Synthesis and Antiviral Evaluation of \(N\)-Carboxamidine-Substituted Analogues of 1-b-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide Hydrochloride


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Ten, hitherto unreported, analogues of 1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide hydrochloride (2a, ribavirin) and methyl carboxamide 5 have been synthesized. These include the N-cyano (2b), N-alkyl (2c-e), N-amino acid (2f-h), N,N'-disubstituted (6, 7a,b), and the N-methylated carboxamide (1f) analogues of ribavirin. In addition, a new facile synthesis of carboxamide 2a was also developed. All compounds were evaluated for biological activity against the following RNA viruses: Punta Toro (PT) and sandfly fever (SF) viruses (bunyaviruses); Japanese encephalitis (JE), yellow fever (YF), and dengue-4 viruses (flaviviruses); parainfluenza virus type 3 (PIV3), respiratory syncytial virus (RSV), and measles viruses (orthomyxoviruses); Venezuelan equine encephalomyelitis virus (VEE, alphavirus); human immunodeficiency virus type-1 (HIV-1, lentivirus); the DNA-containing vaccinia (VV) virus (poxvirus); and adenovirus type 5 (Ad5) viruses. All of the compounds except for 2b and 7a,b exhibited activity against the bunyaviruses such as that observed with 2a; however, higher IC\(_{50}\) values were generally observed. Glycine analogue 2f showed increased survival and decreased markers of viral pathogenicity. Carboxamide 2a, carboxamide 5, and dimethyl amide 6 exhibited activity against dengue type-4 virus. Monomethyl amide 2c demonstrated activity against RSV, PIV3, and, to a lesser extent, influenza A and B. Activity of 2c generally required higher IC\(_{50}\) values than unsubstituted 2a. The latter exhibited hitherto unreported activity against RSV; therapeutic indices for 2a against RSV and PIV3 were >64 and >21. No substantial in vitro activity was observed for any of the compounds tested against Ad5, measles, JE, YF, VEE, or HIV-1. In addition, evidence is presented which argues in favor of a distinct antiviral mechanism of action for carboxamidines, e.g., in contrast to a role as a carboxamide precursor.

Compounds possess efficacy against a broad array of DNA and RNA viruses,\(^5\) are known inhibitors of inosine monophosphate dehydrogenase (IMP) after adenosinekinase dependent conversion to nucleotides, and, in general, possess similar mechanisms of action.\(^5,6\) In addition to IMP dehydrogenase, 2a is an effective competitive inhibitor of purine nucleoside phosphorylase.\(^5,6\) A ribavirin analogue, 5-ethynyl-1-b-D-ribofuranosylimidazole-4-carboxamide (3, EICAR)\(^7\) has recently been reported to

![Diagram](image)

...ibavirin, 1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1a), and its 3-carboxamide hydrochloride analogue 2a are broad-spectrum antiviral agents which were synthesized and developed nearly concurrently.\(^1,2\) Both

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provide N-substituted amidines. Amidines conjugated to amino acids may exhibit facilitated cellular transport or uptake. Therefore, the amino acids glycine (Gly), glutamine (Gln), and asparagine (Asp) were condensed with methyl carbobazimide 5 in methanol at 35–50 °C to give the N-substituted amidines 2f (Gly), 2g (Asp), and 2h (Gln). Analytical and NMR spectral data indicated that 2f–h were obtained as zwitterions. Coupling of a hydrophobic “tail” onto a hydrophilic, biologically-active molecule can often affect its biological properties. Thus, methylamine hydrochloride, n-butylamine, and n-octylamine were condensed with imidate 5 in methanol to produce the mono-N-methyl hydrochloride (2c), n-butyl (2d), and n-octyl (2e) alkylated carboxamidines. Methylation at both amidine nitrogens was accomplished by treating carboxamidine 2a with excess methylamine in anhydrous methanol in a reaction bomb heated to 60 °C for 6 days to produce 6 as a hydrated hydrochloride salt. In order to determine the effect of conjugating the amidine moiety of 2a within the skeletal framework of a 6-membered ring, the 1,4,5,6-tetrahydro-pyrimidin-2(1H)-one 7a,b were prepared from carboxamidine 2a by treatment with 1,3-diaminopropene and its hydrated hydrochloride analogues) in refluxing absolute ethanol.16

Stability Studies Leading to If. Amidines and their N-substituted analogues undergo hydrolysis at various rates and could thus serve as potential precursors of carboxamidines.17 Studies were undertaken to evaluate the stability of carboxamidine 2a, dimethylamilde 6, and glycine conjugate 2f to hydrolysis. The rate of conversion of carboxamidine hydrochloride 2a to ribavirin 1a in D2O (referred to TSP) was monitored by quantitation of the 1H-NMR absorptions of the C7-H (proton) at δ 8.91 and 8.77 for 2a and 1a, respectively, as well as by observing the appearance of the carbonyl (CONH2) absorption at δ 165.5 in the 13C-NMR spectrum of 1a. No interconversion to ribavirin was observed within 2 weeks, 12% conversion being observed after 7 weeks. Thin-layer chromatographic studies revealed the N,N'-dimethyl analogue 6 to be stable to hydrolysis in pH 7.4 phosphate buffer solution at room temperature for 1, 5, and 8 h. However after 24 h, TLC showed complete conversion of 6 (Rf = 0.45) to the N-methyl carboxamidine derivative N-methyl-1,2,4-triazole-3-carboxamide (1f, N-methylribavirin, Rf = 0.69). This conversion was confirmed by FAB mass spectrometric (FAB/MS) analysis of the lyophilized crude reaction mixture which showed (M + 1) = 259 corresponding to the methyl carboxamide as opposed to (M + 1) = 272 corresponding to the N,N'-dimethyl amidine starting material. Since the sample of 1f obtained by hydrolysis represented a hitherto unreported analogue of ribavirin, it was independently synthesized and characterized by treating carboxamidine 2a with ethanolic methylamine. Similarly, glycine analogue 2f was converted quantitatively to ribavirin 1a in 1 h in phosphate-buffered saline (pH 7.4).

Recently, it became necessary to prepare kilogram quantities of the carboxamidine 2a-HCl. The Pinner synthesis involves treating a nitrile with dry ethanolic

hydrogen chloride to give the imidate followed by ammonia or an amine in absolute ethanol to yield the amidine.\textsuperscript{12,17} The preparation of kilogram quantities of carboxamidine 2a by treatment of the methyl carboximide 5 with a saturated methanolic ammonia solution in a pressure bottle containing ammonium chloride has been described.\textsuperscript{12} The use of ammonia in a pressure bottle can be eliminated by treating the methyl carboximide 5 (prepared but not isolated from the reaction of acetylated cyanotriazole 3 with methanolic sodium methoxide followed by neutralization with ion-exchange resin) with anhydrous ammonium chloride in refluxing methanol. Carboximide hydrochloride 2a was thus prepared in 86% yield and characterized by \textsuperscript{1}H and \textsuperscript{13}C NMR\textsuperscript{18,19} in both DMSO-\textsubscript{d}\textsubscript{6} and D\textsubscript{2}O. The \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra of the carboxamidine 2a exhibited marked solvent dependence. Changing solvent from DMSO-\textsubscript{d}\textsubscript{6} to D\textsubscript{2}O shifted all proton absorptions to lower field by 0.25–0.37 ppm except that for the C-5-triazole proton, which was shifted upfield from \( \delta \) 9.21 to 8.91 ppm. Similarly, all \textsuperscript{13}C absorptions appeared at lower field by 2.0–3.5 ppm in D\textsubscript{2}O.

**Antiviral Activity**

**In Vitro Studies.** 1-β-d-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (2a), its N-cyano (2b), N-alkyl (2c–e), and N-amino acid (2f–h) analogues, the N,N'-disubstituted analogues 6, 7a,b, methyl carboximide 5, and N-methyl ribavirin derivative 1f were evaluated in vitro to determine their inhibitory properties against the following RNA viruses: flaviviruses (family Flaviviridae) Japanese encephalitis (JE), yellow fever (YF), and dengue type-4 viruses; phleboviruses (family Bunyaviridae) Punta Toro (PT) and sandfly fever-Sicilian (SF) viruses; the alphavirus (family Togaviridae) Venezuelan equine encephalomyelitis (VEE) virus; paramyxoviruses (family Paramyxoviridae) respiratory syncytial (RSV), measles, and parainfluenza type 3 (PIV3) viruses; orthomyxoviruses (family Orthomyxoviridae) influenza A and influenza B viruses; and the lentivirus human immunodeficiency virus type 1 (HIV-1). Activity against the DNA-containing vaccinia virus (VV, Poxviridae) and adenotype 5 (Ad5, Adenoviridae) virus was also evaluated. The antiviral quantitative MTT assay\textsuperscript{20–22} was used to

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\textsuperscript{(19)} King-Morris, M. J.; Serianni, A. S. \textsuperscript{13}C NMR Studies of [1-\textsuperscript{13}C]Aldoses: Empirical Rules Correlating Pyranose Ring Configuration and Conformation with \textsuperscript{13}C Chemical Shifts and \textsuperscript{13}C-\textsuperscript{13}C Spin Couplings. *J. Am. Chem. Soc.* 1987, 109, 3501-3508.


in vivo efficacy against above, ribavirin has demonstrated exceptional in vitro and values of >1000 \mu g/mL, and viruses. Against RNA viruses such as those described stratted by therapeutic indices of >64, >20, and >20, against DNA viruses such as vaccinia virus (VV) and RNA RSV, influenza A, and influenza B viruses (as demon-

- **Table I.** In Vitro Antiviral (RNA) Evaluation of Carboxamidine Analouges

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<td>inactive</td>
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<td>800</td>
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<td>2c</td>
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<td>7a</td>
<td>&gt;3200</td>
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*TI (therapeutic index) was calculated as the ratio of \(\text{TC}_{50}/\text{IC}_{50}\), inactive against dengue-4 virus, except for dengue virus, where activity was determined by a plaque-reduction assay. Paramyx- and orthomyxoviruses were assayed by inhibition of virus-induced syncytia formation using ribavirin as the positive control. The concentration of test compound which was cytotoxic to 50% of uninfected cells (\(\text{TC}_{50}\)) was also determined, as was the therapeutic index, \(\text{TI}_{50}\), a ratio of these two values (\(\text{TC}_{50}/\text{IC}_{50}\)).

Ribavirin exhibits a broad spectrum of antiviral activity against DNA viruses such as vaccinia virus (VV) and RNA viruses. Against RNA viruses such as those described above, ribavirin has demonstrated exceptional in vitro and in vivo efficacy against bunyaviruses, paramyx- and orthomyxoviruses, however, its activity against the flaviviruses (JE and YF) and alphavirus (VEE) is 10-100-fold less in vitro, and in vivo activity is not demonstrate. The carboxamidine analogue of ribavirin (2a) demonstrates a similar spectrum of activity with exceptional in vitro and in vivo activity against Punta Toro virus and parainfluenza, and influenza A virus. In the current study, 2a (a) showed activity against VV but was inactive against Ad5 virus; (b) was inactive against HIV-1 and measles viruses; (c) exhibited good activity against RSV, influenza A, and influenza B viruses (as demonstrated by therapeutic indices of >64, >20, and >20, \(\text{TC}_{50}\) values of >1000 \mu g/mL, and \(\text{IC}_{50}\) values of 16, 48, and 48 \mu g/mL, respectively); (d) was marginally active against the JE, YF, and VEE viruses, inhibiting viral cytopathic effect (CPE) by 25-40% at 320 \mu g/mL; (e) was cytotoxic to Vero cells at 1000 \mu g/mL; (f) was active against dengue-4 virus, exhibiting 99% plaque reduction at concentrations of 100 and 250 \mu g/mL, although 2a was cytotoxic to MK-2 cells (these being more sensitive to cytotoxicity than are Vero cells at 1000 \mu g/mL). It is worth noting that the antiviral activity of ribavirin is not limited to RNA viruses, as it also exhibits activity against DNA viruses such as vaccinia virus, with \(\text{TC}_{50}\) values of 16, 48, and 48 \mu g/mL, respectively.
cells) at the higher (250 μg/mL) concentration; and (g) 
reinforced its efficacy against PIV3, PT, and SF viruses.2r
A therapeutic index of >21 was indicative of PIV3 virus
inhibition (50%) at a concentration of 2a corresponding
to 48 μg/mL. SF- and PT-virus-induced CPE were in-
hibited by 100% and 50–100%, at drug concentrations of
100 and 100–320 μg/mL, respectively. In general, com-
parisons of IC50 values of ribavirin and carboxamidine 2a
against bunyaviruses reveal that higher IC50 values are
required for 2a to achieve similar antiviral efficacy.
This pattern is duplicated against paramyxoviruses.

The in vitro activity of the compounds studied is listed
in Table I. No substantial in vitro activity was observed
for any of the compounds tested against flaviviruses, an
alphavirus, or HIV-1. Substitution of a cyano substituent
at the amidine nitrogen to give 2b eliminated all in vitro
activity observed for carboxamidine 2a. Incorporation
of the amidine moiety within a tetrahydropyrimidine ring
(as in 7a,b) gave similar results. In the case of 7a,b, the
presence of a second ring at the 3-position may induce
conformational changes and thereby preclude phospho-
rylation of the nucleoside. Imidate 5 exhibited significant
in vivo antitumor activity12 against murine leukemia L1210.
However, its antiviral (RNA) activity was limited to
the bunyaviruses PT and SF and dengue-4 virus. In
each case, higher IC50 values were observed in comparison
to those of ribavirin. Activity against PT and SF viruses
was observed at 320 μg/mL with accompanying Vero cell
toxicity at 1000 μg/mL. Significant host cell (MK-2) cell
toxicity was observed at 250 μg/mL in the dengue virus
assay. Imidate 5 was inactive against HIV, VV, JE, YF,
and VEE. Substitution of an N-amino acid such as glycine
(2f), asparagine (2g), or glutamine (2h) at the car-
boxamidino carbon (C=) in ribavirin dimethyl analogue 6
inhibits the activity of ribavirin under the conditions of in vitro evaluation. Glycyamine 2f (glycine) produced 40% and 70% reductions in PIV3 toxicity at 1000 μg/mL. Significant host cell (MK-2) toxicity was observed at 250 μg/mL in the dengue virus assay. Imidate 5 was inactive against HIV, VV, JE, YF, and VEE.

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biologically responsible for the activity against dengue-4 virus and that in situ hydrolysis is not a major factor.

In vivo studies. Ribavirin and its carbamidines and analogues22 have demonstrated consistent and potent antiviral activity against PT virus, both in vitro and in a mouse model in which the principal pathogenic process involved hepatitis. Efficacy was measured in terms of survivor numbers and survival times. In addition, viral pathogenicity and the development of infectious virus in sera and livers were correlated with the degree of hepatic icterus (liver score) and with elevations in serum glutamic oxalacetic and pyruvic acid transaminases, SGOT and SGPT, respectively. N-Methyl- and glycy1-substituted carbamidine analogues were tested in PTV-infected mice. All (except 7a) were administered in doses of 1200, 600, and 300 mg/kg per day, subcutaneously (sc) in saline, twice daily for 4 days (b.i.d. × 4), beginning 4 h pre virus inoculation. The dimethyl derivative 6 was ineffective at any dose used in the study. The monomethyl analogue 2c was marginally effective at the highest nontoxic dose (1200 mg), producing slight increases in mean survival times and decreased levels of SGOT, SGPT, and mean liver virus titers. N-Glycyl 2f produced a 90% survival rate (cf. 30% survival in the control mice) when administered at a dose (1200 mg) approaching its maximum tolerated dose (MTD). Significant reductions in mean liver score and SGOT and SGPT levels and moderate decreases in liver and serum virus titers were observed. It is not possible to ascribe these results to the activity of 2f since it is known that 2f is converted to ribavirin 1a in phosphate-buffered saline in ca. 1 h. Administration of 1,4,5,6-tetrahydropyrimidine 7a (sc, b.i.d. × 5) at nontoxic doses of 600, 300, 150, and 75 mg/kg per day resulted only in slight increases in mean survival times at 300 and 150-mg dose levels. Under similar conditions, ribavirin (administered at 75 mg/kg per day) and carbamidine 2a (administered at 62.5 and 125 mg/kg per day) produced 100% survival for >21 days. The MTD values for ribavirin and 2a are 100 and 1000 mg/kg per day, respectively.22

Experimental Section

Chemistry. All solvents were distilled before use and dried with calcium chloride and all chemicals were reagent grade. Evaporation was conducted at bath temperatures ≤30 °C with a Buchi rotary evaporator under water aspirator or mechanical oil pump vacuum. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Results agreed within ±0.4% of the theoretical values. The 'H NMR and 13C NMR spectra were recorded on a Varian XL200 spectrometer with 1025, 900 cm−1; 'H NMR (DMSO-d6) δ 10.1 (br, 1 H, 3'-OH), 5.36-5.21 (d, 1 H, 3'-OH), 4.93 (t, 1 H, 5'-OH), 4.36 (m, 1 H, H-2'), 4.14 (m, 1 H, H-3'), 3.96 (m, 1 H, H-4'), 3.64, 3.49 (m, 2 H, H-5'), 2.78 (d, 3 H, NHCH3). Anal. (C8H8N2O4) C, H, N.

1,2-Di-m-bromo-1,2,3-triazole-3-carboxamidine (2a). Cyanamidine derivative (7a) (4.5 g) was suspended in methanol (12 L) in a 22-L round-bottomed flask. Sodium methoxide (45 g) was slowly introduced and the reaction mixture was stirred for 2 h at 25 °C, pH 8.5-9.0. When thin-layer chromatography (6:1 CHCl3/MeOH, Rf = 0.2) indicated complete deacetylation of the ribose ring as well as imidate formation, the sodium ions in solution were neutralized by the addition of Amberlite IR 120 H+ ion-exchange resin. The mixture was filtered and anhydrous ammonium chloride (107 g, 2 mol) was added to the filtrate. The stirred mixture was gently refluxed for 3 h. Upon completion (by TLC) of amine formation, charcoal (15 g) was added. The mixture was stirred for 15 min then filtered through a Celite pad. The filtrate was concentrated in vacuo to one-third its volume and left overnight. The crystallized product 2a-HCl was collected by filtration, and the mother liquor concentrated further to provide a second crop. The two batches were combined and recrystallized from methanol to give 480 g (85.8%) of pure amidine hydrochloride 2a. This sample was identical in all respects with an authentic sample.12

1,2-Di-bromo-1,1,2,4-triazole-3-N-cyanocarbamidine (2b). Cyanamidine (1.8 g, 42.6 mmol) and a solution of freshly-prepared methanolic sodium methoxide (42.6 mL) were successively added to a methanolic solution of methyl 1,2-di-bromoteracarbamidine-1,2,4-triazole-3-carboxamidine11 (5) (3.95 g, 15.2 mmol). The resulting solution was stirred at room temperature for 6 h, acidified with acetic acid to pH 4, and concentrated to dryness in vacuo. The residue was chromatographed over silica gel (flash chromatography) with 95% methanol in diethyl ether as eluent to yield 2b as an amorphous solid (1.63 g, 40%). 'H NMR (DMSO-d6) δ 8.80-9.10 (2a, 3 H, amidine NH, C-5-H), 5.85 (d, 1 H, J = 3.1 Hz, H-1'), 4.89, 5.20, 5.61 (t, d, d, 3 H, OH), 4.35 (d, 1 H, H-2'), 4.16 (d, 1 H, H-3'), 3.96 (m, 1 H, H-4'), 3.57 (m, 2 H, H-5'), 13C NMR (DMSO-d6) δ 61.75, 70.42, 75.07, 89.05, 92.50, 116.81, 146.23, 155.61, 160.83. Anal. (C8H8N2O4) C, H, N.

1,2-Di-bromo-1,2,4-triazole-3-N-methylcarbami- midine Hydrochloride (2c). Methyl carbamidine (5 g, 7.75 mmol) was combined with methylamine hydrochloride (0.55 g, 7.75 mmol) followed by the addition of methanol (40 mL). The stirred solution was heated at gentle reflux until TLC monitoring (6:1 CHCl3/MeOH, Rf = 0.2) indicated complete dissolution of the solid form which was dissolved in ethanol (15 mL). The ethanolic solution was concentrated in vacuo until a semisolid product appeared. Trituration of the mixture with 4:1 ether/ethanol (50 mL) gave N-methyl carbamidine hydrochloride 2c (2.0 g, 89%). mp 175-177 °C; IR (KBr) 3420-3060 (br), 1690, 1650, 1530, 1230, 1100 cm−1; 'H NMR (DMSO-d6) δ 3.05 (s, 3 H, NCH3), 5.85 (d, 1 H, J = 5.36 Hz, 2'-OH), 5.32 (d, 1 H, J = 5.53 Hz, 3'-OH), 5.10 (t, 1 H, J = 5.31 Hz, 5'-OH), 4.44 (dd, 1 H, J = 4.62, 4.13, 4.62 Hz, H-2'), 4.23 (dd, 1 H, J = 5.1, 5.0, 5.13 Hz, H-3'), 4.03 (dd, 1 H, J = 4.3, 4.5, 4.4 Hz, H-4'), 3.73-3.45 (m, 2 H, H-5'), 3.07 (s, 3 H, NCH3), 13C NMR (DMSO-d6) δ 61.01, 61.45, 61.75, 70.42, 75.07, 89.05, 92.50, 116.81, 146.23, 155.61, 160.83. Anal. (C8H10N2O4Cl) C, H, N, Cl.

1,2-Di-bromo-1,2,4-triazole-3-N-butyrlcarbamidine (2d). A solution of methyl imidate 5 (0.3 g, 1.2 mmol) and butylamine (100 mg, 1.37 mmol) in anhydrous ethanol (40 mL) was flushed with argon, sealed, and stirred at room temperature for 5 days. The solvent was removed in vacuo and the residue was stirred overnight with anhydrous ether (50 mL). The ether was decanted and the procedure repeated with a fresh portion of anhydrous ether. Removal of the solvent in vacuo gave 2d as a glassy solid: 316 mg (67%); mp 63-66 °C; 'H NMR (DMSO-d6) δ 8.86 (s, 1 H, H-1'), 4.89, 5.36, 5.48, 5.58 and 5.0 (brs, 5 H, OH, NH), 4.36 (dd, 1 H, J = 3.8, 4.9 Hz, H-2'), 4.147 (dd, 1 H, J = 4.9, 3.8 Hz, H-3'), 3.955 (dd, 1 H, J = 4.9, 3.8 Hz, H-3').
1-b-D-Ribofuranosyl-1,2,4-triazole-3-N-acetylcarboxamidine (2f). The procedure for the synthesis of 2g was followed except for reaction time (1 h) and temperature (50 °C) to give a TLC Rf 0.35 (CHCl3/CH3OH, 2:1; i.e., 2f). The reaction was repeated three to five times with fresh portions of anhydrous ether. The solvent was removed in vacuo and the residue was stirred overnight with anhydrous ether (50 mL). The ether was decanted and the procedure was repeated three to five times with fresh portions of anhydrous ether.

Removal of the residue in vacuo gave 2g (424 mg, >95%) as white crystals; mp 151–153 °C; H NMR (DMSO-d6) δ 8.94 (s, 1 H, H-5), 8.10 (brs, 2 H, NH), 7.77 and 7.10 (each d, 2 H, J = 1.7, 1.7 Hz, CONH4), 5.90 (d, 1 H, J = 3.5 Hz, H-1'), 5.9, 5.5, 5.0 (3 x brs, 3 x H-3, 3 x H-4), 4.39 (dd, 1 H, J = 3.5, 4.8 Hz, H-2'), 4.21 (dd, 1 H, J = 3.5, 7.5 Hz, N(1)-CH2), 4.17 (dd, 1 H, J = 5.4, 4.7 Hz, H-3'), 3.99 (dd, 1 H, J = 3.5, 4.8, 5.4 Hz, H-4'), 3.66 (dd, 1 H, J = 3.5, 4.8 Hz, H-5'), 3.54 (dd, 1 H, J = 4.2, 12.1 Hz, H-5''), 2.96 (s, 2 H, AB, J = 17.4 Hz, H-2'), 2.67 (s, 2 H, AB, J = 7.1, 16.5 Hz, H-2''). MS (FAB) m/z 344 (M+H), 329 (M+Na), 291 (M+K), 286 (M+K2), 273 (M+Ac), 225 (M+N,N'), 170 (M+CO2H).

The product was purified by TLC (CHCl3/CH3OH, 2:1) with a Rf value of 0.35 (2f). The compound was stored at 5 °C.

Hydrolysis Studies of 2f and 6. 1-b-D-Ribofuranosyl-1,2,4-triazole-3-N-acetoxy carbamidine (2f) (6 mg) and N,N'-dimethyl-1-b-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid hydrochloride (6) (200 mg) were added to 3 and 5 mL respectively, of a pH 7.4 phosphate buffer solution at room temperature. The solutions were stirred and monitored by thin-layer chromatography (mobile phase: 0.155 CHCl3/CH3OH/HOAc) over a 24-h period. Hydrolysis products were determined by TLC comparison with products ribavin (Rf = 0.88) and N-methyl analogue 1f (Rf = 0.80).

1-b-D-Ribofuranosyl-1,2,4-triazole-3-N-acetoxy carbamidine (2f), 1-b-D-Ribofuranosyl-1,2,4-triazole-3-N-acetoxy carbamidine (2f) (2g), 1-b-D-Ribofuranosyl-1,2,4-triazole-3-N-1-(2-bromoethyl) carbamidine (2g), (2 g, H2O) or N-(1-(1-b-D-Ribofuranosyl-1,2,4-triazole-3-yl)methyl)methylene)glutamine. The procedure for the preparation of 2g was followed, allowing imidate solutions were stirred and monitored by thin-layer chromatography (mobile phase, 1:1:0.5 CHCl3/CH3OH/HOAc) and found to give a hygroscopic, light brown foam (3.0 g, 92.3%). Attempts to obtain a melting point using a nitrogen-filled glove box led to the observations that the crystals became gummy at 80 °C with melting occurring at 95–115 °C. MS (FAB) m/z = 272 (M + H); IR (KBr) δ 3330 (broad), 3100, 2945, 1600, 1500 cm−1. Anal. Calcd for C18H18N4OsCl: C, 54.6; H, 4.8; N, 26.9; Cl, 6.2. Found: C, 54.2; H, 4.8; N, 26.5; Cl, 6.0.

The solvent was removed in vacuo and the residue was dried in vacuo over P2O5 to give a hygroscopic, light brown foam (3.0 g, 92.3%). Attempts to obtain a melting point using a nitrogen-filled glove box led to the observations that the crystals became gummy at 80 °C with melting occurring at 95–115 °C. MS (FAB) m/z = 272 (M + H); IR (KBr) δ 3330 (broad), 3100, 2945, 1600, 1500 cm−1. Anal. Calcd for C18H18N4OsCl: C, 54.6; H, 4.8; N, 26.9; Cl, 6.2. Found: C, 54.2; H, 4.8; N, 26.5; Cl, 6.0.

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medium and incubated for 6 days, at which time they are stained by addition of 2 mL of 5% neutral red. Wells are decanted after 4 h and plaques counted. The IC50 is determined as the concentration of drug reducing plaques by 50% over the untreated control, while the minimum toxic concentration (MTC) is estimated visually by inspection of uninfected drug-treated wells.

Basic measurements and definitions used throughout these studies include (a) 50% cellular toxicity concentration, TC50, the drug concentration (μg/mL) that reduces the cell number and their metabolic activity by 50% as compared to the viability of uninfected control cells in duplicate test wells in the MTT assay; (b) 50% viral inhibitory concentration, IC50, the drug concentration (μg/mL) at which 50% reduction of viral cytopathic effect (CPE) is observed in triplicate test wells; the therapeutic (or antiviral) index, Tl50, a value proportional to the overall in vitro activity calculated as a ratio of TC50/IC50. It is a single drug concentration measurement of the relative antiviral and antiviral effectiveness of a compound during the same test and time period. All in vitro MTT assay results given represent an average of two to six individual test results.

In Vivo Evaluation in the Murine Punta Toro Model. Compounds were evaluated in Punta Toro virus-infected mice as previously described for ribavirin and carboxamidine.2a,2b

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