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Radiosynthesis and *in vitro* evaluation of 1-(tetrahydro-5-hydroxy-6-(hydroxymethyl)-2H-pyran-3-yl)-5-[^{125}I]iodouracil: A new potential agent for HSV1-tk reporter gene monitoring

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Synthesis, radiolabelling, and *in vitro* evaluation of a new ^{125}I-labelled iodouracil hexitol nucleoside analogue are reported. The target compound was successfully synthesized by an iodination–destannylation method and then purified by reverse phase HPLC. The radiochemical purity of the product was >99% with decay-corrected yields of 48±3%. *In vitro* cellular uptake testing was carried out using MCA and MCA-tk cell lines for comparison of compound 1 with [^{18}F]FHBG. The newly synthesized compound 1 showed higher accumulation in herpex simplex virus type 1 thymidine kinase (HSV1-tk) gene expression cell line (MCA-tk cell line) than in the wild type MCA cell line compared with [^{18}F]FHBG. The MCA-tk to MCA cellular uptake ratio for compound 1 was higher than that of [^{18}F]FHBG from 2 h after incubation. The radioiodine-labelled compound 1 (I-125, $t_{1/2}$ = 59.37 days) has a longer physical half-life than F-18-($t_{1/2}$ = 110 min) labelled FHBG. Radioiodine-labelled compound 1 could be used for monitoring gene expression for a long time. The selectivity for MCA-tk cell line makes compound 1 a promising imaging agent for HSV1-tk expression.

Keywords: HSV1-tk; nucleoside; hexitol; iodouracil; MCA-tk; MCA; iodine-125; imaging agents

Introduction

Gene therapy is a technique that is developing rapidly for the treatment of a number of different cancers. Among the various gene therapy approaches, the prodrug strategy is the most popular.[1] This strategy involves the delivery of a suicide gene (or reporter gene) to the target cells and administering prodrugs. The suicide gene encodes novel nonmammalian enzymes, such as herpes simplex virus type-1 thymidine kinase (HSV1-tk), that can convert a relatively nontoxic prodrug (e.g. ganciclovir) into a highly toxic agent.[2] Optimization of the therapeutic effect requires an adequate radiolabelled probe to image the reporter gene. Thus, these probes are of high interest in the fields of radiopharmaceuticals and nuclear medicine.[3]

HSV1-tk is a multifunctional protein that can phosphorylate pyrimidine and purine nucleoside analogues.[4–6] In contrast, human cellular thymidine kinase, because of restricted changes in the sugar moiety,[4,5] has a higher specificity, phosphorylating only pyrimidine substrate analogues. Generally, HSV1-tk exhibits higher activity with unnatural nucleoside analogues at the first two steps of phosphorylation than the cellular kinase, which is stimulated at the final step.[7–9] The reporter gene concept for molecular imaging has become a standard in various molecular biology protocols using the HSV1-tk gene.[10–13]

In previous studies, it was found that pyrimidine nucleoside analogues, such as radioiodine-labelled 1-(2′-fluoro-2′-deoxy-D-arabinofuranosyl)-5-iodouracil (FIAU), showed better uptake than purine analogues, such as 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG), for HSV1-tk gene expression imaging.[14,15] Various radiolabelled nucleoside analogues with

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pyrimidine ring have been synthesized and evaluated for HSV1-tk imaging.[3,15–20]

Herein, we report the synthesis and preliminary *in vitro* evaluation of a new [^{125}I]iodouracil hexitol nucleoside analogue **1** for use as a potential probe for monitoring of HSV1-tk gene expression.

Experimental

Materials

All reagents and solvents were purchased from Aldrich Chemical Co. and used without further purification. The solid phase extraction cartridge (Sep-pak, silica) was purchased from Waters Associates (Milford, MA). The QMA cartridge (SPE cartridge Chromafix 30-PS-HCO_3) was purchased from Macherey-Nagel Inc. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica plates. Radio-TLC was monitored on a Bioscan AR-2000 imaging scanner (Washington, DC) and high-performance liquid chromatograph (HPLC) was performed on a Waters system using a 515 pump, 2487 UV detector (254 nm), and Raytest GABI γ-detector using a semi-preparative C18 reverse phase column (7.9 × 250 mm, deltapack; Waters). HPLC grade solvents were used for chromatography. Sodium [^{125}I]iodide solution was produced by PerkinElmer. The radioactivity was measured with a dose calibrator (Capintec). The MCA-RH7777 (MCA, CRL1601) rat hepatoma cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD). Thymidine kinase transduced MCA cell line (MCA-tk) was derived from HSV1-tk expressing cells using a retroviral vector. It was kindly provided by Dr Kwon, the Molecular Oncology Laboratory of Korea Institute of Radiological and Medical Sciences (KIRAMS). [^{18}F]FHBG was prepared according to the literature method illustrated by Alauddin and Conti.[21]

Synthesis of 1-(hexahydro-2-phenylpyrano[3,2-d][1,3]dioxin-7-yl)-5-iodopyrimidine-2,4-(1H,3H)-dione (3a)[22]

A suspension of the sodium salt of 5-iodouracil (2.60 g, 10 mmol) and **2** (1.95 g, 5 mmol) in dry DMF (80 ml) was heated at 100°C for 20 h under N_2. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution was washed with aqueous $NaHCO_3$ (3 × 50 ml). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 20 ml). The combined organic layer extracts were washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was purified by using flash column chromatography (silica gel, hexanes–ethyl acetate 5:1 v/v) to yield compound **3a** (0.85 g, 38%).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm): 9.43 (brs, 1H), 8.57 (s, 1H), 7.50–7.36 (*m*, 5H), 5.65 (s, 1H), 4.72 (brs, 1H), 4.37 (dd, *J* = 4.8 and 10.6 Hz, 1H), 4.28–4.21 (*m*, 1H), 4.04 (dd, *J* = 3.5 and 14.2 Hz, 1H), 3.80 (*t*, *J* = 10.6 Hz, 1H), 3.74–3.68 (*m*, 1H), 3.57–3.49 (*m*, 1H), 2.48–2.42 (*m*, 1H), 2.09–2.02 (*m*, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm): 161.3, 156.8, 150.5, 147.0, 137.3, 129.3, 128.4, 126.1, 126.0, 102.1, 74.33, 73.5, 68.9, 68.8, 68.7, 52.2, 32.7. LC-MS (ES+): *m/z* 457.40 $(M+H)^+$.

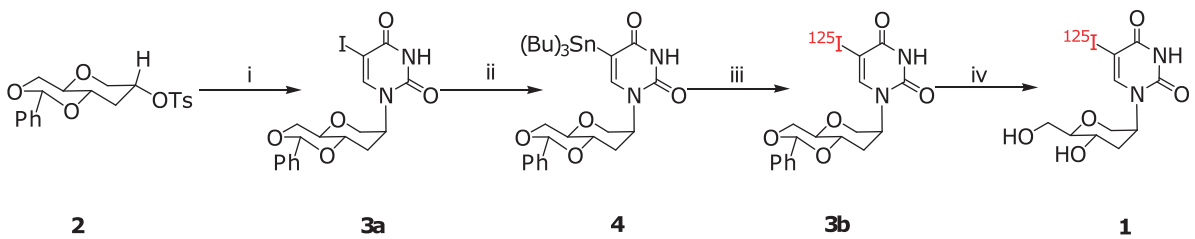
Synthesis of 5-(tributylstannyl)-1-(hexahydro-2-phenylpyrano[3,2-d][1,3]dioxin-7-yl)pyrimidine-2,4(1H,3H)-dione (4)

A mixture of compound **3a** (90 mg, 0.19 mmol), hexabutyltin (0.22 ml, 0.43 mmol), and bis(triphenylphosphine)palladium dichloride (7 mg, 9.97 μmol) in anhydrous dioxane (5 ml) was heated under reflux for 8 h under N_2. The reaction mixture was evaporated under reduced pressure, and the remaining oily residue was purified by using column chromatography (silica gel, hexanes–ethyl acetate 3:1 v/v) to yield compound **4** (54.2 mg, 52%).

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm): 8.09 (s, 1H), 7.61–7.37 (*m*, 5H), 5.55 (s, 1H), 4.76–4.70 (*m*, 1H), 4.39–4.35 (*m*, 1H), 4.22–4.20 (*m*, 1H), 4.06–4.01 (*m*, 1H), 3.74–3.67 (*m*, 2H), 3.64–3.46 (*m*, 1H), 2.43–2.36 (*m*, 1H), 2.22–1.96 (*m*, 1H), 1.39–1.12 (*m*, 12H), 1.12–1.06 (*m*, 6H), 0.92 (*t*, *J* = 10.6 Hz, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ(ppm): 160.5, 156.8, 146.5, 137.0, 131.0, 130.6, 129.3, 128.4, 126.0, 102.1, 74.0, 73.2, 68.4, 68.3, 67.9, 52.0, 32.6, 27.8, 27.4, 13.8, 9.9. LC-MS (ES+): *m/z* 620.82 $(M+H)^+$.

Iodine-125 labelling method

A solution of compound **4** (1 mg, 1.6 μmol) in ethanol (50 μl) was added to sodium [^{125}I]iodide solution (100 μCi/10 μl) at pH ~ 4–5, adjusted with 3% H_2O_2 (50 μl) and 1 M HCl (1 μl), in a 5-ml volume reaction vial. The mixture was kept at room temperature for 30 min. After adding 80% acetic acid (300 μl), the reaction vial was sealed and placed in a heating block at 80°C for 10 min. The reaction vial cover was slowly opened, and the mixture was added to CH_3CN (300 μl) and then dried at 80–100°C under supplying nitrogen gas for 20 min. The mixture was washed with 10% ethanol, and then purified by reverse phase HPLC using a semi-preparative C18 column (7.9 × 250 mm, deltapack; Waters) including installed γ-radioactivity detector with 5% EtOH/H_2O at a flow rate of 2.0 ml/min. The product (average 48 μCi, compound **1**) was collected at 10.9–12.3 min (Figure 2) with radiochemical yield of 48 ± 3%. The product was added to ascorbic acid (1 mg). The radiochemical purity determined by



Reaction conditions: i) Na^+ salt of 5-iodouracil, DMF, 100°C, 38%; ii) $(Bu_3Sn)_2$, $Pd(PPh_3)_2Cl_2$, 1,4-Dioxane, reflux, 52%; iii) $Na^{125}I$, 1N HCl, 3% H_2O_2 iv) 80% AcOH, 80°C

Figure 1. Synthesis of the target compound **1**.

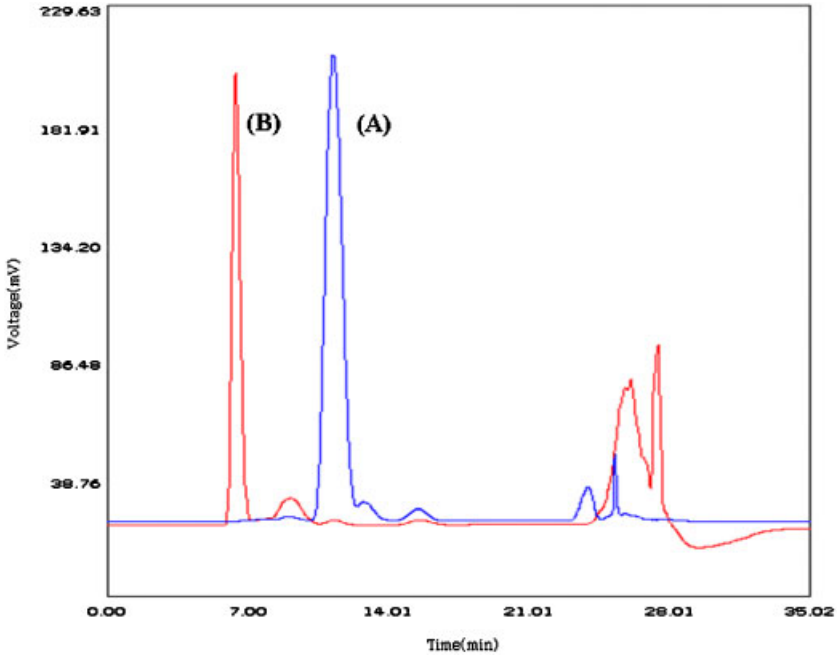


Figure 2. HPLC chromatograms of purified ^{125}I-labelled compound 1 by γ-radioactivity detector (A) and ascorbic acid (additive for radioprotection) at 254 nm by UV detector (B) (Waters; deltapack RP-18, 10 μ, 7.9 × 250 mm, EtOH/H_2O = 5:95 (v/v), flow rate: 2.0 ml/min, retention time of compound **1**: 10.9–12.3 min).

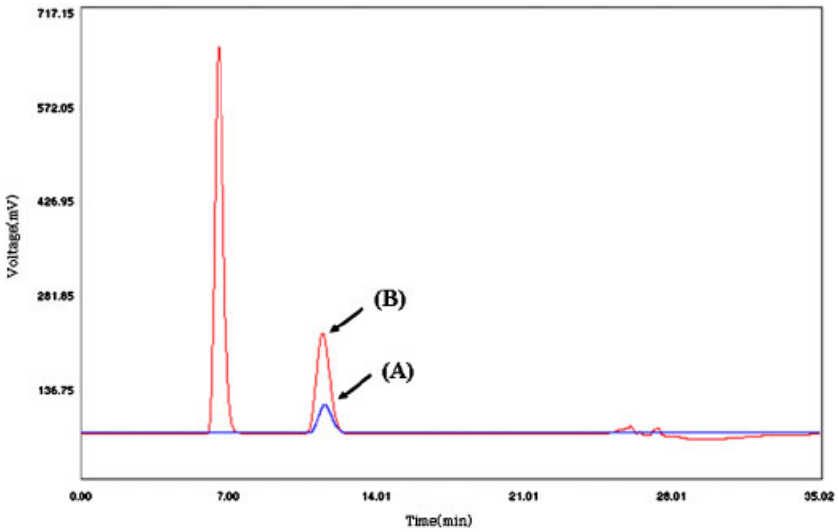


Figure 3. HPLC chromatograms of co-injection of purified ^{125}I-labelled compound **1** (A; γ-radioactivity detector) and standard compound (B; UV detector). (Waters; deltapack RP-18, 10 μ, 7.9 × 250 mm, EtOH/H_2O = 5:95 (v/v), flow rate: 2.0 ml/min, retention time: 10.9–12.3 min).

radio-TLC scanner was >99%. The purified product **1** was confirmed by reverse phase HPLC by co-injection with the standard compound (Figure 3).

Cell lines and cultures

MCA and MCA-tk cells were grown in Dulbecco's Modified Eagle's medium (DMEM; Welgene, Seoul, Republic of Korea), supplemented with 20% horse serum (Gibco, Carlsbad, CA), 5% fetal bovine serum (FBS; JHR Biosciences, Lenexa, KS), and 1% penicillin–streptomycin (Gibco, Carlsbad, CA). The medium was changed twice or three times per week. The cells were cultured at 37°C in a 5% CO_2 atmosphere. The MCA-tk cell line was selected in the presence of G418 (600 μg/ml; Gibco, Carlsbad, CA).

Cellular uptake test

MCA and MCA-tk cells were grown to 5×10^5 cells/well in six-well culture plates and incubated at 37°C for 24 h. Compound **1** was added to each well (1 μCi/2 ml) and the mixture was incubated for 0.5, 1, 2, 4, and 8 h at 37°C in 5% CO_2 atmosphere. Accordingly, [^{18}F]FHBG was added to each well (20 μCi/2 ml) and the mixture was incubated for 0.5, 1, and 2 h at the same conditions. After that, the media were removed, the cells were rinsed with Dulbecco's phosphate-buffered saline (DPBS), and the adherent cells were harvested. Finally, the radioactivity was determined by using gamma counter. Triplicate cellular uptake assay at each time point was performed Figure 4.

Results and discussion

Chemistry

Synthesis of the target compound **1** was achieved according to the sequence illustrated in Figure 1. Hexahyrdo-2-phenylpyrano[3,2-d][1,3]dioxin-7-yl *p*-toluenesulfonate (**2**) was synthesized according to a previously described seven-steps procedure.[23] 1-(Hexahydro-2-phenylpyrano[3,2-d][1,3]dioxin-7-yl)-5-iodopyrimidine-2,4-(1H,3H)-dione (**3a**) was prepared by heating the tosyl compound **2** with sodium salt of 5-iodouracil in dry DMF.[22] The iodine atom of **3a** was replaced by a tributyl–stannyl group by refluxing with hexabutylditin in the presence of a palladium catalyst, $Pd(PPh_3)_2Cl_2$, to afford the tributyl–stannyl derivative **4**. Labelling with I-125 was carried out using sodium [^{125}I]iodide in the presence of hydrogen peroxide at pH ~4–5 inside a reactor. The benzylidene-protected compound **3b** was deprotected by heating with 80% acetic acid to give the target hexitol product **1**.

Purification by HPLC

The product was purified by reverse phase HPLC using 5% EtOH/H_2O as a mobile phase system to yield the target ^{125}I-labelled compound **1** with radiochemical purity >99% and decay-corrected yields of 48 ± 3%.

In vitro cellular uptake testing

The cellular uptake profile of the hexitol compound **1** was compared with that of [^{18}F]FHBG using the same cell lines, MCA and MCA-tk. Upon comparing the cellular uptake of both compounds in MCA cell line, we find that the uptake of [^{18}F]FHBG into MCA and MCA-tk cell lines was continuously increasing through 2 h (Table 2). On the other hand, no significant increase in the uptake of compound **1** in MCA cell line after 1, 2, and 4 h (Table 1). This limited cellular uptake of compound **1** into MCA may contribute to its increased selectivity to HSV1-tk gene expression cell line (MCA-tk).

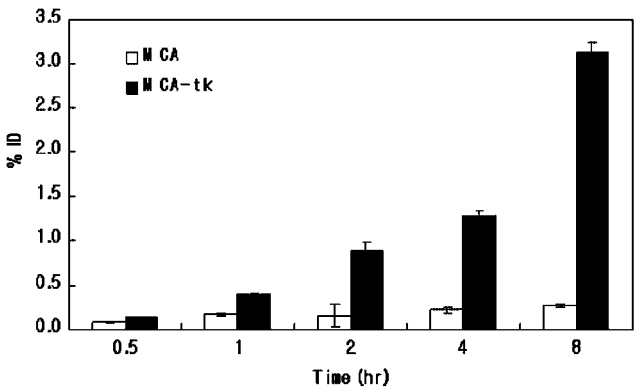


Figure 4. Results of cellular uptake of compound **1** in MCA (HSV1-tk negative) and MCA-tk (HSV1-tk positive) cell lines. Cells were incubated with 2 ml of incubation medium containing 1 μCi of compound **1**. The uptake is expressed as mean %injected dose ± standard error of mean ($n = 3$).

MCA-tk/MCA uptake ratios of compound **1** and [^{18}F]FHBG at different testing times are summarized in Table 3. In case of [^{18}F]FHBG, this ratio was high at 0.5 and 1 h (7.47 and 8.44, respectively) then suddenly decreased at 2 h to reach 3.77. This sudden decrease may be attributed to the relatively short half-life of fluorine-18 ($t_{1/2} = 110$ min) compared with that of iodine-125. However, the MCA-tk/MCA uptake ratio in case of compound **1** was continuously increasing through the whole testing period to reach 11.63 at 8 h. In addition, the MCA-tk/MCA ratio of the newly synthesized hexitol compound **1** at 2 h was 5.56, 1.5 times higher than that of [^{18}F]FHBG.

[^{18}F]FHBG is a purine type nucleoside. However, compound **1** contains an iodouracil base. The size of iodouracil is nearly similar to purine but the iodine group is not charged and its polarity is very low. According to the literature, the pyrimidine nucleoside analogues have shown better cellular uptake than purine types nucleosides for HSV1-tk gene expression imaging.[14,15] One can conclude that iodouracil base is a good base for HSV1-tk gene imaging probe.

According to the *in vitro* cellular uptake data, the ^{125}I-labelled iodouracil compound **1** demonstrated higher selectivity for HSV1-tk gene expression cell line (MCA-tk) over the wild type MCA, compared with [^{18}F]FHBG. This selectivity for MCA-tk

Table 2. *In vitro* cellular uptake of [^{18}F]FHBG (20 μCi) in MCA and MCA-tk cell lines

Time (h)	0.5	1	2
MCA	0.17 ± 0.02	0.32 ± 0.01	1.63 ± 0.27
MCA-tk	1.27 ± 0.04	2.7 ± 0.37	6.14 ± 0.14

Values are expressed as mean of the %ID of [^{18}F]FHBG cellular uptake. %ID is the %injected dose of [^{18}F]FHBG into the wells of MCA and MCA-tk cell lines.

Table 3. Comparison of MCA-tk/MCA uptake ratio

	MCA-tk/MCA uptake ratio	
Time (h)	Hexitol compound 1	[^{18}F]FHBG
0.5	1.75	7.47
1	2.29	8.44
2	5.56	3.77
4	5.82	—
8	11.63	—

Table 1. *In vitro* cellular uptake of compound **1** (1 μCi) in MCA and MCA-tk cell lines

Time (h)	0.5	1	2	4	8
MCA	0.08 ± 0.01	0.17 ± 0.02	0.16 ± 0.13	0.22 ± 0.03	0.27 ± 0.01
MCA-tk	0.14 ± 0.01	0.39 ± 0.01	0.89 ± 0.01	1.28 ± 0.05	3.14 ± 0.12

Values are expressed as mean of the %ID of compound **1** cellular uptake. %ID is the %injected dose of [^{125}I]hexitol compound **1** into the wells of MCA and MCA-tk cell lines.

makes compound **1** a promising imaging agent for HSV1-tk expression.

Conclusions

A novel ^{125}I-labelled iodouracil hexitol nucleoside analogue **1** was successfully synthesized by an iodination–destannylation method and was then purified by using reverse phase HPLC. The radiochemical purity of the product was $>99\%$ with decay-corrected yields of $48 \pm 3\%$. *In vitro* cellular uptake testing was carried out using MCA and MCA-tk cell lines for comparison of compound **1** with [^{18}F]FHBG. The newly synthesized compound **1** demonstrated higher accumulation in MCA-tk cell line than in the wild-type MCA cell line from 2 h after incubation compared with [^{18}F]FHBG. The longer physical half-life of the radioiodine-labelled compound 1 (I-125, $t_{1/2} = 59.37$ days) than F-18 ($t_{1/2} = 110$ min) labelled FHBG may enable compound 1 to be used for monitoring gene expression for a long time. In addition, the selectivity for MCA-tk cell line makes compound **1** a promising imaging agent for HSV1-tk expression.

Acknowledgements

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