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Aminothiazole inhibitors of HCV RNA polymerase

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Abstract—Aminothiazole-based inhibitors designed for HCV polymerase display low micromolar potencies in biochemical assays. These compounds show a stringent preference for a cyclohexyl hydrophobe at the 2-amino position. The composition of these compounds suggests that they may be interacting at a recently discovered allosteric site on the polymerase.

The hepatitis C virus (HCV) chronically infects approximately 3% of the world's population and is a leading cause of liver transplantation in the United States.[1] Because the virus often results in fibrosis and cirrhosis of the liver over a time course that can last decades, it is projected that the societal costs of this disease will increase over the next 20 years.[2] Current treatment options are limited, with interferon α-2b/ribavirin combinations having the most widespread application. Unfortunately this combination does not always produce sustained responses across all viral genotypes, particularly genotype 1. Therefore, the development of new inhibitors targeting HCV becomes urgent.

HCV is a single stranded RNA virus of the *Flaviviridae* family, whose genome encodes for a polyprotein consisting of both structural and nonstructural proteins.[3] Of the six nonstructural targets the two that have been pursued most vigorously are the NS3 serine protease and the NS5B RNA dependent RNA polymerase. Known polymerase inhibitors can be grouped into three categories (Fig. 1): generally hydrophobic heterocyclic compounds, which contain an acidic functionality (**1**–**3**),[4–6] ketoacids or their isosteres that likely target the active site (**4**, **5**)[7,8] and a third series (**6**), which has been recently disclosed by GSK.[9] The first category of inhibitors (i.e., **3**) has recently been linked to an allosteric site within a narrow cleft on the protein's surface in the 'thumb' domain.[10]

In this letter we describe a novel series of HCV RNA polymerase inhibitors developed from an original hit **7a** identified by screening our library using NeoGenesis ALIS™ (automated ligand identification system) technology. Compound **7a** showed approximately 20 μM activity in the biochemical assay and the initial optimization of **7a** resulted in modest potency improvement. After a comparison with other inhibitors reported in the literature (**1**, **2**) we decided to shift the cyclohexyl group to the amide nitrogen placing the two hydrophobic groups adjacent to each other (**7b**). Although the initial change resulted in loss of activity, the subsequent replacement of pyrazole with thiazole generated inhibitor **8** with an IC_{50} of 7 μM. Herein, we would like to report the synthesis and optimization of a series of HCV polymerase inhibitors based on aminothiazole **8**. Several compounds were optimized to low micromolar potencies in the biochemical assay. The SAR trends of these compounds clearly reveal that hydrophobicity at the 2-amino position is important for inhibition in the biochemical assay.

The general synthesis of the aminothiazole inhibitors is illustrated in Scheme 1. The acylation step proceeded with high efficiency in most cases, while the yields of the Mitsunobu reactions were modest (10–50%). Alternate synthetic schemes, starting with alkylation of the aminothiazole, followed by acylation were less successful. The final deprotection of the ethyl ester with LiOH in THF generated the test compounds in 80–90% yields.[11]

Keywords: HCV; Aminothiazole.
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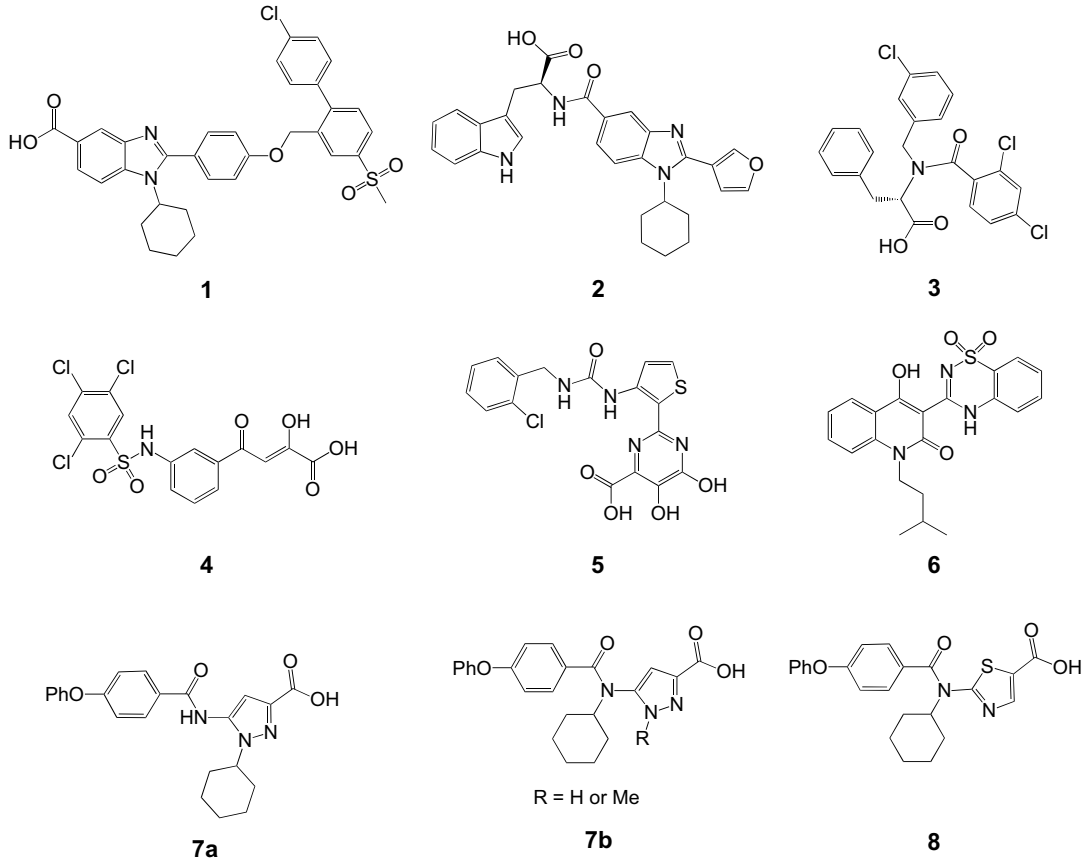
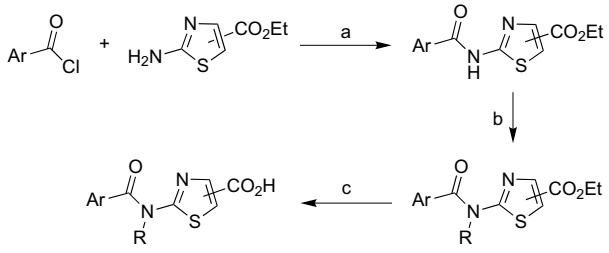


Figure 1.



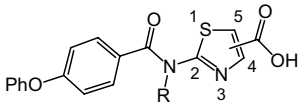
Scheme 1. General synthetic sequence for HCV polymerase inhibitors. Reagents and conditions: (a) DMAP, 10 equiv pyridine, dichloromethane, 0 °C–rt, 2 h; (b) triphenylphosphine, diisopropyl azodicarboxylate, ROH, THF, 0 °C–rt, 4 h; (c) 5 equiv 1 M LiOH in THF, rt, 12 h.

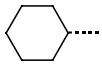
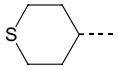
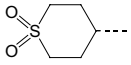
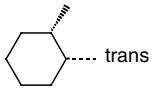
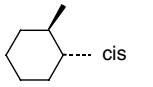
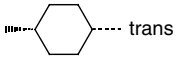
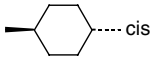
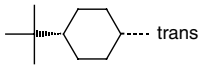
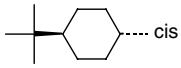
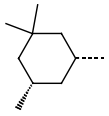
To measure the efficacy of these compounds an SPA-based RNA polymerase assay was performed using radiolabeled GTP, a poly C oligo G template/primer and the Δ-55 construct of NS5B according to a modified literature procedure.[12] In some cases compounds were also assayed using the Δ-21 (BK isolate) construct of the enzyme and similar, albeit usually lower, IC_{50} values were determined.[13]

The importance of the cyclohexyl substituent at the N2-position was explored in both the 4 and the 5 substituted aminothiazole series and is highlighted in Table 1 using data from the Δ-55 construct. Overall, both series showed similar SAR trends. The removal of the cyclohexyl group resulted in complete loss of activity (**9**, **21**). Replacing the cyclohexane ring with smaller branched alkyl groups such as isopropyl (**10**, **22**) also obliterated the inhibitory activity. While introduction of a sulfide group into the ring caused a slight loss of activity (**11**), the oxidation of sulfide into sulfone (**12**) resulted in complete loss of activity (**12**). Substitution of the cyclohexane ring with a methyl group at the 2-position had modest impacts on the potency, as the *cis* was slightly favored (**14**, **24**) over the *trans* form (**13**, **23**). Substitution of cyclohexane at 4-position with a methyl group had little impact on the activity in the 5-carboxylic acid series (**15**, **16**) but for the 4-carboxylic acid series, twofold activity improvement was observed (**25**, **26**). Substitution with a much larger *t*-butyl group, particularly in the *cis*-form, resulted in a twofold improvement in potency in the 5-carboxylic acid series (**18**) and threefold in the 4-carboxylic acid series (**28**).

In our initial optimization of **7**, we found that 4-phenoxy or 4-benzoxy hydrophobic motifs are necessary for activity. Attempts to replace the oxygen linker with nitrogen or carbon caused a loss of activity. Directly linking the distal ring to the phenyl group also resulted in loss of activity. From this initial SAR, it became clear that the 4-phenoxy and benzyloxy compounds were of greater interest. Since both 4-, and 5-carboxylic acid series showed similar SAR trends in the previous optimiza-

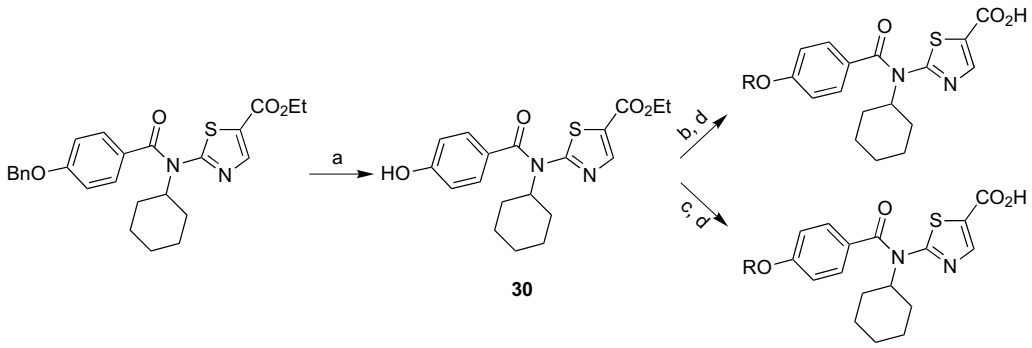
Table 1. Modification of cyclohexyl group



R	HCV NS5B IC_{50} (μM)			
	Compd	5-Carboxylic acid	Compd	4-Carboxylic acid
	**8**	7 ± 0.3	**20**	10 ± 1.0
H	**9**	>50	**21**	>50
	**10**	>50	**22**	>50
	**11**	13 ± 0.5		
	**12**	>50		
	**13**	9.4 ± 1.6	**23**	11 ± 2.0
	**14**	6.1 ± 1.0	**24**	7.6 ± 0.5
	**15**	5.0 ± 1.0	**25**	5.4 ± 0.8
	**16**	6.6 ± 1.4	**26**	5.7 ± 0.6
	**17**	6.0 ± 0.3	**27**	4.7 ± 0.9
	**18**	3.4 ± 0.4	**28**	2.8 ± 0.3
	**19**	6.0 ± 2.0	**29**	5.5 ± 0.8

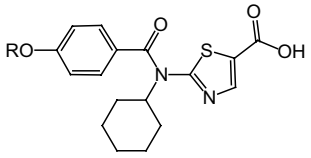
tion, the SAR exploration on the distal group was mainly focused on the 5-carboxylic acid series. In an effort to expedite the compound synthesis, advanced intermediates containing a readily functionalized phenol group were prepared. General alkylation and copper arylation procedures were then used to synthesize analogs (Scheme 2). A large number of substituted phenyl boronic acids and benzyl bromides were used to react with **30**, and some examples are shown in Table 2. In the phenoxy analogs, it was apparent that substitution of the phenyl ring at 3 or 4-position led to more active compounds compared to **8**, with the 3-position being favorable (**33**–**35**). This SAR pattern was conserved in the benzyloxy series as well, where compound **43** had approximately fourfold improved potency compared to **40**. The improvement in potency with the increase of hydrophobicity of the distal ring led us to speculate that this distal group might be occupying a hydrophobic pocket during its binding to the protein. Indeed, when the distal phenyl group in **8** was replaced with a cyclohexane ring (**31**), the inhibitory activity was retained. However, when a more polar group (e.g., pyridine) was used in the place of phenyl (**41**), its potency was reduced compared to the parent compound (**40**).

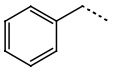
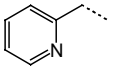
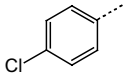
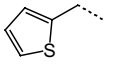
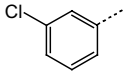
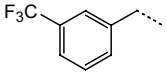
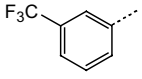
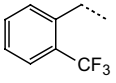
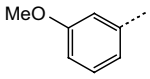
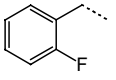
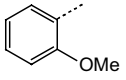
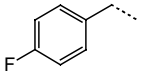
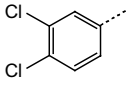
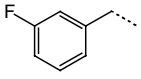
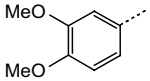
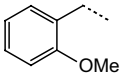
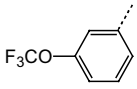
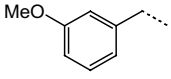
A preliminary evaluation of in vitro DMPK (drug metabolism and pharmacokinetics) properties was performed on a few selected compounds and favorable results were obtained. For example, compound **43** had 147 nM/s permeability in the CACO2 experiment and was found to be 5.0 μL/min/million clearance rate in human hepatocytes. It was also found to be selective against CYP's and had an IC_{50} of over 30 μM in both CYP 3A4 and CYP 2D6 assays.



Scheme 2. Synthetic routes used for discrete syntheses. Reagents and conditions: (a) BCl_3, dichloromethane, −78 °C; (b) copper(II) acetate, boronic acid, pyridine, air, 48 h; (c) cesium carbonate, benzyl bromides or chlorides, MeCN, 55 °C, overnight; (d) 5 equiv 1 M LiOH in THF, rt, 12 h.

Table 2. SAR at the distal hydrophobic group



R	Compd	HCV NS5B IC_{50} (μM)	R	Compd	HCV NS5B IC_{50} (μM)
	**8**	7.0 ± 0.3		**40**	6.5 ± 1.0
	**31**	5.5 ± 0.4		**41**	15 ± 1.0
	**32**	4.2 ± 0.8		**42**	7.0 ± 1.0
	**33**	2.8 ± 0.2		**43**	1.4 ± 0.3
	**34**	4.4 ± 1.0		**44**	5.1 ± 0.5
	**35**	3.1 ± 0.4		**45**	4.0 ± 1.0
	**36**	8.5 ± 1.6		**46**	3.9 ± 0.2
	**37**	2.6 ± 0.2		**47**	6.5 ± 1.0
	**38**	3.5 ± 0.5		**48**	8.5 ± 1.6
	**39**	5.2 ± 0.7		**49**	3.2 ± 0.5

In conclusion, suitably substituted aminothiazoles have been shown to be active against the NS5B RNA dependent RNA Polymerase. The need for a branched aliphatic hydrophobe on the 2-amino group was quite conserved across all analogs. There was some variability in the orientation of the distal aromatic hydrophobes, with a slight preference for *meta* substitution. The position of the carboxyl group was also variable with no significant preference for either the 4 or 5 positions on the thiazole ring. One explanation for this lack of preference may be that the ring heteroatoms are not forming close or highly directional contacts with NS5B. The composition

of these compounds suggests that they may be interacting at the recently discovered allosteric site on the polymerase. Select compounds have been analyzed in DMPK studies and their profiles in these assays are encouraging. This series is currently undergoing more extensive evaluation and it is hoped that further efforts on this novel HCV series will produce more efficacious compounds.

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11. All compounds in this study were characterized by ^{1}H NMR and MS. Purity was assessed by HPLC.
12. Briefly, 50 μL reaction mixture containing 20 mM HEPES (pH 7.3), 7.5 mM DTT, 20 units/mL RNasIN, 0.5 μg/mL biotinylated oligoG_{12}, 5 μg/mL polyC, 0.5 μM GTP, 1 μCi/mL [^{3}H]-GTP, 10 mM $MgCl_2$, 60 mM NaCl, 100 μg/mL BSA, and 6 nM NS5B (Δ55) were incubated at room temperature for 50 min in 96-well plates with or without test compounds. Assay was terminated by the addition of 50 μL, 10 mg/mL streptavidin-coated SPA beads supplemented with 100 mM EDTA, and the incorporation of labeled GTP determined by a TopCount Scintillation Counter. IC_{50} values were calculated from single experiments using 11 serial 2-fold dilutions (0.05–50 μM), and data were considered reliable only when the IC_{50} value of a positive internal control was within standard deviation range.
13. Assay was identical to that described in (12) except 5 μM GTP, 20 μCi/mL [^{3}H]-GTP, and 50 nM NS5B (Δ-21) enzyme form were used in a 3 h reaction at room temperature.