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The Design and Synthesis of a New Tumor-Selective Fluoropyrimidine Carbamate, *Capecitabine*

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Abstract—To identify an orally available fluoropyrimidine having efficacy and safety profiles greatly improved over those of parenteral 5-fluorouracil (5-FU: **1**), we designed a 5-FU prodrug that would pass intact through the intestinal mucisa and be sequentially converted to 5-FU by enzymes that are highly expressed in the human liver and then in tumors. Among various N^4-substituted 5′-deoxy-5-fluorocytidine derivatives, a series of N^4-alkoxycarbonyl derivatives were hydrolyzed to 5′-deoxy-5-fluorocytidine (5′-DFCR: **8**) specifically by carboxylesterase, which exists preferentially in the liver in humans and monkeys. Particularly, derivatives having an N^4-alkoxylcarbonyl moiety with a C4–C6 alkyl chain were the most susceptible to the human carboxylesterase. Those were then converted to 5′-deoxy-5-fluorouridine (5′-DFUR: **4**) by cytidine deaminase highly expressed in the liver and solid tumors and finally to 5-FU by thymidine phosphorylase (dThdPase) preferentially located in tumors. When administered orally to monkeys, a derivative having the N^4-alkoxylcarbonyl moiety with a C5 alkyl chain (capecitabine: **6**) The highest AUC and Cmax for plasma 5′-DFUR. In tests with various human cancer xenograft models, capecitabine was more efficacious at wider dose ranges than either 5-FU or 5′-DFUR and was significantly less toxic to the intestinal tract than the others in monkeys.

Introduction

Most antitumor agents are cytotoxic and are associated with severe side effects, including myelo-suppression, intestinal toxicity and skin toxicity. There have been several attempts to reduce such side effects by means of prodrug strategies for the tumor-selective delivery of cytotoxic agents,[1] such as (a) prodrugs activated either by endogenous enzymes preferentially localized in tumor tissues or (b) exogenous enzymes specifically introduced into tumor tissues by gene technology, and (c) conjugates of cytotoxic drugs and molecules selectively bound to tumors. To our knowledge, however, none of them has yet to be used clinically. The present study describes the tumor-selective delivery of 5-fluorouracil (5-FU: **1**) by sequential conversion of the new fluoropyrimidine carbamates (**7**: R = alkoxy) by endogenous enzymes preferentially localized in human liver and tumor tissues.

5-FU (**1**) is one of the antitumor agents most frequently used for treating solid tumors, such as breast, colorectal, and gastric cancers, in either monotherapy or combination therapy with various cytotoxic drugs and modulators, such as leucovorin and methotrexate.[2] To optimize the efficacy of 5-FU (**1**), it is often administered by continuous infusion,[3] because of its short plasma half-life, as well as in combination with other cytotoxics or with biochemical modulators, such as leucovorin. 5-FU is poorly tumor selective, however, and its therapy causes high incidences of toxicity in the bone marrow, gastrointestinal tract, central nervous system and skin. Several oral 5-FU derivatives, such as tegafur (**2**), carmofur (**3**), and doxifluridine (5′-DFUR: **4**) (Fig. 1), have been developed in Japan to improve such side effects and are being used clinically in several countries. Nevertheless, they still need to be improved in terms of tumor selectively, efficacy, and safety.[2]

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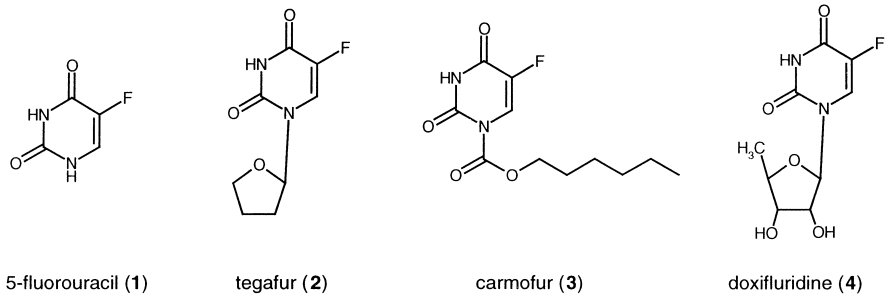


Figure 1. 5-Fluorouracil and its prodrugs.

5′-DFUR (**4**), which we previously developed for the treatment of breast, colorectal and other cancers, shows a tumor-selective activity as a result of its selective conversion to 5-FU by pyrimidine nucleoside phosphorylases (PyNPase) preferentially localized in tumors: uridine phosphorylase in mice[4] and thymidine phosphorylase (dThdPase) in humans.[5,6] These enzymes, however, also exist in the intestinal tract,[4,5] and generate 5-FU there. As a consequence, **4** causes its dose-limiting toxicity, diarrhea, when orally given at high doses for long periods.[7] To improve on the efficacy and safety profiles of the oral fluoropyrimidine 5′-DFUR (**4**), capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine; **6**), a new orally available fluoropyrimidine carbamate, was synthesized so that it passes intact through the intestinal mucosa and then selectively delivers 5-FU to tumor tissues following its enzymatic conversion: first to 5′-deoxycytidine (5′-DFCR: **8**) by carboxylesterase in the liver, then to 5′-deoxy-5-fluorouridine (5′-DFUR: **4**) by cytidine deaminase,[8] and finally to 5-FU by dThdPase. The latter two enzymes are preferentially localized in tumor tissues.[5] It has been approved in the USA, Canada, and other countries for the treatment of metastatic breast cancer that is resistant to standard chemotherapy with paclitaxel and an anthracycline-containing regimen.

In this paper, we describe our studies on the design, synthesis, and assessment of N^4-(substituted)-5′-deoxy-5-fluorocytidine derivatives, including capecitabine.

Design and Synthesis of Prodrugs

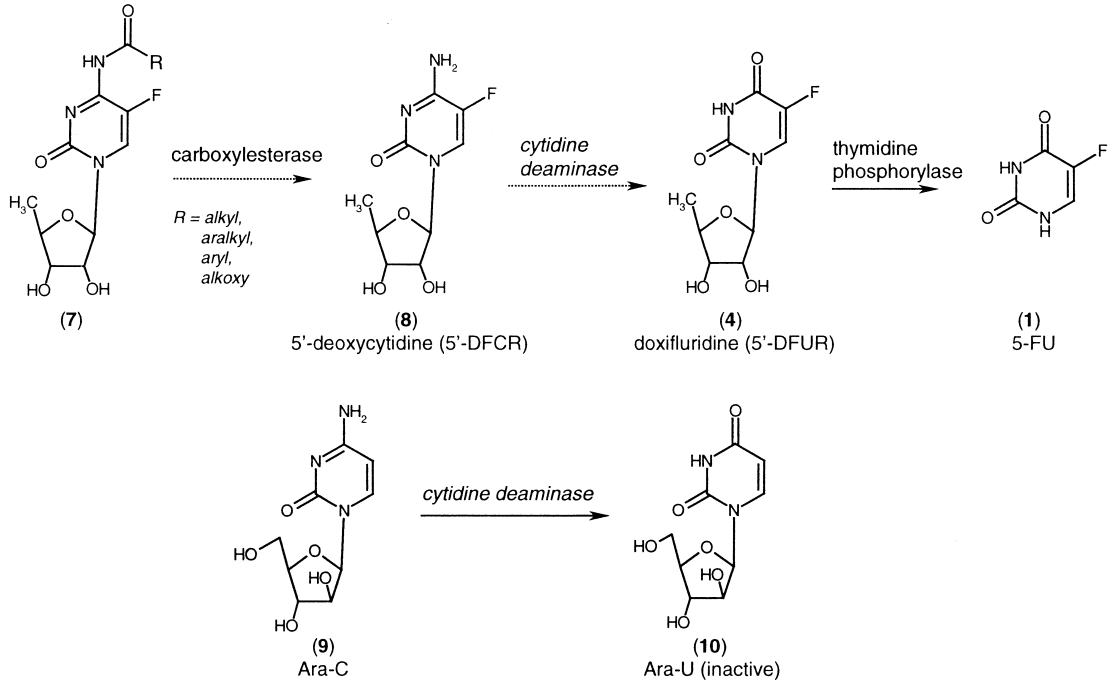
Design

We set out to design 5′-deoxycytidine (**8**)-type prodrugs of doxifluridine (**4**) with high tumor-selective activity based on our experience and knowledge of anticancer drug development. It is well known that Ara-C (**9**), a potent DNA polymerase α inhibitor, is active against leukemia but inactive against solid tumors.[9] This efficacy profile was suggested to result from the physiological localization of cytidine deaminase, which converts Ara-C to its inactive metabolite Ara-U (**10**), whereby low levels are found in leukemic cells and high levels in solid tumors.[10,11] Moreover, we observed that the enzyme activity was higher in various types of human cancer tissues than in the healthy tissues adjacent to the cancer tissues.[5] The enzyme activity was also reported to be low in growing bone marrow cells.[12] We, therefore, assumed that 5′-DFCR (**8**) would be efficiently converted to 5′-DFUR (**4**) in solid tumors, showing tumor selective action, but only slightly converted in the growing bone marrow cells, without severe bone marrow toxicity. In addition, acylation of the N^4-amino group of 5′-DFCR could no longer render it a substrate for dThdPase in the intestinal tissues, allowing it to pass through the intestinal mucosa as an intact molecule without causing intestinal toxicity. The key to the success of this approach was to find a liver-specific enzyme for the hydrolysis of the N^4-acyl group (Scheme 1).

Our strategy was to identify N^4-(substituted)-5′-DFCR derivatives **7** that pass intact through the intestinal mucosa and then are metabolized to 5′-DFCR (**8**) in the liver. First, we screened various N^4-substituted 5′-DFCR derivatives to identify compounds susceptible only to enzymes in the liver, but not to those in the intestine or other organs, from humans and monkeys. Among such compounds identified, we then selected compounds with high oral bioavailability in monkeys while, in parallel, assessing their antitumor efficacy in human cancer mouse xenograft models and their intestinal toxicity in monkeys. We selected human and monkey enzyme systems for this screening, because we had previously found a significant species difference in the enzyme susceptibility of N^4-substituted-5′-DFCR derivatives between rodents and primates. We previously synthesized galocitabine (5′-deoxy-5-fluoro-N^4-(3,4,5-trimethoxybenzoyl)cytidine: **5**) (Fig. 2), which was selected from among various types of N^4-acyl-5′-DFCR derivatives synthesized based on its efficacy and safety in mouse tumor models. Galocitabine (**5**), however, had low bioavailability in humans and monkeys; it was not well converted to 5′-DFCR in these species, although it generated 5′-DFCR and was highly effective and safe in mice.[13]

Synthesis

The synthesis of the new N^4-(substituted)-5′-DFCR (**7**) is outlined in Scheme 2. Various N^4-(acyl or alkoxycarbonyl)-5′-DFCR (**7**) derivatives were synthesized from 5-fluorocytosine (**11**) in 3 steps: (i) glycosidation of **11** with 1,2,3-tri-*O*-acetyl-5-deoxyribose[14] with stannic tetrachloride[15] in dichloromethane (at 15–20 °C) or in situ generated trimethysilyl iodide[16] in acetonitrile (at



Scheme 1. Design of cytidine-type prodrugs of 5-FU.

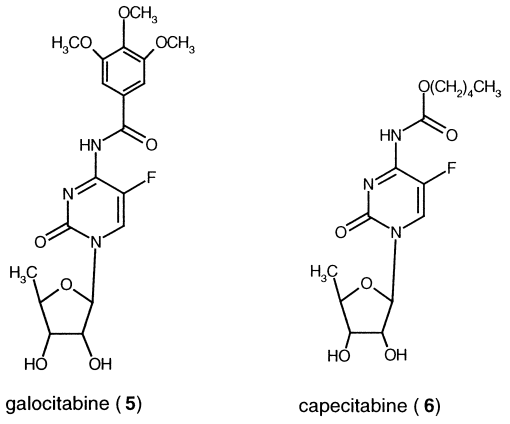


Figure 2.

0 °C), (ii) acylation of the N^4-amino group of the resulting 5′-DFCR derivative **12** with acid chlorides or alkoxycarbonyl chlorides in 2 equiv pyridine/dichloromethane, and (iii) alkaline hydrolysis of the acetyl group of **13** at 0 °C.

Results and Discussion

Structure–enzyme susceptibility relationship

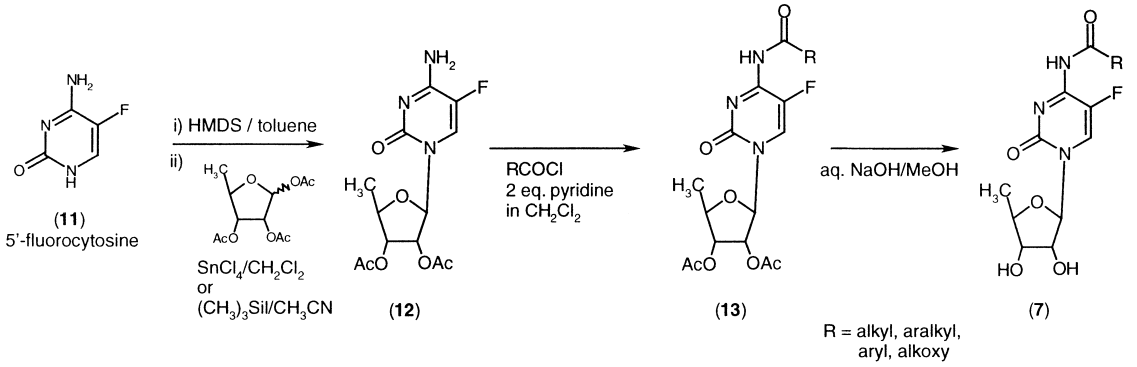
The susceptibility of N^4-(substituted)-5′-DFCR derivatives to enzymes converting them to 5′-DFCR was measured as described in our previous report.[5] Briefly, the derivatives were incubated with extracts from human and monkey tissue samples in the presence of a cytidine deaminase inhibitor, tetrahydrouridine, at 37 °C for 60 min, and the amount of the product 5′-DFCR was measured by HPLC.

Figure 3 shows the initial screening results for susceptibility of all the 5′-DFCR derivatives to the crude enzyme extract from human liver and intestine; the detailed data for the N^4-(C1 to C9)alkoxycarbonyl derivatives are shown in Table 1. The carbamate derivatives showed higher specificity to human liver enzyme over the intestinal enzyme, while most of the amide derivatives showed very poor specificity except for a few derivatives. Namely, all the carbamate derivatives having a normal alkyl, branched chain alkyl, cycloalkyl, or aralkyl group in the structure were susceptible only to the liver enzymes. In contrast, only the three amide derivatives having a 3,5-disubstituted benzoyl group, namely compounds **22** (N^4-3,5-dimethoxybenzoyl), **23** (N^4-3,5-dimethylbenzoyl), and **24** (N^4-3,5-dichlorobenzoyl) showed preferential susceptibility to the liver enzymes; their susceptibilities (nmol/mg protein/h), liver/intestine, were $32/<10$, 270/22, and $38/<10$, respectively (Fig. 3, Table 1).

Figure 4 shows the relationship between the alkyl chain length of the carbamates (only normal alkyl: C1–C9) and the susceptibility to the human and monkey carboxylesterases (crude enzymes from the liver and the intestine). The carbamate derivatives showed extremely high specificity to the liver enzyme over the intestinal enzyme for both humans and monkeys, suggesting their efficient biotransformation to 5′-DFCR in the liver after passing intact through the intestinal mucosa when given orally to humans.

The optimal chain lengths for the human liver enzyme were C5 and C6, while that for the monkey enzyme was C8.

As a reference, the susceptibilities of the same carbamate derivatives to the mouse liver and intestinal enzymes are



Scheme 2. Synthesis of N^4-(acyl or alkoxycarbonyl)-5′-DFCR derivatives.

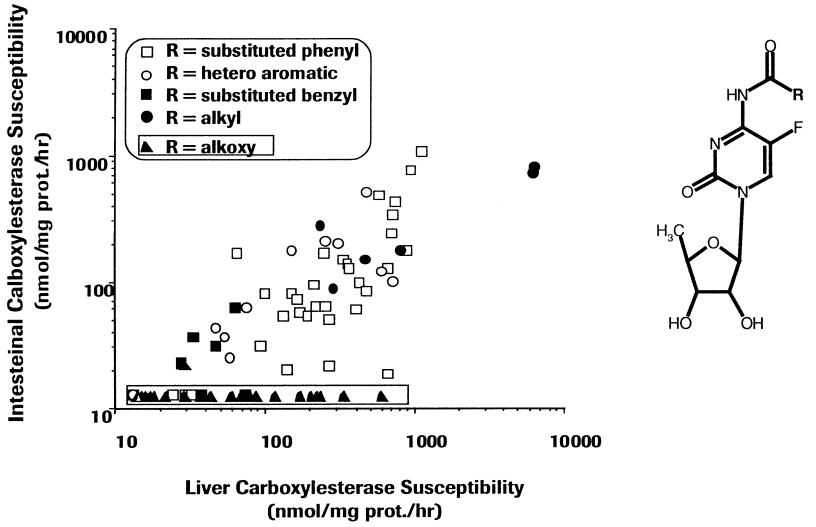


Figure 3. Susceptibility of 5′-DFCR derivatives to human liver and intestinal carboxylesterase (crude enzyme).

shown in Figure 5, which emphasizes the species difference in the enzyme susceptibility as compared with that in humans and monkeys. Actually, no liver enzyme selectivity was seen in mice. Therefore, it should be emphasized that the carbamate type prodrugs could never have been selected without human enzyme assay systems (Figs 4 and 5).

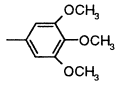
Pharmacokinetic profiles in monkeys

Correlation between the C_{max} or AUC of 5′-DFUR (**4**) and the susceptibility to the monkey liver carboxylesterase as well as the alkyl chain length of the carbamates.

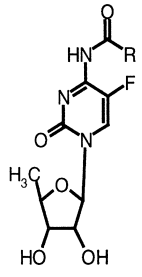
The N^4-(alkoxycarbonyl)-5′-DFCR derivatives **7** were orally administered into groups of five cynomolgous monkeys. At various times after the administration, blood was withdrawn for determination of plasma concentrations of intact molecules and their active metabolite 5′-DFUR by the HPLC method. Figure 6 shows the 2nd order correlation between the C_{max} or AUC of 5′-DFUR (**4**) generated from the N^4-(alkoxycarbonyl)-5′-DFCR derivatives in monkeys and their susceptibility to the monkey liver carboxylesterase as well as that of the alkyl chain length of the carbamates. As shown in Figure 6 and Table 1, N^4-(alkoxycarbonyl)-5′-DFCR derivatives with the appropriate susceptibility to carboxylesterase showed better pharmacokinetic profiles. Namely, those having C4 (**17**), C5 (**6**), and C6 (**18**) alkyl chains in the N^4-alkoxycarbonyl moiety, whose enzyme susceptibilities range from 20 to 50 nmol/mg protein/h, gave high 5′-DFUR AUC and C_{max}. Among them, N^4-(*n*-pentyloxycarbonyl)-5′-DFCR (capecitabine: **6**) with the highest AUC and C_{max} of 5′-DFUR was selected as a candidate for further development (Fig. 6).

The enzyme susceptibilty and the PK parameters of capecitabine (**6**) were compared with those of galocitabine (**5**) after single oral administration of **5** and **6** at 45.5 μmol/kg in monkeys (Table 2). Capecitabine showed clearly better oral bioavailability than galocitabine: higher C_{max} and AUC value of 5′-DFUR (**4**) (5.3-fold higher C_{max} and 4.6-fold higher AUC as compared with galocitabine administration) and lower levels of intact molecule in the plasma and urine were achieved with the oral capecitabine. The pharmacokinetic profiles of capecitabine indicate that it can pass through the intestinal mucosa unchanged and then be hydrolyzed into 5′-DFCR (**8**) by the hepatic carboxylesterase.[5] In separate experiments, the higher tumor selectivity of

Table 1. Susceptibility of 5′-DFCR derivatives to carboxylesterase and their pharmacokinetic profiles in monkeys

		Susceptibility to carboxylesterase (nmol/mg protein/h)						Pharmacokinetic profile in monkeys[a]			
		Mouse		Monkey		Human		Drug concentration in plasma			
		Liver	Intestine	Liver	Intestine	Liver	Intestine	Intact drug		5′-DFUR (**4**)	
Compound no.	R							C_{max}[b]	AUC[c]	C_{max}[b]	AUC[c]
14	OCH_3	10	10	2	< 10	10	< 10	7.2	17.5	0.1	0.3
15	OCH_2CH_3	10	28	5	< 10	10	< 10	9.7	19.3	0.4	0.8
16	$O(CH_2)_2CH_3$	24	93	10	< 10	35	< 10	6.9	16.9	1.7	2.6
17	$O(CH_2)_3CH_3$	41	160	20	< 10	71	< 10	3.3	4.5	2.0	2.6
Capecitabine (**6**)	$O(CH_2)_4CH_3$	38	99	29	13	**190**	**< 10**	**2.4**	**2.1**	**2.3**	**2.8**
18	$O(CH_2)_5CH_3$	44	76	47	17	220	< 10	1.8	2.1	1.9	2.6
19	$O(CH_2)_6CH_3$	55	100	59	16	110	< 10	1.2	3.9	0.6	1.0
20	$O(CH_2)_7CH_3$	43	42	66	16	73	< 10	1.2	3.2	0.4	1.2
21	$O(CH_2)_8CH_3$	36	34	61	< 10	56	< 10	0.4	1.4	0.3	0.7
Galocitabine (**5**)		280	< 10	1.4	< 0.5	**3.7**	**< 0.8**	**33.0**	**66.6**	**0.4**	**0.7**
22		1300	1800	14	16	32	< 10	10.0	20.9	0.9	1.7
23		1500	7200	67	22	270	22	6.1	10.0	1.5	2.2
24		270	82	18	< 10	38	< 10	3.2	6.0	1.0	2.8

[a]Single oral administration of **5** and **6** (45.5 μmol/kg).
[b]C_{max} μg/mL.
[c]AUC: μg h/mL.



orally administered (po) capecitabine versus 5-FU (ip) in the HCT116 human colon cancer xenograft model in mice was actually confirmed by measuring 5-FU levels in tumor, muscle, and plasma after a single administration.[17] In accordance with these results, capecitabine showed only mild toxicity to both the intestinal tract and bone marrow in monkeys. The degree of toxicity, which will be reported in detail elsewhere, was less than that of 5′-DFUR administration (po), even though capecitabine gave higher AUC and C_{max} of 5′-DFUR in the plasma.[18] Moreover, capecitabine exhibited higher efficacy than 5′-DFUR and 5-FU in various human cancer xenograft models in nude mice, including colon cancer/CXF280, HCT116; gastric cancer/GXF97, MKN-45; and breast cancer/MX-1, MAXF401.[19] This sequential metabolic activation has also been supported in humans by the results of clinical trials.[20]

The antitumor activity of capecitabine in mice depended on the activity of thymidine phosphorylase (dThdPase) in tumor tissues as well as the ratio of dThdPase and dihydropyrimidine dehydrogenase, a catabolic enzyme of 5-FU.[21] Some inflammatory cytokines, such as TNFα, IFNγ, and IL-1α, up-regulated the expression of dThdPase in tumor cells making the tumor cells more susceptible to 5′-DFUR.[22] Consequently, cancer treatments that up-regulate these cytokines, such as taxane (taxotere/taxol) administration[23] and X-ray irradiation,[24] enhanced the dThdPase levels in human cancer xenografts and the efficacy of 5′-DFUR and its precursor capecitabine. Thus, if this process were also the case in humans, the efficacy of capecitabine would be optimized by selecting appropriate patients whose tumors highly express dThdPase or by combining it with dThdPase up-regulators.

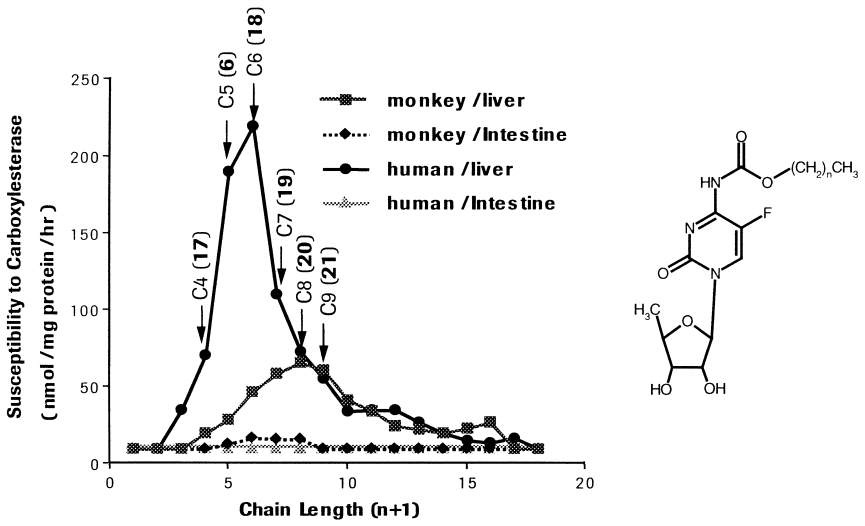


Figure 4. Correlation between the chain length and the susceptibility to carboxylesterase (monkey and human).

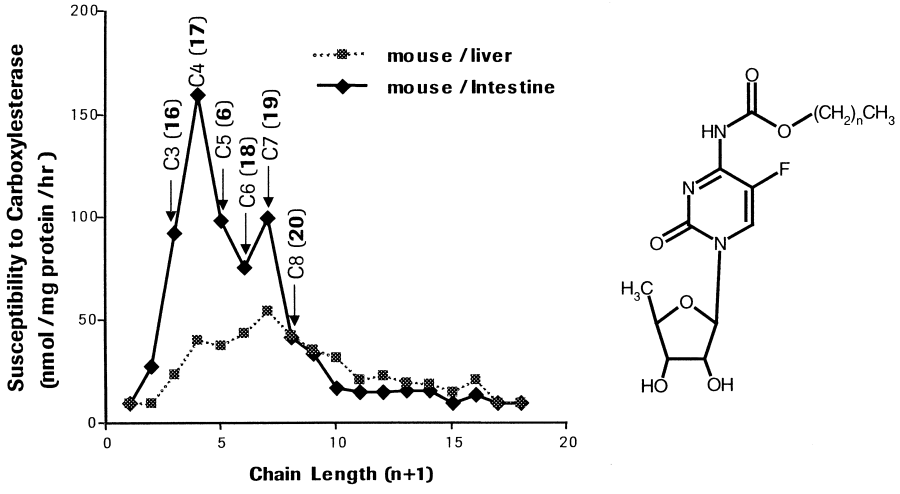


Figure 5. Correlation between the chain length and the susceptibility to carboxylestesterase (mice).

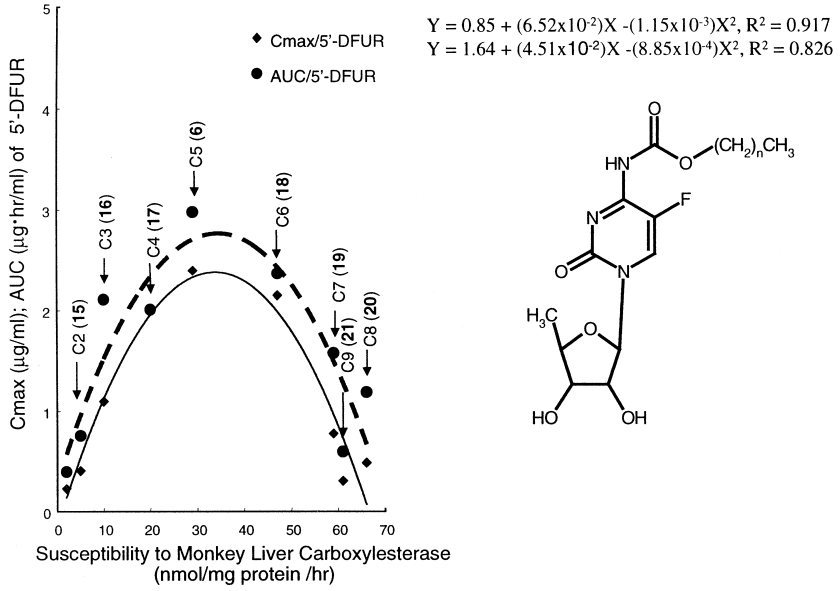


Figure 6. Correlation between enzyme susceptibility and C_{max} or AUC of 5′-DFUR (**4**) after oral administration of the C1–C9 normal alkyl carbamates (20 mg/kg) in monkeys.

Table 2. Pharmacokinetic profile of capecitabine (**6**) and galocitabine (**5**)[a]

	Drug concentration in plasma						Urinary excretion[d]	
	Intact		5′-DFCR (8)		5′-DFUR (**4**)		Intact drug	< 5-FU[e]
Compound no.	C_{max}[b]	AUC[c]	C_{max}[b]	AUC[c]	C_{max}[b]	AUC[c]		
Capecitabine (**6**)	2.3	2.0	0.15	0.36	2.1	2.9	< 0.2	66
Galocitabine (**5**)	33	67	0.76	1.88	0.41	0.69	27	34

[a]Single oral administration of **5** or **6** (45.5 μmol/kg) in monkeys.
[b]C_{max}: (μg/mL).
[c]AUC: μg h/mL.
[d]% of dose.
[e]< 5-FU: metabolites after 5-FU.

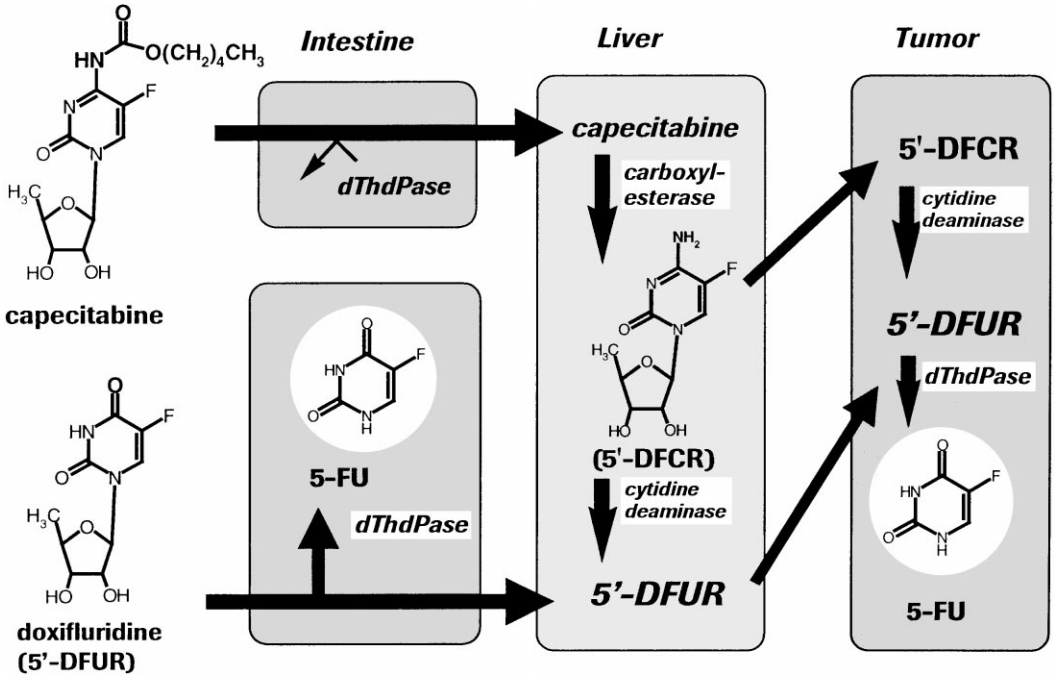


Figure 7. Conversion of capecitabine into 5-FU in humans.

Conclusion

We identified a new fluoropyrimidine carbamate, capecitabine, which would be sequentially converted to 5′-DFCR by carboxylesterase in the liver, to 5′-DFUR by cytidine deaminase highly expressed in the liver and tumor tissues, and finally to 5-FU by dThdPase preferentially located in various types of cancer tissues (Fig. 7). As a result of the unique localization of these enzymes in humans, capecitabine is expected to deliver the active drug 5-FU selectively to tumor tissues and to have better efficacy and safety profiles than do 5-FU and doxifluridine. This study thus demonstrates that the approach utilizing enzymes that are specifically expressed in certain tissues or tumors for prodrug activation is useful for the targeting of cytotoxic drugs to tumor tissues.

Experimental

NMR spectra were obtained on a JEOL SX270 spectrometer and are referenced to Me_4Si. FAB Mass spectra and high resolution FAB–MS spectra (matrix: *m*-nitrobenzoic acid) were obtained on a JEOL SX102/102. Melting points were determined on a micro melting point apparatus YANACO model MP. All products were judged to be > 98% pure by reverse-phase HPLC analysis with diode array detection. 5-Fluorocytosine was purchased from Aldrich.

2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine (12). Method (a). 5-Fluorocytosine (25.8 g, 0.2 mol) was suspended in toluene (103 mL) and hexyamethyl-disilazane (32.3 g, 0.2 mol). The mixture was heated at 100 °C for 3 h. After concentrating the reaction mixture under reduced pressure, methylene chloride (330 mL) and 5-deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranoside (59.3 g, 0.23 mol) were added to the residue. Then, anhydrous stannic chloride (62.5 g, 0.24 mol) was added dropwise to the ice-cooled reaction mixture over a period of 10 min. After stirring the mixture at room temperature for an additional 2 h, sodium bicarbonate (101 g, 1.2 mol) was added, followed by the dropwise addition of water (35 mL) over a period of 20 min. After stirring the resulting mixture at

room temperature for 3 h, insoluble material was filtered off and the filtrate was washed with 4% sodium bicarbonate solution (100 mL). The solvent was removed under reduced pressure, and the residue was recrystallized from isopropanol (180 mL) to give 5′-deoxy-2′,3′-di-*O*-acetyl-5-fluorocytidne (**12**) (49.9 g (y. 76%)): mp 191.5–192.3 °C. HRMS [FAB, MH^+] calcd for $C_{13}H_{17}FN_3O_6$ *m/z* 330.1097, found 330.1119; ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, d, J = 6.6 Hz), 2.04 (3H, s), 2.06 (3H, s), 4.04 (1H, d. q, J = 6.6 Hz), 5.09 (1H, t, J = 6.6 Hz), 5.43 (1H, d. d, J = 6.6 and 5.1 Hz), 5.76 (1H, d, J = 5.1 Hz), 7.67 (1H, br. s), 7.94 (1H, br. s), 8.01 (1H, d, J = 7.3 Hz).

Method (b). A solution of sodium iodide (3.6 g, 24 mmol) and chlorotrimethylsilane (3 mL, 24 mmol) in dry acetonitrile (15 mL) was stirred with molecular sieves 4A (200 mg) at 0 °C for 5 min (colorless sodium chloride deposited during stirring). 1,2,3-Tri-*O*-acetyl-5-deoxy-β-D-ribofuranose (2.0 g, 7.7 mmol) was added and the mixture was stirred at 0 °C for 30 min. Then, a solution of the trimethylsilylated 5-fluorocytosine, freshly prepared from 5-fluorocytosine (1.12 g, 8.68 mmol), in dry acetonitrile (5 mL) was added at 0 °C and stirring was continued for 3 h at room temperature. The mixture was filtered, the filtrate was concentrated in vacuo, and the residue was partitioned between dichloromethane and saturated aq. sodium bicarbonate solution. The aqueous layer was extracted with CH_2Cl_2:MeOH (10:1). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel chromatography using CH_2Cl_2:MeOH (15:1) as an eluent, followed by recrystallization from isopropanol to give 1.24 g (yield 49%) of 2′,3′-di-*O*-acetyl-5′-deoxy-5-fluorocytidine.

2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine (13a). 2′,3′-Di-*O*-acetyl-5′-deoxy-5-fluorocytidine (800 g, 2.43 mol) was dissolved in dry CH_2Cl_2 (2000 mL) and dry pyridine (394 mL). To the stirred solution on an ice-salt bath, *n*-pentyl chloroformate (493 mL, 3.38 mol) was added dropwise over a period of 90 min so that the reaction temperature was kept between −20 and −5 °C. After stirring the mixture for an additional 30 min at room temperature, MeOH (30 mL) was added in one portion, and the mixture was evaporated to a heavy syrup under reduced pressure. To the heavy syrup residue, Et_2O (1500 mL) was added, and the suspension was stirred for 10 min at room temperature. The insoluble white powder (pyridine hydrochloride) was filtered off and washed with Et_2O (2000 mL). The filtrate and washings were combined, dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure to give the crude 2′,3′-di-*O*-acetyl-5′-deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine, **13a** (1311 g) as a pale yellow syrup.

HRMS [FAB, MH^+] calcd for $C_{19}H_{27}FN_3O_8$ *m/z* 444.1775, found 444.1752; ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.3 Hz), 1.30–1.34 (4H, m), 1.36 (3H, d, J = 6.3 Hz), 1.61 (2H, m), 2.06 (3H, s), 2.07 (3H, s), 4.07–4.14 (3H, m), 5.11 (1H, t, J = 6.3 Hz), 5.47 (1H, d. d, J = 6.3 and 4.6 Hz), 5.80 (1H, d, J = 4.6 Hz), 8.28 (1H, br. s), 10.63 (1H, br. s).

5′-Deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine, capecitabine (6). The crude 2′,3′-di-*O*-acetyl-5′-deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine, **13a** (300 g) obtained above was dissolved in MeOH (300 mL). To the stirred mixture on an ice–salt bath, a solution of NaOH (108.4 g, 2.71 mol) in water (150 mL) was added dropwise over a period of 35 min so that the reaction temperature was kept between −20 and −5 °C. Then, to the reaction mixture on the ice–salt bath, concentrated HCl (ca. 226 mL) was added dropwise over a period of 25 min with stirring and cooling between −20 and −5 °C. Then the pH of the reaction mixture was adjusted from 4 to 5. The mixture was partitioned between CH_2Cl_2 (1500 mL) and water (300 mL). The organic layer was washed with water (300 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness under reduced pressure. The oily residue was dissolved in AcOEt (200 mL). To the solution, *n*-hexane (400 mL) was added dropwise over a period of 20 min with stirring, and the solution was allowed to stand at room temperature for 4 h. The colourless needle crystals precipitated were collected by filtration and washed with a mixed solvent of *n*-hexane:AcOEt (5:2) (900 mL) to give 5′-deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine (**6**) (170 g: y. 75% from 2′,3′-di-*O*-acetyl-5′-deoxy-5-fluorocytidine, (**12**)) as colorless needle crystals: mp 119–121 °C, [FAB, MH^+] calcd for $C_{15}H_{23}FN_3O_6$ *m/z* 360.1565, found 360.1592; ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 6.6 Hz), 1.22–1.42 (4H, m), 1.30 (3H, d, J = 6.3 Hz), 1.61 (2H, t. t, J = 6.6 Hz), 3.67 (1H, q, J = 5.6 Hz), 3.87 (1H, d. q, J = 6.3 Hz), 4.04–4.11 (3H, m), 5.02 (1H, d, J = 5.6 Hz), 5.37 (1H, d, J = 5.3 Hz), 5.66 (1H, d, J = 3.6 Hz), 8.00 (1H, br. s), 10.54 (1H, br. s).

The following compounds were obtained according to the method similar to that described above.

5′-Deoxy-5-fluoro-*N*^{4}-(methoxycarbonyl)cytidine (14). HRMS [FAB, MH^+] calcd for $C_{11}H_{15}FN_3O_6$ *m/z* 304.0911, found 304.0970; ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, d, J = 6.6 Hz), 3.66–3.69 (4H, m), 3.89 (1H, m), 4.06 (1H, m), 5.05 (1H, J = 5.9 Hz), 5.41 (1H, d, J = 5.1 Hz), 5.67 (1H, d, J = 3.7 Hz), 8.03 (1H, br. s), 10.60 (1H, br. s).

5′-Deoxy-5-fluoro-*N*^{4}-(ethoxycarbonyl)cytidine (15). Mp 119.5–121 °C, HRMS [FAB, MH^+] calcd for $C_{12}H_{17}FN_3O_6$ *m/z* 318.1097, found 318.1143; ^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 6.9 Hz), 1.30 (3H, d, J = 6.3 Hz), 3.67 (1H, m), 3.89 (1H, d.q, J = 6.3 Hz), 4.06 (1H, d.d.d, J = 4.6 Hz), 4.13 (2H, q, J = 6.9 Hz), 5.03 (1H, d, J = 5.9 Hz), 5.38 (1H, d, J = 4.6 Hz), 5.66 (1H, d, J = 4.0 Hz), 7.99 (1H, br. s), 10.96 and 11.75 (total 1H, br. s each).

5′-Deoxy-5-fluoro-*N*^{4}-(propoxycarbonyl)cytidine (16). Mp 125–126.5 °C, HRMS [FAB, MH^+] calcd for $C_{13}H_{19}FN_3O_6$ *m/z* 332.1253, found 332.1280; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, t, J = 7.3 Hz), 1.30 (3H, d, J = 6.3 Hz), 1.62 (2H, q. t, J = 7.3 Hz), 3.67 (1H, q, J = 5.9 Hz), 3.89 (1H, d. q, J = 5.9 Hz), 4.02–4.07 (3H, m),

5.02 (1H, d, $J=5.9$ Hz), 5.38 (1H, d, $J=5.3$ Hz), 5.66 (1H, d, $J=3.6$ Hz), 8.00 (1H, br. s), 10.58 (1H, br. s).

5′-Deoxy-5-fluoro-N^4-(butoxycarbonyl)cytidine (17). Mp 119–120 °C, HRMS [FAB, MH^+] calcd for $C_{14}H_{21}FN_3O_6$ m/z 346.1409, found 346.1418; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, t, $J=7.3$ Hz), 1.31 (3H, d, $J=5.9$ Hz), 1.36 (2H, m), 1.59 (2H, t. t, $J=7.3$ Hz), 3.68 (1H, q, $J=5.9$ Hz), 3.89 (1H, m), 4.02–4.12 (3H, m), 5.06 (1H, d, $J=5.9$ Hz), 5.41 (1H, br. d), 5.67 (1H, d, $J=3.7$ Hz), 8.04 (1H, br. s), 10.53 and 11.67 (total 1H, br. s each).

5′-Deoxy-5-fluoro-N^4-(hexyloxycarbonyl)cytidine (18). Mp 114–116 °C, HRMS [FAB, MH^+] calcd for $C_{16}H_{25}FN_3O_6$ m/z 374.1721, found 374.1721; ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, $J=6.3$ Hz), 1.22–1.41 (6H, m), 1.30 (3H, d, $J=5.9$ Hz), 1.60 (2H, t. t, $J=6.3$ Hz), 3.67 (1H, q, $J=5.9$ Hz), 3.89 (1H, d. q, $J=5.9$ Hz), 4.06–4.11 (3H, m), 5.02 (1H, d, $J=5.9$ Hz), 5.37 (1H, d, $J=5.3$ Hz), 5.66 (1H, d, $J=3.5$ Hz), 8.00 (1H, br. s), 10.51 (1H, br. s).

5′-Deoxy-5-fluoro-N^4-(heptyloxycarbonyl)cytidine (19). Mp 115.5–117.5 °C; HRMS [FAB, MH^+] calcd for $C_{17}H_{27}FN_3O_6$ m/z 388.1877, found 388.1883; ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, $J=6.1$ Hz), 1.21–1.40 (11H, m), 1.60 (2H, t. t, $J=6.6$ Hz), 3.67 (1H, q, $J=5.9$ Hz), 3.89 (1H, d. q, $J=5.9$ Hz), 4.04–4.10 (3H, m), 5.02 (1H, d, $J=5.9$ Hz), 5.38 (1H, d, $J=4.9$ Hz), 5.66 (1H, d, $J=4.0$ Hz), 7.99 (1H, br.s), 10.52 (1H, br. s).

5′-Deoxy-5-fluoro-N^4-(octyloxycarbonyl)cytidine (20). HRMS [FAB, MH^+] calcd for $C_{18}H_{29}FN_3O_6$ m/z 402.2033, found 402.2057; ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, $J=6.4$ Hz), 1.26–1.32 (10H, m), 1.30 (3H, d, $J=6.3$ Hz), 1.60 (2H, t. t, $J=6.3$ Hz), 3.67 (1H, q, $J=5.6$ Hz), 3.89 (1H, d. q, $J=5.9$ Hz), 4.04–4.10 (3H, m), 5.02 (1H, d, $J=6.3$ Hz), 5.37 (1H, d, $J=5.3$ Hz), 5.66 (1H, d, $J=2.6$ Hz), 8.00 (1H, br. s), 10.50 (1H, br. s).

5′-Deoxy-5-fluoro-N^4-(nonyloxycarbonyl)cytidine (21). Mp 120–122 °C, HRMS [FAB, MH^+] calcd for $C_{19}H_{31}FN_3O_6$ m/z 416.2189, found 416.2203; ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, $J=6.3$ Hz), 1.25–1.32 (12H, m), 1.30 (3H, d, $J=6.3$ Hz), 1.60 (2H, t. t, $J=6.3$ Hz), 3.67 (1H, q, $J=5.6$ Hz), 3.89 (1H, d. q, $J=5.9$ Hz), 4.04–4.10 (3H, m), 5.02 (1H, d, $J=5.9$ Hz), 5.37 (1H, d, $J=5.3$ Hz), 5.66 (1H, d, $J=4.0$ Hz), 8.00 (1H, br. s), 10.53 (1H, br. s).

5′-Deoxy-5-fluoro-N^4-(3,4,5-trimethoxybenzoyl)cytidine, Galocitabine (5). Mp 170–171 °C, HRMS [FAB, MH^+] calcd for $C_{19}H_{23}FN_3O_8$ m/z 440.1463, found 440.1475; ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, d, $J=6.3$ Hz), 3.74 (1H, q, $J=5.6$ Hz), 3.75 (3H, s), 3.85 (6H, s), 3.91 (1H. m), 4.12 (1H m), 5.06 (1H, d, $J=5.9$ Hz), 5.41 (1H, d, $J=4.9$ Hz), 5.72 (1H, d, $J=3.6$ Hz), 7.35 (2H, s), 8.12 (1H, br. s), 11.75 and 12.42 (total 1H, br. s each).

5′-Deoxy-N^4-(3,5-dimethoxybenzoyl)cytidine (22). Mp 159–161 °C, HRMS [FAB, MH^+] calcd for $C_{18}H_{21}FN_3O_7$ m/z 410.1358, found 410.1372; ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, d, $J=6.3$ Hz), 3.71 (1H, q, $J=5.6$ Hz), 3.80 (6H, s), 3.91 (1H, m), 4.12 (1H, m), 5.06 (1H, d, $J=5.9$ Hz), 5.41 (1H, d, $J=4.9$ Hz), 5.71 (1H, d, $J=3.3$ Hz), 6.75 (1H, br. s), 7.14 (3H, s), 7.15 (3H, s), 8.10 (1H, br. s), 11.69 & 12.50 (total 1H, br. s each).

5′-Deoxy-N^4-(3,5-dimethybenzoyl)cytidine (23). Mp 168–169 °C, MS [FAB, MH^+] m/z 378; HRMS [FAB, MH^+] calcd for $C_{18}H_{21}FN_3O_5$ m/z 378.1460, found 378.1463; ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, d, $J=6.3$ Hz), 2.34 (6H, s), 3.71 (1H, q, $J=5.6$ Hz), 3.91 (1H, m), 4.12 (1H, m), 5.06 (1H, d, $J=5.9$ Hz), 5.42 (1H, br. d,), 5.71 (1H, d, $J=3.6$ Hz), 7.27 (1H, s), 7.62 (2H, s), 8.09 (1H, br. s), 11.20 (1H, br. s).

5′-Deoxy-N^4-(3,5-dichlorobenzoyl)cytidine (24). Mp 156–157 °C, MS [FAB, MH^+] m/z 418; HRMS [FAB, MH^+] calcd for $C_{16}H_{15}{}^{35}Cl\ {}^{37}ClFN_3O_5$ m/z 420.0340, found 420.0443; calcd for m/z $C_{16}H_{15}{}^{35}Cl_2FN_3O_5$ 418.0370, found 418.0428; ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, d, $J=6.3$ Hz), 3.71 (1H, q, $J=5.6$ Hz), 3.91 (1H, d. q, $J=5.9$ Hz), 4.12 (1H, m), 5.07 (1H, d, $J=5.6$ Hz), 5.40 (1H, d, $J=4.9$ Hz), 5.70 (1H, d, $J=3.6$ Hz), 7.90 (1H, s), 7.96 (2H, s), 8.09 (1H, br. s), 12.51 (1H, br. s).

Enzyme assay

The enzyme assay method as well as the method for the preparation of crude enzyme extracts of the liver and intestinal tissues from mice, monkeys, and humans are described in ref 5.

Pharmacokinetic studies

The pharmacokinetic studies of N^4-(alkoxycarbonyl)-5′-DFCR derivatives were carried out in cynomolgus monkeys (3–4 kg; five monkeys in each group). The blood samples were taken at various time points after single oral administration of the test compounds (20 mg/kg). The intact drug levels in plasma were measured by the reverse-phase HPLC-UV method (column: YMC-Pack AM-212 C-8 (150×6 mm I.D., 5 μM, YMC K.K., Tokyo Japan); mobil phase; acetonitrile:citrate buffer (30:70–40:60, v/v); Flow rate: 1.0 mL/min; detection: 310 nM). The 5′-DFCR (**8**) and 5′-DFUR (**4**) levels in the plasma were measured by the reverse-phase HPLC-UV method (column: Inertsil ODS-2 (250×4.6 mm I.D., 5 μM, GL Science, Tokyo, Japan); mobil phase; acetonitrile:methanol:citrate buffer (6:20:174, v/v/v); flow rate: 0.8 mL/min; detection: 267 nM; retention time: 9.3 min for 5′-DFCR, 12.8 min for 5′-DFUR, 16.5 min for tegafur (internal standard)) with quantification limits of 0.02–0.1 μg/mL. The urinary concentration (0–24 h) of the intact drug, 5-FU, and a range of further metabolites (5,6-dihydro-5-fluorouracil, α-fluoro-β-ureido propionic acid, a-fluoro-β-alanine (FBAL), *N*-carboxyl-FBAL) were determined simultaneously by ^{19}F NMR with quantification limits of 6.5–20 mg/mL for 10 mL of urine.

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