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Synthesis of new pyrimidine nucleoside derivatives with nitric oxide donors for antiviral activity

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Abstract

New pyrimidine nucleoside derivatives with nitric oxide (NO) donor were systematically synthesized. The antivirus activities of these nucleoside analogues against vesicular stomatitis virus (VSV) in Wish cell were evaluated. It was demonstrated that most of compounds had stronger antiviral acitivity than acyclovir, while their toxicities were similar or lower to acyclovir.

Keywords: Pyrimidine nucleoside; NO-donor; Antivirus; Furoxans; Oximes; Synthesis

Structural analogues of nucleosides, nucleoside analogues (NA), together with nucleobases and nucleotide analogues, are commonly used in the treatment of viral infection and cancer. In both cases, they act as antimetabolite agents and interfere with the synthesis of cellular or viral nucleic acids [1,2]. Most of these agents are hydrophilic molecules and therefore require specialized transporter proteins to enter cells. Once inside, they are activated by intracellular metabolic steps to triphosphate derivatives. Active derivatives of nucleoside analogues can exert cytotoxic activity by being incorporated into and altering the DNA and RNA macromolecules or by interfering with various enzymes involved in synthesis of nucleic acids, such as DNA polymerases and ribonucleotide reductase. These actions result in inhibition of DNA synthesis and apoptotic cell death [3,4].

NAs such as didanosine (ddI), zalcitabine (ddC), lamivudine (3TC), zidovudine (AZT), stavudine (d4T) and abacavir (ABC) are used in antiviral treatment. These compounds belong to the class of nucleoside reverse transcriptase (RT) inhibitors (NRTI) [2]. Major problems in the treatment of viral and cancer diseases with NAs are acquirement of resistance and side effects such as delayed cytotoxicity. Accordingly, its therapeutic efficacy is limited, the development of new NAs with potent anti-virus and anti-tumor activity should be of great significance.

Nitric oxide (NO) is one of the most important signaling molecules within physiology and pathophysiology [5]. NO is produced by a group of enzymes called nitric oxide synthases (NOS). It is generally accepted that there are three main isoforms of the NOS enzyme, endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible (iNOS) [6].

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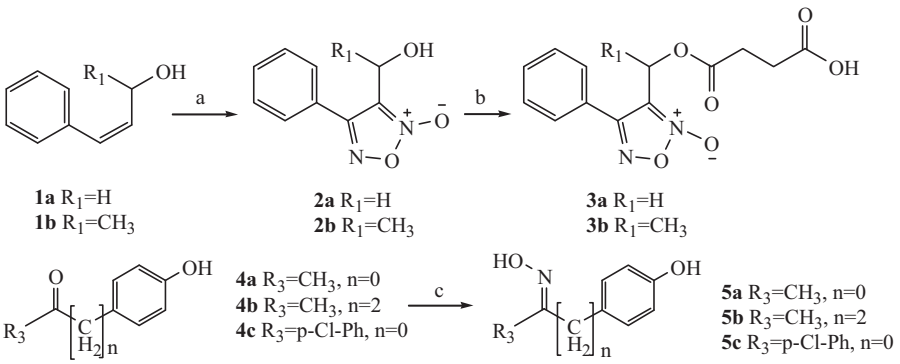


Scheme 1. General method for the synthesis of NO donors **3a–b**, **5a–c**. Reagents and conditions: (a) $NaNO_2$, AcOH, rt. 3 h; (b) succinic anhydride, ACN, Py, rt. 24 h; (c) NH_2OH HCl (aq), KOH, EtOH, reflux, 3 h.

Indeed, previous studies have shown that synthesized NO-releasing nucleoside analogues have strong cytotoxicity against human carcinoma cells or have strong antiviral activity *in vitro* [7–9]. Furoxans and alkyl- or aryloximes are thermally stable compounds and represent an important class of NO-donors, which can produce high levels of NO *in vitro*, and inhibit the growth of tumors *in vivo* [7,10].

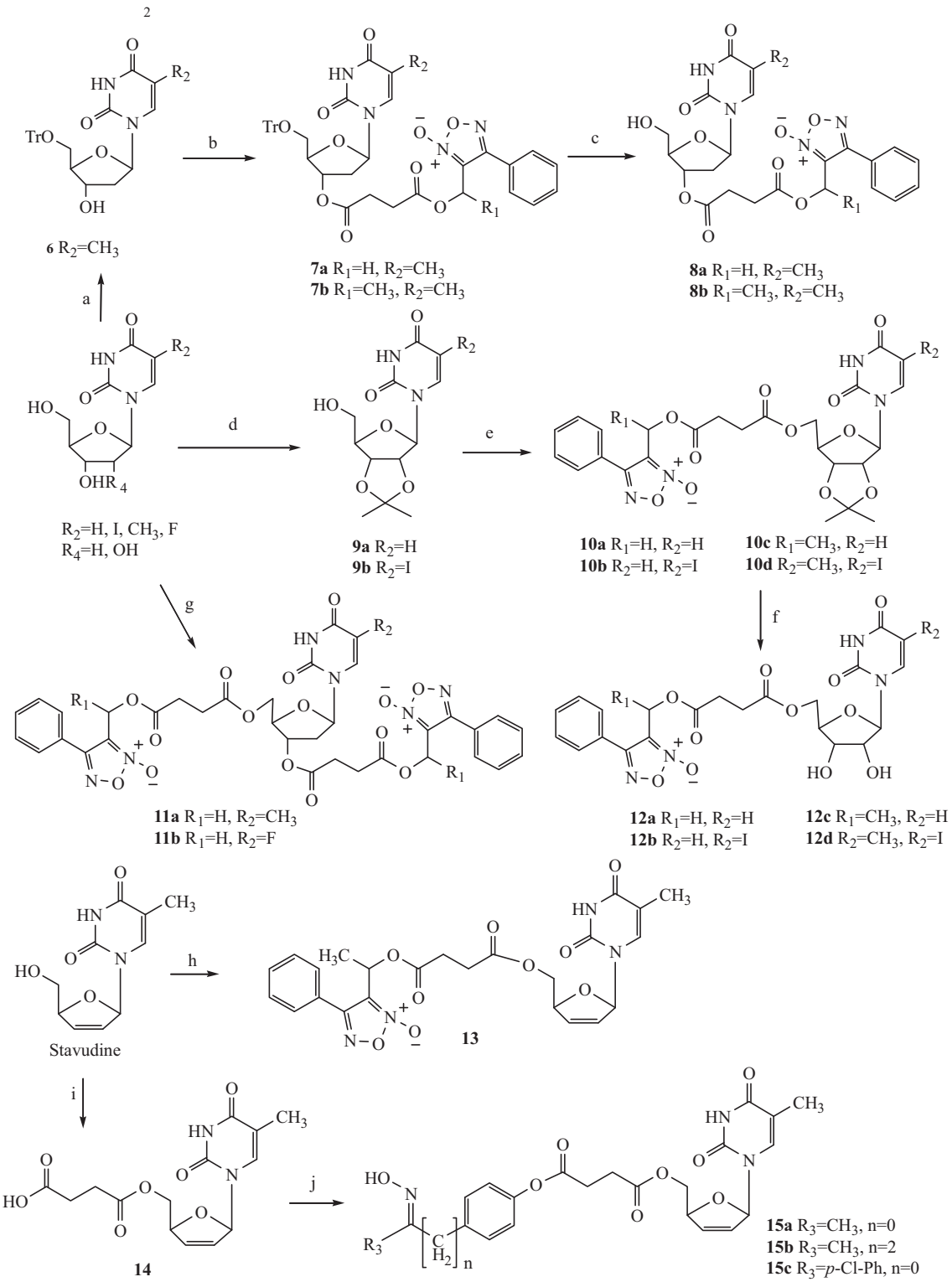
In order to get more potent antiviral and/or antitumor NAs, a series of new stavudine and β-thymidine derivatives combined NO donors, namely furoxans or aryloximes, were designed and synthesized.

The synthetic routes of substituted furoxan or aryloxime NO donors **3a–b, 5a–c** are outlined in Scheme 1. The substituted furoxans were prepared in a three-step sequence. The starting material cinnamyl alcohol derivates **1a**, **1b** were converted to **2a**, **2b** by treatment with $NaNO_2$ and glacial acetic acid in 40–90% yield [11]. **2a**, **2b** were acylated by succinic anhydride to generate succinic acid mono ester **3a**, **3b**. The substituted oxime NO donors were synthesized from the ketone derivates **4a–c**, treated with $NH_2OH{\cdot}HCl$ and KOH to produce the oxime derivates **5a–c** in 50–66% yield [12].

The target compounds **8a–b**, **10a–d**, **11a–b**, **12a–d**, **13**, **15a–c**, so called NO donor/NA hybrids, were synthesized as outlined in Scheme 2. To prepare compounds **8a–b**, β-thymidine was treated with triphenylmethyl chloride (TrCl) in dry pyridine to yield **6**, then reacted with **3a** or **3b** in the presence of *N*′,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 to lead the compounds **7a–b**. Finally, the protected groups of **7a–b** were removed with CF_3COOH to give the compounds **8a–b** [13]. The compounds **10a–d**, **12a–d** were preparated by using uridine or 5-iodouridine as starting material. It was respectively converted to 2′,3′-*O*-isopropylideneuridine **9a–b** by treatment with 4-methylbenzenesulfonic acid, triethoxymethane and acetone at room temperature in 60–70% yield [14]. The esterification of compounds **3a** or **3b** with **9a** or **9b** in the above-mentioned reaction conditions gave the compounds **10a–d** with yields in the range of 40–76%. Subsequently, treatment of **10a–d** with CF_3COOH cleaved the 2′,3′-*O*-isopropylidene-protected group to give the compounds **12a–d** [13]. β-Thymine or 2′-deoxy-5-fluorouridine was treated with two equilibrium of **3a** in the presence of DCC and DMAP in CH_2Cl_2 to generate the compounds **11a–b** in yield of 50–69%, respectively. Stavudine was reacted with **3b** to form the compound **13** [13] in 42.1% yield. In order to get **15a–c**, stavudine was reacted with succinic anhydride in pyridine to bring the corresponding ester **14**. The esterification of compounds **5a**, **5b** or **5c** with **14** in the presence of DCC and DMAP in CH_2Cl_2 lead to the compounds **15a–c**, respectively [13].

The antiviral activity of synthesized NO releasing nucleosides against vesicular stomatitis virus(VSV) in Wish cell was evaluated by previously reported method [15]. Acyclovir and stavudine were selected as positive control compounds. The TC0 (maximal atoxic concentration) values of individual compounds for Wish cell and ED50 values against VSV are presented in Table 1.

As shown in Table 1, ten of prepared nucleosides showed potent anti-VSV activities which were stronger than that of acyclovir, while most of their toxicities were similar to or lower than that of acyclovir, except **10b**, **10d**, **13**. Although stavudine is used as a clinical antiviral agent, anti-VSV activities of stavudine derivatives **13** and **15b** were not higher than other thymidine derivatives. It showed that the combined NO donors at 5′-position of sugar may play an important role in enhancing their antiviral activities.



Scheme 2. General method for the synthesis of **8a–b**, **10a–d**, **11a–b**, **12a–d**, **13**, **15a–c**. Reagents and conditions: (a) TrCl, Py, reflux, 45 min; (b) **3a** or **3b**, DCC, DMAP, CH_2Cl_2, rt. 24 h; (c) CF_3COOH, rt. 1 h; (d) uridine, 4-methylbenzenesulfonic acid, triethoxymethane, acetone, rt. 24 h; (e) **3a** or **3b**, DCC, DMAP, CH_2Cl_2, rt. 24 h; (f) CF_3COOH, rt. 1 h; (g) **3a**, DCC, DMAP, CH_2Cl_2, rt. 24 h; (h) **3b**, DCC, DMAP, CH_2Cl_2, rt. 24 h; (i) succinic anhydride, Py, reflux, 3 h; (j) **5a**, **5b** or **5c**, DCC, DMAP, CH_2Cl_2, rt. 24 h.

Table 1
Toxicity for Wish cell and anti-VSV effect of control compounds and NO-NA derivatives **8a–b**, **10a–d**, **11a–b**, **12a–d**, **13**, **15a–c**.

Compound	Acyclovir	Stavudine	**8a**	**8b**	**10a**	**10b**	**10c**	**10d**	**11a**
(TC0) (μmol/L)	2775	2788	1210	589	560	4566	546	8949	765
ED50 (μmol/L)	830.8	N.D.	N.D.	180.2	229.7	172.1	186.9	284.1	282.0

Compound	**11b**	**12a**	**12b**	**12c**	**13**	**15a**	**15b**	**15c**
(TC0) (μmol/L)	762	603	485	1174	6098	1366	1287	70
ED50 (μmol/L)	301.0	N.D.	N.D.	460.3	238.6	N.D.	278.7	N.D.

Note: N.D.: not determined.

Further NO-release and anti-tumor studies of these new nucleosides in correlated cells are currently in progress. Additional results will be reported in due course.

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[13] The data of selected compounds. **8a**: white foam; mp 72–74 °C; EMS-MS: 539[M+Na]$^+$, 517[M+H]$^+$, 515[M−1]$^+$, $C_{23}H_{24}N_4O_{10}$(Mr: 516.46); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.1(s, 1H, NH), 7.52–7.71 (m, 6H, ArH, 6-CH), 6.20 (q, 1H, 1′-CH), 5.37 (t, 1H, 4′-CH), 5.19 (s, 2H, O-CH_2), 4.10 (q, 1H, 3′-CH), 3.89 (d, 2H, J = 3 Hz, 5′-CH_2), 2.68 (s, 5H, 2×CH_2, OH), 2.34–2.45 (m, 2H, 2′-CH_2), 1.91 (s, 3H, CH_3); Anal. Calcd. for. $C_{23}H_{24}N_4O_{10}$ + 0.2$CH_3COOCH_2CH_3$(534.08): C 53.52, H 4.83, N 10.49; found: C 53.76, H 4.82, N 10.21. **12a**: white foam; mp 68–70 °C; EMS-MS: 541[M+Na]$^+$, 519[M+H]$^+$, $C_{22}H_{22}N_4O_{11}$ (Mr: 518.43); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.78 (s, 1H, NH), 7.52–7.70 (m, 6H, ArH, 6-CH), 5.75–5.79 (m, 2H, 5-CH, 1′-CH), 5.16 (s, 2H, O-CH_2), 4.19–4.38 (m, 5H, 2′-CH, 3′-CH, 4′-CH, 5′-CH_2), 2.9 (s, 2H, 2×OH), 2.67 (s, 4H, 2×CH_2); Anal. Calcd. for $C_{22}H_{22}N_4O_{11}$ + 0.25$CH_3COOCH_2CH_3$(540.45): C 51.11, H 4.48, N 10.37; found: C 50.61, H 4.70, N 9.91. **13**: white foam; mp 69–72 °C; EMS-MS: 535[M+Na]$^+$, 513[M+H]$^+$, $C_{24}H_{24}N_4O_9$ (Mr: 512.47); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (s, 1H, NH), 7.53–7.72 (m, 5H, ArH), 7.21 (d, 1H, 1′-CH), 6.98 (d, 1H, J = 3 Hz, 4′-CH), 6.24 (q, 1H, O-CH), 5.89 (d, 2H, O-CH_2), 5.01 (s, 1H, 6-CH), 4.41–4.21 (m, 2H, –CH═CH–), 2.60–2.61 (m, 4H, 2×CH), 1.90 (d, 3H, CH_3), 1.62 (s, 3H, CH_3); Anal. Calcd. for $C_{24}H_{24}N_4O_9$ + $CH_3COOCH_2CH_3$ (600.58): C 55.90, H 5.52, N 9.31; found: C 55.85, H 5.07, N 9.48. **15a**: white foam; mp 78–80 °C; EMS-MS: 480[M+Na]$^+$, 456[M−1]$^+$, $C_{22}H_{23}N_3O_8$(Mr: 457.43); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (s, 1H, NH), 7.59 (d, 2H, ArH), 7.28 (d, 1H, 1′-CH), 6.96 (d, 1H, 4′-CH), 6.85 (d, 2H, ArH), 5.91–6.3 (dd, 2H, 5′-CH_2), 5.05 (s, 1H, 6-CH), 4.22–4.52 (m, 2H, –C═C–), 2.67–2.94 (m, 4H, 2×CH_2), 2.33 (s, 3H, CH_3), 1.93 (s, 3H, CH_3); Anal. Calcd. for $C_{22}H_{23}N_3O_8$ + 1.75H_2O (488.93): C 54.04, H 5.46, N 8.59; found: C 53.99, H 5.01, N 8.34.
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