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Design, synthesis, and anticancer activities of novel perfluoroalkyltriazole-appended 2′-deoxyuridines

Sun Min Park [a], Heeju Yang [a], Song-Kyu Park [b], Hwan Mook Kim [b], Byeang Hyean Kim [a,*]

[a] Department of Chemistry, BK School of Molecular Science, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Republic of Korea
[b] Bio-Evaluation Center, KRIBB, 685-1 Ochang, Cheongwon, Chungbuk 363-883, Republic of Korea

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ABSTRACT

We have focused on the C5-modification of 2′-deoxyuridine with substituted heterocycles for bioactivity, such as antiviral or anticancer activity. Herein, we report a novel class of nucleoside analogues with perfluoroalkyltriazole moiety as an anticancer drug candidate.

Cancer is one of the leading causes of death in developing and developed countries. It is estimated that cancer led to more than 7.6 million people losing their lives in 2007. General methods for the treatment of cancer include surgery, chemotherapy, and radiotherapy—often used in combination for better effect.

Nucleoside analogues comprise a significant class of anticancer agents; many of them are halogenated nucleosides or nucleobases.[1] Ever since the demonstrated effectiveness of 5-fluorouracil,[2] many fluorinated nucleic acid analogues have been developed, including floxuridine, capecitamine, gemcitabine, and fludarabine (Fig. 1), to improve the bioavailability, metabolic stability, and interactions with enzymes.

The fluorine atom has unique stereoelectronic properties that make it an effective bioisostere for both hydrogen and oxygen atoms. The electronegativity and van der Waals radius of fluorine (4.0, 1.47 Å) are similar to those of oxygen (3.5, 1.57 Å), making it an effective mimic of the hydroxyl groups,[3] even though C–F bonds participate in hydrogen bonds only very weakly. Nevertheless, the presence of fluorine atoms contributes to the electrostatic interactions and improved binding affinity of a substrate in the active site of an enzyme.[4]

In this Letter, we report the synthesis and evaluation of new perfluorinated (i.e., the 1*H*,1*H*,2*H*,2*H*-perfluorobutyl, 1*H*,1*H*,2*H*,-2*H*-perfluorohexyl, 1*H*,1*H*,2*H*,2*H*-perfluorooctyl, 1*H*,1*H*,2*H*,2*H*-perfluorodecyl, and 1*H*,1*H*,2*H*,2*H*-perfluorododecyl-substituted triazole units) nucleosides for use as anticancer drugs. In previous studies,[5,6] we have modified nucleic acids (nucleobases, nucleosides, oligonucleotides) for potential use in biomedical applications—namely as drugs, drug delivery agents, and DNA/RNA probes. In particular, we studied nucleoside-based drugs derived from 2′-deoxyuridine modified with isoxazole[5] and triazole[6] heterocycles for their biological activity. In this study, however, we turned our attention toward fluorinated nucleoside-based anticancer drugs. All of the anticancer drugs developed to date contain fewer than two fluorine atoms, although several results have been obtained for nucleosides appended with relatively short perfluoroalkyl chains. In this study, we introduced long perfluoroalkyl chains via triazole rings at the C5 position of 2′-deoxyuridine. Scheme 1 outlines the syntheses of our target compounds.

The acetylated 2′-deoxyuridine was synthesized in 98% yield from 2′-deoxyuridine, acetic anhydride and pyridine. It was subjected to Sonogashira coupling with trimethylsilylacetylene (TMS-acetylene) in the presence of $Pd(PPh_3)_2Cl_2$ and CuI to give C5-TMS-ethynyl–modified 3′,5′-diacetyl-2′-deoxyuridine in 95% yield.[7] Desilylation was performed using tetrabutylammonium fluoride (TBAF) to provide the key intermediate, C5-ethynyl-3′,5′-diacetyl-2′-deoxyuridine, in 86% yield.[6b] Several perfluoroalkyl azides for use in cycloaddition reactions were prepared through simple substitution reactions. Under the conditions of the Sharpless click reaction,[8] we synthesized a series of perfluoroalkyltriazole-appended acetylated 2′-deoxyuridines. After isolation through using column chromatography, we obtained C5-modified nucleosides featuring seven different perfluoroalkyltriazoles (yields: 37–97%).

* Corresponding author. Tel.: +82 54 279 5856; fax: 82 54 279 3399.
E-mail address: bhkim@postech.ac.kr (B.H. Kim).

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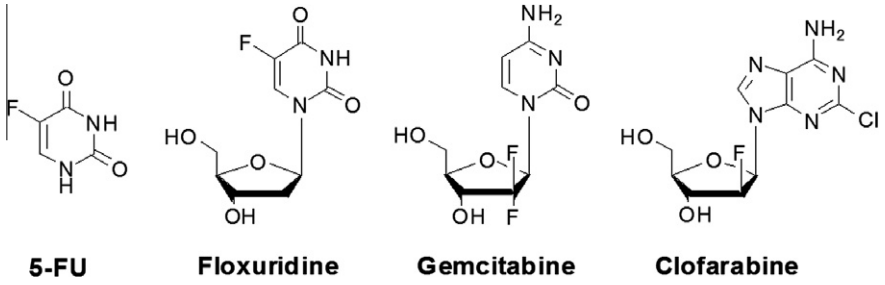
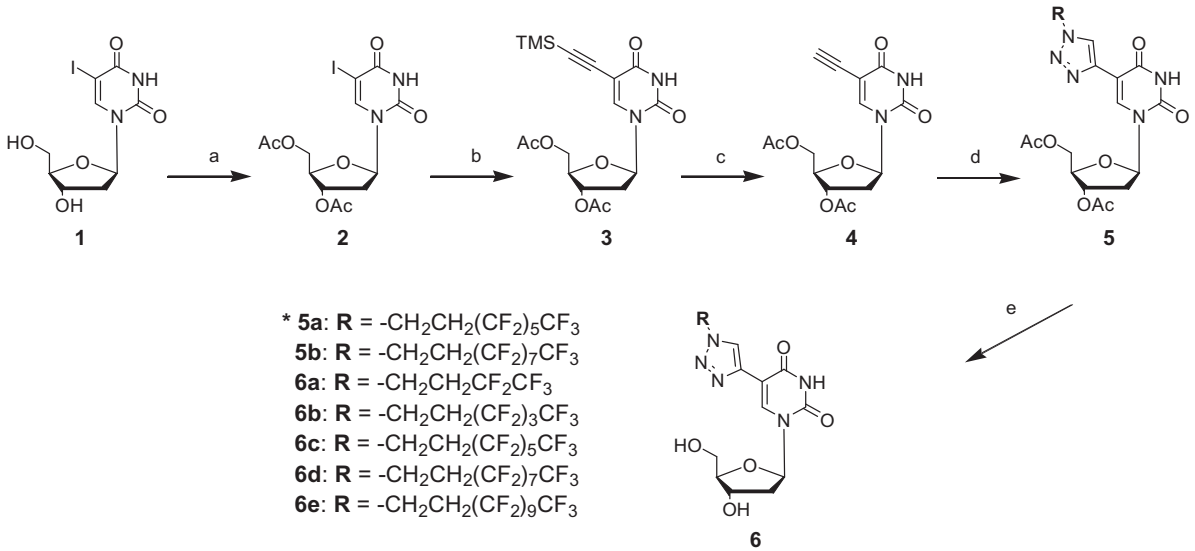


Figure 1. Representative fluoronucleoside-based anticancer drugs.



Scheme 1. Synthesis of 2′-deoxyuridines appended with perfluoroalkyltriazole rings. (a) $(AcO)_2O$, pyridine, rt, 98%; (b) TMS-acetylene, $Pd(PPh_3)_2Cl_2$, CuI, DIPEA, DMF, 50 °C, 95%; (c) TBAF, THF, rt, 86%; (d) 1*H*,1*H*,2*H*,2*H*-perfluoroalkyl azide, $Cu(OAc)_2$, Na-ascorbate, CH_2Cl_2/H_2O, rt, **5a** (87% yield), **5b** (97% yield); (e) LiOH, MeOH/H_2O, rt, **6a** (37% yield), **6b** (58% yield), **6c** (74% yield), **6d** (86% yield), **6e** (82% yield).

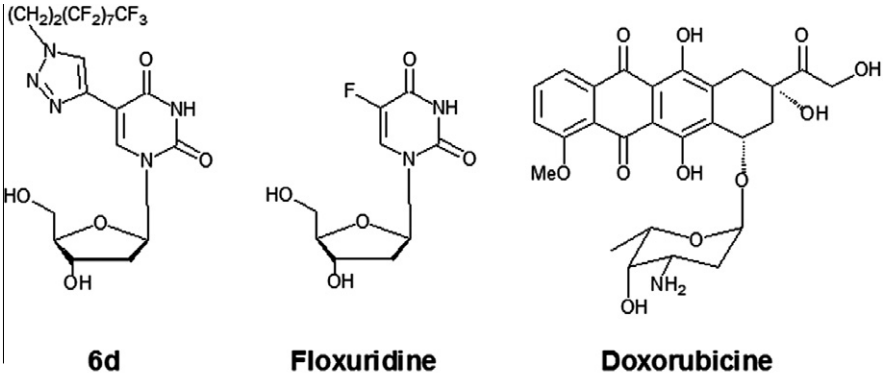
We used the sulforhodamine B screening assay to test the bioactivities of these synthesized perfluoroalkyltriazole-appended nucleosides against three cancer cell lines (PC-3, MDA-MB-231, ACHN) and a normal cell line.[9] Their inhibitory effects on cell growth were evaluated through comparisons with those of floxuridine and doxorubicin, two well-known anticancer drugs. Table 1 lists the anticancer activities of our nucleoside analogues. At first, we tested only the acetyl-free perfluoroalkyltriazole-appended nucleoside analogues (i.e., the 1*H*,1*H*,2*H*,2*H*-perfluorobutyl, 1*H*,1*H*,2*H*,2*H*-perfluorohexyl, 1*H*,1*H*,2*H*,2*H*-perfluorooctyl, 1*H*,1*H*,2*H*,2*H*-perfluorodecyl, and 1*H*,1*H*,2*H*,2*H*-perfluorododecyl-substituted triazole units) for identification of their anticancer activities. Among these five analogues, the most potent was **6d**, which features a perfluorodecyl-substituted triazole unit; it inhibited the cancer cell (PC-3, MDA-MB-231, ACHN) growth rate by more than 80% at a concentration of 10 μM. The difference in the values of GI_{50} (Fig. 2) for the reference drug floxuridine, which contains a fluorine atom, and **6d** was less than twofold (1.6-fold induction). The compounds presenting longer (e.g., 1*H*,1*H*,2*H*,2*H*-perfluorododecyl) or shorter (e.g., perfluorooctyl) perfluoroalkyl chains exhibited highly reduced or no anticancer activity.

Table 1
Cancer growth rates of human tumor cell lines[a]

Compounds	PC-3	MDA-MM-231	ACHN
5a	89.4 ± 2.5	99.4 ± 1.8	113.8 ± 6.9
5b	77.9 ± 3.7	99.1 ± 2.9	110.8 ± 9.9
6a	96.4 ± 6.2	98.7 ± 2.7	96.1 ± 6.2
6b	97.8 ± 3.7	109.7 ± 5.6	116.0 ± 5.1
6c	114.5 ± 2.0	106.5 ± 5.5	99.3 ± 6.0
6d	16.7 ± 1.9	15.9 ± 1.4	14.2 ± 3.5
6e	89.4 ± 7.2	93.7 ± 9.1	90.8 ± 4.7
Floxuridine	40.7 ± 4.6	N.D.	25.8 ± 7.3
Doxorubicin	28.5 ± 2.8	16.5 ± 1.3	12.3 ± 1.9

[a] SRB (sulforhodamine B) assay using doxorubicin as positive control. Human cancer cell lines: PC-3 (prostate), MDA-MB-231 (breast), ACHN (renal). Cell growth inhibition was determined at 10 μM [except for doxorubicin (1 μM)].

For their potential use as prodrugs, we also tested the performance of the acetylated nucleosides presenting 1*H*,1*H*,2*H*,2*H*-perfluorooctyl and 1*H*,1*H*,2*H*,2*H*-perfluorodecyl chains. Typically, acetyl groups increase the rate of cellular delivery by enhancing the lipophilicity of the drugs; they are subsequently cleaved by carboxyesterase within the cell.[10] Our results were, however, surprising; the acetylated analogues (**5a** and **5b**) did not show enhanced anticancer effects, even decreased. In case of 1*H*,1*H*,2*H*,2*H*-perfluorooctyltriazole substitution, the acetylated nucleoside analogue **5a** exhibited slightly better anticancer activity than its acetyl-free analogue **6c**. The most effective acetyl-free compound (**6d**), however, exhibited dramatically decreased or no anticancer activity when modified with acetyl groups (**5b**). The fluorinated nucleoside analogues are already lipophilic themselves; therefore, there may be no effects of acetyl group. These results suggest that the anticancer activities of the fluorine-modified nucleosides correlate not only with the lipophilicity but also with the bulkiness of the substituted groups.



	PC-3	MDA-MB-231	ACHN
6d	7.75	8.67	8.24
Floxuridine	4.97	0.16	2.10
Doxorubicin	0.28	0.18	0.22

Figure 2. Structures and values of GI_{50} (μM) of **6d**, floxuridine, and doxorubicin.

To investigate the effects of the perfluorodecyltriazole unit on cancer growth, we also investigated the behavior of a 2′-deoxyadenosine analogue (not shown here). The acetyl-free 2′-deoxyadenosine analogue exhibited anticancer activity against the prostate (PC-3) and breast (MDA-MB-231) cancer cells, almost comparable with that of **6d**. Therefore, it appears that the perfluorodecyltriazole unit plays an important role in inhibiting cancer cell growth. Although we cannot say for sure about

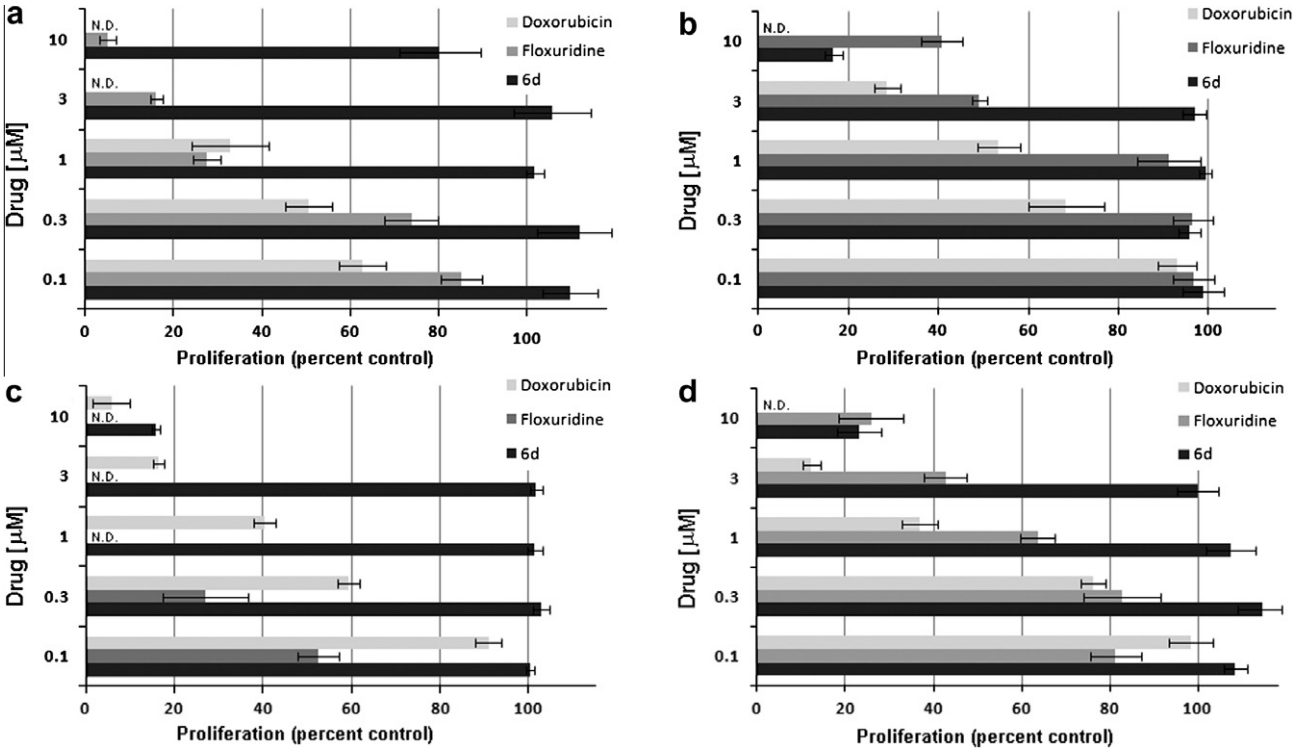


Figure 3. Effects of **6d**, floxuridine, and doxorubicin on the proliferation of (a) normal lung fibroblast cells (CCD-33Lu), (b) prostate cancer cells (PC-3), (c) breast cancer cells (MDA-MB-231), and (d) renal cancer cells (ACHN), which were treated varying concentrations of the drugs (0.1–10 μM) for 48 h.

the mode of action of **6d**, among the several metabolic effects of fluoropyrimidines, the efficacy of compound **6d** might be related to its inhibition of the key enzyme thymidylate synthase (TS).

To determine the selective toxicity of the perfluoroalkyltriazole nucleoside analogues toward cancer cells, we performed a proliferation assay using CCD-33Lu (lung fibroblast normal) cells (Fig. 3).

Interestingly, none of the perfluoroalkyltriazole nucleoside analogues exhibited any toxicity toward normal lung fibroblast cells, even at 10 μM. In contrast, the reference drugs floxuridine and doxorubicin displayed serious toxicities toward normal cells, with values of GI_{50} greater than 100-fold of those of the perfluoroalkyltriazole nucleoside analogues.

In summary, we have investigated perfluoroalkyltriazole-presenting nucleosides as anticancer drug candidates. A 2′-deoxyuridine derivative (**6d**) featuring an appended perfluorodecyl-substituted triazole unit displayed significant anticancer effects against selected cancer cell lines (PC-3, MDA-MB-231, ACHN) and superior cancer cell-selective antiproliferation effects relative to reference drugs (floxuridine and doxorubicin). Although we lack evidence regarding the cancer cell inhibitory mechanism of **6d**, we suspect that it involves interactions with the enzyme TS. To date, most perfluorocarbons have been applied in materials science because of their interesting physical properties. Our findings are meaningful because these perfluoroalkyltriazole-substituted nucleosides are the first to have found potential medicinal applications. We are continuing our search for more effective anticancer drugs based on perfluoroalkyltriazole nucleosides and their mechanisms of action.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.126.

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