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Synthesis and cytotoxicity of novel fatty acid-nucleoside conjugates

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Abstract—N^3-Hydroxyethyltegafur (**3**) was synthesized in 91.0% yield under a new condition. A series of novel fatty acid esters of **3** were synthesized. These fatty acid-nucleoside conjugates have shown cytotoxicities against Ec9706 cells and A549 cells, and the structure activity relationship was discussed.

Tegafur [5-fluoro-1-(tetrahydro-2-furyl) uracil (**1**)], an important antitumor drug, has been used to treat adenocarcinomas, including carcinomas of the breast and gastrointestinal tract.[1] As a prodrug of 5-fluorouracil (5-FU), **1** has a similar antitumor mechanism to that of 5-FU. It has a higher lipid-solubility, quicker absorption rate in stomach and intestines, and longer half-time than 5-FU. Its side-effect is only one seventh of that of 5-FU, but the chemotherapeutic index is twice as much as that of 5-FU.[2] However, **1** still exhibits some defects in clinical use such as marrow toxicity, gastrointestinal discomfort, accidental damage of liver and kidney function, and low selectivity.[3] In order to improve its antitumor activities and reduce its side-effects, **1** was modified in various ways, among which the studies on phospholipids–nucleoside conjugates are particularly of interest.[4–7] For example, glycerothiophospholipid–nucleoside conjugates (Fig. 1) showed antitumor activity against bladder cancer cells.[7]

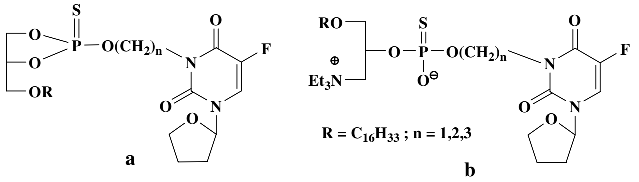

Figure 1. Structure of glycerothiophospholipids-nucleoside conjugates (**a** and **b**).

Fatty acids, especially polyunsaturated fatty acids (PUFAs), as a kind of components of cell membrane, have strongly biological activities such as antitumor and immunological enhancement.[8] In this study, **1** was conjugated with fatty acids in order to further improve its lipid-solubility, increase its bioavailability, enhance its activity, and decrease its side-effects. Moreover, the two moieties of the conjugate, which should be released in the cell via enzymatic actions, were expected to have synergistic effect so that an increase to the chemotherapeutic index is possible.[9] In the present work, five novel fatty acid-nucleoside conjugates (**5a**–**5e**) were synthesized and structurally confirmed by ^{1}H NMR and HR-MS. Compounds **5c**, **5d**, and **5e** showed cytotoxicities against human esophageal cancer Ec9706 cells and the human lung cancer A549 cells.

The synthetic route of compound **3** is shown in Scheme 1. Xu[10] and co-workers reported the synthesis of **3** by the reaction of **1** with 2-chloroethanol (**2**) in the presence of $NaHCO_3$ in acetonitrile at 80 °C with an 89% yield (the molar ratio of **1** to **2** was 1:6). In our work, because of its better solubility than that of $NaHCO_3$ in acetonitrile, triethylamine was used as a base in place of $NaHCO_3$ in the reaction. The yield of compound **3** was enhanced to 91.0%. Some of the unreacted **1** was recovered (about 7%). A small amount of compound **4** was also formed and not separated from the resulting reaction mixture.

N^3-Acyloxyethyltegafurs (**5a**–**5e**), novel fatty acid-nucleoside conjugates, were synthesized according to Scheme 2. Compound **5a** was obtained in higher yield (77.6%) than compounds **5b**–**5e** (44.4–53.0%) due to the less steric effect of lauric acid in the reaction.

Keywords: Tegafur; Fatty acid; Conjugates; Synthesis; Cytotoxicity.

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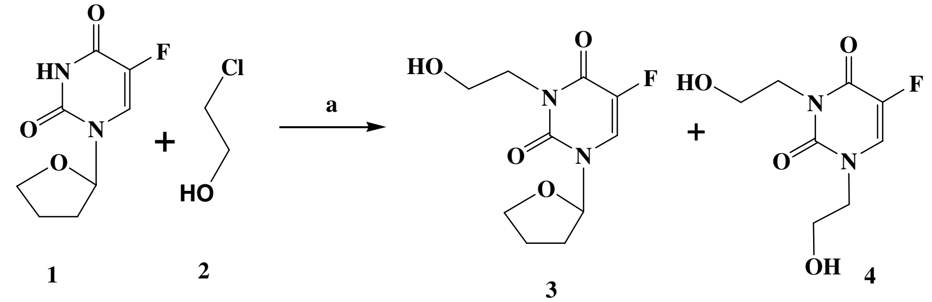
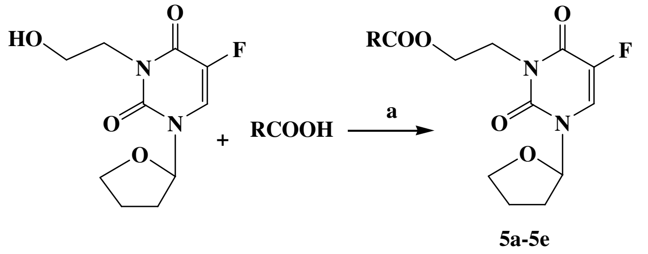


Scheme 1. Reagents and conditions: (a) MeCN, Et_3N, 80 °C, 6 h, isolated yield 91.0% (compound **3**).



Scheme 2. Reagents and conditions: (a) DCC/DMAP, MeCN, rt, 12–24 h. **5a**: RCO = lauroyl, isolated yield 77.6%; **5b**: RCO = stearoyl, isolated yield 44.4%; **5c**: RCO = oleoyl, isolated yield 45.5%; **5d**: RCO = linoleoyl, isolated yield 46.9%; **5e**: RCO = arachidonoyl, isolated yield 53.0%.

Compounds **5c–5e**, unsaturated fatty acid–nucleoside conjugates, are oil at rt, conforming to the common physical state of unsaturated fatty acid ester. As a catalyst, 4-(dimethylamino) pyridine (DMAP) is essential to the esterification, about 10% (mol %) of which is efficient and economical. Due to the instability of PUFAs,[11] the synthesis and workup of compounds **5c–5e** were carried out under 50 °C. Because of the low solubility of DCU in common reaction solvents, small quantities of this urea tend to come out of solution even after repeated filtration, due to the slow hydrolysis of the remaining dicyclohexylcarbodiimide (DCC) in the crude product solution. Since carbodiimides are far more easily hydrolyzed in acidic media, treating the reaction mixture prior to filtration sequentially with hydrochloric acid, saturated $NaHCO_3$, saturated brine, and distilled water eliminated any remaining DCC. We previously used fatty acylchlorides as acylating agents to synthesize N^3-acyloxyethyltegafur, but these target compounds were not easy to be separated from impurities. The DCC/DMAP method, therefore, is a more suitable synthetic route with mild reaction conditions, higher yield, and convenient product purification.[12]

The cytotoxicity was assessed by MTT assay. Human esophageal cancer cell line Ec9706 or human lung cancer cell line A549, cultured in RPMI-1640 medium supplemented with 10% FBS, 100 IU/mL of penicillin, and 100 µg /mL of streptomycin at 37 °C in humidified air atmosphere of 5% CO_2, was plated into 96-well plate (1×10^4 cells/well). The next day, the compounds diluted in culture medium were added (200 µL/well) to the wells. 48 h later 20 µL MTT (0.5 mg/mL MTT in PBS) was added and cells were incubated for a further 4 h. DMSO (0.20 mL) was added to each well to dissolve the reduced MTT crystals. The MTT- formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a micro plate reader. As demonstrated by MTT cytotoxicity assay (Table 1), the cytotoxicities of **1** and **3** in Ec9706 cells and A549 cells increased in a dose-dependent manner at concentration range of 0–400 µM, and the cytotoxicity of **1** is higher than that of **3**. Except **5a** and **5b**, these fatty acid-nucleoside conjugates (**5c–5e**) have shown higher cytotoxicity than compound **3**. When RCO is a polyunsaturated fatty acyl, especially arachidonoyl, the conjugates **5d** and **5e** have shown higher antitumor activities in vitro in a dose-dependent manner.

In summary, compound **3** was synthesized in a good yield and five unreported compounds **5a–5e** have been obtained in an efficient way. Their structures were confirmed by ^1H NMR and HR-MS. Among them, **5d** and **5e** exhibited better cytotoxicity activities, which was proportional to the degree of unsaturation of PUFA portion. Although the mechanism of action is unclear, the cytotoxicities of PUFA-nucleoside conjugates may be related to cellular membrane-activity including the enzymes, receptors, and ion channels[13]

Table 1. Cytotoxicities of compounds to Ec9706 and A549 cells

Compound	Inhibition percentage (%)			
	50 µM	100 µM	200 µM	400 µM
1		—[a]	7.8[c]	17.4[c]
		12.7[d]	15.2[d]	17.8[d]
3		—[a]	—[a]	8.5[c]
		—[a]	—[a]	—[a]
5a	—[a]	—[a]	—[b]	—[b]
	—[a]	—[a]	—[b]	—[b]
5b	—[a]	—[a]	—[b]	—[b]
	—[a]	—[a]	—[b]	—[b]
5c	3.7[c]	4.9[c]	—[b]	—[b]
	2.5[d]	4.8[d]	—[b]	—[b]
5d	—[a]	14.4[c]	31.9[c]	54.4[c]
	1.8[d]	11.0[d]	24.7[d]	90.7[d]
5e	19.3[c]	30.8[c]	59.9[c]	69.9[c]
	1.6[d]	33.8[d]	78.2[d]	92.1[d]

[a] No inhibition.

[b] Undetermined.

[c] To Ec9706 cells.

[d] To A549 cells.

perhaps due to the high compatibility of the PUFA portion of those compounds with cell membrane.

Acknowledgments

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12. N^3-Acyloxyethyltegafurs (**5a**–**5e**, **5a** as a synthetic example). Lauric acid (401 mg, 2 mmol) and **3** (244 mg, 1 mmol) were dissolved in 10 mL of acetonitrile to give a solution A; 413 mg DCC (2 mmol) and 24 mg (0.2 mmol) DMAP were dissolved in 20.0 mL acetonitrile to give another solution B. The solution B was dropped into the solution A within 30 min at r.t., and the reaction system was stirred for 24 h. After filtering off DCU, the filtrate was concentrated *in vacuo* to afford a yellow syrup. The syrup was dissolved in 20.0 mL ethyl acetate and subsequently washed with 1 M HCl (3× 60 mL), saturated $NaHCO_3$ (3× 60 mL), saturated brine (3× 60 mL), and distilled water (2× 60 mL), respectively. The ethyl acetate phase was dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The residue was purified via flash column chromatography with a mixed eluent (petroleum ether/ethyl acetate/acetic acid = 40:8:1, v/v/v) to give 331 mg **5a**, 77.6%, mp: 35–36 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.6$ Hz), 1.25 (br s, 16H), 1.56 (d, 2H, $J = 6.8$ Hz), 1.94 (m, 1H), 2.07 (m, 2H), 2.26 (t, 2H, $J = 7.5$ Hz), 2.40 (m, 1H), 3.99 (q, 1H, $J = 5.7$ Hz), 4.24 (m, 3H), 4.31 (m, 2H), 5.98 (d, 1H, $J = 5.3$ Hz), 7.43 (d, 1H, $J = 5.8$ Hz). ESI-HR-MS (*m*/*z*), $(M+Na^+)$: 449.2426, Found: 449.2428. **5b**, 44.4%, mp: 54–55 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.6$ Hz), 1.28 (br s, 28H), 1.58 (t, 2H, $J = 7.0$ Hz), 1.93 (m, 1H), 2.06 (m, 2H), 2.26 (t, 2H, $J = 7.5$ Hz), 2.42 (m, 1H), 4.01 (q, 1H, $J = 5.0$ Hz), 4.23 (m, 3H), 4.34 (m, 2H), 5.98 (m, 1H), 7.40 (d, 1H, $J = 5.8$ Hz). ESI-HR-MS (*m*/*z*), $(M+Na^+)$: 533.3358, Found: 533.3367. **5c**, 45.5%, faint yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.7$ Hz), 1.32 (br s, 20H), 1.58 (m, 2H), 2.04 (m, 7H), 2.26 (t, 2H, $J = 7.5$ Hz), 2.40 (m, 1H), 4.00 (q, 1H, $J = 4.0$ Hz), 4.24 (m, 3H), 4.33 (m, 2H), 5.36 (m, 2H), 5.98 (m, 1H),7.40 (d, 1H, $J = 5.8$ Hz). ESI-HR-MS (m/z), $(M + Na^+)$: 531.3218, Found: 531.3210. **5d**, 46.9%, faint yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.33 (br s, 14H), 1.58 (t, 2H, $J = 6.8$ Hz), 1.96 (m, 7H), 2.26 (t, 2H, $J = 7.5$ Hz), 2.41 (m, 1H), 2.76 (t, 2H, $J = 6.4$ Hz), 4.01 (q, 1H, $J = 4.0$ Hz), 4.24 (m, 3H), 5.34 (m, 4H), 5.98 (d, 1H, $J = 5.3$ Hz), 7.41 (d, 1H, $J = 5.8$ Hz). ESI-HR-MS (*m*/*z*),$(M+Na^+)$: 529.3049, Found: 529.3054. **5e**, 53.0%, faint yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.8$ Hz), 1.32 (br s, 6H), 1.67 (t, 2H, $J = 7.4$ Hz), 1.93 (m, 1H), 2.06 (br s, 6H), 2.28 (t, 2H, $J = 7.7$ Hz), 2.41 (m, 1H), 2.82 (br s, 6H), 4.00 (q, 1H, $J = 6.9$ Hz), 4.24 (m, 3H), 4.33 (m, 2H), 5.32 (br s, 8H), 5.98 (d, 1H, $J = 5.3$ Hz), 7.41 (d, 1H, $J = 6.0$ Hz). ESI-HR-MS (*m*/*z*), $(M + Na^+)$: 553.3058, Found: 553.3054.
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