New Analogues of Acyclovir – Synthesis and Biological Activity

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New acyclovir esters with peptidomimetics were synthesized and evaluated *in vitro* for their antiviral activity against the replication of Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). The influence of peptidomimetics containing oxazole and thiazolyl-thiazole moieties on the antiviral activity is also reported. The esters were synthesized using the coupling reagents *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) as a catalyst.

Key words: Peptidomimetics, Acyclovir, HSV

Introduction

The discovery of acyclovir, 9-[(2-hydroxyethoxy)methyl] guanine (ACV) as a selective antiherpes agent heralded a new era in antiviral chemotherapy (Elion *et al*., 1977). ACV is an acyclic nucleoside analogue of guanosine. The problem with ACV is its high lipophilicity and, from this, its low bioavailability. Its limited absorption (15%–20%) in humans after oral administration prompted the search for prodrugs (De Clercq *et al.* 2006; Balzarini *et al.* 2004). A possible way to increase the bioavailability is by modifying the known antiviral drugs with various amino acids (Beauchamp *et al*., 1992; Zacchigna *et al*., 2002; Anand *et al*., 2003, 2004a; Nashed and Mitra, 2003). Amino acid ester prodrugs of nucleoside antiviral drugs have been employed to increase the oral bioavailability of the parent drugs.

The L-valyl ester of acyclovir (valacyclovir) is obtained in this manner (Beauchamp and Krenitsky, 1993). Valacyclovir is such a prodrug, which is derived from ACV by esterifying ACV with L-valine. Upon administration valacyclovir is rapidly and completely converted to ACV, the active parent drug, by enzymatic hydrolysis (Anand *et al,* 2004a, b; Anand and Mitra, 2002). The prodrug increases the oral bioavailability of ACV in humans three- to five-fold. Enhanced oral absorption of ACV has been attributed to the human peptide transporter-mediated transport of valacyclovir. The compound is recognized as a peptidyl derivative and absorbed by peptide transporters, even though there is no peptide bond in its structure (Spruance *et al.,* 2002; Painter and Hostetler, 2004; Field *et al.,* 2003).

Modification of anti-herpes agents like ACV by peptidomimetics, whose chemical structures are different from those of the natural peptides but have the same ability to interact with specific receptors, is of definite interest (Field *et al.* 2003; Vabeno *et al*., 2004a, b).

Considering all these facts, we have been interested in looking for other esters of ACV. Here we report the synthesis of oxazole- and thiazolyl-thiazole-containing amino acid esters of ACV and exploration of their activity on the Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2).

Results and Discussion

In the last two decades, inprecedented biologically active natural products containing directly linked azoles have been isolated from natural sources. Many of these compounds are candidates for drug development. In particular thiazole, oxazole and imidazole amino acids that may play a key role in biological activities of unusual peptides are important intermediates for natural product synthesis and peptidomimetics.

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ACV modified with amino acids and peptides is found, but ACV containing peptidomimetics is not know till now. In order to obtain analogues with more desirable characteristics, we synthesized new esters of ACV containing Boc-2-aminomethyl-oxazole-4-carboxylic acid and Fmoc-2-(2'-aminomethyl-thiazol-4'-yl)-thiazole-4-carboxylic acids.

Synthesis of esters of ACV

A mixture of Boc-2-aminomethyl-oxazole-4-carboxylic acid (**1a**) or Fmoc-2-(2'-aminomethyl-thiazol-4'-yl)-thiazole-4-carboxylic acid (**1b**) and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in dimethylformamide (DMF) with the EDC/amino acid ratio 1:2 was stirred for 1 h at 0 °C under nitrogen atmosphere (Nakajima and Ikada, 1995).

A solution of ACV (**2**) (Fig. 1) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) was added to the reaction mixture and stirred for 24 h. Then DMF was evaporated *in vacuo,* and the residue was chromatographed on silica gel, using MeOH/CH_2Cl_2 (1:4). The ^1H, ^{13}C NMR and mass spectra of the compounds were consistent with the desired structures.

Antiviral activity

The two esters of ACV, **3a** and **3b**, were explored against HSV-1 and HSV-2. They were tested in the following concentrations: 100, 40, 20, 10, 5 and 1 μg/ml. The two modifications of ACV slightly affected the replication of HSV-1 in the same mode (Fig. 2a). Applied in the maximal tested dose (100 μg/ml) they suppressed the virus by 60% and 49%. Their effects at 10 μg/ml were same – within 20% and 5%. The ED_{50} value of **3b** was 78.4 μg/ml, whereas the ED_{50} value of ACV, 1.2 μg/ml, differed considerably. The referent drug in same dose inhibited completely the replication (Golankiewicz *et al.*, 2001). The influence of these esters on the replication of HSV-2 were analogical (Fig. 2b). The established activities were correlative with our results for application of similar prodrugs against the replication on these viral strains (Stankova *et al.*, 2007).

In conclusion, in this study we extended the scope on modification of ACV with various peptidomimetics.

First, two novel esters with peptidomimetics of ACV were synthesized. One oxazole-containing dipeptide mimetic and one tripeptide mimetic with two fused 5-ring heterocycles derived from glycine were used. The ESI-MS and NMR analyses proved the identity of the final products **3a** and **3b**.

Second, the results of the antiviral activity test showed that compounds **3a** and **3b** affect slightly the replication of HSV-1 and HSV-2.

Third, the results of our investigations showed that modification of ACV with amino acids containing oxazole and thiazolyl-thiazole reduce the antiviral effect in comparison with modifications of ACV with natural amino acids (Beauchamp *et al.*, 1992).

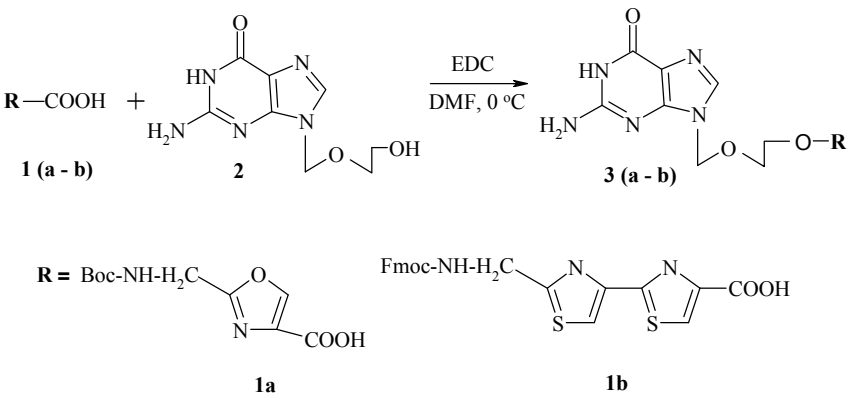

Fig. 1. Synthesis of *N*-α-*tert*-Boc-2-aminomethyl-oxazol-4-yl-acyclovir (**3a**) and *N*-α-Fmoc-2-(2'-aminomethyl-thiazol-4'-yl)-thiazol-4-yl-acyclovir (**3b**).

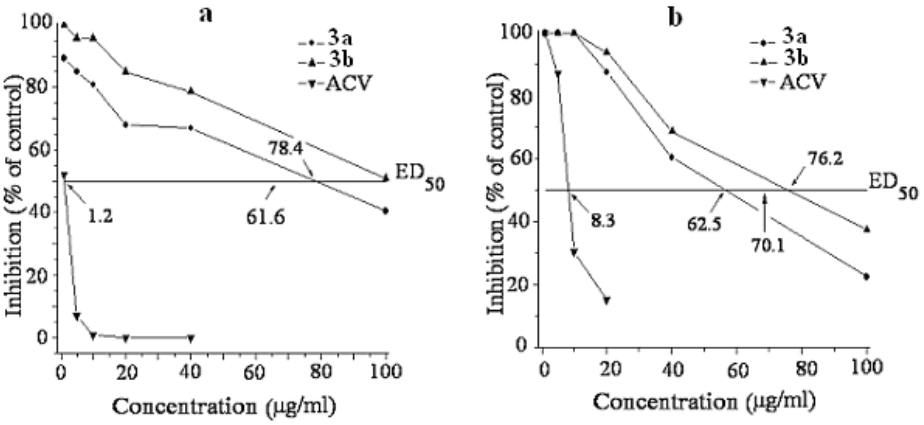

Fig. 2. *In vitro* antiviral activity of **3a** and **3b** (a) on the replication on HSV-1 and (b) on the replication on HSV-2.

Material and Methods

Chemicals

The amino acids were purchased from Sigma, DMAP and EDC were purchased from Merck.

TLC analysis was performed on aluminium silica gel 60 F_{254} plates (Merck) and detection was performed using an UV lamp at 254 nm.

NMR spectroscopy: Bruker Avance DRX-600 spectrometer; chemical shifts referenced to the solvent peaks [δ (^{1}H, [D$_6$]-DMSO) = 2.49 and δ (^{13}C, [D$_6$]-DMSO) = 39.5].

Mass spectrometry: API III triple quadrupole mass spectrometer equipped with an electrospray ion source at atmospheric pressure (Sciex, Thornhill, Canada); electrospray ionization (EI) mass spectra were recorded in the positive ion mode.

*Synthesis of **1a** and **1b***

1a and **1b** were prepared according to Videnov *et al.* (1996) and Stankova *et al.* (1999).

*N-α-tert-Boc-2-aminomethyl-oxazol-4-yl-acyclovir (**3a**)*

A mixture of **1a** (0.480 g, 2 mmol) and EDC (0.191 g, 2 mmol) in DMF was stirred for 1 h at 0 °C under nitrogen atmosphere. A solution of ACV (**2**) (0.225 g, 1 mmol) and DMAP (0.244 g, 2 mmol) was added to the reaction mixture and stirred for 24 h. Then DMF was evaporated *in vacuo,* and the residue was chromatographed on silica gel, using MeOH/ CH_2Cl_2 (1:4).

Yield: 0.187 g (40%). – ^{1}H NMR ([D$_6$]-DMSO): δ = 1.36 (s, 9H, 3CH), 3.47 (m, 2H, CH_2O, ACV), 4.22 [m, 2H, $CH_2OC(O)$, ACV)], 4.34 (d, 2H, CH_2), 5.33 (s, 2H, N-CH_2-O, ACV), 5.36 (br t, 1H, NH), 6.83 (s, 2H, 2-NH_2, ACV), 7.94 (s, 1H, H-8, ACV), 8.15 (s, 1H, CH_{Oxa}), 10.62 (s, 1H, ACV-NH). – ESI-MS: *m/z* = 468 $[M+H]^+$.

*N-α-Fmoc-2-(2'-aminomethyl-thiazol-4'-yl)-thiazol-4-yl-acyclovir (**3b**)*

A mixture of **1b** (0.371 g, 8 mmol) and EDC (0.764 g, 8 mmol) in DMF was stirred for 1 h at 0 °C under nitrogen atmosphere. A solution of ACV (**2**) (0.900 g, 4 mmol) and DMAP (0.976 g, 8 mmol) was added to the reaction mixture and stirred for 24 h. DMF was evaporated *in vacuo*, and the residue was chromatographed on silica gel, using MeOH/CH_2Cl_2 (1:4).

Yield: 0.081 g (30%). – ^{1}H NMR ([D$_6$]-DMSO): δ = 3.44 (t, H-Fmoc), 3.48 (d, 2H-Fmoc), 3.51 (m, 2H, CH_2O, ACV), 3.81 [m, 2H, $CH_2OC(O)$, ACV], 4.46 (d, 2H, CH_2), 5.34 (s, 2H, N-CH_2-O, ACV), 6.50 (s, 2H, 2-NH_2, ACV), 7.29 (t, 2H-Fmoc), 7.39 (t, 2H-Fmoc), 7.55 (br m, 2H-Fmoc), 7.75 (d, 2H-Fmoc), 7.81 (s, 1H, H-8, ACV), 7.94 (t, 1H, NH), 8.12, 8.11 (CH_{Txz}), 10.8 (s, 1H, ACV-NH). – ^{13}C NMR ([D$_6$]-DMSO): δ = 42.0 (CH_2), 47.86 (CH_2-CH_2O, Fmoc), 47.86 (CH_2-CH_2O, Fmoc), 64.13 (CH_2OCO, ACV), 66.28 (CH_2O, ACV), 67.60 (Fmoc), 71.68 (NCH_2O), 116.84 (C-5, ACV), 117.9 ($C_{Thz}^{5'}$), 120.46 (2C, Fmoc), 125.60 (2C, Fmoc), 127.72 (2C, Fmoc), 128.21 (2C, Fmoc), 128.9 (C_{Thz}^{5}), 137.55 (C-8, ACV), 141.87 (2C, Fmoc), 144.68 (2C, Fmoc), 147.3 ($C_{Thz}^{4'}$), 148.2 (C_{Thz}^{4}),

151.08 (C-4, 151.08), 156.63 (C-6, ACV), 157.05 (C-O, Fmoc), 162.0 ($C_{Thz}^{2'}$), 162.2 (C_{Thz}^{2}), 168.71 (C=O, ACV). – ESI-MS: m/z = 671 $[M+H]^+$.

*Antiviral activity of **3a** and **3b** against HSV-1 and HSV-2*

Viruses and cells

The two laboratory strains, DA (HSV-1) and Bja (HSV-2), were kindly provided by Prof. S. Dundarov (National Center of Infectious and Parasitic Diseases, NCIPD, Bulgaria). Madin-Darby bovine kidney (MDBK) cells were cultured at 37 °C as monolayers in RPMI-1640 medium (Flow Laboratories, USA) supplemented with antibiotics (penicillin and streptomycin) and 10% bovine serum (NCIPD). Serum concentration was reduced to 5% for growth of viruses and for testing the compounds.

Cytotoxicity assay – determination of the maximal tolerate concentration (MTC)

To compare the MTC values of substances to that of ACV, confluent monolayers were covered with media containing different concentrations of compounds or reference substance (ACV) and cultured at 37 °C for 96 h. Samples of cells grown in test prodrug-free medium served as a control. The maximal concentration, which did not alter neither the morphology nor viability of the cells, was recognized as MTC.

Antiviral assay

Experiments were done under multicycle growth conditions. Confluent cell monolayers were washed and infected with 320 cell culture infectious doses ($CCID_{50}$) per 0.1 ml of the appropriate virus strain. After 1 h, cells were covered with maintenance media including test drugs at tested concentrations. The effect on viral replication was determined after 48 h (for strains DA and Bja) of culturing at 37 °C by reduction of infectious virus titres as compared to that of the untreated viral control. The 50% inhibitory concentration (IC_{50}) for virus-induced cytopathic effect (CPE) was determined by a dose-response curve. To calculate the standard deviation of IC_{50}, each experiment was done in triplicate (for HSV-1 strain DA) or duplicate (for HSV-2 strain Bja).

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Anand B. S. and Mitra A. K. (2002), Mechanism of corneal permeation of L-valyl ester of acyclovir: targeting the oligopeptide transporter on the rabbit cornea. Pharm. Res. **19**, 1194–1202.

Anand B. S., Nashed Y. N., and Mitra A. K. (2003), Novel dipeptide prodrugs of acyclovir for ocular herpes infection: Bioreversion, antiviral activity and transport across rabbit cornea. Curr. Eye Res. **26**, 151–163.

Anand B. S., Katragadda S., Nashed Y. E., and Mitra A. K. (2004a), Amino acid prodrugs of acyclovir as possible antiviral agents against ocular HSV-1 infection: interaction with the neutral and cationic amino acid transporter on the cornel epithelium. Curr. Eye Res. **29**, 153–166.

Anand B. S., Katragadda S., and Mitra A. K. (2004b), Pharmacokinetics of novel dipeptide ester prodrugs of acyclovir after oral administration: intestinal absorption and liver metabolism. J. Pharmacol. Exp. Ther. **311**, 659 – 667.

Balzarini J., Schols D., Baba I., Field H. J., and de Clercq E. (2004), Antiviral drugs – a short history of their discovery and development. Microbiol. Today **31**, 58–61.

Beauchamp L. M. and Krenitsky T. A. (1993), Acyclovir prodrugs: the road to valacyclovir. Drugs Future **18**, 619–628.

Beauchamp L. M., Orr G. F., de Miranda P., Burnette T., and Krenitsky T. A. (1992), Amino acid ester prodrugs of acyclovir. Antiv. Chem. Chemoth. **3**, 157–164.

De Clercq E., Field J., and Hugh V. (2006), Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy. Br. J. Pharmacol. **147**, 1–11.

Elion G. B., Furman P. A., Fyfe J. A., de Miranda P., Beauchamp L., and Schaeffer H. J. (1977), Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. USA **74**, 5716–5720.

Field H. J., Dejesus E., Wald A., Warren T., Schacker T. W., Trottier S., Shahmanesh M., Hill J. L., and Brennan C. A. (2003), Valacyclovir for the suppression of recurrent genital herpes inhuman immunodeficiency virus-infected subjects. J. Infect. Dis. **188**, 1009–1016.

Golankiewicz B. T., Ostrowski T., Goslinski P., Januszczyk J., Zeidler D., Baranowski S., and de Clercq E. (2001), Fluorescent tricyclic analogues of acyclovir and gancyclovir. A structure-antiviral activity study. J. Med. Chem. **44**, 4284–4287.

Nakajima N. and Ikada Y. (1995), Mechanism of amide formation by carbodiimide for bioconjugation in aqueous media. Bioconjug. Chem. **6**, 123–130.

Nashed Y. E. and Mitra A. K. (2003), Synthesis and characterization of novel dipeptide ester prodrugs of acyclovir. Spectrocim. Acta A **59**, 2033–2039.

Painter G. R. and Hostetler K.Y. (2004), Design and development of oral drugs for the prophylaxis and treatment of smallpox infection. Trends Biotechnol. **22**, 423–427.

Spruance S. L., Jones T. M., Blatter M. M., Vargas-Cortes M., Barber J., Hill J., Goldstein D., and Schultz M. (2002), Valacyclovir cold sore study group. High-dose, short duration, early valacyclovir therapy for episodic treatment of cold sores: results of two randomized, placebo-controlled, multicenter studies. Antimicrob. Agents Chemother. **47**, 1072–1080.

Stankova I. G., Videnov G. I., Golovinsky E. V., and Jung G. (1999), Synthesis of thiazole, imidazole and oxazole containing amino acids for peptide backbone modification. J. Peptide Sci. **5**, 392–398.

Stankova I. G., Dzimbova T., Shishkov St., Kostova K., and Galabov A. (2007), Synthesis and biological activity of amino acid ester prodrugs of acyclovir. Peptides 2006, Proceedings of the 29th European Peptide Symposium (Rolka K., Rekowski P., and Silberring J., eds.). Escom, Leiden, pp. 226–227.

Vabeno J., Lejon T., Nielsen C. U., Steffansen B., Chen W., Quyang H., Borchard R., and Luthman K. (2004a), Phe-Gly dipeptidomimetics designed for di/tri transporters PEPT1 and PEPT 2; synthesis and biological investigation. J. Med. Chem. **47**, 1060–1069.

Vabeno J., Nielsen C. U., Ingebrigtsen T., Lejon T., Steffansen B., and Luthman K. (2004b), Dipeptidomimetics ketomethylene isosters as pro-moieties for drugs transport via the human intestinal di-/tripeptide transporter hPEPT1: design, synthesis, stability and biological investigation. J. Med. Chem. **47**, 4755–4765.

Videnov G., Kaiser D., Kempter C., and Jung G. (1996), Synthesis of naturally occurring conformationally restricted oxazole and thiazole containing di- and tripeptide mimetics. Angew. Chem. **108**, 1604–1607; Angew. Chem. Int. Ed. Engl. **35**, 1503–1506.

Zacchigna M., Di Luca, Maurich G. V., and Boccu E. (2002), Syntheses, chemical and enzymatic stability of new poly(ethyleneglycol)-acyclovir prodrugs. Farmaco **57**, 207–214.