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Synthesis and Biological Activity of the New 5-Fluorocytosine Derivatives, 5′-Deoxy-*N*-alkyloxycarbonyl-5-fluorocytosine-5′-carboxylic Acid

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Abstract—A series of 5-fluorocytosine derivatives, 5′-deoxy-*N*-alkyloxycarbonyl-5-fluorocytosine-5′-carboxylic acid **6**, were synthesized and evaluated for their antitumor activity.

Introduction

Since 5-fluorouracil (5-FU) was introduced as an antitumor agent acting as thymidylate synthase inhibitor in the 1960s, it is one of the most used and a standard chemotherapeutic agent for some cancers. 5-FU, however, possesses toxicity and shows side effects. So, a large number of its chemotherapy and derivatives with promising antineoplastic activity and low toxicity, such as 5-fluoro-1-(2-tetrahydrofuryl)-2,4 (1H,3H)-pyrimidinedione (tegafur), 5′-deoxy-5-fluoro-uridine (doxifluridine), and *N*-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine (capecitabine), have been developed in past decades (Fig. 1).[1]

In order to develop more active and less toxic analogues, we also designed and synthesized new derivatives of 5-fluorocytosine, 5′-deoxy-*N*-alkyloxycarbonyl-5-fluorocytosine-5′-carboxylic acid **6**, as new compounds having potent antitumor activity and low toxicity.

Synthesis

A synthetic route for new 5-fluorocytosine derivatives, 5′-deoxy-*N*-alkyloxycarbonyl-5-fluorocytosine-5′-carboxylic acid **6** is outlined in Scheme 1. Trimethylsilylated 5-fluorocytosine **1**, derived from 5-fluorocytosine, 1,1,1,3,3,3,-hexamethyldisilazane, and catalytic amount of ammonium sulfate in toluene,[2] were coupled with β-D-ribofuranose 1,2,3,5-tetraacetate **2** in acetonitrile in the presence of tin(IV) chloride to afford 2′,3′,5′-tri-*O*-acetyl-5-fluorocytidine **3**.[3] This compound **3** was used as a common intermediate herein. Then, alkyloxycarbonylation of **3** by means of *N,N*-diisopropylethylamine and alkyl chloroformate in methylene chloride gave 2′,3′,5′-tri-*O*-acetyl-5-fluoro-*N*-(alkyloxycarbonyl)-cytidine **4**. The hydrolysis of triacetate **4** by sodium methoxide in methanol gave triol, 5-fluoro-*N*-(alkyloxycarbonyl)cytidine **5**. Finally, selective oxidation of primary alcohol **5** with oxygen and platinum oxide gave the carboxylic acid **6**.[4]

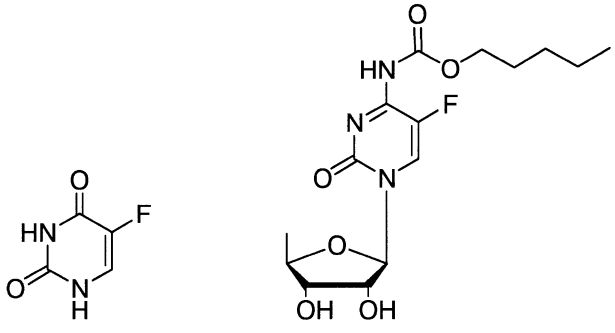

Figure 1. 5-FU and capecitabine.

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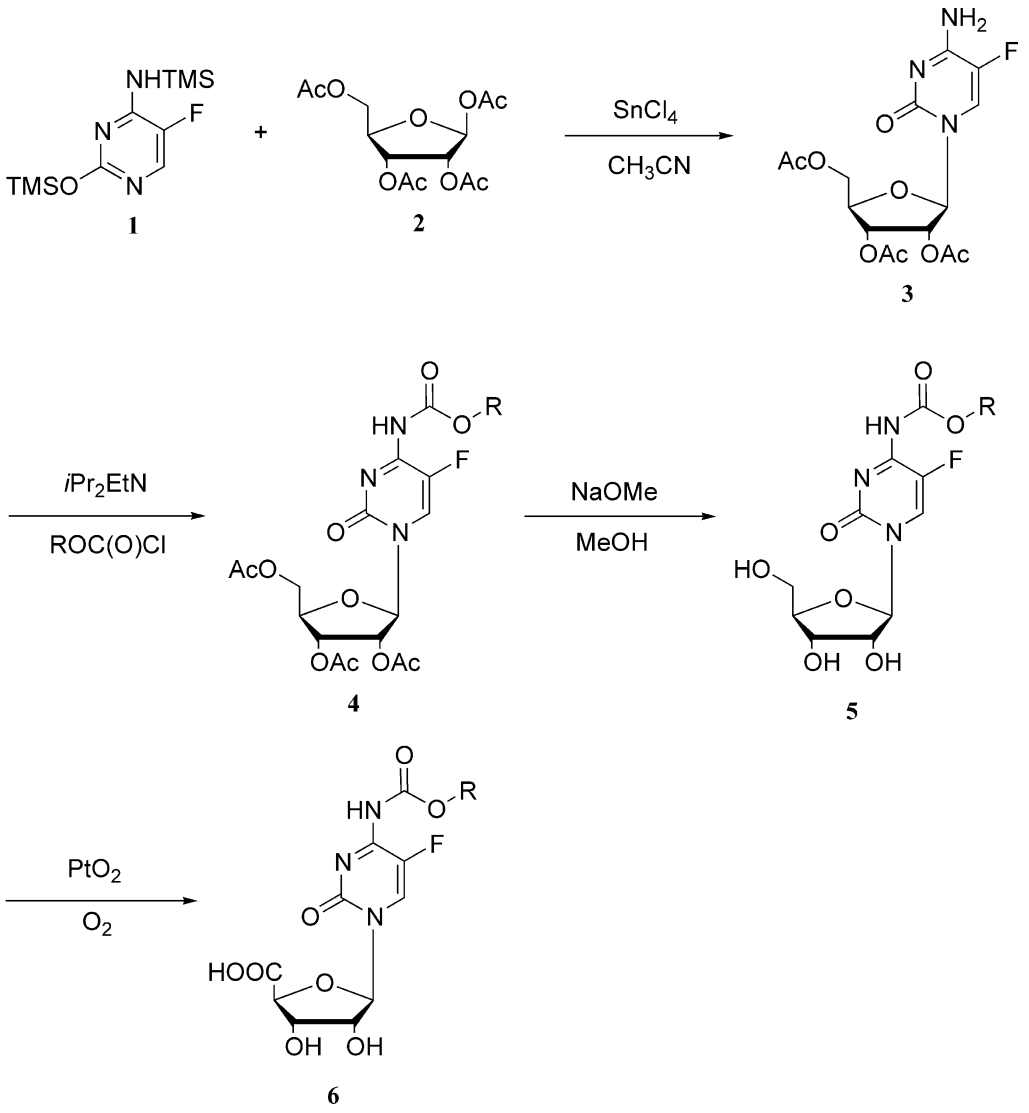

Scheme 1. Reagents and conditions: (a) $SnCl_4/CH_3CN$, 0 °C to rt, 81%; (b) $iPr_2EtN/ROC(O)Cl/CH_2Cl_2$, rt, 65–90%; (c) NaOMe/MeOH, rt, 80–95%; (d) $PtO_2/O_2/NaHCO_3$ buffer (pH 8–10), 56–77%.

Biological Activity

Cytotoxicity of the 5-fluorocytosine derivatives **6a–f** was examined by using cells of human lung (A549), ovarian (SK-OV-3), colon (HCT15), and melanoma (SK-MEL-2) cancers and expressed in terms of inhibitory activity (IC_{50}, μg/mL) obtained by the MTT method.[5] The results are summarized in Table 1.

The in vivo antitumor activity of compounds **6** was investigated with murine leukemia L1210 and the results are listed in Tables 2 and 3.[6] The activity was evaluated in terms of increase of life span (ILS, %). The results in Table 2 were carried out by the following protocol A. L1210 cells were performed every 2 weeks into male BDF1 mice, and then ascites cells were collected by centrifugation. Collected cells were resuspended in phosphate buffered saline (PBS) to a concentration of 1×10^6/mL. Then, L1210 cells (1×10^5/mL) were inoculated intraperitoneally (ip) into male BDF1 mice on day 0. The test compound **6b** and one reference compound, capecitabine, were orally (po) administered daily for 2 weeks. Another reference compound, 5-FU, was intravenously (iv) administered daily also for 2 weeks.

Table 1. Cytotoxicities of new 5-fluorocytosine derivatives against tumor cells

Compd	R	IC_{50} (μg/mL)[a]			
		A549	SK-OV-3	HCT15	SK-MEL-2
5-FU	—	0.26	0.03	0.11	0.63
6a	Ethyl	0.49	0.16	0.47	0.16
6b	Pentyl	0.17	0.03	0.12	0.03
6c	Allyl	0.040	0.008	0.032	0.008
6d	Propargyl	0.005	0.005	0.040	0.007
6e	Phenyl	0.55	0.12	0.39	0.10
6f	Benzyl	0.37	0.07	0.17	0.10

[a]Activities against tumor cells (A549, human lung; SK-OV-3, human ovarian; HCT15, human colon; SK-MEL-2, human melanoma) were measured by MTT assay after 3 days of incubation.

Table 2. In vivo activities of new 5-fluorocytosine derivatives against murine leukemia L1210 by protocol A

Compd (po)	Dose[a] (mmol/kg/day)	MST[b] (day)	ILS[b] (%)
Control		8.8	
5-FU	0.23	16.5	87.5
	0.15	14.3	62.5
Capecitabine	1.5	12.5	42.0
	0.67	9.6	9.1
	0.13	9.4	6.8
6b	1.5	16.8	90.9
	0.67	11.3	28.4
	0.13	9.1	3.4

[a]L1210 cells (1×10^5) by protocol A were implanted intraperitoneally (ip) into BDF1 male mice (6 weeks old) on day 0 and the mice were divided into several groups (8 mice per group) on day 1. The 5-FU was suspended in 0.5% CMC and intravenously (iv) administrated daily for 2 weeks. The other test compounds were dissolved in saline and administrated per os (po) daily for 2 weeks.
[b]Survival number was monitored daily and the increase in life span (ILS, %) was calculated from [(mean survival time of treated group)/(mean survival time of control group)−1]×100.

Table 3. In vivo activities of new 5-fluorocytosine derivatives against murine leukemia L1210 by protocol B

Comp (po)	Dose[a] (mmol/kg/day)	MST[b] (day)	ILS[b] (%)
Control		17.8	
Capecitabine	2.00	22.8	28.1
	0.40	20.1	12.9
	0.080	20.2	13.5
	0.016	19.2	7.9
	0.0032	18.9	6.2
6c	2.00	5.1	−71.3
	0.40	16.2	−9.0
	0.080	25.7	44.4
	0.016	24.8	39.3
	0.0032	22.1	24.2

[a]L1210 cells (1×10^6) by protocol B were implanted intraperitoneally (ip) into BDF1 male mice (6 weeks old). The test compounds were dissolved in saline and orally (po) administrated 15 times for 3 weeks.
[b]Survival number was monitored daily and the increase in life span (ILS, %) was calculated from [(mean survival time of treated group)/(mean survival time of control group)−1]×100.

The results in Table 3 were carried out by following protocol B. L1210 cell line for implantation was maintained at 37 °C under an atmosphere of 5% CO_2 in a 75 cm^2 culture flask and subcultured once or twice per week in RPMI 1640 medium containing 10% fetal bovine serum. The tumor cells were resuspended in PBS to a concentration of 1×10^7/mL. Similarly, L1210 cells (1×10^6/mL) were inoculated intraperitoneally (ip) into male BDF1 mice on day 0. The test compound **6c** and the reference compound, capecitabine, were orally administered (po) 15 times for 3 weeks and the maximum doses were decided by considering acute toxicity.

Results and Discussion

As shown in Table 1, all the new 5-fluorocytosine derivatives **6** were potent to inhibit the proliferation of A549, SK-OV-3, HCT15, and SK-MEL-2 cells. Among the compounds, **6c** and **6d** showed excellent inhibitory activity on the above cell lines, with IC_{50} values in the range of 0.005∼10.040 μg/mL. It is evident that the in vitro potency of those bearing unsaturated alkyl groups (**6c**, R = allyl; **6d**, R = propargyl) is better than that of those bearing saturated alkyl and aromatic groups (**6a**, R = ethyl; **6b**, R = pentyl, **6e**, R = phenyl; **6f**, R = benzyl). We initially evaluated that the in vivo activity test of the new 5-fluorocytosine derivative **6b** and the reference compounds, 5-FU and capecitabine. As shown in Table 2, the antitumor activity of **6b** against L1210 leukemia in mice showed the best ILS value at high dose range, 90.9% at 1.5 mmol/kg/day, after po administration compared to ILS of 5-FU and capecitabine, 87.5% at 0.23 mmol/kg/day (iv) and 42.0% at 1.5 mmol/kg/day (po), respectively.[7]

The toxicity (LD_{50}) of **6c** by oral administration was 650 mg/kg for single dose and 22 mg/kg/day daily for 21 days in mice. This value for single dose is very low compared to 115 mg/kg of 5-FU.[8] In case of **6d**, the LD_{50} value was 12 mg/kg for single dose and it is higher than that of **6c**.

As also shown in Table 3, the new 5-fluorocytosine derivative **6c** gave good antitumor activity over a broad dose range compared to capecitabine.[9] The compound **6c** showed ILS ranging from 24.2% at 0.0032 mmol/kg/day to 44.4% at 0.080 mmol/kg/day. ILS of capecitabine had a range of 6.2% at 0.0032 mmol/kg/day to 28.1% at 2.00 mmol/kg/day.

Evidently, the in vivo antitumor activity of **6b** and **6c** is better than that of 5-FU and capecitabine. Especially, in case of **6c**, it shows that the similar antitumor activity at much lower dose range compared to that of capecitabine.

In conclusion, we have synthesized the new 5-fluorocytosine derivatives, 5′-deoxy-*N*-alkyloxy-carbonyl-5-fluorocytosine-5′-carboxylic acid **6**, and they showed better antitumor activity than that of 5-FU or capecitabine. In particular, **6c** showed potent antitumor activities against L1210 leukemia and low toxicity. We selected **6c** for further pharmacological evaluation.

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9. Three of treatment groups (**6c** at 2.00 and 0.40 mmol/kg/day, and capecitabine at 2.00 mmol/kg/day) showed a small but significant decrease in body weight relative to the group without tumor cells.