NEW NEPLANOCIN ANALOGUES. IV. 2-FLUORONEPLANOCIN A: AN ADENOSINE DEAMINASE-RESISTANT EQUIVALENT OF NEPLANOCIN A [1]

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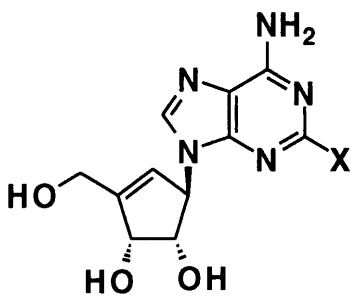
2-Fluoro- and 2-chloroneplanocin A's (**2** and **3**) were synthesized as an adenosine deaminase resistant-equivalent of neplanocin A, and evaluated for their antitumor and antiviral activities. Of these, **2** was completely resistant to adenosine deaminase and showed more significant antitumor and antiviral activities than neplanocin A.

KEYWORDS neplanocin A; nucleoside antibiotic; adenosine deaminase; antiviral activity; antitumor activity

Neplanocin A (NPA, **1**), a carbocyclic nucleoside antibiotic,[2] has significant antitumor[3] and antiviral[4] activities. The mechanism of action has been extensively explored and elucidated; the antitumor effect could be derived from, for the most part, phosphorylation of the primary hydroxyl group at its 6'-position by adenosine kinase and subsequent metabolism by cellular enzymes,[5] while the antiviral effect would be due to the inhibition of adenosylhomocystein (AdoHcy) hydrolase via disturbing virus mRNA maturation.[4,6] NPA has also been known to be rapidly deaminated by adenosine deaminase into a chemotherapeutically inactive inosine congener,[2,4,7a] which would reduce the therapeutic potency of NPA especially *in vivo*. Based on these observations, chemical modifications of NPA have been extensively studied to develop efficient antitumor or antiviral agents by us[7] and others.[8]

We report here the synthesis of 2-fluoro- and 2-chloro-NPA's (**2** and **3**, respectively), which were designed to be an adenosine deaminase-resistant equivalent of NPA.[9] These were expected to serve as substrates for both adenosine kinase and AdoHcy hydrolase, because these compounds preserved all functional groups of NPA to be recognized by the two enzymes,[10,11] while the presence of a fluorine or a chlorine atom at the 2-position of the adenine moiety would invest them with resistance to adenosine deaminase.[9]

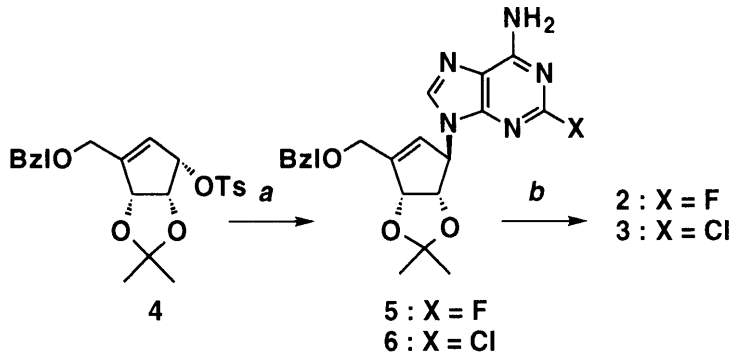
Because practical methods for introducing a substituent at the 2-position of adenine nucleosides directly were not known, we synthesized the target compounds by applying a total synthetic method for NPA reported by Marquez and coworkers.[12] A *p*-toluenesulfonyloxycyclopentene derivative **4**,[12] which was prepared from D-ribonolactone, was treated with 2-fluoroadenine[13] and K_2CO_3 in the presence of 18-crown-6 in DMF at 75 °C to give the desired 2-fluoro-NPA derivative **5** in 30% yield. In the same way, 2-chloro derivative **6** was obtained in 43% yield. Deprotections of **5** and **6** were done by treating them with $AlCl_3$/anisole in CH_2Cl_2[14] at room temperature to give **2** and **3** in 66% and 62% yields, respectively.[15]



1 (NPA) : X = H
2 : X = F
3 : X = Cl

The susceptibilities of **2** and **3** to adenosine deaminase from calf intestine were studied, and the results are shown in Fig. 1. As expected, both compounds were completely resistant to the deamination by the enzyme, though NPA was deaminated rapidly into the inactive inosine congener under the same reaction conditions.

Antiproliferative effects of the compounds against the mouse P388 leukemia cell line were investigated. 2-Fluoro derivative **2** significantly inhibited the growth of



Reagents: *a*) 2-F-(or 2-Cl)adenine, K_2CO_3, 18-crown-6, DMF; *b*) $AlCl_3$, anisole, CH_2Cl_2.

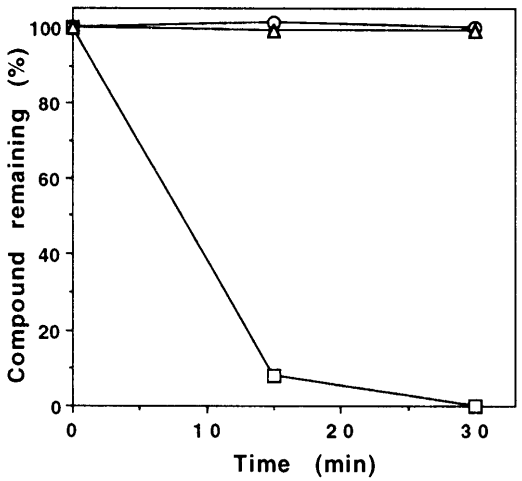


Fig. 1. The Effects of Calf Intestinal Adenosine Deaminase on NPA (□), **2** (Δ), and **3** (○). The assay was done as previously described (Ref. 7a).

the cells (IC_{50} = 0.025 μg/mL), while NPA had a more notable effect (IC_{50} = 0.001 μg/mL). 2-Chloro derivative **3** was much less active (IC_{50} = 17 μg/mL) in this evaluation system. Therefore, we thought **2** should be further pursued for its therapeutic potential as an antitumor agent. It has been known that NPA is almost inactive against solid tumors *in vivo*, in spite of its marked antitumor effects on leukemia or ascitic tumors in mice.[3,7c] Inhibitory effects of **2** and NPA on solid tumor were investigated with sc-implanted Meth-A fibrosarcoma in mice, and the results are shown in Table I. Compound **2**, administered ip on day 1-5, distinctly inhibited the growth of sc-implanted Meth A fibrosarcoma in mice (T/C = 37% at 30 mg/kg/day, significantly different from the control at $P<0.05$), though NPA had only an insignificant effect on this tumor. The improvement in the antitumor potency of **2** might be due to the resistance to adenosine deaminase, because it was known that NPA itself was deaminated quite rapidly and was almost undetectable in the blood after ip-administration of NPA in mice.[3]

Table I. Antitumor Effects of **2**, **3**, and NPA on sc-Implanted Meth A Fibrosarcoma in Mice[a]

Dose[b] (mg/kg/day)	T/C (%)[c]	
	2	NPA
1	NT[d]	101
3	63	63
10	58	toxic
30	37	NT

a)Assay was done by a reported method (Ref. 7c). b)Administered ip on day 1-5. c)Antitumor activity was evaluated from the average tumor volume of treated mice (T) over that of control mice (C). d)Not tested.

The inhibitory effects of compounds on AdoHcy hydrolase, which has been recognized to be correlated with the antiviral potency, were evaluated in a cell-free system with an enzyme from rabbit erythrocyte.[17] Compound **2** apparently inhibited the enzyme (IC_{50} = 0.20 μg/mL), while NPA had a more potent inhibitory effect (IC_{50} = 0.004 μg/mL). The 2-chloro derivative **3** was again inactive in this system.

Next, the compounds were evaluated for antiviral activity against a variety of pathogenic RNA viruses *in vitro*, together with ribavirin,[18] an anti-RNA virus drug used in the clinic, as a positive control. The results are summarized in Table II. Compound **2** had excellent activity against all RNA viruses tested (ED_{50} = 0.25 - 7.7 μg/mL) while not being toxic to the normal host cells in stationary phase, at concentrations up to 100 μg/mL. This surpassed the antiviral potency of ribavirin. Although NPA showed a comparable antiviral effect to **2**, it was apparently cytotoxic to host cells. These results suggested that *in vivo* antiviral effects of **2** would be of interest because of its adenosine deaminase-resistant property and reduced cytotoxicity to the host cells compared to NPA. This is under investigation.

Table II. Anti-RNA Virus Activities and Cytotoxicity on Host Cells of **2**, **3**, NPA, and Ribavirin[a)]

Compound	Anti-RNA viruses activity, ED_{50} (μg/mL)[b)]						Cytotoxicity, IC_{50} (μg/mL)[c)]		
	VSV	Measles (Sugiyama)	Mumps (WV-3)	PV-3 (C 243)	RSV (Long)	Influenza V-A (Ishikawa)	Vero	Hela	MDCK
2	0.25	<0.9	1.2	3.0	5.2	7.7	>200	>100	>100
3	63	NT[d)]	NT	NT	NT	NT	>200	NT	>100
NPA	0.25	NT	NT	1.6	3.9	15.9	155	>40[e)]	>20[e)]
Ribavirin	12.6	9.6	17.9	5.0	7.7	2.0	>200	>100	>100

a)Assay was done by a reported method (Ref. 7b). Abbreviations: VSV, vesicular stomatitis virus; PV-3, parainfluenza virus-3; RSV, respiratory syncytial virus. b)Concentration required to inhibit virus-induced cytopathogenicity by 50%. c)Concentration required to reduce the number of viable cells by 50%. d)Not tested. e)Data were taken from Ref. 7a.

In conclusion, 2-fluoro-NPA (**2**) was completely resistant to adenosine deaminase and had a more significant potency both in antitumor and antiviral effects than NPA. This compound should be pursued for its therapeutic potential as an antiviral and antitumor agent as well as for its mechanism of action.

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(Received May 20, 1994; accepted June 27, 1994)