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Inhibition of Dengue virus and West Nile virus proteases by click chemistry-derived benz[*d*]isothiazol-3(2*H*)-one derivatives

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ABSTRACT

Two click chemistry-derived focused libraries based on the benz[*d*]isothiazol-3(2*H*)-one scaffold were synthesized and screened against Dengue virus and West Nile virus NS2B-NS3 proteases. Several compounds (**4l**, **7j–n**) displayed noteworthy inhibitory activity toward Dengue virus NS2B-NS3 protease in the absence and presence of added detergent. These compounds could potentially serve as a launching pad for a hit-to-lead optimization campaign.

1. Introduction

The genus *Flavivirus* in the family *Flaviviridae* comprises an array of major viral pathogens, including West Nile virus (WNV), Dengue virus (DENV), Hepatitis C virus (HCV), Yellow Fever virus (YFV), and Japanese encephalitis virus (JEV).[1] DENV is a mosquito-borne virus that has four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Worldwide, DENV is the cause of 50–100 million infections annually, with approximately 500,000 cases progressing to dengue hemorrhagic fever/dengue shock syndrome, resulting in ~25,000 deaths.[2,3] There are no vaccines or small molecule therapeutics for the treatment of DENV infection; consequently, there is currently an urgent and unmet need for the discovery and development of therapeutic agents for DENV infection.

DENV is a small, enveloped virus with a single-stranded, positive sense 11-kb RNA genome, which encodes a polyprotein precursor. Co- and post-translational cleavage of the polyprotein produces three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) by the concerted action of host proteases (furin, signalase) and the two-component trypsin-like protease NS2B-NS3 (NS2B-NS3pro).[4,5] Processing of the polyprotein by NS2B-NS3pro is essential for viral replication; consequently, NS2B-NS3pro has emerged as an attractive target for the discovery and development of therapeutics for DENV infection.[6,7] NS3 is a multifunctional protein that comprises a protease (NS3pro), an RNA helicase, a nucleoside triphosphatase, and 5′-RNA triphosphatase activities.[7] NS3pro is a serine endoprotease with a His51-Asp75-Ser135 catalytic triad and a strongly-preferred substrate specificity for a -X-K-R-R-G/S- sequence corresponding to the subsites -S_4-S_3-S_2-S_1-S_1'-.[8] Cleavage is at the P_1–P_1' scissile bond (R–G/S). The hydrophilic core of the NS2B protein cofactor is required for optimal catalytic efficiency.[9,10] X-ray crystal structures of NS2B-NS3pro of WNV and DENV-2 and DENV-1 have been reported.[10–13] Inhibitors of NS2B-NS3 protease containing a highly charged peptidyl recognition element with a dibasic motif at P_1–P_2 coupled to an array of electrophilic warheads (aldehydes, trifluoromethyl ketones, and boronic acids)[14–16] or non-peptidyl α-ketoamides,[17] and others[18,19] have been described.

We describe herein the results of synthetic and biochemical studies related to the inhibition of DENV NS2B-NS3 protease by click chemistry-derived benz[*d*]isothiazol-3(2*H*)-one derivatives (I–II) (Fig. 1).

1.1. Chemistry

The synthesis of compounds **4a–p** and **7a–p** is summarized in Scheme 1. Thus, starting acid **1**[20] was activated with 1,1′-carbonyldiimidazole (CDI) and then coupled to propargylamine to yield

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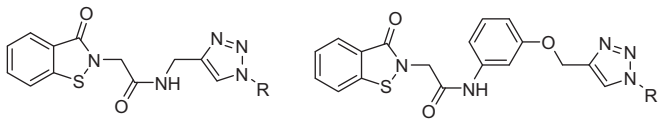


(I) (II)

Figure 1. General structures of DENV2 NS2B-NS3pro inhibitors (I–II).

intermediate **2**. Click chemistry methodology was employed to generate the desired triazole derivatives **4a–p** by coupling compound **2** to an array of structurally diverse azides **3a–p**. The latter were readily synthesized from the corresponding halides and sodium azide in dimethyl sulfoxide.[21] Intermediate **1** was also coupled to aniline derivative **5**[22] to yield compound **6**, which was subsequently converted to compounds **7a–p** via reaction with a series of azides **3a–p** under click chemistry conditions. Compounds **4p** and **7p** were obtained by hydrolyzing compounds **4o** and **7o**, respectively.

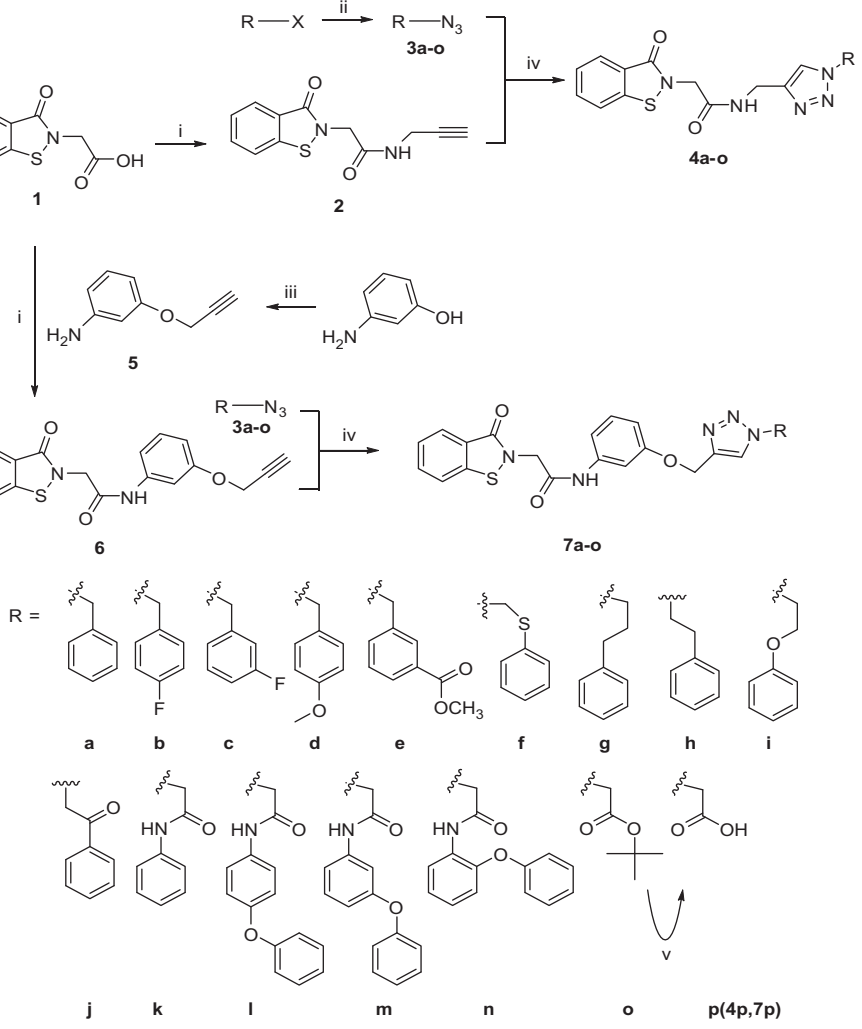
1.2. Biochemistry

The expression and purification of DENV NS2B-NS3pro and WNV NS2B-NS3pro have been previously described.[23,24] Enzyme assays and inhibition studies were carried out as previously described[25–28] and the results are summarized in Figures 2 and 3.

2. Results and discussion

Diseases caused by Dengue and related flaviviruses constitute a world-wide health problem for which there are currently no effective vaccines or small-molecule therapeutics. Consequently, there has been an increasing interest in the discovery and development of agents for DENV infection.[18]

As part of a program related to the discovery of DENV NS2B-NS3pro inhibitors, screening of a representative subset of compounds from a pharmacologically-rich in-house library of compounds lead to the identification of a benz[*d*]isothiazol-3(2*H*)-one derivative that exhibited inhibitory activity against DENV NS2B-NS3pro. This finding provided the impetus for the synthesis of two focused libraries based on general structures (I) and (II) (Fig. 1). The high synthetic tractability of (I) and (II) made possible the facile synthesis of the desired compounds (Scheme 1). Considerations associated with the design strategy employed in the construction of the libraries included using click chemistry to generate an electron-rich ring with hydrogen acceptor capabilities and enhancing binding by probing the nature of R (Scheme 1, compounds **4a–p**). The active site of DENV NS2B-NS3pro is rather shallow;



Scheme 1. Reagents and conditions: (i) CDI/THF/reflux then propargylamine or compound **5**; (ii) NaN_3/DMSO; (iii) NaOH/CH_3OH, removal of CH_3OH, then propargyl bromide/CH_3CN; (iv) sodium ascorbate/$CuSO_4$/BuOH/H_2O; (v) TFA.

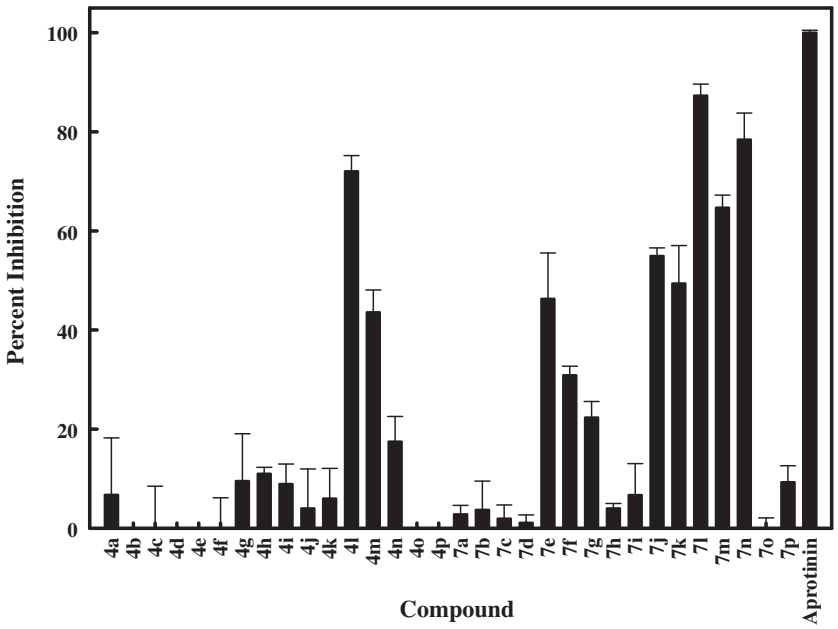


Figure 2. Inhibition of DENV2 NS2B-NS3pro by derivatives of compounds (I–II). The concentrations of the tested compounds and DENV2 NS2B-NS3 protease were 25 μM and 25 nM, respectively. The buffer used contained 200 mM Tris hydrochloride, 6 mM NaCl, and 30% glycerol, pH 9.5. The percent values were calculated from the relative fluorescence units obtained in the presence and absence of tested compound. All assays were performed in triplicate and the average values are shown. The assays were performed as described in Section 1.2.

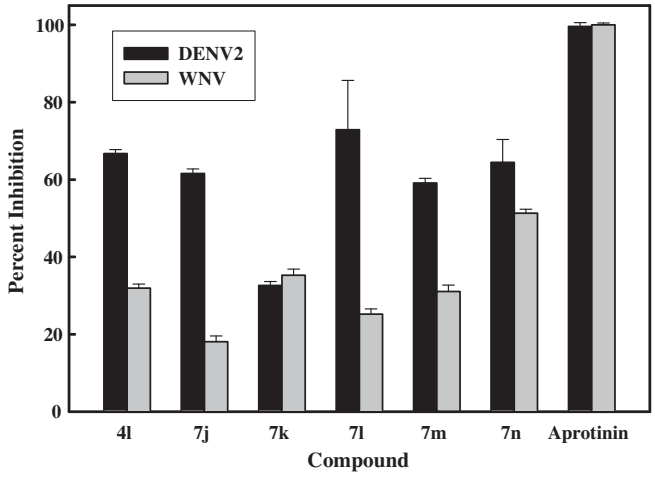


Figure 3. Inhibition of DENV2 and WNV NS2B-NS3pro by selected derivatives of (I–II) at 25 μM. The concentrations of DENV2 and WNV NS2B-NS3 proteases were 25 and 28 nM, respectively. The buffer used contained 200 mM Tris–HCl, 6 mM NaCl, and 30% glycerol and 0.1% CHAPS, pH 9.5. The percent values were calculated from the relative fluorescence units obtained in the presence and absence of tested compound. All assays were performed in triplicate and the average values are shown. The assays were performed as described in Section 1.2.

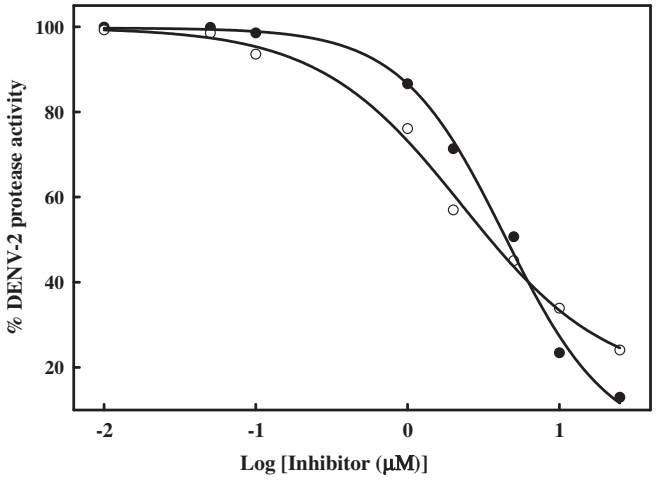


Figure 4. Determination of IC_{50} values of selected benz[*d*]isothiazol-3(2*H*)-one derivatives for inhibition of DENV2 NS2B-NS3 protease. The inhibitors were incubated with DENV2 NS2B-NS3 protease (50 nM) in buffer (200 mM Tris–HCl, 6 mM NaCl and 30% glycerol, pH 9.5) for 15 min. Bz-Nle-Lys-Arg-Arg-AMC (5.0 μM) was added to the mixture in a final volume of 100 μL. The fluorescence intensity was measured at 460 nm with excitation at 380 nm and converted to the percentage of protease activity in the absence and the presence of inhibitors. The inhibitors analyzed were as follows: (●) 7 l; (○) 7n. The solid line is the theoretical fitting curve based on the Sigmoidal Equation. All the spectra were recorded at 37 °C and shown after subtraction of the buffer spectrum. The apparent IC_{50} for compounds 7 l and 7n were 4.45 ± 0.06 and 3.48 ± 0.05, respectively.

consequently, we envisaged tethering the benz[*d*]isothiazol-3(2*H*)-one ring to the triazole ring via a *m*-aminophenol linker to provide additional favorable binding interactions with the enzyme (Scheme 1, compounds **7a–p**).

The results related to the interaction of the synthesized compounds with DENV NS2B-NS3pro are shown in Figure 2. With the exception of compounds **4l** and **4m**, the rest of the compounds based on structure (I) were inactive. However, several derivatives based on structure (II) showed noteworthy inhibitory activity (compounds **7e** and **7j–n**, Fig. 2). The IC_{50} values of compounds **4l** and **7j–n** were subsequently determined (Fig. 4) and are listed in Table 2. The kinetics of the interaction of a representative inhibitor **7n** against DENV2 NS2B-NS3pro was investigated in greater detail by determining K_m and V_{max} (Figs. 5 and 6, Table 2), as well as the K_i (determined to be 4.77 ± 0.05 μM). Because at micromolar concentrations many small molecules self–associate and the resulting colloid-like aggregates can lead to non-specific inhibition,[29,30] the synthesized compounds were re-screened in the presence of 0.1% CHAPS to confirm the activity of the compounds and to eliminate the possibility that the compounds acted as promiscuous inhibitors.[31,32] Indeed, the inhibitory activity of all

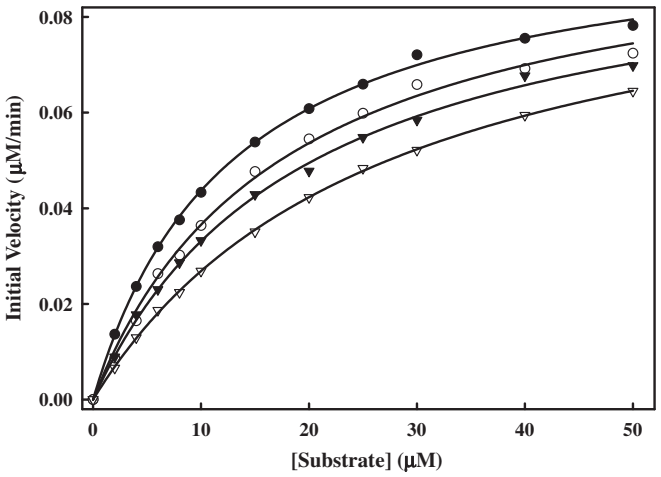


Figure 5. DENV2 NS2B-NS3pro activity in the absence and presence of inhibitor **7n**. The in vitro protease assays were performed as described in Section 3. The initial reaction rates of the *tetra*-peptide substrate cleavage catalyzed by 0.025 μM DENV2 NS2B-NS3 protease in the absence (●–●) and the presence of 1.0 μM (o-o), 2.0 μM (▼–▼) and 5.0 μM (▽–▽) were determined by varying concentrations of *tetra*-peptide substrate (0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40 and 50 μM range). The reactions were initiated by the addition of DENV2 NS2B-NS3pro and the fluorescence intensity at 460 nm was monitored with an excitation at 380 nm. Reactions were less than 5% completion in all cases to maintain valid steady-state measurements. The calculated K_m^{app} and V_{max} values are shown in Table 2.

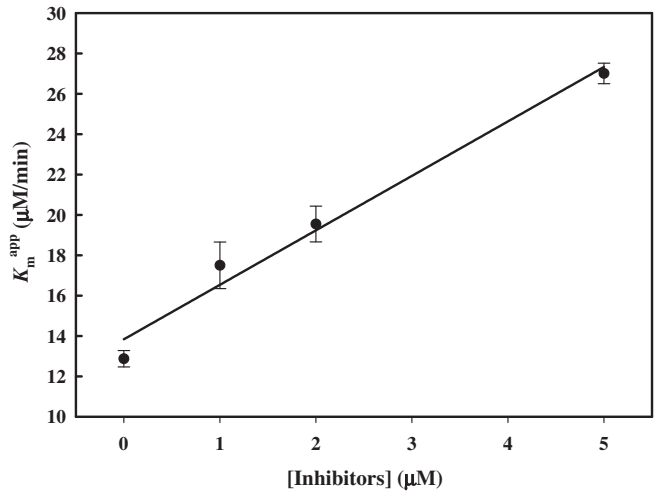


Figure 6. Plot of K_m values versus concentration of compound **7n**. K_m values with no inhibitor and at different concentrations of inhibitor were calculated and plotted versus different concentrations of inhibitor. From these data, the slope = 2.6988 ± 0.2712 μM and the intercept = 12.8732 ± 0.7427 μM were obtained and the K_i value calculated from the equation intercept/slope is 4.77 ± 0.05 μM.

Table 1
IC_{50} values against DENV-2 protease[a]

Compound	IC_{50} (μM)
4l	4.87 ± 0.07
7j	6.22 ± 0.09
7k	13.36 ± 0.21
7l	4.45 ± 0.06
7m	4.59 ± 0.07
7n	3.48 ± 0.05

[a] The IC_{50} values were determined as described under Section 3.

Table 2
Kinetics of **7n** against DENV2 NS2B-NS3pro

Inhibitor [**7n**] (μM)	K_m (μM)	V_{max} (μM/min)
0	12.8732 ± 0.4064	0.0999 ± 0.0012
1	17.5003 ± 1.1541	0.1006 ± 0.0029
2	19.5461 ± 0.8851	0.0978 ± 0.0020
5	27.0101 ± 0.5086	0.0994 ± 0.0010

active compounds remained essentially the same in the presence of detergent. It is evident from the results shown in Table 1 that the presence of a (phenoxy)phenyl moiety enhances inhibitory activity, however, the gain in potency is accompanied by a significant increase in molecular weight and hydrophobicity which are likely to impact adversely ADMET and drug-like characteristics.[33–35]

The closely related West Nile virus (WNV), a member of the *Flavivirus* genus of the *Flaviviridae* family, is an emergent viral pathogen, for which there are no effective vaccines or antiviral agents.[18,36,37] Consequently, the synthesized compounds were also screened against WNV NS2B-NS3pro[26] and the results are summarized in Figure 3. Compounds **4l** and **7j–n** displayed weak inhibitory activity toward the enzyme.

Inspection of the structures of the six most active derivatives of compound (II) toward DENV NS2B-NS3pro suggests that they have in common an R group (keto, amide or ester carbonyl) capable of engaging in hydrogen bonding, as well as hydrophobic or π–π interactions. A plausible mode of binding of energy-minimized inhibitor **7j** to the active site of DENV2 NS2B-NS3pro (homology model derived by Wichapong et al. from the corresponding WNV homolog[10]) is shown in Figure 7. This bound conformation, derived from molecular docking simulations, predicts that the ligand will occupy space adjacent to catalytic triad residues Ser135 and His51, with the core benz[*d*]isothiazol-3(2*H*)-one engaging in lipophilic interactions with the His51 and Trp50 side chains. The ligand triazole also accepts H-bonds from the backbone amide proton of Gly153 and the hydroxyl proton of Tyr161, donates a H-bond to the hydroxyl oxygen of Ser83, and engages in favorable lipophilic interactions with Pro132, Tyr150 and Tyr161. The terminal phenyl group on the ligand appears to be somewhat sterically limited by its presence in a relatively small lipophilic pocket, which might limit the prospects for lead optimization via aryl substituents, however the torsional flexibility availed from an aliphatic carbon immediately adjacent to the triazole (as per compounds **7k,l,m,n**) may permit the ligand to bending downwards to engage with other lipophiles in the more spacious hydrophobic pocket marked by the nearby Tyr161.

In conclusion, the synthesis and in vitro evaluation of two focused libraries of compounds have resulted in the identification of several inhibitors of DENV2 NS2B-NS3pro. The novelty of the structures and high synthetic tractability provide a reasonable basis for a hit-to-lead optimization campaign.

3. Experimental section

3.1. General

The ^{1}H spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. Melting points were determined on a Mel-Temp apparatus and are uncorrected. High resolution mass spectra (HRMS) were performed at the University of Kansas Mass Spectrometry Lab. Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, and Bachem). Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates.

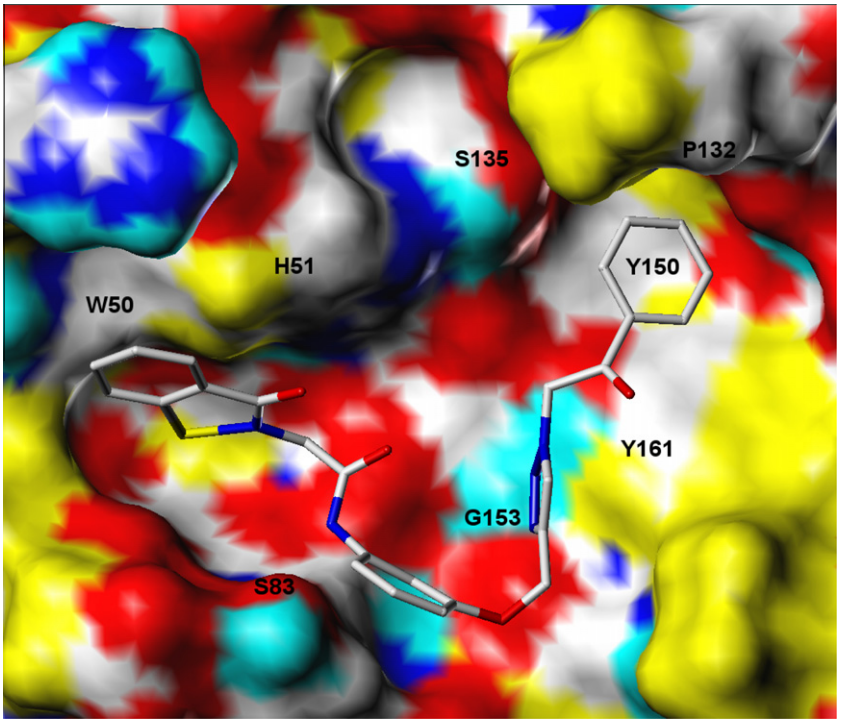


Figure 7. Conformation for inhibitor **7j** bound to the catalytic site of DENV2 NS2B-NS3 protease, as predicted from molecular docking simulations. The receptor surface is colored as follows: red = polar O, blue = polar N, cyan = polar (donatable) H, white = weakly polar aliphatic or aryl CH groups, yellow = nonpolar lipophiles. The ligand is rendered in stick form according to standard CPK coloring.

The TLC plates for the final compounds were eluted using two different solvent systems and were visualized using iodine chamber and/or UV light. Each individual compound was identified as a single spot on TLC plate (purity greater than 95%). DENV2 NS2B-NS3 protease (or WNV NS2B-NS3pro) substrate Bz-Nle-Lys-Arg-Arg-AMC was purchased from Bachem, Torrance, CA. or custom synthesized by NeoBioScience, Cambridge, MA.

3.2. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(prop-2-ynyl)acetamide (2)

To a solution of compound **1** (10.45 g; 50 mmol) in dry THF (100 mL) was added portionwise a suspension of 1,1′-carbonyldiimidazole (8.10 g; 50 mmol) in THF (25 mL). The resulting solution was stirred at room temperature for 20 min and refluxed for 10 min. A solution of propargylamine (2.75 g; 50 mmol) in THF (25 mL) was added and the solution was allowed to stir at room temperature overnight. A precipitate formed which was collected by vacuum filtration, leaving compound **2** as a white solid (5.71 g; 46% yield), mp 156–158 °C. ^{1}H NMR ($CDCl_3$): δ 2.20 (t, J = 4.2 Hz, 1H), 4.05 (dd, J = 7.9, 7.9 Hz, 2H), 4.50 (s, 2H), 6.62 (s, 1H), 7.45 (t, J = 11.1 Hz, 1H), 7.60 (d, J = 4.3 Hz, 1H), 7.68 (t, J = 11.1 Hz, 1H), 8.06 (d, J = 4.3 Hz, 1H).

3.3. Azides 3a–j were synthesized as described below using standard literature procedures[21]

3.3.1. Benzyl azide (3a)

To a solution of sodium azide (4.32 g; 66 mmol) in dry dimethyl sulfoxide (120 mL) was added benzyl chloride (7.62 g; 60 mmol), and the reaction mixture was stirred at room temperature overnight. Water (80 mL) was carefully added to the reaction mixture and the aqueous layer was extracted with diethyl ether (2 × 150 mL). The combined organic extracts were washed with water (50 mL) and the organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was concentrated, leaving compound **3a** as a yellow oil (6.31 g; 79% yield). ^{1}H NMR ($CDCl_3$): δ 4.38 (s, 2H), 7.27–7.44 (m, 5H).

3.3.2. *p*-Fluorobenzyl azide (3b)

Yellow oil (99% yield). ^{1}H NMR ($CDCl_3$): δ 4.31 (s, 2H), 7.00–7.12(m, 2H), 7.24–7.35 (m, 2H).

3.3.3. *m*-Fluorobenzyl azide (3c)

Yellow oil (90% yield). ^{1}H NMR ($CDCl_3$): δ 4.38 (s, 2H), 6.98–7.13 (m, 3H), 7.33–7.40 (m, 1H).

3.3.4. *p*-Methoxybenzyl azide (3d)

Colorless oil (98% yield). ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H), 4.23 (s, 2H), 6.90 (d, J = 6.9 Hz, 2H), 7.25 (d, J = 6.9 Hz, 2H).

3.3.5. Methyl 3-(azidomethyl)benzoate (3e)

Colorless oil (71% yield). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 3H), 4.41 (s, 2H), 7.42–7.59 (m, 3H), 8.00–8.15 (m, 1H).

3.3.6. (Azidomethyl)(phenyl)sulfane (3f)

Colorless oil (98% yield). ^{1}H NMR ($CDCl_3$): δ 4.58 (s, 2H), 7.25–7.39 (m, 3H), 7.50 (d, J = 8.3 Hz, 2H).

3.3.7. (3-Azidopropyl)benzene (3g)

Yellow oil (79% yield). ^{1}H NMR ($CDCl_3$): δ 1.85–2.00 (m, 2H), 2.72 (t, J = 25.0 Hz, 2H), 3.28 (t, J = 25.0 Hz, 2H), 7.18–7.40 (m, 5H).

3.3.8. (2-Azidoethyl)benzene (3h)

Yellow oil (41% yield). ^{1}H NMR ($CDCl_3$): δ 2.90 (t, J = 8.3 Hz, 2H), 3.50 (t, J = 8.3 Hz, 2H), 7.20–7.38 (m, 5H).

3.3.9. (2-Azidoethoxy)benzene (3i)

Yellow oil (69% yield). ^{1}H NMR ($CDCl_3$): δ 3.89 (t, J = 20.3 Hz, 2H), 4.29 (t, J = 20.3 Hz, 2H), 6.90–7.03 (m, 3H), 7.25–7.39 (m, 2H).

3.3.10. 2-Azido-1-phenylethanone (3j)

Reddish oil (85% yield). ^{1}H NMR ($CDCl_3$): δ 4.59 (s, 2H), 7.43–7.70 (m, 3H), 7.95 (d, J = 8.3 Hz, 2H).

3.4. Representative synthesis of compound 3k–o

3.4.1. 2-Azido-N-phenylacetamide (3k)

To a solution of aniline (9.3 mL; 100 mmol) in acetone (20 mL) kept in an ice bath was added 2-chloroacetyl chloride (4.1 mL; 50 mmol) dropwise with stirring. After the addition the ice bath was removed and the reaction mixture was stirred at room temperature for 4 h. Aqueous hydrochloric acid (10% v/v; 40 mL) was added to the reaction mixture, whereupon a white solid formed. The white precipitate was collected by vacuum filtration and washed with 5% hydrochloric acid (3 × 40 mL) and water (2 × 40 mL). The solid was allowed to air dry overnight (7.8 g; 92% yield), mp 122–124 °C. The dry solid (7.6 g; 45 mmol) was added to a solution of sodium azide (3.24 g; 49.5 mmol) in dry dimethyl sulfoxide (100 mL) and the reaction mixture was stirred at room temperature overnight. Water (50 mL) was added to the reaction mixture and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined extracts were washed with water (50 mL) and dried by anhydrous sodium sulfate. The drying agent was filtered off and the filtrate concentrated, leaving compound **3k** as a white solid (8.7 g; 98% yield), mp 57–59 °C. ^{1}H NMR ($CDCl_3$): δ 4.18 (s, 2H), 7.18 (t, J = 6.9 Hz, 1H), 7.35 (t, J = 8.6 Hz, 2H), 7.57 (d, J = 5.2 Hz, 2H), 8.00 (s, 1H).

3.4.2. 2-Azido-*N*-(4-phenoxyphenyl)acetamide (3l)

Reddish oil (80% total yield). ^{1}H NMR ($CDCl_3$): δ 4.18 (s, 2H), 7.00 (m, 4H), 7.09 (t, J = 6.7 Hz, 1H), 7.35 (t, J = 6.7 Hz, 2H), 7.50 (d, J = 4.9 Hz, 2H), 8.03 (s, 1H).

3.4.3. 2-Azido-*N*-(3-phenoxyphenyl)acetamide (3m)

Reddish oil (56% total yield). ^{1}H NMR ($CDCl_3$): δ 4.17 (s, 2H), 6.80 (m, 1H), 7.02 (d, J = 6.9 Hz, 2H), 7.15 (t, J = 8.6 Hz, 1H), 7.24–7.40 (m, 5H), 8.00 (s, 1H).

3.4.4. 2-Azido-*N*-(2-phenoxyphenyl)acetamide (3n)

Brown oil (93% total yield). ^{1}H NMR ($CDCl_3$): δ 4.18 (s, 2H), 6.90 (d, J = 5.5 Hz, 1H), 7.00–7.20 (m, 6H), 7.39 (t, J = 9.1 Hz, 2H), 8.62 (s, 1H).

3.4.5. *tert*-Butyl 2-azidoacetate (3o)

Colorless oil (99% total yield). ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 9H), 3.79 (s, 2H).

3.5. Representative synthesis of compounds 4a–p

3.5.1. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo-[*d*]isothiazol-2(3*H*)-yl)acetamide (4a)

To a solution of compound **2** (0.25 g; 1.0 mmol) and compound **3a** (0.33 g; 2.5 mmol) in *tert*-butyl alcohol and water (1:1) (15 mL) were added sodium ascorbate (0.04 g; 0.2 mmol) and copper(II) sulfate pentahydrate (0.005 g; 0.02 mmol) and the reacting mixture was stirred at room temperature overnight. The disappearance of compound **2** was monitored by TLC. Water (25 mL) was added and the reaction mixture was stirred for 5 min. The precipitate was collected by vacuum filtration and washed with water (25 mL) and diethyl ether (30 mL), leaving compound **4a** as a white solid (0.30 g; 79% yield), mp 193–195 °C. ^{1}H NMR ($CDCl_3$): 4.50 (s, 2H), 4.51 (s, 2H), 5.48 (s, 2H), 6.82 (s, 1H), 7.32–7.44 (m, 6H), 7.59 (d, J = 5.1 Hz, 1H), 7.64 (t, J = 6.9 Hz, 1H), 8.02 (s, 1H). HRMS (ESI): calcd for $C_{19}H_{17}N_5O_2S$ $[M+Na]^+$ 402.1001; found 402.0992.

3.5.2. *N*-((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (4b)

White solid (95% yield), mp 187–189 °C. ^{1}H NMR ($CDCl_3$): δ 4.49 (s, 2H), 4.51 (d, J = 5.2 Hz, 2H), 5.44 (s, 2H), 6.80 (s, 1H), 7.05 (t, J = 6.9 Hz, 2H), 7.26 (m, 1H), 7.43 (m, 2H), 7.59 (d, J = 5.2 Hz, 1H), 7.68 (d, J = 5.2 Hz, 1H), 8.02 (d, J = 5.2 Hz, 1H). HRMS (ESI): Calculated for $C_{19}H_{16}FN_5O_2S$ $[M+Na]^+$ 420.0906; found 402.0903.

3.5.3. *N*-((1-(3-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (4c)

White solid (78% yield), mp 191–193 °C. ^{1}H NMR ($CDCl_3$): δ 4.49 (s, 2H), 4.51 (d, J = 5.2 Hz, 2H), 5.44 (s, 2H), 6.80 (s, 1H), 6.98 (d, J = 6.9 Hz, 1H), 7.05 (d, J = 6.9 Hz, 1H), 7.36 (m, 1H), 7.43 (m, 2H), 7.59 (d, J = 5.2 Hz, 1H), 7.68(d, J = 5.2 Hz, 1H), 8.02 (d, J = 5.2 Hz, 1H). HRMS (ESI): calcd for $C_{19}H_{16}FN_5O_2S$ $[M+H]^+$ 398.1087; found 398.1068.

3.5.4. *N*-((1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (4d)

White solid (92% yield), mp 188–190 °C. ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H), 4.49 (s, 4H), 5.40 (s, 2H), 6.89 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H), 7.41 (m, 2H), 7.58 (d, J = 6.9 Hz, 1H), 7.63 (d, J = 6.9 Hz, 1H), 8.02 (d, J = 6.9 Hz, 1H). HRMS (ESI): calcd for $C_{20}H_{19}N_5O_3S$ $[M+H]^+$ 410.1287; found 410.1297.

3.5.5. Methyl 4-((4-((2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzoate (4e)

White solid (10% yield), mp 138–140 °C. ^{1}H NMR ($CDCl_3$): δ 3.96 (s, 3H), 4.45 (s, 2H), 4.47 (d, J = 5.2 Hz, 2H), 5.55 (s, 2H), 6.80 (s, 1H), 7.40–7.49 (m, 8H), 8.01 (d, J = 5.2 Hz, 1H). HRMS (ESI): calcd for $C_{21}H_{19}N_5O_4S$ $[M+Na]^+$ 460.1055; found 460.1044.

3.5.6. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-((1-(phenylthiomethyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (4f)

White solid (49% yield), mp 176–178 °C. ^{1}H NMR ($CDCl_3$): δ 4.49 (s, 2H), 4.51 (s, 2H), 5.58 (s, 2H), 6.79 (s, 1H), 7.31(s, 3H), 7.41 (t, J = 3.4 Hz, 2H), 7.58 (d, J = 3.4 Hz, 1H), 7.65 (t, J = 3.4 Hz, 2H), 8.03 (d, J = 3.4 Hz, 1H). HRMS (ESI): calcd for $C_{19}H_{17}N_5O_2S_2$ $[M+H]^+$ 412.0902; found 412.0909.

3.5.7. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-((1-(3-phenylpropyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (4g)

Brown solid (75% yield), mp 143–145 °C. ^{1}H NMR ($CDCl_3$): δ 2.19–2.25 (m, 2H), 2.65 (t, J = 12.1 Hz, 2H), 4.34 (t, J = 8.6 Hz, 2H), 4.51 (s, 2H), 4.53 (d, 2H), 6.80 (s, 1H), 7.18 (d, J = 5.2 Hz, 2H), 7.20–7.35 (m, 2H), 7.42 (t, J = 8.6 Hz, 1H), 7.49–7.60 (m, 2H), 7.63 (t, J = 8.6 Hz, 1H), 8.01 (s, 1H). HRMS (ESI): calcd for $C_{21}H_{21}N_5O_2S$ $[M+H]^+$ 408.1494; found 408.1475.

3.5.8. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-((1-phenethyl-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (4h)

Grey solid (78% yield), mp 173–175 °C. ^{1}H NMR ($CDCl_3$): δ 3.20 (t, J = 8.6 Hz, 2H), 4.46–4.59 (m, 6H), 6.81 (s, 1H), 7.10 (d, J = 6.9 Hz, 2H), 7.22–7.35 (m, 3H), 7.43 (t, J = 8.6 Hz, 2H), 7.59 (d, J = 5.2 Hz, 1H), 7.66 (t, J = 8.6 Hz, 2H), 8.02 (d, J = 5.2 Hz, 1H). HRMS (ESI): calcd for $C_{20}H_{19}N_5O_2S$ $[M+H]^+$ 394.1338; found 394.1333.

3.5.9. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-((1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (4i)

Gray solid (82% yield), mp 168–170 °C. ^{1}H NMR ($CDCl_3$): δ 4.33 (t, J = 6.9 Hz, 2H), 4.51 (s, 2H), 4.53 (d, J = 5.2 Hz, 2H), 4.75 (t, J = 6.9 Hz, 2H), 6.80 (s, 1H), 6.88 (d, J = 6.9 Hz, 2H), 7.00 (t, J = 8.6 Hz, 1H), 7.25–7.32 (m, 1H), 7.42 (t, J = 8.6 Hz, 2H), 7.53 (d, J = 6.9 Hz, 1H), 7.63 (t, J = 8.6 Hz, 2H), 7.75 (s, 1H), 8.02 (d, J = 5.2 Hz, 1H). HRMS (ESI): calcd for $C_{20}H_{19}N_5O_3S$ $[M+Na]^+$ 432.1106; found 410.1120.

3.5.10. *N*-((1-(2-Oxo-2-phenylethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (4j)

Brown solid (90% yield), mp 180–182 °C. ^{1}H NMR (DMSO): δ 4.40 (d, J = 3.7 Hz, 2H), 4.50 (s, 2H), 6.18 (s, 2H), 7.42 (t, J = 9.1 Hz, 1H), 7.60–7.79 (m, 5H), 7.85–8.10 (m, 4H), 8.80 (t, J = 9.1 Hz, 1H). HRMS (ESI): calcd for $C_{20}H_{17}N_5O_3S$ $[M+H]^+$ 408.1130; found 408.1150.

3.5.11. *N*-((1-(2-Oxo-2-(phenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)-methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (4k)

White solid (74% yield), mp 176–178 °C. ^{1}H NMR (DMSO): δ 4.40 (d, J = 4.2 Hz, 2H), 4.51 (s, 2H), 5.38 (s, 2H), 7.10 (t, J = 8.3 Hz, 1H), 7.39 (t, J = 8.3 Hz, 2H), 7.45 (t, J = 8.3 Hz, 1H), 7.60 (d, J = 6.5 Hz, 1H), 7.70 (t, J = 8.3 Hz, 1H), 7.91 (d, J = 6.5 Hz, 1H), 8.00 (d, J = 6.5 Hz, 2H), 8.82 (s, 1H), 10.51 (s, 1H). HRMS (ESI): calcd for $C_{20}H_{18}N_6O_3S$ $[M+Na]^+$ 445.1059; found 445.1068.

3.5.12. *N*-((1-(2-Oxo-2-(4-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamide (4l)

Gray solid (65% yield), mp 240–242 °C. ^{1}H NMR (DMSO): δ 4.39 (d, J = 4.3 Hz, 2H), 4.50 (s, 2H), 5.30 (s, 2H), 6.95–7.05 (m, 3H), 7.10 (t, J = 6.5 Hz, 2H), 7.38–7.45 (m, 3H), 7.60 (d, J = 6.5 Hz, 1H), 7.69 (t, J = 6.5 Hz, 2H), 7.90 (d, J = 4.3 Hz, 1H), 8.00 (d, J = 4.3 Hz, 2H), 8.78 (t, J = 6.5 Hz, 1H), 10.51 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{22}N_6O_4S$ $[M+Na]^+$ 537.1321; found 537.1338.

3.5.13. *N*-((1-(2-Oxo-2-(3-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamide (4m)

Gray solid (66% yield), mp 220–222 °C. ^{1}H NMR (DMSO): δ 4.39 (d, J = 4.3 Hz, 2H), 4.50 (s, 2H), 5.31 (s, 2H), 6.78 (d, J = 4.3 Hz, 2H), 7.03 (d, J = 4.3 Hz, 2H), 7.39 (t, J = 6.5 Hz, 2H), 7.29–7.48 (m, 4H), 7.70 (t, J = 6.5 Hz, 1H), 7.89 (d, J = 4.3 Hz, 1H), 7.98 (d, J = 4.3 Hz, 2H), 8.79 (t, J = 6.5 Hz, 1H), 10.56 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{22}N_6O_4S$ $[M+Na]^+$ 537.1321; found 537.1355.

3.5.14. *N*-((1-(2-Oxo-2-(2-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamide (4n)

Gray solid (77% yield), mp 213–215 °C. ^{1}H NMR (DMSO): δ 4.38 (d, J = 4.3 Hz, 2H), 4.50 (s, 2H), 5.39 (s, 2H), 6.87 (m, 1H), 7.02–7.21 (m, 6H), 7.40–7.46 (m, 3H), 7.70 (t, J = 6.9 Hz, 1H), 7.85–8.05 (m, 3H), 8.79 (t, J = 5.2 Hz, 1H), 10.09 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{22}N_6O_4S$ $[M+Na]^+$ 537.1321; found 537.1339.

3.5.15. *tert*-Butyl 2-(4-((2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)acetate (4o)

White solid (80% yield), mp 177–179 °C. ^{1}H NMR ($CDCl_3$): δ 4.52 (s, 2H), 4.59 (d, J = 3.4 Hz, 2H), 5.01 (s, 2H), 6.79 (s, 1H), 7.43 (t, J = 6.8 Hz, 1H), 7.56–7.70 (m, 3H), 8.04 (d, J = 5.2 Hz, 1H). HRMS (ESI): calcd for $C_{18}H_{21}N_5O_4S$ $[M+H]^+$ 404.1393; found 404.1391.

3.5.16. 2-(4-((2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamido)-methyl)-1*H*-1,2,3-triazol-1-yl)acetic acid (4p)

A solution of compound **4o** (0.21 g; 0.5 mmol) in TFA (15 mL) was allowed to stir at rt for 5 h while monitoring the disappearance of the starting material by TLC. TFA was evaporated off and the residue was treated with diethyl ether (75 mL) and stirred for 30 min at rt. A white solid formed which was collected by suction filtration (0.13 g; 75% yield), mp 230–232 °C. ^{1}H NMR (DMSO): δ 4.38 (d, J = 5.6 Hz, 2H), 4.50 (s, 2H), 5.25 (s, 2H), 7.43 (t, J = 5.4 Hz, 1H), 7.71 (t, J = 5.4 Hz, 1H), 7.83–8.00 (m, 3H), 8.79 (t, J = 5.4 Hz, 1H), 13.38 (s, 1H). HRMS (ESI): calcd for $C_{14}H_{14}N_5O_4S$ $[M+Na]^+$ 348.0767; found 348.0779.

3.6. 3-(Prop-2-ynyloxy)aniline (5)

Sodium hydroxide (4.30 g; 107.5 mmol) and 3-aminophenol (11.0 g; 100.9 mmol) were dissolved in methanol (200 mL) at gentle heating. The volatiles were evaporated off and the residual water was removed by four successive co-distillations with absolute ethanol (80 mL). The residue was dissolved in dry acetonitrile (200 mL) and propargyl bromide (14.24 g; 119.7 mmol) was added in three portions over 1 h period. The reaction mixture was stirred overnight, concentrated, and partitioned between 0.1 M sodium hydroxide (200 mL) and diethyl ether (200 mL). The layers were separated and the aqueous layer was extracted with diethyl ether (2×200 mL). The combined organic extracts were washed with brine (100 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent left a brown oily residue which was dissolved in methanol (160 mL) and treated with 4 M hydrochloric acid in dioxane (54 mL; 215.3 mmol). The volatiles were removed and the resulting brownish solid was suspended in boiling ethyl acetate (140 mL). Cooling the suspension to room temperature yielded a white crystalline solid which was collected by suction filtration and dried. The solid was dissolved in water (50 mL) and the pH was adjusted to ~11 using 10% aqueous sodium hydroxide (35 mL). The aqueous layer was extracted with ethyl acetate (2×100 mL), dried over sodium sulfate, filtered, and concentrated, leaving a yellow oil (10.3 g; 70% yield). ^{1}H NMR ($CDCl_3$): δ 2.50 (t, J = 3.1 Hz, 1H), 3.65 (br s, 2H), 4.63 (d, J = 3.1 Hz, 2H), 6.29–6.40 (m, 3H), 7.05 (t, J = 7.8 Hz, 1H).

3.7. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(3-(prop-2-ynyloxy)-phenyl)acetamide (6)

To a solution of compound **1** (6.27 g; 30 mmol) in dry THF (60 mL) was added portion wise a suspension of carbonyldiimidazole (4.86 g; 30 mmol) in THF (15 mL) and the mixture was stirred at rt for 20 min and then refluxed for 10 min. A solution of compound **5** (4.41 g; 30 mmol) in THF (15 mL) was added and the reaction mixture was stirred at rt overnight. A white precipitate formed which was collected by suction filtration (4.46 g; 44% yield), mp 173–175 °C. ^{1}H NMR ($CDCl_3$): δ 2.40–2.60 (m, 1H), 4.60 (s, 2H), 4.65 (s, 2H), 6.30–6.42 (m, 1H), 6.75 (d, J = 5.5 Hz, 1H), 7.00–7.30 (m, 2H), 7.45 (t, J = 7.3 Hz, 2H), 7.60 (d, J = 3.6 Hz, 1H), 7.70 (d, J = 3.6 Hz, 1H), 8.10 (d, J = 3.6 Hz, 1H), 8.63 (s, 1H).

3.8. Representative synthesis of compounds 7a–p

3.8.1. *N*-(3-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (7a)

A solution of compound **6** (0.34 g; 1.0 mmol) and compound **3a** (0.33 g; 2.5 mmol) in *tert*-butyl alcohol and water (1:1) (20 mL was treated with sodium ascorbate (0.20 g; 1.0 mmol) and copper(II) sulfate pentahydrate (0.02 g; 0.10 mmol) and the reacting mixture was stirred at room temperature overnight. TLC analysis showed the presence of unreacted compound **6**. An extra portion of compound **3a** (0.33 g; 2.5 mmol) was added and the reaction mixture was stirred at 40 °C overnight. Water (35 mL) was added and the resulting mixture was stirred for 5 min while kept in an ice bath. A precipitate formed which was collected by vacuum filtration and washed with water (25 mL) and diethyl ether (100 mL), leaving compound **7a** as a gray solid (0.33 g; 70% yield), mp 185–187 °C. ^{1}H NMR ($CDCl_3$): δ 4.60 (s, 2H), 5.18 (s, 2H), 5.50 (s, 2H), 6.70 (d, J = 5.5 Hz, 1H), 7.01 (d, J = 5.5 Hz, 1H), 7.15–7.40 (m, 5H), 7.41–7.72 (m, 6H), 8.09 (d, J = 5.5 Hz, 1H), 8.75 (s, 1H). HRMS (ESI): calcd for $C_{25}H_{21}N_5O_3S$ $[M+Na]^+$ 494.1263; found 494.1258.

3.8.2. *N*-(3-((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (7b)

Gray solid (94% yield), mp 185–187 °C. ^{1}H NMR ($CDCl_3$): δ 4.60 (s, 2H), 5.18 (s, 2H), 5.49 (s, 2H), 6.71 (d, J = 6.3 Hz, 1H), 6.98–7.10 (m, 3H), 7.18–7.37 (m, 4H), 7.44 (t, J = 5. 6 Hz, 1H), 7.55–7.74 (m, 4H), 8.10 (d, J = 4.3 Hz, 1H), 8.63 (s, 1H). HRMS (ESI): calcd for $C_{25}H_{20}FN_5O_3S$ $[M+Na]^+$ 512.1169; found 512.1190.

3.8.3. *N*-(3-((1-(3-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (7c)

Gray solid (93% yield), mp 198–200 °C. ^{1}H NMR ($CDCl_3$): δ 4.60 (s, 2H), 5.20 (s, 2H), 5.52 (s, 2H), 6.72 (d, J = 5.5 Hz, 1H), 6.95–7.10 (m, 4H), 7.19–7.40 (m, 3H), 7.45 (t, J = 7.4 Hz, 1H), 7.60 (d, J = 5.5 Hz, 2H), 7.69 (t, J = 7.4 Hz, 1H), 8.10 (d, J = 5.5 Hz, 1H), 8.62 (s, 1H). HRMS (ESI): calcd for $C_{25}H_{20}FN_5O_3S$ $[M+H]^+$ 490.1349; found 490.1345.

3.8.4. *N*-(3-((1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (7d)

White solid (68% yield), mp 163–165 °C. ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H), 4.59 (s, 2H), 5.17 (s, 2H), 5.45 (s, 2H), 6.70 (d, J = 5.4 Hz, 1H), 6.92 (d, J = 5.4 Hz, 2H), 7.00 (d, J = 3.6 Hz, 1H), 7.18–7.35 (m, 4H), 7.45–7.55 (m, 2H), 7.60 (d, J = 3.6 Hz, 1H), 7.68 (d, J = 3.6 Hz, 1H), 8.10 (d, J = 5.4 Hz, 1H), 8.60 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{23}N_5O_4S$ $[M+H]^+$ 502.1549; found 502.1526.

3.8.5. Methyl 4-((4-((3-(2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamido)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-benzoate (7e)

White solid (15% yield), mp 98–100 °C. ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 3H), 4.60 (s, 2H), 5.19 (s, 2H), 5.59 (s, 2H), 6.70 (d, J = 5.6 Hz, 1H), 7.00 (d, J = 5.6 Hz, 1H), 7.10–7.40 (m, 3H), 7.40–7.72 (m, 5H), 8.00–8.20 (m, 3H), 8.61 (s, 1H). HRMS (ESI): calcd for $C_{27}H_{23}N_5O_5S$ $[M+Na]^+$ 552.1318; found 552.1302.

3.8.6. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(3-((1-(phenylthio-methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide (7f)

White solid (60% yield), mp 158–160 °C. ^{1}H NMR ($CDCl_3$): δ 4.60 (s, 2H), 5.18 (s, 2H), 5.60 (s, 2H), 6.71 (d, J = 5.6 Hz, 1H), 7.01 (d, J = 5.6 Hz, 1H), 7.20–7.39 (m, 5H), 7.48 (t, J = 7.7 Hz, 1H), 7.60–7.75 (m, 4H), 8.11 (d, J = 3.7 Hz, 1H), 8.62 (s, 2H). HRMS (ESI): calcd for $C_{25}H_{21}N_5O_3S_2$ $[M+Na]^+$ 526.0984; found 526.0969.

3.8.7. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(3-((1-(3-phenyl-propyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide (7g)

White solid (40% yield), mp 169–171 °C. ^{1}H NMR ($CDCl_3$): δ 2.20–2.31(m, 2H), 2.63 (t, J = 8.9 Hz, 2H), 4.35 (t, J = 7.1 Hz, 2H), 4.59 (s, 2H), 5.20 (s, 2H), 6.75 (d, J = 3.6 Hz, 1H), 7.00 (d, J = 3.6 Hz, 1H), 7.14–7.40 (m, 7H), 7.45 (t, J = 5.4 Hz, 1H), 7.58–7.73 (m, 3H), 8.07 (d, J = 3.6 Hz, 1H), 8.60 (s, 1H). HRMS (ESI): calcd for $C_{27}H_{25}N_5O_3S$ $[M+H]^+$ 500.1756; found 500.1748.

3.8.8. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(3-((1-phenethyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide (7h)

White solid (52% yield), mp 175–177 °C. ^{1}H NMR ($CDCl_3$): δ 3.20 (t, J = 8.9 Hz, 2H), 4.59 (t, J = 8.9 Hz, 2H), 4.60 (s, 2H), 5.16 (s, 2H), 6.69 (d, J = 5.4 Hz, 2H), 7.00 (d, J = 5.4 Hz, 1H), 7.03–7.40 (m,8H), 7.45 (t, J = 7.1 Hz, 1H), 7.60 (d, J = 3.6 Hz, 1H), 7.65 (d, J = 3.6 Hz, 1H), 8.09 (d, J = 3.6 Hz, 1H), 8.62 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{23}N_5O_3S$ $[M+H]^+$ 486.1600; found 486.1597.

3.8.9. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(3-((1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetamide (7i)

Gray solid (96% yield), mp 163–165 °C. ^{1}H NMR (DMSO-d_6): δ 4.39 (t, J = 4.4 Hz, 2H), 4.63 (s, 2H), 4.79 (t, J = 4.4 Hz, 2H), 5.10 (s, 2H), 6.78 (d, J = 4.3 Hz, 1H), 6.89–7.00 (m, 3H), 7.12–7.38 (m, 5H), 7.42 (t, J = 6.5 Hz, 1H), 7.70 (t, J = 6.5 Hz, 1H), 7.90 (d, J = 4.3 Hz, 1H), 8.00 (d, J = 4.3 Hz, 1H), 8.29 (s, 1H), 10.28 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{23}N_5O_4S$ $[M+H]^+$ 502.1549; found 502.1547.

3.8.10. *N*-(3-((1-(2-Oxo-2-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamide (7j)

Gray solid (76% yield), mp 165–167 °C. ^{1}H NMR ($CDCl_3$): δ 4.60 (s, 2H), 5.22 (s, 2H), 5.82 (s, 2H), 6.77 (d, J = 4.3 Hz, 1H), 7.02 (d, J = 4.3 Hz, 1H), 7.15–7.35 (m, 3H), 7.40–7.70 (m, 6H), 7.80 (s, 1H), 8.00 (d, J = 4.3 Hz, 1H), 8.09 (d, J = 4.3 Hz, 1H), 8.60 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{21}N_5O_4S$ $[M+H]^+$ 500.1393; found 500.1377.

3.8.11. *N*-(3-((1-(2-Oxo-2-(phenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamide (7k)

White solid (74% yield), mp 205–207 °C. ^{1}H NMR (DMSO): δ 4.66 (s, 2H), 5.12 (s, 2H), 5.38 (s, 2H), 6.80 (d, J = 4.2 Hz, 1H), 7.02–7.39 (m, 7H), 7.41 (t, J = 6.5 Hz, 1H), 7.59 (d, J = 4.2 Hz, 2H), 7.69 (t, J = 6.5 Hz, 1H), 7.90 (d, J = 4.2 Hz, 1 h), 8.00 (d, J = 4.2 Hz, 1H), 8.22 (s, 1H), 10.38 (s, 1H). HRMS (ESI): calcd for $C_{26}H_{22}N_6O_4S$ $[M+H]^+$ 515.1502; found 515.1505.

3.8.12. *N*-(3-((1-(2-Oxo-2-(4-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2-(3*H*)-yl)acetamide (7l)

Brown solid (90% yield), mp 198–200 °C. ^{1}H NMR (DMSO): δ 4.66 (s, 2H), 5.13 (s, 2H), 5.36 (s, 2H), 6.80 (d, J = 4.3 Hz, 1H), 6.92–7.03 (m, 2H), 7.08–7.30 (m, 4H), 7.32–7.49 (m, 5H), 7.60 (d, J = 6.3 Hz, 1H), 7.70 (t, J = 6.5 Hz, 1H), 7.91 (d, J = 4.3 Hz, 1H), 8.00 (d, J = 4.3 Hz, 1H), 8.24 (s, 1H), 10.39 (s, 1H). HRMS (ESI): calcd for $C_{32}H_{26}N_6O_5S$ $[M+Na]^+$ 629.1583; found 629.1583.

3.8.13. *N*-(3-((1-(2-Oxo-2-(3-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (7m)

Gray solid (22% yield), mp 230–232 °C. ^{1}H NMR (DMSO): δ 4.65 (s, 2H), 5.11 (s, 2H), 5.30 (s, 2H), 6.78 (t, J = 10.6 Hz, 2H), 7.02 (d, J = 6.7 Hz, 1H), 7.17 (d, J = 6.7 Hz, 1H), 7.20–7.44 (m, 9H), 7.70 (t, J = 8.5 Hz, 1H), 7.91 (d, J = 4.3 Hz, 1H), 8.00 (d, J = 4.3 Hz, 1H), 8.20 (s, 1H), 10.33 (s, 1H), 10.52 (s, 1H). HRMS (ESI): calcd for $C_{32}H_{26}N_6O_5S$ $[M+Na]^+$ 629.1583; found 629.1586.

3.8.14. *N*-(3-((1-(2-Oxo-2-(2-phenoxyphenylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(3-oxobenzo[*d*]isothiazol-2-(3*H*)-yl)acetamide (7n)

White solid (94% yield), mp 185–187 °C. ^{1}H NMR ($CDCl_3$): δ 4.59 (s, 2H), 5.13 (s, 2H), 5.19 (s, 2H), 6.70 (d, J = 5.4 Hz, 1H), 6.80–6.95 (m, 3H), 6.95–7.38 (m, 7H), 7.45 (t, J = 5.4 Hz, 1H), 7.59–7.75 (m, 3H), 8.10 (m, 2H), 8.32 (d, J = 5.4 Hz, 1H), 8.61 (s, 1H). HRMS (ESI): calcd for $C_{32}H_{26}N_6O_5S$ $[M+Na]^+$ 629.1583; found 629.1573.

3.8.15. *tert*-Butyl 2-(4-((3-(2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-acetamido)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetate (7o)

White solid (82% yield), mp 188–190 °C. ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H), 4.60 (s, 2H), 5.03 (s, 2H), 5.20 (s, 2H), 6.75 (d, J = 5.4 Hz, 1H), 7.00 (d, J = 5.4 Hz, 1H), 7.18–7.38 (m, 3H), 7.44 (t, J = 7.3 Hz, 1H), 7.58–7.75 (m, 2H), 8.10 (d, J = 5.4 Hz, 1H), 8.60 (s, 1H). HRMS (ESI): calcd for $C_{24}H_{25}N_5O_5S$ $[M+H]^+$ 496.1655; found 496.1641.

3.8.16. 2-(4-((3-(2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamido)-phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetic acid (7p)

Compound **7o** (0.25 g; 0.5 mmol) was treated with TFA (15 mL) and the solution was stirred at RT for 5 h. TFA was evaporated off

and the residue was treated with diethyl ether (100 mL) and stirred at RT for 30 min. The solid was collected by suction filtration, leaving a white powder (0.11 g; 50% yield), mp 115–117 °C. ^{1}H NMR (DMSO): δ 4.65 (s, 2H), 5.11 (s, 2H), 5.30 (s, 2H), 6.79 (d, J = 5.6 Hz, 1H), 7.15–7.28 (m, 2H), 7.35 (s, 1H), 7.44 (t, J = 5.6 Hz, 1H), 7.76 (t, J = 5.6 Hz, 1H), 7.90 (d, J = 4.2 Hz, 1H), 8.00 (d, J = 4.2 Hz, 1H), 8.20 (s, 1H), 10.39 (s, 1H). HRMS (ESI): calcd for $C_{20}H_{17}N_5O_5S$ $[M+H]^+$ 440.1029; found 440.1024.

3.9. DENV2 NS2B-NS3pro expression and purification

The construction of the pQE30-NS2BH(QR)NS3pro expression plasmid has been described previously.[24] The protease was expressed from *Eschemrichia* coli strain Top 10 F′ (Invitrogen) transformed by pQE30-NS2BH(QR)-NS3pro plasmid and purified as described.[25] DENV2 NS2B-NS3pro contains the hydrophilic NS2B cofactor peptide (NS2BH) linked to the N-terminal NS3 protease domain via Q-R (P2 and P1 residues at the NS2B-NS3 junction site).

3.10. WNV NS2B-NS3pro expression and purification

The expression and purification of the WNV NS2BH-NS3pro containing the 5 amino acid spacer between the NS2B and NS3pro domains was previously described.[24]

3.11. In vitro DENV2 and WNV NS2B-NS3pro assays and inhibition studies

The compounds were dissolved in dimethylsulfoxide (DMSO) to make 50 mM stock solutions. The compounds were screened at 25 μM in 1% v/v DMSO in the final reaction mixture. Protease assays were performed in triplicates in Greiner Black 96 well plates. Each assay consisted of the reaction mixture of 100 μL containing 200 mM Tris–HCl buffer, pH 9.5, 30% glycerol, 25 nM DENV2 NS2B-NS3 protease (or 28 nM WNV NS2BH-NS3pro) and the compound. The enzyme and the compound were pre-incubated at room temperature prior to addition of the substrate (5 μM), Bz-Nle-Lys-Arg-Arg-AMC. The time course of the reaction at 37 °C was followed at every 90 s intervals for up to 30 min in a monochrometer-based spectrofluorometer (Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 380 and 460 nm, respectively. The percent inhibition for each compound at 25 μM was first determined. For determining IC_{50} values, the range of 10 nM, 50 nM, 0.1, 1, 2, 5, 10, and 25 μM concentrations of selected compounds were used. IC_{50} values were calculated using the SigmaPlot software.

3.12. Molecular modeling

Molecular docking calculations were carried out via the Surflex program[38] requesting 5 distinct randomized starting conformations for the ligand, and 100 final docked poses to select from. For these calculations, ligands were sketched and optimized (according to default molecular mechanics constraints and force fields) via the SYBYL 8.1 program (Tripos Associates, St. Louis, MO, 2009), and the receptor was modeled based on the homology model generated for DENV NS3/NS2Bpro[10] from the corresponding WNV homolog (the protein protonation state used herein presumed anionic aspartate and glutamate groups, and cationic arginines and lysines).

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