Bioorganic & Medicinal Chemistry Letters 21 (2011) 916–919

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and antitumor activity of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group

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ARTICLE INFO

Article history:
Received 2 November 2010
Revised 12 December 2010
Accepted 15 December 2010
Available online 19 December 2010

Keywords:
Ribonucleoside
Pyrimidine
Fatty amine
Anticancer
Nucleophilic substitution

ABSTRACT

A series of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group were synthesized by successively nucleophilic substitutions of 5′-deoxy-5′-amino-ribonucleosides with 2,4-dichloropyrimidine and then with various fatty amines under microwave irradiation. Their anticancer activities in vitro were preliminarily evaluated. Compounds **7a** and **8a** only exhibited anticancer activity against A549 cell line with the IC_{50} values of 10.73 and 10.99 μM, respectively. In addition, **7h** and **8h** showed potent activities against both A549 and Hela cell lines with the IC_{50} values of 12.71, 8.55 and 8.44, 5.55 μM, respectively.

Nucleoside analogs constitute an important class of therapeutic compounds with significant anticancer or antiviral properties. Zidovudine, Floxuridine, Didanosine, Fludarabine, Clofarabine, and Cladribine (Fig. 1) are a few notable examples.[1] Since these

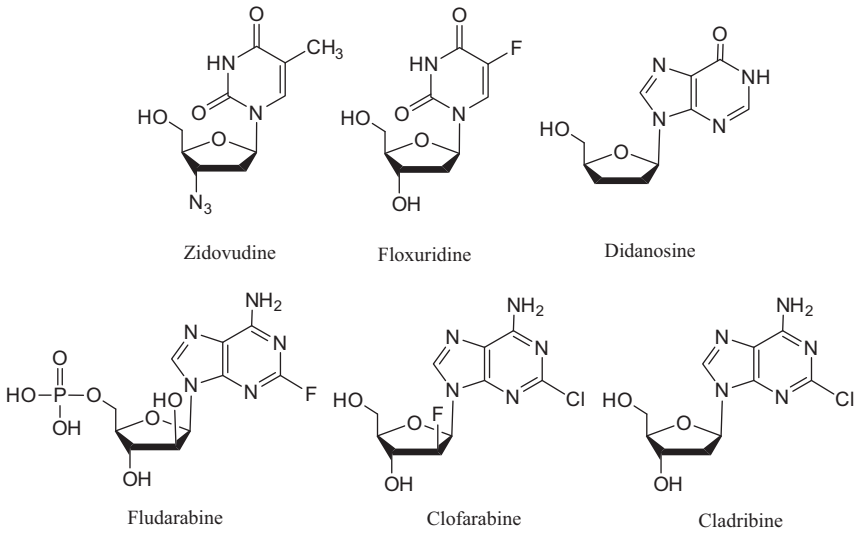

Figure 1. The structures of nucleoside anticancer and antiviral drugs.

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doi:10.1016/j.bmcl.2010.12.069

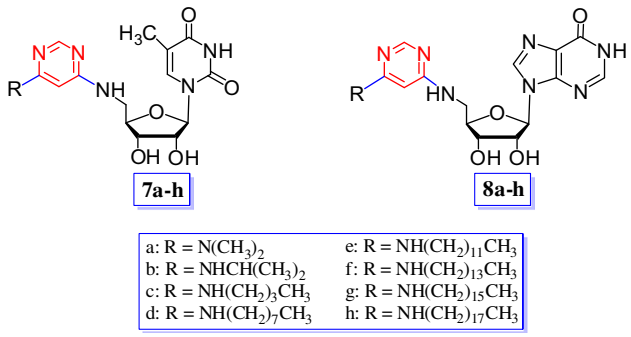


Figure 2. The structures of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group.

nucleotides are essential molecules for the synthesis of ribo- and deoxyribo-nucleic acids during replication, the main action of nucleoside analogs is to ultimately hinder the synthesis of nucleic acids in the fast replicating cancer cells by mimicking various metabolites or inhibiting an essential enzyme.[2] Therefore, the inclusion of nucleoside analog structures has become a very attractive approach for anticancer or antiviral drug design.

Though promising, nucleoside analogs suffered from serious limitations as therapeutic agents, such as poor stability and high hydrophilicity in vivo. These drawbacks mainly reduce their intracellular diffusion. To overcome these drawbacks, a lot of chemically modified nucleotides in the sugar moiety or in the base moiety have been developed.[3] Among them, the conjugation of nucleoside analogs with heterocycles or alkyl chains is hailed as a bright prospect towards enhanced activities.[4] For example, a series of 5′-substituted ribonucleosides modified by morpholine, piperidine, and pyrrolidine have been recently investigated as Ribonuclease A inhibitor, and all of the modifications on the 5′ position of the ribonucleosides could generate profound alterations to its interaction mode with the protein target.[5] Recently, Bourgaux[6] and Couvreur[7] reported that the gemcitabine modified with squalene showed remarkably improved anticancer activity and they suggested that gemcitabine–squalene might be able to penetrate the cell membrane more easily and promote the formation of non-lamellar structures due to the presence of the lipophilic alkyl moiety in the structure.[4c,d,8]

Table 1
Determination of optimum reaction conditions for the synthesis of **3**[a]

Entry	Bases (10 equiv)	Solvents	Conditions	Yield%
1	TEA	DCM	rt, 24 h	58
2	TEA	*n*-BuOH	60 °C, 8 h	68
3	TEA	*n*-BuOH	80 °C, 5 h	72
4	TEA	*n*-BuOH	115 °C, 5 h	62
5	TEA	THF	Reflux, 3 h	73
6	DIPEA	*n*-BuOH	80 °C, 5 h	75
7	DIPEA	DCM	rt, 24 h	63
8	DIPEA	THF	Reflux, 3 h	83

[a] 1.3 equiv of 2,4-dichloropyrimidine were used.

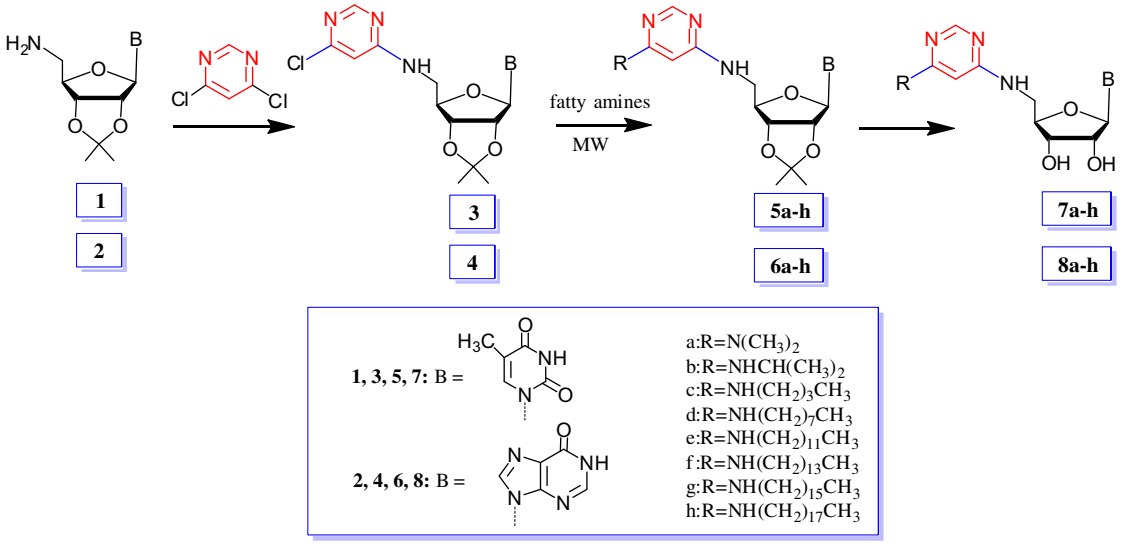
Table 2
The microwave assisted synthesis of compounds **5a–h** and **6a–h**

Entry	Amines (10 equiv)	Solvents	Conditions (°C, min)	Yield%
5a	$N(CH_3)_2$	Xylene	210, 20	94
5b	$NHCH(CH_3)_2$	Xylene	210, 30	68
5c	$NH(CH_2)_3CH_3$	Xylene	210, 20	75
5d	$NH(CH_2)_7CH_3$	Xylene	210, 20	79
5e	$NH(CH_2)_{11}CH_3$	Xylene	210, 30	65
5f	$NH(CH_2)_{13}CH_3$	Xylene	210, 30	73
5g	$NH(CH_2)_{15}CH_3$	Xylene	210, 30	70
5h	$NH(CH_2)_{17}CH_3$	Xylene	210, 40	72
6a	$N(CH_3)_2$	Diglyme	190, 20	78
6b	$NHCH(CH_3)_2$	Diglyme	190, 40	65
6c	$NH(CH_2)_3CH_3$	Diglyme	190, 20	78
6d	$NH(CH_2)_7CH_3$	Diglyme	190, 20	79
6e	$NH(CH_2)_{11}CH_3$	Diglyme	190, 30	75
6f	$NH(CH_2)_{13}CH_3$	Diglyme	210, 30	70
6g	$NH(CH_2)_{15}CH_3$	Diglyme	210, 30	72
6h	$NH(CH_2)_{17}CH_3$	Diglyme	210, 40	65

Table 3
The yields of the novel ribonucleosides (**7a–h**) and (**8a–h**)

Yield%	a	b	c	d	e	f	g	h
7	87	90	94	93	95	85	85	83
8	83	78	85	83	80	85	82	82

It is well known that pyrimidine derivatives constitute a very important class of compounds, including a large number of natural



Scheme 1. General reaction strategy for the synthesis of novel ribonucleosides **7a–h** and **8a–h**.

Table 4
Anticancer activity against A549 and Hela in vitro of novel ribonucleosides

Compounds	IC_{50} (μM)		Compounds	IC_{50} (μM)	
	A549	Hela		A549	Hela
7a	10.73 ± 0.55	>100	**8a**	10.99 ± 0.92	>100
7b	23.23 ± 0.61	64.54 ± 1.00	**8b**	16.73 ± 1.77	>100
7c	29.51 ± 2.22	52.62 ± 0.78	**8c**	55.06 ± 4.00	>100
7d	>100	>100	**8d**	>100	>100
7e	>100	>100	**8e**	>100	61.54 ± 2.91
7f	>100	>100	**8f**	>100	97.45 ± 3.42
7g	>100	>100	**8g**	>100	82.66 ± 6.70
7h	12.71 ± 0.37	8.44 ± 0.17	**8h**	8.55 ± 0.13	5.55 ± 0.22
Cisplatin	19.41 ± 5.67	20.01 ± 1.36			

products, pharmaceuticals, and functional materials. They are robust pharmacophores endowed with drug like properties and a wide range of pharmacological activities.[9,10] In this letter, we synthesized a series of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group based on 5-methyl-uridine and inosine (Fig. 2). Their anticancer activities in vitro were also preliminarily evaluated.

The novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group were synthesized by stepwise nucleophilic substitutions of 5′-deoxy-5′-amino-2′,3′-isopropylideneyl-ribonucleosides[11] with 2,4-dichloropyrimidine and then with various fatty amines under microwave irradiation, as shown in Scheme 1. The 5′-deoxy-5′-amine-2′,3′-isopropylideneyl-ribonucleosides (**1**) and (**2**) firstly reacted with 2,4-dichloropyrimidine to afford the intermediates **3** and **4**, respectively. The reaction was optimized under different acid binding agents, solvents, reaction temperatures and times, as described in Table 1. It was found that the synthesis of **3** could proceed well by using *N*,*N*-diisopropylethylamine (DIPEA) as the acid binding agent in THF, which afforded the desired product (**3**) in the yield of 83% after 3 h refluxing (entry 8 of Table 1). Similarly, compound **4** could be synthesized with a yield of 72% by using *n*-butanol as the solvent.

Then the second step of substitution was performed for the intermediates (**3**) and (**4**) with a series of fatty amines, respectively. Under the conventional conditions (heating and reflux), no reaction products were formed. The reaction was found to undergo smoothly and efficiently under microwave irradiation[12] at the reaction temperature of 190–210 °C, and provided the corresponding conjugated products (**5**) and (**6**), respectively, in good to excellent yields, as shown in Table 2. Obviously, for the amine with a longer alkyl chain, a prolonged reaction time was necessary to make the reaction complete. One possible reason could be the decreased reactivity due to the increase of alkyl chain. In addition, the synthesis for compounds **5b** and **6b** also required longer reaction time than other compounds, likely due to the steric effect which could lower the reactivity of the amine.

The removal of protecting isopropenyl groups of **5a–h** and **6a–h** was performed effectively under the conditions of TFA/H_2O (9:1) at room temperature for 20 min, and afforded the corresponding target products **7a–h** and **8a–h** with high yields (Table 3). All of the final compounds (**7a–h** and **8a–h**) were characterized by NMR and HR-MS spectra.

The anticancer activities of the novel ribonucleosides **7a–h** and **8a–h** against cancer cell lines (A549 and Hela) in vitro were measured with comparison to the control drug cisplatin. As shown in Table 4, compounds **7a**, **8a**, **7h**, and **8h** exhibited potent anticancer activity, even better than the positive control drug (cisplatin).

It is interesting to find that **7a** and **8a** exhibited anticancer activity against A549 cell line with the IC_{50} values of 10.73 and 10.99 μM, respectively, which can be attributed to that the pyrimidine and *N*,*N*-dimethyl moiety as the second heterocyclic base in the molecule could effectively enhance their anticancer activity by increasing their affinity.[9,10] In addition, **7h** and **8h** showed potent activities against both A549 and Hela cell lines with the IC_{50} values of 12.71, 8.55 and 8.44, 5.55 μM, respectively. This is likely due to the increased membrane permeability, consistent with the reported results.[7,8] However, other compounds, such as **7g** and **8g**, exhibited no anticancer activity in the test cell lines, though they contained similar alkyl chain length compared with **7h** and **8h**. These results indicated that even a slightly structural difference in the substituent group could remarkably influence the anticancer activity of a compound.

In conclusion, a series of novel ribonucleosides with C-5 OH replaced by a diaminopyrimidinyl group based on 5-methyl-uridine and inosine were synthesized with satisfactory yields by stepwise nucleophilic substitutions for 5′-deoxy-5′-amino-ribonucleosides with 2,4-dichloropyrimidine and then with various fatty amines under microwave irradiation. Their anticancer activities, in vitro evaluation, in comparison with cisplatin showed that compounds **7a** and **8a** exhibited anticancer activity against A549 cell line with the IC_{50} values of 10.73 and 10.99 μM, respectively, and **7h** and **8h** gave potent activity to both A549 and Hela cell lines with the IC_{50} values of 12.71, 8.55 and 8.44, 5.55 μM, respectively, which were better than that of the positive control drug (cisplatin). The results here suggest that these novel compounds merited the further investigation as potential anticancer agents.

Acknowledgments

This work was supported by the 973 Program (2010CB534913), the Natural Science Foundation of Hebei Province (B2008000588), the State Key Laboratory of Natural and Biomimetic Drugs of Peking University (20080205) and the Program of Science and Technology of Hebei Program (09276418D-13).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.069.

References and notes

1. (a) Clercq, E. D. *J. Clin. Virol.* **2004**, *30*, 115; (b) Pui, C. H.; Jeha, S.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 369; (c) Bonate, P. L.; Arthaud, L.; Cantrell, W. R.; Stephenson, K.; Secrist, J. A.; Weitman, S. *Nat. Rev. Drug Disc.* **2006**, *5*, 855; (d) Issa, J. P.; Kantarjian, H.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 275; (e) Gore, S. D.; Jones, C.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2006**, *5*, 891.
2. (a) Galmarini, C. M.; Mackey, J. R.; Dumontet, C. *Lancet Oncol.* **2002**, *3*, 415; (b) Ewald, B.; Sampath, D.; Plunkett, W. *Oncogene* **2008**, *27*, 6522; (c) Sampath, D.; Rao, V. A.; Plunkett, W. *Oncogene* **2003**, *22*, 9063; (d) Damaraju, V. L.; Damaraju, S.; Young, J. D.; Baldwin, S. A.; Mackey, J.; Sawyer, M. B.; Cass, C. E. *Oncogene* **2003**, *22*, 7524.
3. (a) Romeo, G.; Chiacchio, U.; Corsaro, A.; Merino, P. *Chem. Rev.* **2010**, *110*, 3337; (b) Amblard, F.; Cho, J. H.; Schinazi, R. F. *Chem. Rev.* **2009**, *109*, 4207.

4. (a) Chen, L.; Wilson, D. J.; Xu, Y.; Aldrich, C. C.; Felczak, K.; Sham, Y. Y.; Pankiewicz, K. W. *J. Med. Chem.* **2010**, *53*, 4768; (b) Poecke, S. V.; Negri, A.; Gago, F.; Daele, I. V.; Solaroli, N.; Karlsson, A.; Balzarini, J.; Calenbergh, S. V. *J. Med. Chem.* **2010**, *53*, 2902; (c) Godeau, G.; Bernard, J.; Staedel, C.; Barthélémy, P. *Chem. Commun.* **2009**, 5127; (d) Park, S. M.; Woo, J.; Jeon, E.; Kim, B. H. *Chem. Commun.* **2010**, *46*, 1523.
5. Samanta, A.; Leonidas, D. D.; Dasgupta, S.; Pathak, T.; Zographos, S. E.; Oikonomakos, N. G. *J. Med. Chem.* **2009**, *52*, 932.
6. Pili, B.; Bourgaux, C.; Amenitsch, H.; Keller, G.; Lepêtre-Mouelhi, S.; Desmaële, D.; Couvreur, P.; Ollivon, M. *Biochim. Biophys. Acta* **2010**, *1798*, 1522.
7. Pili, B.; Reddy, L. H.; Bourgaux, C.; Lepêtre-Mouelhi, S.; Didier Desmaëleb, D.; Couvreur, P. *Nanoscale* **2010**, *2*, 1521.
8. Manfredini, S.; Simoni, D.; Ferroni, R.; Bazzanini, R.; Vertuani, S.; Hatse, S.; Balzarini, J.; Clercq, E. D. *J. Med. Chem.* **1997**, *40*, 3851.
9. (a) Radi, M.; Schenone, S.; Botta, M. *Org. Biomol. Chem.* **2009**, *7*, 2841; (b) Deng, X.; Nagle, A.; Wu, T.; Sakata, T.; Henson, K.; Chen, Z.; Kuhen, K.; Plouffe, D.; Winzeler, E.; Adrian, F.; Tuntland, T.; Chang, J.; Simerson, S.; Howard, S.; Ek, J.; Isbell, J.; Tully, D. C.; Chatterjee, A. K.; Gray, N. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4027; (c) Parent, A. A.; Gunther, J. R.; Katzenellenbogen, J. A. *J. Med. Chem.* **2008**, *51*, 6512; (d) Hill, M. D.; Movassaghi, M. *Chem. Eur. J.* **2008**, *14*, 6836.
10. (a) Temple, C.; Kussner, C. L.; Montgomery, J. A. *J. Med. Chem.* **1962**, *5*, 866; (b) Israel, M.; Protopapa, H. K.; Schlein, H. N.; Modest, E. J. *J. Med. Chem.* **1964**, *7*, 792; (c) Baindur, N.; Chadha, N.; Player, M. R. *J. Comb. Chem.* **2003**, *5*, 653.
11. (a) Ishido, Y. *J. Chem. Soc., Perkin. II* **1979**, 2088; (b) Hampton, A. *J. Am. Chem. Soc.* **1961**, *83*, 3640; (c) Babic, A.; Gobec, S.; Gravier-Pelletier, C.; Merrer, Y. L.; Pečar, S. *Tetrahedron* **2008**, *64*, 9093; (d) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C. H. *J. Am. Chem. Soc.* **2002**, *124*, 10773.
12. (a) Roberts, B. A.; Strauss, C. R. *Acc. Chem. Res.* **2005**, *38*, 653; (b) Polshettiwar, V.; Varma, R. S. *Acc. Chem. Res.* **2008**, *41*, 629; (c) Larhed, M.; Moberg, C.; Hallberg, A. *Acc. Chem. Res.* **2002**, *35*, 717; (d) Dallinger, D.; Kappe, C. O. *Chem. Rev.* **2007**, *107*, 2563.