



Drug Discovery Group

Synthesis and Anti-HBV Activity of 7-Deaza Neplanocin Analogs

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Abstract

Four anti-HBV nucleosides (Lamivudine, Adefovir dipivoxil, Entecavir & Telbivudine) have been approved by the US FDA for the treatment of chronic HBV infection. In addition to these drugs, several other nucleosides such as Clevudine (LFMAU), Valantitabine (LdC) and Tenofovir are currently under various stages of clinical evaluation. However, a significant number of patients develop drug resistance during the long-term use of these agents. Thus, there is a critical need to continue discovering and developing safe and effective novel anti-HBV agents to cope with the drawbacks of the current agents.

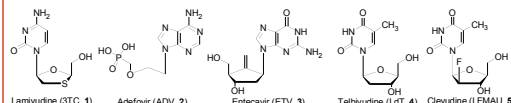
As part of our antiviral drug discovery program, it was of interest to synthesize less toxic neplanocin A analogs as potential anti-HBV agents. For the synthesis of the analogs, we utilized a key cyclopentylidene carboxylic intermediate, which was previously developed in our laboratory. From the synthesis, we prepared ten 7-deaza-neplanocin analogs.

Among the 7-deaza neplanocin A analogs, two analogs exhibited significant anti-HBV activity with EC₅₀ values of 0.43 and 0.32 μM, respectively based on extracellular HBV virions with negligible cytotoxicity (CC₅₀ > 300 μM). In addition, these analogs also showed significant anti-HBV activity against variety of clinically relevant lamivudine-resistant mutants. (Supported by NIH AI056540 & N01-AI-30046).

Introduction

Hepatitis B Virus (HBV):

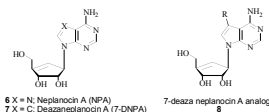
More than 350 million people are chronically-infected with hepatitis B virus. Lamivudine (1), Adefovir dipivoxil (2), Entecavir (3) and Telbivudine (4) are approved anti-HBV agents for the treatment of chronic HBV infection. Recently we have discovered a clinical candidate, Clevudine (LFMAU, 5), which has been approved for the treatment of chronic hepatitis B virus infection in South Korea in November 13, 2006 and is currently undergoing Phase III clinical trials in US and China. Upon entry into the cell, all of these nucleoside reverse transcriptase inhibitors (NRTIs; 1-5) require subsequent phosphorylation by cellular enzymes to generate their triphosphate-forms as active moieties. NRTIs inhibit an enzymatic target of HBV, the viral polymerase, having a DNA-polymerase function and inhibition leads to viral DNA-chain termination.



A significant number of patients develop drug resistance during the long-term use of these agents. Thus, there is a critical need to continue discovering and developing safe and effective novel anti-HBV agents to cope with the drawbacks of the current agents. In lieu of this, our groups are involved in a discovery of a new class of interesting carbocyclic nucleosides as a potential antiviral.

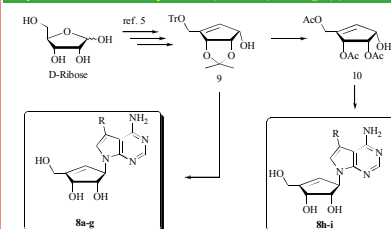
Carbocyclic Nucleosides:

A potential molecule neplanocin A (6) [S-adenosyl-L-homocysteine hydrolase inhibitor (SAH Inhibitor)] possess multifunctional property, however, the therapeutic utility of NPA has been limited by its cytotoxicity. Its not specific/selective antiviral,¹ as it can also serve as a substrate for adenosine deaminase and/or adenosine kinase² and could be the another reason of its cytotoxicity.³ Several attempts are under process to synthesize a suitable carbocyclic nucleosides that could serve as selective potential non-toxic antiviral.



Recently, our groups found significant antiviral activity of 7-deazaneplanocin A (7-DNPA, 7) against orthopoxviruses.⁴ Further screening of the compound revealed significant anti-HBV activity with low cytotoxicity. Consequently, significant anti-HBV activity prompted us to explore structure activity relationship along with a molecular modeling approach to understand the possible mechanism for 7-deaza neplanocin A analogs against HBV.

Synthesis of 7-Deazaneplanocin (7-DNPA) Analogs (8)



~ Key intermediate **9** and **10** used to synthesize all the analogs of 7-Deazaneplanocin (**8**).

In vitro activity against wild type Hepatitis B Virus and Cytotoxicity of 7-DNPA analogs

Compound	R	Anti-HBV Activity		Cytotoxicity (μM)
		EC ₅₀ (μM) Virion	EC ₅₀ (μM) HBV-RT*	
7	H	0.60	ND	22
8a	F	>10	ND	>300
8b	Cl	>10	ND	>300
8c	Br	>10	ND	>300
8d	I	0.43	1.6	>300
8e	CN	>10	ND	>300
8f	CONH ₂	>10	ND	>300
8g	NO ₂	>10	ND	>300
8h	Vinyl	3.3	10	>300
8i	Ethynyl	0.32	1.8	>300
3TC (1)	-	0.048	0.15	>2000

*RT: Intracellular HBV-DNA replication assay

In vitro activity of compound **8i** against HBV-mutant

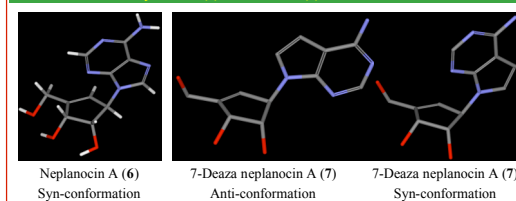
Mutant Strain	Anti-HBV Activity (RT)*		
	8i EC ₅₀ (μM)	3TC EC ₅₀ (μM)	ADV EC ₅₀ (μM)
WT	2.5	0.2	1.3
rt L180M	2.0	10.2	1.6
rt L180M/M204V	2.8	>100	1.2
rt M204I	3.0	>100	1.8
rt M204V	2.1	>100	1.5
rt N236T	5.6	0.3	7.7

*RT: Intracellular HBV-DNA replication assay

~Most active compound **8i** (7-ethynyl derivative) did not confer any resistance to the lamivudine related mutants with an EC₅₀ value comparable to that of adefovir (ADV), while two fold resistance was observed toward the ADV-mutant (rtN236T). However, the second potent compound **8d** (iodo-derivative) was not active against the lamivudine as well as adefovir resistant mutants (data not shown).

Results and Discussions

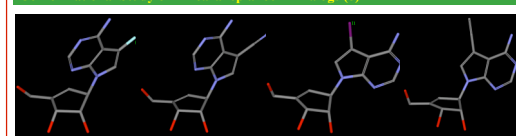
Conformational Study of NPA (6) and 7-DNPA (7)



~ Conformation search results indicate the Syn-base disposition is highly favored in the case of Neplanocin A (**6**), while 7-Deazaneplanocin A (**7**) may adopt both Syn and Anti-conformation.

~ Chemically, both molecules (**6** & **7**) are very close, but 7-position significantly affects the conformational nature of the molecules that leads to the differentiation between the conformational properties of the above two molecules.

Conformational Study of 7-Deazaneplanocin Analogs (8)



~ Only iodo substituted 7-DNPA analogs (**8d**) show exclusively anti-conformation, while F, Cl, and Br substituted analogs shows syn conformation.

~ 7-DNPA analogs (**8**): Cyano (**8e**), Nitro (**8f**) and Amide (**8g**) show mixture of syn and anti-conformation, where top low energy structure was found in syn-conformation.

~ Interestingly, 7-vinyl (**8h**) and 7-ethynyl (**8i**) substituted 7-DNPA analogs show exclusively anti-conformation.

Above results clearly reveal that 7-iodo, 7-vinyl and 7-ethynyl substituted 7-DNPA analogs favor exclusively anti-conformation, while other analogs possess syn-conformation or possibility to adopt both conformations. These results further support the significant in-vitro anti-HBV activity by conformational-distinguished compounds (**8d**, **8h** and **8i**) and it seems that anti-conformation may be the suitable form to show anti-HBV activity. Conformational details are shown in Table 1.

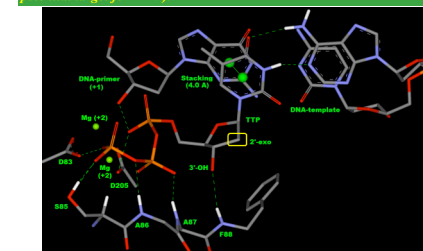
Conclusion

~ A series of novel 7-deazaneplanocin A analogues have been synthesized and their anti-HBV activity were determined *in vitro*. Among synthesized nucleosides, the most potent analog was the 7-ethynyl compound **8i** without any cytotoxicity.

~ Ethynyl derivative **8i** is found to be the most active against wild-type, and maintained the anti-HBV activities against drug-resistant mutants (L180M, M204V, M204I, and L180M/M204V) also. It showed two fold resistance by the ADV-specific mutant rtN236T.

~ The present findings warrant the future investigation on the mechanism of action as well as additional biological evaluation of these analogues.

Binding mode of natural substrate (TTP) in HBV-polymerase (a known potential target for HBV).



Thymidine-triphosphate (TTP) binding mode in HBV-polymerase showing the anti-north conformation (2'-exo) for TTP, which is suitable for fitting properly in the active site.

Table 1: Conformational Details and Pseudorotational Angle for 7-DNPA analogs

	Y	X	v	P	Conformation
NPA (6)	-57	70	15.5	345	Syn
7-DNPA (7)	61	74	16	347	Syn
8a	60	74	16	347	Syn
8b	61	74	16	347	Syn
8c	61	74	16	347	Syn
8d	63	-144	22	345	Anti
8e	60	74	16	347	Syn
8f	60	74	16	347	Syn
8g	60	74	16	347	Syn
8h	62	-144	22	345	Anti
8i	63	-143	22	345	Anti

~ According to pseudorotational angle, all above compound have north conformation. Compound **8d**, **8h** and **8i** also show north conformation but are distinguished in terms of base disposition (χ) and pseudo-sugar ring puckering (ν) in comparison to other synthesized analogs (**8**).

~ Potential nucleoside reverse transcriptase inhibitor possess anti-north conformation, consequently there is a possibility that above three molecules may be the inhibitor of HBV-polymerase (a potential target for Hepatitis B Virus)

References

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