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A new synthetic analogue of thymidine, 7-(3-bromo-phenoxy)-thymidine, inhibits the proliferation of tumor cells

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Abstract—Modified thymidine analogues have been highlighted as useful agents for the treatment of cancer and viral diseases due to their potent biological activities. In the present study, we synthesized a new thymidine analogue, 7-(3-bromo-phenoxy)-thymidine (**4a**), as a potential lead for anti-tumor agent. Compound **4a** potently inhibits HeLa cell proliferation with an IC_{50} of 15 μM.

1. Introduction

Thymidine is a component of thymonucleic acid[1–4] and modified thymidine nucleosides have been of great interest as potential drugs for the treatment of viral diseases and cancer.[5,6] As such, 5-R-2′-deoxyuracils (R = Et, Pr, $CHMe_2$, CH_2OH, CH_2SMe) are known as antiviral agents and 3′-azido-3′-deoxythymidine (AZT) is the first drug against human immunodeficiency virus (HIV).[7] In addition, several pyrimidine modified thymidine derivatives have shown anticancer activities. These include 5-iodo-2′-deoxy-uridine,[8–10] 5-trifluoromethyl-2′-deoxyl-uridine,[11] and its precursor compound, 5-fluorouracil.[12,13] In particular, attention has been focused on the pyrimidine moiety of the compound for structure activity related synthesis. Based on this idea, we have synthesized several analogues of thymidine and investigated the structure activity relationship of 5-methyl group modified thymidine analogues for their antiproliferative activity to cancer cells. In this paper, the synthetic strategy and biological activity of a new thymidine analogue, 7-(3-bromo-phenoxy)-thymidine (**4a**), are described.

2. Chemistry

Several hydrophobic substituents were introduced into 5-methyl group of thymidine and new thymidine analogues were obtained by the synthetic sequence described in Scheme 1.

The condensation of thymidine **1** with acetic anhydride provided the 3′,5′-di-acetyl-thymidine **2** in 91% yield. Compound **2** was isolated by flash column chromatography (hexane–ethyl acetate = 2:1) followed by recrystallization (Fig. 1).

At the next reaction (**b**), bromination was achieved using *N*-bromosuccinimide and benzoyl peroxide under reflux (80–100 °C) condition in chloroform. Residual *N*-bromosuccinimide and succinimide in chloroform were removed by flash column chromatography.[14] Reaction (**c**), in the presence of 1 M equiv of TEA, smoothly proceeded to afford the corresponding compounds **Ac-4-(c–i)** in 15–70% yield.

Different reaction condition (**d**) was used for the synthesis of **Ac-4a**, **Ac-4b**, and **Ac-4j**. The reaction was performed in the presence of 1.05–1.2 M equiv of K_2CO_3 to afford the corresponding compounds **Ac-4a**, **Ac-4b**, and **Ac-4j** in 35–90% yield. Finally, acetyl protecting group of **Ac-4-(a–j)** was removed by NaOMe, resulting in deprotected thymidine analogues **4-(a–j)**.[15,16]

Keywords: Thymidine; Antitumor activity.
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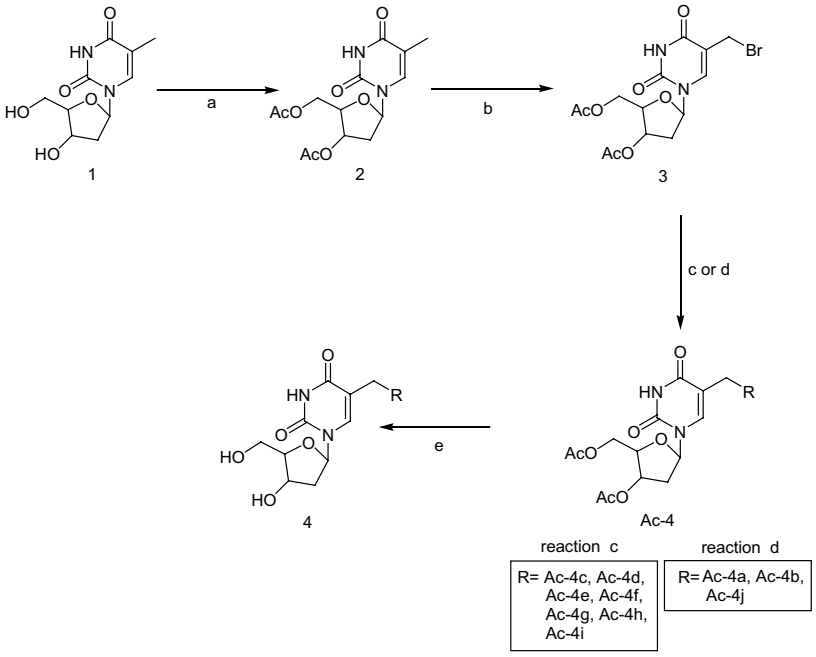


Scheme 1. Reagent and conditions: (a) acetic anhydride, Py, DMAP, rt, 3 h; (b) NBS, $(BzO)_2$, $CHCl_3$, reflux, 4 h; (c) R–OH, TEA, $CHCl_2$, rt, 7 h; (d) R–OH or R–SH, K_2CO_3, DMF, reflux, 5 h; (e) NaOMe, MeOH, rt, 24 h.

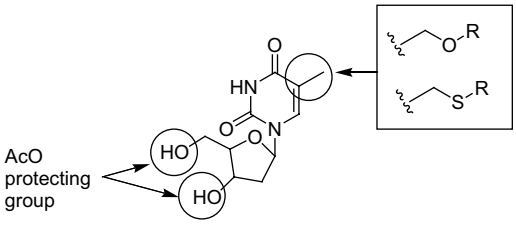
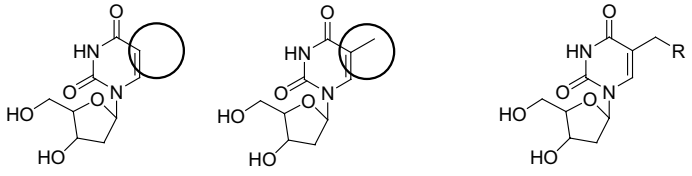


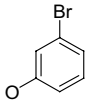
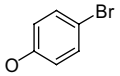
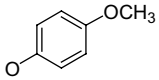
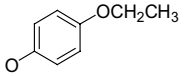
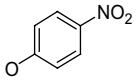
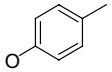
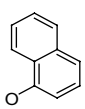
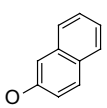
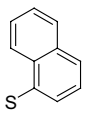
Figure 1. Synthesis strategy of several analogues of thymidine.

3. Biological activity

We investigated the effect of new thymidine analogues **4-(a–j)**, thymidine, and 2′-deoxy-uridine on the growth of HeLa cells using MTT (1-(4, 5-dimethylthiazol-2-yl)-3,5-diphenylformazan) assay.[17,18] As shown in Table 1, thymidine analogues **4-(a–j)** exhibited higher inhibitory activity toward HeLa cell proliferation than those of 2′-deoxy-uridine and thymidine. Among these thymidine analogues, **4a** potently inhibited the proliferation

Table 1. Cell growth inhibitory activity of 5-methyl group modified thymidine analogues



R	Compd	IC_{50} (μM)	R	Compd	IC_{50} (μM)	R	Compd	IC_{50} (μM)
2′-Deoxy-uridine		NA	Thymidine	**1**	4000		**4a**	15
	**4b**	25		**4c**	200		**4d**	250
	**4e**	250		**4f**	250		**4g**	200
	**4h**	50		**4i**	50		**4j**	500

NA=not active.

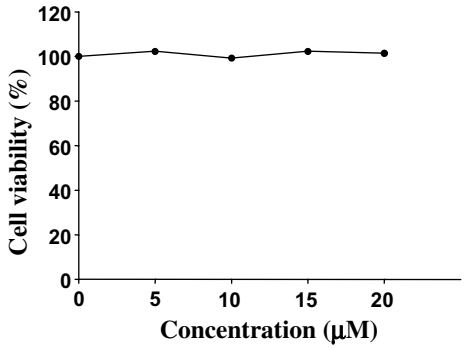


Figure 2. Cell viability assay. **4a** (5–20 μM) was applied to the cell and incubated for up to 72 h. The cells were then stained with trypan blue and counted by hemocytometer.

of HeLa cell with IC_{50} value of 15 μM, suggesting that the increased hydrophobic property at 5-methyl group of pyrimidine may contribute to enhance biological activity of the compound. From this result, we speculate that the position of bromide in phenoxy ring and hydrophobic property of the substitution can affect the biological activity of analogues. In addition, it would be interesting to isolate the specific binding protein for **4a**. The selected candidate, **4a**, was further studied for the effect on the viability of the tumor cells using the trypan blue exclusion method.[19,20]

At the end of the assay the relative cell viability was calculated by comparison with total cells counted. Compound **4a** showed no cytotoxicity against HeLa cells even at the concentration ranges in which the proliferation of the cells was inhibited by the compound (Fig. 2).

In conclusion, we synthesized a new thymidine analogue with increased hydrophobic property at 5-methyl group of pyrimidine and this compound will be a promising candidate for development of thymidine based antitumor agent. Target identification using biotinylated **4a** will be our next challenge for deciphering its mode of action in anti-proliferative activity of tumor cells.

Acknowledgements

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15. Synthetic procedure for **4a**; (compound **Ac-4a**); Compound **3** (370 mg, 0.959 mmol) was dissolved in DMF (5 mL, dry) containing K_2CO_3 (132.5 mg, 0.959 mmol), 3-bromo-phenol (112.8 μL, 0.913 mmol) at 0 °C. The reaction mixture was preceded at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate (20 mL), washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash column chromatography (1.2:1 = *n*-hexane–EtOAc) to give **Ac-4a** as a solid in 21% yield. (Compound **4a**); Compound Ac-**4a** was dissolved in $CHCl_2$ (3 mL) and MeOH (2 mL) containing NaOMe (12 mg, 0.153 mmol) at 0 °C. After 24 h at room temperature, the reaction mixture was neutralized at 0 °C using 10% HOAc in MeOH and concentrated in vacuo. The crude product was directly purified by flash column chromatography (20:1 = CH_2Cl_2–MeOH) to give **4a** as a solid in 97% yield.
16. Analytical data for **Ac-4a**; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.11 (m, 3H), 6.80 (m, 1H), 6.24 (dd, J = 5.8 Hz), 4.79 (q, 2H, J = 1.0 Hz), 4.34–4.26 (m, 4H), 2.61–1.99 (m, 2H), 1.22 (dd, 6H, J = 3.6 Hz, J = 14 Hz). HRMS (FAB, M+Na) Calcd $C_{20}H_{21}O_8N_2Br$: 519.0379, found = 519.0381: **4a**; mp 74–76 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.54 (s, 1H), 7.64–7.60 (m, 2H), 7.52 (d, 1H, J = 6.0 Hz), 7.38 (dd, 1H, J = 1.0 Hz, J = 4.0 Hz), 4.17 (q, 1H, J = 2.0 Hz), 3.95 (s, 1H), 2.9–2.7 (m, 2H). HRMS (FAB, M+Na) Calcd $C_{16}H_{17}O_6N_2Br$: 435.017, found = 435.167.
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18. HeLa (cervical carcinoma) cells were maintained in DMEM and were grown at 37 °C in a humidified atmosphere of 5% CO_2. Cell proliferation was measured by using MTT assay. Briefly, HeLa cells were inoculated at a density of 4×10^3 cells/well in 96-well plates. Various doses of thymidine analogues were added to each well and inoculated for 72 h. MTT (50 μM, 2 mg/mL) was added and the plate was incubated for 4 h. The absorbance of MTT-formazan was measured using a 540 nm filter-equipped mircroplate reader (Bio-Tek instruments, Inc., Winooski, VT).
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20. The effect of thymidine analogues on the viability of cancer cells was investigated. HeLa cells were inoculated at a density of 5×10^4 cells/well in a 24-well plate. The cells were cultivated and exchanged with fresh media. Thymidine analogues (5–20 μM) was applied to each well and incubated for up to 72 h. The cells were then stained with trypan blue and counted by hemocytometer.