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Synthesis and antiviral activity of 7-deazaneplanocin A against orthopoxviruses (vaccinia and cowpox virus)

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Abstract

An efficient method for the synthesis of 7-deazaneplanocin A (**2**) has been accomplished by the condensation of cyclopentenol **3** with 6-chloro-7-deazapurine followed by subsequent functional group manipulations. The synthesized 7-deazaneplanocin A (**2**) exhibited potent antiviral activity against cowpox and vaccinia viruses without cytotoxicity in HFF cells.

Keywords

7-Deazaneplanocin A; Vaccinia; Cowpox; Orthopoxviruses

Neplanocin A (NPA)¹ (**1**) is a naturally occurring carbo-cyclic nucleoside, which has a cyclopentenyl sugar-substituted moiety. NPA has attracted much attention because it showed interesting biological activities including antitumor and broad spectrum antiviral activities.² NPA's biological activity may be explained in part by the inhibition of S-adenosyl-L-homocysteine (AdoHcy) hydrolase, which is essential for viral mRNA capping methylation.³ Structural modifications of NPA have yielded a number of biologically active compounds. Among them, modification on the C6'-position of NPA has produced potent analogues against malaria, hepatitis B virus, and hepatitis C virus.^{4,5} Base (adenine) modified NPA analogues such as 2-fluoro-⁶ and 3-deaza-⁷ derivatives showed potent antiviral activity. Recently, 7-deazaadenine moiety was introduced in place of adenine in 2'-C-ribonucleoside.⁸ According to the report, the introduction of 7-deazaadenine moiety resulted in good enzymatic stability as well as a significant anti-HCV activity. These results prompted us to pursue the synthesis and biological evaluation of a 7-deazaneplanocin A analogue (**2**), which has not been reported so far.

Variola, monkeypox, cowpox, and vaccinia viruses are orthopoxviruses, which can infect humans.⁹ Among orthopoxviruses, variola virus is the most dangerous virus and smallpox was responsible for serious illness and death until the development of a successful vaccine. The need for the therapeutic agent for the treatment of orthopoxvirus infections was not urgent since the eradication of smallpox from the world in 1980. However, recent concern

on bioterrorism rekindled the interest on the treatment of patients, who were infected with the orthopoxviruses, including variola virus. The antiviral activities of neplanocin A and its analogues (neplanocin C, neplanocin D, 2'-deoxy NPA, 3-deaza NPA, and 5'-nor NPA) against surrogate vaccinia virus infections were reported in the literature.^{2,10} Recently, several carbocyclic analogues also showed antiviral activity against orthopoxviruses.¹¹

In order to investigate the structure–activity relationships of NPA, a more convenient and facile synthetic methodology for key intermediates is required. During the course of drug discovery program for carbocyclic nucleosides, we developed a convergent method for the synthesis of cyclopentenyl carbocyclic nucleosides.¹² The synthetic strategy employed was the coupling of cyclopentenol and heterocyclic bases to afford various enantiomerically pure carbocyclic nucleosides. Herein, we report the synthesis and antiviral activity of 7-deazaneplanocin A (**2**) (Fig. 1).

The preparation of 7-deazaneplanocin A (**2**) is shown in Scheme 1. The cyclopentenol **3**, which was prepared according to the literature with slight modifications of reported method,¹² was coupled with 6-chloro-7-deazapurine¹³ under the Mitsunobu reaction conditions to give compound **4** in 81% yield. Treatment of **4** with methanolic ammonia at 100 °C for 12 h provided compound **5** in 83% yield. Deprotection in acidic conditions of **5** in 10% HCl in methanol followed by neutralization with NaHCO₃ gave 7-deazaneplanocin A (**2**)¹⁴ in 63% yield.

The antiviral activity of 7-deazaneplanocin A (**2**) was evaluated against a wide variety of viruses, including cowpox, vaccinia, yellow fever, dengue type 2, Punta Toro A, SARSCoV, Tacaribe, VEE, and West Nile. Among the tested viruses, 7-deazaneplanocin A exhibited potent activity against cowpox and vaccinia viruses in a CPE reduction assay without any significant cytotoxicity in HFF cells as shown in Table 1.

Although Neplanocin A has potent broad spectrum antiviral activity including orthopoxviruses,² significant cytotoxicity of NPA limited its usefulness as an antiviral agent.¹⁵ However, 7-deaza NPA (**2**) did not show any cytotoxicity up to 300 µM in HFF cells in a neutral red assay. Furthermore, 7-deaza NPA was more potent than that of cidofovir in this assay, which has been known to be one of the most potent agents against orthopoxviruses.¹⁶

In summary, we have synthesized 7-deazaneplanocin A (**2**) by the coupling of functionalized cyclopentenol (**3**) with 7-deazapurine. The synthesized 7-deazaneplanocin A (**2**) showed potent antiviral activity against orthopoxviruses (cowpox and vaccinia) without any significant cytotoxicity. Further biological evaluation to delineate the mode of action as well as study of animal models to assess the full potential of 7-deazaneplanocin A is warranted.

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13. 6-Chloro-7-deazapurine is commercially available at Toronto Research Chemicals, Inc.
14. Data for compound **2**: mp 196–198 °C; $7[\alpha]_D^{23}$ –43.80 (*c* 0.10, MeOH); ^1H NMR (500 Hz, DMSO- d_6) δ 8.04 (s, 1H), 7.00 (d, 3.5 Hz, 1H), 6.94 (br s, 2H), 6.54 (d, 3.5 Hz, 1H), 5.58 (m, 1H), 5.52 (m, 1H), 4.99 (br s, 1H), 4.88 (br s, 2H), 4.40 (d, 4.5 Hz, 1H), 4.05–4.15 (m, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) 157.9, 151.9, 150.4, 2150.2, 124.9, 121.8, 103.2, 99.4, 77.6, 72.9, 64.2, 59.0; UV (H₂O) λ_{max} 273.0 nm (ϵ 9281, pH 2), 272.0 nm (ϵ 9068, pH 7), 272.0 nm (ϵ 9244, pH 11); Anal. Calcd for C₁₂H₁₄N₄O₃·H₂O: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.85; H, 5.22; N, 19.67.
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Figure 1.
Structures of NPA and 7-deaza NPA.

Scheme 1.

Synthesis of 7-deazaneplanocin A (**2**). Reagents and conditions: (a) 6-chloro-7-deazapurine, PPh_3 , DIAD, THF, 81%; (b) NH_3 , MeOH, 100 °C, 12 h, 83%; (c) 1—HCl, MeOH, 2— NaHCO_3 63%.

Table 1
Antiviral activity of 7-deazaneplanocin A (2) against cowpox and vaccinia viruses

Virus	Cell line	EC ₅₀ (μM)	EC ₉₀ (μM)	Cytotoxicity CC ₅₀ (μM)	Cidofovir ^a EC ₅₀ (μM)
Cowpox	HFF cells	1.2	4.6	>300	5.0
Vaccinia	HFF cells	3.4	21.5	>300	4.8

^a Positive control.