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In Vitro Inhibition of the Measles Virus by Novel Ring-Expanded ('Fat') Nucleoside Analogues Containing the Imidazo[4,5-*e*][1,3]diazepine Ring System

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Abstract—The synthesis and in vitro anti-measles virus (anti-MV) activity of a class of ring-expanded ('fat') nucleoside analogues (**1**–**4**) containing the title heterocyclic ring system are reported. The target compounds were synthesized by base-catalyzed condensations of 4,5-dicarboxylic acid esters of the appropriately substituted imidazole-1-ribosides with suitably substituted guanidine derivatives. Compounds were screened for anti-MV activity in African green monkey kidney cells (CV-1), employing ribavirin as the control standard. While the parent compound **1** itself failed to show any significant antiviral activity against MV, its analogues containing hydrophobic substituents at the 2-position (**2**) or the 6-position (**4**) showed promising antiviral activity at submicromolar or micromolar concentration levels with no apparent toxicity to the host cell line. Both compounds showed higher anti-MV activity than the control drug ribavirin.
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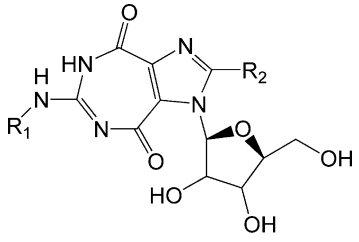
With over one million child deaths per year, measles virus (MV) is a major human pathogen, and ranks 8th as the cause of death worldwide, especially in the developing countries.[1] Despite large vaccination campaigns, MV is still resisting eradication, and there is no available therapeutic treatment. The MV infection causes a respiratory disease which is, more often than not, controlled solely by the immune response. However, MV infection can lead to a severe immunosuppression that is responsible for additional opportunistic infections.[2] Furthermore, in certain cases, MV establishes persistent infection of the brain leading to neurological complications.[3] MV is an enveloped, negative, single-stranded RNA virus belonging to the *Paramyxoviridae* family.[4] The intricate transcription and genome replication mechanisms of MV, coupled with the fine tuning of expression of its gene products, make MV one of the most sophistcated and clinically attractive viruses to study the life cycle as well as structural and biochemical basis of viral gene expression. The discovery of potent, new MV inhibitors may pave the way to novel approaches to combat the viral epidemic. Such inhibitors may also facilitate the in-depth structural and molecuar biological investigations that could further assist in studies of other similar human pathogens such as the Ebola virus. We report here a novel class of selective anti-MV agents (**1**–**4**) that can be considered as ring-expanded ('fat') analogues of purine nucleosides, and contain the title 5:7-fused imidazo[4,5-*e*][1,3]diazepine ring system.



1: $R_1=R_2=H$
2: $R_1=H$; $R_2=Ph$
3: $R_1=CH_3$; $R_2=H$
4: $R_1=C_2H_5$; $R_2=H$

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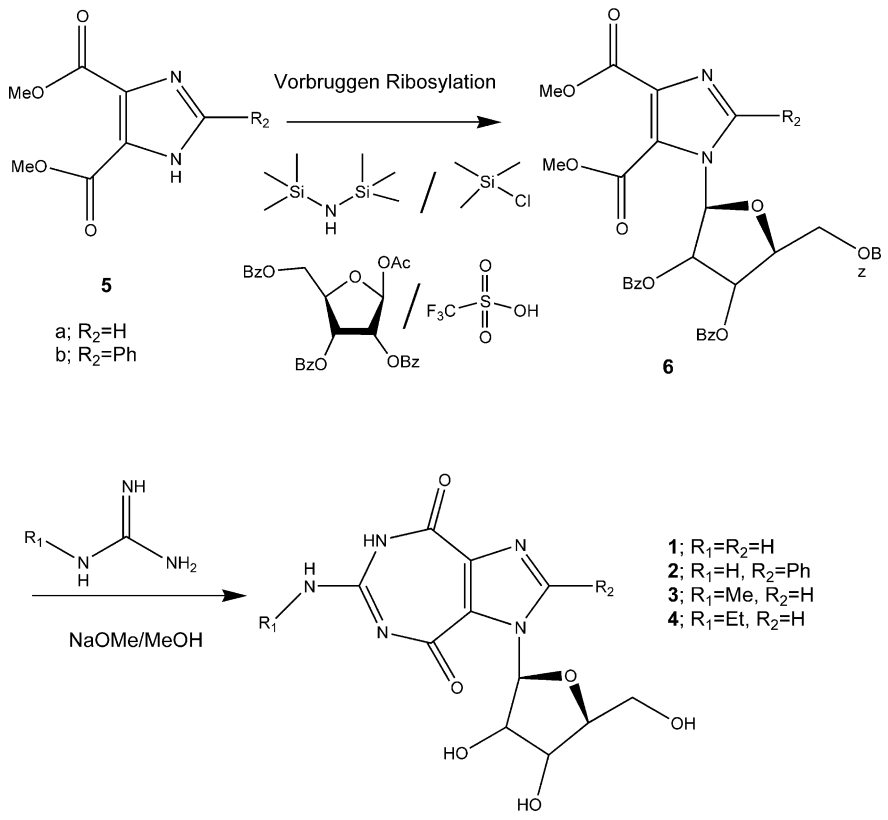

Scheme 1.

The general procedure for the synthesis (see Scheme 1) of the target nucleosides involved glycosylation of the appropriate 2-substituted imidazole analogues containing carboxylic acid ester groups at the 4- and 5-positions, followed by condensation of the resulting imidazole nucleosides with the appropriately substituted guanidine derivatives. We have already reported the synthesis of the parent nucleoside **1**.[5] The synthesis of the necessary 2-phenylimidazole precursor **5b** was accomplished by esterification of the corresponding 2-phenyl-4,5-imidazole- dicarboxylic acid[6–9] with anhydrous methanolic hydrogen chloride. The diacid, in turn, was prepared by one of the following two known methods, including (a) nitration of D-tartaric acid, followed by ring-closure of the resulting dinitrate by treatment with benzaldehyde and ammonium hydroxide,[6,7] or (b) basic hydrolysis (6 N NaOH) of 2-phenyl-4,5-dicyanoimidazole. The latter was synthesized either by ring-closure of *N*-benzylidenediaminomaleonitrile with *N*-chlorosuccinimide, and nicotinamide in dimethylformamide[8] or by diazotization of 2-amino-4,5-dicyanoimidazole with sodium nitrite and hydrochloric acid, followed by replacement of the resulting diazo functionality by phenyl group by heating with benzene at reflux.[9] The overall product yield by either of the two methods, *a* or *b*, was nearly identical although method *b* with 2-amino-4-cyanoimidazole as the starting material was our method of choice because of its simplicity, convenience, and ease of preparation. Nucleosides **3** and **4** were synthesized by condensation of **6a** with methyl- and ethylguanidine, liberated from the respective commercially available hydrochloride and sulfate salts by treatment with sodium methoxide in methanol. All final products and intermediates were fully characterized by NMR and mass spectral data, coupled with elemental microanalyses.[10]

Antiviral efficacy of compounds **1**–**5** was assessed in vitro by two different methods, including the Cytopathic Effect (CPE) inhibition assay and Neutral Red (NR) uptake assay.[11] African green monkey kidney cells (CV-1) were employed for both assays, using ribavirin as a positive control drug. Ribavirin, a broad spectrum antiviral nucleoside analogue containing a triazole moiety, is known to mimic guanosine.[12] The EC_{50} and IC_{50} values, along with a selective index (SI) for each compound, are collected in Table 1.

Table 1. Anti-measles virus activity of compounds **1**–**4** in vitro (CV-1 cell)

Compd	Assay used	EC_{50} (μM)	IC_{50} (μM)	SI (IC_{50}/EC_{50})
1	CPE inhibition	>100	>100	>0
	Neutral red	>100	>100	>0
2	CPE inhibition	10	>100	>10
	Neutral red	0.5	>100	>200
3	CPE inhibition	>100	>100	>0
	Neutral red	>100	>100	>0
4	CPE inhibition	2	>100	>50
	Neutral red	1.2	>100	>83
Ribavirin (Control)	CPE inhibition	20	1160	58
	Neutral red	16	612	38

While the parent compound **1** failed to show any significant anti-MV activity, data from Table 1 suggest that the introduction of the hydrophobic phenyl group at position-2 of the heterocyclic ring, as in **2**, results in antiviral activity. Antiviral activity also comes from introduction of a hydrophobic substituent bulkier than methyl group at position-6 as in compound **4**. These initial findings suggest that a few more analogues of **1**, containing substituents with increasing hydrophilicity at position-2 and/or position-6 need to be synthesized and screened against the measles virus in order to fully explore the structure–activity relationship (SAR) and the antiviral potential of these novel class of ring-expanded nucleoside analogues. Such an endeavor is currently in progress.

Conclusion

Ring-expanded ('fat') nucleoside analogues represent a new class of compounds that show promising anti-measles virus activity at submicromolar or micromolar concentration levels with no apparent toxicity to the host cell line. Both compounds showed higher anti-MV activity than the control drug ribavirin. Nucleosides **2** and **4** are promising candidates for further extensive structure–activity relationship (SAR) studies and drug development

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10. **General procedure for preparation of 6 by ribosylation of 5**: A solution of the appropriately substituted methyl 4,5-imidazoledicarboxylate[13] **(5)** (10 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-β,-D-ribofuranose (5.04 g, 10 mmol) in dry acetonitrile (50 mL) was placed into a flame-dried 100-mL round-bottom flask. The solution was stirred in an ice bath for 10 min. Then, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (7 mL, 33 mmol), chlorotrimethylsilane (TMSCl) (4.5 mL, 36 mmol) and trifluoromethanesulfonic acid (TFMSA) (3 mL, 36 mmol) were consecutively added to the above solution. The resulting solution was stirred in an ice-bath for 1 h. The reaction was complete as shown by TLC analysis [silica gel plates, chloroform/methanol (30:1)]. The reaction mixture was evaporated to dryness in vacuo. The resulting residue was dissolved in chloroform, and washed with saturated aqueous sodium bicarbonate and water respectively. After drying over anhydrous Na_2SO_4 and filtering, the chloroform solution was evaporated to dryness in vacuo to obtain the product **6** either as a foam or solid, which was recrystallized from the appropriate solvent or purified by silica gel flash chromatography to give the pure product **6**. The solvent of recrystallization and/or eluting solvent for column or prep-TLC chromatography, along with physical, spectral, and analytical data for **6** are given below:

Methyl 2-phenyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4,5-imidazoledicarboxylate (6b): Foam, recrystallized from methanol, colorless crystals (6.0 g, 85%), R_f 0.74 (chloroform-methanol (30:1)), mp 139–140 °C, ^{1}H NMR ($CDCl_3$) δ, 7.24–8.06 (m, 20H, ArH), 6.32 (dd, 1H, J = 5.7 and 6.9 Hz, 2′-H), 6.22 (d, 1H, J = 5.7 Hz, 1′-H), 5.82 (dd, 1H, J = 6.3 and 6.9 Hz, 3′-H), 4.71 (dd, 1H, J = 3.6 and 11.7 Hz, 5′-H), 4.58 (dd, 1H, J = 6.9 and 11.7 Hz, 5′-H), 4.52 (m, 1H, 4′-H), 3.96 (s, 3H, OMe), 3.93 (s, 3H, OMe), ^{13}C NMR ($CDCl_3$) δ, 52.32 (CH_3), 53.18 (CH_3), 63.69 (5′-C), 70.22 (3′-C), 74.12 (2′-C), 79.96 (4′-C), 89.54 (1′-C), 126.22, 126.22, 128.43, 128.43, 128.29 (Ph-3′, 5′, 2′, 6′, 4′), 128.34, 128.34, 128.39, 128.39, 128.55, 128.55 (Ph-*m*), 129.68, 129.68, 129.68, 129.73, 129.73, 129.73, 129.84, 129.84, 129.84 (Ph-*o* or *p*), 130.29, 130.29, 130.37 (imidazole), 133.22, 133.46, 133.66 (Ph-C1), 150.69 (Ph-1′), 161.54 (C=O), 162.56 (C=O), 164.70, 164.83, 166.12 (PhC=O), Anal. calcd for $C_{39}H_{32}N_2O_{11}$: C, 66.47, H, 4.58, N, 3.96. Found: C, 66.45, H, 4.66, N, 3.96.

General procedure for preparation of nucleosides 2–4 by condensation of 6 with guanidine: Guanidine hydrochloride (0.38 g, 4 mmol) was added to 4 mL of 2.3 M sodium methoxide solution resulting from sodium (0.75 g) dissolved in 15 mL of absolute methanol. The mixture was stirred in an ice-bath for 30 min. The precipitated sodium chloride was removed and the filtrate was poured into a solution of appropriately substituted methyl 1-(2,3,5-tri-*O*-benzoyl-β,-D-ribofuranosyl)-4,5-imidazoledicarboxylate (**6**) (1 mmol) in 20 mL of absolute methanol. The mixture was stirred at room temperature for 24–48 h, until when TLC showed the completion of reaction. The reaction mixture was filtered if necessary. The clear filtrate was treated with a solution of methanol saturated with HCl gas to pH 6.5–7.0. The resulting precipitate was filtered and washed with water and methanol to give the product. The solvent of recrystallization and/or eluting solvent for column or prep-TLC chromatography, along with physical, spectral, and analytical data for **2**–**4** are given below: **6-Amino-4,5-dihydro-8*H*-2-phenyl-1-(β-D-ribofuranosyl)imidazo[4,5-*e*][1,3] diazepine-4,8-dione (2):** Yield 85%, an analytical sample was prepared by preparative TLC on silica gel plate, using a mixture of acetonitrile–water (4:1) as a developing solvent, R_f 0.26 [chloroform-methanol-30% ammoniun hydroxide (2:1:0.3)), mp >250 °C, ^{1}H NMR (DMSO-d_6) δ, 10.71 (br s, 1H, NH, exchangeable with D_2O), 7.75–7.47 (m, 5H, Ph), 7.47 (br s, 1H, NH, exchangeable with D_2O), 6.53 (brs, 1H, NH, exchangeable with D_2O), 5.84 (d, 1H, J = 5.7 Hz, 1′-H), 5.33

(d, 1H, $J=6.3$ Hz, OH, exchangeable with D_2O), 4.96 (d, 1H, $J=6.3$ Hz, OH, exchangeable with D_2O), 4.72 (t, 1H, $J=5.4$ Hz, OH, exchangeable with D_2O), 4.44 (q, 1H, $J=6.0$ Hz, 2′-H, it became t after D_2O exchange), 3.70 (dd, 1H, $J=11.7$ and 5.7 Hz, 5′-H), 3.62 (dd, 1H, $J=11.7$ and 6.3 Hz, 5′-H), 3.52–3.36 (m, 2H, 3′,4′-H), ^{13}C NMR (DMSO-d_6) δ, 61.55 (C-5′), 69.02 (C-3′), 72.14 (C-2′), 85.19 (C-4′), 90.48 (C-1′), 128.12, 128.12 (Ph-3′,5′), 129.88, 129.88 (Ph-2′,6′), 129.40 (Ph-4′), 130.50, 131.16 (C-3a,8a), 140.75 (C-2), 149.92 (Ph-1′), 161.81, 162.54 (C-4,8), 166.76 (C-6), HRMS (FAB): Calcd. for $C_{17}H_{18}N_5O_6$ 388.1257, found 388.1276, Anal. calcd for $C_{17}H_{17}N_5O_6CH_3CN{\cdot}2/3H_2O$: C, 51.82, H, 4.88, N, 19.08. Found: C, 51.66, H, 4.43, N, 19.04.

4,5-Dihydro-8*H*-2-(*N*-methyl)amino-1-(β,-D-ribofuranosyl)-imidazo[4,5-*e*][1,3]diazepine-4,8-dione (3): Yield 93%, Mp > 210 °C (dec.), ^1H NMR (DMSO-d_6): δ, 10.63 (brs, 1H, NH, exchangeable with D_2O), 8.54 (s, 1H, imidazole), 7.01 (brs, 1H, NH, exchangeable with D_2O), 6.36 (d, $J=2.6$ Hz, 1H, 1′-H), 5.47 (d, $J=5.1$ Hz, 1H, OH, exchangeable with D_2O), 5.16 (t, $J=4.9$ Hz, 1H, OH, exchangeable with D_2O), 5.07 (d, $J=5.5$ Hz, 1H, OH, exchangeable with D_2O), 4.06 (m, 2H, 2′- and 3′-H), 3.90 (m, 1H, 4′-H), 3.73 (dd, $J_1=12.1$ Hz, $J_2=4.0$ Hz, 1H, 5′-H_1), 3.58 (dd, $J_1=11.7$ Hz, $J_2=4.0$ Hz, 1H, 5′-H_2), 2.75 (d, $J=4.0$ Hz, 3H, CH_3), Anal. calcd for $C_{12}H_{15}N_5O_6AH_2O$: C, 41.98, H, 4.99, N, 20.40 Found: C, 42.30, H, 4.79, N, 20.35

4,5-Dihydro-8*H*-2-(*N*-ethyl)amino-1-(β,-D-ribofuranosyl) imidazo[4,5-*e*][1,3]diazepine-4,8-dione (4): Yield 90%, Mp > 219 °C (dec.), ^1H NMR (DMSO-d_6) δ, 10.48 (brs, 1H, NH, exchangeable with D_2O), 8.55 (s, 1H, imidazole), 7.07 (brs, 1H, NH, exchangeable with D_2O), 6.36 (d, $J=2.9$ Hz, 1H, 1′-H), 5.50 (d, $J=4.8$ Hz, 1H, OH, exchangeable with D_2O), 5.18 (t, $J=4.8$ Hz, 1H, OH, exchangeable with D_2O), 5.08 (d, $J=5.1$ Hz, 1H, OH, exchangeable with D_2O), 4.06 (m, 2H, 2′- and 3′-H), 3.90 (m, 1H, 4′-H), 3.73 (dd, $J_1=11.7$ Hz, $J_2=4.4$ Hz, 1H, 5′-H_1), 3.58 (dd, $J_1=11.9$ Hz, $J_2=4.2$ Hz, 1H, 5′-H_2), 3.24 (m, 2H, CH_2), 2.75 (t, $J=7$ .1 Hz, 3H, CH_3), Anal. calcd for $C_{13}H_{17}N_5O_6$: C, 46.02, H, 5.05, N, 20.64. Found: C, 45.59, H, 5.14, N, 20.43.

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