Biochemical screening for SARS-CoV-2 main protease inhibitors

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Supporting Information

S1 Table. Kinetics of SARS-Cov-2 M^{pro} inhibitors. IC$_{50}$, inhibitory (dissociation) constants (K$_i$), Michaelis Menten constants (K$_m$), turnover numbers (k$_{cat}$), and different kinetic model candidates (together with acceptance criteria values) of SARS-Cov-2 M^{pro} inhibitors are given.

Compound	IC$_{50}$ (μM)*	Ki (μM)*	Km (μM)*	k$_{cat}$ (s^{-1})*	Inhibition mechanism**	SSQ§	ΔAIC$^\&$	ΔBIC$^\#$
Thimerosal (1)	0.6 ± 0.1	0.6 ± 0.2	47 ± 8	0.010 ± 0.0011	**Mixed** non-competitive	1 1.4	0 2.4	0 1.5
Phenylmercuric acetate (2)	0.4 ± 0.06	0.11 ± 0.03	52 ± 19	0.013 ± 0.0024	**Competitive** non-competitive	1 1.1	0 2.0	0 2.0
Bronopol (3)	4.4 ± 0.6	2.5 ± 0.3	45 ± 9	0.010 ± 0.0013	**non-competitive** mixed	1 1	0 3.0	0 3.9
Tannic acid (4)	2.1 ± 0.2	1.4 ± 0.14	47 ± 8	0.010 ± 0.0011	**Competitive** non-competitive	1 1.2	0 4.1	0 4.1
Hematoporphyrin (5)	3.9 ± 0.6	5.9 ± 0.5	50 ± 8	0.011 ± 0.0011	**non-competitive** mixed competitive	1 1 1.2	0 3.0 3.2	0 4.2 3.2
3,4-Didesmethyl-5-deshydroxy-3'-ethoxyscleroin (6)	10.6 ± 1.3	5.6 ± 0.5	51 ± 10	0.011 ± 0.0014	**non-competitive** competitive mixed	1 1.1 1	0 2.1 3.3	0 2.1 4.4
Evans blue (11)	0.2 ± 0.06	0.21 ± 0.022	56 ± 8	0.010 ± 0.0008	**Competitive**	1	0	0
Chicago Sky Blue (12)	7.7 ± 1.6	1.3 ± 0.2	56 ± 12	0.013 ± 0.0015	**Competitive** noncompetitive mixed	1 1.2 1	0 3.2 3.4	0 3.2 4.4

*Error values are expressed as standard error of the mean (SEM).
**Most probable mechanism according to SSQ, ΔAIC and ΔBIC analyses in bold.
§Summed squared deviation.
$^\&$Second order Akeike information criterion.
$^\#$Bayesian information criterion.

S2 Table. Toxicity of SARS-Cov-2 M^{pro} inhibitors.

Compound	LD$_{50}$*	Source
Thimerosal (1)	75 mg/kg (oral, rat)	Pesticide Chemicals Official Compendium, Association of the American Pesticide Control Officials, Inc., 1966, (1130), 1966.
Phenylmercuric acetate (2)	41 mg/kg (oral, rat)	Acute Toxicity Data. Journal of the American College of Toxicology, Part B., 1(175), 1992.
Bronopol (3)	180 mg/kg	Pesticide Index, Frear, E.H., ed., State College, PA, College Science Pub., 1969, 5(30), 1976.
Tannic acid (4)	No data available	
Hematoporphyrin (5)	307 mg/kg (IV, mouse)	Nippon Yakurigaku Zasshi. Japanese Journal of Pharmacology., 57(219), 1961.
3,4-Didesmethyl-5-deshydroxy-3'-ethoxyscleroin (6)	No data available	
2,3,4-Trihydroxy-4'-ethoxybenzophenone (7)	No data available	
Chloranil (8)	4 g/kg (oral, rat)	Pesticide Chemicals Official Compendium, Association of the American Pesticide Control Officials, Inc., 1966, (218), 1966.
Plumbagin (9)	16 mg/kg (oral, mouse)	Indian Journal of Experimental Biology, 18(876), 1980. [PMID:7461745]
Vanitiolide (10)	No data available	
Evans blue (11)	340 mg/kg (IP, mouse)	Biochemical and Biophysical Research Communications., 136(64), 1986. [PMID:3010977].
Chicago Sky Blue (12)	2260 mg/kg(IV, mouse)	Science, 114(41), 1951. [PMID:14854894]

*LD$_{50}$ – median lethal dose, as reported in www.pubchem.gov

S1 Figure: **Inhibition kinetics of SARS-Cov-2 M^{pro} inhibitors.** Enzyme kinetics experiments of selected inhibitory compounds were performed with the MCA-AVLQSGFR-K(Dnp)-K-NH2 fluorescent peptide as substrate (S) and using 0.5 μM of recombinant M^{pro} and the shown inhibitor concentrations (given in μM). Fluorescence values were converted using a calibration curve with MCA to molar values and corrected for IFE (internal filter effect) to obtain enzymatic rates.