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Mechanism of Inhibition of SARS-CoV-2 M^{pro} by N3 Peptidyl Michael Acceptor Explained by QM/MM Simulations and Design of New Derivatives with Tunable Chemical Reactivity

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Computational Methods

Computational details of the molecular models set up. The coordinates of atoms of SARS-CoV-2 M^{pro} were adapted from the X-Ray structure of its complex with the **N3** inhibitor, as available in Protein Data Bank (PDB ID 6LU7).[1] Biological assemble (homodimer) was build using Discovery Studio Visualizer 19. Inhibitor **N3** was replaced by two Michael acceptor inhibitors (compounds **B1** and **B2**) to create two new enzyme-inhibitor models. Missing force field parameters for Michael acceptor inhibitors were generated using the Antechamber program[2] available in AmberTools package. The protonation state of titratable residues was determined at pH 7 by estimating pKa shifts generated by the local environment on titratable groups using the empirical program PropKa ver. 3.0.3.[3] After a detailed inspection of surrounding of each histidine residue, it was concluded that all should be neutral. However catalytically important His41 and His80 should be protonated in Nδ position, while remaining His64, His163, His164, His172, and His246 on Nε. Presence of S-S linkage between Cys residues was not detected. According to the determined pKa values, all Cys residues should be protonated except the catalytic Cys145 that was treated quantum mechanicaly and its protonation state is studied in detail in the first step of the reaction. After adding missing hydrogen atoms, the systems were neutralized by addition of 8 sodium counter ions and placed in the box of 9.370 × 10.010 × 11.130 nm^3 of TIP3P[4] water molecules. Such a prepared model was further optimized, heated up to 310 Kelvins with 0.001 K temperature increment. and equilibrated using Molecular Dynamic (MD) simulations under NPT conditions with AMBER ff03 force field,[5] as implemented in NAMD software.[6] Subsequently, 10 ns NVT MD simulations were performed in the inhibitor-protein covalently bound complex. The constant temperature during MD simulation was controlled using the Langevin thermostat.[7]

Analysis of the time evolution of the root-mean-square deviations (RMSDs) of the backbone atoms of the protein models confirmed that all the systems were equilibrated (see Figures S1a, S2a, and S3a). Analysis was done using cpptraj software.[8] After optimization of the system, those residues located 25 Å beyond of the double C_β-Cα bond of the inhibitor were kept frozen in the remaining calculations. Potential energy surfaces, free energy surfaces and spline corrections have been performed using fDynamo library[9] together with implemented AMBER force field.[10] A Cut-off for nonbonding interactions was applied using a smooth switching function between 14.5 to 16 Å.

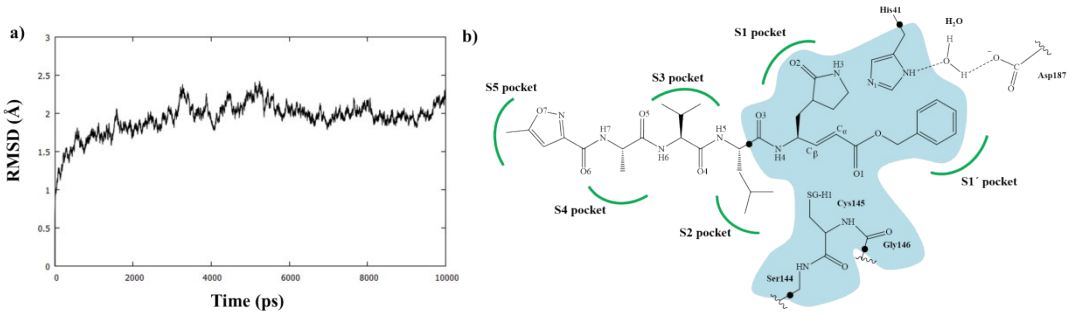

Figure S1. a) RMSD along the classical 10 ns MD simulation for the backbone atoms of the SARS-CoV-2 M^{pro} cysteine protease. Simulations performed on the **E-I** state corresponding to the enzyme-inhibitor **N3** model. b) Details of the atoms included in the QM region (blue region) in QM/MM calculations. The black dots represent the hydrogen link atoms.

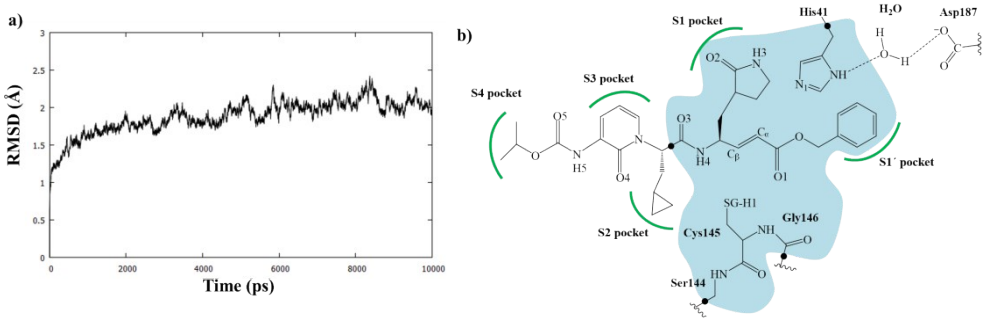

Figure S2. a) RMSD along the classical 10 ns MD simulation for the backbone atoms of the SARS-CoV-2 M^{pro} cysteine protease. Simulations performed on the **E-I** state corresponding to the enzyme-compound **B1** model. b) Details of the atoms included in the QM region (blue region) in QM/MM calculations. The black dots represent the hydrogen link atoms.

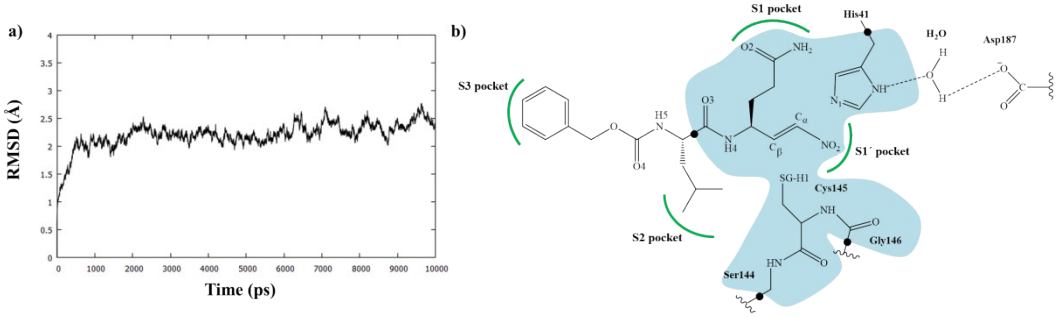

Figure S3. a) RMSD along the classical 10 ns MD simulation for the backbone atoms of the SARS-CoV-2 M^{pro} cysteine protease. Simulations performed on the **E-I** state corresponding to the enzyme-compound **B2** model. b) Details of the atoms included in the QM region (blue region) in QM/MM calculations. The black dots represent the hydrogen link atoms.

Computational details of the QM/MM simulations. In this work, an additive hybrid QM/MM scheme was employed for the construction of the total Hamiltonian where the total energy is obtained from the sum of each contribution to the energy.

$$E_{QM/MM} = E_{QM} + E_{QM/MM}^{elect} + E_{QM/MM}^{vdW} + E_{MM} \quad \text{(S1)}$$

Here, E_{QM} describes the atoms in the QM region, $E_{QM/MM}$ defines the interaction between the QM and MM region and E_{MM} describes the rest of the MM region. The QM subset of atoms includes the P1´ and P1 positions of the inhibitor, together with Cys145 and His41 residues of the protein. Four link atoms were inserted where the QM/MM boundary intersected covalent bonds in the positions indicated on Figures S1b, S2b and S3b. Thus, QM part consisted of 75 atoms for the inhibitors **N3** and compound **B1**, and 57 atoms for the compound **B2**. The Austin Model 1 (AM1)[11] semiempirical and the Minnesota Functional M06-2X[12] with the standard 6-31+G(d,p) basis set[13] were used to treat the QM sub-set of atoms, as implemented in Gaussian09 program.[14] The protein of the two monomers and solvent water molecules were treated with the AMBER[5] , as implemented in fDynamo,[10] and TIP3P[4] force fields.

Contribution of each residue of the protein to the interaction energy with defined part of substrate was computer using the following expression:

$$E_{QM/MM}^{Int} = \sum \left\langle \Psi \left| \frac{q_{MM}}{r_{e,MM}} \right| \Psi \right\rangle + \sum\sum \frac{Z_{QM} q_{MM}}{r_{QM,MM}} + E_{QM/MM}^{vdW} \quad \text{(S2)}$$

This interaction energy can be exactly decomposed in a sum over residues provided that the polarized wave function (Ψ) is employed to evaluate this energy contribution. The global polarization effect can be obtained from the gas phase energy difference between the polarized, Ψ, and non-polarized, Ψ_0, wave functions.

Potential Energy Surfaces. Potential energy surfaces (PESs) were obtained by grid scanning of distinguished reaction coordinates most suitable for describing each of the chemical step, as described below. A harmonic constraint was used to maintain the proper interatomic distances along the reaction coordinate, and a series of conjugate gradient optimizations and L-BFGS-B optimization algorithms were applied to obtain the final potential energy of the minimized constrained geometry. A micro-macro iteration

optimization algorithm[15, 16] was used to localize, optimize, and characterize the key TS structures using a Hessian matrix containing all the coordinates of the QM subsystem at M06-2X/MM level of theory with the standard 6-31+G(d,p) basis set. The gradient norm of the remaining movable atoms was maintained less than 0.01 kcal·mol^{-1}·Å^{-1}. Intrinsic reaction coordinates (IRCs) were traced down from located TSs to the valleys of the reactants, intermediates and products in mass-weighted Cartesian coordinates.

Free Energy Surfaces. FESs were obtained, in terms of Potentials of Mean Force (PMF), for every step of the reaction using the Umbrella Sampling approach[17] combined with the Weighted Histogram Analysis Method (WHAM).[18] Series of MD simulations, with the QM subset of atoms described at AM1 level and the remaining atoms of the protein and water molecules with AMBER and TIP3P force fields, respectively, were performed adding a constraint along the selected reaction coordinates with an umbrella force constant of 2500 kJ·mol^{-1}·Å^{-2}. In every window, QM/MM MD simulations were performed with a total of 5 ps of equilibration and 20 ps of production at 310 K with a time step of 1 fs. According to the overlapping of the sampling in consecutive windows (see Figures S4 and S5) this selection appears to be well justified.

The estimated error associated to this technique for these kind of systems is usually accepted to be ca 1 kcal·mol^{-1}.[19] Additionally, statistical errors were computed using the bootstrapping analysis[20] by means of the code written by Grossfield.[21] 200 PMFs per step, randomly generated from the original PMFs, render similar magnitudes of statistical errors, as reported in Table S1.

Regarding the selection of the reaction coordinates for the exploration of every step, based on previous experience and testing for this and similar reactions, the study of the activation of the Cys145 by His41 was carried out by a monodimensional PMF (1D-PMF) at the AM1/MM level using as ζ the antisymmetric combination of two distances defined for the proton transfer from the Cys145 to the His41, d(N1-H1)-d(SG-H1) for the three studied inhibitors. The first step required series of 31, 48 and 36 simulation windows for the **N3**, **B1**, and **B2** inhibitors, respectively. For the second step of the inhibition process, the sulfur attack on the C_β, a 1D-PMF at AM1/MM level was generated with the bond-forming distance, d(SG-Cβ) as ζ. The second step required series of 66, 36 and 41 simulation windows for the **N3**, **B1**, and **B2** inhibitors, respectively. The last step of the inhibition process, the protonation of the E-I$^{(-)}$ intermediate, 1D-PMF was generated using as ζ the antisymmetric combination of the distances defined for the proton transfer

from the His41 to the Cα, d(Cα-H1)-d(N1-H1). This step required series of 66, 86 and 81 simulation windows for the **N3**, **B1**, and **B2** inhibitors, respectively.

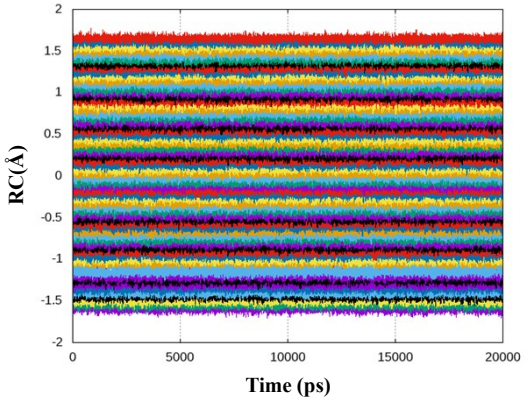

Figure S4. Overlapping of the sampling in consecutive windows during the 1D-PMF AM1/MM corresponding to the step 3 of the inhibition of SARS-CoV-2 M^{pro} cysteine protease by **N3**.

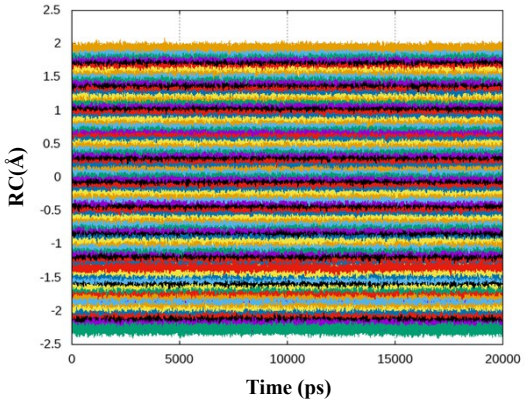

Figure S5. Overlapping of the sampling in consecutive windows during the 1D-PMF AM1/MM corresponding to the step 3 of the inhibition of SARS-CoV-2 M^{pro} cysteine protease by **B1**.

Table S1. Statistical errors on the activation and reaction free energies ($\Delta G^{\ddagger}$ and ΔG, respectively, in kcal·mol^{-1}) computed on the AM1/MM PMFs of the three steps involved in the inhibition of SARS-CoV-2 M^{pro} cysteine protease by the three studied inhibitors (N3, B1 and B2) using the bootstrapping analysis.

	N3	**B1**	**B2**
$\Delta G_1^{\neq}$ ΔG_1	± 0.12 ± 0.12	± 0.05 ± 0.04	± 0.07 ± 0.07
$\Delta G_2^{\neq}$ ΔG_2	± 0.02 ± 0.02	± 0.04 ± 0.04	--- ± 0.04
$\Delta G_3^{\neq}$ ΔG_3	± 0.14 ± 0.13	± 0.24 ± 0.24	± 0.12 ± 0.11

Spline Corrections. A correction term is interpolated to any value along the reaction coordinates in the AM1/MM FES. A continuous energy function is used to obtain the corrected PMFs:

$$E = E_{LL/MM} + S\left[\Delta E_{LL}^{HL}(\xi)\right] \quad \text{(S3)}$$

where S is a one-dimensional spline function and is the difference between the energies obtained at low-level (LL) and high-level (HL) of theory of the QM part. The AM1 semiempirical Hamiltonian was used as LL method, while a density functional theory (DFT)-based method was selected for the HL energy calculation. In particular, HL energy calculations were performed by means of the hybrid M06-2X functional using the standard 6-31+G(d,p) basis set. The resulting spline functions for all the computed FESs are shown in Figure S6.

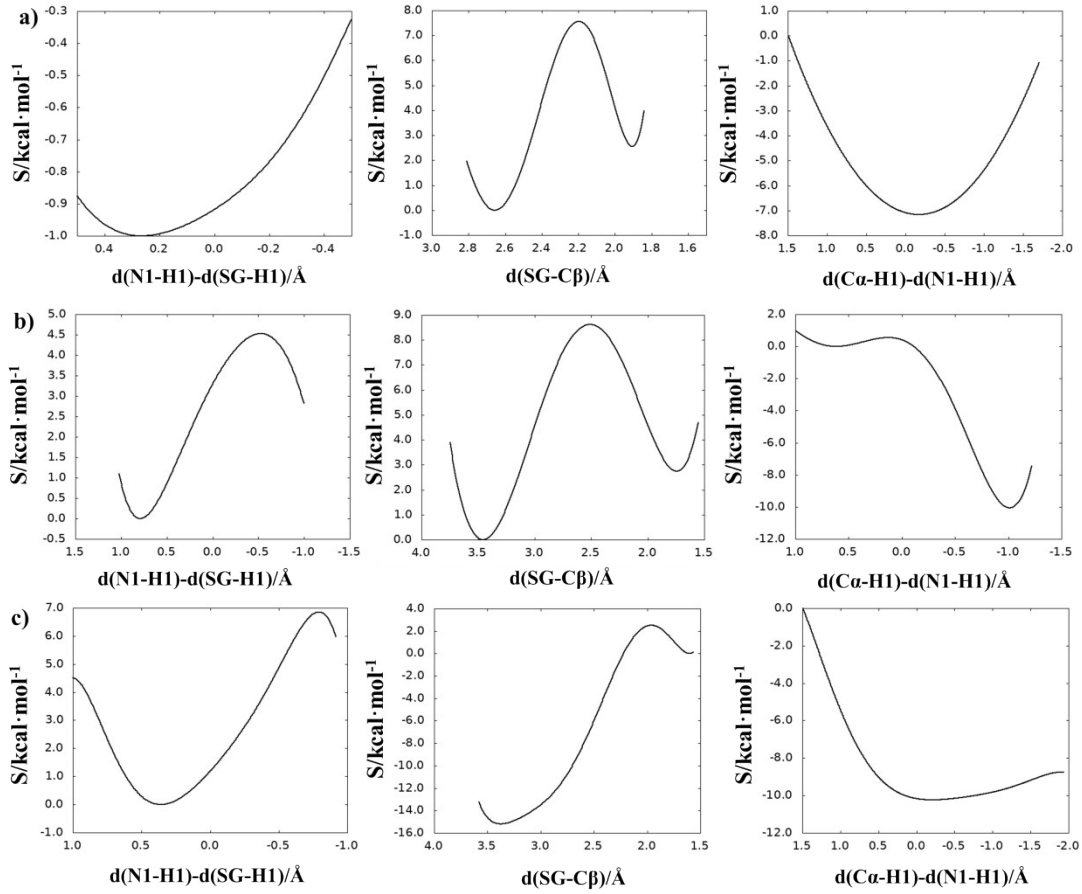

Figure S6. Spline function of the three steps involved in the inhibition of SARS-CoV-2 M^{pro} cysteine protease by the three studied inhibitors: a) **N3**; b) **B1**; and c) **B2**.

Parameters for the inhibitors

Table S2. Atom types, charges and parameters obtained for the inhibitor **N3** generated on the **E-I** covalent complex using antechamber package included in AmberTools.

Atom name	Atom type	Charge	Parameters
Cβ	c3	0.004474	**NONBON**
C33	c3	0.112563	n3 1.8240 0.1700
N29	n	-0.545563	hn 0.6000 0.0157
C23	c	0.643753	c3 1.9080 0.1094
C22	c3	0.038334	n 1.8240 0.1700
N21	n	-0.523933	c 1.9080 0.0860
C16	c	0.639686	cd 1.9080 0.0860
C15	c3	0.044435	nc 1.8240 0.1700
N14	n	-0.541630	os 1.6837 0.1700
C11	c	0.647820	cc 1.9080 0.0860
C10	c3	0.037318	ha 1.4590 0.0150
N9	n	-0.529832	hc 1.4870 0.0157
C7	c	0.607656	o 1.6612 0.2100
C3	cd	0.268239	**BOND**
N2	nc	-0.320809	n3-hn 394.10 1.018
O7	os	-0.109231	n3-c3 320.60 1.470
C5	cc	0.261325	c3-c3 303.10 1.535
C4	cd	-0.306357	c3-h1 335.90 1.093
H09	ha	0.199298	c3-c 328.30 1.508
C6	c3	-0.137054	c3-n 330.60 1.460
H12	hc	0.070873	n -c 478.20 1.345
H13	hc	0.066806	n -hn 410.20 1.009
H14	hc	0.070873	c -o 648.00 1.214
O6	o	-0.588036	c -cd 377.40 1.462
H7	hn	0.347246	cd-nc 494.60 1.335
H18	h1	0.105445	cd-cd 418.30 1.429
C13	c3	-0.106281	nc-os 414.90 1.395
H21	hc	0.059688	os-cc 376.10 1.370
H22	hc	0.053587	cc-cd 504.00 1.371
H23	hc	0.055620	cc-c3 337.30 1.499
O5	o	-0.599834	cd-ha 347.20 1.085
H6	hn	0.333011	c3-hc 337.30 1.092
C18	c3	-0.073443	c -os 411.30 1.343
C20	c3	-0.087601	os-c3 301.50 1.439
H32	hc	0.043419	c3-ca 323.50 1.513
H33	hc	0.038334	ca-ca 478.40 1.387
H34	hc	0.045452	ca-ha 344.30 1.087
H28	hc	0.068839	C -n3 490.0 1.335
C19	c3	-0.103331	c -N 490.0 1.335
H29	hc	0.042402	**ANGLE**
H30	hc	0.050536	n3-c3-c3 66.180 110.380
H31	hc	0.040368	n3-c3-h1 49.390 109.920
H25	h1	0.103411	n3-c3-c 66.590 111.140
O4	o	-0.602783	hn-n3-c3 47.130 109.920
H5	hn	0.325893	c3-c3-h1 46.360 110.070
H36	h1	0.100361	c3-c -o 68.030 123.110
C25	c3	-0.089862	c3-c3-c 63.790 110.530
C26	c3	-0.061645	c3-c3-n 65.850 112.130
C27	c3	-0.089567	c3-c3-c3 63.210 110.630
H45	hc	0.039351	c3-c3-hc 46.370 110.050
H46	hc	0.033250	c3-n -c 63.920 121.350
H47	hc	0.036301	c3-n -hn 46.040 116.780
H41	hc	0.052570	n -c3-h1 49.820 109.320
C28	c3	-0.090550	n -c -c3 67.860 115.150
H42	hc	0.038334	n -c -o 75.830 122.030

H43	hc	0.035284
H44	hc	0.037318
H39	hc	0.056637
H40	hc	0.085108
O3	o	-0.630312
H4	hn	0.338095
H52	h1	0.099344
C37	c3	-0.083963
H57	hc	0.062738
H58	hc	0.072906
C38	c3	-0.133417
C42	c	0.710254
N30	n	-0.578991
H3	hn	0.337078
C41	c3	0.092531
C39	c3	-0.095761
H60	hc	0.084092
H61	hc	0.057654
H62	h1	0.041385
H63	h1	0.040368
O2	o	-0.629722
H59	hc	0.091209
H53	h1	0.110529
Cα	c3	-0.122307
H54	hc	0.083075
C36	c	0.654938
O1	o	-0.537796
O44	os	-0.426599
C43	c3	0.186791
H65	h1	0.081041
H66	h1	0.071890
C50	ca	-0.119259
C45	ca	-0.107166
C46	ca	-0.134695
H20	ha	0.133204
C47	ca	-0.118964
H37	ha	0.133204
C48	ca	-0.132728
H19	ha	0.135238
C49	ca	-0.109132
H17	ha	0.155575
H15	ha	0.134221

c -n -hn	49.210	118.460	
c -c3-n	66.670	111.560	
c -c3-h1	47.630	107.660	
n -c -cd	70.190	111.860	
c -cd-nc	67.450	121.860	
c -cd-cd	63.720	122.690	
cd-c -o	68.910	125.710	
cd-nc-os	73.170	104.300	
cd-cd-cc	68.160	114.190	
cd-cd-ha	47.020	121.510	
nc-cd-cd	71.150	112.560	
nc-os-cc	68.120	107.230	
os-cc-cd	69.960	120.300	
os-cc-c3	67.480	117.090	
cc-cd-ha	48.350	122.890	
cc-c3-hc	47.200	110.860	
cd-cc-c3	64.810	119.450	
hc-c3-hc	39.430	108.350	
c -c3-hc	47.200	109.680	
h1-c3-h1	39.180	109.550	
c3-c -os	69.260	111.960	
c -os-c3	63.630	115.140	
o -c -os	75.930	123.330	
os-c3-h1	50.840	108.820	
os-c3-ca	68.190	108.890	
c3-ca-ca	63.840	120.630	
h1-c3-ca	46.780	110.950	
ca-ca-ca	67.180	119.970	
ca-ca-ha	48.460	120.010	
n3-C -O	80.0	122.90	
N-c -o	80.0	122.90	
CT-C -n3	70.0	116.60	
c3-c -N	70.0	116.60	
c -N -H	50.0	120.00	
c -N -CT	50.0	121.90	
c3-c -N	70.0	116.60	
C -n3-c3	50.0	121.90	

IMPROPER

c -c3-n -hn	1.1	180.0	2.0
c3-n -c -o	10.5	180.0	2.0
cd-n -c -o	10.5	180.0	2.0
c -cd-cd-nc	1.1	180.0	2.0
c3-cd-cc-os	1.1	180.0	2.0
cc-cd-cd-ha	1.1	180.0	2.0
c3-o -c -os	10.5	180.0	2.0
c3-ca-ca-ca	1.1	180.0	2.0
ca-ca-ca-ha	1.1	180.0	2.0

Parameters

DIHEDRALS

n3-c3-c3-h1	1	0.156	0.000	3.000
n3-c3-c -o	1	0.000	180.000	2.000
hn-n3-c3-c3	1	0.300	0.000	3.000
hn-n3-c3-h1	1	0.300	0.000	3.000
hn-n3-c3-c	1	0.300	0.000	3.000
c3-c3-c -o	1	0.000	180.000	2.000
c3-c3-n -c	1	0.500	180.000	-4.000
c3-c3-n -c	1	0.150	180.000	-3.000
c3-c3-n -c	1	0.530	0.000	1.000
c3-c3-n -hn	1	0.000	0.000	2.000
c3-c3-c3-hc	1	0.160	0.000	3.000
c3-c3-c3-c3	1	0.180	0.000	-3.000
c3-c3-c3-c3	1	0.250	180.000	-2.000
c3-c3-c3-c3	1	0.200	180.000	1.000
c3-c3-c -os	1	0.000	180.000	2.000
c3-c3-c3-c	1	0.156	0.000	3.000

DIHEDRALS

cd-c -n -hn	1	2.500	180.000	2.000
cd-nc-os-cc	1	4.800	180.000	2.000
cd-cd-cc-os	1	4.000	180.000	2.000
cd-cd-cc-c3	1	4.000	180.000	2.000
nc-cd-c -o	1	2.875	180.000	2.000
nc-cd-cd-cc	1	4.000	180.000	2.000
nc-cd-cd-ha	1	4.000	180.000	2.000
nc-os-cc-cd	1	1.050	180.000	2.000
nc-os-cc-c3	1	1.050	180.000	2.000
os-nc-cd-cd	1	4.750	180.000	2.000
os-cc-cd-ha	1	4.000	180.000	2.000
os-cc-c3-hc	1	0.000	0.000	3.000
cd-cd-c -o	1	2.875	180.000	2.000
cd-cc-c3-hc	1	0.000	0.000	3.000
ha-cd-cc-c3	1	4.000	180.000	2.000
o -c -n -hn	1	2.500	180.000	-2.000

c3-n -c -c3	1	2.500	180.000	2.000	o -c -n -hn	1	2.000	0.000	1.000
c3-n -c -o	1	2.500	180.000	2.000	hn-n -c3-h1	1	0.000	0.000	2.000
n -c3-c3-h1	1	0.156	0.000	3.000	h1-c3-c -o	1	0.800	0.000	-1.000
n -c3-c3-c3	1	0.156	0.000	3.000	h1-c3-c -o	1	0.080	180.000	3.000
n -c3-c3-hc	1	0.156	0.000	3.000	h1-c3-c3-hc	1	0.156	0.000	3.000
n -c -c3-n	1	1.700	180.000	-1.000	c3-c3-c3-h1	1	0.156	0.000	3.000
n -c -c3-n	1	2.000	180.000	2.000	hc-c3-c3-hc	1	0.150	0.000	3.000
n -c -c3-h1	1	0.000	180.000	2.000	h1-c3-c3-h1	1	0.156	0.000	3.000
n -c -c3-c3	1	0.100	0.000	-4.000	n -c -c3-hc	1	0.000	180.000	2.000
n -c -c3-c3	1	0.070	0.000	2.000	o -c -c3-hc	1	0.800	0.000	-1.000
c -n -c3-h1	1	0.000	0.000	2.000	o -c -c3-hc	1	0.080	180.000	3.000
c -c3-n -c	1	0.850	180.000	-2.000	h1-c3-c3-c	1	0.156	0.000	3.000
c -c3-n -c	1	0.800	0.000	1.000	c3-c -os-c3	1	2.700	180.000	2.000
c -c3-n -hn	1	0.000	0.000	2.000	hc-c3-c -os	1	0.000	180.000	2.000
c -c3-c3-hc	1	0.156	0.000	3.000	c -os-c3-h1	1	0.383	0.000	3.000
c3-c -n -hn	1	2.500	180.000	2.000	c -os-c3-ca	1	0.383	0.000	3.000
n -c3-c -o	1	0.000	180.000	2.000	o -c -os-c3	1	2.700	180.000	-2.000
c3-n -c -cd	1	2.500	180.000	2.000	o -c -os-c3	1	1.400	180.000	1.000
n -c -cd-nc	1	2.875	180.000	2.000	os-c3-ca-ca	1	0.000	0.000	2.000
n -c -cd-cd	1	2.875	180.000	2.000	c3-ca-ca-ca	1	3.625	180.000	2.000
c -cd-nc-os	1	4.750	180.000	2.000	c3-ca-ca-ha	1	3.625	180.000	2.000
c -cd-cd-cc	1	4.000	180.000	2.000	h1-c3-ca-ca	1	0.000	0.000	2.000
c -cd-cd-ha	1	4.000	180.000	2.000	ca-ca-ca-ha	1	3.625	180.000	2.000
ha-ca-ca-ha	1	3.625	180.000	2.000	ca-ca-ca-ca	1	3.625	180.000	2.000

Table S3. Atom types, charges and parameters obtained for the compound **B1** generated on the **E-I** covalent complex using antechamber package include in AmberTools.

Atom name	Atom type	Charge	Parameters
Cβ	c3	0.014674	**NONBON**
C33	c3	0.117898	n3 1.8240 0.1700
N29	n	-0.564960	hn 0.6000 0.0157
C23	c	0.658374	c3 1.9080 0.1094
C22	c3	0.054720	n 1.8240 0.1700
C01	c3	-0.076911	c 1.9080 0.0860
C06	cx	-0.127236	cx 1.9080 0.0860
C05	cx	-0.136752	hc 1.4870 0.0157
H16	hc	0.070005	o 1.6612 0.2100
C07	cx	-0.121056	cc 1.9080 0.0860
H19	hc	0.074081	os 1.6837 0.1700
H20	hc	0.077138	cd 1.9080 0.0860
H17	hc	0.072043	h4 1.4090 0.0150
H18	hc	0.089366	ha 1.4590 0.0150
H07	hc	0.075100	h1 1.3870 0.0157
H08	hc	0.098537	ca 1.9080 0.0860
N02	n	-0.246624	**BOND**
C21	c	0.622097	n3-hn 394.10 1.018
O4	o	-0.637162	n3-c3 320.60 1.470
C20	cc	-0.032766	c3-c3 303.10 1.535
N01	n	-0.418987	c3-h1 335.90 1.093
C02	c	0.762311	c3-c 328.30 1.508
O03	os	-0.439392	c3-n 330.60 1.460
C03	c3	0.190349	n -c 478.20 1.345
C04	c3	-0.122724	n -hn 410.20 1.009
H13	hc	0.073062	c -o 648.00 1.214
H14	hc	0.045549	c3-cx 322.50 1.514
H15	hc	0.047587	c3-hc 337.30 1.092
C08	c3	-0.092312	cx-cx 337.30 1.499
H21	hc	0.051663	cx-hc 345.80 1.086
H22	hc	0.050644	n -cc 426.00 1.380
H23	hc	0.047587	c -cc 377.40 1.462
			cc-cd 594.00 1.371

C09	c3	-0.120762	c -os 411.30 1.343
H24	hc	0.074081	os-c3 301.50 1.439
H25	hc	0.047587	cd-cd 418.30 1.429
H26	hc	0.043511	cd-ha 347.20 1.085
O5	o	-0.575849	cc-h4 350.10 1.083
H5	hn	0.357158	c3-ca 323.50 1.513
C19	cd	-0.088290	ca-ca 478.40 1.387
C18	cd	-0.202087	ca-ha 344.30 1.087
C17	cc	-0.022563	C -n3 490.0 1.335
H27	h4	0.157944	c -N 490.0 1.335
H28	ha	0.157944	**ANGLE**
H29	ha	0.182400	n3-c3-c3 66.180 110.380
H35	h1	0.078157	n3-c3-h1 49.390 109.920
O3	o	-0.585757	n3-c3-c 66.590 111.140
H4	hn	0.342892	hn-n3-c3 47.130 109.920
H42	h1	0.091404	c3-c3-h1 46.360 110.070
C37	c3	-0.087702	c3-c -o 68.030 123.110
H47	hc	0.058796	c3-c3-c 63.790 110.530
H48	hc	0.083252	c3-c3-n 65.850 112.130
C38	c3	-0.133122	c3-c3-c3 63.210 110.630
C42	c	0.708712	c3-c3-hc 46.370 110.050
N30	n	-0.573789	c3-n -c 63.920 121.350
H3	hn	0.335759	c3-n -hn 46.040 116.780
C41	c3	0.091710	n -c3-h1 49.820 109.320
C39	c3	-0.092607	n -c -c3 67.860 115.150
H50	hc	0.083252	n -c -o 75.830 122.030
H51	hc	0.052682	c -n -hn 49.210 118.460
H52	h1	0.040454	c -c3-n 66.670 111.560
H53	h1	0.043511	c -c3-h1 47.630 107.660
O2	o	-0.624409	c3-c3-cx 63.300 111.820
H49	hc	0.092423	c3-n -cc 63.380 120.810
H43	h1	0.095480	c3-cx-cx 61.820 120.060
Cα	c3	-0.128904	c3-cx-hc 46.120 114.160
H45	hc	0.096499	cx-c3-hc 46.920 110.200
C36	c	0.658374	cx-cx-hc 45.790 117.920
O1	o	-0.557210	cx-cx-cx 87.900 60.000
O44	os	-0.416828	hc-cx-hc 38.580 114.470
C43	c3	0.182095	hc-c3-hc 39.430 108.350
H55	h1	0.086309	n -c -cc 70.190 111.860
H56	h1	0.073062	n -cc-cd 70.720 115.520
C50	ca	-0.121939	n -cc-h4 50.390 117.620
C45	ca	-0.103005	c -n -cc 65.240 124.190
C46	ca	-0.133416	c -cc-n 68.110 116.330
H36	ha	0.135527	c -cc-cd 65.230 121.510
C47	ca	-0.116739	o -c -cc 68.910 125.710
H11	ha	0.134508	cc-n -hn 48.080 118.710
C48	ca	-0.133416	cc-cd-cd 68.160 114.190
H12	ha	0.135527	cc-cd-ha 48.350 122.890
C49	ca	-0.109872	n -c -os 76.680 109.280
H10	ha	0.150811	c -os-c3 63.630 115.140
H34	ha	0.138584	os-c -o 75.930 123.330

Parameters

ANGLE

os-c3-c3	67.780	108.420
cd-cd-ha	47.020	121.510
cd-cc-h4	47.190	129.110
c -c3-hc	47.200	109.680
h1-c3-h1	39.180	109.550
c3-c -os	69.260	111.960
os-c3-h1	50.840	108.820
os-c3-ca	68.190	108.890
c3-ca-ca	63.840	120.630
h1-c3-ca	46.780	110.950
ca-ca-ca	67.180	119.970

DIHEDRALS

hc-cx-cx-hc	1	0.156	0.000	3.000
hc-cx-c3-hc	1	0.150	0.000	3.000
hc-c3-c3-h1	1	0.156	0.000	3.000
n -c3-c -o	1	0.000	180.000	2.000
n -c -cc-n	1	2.875	180.000	2.000
n -c -cc-cd	1	2.875	180.000	2.000
n -cc-cd-cd	1	4.000	180.000	2.000
n -cc-cd-ha	1	4.000	180.000	2.000
c -n -cc-cd	1	1.650	180.000	2.000
c -n -cc-h4	1	1.650	180.000	2.000
c -cc-n -c	1	1.650	180.000	2.000

ca-ca-ha	48.460	120.010	
n3-C -O	80.0	122.90	
N-c -o	80.0	122.90	
CT-C -n3	70.0	116.60	
c3-c -N	70.0	116.60	
c -N -H	50.0	120.00	
C -n3 -hn	50.0	120.00	
c -N -CT	50.0	121.90	
c3-c -N	70.0	116.60	
C -n3-c3	50.0	121.90	

DIHEDRALS

n3-c3-c3-h1	1	0.156	0.000	3.000
n3-c3-c -o	1	0.000	180.000	2.000
hn-n3-c3-c3	1	0.300	0.000	3.000
hn-n3-c3-h1	1	0.300	0.000	3.000
hn-n3-c3-c	1	0.300	0.000	3.000
c3-c3-c -o	1	0.000	180.000	2.000
c3-c3-n -c	1	0.500	180.000	-4.000
c3-c3-n -c	1	0.150	180.000	-3.000
c3-c3-n -c	1	0.530	0.000	1.000
c3-c3-n -hn	1	0.000	0.000	2.000
c3-c3-c3-hc	1	0.160	0.000	3.000
c3-c3-c3-c3	1	0.180	0.000	-3.000
c3-c3-c3-c3	1	0.250	180.000	-2.000
c3-c3-c3-c3	1	0.200	180.000	1.000
c3-c3-c -os	1	0.000	180.000	2.000
c3-c3-c3-c	1	0.156	0.000	3.000
c3-n -c -c3	1	2.500	180.000	2.000
c3-n -c -o	1	2.500	180.000	2.000
n -c3-c3-h1	1	0.156	0.000	3.000
n -c3-c3-c3	1	0.156	0.000	3.000
n -c3-c3-hc	1	0.156	0.000	3.000
n -c -c3-c3	1	0.100	0.000	-4.000
n -c -c3-c3	1	0.070	0.000	2.000
n -c -c3-n	1	1.700	180.000	-1.000
n -c -c3-n	1	2.000	180.000	2.000
n -c -c3-h1	1	0.000	180.000	2.000
c -n -c3-h1	1	0.000	0.000	2.000
c -c3-c3-cx	1	0.156	0.000	3.000
c -c3-c3-hc	1	0.156	0.000	3.000
c -c3-n -c	1	0.850	180.000	-2.000
c -c3-n -c	1	0.800	0.000	1.000
c -c3-n -cc	1	0.000	0.000	2.000
c3-c -n -hn	1	2.500	180.000	2.000
c3-c3-cx-cx	1	0.156	0.000	3.000
c3-c3-cx-hc	1	0.160	0.000	3.
c3-n -c -cc	1	2.500	180.000	2.000
c3-n -cc-cd	1	1.650	180.000	2.000
c3-n -cc-h4	1	1.650	180.000	2.000
c3-c3-n -cc	1	0.000	0.000	2.000
c3-cx-cx-hc	1	0.156	0.000	3.000
c3-cx-cx-cx	1	0.156	0.000	3.000
cx-c3-c3-n	1	0.156	0.000	3.000
cx-c3-c3-h1	1	0.156	0.000	3.000
cx-cx-cx-cx	1	0.156	0.000	3.000
cx-cx-cx-hc	1	0.156	0.000	3.000
cx-cx-c3-hc	1	0.156	0.000	3.000
c -cc-n -hn	1	1.650	180.000	2.000
c -cc-cd-cd	1	4.000	180.000	2.000
c -cc-cd-ha	1	4.000	180.000	2.000
o -c -n -cc	1	2.500	180.000	2.000
o -c -cc-n	1	2.875	180.000	2.000
o -c -cc-cd	1	2.875	180.000	2.000
cc-c -n -cc	1	2.500	180.000	2.000
cc-n -c -os	1	2.500	180.000	2.000
cc-cd-cd-cc	1	4.000	180.000	2.000
cc-cd-cd-ha	1	4.000	180.000	2.000
n -c -os-c3	1	2.700	180.000	2.000
c -os-c3-c3	1	0.383	0.000	-3.000
c -os-c3-c3	1	0.800	180.000	1.000
os-c -n -hn	1	2.500	180.000	2.000
os-c3-c3-hc	1	0.000	0.000	-3.000
os-c3-c3-hc	1	0.250	0.000	1.000
c3-os-c -o	1	2.700	180.000	-2.000
c3-os-c -o	1	1.400	180.000	1.000
o -c -n -hn	1	2.500	180.000	-2.000
o -c -n -hn	1	2.000	0.000	1.000
hn-n -cc-cd	1	1.650	180.000	2.000
cd-cd-cc-h4	1	4.000	180.000	2.000
cc-n -c3-h1	1	0.000	0.000	2.000
h4-cc-cd-ha	1	4.000	180.000	2.000
ha-cd-cd-ha	1	4.000	180.000	2.000
h1-c3-c -o	1	0.800	0.000	-1.000
h1-c3-c -o	1	0.080	180.000	3.000
hn-n -c3-h1	1	0.000	0.000	2.000
h1-c3-c3-h1	1	0.156	0.000	3.000
h1-c3-c3-c3	1	0.156	0.000	3.000
hc-c3-c3-hc	1	0.150	0.000	3.000
n -c -c3-hc	1	0.000	180.000	2.000
o -c -c3-hc	1	0.800	0.000	-1.000
o -c -c3-hc	1	0.080	180.000	3.000
h1-c3-c3-c	1	0.156	0.000	3.000
c3-c -os-c3	1	2.700	180.000	2.000
hc-c3-c -os	1	0.000	180.000	2.000
c -os-c3-h1	1	0.383	0.000	3.000
c -os-c3-ca	1	0.383	0.000	3.000
os-c3-ca-ca	1	0.000	0.000	2.000
c3-ca-ca-ca	1	3.625	180.000	2.000
c3-ca-ca-ha	1	3.625	180.000	2.000
h1-c3-ca-ca	1	0.000	0.000	2.000
ca-ca-ca-ha	1	3.625	180.000	2.000
ca-ca-ca-ca	1	3.625	180.000	2.000
ha-ca-ca-ha	1	3.625	180.000	2.000

IMPROPER

c -c3-n -hn	1.1	180.0	2.0
c3-n -c -o	10.5	180.0	2.0
c -c3-n -cc	1.1	180.0	2.0
cc-n -c -o	10.5	180.0	2.0
c -cd-cc-n	1.1	180.0	2.0
c -cc-n -hn	1.1	180.0	2.0
n -o -c -os	10.5	180.0	2.0
cc-cd-cd-ha	1.1	180.0	2.0
cd-h4-cc-n	1.1	180.0	2.0
c3-o -c -os	10.5	180.0	2.0
c3-ca-ca-ca	1.1	180.0	2.0
ca-ca-ca-ha	1.1	180.0	2.0

Table S4. Atom types, charges and parameters obtained for the compound **B2** generated on the **E-I** covalent complex using antechamber package include in AmberTools.

Atom name	Atom type	Charge	Parameters
Cβ	c3	-0.012277	**NONBON**
C33	c3	0.107381	n3 1.8240 0.1700
N29	n	-0.538740	hn 0.6000 0.0157
C23	c	0.649313	c3 1.9080 0.1094
C22	c3	0.043794	n 1.8240 0.1700
N21	n	-0.486123	c 1.9080 0.0860
C16	c	0.755977	os 1.6837 0.1700
O01	os	-0.418890	ca 1.9080 0.0860
C01	c3	0.202763	ha 1.4590 0.0150
C07	ca	-0.097731	h1 1.3870 0.0157
C02	ca	-0.114004	o 1.6612 0.2100
H17	ha	0.136406	hc 1.4870 0.0157
C03	ca	-0.127645	no 1.8240 0.1700
H13	ha	0.137432	
C04	ca	-0.117901	**BOND**
H14	ha	0.136406	n3-hn 394.10 1.018
C05	ca	-0.127645	n3-c3 320.60 1.470
C06	ca	-0.097439	c3-c3 303.10 1.535
H10	ha	0.147688	c3-h1 335.90 1.093
H09	ha	0.138457	c3-c 328.30 1.508
H07	h1	0.061229	c3-n 330.60 1.460
H08	h1	0.065331	n -c 478.20 1.345
O4	o	-0.607045	n -hn 410.20 1.009
H5	hn	0.338964	c -o 648.00 1.214
H23	h1	0.089946	c -os 411.30 1.343
C25	c3	-0.087111	os-c3 301.50 1.439
C26	c3	-0.063043	c3-ca 323.50 1.513
C27	c3	-0.088767	ca-ca 478.40 1.387
H29	hc	0.033537	ca-ha 344.30 1.087
H30	hc	0.036614	c3-hc 337.30 1.092
H31	hc	0.038665	c3-no 265.40 1.533
H28	hc	0.054050	no-o 761.20 1.219
C28	c3	-0.090716	C -n3 490.0 1.335
H32	hc	0.040717	c -N 490.0 1.335
H33	hc	0.036614	
H34	hc	0.040717	**ANGLE**
H26	hc	0.067383	n3-c3-c3 66.180 110.380
H27	hc	0.090972	n3-c3-h1 49.390 109.920
O3	o	-0.592527	n3-c3-c 66.590 111.140
H4	hn	0.330759	hn-n3-c3 47.130 109.920
H40	h1	0.101228	c3-c3-h1 46.360 110.070
C37	c3	-0.093931	c3-c -o 68.030 123.110
H44	hc	0.073536	c3-c3-c 63.790 110.530
H45	hc	0.068408	c3-c3-n 65.850 112.130
C38	c3	-0.147523	c3-c3-c3 63.210 110.630
C42	c	0.672902	c3-c3-no 65.210 109.270
N30	n	-0.658688	c3-n -c 63.920 121.350
H36	hn	0.318452	c3-n -hn 46.040 116.780
H37	hn	0.338964	c3-c3-hc 46.370 110.050
O2	o	-0.612989	n -c3-h1 49.820 109.320
H46	hc	0.080715	n -c -c3 67.860 115.150
H47	hc	0.069434	n -c -o 75.830 122.030
H41	h1	0.119689	c -n -hn 49.210 118.460
Cα	c3	-0.067915	c -c3-n 66.670 111.560
N01	no	0.245428	c -c3-h1 47.630 107.660
O31	o	-0.217289	n -c -os 76.680 109.280
O44	o	-0.201699	c -os-c3 63.630 115.140
H43	h1	0.112509	os-c -o 75.930 123.330
			os-c3-ca 68.190 108.890

Parameters

ANGLE

os-c3-h1	50.840	108.820
c3-ca-ca	63.840	120.630
ca-c3-h1	46.780	110.950
ca-ca-ha	48.460	120.010
ca-ca-ca	67.180	119.970
h1-c3-h1	39.180	109.550
hc-c3-hc	39.430	108.350
c -c3-hc	47.200	109.680
hn-n -hn	39.730	117.850
c3-no-o	66.960	116.560
no-c3-h1	48.660	105.150
o -no-o	77.150	125.130
n3-C -O	80.0	122.90
N-c -o	80.0	122.90
CT-C -n3	70.0	116.60
c3-c -N	70.0	116.60
c -N -H	50.0	120.00
C -n3 -hn	50.0	120.00
c -N -CT	50.0	121.90
c3-c -N	70.0	116.60
C -n3-c3	50.0	121.90

DIHEDRALS

n3-c3-c3-h1	1	0.156	0.000	3.000
n3-c3-c -o	1	0.000	180.000	2.000
hn-n3-c3-c3	1	0.300	0.000	3.000
hn-n3-c3-h1	1	0.300	0.000	3.000
hn-n3-c3-c	1	0.300	0.000	3.000
c3-c3-c -o	1	0.000	180.000	2.000
c3-c3-n -c	1	0.500	180.000	-4.000
c3-c3-n -c	1	0.150	180.000	-3.000
c3-c3-n -c	1	0.530	0.000	1.000
c3-c3-n -hn	1	0.000	0.000	2.000
c3-c3-c3-hc	1	0.160	0.000	3.000
c3-c3-c3-c3	1	0.180	0.000	-3.000
c3-c3-c3-c3	1	0.250	180.000	-2.000
c3-c3-c3-c3	1	0.200	180.000	1.000
c3-c3-no-o	1	0.000	0.000	2.000
c3-c3-c3-no	1	0.156	0.000	3.000
c3-c3-c3-h1	1	0.156	0.000	3.000
c3-n -c -c3	1	2.500	180.000	2.000
c3-n -c -o	1	2.500	180.000	2.000
c3-c3-c3-c	1	0.156	0.000	3.000
n -c3-c3-h1	1	0.156	0.000	3.000
n -c3-c3-c3	1	0.156	0.000	3.000
n -c3-c3-hc	1	0.156	0.000	3.000
n -c -c3-n	1	1.700	180.000	-1.000
n -c -c3-n	1	2.000	180.000	2.000

DIHEDRALS

n -c -c3-h1	1	0.000	180.000	2.000
n -c -c3-c3	1	0.100	0.000	-4.000
n -c -c3-c3	1	0.070	0.000	2.000
c -n -c3-h1	1	0.000	0.000	2.000
c -c3-n -c	1	0.850	180.000	-2.000
c -c3-n -c	1	0.800	0.000	1.000
c -c3-n -hn	1	0.000	0.000	2.000
c -c3-c3-hc	1	0.156	0.000	3.000
c3-c -n -hn	1	2.500	180.000	2.000
c3-n -c -os	1	2.500	180.000	2.000
n -c3-c -o	1	0.000	180.000	2.000
n -c -os-c3	1	2.700	180.000	2.000
c -os-c3-ca	1	0.383	0.000	3.000
c -os-c3-h1	1	0.383	0.000	3.000
os-c -n -hn	1	2.500	180.000	2.000
os-c3-ca-ca	1	0.000	0.000	2.000
c3-os-c -o	1	2.700	180.000	-2.000
c3-os-c -o	1	1.400	180.000	1.000
c3-ca-ca-ha	1	3.625	180.000	2.000
c3-ca-ca-ca	1	3.625	180.000	2.000
ca-ca-ca-ha	1	3.625	180.000	2.000
ca-ca-ca-ca	1	3.625	180.000	2.000
ca-ca-c3-h1	1	0.000	0.000	2.000
ha-ca-ca-ha	1	3.625	180.000	2.000
o -c -n -hn	1	2.500	180.000	-2.000
o -c -n -hn	1	2.000	0.000	1.000
hn-n -c3-h1	1	0.000	0.000	2.000
h1-c3-c -o	1	0.800	0.000	-1.000
h1-c3-c -o	1	0.080	180.000	3.000
h1-c3-c3-hc	1	0.156	0.000	3.000
hc-c3-c3-hc	1	0.150	0.000	3.000
h1-c3-c3-h1	1	0.156	0.000	3.000
n -c -c3-hc	1	0.000	180.000	2.000
o -c -c3-hc	1	0.800	0.000	-1.000
o -c -c3-hc	1	0.080	180.000	3.000
h1-c3-c3-no	1	0.156	0.000	3.000
o -no-c3-h1	1	0.000	0.000	2.000
h1-c3-c3-c	1	0.156	0.000	3.000

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c -c3-n -hn	1.1	180.0	2.0
c3-n -c -o	10.5	180.0	2.0
n -o -c -os	10.5	180.0	2.0
c3-ca-ca-ca	1.1	180.0	2.0
ca-ca-ca-ha	1.1	180.0	2.0
c -hn-n -hn	1.1	180.0	2.0

Computational details of the estimation of inhibitor:M^{pro} binding poses

Docking studies with compounds **N3**, **B1** and **B2** were performed with Glide software[22] starting from the atomic coordinates of the Mpro-inhibitor complexes generated by QM/MM simulations and corresponding to the reactant state denoted as **E:I**. Before running calculations all the water molecules were deleted from each enzyme-inhibitor complex and an energy minimization of the resulting system was performed with OPLS3e force field to an energy gradient of 0.05 kcal·mol^{-1}·Å^{-1}. Docking grids (one for each complex) were centered in the channel of the M^{pro} enzyme. Dimensions of enclosing and bounding boxes were set to 20 and 10 Å on each side, respectively, and van der Waals radii of protein atoms were not scaled during grid generation. The structures of **N3**, **B1** and **B2** were extracted from QM/MM complexes in **E:I** configuration and then energy-minimized in gas phase with MacroModel[23] applying the OPLS3e force field[24] to an energy gradient of 0.01 kcal·mol^{-1}·Å^{-1}). The compounds were thus re-docked in the active site of M^{pro} using Glide applying Standard Precision (SP) mode without applying scaling factor to van der Waals radii of the ligands.[25] During docking runs, the structure of the protein was kept rigid while the ligands were free to adopt alternative conformations according to their internal degrees of freedom. For each ligand (**N3**, **B1** and **B2**) the best solution in term of SP Gscore has been reported (see Figure S13).

Results for N3

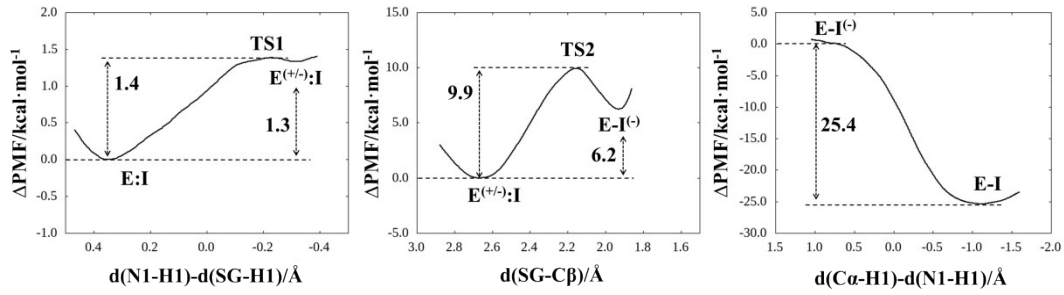


Figure S7. M06-2X/6-31+G(d,p):AM1/MM FESs corresponding to the inhibition of SARS-CoV-2 M^{pro} cysteine protease by **N3.**

Table S5. Average distances (in Å) for the states located along the inhibition mechanism of SARS-CoV-2 M^{pro} by the inhibitor **N3**, obtained from AM1/MM MD simulations on the stationary points extracted from the M06-2X/6-31+G(d,p):AM1/MM free energy surfaces.

Distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	E-I
SG-C_β	3.20±0.32	3.35±0.20	2.67±0.03	2.11±0.03	1.93±0.05	1.86±0.04
Cα-C_β	1.34±0.02	1.34±0.02	1.34±0.02	1.41±0.03	1.46±0.03	1.51±0.03
Cα–H1	3.36±0.24	3.30±0.16	3.54±0.20	3.33±0.21	1.78±0.03	1.14±0.03
N1-H1	1.86±0.04	1.37±0.04	1.03±0.02	1.02±0.02	1.01±0.02	2.24±0.03
SG-H1	1.51±0.03	1.63±0.03	1.97±0.08	2.11±0.11	2.83±0.19	2.69±0.13
H2-Ow	2.18±0.21	2.14±0.19	2.09±0.23	1.96±0.15	2.25±0.26	2.32±0.24
OD2(D187)-Hw	1.77±0.13	1.81±0.15	1.79±0.14	1.71±0.12	2.02±0.30	1.94±0.24
S1´ pocket						
O1-H(G143)	3.79±0.36	3.82±0.33	4.25±0.30	4.04±0.27	3.54±0.26	3.21±0.35
O1-H(S144)	3.35±0.33	3.07±0.37	3.19±0.29	2.94±0.39	4.20±0.41	3.88±0.45
O1-H(C145)	2.63±0.31	2.61±0.26	2.49±0.30	2.24±0.29	2.91±0.51	2.77±0.34
S1 pocket						
O2-HG(S144)	3.84±0.26	3.94±0.30	4.00±0.28	4.67±0.35	3.68±0.48	3.20±0.41
O2-HE2(H163)	2.10±0.16	2.04±0.17	2.05±0.14	2.00±0.14	2.11±0.21	2.16±0.25
O2-HE2(H172)	3.70±0.26	3.84±0.30	3.57±0.27	3.45±0.27	3.52±0.29	3.51±0.32
H3-O(F140)	2.90±0.33	3.00±0.27	3.12±0.32	3.55±0.39	3.76±0.44	3.88±0.31
H3-OE1(E166)	3.73±0.23	3.49±0.27	3.70±0.25	3.51±0.26	3.58±0.32	3.22±0.42
H3-OE2(E166)	2.04±0.20	2.03±0.20	1.95±0.17	2.09±0.22	2.20±0.26	2.15±0.22
H4-O(H164)	2.45±0.23	2.28±0.25	2.15±0.19	2.21±0.19	2.15±0.21	2.09±0.17
S2 pocket						
H5-OE1(Q189)	1.87±0.10	1.92±0.13	1.86±0.12	1.86±0.08	1.87±0.12	1.88±0.10
S3 pocket						
O4-H(E166)	2.00±0.16	1.94±0.10	1.99±0.13	1.93±0.11	1.90±0.11	1.89±0.10
H6-O(E166)	2.03±0.16	1.99±0.12	1.95±0.12	2.09±0.17	1.95±0.12	1.93±0.10
S4 pocket						
O5-HE22(Q192)	4.14±0.39	3.90±0.47	4.06±0.39	3.96±0.43	4.02±0.34	4.22±0.35
H7-O(Q192)	2.74±0.41	2.20±0.20	2.08±0.23	2.54±0.31	2.10±0.18	2.03±0.16
S5 pocket						
N7-H(Q192)	2.63±0.30	2.53±0.26	2.28±0.22	2.38±0.22	2.31±0.18	2.39±0.23

Table S6. Key distances (in Å) for the states located along the inhibition mechanism of SARS-CoV-2 M^{pro} by the inhibitor **N3**, optimized at M06-2X/6-31+G(d,p)/MM level of theory.

Distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	E-I
SG-C_β	3.48	3.59	3.53	2.45	1.91	1.82
Cα-C_β	1.34	1.34	1.34	1.38	1.47	1.53
Cα–H1	3.11	3.57	3.57	3.38	2.91	1.10
N1-H1	2.12	1.33	1.07	1.03	1.02	2.49
SG-H1	1.36	1.67	2.11	2.70	3.41	2.77
H2-Ow	1.88	1.88	1.83	1.82	1.87	1.90
OD2(D187)-Hw	1.63	1.68	1.64	1.70	1.62	1.79
S1´ pocket						
O1-H(G143)	3.43	3.48	3.46	4.80	4.68	2.88
O1-H(S144)	4.80	4.58	4.82	3.76	3.81	3.34
O1-H(C145)	3.90	3.61	3.96	3.05	2.83	2.44
S1 pocket						
O2-HG(S144)	2.22	2.39	2.18	4.03	4.00	3.29
O2-HE2(H163)	1.79	1.80	1.79	1.77	1.77	2.00
O2-HE2(H172)	3.62	3.47	3.63	3.38	3.38	3.33
H3-O(F140)	3.34	3.27	3.32	3.51	3.94	4.03
H3-OE2(E166)	1.75	1.73	1.76	1.77	1.80	1.83
H4-O(H164)	1.82	1.82	1.84	1.79	1.85	1.98
S2 pocket						
H5-OE1(Q189)	1.93	1.88	1.92	1.95	1.84	1.90
S3 pocket						
O4-H(E166)	1.92	1.92	1.92	1.85	1.90	1.86
H6-O(E166)	1.89	1.89	1.88	1.88	1.91	1.91
S4 pocket						
O5-HE22(Q192)	3.69	3.69	3.70	4.03	4.05	3.93
H7-O(Q192)	2.14	2.25	2.20	1.98	2.23	2.10
S5 pocket						
N7-H(Q192)	2.08	2.16	2.08	2.38	2.27	2.09

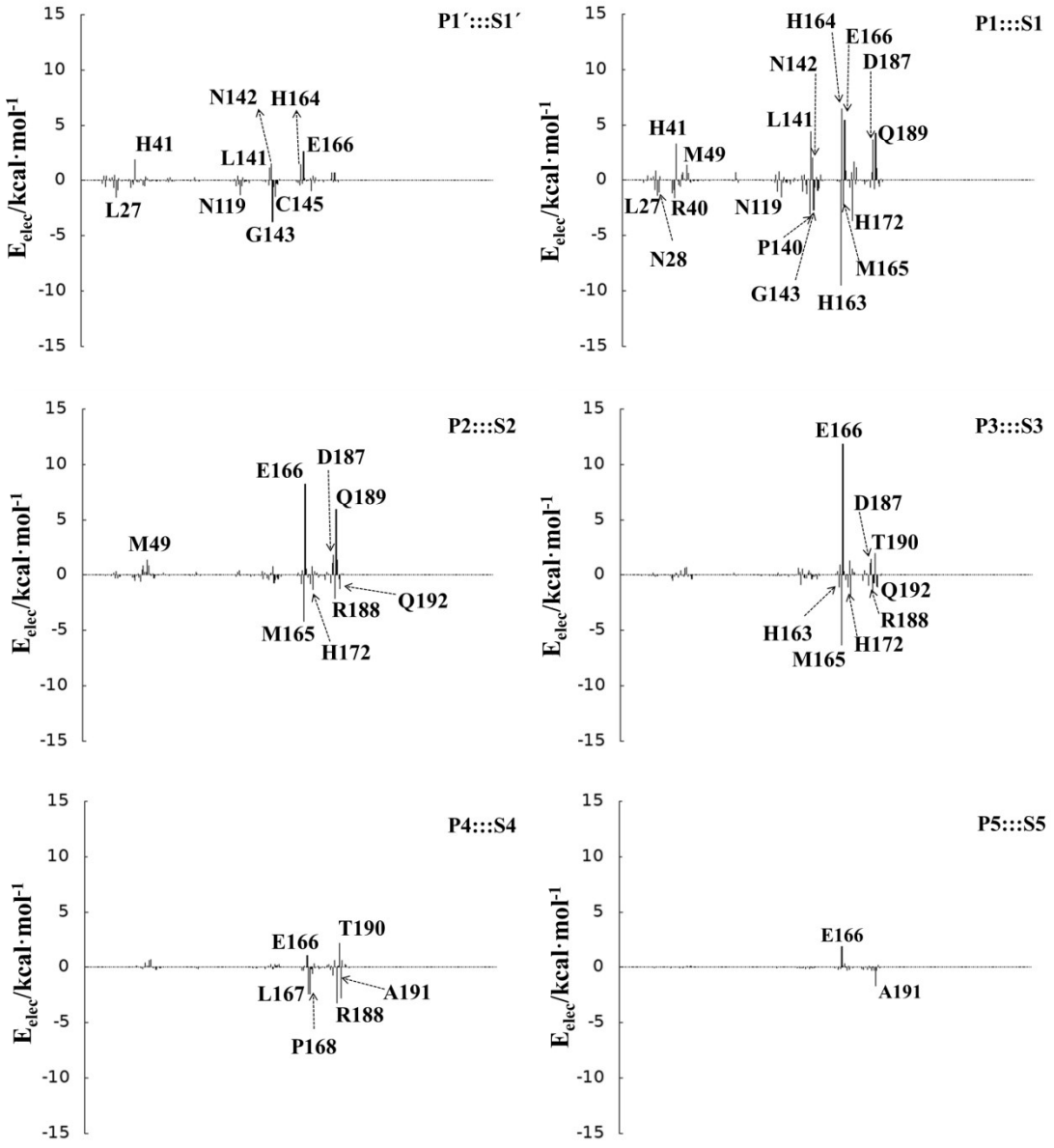


Figure S8. Total averaged electrostatic interaction energies between residues of Chain-A and each fragment of the inhibitor **N3** in **E:I**, obtained as an average over 1000 structures of the AM1/MM MD simulations. Only residues showing electrostatic interaction energies higher than 1.5 kcal·mol^{-1}, in absolute value, are labelled in the panels.

Table S7. Cartesian coordinates (in Å) of QM atoms for TS1, TS2, E:I and E-I of the inhibition process by the inhibitor **N3** optimized at M06-2X/6-31+G(d,p)/MM level.

TS1 (ν_i = 1020.4i cm^{-1})				TS2 (ν_i = 129.8i cm^{-1})			
Atoms	x	y	z	Atoms	x	y	z
C	12.354409	4.210624	24.173561	C	12.230094	4.198298	24.119723
H	11.440637	3.887503	24.678495	H	11.257772	4.004606	24.578220
H	13.145470	3.553450	24.546690	H	12.933815	3.533950	24.626728
C	12.188786	3.992293	22.688190	C	12.179439	3.802983	22.665679
N	10.937532	3.740569	22.160885	N	10.979886	3.659181	21.985642
H	10.056821	3.891979	22.664101	H	10.064283	3.937738	22.364054
C	11.057337	3.466587	20.851434	C	11.194020	3.180950	20.760272
H	10.235582	3.256427	20.184172	H	10.450964	2.976091	20.004398
N	12.325389	3.527803	20.489595	N	12.502977	3.013636	20.617778
C	13.051437	3.844897	21.625161	C	13.144112	3.386798	21.785036
H	14.128118	3.921907	21.609045	H	14.213359	3.311783	21.909451
C	12.405187	2.551135	12.914327	C	12.498487	2.461924	12.851767
O	11.350053	2.911594	12.373817	O	11.386264	2.524438	12.309819
N	12.660197	2.854535	14.204372	N	12.665097	2.710910	14.158807
H	13.554856	2.604037	14.602259	H	13.615698	2.843027	14.491223
C	11.771490	3.723259	14.997589	C	11.619796	3.354097	14.945989
C	12.369441	4.051093	16.383675	C	12.072486	3.437253	16.399666
S	12.240777	2.726091	17.647715	S	12.194675	1.838240	17.263657
C	14.872185	0.528389	16.570416	C	14.312487	0.712555	16.764811
C	14.361656	-0.879781	16.720362	C	13.921084	-0.753039	16.852952
N	13.600336	-1.038951	17.936831	N	13.242406	-1.044055	18.101781
C	14.192430	-1.530274	19.029469	C	13.730360	-1.919718	18.991666
O	15.404476	-1.799385	19.066740	O	14.770190	-2.567231	18.785615
H	12.620733	-0.737106	17.979315	H	12.339643	-0.597927	18.295033
C	13.651959	-1.497931	15.530111	C	13.200381	-1.389500	15.660783
C	13.518835	-3.004810	15.724158	C	13.333869	-2.910913	15.760450
C	14.837625	-3.778544	15.877265	C	14.760366	-3.469455	15.659034
C	14.530671	-5.159231	15.279804	C	14.556747	-4.902444	15.146740
N	13.490737	-4.850550	14.300946	N	13.323501	-4.786861	14.373567
C	12.855015	-3.688626	14.541636	C	12.584715	-3.707131	14.705071
O	11.878160	-3.268836	13.918215	O	11.466388	-3.453851	14.259536
H	13.014718	-5.579118	13.756077	H	12.875727	-5.585460	13.911001
H	15.403521	-5.611378	14.804488	H	15.383618	-5.249306	14.522096
H	14.157236	-5.851343	16.043347	H	14.433399	-5.610301	15.976001
H	15.188298	-3.848619	16.906841	H	15.292489	-3.436488	16.605598
H	15.620286	-3.294900	15.282034	H	15.325610	-2.888348	14.926542
H	12.872657	-3.210819	16.591020	H	12.890265	-3.238162	16.714906
H	14.255588	-1.315430	14.635686	H	13.675938	-1.052869	14.728979
H	12.673438	-1.035083	15.371849	H	12.149389	-1.080928	15.641351
H	15.269805	-1.478684	16.884546	H	14.880956	-1.272627	16.940536
C	15.015196	1.362765	17.604306	C	15.183439	1.205231	17.710241
C	15.947413	2.514224	17.544088	C	15.937739	2.393187	17.445298
O	16.577294	2.855405	16.560254	O	16.023115	2.972202	16.365873
O	16.131402	3.034053	18.761801	O	16.609179	2.806041	18.560844
C	17.279718	3.877363	18.943540	C	17.852734	3.495262	18.362206
C	18.534292	3.076808	19.226895	C	18.966610	2.487128	18.222069
C	19.777001	3.597676	18.853693	C	19.376107	2.070347	16.945189
C	20.965545	2.962885	19.210978	C	20.379482	1.111600	16.804480

C	20.920075	1.766212	19.922930	C	21.003081	0.584845	17.934389
C	19.684729	1.214386	20.254318	C	20.613399	0.998209	19.208274
C	18.497530	1.869026	19.928008	C	19.588283	1.933768	19.353024
H	17.545116	1.433131	20.216602	H	19.292544	2.252114	20.348817
H	19.647076	0.265691	20.774109	H	21.109781	0.609983	20.090548
H	21.835204	1.266732	20.222471	H	21.800543	-0.141093	17.818363
H	21.921225	3.395413	18.929912	H	20.684145	0.785752	15.817912
H	19.808328	4.503964	18.259430	H	18.906201	2.515483	16.072488
H	17.010577	4.491178	19.805566	H	17.987554	4.107101	19.256633
H	17.413591	4.516569	18.069641	H	17.792174	4.133068	17.481064
H	14.602899	1.139935	18.579077	H	15.218146	0.786634	18.712237
H	12.490486	3.328153	19.187559	H	12.899108	2.656679	19.737282
H	15.293829	0.821147	15.610833	H	14.371416	1.132919	15.764267
H	11.833489	4.933343	16.738868	H	11.313002	4.015582	16.929815
H	13.413931	4.344562	16.235573	H	13.021409	3.986991	16.440644
H	10.804810	3.225641	15.119664	H	10.703656	2.762142	14.875568
C	11.610523	5.074799	14.261061	C	11.422510	4.771348	14.363929
O	12.637310	5.718651	14.004863	O	12.433196	5.419349	14.059449
N	10.373498	5.486140	13.984857	N	10.175214	5.215650	14.214535
H	9.622790	4.846857	14.213012	H	9.423004	4.619939	14.541855
C	10.041296	6.696427	13.179344	C	9.849369	6.478473	13.495558
H	10.860850	7.396188	13.330466	H	10.614452	7.204326	13.765328
H	9.157087	7.139170	13.644181	H	8.904560	6.831451	13.912693
H	9.851214	6.521566	12.213273	H	9.750657	6.380219	12.505305
H	13.599506	-1.647186	19.826195	H	13.206358	-2.001283	19.839469
H	13.045398	2.012225	12.366873	H	13.320407	2.234300	12.329622
H	12.540660	5.136787	24.501483	H	12.486410	5.138780	24.342873

E:I				E-I			
Atoms	x	y	z	Atoms	x	y	z
C	12.297524	4.202607	24.258199	C	12.683710	4.248307	24.265626
H	11.351487	3.953607	24.743968	H	11.748004	3.936485	24.735999
H	13.055174	3.564856	24.721538	H	13.474028	3.670997	24.753563
C	12.214228	3.894424	22.795836	C	12.657461	3.893999	22.811384
N	11.009971	3.784487	22.141366	N	11.478014	3.677499	22.141011
H	10.092410	3.999951	22.543747	H	10.553589	3.850333	22.540865
C	11.266125	3.434484	20.856444	C	11.785477	3.279958	20.877213
H	10.479630	3.286923	20.129524	H	11.032399	3.019100	20.147680
N	12.560184	3.310745	20.638040	N	13.086545	3.224791	20.690952
C	13.160275	3.591496	21.845476	C	13.638691	3.602696	21.894461
H	14.232892	3.546283	21.976211	H	14.708877	3.624816	22.049191
C	12.291879	2.504285	12.836578	C	12.666489	2.750776	13.206995
O	11.226981	2.870820	12.329901	O	11.632568	3.239265	12.737301
N	12.595160	2.817464	14.110944	N	12.918722	2.857527	14.520140
H	13.507409	2.578568	14.472963	H	13.861102	2.729230	14.875112
C	11.725823	3.685174	14.905672	C	11.909456	3.510331	15.335225
C	12.350870	3.973117	16.269045	C	12.230527	3.363451	16.812604
S	12.143855	2.557500	17.386937	S	12.075095	1.652503	17.386261
C	14.893296	0.582472	16.560778	C	13.780563	1.032588	17.204655
C	14.341367	-0.812172	16.705801	C	13.794036	-0.488341	17.400682
N	13.576793	-0.950181	17.920966	N	13.232908	-0.845009	18.693841
C	14.151389	-1.483063	19.006730	C	13.938270	-1.517566	19.614855

O	15.348180	-1.804955	19.029414	O	15.108589	-1.896782	19.443705
H	12.597371	-0.651198	17.961606	H	12.306916	-0.494827	18.956564
C	13.635166	-1.432883	15.512257	C	13.148140	-1.249279	16.244502
C	13.489566	-2.941557	15.703595	C	13.305764	-2.764774	16.325061
C	14.798231	-3.727151	15.873841	C	14.715351	-3.331239	16.552727
C	14.478561	-5.114915	15.300421	C	14.690918	-4.689162	15.827242
N	13.445187	-4.811457	14.312006	N	13.719487	-4.463201	14.760947
C	12.832707	-3.634591	14.517373	C	12.871729	-3.432219	15.021899
O	11.872243	-3.209212	13.868183	O	11.910212	-3.115680	14.335249
H	12.981810	-5.534242	13.747109	H	13.369391	-5.219804	14.165283
H	15.346486	-5.583967	14.836025	H	15.665834	-4.966037	15.420493
H	14.096834	-5.789248	16.075011	H	14.362527	-5.490966	16.501125
H	15.143650	-3.783284	16.904647	H	14.964380	-3.423100	17.610027
H	15.589720	-3.265434	15.273753	H	15.465050	-2.673576	16.099000
H	12.834167	-3.141221	16.565415	H	12.635578	-3.162220	17.102942
H	14.242925	-1.254024	14.619615	H	13.613271	-0.894184	15.316994
H	12.659720	-0.964735	15.350999	H	12.087134	-0.995460	16.172564
H	15.242251	-1.421405	16.878396	H	14.847419	-0.785605	17.449770
C	15.058985	1.414866	17.593530	C	14.795316	1.723721	18.125064
C	16.037939	2.529611	17.554358	C	15.988200	2.191921	17.325324
O	16.762724	2.794450	16.607397	O	15.931939	2.486192	16.140466
O	16.120513	3.108725	18.743703	O	17.093081	2.240233	18.051295
C	17.262733	3.947861	18.998476	C	18.326897	2.634358	17.409081
C	18.509030	3.136658	19.275423	C	19.472226	1.952444	18.104379
C	19.754855	3.582897	18.825056	C	20.518766	1.415079	17.351429
C	20.927047	2.914792	19.178379	C	21.632453	0.857983	17.987330
C	20.860962	1.764918	19.960845	C	21.692193	0.820541	19.380047
C	19.617580	1.288659	20.372118	C	20.639005	1.342615	20.130990
C	18.449697	1.976092	20.052023	C	19.537020	1.906606	19.497500
H	17.491204	1.605722	20.405934	H	18.717026	2.304569	20.088662
H	19.558333	0.375760	20.949968	H	20.671831	1.303745	21.211114
H	21.765290	1.241150	20.250557	H	22.549798	0.384025	19.882768
H	21.887769	3.283029	18.831031	H	22.454548	0.464552	17.395314
H	19.808680	4.445222	18.169855	H	20.469023	1.443339	16.266018
H	16.952811	4.509886	19.880519	H	18.403363	3.722893	17.483836
H	17.408176	4.629152	18.161895	H	18.287743	2.359833	16.354215
H	14.612818	1.221153	18.562044	H	15.136593	1.058063	18.924844
H	12.627045	3.101823	18.532534	H	14.358162	2.584105	18.651187
H	15.349288	0.856719	15.612243	H	14.071655	1.201295	16.167918
H	11.845913	4.842059	16.688868	H	11.482731	3.914541	17.380476
H	13.406843	4.225641	16.134285	H	13.218777	3.764661	17.062421
H	10.757883	3.192422	15.031787	H	10.963190	2.994569	15.144376
C	11.552155	5.045468	14.213257	C	11.748564	4.979418	14.897485
O	12.572861	5.694985	13.951882	O	12.743810	5.615000	14.528911
N	10.307637	5.453578	13.992443	N	10.504373	5.448508	14.931427
H	9.565332	4.811256	14.242267	H	9.774091	4.819638	15.252734
C	9.968110	6.657016	13.194741	C	10.099052	6.731501	14.314267
H	10.781441	7.364264	13.343958	H	10.951052	7.406730	14.406711
H	9.080587	7.097105	13.653827	H	9.288158	7.135527	14.924491
H	9.786621	6.472951	12.228728	H	9.794534	6.657383	13.364648
H	13.550396	-1.594107	19.798233	H	13.455383	-1.701529	20.470996
H	12.912568	1.943321	12.288797	H	13.271370	2.215977	12.616987
H	12.504126	5.153090	24.490359	H	12.820506	5.212613	24.492343

Results for Compound B1

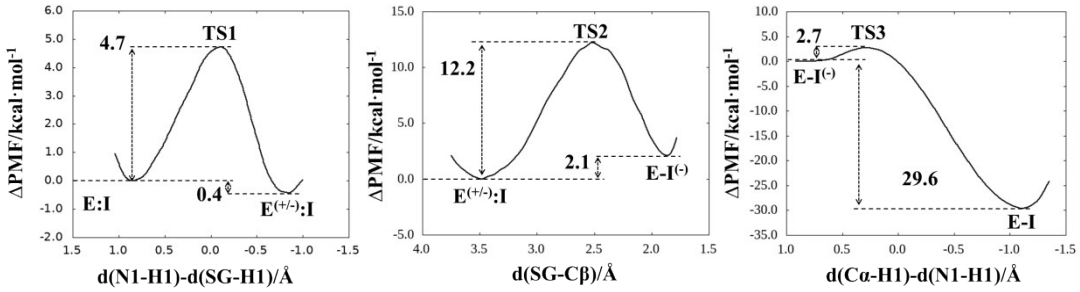


Figure S9. M06-2X/6-31+G(d,p):AM1/MM FESs corresponding to the inhibition of SARS-CoV-2 M^{pro} cysteine protease by **B1.**

Table S8. Average distances (in Å) for the states located along the inhibition mechanism of SARS-CoV-2 M^{pro} by the compound **B1**, obtained from AM1/MM MD simulations on the stationary points extracted from the M06-2X/6-31+G(d,p):AM1/MM free energy profiles.

Inter-atomic distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	TS3	E-I
SG-C_β	3.60 ± 0.30	3.69 ± 0.30	3.50 ± 0.02	2.49 ± 0.02	1.90 ± 0.02	1.90 ± 0.02	1.89 ± 0.02
Cα-C_β	1.34 ± 0.02	1.34 ± 0.02	1.34 ± 0.02	1.36 ± 0.02	1.45 ± 0.03	1.48 ± 0.03	1.51 ± 0.03
Cα–H1	4.78 ± 0.34	4.70 ± 0.37	4.08 ± 0.99	4.13 ± 0.22	1.86 ± 0.03	1.58 ± 0.04	1.10 ± 0.03
N1-H1	2.32 ± 0.04	1.49 ± 0.04	1.03 ± 0.02	1.02 ± 0.02	1.06 ± 0.03	1.28 ± 0.04	2.26 ± 0.04
SG-H1	1.47 ± 0.04	1.59 ± 0.03	1.93 ± 0.07	1.98 ± 0.08	2.86 ± 0.24	2.03 ± 0.02	2.84 ± 0.12
H2-Ow	2.02 ± 0.15	1.96 ± 0.13	2.03 ± 0.15	1.97 ± 0.15	2.13 ± 0.20	2.26 ± 0.26	2.43 ± 0.33
OD2(D187)-Hw	1.77 ± 0.14	1.78 ± 0.15	1.72 ± 0.12	1.70 ± 0.11	1.74 ± 0.12	1.85 ± 0.22	1.79 ± 0.46
S1´ pocket							
O1-H(G143)	3.24 ± 0.37	3.75 ± 0.55	2.72 ± 0.32	3.65 ± 0.38	2.60 ± 0.31	3.79 ± 0.97	2.71 ± 0.46
S1 pocket							
O2-HE2(H163)	2.14 ± 0.21	2.20 ± 0.34	2.03 ± 0.14	2.02 ± 0.14	2.19 ± 0.23	2.11 ± 0.19	2.24 ± 0.27
O2-H(E166)	3.16 ± 0.30	3.34 ± 0.33	3.46 ± 0.27	3.25 ± 0.35	3.22 ± 0.28	2.99 ± 0.30	3.02 ±0.27
H3-O(F140)	3.87 ± 0.43	4.01 ± 0.38	4.32 ± 0.44	4.31 ± 0.44	3.84 ± 0.60	4.62 ± 0.37	4.39 ± 0.61
H3-OE1(E166)	2.58 ± 0.34	2.46 ± 0.33	2.74 ± 0.38	2.47 ± 0.31	2.71 ± 0.65	2.42 ± 0.33	2.96 ± 0.50
H4-O(H164)	2.94 ± 0.29	2.91 ± 0.29	3.70 ± 0.43	2.53 ± 0.23	2.16 ± 0.16	2.64 ± 0.26	2.11 ± 0.16
S4 pocket							
O5-HE22(Q192)	3.59 ± 0.33	3.64 ± 0.32	3.86 ± 0.24	3.74 ± 0.32	3.60 ± 0.33	3.87 ± 0.34	3.66 ± 0.24

Table S9. Key distances (in Å) for the states located along of the inhibition mechanism of SARS-CoV-2 M^{pro} by the compound **B1**, optimized at M06-2X/6-31+G(d,p)/MM level of theory.

Inter-atomic distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	TS3	E-I
SG-C_β	3.67	3.79	3.83	2.32	1.92	1.86	1.83
Cα-C_β	1.33	1.33	1.32	1.40	1.48	1.53	1.53
Cα–H1	3.92	4.32	4.29	3.63	3.34	1.66	1.10
N1-H1	2.13	1.34	1.07	1.04	1.03	1.22	2.07
SG-H1	1.36	1.68	2.19	2.55	2.76	3.32	2.78
H2-Ow	1.82	1.83	1.78	1.81	1.81	1.84	1.77
OD2(D187)-Hw	1.67	1.67	1.67	1.69	1.67	1.74	1.71
S1´ pocket							
O1-H(G143)	2.21	2.19	2.09	2.18	2.00	3.47	3.41
S1 pocket							
O2-HE2(H163)	2.06	2.12	2.14	2.07	2.08	1.74	1.72
O2-H(E166)	3.12	3.09	3.16	3.19	3.27	4.01	4.65
H3-O(F140)	4.24	4.35	4.15	4.62	4.23	3.21	2.55
H3-OE1(E166)	1.78	1.85	1.70	1.98	1.71	2.68	3.30
H4-O(H164)	1.99	2.03	2.01	1.84	1.87	1.81	1.86
S4 pocket							
O5-HE22(Q192)	3.69	3.66	3.69	4.01	3.67	3.89	3.45

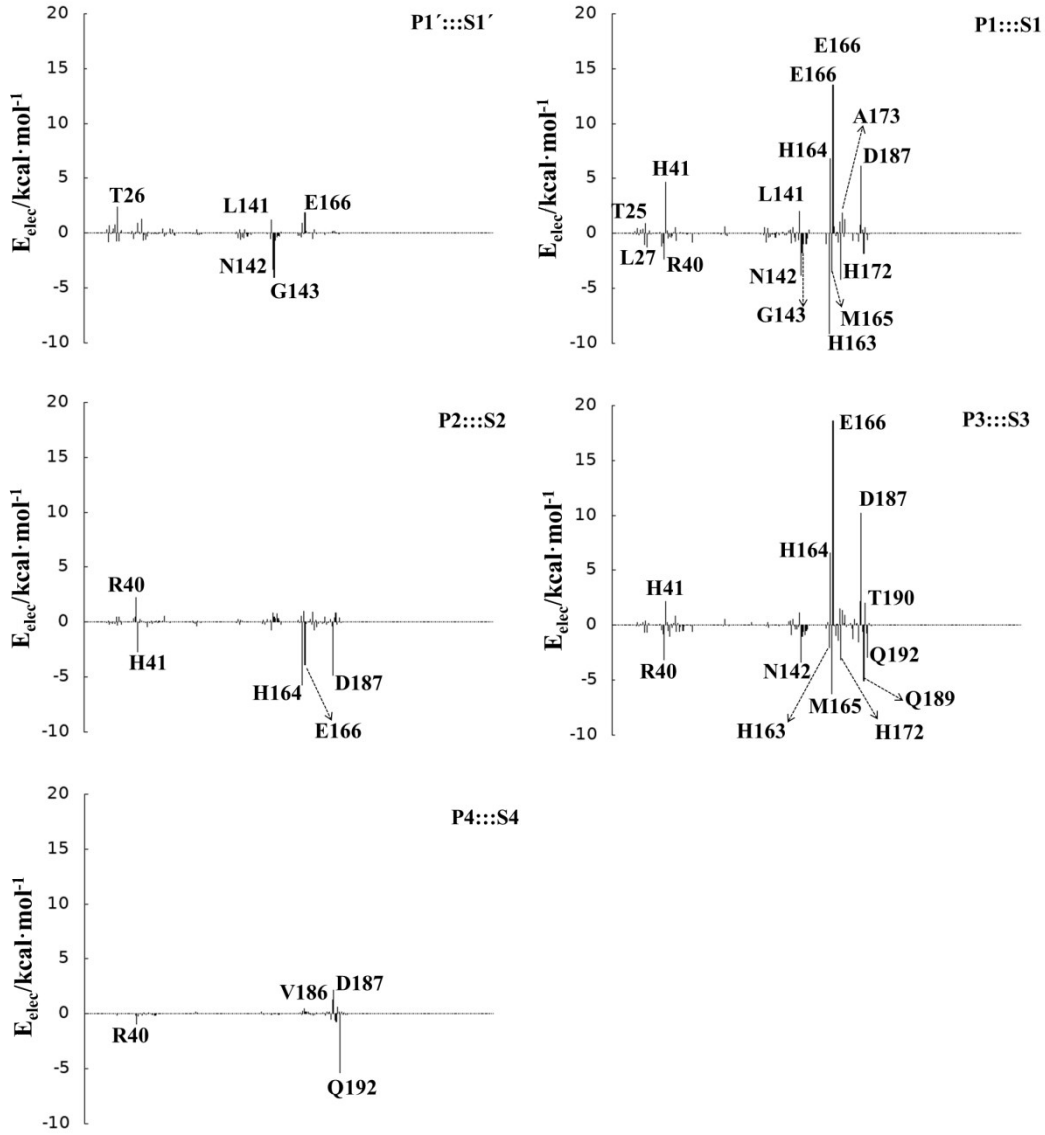


Figure S10. Averaged electrostatic interaction energies between residues of Chain-A and each fragment of the compound **B1** in **E:I**, obtained as an average over 1000 structures of the AM1/MM MD simulations. Only residues showing electrostatic interaction energies higher than 1.5 kcal·mol^{-1}, in absolute value, are labelled in the panels.

Table S10. Cartesian coordinates (in Å) of QM atoms for TS1, TS2, TS3, E:I and E-I of the inhibition process by the compound **B1** optimized at M06-2X/6-31+G(d,p)/MM level.

TS1 (ν_i = 1013.4i cm^{-1})				TS2 (ν_i = 160.7i cm^{-1})			
Atoms	x	y	z	Atoms	x	y	z
C	7.160424	14.710739	24.241316	C	7.190814	14.754559	24.397871
H	6.450962	14.202273	24.899364	H	6.448184	14.241006	25.012050
H	8.153073	14.503180	24.648159	H	8.162204	14.558410	24.857504
C	7.065869	14.119538	22.850516	C	7.185434	14.182063	23.006919
N	5.988951	13.338767	22.477096	N	6.113263	13.448740	22.527756
H	5.122214	13.231281	23.020391	H	5.232128	13.304483	23.042131
C	6.201716	12.859592	21.239265	C	6.380215	12.985353	21.305990

H	5.505505	12.239670	20.695889	H	5.727254	12.381785	20.694694
N	7.359411	13.294348	20.772990	N	7.594701	13.407281	20.969787
C	7.918053	14.081040	21.768422	C	8.123317	14.154000	22.007164
H	8.881475	14.556224	21.654796	H	9.109653	14.592056	21.964885
C	7.208727	11.823520	13.216074	C	7.270953	11.758848	13.217708
O	6.026812	11.489292	13.093532	O	6.077165	11.484861	13.075858
N	7.697999	12.122553	14.450861	N	7.747713	12.063132	14.450112
H	8.594207	12.593344	14.462363	H	8.666574	12.489065	14.471231
C	6.730510	12.610564	15.452366	C	6.797155	12.598803	15.429757
C	7.416380	13.196151	16.706018	C	7.546729	13.090545	16.678911
S	7.731087	12.029835	18.093491	S	8.031679	11.803430	17.894817
C	11.300475	10.841308	17.604762	C	10.312235	11.460506	17.646677
C	10.583995	9.668858	18.222238	C	10.354134	9.994348	18.101906
N	9.930237	9.948120	19.489854	N	9.879344	9.839209	19.488049
C	10.722613	10.227655	20.549156	C	10.673466	10.227896	20.527490
O	11.951362	10.135957	20.468757	O	11.898189	10.280262	20.434058
H	8.914143	10.007396	19.556898	H	8.869548	9.793722	19.650245
C	9.721012	8.919858	17.196201	C	9.710534	9.005987	17.100160
C	10.575569	7.847396	16.493420	C	10.669645	7.921998	16.552941
C	10.957782	7.958449	15.015532	C	11.071241	7.885259	15.069706
C	11.360398	6.511706	14.668748	C	11.618183	6.450796	14.918896
N	10.475060	5.724594	15.536857	N	10.792259	5.690561	15.869448
C	9.908905	6.471836	16.505660	C	10.094898	6.507425	16.695459
O	9.012926	6.135737	17.278438	O	9.147754	6.191084	17.410617
H	10.310133	4.718349	15.418973	H	10.607313	4.685937	15.770393
H	11.183102	6.269919	13.619515	H	11.491609	6.064858	13.905525
H	12.413800	6.323645	14.908357	H	12.677875	6.395616	15.191965
H	11.773551	8.659651	14.842724	H	11.828948	8.632177	14.837158
H	10.093386	8.266310	14.419613	H	10.206110	8.038708	14.417366
H	9.264446	9.624376	16.493376	H	9.277135	9.579124	16.274042
H	8.899008	8.425201	17.727500	H	8.869803	8.507182	17.595422
H	11.400175	8.984093	18.491203	H	11.426328	9.775429	18.150289
C	11.651922	11.965788	18.220149	C	11.120603	12.382360	18.322629
C	12.560563	12.919859	17.549848	C	11.972287	13.244494	17.593607
O	12.435629	13.254055	16.387746	O	11.889719	13.537993	16.394703
O	13.552900	13.427802	18.301796	O	13.048835	13.768191	18.295508
C	14.145606	12.615488	19.352869	C	13.835674	12.870060	19.100292
C	14.118217	13.283081	20.703549	C	13.879679	13.230467	20.566724
C	13.225701	14.302021	21.032509	C	13.548137	14.501720	21.030620
C	13.227141	14.849251	22.318010	C	13.736626	14.840797	22.371433
C	14.118950	14.376017	23.277449	C	14.258107	13.904030	23.260477
C	15.018000	13.359299	22.951339	C	14.572505	12.620766	22.809614
C	15.017032	12.817028	21.670392	C	14.379228	12.285789	21.471915
H	15.727926	12.033303	21.419550	H	14.625842	11.284828	21.124822
H	15.740285	13.006063	23.680731	H	14.981476	11.885101	23.495601
H	14.126924	14.808607	24.273720	H	14.438970	14.178967	24.294830
H	12.548035	15.659363	22.559469	H	13.504445	15.843647	22.709687
H	12.543674	14.692188	20.281516	H	13.162465	15.239286	20.336940
H	15.177084	12.445648	19.035633	H	14.850222	12.916084	18.689082
H	13.633000	11.649804	19.400207	H	13.464755	11.848327	18.979813
H	7.636675	12.898584	19.522160	H	8.024793	13.155487	20.061904
H	11.381539	12.186430	19.247047	H	11.316943	12.284226	19.381082
H	11.668848	10.664886	16.593686	H	10.343062	11.556135	16.558989

H	6.761397	13.983829	17.072832	H	6.879695	13.778056	17.200380
H	8.354787	13.675782	16.408915	H	8.432437	13.668453	16.384939
H	6.068847	11.791945	15.748063	H	6.093046	11.812751	15.717967
C	5.915551	13.719926	14.736261	C	6.041513	13.762537	14.712975
O	6.498256	14.461817	13.946078	O	6.695898	14.524484	14.004584
N	4.610251	13.783679	15.014042	N	4.713785	13.831559	14.892364
H	4.216120	13.075671	15.621731	H	4.278771	13.077922	15.408545
C	3.712292	14.730760	14.316187	C	3.831152	14.801702	14.183923
H	4.268087	15.662505	14.216783	H	4.450467	15.676789	14.007415
H	2.870017	14.920716	14.986106	H	3.051103	15.092464	14.891208
H	3.362923	14.423117	13.431146	H	3.411005	14.470166	13.339198
H	11.437023	7.752641	16.992337	H	11.561397	7.953530	17.004365
H	10.275252	10.526124	21.392236	H	10.223161	10.481217	21.383676
H	7.781651	11.851757	12.396951	H	7.881536	11.749976	12.425806
H	6.991319	15.693079	24.321386	H	7.008008	15.736130	24.453546

TS3 (ν_i = 894.2i cm^{-1})			
Atoms	x	y	z
C	7.469646	14.439581	24.122143
H	6.713318	13.889180	24.687118
H	8.423386	14.235353	24.615195
C	7.543494	13.900958	22.709166
N	6.452154	13.299066	22.114364
H	5.534596	13.226589	22.569340
C	6.801848	12.813860	20.912644
H	6.146955	12.280943	20.240513
N	8.082372	13.072893	20.686470
C	8.563440	13.751829	21.797144
H	9.588162	14.083014	21.866899
C	7.214593	11.525769	13.202949
O	6.364508	10.623084	13.128964
N	7.327823	12.324970	14.278412
H	7.878220	13.175787	14.152343
C	6.292999	12.435837	15.302035
C	6.879383	12.455621	16.723518
S	7.225558	10.777042	17.371770
C	9.073559	10.853920	17.559544
C	9.666980	9.423044	17.847394
N	9.599011	9.167618	19.307552
C	10.698476	9.470589	20.047255
O	11.836354	9.223240	19.617289
H	8.687567	9.373832	19.736386
C	9.105747	8.258306	16.984823
C	10.092023	7.173059	16.490636
C	11.174190	7.605108	15.499762
C	11.589396	6.270805	14.855503
N	10.330516	5.531900	14.869715
C	9.433418	6.009929	15.757670
O	8.348685	5.509342	16.017806
H	10.178988	4.623478	14.450633
H	11.966743	6.382060	13.838162

H	12.346828	5.763507	15.469240
H	12.017894	8.116089	15.966259
H	10.722831	8.259088	14.752516
H	8.660154	8.705097	16.087976
H	8.274481	7.782183	17.517061
H	10.735373	9.525470	17.634668
C	9.589636	11.911992	18.537139
C	10.397591	12.941100	17.904605
O	10.247762	13.446513	16.799765
O	11.428751	13.318651	18.730356
C	12.221274	14.467226	18.369616
C	13.212693	14.695920	19.482616
C	14.580971	14.757933	19.219801
C	15.487969	15.035703	20.243944
C	15.030749	15.242045	21.541917
C	13.665616	15.161388	21.820781
C	12.765548	14.887514	20.795381
H	11.699741	14.855263	21.004446
H	13.305236	15.344454	22.827911
H	15.734495	15.475439	22.334270
H	16.548266	15.095016	20.023475
H	14.942388	14.596509	18.208192
H	11.551588	15.323910	18.237894
H	12.725538	14.285705	17.416188
H	8.669873	12.654019	19.702283
H	10.211102	11.485871	19.320049
H	9.419124	11.113610	16.550431
H	6.150040	12.911110	17.394914
H	7.803591	13.044915	16.759940
H	5.633847	11.573248	15.193572
C	5.529482	13.739089	14.951054
O	6.167920	14.687135	14.504026
N	4.194407	13.713854	15.088709
H	3.783182	12.871224	15.469012
C	3.290180	14.703574	14.439970
H	3.856172	15.632115	14.374153
H	2.456421	14.875807	15.123760
H	2.945405	14.394251	13.553714
H	10.553147	6.601366	17.169261
H	10.572112	9.868208	20.956063
H	7.855318	11.676668	12.450154
H	7.270921	15.413593	24.230815

E:I				E-I			
Atoms	x	y	z	Atoms	x	y	z
C	7.125760	14.706326	24.316988	C	7.341304	14.403289	24.340082
H	6.384379	14.237205	24.968683	H	6.567434	13.887781	24.912739
H	8.101007	14.506808	24.768127	H	8.275652	14.269361	24.890945
C	7.079809	14.094685	22.946998	C	7.497341	13.786986	22.984026
N	6.001349	13.369190	22.499614	N	6.460221	13.172506	22.328528
H	5.108803	13.271758	22.995885	H	5.505454	13.123999	22.689569
C	6.297626	12.896398	21.264392	C	6.927324	12.686403	21.155445

H	5.600871	12.299681	20.694362	H	6.318613	12.126109	20.460788
N	7.501161	13.274394	20.879041	N	8.209870	12.951787	21.008738
C	7.996919	14.026590	21.923301	C	8.575563	13.640692	22.144866
H	8.980196	14.476218	21.893756	H	9.581779	14.001860	22.302602
C	7.182109	11.819506	13.197515	C	7.224408	11.432508	13.215294
O	6.007150	11.462024	13.093440	O	6.312128	10.603663	13.096776
N	7.663857	12.153386	14.424459	N	7.414952	12.138367	14.347821
H	8.535609	12.668834	14.422745	H	7.991287	12.971825	14.218764
C	6.669541	12.627852	15.394388	C	6.323439	12.348434	15.287148
C	7.340934	13.139577	16.665587	C	6.867066	12.570911	16.697134
S	7.800787	11.771227	17.783487	S	7.197323	11.012291	17.556471
C	11.346319	10.817390	17.622498	C	9.025514	10.957628	17.496185
C	10.602982	9.662214	18.236387	C	9.492980	9.500172	17.750961
N	9.947566	9.972644	19.498558	N	9.459021	9.242566	19.234032
C	10.746086	10.255991	20.554515	C	10.598719	9.507825	19.975514
O	11.973756	10.167264	20.475226	O	11.709059	9.247881	19.491846
H	8.933795	9.932607	19.614894	H	8.565798	9.472866	19.692296
C	9.734301	8.935762	17.198722	C	8.734200	8.554840	16.733597
C	10.577348	7.863752	16.487811	C	9.489047	7.289004	16.254317
C	10.952877	7.973006	15.009529	C	10.995172	7.524755	16.151288
C	11.374630	6.528614	14.686113	C	11.555065	6.403285	15.274815
N	10.462794	5.748069	15.530135	N	10.414326	6.127401	14.400928
C	9.895684	6.494936	16.493858	C	9.243187	6.383501	15.043310
O	8.979667	6.171297	17.250202	O	8.242882	5.681616	14.978520
H	10.305893	4.739731	15.412075	H	10.405178	5.305958	13.807809
H	11.234783	6.274943	13.635788	H	12.439337	6.704954	14.711263
H	12.418953	6.349311	14.967020	H	11.806815	5.511128	15.865261
H	11.759228	8.683612	14.833152	H	11.495023	7.678480	17.102928
H	10.082969	8.258862	14.411080	H	11.111742	8.467876	15.603075
H	9.296030	9.650941	16.496445	H	8.515679	9.197220	15.876043
H	8.899542	8.447558	17.718290	H	7.767128	8.282006	17.160949
H	11.404453	8.961671	18.510440	H	10.555316	9.475356	17.508451
C	11.689590	11.949317	18.227846	C	9.683657	11.957785	18.445677
C	12.611872	12.884383	17.548942	C	10.434491	13.073599	17.775759
O	12.468524	13.220043	16.388978	O	10.281566	13.435034	16.627432
O	13.627478	13.365103	18.280957	O	11.338027	13.552977	18.620648
C	14.180828	12.574306	19.370600	C	12.317095	14.518255	18.178308
C	14.124029	13.279721	20.701249	C	13.274156	14.721982	19.325013
C	13.194674	14.278452	20.990276	C	14.641677	14.841476	19.083247
C	13.156718	14.856628	22.260922	C	15.522074	15.104480	20.132394
C	14.047738	14.436619	23.245620	C	15.039464	15.230388	21.432150
C	14.984908	13.442863	22.959427	C	13.674638	15.091522	21.684753
C	15.021987	12.867972	21.692507	C	12.796640	14.836745	20.635102
H	15.761499	12.101293	21.473672	H	11.731750	14.744951	20.827840
H	15.706664	13.131988	23.708647	H	13.291680	15.215662	22.691833
H	14.022841	14.893587	24.230555	H	15.724455	15.452097	22.243951
H	12.445033	15.646593	22.473505	H	16.581062	15.220750	19.928625
H	12.514331	14.630281	20.219117	H	15.023714	14.726063	18.074379
H	15.217416	12.383963	19.083805	H	11.791649	15.436560	17.899175
H	13.653770	11.616819	19.426956	H	12.825773	14.130388	17.291119
H	7.871412	12.494306	18.935188	H	8.982017	12.406275	19.168049
H	11.402270	12.192864	19.245140	H	10.424387	11.489784	19.094172
H	11.736425	10.622524	16.623268	H	9.303746	11.197311	16.463077

H	6.644815	13.785037	17.195762	H	6.132829	13.091617	17.303170
H	8.224352	13.732309	16.415318	H	7.780465	13.174284	16.672365
H	5.989414	11.809874	15.646034	H	5.687897	11.460923	15.271616
C	5.874635	13.758615	14.697841	C	5.546370	13.580541	14.773313
O	6.468761	14.479137	13.898815	O	6.157073	14.419450	14.111573
N	4.577148	13.847470	14.995971	N	4.239474	13.628377	15.046866
H	4.175617	13.144373	15.604825	H	3.835509	12.867917	15.583756
C	3.684640	14.797619	14.295256	C	3.344998	14.640294	14.415449
H	4.259623	15.710062	14.164504	H	3.900702	15.575494	14.401005
H	2.859513	15.020788	14.974676	H	2.488061	14.753582	15.079840
H	3.322784	14.475122	13.420581	H	3.028681	14.384183	13.502020
H	11.449622	7.770310	16.967818	H	9.072293	6.512543	16.726994
H	10.299679	10.553408	21.398473	H	10.538537	9.884618	20.899854
H	7.761592	11.847047	12.382996	H	7.865497	11.597655	12.465806
H	6.969328	15.693725	24.340904	H	7.117667	15.377902	24.329295

Results for Compound B2

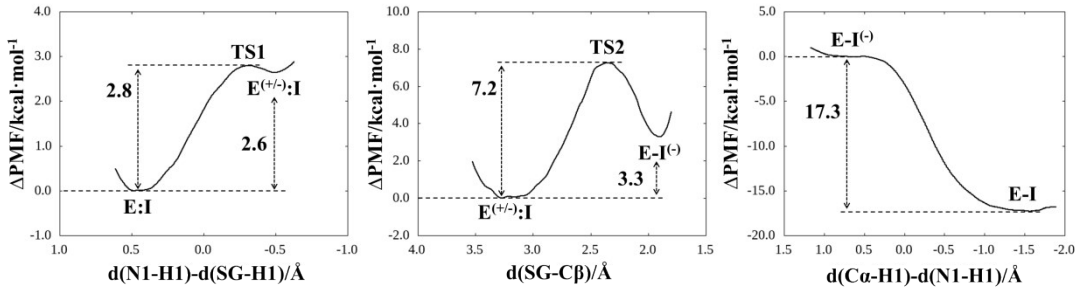


Figure S11. M06-2X/6-31+G(d,p):AM1/MM FESs corresponding to the inhibition of SARS-CoV-2 M^{pro} cysteine protease by **B2.**

Table S11. Average distances (in Å) for the states located along the inhibition mechanism of SARS-CoV-2 M^{pro} by the compound **B2**, obtained from 20 ps of AM1/MM MD simulations on the stationary points extracted from the M06-2X/6-31+G(d,p):AM1/MM free energy profiles.

Distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	E-I
SG-C_β	3.07 ± 0.23	3.18 ± 0.15	3.25 ± 0.02	2.33 ± 0.02	1.90 ± 0.01	1.85 ± 0.04
Cα-C_β	1.34 ± 0.02	1.34 ± 0.02	1.34 ± 0.02	1.39 ± 0.02	1.45 ± 0.03	1.52 ± 0.03
Cα–H1	3.72 ± 0.31	4.28 ± 0.30	3.28 ± 0.27	3.57 ± 0.24	1.88 ± 0.03	1.14 ± 0.03
N1-H1	1.99 ± 0.04	1.33 ± 0.04	1.03 ± 0.02	1.02 ± 0.02	1.06 ± 0.03	2.69 ± 0.04
SG-H1	1.49 ± 0.04	1.64 ± 0.03	1.94 ± 0.08	2.00 ± 0.08	2.53 ± 0.20	2.68 ± 0.14
H2-Ow	2.11 ± 0.17	2.05 ± 0.15	1.98 ± 0.13	2.01 ± 0.14	2.10 ± 0.16	2.20 ± 0.22
OD2(D187)-Hw	1.77 ± 0.15	1.78 ± 0.16	1.71 ± 0.12	1.79 ± 0.17	1.87 ± 0.21	2.00 ± 0.42
S1´ pocket						
NO_2-H(G143)	2.37 ± 0.28	2.45 ± 0.24	2.85 ±0.33	2.67 ± 0.30	2.72 ± 0.31	2.39 ± 0.37
S1 pocket						
O2-HE2(H163)	1.98 ± 0.14	1.93 ± 0.10	2.00 ± 0.14	1.95 ± 0.13	2.03 ± 0.15	1.96 ± 0.17
NH_2-O(F140)	2.35 ± 0.27	2.33 ± 0.22	2.73 ± 0.49	2.71 ± 0.29	2.87 ± 0.28	2.85 ± 0.33
NH_2-OE1(E166)	3.97 ± 0.87	2.88 ± 0.42	3.05 ± 0.46	3.47 ± 0.35	2.48 ± 0.42	3.59 ± 0.54
H4-O(H164)	2.13 ± 0.18	2.26 ± 0.20	2.17 ± 0.20	2.04 ± 0.16	2.06 ± 0.14	1.99 ± 0.13
S3 pocket						
O4-H (E166)	2.23 ± 0.25	2.73 ± 0.31	2.21 ± 0.20	1.98 ± 0.13	1.97 ± 0.15	2.60 ± 0.33

Table S12. Key distances (in Å) for the states located along of the inhibition mechanism of SARS-CoV-2 M^{pro} by the compound **B2**, optimized at M06-2X/6-31+G(d,p)/MM level of theory.

Distances	E:I	TS1	$E^{(+/-)}$:I	TS2	E-$I^{(-)}$	E-I
SG-C_β	3.66	3.67	3.54	2.52	1.91	1.82
Cα-C_β	1.33	1.33	1.33	1.37	1.47	1.54
Cα–H1	4.31	4.88	4.86	4.42	4.24	1.09
N1-H1	2.06	1.32	1.09	1.05	1.03	2.73
SG-H1	1.36	1.66	2.05	2.32	2.74	2.76
H2-Ow	1.80	1.82	1.78	2.26	1.86	1.83
OD2(D187)-Hw	1.65	1.73	1.63	1.65	1.64	1.68
S1´ pocket						
NO_2-H(G143)	2.43	2.43	2.37	3.41	3.06	2.45
S1 pocket						
O2-HE2(H163)	1.81	1.85	1.81	1.84	1.84	1.92
NH_2-O(F140)	2.24	2.21	2.22	3.15	2.87	3.33
NH_2-OE1(E166)	2.76	3.58	2.81	1.84	1.85	1.85
H4-O(H164)	2.01	2.07	2.01	1.95	1.80	1.86
S3 pocket						
O4-H (E166)	2.06	2.05	2.06	2.05	2.36	2.51

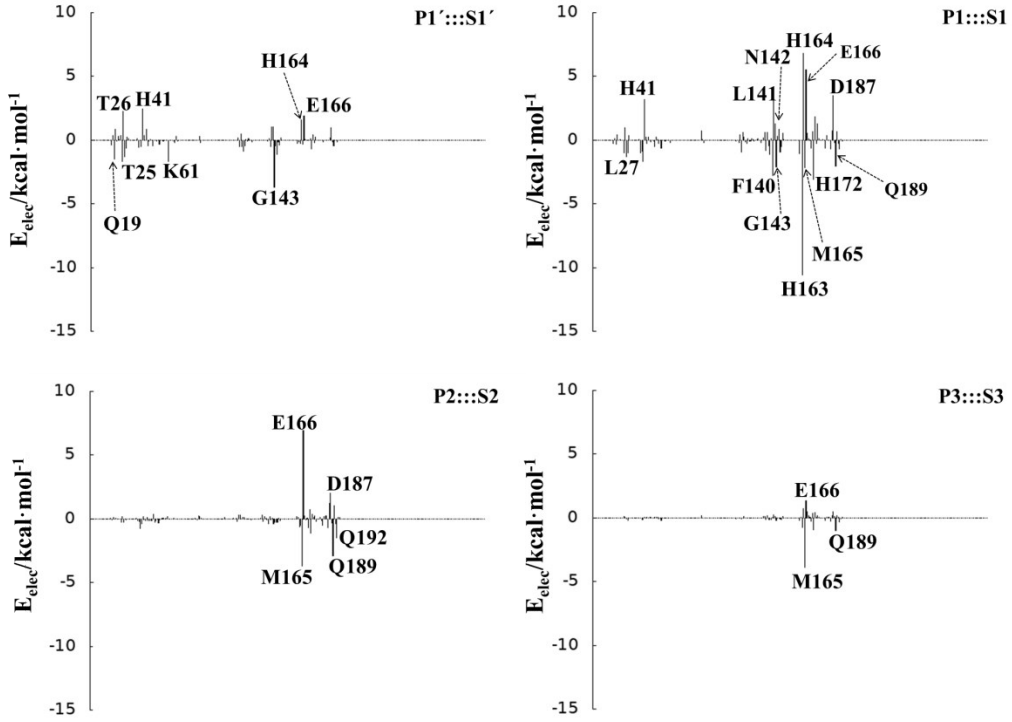


Figure S12. Averaged electrostatic interaction energies between residues of Chain-A and each fragment of the compound **B2** in **E:I**, obtained as an average over 1000 structures of the AM1/MM MD simulations. Only residues showing electrostatic interaction energies higher than 1.5 kcal·mol^{-1}, in absolute value, are labelled in the panels.

Table S13. Cartesian coordinates (in Å) of QM atoms for TS1, TS2, E:I, and E-I of the inhibition process by the compound **B2** optimized at M06-2X/6-31+G(d,p)/MM level.

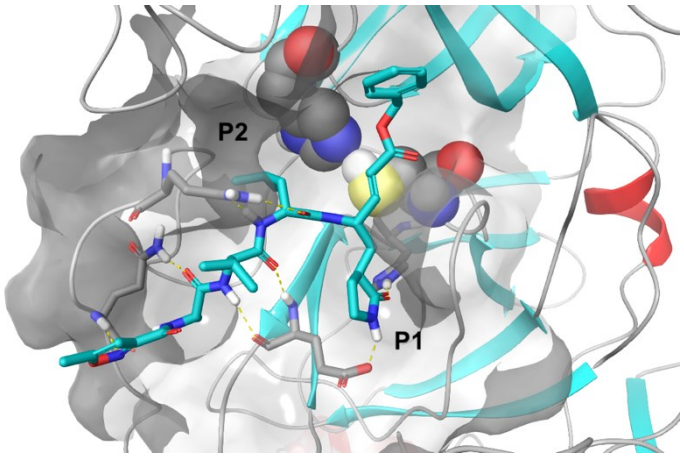
TS1 (ν_i = 927.7i cm^{-1})				TS2 (ν_i = 150.3i cm^{-1})			
Atoms	x	y	z	Atoms	x	y	z
C	13.712747	3.177925	23.954806	C	13.057600	3.313555	24.468392
H	12.919789	2.531558	24.340874	H	12.203166	2.846209	24.964708
H	14.659552	2.697244	24.216944	H	13.946204	2.763741	24.787382
C	13.614442	3.263815	22.454999	C	12.919542	3.177010	22.975236
N	12.393610	3.230660	21.816101	N	11.690660	3.029178	22.356616
H	11.491905	3.135728	22.292469	H	10.788776	3.018270	22.847533
C	12.594694	3.281008	20.488551	C	11.860564	2.916988	21.037525
H	11.801336	3.260636	19.757485	H	11.084959	2.809772	20.297737
N	13.887727	3.352281	20.231824	N	13.157510	2.992934	20.779741
C	14.544523	3.342206	21.446033	C	13.844512	3.150252	21.965903
H	15.622359	3.379209	21.516907	H	14.919806	3.231876	22.006596
C	13.226951	3.899965	13.167570	C	13.381318	4.079769	12.983961
O	12.132452	4.345462	12.807346	O	12.220453	4.105813	12.582893
N	13.464827	3.689269	14.474382	N	13.665609	4.038371	14.317582
H	14.400796	3.508231	14.817487	H	14.599660	4.335497	14.594015
C	12.461280	4.088022	15.444295	C	12.623641	4.336539	15.301148
C	12.814899	3.591964	16.860248	C	13.136694	4.268406	16.747319
S	14.571655	3.735219	17.366894	S	13.385924	2.622034	17.498454
C	16.736718	0.863261	16.637681	C	15.380056	1.936511	16.118007
C	16.099091	-0.499550	16.512675	C	15.215685	0.429811	16.104210
N	15.284789	-0.777708	17.694603	N	14.651122	-0.123440	17.323789
C	15.870503	-1.357302	18.764164	C	15.404532	-0.922774	18.077196
O	17.095970	-1.545196	18.804158	O	16.615140	-1.101444	17.848757
H	14.287256	-0.559465	17.704589	H	13.667201	0.033437	17.554419
C	15.315223	-0.697451	15.215931	C	14.517477	-0.177868	14.882404
C	14.941850	-2.178819	15.044815	C	15.014899	-1.616925	14.747786
C	14.438615	-2.459884	13.641726	C	14.342084	-2.455966	13.679201
N	15.313218	-3.078186	12.818484	N	15.103946	-3.462026	13.204483
H	15.000691	-3.363394	11.897332	H	14.675175	-4.196419	12.639687
H	16.137103	-3.529830	13.188180	H	16.021053	-3.637684	13.600424
O	13.320001	-2.111328	13.273891	O	13.186403	-2.251345	13.323109
H	14.149233	-2.456053	15.745097	H	14.846227	-2.147322	15.694608
H	15.817588	-2.798084	15.264829	H	16.099256	-1.624682	14.593382
H	15.960464	-0.389669	14.384210	H	14.765237	0.393249	13.981431
H	14.423980	-0.059602	15.184082	H	13.431093	-0.141750	15.004639
H	16.907277	-1.236501	16.524809	H	16.234818	0.038080	16.105073
C	17.995773	0.967277	17.058011	C	16.562162	2.395087	16.640485
N	18.582353	2.282397	17.264355	N	16.949873	3.728773	16.490987
O	19.506596	2.358054	18.065135	O	17.969345	4.103546	17.065658
O	18.148588	3.239420	16.650816	O	16.285040	4.487105	15.758621
H	14.294879	3.490832	18.987786	H	13.512579	2.941187	19.792178
H	18.644980	0.161797	17.379610	H	17.209960	1.825033	17.292856
H	16.131075	1.757342	16.467413	H	14.879365	2.499043	15.341755
H	12.534184	2.535502	16.904952	H	12.372970	4.765049	17.348088
H	12.172540	4.144327	17.551994	H	14.052191	4.860404	16.809651
H	11.533965	3.576811	15.167183	H	11.789578	3.644457	15.161201
C	12.171313	5.602904	15.284138	C	12.191417	5.796175	15.083250

O	13.081234	6.388955	15.003109	O	13.065197	6.631566	14.842805
N	10.882313	5.936195	15.405601	N	10.895470	6.074015	15.221304
H	10.234484	5.196502	15.653783	H	10.265082	5.312543	15.441124
C	10.317025	7.259533	15.051944	C	10.340317	7.389132	14.839394
H	11.118473	7.983505	15.191189	H	11.088915	8.133444	15.109162
H	9.559046	7.491362	15.804984	H	9.467301	7.564952	15.471660
H	9.899384	7.301681	14.144310	H	10.078449	7.450971	13.876274
H	15.279932	-1.673772	19.506506	H	14.947094	-1.397805	18.828924
H	13.912039	3.654815	12.481599	H	14.152637	4.104389	12.347989
H	13.643211	4.054053	24.431843	H	13.129408	4.257191	24.791495

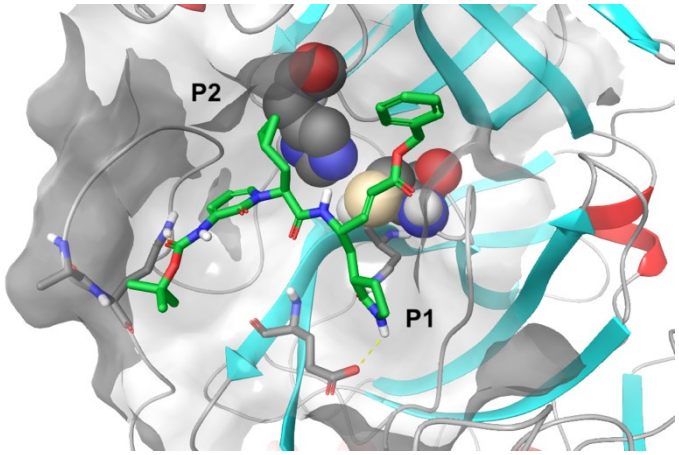
E:I				E-I			
Atoms	x	y	z	Atoms	x	y	z
C	13.573515	3.191211	23.997584	C	14.161021	3.505417	24.585146
H	12.748065	2.614873	24.424534	H	13.437373	2.930570	25.173282
H	14.496486	2.713570	24.338338	H	15.146441	3.116836	24.852687
C	13.538231	3.152434	22.497756	C	13.932744	3.249663	23.118134
N	12.360180	3.214379	21.794069	N	12.722014	2.835785	22.610091
H	11.427924	3.238263	22.213362	H	11.826918	2.829227	23.106047
C	12.659845	3.161405	20.474605	C	12.881044	2.617356	21.277798
H	11.894929	3.172352	19.712880	H	12.066639	2.284447	20.650488
N	13.958123	3.076364	20.276974	N	14.114724	2.856533	20.889614
C	14.518396	3.071259	21.535831	C	14.774900	3.259872	22.028784
H	15.586668	3.000520	21.679776	H	15.807554	3.569824	21.987418
C	13.084283	3.876035	13.051478	C	13.788546	3.915023	12.968681
O	11.944753	4.199752	12.725566	O	12.601418	4.020781	12.654930
N	13.348761	3.564650	14.341234	N	14.136689	3.888970	14.282783
H	14.319066	3.549605	14.629822	H	15.072647	4.213010	14.511216
C	12.381600	3.966886	15.351524	C	13.086723	4.074836	15.275468
C	12.837229	3.506380	16.737486	C	13.580074	3.765681	16.685259
S	14.586792	3.922120	17.072276	S	13.608405	1.986131	16.996984
C	16.682438	0.961249	16.571222	C	15.349535	1.549862	16.685871
C	16.012710	-0.378833	16.426663	C	15.427740	0.033416	16.442199
N	15.206531	-0.620055	17.616844	N	14.869118	-0.669732	17.596643
C	15.800425	-1.198124	18.678424	C	15.559609	-1.644271	18.206106
O	17.027113	-1.386201	18.709408	O	16.665132	-2.007218	17.773448
H	14.199122	-0.450408	17.621538	H	13.925084	-0.443548	17.929239
C	15.207890	-0.559915	15.147649	C	14.809049	-0.466305	15.128484
C	14.727248	-2.017305	15.040997	C	15.261630	-1.899471	14.850194
C	14.441272	-2.387293	13.599800	C	14.517251	-2.596336	13.717978
N	15.443401	-2.994782	12.938868	N	15.130654	-3.697505	13.236429
H	15.229475	-3.391112	12.027867	H	14.595734	-4.354380	12.662378
H	16.247135	-3.353687	13.443315	H	15.988196	-4.042471	13.670504
O	13.368486	-2.113831	13.063964	O	13.425490	-2.199829	13.312709
H	13.812612	-2.167687	15.617767	H	15.094951	-2.519436	15.739960
H	15.489263	-2.685408	15.454510	H	16.340652	-1.948137	14.665275
H	15.865204	-0.325493	14.302264	H	15.114151	0.195469	14.307584
H	14.361804	0.137320	15.106413	H	13.717518	-0.419349	15.180587
H	16.798908	-1.139899	16.431939	H	16.491771	-0.229544	16.437099
C	17.921310	1.034860	17.051573	C	16.260999	1.965416	17.853077
N	18.502830	2.335180	17.330809	N	17.134326	3.158379	17.540821

O	19.451559	2.354076	18.104628	O	17.562981	3.783679	18.478959
O	18.041029	3.334701	16.813027	O	17.409657	3.407220	16.367701
H	14.587773	3.440416	18.346724	H	15.716222	2.236176	18.763013
H	18.541279	0.212211	17.386881	H	16.997413	1.190087	18.099483
H	16.109614	1.871234	16.383019	H	15.674411	2.049480	15.769980
H	12.722075	2.421071	16.802634	H	12.870964	4.173644	17.409436
H	12.195025	3.976417	17.483883	H	14.551904	4.227772	16.893186
H	11.443476	3.452559	15.135359	H	12.283991	3.374906	15.029745
C	12.138725	5.494522	15.205449	C	12.536834	5.499627	15.231826
O	13.095363	6.230600	14.930136	O	13.323738	6.441037	15.051189
N	10.876315	5.894591	15.334062	N	11.230774	5.619858	15.426304
H	10.189819	5.192286	15.597028	H	10.687631	4.789293	15.657556
C	10.401992	7.265184	15.006851	C	10.517706	6.897502	15.254572
H	11.242070	7.932930	15.191909	H	11.180607	7.693795	15.596987
H	9.627986	7.511750	15.736631	H	9.657119	6.856848	15.926880
H	10.026764	7.361070	14.084891	H	10.191393	7.063821	14.324057
H	15.207278	-1.524806	19.414260	H	15.151671	-2.038227	19.029747
H	13.819973	3.853083	12.374549	H	14.504389	3.822858	12.276529
H	13.524586	4.115320	24.376568	H	14.091722	4.460126	24.874508

a)



b)



c)

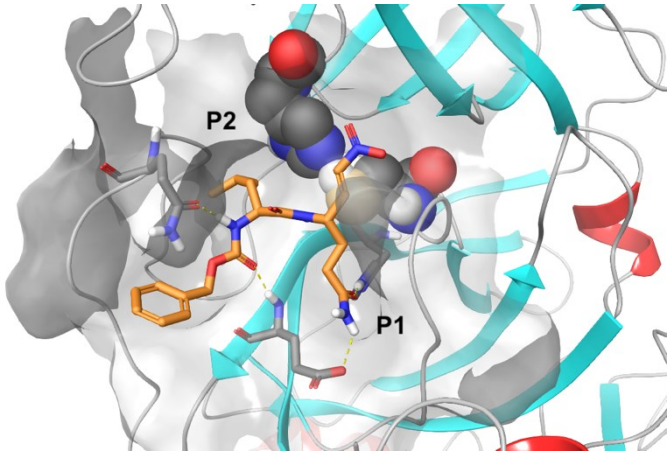


Figure S13. Compounds docked into the active site of SARS-CoV-2 M^{pro} (grey carbon atoms): a) **N3** (cyan carbon atoms); b) **B1** (green carbon atoms); and c) **B2** (orange carbon atoms). The catalytic residues Cys145 and His41 are displayed with a space fill representation. The P1 and P2 residues are labelled. The reference N3 inhibitor gave a Gscore values of –14.4 kcal·mol^{-1}, consistent with the high number of hydrophilic and lipophilic contacts formed by this compound within the M^{pro} active site. Compounds B1 and B2 also predicted to bind with good affinity: Gscore values of –10.6 and –8.6 kcal·mol-1, respectively. The less negative score of these putative inhibitors is due to their smaller size compared to N3, but better pharmacokinetic properties can be predicted.

References

1. Z. Jin, X. Du, Y. Xu, Y. Deng, M. Liu, Y. Zhao, B. Zhang, X. Li, L. Zhang, C. Peng, Y. Duan, J. Yu, L. Wang, K. Yang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, X. Yang, F. Bai, H. Liu, X. Liu, L. W. Guddat, W. Xu, G. Xiao, C. Qin, Z. Shi, H. Jiang, Z. Rao and H. Yang, *Nature*, 2020, **582**, 289-293.
2. J. Wang, W. Wang, P. A. Kollman and D. A. Case, *Journal of Molecular Graphics & Modelling*, 2006, **25**, 247-260.
3. M. H. M. Olsson, C. R. Sondergaard, M. Rostkowski and J. H. Jensen, *Journal of Chemical Theory and Computation*, 2011, **7**, 525-537.
4. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *Journal of Chemical Physics*, 1983, **79**, 926-935.
5. Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. M. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. M. Wang and P. Kollman, *Journal of Computational Chemistry*, 2003, **24**, 1999-2012.
6. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé and K. Schulten, *Journal of Computational Chemistry*, 2005, **26**, 1781-1802.
7. G. S. Grest and K. Kremer, *Physical Review A*, 1986, **33**, 3628-3631.
8. D. R. Roe and T. E. Cheatham, *Journal of Chemical Theory and Computation*, 2013, **9**, 3084-3095.
9. M. J. Field, M. Albe, C. Bret, F. Proust-De Martin and A. Thomas, *J. Comp. Chem.*, 2000, **21**, 1088-1100.
10. A. Krzeminska, P. Paneth, V. Moliner and K. Swiderek, *Journal of Physical Chemistry B*, 2015, **119**, 917-927.
11. M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902-3909.
12. Y. Zhao and D. G. Truhlar, *Theoretical Chemistry Accounts*, 2008, **120**, 215-241.
13. W. J. Hehre, L. Radom, P. v. R. Schleyer and J. A. Pople, *Ab Initio Molecular Orbital Theory*, New York, 1986.
14. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson and H. C. Nakatsuji, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. , *Gaussian 09 (Revision A.1)*, 2009.
15. A. J. Turner, V. Moliner and I. H. Williams, *Physical Chemistry Chemical Physics*, 1999, **1**, 1323-1331.
16. S. Martí, V. Moliner and I. Tuñón, *Journal of Chemical Theory and Computation*, 2005, **1**, 1008-1016.
17. G. M. Torrie and J. P. Valleau, *Journal of Computational Physics*, 1977, **23**, 187-199.

18. S. Kumar, D. Bouzida, R. H. Swendsen, P. A. Kollman and J. M. Rosenberg, *Journal of Computational Chemistry*, 1992, **13**, 1011-1021.
19. J. Kästner and W. Thiel, *Journal of Chemical Physics*, 2006, **124**, 234106.
20. J. S. Hub, B. L. de Groot and D. van der Spoel, *Journal of Chemical Theory and Computation*, 2010, **6**, 3713-3720.
21. A. Grossfield, "WHAM: the weighted histogram analysis method", version 2.0.10, http://membrane.urmc.rochester.edu/wordpress/?page_id=126).
22. *Glide, Schrödinger, LLC, New York, NY*, 2019.
23. *MacroModel, Schrödinger, LLC, New York, NY*, 2019.
24. *Schrödinger, LLC, New York, NY*, 2019.
25. R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, D. E. Shaw, P. Francis and P. S. Shenkin, *Journal of Medicinal Chemistry*, 2004, **47**, 1739-1749.