ELSEVIER

International Journal of Pharmaceutics 200 (2000) 181–185

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Stability of some novel thymidine, 5-bromo-2′-deoxyuridine and 3′-azido-2′-3′-dideoxythymidine analogues

Zvetanka Zhivkova [a,*], Ivanka Stankova [b]

[a] *Department of Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav, 1000 Sofia, Bulgaria*
[b] *South-West University 'N. Rilski', Ivan Michailov 66, 2700 Blagoevgrad, Bulgaria*

Received 11 October 1999; received in revised form 5 January 2000; accepted 7 February 2000

Abstract

In the search of new prodrugs effective against herpes simplex virus series of thymidine, 5-bromo-2′-deoxyuridine esters with amino acid and peptide chains and 3′-azido-2′,3′-dideoxythymidine derivatives have been synthesized and evaluated for antiviral activity. The chemical stability of some of them containing different residues was studied at pH 1 and 7.4 and temperature of 37°C. An HPLC method was developed for quantification of the unchanged ester concentration. It was proved that esters with simple aliphatic straight side chain (containing alanyl-, glycyl-, or glycyl-glycyl-glycyl-residues) are relatively stable both at acidic and neutral media, 37°C. Some of them undergo negligible hydrolysis with half lifes ranging between 6 and 23 h. In contrast, more complex esters with branched side chain (valyl-), with phenyl residue (phenylalanyl-), as well as containing thiazol ring are rather unstable especially at acidic conditions and undergo rapid hydrolysis resulting in the respective chemical precursor. The stability of the former group esters outlines them as suitable candidates for prodrugs: with higher lipophilicity facilitating po absorption, satisfying chemical stability and possibility to release the active moiety following enzymatic hydrolysis.

Keywords: Prodrugs; Pyrimidine 2′-deoxynucleosides; Amino acids; Peptides

1. Introduction

Extensive studies are worldwide underway in the search of chemotherapeutic agents effective against herpes virus infections (herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV)). One of the widely used drugs for prophylactics and treatment of infections caused by herpes group viruses is acyclovir (O'Brien and Campoli-Richards, 1989). The limited oral bioavailability of acyclovir (15–21%) results in low plasma levels, ineffective against the less sensitive viruses (De Miranda and Blum, 1983). In some clinical situations the intravenous route of application appears to be the only alternative for achievement of therapeutic effect.

* Corresponding author. Tel.: +359-2-9883143(214); fax: +359-2-9879874.
E-mail address: tzzhivkova@mbox.pharmfac.acad.bg (Z. Zhivkova)

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00367-7

During the last decade the efforts are directed towards the development of effective and safe prodrugs of acyclovir improving the po bioavailability of the drug (Colla et al., 1983; Selby et al., 1984; Welch et al., 1985; Kumar et al., 1988; Bundgaard et al., 1989; Stimac and Kobe, 1990; Bundgaard et al., 1991). On the basis of their studies on a small group of amino acid esters (mainly simple derivatives: glycyl, L-alanyl, β-alanyl, prepared as hydrochloride salts) Colla et al., (1983) supposed that these water soluble amino acid esters are suitable prodrugs for ophthalmic and intramuscular administration. Beauchamp et al. (1992) reported that amino acid esters of acyclovir with more complex side chains are promising candidates for prodrugs for oral use. The gastrointestinal absorption of these esters exceeds considerably that of the parent drug as well as those of the simpler amino acid esters. Moreover, the complex amino acid esters have better chemical stability in aqueous solution.

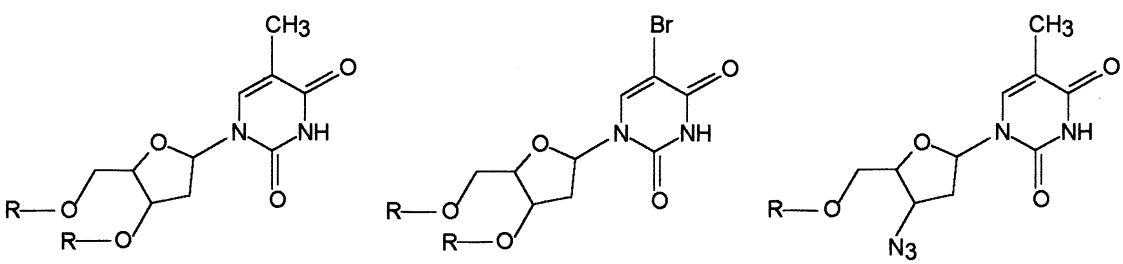
In the search of new prodrugs effective against herpes simplex virus series of thymidine, 5-bromo-2′-deoxyuridine analogues with amino acid and peptide chains (Stankova et al., 1999a) and 3′-azido-2′,3′-dideoxythymidine (AZT) derivatives (Stankova et al., 1999b) were synthesized and evaluated for antiviral activity. Three 5-bromo-2′-deoxyuridine derivatives containing glycyl-, glycyl-glycyl- and glycyl-glycyl-glycyl- residues show high activity against herpes virus (pseudo rabies virus (PsRV)), and only moderate one versus HSV-1. The corresponding thymidine analogues are considerably less effective, and only thymidine esters containing alanyl- and phenylalanyl-residues show borderline activity against PsRV. The activity of 3′-azido-2′,3′-dideoxythymidine against HIV-1 is almost equal to that of its derivatives while the toxicity of the later is proved to be considerably lower.

The suitability of certain structures as prodrugs candidates depends on their chemical stability in gastrointestinal tract, especially in the acidic gastric media. The present study deals with the stability of some derivatives of thymidine, 5-bromo-2 deoxyuridine and 3′-azido-2′,3′-dideoxythymidine in aqueous solution at pH and temperature of biological relevance.

2. Materials and methods

2.1. Chemicals

The structures of the compounds under investigation are presented in Fig. 1. The ester prodrugs are synthesized as previously described (Stankova et al., 1999a). Propan-1-ol for HPLC, dimethyl-



1. R = N-α-Benzyloxycarbonyl-alanyl-

2. R = N-α-Benzyloxycarbonyl-valyl-

3. R = N-α-Benzyloxycarbonyl-glycyl-glycyl-glycyl

4. R = N-α-Benzyloxycarbonyl-glycyl-

5. R = N-α-Benzyloxycarbonyl-phenylalanyl-

6. R = N-tert-Butoxycarbonyl-2-aminomethyl-thiazolyl-4-carboxyl-

7. R will be reported later because patenting is in progress.

Fig. 1. Structures of derivatives of thymidine, 5-bromo-2′-deoxyuridine and 3′-azido-2′,3′-dideoxythymidine.

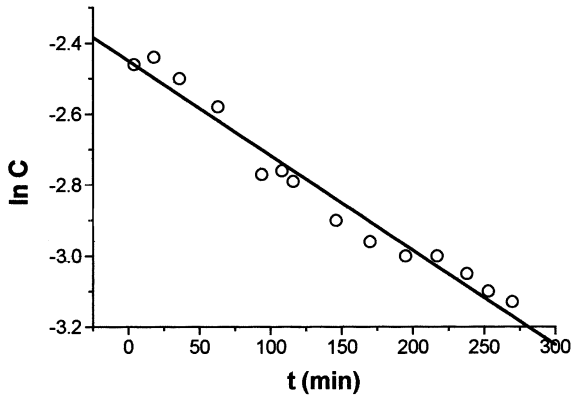


Fig. 2. Semilogarithmic plot of unchanged ester concentration versus time for compound 2, pH 1, 37°C.

sulfoxide (DMSO), as well as buffer components HCl, Na_2HPO_4 and NaH_2PO_4 of purest grade are purchased from Merck (Germany). The chromatographic column Hibar LiChrospher 100RP-18 (10 μm) is a product of Merck (Germany).

2.2. Chromatography

Chromatography is carried out isocratically, on modular HPLC system LC-10A Shimadzu (Japan) arranged of a LC-10A pump, solvent degaser DGU-3A, Rheodyne injector with 20 μl loop, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. The analysis is controlled and the data are acquired with CLASS LC-10 software. The mobile phase consisted of propan-1-ol/distilled water in the ratio 30:70 or 50:50 v/v depending on the polarity of the compound. The flow rate was 1 ml/min, and the temperature was maintained at 30 ± 1°C. Detection was performed at relevant λ_{max} for the respective compound (range 258–262nm).

2.3. Kinetic study

The chemical stability of thymidine, 5-bromo-2′-deoxyuridine and 3′-azido-2′,3′-dideoxythymidine derivatives was studied at pH 1 (0.1 M HCl) and pH 7.4 (phosphate buffer) both at 37°C (thermostatically controlled) and at ambient temperature (20°C). Because of the poor water stability of the investigated compounds an accurately weighted amount of them was dissolved in DMSO to produce an initial concentration of 1 mM, and then diluted with the respective buffer until concentration of 0.1 mM. Ten milliliter samples were incubated in closed vials at 37°C for 5 h with natural light exposure. Samples from the reaction solutions were withdrawn at appropriate time intervals, and immediately analyzed by HPLC. The quantification was made according previously prepared standard curves. Samples taken 24 h following the start of the study at ambient temperature (20°C) were also analyzed.

3. Results and discussion

Chemical stability of 3′,5′-bis-*O*-*N*-α-benzyloxycarbonyl-alanyl, 3′,5′-bis-*O*-*N*-α-benzyloxycarbonyl-valyl and 3′,5′-bis-*O*-*N*-α-benzyloxycarbonyl-glycyl-glycyl-glycine esters of thymidine (**1**, **2**, **3**), 3′,5′-bis-*O*-*N*-α-benzyloxy-carbonyl-glycyl, 3′,5′-bis-*O*-*N*-α-benzyloxycarbonyl-phenylalanyl and 3′,5′-bis-*O*-*N*-t-butoxycarbonyl-2-aminomethyl-thiazolyl-4-carboxyl esters of 5-bromo-2′-deoxyuridine (**4**, **5**, **6**), and of compound **7** (Fig. 1) was studied at experimental conditions of biological relevance, i.e. at pH 1 and 7.4, and temperature of 37°C. Analysis after 24 h at ambient temperature (20°C) aimed rather to support the results obtained at 37°C.

It was established that under the described experimental conditions some esters undergo decomposition by hydrolysis. The hydrolysis followed an apparent first order kinetics, and the rate constants (K) were obtained as slopes from the semilogarithmic plots of the unchanged ester concentration versus time. A representative kinetic curve is shown in Fig. 2. The chemical stability was assessed by means of the decomposition half-times ($t_{1/2} = \ln 2/K$) which values are listed in Table 1.

It was proved that compounds **1**, **4** and **7** are fairly stable at the described experimental conditions. Compounds **1** and **4** undergo a stepwise hydrolysis (via two monoesters) at a very low rate leading to thymidine and 5-bromo-2′-de-

Table 1
Kinetic data for chemical hydrolysis of the compounds 1–7

Compound number	pH 1[a]		pH 7.4[b]	
	37°C (Termostat)	Ambient (20°C)	37°C (Termostat)	Ambient (20°C)
1	Stable	$t_{1/2}>48$ h	Stable	$t_{1/2}\approx 40$ h
2	$t_{1/2}=4.33$ h	$t_{1/2}=22$ h	Stable	$t_{1/2}>100$ h
3	$t_{1/2}=11.55$ h	$t_{1/2}=31.4$ h	$t_{1/2}=6.08$ h	$t_{1/2}=17.6$ h
4	Stable	$t_{1/2}>48$ h	$t_{1/2}=22.65$ h	$t_{1/2}\approx 37$ h
5	$t_{1/2}=1.8$ h	$t_{1/2}=22$ h	$t_{1/2}=1.45$ h	$t_{1/2}=17.6$
6	$t_{1/2}=50$ min	$t_{1/2}=5$ h	Stable for 2.5h	Stable for 2.5h
7	$t_{1/2}=21.7$ h	$t_{1/2}\approx 70$ h	$t_{1/2}=11.55$ h	$t_{1/2}=39$ h

[a] Hydrochloric acid (0.1 M).
[b] Phosphate buffer (0.1 M).

oxyuridine respectively. Compound **7** is also considered as stable although its slow conversion to 3′-azido-2′,3′-dideoxythymidine. Ester **3** manifests lower stability and decomposes partially via two monoesters to thymidine. The lower stability should not be a problem by po administration since the residence time in the acidic pH gastric media normally does not exceed 4–5 h. Ester **2** is relatively unstable in acidic media undergoing hydrolysis to thymidine, but surprisingly the compound is fairly stable at pH 7.4. Ester **6** undergoes rapid hydrolysis at pH 1, 37°C to 5-bromo-2′-deoxyuridine, and only traces of both monoesters are detected. The stability of **6** at pH 7.4, 37°C was not fully clarified. No visible change in initial ester concentration was observed for 2.5 h of incubation followed by the instantanteneous decomposition to the respective monoesters. Ester **5** is very unstable both in acidic and neutral media.

4. Conclusions

The chemical stability of amino acids and peptides esters of thymidine, 5-bromo-2′-deoxyuridine and 3′-azido-2′,3′-dideoxythymidine was studied in experimental conditions simulating some relevant biological medias (pH 1 and 7.4, 37°C). Those with simple aliphatic straight side chain-**1**, **3** and **4** (containing Ala-, Gly-, Gly-Gly- and Gly-residues) are relatively stable both at acidic and neutral media and temperature of 37°C. More complex esters with branched side chain: methyl group substitution at the beta carbon of the amino acid (**2**), with phenyl residue (**5**), as well as containing thiazol ring (**6**) are rather unstable especially at acidic conditions. They undergo rapid hydrolysis resulting in the respective chemical precursor. The stability of the former group of esters outlines them as suitable candidates for prodrugs: with higher lipophilicity facilitating po absorption, satisfying chemical stability and a possibility to release the active moiety following enzymatic hydrolysis. Further investigations are necessary to establish the stability in vivo and to correlate it with both biological activity and toxicity.

Acknowledgements

We thank Professor Dr E. Golovinsky, Institute of Molecular Biology, Bulgarian Academy of Sciences, for the initiation of this study and for helpful discussion.

References

Beauchamp, M.L., Orr, F.G., deMiranda, P., Burnette, T., Krenitsky, A.T., 1992. Amino acid ester prodrugs of acyclovir. Antiviral. Chem. Chemother. 3 (3), 157–164.

Bundgaard, H., Falch, E., Jensen, E., 1989. A novel solution-stable, water soluble prodrug type for drugs containing a hydroxyl or an NH-acid group. J. Med. Chem. 32, 2503–2507.

Bundgaard, H., Jensen, E., Falch, E., 1991. Water soluble, solution stable, and biolabile *N*-substituted (aminomethyl) benzoate ester prodrugs of Acyclovir. Pharm. Res. 8, 1087–1093.

Colla, L., De Clercq, E., Busson, R., Vanderhaeghe, H., 1983. Synthesis and antiviral activity of water soluble esters of acyclovir (9-((2-hydrox-ethoxy) methyl))-9H-guanine. J. Med. Chem. 26, 602–604.

Kumar, S., Oakes, F.T., Wilson, S.R., Leonard, N.J., 1988. Synthesis and structure of a fluorescent, tricyclic analogue of 2′-deoxyadenosine and of a prodrug by N-annelation of 2′-deoxyguanosine and (9-(2hydroxetoxy)methyl)) guanine (acyclovir), respectively. Heterocycles 27, 2891–2901.

De Miranda, P., Blum, M.R., 1983. Pharmacokinetics of acyclovir after intravenous and oral administration. J. Antimicrob. Chemother. 12, 29–37.

O'Brien, J.J., Campoli-Richards, D.M., 1989. Acyclovir: An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs 37, 233–309.

Selby, P., Powles, R.L., Blake, S., et al., 1984. Amino(hydroxethoxymethyl) purine: A new well absorbed prodrug of acyclovir. Lancet 2, 1428–1430.

Stankova, G.I., Simeonov, F.M., Maximova, V., Galabov, S.A., Golovinsky, V.E., 1999a. Synthesis and anti-virus activity of some nucleosides analogues. Zeitschrift fur Naturforsch 1-2, 75–83.

Stankova, G.I., Bechkov, D., Golovinsky, V.E., 1999. (Unpublished results).

Stimac, A., Kobe, J., 1990. A new synthesis of acyclovir prodrugs. N2-acetylacyclovir and 6-deoxyacyclovir. Synthesis 6, 462–464.

Welch, C.J., Larsson, A., Ericson, A.C., et al., 1985. The chemical synthesis and antiviral properties of an acyclovir-phospholipid conjugate. J. Acta Chem. Scand. 39, 47–54.