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Synthesis and SAR of pyridothiazole substituted pyrimidine derived HCV replication inhibitors

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ABSTRACT

Introduction of a nitrogen atom into the benzene ring of a previously identified HCV replication (replicase) benzothiazole inhibitor **1**, resulted in the discovery of the more potent pyridothiazole analogues **3**. The potency and PK properties of the compounds were attenuated by the introductions of various functionalities at the R^1, R^2 or R^3 positions of the molecule (compound **3**). Inhibitors **38** and **44** displayed excellent potency, selectivity (GAPDH/MTS CC_{50}), PK parameters in all species studied, and cross genotype activity.

Hepatitis C (HCV) is the leading cause of liver cirrhosis, hepatocellular carcinoma, and chronic liver failure.[1] An estimated 180 million people worldwide are infected with HCV. The standard of care for HCV infection until recently provided low response rates (less than 50% response rates among patients infected with the most prevalent genotype 1 virus), genotype variability, viral resistance, and side effect profiles that resulted in low patient compliance.[2] The recent success of two NS3 protease inhibitors, boceprevir and telaprevir, has inspired further efforts to discover novel methods of treating the disease.[3] In addition to several other protease inhibitors undergoing clinical trials, novel compounds targeting the NS5b polymerase, the NS5a protein, and other cellular targets are in clinical studies.[4] Since the emergence of resistance is a major obstacle in HCV treatment, it is important to discover therapies with novel modes of action and unique resistance profiles.

Our early research efforts on small molecule HCV replication inhibitors led to a series of pyrimidine compounds.[5] The lead compound (Fig. 1, **1**) was a benzothiazole substituted pyrimidine derivative that had reasonable potency (replicon assay) and selectivity (GAPDH/MTS CC_{50}).[6] Our earlier SAR[5a,b] concluded that the 4-carbasugar and 6-Me group were optimal with regard to replicon activity; therefore, our goal was to retain these groups and focus attention on modifications to the benzothiazole ring and the 2-position of pyrimidine. One of the modifications described in the earlier publication[5b] was the addition of the methylamino functionality to the 5′-position of the benzothiazole (**2**). This compound retained potency but displayed suboptimal pharmacokinetic properties. Due to the tolerance of polar functionality at the 5′-position, we hypothesized that pyridothiazole compound **3** could retain the potency and PK advantages of benzothiazole **1**.

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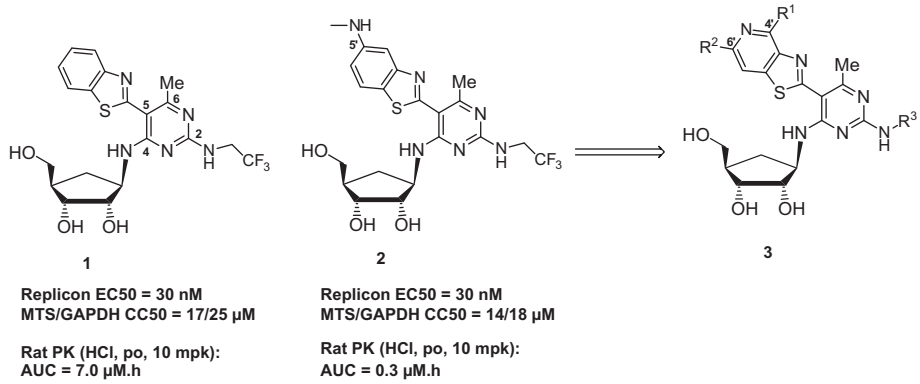


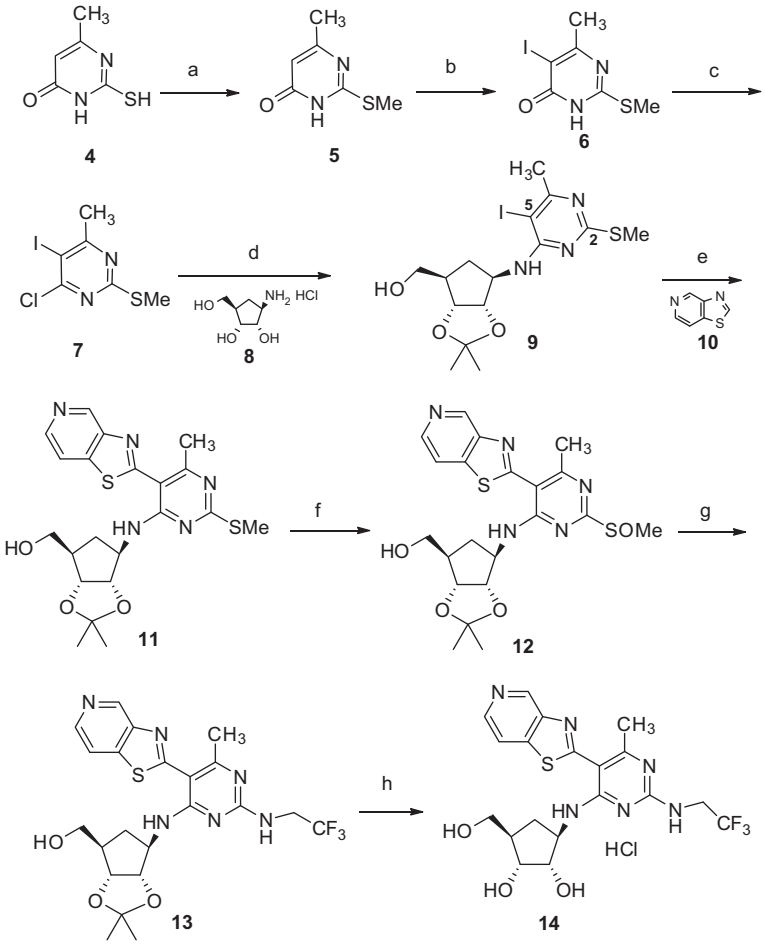
Figure 1. Optimization of the benzothiazole series.

The sterics of the methylamino functionality suggested that further substitutions at the 4′- and 6′-positions might also be tolerated.

Previously it had been determined that the 2-position of the pyrimidine could be varied with functionalized amines in order to modulate the potency, selectivity and PK properties of the compounds. Therefore, we developed a synthetic procedure that allowed for the facile incorporation of amines to the 2-position of the pyrimidine at the end of the synthesis.

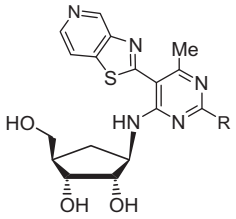
Our synthesis began with methylation of commercially available 2-mercapto-6-methylpyrimidin-4(3H)-one (**4**) using iodomethane and potassium carbonate to provide compound **5** (Scheme 1). The open position of the ring was iodinated using iodine and sodium hydroxide and further treatment with phosphorus oxychloride gave compound **7**. The chloro group was then displaced with commercially available carbasugar **8**, followed by protection of the free secondary alcohols as an acetonide to afford compound **9**. This intermediate was crucial to the convergent synthesis of analogs since it allowed for the incorporation of groups at both the 2 and 5-positions late in the synthesis.

The next step in the synthesis was the crucial arylation step that would allow for optimization of the 5-position. Previously, our group was the first to report the direct arylation of pyridothiazoles



Scheme 1. Reagents and conditions: (a) Iodomethane, K_2CO_3, DMSO 70%; (b) iodine, NaOH 83%; (c) $POCl_3$, 120 °C, 88%; (d) (1) triethylamine, EtOH, reflux; (2) 2,2-dimethoxypropane, methanesulfonic acid, acetone (74% over two steps); (e) $Pd(PPh_3)_4$, Cs_2CO_3, CuI, DMF 62%; (f) *m*-CPBA, methylene chloride 95%; (g) 2,2,2-trifluoroethylamine, acetonitrile 85%; (h) 4 M HCl dioxane, MeOH 95%.

Table 1
Modifications to the 2-position of pyrimidine



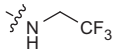
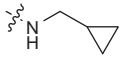
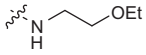
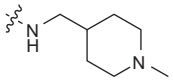
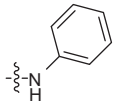
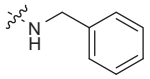
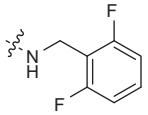
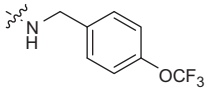
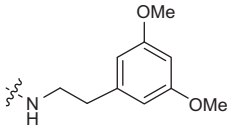
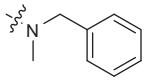
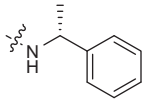
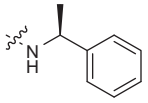
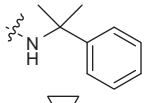
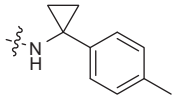
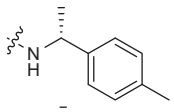
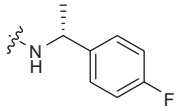
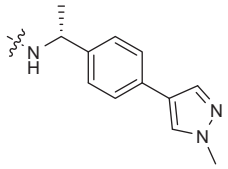
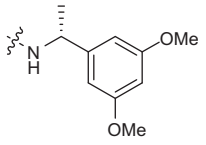
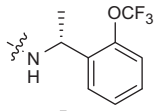
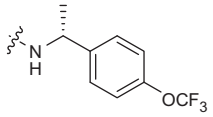
Compd	R	EC_{50} (nM)	MTS/GAPDH CC_{50} (μM)	Rat $AUC_{(0-6\ h)}$[a] (μM h)	Liver C6h[b] (ng/g)
14		16	>25/25	0	0
15		124	18/25	0	0
16		52	20/14	0.3	0
17		557	>25/25	7.5	1395
18		8	5/3	0.4	192
19		9	16/25	0.2	368
20		125	19/25	1.4	276
21		1683	>25/25	—[c]	—[c]
22		168	>25/25	0.2	29
23		3	14/25	0	0
24		1	10/4	0	0
25		3	25/25	0	0
26		12	19/25	0.1	100
27		301	>25/25	0.2	100
28		7	>25/25	0.2	104
29		222	>25/25	0.1	77
30		6204	>25/25	0	0
31		3165	>25/25	—[c]	—[c]

Table 1 (*continued*)

Compd	R	EC_{50} (nM)	MTS/GAPDH CC_{50} (μM)	Rat $AUC_{(0-6\ h)}$[a] (μM h)	Liver C6h[b] (ng/g)
32		114	>25/25	0.1	116
33		222	25/25	0	0
34		17	25/25	0	0
35		37	25/25	0.1	17
36		17	23/24	0.5	506
37		16	>25/25	1.6	1800

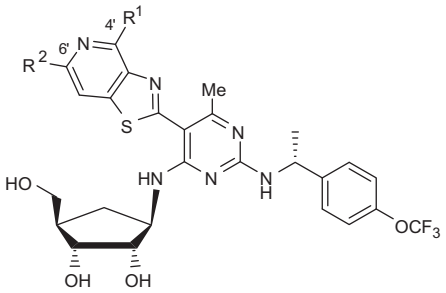
[a] $AUC_{0-6\ h}$, po (10 mpk), vehicle–0.4% mc.
[b] Liver concn at 6 h.
[c] Not measured.

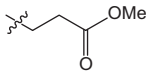
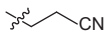
using both aryl and heteroaryl bromides and iodides.[7] Using this chemistry, the pyridothiazole **10** was efficiently coupled to the pyrimidine **9** using $Pd(PPh_3)_4$, copper iodide and cesium carbonate to supply the desired product **11**. The use of both palladium and copper sources were found to be instrumental in the reaction as well as the choice of base. This intermediate made it possible to study several substituted amines at the 2-position in order to optimize for potency, selectivity and PK properties. The thiomethyl group was oxidized to the corresponding sulfoxide using *m*-CPBA followed by displacement with a variety of amines to provide compounds of type **13**. Deprotection of the acetonide under acidic conditions gave access to the desired inhibitors in good yield.

With an efficient synthesis in hand, our first goal was to optimize the 2-position functionality and then to study modifications to the pyridothiazole. Table 1 displays the optimization of the 2-amino group of the pyrimidine ring. Compound **14** was synthesized for direct comparison with compounds **1** and **2** and demonstrated an improved potency (2-fold) compared to compound **1**, but improvements in PK[8] were necessary. As can be seen from compounds **15** and **16**, there is a significant loss in potency when the amino group at the 2- position is unsubstituted or has a smaller methyl substitution.

Compound **17** demonstrated that disubstitution of the nitrogen was not tolerated with respect to potency, but there was a marked improvement in PK. Compounds **18**–**19** showed that the introduction of substituted alkyl groups gave improved potency and provided a modest improvement to PK when compared to **14**. α-Branched alkyl groups such as cyclopropyl amino compound **20** demonstrated a loss in potency, but a significant improvement to PK properties. Compound **21** is a representative example that demonstrated that basic substitution was not tolerated. Similarly *N*-aryl substitution, such as compound **22**, was not tolerated. Benzyl substituted compounds **23**–**25** displayed a significant improvement in potency, but unfortunately did not provide desirable PK properties. Phenethylamines such as compound **26** maintained reasonable activity, but provided no improvements to selectivity or PK. It was evident from compound **20** that alkyl substitution adjacent to the nitrogen improved PK properties but lowered potency. Since the benzyl substitutions provided excellent potency, but lacked adequate PK properties, we decided to investigate further substitutions in this series. Incorporation of the *N*-methyl group (**27**) in the more potent benzyl series caused a significant loss in potency and confirmed the necessity of the N–H for activity. Compounds **28** and **29** incorporated an alpha-methyl to the benzylic position. The potency discrepancy between the two isomers clearly indicated that appropriate stereochemistry at the α-position is important. These compounds also provided improvement in the selectivity window. The more active compound **28** also exhibited a modest improvement to PK when compared to benzylic compounds **23**–**25**. Di-methyl substitution at the benzylic position (**30**) as well as the spirocyclic cyclopropyl **31** were attempted in order to further improve PK properties, but these modifications caused a loss in potency. We decided to modulate the PK of the molecule via substitution of the aromatic ring. Para-substituted analogs **32**–**34** retained potency and selectivity but did not improve PK properties. Meta-substituted analogs such as **35** lost potency. Addition of the trifluoromethoxy group at the ortho position (**36**) provided comparable potency to compound

Table 2
Optimization of Pyridothiazole



Compd	R^1	R^2	EC_{50} (nM)	MTS/GAPDH CC_{50} (μM)	rAUC[a] (μM h)	Liver C6h[b] (ng/g)
37	H	H	16	>25/25	1.6	1800
38	Me	H	16	>25/25	1.1[c]	2816
39	Et	H	20	7/7	5.8	6000
40	Pr	H	20	13/13	0.8	1259
41	i-Pr	H	26	10/11	3.2	8500
42		H	26	13/10	3.2	4400
43	Cyclobutyl	H	16	5/5	3.3	4900
44	Cyclopropyl	H	60	>25/25	5.2[c]	11,000
45		H	59	14/23	–[d]	–[d]
46		H	34	12/12	0.4	16
47	NH_2	H	37	>25/25	0	0
48	H	Me	38	>25/25	–[d]	–[d]
49	Me	Me	43	12/20	5.6	9800
50	Et	Me	264	24/23	25.0	257
51	Cyclopropyl	Me	703	24/21	–[d]	–[d]
52	Et	Et	69	13/12	–[d]	–[d]

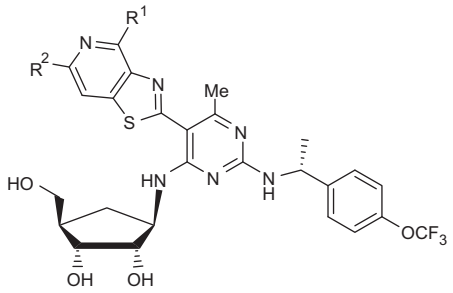
[a] $AUC_{0-6\ h}$ po (10 mpk), vehicle–0.4% mc.
[b] Liver conc at 6 h.
[c] $AUC_{0-24\ h}$, po (10 mpk).
[d] Not measured.

14 as well as measurable improvements to PK. When the trifluoromethoxy group was moved to the para position we were encouraged to find that compound **37** maintained potency and displayed improved PK properties. At this point, we decided to continue our optimization by maintaining the 2-position (*R*)-4-trifluoromethoxy-α-methylbenzylamino substitution and turning our attention to the optimization of the pyridothiazole functionality.

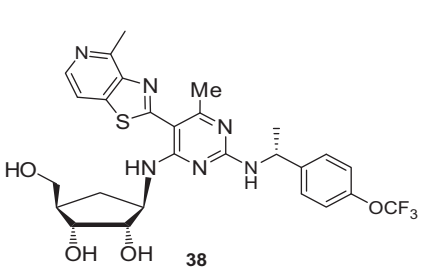
In a previous paper we described the synthesis of a variety of 4′ and 6′-substituted pyridothiazoles.[7,9] These substituted pyridothiazoles were introduced using chemistry described in Scheme 1 in order to optimize the series for potency, selectivity and PK. The data is summarized in Table 2. Incorporation of a methyl group at the 4′-position of pyridothiazole (compound **38**) retained the potency and PK properties of compound **37** including a measurable improvement to the concentration in the liver at 6 h.[10] The corresponding ethyl analog **39** maintained the potency and improved PK, but displayed lower selectivity. This was also true with other alkyl functionalities such as **40**–**43**. The exception to this trend was the cyclopropyl compound **44** which maintained excellent selectivity while showing significant improvements in PK. Incorporation of esters or nitriles at the 4′-position was not optimal as demonstrated by compounds **45** and **46**. Incorporation of a polar functionality, exemplified by amino compound **47**, provided reasonable potency and selectivity, but caused a loss in PK. Substitution at the 6′-position of the pyridothiazole, such as compound **48**, demonstrated a slight loss of potency. Compounds **49**–**52** all contain di-substituted pyridothiazole moeities and displayed a loss in potency and selectivity.

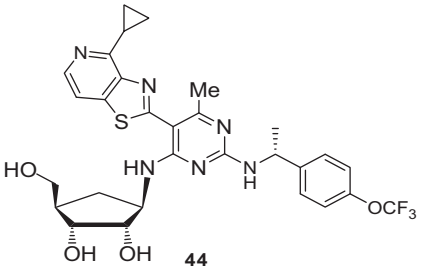
Tables 1 and 2 include several compounds that demonstrate good potency, PK, and selectivity in the MTS/GAPDH assay. In order to further prioritize the compounds for advancement, the more stringent thymidine incorporation assay (DNA CC50) was utilized. This assay measured the amount that these compounds inhibited DNA synthesis.[11] Table 3 shows the DNA CC50 values for selected compounds (from Tables 1 and 2) with the best potency, PK or MTS/GAPDH values. Although, compounds **37**, **38**, and **44** all had promising replicon potency and MTS/GAPDH values, the DNA CC50 selectivity window was much lower for compound **37** and, therefore, compounds **38** and **44** displayed the best overall profiles. Compound **38** had encouraging PK in rat and dog, and had a good selectivity profile (Fig. 2). Although compound **44** was slightly less potent in the replicon assay, the compound exhibited a higher liver

Table 3
Profiles of lead compounds



Compd	R^1	R^2	EC_{50} (nM)	MTS/GAPDH CC_{50} (μM)	DNA CC50 (μM)
37	H	H	16	>25/25	6
38	Me	H	16	>25/25	>25
44	Cyp	H	60	>25/25	>25





Replicon (1b) EC50 = 16 nM
Replicon (1a) EC50 = 70 nM
Replicon (2a) EC50 = 80 nM
MTS/GAPDH CC50 >25/25 μM
DNA CC50 = >25 uM

Full PK Data:
Rat (HCl, po, 10 mpk):
AUC = 1.1 μM.h; F = 15%
t1/2 = 2.5 hr; Cl = 40 mL/min/kg
Liver C(6h) = 2816 ng/g

Monkey (HCl, po, 3 mpk):
AUC = 0.3 μM.h; F = 7%
t1/2 = 3.3 hr; Cl = 20 mL/min/kg

Dog (HCl, po, 3 mpk):
AUC = 2.9 μM.h; F = 31%
t1/2 = 4.2 hr; Cl = 9.8 mL/min/kg

Replicon (1b) EC50 = 60 nM
Replicon (1a) EC50 = 400 nM
Replicon (2a) EC50 = 180 nM
MTS/GAPDH CC50 >25/25 μM
DNA CC50 = >25 uM

Full PK Data:
Rat (HCl, po, 10 mpk):
AUC = 5.2 μM.h; F = 18%
t1/2 = 5 hr; Cl = 11 mL/min/kg
Liver C(6h) = 11000 ng/g

Monkey (HCl, po, 3 mpk):
AUC = 1.3 μM.h; F = 12%
t1/2 = 4.4 hr; Cl = 7.2 mL/min/kg

Dog (HCl, po, 3 mpk):
AUC = 5.1 μM.h; F = 32%
t1/2 = 5.2 hr; Cl = 4.2 mL/min/kg

Figure 2. Overall profile of compounds **38** and **44**.

concentration at 6 h post dose as well as better overall PK in all species tested compared to compound 38. Both compounds also demonstrated activity against other genotypes, but with a modest shift. Inhibitors **38** and **44** had no issues with CYP inhibition (3A4, 2D6, 2C9 >20 μM), and were clean in an in-house kinase panel counterscreen (22 kinases, IC_{50} >30 μM).

In summary, we have developed a novel class of HCV replication inhibitors with good potency, selectivity and PK. Improvements to potency were achieved by adding the pyridothiazole group as a replacement for the benzothiazole moeity. These modifications led to the discovery of compounds **38** and **44** which displayed good potency and selectivity profiles while demonstrating good PK in multiple animal species. Further optimization of the profile of these inhibitors by modifications to the carbasugar ring system will be reported in future publications.

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6. To measure cell-based anti-HCV activity, replicon cells (1b-Con1) are seeded at 5000 cells/well in 96-well plates one day prior to inhibitor treatment. Various concentrations of an inhibitor in DMSO are added to the replicon cells, with the final concentration of DMSO at 0.5% and fetal bovine serum at 10% in the assay media. Cells are harvested three days post dosing. The replicon RNA level is determined using real-time RT-PCR (Taqman assay) with GAPDH RNA as endogenous control. EC50 values are calculated from experiments with 10 serial twofold dilutions of the inhibitor in triplicate. To measure cytotoxicity in replicon cells of an inhibitor, an MTS assay is performed according to the manufacturer's protocol for CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Cat # G3580) three days post dosing on cells treated identically as in replicon activity assays. CC50 is the concentration of inhibitor that yields 50% inhibition compared to vehicle-treated cells.
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10. The animals were sacrificed at 6 h and the liver was harvested and processed to measure the compound concentration (liver C6 h).
11. Effect of an inhibitor on cellular DNA synthesis is determined by a scintillation proximity assay. Replicon cells are seeded in 96-well Cytostar-T Scintillating Microplates (PerkinElmer, Cat # RPNQ0163) one day prior to inhibitor treatment. Various concentrations of an inhibitor in triplicate are added with [methyl-14C]-thymidine (PerkinElmer, Cat # NEC568050UC, final concentration 0.5 uCi/mL media) and incubated for three days. DNA CC50 is the inhibitor concentration that yields 50% inhibition of labeled thymidine incorporation as measured by Packard TopCount compared to vehicle-treated cells.