s, 3H), 1.84 and 2.46 (2m, 2H), 3.77 (s, 1H), 3.94 (d, J = 7.2 Hz, 1H), 4.17 (s, 2H), 4.24 (m, 1H), 4.32 (m, 1H), 4.35 (s, 1H), 4.33 and 4.50 (2m, 2H), 4.66 (m, 1H), 4.91 (d, J = 6.6 Hz, 1H), 4.97 (d, J = 7.8 Hz, 1H), 5.24 (s, 1H), 5.24 (m, 1H), 5.69 (d, J = 7.5 Hz, 1H), 6.20 (*t*, J = 8.4 Hz, 1H), 6.82 (d, J = 9.3 Hz, 1H), 7.17 (*t*, J = 8.7 Hz, 2H), 7.54 (m, 2H), 7.56 (*t*, J = 7.8 Hz, 2H), 7.66 (*t*, J = 7.5 Hz, 1H), 8.10 (d, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.4, 13.5, 20.4, 21.6, 21.7, 22.1, 26.1, 36.0, 36.7, 43.3, 46.6, 57.7, 71.4, 71.6, 74.0, 74.3, 75.0, 76.0, 77.7, 79.9, 80.9, 84.1, 86.0, 87.7, 115.0, 128.5, 129.2, 129.9, 130.4, 133.1, 136.7, 137.5, 155.0, 165.7, 170.0, 210.6; ESIMS *m/z* 866.5 $[M + Na]^+$; HRMS (MALDI) *m/z* calcd for $C_{43}H_{51}NO_{14}F_2Na^+$: 866.3154 $[M + Na]^+$, found 866.31699.

*4.2.4.2. N-De-t-butoxycarbonyl-N-[2-(1,1-difluoro-2-methyl)propyloxycarbonyl]-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**3b**).* Yield 60.4%; mp 180–182 °C; [α]20D −16.8° (*c* 0.43 MeOH); ^{1}H NMR (300 MHz, CD3COCD3): δ 1.16 (s, 3H), 1.21 (s, 3H), 1.39 (s, 6H), 1.73 (s, 3H), 1.90 (s, 3H), 2.31 (m, 2H), 2.41 (s, 3H), 1.84 and 2.46 (2m, 2H), 3.78 (s, 1H), 3.94 (d, J = 7.2 Hz, 1H), 4.17 (s, 2H), 4.24 (m, 1H), 4.30 (m, 1H), 4.35 (s, 1H), 4.67 (m, 1H), 4.95 (s, 1H), 4.96 (d, J = 7.5 Hz, 1H), 5.22 (m, 1H), 5.24 (s, 1H), 5.69 (d, J = 7.5 Hz, 1H), 6.14 (*t*, J = 57.0 Hz, 1H), 6.20 (*t*, J = 9.0 Hz, 1H), 7.04 (d, J = 9.6 Hz, 1H), 7.18 (*t*, J = 8.7 Hz, 2H), 7.55 (m, 2H), 7.56 (*t*, J = 7.8 Hz, 2H), 7.67 (*t*, J = 7.5 Hz, 1H), 8.10 (d, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, CDCl3): δ 9.4, 13.5, 19.1, 19.3, 20.4, 22.1, 26.1, 36.0, 36.7, 43.3, 46.6, 56.7, 57.7, 71.4, 71.6, 73.9, 74.3, 75.0, 76.0, 77.7, 79.9, 80.9, 84.1, 115.04, 115.4, 128.5, 129.2, 129.3, 129.9, 130.4, 133.2, 136.7, 137.4, 154.5, 162.2, 165.7, 170.0, 172.5, 210.6; ESIMS *m/z* 884.4 $[M + Na]^+$; HRMS (MALDI) *m/z* calcd for C43H50NO14F3Na$^+$: 884.3071 $[M + Na]^+$, found 884.30756.

*4.2.4.3. N-De-t-butoxycarbonyl-N-[2-(1,1,1-trifluoro-2-methyl)propyloxycarbonyl]-3′-dephenyl-3′-(4-fluorophenyl)docetaxel (**3c**).* Yield 52.5%; mp 166–168 °C; [α]20D −23.8° (*c* 0.53 MeOH); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 1.15 (s, 3H), 1.20 (s, 3H), 1.58 (s, 6H), 1.72 (s, 3H), 1.90 (s, 3H), 2.29 (m, 2H), 2.40 (s, 3H), 1.84 and 2.46 (2m, 2H), 3.78 (s, 1H), 3.93 (d, J = 7.2 Hz, 1H), 4.17 (s, 2H), 4.23 (m, 1H), 4.30 (m, 1H), 4.35 (s, 1H), 4.67 (m, 1H), 4.96 (m, 1H), 4.99 (d, J = 7.8 Hz, 1H), 5.23 (m, 1H), 5.24 (s, 1H), 5.68 (d, J = 7.2 Hz, 1H), 6.10 (*t*, J = 7.8 Hz, 1H), 7.18 (*t*, J = 8.7 Hz, 2H), 7.19 (m, 1H), 7.54 (m, 2H), 7.56 (*t*, J = 7.5 Hz, 2H), 7.67 (*t*, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.4, 13.5, 18.9, 20.4, 22.1, 22.5, 26.1, 36.0, 36.7, 43.3, 46.6, 57.7, 71.6, 74.0, 74.3, 75.0, 76.0, 80.9, 84.1, 115.1, 128.5, 129.3, 129.9, 130.4, 133.1, 136.8, 165.8, 170.0, 172.5, 210.5; ESIMS *m/z* 902.3 $[M + Na]^+$; HRMS (MALDI) *m/z* calcd for $C_{43}H_{49}NO_{14}F_4Na^+$: 902.2970 $[M + Na]^+$, found 902.29814.

4.3. In vitro cytotoxicity

Potential cytotoxicity was evaluated against five human cancer cell lines, i.e., breast carcinoma (MDA-MB-468), ovarian carcinoma (HO-8910), non-small cell lung carcinoma (A549), and hepatoma carcinoma (BEL-7402). The compounds were pre-dissolved in DMSO and diluted with cell culture medium to at least five required concentrations. The content of DMSO in the final concentrations did not exceed 0.1%. At this content, DMSO was found to be nontoxic to the cells tested. All cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, at 37 °C in a humidified atmosphere of 5% CO_2, The cells were seed at a density of 0.4–1.0 × 10^4 cells per well in 96-well microplates. After 24 h, the cells were treated with 10 times diluted concentration test compound. The cells were exposed to drugs for 72 h. Cell growth was assayed using sulforhodamine B (SRB). The optical density (OD) was read 520 nm. All cytotoxicity tests were performed three times in quadruplicate. The IC_{50} values were calculated from curves constructed by plotting cell survival (%) versus compound concentration (in μM).

4.4. Metabolic stability [17–19]

0.1M potassium phosphate buffer (pH 7.4) stock solutions of test compound usually in DMSO or water) were prepared. Stock solution of compounds was diluted into buffer to give a concentration of 5 μmol/L with 0.5 mg/mL pooled human liver microsome (Lot 46262, BD Gentest, US) and 1 mM NADPH in 200 μL incubation solution. All samples were incubated at 37 °C and incubation was ended by adding 200 μL acetonitrile at 0, 5, 10, 15, 30, 60 min. Then the extraction solvent (n-hexane : dichloromethane : isopropanol = 300:150:15, v/v/v) was added and the proteins were removed by centrifugation. The supernatant was analyzed for the amount of remaining parent compound by HPLC-MS. The HPLC/ITMS experiment was carried out on an Agilent 6330LC/MSD Trap XCT ultra (Agilent Technologies, Waldbronn, Germany). The mass spectrometer was equipped with electrospray ionization (ESI) sources. The ionization mode was positive. The interface and MSD parameters of the ESI source were as follows: nebulizer pressure 40 psi (N_2), drying gas 12 mL/min (N_2), drying gas temperature 350 °C, spray capillary voltage 3500 V, skimmer voltage 40 V, ion transfer capillary exit 124 V, scan range *m/z* 100–1000. The Agilent 1200 high-performance liquid chromatography (HPLC) system was equipped with a reversed-phase column (Capcell-CN UG-120 column; 6 × 100 mm i.d., 5 μm) protected by a 4.0 × 3.0 mm i.d. Security Guard (5 mm) C_{18} guard column (Phenomenex, Torrance, CA, USA). The mobile phase was a gradient of a mixture of acetonitrile : 5 mM ammonium acetate (55 : 45. v/v). The flow rate was 0.6 mL/min and the injection volume was 20 μL.

4.5. Pharmacokinetics

The test compounds were dissolved in the mixture of cremophor:etbanol (1:1), and then diluted further with sterile 0.154 M NaCl such that i.v. dose of 5 mg/kg test compounds could be delivered in a volume of 10 ml/kg. Sprague–Dawley male rats (200–220 g) with five rats in each group received food and water ad libitum except on the evening prior to dosing, when all food was removed and withheld until 2 h after dosing. Serial specimens were collected via the retrobulbar vein at 5, 15, 30, 60, 120, 180, 240, 360, 480, 600, and 1440 min after dosing. Blood was collected by cardiac puncture into heparinized syringes and stored on ice until centrifuged at 3000 rpm for 10 min to obtain plasma. Plasma and dosing solutions were stored frozen at −20 °C until analysis. The concentrations of the test compounds in the plasma were detected by LC-MS-MS, and pharmacokinetic parameters were calculated from the mean plasma concentration by non-compartmental analysis.

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