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SYNTHESIS AND ANTIVIRAL ACTIVITY OF AMINO ACID ESTERS OF RIBAVIRIN

R.D.Zakharieva*[a], A.S.Galabov[b] and N.Nikolova[b]

[a]*Institute of Molec. Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

[b]*Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

Abstract: The synthesis and antiviral activity of amino acid esters of 1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin,**1**) is described.

To date a number of nucleoside derivatives have been found which are active inhibitors of viral replication. Among them 1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin, **1**) has shown in vitro antiviral activity against RNA and DNA viruses[1]. It inhibits also the replication of HIV in human T lymphocytes[2]. The biochemistry and clinical application of ribavirin has been reviewed[1].

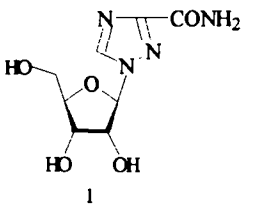
Several derivatives of ribavirin, such as the 2',3',5'-triacetate[3], 5'-acid esters[4] and some phosphates[5] have also been shown to exhibit promising in vitro effect. It is suggested that this antiviral activity probably is due to intracellular hydrolysis of the analog back to active ribavirin.

There are several strategies for designing prodrugs of biologically active nucleosides. A general approach is the synthesis of amino acid or peptide analogs. These modification usually result in increased stability, lipofilicity and permeation of the active compound through plasma membranes. This approach has been successively exploited for preparation of prodrugs of AZT[6], 5-fluorouridine[7] and ara-C[8]

In this report we describe the synthesis and in vitro evaluation of 5'-amino acid esters of 2',3'-isopropylidene ribavirine **3-7** as inhibitors of several viruses in vitro

2',3'-Isopropylidene ribavirin **2** was prepared according to the method of Schmidt[9], with slight modification, consisting in replacement of P_2S_5 for p-methoxy thiophosphine sulfide (Lawesson's reagent) for thioation of oxamic acid. We found that the use of P_2S_5 in toluene at 90-100°C afforded poor yields (5-10%) both for 2 hrs[9] or more. Lawesson's reagent[10] in toluene reacted with oxamic acid for 30 min at 80°C in 87% yield after silica gel column chromatography ($CHCl_3$).

The amino acid esters **3-7** were synthesized by coupling of the t-BOC-L-amino acids with **2** in the presence of 1.5 molar equivalents of DMAP and DCC in dry CH_3CN in moderate to high yields (40-82%) after purification by column chromatography (SiO_2, $CHCl_3$/MeOH). All compounds were characterized by ^1H NMR after purification by reversed phase HPLC[11]. Satisfactory elemental analysis were obtained.



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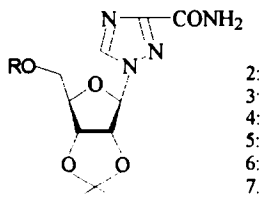

2: R=H
3: R=N-α-BOC-L-Phe
4: R=N-α-BOC-L-Leu
5: R=N-α-BOC-L-Gly
6: R=N-α-BOC-L-Trp
7. R=N-α-BOC-L-Pro

All attempts to deprotect the t-BOC- and isopropylidene groups resulted in deisopropylidenation with simultaneous hydrolysis of the ester bond giving the parent ribavirin **1**

The target compounds **3-7** were evaluated for activity against influenza virus A (chicken/Germany/27/Weybridge) (H7N7) (FPV), Newcastle disease virus (Russeff)(NDV) and pseudorabies virus (Anjeszky A_2 strain) (PsRV) by agar-diffusion plaque-inhibition method in cylinders[12,13]. They were grown in primary chick embryo fibroblasts cultures (CEC). The antiviral effect ($E=\phi_i-\phi_t$) of a given compound (0.1 ml of 4.10^{-2} mol/l solutions in DMSO) was determined by the basis of the size (diameter, ϕ in mm) of the zone of plaque inhibition (ϕ_i) and the zone of cytotoxicity (ϕ_t) designated as follows: (-), E=5 mm; (±), E=6-10 mm; (+), E=11-12 mm; (++), E=21-40 mm; (+++), E>40 mm. A compound was considered to have a marked antiviral effect if E is greater than 10 mm.

Table 1. Antiviral activity of 5'-amino acid esters of ribavirin by the agar-diffusion plaque inhibition test.

Compound	FPV			NDV			PsRV		
	ϕ_i	ϕ_t	E	ϕ_i	ϕ_t	E	ϕ_i	ϕ_t	E
Ribavirin	29.2	9.5	+	52.0	15 0	++	47.0	16.0	++
3	19.3	9.2	+	0	16.5	-	0	16.5	-
4	16.8	8.3	±	28.0	16.0	+	18.0	16.0	-
5	0	10.2	-	31.5	19.0	+	23.5	15.0	±
6	15.7	10.7	-	0	10.7	-	0	9.0	-
7	22.8	14.0	±	16.5	8.7	±	0	10.0	-

Some of the compounds showed high antiviral activity (3 against FPV, 4 and 5 against NDV) (table 1) but they are less potent than ribavirin. This lack of higher antiviral activity by esters **3-7** may be related to their incomplete hydrolysis under the in vitro conditions.

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11. **3**: ^{1}H NMR (DMSO-d_6) 8.81(s,1H), 7.84(s,1H), 7.67(s,1H), 7.22 (m,5+1H), 6.31(s,1H), 5.16 (d,1H), 4.93(d,1H), 4.36(t,1H), 4.11-4.04(m,2H), 2 86(m,2H), 1.50(s,3H), 1 32(s,3H), 1 30(s,9H)
4: ^{1}H NMR (DMSO-d_6) 8 79(s,1H), 7.81 and 7.66(2s,2H), 6.31(s,1H), 5.19(d,1H), 4.99(t,1H), 4.39(t,1H), 4 28-4.08 (m,2H), 3.92(m,1H), 3.45(m,2H), 1 52(s,3H), 1.35(s,9H), 1.32(m,6H), 1.29 (s,3H).
5. ^{1}H NMR (DMSO-d_6) 8 80(s,1H), 7.87 and 7.67(2s,2H), 7.16(s,1H), 6.29(s,1H), 5.20(d,1H), 5.01(t,1H), 4.41(q,1H), 4.24(m,1H), 4.09(m,1H), 3.64(d,2H), 1 51(s,3H), 1.36(s,9H),1.32 (s,3H)
6: ^{1}H NMR (DMSO-d_6) 10.90(s,1H), 8.80(s,1H), 7 42-6.92 (m,6H), 6.31(s,1H), 5.12(d,1H), 4.87(d,1H), 4.35(t,1H), 4 24-4.04(m,3H), 3.03(m,1H), 2.43(m,2H), 1.72(s,3H), 1.50(s,3H), 1.31(s,9H).
7. ^{1}H NMR (DMSO-d_6) 8.80(s,1H), 7.82 and 7 66(2s,2H), 6.32(d,1H), 5.21(d,1H), 4.99(t,1H), 4.39(t,1H), 4,25(m,1H), 4.23-4 08(m,2H), 3 28(m,2H), 2.40(m,2H), 1.76(m,2H), 1.54 (s,3H), 1 36(s,3H), 1.25(s,9H)
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