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Structure-Based Design of a Benzodiazepine Scaffold Yields a Potent Allosteric Inhibitor of Hepatitis C NS5B RNA Polymerase§

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Abstract: HCV NS5B polymerase, an essential and virus-specific enzyme, is an important target for drug discovery. Using structure-based design, we optimized a 1,5-benzodiazepine NS5B polymerase inhibitor chemotype into a new sulfone-containing scaffold. The design yielded potent inhibitor (*S*)-**4c** (K_D = 0.79 nM), which has ~20-fold greater affinity for NS5B than its carbonyl analogue (*R*)-**2c**.

It is estimated that 170 million people, 3% of the world's population, are infected with hepatitis C virus (HCV[a]), a condition that poses a high risk of ultimately leading to liver cirrhosis and hepatocellular carcinoma.[1] The current treatment of HCV infection, involving a combination of pegylated interferon-α and ribavirin, is associated with numerous side effects and yields at best a 50% sustained virological response (SVR) for genotype 1 infected patients.[2] Ongoing research efforts anticipate that a combination of small-molecule drugs targeting different essential viral proteins, in an approach similar to the current standard of care for HIV therapy, will lead to improved therapeutic outcomes for HCV patients in the future.[2]

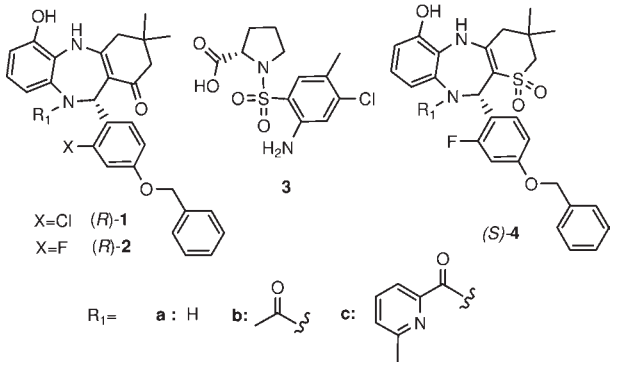

Figure 1. 1,5-Benzodiazepine and proline sulfonamide inhibitors of HCV NS5B.[3,4]

Inhibition of the essential, virus-specific nonstructural protein 5B RNA-dependent RNA polymerase of HCV (HCV NS5B) with small molecules is a compelling target for drug discovery, and several HCV NS5B inhibitors are presently undergoing clinical evaluation.[2a,5] The current state of the art suggests that targeting the active site (nucleoside inhibitors) or one of four distinct allosteric binding sites (non-nucleoside inhibitors) of this enzyme represents a viable approach to a new mode of HCV chemotherapy.[6] We have recently described a new class of 1,5-benzodiazepine (BZD, Figure 1)[3] inhibitors of HCV NS5B that bind to an allosteric site near the interface of the palm and thumb subdomains. Other chemotypes known to bind at or near this site include benzothiadiazines,[7] pyrrolidines,[8] acrylic acids,[9] rhodanines,[10] isothiazoles,[11] benzofurans,[12] and proline sulfonamides.[4,13] Using structure-based design, we have extracted interaction information from the publicly available 3D structure of an NS5B−inhibitor complex with an unrelated chemotype and applied this to our BZD series.

We determined the 2.75 Å resolution crystal structure of the NS5B-(*R*)-**1b** complex (PDB code 3GNV), and observed that the carbonyl O of bound (*R*)-**1b** forms an intermolecular hydrogen bond with Tyr448:N (Figure 2). Superimposition of the published structure of the NS5B complex of **3** (PDB code 2GC8),[4] an inhibitor chemotype distinct from that of **1**, revealed that one sulfonamide oxygen of bound **3** makes a similar contact with Tyr448:N (Figure 2). In an adjacent region of the binding site, a carboxylate O of bound **3** forms another intermolecular hydrogen bond, in this case with Gly449:N (Figure 2).

Our optimization effort had by this time progressed from the chloro series to the closely related fluoro series, with larger *N*-acyl substituents (cf. **1b** and **2c** in Figure 1), and we had established that the Cl-to-F substitution did not appear to affect the inhibitor binding mode.[3,14] Thus, **2c** is derived from

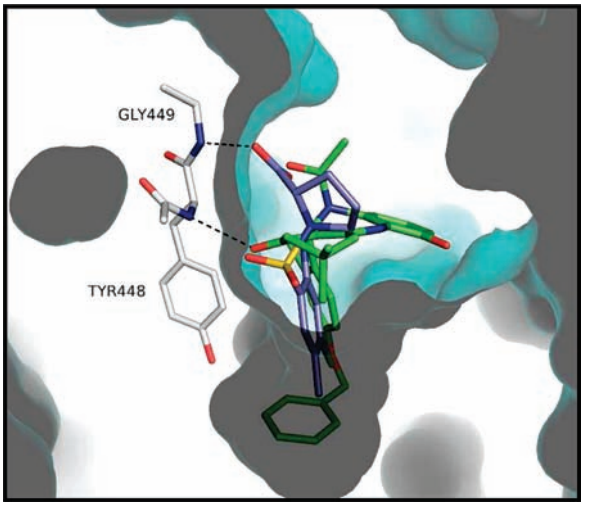

Figure 2. Overlay of the 2.75 Å resolution crystal structure of BZD (*R*)-**1b** (green, by atom) bound to HCV NS5B (white, by atom) with that of proline sulfonamide **3** (purple, by atom), indicating the common hydrogen bond with Tyr448:N and the additional hydrogen bond of **3** with Gly449:N.

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§The atomic coordinates and structure factors (PDB codes 3GNV and 3GNW for NS5B-(*R*)-**1b** and NS5B−(*S*)-**4c**, respectively) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

[a] Abbreviations: HCV, hepatitis C virus; SVR, sustained virological response; BZD, 1,5-benzodiazepine; TFA, trifluoroacetic acid.

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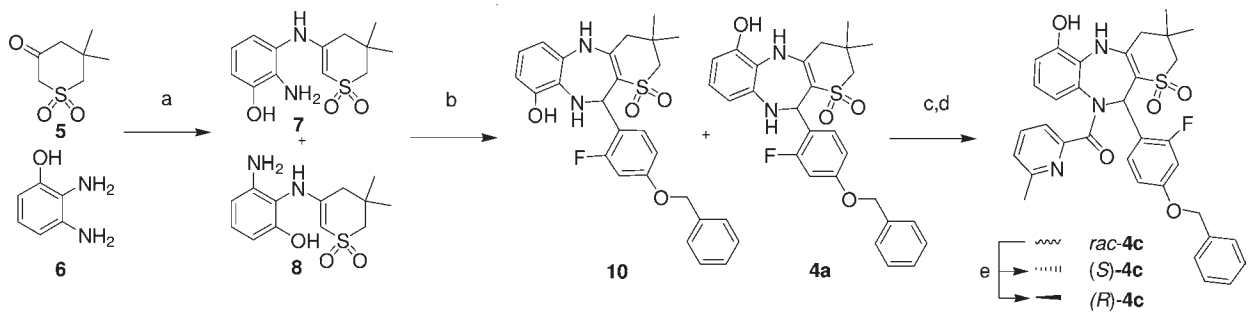
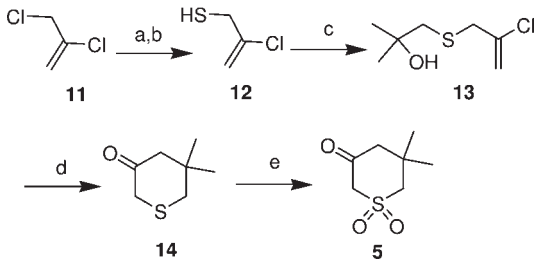
Scheme 1. Preperation of Sulfone-BZD Compounds **4a**,**c**[a]



[a] Reagents: (a) 1 equiv TFA, DMF, 50 °C, 6 h; (b) 0.9 equiv of $NaHCO_3$, 4-benzyloxy-2-fluorobenzaldehyde (**9**), 50 °C, 12 h; overall yield for **4a** (a and b) of 18%; (c) 6-methyl-2-pyridinecarbonyl chloride, DIPEA, THF, room temp, 12 h; (d) LiOH, H_2O/THF, room temp, 3 h, combined yield of *rac*-**4c** (c and d) of 82%; (e) Chiralpak AD, hexane/2-propanol/MeOH (7 N NH_3) 60/30/10.

1b.[3] On the basis of our structural observations, we hypothesized that replacement of the carbonyl of **2c** by a sulfone moiety would yield a benzodiazepine inhibitor (**4c**) that forms an additional intermolecular hydrogen bond, maintaining the interaction with Tyr448:N and gaining the Gly449:N contact, thus improving binding affinity. Small-molecule conformational analysis supported the conclusion that the bound conformation observed for **1b** (and predicted for **2c**) was equally accessible to **4c** (see below), and calculated interaction energies suggested improved affinity for the sulfone.[14]

Scheme 2. Preparation of Ketosulfone **5**[a,14,15]



[a] Reagents: (a) thiourea, EtOH, reflux, 16 h, 95%; (b) NaOH, H_2O, 90 °C, 4 h, 70%; (c) NaOMe, 2,2-dimethyloxirane, MeOH, room temp, 16 h; 95%; (d) formic acid, 80 °C, 1 h, 34%; (e) Oxone, CH_2Cl_2, H_2O, room temp, 4 h, 36%.

The synthetic route for this previously unreported tricyclic benzodiazepine system is outlined in Scheme 1. Condensation of ketosulfone **5**, obtained in five steps from commercial 2,3-dichloropropene (Scheme 2)[14,15] with 2,3-diaminophenol **6** was performed in DMF in the presence of 1 equiv of TFA, yielding a mixture of **7** and **8**, which was then converted into a mixture of **4a** and **10** by reaction with aldehyde **9**. This synthetic sequence differs from that of the corresponding carbonyl BZD (e.g., **1**);[3] the original Dean−Stark conditions were modified, for the enamines **7** and **8**, to a lower reaction temperature (50 °C) in the presence of TFA to avoid the formation of side products. Interestingly, TFA also catalyzed the formation of the isomeric mixture of sulfone-BZD (**4a**/**10**, ~6/4), allowing omission of the intermediate isolation of **7** and **8**. After separation of regioisomers **4a** and **10**, scaffold **4a**, obtained in 18% isolated yield after a one-pot, two-step procedure, was converted to the racemate **4c**, which was then separated into enantiomers (*S*)-**4c** and (*R*)-**4c** by chiral chromatography. The absolute configuration of (*S*)-**4c** was established by single crystal X-ray crystallography (see below).[14]

The rationally designed sulfone (*S*)-**4c** exhibits a marked 19-fold increase in NS5B binding affinity over the parent carbonyl compound (*R*)-**2c** (Table 1, K_D values determined by surface plasmon resonance[14]). Importantly, most of the potency enhancement translates to antiviral activity, based on the observed 14-fold improvement in replicon activity (Table 1). The contrasting 3-fold improvement in IC_{50} is attributed to the low sensitivity of the enzyme assay, which often precludes discrimination of highly active compounds by this method. The greater discrepancy between replicon activity and binding affinity for (*S*)-**4c** compared to (*R*)-**2c** (Table 1) is also fully consistent with the increased polar surface area of the designed compound.

Table 1. NS5B Polymerase Affinity (K_D), Inhibition (IC_{50}), and Replicon Activity (EC_{50}) of BZD Inhibitors

compd	K_D (μM)	IC_{50} (μM)	EC_{50} (μM)
(*R*)-**1b**	nd	0.093	1.2
(*R*)-**2c**	0.015	0.074	0.40
rac-**4c**	nd	0.12	0.052
(*R*)-**4c**	nd	6.7	4.8
(*S*)-**4c**	0.00079	0.026	0.029

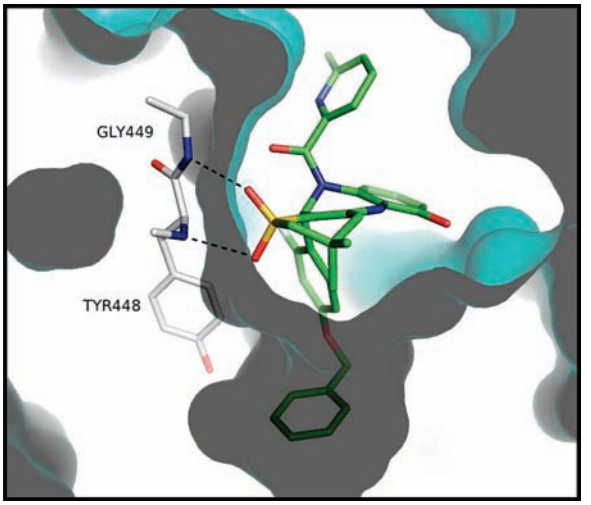


Figure 3. The 2.4 Å resolution crystal structure of (*S*)-**4c** bound to HCV NS5B polymerase.

We determined the 2.4 Å resolution crystal structure of the NS5B−(*S*)-**4c** complex (PDB code 3GNW). Consistent with our structure-based design rationale, the observed binding contacts include the predicted intermolecular hydrogen bonds for the sulfone moiety, the designed contact with Gly449 and the "conserved" (from the original carbonyl analogue (*R*)-**2c**)

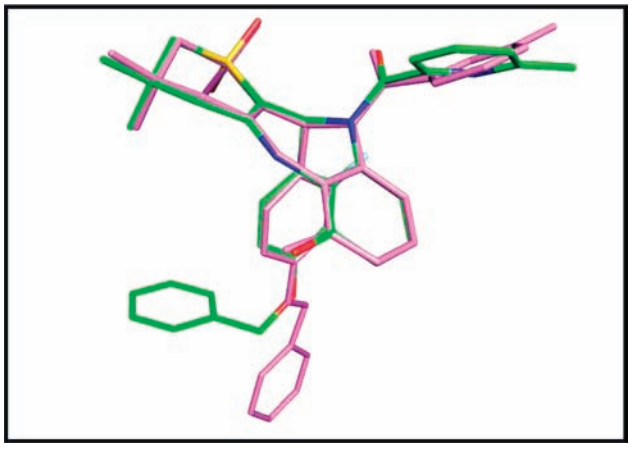


Figure 4. Overlay of the small molecule crystal structure (pink) and the NS5B-bound structure (color by atom) of (*S*)-**4c**.

interaction with Tyr448:N (Figure 3). *The designed sulfone group captures the intermolecular hydrogen bonds of the sulfone and carboxylate moieties of bound* ***3***. In all other respects, and as expected, the binding mode of (*S*)-**4c** (PDB code 3GNW) is essentially identical to that of the parent carbonyl (*R*)-**1b** (PDB code 3GNV).[16]

Small-molecule conformational analysis indicated that the benzodiazepine scaffold is quite rigid, and much of the calculated conformational diversity stems from the relatively flexible benzyloxy group.[14] These studies suggest that, with the exception of the relatively flexible benzyloxy moiety, the required binding conformation for (*R*)-**2c** and (*S*)-**4c** is close to energetically optimal. The benzyloxy group is predicted to prefer a fully extended conformation rather than the more compact conformation observed to bind to NS5B. The small-molecule crystal structure of (*S*)-**4c** is also consistent with a preference of the benzyloxy group for a fully extended conformation over the more compact conformation seen in the NS5B complex (Figure 4).[14] Overall, we conclude that the NS5B-bound conformation of (*S*)-**4c** and related compounds in these chemical series is relatively favorable and readily accessible. Binding to NS5B requires adoption of a suboptimal conformation for the flexible benzyloxy moiety of the inhibitor.

In summary, a structure-based design approach generated a novel sulfone-BZD chemotype that enabled further progress in HCV drug discovery, yielding specific inhibitors of HCV NS5B polymerase with low nanomolar potencies in biochemical and cell-based assays. Crystallography validated our design process, clearly underscoring the value of public and proprietary 3D structural information in the drug discovery process. Further characterization of molecules in this series is ongoing, and these results will be reported in due course.

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Supporting Information Available: Experimental and computational details and crystallographic data for (*S*)-**4c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The impact on affinity and activity of the larger R_1 group of (*S*)-**4c** compared to that of (*R*)-**1b** is accounted for in the design "parent" (*R*)-**2c** (Figure 1, Table 1). On the basis of an extensive set of 3D complex structures in this series, both published[3] and unpublished, and related computational studies,[14] we have determined that the nature of the R_1 group (Figure 1) does not affect the binding mode observed for the remainder of the inhibitor structure.