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Preparation of 5-(2,6-Dideoxy-2-fluoro-α-L-talopyranosyloxy)-6-hydroxynaphtho[2,3-*f*]quinoline-7,12-dione (FT-Alz), a New-Type, Potentially Antitumor Substance with Various Biological Activities

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Abstract—The title compound (**6**), its structure being imaginatively created, has been prepared through coupling of alizarine blue (**2**), a classical dye, and 3,4-di-*O*-acetyl-2,6-dideoxy-2-fluoro-α-L-talopyranosyl bromide (**3**). Compound **6** has considerably higher and different antitumor activity from that of doxorubicin or its analogue (**10**), and, further, has properties to reverse multidrug resistance (by P-glycoprotein), to inhibit topoisomerase II, and to induce apoptosis.

Introduction

Clinically important antitumor substances such as vincristine, camptothecin analogues, doxorubicin, etoposide, taxol, bleomycin, and folic acid analogues (methotrexate), except cisplatin, are all of natural origin, and considerable efforts have been made to improve the biological activities of these compounds through structural modification. However, such efforts have been little rewarded from a viewpoint of dramatic alteration of the key biological characters, such as antitumor spectrum, type of action, and drug resistance, except for antitumor potency and toxicity. This means that the fundamental biological characters originally present in the parent compounds are difficult to alter by simple chemical modification. We therefore aimed to produce new kinds of compounds by creating new structures artificially, that is, by constructing the structure a priori. To pursue this idea, we have chosen a polyphenol(aglycon)–sugar structure as the most fundamental building frame in our approach, based on the expectation that the polyphenol-aglycon will give the new compounds tumor cell-killing ability, and the sugar portion, tumor cell-recognizing ability, although a combination of both is thought to be the most important. Numerous polyphenols have been reported to show (weak) antitumor activities by themselves.

As the first trial along this line, we prepared a phenolphthalol derivative (**1**) bringing 2,6-dideoxy-2-fluoro-α-L-talopyranose (FT)[1,2] at the side chain, the fluorosugar being chosen on the basis of our finding that substitution of the sugar portion of several antitumor anthracyclines with FT often gives compounds with considerably enhanced activity.[1] However, compound **1** showed neither antitumor nor any other biological activities within our testing. At this stage, we felt the need to adjust our strategy. We tried, therefore, to unite quinone and polyphenol structures with an additional seasoning of the resulting quinone–polyphenol structure by fusing a heterocyclic ring to it. Incidentally, some quinones have been reported[3,4] to show antitumor activities more or less. The heterocyclic ring to be fused was arbitrarily chosen, based on intuition, in the hope that it would give the new compounds a novel biological property. Based on the above concept, we searched for an appropriate compound in the range of polyhydroxyanthraquinones having a pyridine ring (or similar), and from the known compounds reported, we chose a seemingly promising structure for our purpose, 5,6-dihydroxynaphtho[2,3-*f*]quinoline-7,12-dione (**2**, alizarine blue; original name, alizarin-blue),[5] a synthetic classical dye,[5–7] not used now.

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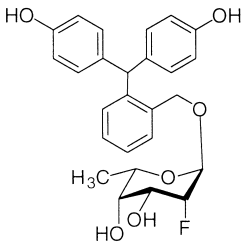

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Chemistry

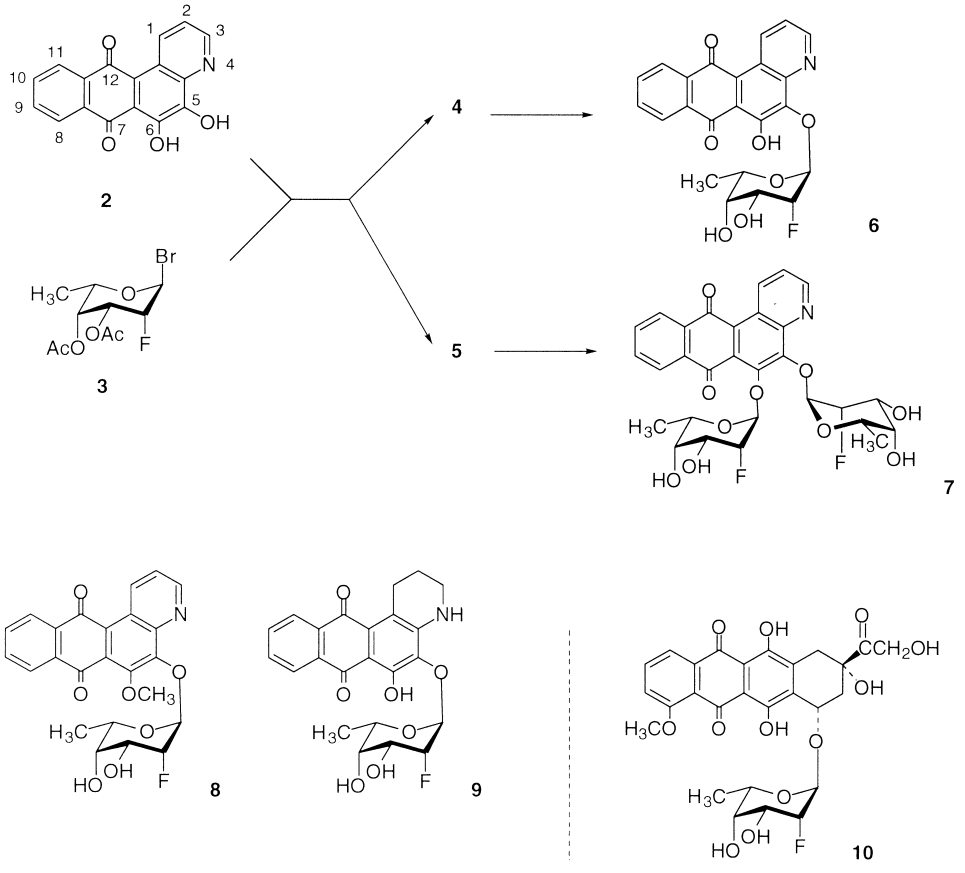
Coupling of alizarine blue (**2**) with FT was carried out under Koenigs–Knorr conditions (HgO, $HgBr_2$, MS3A, benzene, reflux 5 h) using **3**[2] (1.2 mol equiv for **2**) and the resulting **4** and the 5,6-diFT derivative (**5**), after separation by preparative TLC, were deacetylated (MeONa/MeOH, THF, rt), respectively, to give a red solid of **6** (54% based on **2**) and a yellow solid of diFT-Alz **7** (6%). No 6-FT-Alz analogue was produced. Compound **6** was reprecipitated from a solution of $CHCl_3$–MeOH (2:1) by gradual addition of isopropyl ether, mp 167–169.5 °C, $[\alpha]_D^{24}$ +10° (c 0.1, pyridine). The structure of **6** was determined by the NMR spectra;[8] the position of FT attached to **2** was decided by a signal of phenolic O*H*, δ 13.1 (br), the low-field shift indicating the hydrogen bonding between O*H*-6 and =O-7. The structure of **6** was further confirmed by X-ray crystallography [the crystal used was prepared from a $CHCl_3$–MeOH (2:1) solution by gradual evaporation (3 days) of the solvents].

Antitumor Activity

Antitumor activities of **6** and **7** were examined in vitro (Table 1), which show that **6** is similarly or more active than doxorubicin (DOX) against the cell lines tested, except for the lines of leukemia (K562, P388, and L1210), but **7** and alizarine blue itself were practically devoid of activity, indicating that 5-*O*-glycosylation is necessary to produce appreciable antitumor activity. In an acute toxicity test, **6** showed approximately 10 times less toxicity than DOX (mice, ip administration).

To investigate the structure–activity relationships, 6-*O*-methyl (**8**) and 1,2,3,4-tetrahydro (**9**) derivatives of FT-Alz were prepared. Methylation of **4** (CH_3I, Ag_2O, CH_3CN) followed by deacetylation gave **8** (^1H NMR in $CDCl_3$, δ 4.09 s, OCH_3); catalytic reduction of **4** (H_2/PtO_2, dioxane) followed by deacetylation (NaOMe/MeOH) of the products gave **9** through air oxidation of the resulting hydroquinone intermediates. The data for ^1H, ^{19}F, and ^{13}C NMR spectroscopy of **9** fully support the structure.[9] Both **8** and **9**, however, showed almost no detectable antitumor activity. This indicates, together with the result of **7** (Table 1), that the free HO-6 group and aromaticity of the molecule are requisite in order to show antitumor activity.

The next problem to be solved was "will **6** be really a new-type anticancer agent?" To approach the problem, we consulted the Japanese Foundation for Cancer Research (JFCR, headed by Professor Takao Yamori)



2 3 4 5 6 7 8 9 10

Table 1. Growth inhibitory concentrations (IC_{50},[a] μM; mean values of duplicate measurements) of FT-Alz (**6**), diFT-Alz (**7**) and alizarine blue (**2**) in comparison with doxorubicin (DOX) on various cell lines in vitro

	MKN-1	PC-14	T24	KB	HMV-1	K562	P388	P388/DOX	L1210
6	0.73	0.71	0.36	0.27	0.59	0.71	0.77	0.82	0.32
7	>8.5	>8.5	>8.5	>8.5	7.5	5.8	>8.5	>8.5	>8.5
2	14	>17	14	3.0	4.1	7.2	6.9	3.3	7.2
DOX[b]	0.64	1.9	0.64	0.22	0.28	0.12	0.02	>0.9	0.05

[a]IC_{50} values (50% inhibition concentration) were determined by MTT method on day-3 cell culture.
[b]Hydrochloride. Abbreviations: MKN-1 human gastric adenocarcinoma, PC-14 human lung carcinoma, T24 human bladder carcinoma, KB human nasopharyngeal carcinoma, HMV-1 human melanoma, K562 human leukemia, P388 murine leukemia, P388/DOX DOX-resistant P388, L1210 murine leukemia.

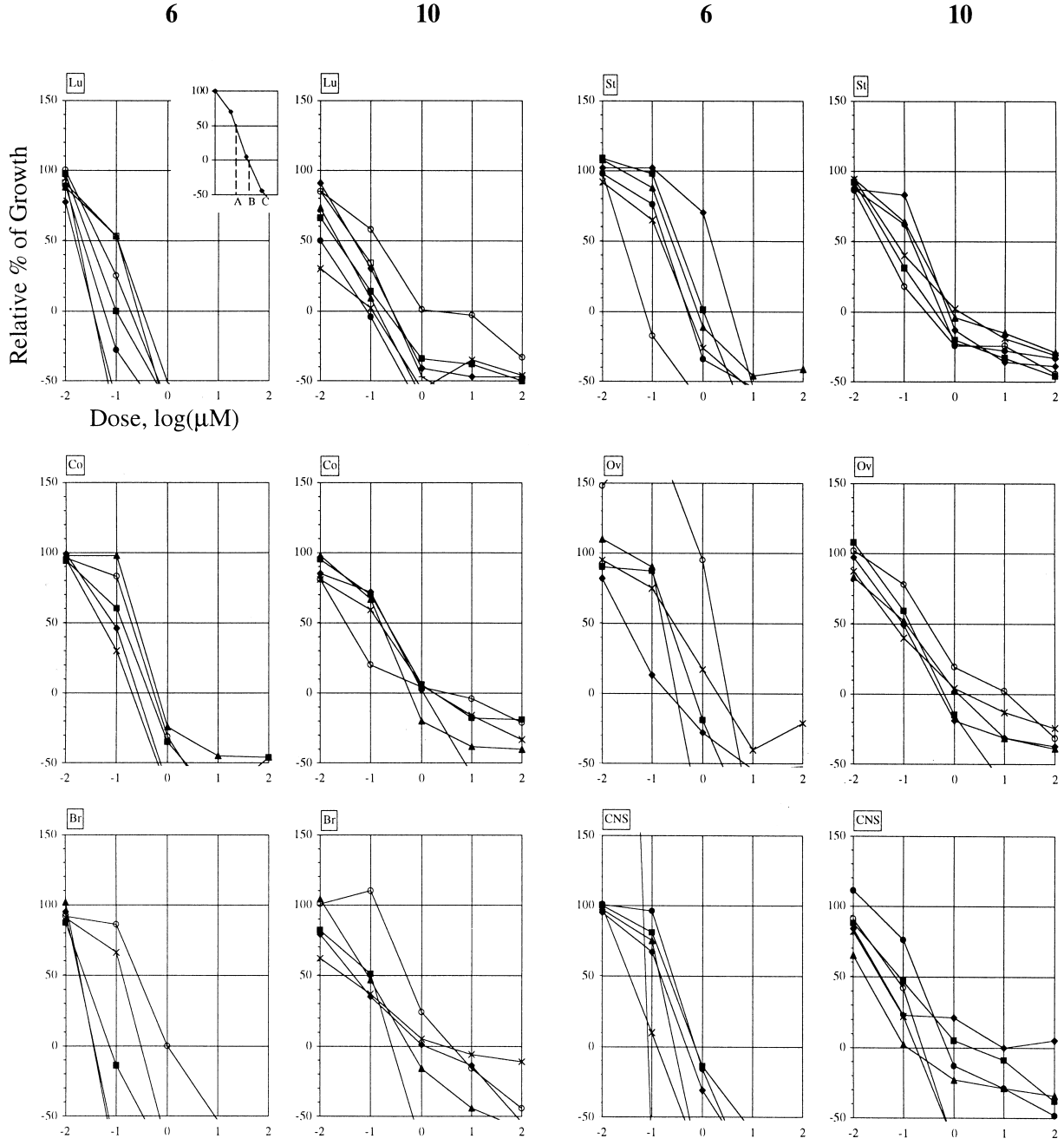


Figure 1. Dose response curve (done by JFCR) of **6** (1st and 3rd columns) and **10** (2nd and 4th columns) for six kinds of cancer classified by organs: lung (Lu, 7 cell lines), stomach (St, 6), colon (Co, 5), ovary (Ov, 5), breast (Br, 5), and central nervous system (CNS, 6), the cell lines in each organ being cited by different marks. The top-left figure illustrates the positions of GI_{50} (A), TGI (B), and LC_{50} (C).

Table 2. LC_{50} values[a] [log(μM)] of **6** and **10** classified by the effectiveness for the cancers of different organs

Cancers from: (number of cell lines)	**6**			**10**		
	< 0	0∼1	1 <	< 0	0∼1	1 <
Lung (7)	7			3		4
Stomach (6)	1	4	1			6
Colon (5)	2	2	1		1	4
Ovary (5)	1	3	1		1	4
Breast (5)	4	1		1		4
Kidney (2)	1	1				2
Central nervous system (6)	3	3		2		4
Prostate (2)	1	1				2
Melanoma (1)	1					1

[a]See Figure 1.

to carry out anticancer drug-screening tests using a diverse panel of cultured human tumor cell lines,[10] the system being based on a pioneering project in a drug-discovery screening system promoted by the National Cancer Institute[11] in USA. The JFCR system adopts 39 human tumor cell lines comprising the cancers of lung (7 cell lines), stomach (6), colon (5), ovary (5), breast (5), kidney (2), central nervous system (6), prostate (2), and melanoma (1), chosen to reflect the current human cancer state in Japan. Compound **6** was therefore subjected to this system together with a doxorubicin analogue having an FT in the molecule, 7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)adriamycinone[1,2] (**10**).

At first, cell growths (measured by cell population density) of the 39 cell lines at the 48 h incubation time (t) were measured in the presence or absence of drugs (**6** or **10**) in various concentrations (10^{-2}–10^{2} μM, in five 10-fold steps) using special panels having incubation wells, designed to coincide with the special screening protocol[12] to gain GI_{50}[13] [the drug concentration to give 50% growth inhibition at time t (drug is added at time zero t_0) compared to the drug-free control at time t], TGI[13] [the drug concentration resulting in total growth inhibition at time t, where cell density (T) is equal to that (T_0) at t_0], and LC_{50}[13] values [the drug concentration to give 50% cell kill at time t relative to the cell density (T_0) at time t_0] (see the dose response curves, Fig. 1). At this stage it was, **6** exhibits remarkably stronger antitumor activity than that of **10** (compound **10** showed, in turn, slightly better activity than DOX[14]), as indicated in Table 2. A characteristic feature directly drawn from the above response curves (Fig. 1) would be that **6** shows, generally, relatively gentle and steep declivities at low (−2–−1) and high (−1–1) concentrations, respectively, compared to those for **10**; in relation to this, it is worthy of note that two cell lines of Ov and CNS showed strong growth enhancement at −1 (190%) and −2 (900%), respectively. After determining the 39 GI_{50} values (also TGI and LC_{50} values), each mean value (in log scale) for these values was calculated, and the deviations of the respective GI_{50} values from the mean GI_{50} value were then bar-graphed,[10,12] the graph being called a "finger-print" for the compound. Substances showing similar finger-prints have been shown to give a similar mechanism of action. The deviation data, together with others, are the basis, by use of the "COMPARE" algorithm,[11,12] to find the compound to give the most (and the second most) similar finger-print pattern to the test compounds (**6** and **10**) among the authorized antitumor substances (more than 100) previously inputted in the program. In our cases, **6** was concluded to resemble mitomycin C with a correlation coefficient (r) of 0.62, and carboquone (r 0.57), both known as DNA alkylating agents; and **10** resembled peplomycin (r 0.84), daunorubicin (r 0.83), and bleomycin (r 0.82), all known as DNA strand-breaking agents. The results for **10** can be understood from the viewpoint of its chemical structure. In contrast, the conclusion for **6** is quite unexpected; this fact, however, may indicate that **6** is a new-type antitumor agent, because it is hard to consider that **6** acts as a usual alkylating agent, judging from its chemical structure and low acute toxicity.

Other Biological Activities

The effect of **6** for multidrug resistance (MDR) was examined. MDR human ovarian carcinoma cell line 2780^{AD} expressing P-glycoprotein multidrug-transporter was cultured in the presence of vincristine (V_c) with or without (control) verapamil[15] (V_p) or **6**, and the cellular accumulations of V_c were measured after 72 h;[16] V_p is known to reduce the efflux of antitumor drugs from MDR cells mediated by P-glycoprotein. In 20 μM concentration of **6** (or V_p), a 160% (560% for control) accumulation of V_c compared to that by V_p (350% for control) was observed. It is noteworthy that **6** shows both strong antitumor and MDR-reversing activities.

The inhibitory effect of **6** to topoisomerase II was assayed based on the ATP-dependent decatenation of kinetoplast DNA in trypanosomatid cells by use of human topoisomerase IIα,[17] essentially according to Marini et al.,[18] using ICRF-193[19] as the positive reference. The 50% inhibitory concentration (IC_{50}) of **6** was shown to be comparative with that of ICRF-193 (IC_{50}: 5 μM),[20] indicating that **6** has strong topoisomerase II-inhibiting ability.

In an apoptosis-inducing test using human monocyte leukemia U937 (3×10^5 cells mL^{-1} in each well), **6** showed large morphological change as well as DNA fragmentation in the concentration of 0.2 μM after 24 h and 2 μM after 4 h (**2** showed the activity at 35 μM after 24 h, and **8** did not).

Other biological effects of **6**, including inhibition of protein kinases, tubulin polymerization, angiogenesis, and metastasis by cancer cells (checked by invasiveness of human fibrosarcoma HT1080 into basement membrane) were all negative.

In summary, the following facts are concluded: (1) FT-Alz (**6**), the structure being imaginatively created, exhibits high and characteristic antitumor activity together with the characters for reversing multidrug resistance by P-glycoprotein, inhibiting topoisomerase II, and inducing apoptosis; (2) the modes of antitumor actions of **6** and **10** with the same sugar portion are considerably

different from each other, which indicates again the importance of latent biological activity of aglycons in glycosides; (3) compound **6** may not be recognized as a useful substance if screened on a classical assay using P388 (or L1210) cells.

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