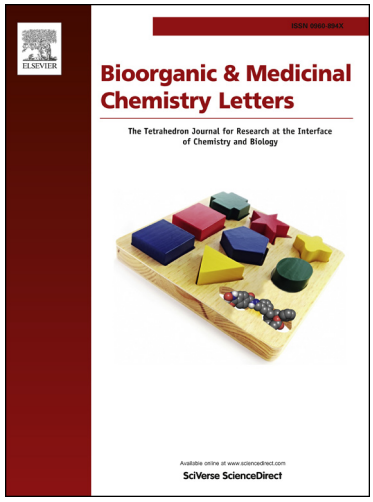


Thiophene-expanded guanosine analogues of Gemcitabine

Zhe Chen, Therese C. Ku, Katherine L. Seley-Radtke

PII: S0960-894X(15)00805-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.07.086
Reference: BMCL 22975

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 18 June 2015
Revised Date: 24 July 2015
Accepted Date: 27 July 2015

Please cite this article as: Chen, Z., Ku, T.C., Seley-Radtke, K.L., Thiophene-expanded guanosine analogues of Gemcitabine, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.07.086

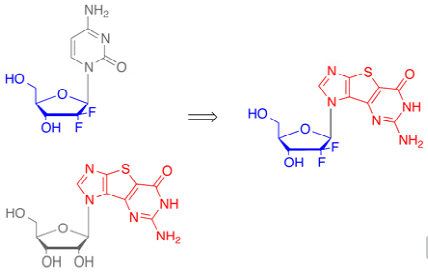
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Thiophene-expanded guanosine analogues of Gemcitabine.

Leave this area blank for abstract info.

Zhe Chen, Therese C. Ku and Katherine L. Seley-Radtke*



Thiophene-expanded guanosine analogues of Gemcitabine

Zhe Chen, Therese C. Ku, and Katherine L. Seley-Radtke*

University of Maryland, Baltimore County, Department of Chemistry & Biochemistry, 1000 Hilltop Circle, Baltimore, MD 21250 USA

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Tricyclic
Nucleosides
Expanded guanosine
2',2'-Difluoro-2'-deoxycytidine
Cancer
* corresponding author: kseley@umbc.edu
1-410-455-8684 (O); 1-410-455-2608 (Fax)

ABSTRACT

The chemotherapeutic drug Gemcitabine, 2',2'-difluoro-2'-deoxycytidine, has long been the standard of care for a number of cancers. Gemcitabine's chemotherapeutic properties stem from its 2',2'-difluoro-2'-deoxyribose sugar, which mimics the natural nucleoside, but also disrupts nucleic acid synthesis, leading to cell death. As a result, numerous analogues have been prepared to further explore the biological implications for this structural modification. In that regard, a thieno-expanded guanosine analogue was of interest due to biological activity previously observed for the tricyclic heterobase scaffold. Several analogues were prepared, including the McGuigan ProTide, however the parent nucleoside exhibited the best chemotherapeutic activity, specifically against breast cancer cell lines (89.53% growth inhibition).

2'-Fluorinated nucleosides have proven to be effective therapeutic candidates against cancers and viruses; 2',2'-difluoro-2'-deoxycytidine, or Gemcitabine (Figure 1), has been FDA ap-proved for the treatment of a broad spectrum of solid tumors. More recently, Sofosbuvir (Figure 1), another 2'-F nucleoside, was been approved to treat hepatitis C.[1,2] Fluorine substitutions on nucleosides were initially pursued due to fluorine's multi-faceted chemical properties: due to its high electronegativity and low polarizability, fluorines can imitate a hydrogen atom from a size perspective, a hydroxyl group from a polarity perspective, and can also act as a hydrogen bond acceptor.[3-5] Moreover, nuc-leosides endowed with a fluorine at C-2' are much more stable to enzymatic cleavage.[4] Gemcitabine's mechanism of action is two-fold; not only does Gemcitabine inhibit DNA and RNA syn-thesis, but it also inhibits ribonucleotide reductase, which further inhibits DNA synthesis.[4,6,7]

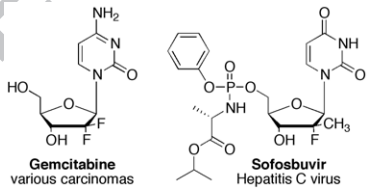


Figure 1. Gemcitabine and Sofosbuvir.

Although Gemcitabine is marketed as a cytidine nucleoside analogue, the guanosine version was also synthesized and show-ed similar activity profile.[7] Our group has long been interested in modified purine nucleoside analogues and has previously syn-thesized various tricyclic thieno-expanded purine nucleosides (Figure 2) that possess a thiophene spacer ring between the imidazole and pyrimidine moieties of the purine.[8-14] These novel nucleosides were designed to further explore the biological potential for bioprobes such as Nelson Leonard's benzene expanded adenosine nucleosides.[15-19] A number of his benzo-ex-panded nucleosides exhibited interesting therapeutic activities but occupied a larger spatial footprint that limited their potential.[15-19] Our thieno-expanded purine nucleosides retain the essential binding elements of the parent nucleosides but also increase the aromaticity and polarizability of the base due to the thiophene spacer.[8-14] The thiophene spacer also decreases the spatial implications of the expanded purine scaffold as compared to Leonard's benzene spacer, thus allowing it to base pair more readily.[20]

The thiophene-expanded analogues were shown to be recog-nized by nucleoside and nucleobase transporters, to exhibit acti-vity against certain cancers, as well as to exhibit activity against hepatitis C.[10,11,13,21,22] As a result, additional structural modifications were pursued to further explore the potential therapeutic profile of the tricyclic expanded guanosine base. In that regard, the 2',2'-difluoro-2'-deoxyribose sugar of Gemcitabine was of interest (Figure 3, **1**) as well as the corresponding McGuigan ProTide (Figure 3, **2**). McGuigan's ProTides have had a major impact on the nucleoside field since many inactive nucleosides that were subsequently converted to their corresponding Protide form show potent activity due to the ability to overcome the rate limiting step of monophosphorylation.[23-27]

Although there are numerous ProTides to choose from, the combination of the L-alanine (L-Ala) amino acid group, the phenyl aryl group and the *i*PrO ester group (Figure 3, **2**) was selected due to its success with similar nucleosides.[28]

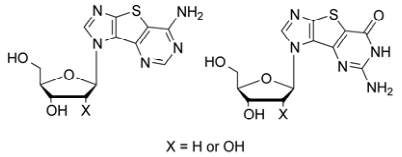


Figure 2. Tricyclic "thieno-expanded" purine nucleosides.

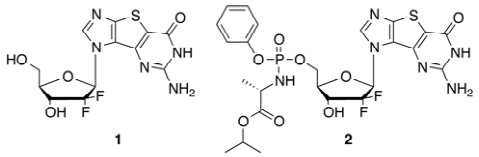
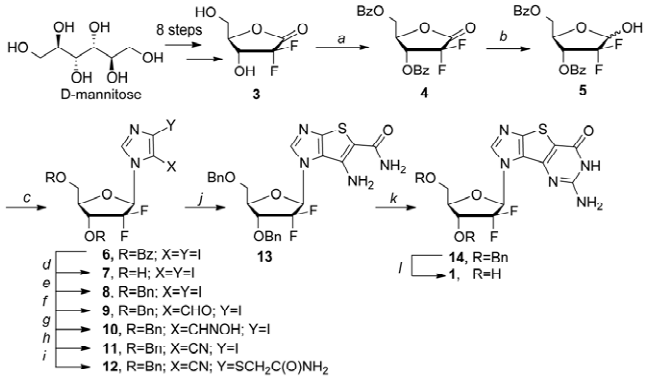


Figure 3. Target compounds **1** and **2**.

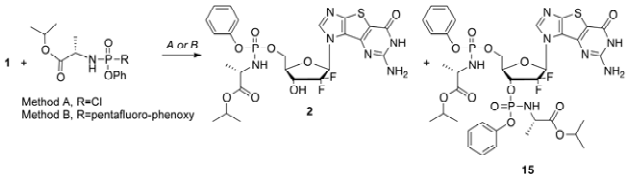
The 2',2'-difluoro-2'-deoxyribose sugar was synthesized following a known route.[29,30] Starting with D-mannitose, the fluorinated pentose sugar **3** can be achieved in 8-steps.



Scheme 1. Reagents and conditions: (a) BzCl, Py, DCM, 0°C, 10 min, then rt, 30 min; (b) $LiAl(O^tBu)_3H$, THF, -20°C, 6 h;(c) 4,5-diiodoimidazole, Ph_3P, DIAD, THF, 0°C to rt, 24 h; (d) NaOMe, MeOH, 0°C, 1 h; (e) (i) NaH, 0°C, 2 h; (ii) BnBr, TBAI, DMF, rt, 4 h; (f) (i) EtMgBr, THF, 0°C, 30 min; (ii) anhydrous DMF, rt, overnight; (g) NH_2OH•HCl, $NaHCO_3$, EtOH, reflux, 5 h; (h) CDI, THF, reflux, 8 h; (i) $NH_2C(O)CH_2SH$, K_2CO_3, DMF, 65°C, 24 h; (j) EtONa, EtOH, reflux, 2 h; (k) (i) NaOH, CS_2, MeOH, 150°C, 18 h; (ii) H_2O_2, MeOH, 0°C, 2 h; (iii) NH_3/MeOH, 130°C, 12 h; (l) BF_3•Et_2O, EtSH, DCM, rt, 72 h.

The 2',2'-difluoro-2'-deoxyribonolactone **3** was then benzoyl-protected before reducing the ketone to give **5** (Scheme 1). Next, 4,5-diiodoimidazole was coupled to the modified sugar **5** to give both the α and β isomers of **6**. The benzoyl protecting groups were removed and replaced with more robust benzyl groups **8,** so as to withstand the rigorous conditions employed while synthesizing the tricyclic base, and the α and β isomers were separated. Construction of the tricyclic ring system followed our previously reported route.[8-13] Finally, benzyl deprotection of **14** yielded the desired nucleoside **1**.

The McGuigan ProTide of **1** was then synthesized following literature procedures (Scheme 2) to give **2** as well as and the bis-ProTide **15**.[31-35]



Scheme 2. Reagents and conditions: (A) ^{t}BuMgCl, DMF, -78 °C, 1h, then r.t., 4 h; (B) ^{t}BuMgCl, DMF, 0 °C, 1 h, then rt, overnight.

Compounds **1**, **2** and **15** were submitted to NCI for screening in their 60 cell line assay system. As shown below in Table 1, the compounds showed inhibitory activity against the MOLT-4 (leukemia), T-47D (breast cancer), LOX IMVI (melanoma) and NCI-H522 (non-small cell lung cancer) cell lines at 15μg/mL.

Table 1. Growth percent of cancer cell lines MOLT-4, T-47D, LOX IMVI and NCI-H522 at 15μg/mL.

Compound	Cell Line (percent growth)			
	MOLT-4	T-47D	LOX IMVI	NCI-H522
1	13.94	10.47	25.60	33.95
2	93.98	64.50	74.06	65.63
15	109.04	87.15	101.93	77.66

The results show that the best activity was observed for compound **1** against the breast cancer cell line T-47D (10.47%). Surprisingly, the McGuigan ProTides **2** and **15** did not demonstrate superior inhibition as compared to **1** as would have been expected. In fact, the therapeutic activity worsened with a second ProTide moiety present. Subsequent to our synthesis, a report appeared in the literature that ultimately confirmed the biological results: Slusarczyk et al. found that the selected combination of the L-Ala amino acid group, the phenyl aryl group and the *i*PrO ester led to a decrease in the activity of Gemcitabine.[27] As a result, future efforts will focus on the ProTide combination they found to be best for the gemcitabine modification: a L-Ala amino acid group, a phenyl aryl group and an OBn ester.[27]

The tricyclic thiophene-expanded guanine base was successfully combined with the 2',2'-difluoro-2'-deoxyribose sugar of Gemcitabine. In addition the corresponding McGuigan ProTide was also made and all three compounds were screened. The synthetic approach was nontrivial as both the sugar and base had to be constructed in a linear fashion, thus the overall yield was quite low. As a result, only preliminary biological testing could be undertaken. While the results from the NCI cancer screen showed promising results against leukemia, melanoma and breast cancer, the McGuigan ProTides were less active as compared to the parent nucleoside. Current efforts are underway to optimize the synthesis of the parent nucleoside **1** so further testing can be undertaken. The results of those efforts will be reported as they become available.

This work was funded in part by the National Institutes of Health [R21AI097685 (K.S.R.) and T32GM066706 (K.S.R. and T.C.K.)].

1. Liu, P.; Sharon, A.; Chu, C. K. *J. Fluorine Chem.* 2008, *129*, 743.
2. Lawitz, E.; Mangia, A.; Wyles, D.; Rodriguez-Torres, M.; Hassanein, T.; Gordon, S. C.; Schultz, M.; Davis, M. N.; Kayali, Z.; Reddy, K. R.; Jacobson, I. M.; Kowdley, K. V.; Nyberg, L.; Subramanian, G. M.; Hyland, R. H.; Arterburn, S.; Jiang, D. Y.; McNally, J.; Brainard, D.; Symonds, W. T.; McHutchison, J. G.; Sheikh, A. M.; Younossi, Z.; Gane, E. J. *New Engl. J. Med.* 2013, *368*, 1878.
3. Gesto, D. S.; Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. *Curr. Med. Chem.* 2012, *19*, 1076.
4. Pankiewicz, K. W. *Carbohyd. Res.* 2000, *327*, 87.
5. Hunter, L. *Beilstein J. Org. Chem.* 2010, *6*.
6. Plunkett, W.; Huang, P.; Gandhi, V. *Nucleosides Nucleotides* 1997, *16*, 1261.
7. Gandhi, V.; Mineishi, S.; Huang, P.; Chapman, A. J.; Yang, Y. D.; Chen, F.; Nowak, B.; Chubb, S.; Hertel, L. W.; Plunkett, W. *Cancer Res.* 1995, *55*, 1517.
8. Zhang, Z. B.; Wauchope, O. R.; Seley-Radtke, K. L. *Tetrahedron* 2008, *64*, 10791.
9. Zhang, Z.; Wauchope, O. R.; Seley-Radtke, K. L. *Tetrahedron* 2008, *64*, 10791.
10. Wauchope, O. R.; Tomney, M. J.; Pepper, J. L.; Korba, B. E.; Seley-Radtke, K. L. *Org. Lett.* 2010, *12*, 4466.
11. Wauchope, O. R.; Johnson, C.; Krishnamoorthy, P.; Andrei, G.; Snoeck, R.; Balzarini, J.; Seley-Radtke, K. L. *Bioorg. Med. Chem.* 2012, *20*, 3009.
12. Seley, K. L.; Zhang, L.; Hagos, A.; Quirk, S. *J. Org. Chem.* 2002, *67*, 3365.

13. Seley, K. L.; Januszczyk, P.; Hagos, A.; Zhang, L.; Dransfield, D. T. *J. Med. Chem.* 2000, *43*, 4877.
14. Wauchope, O. R.; Velasquez, M.; Seley-Radtke, K. *Synthesis* 2012, *44*, 3496.
15. Leonard, N. L.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* 1975, *40*, 356.
16. Leonard, N. J. *Acc. Chem. Res.* 1982, *15*, 128.
17. Leonard, N. J.; Hiremath, S. P. *Tetrahedron* 1986, *42*, 1917.
18. Leonard, N. J.; Scopes, D. I.; VanDerLijn, P.; Barrio, J. R. *Biochemistry* 1978, *17*, 3677.
19. Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. *J. Am. Chem. Soc.* 1976, *98*, 3987.
20. O'Daniel, P. I.; Jefferson, M.; Wiest, O.; Seley-Radtke, K. L. *J. Biomol. Struct. Dyn.* 2008, *26*, 283.
21. Chen, Z.; Jochmans, D.; Ku, T.; Paeshuyse, J.; Neyts, J.; Seley-Radtke, K. L. *ACS Inf. Dis.* 2015.
22. Wallace, L. J. M.; Candlish, D.; Hagos, A.; Seley, K. L.; de Koning, H. P. *Nucleosides, Nucleotides and Nucleic Acids* 2004, *23*, 1441.
23. Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* 2004, *4*, 371.
24. McGuigan, C.; Shackleton, J. M.; Tollerfield, S. M.; Riley, P. A. *Nucleic Acids Res.* 1989, *17*, 10171.
25. Balzarini, J.; Karlsson, A.; Aquaro, S.; Perno, C. F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C. *Proc. Natl. Acad. Sci. U. S. A.* 1996, *93*, 7295.
26. McGuigan, C.; Harris, S. A.; Daluge, S. M.; Gudmundsson, K. S.; McLean, E. W.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* 2005, *48*, 3504.
27. Slusarczyk, M.; Lopez, M. H.; Balzarini, J.; Mason, M.; Jiang, W. G.; Blagden, S.; Thompson, E.; Ghazaly, E.; McGuigan, C. *J. Med. Chem.* 2014, *57*, 1531.
28. Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* 2010, *53*, 7202.
29. Hertel, L. W.; Kroin, J. S.; Misner, J. W.; Tustin, J. M. *J. Org. Chem.* 1988, *53*, 2406.
30. 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.
31. McGuigan, C.; Daverio, F.; Najera, I.; Martin, J. A.; Klumpp, K.; Smith, D. B. *Bioorg. Med. Chem. Lett.* 2009, *19*, 3122.
32. McGuigan, C.; Kelleher, M. R.; Perrone, P.; Mulready, S.; Luoni, G.; Daverio, F.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Martin, J. A.; Klumpp, K.; Smith, D. B. *Bioorg. Med. Chem .Lett.* 2009, *19*, 4250.
33. Perrone, P.; Daverio, F.; Valente, R.; Rajyaguru, S.; Martin, J. A.; Leveque, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* 2007, *50*, 5463.
34. Perrone, P.; Luoni, G. M.; Kelleher, M. R.; Daverio, F.; Angell, A.; Mulready, S.; Congiatu, C.; Rajyaguru, S.; Martin, J. A.; Leveque, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* 2007, *50*, 1840.
35. Ross, B. S.; Reddy, P. G.; Zhang, H. R.; Rachakonda, S.; Sofia, M. J. *J. Org. Chem.* 2011, *76*, 8311.

SUPPLEMENTAL MATERIAL

Synthetic procedures and structural characterization.

Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Thiophene-expanded guanosine analogues of Gemcitabine.

Leave this area blank for abstract info.

Zhe Chen, Therese C. Ku and Katherine L. Seley-Radtke*