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C5-Modified nucleosides exhibiting anticancer activity

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ABSTRACT

We describe (i) a simple method for the synthesis of C5-modified nucleosides from 5-iodo-2′-deoxyuridine and (ii) their activity against six types of human cancer cell lines (HCT15, MM231, NCI-H23, NUGC-3, PC-3, ACHN). We generated nitrile oxides in situ from oximes using a commercial bleaching agent; their cycloadditions with 5-ethynyl-2′-deoxyuridine yielded isoxazole derivatives possessing activity against the cancer cell lines. We synthesized several azides from benzylic bromides and their click reactions with 5-ethynyl-2′-deoxyuridine provided triazole derivatives.

About 13% of all human deaths throughout the world are caused by cancers—diseases characterized by uncontrolled cell growth, metastasis, and invasion.[1] Although the risk of cancer increases with age, people of all ages—even fetuses—can be affected by the disease. The five most fatal cancers are lung, stomach, liver, colon, and breast cancer. Several carcinogens, including tobacco smoke, radiation, chemicals, and infectious agents, cause abnormalities of the genetic material, which can induce the onset of cancer.[1] For example, infections from hepatitis B or C are known to cause liver cancer.[2] Several nucleoside drugs have been developed as cancer treatment agents: cladribine, clofarabine, capecitabine, cytarabine, fludarabine, gemcitabine, decitabine (**1a**), and floxuridine (**1b**).[3] Indeed, nucleoside analogs were among the first oncology drugs ever developed. Using Sonogashira coupling, Dembinski's group discovered that several C5-ethynyl-modified nucleosides (**1c**) exhibit anticancer activity (Fig. 1).[4] We became interested in using such C5-ethynyl nucleosides as partners in [3+2] cycloadditions, which are powerful reactions for the modification of nucleosides and nucleotides as well as for the synthesis of a diverse range of heteroaromatic five-membered rings. Previously, we prepared a small library of isoxazoles that we tested against 12 types of viruses.[5] In this present study we synthesized two scaffolds from a series of C5-ethynyl-2′-deoxyuridines—the isoxazoles **2a** and triazoles **2b**—through reactions with oximes and azides, respectively (Fig. 2). Click reactions have been used widely for the synthesis of modified nucleosides and DNA strands ever since our group first applied them to nucleoside chemistry in 2003.[6] The Sharpless click reaction is a very simple and useful transformation that does not generate any sideproducts or byproducts, unlike other [3+2] cycloadditions.[7] The click reactions that have been applied to modify the C-5 positions of pyrimidine nucleosides have also been used to modify oligonucleotides.[8] Several antiviral drugs, such as BVDU ((*E*)-5-(2-bromovinyl)-2′-deoxyuridine) are C5-modified pyrimidine nucleosides. Although the triazole nucleosides that we synthesized through click chemistry appear to be inactive against viruses, they exhibit no toxicity unlike their isoxazole congeners, which displayed cytotoxicity against several human cell lines. Because the triazole nucleosides readily formed very good hydrogels, we suspect that they might be useful as carrier in drug delivery systems.

We have been exploring the behavior of our C5-modified nucleosides in several biological studies; in this Letter, we describe their anticancer activities. For the cell growth inhibition assays, we tested all of our compounds against six human cancer cell lines of

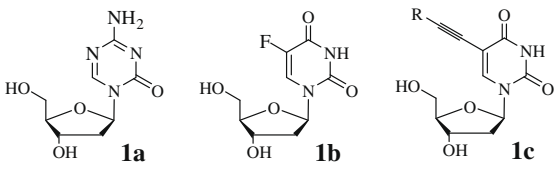

Figure 1. Nucleoside anticancer drugs.

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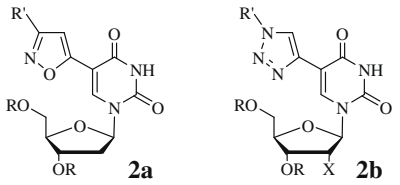


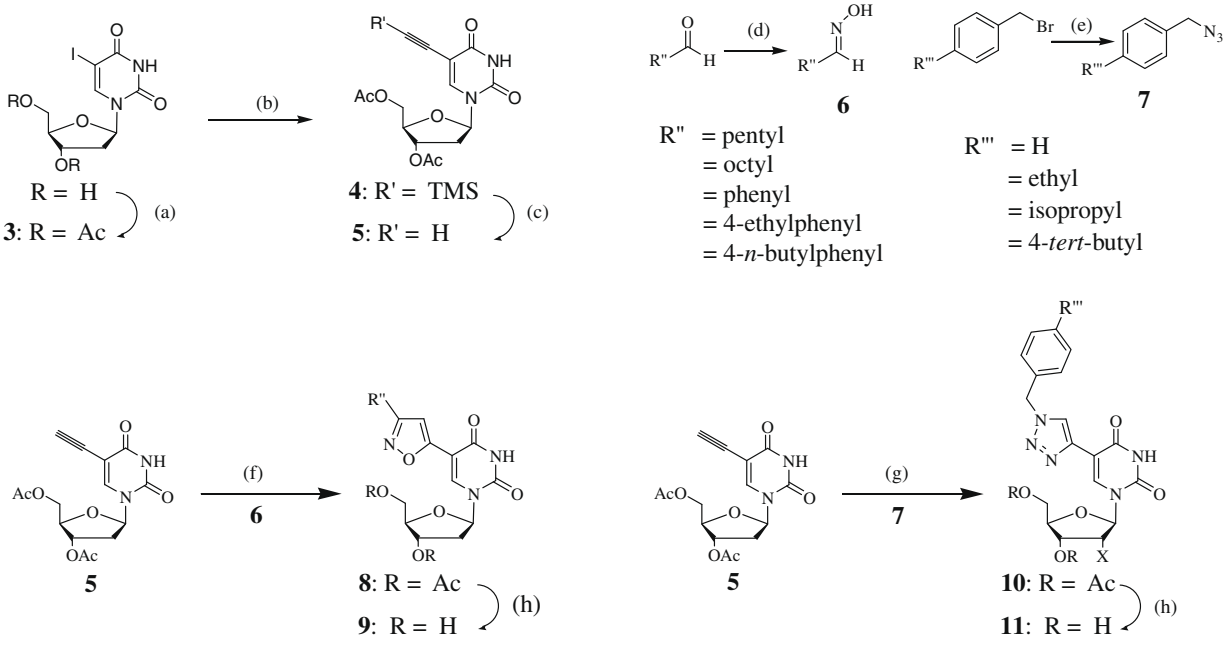
Figure 2. C5-ethynyl, -isoxazole, and -triazole nucleosides.

various origins—HCT15, MM231, NCI-H23, NUGC-3, PC-3, and ACHN—and use the sulforhodamine B screening assay to determine their degrees of cancer growth inhibition. The cells were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum at 37 °C in humidified air containing 5% CO_2. The bioassay protocols have been described previously[9].

The experimental conditions that we used for the cycloadditions leading to the isoxazoles **8b**, **8e**–**g**, and **9b** and the triazoles have been reported previously.[5,10] We prepared the requisite dipolarophile, the acetyl-protected 5-ethynyl-2′-deoxyuridine **5**, through Sonogashira coupling (87% yield) from C5-iodo-2′-deoxyuridine and trimethylsilylacetylene.[11,12] We obtained a series of oximes **6** from the reactions of several aldehydes with hydroxylamine; the azides **7** were acquired from the reactions of benzylic bromides with sodium azide. We isolated the triazole nucleosides after performing Sharpless click reactions of the azides with the dipolarophile **5**, using copper sulfate and sodiumascorbate as catalysts in a mixed solvent of *n*-butanol and water (Scheme 1).[7]

Table 1 lists the anticancer activities of our 16 synthesized compounds[13] (see Fig. 3) against the six types of human cancer cell lines. Three of the acetylated isoxazole nucleosides (**8b**, **8d**, and **8e**) inhibited the cancer growth rate by 50% or more at a concentration of 10 μM. These three compounds, which feature isopropyl-, *n*-butyl-, and *tert*-butyl-substituted phenyl-isoxazoles units, exhibited their highest activities against the NUGC-3 (stomach) and PC-3 (prostate) cancer cell lines. Among these three compounds, **8e** contains the most bulky (*tert*-butyl) group and displayed the greatest anticancer activity. The compounds substituted by smaller R groups—for example, a proton (**8a**) or an ethyl group (**8c**)—displayed dramatically reduced anticancer activities. Compounds **8f** and **8g**, which possess short alkyl group-substituted isoxazole rings, exhibited no anticancer activity at all. In contrast, the pentyl-substituted isoxazole **8h** displayed minimal activity against four of the cancer cell lines (MM231, NCI-H23, NUGC-3, PC-3). The acetyl-free isoxazole nucleosides **9a** and **9b** provided lower activities than any of the acetylated nucleosides **8**. Indeed, the hydrolyzed compound **9a** had only about half the activity of **8e**. Acetyl groups typically increase the lipophilicity of drugs, thereby supporting their cell penetration; subsequent in-cell cleavage of the acetyl groups is mediated by carboxyesterase. Our assays suggest that the anticancer activities of the pyrimidine isoxazole nucleosides increased upon increasing the bulkiness of the nonpolar substituent.

Among our triazole-appended nucleosides, compound **10c** exhibited the highest anticancer activity. Although we observed no relationship between the activity and the size of the substituent on the triazole unit, we found that the acetyl-free triazole nucleosides (e.g., **11**) exhibited slight lower anticancer activities than their acetylated congeners. In general, our triazole nucleosides provided lower anticancer activities relative to those of the isoxazole compounds. On the other hand, the triazole nucleosides exhibited no cytotoxicity at 300 μM concentration (CC_{50} for three human cell lines). The cytotoxicity for three human cell lines (Vero, MT-4, and HeLa) was determined during the antiviral assay. The isoxazole compounds have cytotoxicity (CC_{50}) in the range of 3–300 μM. Therefore we believe that the triazole nucleosides have a better chance as drug candidates. The triazole scaffold contains an extra carbon atom between the triazole ring and phenyl ring. In other words, this benzylic carbon atom changes the relative positions of the five-membered heterocyclic ring and the phenyl ring. Thus, to better understand the structure–activity relationships between the two scaffolds, it will be necessary for us to prepare series of compounds with and without the benzylic carbon atom.



Scheme 1. Synthesis of compounds **3**–**11**. Reagents and conditions: (a) Ac_2O, pyridine, rt, 8 h (92% yield); (b) TMS–acetylene, $Pd(PPh_3)_4$, Et_3N, DMF, 40 °C, 8 h (87% yield); (c) KF, 10% MeOH in CH_2Cl_2 (80% yield); (d) $HONH_2$·HCl, 1 N NaOH, THF (1:1), rt, 6 h (90–99% yield); (e) NaN_3, DMF, 40 °C, 6 h (86–97% yield); (f) 4% NaOCl, THF, slow dropwise addition, rt, 10 h (59–80% yield); (g) $CuSO_4$·$5H_2O$, sodium ascorbate, *n*-BuOH, water, rt, 10 h (71–87% yield); (h) LiOH, MeOH/water (3:1), rt, 10 h (71–90% yield).

Table 1
Cancer growth rates of human tumor cell lines

	HCT15	MM231	NCI-H23	NUGC-3	PC-3	ACHN
8a	92.2	95.0	91.4	87.6	85.5	97.6
8b	80.9	83.2	107.8	56.4	50.3	90.1
8c	92.2	97.4	92.7	72.6	62.8	92.0
8d	71.4	72.2	97.4	39.8	30.4	80.5
8e	53.8	69.3	111.9	32.8	27.5	74.7
8f	101.7	106.1	101.0	90.8	92.4	96.4
8g	98.8	102.0	96.9	89.1	88.1	99.5
8h	98.7	91.5	88.9	91.9	93.9	99.8
9a	88.2	96.7	119.7	76.4	77.3	95.6
9b	79.8	102.2	98.9	88.0	87.9	94.2
10a	92.3	102.3	98.2	88.7	92.1	99.0
10b	94.2	104.6	105.6	94.2	90.4	97.2
10c	88.0	97.7	77.2	94.3	91.2	98.4
10d	96.5	98.1	86.7	95.4	91.3	92.2
11a	88.3	102.8	97.5	95.6	92.1	98.7
11b	96.9	105.6	95.0	94.8	92.3	101.7
Doxorubicin GI_{50} in (μM)	1.64	0.61	0.51	0.25	0.73	0.41

SRB (sulforhodamine B) assay using adriamycin as a positive control. Human cancer cell lines: ACHN (renal), NCI-H23 (lung), MDA-MB-231 (breast), HCT-15 (colon), NUGC-3 (stomach), and PC-3 (prostate). The cell growth inhibition assay was determined at 10 μg/mL. Doxorubicin was used as reference drug.

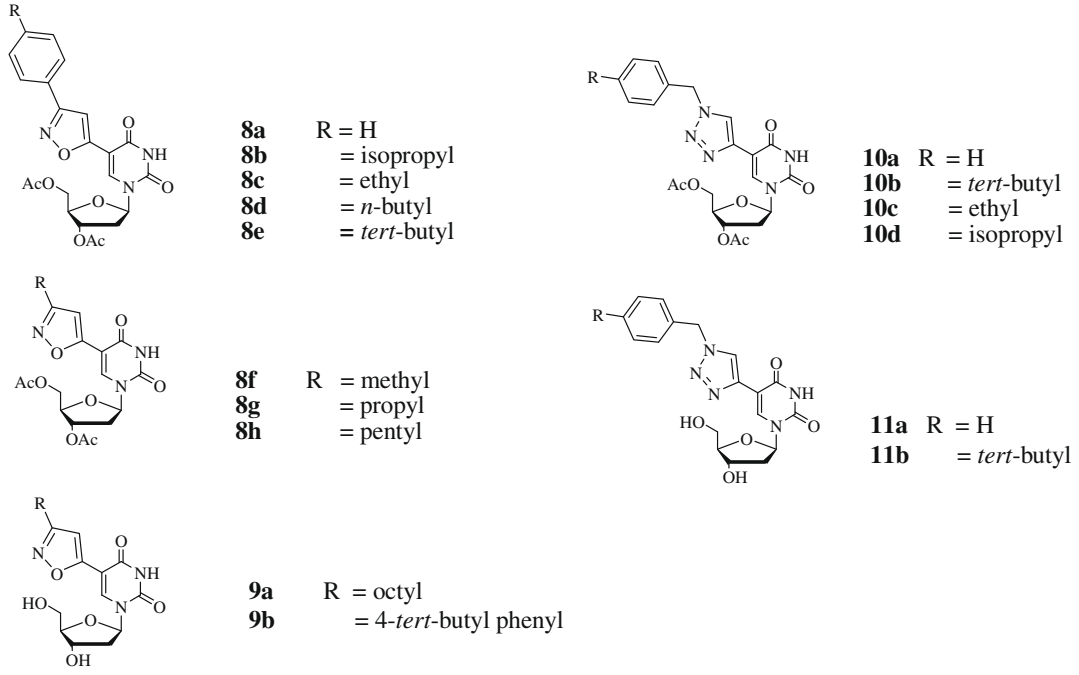


Figure 3. Isoxazole (**8** and **9**) and triazole nucleosides (**10** and **11**) tested for their anticancer properties against six types of cancer cell lines.

Acknowledgment

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References and notes

1. (a) For statistical information about cancer, see: World Health Organisation http://www.who.int/mediacentre/factsheets/fs297/en/; (b) Cancer Research UK http://info.cancerresearchuk.org/cancerstats/incidence/age/.
2. Duelli, D.; Lazebnik, Y. *Nat. Rev. Cancer* **2007**, *7*, 968.
3. (a) Lauria, F.; Benfenati, D.; Raspadori, D.; Rondelli, D.; Zinzani, P. L.; Tura, S. *Leuk. Lymphoma* **1993**, *11*, 399; (b) Pui, C. H.; Jeha, S.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 369; (c) Bonate, P. L.; Arthaud, L.; Cantrell, W. R.; Stephenson, K.; Secrist, J. A.; Weitman, S. *Nat. Rev. Drug Disc.* **2006**, *5*, 855; (d) Issa, J.-P.; Kantarjian, H.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 275; (e) Gore, S. D.; Jones, C.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2006**, *5*, 891.
4. Meneni, S.; Ott, I.; Sergeant, C. D.; Sniady, A.; Gust, R.; Dembinski, R. *Bioorg. Med. Chem.* **2007**, *15*, 3082.
5. Lee, Y. S.; Park, S. M.; Kim, B. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1126.
6. Park, S. M.; Lee, Y. S.; Kim, B. H. *Chem. Commun.* **2003**, 2912.
7. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
8. Gramlich, P. M. E.; Wirges, C. T.; Gierlich, J.; Carell, T. *Org. Lett.* **2008**, *10*, 249.
9. (a) Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1993**, *36*, 1146; (b) Lee, H.-S.; Lee, J. H.; Won, H.; Park, S.-K.; Kim, H. M.; Shin, H. J.; Park, H. S.; Sim, C. J.; Kim, H.-K. *Lipids* **2009**, *44*, 71.
10. (a) Lee, Y.-S.; Kim, B. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1395; (b) Kim, S. J.; Lee, J. Y.; Kim, B. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1313; (c) Kong, J. R.; Kim, S. K.; Moon, B. J.; Kim, S. J.; Kim, B. H. *Nucleosides, Nucleotides, Nucleic Acids* **2001**, *20*, 1751.
11. McGuigan, C.; Yarnold, C. J.; Jones, G.; Velazquez, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1991**, *34*, 2275.
12. (a) Cristofoli, W. A.; Wiebe, L. I.; De Clercq, E.; Andrei, G.; Snoeck, R.; Balzarini, J.; Knaus, E. E. *J. Med. Chem.* **2007**, *50*, 2851; (b) Terrence, P. F.; Spencer, J. W.;

Bobst, A. M.; Descamps, J.; De Clercq, E. *J. Med. Chem.* **1978**, *21*, 228; (c) Robins, M. J.; Manfredini, S.; Wood, S. G.; Wanklin, R. J.; Rennie, B. A.; Sacks, S. L. *J. Med. Chem.* **1991**, *34*, 2275.

13. *Selected spectroscopic data*. **8c**: ^{1}H NMR (300 MHz, acetone-d_6) δ 10.46 (br, 1H), 8.84 (s, 1H), 7.76–7.67 (m, 2H), 7.28–7.25 (m, 2H), 6.97 (s, 1H), 6.29 (t, J = 6.21 Hz, 1H), 4.50 (br, 2H), 3.97 (br, 1H), 3.83–3.79 (m, 2H), 2.64–2.57 (m, 2H), 2.33–2.30 (m, 2H), 2.25 (s, 3H), 2.12 (s, 3H), 1.15 (t, J = 9.12 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.6, 163.6, 161.8, 159.4, 149.4, 147.0, 137.6, 128.8, 127.4 105.0, 101.6, 86.1, 83.4, 74.6, 64.1, 29.1, 21.1, 15.6; MS (EI+) m/z found 483.16, calcd 483.164. *Compound* **8d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (br, 1H), 8.69 (s, 1H), 7.73 (d, J = 7.97 Hz, 2H), 7.25–7.23 (m, 3H), 6.42 (t, J = 6.01 Hz, 1H), 5.27 (d, J = 5.76 Hz, 1H), 4.48–4.43 (m, 1H), 4.35–4.32 (m, 2H), 2.64–2.54 (m, 3H), 2.30–2.28 (m, 1H), 2.26 (s, 3H), 2.11 (s, 3H), 1.61–1.54 (m, 2H), 1.37–1.30 (m, 2H), 0.97 (t, J = 7.22 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 170.6, 163.6, 161.8, 159.7, 149.6, 145.7, 137.6, 129.3, 127.2, 105.0, 101.6, 86.1, 83.4, 74.7, 64.2, 38.8, 35.9, 33.7, 22.7, 21.2, 14.2; MS (EI+) m/z found 512.3, calcd 511.195. *Compound* **10a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (br, 1H), 8.53 (s, 1H), 8.23 (s, 1H), 7.32–7.19 (m, 5H), 6.43 (t, J = 5.73 Hz, 1H), 5.47 (s, 2H), 5.25 (d, J = 6.00, 1H) 4.39–4.31 (m, 2H), 4.25 (br, 1H), 2.49–2.32 (m, 2H), 2.27 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 170.5, 170.3, 161.8, 150.0, 145.0, 139.9, 135.2, 128.5, 128.3, 125.0, 123.1, 105.9, 87.9, 85.8, 71.1, 61.8, 53.9, 49.1, 21.1, 21.0; MS (EI+) m/z found 469.16, calcd 469.16. *Compound* **10c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (br, 1H), 8.55 (s, 1H), 8.10 (s, 1H), 7.24–7.17 (m, 4H), 6.45 (t, J = 5.69 Hz, 1H), 5.46 (s, 2H), 5.28 (d, J = 6.23, 1H) 4.42–4.34 (m, 2H), 4.28 (br, 1H), 2.65–2.57 (m, 2H), 2.47–2.33 (m, 2H), 2.31 (s, 3H), 2.10 (s, 3H), 1.19 (t, J = 7.37, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 170.7, 170.4, 162.0, 150.1, 145.1, 139.9, 137.1, 133.2, 128.7, 128.6, 123.1, 105.9, 88.0, 86.0, 71.3, 61.8, 53.9, 49.1, 40.4, 28.5, 21.2, 21.0, 15.4. MS (EI+) found m/z 497.19, calcd 497.191. *Compound* **11a**: ^{1}H NMR (300 MHz, CD_3OD) δ 8.59 (s, 1H), 8.28 (s, 1H), 7.33 (br, 5H), 6.31 (t, J = 6.61 Hz, 1H), 5.59 (s, 2H), 4.41 (br, 1H), 3.94 (br, 1H), 3.84–3.71 (m, 2H), 2.31–2.20 (m, 2H); MS (EI+) m/z found 385.138, calcd 385.139.