SYNTHESIS AND ANTIVIRAL ACTIVITY OF ADENOSINE DEAMINASE-RESISTANT OXETANOCIN A DERIVATIVES: 2-HALOGENO-OXETANOCIN A

Sir:

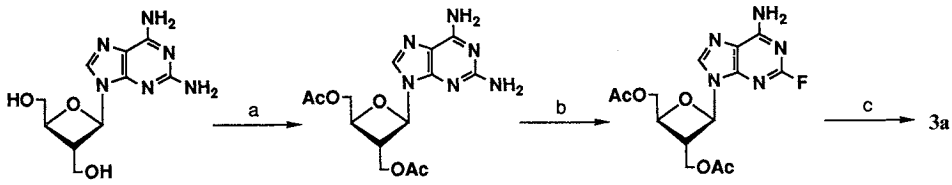
A number of adenosine analogues with various types of sugar moiety have been found and synthesized by pioneering researchers in nucleoside chemistry.[1~4] Several analogues are now identified as potent antiviral agents, such as 2',3'-dideoxyadenosine (DDA) against human immunodeficiency virus (HIV)[5] and 9-β-D-arabinofuranosyladenine (araA) against hepatitis B virus (HBV).[6] Oxetanocin A (OXT-A, **1**), which was isolated as a natural product, is a nucleoside analogue with adenine as base moiety as similar as DDA and araA. It shows antiviral activities against HIV, human cytomegalovirus (HCMV), and herpes simplex virus type 1 and 2 (HSV-1,2).[7] Its usefulness is, however, limited by several drawbacks. A major drawback of OXT-A is a short half-life time in plasma partly due to the deamination by adenosine deaminase; the deamination of OXT-A produces chemotherapeutically inactive oxetanocin H (OXT-H, **2**).[8] It has been shown that some adenosine analogues substituted at the C-2 position of base moiety by fluoro, chloro or alkynyl groups are substrate analogues resistant to the action of adenosine deaminase,[8~11] and that some of them have various bioactivities. For example, 2-chloro-2'-deoxyadenosine is remarkably toxic to many leukemia cell specimens.[9]

In the present communication, we report the synthesis and antiviral activity *in vitro* of the adenosine deaminase-resistant derivatives of OXT-A, namely, 2-fluoro-oxetanocin A (2-F-OXT-A, **3a**), 2-chloro-oxetanocin A (2-Cl-OXT-A, **3b**), 2-bromo-oxetanocin A (2-Br-OXT-A, **3c**), and 2-iodo-oxetanocin A (2-I-OXT-A, **3d**). We also describe antiviral activity of 2-F-OXT-A against HSV-2 *in vivo*.

Chemistry

2-Amino-oxetanocin A (2-amino-OXT-A, **4**) served as starting material in the synthesis of the 2-halogeno compounds.[11] 2-F-OXT-A was synthesized with the selective fluoro substitution process at the C-2 position of the purine ring by the method reported previously.[12] The hydroxyl groups of 2-amino-OXT-A was protected by acetyl group to

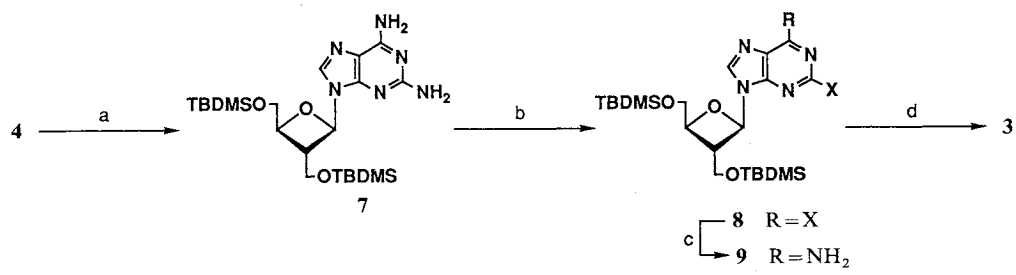
Scheme 1.



4 5 6

(a) Ac_2O, DMAP, pyridine; (b) *tert*-BuONO, 60% HF/pyridine, −30°C; (c) concentrated NH_4OH, MeOH.

Scheme 2.



b series X=Cl, **c** series X=Br, **d** series X=I

(a) TBDMS-Cl, imidazole, DMF; (b) isoamyl nitrite in CCl_4 for **8b**, $CHBr_3$ for **8c**, CH_2I_2 for **8d**; (c) NH_3-MeOH; (d) TBAF, THF.

afford **5**. Treatment of **5** with *tert*-butyl nitrite in 60% HF/pyridine at −30°C gave 2-fluoro derivatives **6** in 55% yield. Deblocking of **6** by concentrated NH_4OH/MeOH gave the target compound 2-F-OXT-A. The other 2-halogeno compounds were obtained as follows: Treatment of 2-amino-OXT-A with *tert*-butyldimethylsilyl chloride in the presence of imidazole in *N,N*-dimethylformamide gave the protected nucleoside **7**. When **7** was heated in halomethane (CCl_4, $CHBr_3$, CH_2I_2) in the presence of isoamyl nitrite, each of the crude 2,6-dihalogeno purine derivatives **8b**～**8d** was obtained.[13] On high temperature condition (above room temperature) diazotiation with alkyl nitrite occurs not only at the C-2 position but at the C-6 position of purine ring. So the obtained **8b**～**8d** were treated with methanolic ammonia to give the desired compounds, the 2-halogeno nucleosides **9b**～**9d** (**9b** in 52%, **9c** in 46%, **9d** in 53% from **7**). **9b**～**9d** were deprotected with tetrabutylammonium fluoride to yield each 2-Cl-OXT-A, 2-Br-OXT-A, and 2-I-OXT-A.

Data of 2-F-OXT-A are representative: MP 235～237°C (H_2O); MS *m/z* 269 (M^+), 153 (Base+H^+); ^1H NMR (Me_2SO-d_6, 200 MHz) δ 8.64 (1H, s, 8-H), 7.90 (2H, brs, 6-NH_2), 6.29 (1H, d, 1′-H, J=5.5 Hz), 5.24 (1H, t, 3′-OH), 5.03 (1H, t, 2′-OH), 4.52 (1H, m, 3′-H), 3.73～3.57 (5H, m, 2′-H, 4′-Ha, b, 2′-CHa, b); UV λ_{max} (H_2O) 260 (ε 14,800); Anal. ($C_{10}H_{12}N_5O_3F$) C, H, N.

Table 1. Effects of adenosine deaminase on 2-Halogeno-OXT-As[a].

Compounds	Remaining rate (%)[b]		
	10 minutes	30 minutes	60 minutes
2-F-OXT-A (**3a**)	98	99	97
2-Cl-OXT-A (**3b**)	100	100	100
2-Br-OXT-A (**3c**)	100	99	99
2-I-OXT-A (**3d**)	100	97	98
OXT-A (**1**)	83	27	0

[a] See ref 15.
[b] % of total compound remaining after incubation.

Effects of Adenosine Deaminase on the 2-Halogeno-OXT-As

2-Halogeno compounds were examined for their susceptibility to deamination by calf intestinal adenosine deaminase, so they proved to be virtually resistant to deamination. In contrast, OXT-A itself was totally deaminated by adenosine deaminase (Table 1).†

Biological Activity

Target compounds were first evaluated for activities against HSV-1 and HCMV.[14,15] Among 2-halogeno-OXT-As tested, 2-F-OXT-A was the most active against both HSV-1 and HCMV *in vitro*, and its IC_{50} (the compound concentration required to inhibit the cytopathic effect of viruses by 50% *in vitro*) value against HCMV (1 μg/ml) was less than 1/15 that of the parental compound OXT-A. However, the anticellular activity of 2-F-OXT-A also increased more than 10-fold compared with that of OXT-A, and hence the selectivity index of the compound against HCMV remained relatively low (data not shown). We next evaluated

Table 2. Antiviral activity in plaque-reduced assays of 2-F-OXT-A (**3a**) against wild-type (TK^+) and thymidine kinase-deficient (TK^-) herpes simplex virus type 2 (HSV-2)[a].

Compounds	CC_{50}[b]	HSV-2 (TK^+)		HSV-2 (TK^-)	
		IC_{50}[c]	S.I.[d]	IC_{50}[c]	S.I.[d]
2-F-OXT-A (**3a**)	201	5.6	36	3.9	52
Acyclovir (ACV)	>50	0.18	>278	12	>4.2
Ganciclovir (DHPG)	>50	0.30	>167	>50	

[a] See ref 16.
[b] The compound concentration (μg/ml) required to supress the growth of target Vero cells, a line of African green monkey kidney cells, by 50% *in vitro*.
[c] The compound concentration (μg/ml) required to reduce viral replication by 50% in plaque reduction assays.
[d] Selectivity index: ratio of CC_{50}/IC_{50}.

† Effects of adenosine deaminase were examined as follows: 5 units of adenosine deaminase prepared from calf intestinal mucosa (SIGMA, 1,800 units/ml was added to 20 ml of phosphate buffer (0.1 M, pH 6.8) containing 0.05 mmol of the test compound, and this solution was incubated at 37°C. After 10, 30, and 60 minutes 2 μl of reaction mixture was sampled and analyzed by reversed phase HPLC (column, Senshu Pac ODS-5121-N, 6 mm i.d. × 150 mm; eluate, 0.1 M citrate buffer - CH_3CN - MeOH (50 : 2 : 1); detector, UV 254 nm).

Table 3. Antiviral activity against human immunodeficiency virus type 1 (HIV-1)[a].

Compound	CC_{50}[b]	HIV-1	
		IC_{50}[c]	S.I.[d]
2-F-OXT-A (**3a**)	5.6	0.016	350
2-Cl-OXT-A (**3b**)	56	0.44	130
2-Br-OXT-A (**3c**)	30	7.2	4.2
2-I-OXT-A (**3d**)	54	72	0.75
OXT-A (**1**)	23	1.7	14
2′,3′-dideoxyinosine (DDI)	980	0.21	4,700

[a] See ref 18.
[b] The compound concentration (μg/ml) required to inhibit the growth of target MT-4 cells by 50% *in vitro*.
[c] The compound concentration (μg/ml) required to inhibit infection of MT-4 cells with the $HTLV\text{-}III_B$ strain of HIV-1 by 50% *in vitro*.
[d] Selectivity index: ratio of CC_{50}/IC_{50}.

Table 4. Efficacy of 2-F-OXT-A (**3a**) against a systemic herpes simplex virus type 2 (HSV-2) infection in mice[a].

Compounds	Dose[b] (mg/kg/day)	Survivors/ Treated[c]	MMD[d]
Control	—	0/15	8.1
2-F-OXT-A (**3a**)	10	8/10	14.0
OXT-G[e]	10	8/10	13.5
Acyclovir (ACV)	10	0/10	10.3
Acyclovir (ACV)	50	0/10	12.2

[a] ICR male 8-week-old.
[b] Mice received drugs ip 6 hours after virus inoculation and thereafter once a day every 24 hours for 5 days.
[c] Calculated on days 21.
[d] Mean day to death of nonsurvivors.
[e] See ref 19.

the activity of these halogeno-OXT-As against wild-type and thymidine kinase-deficient (TK^-) HSV-2.[14] As shown in Table 2, the activity of the most potent compound 2-F-OXT-A against TK^- HSV-2 was significantly higher than those of acyclovir (ACV) and ganciclovir, and the compound was found to have significant selectivity against TK^- HSV-2. The 2-halogeno compounds were also evaluated against human immunodeficiency virus type 1 (HIV-1) in MT-4 cells (Table 3).[16] As described above, the fluoro-substitution at the C-2 position of the purine ring similarly enhanced the anti-HIV activity of the parental compound. At the same time, the substitution also significantly improved antiviral selectivity.

We then evaluated the therapeutic activity of 2-F-OXT-A using a systemic HSV-2 infection in mice.[17] Eight-week-old male ICR mice were inoculated intraperitoneally (ip) with HSV-2 at 2.5×10^5 PFU/0.2 ml/mouse. When mice were given this dose of HSV-2, the virus replicated well in the intraperitoneal organs including liver, spleen, and adrenal glands, and invaded the central nervous system, and thereby mice were killed by encepalitis. 2-F-OXT-A and control drugs were administrated ip once a day (at 24 hours intervals) with the indicated doses for 5 days starting 6 hours after infection, and survival of mice was monitored for 3 weeks after infection. As shown in Table 4, the administration of 2-F-OXT-A gave the similar result with that of oxetanocin G (OXT-G) against the systemic HSV-2 infection in mice.[17] The mortality rate was reduced from 100% to 20% by the administration of 2-F-OXT-A at a dose of 10 mg/kg/day, whereas ACV had no effect on the mortality rate even at a dose of 50 mg/kg/day. Thus 2-F-OXT-A was shown to have potent antiviral activity *in vivo*, too.

In conclusion, the results obtained in the present study clearly indicate that 2-F-OXT-A, which are resistant to the action of adenosine deaminase, manifests its anti-HIV-1 activity *in vitro*, and its IC_{50} value is less than 1/10 that of DDI. 2-F-OXT-A also shows significant antiviral activity against herpes viruses, particularly against TK-HSV-2. And the *in vivo* study has shown that 2-F-OXT-A is highly effective against HSV-2 infection, whereas ACV has no effect on the same condition.

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