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Novel thiazolones as HCV NS5B polymerase allosteric inhibitors: Further designs, SAR, and X-ray complex structure

Shunqi Yan,* Gary Larson, Jim Z. Wu, Todd Appleby, Yili Ding, Robert Hamatake, Zhi Hong and Nanhua Yao*

Valeant Pharmaceuticals Research and Development, 3300 Hyland Ave., Costa Mesa, CA 92626, USA

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Abstract—Structure–activity relationships (SAR) of **1** against HCV NS5B polymerase were described. SAR explorations and further structure-based design led to the identifications of **2** and **3** as novel HCV NS5B inhibitors. X-ray structure of **3** in complex with NS5B polymerase was obtained at a resolution of 2.2 Å, and confirmed the design.

Hepatitis C virus (HCV) was identified in 1989 as the leading pathogen for non-A, non-B viral hepatitis.[1] More than 170 million individuals worldwide including 4 million people in the United States are believed to have been infected.[2] The current FDA approved standard therapy is effective for about half of the patient population.[3] Therefore, newer and more efficacious therapies directly targeting HCV are still an unmet medical need.

HCV NS5B is a virus-encoded RNA-directed RNA polymerase (RdRp). It is responsible for HCV viral replication,[4] and thus represents a valid target for antiviral therapy against HCV.[5] During polymerization, NS5B sub-domains undergo conformational changes as it binds to its substrates and performs the subsequent catalytic reactions. Recently, there has been growing interest in discovery of scaffolds that target an allosteric binding site of NS5B located on the thumb subdomains distant from the polymerase active site.[6] Inhibitors bound to such allosteric sites prevent elongation by locking in the conformation of NS5B. Allosteric inhibitors may have fewer off-target side-effects than nucleoside analogs due to the absence of binding to homologous cellular enzymes.

Previously, we disclosed a successful structure-based design of a novel thiazolone scaffold represented by compound **1** as an example of this type of HCV NS5B inhibitor. It has an IC_{50} of 3.0 μM and was established by X-ray crystallography as an allosteric inhibitor that binds to the thumb subdomain.[7] Further structure-based design and SAR exploration of **1** led to the identification of new scaffolds exemplified by compounds **2** and **3** as novel inhibitors of HCV NS5B polymerase. In this paper, we report a more detailed SAR analysis of **1**, as well as further designs, and synthesis of **2** and **3**. The X-ray complex structure of **3** with HCV NS5B is also described.

The synthesis of thiazolone derivatives discussed in this paper was carried out according to Schemes 1 and 2. For compounds **1**, **15**–**38**, the synthesis started with commercially available rhodanine **4** and aldehyde **5** (Scheme 1).[7] Thus, **4** was condensed with **5** to form **6**, which was subsequently coupled with MeI to give **7**. Treatment of **7** with a suitable amino-containing reagent (**8**) in the presence of DIEA afforded the desired products in a good yield.

The tetrazole derivatives, **39**–**42**, were prepared from the known amino acids (**9**) over seven steps by way of isolated intermediates **10**–**13** (Scheme 2). Thus, the amino group of an amino-acid (**9**) was first protected by a Boc group to give **10**, which was treated with ethyl chloroformate in the presence of TEA, followed by the introduction of a stream of NH_3 (gas) to afford amide **11**.[8] Treatment of **11** with TFA in the presence of TEA gave nitrile **12** in a quantitative yield. The resulting nitrile **12** was allowed

Keywords: HCV; NS5B; NS5B inhibitor; HCV NS5B polymerase; HCV NS5B inhibitors; Thiazolone; Tetrazole.
* Corresponding authors. Tel.: +1 714 545 0100; fax: +1 714 668 3142; e-mail addresses: syan@valeant.com; nyao@valeant.com

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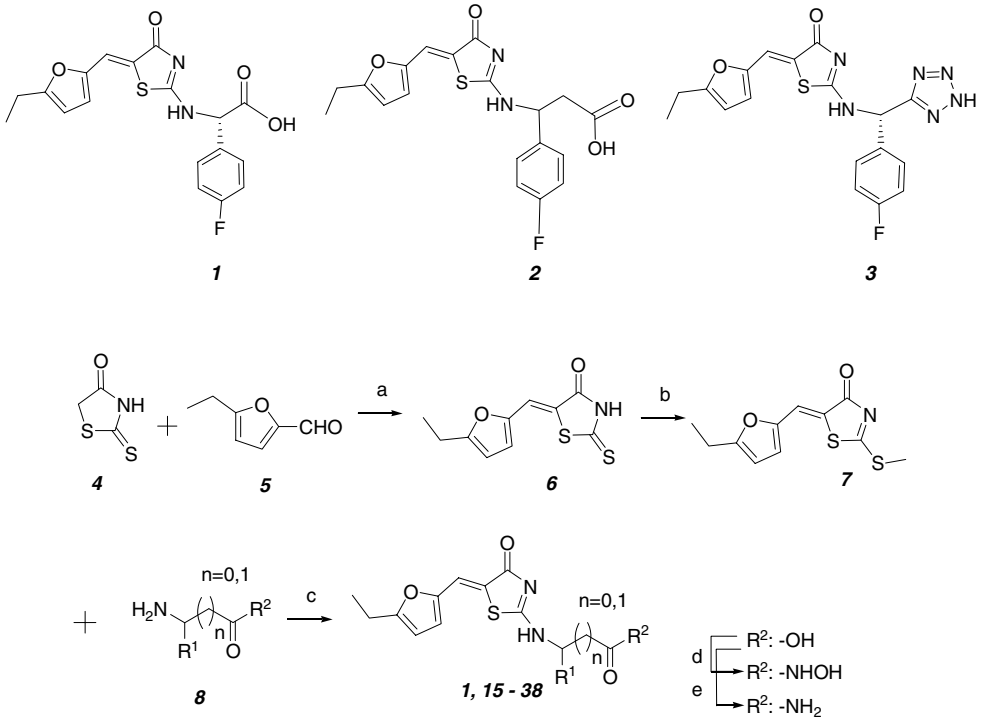
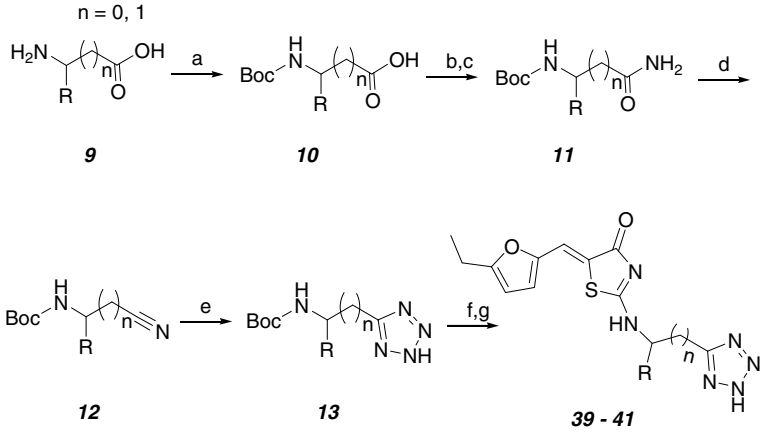


Scheme 1. General procedures for the synthesis **1**, **15**–**38**. Reagents and conditions: (a) EtONa, HOAc, 95 °C, 12 h, 93%.; (b) MeI, EtOH, DIEA, rt, 10–16 h, 95%; (c) i—EtOH, DIEA, 80 °C, 21 h; ii—NaOH (aq, 1 N), 100 °C, 30 min; iii—HCl (aq, 1 N), 93%; (d) HOBt, PS-Carbodiimide, DMF, $NH_2OH{\cdot}HCl$, DMF, rt, 16 h, 70%; (e) i—**11**, HCl (4 N in dioxane), rt overnight, 90%; ii—(c), **7** + de-Boc-11, 63%.



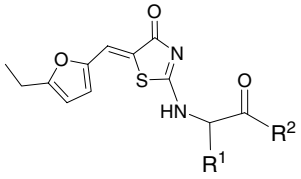
Scheme 2. General procedure for ester synthesis of **39**–**41**. Reagents and conditions: (a) Boc_2O, NaOH (1 N, aq), dioxane, rt, 12 h, 67%; (b) TEA, $ClCO_2Et$, THF, −10 °C, 30 min; (c) NH_3 (gas), THF, <0 °C to >12 h, 90%; (d) $(CF_3CO)_2O$, TEA, THF, rt, 14 h, 100%; (e) NaN_3, $Et_3N{\cdot}HCl$, toluene, 95 °C, 16 h, 83%; (f) HCl (3 N), rt, 16 h, 85%; (g) **7**, DIEA, EtOH, 75 °C, 12 h, 96%.

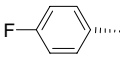
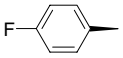
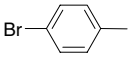
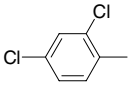
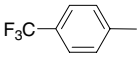
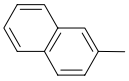
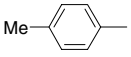
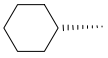
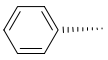
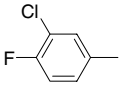
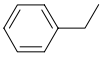
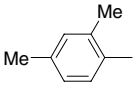
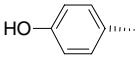
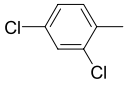
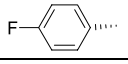
to react with NaN_3 and $TEA{\cdot}HCl$ in toluene to form tetrazole **13** in a good yield.[9] Subsequently, Boc group of **13** was removed and the resulted intermediate was coupled with **7** to afford the desired compound **39**–**41**.

The synthesized compounds were then evaluated for their ability to inhibit HCV NS5B polymerase by an in vitro NS5B RdRp-catalyzed elongation assay following the protocol previously described.[7,10] The resulting IC_{50} values are shown in Tables 1–3.

The most potent compounds of these α-amino acid derivatives **1**, **15**–**29** are in the low micro-molar range (Table 1). Since replacement of the 5-ethyl furan moiety with other groups did not result in significant variation of IC_{50} values (data not shown), we have focused on the substitution patterns on R^1 and R^2. The (*S*)-configuration (**1**) is approximately 17-fold more potent than the (*R*)-isomer (**2**) and about 18-fold more potent than the unsubstituted compound (**22**). The preference of the (*S*)-isomer **1** could be due to the overall favorable protein–ligand interactions from the 4-*F*-phenyl and carboxylic group of **1**.[7] The X-ray complex structure of **1** described in the previous paper confirmed that the 4-*F*-phenyl is buried into a hydrophobic pocket defined by Leu 419, Met 423, Tyr 477, and Trp 528, and that the

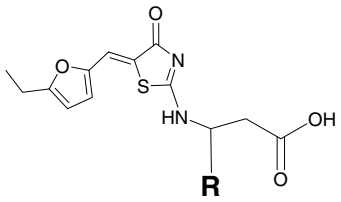
Table 1. IC_{50} values (μM) of **1**, **15**–**29**

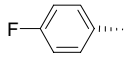
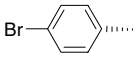
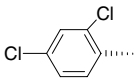
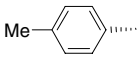
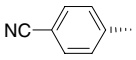
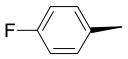
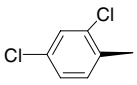


Compound	R^2 –OH		
	R^1		IC_{50}
1			3.0
15			50.0
16			6.0
17			7.6
18			21.0
19			14.0
20			11.0
21			25.0
22			55.0
23			>100.0
24			24.0
25			68.0
26			12.0
27			43.0
28	**R^1:** 	**R^2: -NHOH**	5.5
29	**R^1:** 	**R^2: -NH_2**	16.0

carboxylic group hydrogen-bonds in an ionic fashion with the basic side-chains of both Arg 501 and Lys 533 (Fig. 1).[7] Noticeably, the 4-*F*-phenyl moiety of **1** is

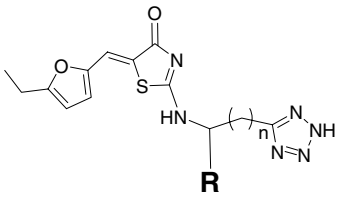
Table 2. IC_{50} values (μM) of **30**–**38**

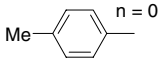
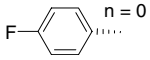
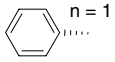


Compound	R	IC_{50}
30		44.0
31		>100.0
32		16.5
33		8.5
34		27.0
35		>100.0
36		59.0
37		13.0
38		9.0

nearly parallel in a π–π stack to the indole ring of Trp 528. This interaction presumably renders the (*S*)-isomer more favorable than the (*R*)-configuration. It is also conceivable that an aryl group, which is positioned in a π–π stack with Trp 528, would be more favorable than an alkyl group for the hydrophobic interactions (Fig. 1). Support for these theories are that compounds with R^1 as alkyl groups, for example, **21**, **23** and benzyl group such as **25**, have weaker or no inhibitory activities against HCV NS5B. We thus focused on the substituted aryl, particularly phenyl, as R^1. A substitution of 4-Br (**16**), or 2,4-Cl (**17**) can be well tolerated, as reflected by their IC_{50} values of 6.0 and 7.6 μM, respectively, which are similar to **1**. The weaker potency of **27** with 4-OH is possibly caused by the hydrophilic nature of 4-OH, while a combination of electronic and steric factors might contribute to the weaker activities of **21** and **24**. Hydrophobicity may be more critical than steric factors as 2-naphthalene (**19**), 4-Me– (**20**), and 2,4-Me– (**26**) are roughly 6-fold more potent than the less hydrophobic unsubstituted **22** but only slightly less potent than the less bulky **1** and **17**.

Table 3. IC_{50} values (μM) of tetrazoles **39**–**42**



Compound	R	IC_{50}
39		19.0
40		14.0
41		9.7
42		24.0

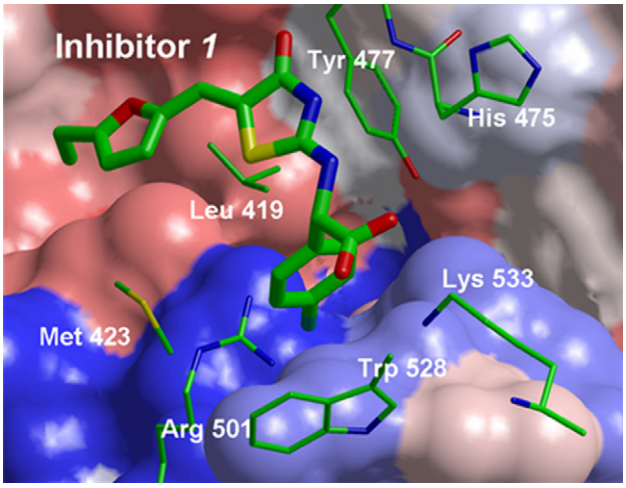


Figure 1. The binding mode of **1** in a complex with NS5B.

Table 1 also shows that the acidic moiety appears to be critical for activities. For example, amide replacement (**29**) of –COOH (**1**) leads to a 5-fold loss of potency compared to **1**. On the other hand, compound (**28**) bearing –CONHOH (5.6 μM), which has similar pK_a to –COOH, is at least as equipotent as **17** (7.6 μM) (Table 1).

Compounds with more flexibility, achieved by increasing the distance of carboxylic acid from the R group with one methylene (–CH_2–) group, were synthesized and examined. The IC_{50} values against HCV NS5B for the corresponding compounds **30**–**38** are shown in Table 2. The most potent compound is **33** with an IC_{50} of 8.5 μM, which is as potent as its other enantiomer **38** and similar in activity to **17** (7.6 μM) (Table 1). The comparable potency indicates that the binding site seems to be able to accommodate the flexibility of the methylene group. As for the effect of different R on IC_{50}s, the activities of 3-pyridyl (**31**) or 4-cyano (**35**) are probably eliminated because of their hydrophilic nature. When R is a substituted phenyl group, compounds bearing 4-Br (**32**), 4-Me (**34**), 4-F (**30**) or 4-H (**36**) have IC_{50} values of 16.5, 27.0, 44.0, and 59.0 μM, respectively. The rank order of these IC_{50} values agrees well with the opposite order of the van der Waals radius (Br > Me > F > H), suggesting that bulkier groups in that position result in more favorable interactions.

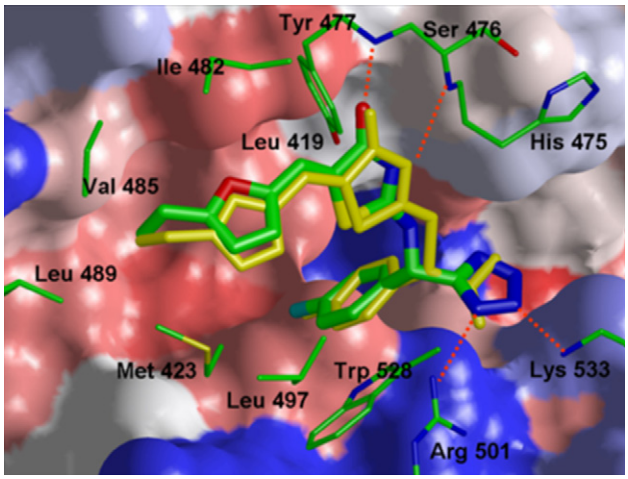


Figure 2. Overlay of X-ray structures of **1** (colored in yellow stick) and GOLD docking pose of tetrazole **41** (colored by atomtypes).

As demonstrated in Tables 1 and 2, a certain degree of flexibility, in addition to the presence of a –COOH head-group and a suitable R^1 or R group, is allowed in order to maintain potency against HCV NS5B. Encouraged by this insight, we turned our attention to the replacement of the –COOH group. The binding mode of **1** in the X-ray complex structure shows that the pocket occupied by the carboxylic group –COOH could accept a hetero-ring moiety, preferably hydrophilic in nature, to accommodate the hydrophilic receptor surroundings defined by His 475, Arg 501, and Lys 533 (Fig. 1).[7] Intuitively, a rational design for the first round would replace the –COOH group by a bio-isosteric tetrazole moiety and GOLD docking predicted a tetrazole replacement could indeed fit well into the pocket (Fig. 2).[11] To test this design, four tetrazole derivatives **39**–**42** were synthesized and their IC_{50} values are shown in Table 3.

As expected, all four tetrazoles are moderately active. The most active compound (**41**) produced an IC_{50} value of 9.7 μM, which is approximately 3-fold less potent than the corresponding carboxylic version (**1**), thus indicating that the carboxylic acid is more suitable for potency, whereas the increased bulkiness of a tetrazole group may decrease activity. 4-Me (**40**) and 4-H (**39**) are about 2-fold weaker than 4-F (**41**). Interestingly, extending the tetrazole group by one –CH_2– (**42**) does not seem to result in any significant loss of activity in comparison to **39**.

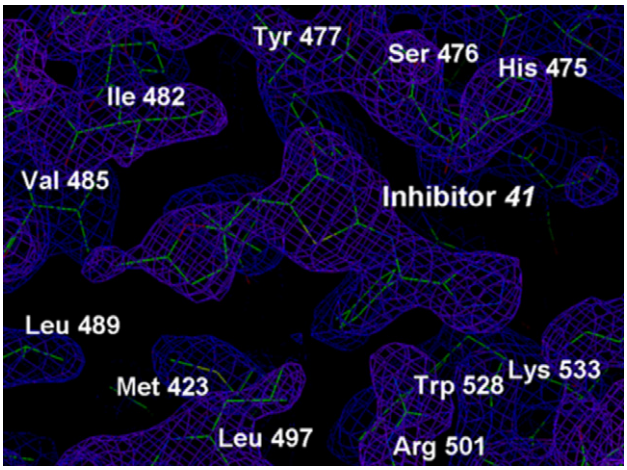


Figure 3. Electron density map of X-ray structure of **41** in complex with HCV NS5B polymerase at a resolution of 2.2 Å.

To confirm the design rationale and further comprehend protein-ligand interactions for future structure-based designs, single crystals of HCV NS5B polymerase in complex with **41** were successfully prepared by soaking crystal. The X-ray crystal structure was established at a resolution of 2.2 Å The electron density was unambiguous for inhibitor **41** and its surrounding amino acid residues. The structure of inhibitor **41** fits satisfactorily into the electron density map (Fig. 3). Inhibitor **41** binds to the 'thumb' sub-domain, which is distant from the enzyme's catalytic center.[12]

The overall structure of the NS5B–**41** complex is basically identical to the previously described complex structure of NS5B–**1**.[7] The binding mode of tetrazole derivative **41** from X-ray coincides with the predicted one and is verymuch comparable with amino-acid derivative **1** (Fig. 2). Similarly to **1**, the 4-*F*-phenyl of **41** is buried within a small deep hydrophobic pocket defined by the side chains of Leu 419, Met 423, Tyr 477, and Trp 528. The ethyl-furan moiety of the inhibitors is bound to the surface of another hydrophobic channel defined by Leu 419, Met 423, Ile 482, Val 485, Leu 489, and Leu 497. The C=O moiety in the thiazolone ring of **41** accepts a hydrogen-bond from the backbone –NH of Tyr 477, while its lone-pair N makes another hydrogen-bond to the backbone –NH of Ser 476. The tetrazole group of **41** retains the same hydrogen bond network as –COOH of **1**. Specifically, one N atom on the tetrazole makes a hydrogen bond with Arg 501, while another picks up one more hydrogen bond with Lys 533 (Figs. 2 and 3).

In conclusion, a SAR study has been performed for **1** as a novel HCV NS5B polymerase inhibitor. Substitution on *para*/*ortho* positions of R^1 with halogens increases activities, while an (*S*)-isomer is preferred for potency. Replacing the –COOH head group by an –NHOH group with a similar pK_a preserves the activity. Based on the binding mode from a complex of **1**, further designs of **2** and **3** were proposed and corresponding compounds were synthesized. Compounds from both series retain comparable potency with **1**. The X-ray complex structure of a tetrazole **41** with HCV NS5B polymerase was solved at a resolution of 2.2 Å and confirmed its binding mode.

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References and notes

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10. All compounds listed in this paper were fully characterized by ^{1}H NMR and MS with more than 95% of purity from HPLC analysis.
11. GOLD v2.1 docking, www.ccdc.cam.ac.uk. The docking was performed using the standard parameters. Structure **1** was used as shape template similarity constraint with a constraint weight of 10.
12. Coordinates for the structure have been deposited at Protein Data Bank (www.rcsb.org) under file name: 2I1R.