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Synthesis and Antiviral Activity of Novel Exomethylene Cyclopropyl Pyrimidine Nucleosides

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A series of novel exomethylene cyclopropyl nucleosides have been synthesized starting from Feist's acid. Classical nucleophilic substitution conditions (K_2CO_3, 18-crown-6) of the tosylate **2** as well as Mitsunobu reaction (DEAD, PPh_3) of alcohol **1** with pyrimidine bases afforded a series of novel cyclopropyl nucleosides. Compound **4b** displayed moderate anti-HBV activity without any cytotoxicity up to 100 μM.

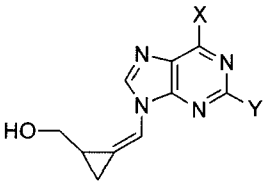
Key words: Exomethylene cyclopropyl nucleoside, Antiviral agent, Feist's acid

INTRODUCTION

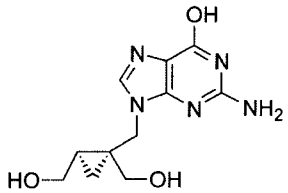
Extensive efforts in the search of chemotherapeutic agents against viral infectious disease have led to the discovery of a variety of biologically active nucleosides analogs, including acyclic nucleosides (El Ashry *et al.*, 1997). Acyclonucleosides can be considered as derivative of classical nucleosides or carbo-nucleosides by omitting any bond from the pentose or cyclopentane rings (Agrofolio *et al.*, 1998). Because of their structural flexibility, many of them possess biological properties despite their lack of chirality such as acyclovir (Elion *et al.*, 1977), ganciclovir (Martin *et al*, 1983), penciclovir (Earnshaw *et al.*, 1992) and famciclovir (Vere Hodge *et al.*, 1989) as antiviral agents.

Recently, novel nucleosides containing a cyclopropane moiety were also synthesized as conformationally constrained analogues of acyclic nucleosides. Among them, the purine derivatives such as synadenol (Qiu *et al.*, 1998a) and synguanol (Qiu *et al.*, 1998b) (Fig. 1) of which the ribofuranoside moiety is replaced with a methylene cyclopropane ring were found to have potent antiviral activity, particularly against human cytomegalovirus (HCMV). Also, the guanine derivative (A-5021) (Fig. 1), which was one of trisubstituted cyclopropane nucleosides with an additional hydroxymethyl group at 1'-position, showed more potent antiviral activity against HSV-1 than acyclovir but ineffective against HIV (Sekiyama *et al.*, 1998).

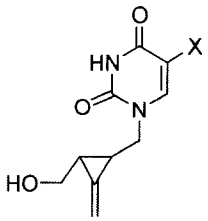
Furthermore, the recent approval of bis(POC) PMPA (Armilli *et al.*, 1997) by FDA as an anti-HIV agent warranted the high possibility of acyclic nucleosides as chem-



Synadenol: X = NH_2, Y = H
Synguanol: X = OH, Y = NH_2



A-5021



4a: X = H
4b: x = CH_3
4c: X = F
4d: X = Cl
4e: X = Br
4f: X = I

Fig. 1. Novel cyclopropyl nucleosides

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other apeutic agents.

Based on these interesting observations of restricted cyclopropyl nucleoside analogs and as part of our drug discovering programs, we have determined to synthesize a novel class of nucleosides comprising a rigid exomethylene cyclopropyl backbone.

MATERIALS AND METHODS

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. Nuclear magnetic resonance (NMR) data for ^{1}H NMR were taken on Varian UNITY *plus* 300 spectrometers and are reported in (ppm) downfield from tetramethylsilane (TMS). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet. Thin layer chromatography (TLC) was carried out using precoated plates with silica gel 60F 254 purchased from Merck.

***Trans*-[1-[(*tert*-Butyldiphenylsilyl)oxymethyl]-2-hydroxymethyl]-3-methylene cyclopropane (1) and *Trans*-1-[(*tert*-Butyldiphenylsilyl)oxymethyl]-2-[(*p*-toluenesulfonyl)oxymethyl]-3-methylenecyclopropane (2):** Silylated cyclopropanol **1** and its tosylate **2** were prepared from Feist acid by previously published method (Kwak *et al.*, 2000).

General procedure A: for the synthesis of protected exomethylene cyclopropyl nucleosides **3a**, **3b** and **3c**: A solution of **2** in DMF was added to the mixture of K_2CO_3, 18-crown-6, and pyrimidine bases (thymine, uracil and 5-substituted uracils) in DMF, and resulting mixture was stirred at 60°C for 2 hr. After concentration under reduced pressure, the residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried (Na_2SiO_4), filtrated, and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography.

General procedure B: for the synthesis of exomethylene cyclopropyl nucleosides **3d**, **3e** and **3f** via Mitsunobu reaction: To a stirred mixture of a nucleoside bases and triphenyphosphine in anhydrous THF under argon was added diethyl azodicarboxylate. The resulting mixture was stirred at room temperature for 10 min, and a solution of **1** in THF was added. The resulting mixture was stirred at room temperature for overnight. After removal of solvent in vacuum, the residue was dissolved in EtOAc, washed with water, dried (Na_2SO_4) and concentrated and separated by silica gel column chromatography.

***Trans*-9-[[[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-uracil (3a):** General procedure A was used; (Hexane-EtOAc = 1:1); yield 17.2%; white solid; mp 140-143°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (1H, br s, NH), 7.71 (1H, d, J = 7.8 Hz, C^6-H), 7.60-7.41 (10H, m, aromatic), 5.52 (1H, dd, J = 2.1, 7.8 Hz, C^5-H), 5.44, 5.45 (each 1H, s, CH_2=C), 3.93 (1H, dd, J = 5.4, 14.1 Hz, CH_2N), 3.80 (1H, dd, J = 5.4, 10.8 Hz, CH_2O), 3.41, 3.22 (each 1H, m, CH_2N, CH_2O), 1.88, 1.70 (each 1H, m, 2×cyPr CH), 0.95 (9H, s, *t*-butyl); IR (KBr) cm^{-1} 1678 (lactam C=O); UV(MeOH) λ_{max} 266 nm (ε 5130).

***Trans*-9-[[[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-thymine (3b):** General procedure A was used; (Hexane-EtOAc = 1:1); yield 20.4%; white solid; mp 190-192°C;^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (1H, br s, NH), 7.60-7.38 (11H, m, aromatic and C^6-H), 5.44 (2H, m, CH_2=C), 3.93 (1H, dd, J = 5.2, 14.1 Hz, CH_2N), 3.81 (1H, dd, J = 5.1, 10.5 Hz, CH_2O), 3.34 (1H, dd, J = 9.3, 14.1 Hz, CH_2N), 3.20 (1H,dd, J = 8.55, 10.5 Hz, CH_2O), 1.99, 1.73 (each 1H, m, 2×cyPr CH), 0.93 (9H, s, *t*-butyl); IR (KBr) cm^{-1}: 1671 (lactam C=O); UV (MeOH); λ_{max} 272 nm (ε 5590).

***Trans*-9-[[[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-5-fluorouracil (3c):** General procedure A was used; (Hexane-EtOAc = 1:1); yield 23.0%; white solid; mp 154-156°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (1H, s, C^6-H), 7.70-7.30 (10H, m, aromatic), 5.49 (2H, dt, J = 2.4, 4.8 Hz, CH_2=C), 3.87 (1H, dd, J = 5.1, 10.8 Hz, CH_2N), 3.80 (1H, dd, J = 5.4, 10.5 Hz, CH_2O), 367 (1H, dd, J = 4.2, 7.5 Hz, CH_2O), 3.36 (1H, dd, J = 8.1, 10.8 Hz, CH_2N), 1.71 (2H, m, 2×cyPr CH), 1.04 (9H, s, *t*-butyl); IR (KBr) cm^{-1}: 1680 (lactam C=O); UV (MeOH) λ_{max} 272 nm (ε 8960).

***Trans*-9-[[[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-5-chlorouracil (3d):** General procedure B was used; (Hexane-EtOAc = 1:1); yield 25.7%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (1H, s, C^6-H), 7.70−7.30 (10H, m, aromatic), 5.48 (2H, dt, J = 8.7, 2.4 Hz, CH_2=C), 3.85 (1H, dd, J = 5.7, 10.8 Hz, CH_2N), 3.76 (1H, dd, J = 8.1, 14.4 Hz, CH_2O), 3.64 (1H, dd, J = 6.6, 14.4 Hz, CH_2O), 3.37 (1H, dd, J = 7.8, 10.8 Hz, CH_2N), 1.72 (2H, m, 2 × cyPr CH), 1.06 (9H, s, *t*-butyl); IR (KBr) cm^{-1}: 1680 (lactam C=O); UV (MeOH) λ_{max} 282 nm (ε 8170).

***Trans*-9-[[[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-5-bromouracil (3e):** General procedure B was used; (Hexane-EtOAc = 1:1); yield 24.2%; white solid; mp 171-174°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (1H, s, C^6-H), 7.68-7.32 (10H, m, aromatic), 5.48 (2H, dt, J = 10.2, 2.4 Hz, CH_2=C), 3.85 (1H, dd, J = 5.1, 11.1 Hz, CH_2N), 3.79 (1H, dd, J = 7.5, 14.1 Hz, CH_2O), 3.61 (1H, dd, J = 6.6, 14.1 Hz, CH_2O), 3.37 (1H, dd, J = 8.1, 11.1 Hz, CH_2N), 1.72 (2H, m, 2 × cyPr CH), 1.06 (9H, s, *t*-butyl); IR (KBr) cm^{-1}: 1680 (lactam C=O); UV (MeOH)

λ_{max} 282 nm (ε 8910).

***Trans*-9-[2-(*tert*-Butyldiphenylsilyl)oxymethyl]-3-methylenecyclopropyl]methyl]-5-iodouracil (3e):** General procedure B was used; (Hexane-EtOAc = 1:1); yield 28.4 %; white solid; mp 149-153°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (1H, s, C^6-H), 7.68-7.30 (10H, m, aromatic), 5.47 (2H, dt, J = 2.4, 12.3 Hz, CH_2=C), 3.84 (1H, dd, J = 4.8, 11.1 Hz, CH_2N), 3.82 (1H, dd, J = 7.2, 14.1 Hz, CH_2O), 3.55 (1H, dd, 6.6, 14.1 Hz, CH_2O), 3.38 (1H, dd, J = 8.1, 11.1 Hz, CH_2N), 1.72 (2H, m, 2×cyPr CH), 1.06 (9H, s, *t*-butyl); IR (KBr) cm^{-1}: 1680 (lactam C=O); UV (MeOH) λ_{max} 288 nm (ε 8420).

General procedure C for the synthesis of exomethylene cyclopropyl nucleosides **4a**, **4b**, **4c**, **4d**, **4e** and **4f**:

A mixture of **3a**, **3b**, **3c**, **3d**, **3e** and **3f** and 1.0 M tetra-*n*-butylammonium fluoride in THF was stirred at room temperature for 2 hr. After the mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography;

***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]uracil (4a):** ($CHCl_3$: MeOH = 5:1); yield 99%; white solid; mp 150-153°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.22 (1H, s, NH), 7.63 (1H, d, J = 7.8 Hz, C^6-H), 5.54 (1H, dd, J = 2.4, 7.8 Hz, C^5-H), 5.46, 5.41 (each 1H, m, CH_2=C), 4.69 (1H, br s, OH), 3.74 (1H, dd, J = 6.0, 14.1 Hz, CH_2N), 3.59 (1H, dd, J = 7.5, 14.1 Hz, CH_2N), 3.44, 3.16 (each 1H, m, CH_2O), 1.67 (2H, m, 2×cyPr CH); IR (KBr) cm^{-1}: 3198-3160 (OH, lactam NH), 1685 (lactam C=O); UV (MeOH) λ_{max} 266 nm (ε 7542).

***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]thymine (4b):** ($CHCl_3$: MeOH = 5:1); white solid; yield 99%; mp 149-151°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.2 (1H, s, NH), 7.53 (1H, s, C^6-H), 5.46, 5.40 (each 1H, s, CH_2=C), 4.69 (1H, br s, OH), 3.72 (1H, dd, J = 5.7, 13.9 Hz, CH_2N), 3.54 (1H, dd, J = 7.8, 13.9 Hz, CH_2N), 3.43, 3.15 (each 1H, m, CH_2O), 1.66 (2H, m, 2×cyPr CH); IR (KBr) cm^{-1}: 3453-3154 (OH, lactam NH), 1680 (lactam C=O); UV (MeOH) λ_{max} 270 nm (ε 24150).

***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]-5-fluorouracil (4c):** ($CHCl_3$:MeOH = 5:1); yield 91.3%; white solid; mp 153-156°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.10 (1H, d, J = 6.9 Hz, C^6-H), 5.45 (2H, d, J = 9.0 Hz, CH_2=C), 3.81 (1H, dd, J = 5.7, 14.4 Hz, CH_2N), 3.48 (1H, dd, J = 5.7, 11.4 Hz, CH_2N), 3.45 (1H, dd, J = 7.8, 14.4 Hz, CH_2N), 3.11 (1H, dd, J = 8.1, 11.4 Hz, CH_2O), 1.69 (2H, m, 2×cyPr CH); IR (KBr) cm^{-1}: 1700 (lactam C =O); UV (MeOH) λ_{max} 273 nm (ε 8960).

***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]-5-chlorouracil (4d):** ($CHCl_3$:MeOH = 5:1); yield 99%; white solid; mp 166-168°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (1H, s, NH), 8.12 (1H, s, C^6-H), 5.43 (2H, d, J = 14.4 Hz, CH_2=C), 3.90-3.00 (4H, m, CH_2N, CH_2O), 1.60 (2H, m, 2 × cyPr CH); IR (KBr) cm^{-1}: 1685 (lactam C=O); UV (MeOH) λ_{max} 282 nm (ε 9420).

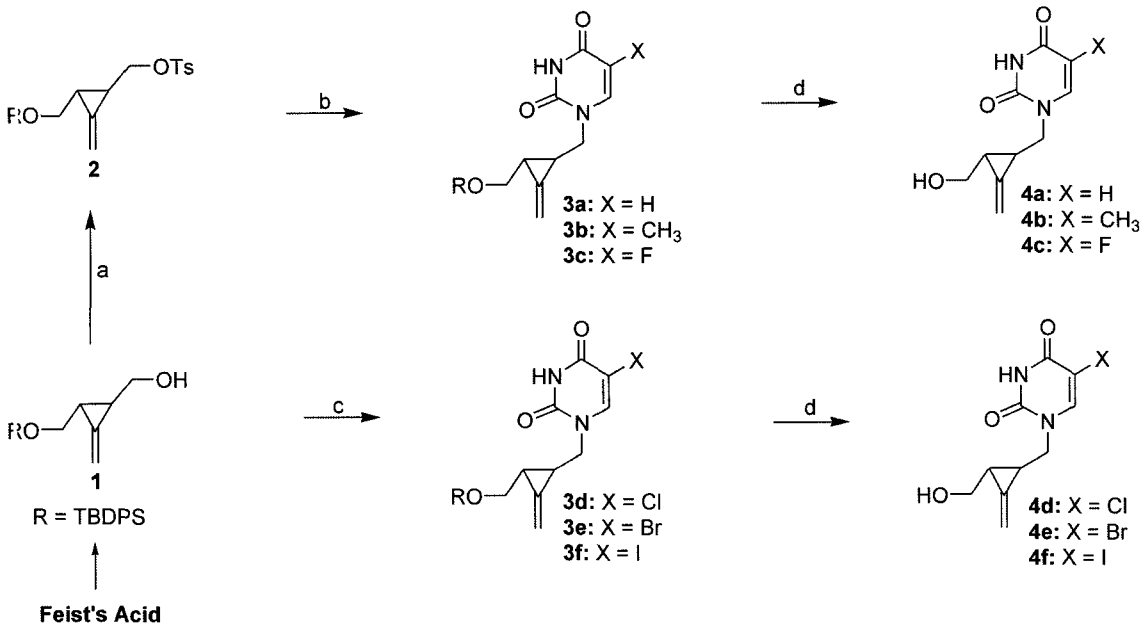
***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]-5-bromouracil (4e):** ($CHCl_3$:MeOH = 5:1); yield 99%; white solid; mp 177-179°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (1H, s, C^6-H), 5.44 (2H, d, J = 15.3 Hz,CH_2=C), 3.84 (1H, dd, J = 5.4, 14.1 Hz, CH_2N), 3.52 (1H, dd, J = 7.8, 14.1 Hz, CH_2N), 3.47 (1H, dd, J = 5.4, 11.4 Hz, CH_2O), 3.10 (1H, dd, J = 8.1, 11.4 Hz, CH_2O), 1.69 (2H, m, 2×cyPr CH); IR (KBr) cm^{-1}: 1680 (lactam C= O); UV (MeOH) λ_{max} 282 nm (ε 9420).

***Trans*-9-[(2-hydroxymethyl-3-methylenecyclopropyl) methyl]-5-iodouracil (4f):** ($CHCl_3$:MeOH = 5:1); yield 95.2%; yellow solid; mp 139-142°C;. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (1H, s, C^6-H), 5.57 (2H, dt, J = 18.6, 2.1 Hz, CH_2=C), 3.93 (1H, dd, J = 6.9, 14.1 Hz, CH_2N), 3.81 (1H, dd, J = 5.1, 11.4 Hz, CH_2O), 3.63 (1H, dd, J = 6.3, 14.1 Hz, CH_2N), 3.42 (1H, dd, J = 7.5, 11.4 Hz, CH_2O), 1.78 (2H, m, 2×cyPr CH); IR (KBr) cm^{-1}: 1680 (lactam C=O); UV (MeOH) λ_{max} 290 nm (ε 7620).

RESULT AND DISCUSSION

For the synthesis of the desired pyrimidine nucleosides, the alcohol derivative **1** was first prepared from Feist's acid by known procedure and then condensed directly with nucleobases by Mitsunobu method as well as activated to tosylate **2** for the condensation using nucleophilic substitution fashion.

In order to synthesize the desired acyclic nucleosides, most of the methods involve the alkylation of the acyclic chain by a suitable halide or equivalent groups. Thus, the requisite acyclic chain was prepared by commencing with acid derivative, which was prepared from ethylacetoacetate in three steps using the well-known procedure (Gilchrist *et al.*, 1968). Feist's acid was transformed to alcohol derivative **1** by two steps using the previously reported procedure (Kwak *et al.*, 2000). The hydroxyl group of **1** was tosylated by treating with *p*-toluenesulfonyl chloride (TsCl) in an anhydrous CH_2Cl_2 solvent to give the key intermediate **2**, which was coupled with the various pyrimidine bases under standard nucleophilic substitution conditions (K_2CO_3, 18-crown-6, DMF) (Jeon *et al.*, 1996; Hossain *et al.*, 1996) to give nucleosides **3a**, **3b** and **3c**. We can directly synthesize some nucleosides **3d**, **3e** and **3f** from alcohol derivative **1** using Mitsunobu conditions (DEAD and PPh_3). Compounds **3a**, **3b**, **3c, 3d, 3e and 3f** were deprotected



Reagents: a) *p*-TsCl, DMAP, CH_2Cl_2; b) pyrimidine bases, K_2CO_3, 18-crown-6, DMF; c) pyrimidine bases, PPh_3, DEAD, THF; d) *n*-Bu_4NF, THF.

Scheme 1. Synthesis of exomethylene cyclopropyl pyrimidine nucleosides

by *n*-tetrabutylammonium fluoride (TBAF) in THF to give the final nucleosides **4a**, **4b**, **4c, 4d, 4e** and **4f**.

In vitro antiviral activity of synthesized nucleosides

The antiviral activities of final pyrimidine nucleosides (**4a**, **4b**, **4c**, **4d**, **4e**, and **4f**) were tested against HSV-I, HSV-II, HCMV, HIV-1, HIV-2 and HBV. Unfortunately, only thymine derivative **4b** showed moderate anti-HBV activity at 30 μM without any cytotoxicity up to 100 μM.

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