Communications to the Editor

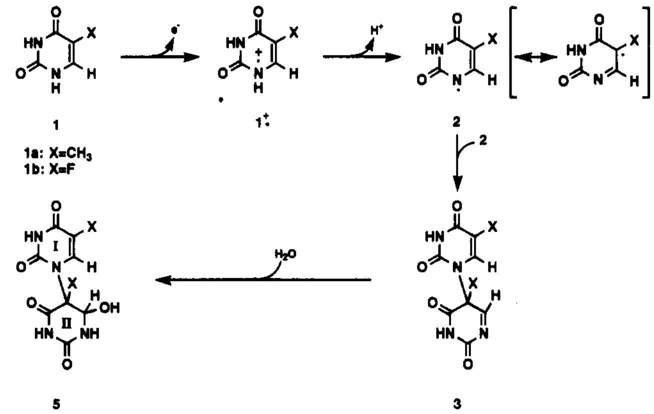
1-(5′-Fluoro-6′-hydroxy-5′,6′-dihydrouracil-5′-yl)-5-fluorouracil, a Novel N(1)–C(5)-Linked Dimer That Releases 5-Fluorouracil by Radiation Activation under Hypoxic Conditions

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 cm^2 in area, 1.6 cm distant), resulting in 97.0% conversion of **1a** after 5 h. HPLC analysis[3] indicated that the N(1)–C(5)-linked dimer 1-(6′-hydroxy-5′,6′-dihydrothymin-5′-yl)thymine (**5a**)[4] was the major product (the yield based on the consumed **1a** at 1-h intervals was 66.0 ± 1.1%), accompanied by an N(1)–C(6)-linked dimer 1-(5′-hydroxy-5′,6′-dihydrothymin-6′-yl)thymine (**6a**; 15.8 ± 0.6%).[5] Minor products involved thymine glycol (**7**; 0.9 ± 0.2%), 5-(hydroxymethyl)uracil (**8**; 4.0 ± 0.6%), N^1-formyl-N^2-pyruvylurea (**9**; 4.6 ± 0.3%), and 5,6-dihydrothymine (**10**; 9.1 ± 0.3%).

Scheme I



Upon electrolysis with a two-compartment cell, **5a** was obtained in the anode cell but not in the cathode cell. Using $NaNO_3$ as a supporting electrolyte, **5a** was also produced with similar selectivity. The dimers are not formed by hydroxyl (•OH) radicals, as confirmed previously in the γ-radiolysis of **1a** in N_2O-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 hydration[8] 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))].

(1) Adams, G. E.; Clarke, E. D.; Flockhart, I. R.; Jacobs, R. S.; Sehmi, D. S.; Straford, I. J.; Wardman, P.; Watts, M. E.; Parrick, J.; Wallace, R. G.; Smithen, C. E. Structure–activity Relationships in the Development of Hypoxic Cell Radiosensitizers. I. Sensitization Efficiency. *Int. J. Radiat. Biol.* **1979**, *35*, 133–150.

(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); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.11 (CH_3, I), 22.68 (CH_3, 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 $C_{10}H_{12}N_4O_5 \cdot 2H_2O$: 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); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.36 (CH_3, I), 15.55 (CH_3, 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 $C_{10}H_{12}N_4O_5$: 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: $G(^{\bullet}OH)$	$G(e^{-}_{aq})$	$G(^{\bullet}H)$	$G(CO_2^{\bullet-})$	decomposition: $G(-\mathbf{5b})$	release:[b] $G(\mathbf{1b})$
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_2O	5.4	0	0.55	0	4.1	0 (0%)
Ar + HCOONa	0	2.7	0	3.25	5.2	1.6 (31%)
N_2O + 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 N_2O for 20 min, except under aerated conditions. [b] The value in the parenthesis is the selectivity of **1b** release ($G(\mathbf{1b}) \times 100/G(-\mathbf{5b})$). [c] Scavenged by O_2 to yield superoxide radical anions ($O_2^{\bullet-}$). [d] Scavenged by O_2 to yield hydroperoxyl radicals ($HO_2^{\bullet}$), which are in equilibrium with $O_2^{\bullet-}$.

Successive anodic oxidation of **4a** at the anode–water interface would produce C(5) and C(6) cations [$\mathbf{4a}^+$(C(5)), $\mathbf{4a}^+$(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) of **1b** (1 mM) 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*-values[11] 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 ($CO_2^{\bullet-}$) enhanced the **1b** release with 31–35% selectivity, while the oxidizing $^{\bullet}$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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(10) For **5b**: mp >190 °C dec; IR (KBr) 3300, 1720, 1675, 1280, 1140 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 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); ^{13}C NMR (100 MHz, DMSO-d_6) 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); ^{19}F NMR (300 MHz, DMSO-d_6, 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 $C_8H_6N_4O_5F_2$: 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.

3-[4-[1-(6-Fluorobenzo[*b*]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

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