Supporting Information

Flaviviral protease inhibitors identied by fragment-based library docking into a structure generated by molecular dynamics

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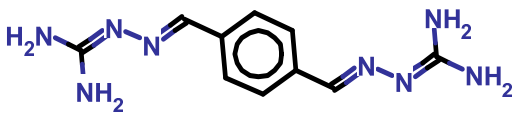
Contents

1 Structures and affinities of known inhibitors with methylguanidinium or 2-phenylimidazoline



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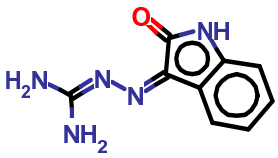

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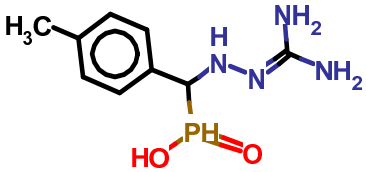

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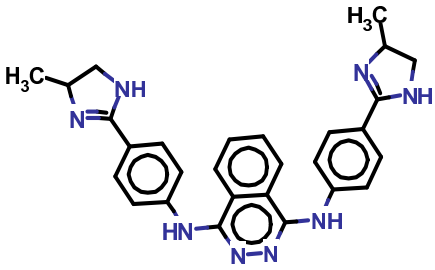

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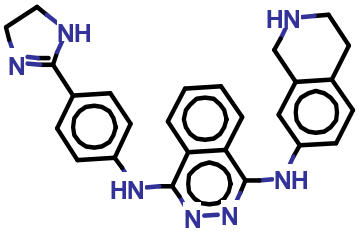

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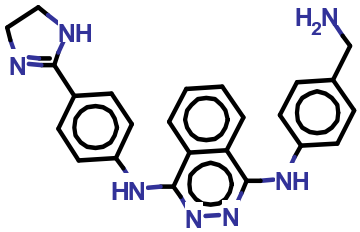

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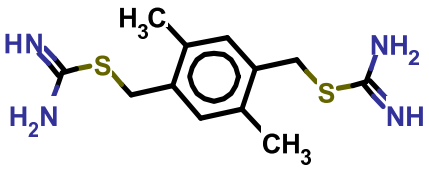

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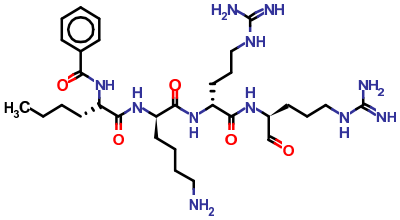


I8

Table S1. WNV NS3pro inhibitors

Compound	**Affinity (μM)**	**Reference**
I1	35 ± 5 [a]	Compound **1** in Ganesh et al. [1]
I2	16 ± 2 [a]	Compound **4** in Ganesh et al. [1]
I3	13 ± 1 [a]	Compound **5** in Ganesh et al. [1]
I4	24.5 [b]	Compound **1** in Bodenreider et al. [2]
I5	5.2 [b]	Compound **4** in Bodenreider et al. [2]
I6	10.6 [b]	Compound **5** in Bodenreider et al. [2]
3	40 [c]	Compound **1** in Ekonomiuk et al. [3]
I8	4.1 [d]	Inhibitor in X-ray structure (PDB code 2fp7) [4]

[a] K_i value.

[b] IC_{50} value.

[c] K_d value. The binding affinity of the compound was determined by measuring the change of ^{1}H chemical shifts of the backbone amide protons of Thr52 and Glu101, which are located in the active site of the protein, as a function of increasing concentration of the compound. K_d values of 30 ± 10 μM and 50 ± 10 μM were derived from the fits to the chemical shift changes of Thr52 and Glu101, respectively

[d] IC_{50} value. The Bz-Nle-Lys-Arg-Arg-H inhibitor is one of the 37 peptide aldehyde inhibitors described in Knox et al. [5]. Notably, most of the peptide aldehyde inhibitors contain arginine residue at P1 and/or P2 position.

2 The five best poses of methylguanidinium

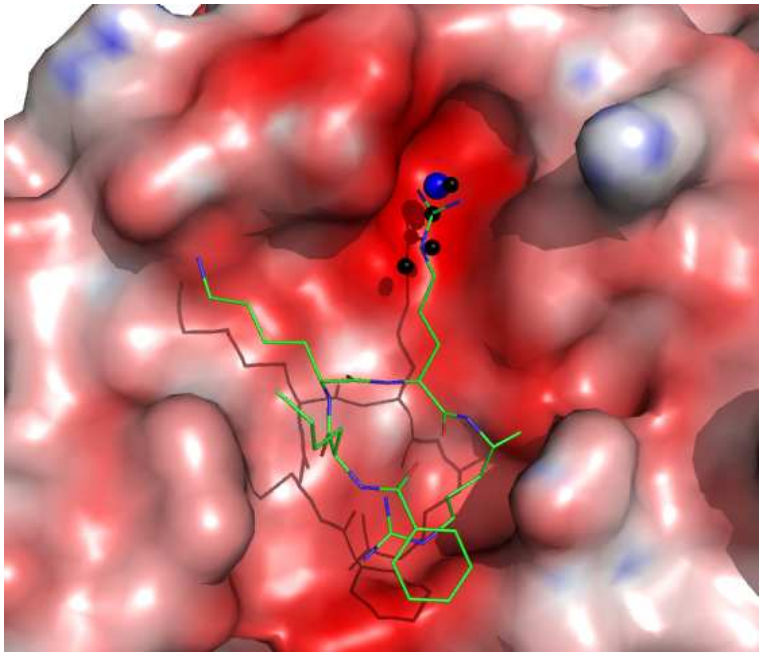
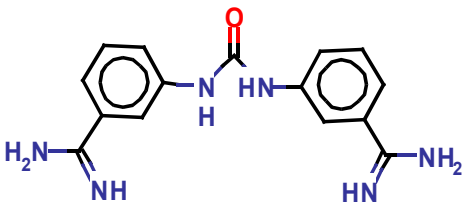


Figure S1: The methylguanidinium fragment is docked to the X-ray conformation of the protease. The geometrical centers of the best poses obtained by the program SEED [6, 7] are represented by spheres. The blue larger sphere corresponds to the best pose of all. This pose and those identified by the four black spheres are the five poses with the most favorable energy, and they are all located in the S2 pocket. The conformation of the inhibitor Bz-Nle-Lys-Arg-Arg-H is shown for reference (green carbon atoms). The surface of the protease is colored according to electrostatic potential. The figure was prepared using PyMOL (Delano Scientific, San Carlos, CA) and the APBS program [8] was used for calculation of the electrostatic surface.

3 Structures of five compounds from docking



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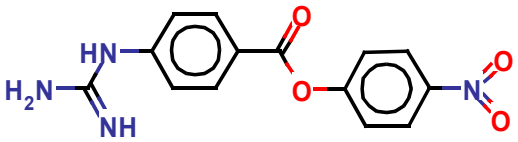

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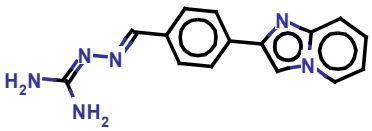

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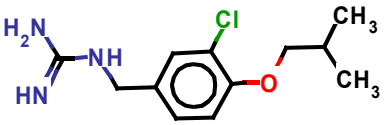

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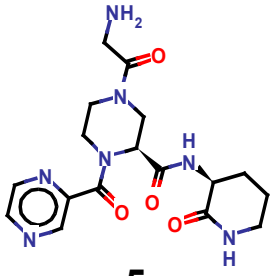

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4 NMR validations

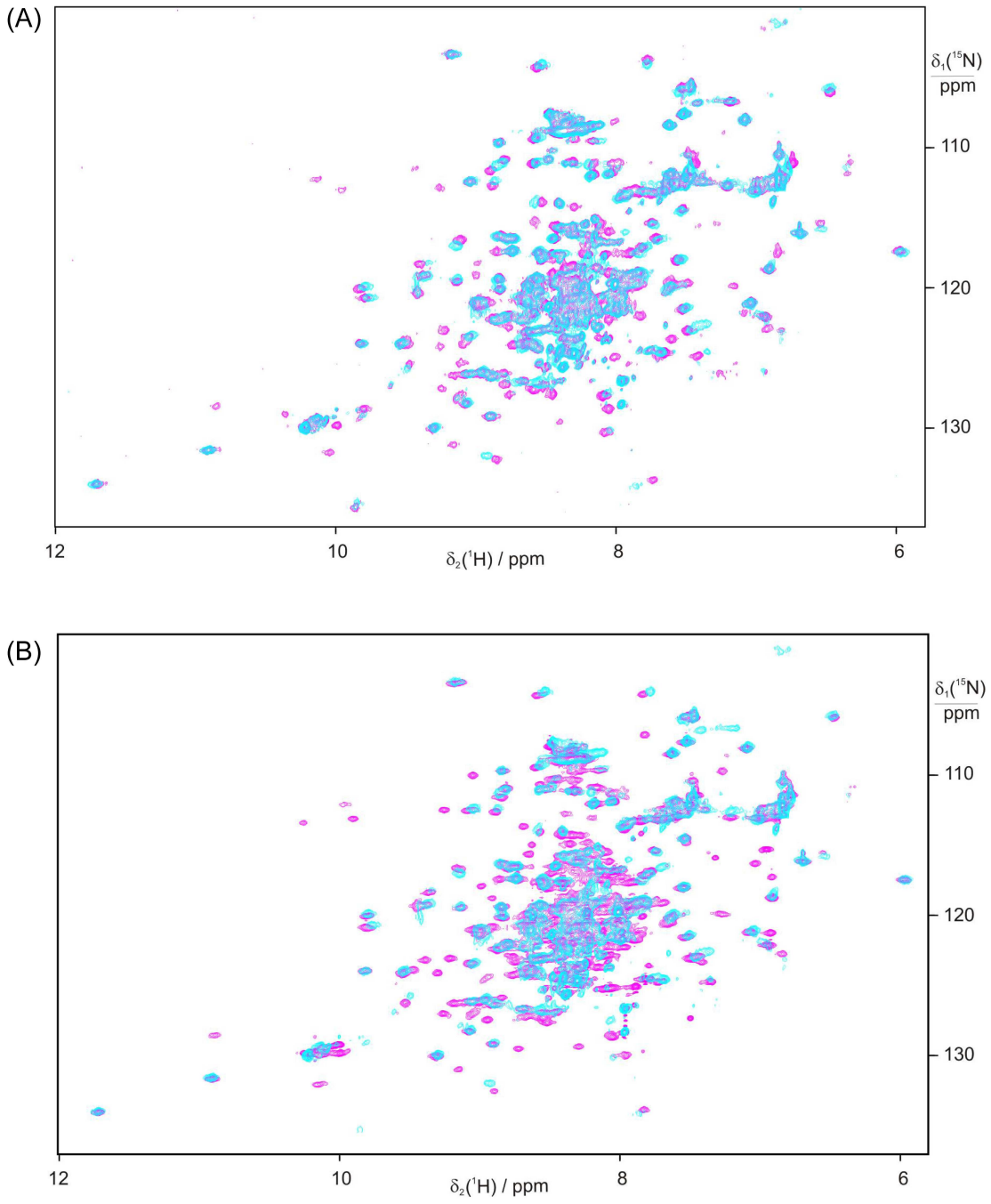


Figure S2: Structure stabilizing effect of compounds **1** and **2**. (A) Superimposition of the ^{15}N-HSQC spectrum of WNV NS2B(K96A)-NS3pro in the absence of an inhibitor (cyan) onto the corresponding spectrum in the presence of an excess of compound **1** (magenta). (B) Same as (A), using compound **2** instead of compound **1**. NMR signals appear in the presence of the inhibitors which, in the absence of inhibitor, are broadened beyond detection due to conformational equilibria in the protein.

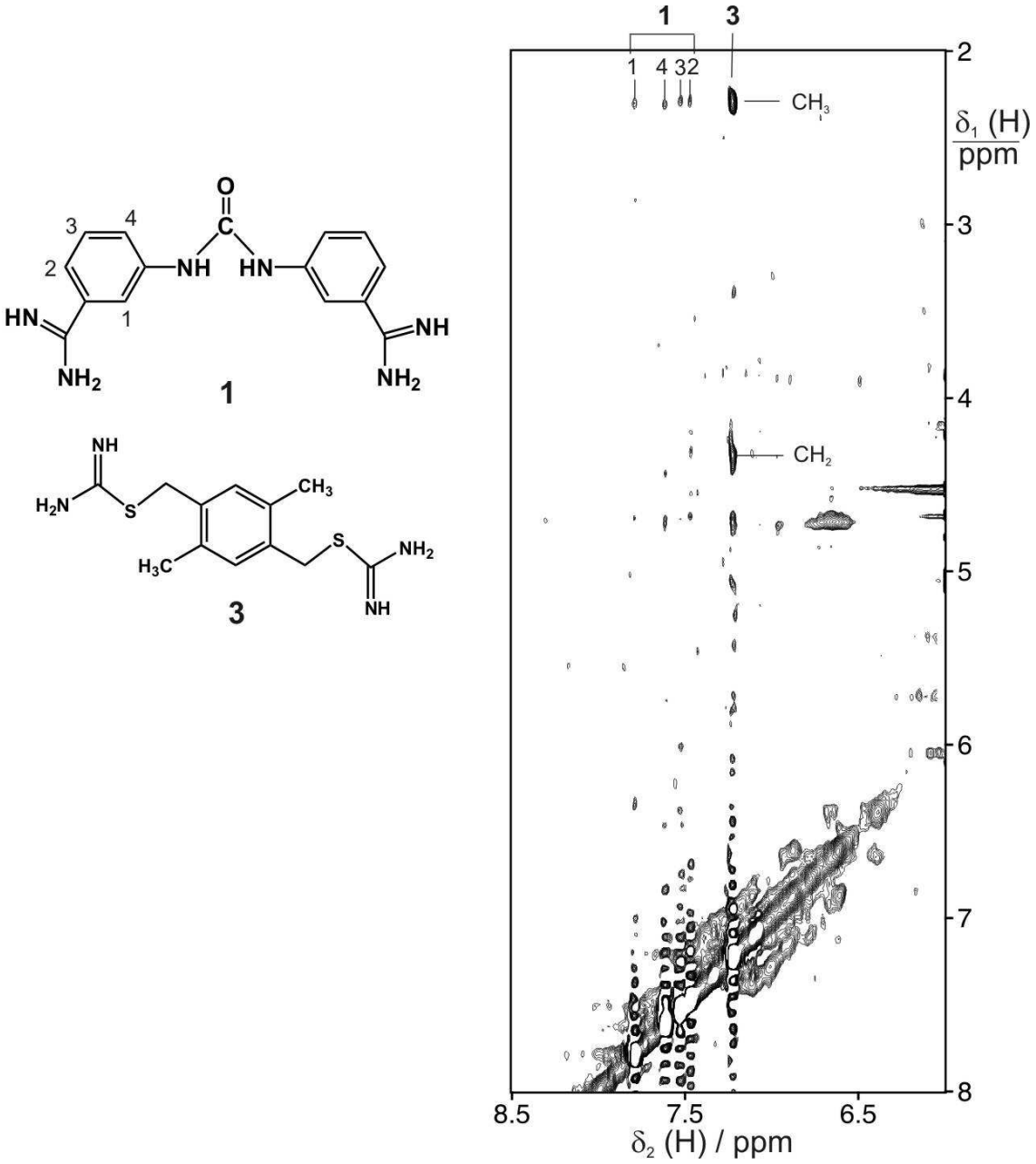
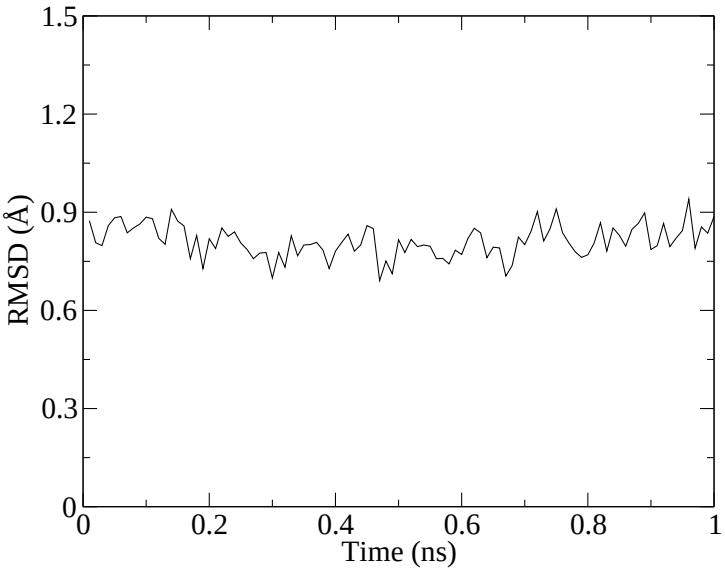
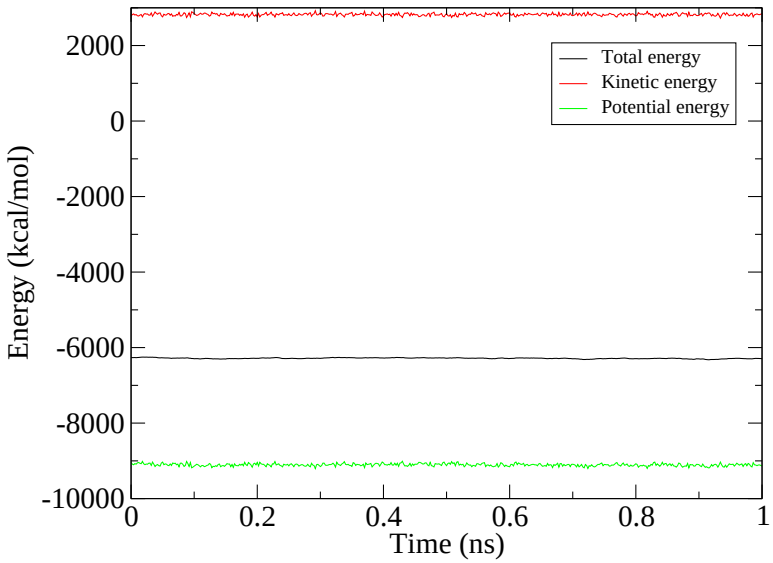


Figure S3: NOESY spectrum recorded with a 0.05 mM solution of WNV NS2B(K96A)-NS3pro in D_2O in the presence of 0.6 mM of compound **1** and 0.6 mM of compound **3**. The spectrum was recorded in 7 hours at 25 ^{o}C on a Bruker 600 MHz NMR spectrometer equipped with a TCI cryoprobe, using a mixing time of 150 ms, $t_{1max} = 18$ ms, and $t_{2max} = 155$ ms. The spectrum shows the transferred NOEs between the aromatic ring proton and the methyl and methylene groups of compound **3**, and cross-peaks between the methyl group of compound **3** and the aromatic ring protons of compound **1** (atom numbering shown with the structures of the compound on the left). A weak cross-peak also correlates the methylene resonance of compound **3** with H2 of compound **1**.

5 Time series of backbone RMSD and energy





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