Supporting Information

Study of [^{18}F]FLT and [^{123}I]IaraU for cellular imaging in HSV1 tk-transfected murine fibrosarcoma cells: Evaluation of the tracer uptake using 5-fluoro, 5-iodo and 5-iodovinyl arabinosyl uridines as competitive probes

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Experimental

1. Chemical reagents and apparatus

All common solvents and reagents of analytical grade were obtained from Acros. Solvents used for water-sensitive reactions (i.e. MeCN, CH_2Cl_2) were reagent grade and refluxed and distilled over CaH_2 prior to use. DMF was stored over 4 Å molecular sieves and distilled before use. Nonradio-halosubstituted arabinosyl uridine derivatives were prepared according to the previous work.[17]The 5-organotin arabinosyl uridine precursor was prepared prior to use. All compounds have been checked for their identity and purity by TLC using multiple detection including UV detection, staining with *p*-anisaldehyde and cerium molybdate.

2. MTT assay

NG4TL4-STK and NG4TL4 cell lines were prepared as described previously.[21] The cells were seeded as a concentration of 3000 cells/well in a 96-well plate, followed by incubation with various

concentrations of compounds (10 μL) in a final volume of 110 μL at 37 ^{o}C for 2 days. The supernatants were removed through washing. MTT reagents were added and the absorbance at 580 nm was recorded.

3. Radiochemical synthesis of [^{18}F]FLT

3.1 General

[^{18}F]F^- was produced by $^{18}O(p,n)^{18}F$ nuclear reaction with an EBCO TR30/15 cyclotron. An irradiation of 60 min with 17 μA of 17MeV protons on 95% ^{18}O-enriched H_2O in an INER 1.2 mL titanium target yielded 4-7GBq of [^{18}F]F^- at EOB (end of bombardment). Radioactivity level were measured with a Capintec CRC712 dose calibrator. Radio thin-layer chromatography (radio-TLC) plates were exposed for 5 min to a Merck TLC Silica gel 60 F254 plate and subsequently scanned with a Bioscan imager.

3.2 Procedure

Step I: Nucleophilic fluorination of precursor 3-Boc-3'-*O*-nosyl-5'-tritylthymidine

(i) [^{18}F] fluoride trapped on a Waters QMA anion exchange cartridge

(ii) [^{18}F] fluoride eluted from the column using 75 mM $Bu_4N^+HCO_3^-$ (0.5 mL) to the reaction vessel

(iii) Bu_4N^+[^{18}F]F^- dried by azeotropic distillation with acetonitrile (1.0 mL)

(iv) 3-Boc-3'-*O*-nosyl-5'-tritylthymidine precursor (10 mg) dissolved in CH_3CN (1.0 mL) was added to the reaction vessel

(v) Radiofluorination (SN_2) reaction carried out at 120 ^oC for 5 min

Step II: Deprotection

(i) The reaction mixture was cooled to 50 ^oC

(ii) 1 N HCl (1 mL) was added to the reaction vessel

(iii) Acid hydrolysis carried out at 120 ^oC for 5 min

(iv) Reaction mixture was cooled to 50 ^oC

Step III: Purification through neutral alumina column

(i) The reaction mixture was passed through Al_2O_3 (neutral), reverse phase cartridge (C18) and silica gel

(ii) The reaction vessel was rinsed with H_2O (1.5 mL) and the column was first washed with 10% EtOH

(iii) [^{18}F]FLT was eluted using H_2O (10 mL) and was collected in the product vial containing 10% NaCl (0.9 mL)

The specific activity (ca. 2 GBq/μmol) was determined by HPLC chromatogram

4. Radiochemical synthesis of [^{123}I]IaraU

To the 5-trimethylstannyl arabinosyl uridine precursor (1 mg) in $CHCl_3$ (200 μL) was added a mixed solution (50 uL), prepared from AcOH (60 μL) and H_2O_2 (20 μL), and NaOH (0.2 N, 0.1 mL), sequentially. A solution of Na[^{123}I]I (255 MBq, 0.1 mL) was added. The mixture was stirred at room temperature for 10 min. After H_2O (1 mL) was added, the aqueous phase was passed through C-18

cartridge and Al_2O_3 (neutral), sequentially. The products trapped in the columns were further washed with a co-solvent of EtOH/H_2O (4 mL, 50:50, v/v). The pure fractions (3 mL, 37 MBq) were collected. For carrying out the cellular accumulation study, a portion of the solution (500μL, 6.3 MBq) was purified through HPLC: Waters 600 Quat pump and UV detector (Waters 2489 UV/Visible Detector) and radioisotope detector (Raytest GABI Star). HPLC was equipped with a semipreparative C18 column (Nomura Devesoil ODS-7, 10×250mm) using a gradient solvent system at a flow rate of 3 mL/min. The gradient began with 0.05 M, pH 5.7 PBS over 1 minute, and was increased linearly to 30% EtOH and 70% PBS over a 10-min period. Following this, the elution continued with 30% EtOH and 70% PBS for an additional 19 min. The fraction corresponding to the desired product was collected (2 mL, 3.5 MBq) implying a total activity of 21 MBq. The radiochemical yield is 8.2%. The radiochemical purity was greater than 99.5%. The specific activity was approximated to be greater than 21 GBq/μmol. The purified fractions were used for subsequent bioassay.

5. The cellular uptake of [^{123}I]IaraU and [^{18}F]FLT

Both [^{123}I]IaraU and [^{18}F]FLT were each diluted by PBS buffer at a concentration of 10 μCi/50 mL in a petri dish. When the cells were grown for 24 h, the growth medium was replaced with diluted [^{123}I]IaraU or [^{18}F]FLT at 0.1 mL per well and the cell were incubated at 37 ^{o}C for 0.25, 0.5, 1.5, 3, 5 h. The time of addition was staggered such that the samples could be harvested at the same time point. At harvesting, the radioactive medium was collected from each of the wells, followed by

rinsing with PBS twice. The medium and rinses were combined into a counting tube, and the radioactivity in it was considered as extracellular radioactivity. Subsequently, the cells were lysed with 0.25% trypsin-EDTA (30 μL) and the wells were rinsed with PBS twice. Both cells and rinses were collected into an additional counting tube for counting intracellular radioactivity. Radioactivity in each tube was determined by a gamma counter and corrected for decay. Triplicate samples were performed at each time point for all uptake studies. The uptake rate was calculated according to the following formula:

Uptake Rate (%) =
$Count_{intracellular}/(Count_{extracellular} + Count_{intracellular})\times 100\%$.

6. Figures for inhibition assay

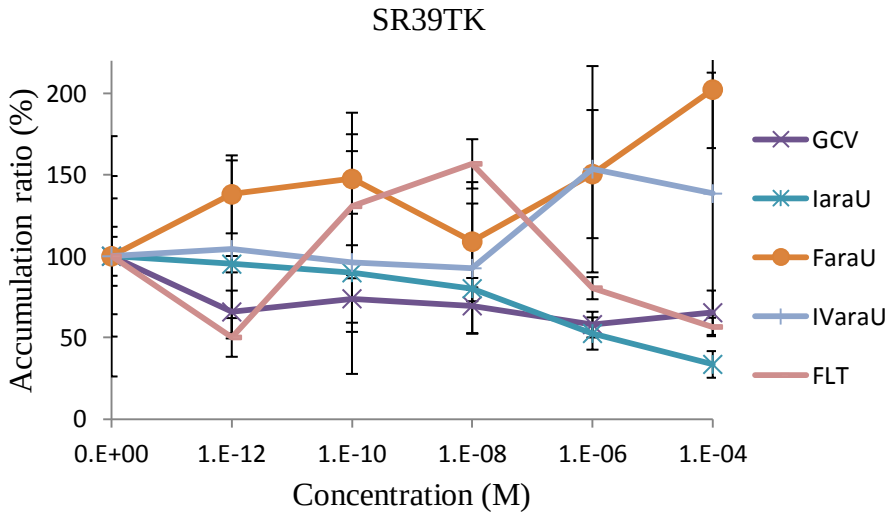
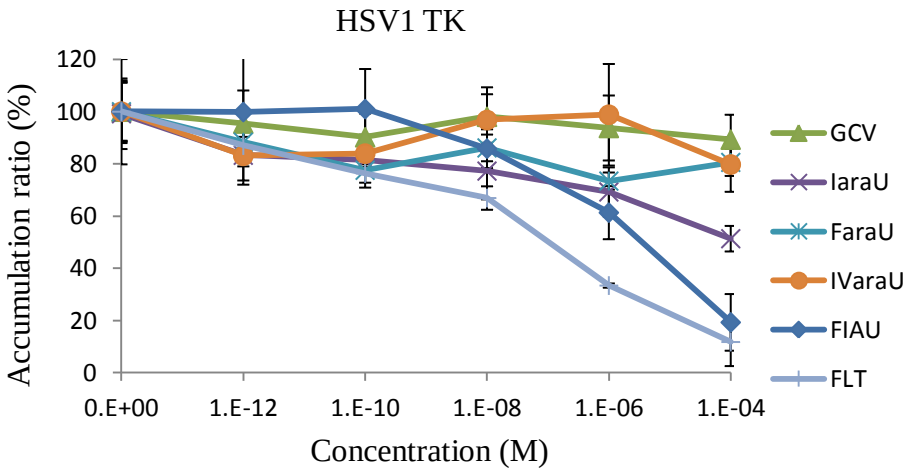


Figure 1. Competitive inhibition by nucleoside analogs under various concentrations against the accumulation of [^{18}F]FLT in NG4TL4-SR39TK cell. *FIAU stimulated a strong radioactivity uptake (200% - 400%).

A



B

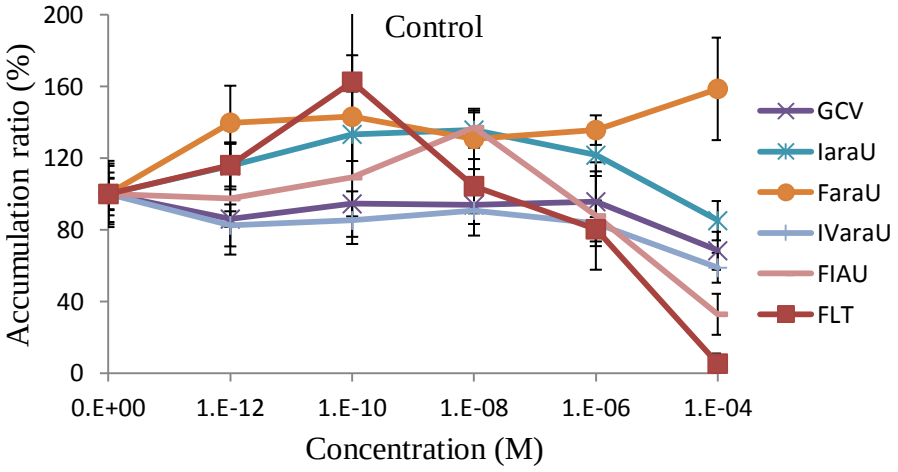


Figure 2. Competitive inhibition against the accumulation of [^{18}F]FLT in (A) NG4TL4-TK and (B) NG4TL4 cells (control) by nucleoside analogs under various concentrations. Intensity was normalized against the cells without treatment of inhibitor.

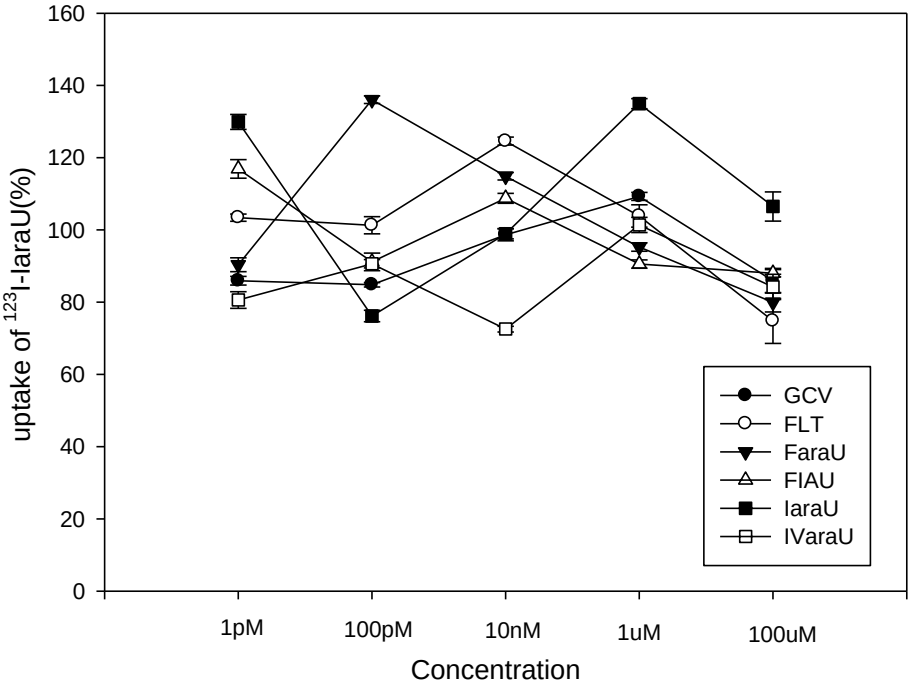


Figure 3. Competitive inhibition against the accumulation of [^{123}I]IaraU in NG4TL4-TK by nucleoside analogs under various concentrations. Intensity was normalized against the cells without treatment of inhibitor.

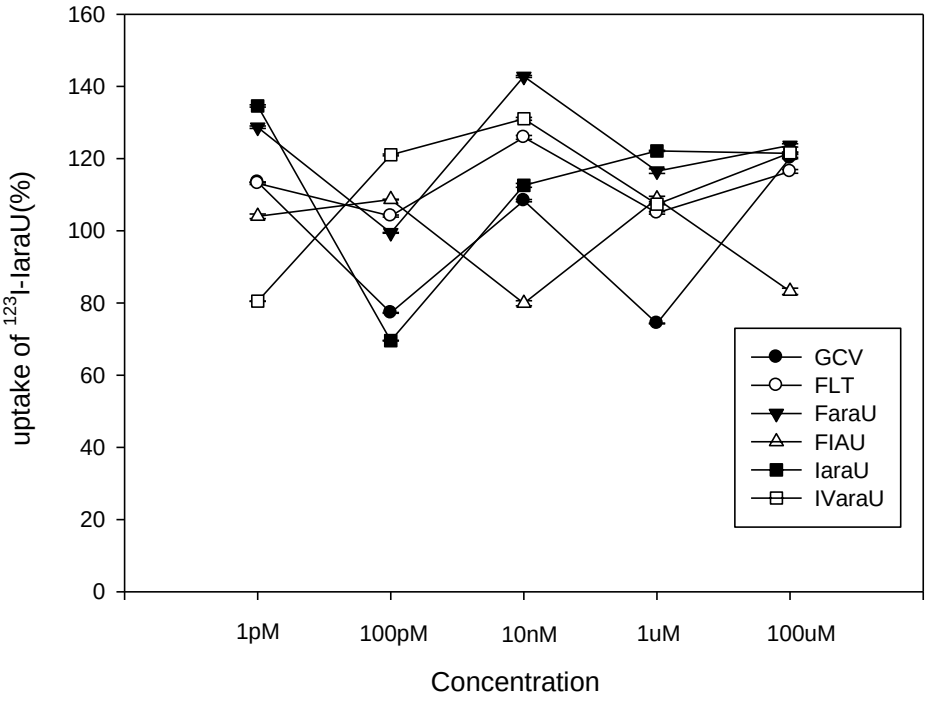


Figure 4. Competitive inhibition against the accumulation of [^{123}I]IaraU in control cell (NG4TL4) by nucleoside analogs under various concentrations

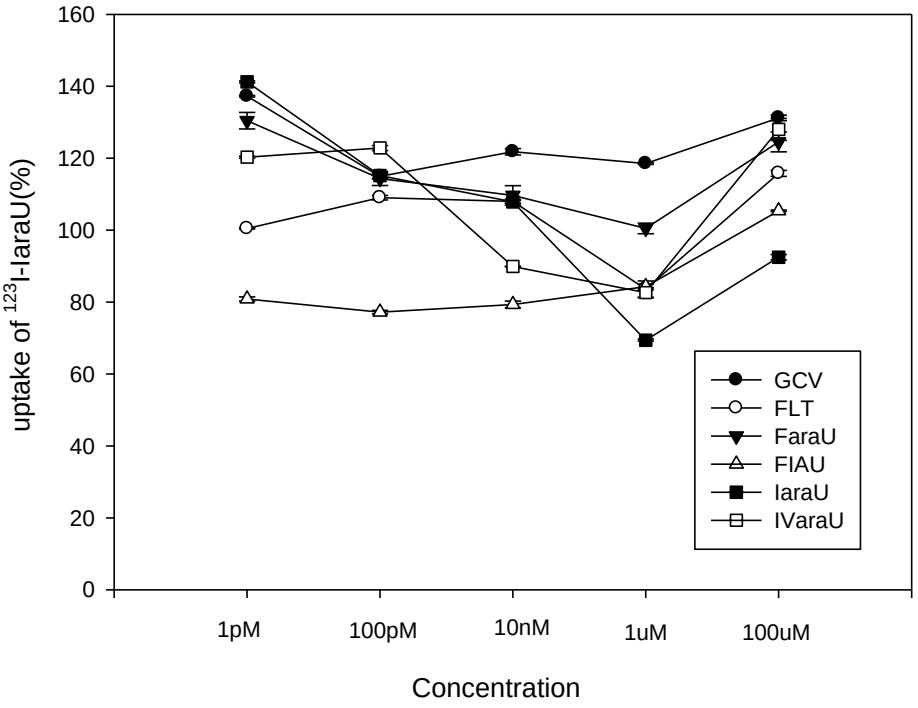


Figure 5. Competitive inhibition by nucleoside analogs under various concentrations against the accumulation of [^{123}I]IaraU in NG4TL4-SR39TK cell.