Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Synthesis and antiviral activity of a series of 1′-substituted 4-aza-7,9-dideazaadenosine C-nucleosides

Aesop Cho *, Oliver L. Saunders, Thomas Butler, Lijun Zhang, Jie Xu, Jennifer E. Vela, Joy Y. Feng, Adrian S. Ray, Choung U. Kim

Gilead Sciences, 333 Lakeside Drive, Foster City, CA 94044, USA

A R T I C L E   I N F O

Article history:
Received 27 January 2012
Revised 27 February 2012
Accepted 29 February 2012
Available online 8 March 2012

Keywords:
C-nucleoside
Antiviral
Polymerase inhibitor

A B S T R A C T

A series of 1′-substituted analogs of 4-aza-7,9-dideazaadenosine C-nucleoside were prepared and evaluated for the potential as antiviral agents. These compounds showed a broad range of inhibitory activity against various RNA viruses. In particular, the whole cell potency against HCV when R = CN was attributed to inhibition of HCV NS5B polymerase and intracellular concentration of the corresponding nucleoside triphosphate.

© 2012 Elsevier Ltd. All rights reserved.

Structural modification of natural N-nucleosides on either the sugar or the base has led to the discovery of a variety of therapeutic agents, which includes antivirals and anticancer agents. This approach has been continuously employed to further identify agents with improved efficacy and safety over existing drugs, overcome issues associated with drug-resistance, and expand into new therapeutic areas.1

Among all the conceivable modifications of the sugar moiety, incorporation of a substitution at 1′-position of the N-nucleosides has been rarely exploited in drug discovery. This is partly due to the chemical instability induced by the 1′-substituent (i.e., ready dissociation of the base and the sugar at lower pH). For example, 1′-C-Me adenosine is rapidly degraded in aqueous solutions at pH < 7.2 However, C-nucleoside, in which the sugar and the base are linked through the C–C bond, should be hydrolytically stable even with a 1′-substituent. Thus, we envisioned that C-nucleoside could be an ideal scaffold to explore various 1′-substituted nucleosides for their therapeutic potential.

As part of an on-going effort to identify new antiviral agents, we were interested in investigating 1′-substituted nucleosides as inhibitors of viral RNA-dependent RNA polymerases. Such nucleoside inhibitors should be converted by intracellular kinases to the triphosphorylated nucleosides, which then function as competitors of the natural nucleoside triphosphates (TP) in RNA synthesis. 7-Deazaadenosine (1, Tubercidin) is a naturally occurring, cytotoxic N-nucleoside. It is triphosphorylated inside the cells and then incorporated into RNA by host RNA polymerases, which is believed to be the main mode of action for the observed cytotoxicity.3 A C-nucleoside analog of 1, 4-aza-7,9-dideazaadenosine (2) has shown equally potent cytotoxicity to cancer cell lines.4 The TP of 2 was also shown to be a substrate of host RNA polymerases and incorporated into RNA (unpublished results). Thus, we chose compound 2 as a template to investigate the effect of the 1′-substituent on antiviral activity and selectivity. Here, we report (1) preparation of a series of novel 1′-substituted analogs of compound 2, (2) their antiviral activities against a panel of RNA viruses, and (3) correlation of the observed anti-HCV potency with both intrinsic enzyme (HCV NS5B) activity and intracellular level of the corresponding TP.
The synthesized nucleosides 3a–3d were evaluated in cell-based assays against a panel of RNA viruses. Anti-HCV activity was obtained using a subgenomic replicon. Viruses tested included representatives of Flaviviridae (HCV, YFV, DENV-2, WNV), Orthomyxoviridae (influenza A), Paramyxoviridae (parainfluenza 3), Picornaviridae (Coxsackie A), and Coronaviridae (SARS-CoV). Antiviral activity (EC50) and cytotoxicity (CC50 for host cells in each assay) are shown in Table 1. Compound 3a (R = CN) displayed broader spectrum activity (HCV, YFV, DENV-2, Influenza A, Parainfluenza 3 and SARS-CoV). Compounds 3b and 3c showed a reduced potency and a narrower spectrum of antiviral activity, and 3d no activity. While exhibiting various levels of antiviral activities, these 1'-substituted nucleosides exerted little or no cytotoxicity.

We then investigated to see if the observed antiviral activity was derived from inhibition of viral RNA dependent RNA polymerases (RdRp). Since readily available, the HCV RdRp was used to determine an enzyme inhibitory activity of TP 8a–8d. Under the established protocol, the IC50 values were obtained (Table 2). These studies revealed a good correlation between the intrinsic enzyme activity and the whole cell HCV potency. For example, compound 3a showed the most potent anti-HCV activity (EC50 of 4.1 μM), and its TP derivative (8a) the most potent inhibitory activity against the enzyme (IC50 of 5.6 μM).

To gain further insights into the mode of the antiviral activity, bis-(SATE) monophosphate produgs were tested in the HCV replicon assay. In addition, intracellular levels of the TPs were measured upon incubation of these produgs with replicon cells, and compared with those from the parent nucleosides. The results are summarized in Table 2. The produgs 9a and 9b showed markedly enhanced replicon activity when compared to the parent nucleosides. These monophosphate produgs afforded >100-fold higher levels of the TP species than the parent nucleosides. Corresponding to the increased potency, an increase in cytotoxicity was also observed for the 4 prepared produgs. The selectivity indices (CC50/EC50) of 9b, 9c and 9d were less than 10-fold making it difficult to differentiate HCV activity from cellular toxicity. The greater potency and selectivity observed for 9a (EC50 of 0.85 μM and selectivity of 40-fold) likely reflects a combination of the potent enzyme activity and the high intracellular TP concentration (4220 pmol/million cells when cells were incubated with 10 μM of 9a). Further structural modification to improve the selectivity of 3a and 9a is warranted.

In summary, a series of novel 1'-substituted analogs of 4-aza-7,9-dideazaadenosine C-nucleoside were prepared and tested in vitro antiviral assays. These compounds showed a broad range of antiviral activity. In particular, the HCV potency of 3a and its bis(SATE) produg 9a in the whole cell replicon assay were correlated with the intrinsic enzyme activity and the intracellular levels of the corresponding TP (8a), which suggests that the antiviral
activity is in part derived from inhibition of RdRp. The current work establishes the potential of 1'-substituted nucleosides as antiviral agents.

References and notes

1. Cihlar, T.; Ray, A. S. *Antivir. Res.* 2010, 85, 39; Robak, T. *Expert Opin. Investig. Drugs* 2011, 20, 343.
2. Sporadic examples of 1'-substituted N-nucleosides prepared for biological evaluation; Franchetti, P.; Cappellacci, L.; Pasqualini, M.; Petrelli, R.; Viñas, P.; Jayaram, H. N.; Horvath, Z.; Szekeles, T.; Grifantini, M. *J. Med. Chem.* 2005, 48, 4983; Damont, A.; Dukhan, D.; Gosselin, G.; Payronnet, J.; Storer, R. *Nucleosides Nucleotides Nucleic Acids* 2007, 26, 1431; Yoshimura, Y.; Kano, F.; Miyazaki, S.; Ashida, N.; Sakada, S.; Haraguchi, K.; Itoh, Y.; Tanaka, H.; Miyasaka, T. *Nucleosides Nucleotides Nucleic Acids* 1996, 15, 305.
3. Cappellacci, P.; Barboni, G.; Palmieri, M.; Pasqualini, M.; Grifantini, M.; Costa, B.; Martini, C.; Franchetti, P. *J. Med. Chem.* 2002, 45, 1196.
4. Olsen, D. B.; Eldrup, A. B.; Bartholomew, L.; Bhat, B.; Bosserman, M. R.; Ceccacci, A.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K. L.; Grobler, J. A.; LaFemina, R. L.; Markel, E. J.; Migliaccino, G.; Pihave, M.; Stablhub, M. W.; Tomassini, J. E.; MacCoss, M.; Hazuda, D. J.; Carroll, S. S. *Antimicrob. Agents Chemother.* 2004, 48, 3944.
5. Patel, S. A.; Otter, B. A.; Klein, R. S. *Tetrahedron Lett.* 1994, 35, 5339.
6. O’Connor, S. J.; Damas, J.; Lee, W.; Dixon, J.; Cantin, D.; Gunn, D.; Burke, J.; Phillips, B.; Lowe, D.; Shelekhin, T.; Wang, G.; Ma, X.; Ying, S.; Mcclure, A.; Achebe, F.; Lobell, M.; Ehrgott, F.; Iwagwu, C.; Parcella, K. WO200756170, 2007.
7. Our work on practical synthesis of Lewis-acid promoted 1'-substitution reactions was recently reported: Metobo, S. E.; Xu, J.; Saunders, O. L.; Butler, T.; Aktodianakis, E.; Cho, L.; Kim, C. *Tetrahedron Lett.* 2012, 53, 484.
8. Analogy to Asbun, W.; Binkley, S. B. *J. Org. Chem.* 1968, 33, 140.
9. Gillerman, I.; Fischer, B. *Nucleos. Nucleot. Nucleic Acids* 2010, 29, 245.
10. Lefebvre, J.; Perigaud, C.; Pompon, A.; Aubertin, A.-M.; Girardet, J.-L.; Kirn, A.; Gosselin, G.; Imbach, J.-L. *J. Med. Chem.* 1995, 38, 3941.
11. All virus EC50 values were measured in cytoprotection effect (CPE) assays. Cytoprotection and compound cytotoxicity are assessed by MTS (CellTitre96) Reagent, Promega, Madison WI) dye reduction. The West Nile virus CPE assay uses Vero cells and WNV strain NY-99. The Dengue Virus CPE assay uses Vero E6 cells and Dengue Virus Type 2 strain New Guinea C. The Yellow Fever Virus CPE assay uses HeLa cells and Yellow Fever Virus strain 17D. The Coxackie A virus CPE assay uses Vero cells and Coxackie A strains A7 or A21. The Parainfluenza CPE assay uses Vero cells and Parainfluenza 3 strain C243. The Influenza A CPE assay uses MDCK cells and H3N2 strain virus. The SARS-CoV CPE assay uses Vero cells and SARS-CoV strain Toronto-2.

Table 2

| Nucleoside | EC50/CC50 (µM) | TP concentration* (pmol/million) | SATE prodrug |
|------------|----------------|----------------------------------|--------------|
| 3a/9a | 4.1>89 | 17.6 (8a) | 0.085/3.2 |
| 3b/9b | 39>89 | 50.8 (8b) | 0.078/0.73 |
| 3c/9c | >89>89 | 2.0 (8c) | 3.43/12 |
| 3d/9d | >89>89 | 0.66 (8d) | 6.01/26 |

* Cmax of intracellular TP (pmol/million cells) upon incubation of 10 µM of nucleosides or prodrugs in Huh-7 cells for 24 h.