

A Helquat-like Compound as a Potent Inhibitor of Flaviviral and Coronaviral Polymerases

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Table S1. Summary of viral polymerases list of polymerases used in this study.

JEV		NP_775674.1	pET28b	N- terminal His _{6x} -TEV
NTAV		YP_006846328.2	pET28b	N- terminal His _{6x} -TEV
TBEV		AKP16371.1	pET26-Ubq	N- terminal Ubiquitin and C-terminal His _{6x}
ZIKV		APP91864.1	pSUMO	N- terminal His _{8x} -SUMO
WNV		YP_001527887.1	pET28b	N- terminal His _{6x} -TEV
YFV		NP_041726.1	pSUMO	N- terminal His _{8x} -SUMO
SARS-CoV-2	nsp12	YP_009725307	pAceBac	C- terminal His _{6x}
	nsp7	YP_009725303	pHGT2	N- terminal His _{6x} -GB1-TEV
	nsp8	YP_009725304	pHGT2	-

Table S2. Exact composition of each polymerase reaction mixture.

	Flavivirus RdRp	SARS-CoV-2 RdRp
Buffer	5 mM Tris 7.4, 10 mM DTT, 2 mM MnCl ₂ , 0.5 % Triton X-100, 10% glycerol	10 mM Tris pH 8.0, 1 mM β-mercaptoethanol, 2 mM MgCl ₂ , 10 mM KCl,
NTPs [μM]	10	10
T/P [μM]	T1/P1 – 0.01	T2/P2 – 0.5
Polymerase [μM]	0.02	1 (nsp12) 3 (nsp7/nsp8 complex)

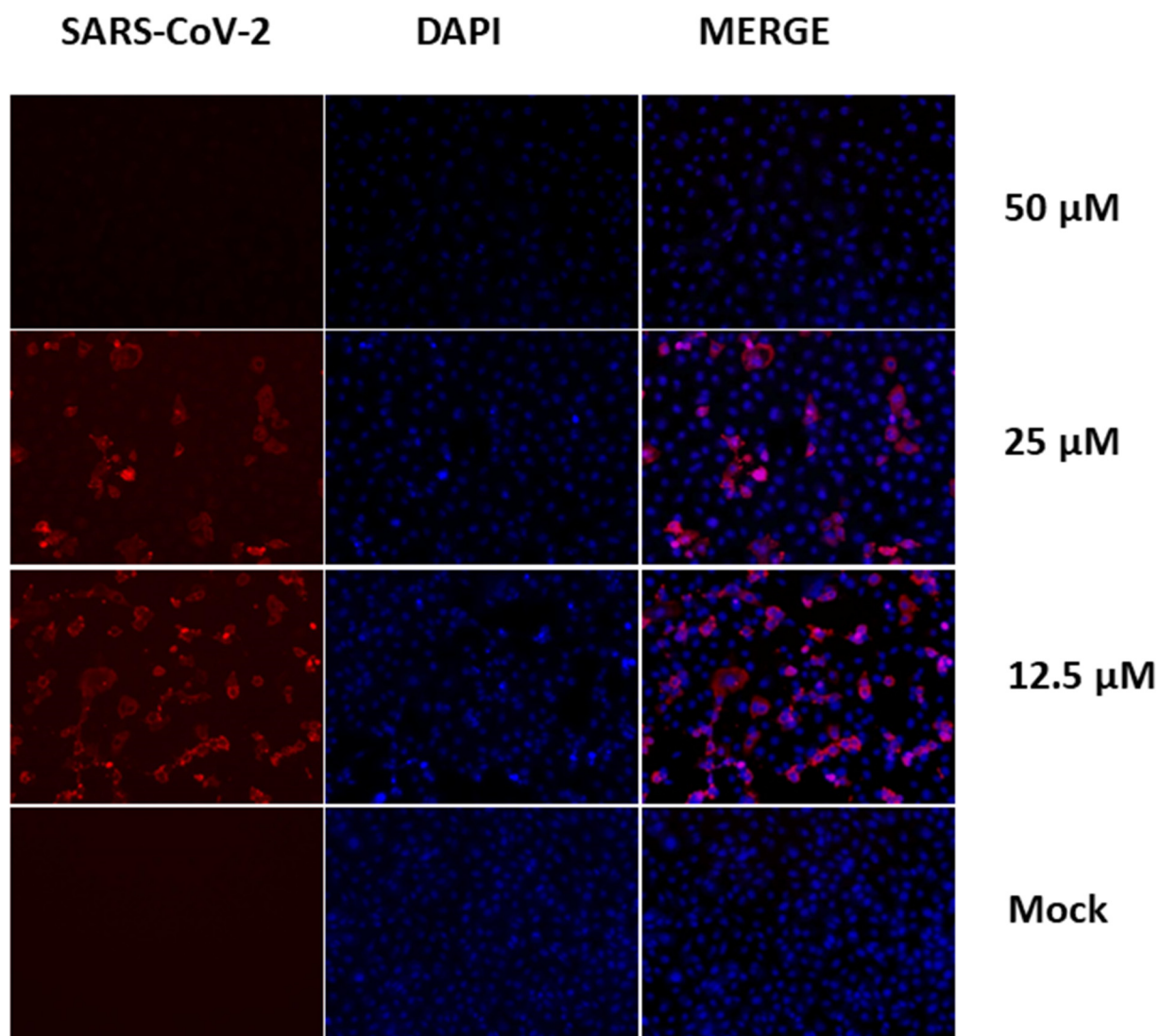


Figure S1. Immunofluorescence microscopy analysis of SARS-CoV-2 nucleoprotein expression after addition of 50 μ M, 25 μ M, and 12.5 μ M of PR673. VERO-E6 cells with and without (Mock) PR673 were infected with SARS-CoV-2 with MOI = 0.02, fixed 3 days post infection, stained with specific SARS-CoV-2 anti-nucleoprotein antibody followed by Cy3-conjugated anti-mouse antibody (red) and counterstained with DAPI (blue).

Synthesis of PR673

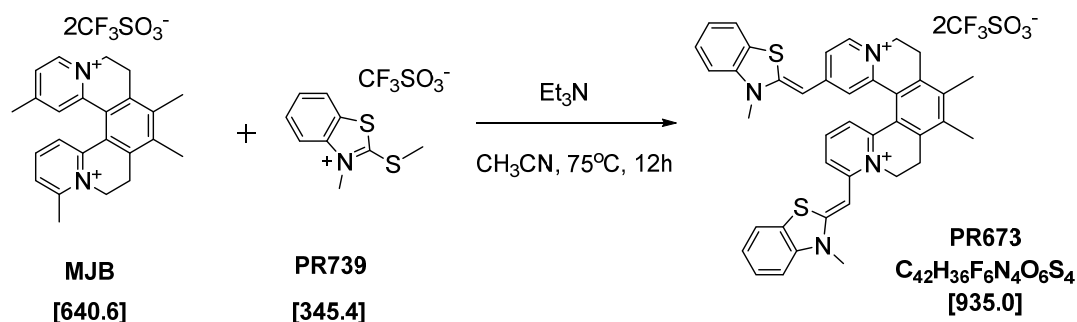
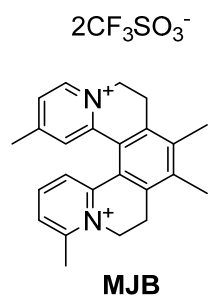


Figure S2. Synthesis of **PR673** by a condensation reaction between the helquat **MJB** and methylbenzothiazole **PR739**.

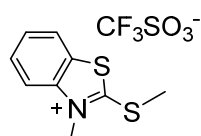
Starting helquat **MJB** (1.0 g, 1.0 equiv, 1.56 mmol), **PR379** (4.3 g, 8.0 equiv, 12.5 mmol), Et_3N (1.26 g, 1.74 mL, 8.0 equiv, 12.5 mmol) and dry CH_3CN (50 mL) were placed into a 100 mL flask and the resulting mixture was stirred under argon for 12 h at 75°C . Progress of the reaction was monitored by thin layer chromatography (mobile phase Stoddart's magic mixture, MeOH : 2 M NH_4Cl : MeNO_2 = 7: 2: 1). Crude product was transferred to 50 mL centrifuge tubes (6 tubes with 8.3 mL of reaction crude each one) and precipitated from reaction mixture by addition of Et_2O (35 mL to each tube). The resulting suspensions were centrifuged and supernatants were removed. Residues were dissolved in a minimum amount of CH_3CN /piperidine solution (60:40, 5 mL added to each tube), afterward Et_2O (35 mL) was added to each tube. Precipitates were centrifuged. This reprecipitation was repeated 6 times more. Centrifuged solids were finally washed with Et_2O . The resulting suspension was centrifuged and Et_2O was removed. The solids were dried under vacuum to get pure **PR673** as a deep red solid in 77% yield (1.12 g, 1.2 mmol).

^1H NMR (400 MHz, DMSO) δ 8.46 (d, J = 7.0 Hz, 1H), 7.76 (dd, J = 10.6, 6.1 Hz, 3H), 7.65 (d, J = 8.3 Hz, 1H), 7.61 – 7.50 (m, 3H), 7.44 (d, J = 8.3 Hz, 1H), 7.29 (dtd, J = 15.7, 8.1, 7.5, 2.8 Hz, 3H), 6.93 (d, J = 2.3 Hz, 1H), 6.85 (t, J = 7.7 Hz, 1H), 6.37 (s, 1H), 6.09 (s, 1H), 5.23 (d, J = 13.9 Hz, 1H), 4.71 (d, J = 12.7 Hz, 1H), 4.50 (t, J = 13.4 Hz, 1H), 4.36 (t, J = 13.6 Hz, 1H), 3.97 (s, 3H), 3.51 – 3.26 (m, 4H), 3.02 (t, J = 16.3 Hz, 2H), 2.42 (d, J = 10.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 157.85, 155.73, 152.70, 148.87, 146.69, 144.87, 141.22, 140.93, 140.26, 139.60, 138.64, 138.20, 138.13, 137.71, 127.72, 127.63, 125.50, 125.04, 123.66, 122.83, 122.64, 122.42, 122.40, 122.28, 122.21, 121.88, 120.27, 119.08, 111.79, 89.48, 83.10, 51.08, 47.62, 33.17, 32.76, 25.91, 25.70, 16.05. MS (ESI) m/z (%): 785.2 (TfO^- , 40), 318.1 (-2TfO^- , $z=2$, 100). HRMS (ESI) m/z : $[(\text{M}-2\text{TfO})]$ ($\text{C}_{40}\text{H}_{36}\text{N}_4\text{S}_2^{2+}$) calc.: 318.11852 ($z=2$), found: 318.11856 ($z=2$).



MJB

^1H NMR (400 MHz, DMSO) δ 9.00 (d, J = 6.4 Hz, 1H), 8.09 (t, J = 8.0 Hz, 1H), 7.97 (dd, J = 8.3, 1.5 Hz, 1H), 7.92 (dd, J = 7.8, 1.5 Hz, 1H), 7.83 (dd, J = 6.4, 1.9 Hz, 1H), 7.79 (d, J = 1.8 Hz, 1H), 5.14 (dd, J = 13.9, 3.8 Hz, 1H), 5.02 – 4.91 (m, 1H), 4.70 (t, J = 14.0 Hz, 1H), 4.37 (td, J = 14.3, 3.6 Hz, 1H), 3.53 – 3.37 (m, 2H), 3.08–2.97 (m, 5H), 2.40 (d, J = 2.8 Hz, 6H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 157.02, 155.07, 147.45, 146.19, 144.16, 142.62, 139.25, 138.95, 129.72, 128.05, 127.83, 126.22, 124.66, 53.11, 48.55, 25.15, 24.90, 21.00, 16.19, 16.15. MS (ESI) m/z (%): 491.2 (TfO $^-$, 10), 341.2 (-2TfO $^-$, 100). HRMS (ESI) m/z : [(M-2TfO $^-$)] ($\text{C}_{24}\text{H}_{26}\text{N}_2^{2+}$) calc.: 171.10425 ($z=2$), found: 171.10423 ($z=2$).



PR739

2-(Methylthio)benzothiazole (5.0 g, 1.0 equiv, 27.6 mmol) was dissolved in dry DCM (100 mL). Then, methyl trifluoromethanesulfonate (5.43 g, 3.63 mL, 1.2 equiv, 33.1 mmol) was added slowly and reaction mixture was stirred under argon for 12 h at RT. Crude product was transferred to 50 mL centrifuge tubes (3 tubes with 33.3 mL of reaction crude each one). The resulting suspensions were centrifuged and DCM was removed. Residues were washed with Et₂O (35 mL) by sonication. Precipitates were centrifuged and Et₂O was removed. This process was repeated 4 times more. The solids were dried under vacuum to get pure **PR739** as a white off solid in 79% yield (7.5 g, 21.8 mmol).

^1H NMR (400 MHz, DMSO) δ 8.38 (dd, J = 8.2, 1.3 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.84 (dd, J = 8.5, 7.3 Hz, 1H), 7.72 (dd, J = 8.5, 7.3 Hz, 1H), 4.11 (s, 3H), 3.12 (d, J = 0.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 181.35, 142.53, 129.18, 128.28, 127.03, 123.94, 115.70, 36.42, 18.02. MS (ESI) m/z (%): 196.0 (- TfO $^-$, 25), 181.0 (- TfO $^-$, - Me, 100). HRMS (ESI) m/z : [(M-TfO $^-$)] ($\text{C}_9\text{H}_{10}\text{NS}_2^+$) calc.: 196.02492, found: 196.02497.

