ACS Medicinal Chemistry Letters

Penn Libraries

Subscriber access provided by University of Pennsylvania Libraries

Letter

Synthesis and Evaluation of 2,6-Modified Purine 2'-C-Methyl Ribonucleosides as Inhibitors of HCV Replication

Longhu Zhou, Hongwang Zhang, Sijia Tao, Maryam Ehteshami, Jong Hyun Cho, Tamara R McBrayer, Philip Tharnish, Tony Whitaker, Franck Amblard, Steven J. Coats, and Raymond Felix Schinazi

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.5b00402 • Publication Date (Web): 23 Nov 2015

Downloaded from http://pubs.acs.org on December 4, 2015

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

ACS Medicinal Chemistry Letters is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Synthesis and Evaluation of 2,6-Modified Purine 2'-*C*-Methyl Ribonucleosides as Inhibitors of HCV Replication

Longhu Zhou,[†] Hongwang Zhang,[†] Sijia Tao,[†] Maryam Ehteshami,[†] Jong Hyun Cho,[†] Tamara R. McBrayer,[§] Philip Tharnish,[§] Tony Whitaker,[§] Franck Amblard,[†] Steven J. Coats,[§] Raymond F. Schinazi[†]*

AUTHOR ADDRESS [†]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Atlanta, Georgia 30322, USA, [§]CoCrystal Pharma, Inc, Tucker, Georgia 30084, USA.

KEYWORDS HCV, Antiviral, phosphoramidate prodrug, purine, nucleoside

ABSTRACT: A variety of 2,6-modified purine 2'-*C*-methylribonucleosides and their phosphoramidate prodrugs were synthesized and evaluated for inhibition of HCV RNA replication in Huh-7 cells and for cytotoxicity in various cell lines. Cellular pharmacology and HCV polymerase incorporation studies on the most potent and selective compound are reported

Hepatitis C virus (HCV) is a global health problem affecting an estimated 170 million individuals worldwide and it is a leading cause of liver cirrhosis and hepatocellular carcinoma.[1,2] Several curative options are now available for HCV infections, but they all require at least two direct acting antiviral agents to result in cure rates of 90 to 100 per cent. Nucleosides inhibitors of HCV NS5B polymerase are favored since they generally have a high genetic barrier to drug resistance and are pan-genotypic activity.[3] Sofosbuvir (PSI/GS-7977) **1**,[4] a 2'-deoxy-2'-α-fluoro-2'-β-*C*-methyl nucleoside monophosphate prodrug was approved by the FDA in December 2013 as a safe and effective anti-HCV agent (Figure 1).[5] IDX-184 **2** and BMS-986094 (INX-189) **3**, two related nucleoside prodrugs, precursors of the same active 2'-β-*C*-methyl guanosine triphosphate, also showed high potency *in vitro* and promising results in early clinical studies, but their development was terminated after low effectiveness in humans for IDX-184, and severe cardiac effects observed during a phase 2b study with BMS-986094. Based on the potential of these 2'-*C*-methyl nucleosides, we present, herein, the synthesis of new 2,6-modified purine 2'-*C*-methyl ribonucleosides that may offer potential alternatives to BMS-986094 and IDX-184 or maybe used in combination with Sofosbuvir.

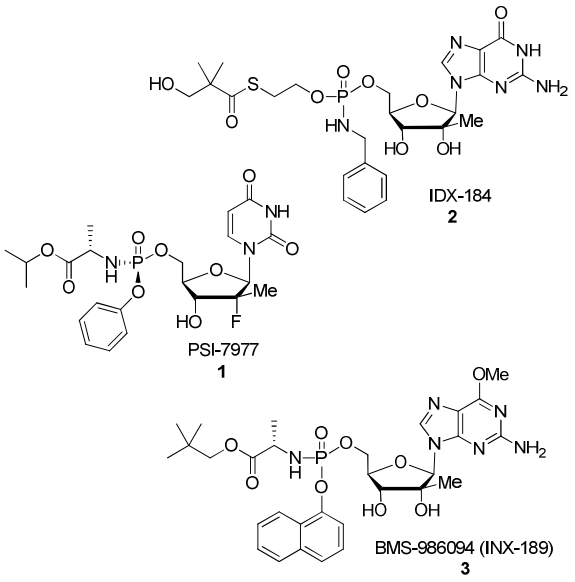
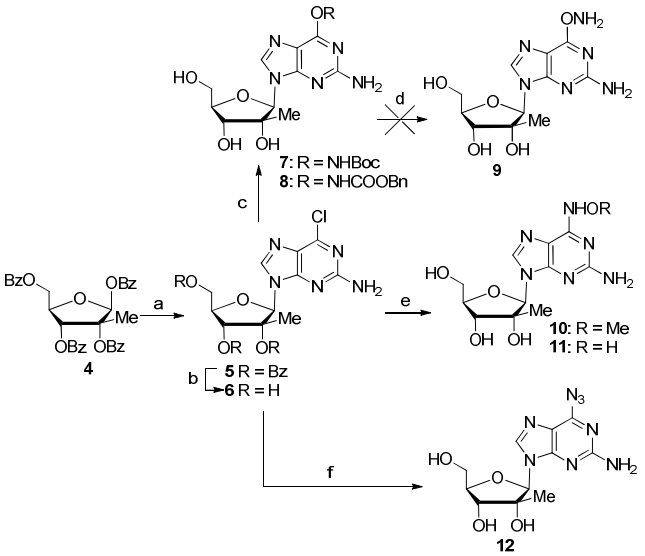


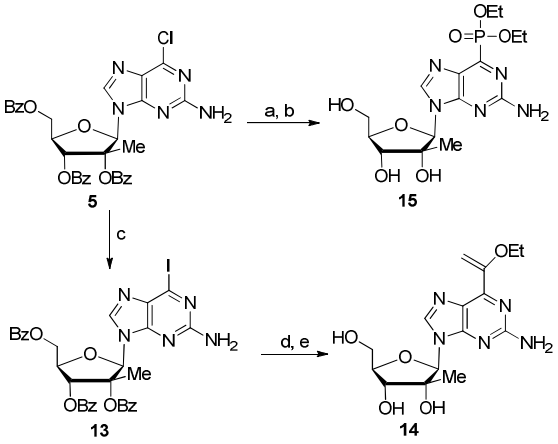
Figure 1. Selected clinical anti-HCV nucleoside analogs.

In the design of 6-position modifications, we strived to maintain groups that retained hydrogen bond accepting characteristics as is present in the 6-position of natural guanosine. Key to the synthesis of 2-amino, 6-modified 2'-*C*-methyl nucleosides was intermediate **5** that was prepared by known methods.[6] Targeted 6-N_3 and 6-NH-O-substituted purines **10-12** were easily prepared from debenzoylated nucleoside **6** by reaction with NaN_3,, methoxyamine and hydroxylamine (Scheme 1). Interestingly, attempts to prepare the 6-ONH_2 compound **9** from *N*-Boc-hydroxylamino and *N*-(benzyloxycarbonyl)hydroxylamino derivatives **7** and **8** were unsuccessful as all attempts to deprotect intermediates **7** and **8**, using acidity (compound **7**) or transition metal catalyzed hydrogenation (compound **8**) lead exclusively to the formation of 2'-*C*-methyl guanosine.



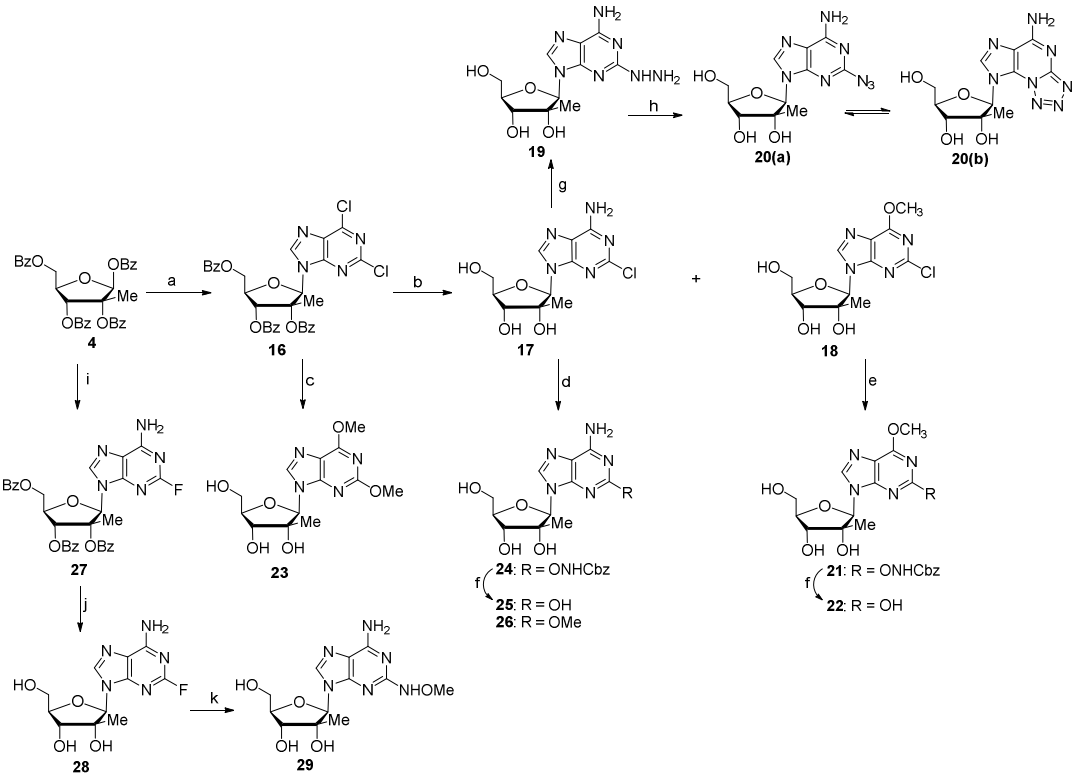
Scheme 1. Reagents and conditions: (a) 2-amino-6-chloropurine, DBU, TMSOTf, -40 to 80 ºC, 5 h, 92%; (b) sat. NH_3/MeOH, rt, overnight, 90%; (c) for **7**: HONHBoc, NaH, THF, rt, 3 h, 69%; for **8**: HONHCbz NaH, THF, rt, 3 h, 88%; (d) for **7**: 80% TFA, H_2O, rt, overnight; for **8**: H_2, Pd/C 10%, MeOH, rt, overnight; (e) for **10**: $MeONH_2$, Et_3N, EtOH/H_2O, 65 ºC, 24 h, 79%; for **11**: NH_2OH, EtOH/H_2O, 35 ºC, 24 h, 51%; (f) NaN_3, DMF, 95 ºC, 2 h, 62%.

Other, more uncommon functionalities such as a phosphonate and an ethoxyvinyl group were also introduced at the 6-position (Scheme 2). Thus, 6-diethylphosphonate derivative **15** was prepared by reaction of 6-chloropurine nucleoside **5** with triethyl phosphite (Scheme 2) at 130 ºC and subsequent deprotection in a saturated solution of ammonia in ethanol. Compound **5** was also reacted with TMSI to generate the more reactive iodo intermediate **13** which was coupled with tributyl(1-ethoxyvinyl)stannane under palladium catalyzed Stille coupling conditions. Final deprotection using a catalytic amount of sodium methoxide in methanol afforded final compound **14**.



Scheme 2. Reagents and conditions: a) $P(OEt)_3$, 130 ºC, overnight, 78%; b) sat. NH_3/EtOH, rt, 4 d, 44%; c) TMSI, CH_2Cl_2, rt, 9 h, 63%; d) tributyl (1-ethoxyvinyl) stannane, $Pd(PhP_3)_2Cl_2$, THF, 80 ºC, 24 h, 54%; e) Cat. NaOMe, CH_3OH, rt, 12 h, 79%.

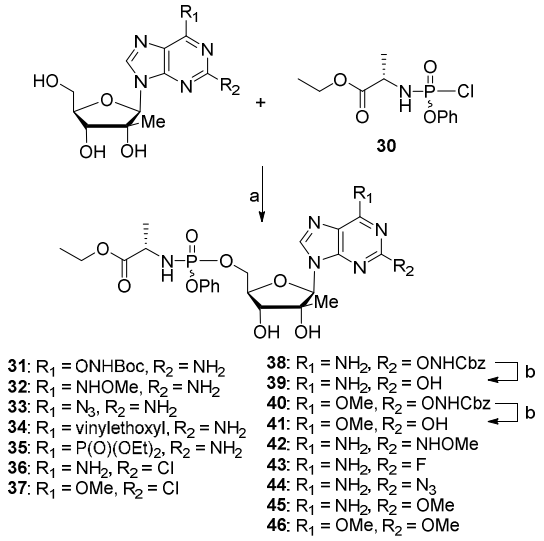
With the knowledge that the 2-position of adenosine is not involved in the hydrogen bonding of base pairing while the 2-amino group of guanosine is, we next turned our attention to the synthesis of 2-modified purine nucleosides. 2-Hydroxylamino-, 2-fluoro-, 2-methoxy- and 2-azido-purine nucleosides were targeted as they potentially offer a variety of steric, electronic, and hydrogen bonding interactions which may enhance recognition by HCV NS5B polymerase in their 5'-triphospate forms. The key tribenzoylated 2,6-dichloropurine-2'-Me nucleoside **16** was prepared in a manner similar to **6** (Scheme 1) with 2,6-dichloropurine, TMSOTf and DBU. Treatment of the 2,6-dichloropurine nucleoside **16** with a saturated solution of ammonia in methanol lead to concomitant formation of 6-amino compound **17** and 6-methoxy compound **18** (Scheme 3). This reaction was preformed at room temperature to avoid displacement of the 2-chloro group and appeared to proceed predominantly by first formation of the 6-methoxy compound which is then converted to the 6-amino via ammonia displacement. The benzoyl group deprotection occurred quite rapidly and was followed by a slow partial conversion of the 6-methoxy nucleoside **18** to the 6-amino nucleoside **17** over the three-day reaction. With **17** and **18** in hand, our initial goal was to prepare the corresponding 2-O-NH_2 derivatives. While the reaction of **17** and **18** with *N*-Cbz hydroxylamine worked well, the subsequent palladium catalyzed hydrogenation of the CBz group generated only 2-hydroxy purine products **22** and **25**. On the other hand, 2,6-dimethoxypurine **23** and 6-amino-2-methoxy purine derivative **26** were successfully synthesized by simple treatment of compounds **16** and **17** with sodium methoxide. 2-azidopurine **20** was prepared in two steps by the reaction of 2-chloro purine derivative **17** with hydrazine hydrate followed by treatment of the resulting hydrazine compound **19** with sodium nitrite in acetic acid.[7,8] As expected, ^{1}H-NMR showed that compound **20** exists as an equilibrium of azido (**20a**) and 1-*N* tetrazole (**20b**) tautomeric forms.[9,10,11] It is worth noting that a two steps sequence was used because direct treatment of **17** with NaN_3 failed to provide 2-azido nucleoside **20**.



Scheme 3. Reagents and conditions: (a) 2, 6-dichloropurine, DBU, TMSOTf, -40 to 80 °C, 4 h, 80%; (b) sat. NH_3/MeOH, rt, 3 days, **17**: 46%, **18**: 21%; (c) K_2CO_3, MeOH, rt, 24 h, 87%; (d) for **24**: HONHCbz, NaH, THF, 50 °C, 24 h, 81%; for **26**: MeONa, MeOH, 65 °C, 24 h, 92%; (e) HONHCbz, NaH, THF, 50 °C, 24 h, 78%; (f) Pd/C, H_2, MeOH, rt, 15 h; for 22, 83%; for 25, 97%; (g) NH_2NH_2, $MeOCH_2CH_2OH$, 110 °C, 5 h, 40%; (h) $NaNO_2$, HOAc, 1 h, 77%; (i) 2-fluoroadenine, DBU, TMSOTf, -40 to 65 °C, 5 h; (j) sat. NH_3/MeOH, rt, 2 d, 72% for two steps; (k) $MeONH_2$, Et_3N, EtOH/H_2O, 110 °C, 15 h, 66%.

Vorbruggen type coupling between tetrabenzoylated sugar 4 and 2-fluoro-6-aminopurine in presence of TMSOTf and DBU afforded compound **27** in 80% (Scheme 3). Deprotection of the three benzyl groups using a saturated solution of ammonia in methanol gave access to 2-fluoro-6-aminopurine nucleoside **28** which was subsequently treated with *O*-methyl hydroxylamine to give the desired 2-*N*-methoxylamine purine derivative **29**.

It has been now well established that nucleosides analogs are often times unable to be intracellularly metabolized to their corresponding nucleoside triphosphates. Therefore, in order to overcome the often rate-limiting first phosphorylation step and improve the antiviral activity of our nucleosides analogs, we prepared their corresponding monophosphate McGuigan type prodrugs.[12] The synthesis of phosphoramidates **31**-**46** was performed following the Uchiyama procedure by reacting the nucleosides **10**-**12**, **14**, **15**, **17**, **18**, **20**, **22**, **23**, **25**, **26**, **28**, **29** with chlorophosphoramidate **30**[13] in the presence of *N*-methylimidazole (Scheme 4).[14,15] It is noteworthy that the use of acetonitrile as a co-solvent improved the solubility of certain nucleosides leading to better overall yields. Attempts to prepare the 2-aminooxypurine nucleoside prodrugs by Cbz removal of compound **38** and **40** were not successful and instead afforded the isoguanosine derivatives **39** and **41**.



Scheme 4. Reagents and conditions: a) NMI, THF/CH_3CN, rt, 2-3 h; b) Pd/C, H_2, MeOH, 15 h.

The nucleosides and phosphoramidate prodrugs were evaluated for inhibition of HCV RNA replication in Huh7 cells using a subgenomic HCV replicon system.[16] Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and cellular ribosomal RNA (rRNA).[17] In addition cytotoxicity was determined in primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid CEM, and African Green monkey Vero cells (Table 1).[18,19] In an initial set of compounds, unusual 6-modifications such as

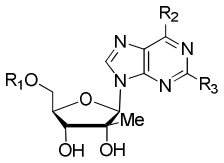
introduction of a phosphonate ester or an ethoxy vinyl group were counterproductive and lead to inactive nucleosides and monophosphate prodrugs **14**, **15**, **34** and **35**. Similarly, purines derivatives **25** (2,6-diMeO), **22** (2-OH, 6-NH_2), **23** (2-OH, 6-MeO), **29** (2-NHOMe, 6-NH_2) and their corresponding phosphoramidate prodrugs **39**, **41**, **46** and **42** showed to be inactive against HCV up to 10 μM. In contrast, 6-substituted purines derivatives **7** (-ONHBoc), **8** (-ONHCbz) and **11** (-NHOH) displayed EC50 values against HCV of 0.9, 2.4, and 0.3 μM, respectively without apparent toxicity up to 10 μM in Huh7 cells and up to 100 μM in human PBM, CEM and Vero cells. Preparation of **31**, the phosphoramidate prodrug of 6-NHBoc substituted compound **7**, even decreased the EC_{90} of **7** by a factor of 10 (8.4 compared to 0.9 μM for **7**). However, it has been well established that 6-modified nucleosides can be substrate of deaminases[20] and therefore we studied the fate of such compounds intracellularly. Thus, compound **7** and its prodrug **31** were incubated in Huh7 cells at 50 μM for 4 h at 37 °C. The cells were washed with phosphate-buffered saline and the intracellular metabolites were extracted with 70% ice-cold methanol in water and identified by LC-MS/MS. In this particular case, the only NTP metabolite observed *in vitro* was a 2'-β-*C*-methylguanosine-5'-triphosphate, a known inhibitor of HCV NS5B polymerase.[3]

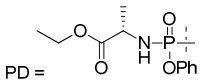
Interestingly, compounds **10** (2-NH_2 6-NHOMe), **12** (2-NH_2, 6-N_3), **17** (2-Cl, 6-NH_2), **18** (2-Cl, 6-OMe), **24** (2-ONHCbz, 6-NH_2), **26** (2-OMe, 6-NH_2), **21** (2-ONHCBz, 6-OMe), **20** (2-N_3, 6-NH_2) and **28** (2-F, 6-NH_2) did not show any activity against HCV in the replicon system when tested up to 10 μM while their corresponding phosphoramidate prodrugs **32**, **33**, **36**, **37**, **38**, **45**, **40**, **44** and **43** revealed their potency and exhibited median effective concentrations (EC_{50}) between 0.3 and 6.3 μM (Table 1). As with compounds **7** and **31**, a cellular pharmacology study of some of the most potent inhibitors **45** (2-OMe, 6-NH_2, 1 μM), **44** (2-N_3, 6-NH_2, 2.3 μM) and **43** (2-F, 6-NH_2, 2.7 μM) was undertaken and intracellular levels of nucleoside-MP, -DP and –TP formed in Huh7 cells were quantified. All phosphoramidates derivatives **45**, **44** and **43** produced high levels of their corresponding NTP along with considerable amounts of NMP and NDP derivatives. No other NTP was observed which implies that **45**, **44** and **43** NTPs are responsible for the anti-HCV activity observed in the replicon system and that 2-position modification of purines, unlike 6-modification, are quite stable *in vitro*.

As a first step toward understanding the difference of antiviral activity observed between inactive nucleoside **28** and its phosphoramidate prodrug **43** (EC_{50} = 2.7 μM), a comparative pharmacology study in Huh7 cells using the LC-MS/MS method described above was performed. While both compounds were found to deliver **28**-TP, prodrug **43** produces ~20 times more 5'-triphosphate than **28**. This discrepancy directly correlates with the anti-HCV activity of these two compounds. Interestingly, we also noted that levels of metabolites produced by **23** were very low in Huh7 cells, even for the nucleoside itself. This seems to imply that, not only can the monophosphate prodrug **43** bypass the first phosphorylation step, but it also allows for a better intracellular penetration of the compound.

To further characterize compound **43**, we decided to study the *in vitro* incorporation of **28**-TP (active metabolite of phosphoramidate **43**) by HCV NS5B polymerase (See Figure 2 in the supporting information section). RNA synthesis by HCV NS5B polymerase was monitored in the presence of increasing concentrations of **28**-TP up to 100 μM. Incorporation of **28**-TP was marked by the appearance of pausing sites opposite uridine residues at positions +11, +13 and +15 of the 20mer RNA template, confirming that **28**-TP behaves as an A analog. Finally, as expected, increased incorporation of **28**-TP correlated with inhibition of full-length 20-mer RNA product formation (K_i = 32 ± 0.07 μM). Finally, since off-target effects can be an issue with nucleoside analogs, we assessed the selectivity of our compound by testing **28**-TP against host RNA polymerase II and human mitochondrial RNA polymerase (POLRMT). At concentrations up to 100 μM **28**-TP did not inhibit host RNA polymerase II (while α-amanitin, used as positive control, had an IC_{50} of 2.5 ± 1.7 nM) and was not significantly incorporated by POLRMT (11% incorporation as normalized to ATP).[21]

Table 1. *In vitro* anti-HCV activity and cytotoxicity of nucleosides and phosphoramidate prodrugs.[a]





Cmpd	R_1	R_2	R_3	Anti-HCV activity (μM)[a]		rRNA (μM)	Cytotoxicity, CC_{50} (μM)		
				EC_{50}	EC_{90}	CC_{50}[b]	PBM	CEM	Vero
7	H	ONHBoc	NH_2	0.9	8.4	> 10	> 100	> 100	> 100
31	PD	ONHBoc	NH_2	0.3	0.9	> 10	> 100	39 ± 4.1	> 100
8	H	ONHCbz	NH_2	2.4	8.0	> 10	88 ± 4.4	> 100	> 100
10	H	NHOMe	NH_2	> 10	> 10	> 10	> 100	> 100	> 100
32	PD	NHOMe	NH_2	0.3	1.0	> 10	> 100	32 ± 17	> 100
11	H	NHOH	NH_2	1.9	5.5	> 10	> 100	> 100	> 100
12	H	N_3	NH_2	> 10	> 10	> 10	> 100	> 100	> 100
33	PD	N_3	NH_2	2.4	7.7	> 10	> 100	> 100	> 100
14	H		NH_2	> 10	> 10	> 10	> 100	> 100	> 100
34	PD		NH_2	> 10	> 10	> 10	> 100	> 100	> 100
15	H	P(O)(OEt)	NH_2	> 10	> 10	> 10	> 100	> 100	> 100
35	PD	P(O)(OEt)	NH_2	> 10	> 10	> 10	> 100	> 100	> 100
17	H	NH_2	Cl	> 10	> 10	> 10	> 100	> 100	> 100
36	PD	NH_2	Cl	1.7	5.3	> 10	> 100	> 100	> 100
18	H	OMe	Cl	> 10	> 10	> 10	> 100	> 100	> 100
37	PD	OMe	Cl	6.3	9.9	> 10	> 100	> 100	> 100
23	H	OMe	OMe	> 10	> 10	> 10	> 100	> 100	> 100
46	PD	OMe	OMe	> 10	> 10	> 10	> 100	> 100	> 100
24	H	NH_2	ONHCbz	> 10	> 10	> 10	> 100	> 100	> 100
38	PD	NH_2	ONHCbz	4.8	10	> 33	> 100	> 100	> 100
26	H	NH_2	OMe	> 10	> 10	> 10	> 100	> 100	> 100
45	PD	NH_2	OMe	1.0	2.8	> 10	> 100	> 100	> 100
25	H	NH_2	OH	> 10	> 10	> 10	> 100	> 100	> 100
39	PD	NH_2	OH	> 10	> 10	> 10	> 100	> 100	> 100
21	H	OMe	ONHCbz	> 10	> 10	> 10	> 100	> 100	> 100
40	PD	OMe	ONHCbz	4.1	9.2	> 33	> 100	24 ± 5.8	> 100
22	H	OMe	OH	> 10	> 10	> 10	> 100	> 100	> 100
41	PD	OMe	OH	> 10	> 10	> 10	> 100	> 100	> 100
20	H	NH_2	N_3	> 10	> 10	> 10	> 100	> 100	> 100
44	PD	NH_2	N_3	2.3	3.0	> 10	> 100	> 100	> 100
28	H	NH_2	F	> 10	> 10	> 10	> 100	> 100	> 100
43	PD	NH_2	F	2.7	8.3	> 10	> 100	> 100	> 100
29	H	NH_2	NHOMe	> 10	> 10	> 10	> 100	> 100	> 100
42	PD	NH_2	NHOMe	> 10	> 10	> 10	> 100	> 100	18 ± 3.0
IDX-184	NA	OH	NH_2	0.3	0.9	> 100	> 100	> 100	> 100
BMS-986094	NA	OMe	NH_2	0.02	0.04	0.8	4.5 ± 3.0	8.0 ± 6.3	14 ± 3.9

[a] The HCV replicon data is run in triplicate and the inter well variability must be less then +/- 0.5 delta Ct; NA: Not Available

Despite the fact that base modifications are often not accepted by polymerases and can lead to undesired toxicity, we identified several substitutions to the purine base that allow their NTP to be recognized by HCV polymerase. Thus, we discovered phosphoramidates **32**, **33**, **36**, **37**, **38**, **45**, **40**, **44** and **43** which displayed EC_{50} values in the low micromolar range against HCV without apparent toxicity in Huh7, PBM, CEM and Vero cells up to 100 μM. Phosphoramidate **43** (2-F, 6-NH_2), one of the most potent compounds, was further characterized. Interestingly, we found that **43,** unlike its corresponding nucleoside **28**, produced high levels of **28**-TP in Huh7 cells. The **28**-TP was shown to be a substrate of HCV NS5B polymerase and was incorporated as an adenosine analog. Further preclinical profiling of compound **43** and exploration of its prodrug portion is in progress. We thus envisage comparing this compound or a related prodrug to Sofosbuvir, INX-189 and IDX-184 in different cellular and animal assays and evaluate the potential therapeutic benefit of such 2-position modification in purine nucleosides and nucleotides.

ASSOCIATED CONTENT

Supporting Information

Biological assays and complete experimental section with full characterization of all new compounds is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*TEL: +1-404-727-1414. Email: rschina@emory.edu

.

Funding Sources

This work was supported in part by NIH grant 5P30-AI-50409 (CFAR) and by the Department of Veterans Affairs.

ACKNOWLEDGMENT

(Dr. Schinazi is the Chairman and a major shareholder of CoCrystal Pharma, Inc. Emory received no funding from CoCrystal Pharma, Inc. to perform this work and vice versa.

ABBREVIATIONS

RNA, ribo nucleic acid; HCV, hepatitis C; FDA, Food and Drug Administration; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TMSOTf, trimethylsilyl trifluoromethanesulfonate; Boc, tert-Butyloxycarbonyl; CBz, benzyloxy carbamate; TFA, trifluoroacetic acid; TMSI, trimethylsilyl iodide; HOAc, acetic acid; NMI, 1-methylimidazole; LC, liquid chromatography; MS, mass spectrometry; NTP, nucleoside triphosphate; NMP, nucleoside monophosphate; NDP, nucleoside diphosphate; TP, triphosphate.

REFERENCES

[1] De Francesco, R.; Migliaccio, G. Challenges and successes in developing new therapies for hepatitis C. *Nature* **2005**, *436*, 953-960.

[2] Sheldon, J.; Barreiro, P.; Soriano, V. Novel protease and polymerase inhibitors for the treatment of hepatitis C virus infection. *Expert Opin. Investig. Drugs* **2007**, *16*, 1171-1181.

[3] Coats S.J.; Garnier-Amblard E. C.; Amblard F.; Ehteshami M.; Amiralaei S.; Zhang H.; Zhou L.; Boucle S. R.; Lu X.; Bondada L.; Shelton J. R.; Li H.; Liu P.; Li C.; Cho J. H.; Chavre S. N.; Zhou S.; Mathew J.; Schinazi R. F. Chutes and ladders in hepatitis C nucleoside drug development. *Antiviral Res.* **2014**, *102*, 119-147.

[4] 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.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. Discovery of a β-D-2'-deoxy-2'-α-fluoro-2'-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* **2010**, *53*, 7202-7218.

[5] For a review of NS5B anti-HCV agents, see: Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase. *J. Med. Chem.* **2012**, *55*, 2481-2531.

[6] Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. Structure-activity relationship of purine ribonucleosides for inhibition of hepatitis C virus RNA-dependent RNA polymerase *J. Med. Chem*. **2004**, *47*, 2283-2294.

[7] Xu, Y.; Ikeda, R.; Sugiyama, H. 8-Methylguanosine: a powerful Z-DNA stabilizer. *J. Am. Chem. Soc*. **2003**, *125*, 13519-13524.

[8] Schaeffer, H. J. US Pat. **1980**, 19804199574, 18 pp

[9] Lioux, T.; Gosselin, G.; Mathé, C. Azido/tetrazole tautomerism in 2-azidoadenine β-D-pentofuranonucleoside derivatives. *Eur. J. Org. Chem*. **2003**, *20*, 3997-4002.

[10] Sodum, R. S.; Fiala, E. S. N2-amination of guanine to 2-hydrazinohypoxanthine, a novel in vivo nucleic acid modification produced by the hepatocarcinogen 2-nitropropane. *Chem. Res. Toxicol*. **1998**, *11*, 1453-1459.

[11] Elzein, E.; Kalla, R.; Li, X.-F.; Perry, T.; Marquart, T.; Micklatcher, M.; Li, Y.; Wu, Y.-Z.; Zeng, D.; Zablocki, J. N6-Cycloalkyl-2-substituted adenosine derivatives as selective, high affinity adenosine A1 receptor agonists. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 161-166.

[12] Pradere, U.; Garnier-Amblard, E. C.; Coats, S. J.; Amblard, F.; Schinazi, R. F. Synthesis of nucleoside phosphate and phosphonate prodrugs, *Chem. Rev.*, **2014**, *114,* 9154-9218.

[13] McGuigan, C.; Ranjith, J.; Pathirana, N.; Balzarini, J.; De Clercq, E. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem*. **1993**, *36*, 1048-1052.

[14] M. Uchiyama, Y. Aso, R. Noyori, Y. Hayakawa, O-selective phosphorylation of nucleosides without N-protection. *J. Org. Chem*. **1993**, *58*, 373-379.

[15] McGuigan, C.; Hassan-Abdallah, A.; Srinivasan, S.; Wang, Y.; Siddiqui, A.; Daluge, S. M.; Gudmundsson, K. S.; Zhou, H.; McLean, E. W.; Peckham, J. P.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Balzarini, J. Application of phosphoramidate ProTide technology significantly improves antiviral potency of carbocyclic adenosine derivatives. *J. Med. Chem*. **2006**, *49*, 7215–7226.

[16] Rondla, R.; Coats, S. J.; McBrayer, T. R.; Grier, J.; Johns, M.; Tharnish, P. M.; Whitaker, T.; Zhou, L.-H.; Schinazi, R. F. Anti-hepatitis C virus activity of novel beta-D-2'-C-methyl-4'-azido pyrimidine nucleoside phosphoramidate prodrugs. *Antivir. Chem. Chemother*. **2009**, *20*, 99-106.

[17] Stuyver, L. J.; Whitaker, T.; McBrayer, T. R.; Hernandez-Santiago, B. I.; Lostia, S.; Tharnish, P. M.; Ramesh, M.; Chu, C. K.; Jordan, R.; Shi, J.;

Rachakonda, S.; Watanabe, K. A.; Otto, M. J.; Schinazi, R. F. Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture. *Antimicrob. Agents Chemother.* **2003**, *47*, 244-254.

[18] Schinazi, R. F.; Sommadossi, J. P.; Saalmann, V.; Cannon, D. L.; Xie, M.-W.; Hart, G. C.; Smith, G. A.; Hahn, E. F. Activities of 3'-azido-3'-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061-1067.

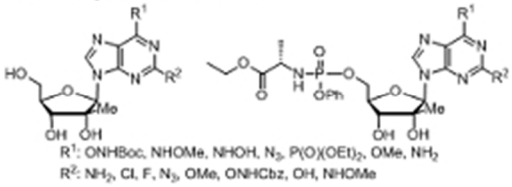
[19] Stuyver, L. J.; Lostia, S.; Adams, M.; Mathew, J.; Pai, B. S.; Grier, J.; Tharnish, P.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C. K.; Otto, M. J.; Schinazi, R. F. Antiviral activities and cellular toxicities of modified 2',3'-dideoxy-2',3'-didehydrocytidine analogues. *Antimicrob. Agents Chemother.* **2002**, *46*, 3854-3860.

[20] Murakami, E.; Bao, H.; Mosley, R. T.; Du, J.; Sofia, M. J.; Furman, P. A. Adenosine deaminase-like protein 1 (ADAL1): Characterization and substrate specificity in the hydrolysis of N^6- or O^6-substituted purine or 2-aminopurine nucleoside monophosphates, *J. Med. Chem.* **2011**, *54*, 5902-5914.

[21] Arnold, J. J.; Sharma; S. D.; Feng, J. Y.; Ray, A. S.; Smidansky, E. D.; Kireeva, M. L.; Cho, A.; Perry, J.; Vela, J. E.; Park, Y.; Xu, Y.; Tian, Y.; Babusis, D.; Barauskus, O.; Peterson, B. R.; Gnatt, A.; Kashlev, M.; Zhong, W.; Cameron, C. E. Sensitivity of mitochondrial transcription and resistance of RNA polymerase II dependent nuclear transcription to antiviral ribonucleosides. *PLoS Pathog.* **2012**; *8*, e1003030

Synthesis and Evaluation of 2,6-Modified Purine 2'-C-Methyl Ribonucleosides as Inhibitors of HCV Replication

Longhu Zhou, Hongwang Zhang, Sijia Tao, Maryam Ehteshami, Jong Hyun Cho, Tamara R. McBrayer, Philip Tharnish, Tony Whitaker, Franck Amblard, Steven J. Coats, Raymond F. Schinazi*



84x39mm (120 x 120 DPI)