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6-Hydrazinopurine 2′-methyl ribonucleosides and their 5′-monophosphate prodrugs as potent hepatitis C virus inhibitors

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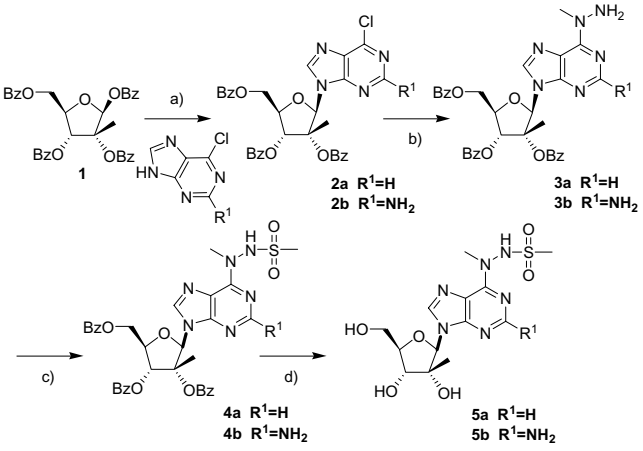
Abstract—A series of 6-hydrazinopurine 2′-methyl ribonucleosides was synthesized and tested for its inhibitory activity against the hepatitis C virus (HCV). The lack of antiviral activity of these nucleosides was associated with a poor affinity for adenosine kinase, which prompted us to synthesize several of their 5′-monophosphate prodrugs. Some of these prodrugs exhibited more than 1000-fold improvement in anti-HCV activity when compared to their parent nucleosides (EC_{50} of 24 nM vs 92 μM for the parent).

Hepatitis C virus (HCV) is a virus that infects approximately 3.5% of the world's population (170 million people). It is primarily a chronic disease that can lead to cirrhosis and hepatocellular carcinoma over time. The current standard of care is a combination of pegylated interferon alpha and ribavirin, but this regimen suffers from various side effects (anemia, CNS toxicity) and shows a limited efficacy in patients with genotype 1 infection (less than 50% of treated patients achieve a sustained viral response).[1] These limitations encouraged our team to search for a more effective nucleoside analogue that would target the HCV polymerase NS5B.

Our group recently published[2] several series of 6-hydrazinopurine 2′-methyl nucleosides, but these compounds suffered from poor chemical stability and/or poor selectivity index. We are now presenting our work on a new series of methylsulfonyl-substituted hydrazine compounds that benefit from much improved chemical stability. Compounds **5a**,**b** (Scheme 1) were obtained by condensation of 1,2,3,4-tetra-*O*-benzoyl-2-methyl-ribofuranose (**1**) with 6-chloropurine or 2-amino-6-chloropurine in the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The resulting protected nucleosides **2a** and **2b** were reacted with 2-methyl hydrazine, followed by methanesulfonyl chloride (MsCl) to obtain **4a** and **4b**. The benzoyl groups were removed with methanolic ammonia to afford **5a** and **5b** in good yields.[3] The HCV replicon activity (Table 1) of these purine nucleoside analogues **5a** and **5b** was rather modest (EC_{50} of 300 and 92 μM, respectively) and most probably explained by the fact that neither nucleoside was a substrate for adenosine kinase, and therefore was probably not converted into the 5′-monophosphate.



Scheme 1. Reagents and conditions: (a) TMSOTf, DBU, THF, 18 h, rt; (b) methyl hydrazine, TEA, THF, 8 h, rt; (c) MsCl, TEA, THF, 8 h rt; (d) NH_3, methanol, 18 h, rt.

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Table 1. Anti-HCV activity of compounds **5a**–**42b**

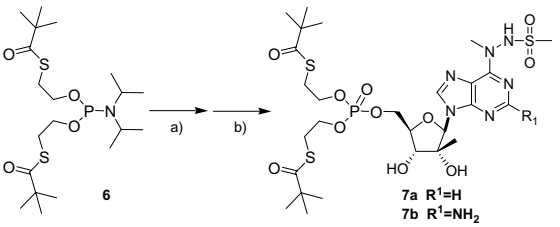
Compound	R^1	R^2	R^3	R^4	R^5	EC_{50}, μM[a]
5a	H	na	na	na	na	300
5b	NH_2	na	na	na	na	92
7a	H	na	na	na	na	0.060
7b	NH_2	na	na	na	na	0.024
34a	H	Me	H	Me	H	26
34b	NH_2	Me	H	Me	H	1.2
35a	H	Me	H	Me	Cl	2.6
35b	NH_2	Me	H	Me	Cl	0.80
36a	H	H	H	Me	H	220
36b	NH_2	H	H	Me	H	22
37a	H	H	H	Me	Cl	39
37b	NH_2	H	H	Me	Cl	8.9
38a	H	Me	H	Bn	H	2.9
38b	NH_2	Me	H	Bn	H	2.2
39a	H	Me	H	Bn	Cl	0.68
39b	NH_2	Me	H	Bn	Cl	0.11
40a	H	Me	H	*i*Pr	Cl	2.5
40b	NH_2	Me	H	*i*Pr	Cl	0.27
41a	H	Me	Me	Me	Cl	1.4
41b	NH_2	Me	Me	Me	Cl	0.26
42b	NH_2	Me	H	*c*Pent	Cl	0.15

[a] Values are means of multiple experiments. na, not applicable.

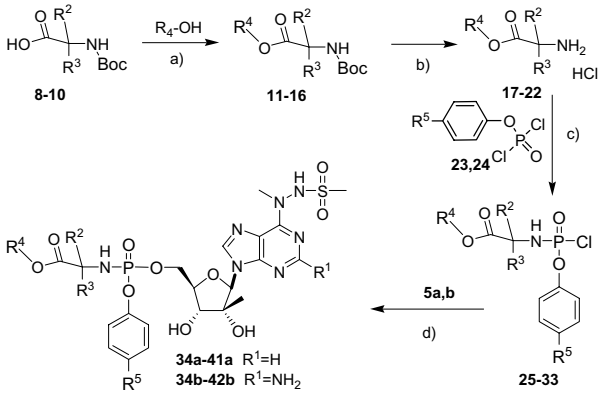
In order to bypass this first enzymatic phosphorylation step, we elected to synthesize the bis(*t*BuSATE) monophosphate prodrugs of these nucleosides following a well-established literature procedure.[4] The phosphoramidite **6** was synthesized and condensed with **5a** and **5b** in the presence of tetrazole in dimethylformamide, followed by oxidation of the resulting phosphite triesters to afford the phosphotriesters **7a** and **7b** (Scheme 2). These two prodrugs were tested for their antiviral activity in the replicon assay (Table 1). Their EC_{50}s were 60 and 24 nM, respectively, which represents a 3 log improvement in antiviral efficacy when compared to their parent nucleosides **5a** and **5b**. However, these SATE prodrugs showed poor stability in human plasma with half-lives of just a few minutes. This characteristic was also observed with similar prodrug moieties attached to cyclic monophosphates.[5] This limitation led us to turn our focus toward a different prodrug approach involving phosphoramidate esters. These prodrugs are also well documented in the literature[6] and have demonstrated some good plasma stability in various species.

The aminoacids **8**–**10** were esterified, then their Boc protecting group was removed before condensation with phosphodichlorides **23** and **24** which yielded the chlorophosphoramidates **25**–**33**. These amidates were condensed with **5a** and **5b** to afford prodrugs **34a**,**b**–**42a**,**b** in decent yields Scheme 3. Each compound was a diastereoisomeric mixture that we did not attempt to separate. These phosphoramidate prodrugs were tested for their anti-HCV activity, and most of them showed submicromolar activity without any cytotoxicity. Several trends were noticeable: the first one was that, as previously noticed with the SATE prodrugs, the 2-amino nucleosides were more active than their 2-hydrogen analogues. This result was interesting because our nucleosides are bearing a heterocyclic base that is a hybrid between a guanine and an adenine. The other observation was that the prodrugs with a methyl group at the R^2 position were generally a lot more potent than those with a hydrogen at the same position, as seen with the pairs **34a**–**36a**, **34b**–**36b** and **35b**–**37b**. Adding a second methyl at R^3 neither improved nor diminished the activity further, as shown with compounds **35a**–**41a** and **35b**–**41b**. The most active compounds (**39b** and **42b**) were 4- to 6-fold less potent than their SATE prodrug counterparts (**7b**), but at this point, we were more interested in pharmacokinetic properties than optimized activities. It is interesting to note that the cyclic monophosphate SATE prodrugs of the same nucleosides exhibited very similar antiviral activities.[5]

Selected compounds were tested for their in vitro stability in multiple assays, including human plasma, human simulated gastric fluid (SGF), and human simulated intestinal fluid (SIF). All were stable in human plasma and SGF for up to 1 h, but the SIF stability proved to be more problematic. As described in the literature,[7] the degradation products observed resulted from the hydrolysis of the amino acid ester followed by a hydrolysis of the phenol group. As seen in Table 2, compounds with R^2 and R^3 as hydrogens performed poorly. This was not an issue for us because they were also poorly active (**36a**,**b**–**37a**,**b**). However, the most active compounds bearing a methyl as R^2 and a hydrogen as R^3 also showed poor stability, especially when R^4 was a benzyl (**38a**,**b**–**39a**,**b**). Interestingly, replacing the benzyl by a more bulky cyclopentyl (**42b**) did not help the situation. However, replacing the R^4 benzyl with an iso-



Scheme 2. Reagents and conditions: (a) **5a** or **5b**, tetrazole, DMF, rt, 2 h; (b) *t*BuOOH, DMF, rt, 3 h.



Scheme 3. Reagents and conditions: (a) TEA, DMAP, isopropenyl chloroformate, DCM, 0 °C to rt, 1–20 h; (b) TFA, DCM, rt, 2 h; (c) TEA, DCM, −10 °C, 2 h; (d) 1-methylimidazole, DCM, rt, 20 h.

Table 2. SIF stability (1 mg/mL, 37 °C)

Compound	% Remain, 30 min	% Remain, 60 min
36a	0	0
36b	0	0
37a	0	0
37b	0	0
38a	0	0
38b	0	0
39a	0	0
39b	0	0
40a	67	48
40b	61	37
41a	100	100
41b	100	100
42b	0	0

propyl group clearly improved the stability of these molecules as seen with compounds **40a**,**b**. The best results were obtained when the hydrogen in R^3 was replaced by a methyl (compounds **41a**,**b**). In these cases, we did not observe any degradation for up to 1 h. Further tests showed no evidence of degradation at the 2 h timepoint.

In conclusion, we synthesized a series of 5′-monophosphate prodrugs of two novel nucleoside analogues. These prodrugs exhibited potent anti-HCV activity with EC_{50}s ranging from 24 nM to several micromolar in our replicon assay. We also tested the in vitro stability of these prodrugs under various conditions. Several compounds were potent and stable under these conditions. In particular, compounds **41a** and **41b** displayed potent EC_{50} values (260 and 150 nM, respectively) and were 100% stable after 1 h incubation in SIF. These favorable characteristics warrant studying the pharmacokinetic properties of these compounds in an in vivo system. The result of these studies will be reported in due course.

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