Supporting Information

NMR study of complexes between low-molecular weight inhibitors and the West Nile virus NS2B-NS3 protease

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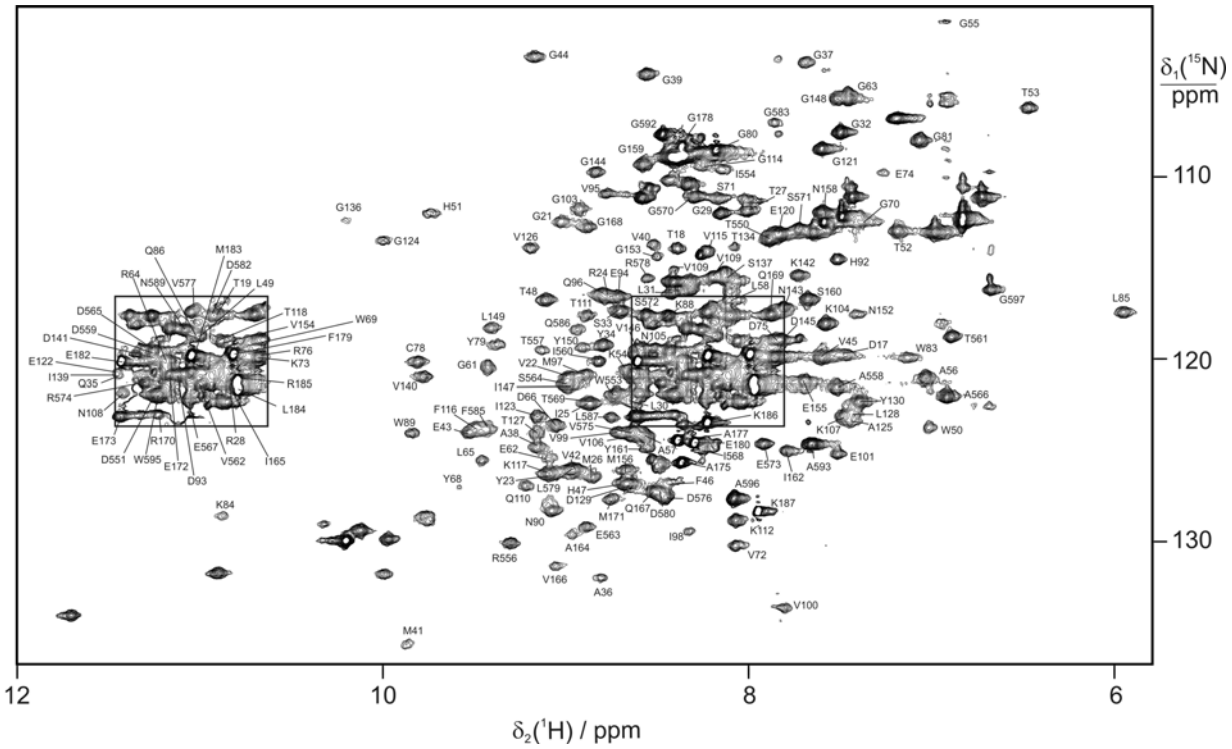
Table S1. PCR primers used in this study to produce different variants of WNV NS2B-NS3pro[1]

1131	5'-PO_4-TTAGCTGGTCGATCCCGCGAAATTAATACG-3' (30-mer)
1132	5'-PO_4-CCAGCTAACAAAAAACCCCTCAAGACCCG-3' (29-mer)
1133	5'-PO_4-TCGATCCCGCGAAATTAATACG-3' (22-mer)
1134	5'-PO_4-CAAAAAACCCCTCAAGACCCG-3' (21-mer)
1307[2]	5'-TTTTTTTTCATATGGCTTCTATGACCGGTCATCACCAT CACCATCACACAGACATGTGGATTGAGAGG-3' (68-mer)
1308[2]	5'- TTTGAATTCTTATTTCTTCCTCAACATTTCAGGTTCGAAGCC-3' (42-mer)
1314[3]	5'-GTGATTGGATTGTATG**C**<u>G</u>AACGGCGTCATC-3' (30-mer)
1315[3]	5'-GATGACGCCGTTC**G**<u>C</u>ATACAATCCAATCAC-3' (30-mer)

[1] Codons of mutated amino acids are underlined. Mutated bases are shown in bold.
[2] Forward and reverse primers to modify the N-terminus to MASMTGH_6-.
[3] Forward and reverse primers to generate the G151A mutant of NS3.

(a)



(b)

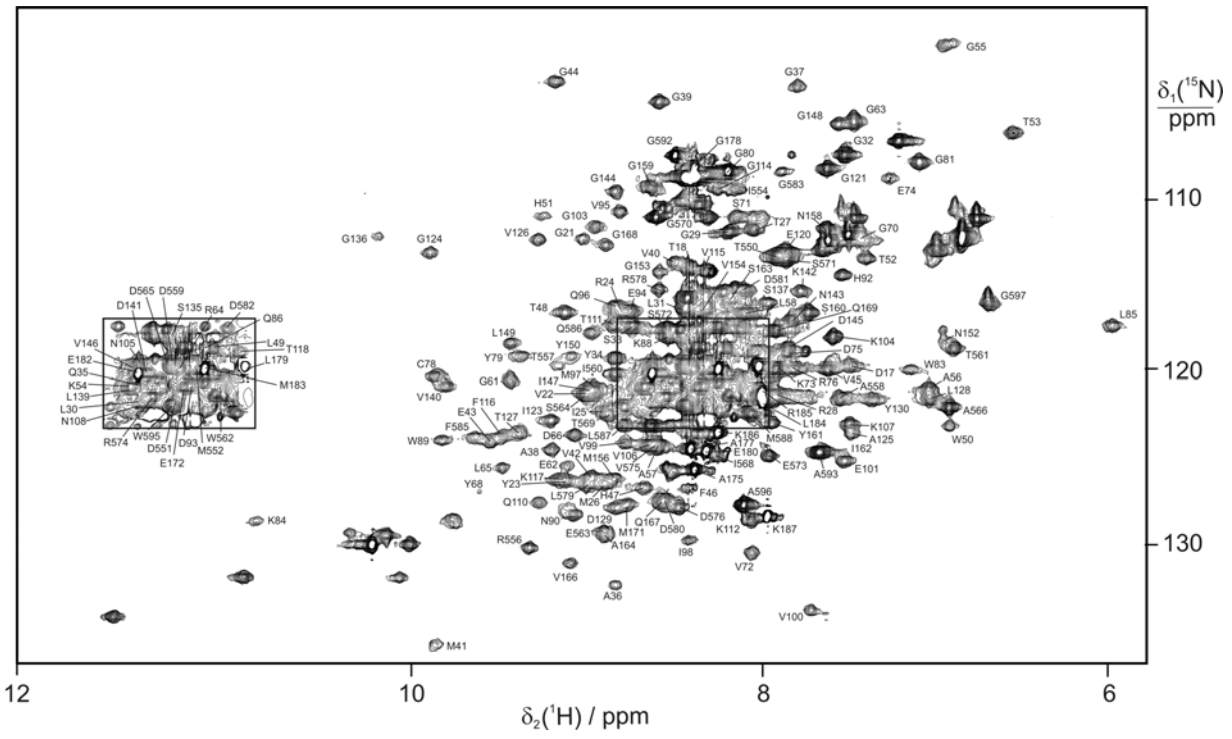


Figure S1. ^{15}N-HSQC spectra of 0.9 mM solutions of ^{15}N-labelled WNV NS2B-NS3pro(K96A) at 25 °C, pH 7.0, in the presence of (a) 3 mM **2** or (b) 3 mM **3**. The backbone amide resonance assignments are indicated. 500 was added to NS2B sequence numbers to distinguish them from those of NS3pro.

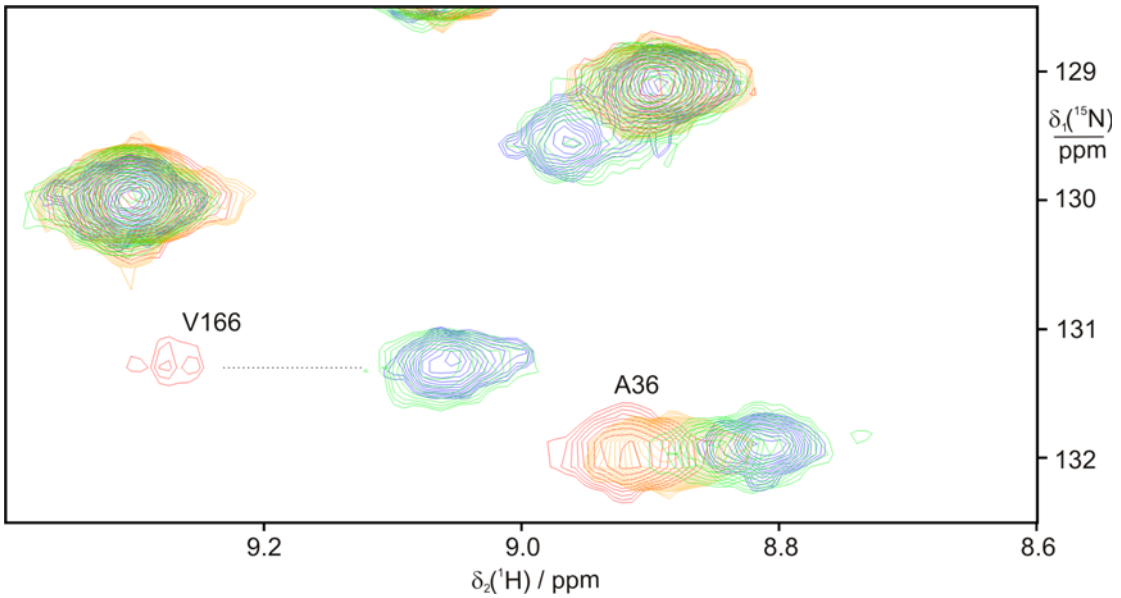


Figure S2. Selected spectral region from ^{15}N-HSQC spectra showing the effect of increasing concentrations of **2** on the NMR spectrum of WNV NS2B-NS3pro(K96A). The spectra were recorded at a ^{1}H NMR frequency of 800 MHz, using a 0.8 mM protein solution in 20 mM HEPES buffer (pH 7.0) at 25 °C. The concentrations of **2** were 0.0 (red), 0.3 (orange), 0.7 (green), and 1.5 mM (blue). The cross-peaks broadened significantly at intermediate titration ratios (orange spectrum). Increasing amounts of **2** shift the cross-peak of Val166 high-field along the dotted line. In the absence of **2**, it is broad due to conformational exchange in the protease (red spectrum) and it is below the lowest contour line plotted at intermediate titration ratios (orange spectrum).

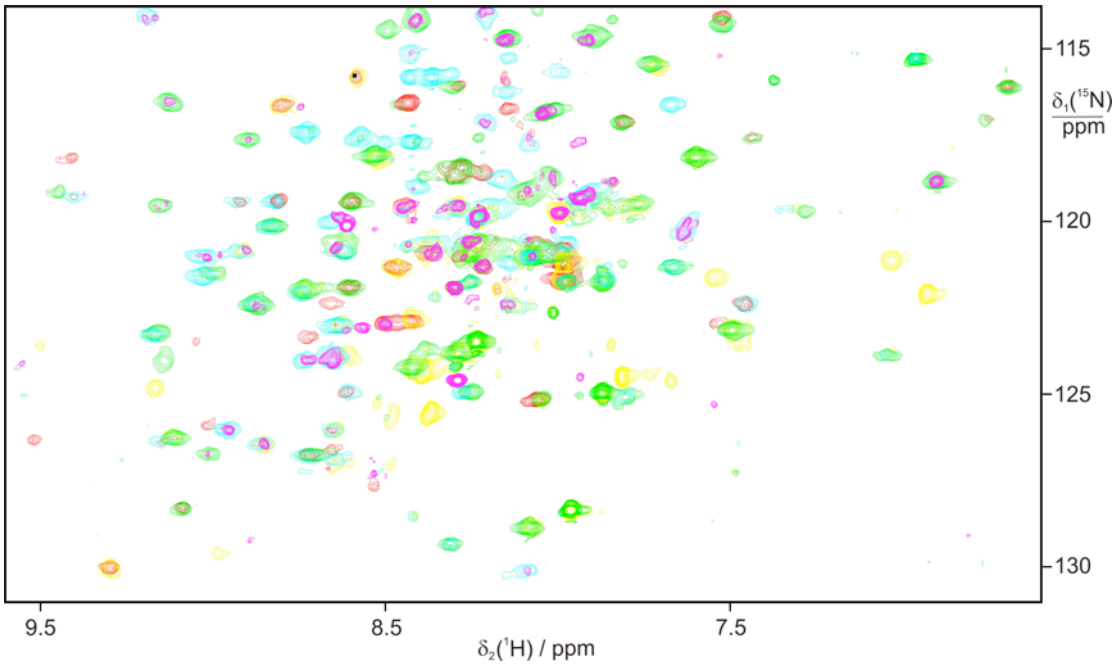


Figure S3. ^{15}N-HSQC spectra of combinatorially ^{15}N-labelled samples of WNV NS2B-NS3pro in the presence of **1**. Each sample contained about 50 μM protease and 200 μM **1** (t_{1max} = 32 ms, t_{2max} = 73 ms, Bruker 800 MHz NMR spectrometer). The five ^{15}N-HSQC spectra are superimposed, where each spectrum is of a sample containing a different set of ^{15}N-labelled amino acids. Red: Leu, Arg, Asp, Asn, Tyr, His, Cys; yellow: Ala, Lys, Arg, Phe, Gln, Met, Cys, Trp; green: Gly, Ile, Lys, Thr, Asn, His, Trp; cyan: Ser, Val, Ile, Gln, Tyr, Met, His, Trp; magenta: Glu, Val, Thr, Asp, Phe, Met, Cys. The spectral region shown contains the most crowded region of the ^{15}N-HSQC spectrum, illustrating the improved spectral resolution obtained by combinatorial labelling.

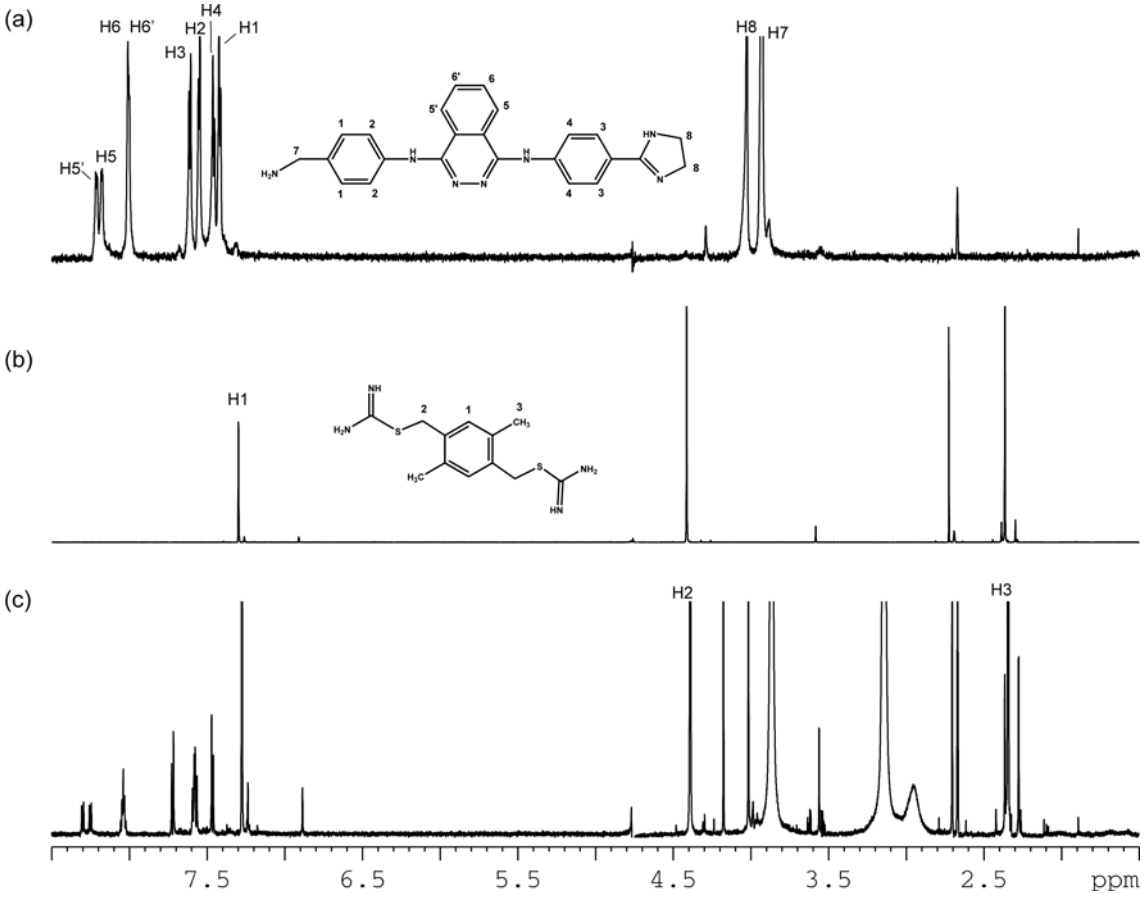


Figure S4. 800 MHz 1D ^{1}H-NMR spectra of the compounds **2** and **3** in the absence and presence of WNV NS2B-NS3pro(K96A) in D_2O solution containing 1.5% d_6-DMSO. The d_6-DMSO was used to dissolve the compounds and generated the resonance at about 2.7 ppm. (a) 0.5 mM **2**. (b) 0.3 mM **3**. (c) 0.6 mM **2** and 0.5 mM **3** in the presence of 30 μM WNV NS2B-NS3pro(K96A). The changes in chemical shifts compared to those in the absence of protease show that bound and free ligands are in fast exchange on the chemical shift time scale. This sample was used for the experiment of Figure 4.

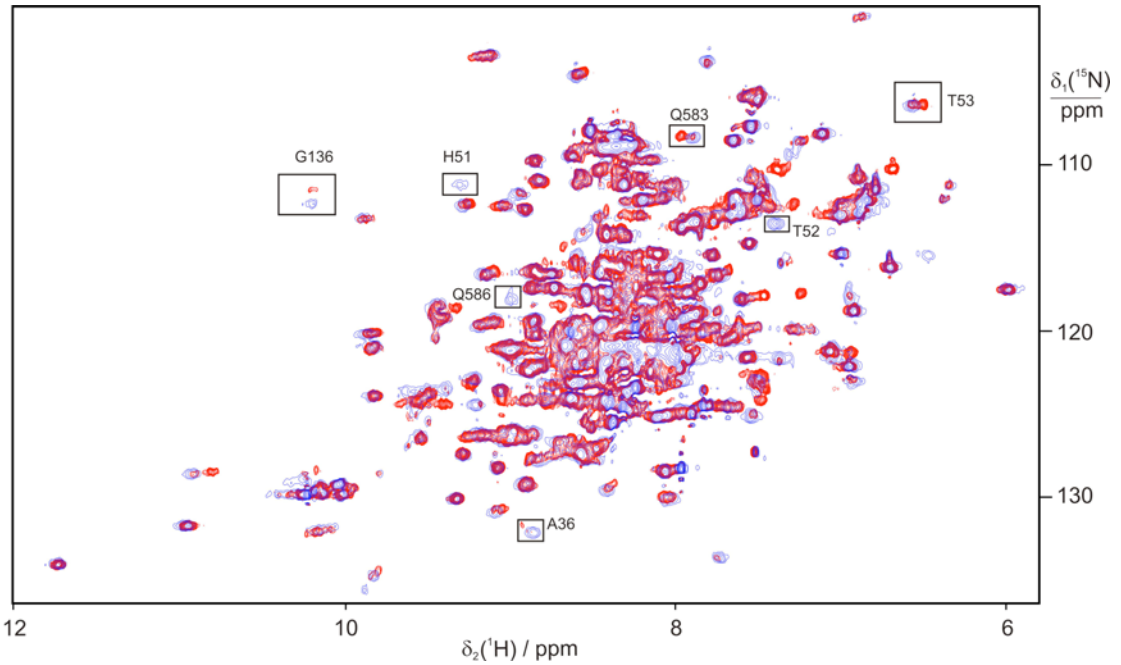


Figure S5. Superimposition of ^{15}N-HSQC spectra of 0.3 mM WNV NS2B-NS3pro(K96A) in the presence of 0.5 mM **3** (blue) and 0.2 mM **3** + 0.4 mM Bz-nKKR-H (red). The peptide out-competes **3** by forming a covalent bond with the active-site serine (Yin et al., *Bioorg Med Chem Lett* **16**, 40-43 (2006); Erbel et al., *Nat Struct Mol Biol* **13**, 372-373 (2006)). The spectra were recorded under the same conditions as those of Figure 3. Boxes highlight cross-peaks of residues lining the binding pocket. NS2B cross-peaks are distinguished from NS3 cross-peaks by adding 500 to their sequence number.

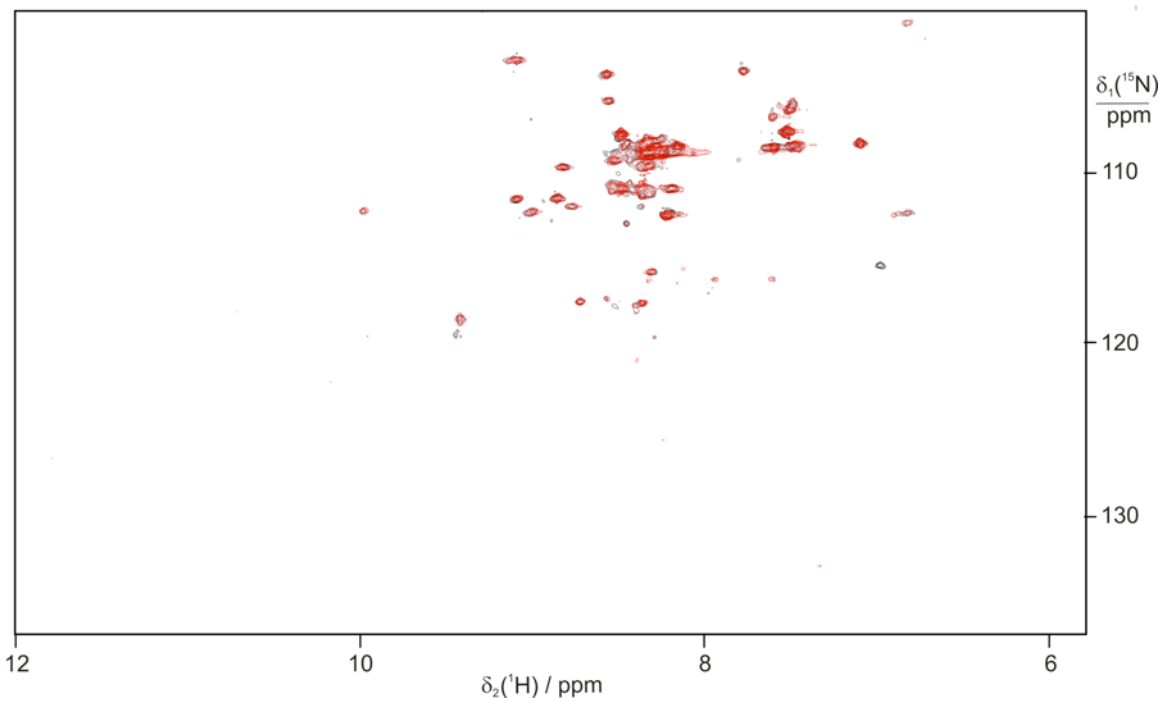


Figure S6. Superimposition of ^{15}N-HSQC spectra of 0.1 mM solutions of selectively ^{15}N-Gly labelled WNV NS2B-NS3pro(G151A) in the absence (red spectrum) and presence (black spectrum) of 0.2 mM **3**. The spectra are indistinguishable, showing that the G151A mutation abolishes binding of **3**.