Bioorganic & Medicinal Chemistry Letters 20 (2010) 7297–7298

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-HCV activity of a new 2′-deoxy-2′-fluoro-2′-*C*-methyl nucleoside analogue

Weidong Hu [a], Ping'an Wang [b], Chuanjun Song [a,*], Zhenliang Pan [c], Qiang Wang [a], Xiaohe Guo [d], Xuejun Yu [d], Zhenhua Shen [a], Shuyang Wang [a], Junbiao Chang [a,*]

[a] Department of Chemistry, Zhengzhou University, Zhengzhou, Henan Province 450001, PR China
[b] College of Chemistry and Chemical Engineering, Henan University, Kaifen, Henan Province 475001, PR China
[c] School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, Henan Province 450001, PR China
[d] Hennan Key Laboratory of Fine Chemicals, Henan Academy of Science, No. 56 Hongzhuan Road, Zhengzhou 450002, PR China

ARTICLE INFO

Article history:
Received 20 July 2010
Revised 27 September 2010
Accepted 15 October 2010
Available online 4 November 2010

Keywords:
2′-Deoxy-2′-fluoro-2′-C-methyl nucleoside
Anti-HCV activity

ABSTRACT

2′-Deoxy-2′-fluoro-2′-*C*-methyl nucleoside analogue **4** was designed and synthesized. Initial biological studies indicated that this compound showed promising activity against HCV replication.

It is estimated that hepatitis C virus (HCV) infect 130 million people worldwide.[1] About 50% of the infected cases will become chronic[2] and about 20% of these chronic patients develop liver cirrhosis that can lead to hepatocellular carcinoma.[3] Unfortunately, no vaccine is currently available to prevent hepatitis C. The standard of care for chronic hepatitis C is combination therapy with an interferon-α and ribavirin, which is effective in only 50% of patients.[4] Clearly, there is an urgent need for effective anti-HCV agent.

Recently, a series of modified nucleosides with potent inhibitory activity against the HCV NS5B polymerase have been identified.[5] Among these, 2′-*C*-methyl substituted nucleoside analogues, such as 2′-*C*-methyladenosine **1**,[6,7] 2′-*C*-methylguanosine **2**,[7] 2′-*C*-methylcytidine **3a**[8] and its 2′-deoxy-2′-fluoro analogue **3b**[8,9] (Fig. 1), are particularly noteworthy, and **3b** is currently in clinical trial. As a continuous research program of our laboratory searching for antiviral agents,[10] we now report the design and synthesis of 2′-deoxy-2′-fluoro-2′-*C*-methyl nucleoside analogue **4**, which shows promising activity against HCV replication.

As shown in Scheme 1, selective reduction of thioxo-pyrrolo[2,3-*d*]pyrimidinone **5** with Raney-Ni gave pyrrolo[2,3-*d*]pyrimidinone **6**, which was treated successively with phosphorus oxychloride and Selectfluor to give compound **8** in good overall yield. Coupling of **8** with 2-deoxy-2-fluoro-2-*C*-methylribofuranose **9**[11] under Mitsunobu conditions gave the protected nucleoside **10** as a mixture of α and β anomers,[12] which could be separated by column chromatography. Displacement of the chloride in the β anomer with ammonia, followed by deprotection, resulted in the formation of the desired nucleoside analogue **4**[13] in 85% isolated yield over the two steps.

The effect of compound **4** on HCV replication was examined using a HCV subgenomic replicon cell culture system (Ava. 5 cells) and a Sip-L assisted authentic HCV infection/replication system (293-Sip-L cells).[14] In replicon system, compound **4** partially suppressed HCV replication at 6 and 0.6 μg/mL. The HCV-RNA level was suppressed to 1/5 of the original level at both concentrations. No suppression effect could be observed at 0.06 μg/mL. In Sip-L assisted HCV replication system, compound **4** suppressed HCV replication in a dose-dependent manner. The results were shown in Figure 2. The estimated EC_{50} of HCV-RNA was 0.8 μg/mL. The IC_{50} calculated using total cellular RNA was 350 μg/mL.

In summary, a new 2′-deoxy-2′-fluoro-2′-*C*-methyl nucleoside analogue **4** was designed and synthesized. Initial biological studies indicated that this compound showed promising activity against HCV replication, thus merited further investigation.

Acknowledgments

We are grateful to the National Natural Science Foundation of China (Outstanding Young Scholarship to J. Chang, #30825043)

* Corresponding authors. Tel.: +86 37167781788.
E-mail addresses: chjsong@zzu.edu.cn (C. Song), changjunbiao@zzu.edu.cn (J. Chang).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.076

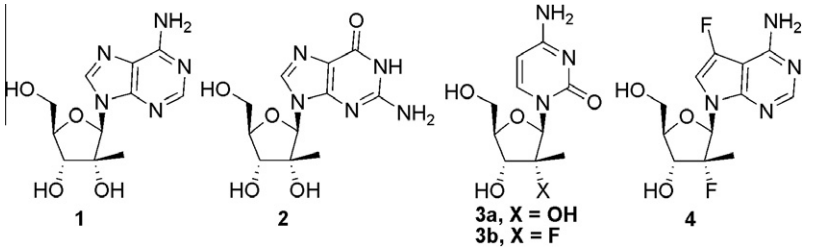
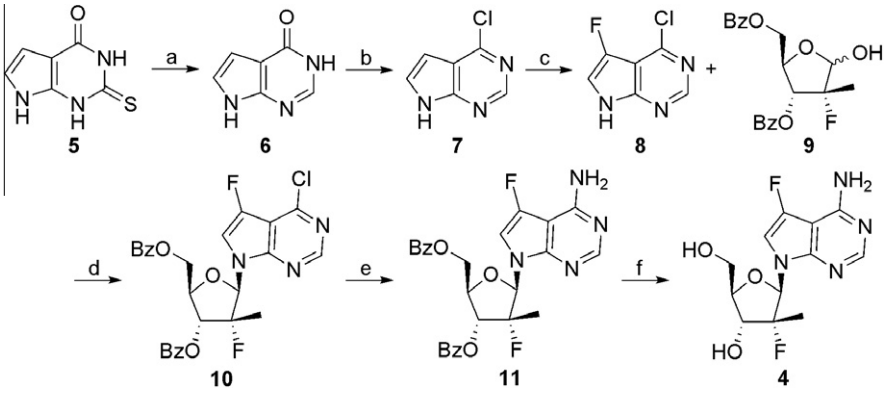


Figure 1. Anti-HCV nucleoside analogues.



Scheme 1. Reagent and conditions: (a) Raney-Ni, $NH_3{\cdot}H_2O$, reflux, 6 h, 85%; (b) $POCl_3$, reflux, 4 h, 91%; (c) Selectfluor, AcOH, CH_3CN, rt, 15 h, 59%; (d) DEAD, PPh_3, CH_3CN, rt, 24 h, 10% for the α anomer, 15% for the β anomer; (e) 0.5 N NH_3, 1,4-dioxane, 80 °C, 24 h; (f) NH_3/MeOH, rt, 24 h, 85% over the two steps.

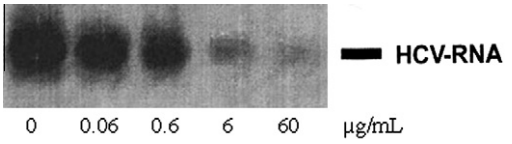


Figure 2. Inhibition of HCV-RNA replication by compound **4**.

and the Outstanding Scholar Foundation of Henan Province (#094100510019) for financial support.

References and notes

1. Later, M. J. *World J. Gastroenterol.* **2007**, *13*, 2436.
2. Cuthbert, J. A. *Clin. Microbiol. Rev.* **1994**, *7*, 505.
3. Di Bisceglie, A. M. *Hepatology* **2000**, *31*, 1014.
4. Poynard, T.; Yuen, M.-F.; Ratziu, V.; Lai, C. L. *Lancet* **2003**, *362*, 2095.
5. For a review of anti-HIV and anti-HCV drugs, see: De Clercq, E. *Nat. Rev. Drug Disc.* **2007**, *6*, 1001.
6. Carroll, S. S.; Tomassini, J. E.; Bosserman, M.; Getty, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. L.; LaFemina, R.; Rutkowski, C. A.; Wolanski, B.; Yang, Z.; Migliaccio, G.; Francesco, R. D.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. *J. Biol. Chem.* **2003**, *278*, 11979.
7. Eldrup, A. B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P. D.; Bennett, C. F.; Carroll, S. S.; Ball, R. G.; Bosserman, M.; Burlein, C.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K.; LaFemina, R. L.; Leone, J.; MacCoss, M.; McMasters, D. R.; Tomassini, J. E.; Langen, D. V.; Wolanski, B.; Olsen, D. B. *J. Med. Chem.* **2004**, *47*, 5284.
8. Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504.
9. Wang, P.; Chun, B.-K.; Rachakonda, S.; Du, J.; Khan, N.; Shi, J.; Stec, W.; Cleary, D.; Ross, B. S.; Sofia, M. J. *J. Org. Chem.* **2009**, *74*, 6819.
10. (a) Wang, S.; Chang, J.; Pan, S.; Zhao, K. *Helv. Chim. Acta* **2004**, *87*, 327; (b) Zhao, B.; Chang, J.; Wang, Q.; Du, Y.; Zhao, K. *Synlett* **2008**, 2993; (c) Chang, J.; Yu, X.; Wang, L.; Wang, Q.; Qi, X.; Dong, C. Faming Zhuanli Shengqing Gongkai Shuomingshu, CN 1626543, 2005; *Chem. Abstr.* **2005**, *144*, 150595.; (d) Chang, J.; Yu, X.; Bao, X.; Ye, Z. Faming Zhuanli Shengqing Gongkai Shuomingshu, CN 1712409, 2005; *Chem. Abstr.* **2005**, *145*, 489507.; (e) Chang, J.; Bao, X.; Wang, Q.; Guo, X.; Wang, W.; Qi, X. Faming Zhuanli Shengqing Gongkai Shuomingshu, CN 101177442, 2008; *Chem. Abstr.* **2008**, *149*, 32518.; (f) Wang, Q.; Li, Y.; Song, C.; Qian, K.; Chen, C.-H.; Lee, K.-H.; Chang, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4053.
11. Clark, J. L.; Mason, J. C.; Hobbs, A. J.; Hollecker, L.; Schinazi, R. F. *J. Carbohydr. Chem.* **2006**, *25*, 461.
12. ^{1}H NMR (300 MHz, $CDCl_3$) for the β-anomer (less polar spot on TLC): 8.67 (1H, s), 8.12–7.40 (10H, m), 7.30 (1H, d, J = 2.4 Hz), 6.66 (1H, d, J = 17.6 Hz), 5.84 (1H, dd, J = 22.0, 9.6 Hz), 4.88 (1H, dd, J = 12.6, 2.8 Hz), 4.74 (1H, m), 4.64 (1H, dd, J = 12.6, 3.6 Hz) and 1.20 (3H, d, J = 22.0 Hz); ^{1}H NMR (300 MHz, $CDCl_3$) for the α-anomer (more polar spot on TLC): 8.62 (1H, s), 8.11–7.36 (11H, m), 6.86 (1H, d, J = 18.2 Hz), 5.81 (1H, dd, J = 21.6, 8.7 Hz), 4.91 (1H, m), 4.72 (1H, dd, J = 12.2, 3.7 Hz), 4.61 (1H, dd, J = 12.2, 4.6 Hz) and 1.54 (3H, d, J = 22.0 Hz). Determination of the stereochemistry was based on a NOE correlation of ribofuranose H-1′ with H-3′ in the α anomer.
13. ^{1}H NMR (300 MHz, DMSO-d_6) 8.10 (1H, s, H-2), 7.44 (1H, d, J = 1.8 Hz, H-6), 7.09 (2H, br s, NH_2), 6.37 (1H, d, J = 17.9 Hz, H-1′), 5.61 (1H, d, J = 6.9 Hz, 3′-OH), 5.25 (1H, t, J = 4.8 Hz, 5′-OH), 4.06 (1H, ddd, J = 26.0, 9.5, 7.7 Hz, H-3′), 3.88–3.38 (3H, m, H-4′ and H-5′) and 0.94 (3H, d, J = 22.3 Hz, CH_3); ^{13}C NMR (DMSO-d_6) 155.9 (C-4), 153.1 (C-2), 145.8 (C-8), 142.8 (C-5, d, J_{FC} = 245.7 Hz), 103.4 (C-6, d, J_{FC} = 26.3 Hz), 101.3 (C-2′, d, J_{FC} = 179.9 Hz), 92.1 (C-9, d, J_{FC} = 16.6 Hz), 87.2 (C-1′, d, J_{FC} = 38.8 Hz), 81.7 (C-4′), 70.4 (C-3′, d, J_{FC} = 17.3 Hz), 59.0 (C-5′) and 16.1 (CH_3, d, J_{FC} = 25.6 Hz); Found: M^++H, 301.1150. $C_{12}H_{15}F_2N_4O_3$ requires 301.1112.
14. (a) Hwang, D.-R.; Lai, H.-Y.; Chang, M.-L.; Hsu, J. T.; Yeh, C.-T. *J. Virol. Methods* **2005**, *129*, 170; (b) Hwang, D.-R.; Tsai, Y.-C.; Lee, J.-C.; Huang, K.-K.; Lin, R.-K.; Ho, C.-H.; Chiou, J.-M.; Lin, Y.-T.; Hus, J. T.; Yeh, C.-T. *Antimicrob. Agents Chemother.* **2004**, *48*, 2876; (c) Yeh, C.-T.; Hwang, D.-R.; Lai, H.-Y.; Hsu, J. T. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 537; (d) Yeh, C.-T.; Lai, H.-Y.; Chen, T.-C.; Chu, C.-M.; Liaw, Y.-F. *J. Virol.* **2001**, *75*, 11017.