Bioorganic & Medicinal Chemistry Letters, Vol.3, No.6, pp. 1061-1066, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

AN UNUSUAL OXETANE NUCLEOSIDE AS A POTENT ANTIVIRAL AGENT+

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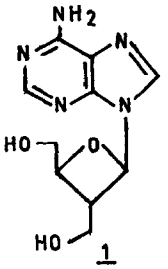
(*Received in USA* 3 *December* 1992)

Abstract: A new oxetane nucleoside (**4**) has been found to afford significant protection to mice experimentally infected with Japanese encephalitis virus (JEV) or Semliki forest virus (SFV).

Modified nucleoside analogues have gained importance in recent years as effective antiviral agents. Of these, an acyclic nucleoside, acyclovir[1], 3'-azido-2',3'-dideoxythymidine (AZT)[2] and 2',3'-dideoxyinosine[3] are recent discoveries of sugar modified nucleosides which have reached clinics for viral chemotherapy. Analysis of current literature, however, reveals the emergence of yet another class of sugar modified nucleosides incorporating oxetane ring in their molecular frame work, as potential antiviral agents. For example, oxetanocin (**1**), a nucleoside isolated from Bacillus megaterium is active against both HIV and HSV[4].



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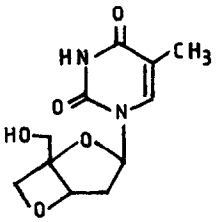

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The bicyclic oxetanothymidine (**2**) obtained as a byproduct during the synthesis of 4'-substituted nucleoside is a potent HIV inhibitor with remarkably low bone marrow toxicity[5]. In the examples mentioned above, the heterocyclic part is comprised of normal purine and pyrimidine bases, thereby suggesting that inhibitory potency could be due to the presence of strained oxetane ring. Chemically, the four-membered heterocyclic ring display transformation reacions with nucleophiles by way of releasing inherent angle strain in the system[6]. This property of the oxetane nucleoside may be responsible for its

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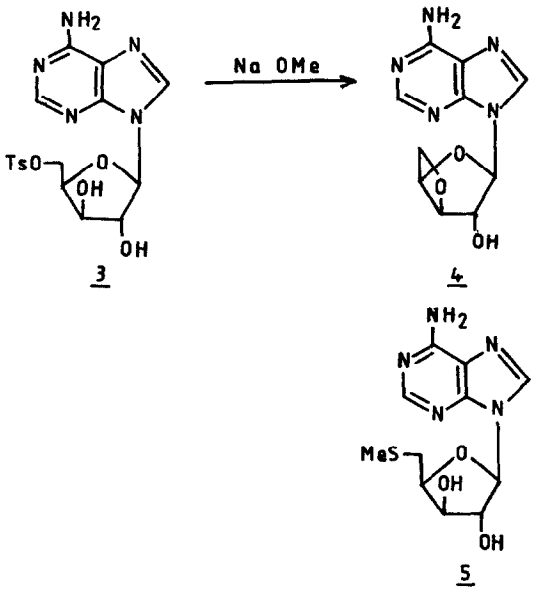
+CDRI Communication No.5073.

inhibitory effect on several enzymes brought about through inhibition of active sites. It is also likely that the oxetane nucleoside may act as substrate analog owing to its structural similarity with furan ring (e.g. **1**). In view of the potent antiviral activity exhibited by these strained ring nucleosides, it was decided to investigate the antiviral activity of a new oxetane nucleoside (**4**). The compound (**4**) was obtained during the synthesis of 9-[5'-(deoxy-5'-methylthio-β -D-xylofuranosyl]adenine (**5**, Xylosyl-MTA) isolated from Mediterranian nuidibranch Doris verrucosa[7].



Chemistry: The synthesis of the compound was carried out as follows: 5'-Deoxy-5'-tosyl-β-D-xylofuranosyladenine (**3**) was obtained by the method reported earlier[8] . During the reaction of (**3**) with sodium methyl mercaptate, a major product was isolated which later was identified as (**4**) along with (**5**) as a minor product. However, the reaction of (**3**) with NaOMe exclusively gave (**4**) which confirmed its structure[9]. The compound **4** has earlier been prepared in connection of its mass spectral studies[10].

Antiviral activity: Randomly bred Swiss albino mice of about 35 days old weighing 14-15 g, maintained in the Division of Laboratory Animals of our Institute were used. Each experimental group consisted of male and female animals in the ratio of 1:1. They were housed in clean steel cages at an ambient temperatmure of about 30 ± 1°C and were fed with standard pellet diet (Hindustan Lever Ltd.) with access to water ad libitum.

Semliki forest virus (Smithburn and Haddow strain) originally obtained from ATCC has been maintained in our laboratory by intracerebral passage in adult swiss mice (35 days old, 14-15 g weight). Japanese encephalitis virus (strain P20728) was originally obtained from the National Institute of Virology, Pune, India. It has been maintained in our laboratory by intracerebral passage in 1-2 day old suckling swiss mice. In both cases, a 20% homogenate of infected mouse brain made in phosphate buffered saline (PBS), pH 7.2 containing 7% bovine serum albumin (Sigma) stored at -20°C after lyophilization served as the stock virus. The subcutaneous LD_{50} of the virus stocks were estimated in mice before each experiment.

The compound was dissolved in PBS, pH 7.2. Treatment was initiated 1 day before challenge with 10 LD_{50} of the virus. The animals received treatment for two more days, one, on the day of challenge and the other, one day after challenge. On each day, animals were given two administrations of the compound, morning and evening. An interferon inducer, a mycoviral dsRNA preparation obtained from fungus Aspergillus ochraceus ATCC 28706 discovered in our laboratory, served the purpose of a standard antiviral substance[11]. Treated and untreated animals were observed for 20 days post infection. Upon termination of the experiment, the percentage mortality, percentage survival, and mean survival time (MST) were computed. The significance of treatment was statistically analysed by the test of proportions using the Student's T test.

Results and Discussion: The results summarised in Table 1 show that the compound (**4**) protected mice significantly from Japanese encephalitis virus as well as from Semliki forest virus infections. In addition, there was also a significant increase in the MST of treated mice compared to the untreated controls. An indication of the consistency of the antiviral activity was evident from the fact that the compound protected mice from both JEV and SFV infections. Another important observation was that there was an increase in the rate of

protection with the increase in dose of the compound with both the virus groups.

Table 1: Antiviral activity of compound **(4)** in mice against experimental infection with Japanese encephalitis virus (JEV)* or Semliki forest virus (SFV)**

Expt. No.	Treatment with	Virus	% survival	p value	MST (days)	p value
	Compound **(4)*****	JEV	37		10.7	
	"	SFV	37		11.6	
	dsRNA+	JEV	80		17.4	
I	"	SFV	90		19.0	
	PBS	JEV	0		6.2	
	"	SFV	0		5.5	
	Compound **(4)**⊕	JEV	67	< 0.05	15.5	< 0.05
	"	SFV	50	< 0.05	11.8	< 0.05
	dsRNA	JEV	70		16.5	
II	"	SFV	80		17.0	
	PBS	JEV	0		6.0	
	"	SFV	0		5.7	

* = 10 LD_{50} of Japanese encephalitis virus injected subcutaneously. Paralytic symptoms of hind/fore limbs started 5 days post infection in untreated groups of animals and all of them succumbed within 10 days post infection.

** = 10 LD_{50} of Semliki forest virus injected subcutaneously. Untreated animals developed paralysis of hind/fore limbs beginning 4 days post infection; all succumbed by 9 days post infection.

*** = 5 mg/kg per administration; two administrations daily, s.c., for 3 days; 1 day before challenge, on the day of challenge and 1 day after challenge.

⊕ = 10 mg/kg per administration; two administrations daily, s.c., for 3 days: 1 day before challenge, on the day of challenge and 1 day after challenge.

\+ = 0.6 mg/kg in a single administration 1 day before challenge.

Our results show that oxetane nucleosides can form a class of compounds having potent antiviral activity against RNA viruses such as togaviruses which are important from the human health point of view.

ACKNOWLEDGEMENT

Financial support from Department of Ocean Development, New Delhi is gratefully acknowledged.

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9. Compound **3** (1.0 g, 2.3 mmol) was dissolved in dry dimethylformamide (30 ml). Freshly prepared sodiummethoxide (12.5 ml) was added and the reaction mixture was heated under stirring at 110° for 8-10 hrs. The reaction mixture was neutralised with dil. acetic acid and the solvent evaporated under reduced pressure. The residue was extracted with chloroform, washed with water, dried (Na_2SO_4) and solvent evaporated. The residue was purified on SiO_2 column. Elution with $CHCl_3$:MeOH (97:3, v/v) gave the product **4** (0.25 g, yield 42%), m.p.222-224°; $[\alpha]_D^{20}$ -72.7 (1%, DMSO); MS (m/e): 249 (M^+); ^1H NMR(DMSO-d_6): 8.35 (s, 1H, H-2), 8.17 (s, 1H, H-8), 7.3 (bs, 2h, NH_2), 6.3 (s, 1H, H-1'); ^{13}CMR(DMSO-d_6): 156.1 (C-6), 152.9 (C-20, 149.9 (C-4), 138.9 (C-8), 118.7 (C-5), 92.7 (C-1'), 90.8 (C-4'), 80.2 (C-3'), 77.3 (C-2'), 76.7 (C-5'). Anal. calcd. for $C_{10}H_{11}N_5O_3.0.25H_2O$: C, 47.33; H, 4.56; N, 27.60; Found: C, 47.44; H, 4.32; N, 27.10.

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