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Potent triazolyl-proline-based inhibitors of HCV NS3 protease

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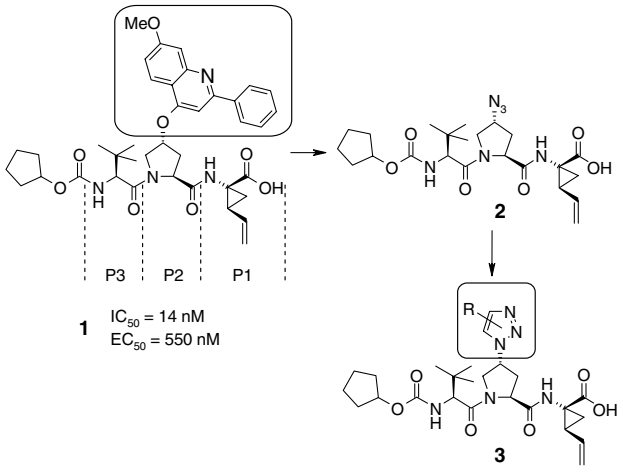
Abstract—The design and synthesis of tripeptide-based inhibitors of the HCV NS3 protease containing a novel P2-triazole is described. Replacement of the P2 quinoline with a triazole moiety provided a versatile handle which could be expediently modified to generate a diverse series of inhibitors. Further refinement by the incorporation of an aryl-substituted triazole and replacement of the P1 acid with an acyl sulfonamide ultimately provided inhibitors with interesting cellular activity.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that can lead to cirrhosis and hepatocellular carcinoma. It is estimated that nearly 200 million individuals worldwide are currently infected with HCV and it is the leading cause of liver transplants.[1] The current standard course of therapy, pegylated interferon-α in combination with ribavarin, suffers from low response rates and severe side effects.[2] Thus, a specific and broadly effective therapeutic agent for the treatment of HCV remains a critical unmet medical need.

HCV is a member of the Flaviviridae family of enveloped, single-stranded, positive-sense RNA viruses. The 9.6-kilobase genome of HCV encodes a single polyprotein that is post-translationally processed into at least 10 structural and nonstructural (NS) proteins by a combination of both host and viral proteases.[3] The NS3 protease has been shown to be essential for viral replication[4] and the first antiviral proof of principle studies in man with BILN2061 demonstrated that the NS3 protease is a relevant clinical target.[5]

We reported previously the discovery of compound **1** containing a 7-methoxy-2-phenyl-4-oxo-quinoline on the P2 proline (Scheme 1).[6] We have demonstrated that the aryl group on the 2-position of the quinoline contributed substantially to the potency of the inhibitor. Effectively, ^{1}H NMR studies demonstrated that the aryl group is positioned above the catalytic triad pair (Asp81 and His57) in the bound conformation, partially shielding the active site from solvent.[7] In our continuing efforts to discover novel NS3 protease inhibitors, we envisioned employing an azide (**2**) as a useful handle to evaluate substituted triazoles at the C4-position of proline (**3**). This would provide a diverse class of inhibitors that could mimic the effect of the aryl-substituted quinoline and allow us to map the large S2 surface binding site on the enzyme.

Introduction of the azide substituent (**2a**, Scheme 2) was straightforward via displacement of the corre-



Scheme 1. General strategy for P2 optimization.

Keywords: HCV NS3 protease; Protease; Hepatitis C; Inhibitors.
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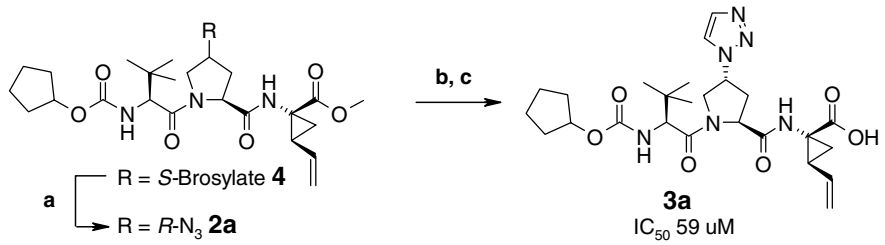


Scheme 2. Initial lead for optimization. Reagents and conditions: (a) NaN_3, THF, 70 °C, 14 h (83%); (b) prop-2-yn-1-oic acid, DME, 100 °C; (c) LiOH (10 equiv), THF, MeOH, H_2O (34%, 2 steps).

sponding brosylate (**4**) with inversion of stereochemistry to yield the desired azide. The unsubstituted triazole was then prepared by thermal cycloaddition of **2a** with prop-2-yn-1-oic acid, followed by hydrolysis of the P1-ester with concomitant decarboxylation to afford triazole **3a** that constituted the initial point of diversity for SAR.

In order to gain insight on the binding conformation of these series of inhibitors and to guide the SAR, compound **3a** was docked and minimized into the active site of the NS3 protease (Fig. 1).[6,7] The docking was based on previously reported structures of related analogues in complex with the NS3 protease. In this model, the P1, P2, and P3 moieties of **3a**, including the C-terminal acid, were docked to invoke the known intermolecular hydrogen bond network with the NS3 protease enzyme. Importantly, the model indicated that the triazole substituent adopts a pseudo axial conformation on the P2 proline ring which induces a large, flat lipophilic surface in the protein defined by the side chains of Asp168 and Arg155.[7] Although the exact orientation of the triazole ring could not be unambiguously established from this docking protocol, it was speculated that C4-substitution would provide a basis for further exploration of this binding area.

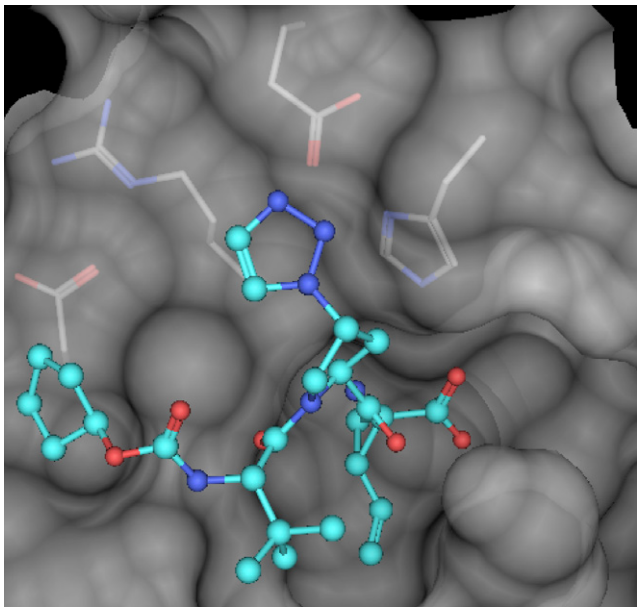


Figure 1. Model of **3a** docked into the NS3 protease active site.

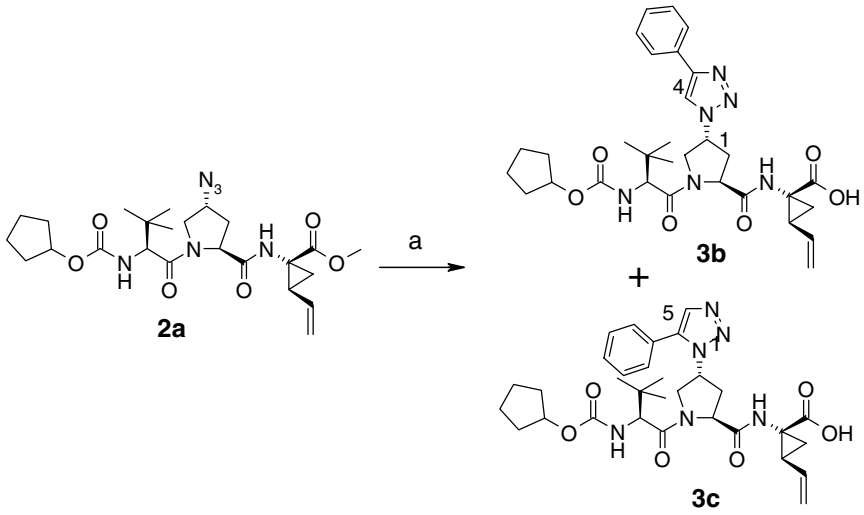
To evaluate this hypothesis, we employed a synthetic strategy that provided both the C4- and C5-arylated inhibitors (Scheme 3). Thermal cycloaddition of azide (**2a**) with phenylacetylene yielded a 1:1 mixture of the two regioisomers, which were separated and hydrolyzed for independent testing.

As predicted, the C4-phenyltriazole (**3b**, Scheme 4) was indeed the most potent of the two isomers and provided an 84-fold improvement in intrinsic potency over the unsubstituted triazole (**3a**).[9] Although the C5-arylated inhibitor (**3c**) also led to an improvement in potency (4-fold) over **3a**, we focused all further efforts on C4-substituted analogues.

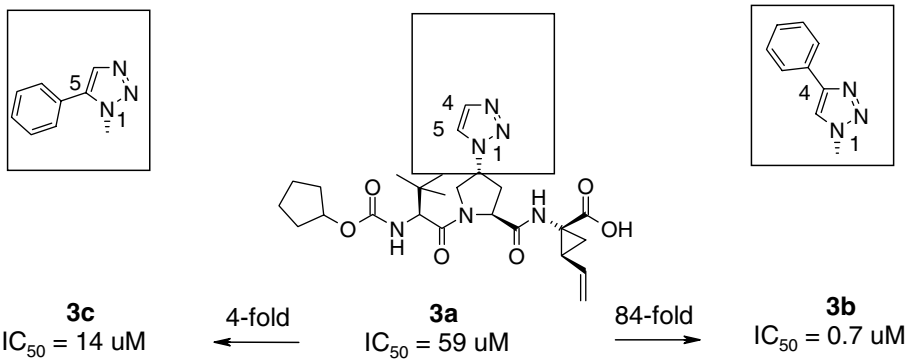
In an effort to further understand the binding mode of these inhibitors, transfer nOe NMR experiments were performed on **3b** in the presence of the NS3 protease (Fig. 2).[7] This study was consistent with the model which was obtained and confirmed that the P2 substituent occupies a pseudo axial conformation on the proline ring in both the free and bound states. Further, the C5–H of the triazole ring of **3b** has specific nOe's with the δ-hydrogens of the proline ring indicating the ring is pointing toward the cyclopentyl carbamate in the bound state. This conformation is likely due to the increased interactions of π–π stacking of the phenyl group with Arg155.

In an effort to further improve the intrinsic potency, we evaluated the effect of the C4-substituent of the triazole (Table 1). Replacement of the phenyl group with a cyclohexyl **3d** or with a 2-pyridyl **3e** substituent led to losses in potency. Increasing the size of the substituent to a bi-phenyl **3f** or naphthyl group **3g** was tolerated. Introduction of a *p*-chloro group **3h** on the second aryl ring had no significant effect, however, a *p*-methoxy group **3i** led to modest improvement in potency which was improved further by the preparation of the related dihydrobenzofuran **3j**.

C-terminal acyl sulfonamides have been disclosed[10] and further evaluated[11] as excellent bioisosteres for the C-terminal carboxylic acids in NS3 protease inhibitors. We were delighted to observe that a similar effect was observed in the triazole series, leading to a further improvement in potency of 260-fold over the corresponding acid (Scheme 5). In addition, compound **5a** demonstrated sub-micromolar potency in the replicon cell-based assay.[12]



Scheme 3. Synthesis of 1,4- and 1,5-disubstituted triazoles.[8] Reagents and conditions: (a) i—phenylacetylene, DME, 100 °C, 5 h; ii—LiOH (20 equiv), THF, MeOH, H_2O (55%, 2 steps).



Scheme 4. Effect of C4 versus C5 substitution.

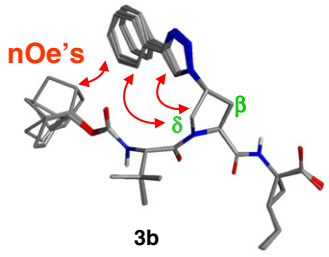
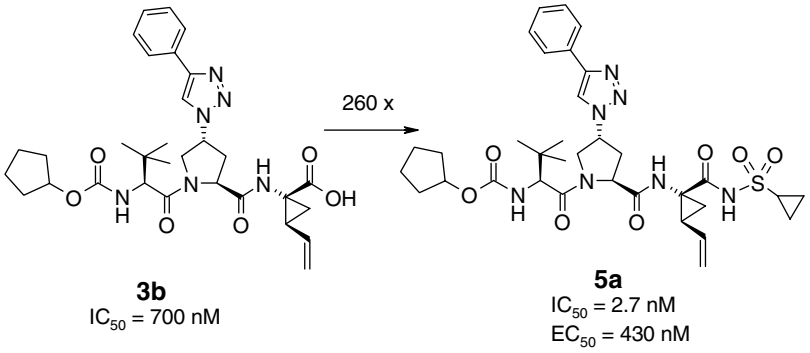


Figure 2. Transfer nOe of **3b** in the presence of NS3 protease.

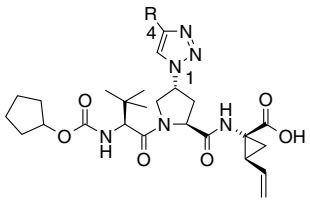
At this point, a regioselective synthesis of 1,4-disubstituted triazoles was implemented to facilitate SAR and avoid the need for regioisomer separation. This was achieved by the copper catalyzed cycloaddition chemistry previously reported for the regiospecific synthesis of 1,4-disubstituted triazoles[13] (see Scheme 6).

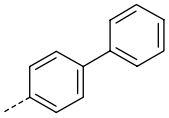
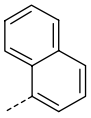
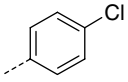
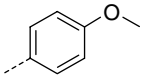
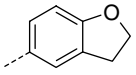
Further modifications were evaluated toward improving cellular activity and a variety of substituted phenyls were prepared (Table 2). Introduction of a dihydrobenzofuran **5b** improved the cellular activity



Scheme 5. Introduction of P1 acyl sulfonamide.

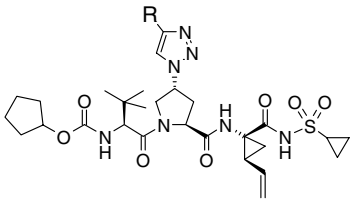
Table 1. Triazole C4 SAR

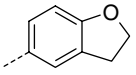
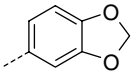
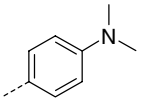
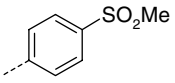
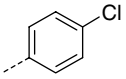
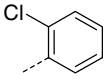
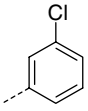
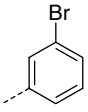
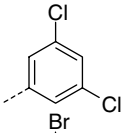
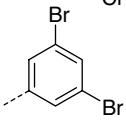


Compound	R	IC_{50} (nM)
3b		700
3d		4300
3e		3700
3f		860
3g		890
3h		790
3i		360
3j		130

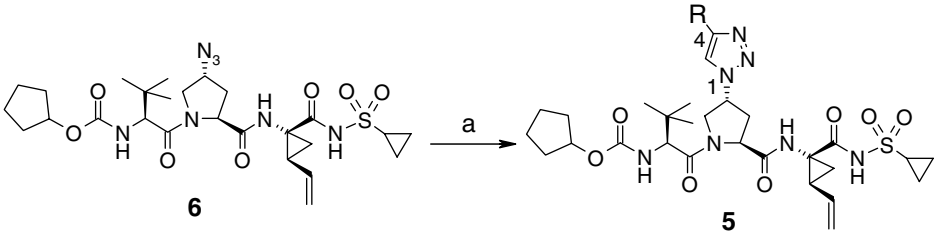
2-fold over **5a**, which was improved slightly when the corresponding acetal **5c** was introduced. Introduction of a NMe_2 **5d** group at the para-position led again to a 2-fold increase in cellular activity while an electron withdrawing sulfone **5e** resulted in complete loss of cellular activity. Replacement of phenyl **5a** with a thiophene **5f** led to a 3-fold loss in potency. The *m*-Cl-substituted phenyl **5i** was more potent than the corresponding isomers **5g** and **5h**. Interestingly, *m*,*m*-disubstitution with Cl led to a further increase in cel-

Table 2. EC_{50} SAR of C4-substituted phenyltriazoles [a,b]



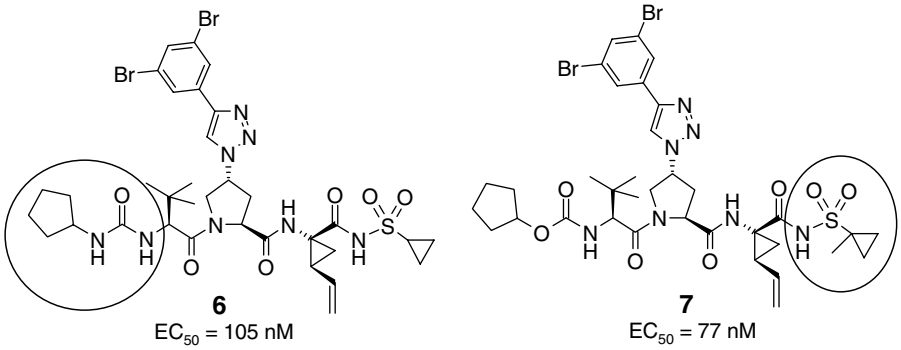
Compound	R	EC_{50} (nM)
5a		430
5b		230
5c		190
5d		270
5e		>7300
5f		1220
5g		680
5h		220
5i		170
5j		210
5k		140
5l		150

[a] All IC_{50} values for this set of inhibitors was <3 nM.
[b] EC50 values were obtained employing a previously described assay.[12]



6 **5**

Scheme 6. Regioselective synthesis of 1,4-disubstituted triazoles. [14] Reagents and conditions: (a) acetylene (1.2 equiv), $CuSO_4$, ascorbic acid, *t*-BuOH, 23 °C, yields: 5–50%.



Scheme 7. Capping group and P1′ modifications.

lular activity **5k**. The same exercise was performed employing di-Br **5l** in place of di-Cl which led to our two most potent inhibitors.

Further improvement in the cellular activity was achieved by modification of the capping group from a carbamate **5l** to the corresponding urea **6** (Scheme 7).[6] Also, the introduction of a methyl-substituted cyclopropane on the acyl sulfonamide **7**[15] improved cellular potency over **5l** and provided an inhibitor with a promising profile for further exploration.

In summary, we have disclosed potent inhibitors of the HCV NS3 protease which contains novel P2 triazole substituents. These inhibitors exhibited good levels of cellular activity while providing a diverse series for further optimization.

Acknowledgments

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8. Representative thermal cycloaddition: phenylacetylene (1.3 equiv) was added to a solution of triazole (1.0 equiv) in DME (0.28 M in triazole) in a sealed tube and was heated at 100 °C for 5 h. The reaction was cooled, concentrated, and diluted with THF/MeOH/H_2O (2:1:1, 0.05 M in triazole) and LiOH was added (20 equiv). The reaction mixture was stirred for 14 h, concentrated and purified by preparative HPLC (CH_3CN/H_2O 0:0.06% TFA) followed by lyophilization to yield the desired compounds. Typical yields, 10–35%.
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14. Typical experimental procedure: azide **2b** (0.05 mmol) was dissolved in *t*-BuOH (0.05 M) followed by the addition of copper sulfate pentahydrate (0.1 M in H_2O, 0.1 equiv), ascorbic acid (0.1 M in H_2O, 0.8 equiv) and aceteylene (1.2 equiv). The resulting mixture was stirred at 23 °C for 14 h, concentrated, and purified by preparative HPLC (CH_3CN/H_2O containing 0.06% TFA) followed by lyophilization yielding the desired compounds, typical yields 5–50%.
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