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Tryptophanyl Phosphoramidates as Prodrugs of Synadenol and Its *E*-isomer: Synthesis and Biological Activity

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Abstract—Phosphorotryptophanates **2c** and **3c** were synthesized and investigated as prodrugs of synadenol (**2a**) and its *E*-isomer **3a**. The antiviral activity of **2c** corresponds to parent analogue **2a** but it is lower than that of phenylphosphoralaninate **2b**. This may indicate an enzymatic cleavage of phosphorotryptophanate **2c** to **2a** before or after entering the host cells. The *E*-isomer **3c** was effective only against EBV with parameters suggesting intracellular delivery of the respective phosphate. Compound **2c** has a moderate but selective activity against solid tumors.

Lipophilic pronucleotides of nucleotide analogues such as phenylphosphoralaninates **1a** and phosphorotryptophanates **1b** have received much attention as antiviral agents and, in the case of **1b**, anticancer agents.[1] However, comparative studies of both triester and diester phosphoramino acid amidate pronucleotides are rather scant[2] and it appears that the pronucleotides of type **1a** and **1b**, most effective in each class, have not been compared. Recently, we have described a new series of nucleoside analogues where a ribofuranose moiety was replaced by a methylenecyclopropane system.[3–6] The *Z*-isomers of purine derivatives of this class such as synadenol **2a** are potent antiviral agents of broad-spectrum activity whereas the *E*-isomers (e.g., **3a**) are effective only in a few cases. Transformation of the purine analogues to lipophilic pronucleotides (e.g., phenylphosphoralaninates **2b** and **3b**) increased the antiviral activity of the parent compounds against several viruses.[5,6]

Therefore, it was of interest to investigate antiviral activity of phosphorotryptophanates **2c** and **3c** and compare their biological potency with the parent analogues **2a** and **3a** and the phosphoralaninates **2b** and **3b**.

Pronucleotides **2c** and **3c** were obtained from synadenol (**2a**) and *E*-isomer[7] **2b** by a procedure described for the corresponding derivatives of AZT[8] (Scheme 1).

Starting analogue **2a** was converted to the respective phosphite[9] **2d** with diphenyl phosphite in pyridine. Intermediate **2d** was then transformed to target phosphoramidate[10] **2c** by a stepwise treatment with trimethylsilyl chloride in pyridine, iodine and tryptophan methyl ester. Pronucleotide **3c** was prepared[11] by a similar procedure from the *E*-isomer **3a** via phosphite **3d**.

The antiviral properties of pronucleotides **2c** and **3c** as well as comparison with parent analogues **2a** and phosphoralaninates **2b** are summarized in Table 1. Overall, the level of antiviral activity of phosphorotryptophanate **2c** corresponded to parent synadenol (**2a**). Potent effects on antiviral activity[5,6] observed in most of the antiviral assays of analogues **2b** were noticeably absent. Against HBV, the activity of **2c** was 4-fold higher than

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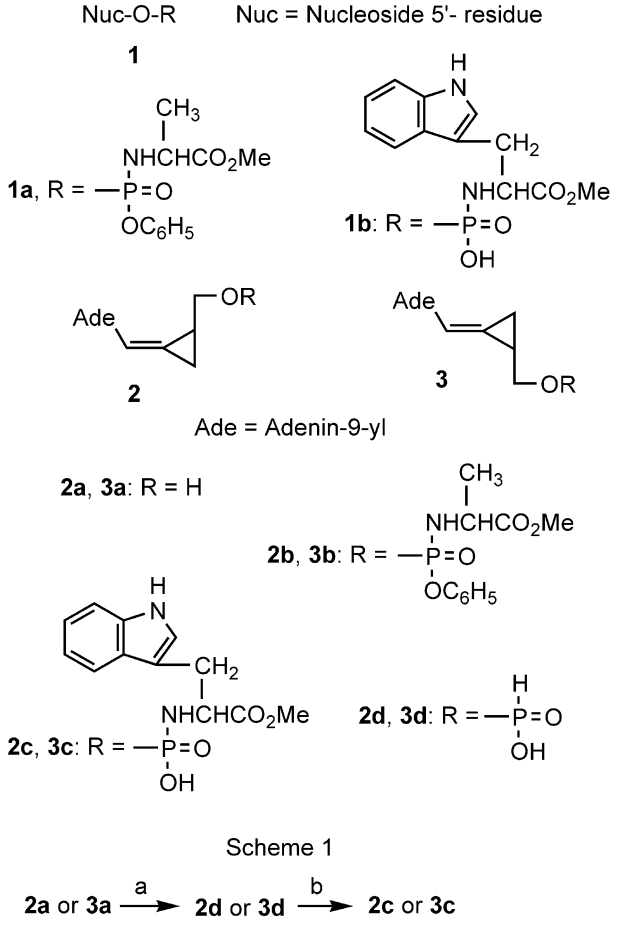
Table 1. Comparison of antiviral activity and cytotoxicity (EC_{50}/CC_{50}, μM) of synadenol (**2a**), phenylphosphoralaninate **2b** and phosphorotryptophanate **2c**

Virus/cells[a]	2a	2b	2c	Control
HCMV (Towne)/HFF[b]	2.1/>100	0.14/2.5	1.5/>100	0.52/>1[i]
HCMV (AD 169)/HFF[c]	1.0/303	1.3/4.5	5.2/90	0.47/>392[i]
HSV-1/BSC-1[d]	26/78	2.5/0.5	15/>100	3.5/>100[i]
HSV-1/HFF[c]	>92/433	<0.07/18	>184/>184	8.4/444[j]
HSV-2/HFF[c]	80/433	1.1/18	>184/>184	3.3/>444[j]
HSV-1/Vero[b,e]	28/>100	>1/<10	31/91	9[j]
HSV-2/Vero[b,e]	59/>100	>1/<10	40/91	25[j]
EBV/Daudi[f]	3.2/368	1.0/>108	>18/57[g]	6.7/>222[j]
VZV/HFF[b]	2.5/368	7.6/101	5.0/111	1.6/>444[j]
HIV-1/MT-2[c]	0.75/32	0.003/0.24	1/10	0.04/>10[k]
HBV/2.2.15[e,h]	2/>100[e]	0.01/0.3	0.49/91[e]	1.4[l]

[a]Values for **2a** and **2b** were taken from refs 3, 5 and 6 or represent unpublished data. Refs 3 and 6 also describe the antiviral assays used.
[b]Plaque reduction assay.
[c]Cytopathic effect inhibition assay.
[d]ELISA, cytotoxicity was determined in KB cells.
[e]Cytotoxicity was determined in CEM cells.
[f]Viral capsid antigen immunofluorescence assay (VCA-IF).
[g]VCA ELISA.
[h]HBV-DNA inhibition assay.
[i]Ganciclovir.
[j]Acyclovir.
[k]AZT.
[l]ddC.



Scheme 1. (a) $(PhO)_2P(O)H$, pyridine; (b) (1) TMSCI, pyridine; (2) I_2, pyridine; (3) Trp (OMe).

that of synadenol (**2a**). With the *E*-isomer **3c** a strong activity not accompanied by cytotoxicity was seen in the EBV/Daudi cell system (EC_{50}/CC_{50} 0.88/>92 μM) which surpassed phenylphosphoralaninate **3b** (EC_{50}/CC_{50} 3.2/65 μM) and it was significantly higher than that of the parent analogue **3a** (EC_{50}/CC_{50} 71/>230 μM).[5] Only a marginal potency was detected against VZV/HFF (EC_{50}/CC_{50} 34/>184 μM). In all other assays, the *E*-isomer **3c** was inactive.

Antitumor activity of pronucleotides **2c** and **3c** was investigated by disk-diffusion assay.[12] The *Z*-isomer **2c** exhibited a moderate effect which was selective for solid tumors (Table 2). By contrast, synadenol (**2a**) and phosphoralaninate **2b** exhibited a nonselective cytotoxicity. The *E*-isomer **3c** was inactive.

The antiviral activity profiles (Table 1) are in accord with a proposition that, in contrast to phosphoralaninate **2b** and pronucleotide **2c**, is not capable of significantly increasing the cellular level of phosphorylated metabolites (monophosphate) beyond that observed with the parent analogue **2a**. It is possible that compound **2c** is enzymatically cleaved to **2a** either before it penetrates the cell membrane or inside the cells. By contrast, results with the *E*-isomer **3c** effective against EBV in Daudi cells argue for an increased intracellular delivery of phosphorylated species. Interestingly, phosphoramidates **2c** and **3c** were not hydrolyzed by porcine liver esterase,[13] an enzyme widely used as a model for intracellular esterase(s).[14,15] This hydrolysis is essential for activation of phosphoralaninates of type **1b**. The mechanism of action of both types of prodrugs must then be different.

Table 2. Comparison of antitumor activity (units/500 μg/disk) of synadenol (**2a**), phosphoralaninate **2b** and phosphoramidate **2c** in disk-diffusion assay[a]

Compd	Leukemia L1210	Mouse colon 38	Human HCT15/Mdr[b]	Normal cells (fibroblasts)
2a	800–950	>950	600–800[c]	800–900
2b	300–350	500	200[d]	100–230
2c	0	550	300–400	0–100
SR271425[e]	0–190	650–750	60–150	0–110

[a]Tumor cells are seeded in soft agar. The drug is placed on a Whatman No. 1 paper disk (6.5 mm). The dried disks are placed on the top of the soft agar midway between the center and the edge of 60-mm plates. The drug diffuses off the disk creating a zone of inhibition of colony formation. The plates are then examined on an inverted microscope for measurement of the zone of inhibition. A zone of inhibition measured from the edge of the disk to the first colony of less than 150 units (1 unit = 32 μm) indicates an agent of insufficient cytotoxic activity. A difference of at least 250 units between the zone for leukemia and solid tumor is indicative of a significant differential effect.[12]
[b]Multiple drug-resistant human colon tumor.[15]
[c]CX-1.
[d]H116.
[e]11 μg/disk.[16]

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9. **Synadenol phosphite (2d, triethylammonium salt)**. Diphenyl phosphite in pyridine (5 mL) was added dropwise over a period of 20 min to a solution of synadenol[7] (**2a**, 370 mg, 1.71 mmol) in pyridine (4 mL) under N_2 at room temperature with stirring. The stirring was continued for 8 h and then at 60 °C for an additional 8 h. Triethylamine (2 mL) was added followed by water (2 mL), the volatile components were evaporated in vacuo and the crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH = 50/20/0.5) to give a white solid **2d** (360 mg, 55%), mp 234–236 °C. UV_{max} (EtOH) 261 nm (ε 12,600), 225 nm (ε 25,200); ^1H NMR (D_2O) δ 8.13 (s, 1H), 7.76 (s, 1H), 6.82 (s, 1H), 4.03–3.97 (m, 1H), 3.67 dd, 1H, J = 19.2 and 8.4 Hz), 3.07 (q, 6H, J = 7.2 Hz), 2.19–2.14 (m, 1H), 1.61–1.56 (m, 1H), 1.31–1.28 (m, 1H), 1.60 (t, 9H, J = 7.2 Hz); ^{13}C NMR 154.7, 152.1, 146.4, 138.5, 117.3, 116.6, 110.0, 65.9, 46.8, 17.4 (d, J = 15.8 Hz), 8.42, 6.70; ^{31}P NMR 6.8; ESI-MS 383 (MeOH, 20.7, M + H), 102 (100.0). Phosphite **3d** (mp 223–225 °C) was prepared from the *E*-isomer[7] **3a** as described for **2d**.
10. **Methyl synadenol phosphorotryptophanate** (**2c**). A stirred suspension of phosphite **2d** (191 mg, 0.5 mmol) in pyridine (20 mL) prepared by sonication was treated with trimethylsilyl chloride (TMSCl, 199 μL, 1.5 mmol) at room temperature. After 5 min, a solution of I_2 (190 mg, 1.5 mmol) in pyridine was added, followed (after 10 min) by tryptophan methyl ester hydrochloride (250 mg, 1 mmol) and Et_3N (1 mL). The reaction mixture was stirred for 30 min, the volatile components were evaporated, the residue was partitioned between 1 N NH_4OH (20 mL) and CH_2Cl_2 (20 mL), the aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and it was evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH = 50/20/0.5) to give product **2c** as a white solid (150 mg, 61%), mp 153–155 °C; UV_{max} (EtOH) 264 nm (ε 11,900), 221 nm (ε 25,200); ^1H NMR (DMSO-d_6 + D_2O) δ 8.69 (s, 1H), 8.15 (s, 1H), 7.92–7.34 (m, 2H), 7.26 (d, 1H, J = 8.0 Hz), 7.05 (d, 1H, J = 9.2 Hz), 6.97 (t, 1H, J = 8.0 Hz), 6.86 (dd, 1H, J = 17.0 and 8.0 Hz), 3.91–3.84 (m, 1H), 3.76–3.68 (m, 1H), 3.44–3.36 (m, 1H), 3.38 and 3.32 (I2s, 3H), 2.98–2.90 (m, 2H), 2.13–1.98 (m, 1H), 1.48–1.43 (m, 1H), 1.16–1.10 (m, 1H); ^{13}C NMR (DMSO-d_6) 175.5, 156.7, 153.7, 148.8, 148.6, 138.5, 136.7, 128.0, 124.4, 121.4, 118.9, 115.4, 112.0, 111.0, 110.4, 66.3, 56.3, 51.9, 31.0, 18.0, 7.0; ^{31}P NMR 4.0; ESI-MS (20% MeOH, NaCl) 498 (82.0, M + H), 520 (21.9, M + Na), 85 (100.0). Anal. calcd for $C_{22}H_{24}N_7O_5P{\cdot}2.5H_2O$: C 49.53, H 5.57, N 17.49, P 5.81. Found: C 49.50, H 5.43, N 17.73, P 6.11.
11. ***E*-isomer 3c**. The procedure described for the *Z*-isomer **2c** was followed starting from phosphite **3d** to give phosphorotryptophanate **3c** as a white solid (170 mg, 69%), mp 148–150 °C; UV_{max} (EtOH) 264 nm (ε 11,900), 221 nm (ε 25,200); ^1H NMR (DMSO + D_2O) δ 8.43 (d, 1H, J = 1.6 Hz), 8.16 (s, 1H), 7.46–7.39 (m, 2H), 7.30–7.24 (m, 1H), 7.01 (s, 1H), 6.97 (t, 1H, J = 8.0 Hz), 6.90 (dd, 1H, J = 15.2 and 7.2 Hz), 3.90 (dd, 1H, J = 14.4, 7.2 Hz), 3.55–3.45 (m, 1H), 3.42 and 3.39 (I2s, 3H), 3.38–3.29 (m, 1H), 3.03–2.92 (m, 2H), 2.13–1.98 (m, 1H), 1.48–1.43 (m, 1H), 1.16–1.10 (m, 1H); ^{13}C NMR 175.5, 156.7, 153.7, 148.8, 137.9, 136.7, 128.0, 124.4, 121.4, 119.0, 118.8, 116.0, 112.0, 111.3, 110.4, 66.1, 56.4, 51.9, 31.0, 16.4,

10.4; ^{31}P NMR 4.7; ESI-MS (20% MeOH, NaCl) 498 (51.5, M + H), 520 (32.9, M + Na), 202 (100.0). Anal. calcd for $C_{22}H_{24}N_7O_5P \cdot 2.5H_2O$: C 49.53, H 5.57, N 17.49, P 5.81. Found: C 49.29, H 5.80, N 17.14, P 6.26.

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13. **Attempted degradation of phosphoramidates 2c and 3c with porcine liver esterase**. Esterase (200 units) was added to a stirred solution of phosphoramidate **2c** or **3c** (0.75 mg, 1.5 μmol) in 0.02 M Na_2HPO_4 (pH 7.4. 0.5 mL). The mixtures were incubated with stirring at 37 °C for 30 h. TLC (CH_2Cl_2/MeOH/NH_4OH = 5/2/0.25) showed only a single spot of starting material.

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