
Digest paper

# Synthesis and antiviral evaluation of novel *N*-6 substituted adenosine analogues

Youwu Hao, Gaohui Zhou, Wei Wu, Yang Zhang, Liming Tao, Jianhua Yao, Wenping Xu

PII: S0040-4039(16)31527-1
DOI: http://dx.doi.org/10.1016/j.tetlet.2016.11.059
Reference: TETL 48345

To appear in: *Tetrahedron Letters*

Received Date: 10 September 2016
Revised Date: 12 November 2016
Accepted Date: 14 November 2016

Please cite this article as: Hao, Y., Zhou, G., Wu, W., Zhang, Y., Tao, L., Yao, J., Xu, W., Synthesis and antiviral evaluation of novel *N*-6 substituted adenosine analogues, *Tetrahedron Letters* (2016), doi: http://dx.doi.org/10.1016/j.tetlet.2016.11.059

Tetrahedron Letters
journal homepage: www.elsevier.com

# Synthesis and antiviral evaluation of novel *N*-6 substituted adenosine analogues

Youwu Hao[a] , Gaohui Zhou[a],Wei Wu[a], Yang Zhang[a], Liming Tao[a], Jianhua Yao[b], Wenping Xu[a,] *

[a]*Shanghai Key Laboratory of Chemical Biology, School of Pharmacy,East China University of Science and Technology, Shanghai 200237, China*
[b]*Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*

ARTICLE INFO

*Article history:*
Received
Received in revised form
Accepted
Available online

*Keywords:*
Adenosine analogues
antiviral activities
Coxsackie virus B3
Herpes simplex virus type 1
higher selectivity index

ABSTRACT

A series of adenosine analogues were synthesized and their biological evaluation was tested against Coxsackie virus B3 (CVB3) and Herpes simplex virus type 1(HSV-1) in HEp-2 cells. The hydrophobic constant, acute toxicity, carcinogenicity and mutagenicity were calculated. Analogues with piperazine derivatives **8b** showed promising activities against CVB3 with a lower $IC_{50}$value and higher selectivity index, their efficacy was better than that of the commercialized medicine, Ribavirin. These described adenosineanalogues exhibit potent antiviral activities against several viruses, and offer new leads for further development.



*Corresponding author. Tel.: +86 2 164 253 756; fax: +86 2 159 883 730; e-mail: xuwp@ecust.edu.cn

## 1. Introduction

Nucleoside analogues are recognized as an important class of biologically active compounds.[1-5] Nucleotides and their derivatives are involved in many cellular processes, including cell growth and division, and they have been successfully used in the treatment of various viral infections and as antitumor agents in clinical settings.[6, 7] For example, didanosine, approved by FDA, is well known for the treatment area of HIV-I infection.[8] Lamivudine, has been widely used in the treatment of HBV since being approved by the FDA in 1998.[9]Dicarmide, has been reported to inhibit CCR5, which is the major co-receptor for HIV-1 entry.[10] An adenosine nucleoside analogue, NITD008, which has been reported to be an antiviral reagent that specifically inhibits flaviviruses, has been reported to effectively suppress the propagation of different strains of EV71 in RD, 293T and Vero cells with a relatively high selectivity index.[11] However, a number of side effects of traditional antiviral drugs have been observed in clinical treatment, including mitochondrial damage, myopathy, kidney injury, and lactic acidosis.[12]In addition to these adverse effects, drug resistances has been frequently reported.[13-15]Tenofovir (TFV), which became commercially available in 2001, and is well known for its use in the treatment of HIV-I infection, may cause treatment failure and is limited in its clinical use due to the rapid emergence of drug resistance.[16]

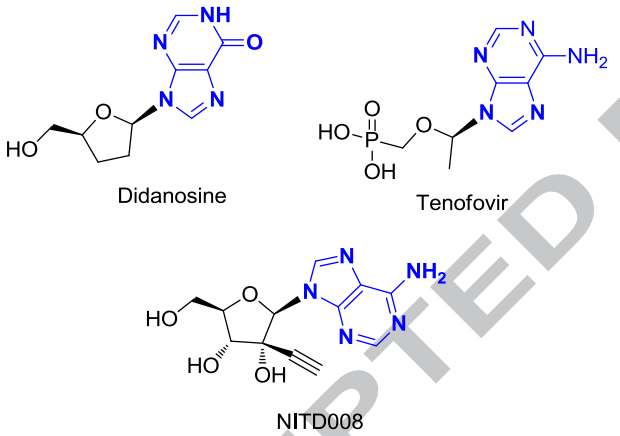



**Fig. 1**Adenosineanalogues of biologically active compounds

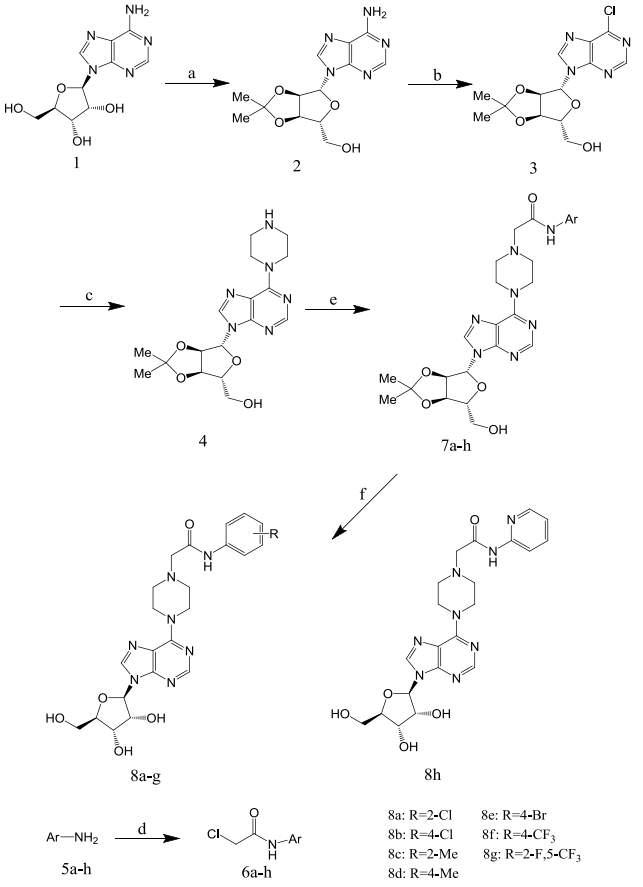
Based on the previous structure of adenosine analoguesand their antiviral activities, with a novel scaffold of adenosine and a heterocyclic ring in mind, we designed numerous compounds using an intermediate derivatization approach. To reduce the number of compounds and the workload, and increase the ratio of the active compounds, we first calculated the hydrophobic constant, acute toxicity, carcinogenic toxicity and mutagenic toxicity of each of the designed compounds and their representative structures. We then excluded compounds that did not obey the "Lipinski's rule of five"[17] or exhibited potential toxicity. Our antileukemic and antiviral activity prediction results prompted us to investigate adenosine substituted with a piperazine-derivatives-based core structure to discover novel adenosine derivatives with potential antitumor or antiviral activities. *N*-6 substituted with piperazine derivatives can increase the drug-likeness of target compounds, because many piperazine designer drugs have already been clinically used in recent decades, and in recent years many newpiperazine active compounds are continually being reported.[18-21] Here, we report the synthesis, calculation, and antiviral evaluation of a new series of adenosine analogues.

## 2. Results and discussion

### *2.1. Chemistry*

Scheme **1** shows the synthetic routes to the target compounds, designed by computer simulation,and their yields and conditions.

Following previously reported procedures,[22,23]we obtained adenosine with an adjacent protected hydroxyl group (compound 2) to improve solubility in organic solvents and for convenience in later reactions. We employed the Sandmeyer reaction to obtain compound 3 from compound 2.[24] To improve the conversion rate, we used both tert-butyl nitrite (*t*-BuONO) and TMCS in this step. Because they are easily decomposed in the light and moist atmosphere, we performed the reaction in dark and dry conditions in an argon-filled reactor. Furthermore, we used the best mole ratio (compound 2: TMCS: *t*-BUNO = 1:7:8.4) to determine the highest yield. We dripped a solution of compound 3 in acetonitrile into a solution of piperazine and $K_2CO_3$ in acetonitrile, and stirred for 6 h at room temperature. We obtained a 90% yield of compound 4 after chromatography purification. From compound 4 to compound 7, the reaction conditions were slightly different due to the different substituent groups. When there are electron-withdrawing groups in the aromatic ring, increasing compound 6 and a longer reaction time will enhance the reaction. To a methanol solution of compounds 6a-h, we added trimethylamine, and stirred this mixture at room temperature; we then added a solution of compound 4 in methanol and refluxed these solutions for 6-8 h. We obtained compounds 7a-h after evaporation and washing. After de-protecting the hydroxyl groups of compounds7a-h, we obtained the final compounds 8a-h by column chromatography and identified the structures using$^1$H nuclear magnetic resonance (NMR), $^{13}$C NMR and high-resolution mass spectrometry (HRMS).



**Scheme 1.**Reagents and reaction conditions:(a) acetone, MeOH, rt, 4 h, 95%; (b) TMCS, tert-Butyl nitrite, DCM, rt, overnight, 60%; (c) $K_2CO_3$, acetonitrile, rt, 8 h, 90%; (d) chloroacetyl chloride, $Et_3N$, DCM, 0℃, 1-6h; 80%-95% (e) 6a-i, MeOH, refluxing, 6-18 h, 50%-70%; (f) HCOOH:$H_2O$(1:1), 50℃, 4-8 h, 39%-61%.

### *2.2. calculation*

Table 1 lists ourcalculations of the hydrophobic constant (log P, predicted by CISOC-log P[25]), acute toxicity (predicted by CISOC-PSAT[26]), carcinogenicity (predicted by CISOC-PSCT[27]), and mutagenicity (predicted by CISOC-PSMT[28]) of some representative and structurally diverse compounds. The calculated log P values were less than 5 and met the log P criteria, and the acute toxicity levels were all within 50≤ LD50 < 500 mg kg−1, with no carcinogenic and mutagenic toxicity risk.

**Table 1.**Calculation results of log P, for acute, carcinogenic and mutagenic toxicities

| Compd | Log P | Acute tox.a | Car. Tox.b | Mutagenic tox.c |
|---|---|---|---|---|
| 8a | 0.6 | 3.64 | N | N |
| 8b | 0.8 | 3.75 | N | N |
| 8c | 0.14 | 3.74 | N | N |
| 8d | 0.51 | 3.8 | N | N |
| 8e | 0.8 | 3.75 | N | N |
| 8f | 1.01 | 3.73 | N | N |
| 8g | 1.15 | 3.68 | N | N |
| 8h | - 0.98 | 3.77 | N | N |

a: Rat, oral, less than 2, LD50 < 1 mg kg−1; equal or more than 2 and less than 3, 1 ≤ LD50 < 50 mg kg−1; equal or more than 3 and less than 4, 50≤ LD50 < 500 mg kg−1; equal or more than 4 and less than 5, 500 ≤ LD50 < 5000 mg kg−1; equal or more than 5, LD50 ≥ 5000 mg kg−1.
b: Rat, oral, P: probability of non carcinogenic is low; N: probability of non carcinogenic is high.
c: Salmonella typhimurium. P: positive; N: negative.

### *2.3. Biological evaluation*

*In vitro* cytotoxicity and antiviral activity assay. Using the commercialized drugRibavirinas a reference compound, we evaluated these new adenosine analogues by MTT assay to determine their cytotoxicities and antiviral activities in HEp-2 cells infected with CVB3 strains (Table 2). Table 2 shows the results, which are expressed as $TC_{50}$ (50% cytotoxic concentration), $IC_{50}$ (50% CVB3 cytoprotective concentration against CVB3 induced cytopathic effect) and TI (selectivity index represented by the $TC_{50}/IC_{50}$ ratio). Seven analogues exhibited promising antiviral activity against CVB3, especially against compound **8b**, for which the $IC_{50}$value was 5.1 mg $L^{-1}$ and the TI value was 41, the values obtained for this compound were better than those obtained for Ribavirin, for which the $IC_{50}$ value was 36.8 mg $L^{-1}$ and TI value was 29.1.

**Table2.** Anti-CVB3 activities and cytotoxicity of compounds in HEp-2 cellsa

| Compound | $TC_{50}$ / mg $L^{-1}$ | $IC_{50}$ / mg $L^{-1}$ | TI |
|---|---|---|---|
| 8a | 103.3±13.0 | 19.0±3.1 | 5.5 |
| 8b | 208.2±18.7 | 5.1±2.3 | 41.0 |
| 8c | 240.5±25.0 | 18.1±1.4 | 13.3 |
| 8d | 239.5±25.9 | 29.1±3.9 | 8.2 |
| 8e | 141.0±12.1 | 17.4±1.0 | 8.1 |
| 8f | 116.2±9.6 | 10.0±0.7 | 11.6 |
| 8g | 148.9±10.1 | 10.6±2.0 | 14.0 |
| 8h | 399.0±2.5 | >60 | — |
| Ribavirin | 1069.9±356.9 | 36.8±9.6 | 29.1 |

a: 4 repeats with 3 independent experiments, mean±SD

*In vitro virus* yield reduction assay.We infected the pre-cultured HEp-2 cells with either CVB3 or HSV-1, washed them with a phosphate-buffered saline (PBS), then added the three compounds (**8b**, **8f**, and **8g**) and Ribavirin at concentration of 32 mg $L^{-1}$. We harvested the infected cells after further incubation for 48 h. We obtained the supematants after they were frozen and thawed three times. We used the Reed-Muench method[29]to calculate the infective virus titer. As shown in Fig.2, the compounds could obviously decrease the titer of both CVB3 and HSV-1, and compound **8f**, in particular, performed better than Ribavirin.

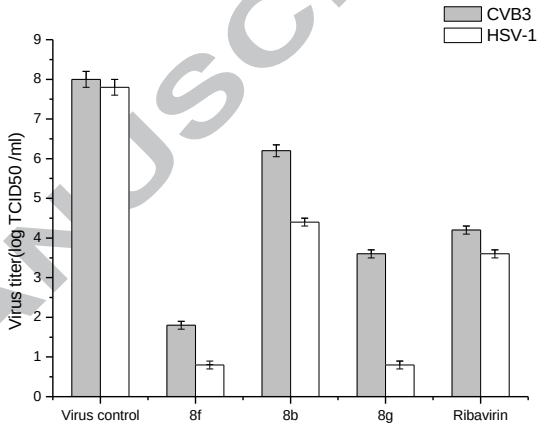



**Fig. 2** The titer of CVB3 and HSV-1 treated with compounds

## 3. Conclusions

In summary, we synthesized a series of novel adenosine analogues as antiviral agents and calculated the hydrophobic constant, acute toxicity, carcinogenicity and mutagenicity of each. Compared with Ribavirin, some of the compounds showed potent inhibitory activities against CVB3 along with low cytotoxicities. Compound **8b**, *N*-6 substituted with piperazine derivatives, exhibited better inhibitory activities against CVB3 and a better selectivity index than Ribavirin. Compounds **8a-g** had lower $IC_{50}$ values than the compound **8h**, which indicates that the phenyl substitution of piperazine moiety may more effectively enhance antiviral activities than pyridine substitution in this series of compounds. This might be related to the lonepair of electrons in the aromatic ring. Furthermore, as shown in the in vitro virus yield reduction assay, compounds **8f** and **8g** can reduce the infective virus titer; better than the compound **8b** and Ribavirin, which might be related to the electron-withdrawing group (trifluoromethyl) or the steric hindrance effects of the $CF_3$ group. These results indicate that these kinds of adenosine analogues could be developed as potent antiviral agents in future.

## 4. Acknowledgements

This work was financially supported by National Key Technologies R&D Program (2011BAE06B04). We are grateful to the colleges of Wuhan University, School of Basic Medical Science, Institute of Medical Virology, for their helpful works in the antiviral assays.

**References and notes**


1. Périgaud, C.; Gosselin, G.; Imbach, J. L. *Nucleosides & Nucleotides***1992**, *11*, 903.
2. Ichikawa, E.; Kato, K. *Current Medicinal Chemistry***2001**, *8*, 385.
3. Shimada, H.; Haraguchi, K.; Hotta, K.; Miyaike, T.; Kitagawa, Y.; Tanaka, H.; Kaneda, R.; Abe, H.; Shuto, S.; Mori, K. *Bioorganic & Medicinal Chemistry***2014**, *22*, 6174.
4. Weiss, A. *Chembiochem***2013**, *14*, 2519.
5. Karran, P. *British Medical Bulletin***2006**, *79-80*, 153.
6. Tuncbilek, M.; Guven, E. B.; Onder, T.; Atalay, R. C. *Journal of Medicinal Chemistry***2012**, *55*, 3058.
7. Bosch, L.; Harbers, E.; Heidelberger, C. *Cancer Research***1958**, *18*, 335.
8. Clercq, E. D. *Future Virology***2016**, *3*, 393.
9. Liu, Y.; Zhou, E.; Yu, K.; Zhu, J.; Zhang, Y.; Xie, X.; Li, J.; Jiang, H. *Molecules***2008**, *13*, 2426.
10. Schul, W.; Chen, Y. L.; Yin, Z.; Keller, T.; Shi, P. Y. *Proceedings of the National Academy of Sciences***2009**, *106*, 20435.
11. Shang, L.; Wang, Y.; Jie, Q.; Bo, S.; Lin, C.; Lou, Z.; Peng, G.; Sun, Y.; Zheng, Y. *Antiviral Research***2014**, *112*, 47.
12. HimaBindu, A.; Naga Anusha, P. *Journal of Antivirals &Antiretrovirals***2011**, *03*, 88.
13. Bani-Sadr, F.; Carrat, F.; Pol, S.; Hor, R.; Rosenthal, E.; Goujard, C.; Morand, P.; Lunel-Fabiani, F.; Salmon-Ceron, D.; Piroth, L. *Jaids Journal of Acquired Immune Deficiency Syndromes***2005**, *40*, 47.
14. Kohler, J. J.; Lewis, W. *Environmental & Molecular Mutagenesis***2007**, *48*, 166.
15. Qian-Qian, H. U.; Shi, L. L.; Tan, C. B.; Zhang, J.; Pan, X. F.; Wei-Ren, X. U.; University, T. M. *Chinese Journal of Pharmacology & Toxicology***2013**, *27*, 885.
16. Iyidogan, P.; Anderson, K. S. *Antiviral Chemistry & Chemotherapy***2014**, *23*, 217.
17. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Advanced Drug Delivery Reviews***2001**, *46*, 3.
18. YANG; Zhang, Y. H.; Yang, L. J.; Wang, L. X.; Xiao-Yu, W. U.; Zhao, X. M.; Yu *Chinese Journal of Organic Chemistry***2008**, *28*, 2095.
19. Westwell, A. D. *Drug Discovery Today***2003**, *8*, 652.
20. Huang, L. H.; Xu, H. D.; Yang, Z. Y.; Zheng, Y. F.; Liu, H. M. *Steroids***2014**, *82*, 1.
21. Fytas, C.; Zoidis, G.; Tsotinis, A.; Fytas, G.; Khan, M. A.; Akhtar, S.; Rahman, K. M.; Thurston, D. E. *European Journal of Medicinal Chemistry***2015**, *93*, 281.
22. Jing, L. I.; Gui-Zhen, A. O.; Chen, J. L.; Qiao, C. H. *Chinese Journal of Synthetic Chemistry***2009**.
23. Townsend, A. P.; Roth, S.; Williams, H. E.; Stylianou, E.; Thomas, N. R. *Organic Letters***2009**, *11*, 2976.
24. Šála, M.; Palma, A. M. D.; Hřebabecký, H.; Dejmek, M.; Dračínský, M.; Leyssen, P.; Neyts, J.; Mertlíková-Kaiserová, H.; Nencka, R. *Bioorganic & Medicinal Chemistry Letters***2011**, *21*, 4271.
25. Liao, Q.; Yao, J.; Yuan, S. *Molecular Diversity***2006**, *10*, 301.
26. J. H. Yao, CISOC-PSAT, version 1.0, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, **2010**.
27. Liao, Q.; Yao, J. H.; Li, F.; Yuan, S. G.; Doucet, J. P.; Panaye, A.; Fan, B. T. *Sar&Qsar in Environmental Research***2004**, *15*, 217.
28. Liao, Q.; Yao, J.; Yuan, S. *Molecular Diversity***2007**, *11*, 59.
29. Jin, J.; Sun, S.; Jiang, C.; Ma, H.; Xu, L.; An, D.; Huang, X.; Kong, W. *Journal of Virological Methods***2013**, *187*, 362.

Highlights:

1 A novel series of adenosine analogs were synthesized as antiviral agents.

2 The phenyl substitution of piperazine moiety may more effective.

3 Compounds 8b exhibited better inhibitory activities against CVB3 than Ribavirin.

4 The target compounds were designed by computer simulation.

5 These kinds of adenosine analogues might be developed as potent antiviral agents.

## Graphical Abstract

**Synthesis and antiviral evaluation of novel *N*-6 substituted adenosine analogues**

Youwu Hao

Leave this area blank for abstract info.