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Potent radiosensitizing agents: 5-Methylselenyl- and 5-phenylselenyl-methyl-2′-deoxyuridine

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ABSTRACT

This Letter describes the novel radiosensitizing agents based on nucleoside base modification. In addition to the known 5-phenylselenide derivative, 5-methylselenide modified thymidine, which has a van der Waals radius smaller than the phenyl group, was newly synthesized. The similar monomer activity of 5-methylselenide derivative under oxidation condition was confirmed by NMR experiments. The cytotoxicity tests and radiosensitizing experiments of both compounds were carried out using the H460 lung cancer cell line. Both the 5-phenylselenide and the 5-methylselenide derivatives showed a relatively low toxicity to the cells. However, in combination with γ-radiolysis, both exerted good radiosensitizing effects to the lung cancer cell lines in vitro. This result confirms that 5-methylselenide modified thymidine could be a useful candidate as a potential radiosensitizing agent in vivo.

Radiation therapy has been the mainstay of nonsurgical treatment of cancer for over a century.[1] Drugs that affect nucleoside and nucleotide metabolism are among the most effective and most widely used agents to sensitize tumor cells in radiotherapy. These include halogenated thymidine analogs, 5-bromo-2′-deoxyuridine[2–4] and 5-iodo-2′-deoxyuridine,[5–7] which were the first nucleoside anti-metabolites shown to enhance ionizing radiation-induced cell killing.[2] More recently, several other nucleoside anti-metabolites have been shown to have radiosensitizing properties in vitro, which have been translated to clinical success for drugs such as fluorouracil,[8–10] 5-fluoro-2′-deoxyuridine,[11–13] 2′,2′-difluoro-2′-deoxycytidine.[14–16]

Interstrand cross-links (ICLs) are among the most toxic DNA lesions and have been suggested as the primary mechanism for the cytotoxic activity of many clinical antitumor drugs, such as nitrogen mustards,[17–19] platinum agents,[20,21] and mitomycin C.[22,23] In addition, interstrand cross-linking agents such as mitomycin C and cisplatin have been used in combination with radiotherapy for the purpose of radiosensitization.[24,25] Therefore, molecules that produce ICLs in the hybridized DNA upon γ-radiolysis might be useful as a novel radiosensitizing agent.

The Greenberg group showed phenylselenide derivative **1** produces an ICL with the opposing dA upon UV photolysis through 5-(2′-deoxyuridinyl) methyl radical or upon oxidation through methide intermediate.[26–31] Moreover, they suggested a modified nucleotide triphosphate **2** that can be incorporated in DNA by DNA polymerases, which produces ICLs with the opposing strand dA when DNA containing it is exposed to γ-radiolysis under O_2-deficient conditions (Fig. 1).[28]

In this Letter we report the radiosensitizing effects of 5-methylselenyl- and 5-phenylselenyl-methyl-2′-deoxyuridine nucleosides in vitro. To function as a radiosensitizer, a modified nucleoside should be incorporated into the cellular DNA in the replication step. The modified nucleoside drugs, which are structurally similar to the natural nucleosides, act as a good substrate for natural enzymes such as DNA polymerase and kinase. The methyl group has a van der Waals radius smaller than the phenyl group, so we expected that replacement of the phenyl group with methyl in phenylselenide derivative **1** would produce methylselenide **3**

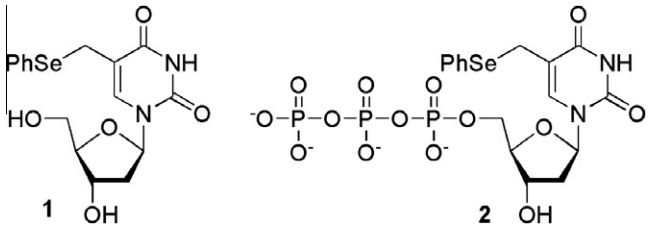

Figure 1. Structures of 5-phenylselenyl-methyl-2′-deoxyuridine and its nucleotide triphosphate.

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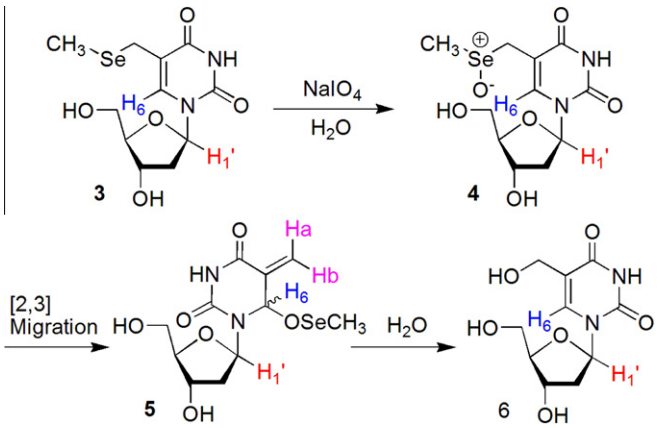

Scheme 1. Decomposition of **3** under oxidative condition.

which would then be a good substrate for mammalian thymidine kinase and DNA polymerase.

Phenylselenide **1** was easily converted to the methide-type intermediate under oxidative conditions such as chemical oxidants, singlet oxygen, γ-radiolysis.[29] Methylselenide derivative **3** was first synthesized from the similar synthetic method of phenylselenide **1**.[33] The reactivity of methylselenide **3** under oxidative condition was checked by NMR experiments. The methylselenide derivative **3** was also easily oxidized to the methylselenoxide derivative **4** after treatment of a chemical oxidant, $NaIO_4$, after which the electrophilic methide-type intermediate **5** was generated by the spontaneous allylic selenoxide rearrangement (Scheme 1).[32] Finally, water attacked the reactive methide intermediate to produce a stable 5-hydroxymethyl 2′-deoxyuridine (HO-mdU, **6**), which was confirmed by independently synthesized authentic HO-mdU.[33]

In contrast to the phenylselenoxide derivative of **1** that was not detected in NMR because of a fast allylic selenoxide rearrangement,[29] the methylselenoxide derivative **4**, which was confirmed by the presence of the diastereomeric H_6 proton, was detected in the NMR spectrum (Fig. 2B). This means that the rate of rearrangement of the methyselenoxide derivative is much slower than that of the phenylselenoxide derivative. The rate of rearrangement of the methylselenoxide **4** was determined by absorption spectroscopy[33] and was $(1.2 \pm 0.01) \times 10^{-4}\ s^{-1}$ ($t_{1/2}$ = 95 min), which was almost 50 times slower than the phenylselenoxide derivative ($(6.1 \pm 0.8) \times 10^{-3}\ s^{-1}$, $t_{1/2}$ = 1.9 min).[31]

After further reaction time, the methide-type intermediate **5** was generated and confirmed by the presence of vinyl hydrogens (H_a δ 6.45, 6.41 ppm and H_b δ 6.09, 6.06 ppm) and upfield shift of diastereomeric H_6 hydrogen (δ 5.88, 5.85 ppm) as shown in Figure 2B and c and was also fully characterized by ^{13}C, COSY, HMBC, and HSQC experiments.[33] Surprisingly, intermediate **5** was at least five times more stable in physiological conditions (Fig. 2D) compared to the methide-type intermediate of **1**, which was converted to **6** during 1 day incubation.[29] As a result, the slow rearrangement of methylselenoxide **4** and the stability of methide-type intermediate **5** are the distinctive differences between the methylselenide **3** and the phenylselenide **1**. These properties of **3** suggest that there may

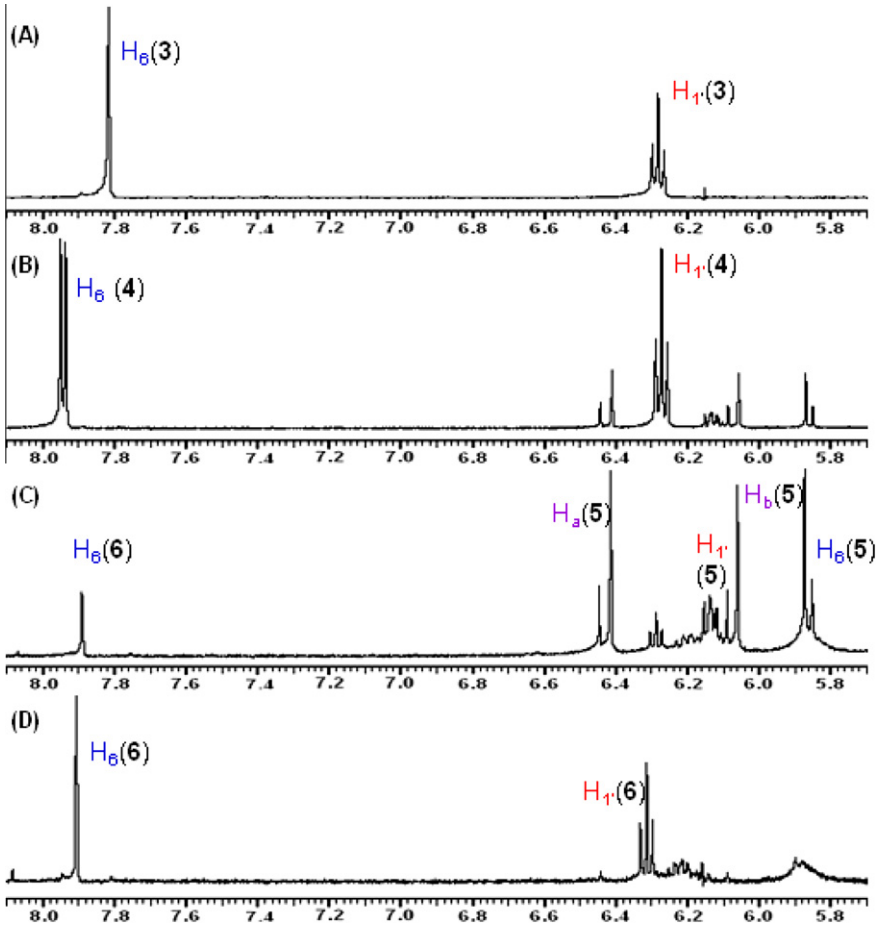

Figure 2. ^1H NMR analysis of the reaction of **3** (10 mM) with $NaIO_4$ (10 mM) in a deuterated phosphate buffer (10 mM, pH 7.0, 25 °C). (A) Before $NaIO_4$ addition, (B) 10 min after $NaIO_4$ addition, (C) 30 h after $NaIO_4$ addition, and (D) 5 days after $NaIO_4$ addition.

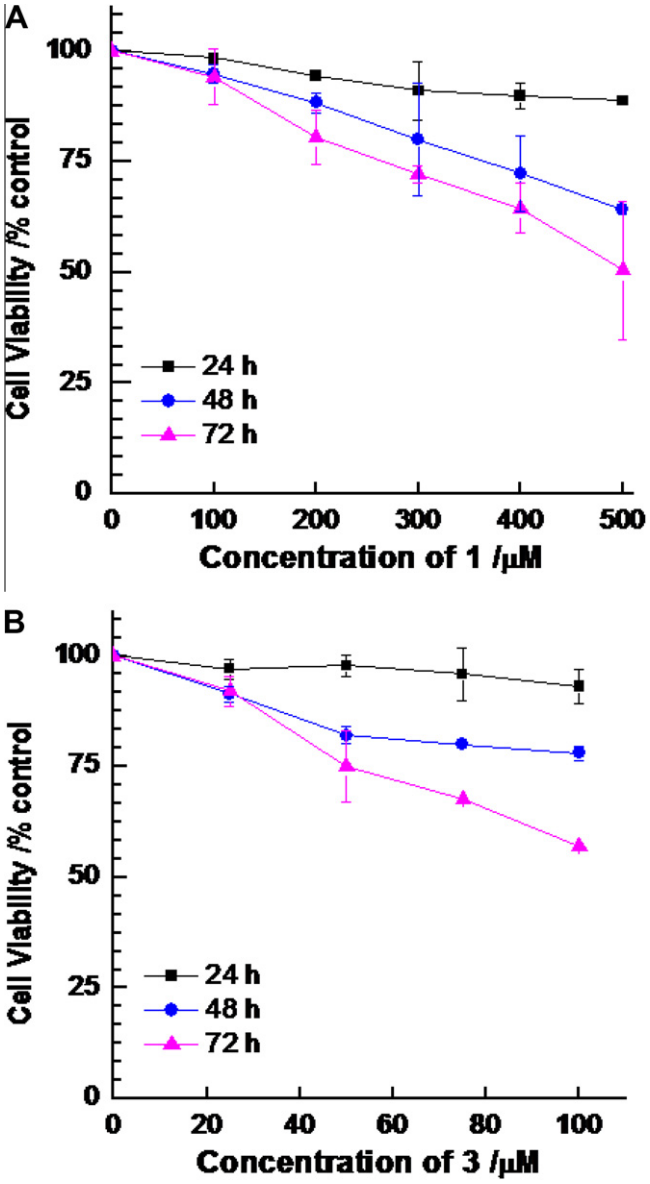

Figure 3. Cell viability curves of H460 cancer cells in the presence of modified nucleoside: (A) dose ranges of 0–500 μM of phenylselenide **1**, (B) dose ranges of 0–100 μM of methylselenide **3**.

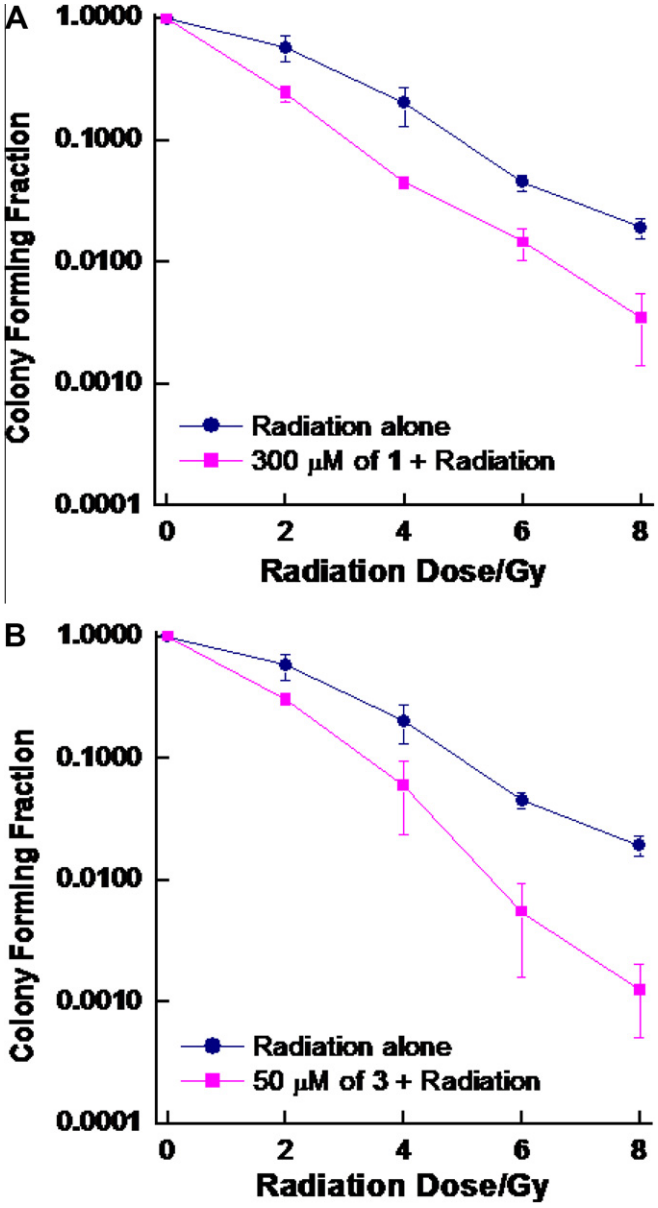

Figure 4. Radiosensitizing effects of **1** and **3** upon γ-ionizing radiation. Colony forming fraction curves of H460 cancer cells exposed to 2, 4, 6 or 8 Gy of γ-radiation with or without dose concentrations: (A) 300 μM of **1** and (B) 50 μM of **3**.

be some differences in the cross-linking efficiency, the cytotoxicity and the radiosensitizing effect between **1** and **3**.

Next, the cytotoxicity tests of phenylselenide **1** and methyl selenide **3** derivatives were carried out to optimize the reaction condition for the radiosensitizing experiments. Figure 3 shows that methylselenide **3** was more toxic (72 h, IC_{50} = 100 μM) than the phenylselenide **1** (72 h, IC_{50} = 500 μM).[33] Detailed studies to reveal a mechanism of single effect to the cytotoxicity of **1** and **3** were in progress.

Finally, the radiosensitizing efficiency of these agents was assessed with a clonogenic assay. The colony forming fraction curves were obtained after exposure of H460 cells with 2, 4, 6 or 8 Gy of γ-radiation with a IC_{20} concentration dose of **1** (300 μM) and a IC_{20} concentration dose of **3** (50 μM) (Fig. 4). The colony forming fractions showed that the dose enhancement ratio (DER) of the phenylselenide analogue **1** was 1.12 and that of the methylselenide nucleoside **3** was 1.2 suggesting that both agents have radiosensitizing effects with increasing radiation doses compared to radiation alone (Fig. 4).[33]

The γ-irradiation of the DNA duplex containing phenylselenide **1** induces interstrand cross-links by the methide intermediate produced through a H_2O_2 oxidation-rearrangement pathway.[28] Based on this observation, our experimental results of the $NaIO_4$ reaction with the methylselenide **3** suggest that the formation of double-strand breaks during nucleotide excision repair of ICLs is a possible key source of the radiosensitizing effect of **1** and **3**.

The $NaIO_4$ oxidation reaction of **3** shows that the methide **5** produced is more stable than the methide produced from **1** with $NaIO_4$. This observation led us to the conclusion that the stability of methide **5** makes the methylselenide **3** more bioavailable in cells than the less stable methide of **1** to produce ICLs which resulted in more efficient radiosensitization by **3** than **1**.

In conclusion, novel radiosensitizing agents based on nucleoside base modification were developed in vitro. Methylselenide **3** and phenylselenide derivative **1** could be useful candidates as a radiosensitizing agents in vivo because a low toxicity of the drug itself

to the cell but a high impact by combination with γ-irradiation are the desired requirements for good radiosensitizers to reduce the side effects of conventional radiosensitizers in radiotherapy. Now more detailed mechanistic approaches to reveal the reaction mechanism are in progress.

Acknowledgments

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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.2010.12.102.

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