

Communications to the Editor

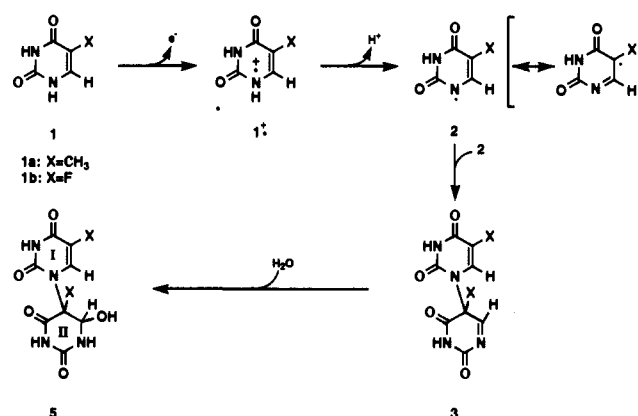
1-(5'-Fluoro-6'-hydroxy-5',6'-dihydrouracil-5'-yl)-5-fluorouracil, a Novel N(1)-C(5)-Linked Dimer That Releases 5-Fluorouracil by Radiation Activation under Hypoxic Conditions

One of the strategies for reducing local failure in radiotherapy of tumors is the use of chemical agents which increase the killing or growth inhibitory effect on hypoxic tumor cells in conjunction with irradiation. Major attention has been hitherto paid to electron-affinic radiosensitizers which provide an oxygen mimetic effect in enhancing radiosensitivity of only hypoxic cells.^{1,2} As a different class of radiation-dose modification, an interesting target would be to develop chemical agents that can be radiation-activated to release a well-specified antitumor drug within hypoxic tumor cells.

We report herein the isolation and characterization of N(1)-C(5)-linked dimers (**5a,b**) from galvanostatic electrolyzed aqueous solution of thymine (**1a**) or 5-fluorouracil (**1b**). The release of a typical antitumor drug (**1b**) from **5b** was observed upon γ -irradiation of oxygen-free aqueous solution. The in vivo tumor-growth-delay assay identified the potential of **5b** as a radiation-dose-modifying prodrug.

Chemistry. Galvanostatic electrolysis (5 mA) of an aqueous solution of **1a** (1 mM, 100 mL) containing NaCl (5 mM) was performed under Ar-bubbling in a one-compartment glass cell (4 cm in diameter, 11 cm high) with Pt electrodes (14 cm² in area, 1.6 cm distant), resulting in 97.0% conversion of **1a** after 5 h. HPLC analysis³ indicated that the N(1)-C(5)-linked dimer 1-(6'-hydroxy-5',6'-dihydrothymine-5'-yl)thymine (**5a**)⁴ was the major product (the yield based on the consumed **1a** at 1-h intervals was 66.0 \pm 1.1%), accompanied by an N(1)-C(6)-linked dimer 1-(5'-hydroxy-5',6'-dihydrothymine-6'-yl)thymine (**6a**; 15.8 \pm 0.6%).⁵ Minor products involved

Scheme I



thymine glycol (**7**; 0.9 \pm 0.2%), 5-(hydroxymethyl)uracil (**8**; 4.0 \pm 0.6%), N¹-formyl-N²-pyruvylurea (**9**; 4.6 \pm 0.3%), and 5,6-dihydrothymine (**10**; 9.1 \pm 0.3%).

Upon electrolysis with a two-compartment cell, **5a** was obtained in the anode cell but not in the cathode cell. Using NaNO₃ as a supporting electrolyte, **5a** was also produced with similar selectivity. The dimers are not formed by hydroxyl (\cdot OH) radicals, as confirmed previously in the γ -radiolysis of **1a** in N₂O-saturated aqueous solution.⁶ Similar electrolyses of 1-methylthymine (**1c**) and thymidine (**1d**) without a dissociative proton at N(1) gave no dimeric products.

The dimerization of **1a** may be rationalized by the reaction pathways outlined in Scheme I. The initial step involves anodic one-electron oxidation of **1a** to thymine cation radical (**1a**⁺). Deprotonation of **1a**⁺ at N(1) occurs to form an allyl-type N(1)-centered radical (**2a**), followed by head-to-tail combination of **2a** into an isopyrimidine (**3a**).⁷ The isopyrimidine undergoes hydration⁸ to give **5a**. The higher yield of **5a**, compared with that of the reported photosensitized reaction,⁹ may arise from enhanced bimolecular encounter of radical **2a** in the vicinity of the anode. An alternative pathway for the byproduct **6a** involves addition of **2a** across the C(5)-C(6) double bond of **1a** to give C(5) and C(6) radicals [**4a**(C(5)), **4a**(C(6))].

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(2) Brown, J. M.; Yu, N. Y.; Brown, D. M.; Lee, W. W. SR-2508: A 2-Nitroimidazole Amide which Should Be Superior to Misonidazole as a Radiosensitizer for Clinical Use. *Int. J. Radiat. Oncol. Biol. Phys.* 1981, 7, 695-703.

(3) The analysis was carried out on an ODS-type column and the phosphate buffer solution (pH 3.0) containing 3 vol % methanol was delivered at a flow rate of 0.6 mL min⁻¹. The products were monitored by UV absorbance at 210 nm.

(4) For **5a**: mp >234 °C dec; IR (KBr) 3472, 1710, 1650, 1310, 1100 cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.72 (s, 3 H), 1.77 (s, 3 H), 4.79 (t, 1 H, *J* = 6.27, 4.27 Hz), 6.32 (d, 1 H, *J* = 6.40 Hz), 7.41 (s, 1 H), 8.33 (d, 1 H, *J* = 4.12 Hz), 10.50 (s, 1 H), 11.25 (s, 1 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 12.11 (CH₃, I), 22.68 (CH₃, II), 63.35 (C(5), II), 77.90 (C(6), II), 108.53 (C(5), I), 139.28 (C(6), I), 151.44 (C(2), I), 151.86 (C(2), II), 164.28 (C(4), I), 169.15 (C(4), II); MS *m/e* 269 (M + 1). Anal. Calcd for C₁₀H₁₂N₄O₅·2H₂O: C, 39.48; H, 5.30; N, 18.41. Found: C, 39.42, H, 5.12; N, 18.55. The structure of **5a** is identical with the one suggested previously for the quinone-sensitized photoreaction (see ref 9).

(5) For **6a**: ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.52 (s, 3 H), 1.82 (s, 3 H), 5.36 (d, 1 H, *J* = 5.86 Hz), 6.62 (d, 1 H, *J* = 5.87 Hz), 7.55 (s, 1 H), 7.88 (s, 1 H), 10.33 (s, 1 H), 11.45 (s, 1 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 12.36 (CH₃, I), 15.55 (CH₃, II), 64.97 (C(5), II), 85.61 (C(6), II), 108.84 (C(5), I), 138.30 (C(6), I), 150.82 (C(2), I), 151.29 (C(2), II), 163.87 (C(4), I), 169.97 (C(4), II). Anal. Calcd for C₁₀H₁₂N₄O₅: C, 44.78; H, 4.51; N, 20.89. Found: C, 43.72; H, 4.37; N, 20.51 (see also ref 9).

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Table I. γ -Radiolysis of 5b (1 mM) To Release 1b in Aqueous Solution (pH 7.0) under Various Conditions

conditions ^a	active species				decomposition: G(-5b)	release: ^b G(1b)
	G(*OH)	G(e ⁻ _{aq})	G(*H)	G(CO ₂ ⁻)		
air	2.7	0 ^c	0 ^d	0	5.7	0.30 (5%)
Ar	2.7	2.7	0.55	0	4.5	0.72 (16%)
N ₂ O	5.4	0	0.55	0	4.1	0 (0%)
Ar + HCOONa	0	2.7	0	3.25	5.2	1.6 (31%)
N ₂ O + HCOONa	0	0	0	5.95	3.4	1.2 (35%)

^a Solution of 5b in triply distilled water, in the absence or presence of HCOONa (100 mM), was purged with Ar or N₂O for 20 min, except under aerated conditions. ^b The value in the parenthesis is the selectivity of 1b release ($G(1b) \times 100/G(-5b)$). ^c Scavenged by O₂ to yield superoxide radical anions (O₂⁻). ^d Scavenged by O₂ to yield hydroperoxyl radicals (HO₂[·]), which are in equilibrium with O₂⁻.

Successive anodic oxidation of 4a at the anode-water interface would produce C(5) and C(6) cations [4a⁺(C(5)), 4a⁺(C(6))], which undergo attack of water to yield 5a and 6a, respectively.

Characteristics of 5b. The present electrochemical method was effective in synthesizing a novel N(1)-C(5)-linked dimer of 5-fluorouracil (1b). On galvanostatic electrolysis (10 mA) in aqueous solution (100 mL, 9 mM NaCl) in air, 87.6% of 1b was converted over 2.5 h to produce a dimer 1-(5'-fluoro-6'-hydroxy-5',6'-dihydrouracil-5'-yl)-5-fluorouracil (5b; 69.9 ± 1.6% at 0.5-h intervals).¹⁰ No N(1)-C(6) linked dimer analogous to 6a was detectable in this electrolysis. For isolation, an aqueous solution of 70 mM 1b (100 mL; 18 mM NaCl) was also electrolyzed at 300 mA for 7 h and evaporated. The residue was dissolved in ice-cooled water (20 mL) and the insoluble solid was repeatedly recrystallized from methanol/water (1:2 v/v) to give 5b as colorless prismatic crystals.

Table I shows the G-values¹¹ for decomposition of 5b and release of 1b in the γ -radiolysis of aqueous solution (pH 7.0) under various conditions. The radiation-activated release of 1b favored oxygen-free conditions in Ar, showing 3 times higher selectivity (16%) over aerated conditions. Furthermore, the reducing species of hydrated electrons (e⁻_{aq}) and carbon dioxide radical anions (CO₂⁻) enhanced the 1b release with 31-35% selectivity, while the oxidizing *OH radicals induced no such a release. Hydrolysis of 5b into 1b did not occur at pH < 8.0. Thus, radiolytic one-electron reduction of 5b occurring more efficiently under oxygen-free conditions accounts for the 1b release. The primary active species produced by radiolysis of intracellular water in hypoxic cells may be similar to those in Ar-saturated aqueous solution, suggesting the potential of 5b as a prodrug that can be activated in the radiotherapy of hypoxic tumors.

A growth-delay assay of 5b was performed using C3H/He mice (female, 8-10-weeks-old, 19-22 g, n = 8) bearing SCCVII tumors (1 cm in mean diameter) in the thigh. The tumor-volume-doubling times were 3.2 days for controls, 3.3 days for 5b (50 mg/kg of body weight

injected iv in the tail vein) alone, 8.0 days for 20-Gy radiation (10 MV X-rays at 5.6 Gy/min) alone, 9.2 days for 1b (50 mg/kg iv) alone, 10.6 days for 20-Gy radiation combined with 5b (50 mg/kg iv 20 min before irradiation), 13.1 days for 20-Gy radiation combined with 1b (50 mg/kg iv 20 min before irradiation), and 13.8 days for 30-Gy radiation alone. Evidently, 5b has no antitumor effect in contrast to 1b, but can potentiate the radiotherapy to inhibit the tumor growth. This effect is presumably attributed to the radiation-activated release of 1b.

Further synthesis and assay of related pyrimidine dimers that release 1b as an antitumor drug component are in progress and will be reported in due course.

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3-[4-[1-(6-Fluorobenz[o]thiophen-3-yl)-4-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone: A New Atypical Antipsychotic Agent for the Treatment of Schizophrenia

The majority of clinically-effective antipsychotic agents in use today exhibit some propensity for the development of extrapyramidal side effects, either acutely (i.e., dystonia, akathisia, pseudo-Parkinsonism) or with a delayed onset (tardive dyskinesia). Clozapine has been classified as an "atypical" neuroleptic agent because this compound is almost devoid of extrapyramidal side-effect liability. However, clozapine demonstrates adverse hematological effects which require selective targeting and careful monitoring of patient populations.¹

In a continuing program to discover and develop new safe antipsychotic agents with an atypical pharmacological

(10) For 5b: mp >190 °C dec; IR (KBr) 3300, 1720, 1675, 1280, 1140 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 5.36 (ddd, 1 H, J = 4.43, 4.42, 4.73 Hz), 7.15 (d, 1 H, J = 4.73 Hz), 8.18 (d, 1 H, J = 6.56 Hz), 8.49 (broad, 1 H), 10.67 (broad, 1 H), 12.21 (broad, 1 H); ¹³C NMR (100 MHz, DMSO-d₆) δ 74.31 (C(6), II, J = 29.34 Hz), 93.80 (C(5), II, J = 220.82 Hz), 124.78 (C(6), I, J = 5.13, 31.55, 5.13 Hz), 140.80 (C(5), I, J = 234.75 Hz), 148.22 (C(2), I), 151.79 (C(2), II), 156.70 (C(4), I, J = 26.41 Hz), 161.71 (C(4), II, J = 24.21 Hz); ¹⁹F NMR (300 MHz, DMSO-d₆, TFA) δ 68.26 (C(5)-F, II), 86.30 (C(5)-F, I, J = 7.10 Hz); MS m/e 277 (M + 1). Anal. Calcd for C₈H₈N₄O₅F₂: C, 34.79; H, 2.19; N, 20.29; F, 13.76. Found: C, 35.03; H, 2.26; N, 20.31; F, 13.76.

(11) The number of molecules produced or changed per 100 eV of energy absorbed by the reaction system.

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