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SYNTHESIS AND EVALUATION OF AMINO ACID ESTER PRODRUGS OF PENCICLOVIR

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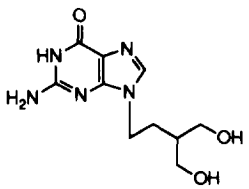
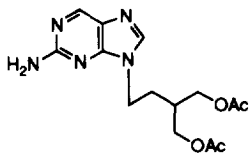
Abstract : The synthesis, aqueous solubility and stability, *in vitro* antiviral activity, and oral bioavailability of the amino acid ester prodrugs of penciclovir, **13–20**, **23**, and **24**, are described. All of the prodrugs were highly soluble (>100 mg/mL) and sufficiently stable in aqueous solution. The oral bioavailability of *O*-acetyl-*O*-L-valylpenciclovir (**13**) (34 %) in mice was comparable to that of famciclovir (33 %) and approximately 4-fold higher than that of penciclovir.

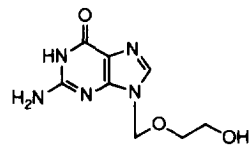
An acyclonucleoside 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (penciclovir) is a potent and highly selective inhibitor of the replication of herpesviruses including herpes simplex virus type 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) in cell cultures and in animals.[1,2] The antiviral spectrum of penciclovir against human herpesviruses is similar to that of 9-(2-hydroxyethoxymethyl)guanine (acyclovir), and both compounds have comparable activity against these viruses.[3] The advantage of penciclovir over



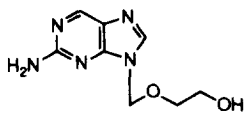
Penciclovir



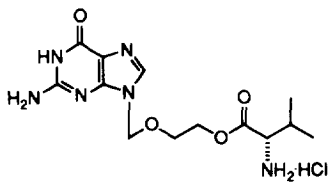
Famciclovir



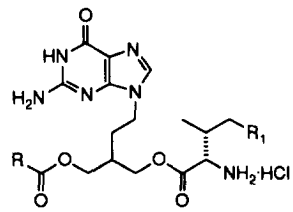
Acyclovir



6-Deoxyacyclovir



Valacyclovir



13–20 : R = Me, Et, *n*-Pr, *i*-Pr
R_1 = H, Me

acyclovir is that its antiviral activity in cell culture is more persistent than that of acyclovir since penciclovir triphosphate is much more stable than acyclovir triphosphate within infected cells.[2,4] However, like other acyclic nucleoside analogs such as acyclovir,[5] ganciclovir,[6] and buciclovir,[7] penciclovir has poor oral bioavailability in mice and rats.[8,9] In order to overcome this inadequate oral bioavailability, 2-amino-9-(4-acetoxy-3-acetoxymethylbut-1-yl)purine (famciclovir), the diacetyl 6-deoxy analog of penciclovir, has been developed as a prodrug of penciclovir.[8] Famciclovir is orally well absorbed and then extensively converted to penciclovir by the enzymatic removal of two *O*-acetyl groups, followed by oxidation at the 6-position of the purine ring by xanthine oxidase in mice,[8] rats,[9] and humans.[10] Famciclovir has recently been approved by FDA for the treatment of herpes zoster (shingles).

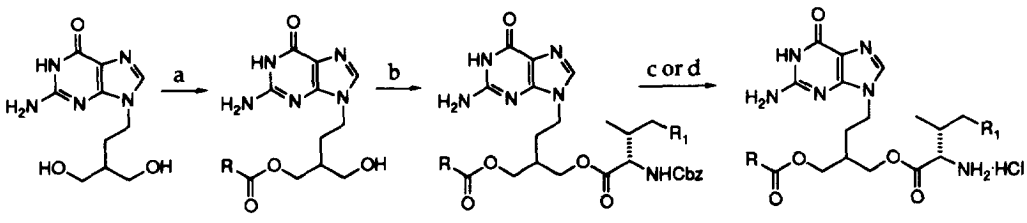
Although 6-deoxyacyclovir is known to be absorbed rapidly after oral administration and extensively oxidized to acyclovir by xanthine oxidase, its chronic toxicity profile in experimental animal models is not as favorable as that of acyclovir itself.[11] The toxicity of 6-deoxyacyclovir observed in laboratory animals was assumed to be the result of phosphorylation of the unconverted prodrug.[11] The L-valyl ester of acyclovir (valacyclovir), a prodrug of acyclovir, is also rapidly and extensively converted to acyclovir after oral administration; the resulting plasma levels of acyclovir in rats and humans are 3 to 5 times higher than those attainable with oral acyclovir itself.[11,12] When adjusted to equivalent plasma levels, valacyclovir showed the same safety profile as acyclovir in a variety of subchronic and chronic toxicity studies in laboratory animals and humans since it could not be phosphorylated before conversion to acyclovir due to the lack of a free hydroxyl group.[11]

In clinical studies, approximately 65 % of the administered dose of famciclovir is excreted in urine as penciclovir (60 %) and 2-amino-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (6-deoxypenciclovir) (5 %).[10] Although a notable toxicity profile of 6-deoxypenciclovir has not been reported yet, there is a possibility that it might show toxicity in long-term treatment since it could also be phosphorylated. Therefore, in this report, we synthesized the *O*-acyl-*O*-amino acid esters of penciclovir as potential prodrugs of penciclovir that hopefully would show the same safety profile as penciclovir. The two naturally occurring branched chain amino acyl groups, L-valyl and L-isoleucyl, have been selected for the protection of one of the two hydroxyl groups since it has been previously shown that they have optimal combination of side chain length and degree of branching for the oral bioavailability and chemical stability in the amino acid ester prodrugs of acyclovir.[11] For further refinement of oral bioavailability, the lower acyl groups, such as acetyl, propionyl, butyryl, or isobutyryl, have been introduced into the other hydroxyl group.

Chemistry

Target compounds, the *O*-acyl-*O*-amino acid esters of penciclovir **13–20** were synthesized as shown in Scheme 1. Penciclovir was first treated with large excess of an appropriate acid anhydride in DMF in the presence of pyridine at room temperature for 24 h to afford the corresponding mono-ester derivatives **1–4** in 37–43 % yields. Further esterification of the mono-ester compounds with *N*-carbobenzyloxy-L-valine or -L-isoleucine using conventional coupling method (DCC/DMAP) in DMF provided the amino acid derivatives **5–12** as a mixture of two

Scheme 1[a]



1 : R = Me (41 %)
2 : R = Et (43 %)
3 : R = *n*-Pr (37 %)
4 : R = *i*-Pr (38 %)

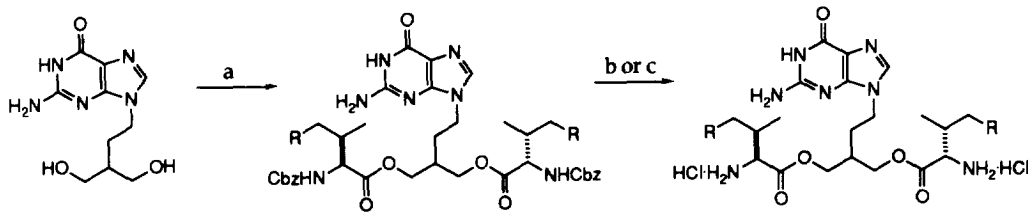
5 : R = Me, R_1 = H (88 %)
6 : R = Et, R_1 = H (84 %)
7 : R = *n*-Pr, R_1 = H (89 %)
8 : R = *i*-Pr, R_1 = H (87 %)
9 : R = R_1 = Me (86 %)
10 : R = Et, R_1 = Me (84 %)
11 : R = *n*-Pr, R_1 = Me (87 %)
12 : R = *i*-Pr, R_1 = Me (88 %)

13 : R = Me, R_1 = H (95 %)
14 : R = Et, R_1 = H (92 %)
15 : R = *n*-Pr, R_1 = H (93 %)
16 : R = *i*-Pr, R_1 = H (90 %)
17 : R = R_1 = Me (97 %)
18 : R = Et, R_1 = Me (92 %)
19 : R = *n*-Pr, R_1 = Me (96 %)
20 : R = *i*-Pr, R_1 = Me (87 %)

[a](a) $(RCO)_2O$ (10 equiv.), pyridine, DMF, rt, 24 h; (b) (i) *N*-Cbz-L-valine or *N*-Cbz-L-isoleucine (1.35 equiv.), DCC (1.62 equiv.), DMAP (0.15 equiv.), DMF, rt, 16 h; (ii) repeat process (i); (c) H_2 (1 atm), 10 % Pd/C, MeOH–H_2O (9:1), rt, 4 h, then 2*N* HCl (aq) (1.5 equiv.), MeOH–$CHCl_3$ (1:1) (for **13–16**); (d) H_2 (1 atm), 10 % Pd/C, 2*N* HCl (aq) (1.5 equiv.), MeOH–H_2O (9:1), rt, 5 h (for **17–20**).

diastereomers in good yields (84–89 %). Reductive cleavage of carbobenzyloxy (Cbz) group and formation of HCl salt were attempted in one-pot reaction by performing hydrogenolysis in the presence of aqueous HCl solution. This attempt turned out to be successful for the isoleucine derivatives, and the desired isoleucine salt products **17–20** were obtained in pure form in good to excellent yields (87–97 %). In contrast, the *O*-L-valinate HCl salt compounds were contaminated with a small amount of unknown impurity which could not be removed by either chromatography or crystallization. Thus, the removal of Cbz group and HCl salt formation for the *O*-L-valinate derivatives were carried out in stepwise fashion. Hydrogenolysis of Cbz group in the absence of aqueous HCl solution and subsequent treatment of the resulting free amine

Scheme 2[a]



21 : R = H (98 %)
22 : R = Me (97 %)

23 : R = H (99 %)
24 : R = Me (99 %)

[a](a) (i) *N*-Cbz-L-valine or *N*-Cbz-L-isoleucine (3.0 equiv.), DCC (3.5 equiv.), DMAP (0.28 equiv.), DMF, rt, 16 h; (ii) repeat process (i); (b) H_2 (1 atm), 10 % Pd/C, MeOH–H_2O (9:1), rt, 4 h, then 2*N* HCl (aq) (3.0 equiv.), MeOH–$CHCl_3$ (1:1) (for **23**); (c) H_2 (1 atm), 10 % Pd/C, 2*N* HCl (aq) (3.0 equiv.), MeOH–H_2O (9:1), rt, 5 h (for **24**).

compounds with aqueous HCl solution afforded the desired HCl salts of *O*-L-valinate derivatives **13–16**, free from any impurities, in excellent yields (90–95 %). It was apparent from their ^1H and ^{13}C NMR spectra that the final compounds **13–20** consisted of two diastereomers in equal amount. Two asymmetric carbon centers and two carbons bridging them were showed up as pairs in their ^{13}C NMR spectra. For example, the ^{13}C NMR spectrum of **13** in DMSO-d_6 showed pairs of two asymmetric carbons at 34.07, 34.14, 57.33, and 57.40 ppm, a pair of methylene carbon at 63.16 and 63.22 ppm, and a pair of carbonyl carbon at 168.41 and 168.53 ppm, respectively. As previously shown in the amino acid ester prodrugs of acyclovir,[11] the stereochemistry of the prodrugs might affect hydrolytic cleavage and absorption. Thus, it seems necessary to separate afore-mentioned diastereomers before biological evaluation. However, since the separation of those isomers was practically difficult even by using HPLC, they were tested without further separation. Penciclovir *O*,*O*-di-L-valinate (**23**)[13] and -L-isoleucinate (**24**) were also prepared for comparison with the *O*-acyl-*O*-amino acid ester derivatives as shown in Scheme 2. *N*-Carbobenzyloxy-L-valine or -L-isoleucine were coupled with penciclovir under typical condition (DCC/DMAP) in DMF to afford the di-ester derivatives **21** and **22** in excellent yields (97–98 %). Hydrogenolysis of Cbz group of **21** and **22** followed by HCl salt formation under the conditions described above produced compounds **23** and **24** in almost quantitative yields.

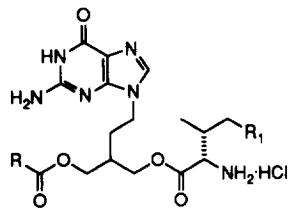
Results and Discussion

All the amino acid ester prodrugs of penciclovir were highly soluble in water (>100 mg/mL) at 25 °C, showing a remarkable increase in aqueous solubility compared with that of penciclovir (3.2 mg/mL). The aqueous stability of the prodrugs was examined at pH 1.0, pH 6.0, pH 7.4, and pH 8.0 at 37 °C, and the calculated half-lives ($t_{1/2}$) are shown in Table 1. The $t_{1/2}$ values at pH 1.0, pH 6.0, pH 7.4, and pH 8.0 for the *O*-acyl-*O*-amino acid esters of penciclovir **13–20** were 19.0–47.5 h, 83.5–121.6 h, 17.3–27.0 h, and 13.2–19.9 h, respectively. It was observed that penciclovir *O*,*O*-di-L-valinate (**23**) and -L-isoleucinate (**24**) were much more stable at pH 1.0, but less stable at pH 6.0, pH 7.4, and pH 8.0 than the *O*-acyl-*O*-amino acid esters of penciclovir **13–20**. The *in vitro* antiviral activity of the prodrugs **13–20**, **23**, and **24** against HSV-1 (KOS strain) in Vero cells was compared with that of penciclovir. Compounds **13**, **14**, **16**, **17**, **18**, and **20** showed no significant antiviral activity at concentrations up to 100 μM. Although compounds **15**, **19**, **23**, and **24** were active against HSV-1 replication with IC_{50} values of 42.0, 24.2, 25.5, and 42.3 μM, respectively, their antiviral activity was approximately 3- to 5-fold lower than that of penciclovir (8.1 μM). Antiviral activity of these prodrugs is probably due to their partial hydrolysis to penciclovir in the test system. No cytotoxicity of the prodrugs to Vero cells was observed even at the maximum concentration of 500 μM.

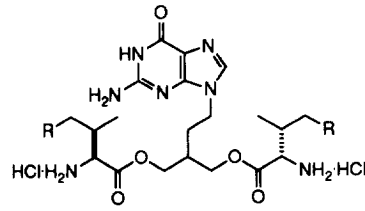
The bioavailability of penciclovir after a single oral administration (0.2 mmol/kg) of the various amino acid ester prodrugs in mice was estimated by determining the total amount of penciclovir recovered in the urine over a 48-h period. Parent prodrugs and their mono-ester type metabolites were also assayed by reversed-phase HPLC with UV detection. However, they were not detected in the urine, suggesting their complete *in vivo* metabolic conversion to penciclovir. Of the prodrugs tested, *O*-acetyl-*O*-L-valylpenciclovir (**13**)[14] achieved the highest penciclovir

bioavailability (34 %), followed by *O*-butyryl-*O*-L-valylpenciclovir (**15**) (29 %), *O*-propionyl-*O*-L-valylpenciclovir (**14**) (25 %), and penciclovir *O,O*-di-L-valinate (**23**) (25 %). It should be stressed that the bioavailability of **13** was comparable to that of famciclovir (33 %) and approximately 4-fold higher than that of penciclovir. As previously observed,[11] the L-isoleucine ester derivatives showed lower bioavailability compared with the L-valine ester derivatives.

Table 1. Stability in Aqueous Solution, *In Vitro* Anti-HSV-1 Activity, and Oral Bioavailability in Mice of Amino Acid Ester Prodrugs of Penciclovir **13–20**, **23**, and **24**



13–20



23 and **24**

compound	R	R_1	half-life[a](h, 37 °C) pH 1.0[b]	pH 6.0[c]	pH 7.4[c]	pH 8.0[c]	EC_{50}[d](μM) KOS	urinary recovery of penciclovir (% dose)[e]
13	Me	H	19.4	108.3	17.6	13.2	>100[f]	34
14	Et	H	19.2	103.5	17.3	13.6	>100	25
15	*n*-Pr	H	33.3	121.6	18.4	14.1	42.0	29
16	*i*-Pr	H	47.5	119.5	19.1	14.4	>100	20
17	Me	Me	19.4	105.0	24.8	19.1	>100	15
18	Et	Me	19.0	83.5	25.0	19.9	>100	20
19	*n*-Pr	Me	34.0	93.7	27.0	19.9	24.2	19
20	*i*-Pr	Me	46.5	83.5	24.9	19.4	>100	13
23	H		>168	30.9	6.7	6.0	25.5	25
24	Me		>168	52.9	11.4	9.8	42.3	9
penciclovir							8.1	9
famciclovir							>100	33

[a]Determined by HPLC using a C_{18} reversed-phase column. [b]HCl/NaCl buffer. [c]Sodium phosphate buffer. [d]Concentration required to inhibit virus-induced CPE by 50 % of the virus-infected control. [e]A single oral dose of test compound (0.2 mmol/kg) was administered to six male ICR mice. The total amount of penciclovir recovered in the urine over a 48-h period was determined by HPLC using a C_{18} reversed-phase column. [f]Values are the mean of at least two independent experiments run in quadruplicate.

On the basis of its high oral bioavailability in mice and its sufficient solubility and stability in aqueous solution, further evaluation of **13** as a hydrochloride salt is presently under way in our laboratory.

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14. **13** : IR (KBr) 3420, 3115, and 2968 (NH), 1740, 1719, and 1691 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6/TMS) δ 0.93 (d, J = 6.6 Hz, 1.5 H, CH_3), 0.94 (d, J = 6.6 Hz, 1.5 H, CH_3), 0.96 (d, J = 6.6 Hz, 1.5 H, CH_3), 0.97 (d, J = 6.6 Hz, 1.5 H, CH_3), 1.92 (m, 2 H, NCH_2CH_2), 2.00 (m, 1 H, CH, overlapped with $COCH_3$), 2.01 (s, 3 H, $COCH_3$), 3.85 (m, 1 H, αCH), 4.06 (m, 2 H, NCH_2), 4.10–4.25 (m, 4 H, 2 CH_2OCO), 7.05 (s, 2 H, NH_2), 8.54 (s, 1 H, H-8), 8.68 (br s, 3 H, NH_3^+), 11.37 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 17.56 ($CHCH_3$), 18.30 ($CHCH_3$), 20.57 ($COCH_3$), 27.55 (NCH_2CH_2), 29.24 ($CH(CH_3)_2$), 34.07 and 34.14 (CH), 41.23 (NCH_2), 57.33 and 57.40 (αC), 63.16 and 63.22 (CH_2OCOCH), 64.70 (CH_2OCOCH_3), 112.12 (C-5), 137.09 (C-8), 150.30 (C-4),154.62 (C-2), 154.95 (C-6), 168.41 and 168.53 (COCH), 170.23 ($COCH_3$); FAB-MS m/z 395 (MH^+); *Anal.* Calcd for $C_{17}H_{27}ClN_6O_5$: C, 47.39; H, 6.32; N, 19.50. Found: C, 47.16; H, 6.51; N, 19.42.

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