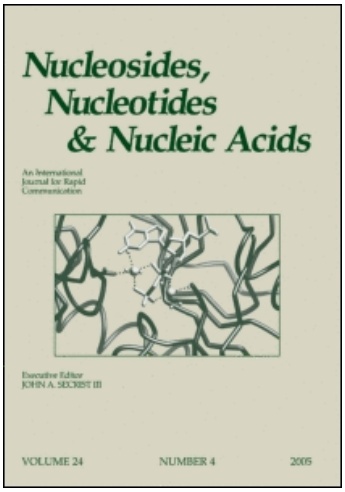

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SYNTHESIS AND BIOLOGICAL EVALUATION OF CARBOCYCLIC ANALOGUES OF RIBAVIRIN

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Abstract : A series of racemic carbocyclic analogues of Ribavirin have been prepared. Their degree of phosphorylation by adenosine kinase and the inhibition of IMPDH and RNA polymerase by their mono and triphosphates respectively is compared with Ribavirin and discussed in the context of anti-influenza activity.

Ribavirin (Virazole, 1) is a broad spectrum antiviral agent that has recently been approved for clinical use in the treatment of infantile Respiratory Syncytial Virus (RSV) infection. The compound also displays potent activity against influenza *in vitro* and *in vivo*. Ribavirin's mode of action is complex and three discrete mechanisms have been implicated. These are a) Ribavirin triphosphate (RTP) mediated selective inhibition of the viral encoded RNA polymerase[1,2]; b) RTP mediated prevention of GTP dependent capping of viral mRNA by inhibition of guanyltransferase and N-7 methyltransferase[2,3]; c) Ribavirin monophosphate (RMP) mediated feedback inhibition of IMPDH[4]. Whilst the first mechanism is virus specific the latter two involve host cell processes and are potentially responsible for the compound's observed toxicity. Compounds which retain mechanism (a) and possibly (b), but lack (c) are of interest as novel drug candidates, since it is believed that IMPDH inhibition is not essential for antiviral activity[5]. IMPDH inhibition might potentially be abolished by modification of the sugar moiety of Ribavirin. Stabilisation of the glycosidic bond is important in this regard since 1,2,4-triazole-3-carboxamide forms rapidly in cells[6] as a result of Ribavirin metabolism

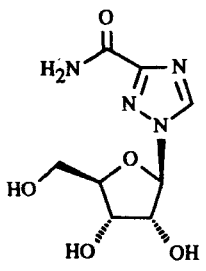
Table 1

Phosphorylation of Ribavirin Analogues & Inhibition of IMPDH		
Analogue	Phosphorylation by Adenosine Kinase[a]	Inhibition of IMPDH (IC_{50})[b]
Ribavirin	1.12	8.5uM
2	< 0.01	250uM
3	0 .014	> 1.2mM
4	< 0.01	> 1.2mM
5	0 .01	4.6uM
6	< 0.01	> 1.2mM

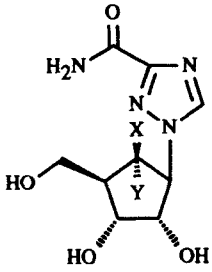
a Incorporation of ^{32}P into compound by Adenosine Kinase purified from Rabbit Liver. Values are the amount of monophosphate formed after 1 hour relative to that obtained with adenosine.

b Inhibition of IMPDH measured from the release of Tritium from ^{3}H-inosine by the action of IMPDH in mouse leukaemia L1210 cells. Cells were washed and suspended at 2.5×10^{7} cells/ml in serum free medium containing the test compound and ^{3}H-inosine. Allopurinol (50uM) was added to block tritium release by the action of xanthine oxidase. After 30 min at 37°C the amount of radioactivity not absorbed by charcoal was used as a measure of IMPDH activity in the cells.

and may recycle to RMP via the reversible action of purine nucleoside phosphorylase. We herein report the preparation[7] of a series of phosphorylase stable, carbocyclic analogues of Ribavirin (2-6) and assess their potential as anti-influenza agents.



1.



2. X = Y = H
3. X = H, Y = OH
4. X = OH, Y = H
5. X = F, Y = H
6. X = H, Y = F

BIOLOGICAL EVALUATION OF NUCLEOSIDE ANALOGUES

The five analogues of ribavirin (i.e. 2-6) were evaluated as substrates for adenosine kinase from rat liver, and as inhibitors of IMPDH in mouse L1210 cells (Table 1). None of the analogues was a good substrate for adenosine kinase, the incorporation of ^{32}P being

Table 2

Inhibition of IMPDH & Influenza RNA Polymerase by 5′-phosphates of Ribavirin & Compound 5		
Analogue	Inhibition of E.coli IMPDH by 5′-monophosphate[a] (IC_{50})	Inhibition of Influenza RNA polymerase by 5′-triphosphate[b] (Ki)
Ribavirin	0.48uM	200uM
5	0.10uM	100uM

a Activity of IMPDH purified from E.coli was measured spectrophotometrically from change in absorbance of IMP at 340nm.

b Assay of RNA polymerase activity of influenza A / Mississipi/1/85 was essentially as described by Eriksson et al[1]. Apparent Kis for inhibitors was determined by limiting a single nucleoside substrate.

less than 5% of that seen with adenosine, whereas ribavirin was as well phosphorylated as adenosine.

Only two of the analogues exhibited any detectable inhibition of IMPDH in the whole cell assay, these being carbocyclic ribavirin (2), which had an IC_{50} of 250μM, and the 6′-β-fluorocarbocycle (5) which had an IC_{50} of 4.6μM, comparable to that of ribavirin (8.5μM) (Table 1).

None of the analogues inhibited replication of influenza A virus *in vitro* (IC_{50} >400μM) in an assay where ribavirin was effective at 26μM.

ENZYME INHIBITION BY NUCLEOTIDE ANALOGUES

That compound (5) inhibited IMPDH, but not influenza virus, in cell culture indicates that inhibition of IMPDH itself does not necessarily lead to inhibition of viral replication. To examine the properties of this analogue further the 5′-monophosphate and 5′-triphosphate derivatives have been synthesised[8,9] and compared to those of ribavirin as inhibitors of IMPDH purified from *E.coli* and influenza A virus RNA polymerase respectively (Table 2).

The 5′-monophosphate of (5) inhibited *E.coli* IMPDH (IC_{50} = 0.1μM), as expected from the results on inhibition of IMPDH in L1210 cells. The compound was more potent than RMP (IC_{50} = 0.48μM).

Ribavirin triphosphate only weakly inhibits the viral polymerase (Ki ~ 0.2mM) and antiviral activity is presumed to result at least in part from the relatively high levels of RTP that accumulate in cells. The 5′-triphosphate of (5) was as potent an inhibitor of the viral RNA polymerase as RTP, with an apparent Ki (measured against ATP) of 100μM.

CONCLUSIONS

Whilst a compound of the desired biological profile was not obtained, the data presented indicates that inhibition of IMPDH in itself does not confer antiviral activity against influenza. Inhibition of IMPDH by RMP may, however, result in the self potentiation of Ribavirin since depression of the GTP pool increases the effective concentration of RTP. The observation that compound 5 is not a substrate for adenosine kinase and that its 5′-triphosphate derivative is a more effective inhibitor of RNA polymerase than RTP itself indicates that activation to the triphosphate level is a prerequisite for antiviral activity against influenza to be exhibited by this class of nucleoside analogue.

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