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Synthesis and evaluation of water-soluble docetaxel prodrugs-docetaxel esters of malic acid

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Abstract—The synthesis of docetaxel esters of malic acid is described. These compounds were found to have greatly improved water solubility and are stable in solution at neutral pH. The C2′ modified compounds **2a–c** and **3a–c** behave as prodrugs, that is, docetaxel is generated upon exposure to human plasma, whereas the C7 and C2′,7,10- l modified derivatives do not. 2′-DL-Malyl docetaxel sodium salt demonstrated enhanced antitumor activity in vitro when compared to docetaxel and showed the inhibitory effect on tumor growth in vivo.

1. Introduction

The diterpenoid natural product paclitaxel (Taxol®) **1a** and its semi-synthetic analogue docetaxel (Taxotere®) **1b** are two important chemotherapeutic drugs widely used today for the treatment of various types of cancer. They are known to exert their therapeutic effect, at least in part, by promoting the assembly of tubulin into microtubules and stabilizing the resulting microtubules. They thereby inhibit microtubule disassembly and normal dynamic reorganization of the microtubular network required for mitosis.[1]

However, the clinical usefulness of these drugs is particularly hampered by their poor solubility. So they should be co-injected with Cremophor EL or Tween 80.

Cremophor EL in paclitaxel for injection frequently causes untoward hypersensitivity reactions (hypotension, bronchospasm, urticaria, etc.). And Tween 80 in docetaxel for injection hampers the clinical usefulness because of its haemolyticus and viscidity, especially for the patients who are hypersensitive to Tween 80.[2] Patients receiving these drugs should be premedicated.[3] To solve the problem of low water solubility, several research groups have synthesized and evaluated water-soluble taxoids through the introduction of solubilizing moieties to C2′-, C7- or/and C10-positions. The solubilizing moieties can be (salts of) carboxylic acids, phosphates, sulfonates, amines, sugar derivatives or polyethylene glycol. In most cases these moieties are coupled via an ester or carbonate linkage.[4]

As we know, docetaxel is a little more water-soluble than paclitaxel, but just 6–7 μg/mL in aqueous solution (paclitaxel: 0.25 μg/mL).[5–8] Until now, just a few attempts have been undertaken to overcome poor solubility of docetaxel, though numerous researches on analogues, prodrugs,[6] and derivatives of paclitaxel have been carried out. Previously, some glycosyl derivatives of docetaxel were prepared and evaluated.[9,10] A direct 7-*O*-glucosyl docetaxel analogue was reported to be twice as water-soluble as paclitaxel and with similar activity.[11] Amino acid conjugates linked to the 2′-hydroxyl of a 3′-cyclopropyl docetaxel analogue were also prepared and tested in vivo.[12] Some showed better activity

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than docetaxel in ILS (Increase of Life Span) on B16 melanoma-bearing mice.

Since our aim was to increase water solubility of docetaxel, we considered converting docetaxel into water-soluble prodrugs, which, after enzymatic cleavage in vivo, will release the active compound. We chose malic acid, a non-cytotoxic acid of the Krebs cycle which could be metabolized easily, as the solubilizing moiety.

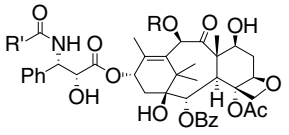
In the present paper, we report the synthesis of eight docetaxel prodrugs, in which the malyl moiety is linked to different hydroxyl groups: C2′-malyl docetaxel **2** and their sodium salts **3** (including C2′-DL-malyl docetaxel **2a**, C2′-L-malyl docetaxel **2b**, C2′-D-malyl docetaxel **2c**, and their sodium salts **3a**–**c**), 2′,7,10-tri-(malyl) docetaxel **4**, and C7-malyl docetaxel **5**. All the target compounds were determined by ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and DEPT135. The stability of synthetic compounds in PBS buffer and plasma and the antitumor activity (in vitro and in vivo) were tested and are described in this paper.

2. Chemistry

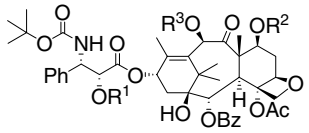
For preparing our target compounds, we protected one of the carboxyl groups of malic acid with acetone to obtain 1,2-*O*-isopropylidene-malic acid,[13] then the latter was reacted with docetaxel in different conditions. Compound **6a** was prepared by condensing docetaxel with protected malic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) at −10 °C. Then deprotection of compound **6a** was effected in a mixture of HOAc–THF–H_2O to provide compound **2a**. Exposure of this compound to aqueous $NaHCO_3$ afforded its sodium salt **3a** (Scheme 1). In the same manner, compounds **2b** and **3b**, **2c** and **3c** were synthesized. The significant downfield shift of the C-2′H (∼δ 5.28) and no great change of C-7H (∼δ 4.24) and C-10H (∼δ 5.28) in **3a**–**c** relative to that in docetaxel in ^{1}H NMR spectrum demonstrated that the malyl was linked to 2′-OH of docetaxel.

The reaction, carried out with an excess of protected malic acid in refluxing dichloromethane replacing DCC by diisopropylcarbodiimide (DIC), afforded 2′,7,10- tri-(malyl) docetaxel **4** instead compared with the result of the literature[3] (Scheme 2). In ^{1}H NMR spectrum, the significant downfield shift of the C-2′H (δ 5.28), C-7H (δ 5.62), and C-10H (δ 6.32) in **4** relative to that in docetaxel and two groups of malyl (δ 4.48, 3H, CH-malyl; δ 2.86, 6H, CH_2-malyl) demonstrated that three malyl groups were linked to 2′-OH, 7-OH, and 10-OH of docetaxel, respectively, since the reacting power of 1-OH is lower than the others of docetaxel.

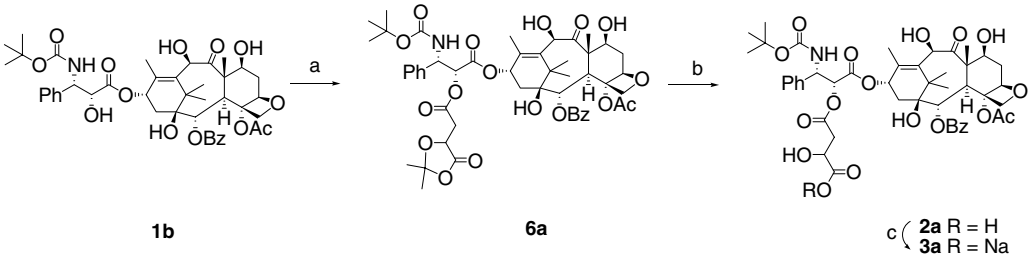
In the preparation of C7-malyl docetaxel **5**, 2′- and 10-OH were protected first by treatment with 2,2,2-Trichloroethylchloroformate (Troc-Cl) at −23 °C under



Paclitaxel **1a** (R = Ac; R' = Ph)
Docetaxel **1b** (R = H; R' = ^{t}BuO)

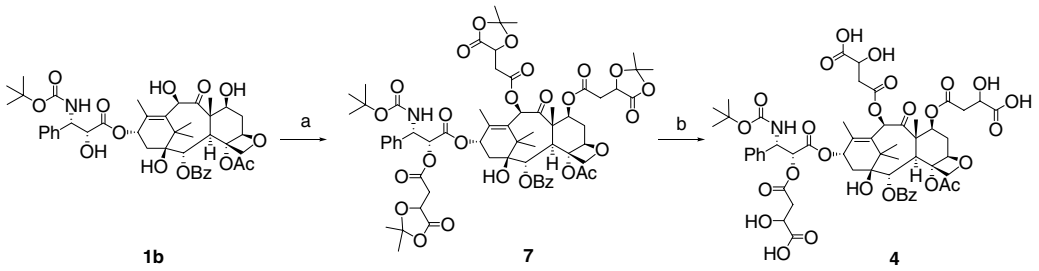


2a R^1 = DL-malyl, R^2 = H, R^3 = H **3a** sodium salt of **2a**
2b R^1 = L-malyl, R^2 = H, R^3 = H **3b** sodium salt of **2b**
2c R^1 = D-malyl, R^2 = H, R^3 = H **3c** sodium salt of **2c**
4 R^1 = DL-malyl, R^2 = DL-malyl, R^3 = DL-malyl
5 R^1 = H, R^2 = DL-malyl, R^3 = H



1b **6a**

Scheme 1. Reagents and conditions: (a) 1,2-*O*-isopropylidene-malic acid, DCC, DMAP, CH_2Cl_2, −10 °C, 7 h, 70%; (b) HOAc:THF:H_2O (1:1:1), rt, 20 h, 86%; (c) $NaHCO_3$, acetone, H_2O, rt, 1 h, 100%.



Scheme 2. Reagents and conditions: (a) 1,2-*O*-isopropylidene-malic acid, DIC, DMAP, CH_2Cl_2, reflux, 5 h, 85%; (b) HOAc:THF:H_2O (4:1:2), 45 °C, 6 h, 68%.

Ar_2 to give compound **8**, which was treated with protected malic acid as described in Scheme 1. Contrarily the protected malic acid was linked at 7-OH. Removal of the Troc groups with zinc dust resulted in the desired compound **10** which was followed by deprotection to provide C7-malyl docetaxel **5** (Scheme 3). In ^{1}H NMR spectrum, the significant downfield shift of C-7H (δ 5.57) and no great change of C-2′H (δ 4.51) and C-10H (δ 5.34) in **5** relative to that in docetaxel demonstrated that the malyl was linked to 7-OH of docetaxel.

3. Biological results

3.1. Solubility and stability

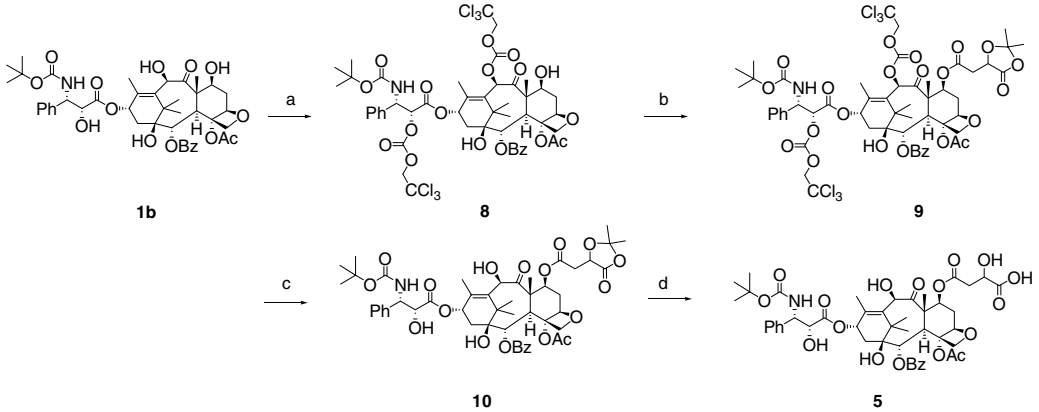
Solubility of synthesized compounds **2**–**5** was measured by HPLC, the results are listed in Table 1. Compounds **2**–**5** demonstrated greatly improved water solubility compared to docetaxel (65–90 times), especially paclitaxel (1720–2480 times).[5–8] According to their water solubility, we considered that it is possible to prepare them in the form of lyophilized powder for injection clinically without any surfactant such as Tween 80.

The test of stability in PBS-buffer showed that all target compounds were proved to be stable when incubated in PBS buffer (pH 7.4) at 37 °C for 48 h, since few liberated docetaxel was detected (HPLC). Otherwise, derivatives **2a**–**c** and **3a**–**c** mostly generated docetaxel when incubated in human plasma in 6 h: 50% of them was degraded to docetaxel in 4 and 3 h, respectively, and 70–80% of docetaxel was generated in 6 h. In contrast, compounds **4** and **5** were too stable to generate docetaxel. Therefore, 2′-malyl docetaxel and their sodium salts act as prodrugs but the 7-malyl and 2′,7,10-tri-malyl derivatives do not. Hydrolysis rate of sodium salts in human plasma is particularly encouraging (Table 1).

3.2. Cytotoxicity

In order to investigate the influence of malyl- on docetaxel in vitro cytotoxicity, all compounds were screened for their cytotoxicity in vitro against human cancer cell lines (A549, K562, KB, and MCF-7). The results are summarized in Table 1. Derivatives **4** and **5** were significantly less active against most tested cancer cell lines. Compound **2a** showed similar activity and compound **3a** showed more activity when compared to docetaxel. On the other hand, 2′-DL-malyl docetaxel **2a** showed more activity than 2′-L-malyl docetaxel **2b** and 2′-D-malyl docetaxel **2c** on the whole, which was consistent with their sodium salts **3a**–**c**. The result was strange and different from forecast. We repeated three times and got similar results. In order to explain this phenomenon, the test of the concentration of compounds **2a**–**c**, **3a**–**c** and docetaxel on cancer cells in vitro with HPLC is in process.

Since compound **3a** has highly anticancer activity in vitro and generated docetaxel when incubated in human plasma in 6 h, we tested its activities in vivo against the



Scheme 3. Reagents and conditions: (a) Troc-Cl, pyridine, CH_2Cl_2, Ar_2, −23 °C, 1.5 h, 65%; (b) 1,2-*O*-isopropylidene-malic acid, DCC, DMAP, CH_2Cl_2, −10 °C, 3 h, 82%; (c) zinc dust, CH_3OH: HOAc (9: 1), rt, 20 min, 88%; (d) HOAc:THF:H_2O (4: 1: 2), 45 °C, 6 h, 86%.

Table 1. Water solubility, stability, and cytotoxicity of malic acid docetaxel derivatives

Compound	Water solubility (μg/mL)	$T_{1/2}$[a] (h)		Cell line[c] (IC_{50} μg/mL)			
		pH 7.4	Plasma	A549	K562	KB	MCF-7
2a				>50	9.5 (4.5–12.0)	3.5 (1.7–6.2)	39.2 (10.2–66.3)
2b	470–480	>48	4	>50	3.9 (1.2–7.0)	>50	>50
2c				>50	0.85 (0.27–3.56)	>50	>50
3a	620–630	>24	3	3.5 (1.1–6.2)	1.5 (1.0–2.1)	0.45 (0.17–1.06)	3.6 (0.7–7.5)
3b				>50	38.1 (12. 5–73.2)	>50	>50
3c				>50	18.8 (8.429.1)	>50	>50
4	550	>48	ND[b]	>50	17.0 (10.4–24.6)	>50	>50
5	430	>48	ND[b]	>50	0.069 (0.025–0.146)	>50	>50
Docetaxel	6–7	—	—	1.4 (0.5–2.5)	6.9 (3.7–10.9)	11.6 (3.2–20.3)	3.3 (1.1–6.4)

[a] Half-life values ($T_{1/2}$), time in which 50% of the derivative is degraded to docetaxel.
[b] No docetaxel detected (HPLC).
[c] Values are means of three experiments, standard deviation is given in parentheses.

Table 2. Effects of **3a** against the murine H22 tumor

Groups	Dose (mg/kg)	No. of animals	Tumor weight (g)	Inhibition rate (%)
Control	0	20	1.20 ± 0.40	—
3a	5	10	0.97 ± 0.40	19.0
3a	10	10	0.85 ± 0.22*	28.9
3a	20	10	0.78 ± 0.17**	35.0
Docetaxel	10	10	0.64 ± 0.16**	46.3

* $P < 0.05$.
** $P < 0.01$ versus control.

murine H22 tumor. The results are depicted in Table 2. It is showed that compound **3a** exhibited inhibitory effect on H22 tumor growth.

4. Conclusion

In conclusion, we have synthesized some novel water soluble docetaxel derivatives in good yields and have demonstrated that 2′-malyl docetaxel **2a**–**c** and their sodium salts **3a**–**c** act as prodrugs. Among them, 2′-DL-malyl docetaxel sodium salt **3a** was found to be more active than docetaxel in vitro and showed the inhibitory effect on tumor growth in vivo, in addition to the excellent water solubility.

5. Experimental

5.1. General procedure

Melting points were determined on BÜCHI B-540. Infrared spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. Optical rotations were measured on a JASCO P-1030 digital polarimeter. NMR spectra were recorded on a Brucker Advance DMX 400 MHz or 500 MHz spectrometer with TMS as the internal standard. Chemical shifts are expressed in parts per million (ppm). Elemental analysis was carried out on Carlo ERBA-1108 analyzer. Mass spectra were recorded on Finnigan LCQ DECA spectrometer. All compounds were at least 90% pure, with some small impurities present, which were integrated as less than 2%.

HPLC: Rheodyne injection valve (20 μL loop); Lichrospher 5RP18 column (250 × 4.6 mM, Phenomenex); UV-detector (Model G1314A, Applied Agilent); eluent: methanol: acetonitrile: H_2O: 15:40:45 in 5 mM NH_4OAc(pH 5.0) and acetonitrile: H_2O: 50:50 in 5 mM KH_2PO_4 (pH 3.0). The detection of the (pro)-drugs was performed at 227 nM, where it is supposed that the extinction coefficients of docetaxel and docetaxel prodrugs are equal. The concentrations were determined by measuring the relative area of docetaxel or the docetaxel prodrugs.

5.1.1. 2′-(1,2-*O*-Isopropylidene-DL-malyl) docetaxel (6a). A solution of docetaxel (80.8 mg, 0.10 mmol)and 1,2-*O*-isopropylidene-malic acid (19.1 mg, 0.11 mmol) in CH_2Cl_2 (2.5 mL) was stirred at −10 °C. Next, DCC (30.9 mg, 0.15 mmol) and DMAP (18.3 mg, 0.15 mmol) were added. After stirring for 7 h at −10 °C, the mixture was filtered, diluted with EtOAc (7.5 mL), and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via column chromatography (EtOAc–petroleum ether, 1:2), yielding **6a** (67.4 mg, 70%) as a colorless sticky oil: IR (KBr, cm^{-1}) 3500, 2995.37, 2925.25, 1790.56, 1724.99, 1389.74, 1275.15, 1221.15, 1128.27, 1019.46, 924.58, 623.53; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.2 Hz, 2H, H-Ph), 7.59 (m, 1H, H-Ph), 7.50 (m, 2H, H-Ph), 7.37 (m, 2H, H-Ph), 7.28 (m, 3H, H-Ph), 6.23 (m, 1H, H-13), 5.68 (m, 1H, H-2), 5.35 (m, 3H, H-10, H-2′, H-3′), 5.21 (s, 1H, NH), 4.95 (d, J = 9.2 Hz, 1H, H-5), 4.64 (m, 1H, CH-malyl), 4.21 (m, 3H, H-7,H-20), 3.93 (d, J = 7.20 Hz, 1H, H-3), 2.89 (m, 2H, CH_2-malyl) 2.58 (m, 1H, H-6a), 2.41 (s, 3H, $OCOCH_3$), 2.30 (m, 1H, H-14a), 2.18 (m, 1H, H-14b), 2.03 (s, 3H, H-18), 1.84 (m, 1H, H-6b), 1.75 (s, 3H, H-19), 1.53 (m, 9H, *t*-Bu-H), 1.23 (m, 12H, H-16, H-17,CH_3-malyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.26, 171.68, 169.67, 168.65, 167.76, 166.90, 155.15, 138.81, 135.57, 133.55, 130.12, 129.20, 128.84, 128.62, 128.17, 126.29, 111.38, 84.24, 80.91, 80.31, 78.72, 76.46, 75.06, 74.90, 74.32, 72.10, 71.70, 70.26, 60.40, 57.50, 46.33, 43.03, 41.85, 36.62, 35.92, 29.60, 28.05, 26.60, 22.55, 20.97, 14.10, 9.86.

5.1.2. 2′-DL-Malyl docetaxel (2a). Compound **6a** (96.4 mg, 0.10 mmol) was dissolved in a mixture of

HOAc–THF–H_2O (5:5:5 mL). The mixture was stirred at room temperature for 20 h. Next, the organic solvents were removed by evaporation in vacuo. The residue was diluted by water (10 mL) and freeze-dried, yielding **2a** (79.5 mg, 86%) as a white solid: $[\alpha]_D^{25}$ −37.14 (*c* 0.70, MeOH); mp 163–166 °C; IR (KBr, cm^{-1}) 3433.64, 2979.48, 2944.88, 1720.19, 1495.53, 1453.10, 1369.21, 1248.68, 1165.76, 1066.44, 983.52, 708.71; ^1H NMR (500 MHz, CD_3OD) δ 8.11 (d, J = 7.30 Hz, 2H, H-Ph), 7.65 (m, 1H, H-Ph), 7.58 (m, 2H, H-Ph), 7.41 (m, 4H, H-Ph), 7.26 (br s, 1H, H-Ph), 6.10 (m, 1H, H-13), 5.45 (m, 1H, H-2), 5.28 (m, 3H, H-10, H-2′, H-3′), 4.99 (d, J = 8.80 Hz, 1H, H-5), 4.36 (m, 1H, CH-malyl), 4.18 (m, 3H, H-7, H-20), 3.72 (br s, 1H, H-3), 2.94 (m, 1H, CH_2-malyl), 2.83 (m, 1H, CH_2-malyl), 2.44 (m, 1H, H-6a), 2.38 (s, 3H, $OCOCH_3$), 2.24 (m, 1H, H-14a), 1.90 (s, 5H, H-14b, H-18, H-6b), 1.68 (s, 3H, H-19), 1.41 (s, 9H, *t*-Bu-H), 1.15 (s, 3H, H-16), 1.12 (s, 3H, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ 210.06, 172.31, 172.01, 171.38, 170.02, 166.68, 158.23, 139.26, 138.59, 137.02, 133.87, 131.41, 131.20, 129.88, 129.68, 129.29, 128.22, 85.80, 82.02, 81.05, 79.13, 77.50, 76.44, 76.40, 75.59, 73.15, 72.55, 69.69, 58.78, 56.25, 47.91, 44.40, 40.89, 37.49, 36.58, 28.69, 26.90, 23.19, 21.66, 14.59, 10.45; Anal. Calcd for $C_{47}H_{57}NO_{18}$ (924): C, 61.10; H, 6.22. Found: C, 61.21; H, 6.32.

5.1.3. 2′-DL-Malyl docetaxel sodium salt (3a). To a solution of 2′-malyl docetaxel (**2a**) (92.4 mg, 0.10 mmol) in acetone (4 mL) was added a solution of $NaHCO_3$ (8.4 mg, 0.1 mmol) in demineralized water (8 mL). The reaction mixture was stirred at room temperature for 1 h. Sodium salt **3a** (94.6 mg, 100%) was isolated after removal of the acetone in vacuo and freeze-drying: $[\alpha]_D^{25}$ −37.30 (*c* 0.70, MeOH); mp 200–203 °C; IR (KBr, cm^{-1}) 3405.67, 2977.55, 2942.84, 1712.48, 1602.56, 1370.18, 1251.58, 1166.72, 1068.37, 708.71; ^1H NMR (500 MHz, CD_3OD) δ 8.12 (d, J = 6.89 Hz, 2H, H-Ph), 7.65 (m, 1H, H-Ph), 7.58 (t, J = 7.47 Hz, 2H, H-Ph), 7.40 (m, 4H, H-Ph), 7.25 (br s, 1H, H-Ph), 6.10 (m, 1H, H-13), 5.64 (m, 1H, H-2), 5.28 (m, 3H, H-10, H-2′, H-3′), 5.00 (d, J = 9.15 Hz, 1H, H-5), 4.36 (m, 1H, CH-malyl), 4.24 (m, 1H, H-7), 4.19 (s, 2H, H-20), 3.87 (br s, 1H, H-3), 2.93 (m, 1H, CH_2-malyl), 2.64 (m, 1H, CH_2-malyl), 2.47 (m, 1H, H-6a), 2.38 (s, 3H, $OCOCH_3$), 2.24 (m, 1H, H-14a), 1.95 (m, 1H, H-14b), 1.90 (s, 3H, H-18), 1.82 (m, 1H, H-6b), 1.66 (s, 3H, H-19), 1.40 (s, 9H, *t*-Bu-H), 1.15 (s, 3H, H-16), 1.12 (s, 3H, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ 211.26, 172.38, 171.49, 170.38, 170.33, 167.76, 158.00, 139.76, 139.11, 137.74, 134.57, 131.42, 131.20, 129.86, 129.71, 129.29, 128.32, 85.97, 82.22, 81.05, 79.13, 77.56, 76.44, 76.40, 75.62, 73.20, 72.55, 69.75, 58.83, 56.25, 47.92, 44.45, 40.92, 37.50, 36.60, 28.69, 26.99, 23.23, 21.66, 14.63, 10.47; Anal. Calcd for $C_{47}H_{56}NNaO_{18}$ (946): C, 59.68; H, 5.97. Found: C, 59.85; H, 6.09; MS (ESI): *m*/*z* 947 $[M+H]^+$, 969 $[M+Na]^+$.

In the same manner, compounds **2b**and **3b**, **2c** and **3c** were synthesized.

5.1.4. 2′-L-Malyl docetaxel (2b). $[\alpha]_D^{25}$ −44.29 (*c* 0.70, MeOH); mp 165–168 °C; ^1H NMR (500 MHz, CD_3OD) δ 8.12 (d, J = 7.30 Hz, 2H, H-Ph), 7.65 (m, 1H, H-Ph), 7.59 (m, 2H, H-Ph), 7.42 (m, 4H, H-Ph), 7.26 (br s, 1H, H-Ph), 6.10 (m, 1H, H-13), 5.65 (m, 1H, H-2), 5.45 (m, 1H, H-2′), 5.33 (m, 1H, H-10), 5.26 (m, 1H, H-3′), 5.00 (d, J = 8.80 Hz, 1H, H-5), 4.48 (m, 1H, CH-malyl), 4.22 (m, 3H, H-7, H-20), 3.72 (br s, 1H, H-3), 2.96 (m, 1H, CH_2-malyl), 2.83 (m, 1H, CH_2-malyl), 2.42 (m, 4H, H-6a, $OCOCH_3$), 2.24 (m, 1H, H-14a), 1.92 (s, 5H, H-14b, H-18, H-6b), 1.68 (s, 3H, H-19), 1.41 (s, 9H, *t*-Bu-H), 1.16 (s, 6H, H-16, H-17); Anal. Calcd for $C_{47}H_{57}NO_{18}$ (924): C, 61.10; H, 6.22. Found: C, 61.29; H, 6.30.

5.1.5. 2′-L-Malyl docetaxel sodium salt (3b). $[\alpha]_D^{25}$ −48.59 (*c* 0.70, MeOH); mp 211–214 °C; ^1H NMR (500 MHz, CD_3OD) δ 7.97 (d, J = 6.89 Hz, 2H, H-Ph), 7.69 (m, 3H, H-Ph), 7.37 (m, 4H, H-Ph), 7.16 (m, 1H, H-Ph), 5.77 (m, 1H, H-13), 5.38 (m, 1H, H-2), 5.08 (m, 3H, H-10, H-2′, H-3′), 4.90 (d, J = 9.20 Hz, 1H, H-5), 4.45 (m, 1H, CH-malyl), 4.24 (m, 1H, H-7), 4.02 (s, 2H, H-20), 3.85 (br s, 1H, H-3), 2.90 (m, 1H, CH_2-malyl), 2.60 (m, 1H, CH_2-malyl), 2.44 (m, 1H, H-6a), 2.38 (s, 3H, $OCOCH_3$), 2.23 (m, 1H, H-14a), 1.95 (m, 1H, H-14b), 1.94 (s, 3H, H-18), 1.82 (m, 1H, H-6b), 1.69 (s, 3H, H-19), 1.43 (s, 9H, *t*-Bu-H), 1.16 (s, 3H, H-16), 1.10 (s, 3H, H-17); Anal. Calcd for $C_{47}H_{56}NNaO_{18}$ (946): C, 59.68; H, 5.97. Found: C, 59.94; H, 6.12; MS (ESI): *m*/*z* 947 $[M+H]^+$, 969 $[M+Na]^+$.

5.1.6. 2′-D-Malyl docetaxel (2c). $[\alpha]_D^{25}$ −29.46 (*c* 0.70, MeOH); mp 171–174 °C; ^1H NMR (500 MHz, CD_3OD) δ 8.05 (d, J = 7.30 Hz, 2H, H-Ph), 7.62 (m, 1H, H-Ph), 7.58 (m, 2H, H-Ph), 7.40 (m, 4H, H-Ph), 7.22 (br s, 1H, H-Ph), 6.05 (m, 1H, H-13), 5.41 (m 1H, H-2), 5.23 (m, 2H, H-10, H-2′), 5.11 (m, 1H, H-3′), 4.91 (d, J = 8.80 Hz, 1H, H-5), 4.30 (m, 1H, CH-malyl), 4.12 (m, 3H, H-7, H-20), 3.70 (br s, 1H, H-3), 2.90 (m, 1H, CH_2-malyl), 2.83 (m, 1H, CH_2-malyl), 2.43 (m, 1H, H-6a), 2.32 (s, 3H, $OCOCH_3$), 2.24 (m, 1H, H-14a), 1.86 (s, 5H, H-14b, H-18, H-6b), 1.60 (s, 3H, H-19), 1.41 (s, 9H, *t*-Bu-H), 1.14 (s, 3H, H-16), 1.12 (s, 3H, H-17); Anal. Calcd for $C_{47}H_{57}NO_{18}$ (924): C, 61.10; H, 6.22. Found: C, 61.27; H, 6.35.

5.1.7. 2′-D-Malyl docetaxel sodium salt (3c). $[\alpha]_D^{25}$ −28.98 (*c* 0.70, MeOH); mp 215–219 °C; ^1H NMR (500 MHz, CD_3OD) δ 8.00 (d, J = 6.89 Hz, 2H, H-Ph), 7.72 (m, 3H, H-Ph), 7.38 (m, 4H, H-Ph), 7.20 (m, 1H, H-Ph), 5.80 (m, 1H, H-13), 5.41 (m, 1H, H-2), 5.10 (m, 3H, H-10, H-2′, H-3′), 4.93 (d, J = 9.20 Hz, 1H, H-5), 4.48 (m, 1H, CH-malyl), 4.27 (m, 1H, H-7), 4.05 (s, 2H, H-20), 3.82 (br s, 1H, H-3), 2.92 (m, 1H, CH_2-malyl), 2.60 (m, 1H, CH_2-malyl), 2.47 (m, 1H, H-6a), 2.38 (s, 3H, $OCOCH_3$), 2.24 (m, 1H, H-14a), 1.97 (m, 1H, H-14b), 1.92 (s, 3H, H-18), 1.80 (m, 1H, H-6b), 1.66 (s, 3H, H-19), 1.40 (s, 9H, *t*-Bu-H), 1.15 (m, 6H, H-16, H-17); Anal. Calcd for $C_{47}H_{56}NNaO_{18}$ (946): C, 59.68; H, 5.97. Found: C, 59.82; H, 6.11; MS (ESI): *m*/*z* 947 $[M+H]^+$, 969 $[M+Na]^+$.

5.1.8. 2′,7,10-Tri-(1,2-*O*-isopropylidene-DL-malyl) docetaxel (7). A solution of docetaxel (80.8 mg, 0.10 mmol) and 1,2-*O*-isopropylidene-malic acid (19.1 mg, 0.11 mmol) in

CH_2Cl_2 (8 mL) was stirred at 0 °C. Next, DIC (173 μL, 1.10 mmol) and DMAP (6.1 mg, 0.05 mmol) were added. After 1 h, the mixture was heated to reflux temperature and stirred for 5 h. The mixture was filtered, diluted with EtOAc (7.5 mL), and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via column chromatography (EtOAc–petroleum ether, 1:2), yielding **7** (108.4 mg, 85%) as a colorless sticky oil: IR (KBr, cm^{-1}) 3383.16, 2923.89, 2852.79, 1795.61, 1749.66, 1379.31, 1270.64, 1171.94, 1127.99, 1065.62, 913.23, 742.14; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.2 Hz, 2H, H-Ph), 7.61 (m, 1H, H-Ph), 7.51 (m, 2H, H-Ph), 7.42 (m, 2H, H-Ph), 7.32 (br s, 3H, H-Ph), 6.31 (m, 1H, H-10), 6.20 (br s, 1H, H-13), 5.63 (m, 5H, H-7, H-2, H-2′, H-3′, NH), 4.31 (d, J = 8 Hz, 1H, H-5), 4.80 (m, 3H, CH-malyl), 4.13 (m, 2H, H-20), 3.92 (br s, 1H, H-3), 3.03 (m, 6H, CH_2-malyl), 2.63 (m, 1H, H-6a), 2.44 (s, 3H, $OCOCH_3$), 2.32 (m, 1H, H-14a), 2.23 (m, 1H, H-14b), 1.97 (s, 3H, H-18), 1.81 (m, 4H, H-6b, H-19), 1.59 (m, 18H, CH_3-malyl), 1.35 (s, 9H, t-Bu-H); 1.25 (m, 3H, H-16), 1.12 (m, 3H, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.15, 171.90, 171.87, 171.77, 171.54, 171.00, 167.96, 167.65, 166.72, 157.03, 142.04, 137.07, 133.58, 131.85, 130.07, 129.07, 128.80, 128.59, 128.17, 126.35, 111.26, 111.01, 110.72, 83.74, 80.58, 80.18, 78.54, 77.48, 76.12, 75.79, 74.79, 74.04, 71.68, 70.56, 70.46, 70.16, 60.23, 55.75, 43.08, 41.81, 36.72, 36.32, 36.05, 33.16, 32.95, 28.03, 25.63, 25.54, 25.42, 23.32, 22.38, 20.83, 14.07, 10.71.

5.1.9. 2′,7,10-tri-(DL-malyl)-docetaxel (4). Compound **7** (127.6 mg, 0.10 mmol) was dissolved in a mixture of HOAc: THF–H_2O (12:3:6 mL). The mixture was stirred at 45 °C for 6 h. Next, the organic solvents were removed by evaporation in vacuo. The residue was diluted by water (20 mL) and freeze-dried, yielding **4** (78.6 mg, 68%) as a white solid: mp 155–158 °C; IR (KBr, cm^{-1}) 3418.21, 2942.84, 1737.55, 1633.41, 1371.14, 1252.54, 1170.58, 1105.98, 1065.48, 708.71; ^1H NMR (500 MHz, CD_3OD) δ 8.11 (d, J = 7.41 Hz, 2H, H-Ph), 7.66 (m, 1H, H-Ph), 7.58 (m, 2H, H-Ph), 7.40 (m, 4H, H-Ph), 7.26 (br s, 1H, H-Ph), 6.32 (s, 1H, H-10), 6.07 (br s, 1H, H-13), 5.62 (m, 2H, H-7, H-2), 5.30 (m, 2H, H-2′, H-3′), 4.99 (d, J = 9.29 Hz, 1H, H-5), 4.48 (m, 3H, CH-malyl), 4.19 (m, 2H, H-20), 3.89 (br s, 1H, H-3), 2.86 (m, 6H, CH_2-malyl), 2.55 (m, 1H, H-6a), 2.40 (s, 3H, $OCOCH_3$), 2.23 (m, 1H, H-14a), 1.98 (m, 1H, H-14b), 1.92 (s, 3H, H-18), 1.78 (m, 4H, H-6b, H-19), 1.41 (s, 9H, t-Bu-H), 1.16 (s, 3H, H-16), 1.12 (s, 3H, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ 203.83, 172.99, 171.65, 171.40, 171.32, 171.05, 170.42, 170.22, 167.64, 157.84, 143.13, 138.84, 134.64, 134.04, 133.95, 131.21, 129.90, 129.72, 129.39, 128.31, 85.23, 81.90, 80.87, 78.99, 77.28, 76.95, 76.55, 75.94,73.46, 72.98, 68.32, 68.14, 57.20, 56.20, 48.04, 44.51, 40.43, 40.08, 39.81, 36.38, 33.97, 28.68, 26.68, 23.16, 22.15, 14.96, 11.45; Anal. Calcd for $C_{55}H_{65}NO_{26}$ (1156): C, 57.14; H, 5.67. Found: C, 57.38; H, 5.90.

5.1.10. 2′,10-Bis-(2,2,2-Trichloroethoxycarbonyl)-docetaxel (8). To docetaxel (80.8 mg, 0.10 mmol) under argon was added CH_2Cl_2 (2 mL) at room temperature. The solution was cooled to −23 °C and pyridine (160 μL) was added. 2,2,2-Trichloroethylchloroformate (14 μL, 0.1 mmol) was slowly added. Stirring was maintained at −23 °C for 45 min, and then more 2,2,2-trichloroethyl- chloroformate (12.6 μL, 0.09 mmol) was added. Stirring at −23 °C was continued for an additional 45 min. The reaction solution was diluted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, concentrated, and purified via column chromatography (EtOAc–petroleum ether, 1:2), yielding **8** (75.3 mg, 65%) as a colorless sticky oil: IR (KBr, cm^{-1}) 3440.96, 2978.53, 1712.01, 1496.98, 1452.26, 1370.83, 1242.92, 1167.00, 1066.47, 1025.50, 987.01, 737.36, 708.65; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 7.6 Hz, 2H, H-Ph), 7.61 (m, 1H, H-Ph), 7.51 (t, J = 7.6 Hz, 2H, H-Ph), 7.39 (m, 2H, H-Ph), 7.33 (m, 3H, H-Ph), 6.28 (m, 1H, H-13), 5.65 (d, J = 7.6 Hz, 1H, H-2), 5.51 (m, 2H, H-2′, NH), 5.38 (s, 1H, H-10), 5.22 (br s, 1H, H-3′), 4.96 (d, J = 8 Hz, 1H, H-5), 4.74 (m, 2H, H-20), 4.24 (m, 5H, H-7, CH_2-Troc),, 3.92 (br s, 1H, H-3), 2.58 (m, 1H, H-6a), 2.46 (s, 3H, $OCOCH_3$), 2.32 (m, 1H, H-14a), 2.19 (m, 1H, H-14b), 1.91 (m, 4H, H-6b, H-18), 1.74 (s, 3H, H-19), 1.33 (s, 9H, t-Bu-H), 1.23 (s, 3H, H-16), 1.12 (s, 3H, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.14, 171.35, 169.83, 167.35, 166.93, 155.04, 153.24, 138.57, 136.82, 135.69, 133.58, 130.12, 129.16, 128.90, 128.63, 128.33, 126.41, 93.88, 84.28, 80.94, 80.31, 78.73, 78.11, 77.40, 77.07, 76.76, 75.00, 74.32, 71.76, 71.13, 61.19, 57.58, 46.35, 43.04, 36.61, 35.54, 28.04, 26.32, 22.59, 20.94, 14.10, 9.86.

5.1.11. 2′,10-Bis-(2,2,2-trichloroethoxycarbonyl)-7-(1,2-*O*- isopropylidene-DL-malyl)-docetaxel (9). A solution of compound **8** (115.9 mg, 0.10 mmol)and 1,2-*O*-isopropylidene-malic acid (19.1 mg, 0.11 mmol) in CH_2Cl_2 (16 mL) was stirred at −10 °C. Next, DCC (30.9 mg, 0.15 mmol) and DMAP (6.1 mg, 0.05 mmol) were added. After stirring for 3 h at −10 °C, the mixture was filtered, diluted with EtOAc (7.5 mL), and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via column chromatography (EtOAc–petroleum ether, 1:2), yielding **9** (107.8 mg, 82%) as a colorless sticky oil: IR (KBr, cm^{-1}) 3434.25, 2983.56, 1714.86, 1497.19, 1452.59, 1383.17, 1243.34, 1174.69, 738.19, 706.56; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 8 Hz, 2H, H-Ph), 7.63 (m, 1H, H-Ph), 7.52 (m, 2H, H-Ph), 7.42 (m, 2H, H-Ph), 7.36 (m, 3H, H-Ph), 6.28 (br s, 1H, H-13), 5.70 (d, J = 6.8 Hz, 1H, H-2), 5.52 (m, 3H, H-10, H-7, H-2′), 5.39 (s, 1H, NH), 5.22 (br s, 1H, H-3′), 4.96 (m, 1H, H-5), 4.74 (m, 3H, H-20, CH-malyl), 4.24 (m, 4H, CH_2-Troc), 3.92 (dd, J = 16, 1.2 Hz, 1H, H-3), 2.88 (m, 1H, CH_2-malyl), 2.73 (m, 1H, CH_2-malyl), 2.56 (m, 1H, H-6a), 2.48 (s, 3H, $OCOCH_3$), 2.36 (m, 1H, H-14a), 2.20 (m, 1H, H-14b), 1.98 (m, 3H, H-18), 1.87 (m, 4H, H-6b, H-19), 1.65 (m, 3H, CH_3-malyl), 1.58 (m, 3H, CH_3-malyl),1.36 (s, 9H, t-Bu-H), 1.23 (s, 3H, H-16), 1.11 (s, 3H, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.68, 171.75, 169.70, 167.98, 167.72, 167.24, 166.93, 154.97, 153.19, 139.12, 136.86, 135.26, 133.63, 130.12, 129.11, 128.92, 128.67, 128.30, 126.37, 111.31, 93.85, 83.56, 80.54, 80.47, 78.82, 78.12, 77.30, 76.98, 76.67, 74.77, 74.39, 72.33, 70.47, 70.30, 56.33, 56.26, 48.86,

42.93, 36.27, 35.47, 33.85, 28.05, 26.83, 26.61, 26.09, 22.51, 20.73, 13.98, 10.89.

5.1.12. 7-(1,2-*O*-Isopropylidene-DL-malyl) docetaxel (10). Compound **9** (131.5 mg, 0.10 mmol) was dissolved in a mixture of CH_3OH: HOAc (7.3:0.8 mL) and zinc dust was added to the mixture. The reaction suspension was stirred at room temperature for 20 min. The zinc dust was removed by filtration. The filtrate was concentrated to dryness, treated with CH_2Cl_2, and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via column chromatography (EtOAc–petroleum ether, 1:2), yielding **10** (84.8 mg, 88%) as a colorless sticky oil: IR (KBr, cm^{-1}) 3434.32, 2962.70, 2882.42, 1576.21, 1475.20, 1382.50, 1205.24, 1031.59, 882.68, 706.87; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, $J = 7.6$ Hz, 2H, H-Ph), 7.61 (m, 1H, H-Ph), 7.50 (m, 2H, H-Ph), 7.35 (m, 5H, H-Ph), 6.20 (m, 1H, H-13), 5.67 (d, $J = 7.2$ Hz, 1H, H-2), 5.54 (m, 2H, H-10, H-3′), 5.29 (m, 2H, NH, H-7), 4.92 (d, $J = 9.2$ Hz, 1H, H-5), 4.65 (m, 2H, CH-malyl, H-2′), 4.31 (d, $J = 8.40$ Hz, 1H, H-20a) 4.19 (d, $J = 9.2$ Hz, 1H, H-20b), 3.96 (m, 2H, H-3, OH), 3.54 (s, 1H, OH), 2.85 (m, 1H, CH_2-malyl), 2.72 (m, 1H, CH_2-malyl), 2.54 (m, 1H, H-6a), 2.39 (s, 3H, $OCOCH_3$), 2.28 (m, 2H, H-14), 1.87 (m, 7H, H-6b, H-18, H-19), 1.34 (m, 9H, *t*-Bu-H), 1.64 (m, 3H, CH_3-malyl), 1.57 (m, 3H, CH_3-malyl),1.21 (s, 3H, H-16), 1.09 (s, 3H, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.64, 171.81, 170.25, 168.05, 167.85, 166.86, 155.29, 138.36, 135.41, 133.66, 130.10, 129.03, 129.01, 128.74, 128.67, 127.92, 126.66, 111.36, 83.50, 80.52, 80.05, 78.71, 77.35, 77.03, 76.71, 74.63, 73.60, 72.71, 70.29, 60.37, 56.36, 45.88, 42.84, 36.23, 35.95, 35.66, 28.12, 26.81, 25.75, 22.41, 21.00, 14.13, 10.86.

5.1.13. 7-DL-Malyl-docetaxel (5). Compound **10** (96.4 mg, 0.10 mmol) was dissolved in a mixture of HOAc: THF–H_2O (12:3:6 mL). The mixture was stirred at 45 °C for 6 h. Next, the organic solvents were removed by evaporation in vacuo. The residue was diluted by water (10 mL) and freeze-dried, yielding **5** (79.4 mg, 86%) as a white solid: mp 164–168 °C; IR (KBr, cm^{-1}) 3439.42, 2979.48, 2942.84, 1731.76, 1496.49, 1452.14, 369.21, 1248.68, 1168.65, 1102.12, 982.55, 708.71; ^1H NMR (500 MHz, CD_3OD) δ 8.10 (d, $J = 7.36$ Hz, 2H, H-Ph), 7.66 (m, 1H, H-Ph), 7.57 (t, $J = 7.17$ Hz, 2H, H-Ph), 7.38 (m, 4H, H-Ph), 7.25 (m, 1H, H-Ph), 6.15 (m, 1H, H-13), 5.65 (dd, $J = 6.45$, 1.71 Hz, 1H, H-2), 5.57 (m, 1H, H-7), 5.34 (s, 1H, H-10), 5.11 (br s, 1H, H-3′), 4.98 (d, $J = 8.99$ Hz, 1H, H-5), 4.51 (br s, 1H, H-2′), 4.42 (br s, 1H, CH-malyl), 4.21 (s, 2H, H-20), 3.92 (m, 1H, H-3), 2.76 (m, 1H, CH_2-malyl), 2.60 (m, 1H, CH_2-malyl), 2.47 (m, 1H, H-6a), 2.35 (s, 3H, $OCOCH_3$), 2.24 (m, 1H, H-14a), 2.04 (m, 1H, H-14b), 1.91 (m, 4H, H-6b, H-18), 1.80 (s, 3H, H-19), 1.40 (s, 9H, *t*-Bu-H), 1.15 (s, 3H, H-16), 1.09 (s, 3H, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ 209.66, 173.19, 170.77, 169.77, 169.68, 166.47, 156.53, 139.43, 138.64, 136.36, 133.39, 130.15, 129.96, 128.49, 128.37, 127.56, 127.00, 83.92, 80.68, 79.54, 77.99, 76.30, 75.06, 74.52, 74.17, 72.63, 71.23, 67.34, 57.36, 56.25, 46.31, 43.19, 39.35, 35.63, 32.94, 27.50, 25.59, 21.87, 20.42, 13.28, 10.27; Anal. Calcd for $C_{47}H_{57}NO_{18}$ (924): C, 61.10; H, 6.22. Found: C, 61.25; H, 6.37.

5.2. Biological activity

5.2.1. Water solubility. Docetaxel and docetaxel derivatives were suspended in water until a concentration was reached of 2 mg/mL. The suspensions were sonicated for 15 min and centrifuged (13,000 rpm) for 10 min. The above fluid was analyzed, using HPLC.

5.2.2. Stability in PBS-buffer and human plasma. The docetaxel derivatives (**2a–c**, **3a–c**, **4**, and **5**) were dissolved in water, sonicated, and centrifuged. 10 μL of the above fluid was mixed with 900 μL of plasma (heparin) or PBS-buffer (pH 7.4). The solutions were incubated at 37 °C and on different points (for **2a–c** and **3a–c**, $T = 0$, 0.5, 1, 1.5, 2, 3, 4, 5, 6 h; for compounds **4** and**5**, $T = 0$, 3, 6, 9, 12, 24, 36, 48 h) 100 μL was precipitated with 900 μL acetonitrile, then extracted with 2000 μL of EtOAc. After mixing for 3 min (using a vortex), this mixture was centrifuged (10 min, 13,000 rpm) and evaporated (4 h, in vacuo). The (pro)drugs were dissolved in 100 μL methanol and analyzed by HPLC.

5.2.3. In vitro cytotoxicity assay. The cytotoxic activity in vitro was measured using the MTT assay. The MTT solution (final concentration: 0.5 mg/ml) in RPMI-1640 (Sigma, St. Louis, MO) was added after cells were treated with the drug for 48 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100 μL DMSO. After 5 min, the plates were read at 570 nm by an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software 'Dose–Effect Analysis with Microcomputers'. The cancer cell lines' panel consisted of A549, K562, KB, and MCF-7. In all of these experiments, three replicate wells were used to determine each point, and all of the experiments were performed in triplicate.

5.2.4. Antitumor activity in ICR mice Hepatoma-22 (H22) tumor. Tumor bearing mice models were established by s.c. injections of H22 cells (5×10^6 cells per animal, subcutaneously into the armpit) in male ICR mice (National rodent laboratory animal resource, Shanghai branch, China). Treatments were initiated 24 h after the injections of tumor cells. Mice in different treatment groups (negative control group containing 20 female mice, other groups containing 10 female mice) were injected intraperitoneally (ip) with the compound **3a** (5, 10, 20 mg/kg) or docetaxel once a day for consecutive 7 days. Docetaxel of 10 mg/kg was used as a positive control and physiological saline as negative control. Tumors were dissected and weighed at day 8, and inhibition rates were calculated as follows: $(C - T)/C \times 100$, T, average tumor weight of treated group; C, average tumor weight of negative control group.

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