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Novel Hepatitis C virus replicon inhibitors: Synthesis and structure–activity relationships of fused pyrimidine derivatives

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ABSTRACT

The synthesis of several pyrido[2,3-*d*]pyrimidine and pyrimido[4,5-*d*]pyrimidine analogs is described with one such analog possessing subnanomolar potency in both genotype 1a and 1b cell culture HCV replicon assays.

Hepatitis C virus (HCV) is a (+)-strand RNA virus of the *Flaviviridae* family that was first identified in 1989.[1] HCV is a common pathogen that can lead to cirrhosis, hepatocellular carcinoma (HCC) and liver failure. It is estimated that 170 million people were infected worldwide in the year 2000, and that the virus is responsible for at least 10,000 deaths annually in the United States alone.[2] HCV has six major genotype classes, with genotypes 1 and 2 being the most prevalent in the United States, Europe, and Japan.[3] Within genotype 1, the majority of patients are infected with subgenotypes 1a or 1b. Recently two new protease inhibitor drugs, boceprevir and telaprevir, were approved by the FDA. However these drugs still must be used in combination with the standard of care which imparts several drug-related toxicities. Thus there is still an urgent need for new HCV drugs with diverse modes of action.[4]

Our research team has been pursuing inhibition of the Hepatitis C virus by targeting several non-structural viral enzymes.[5] We have also initiated a broad screening program towards identifying novel leads that have antiviral potency in a HCV subgenomic replicon assay.[6] One such lead was a substituted naphthyridine derivative (**1**), shown in Table 1. We were pleased with the genotype 1b replicon potency of (EC_{50} 18 nM) as a starting point and hoped to improve upon the EC_{50} for genotype 1a, which was 100-fold higher than in 1b. Thus we initially explored the effect of replacing each of the nitrogen atoms independently in the naphthyridine ring system with carbon atoms to generate quinoline derivatives **2** and **3** (Scheme 1). The data indicate that both nitrogens are required for obtaining sub micromolar potency against the 1b replicon strain in this series. In an effort to obtain sub micromolar potency against both 1a and 1b virus strains and to decrease the cytotoxicity associated with the lead structures (MTT values), we embarked upon an effort to explore further modifications to the fused ring system.

We were able to construct a series of substituted pyrido [2,3-*d*]pyrimidine analogs by employing a short synthetic sequence (Scheme 2).[7] A variety of methyl ketones **14** were treated with sodium metal and ethyl formate to generate the sodium salt of alkyl substituted hydroxyl enal compounds **15** which were cyclized with 2-cyanoacetamide to form the corresponding substituted oxopyridines **16**. Conversion to the corresponding bromopyridines **17** and subsequent reaction with ammonia under high pressure generated the aminopyridines **18**, of which the amino group could be converted to the dimethylamidine functionality. Reaction of these substituted pyrido dimethylamidines **19** with the substituted phenylsulfanyl anilines **5–10** from Scheme 1 in the presence of hot acetic acid generated a wide variety of the desired pyrido[2,3-*d*]pyrimidines **20–47**.[8] The data from Table 2 show that when the methyl substituted pyrido[2,3-*d*]pyrimidine **20** was compared to napthyridine analog **1**, analog **20** was less potent but displayed somewhat lower toxicity in the assay. Within the pyrido[2,3-*d*]pyrimidine series we also generated several analogs that had substituents on the para position of the phenyl thio ring (R^2 group in Scheme 2). In several cases, a hydroxyl group

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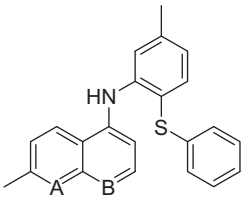
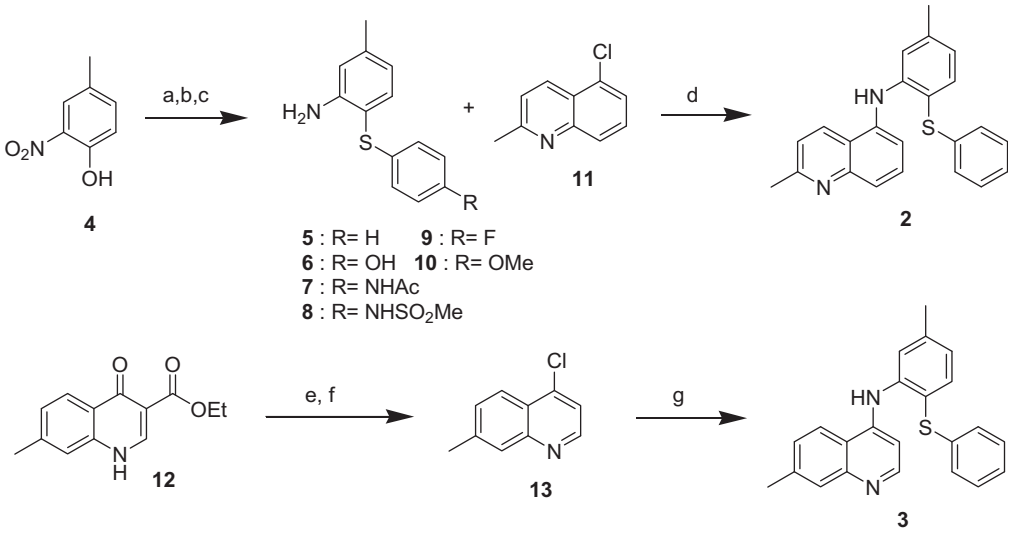
Table 1
Cell culture replicon potency of analogs **1**–**3**

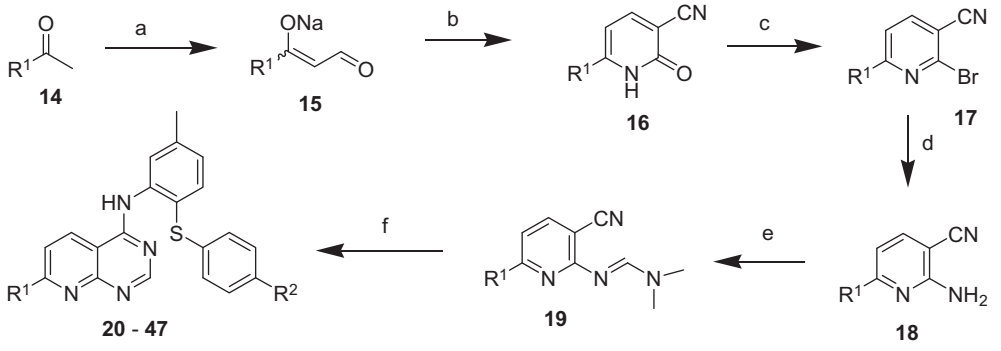


Compd	A	B	Replicon 1a EC_{50}[a,b] (μM)	Replicon 1b EC_{50}[a,b] (μM)	MTT[c] (μM) TD_{50}
1	N	N	1.8	0.018	8.6
2	N	C	15.7	9.9	15.9
3	C	N	—	1.8	2.6

[a] EC_{50} values in all tables are means of at least two independent determinations, with a maximum of threefold value variations.
[b] Assay run with 5% fetal calf serum.
[c] Cytotoxicity measured in Huh-7 cells.



Scheme 1. Reagents and conditions: (a) trifluoroacetic anhydride, Et_3N, CH_2Cl_2, 24 h, 90–100%; (b) Na_2CO_3, DMF, 80 °C, 18 h, 90–98%; (c) 10 equiv Fe, 10 equiv NH_4Cl, MeOH, water, reflux, 2 h, 79–100%; (d) compound **5**, NaOtBu, $Pd_2(dba)_3$, 2,8,9-tri-iso-butyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3] undecane, toluene, 100 °C, 18 h, 25%; (e) NaOH, water, 150 psi, 180 °C, 16 h, 86%; (f) excess $POCl_3$, 60 °C, 2.5 hrs, 88%; (g) compound **5**, EtOH, 80 °C, 24 h, 11%.

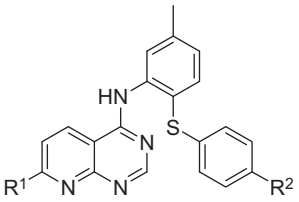


Scheme 2. Reagents and conditions: (a) ethyl formate, Na°, Et_2O, 0 °C to 25 °C, 18 h, 45–63%; (b) 2-cyanoacetamide, water, piperidine acetate, reflux, 2 h, 55–78%; (c) tetrabutylammonium bromide, P_2O_5, toluene, reflux, 5 h, 90–98%; (d) ammonia, EtOH, sealed tube, 130 °C, 20 h, 82–93%; (e) *N*,*N*-dimethylforamide dimethyl acetal, toluene, reflux, 3 h, 98–100%; (f) compound **5**–**10**, acetic acid solvent, reflux, 1.5 h, 10–54%.

at this position provided the greatest potency boost when all other parts of the analogs were the same.

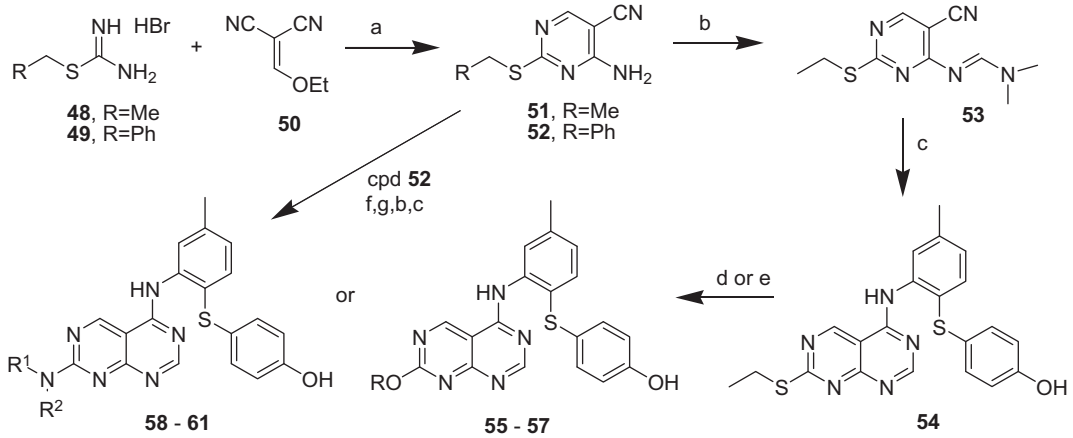
This was especially true in the cases of analogs **22** and **33**. Analog **33** was the most potent of the pyrido[2,3-*d*]pyrimidines prepared, having submicromolar potency against both subtype 1a and 1b replicons. Seemingly small modifications were made to this isopropyl analog **33**, such as changing the isopropyl group to a cyclopropyl (compound **39**) or isobutyl (compound **45**), which produced a potency loss of at least a 100-fold in the 1b replicon and roughly 50-fold in the 1a replicon. In the absence of knowledge

Table 2
Cell culture replicon potency of analogs **20–47**



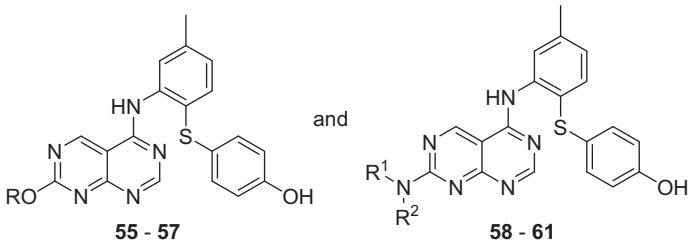
Compd	R^1	R^2	Replicon 1a EC_{50} (μM)	Replicon 1b EC_{50} (μM)	MTT (μM)
20	Methyl	H	13.3	3.84	22.2
21	Methyl	NHAc	>50	>50	>50
22	Methyl	OH	23.4	0.05	>100
23	Methyl	OMe	44.5	13.3	28.6
24	Methyl	F	10.5	3.0	6.1
25	Ethyl	H	18.9	3.4	22.7
26	Ethyl	NHAc	>12.5	6.9	>12.5
27	Ethyl	OH	62.2	10.0	78.0
28	*n*-Propyl	H	9.5	0.32	11.8
29	*n*-Propyl	NHAc	13.8	0.63	>25
30	*n*-Propyl	OH	7.1	0.25	31.1
31	*i*-Propyl	H	10.3	3.4	8.0
32	*i*-Propyl	NHAc	2.6	13.8	28.8
33	*i*-Propyl	OH	0.17	0.016	22.9
34	*i*-Propyl	OMe	11.4	1.7	8.8
35	*i*-Propyl	F	3.2	1.8	4.8
36	*i*-Propyl	$NHSO_2Me$	18.9	11.2	>50
37	Cyclopropyl	H	10.4	3.7	9.9
38	Cyclopropyl	NHAc	15.2	1.7	29.8
39	Cyclopropyl	OH	10.5	1.6	17.4
40	Cyclobutyl	H	6.1	1.6	3.6
41	Cyclobutyl	NHAc	15.8	3.7	49.2
42	Cyclobutyl	OH	11.3	3.1	31.8
43	*i*-Butyl	H	13.3	1.0	7.5
44	*i*-Butyl	NHAc	27.4	3.3	38.5
45	*i*-Butyl	OH	14.3	2.4	11.2
46	*tert*-Butyl	OH	4.6	0.47	7.1
47	Cyclohexyl	OH	2.7	0.88	>3.12

of the mechanism of inhibition and structural information on inhibitor binding, it is difficult to rationalize these results. Other difficult to interpret SAR data include the good potency of analogs **22** and **30** against replicon 1b, verses the lack of potency for analog **27**. Another large 1b potency difference is seen between analogs **21** and **29**. Some calculated properties of the analogs from Table 2 were obtained in order to help find some correlation between the observed SAR. The *c*Log*P* of these analogs varied between 5 and 8, and the PSA was in the 50–80 range. Unfortunately these values did not help us to find any reasonable correlations within the SAR. We also generated other analogs that contained a pyrimido[4,5-*d*]pyrimidine bicyclic ring system (Scheme 3).[7] The reaction of 2-alkyl-2-thiopseudourea hydrobromide **48** or **49** with (ethoxymethylene)malononitrile (**50**) generated an amino-(alkylsulfanyl)pyrimidine **51** or **52**. The amine in **51** was converted to an amidine functionality and cyclized with aniline **6** in hot acetic



Scheme 3. Reagents and conditions: (a) Hunig's base, ethanol, 4 h, 39–45%; (b) *N*,*N*-dimethylforamide dimethyl acetal, toluene, reflux, 3 h, 100%; (c) **6**, acetic acid solvent, reflux, 1.5 h, 15–45%; (d) excess sodium alkoxide, EtOH, heating, 32%; (e) excess morpholine or piperidine, microwave irradiation, 180 °C, 2 h, 38–65%; (f) mCPBA, CH_2Cl_2, 95%; (g) excess ethyl amine or methyl amine, EtOH, heating, 37–44%.

Table 3
Cell culture replicon potency of analogs **54**–**61**



55 - 57 **58 - 61**

Compd	R	R^1	R^2	Replicon 1a EC_{50} (μM)	Replicon 1b EC_{50} (μM)	MTT (μM)
54	see Scheme 3			4.8	0.01	46.9
55	Methyl	—	—	>8	>8	>8
56	Ethyl	—	—	7.3	12.0	29.7
57	Phenyl	—	—	5.3	3.5	17.1
58	—	Morpholine		14.1	7.3	>50
59	—	Piperidine		22.0	2.8	>50
60	—	Ethyl	H	47.6	30.0	>50
61	—	Methyl	Methyl	>50	>50	>50

acid to provide the ethylsulfanyl analog **54**. The ethylsulfanyl functionality could be displaced with other nucleophiles to provide the corresponding ether **55**–**57** and amine **58**, **59** analogs directly. Alternately, the acyclic amine analogs **60** and **61** were generated by first oxidizing the benzyl mercapto group in **52** to the corresponding benzyl sulfone followed by displacement of this group with the corresponding amines. Amidine formation and cyclization provided the desired amine analogs **60** and **61**. The ethylsulfanyl-pyrimidopyrimidine analog **54** displayed excellent 1b replicon potency (EC_{50} 10 nM), however it was much weaker against the 1a replicon (EC_{50} 4.8 μM, Table 3). Unfortunately the EC_{50}s of all of the ether **55**–**57** and amine **58**–**61** analogs were greater than 2 micromolar in both assays.

The most potent analog, iso-propyl pyrido[2,3-*d*]pyrimidine **33**, was retested in the replicon assay in the presence of 40% human serum to access how much potency loss would occur due to unproductive protein binding. The potency loss was 10-fold in the 1b replicon assay (16 to 170 nm). We also evaluated the pharmacokinetic properties of analog **33** after i.v. and oral administration in rats (2.5 mg/kg as a solution). Compound **33** displayed high clearance (1.58 L/h/kg) and a relatively short half-life (3.1 h). C_{max} (0.07 μM), plasma AUC (1.58 μg h/mL) and bioavailability (10%) after oral administration were low. In a future publication we will disclose our efforts related to improving upon this pharmacokinetic profile. Finally, based on resistance studies which will be published in the future,[9] we believe that the target of these HCV replicon inhibitors is the HCV NS5A protein which has been shown to be critical for viral replication.[10]

In summary, a series of substituted pyrido[2,3-*d*]pyrimidine and pyrimido[4,5-*d*]pyrimidine analogs were synthesized and assessed for inhibitory potency in genotypes 1a and 1b HCV replicon assays. Overall we observed that the SAR was very sensitive to seemingly small structural modifications and that the most potent analog displayed submicromolar potency in one cell culture assay containing 40% human serum.

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