Futasoline and Its Derivatives, New Nucleoside Analogues

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Futasoline, a new nucleoside analog, was isolated from a fermentation broth of Streptomyces sp. MK359-NF1. Some chemical derivatives of futasoline were prepared. 6-O-Methylyfutasolone methyl ester inhibited growth of HeLa-S3 cells in vitro (IC₅₀ = 19.5 μg/ml) in contrast to the weak activity of futasoline. 6-O-Methylfutasolone methyl ester at concentrations higher than 10 μg/ml inhibited incorporation of [³H]-TdR and [³H]-UR but not [³H]-Leu into the acid-soluble fractions of HeLa-S3 cells.

Key words: futasoline; nucleoside analog; HeLa-S3.

Experimental

Physicochemical Properties of Futasolone (1) [δ₀] +15.7° (c = 0.40, H₂O) IR νmax cm⁻¹: 3200, 1700, 1605, 1560, 1395. UV (H₂O) λmax nm (log e): 245 (3.96). HR-FAB-MS (m/z): 413 (M⁺). Found 413.1122, Calcd 413.1097 (C₁₇H₂₁N₂O₅).

Preparation of 6-O-Methylyfutasolone Methyester (2): To prepare the compound 2, 16 mg (38 nmol) of 1 was dissolved in MeOH (1.0 ml) and mixed with an excess of trimethylsilyldiazomethane (Me₃SiCNH) in diethyl ether. After the reaction mixture was kept at 24°C for 2 h, the solution was concentrated in vacuo, the residue was dissolved in MeOH (0.5 ml) and the solution was subjected to preparative HPLC. 1-N-Methylfutasolone methyl ester was eluted at a retention time of 20 min, while 2 was eluted at a retention time of 40 min. By evaporation in vacuo, 2 was obtained as a colorless powder (4.0 mg, 25% yield).

[β₀] +13.6° (c = 0.22, MeOH) IR νmax cm⁻¹: 3400, 2950, 1730, 1695, 1605. UV (MeOH) λmax nm (log e): 240 (4.16), 285 (3.12). HR-FAB-MS (m/z): 443 (M⁺). Found 443.1588, Calcd 443.1576 (C₁₇H₂₁N₂O₅). 1H-NMR (in CDCl₃) δ: 4.17 (3H, s), 3.92 (3H, s), 1.43 (6H, 5.9% in CMCD) δ: 54.81 (-OCH₃), 162.23 (C-6), 52.88 (methyl group).

Determination of [³H]-TdR, [³H]-UR and [³H]-Leu in Acid-Soluble and Acid-Insoluble Fractions of Cells

Cells were seeded at a density of 2.0×10⁶ cells/ml medium/2 cm² well at 37°C for 24 h. Then, the culture medium was removed, 0.5 ml/well of the serum free medium including a test compound was added, the cultures were incubated at 37°C for 20 min, and thereafter labeled with 25 μCi/well each of [³H]-TD, [³H]-UR, or [³H]-Leu at 37°C for 60 min. The medium was removed, the cells were washed three times with 1 ml/well of cold phosphate buffered saline (PBS) (—), 0.5 ml/well of cold 5% trichloroacetic acid (TCA) was added, the mixtures were kept cool for 40 min, and the supernatant (acid-soluble fraction) was taken. The remainder was washed once with 1 ml/well of 5% TCA and dissolved in 0.5 ml/well of 0.5% KOH by allowing it to stand at room temperature for 40 min (acid-insoluble fraction).

Analytical Procedures

An HPLC system (3520, SSC Co., Ltd., Tokyo) was used with a column (ODS-2151-A, 6×150 mm, SSC Co., Ltd.) was developed with 30% MeOH/12 ms phosphate buffer (pH 2.4) at a flow rate of 2 ml/min at 40°C, and the elution was monitored at 250 nm. 1 and 2 was eluted at a retention time of 7 and 18 min, respectively. α₅ values on TLC (Kieselgel 60 F₂₅₄, Art, 5715, Merck) of 1 were 0.57 (r-ProH: H₂O: 1: 1: NH₄OH = 10: 1: 1) and 0.48 (n-ButOH: AcOH: H₂O = 4: 1: 1). The α₅ values of 2 were 0.61 (CHCl₃: MeOH = 5: 1) and 0.57 (n-ButOH: MeOH: H₂O = 5: 1: 2), and the spots on the TLC were detected with phosphomolybdate-H₂SO₄.

Preparative HPLC

A reverse-phase column (Capcell Pack C₁₈, 5 μm, 100 Å, 20×150 mm, Shiseido Co., Ltd., Japan) was used for separation employing the same conditions as the analytical HPLC system, except that the flow rate is 9.9 ml/min.

General

UV spectra were recorded on a Hitachi U-3201 spectrophotometer and IR spectra on a Hitachi 260-10 spectrophotometer. NMR spectra were recorded on a JEOL-JNM-SX-102 mass spectrometer. Optical rotation was measured with a Perkin-Elmer 241 polarimeter.

Results and Discussion

The futasoline producing strain, Streptomyces sp. MK359-NF1 was cultured by shaking at 27°C for 7 d in 500-ml Erlenmeyer flasks each containing 110 ml of a medium composed of 3% starch, 0.2% yeast extract, 1.5% soybean meal, 0.3% NaCl, 0.05% MgSO₄, 7H₂O, 0.03% CaCO₃, 0.0005% CuSO₄, 5H₂O, 0.0005% MnCl₂, 4H₂O, 0.0005% ZnSO₄, 7H₂O and water to volume (pH 7.0 before sterilization). Futasoline was isolated from the fermentation broth according to the procedures as shown in Fig. 1.

The UV absorption maximum of futasoline (1) at 245 nm (log e 3.96) in H₂O suggested the presence of a hypoxanthine chromophore in the molecule. The molecular formula of 1

Fermentation broth of Streptomyces sp. MK359-NF1

1. broth filtrate, 5 liters
   2. adsorption to Diaion HP-20 eluted with 50% MeOH (pH 9.0)
   3. adsorption to Amberlite IRA-400 eluted with 1N HCl
   4. adsorption to Diaion HP-20 eluted with 50% MeOH (pH 9.0)
   5. brown powder (200 mg)
   6. Sephadex LH-20 chromatography eluted with H₂O
   7. yellow powder (80 mg)
   8. ODS column chromatography eluted with 30% acq. MeOH/12 ml phosphate buffer (pH 2.4), 9.9 ml/min, monitor at 250 nm.
   9. yellow powder (30 mg)
   10. preparative HPLC

Fig. 1. Isolation Procedures for Futasoline

a) Chromatorex®, 2×30 cm, SSC Co., Ltd., Japan. b) See preparative HPLC.

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was established as C_{19}H_{18}N_{3}O_{7} (MW 414) by HR-FAB-MS [Obs. m/z 413.1122, (M−H)^{−}]. Calculated for C_{19}H_{18}N_{3}O_{7}, m/z 413.1097]. The 1H- and 13C-NMR data (Table 1) and heteronuclear multiple bond correlation (HMBC) experiment showed that futulosine was an analog of a nucleoside which was coupled to an isophthalic acid moiety. Analyses of the 1H-1H correlated spectroscopy (COSY) and HMBC spectra of 1 indicated that a methylene (δH 3.08, 3.12) at the C-6' position in the nucleoside was directly linked to one carbonyl group of the isophthalic acid moiety, and that the other carboxyl group was free (Fig. 3). The 1H-NMR spectrum of 1 in dimethyl sulfoxide (DMSO)−d_{6} showed no signals arising from exchangeable protons at C-6, N-1, 2′ or 3′ in the nucleoside moiety or from the carboxyl group (C-14) in the isophthalic acid moiety. In order to confirm the structure of 1, a methyl derivative (2) was prepared by the treatment of 1 with trimethylsilyldiazomethane. The NMR spectral data of 2 and 1-N-methylfutulosine methylster, which was obtained together with 2 by methylation of 1 in DMSO−d_{6} (data not shown) supported the presence of the hypoxanthine ring and the free carboxyl group at C-14 of 1. The NMR spectra of a diacetyl derivative which was prepared by the treatment of 1 with acetic anhydride and pyridine revealed the presence of the hydroxy groups at 2′ and 3′ positions. Moreover, difference nuclear Overhauser effects (NOEs) were observed between H-8 and H-1′, H-8 and H-2′, and H-2′ and H-3′ of 1 in D_{2}O. This NOE data suggested the hydroxy groups at 2′ and 3′ were attached to the sugar moiety. The coupling constants of the sugar moiety in 1 were similar to those of the ribose moiety in oxanosine.31 But the stereochemistry of the sugar moiety remained to be determined. Thus, the planar structure of futulosine was determined as shown in Fig. 2.

Compound 2 inhibited the growth of HeLa-S3 (IC_{50} = 19.5 μg/ml) in vitro in contrast to the weak activity of futulosine (IC_{50}>100 μg/ml). Cell growth was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method and the inhibition caused by the samples was calculated as reported.2) 2 at 10 μg/ml inhibited incorporation of 3H-thymidine and 3H-uridine but not 3H-lysine into the acid-soluble fraction of HeLa-S3 cells, over a 60 min labeling period (Fig. 4). 1 in contrast, hardly inhibited radiolabel incorporation even at a concentration as high as 100 μg/ml. The difference in the effect between the two compounds may be a reflection of their different affinities to nucleoside transporters,35 although an effect on other intracellular metabolic processes is possible. Incorporation into the acid-insoluble fraction of 1H-thymidine and 3H-uridine were also inhibited by 2, which may reflect the lowered radioactivities in the acid-soluble pools in the cells treated with 2. Some inhibition by 2 of the incorporation 3H-lysine into the acid-insoluble fraction, accompanying by the corresponding enhancement of the incorporation into the acid-soluble pool (134% of the control) may be due to inhibition of the polymerization step of protein synthesis. Additional experiments also showed that 2 was not a simple inhibitor of the nucleoside transporter; dipyridamole, an inhibitor of this process, was antagonistic to FUdR while 2 was rather synergistic to FUdR (data not shown). Furthermore, the first 20 s labeling in vitro of HeLa-S3 cells with 3H-TdR at 25°C (regarded as the membrane transport of the nucleoside) was inhibited by 2 only 1/10 as strongly as was inhibited by dipyridamole when each was compared at 50% growth-inhibitory concentration for HeLa-S3 cells (data not shown).

No sign of acute toxicity for 1 and 2 was detectable at 150 mg/kg in mice (i.v.). 1 and 2 did not inhibit in vitro growth of any bacteria, fungi or yeasts at 100 μg/ml.
Fig. 4. Effect of 6-O-Methylfutalosine Methylester on Incroporation of [³H]-Thymidine, -Uridine and -Leucine in Acid-Soluble and Acid-Insoluble Fractions of HeLa-S3 Cells

A test compound dissolved in 0.5 ml of serum-free medium was added to 3×10⁴ cells (one-day precultured) in a 2 cm²-well. After standing at 37°C for 20 min, the culture received 20 μl solution of either ³H-Tdr, -UR or -Leu (0.25 μCi each) and was kept another 60 min at 37°C. Labeling was terminated by removal of the radioactive medium and washing the cell layers with cold PBS (−). 0.5 ml of cold 5% TCA was added to each well and the labeled cells were fractionated into acid-soluble and -insoluble fractions, whose radioactivities were measured. Closed bars and open bars stand for acid-soluble and -insoluble fractions, respectively. Radioactivities of the control run were taken as 100%. Adrimycin and cycloheximide (inhibitors of the polymerization steps of DNA synthesis and protein synthesis, respectively) were included in the experiment for comparison.

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References