

Supplementary material

Carrageenans and the carrageenan-echinochrome complex as anti-SARS-CoV-2 agents

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Table S1. Contacts of CRG tetrasaccharides with the human ACE2 domain according to molecular docking data

CRG	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
κ-CRG	O1 14	O	ALA 387 (A)	H-donor	2.97 -1.9
	O2 58	OD1	ASP 38 (A)	H-donor	2.90 -2.7
	O6 82	O	HIS 34 (A)	H-donor	3.14 -0.7
	O2 16	NH2	ARG 393 (A)	H-acceptor	3.14 -1.8
	O4 37	NZ	LYS 353 (A)	H-acceptor	3.56 -0.8
	O8 43	NZ	LYS 353 (A)	H-acceptor	2.91 -12.6
	O2 58	NZ	LYS 353 (A)	H-acceptor	2.96 -3.7
	O3 60	NZ	LYS 353 (A)	H-acceptor	3.24 -6.8
	O7 85	NZ	LYS 31 (A)	H-acceptor	4.02 -0.8
	O9 87	NZ	LYS 31 (A)	H-acceptor	2.89 -11.4
	O8 43	NZ	LYS 353 (A)	ionic	2.91 -5.1
	O9 87	NZ	LYS 31 (A)	ionic	2.89 -5.2
β-CRG	C2 65	5-ring	HIS 34 (A)	H-pi	4.35 -0.6
	C5 9	OE1	GLU 37 (A)	H-donor	3.46 -0.9
	O2 34	OE1	GLU 37 (A)	H-donor	2.82 -1.9
	O2 55	OD1	ASP 38 (A)	H-donor	3.14 -1.4
	O5 20	NH2	ARG 393 (A)	H-acceptor	3.46 -1.2
	O2 55	NZ	LYS 353 (A)	H-acceptor	3.01 -7.5
	O3 75	NZ	LYS 31 (A)	H-acceptor	3.14 -2.9
λ-CRG	O4 77	NZ	LYS 31 (A)	H-acceptor	3.08 -7.3
	C 4	ND1	HIS 34 (A)	H-donor	3.53 -0.7
	O 55	OD1	ASP 38 (A)	H-donor	2.78 -2.5
	O 55	NZ	LYS 353 (A)	H-acceptor	2.95 -5.2
	O 69	CB	ASN 33 (A)	H-acceptor	3.70 -0.9
	O 69	CA	PRO 389 (A)	H-acceptor	3.52 -0.9
	O 70	CB	ASN 33 (A)	H-acceptor	3.58 -0.8
	O 71	CA	PRO 389 (A)	H-acceptor	3.51 -1.0
	O 71	NH2	ARG 393 (A)	H-acceptor	3.34 -3.0
	O 75	CA	LYS 353 (A)	H-acceptor	3.22 -0.7
	O 77	CB	ALA 386 (A)	H-acceptor	3.59 -0.6
	O 78	NE2	HIS 34 (A)	H-acceptor	3.17 -1.7
	O 86	NZ	LYS 353 (A)	H-acceptor	2.92 -12.3
	O 87	NH2	ARG 393 (A)	H-acceptor	3.28 -1.0
	O 97	NE2	HIS 34 (A)	H-acceptor	3.13 -1.5
	O 69	NH2	ARG 393 (A)	ionic	3.34 -2.6
	O 71	NH2	ARG 393 (A)	ionic	3.34 -2.6
	O 76	NH1	ARG 393 (A)	ionic	3.18 -3.4
	O 76	NH2	ARG 393 (A)	ionic	3.50 -1.9
	O 86	NZ	LYS 353 (A)	ionic	2.92 -5.0

Table S2. Contacts of CRG tetrasaccharides with the SARS-CoV-2 Delta variant RBD according to molecular docking data

CRG	Ligand	Receptor	Interaction	Distance	E (kcal/mol)	
κ -CRG	O3 18	N	GLY 496	H-acceptor	3.07	-0.9
	O2 34	OH	TYR 505	H-acceptor	3.02	-1.4
	O2 76	NH2	ARG 403	H-acceptor	2.90	-3.6
	O7 85	NH2	ARG 408	H-acceptor	2.98	-5.7
	O7 85	NH2	ARG 408	ionic	2.98	-4.6
β -CRG	O9 87	NH2	ARG 408	ionic	3.35	-2.5
	O4 77	OD2	ASP 405	H-donor	2.90	-3.6
	O5 59	NH1	ARG 403	H-acceptor	3.08	-1.7
	O6 80	NH2	ARG 408	H-acceptor	2.98	-3.5
λ -CRG	O 53	OE2	GLU 406	H-donor	2.79	-3.9
	O 75	NH1	ARG 403	H-acceptor	3.09	-5.5
	O 77	N	GLY 496	H-acceptor	3.25	-2.3
	O 80	NH2	ARG 408	H-acceptor	2.96	-5.9
	O 82	OH	TYR 505	H-acceptor	2.78	-3.3
	O 75	NH1	ARG 403	ionic	3.09	-3.9
	O 76	NH1	ARG 403	ionic	3.98	-0.6
	O 78	NH2	ARG 408	ionic	3.43	-2.2
	O 80	NH2	ARG 408	ionic	2.96	-4.7
	O 82	NH1	ARG 403	ionic	3.68	-1.3

Table S3. Anti-SARS-CoV-2 activity of different types of carrageenans

Compounds	CC ₅₀ (μ g/mL)	CPE Inhibition assay		RT-PCR assay		
		IC ₅₀ (μ g/mL)	SI	Ct _{ci}	Ct _{ci} - Ct _i	IC, %
κ -CRG	≥ 2000	61.0 $\pm$ 6.3	33.0 $\pm$ 3.0	25.7 $\pm$ 2.7	8.2 $\pm$ 0.9	35.9 $\pm$ 3.1
λ -CRG	≥ 2000	120.0 $\pm$ 13.2	16.7 $\pm$ 1.6*	21.2 $\pm$ 2.5	3.7 $\pm$ 0.4	14.9 $\pm$ 1.8*
Σ -CRG (κ + λ)	≥ 2000	85.0 $\pm$ 8.3	23.5 $\pm$ 2.1*	23.8 $\pm$ 2.6	6.3 $\pm$ 0.7	26.6 $\pm$ 2.2*
κ/β -CRG	≥ 2000	160.0 $\pm$ 17.6	12.5 $\pm$ 1.5*	20.3 $\pm$ 2.4	2.8 $\pm$ 0.3	11.0 $\pm$ 1.3*
Ribavirin®	730 $\pm$ 88	207 $\pm$ 25	3.5 $\pm$ 0.4	19.3 $\pm$ 2.5	1.8 $\pm$ 0.2	9.7 $\pm$ 1.2
Remdesivir®	72 $\pm$ 9	1.4 $\pm$ 0.1	51.4 $\pm$ 5.6	28.4 $\pm$ 3.4	10.9 $\pm$ 1.2	49.5 $\pm$ 6.1
Virus control (DMSO)				17.5 $\pm$ 1.9		
Cell control (DMSO)				36.0		

Note: Vero E6 cells were infected with virus (2.0 lg TCID₅₀/mL) and simultaneously treated with tested compounds. CC₅₀ - 50% cytotoxic concentration; IC₅₀ - 50% inhibitory concentration; SI - selectivity indices (CC₅₀/IC₅₀); IC (%) - inhibition coefficient; Ct – the threshold PCR cycle number; Ct_o - threshold PCR cycle number for uninfected cells (cell control); Ct_i - threshold PCR cycle number for infected cells without the drug (virus control); Ct_{ci} - threshold PCR cycle number for the infected cells treated by the investigated compound. Data represent mean $\pm$ SD from three independent experiments. * Significance of the differences between the parameters of κ -CRG compared to CRG polysaccharides (λ -CRG, Σ -CRG (κ + λ), and κ/β -CRG) ($p \leq 0.05$)

Table S4. Anti-SARS-CoV-2 action of the κ -CRG/Ech complex (RT-PCR assay)

Compounds	Pretreatment of virus			Pretreatment of cells			Simultaneous treatment			Treatment of infected cells		
	Ct _{si}	Ct _{si} - Ct _i	IC, %	Ct _{si}	Ct _{si} - Ct _i	IC, %	Ct _{si}	Ct _{si} - Ct _i	IC, %	Ct _{si}	Ct _{si} - Ct _i	IC, %
κ-CRG	20.3±2.2	3.4±0.4	13.1±1.6*	26.4±3.0	9.5±1.0	41.1±4.5*	24.2±2.7	7.3±0.8	30.3 ±3.1*	21.2±2.3	4.3±0.5	16.9±2.2
Ech	23.8±3.1	6.9±0.8	28.4±3.7*	18.4±2.0	1.5±0.2	5.5±0.6*	20.7±2.2	3.8±0.4	14.7±1.8*	18.1±2.1	1.2±0.3	4.4±0.9*
κ-CRG/Ech	27.2±3.3	10.3±1.2	45.3±5.9	22.0±2.8	5.1±0.6	20.3±2.4	26.1±2.9	9.2±1.0	39.7±4.2	21.7±2.4	4.8±0.5	19.0±2.3
Ribavirin®	NA	NA	NA	NA	NA	NA	19.3±2.3	2.4±0.3	9.1±1.1	19.8±2.2	2.9±0.3	11.1±1.2
Remdesivir®	NA	NA	NA	NA	NA	NA	28.0±3.0	11.1±1.2	49.6±5.4	29.2±3.3	12.3±1.2	56.3±6.7
Virus control	16.9 ± 2.0											
Cell control	36.0											

Note: Vero E6 cells were infected with virus (2.0 lg TCID₅₀/mL) and treated with tested compounds in various schemes. The results of the RT-PCR assay were evaluated using the IC (%) - inhibition coefficient; Ct – the threshold cycle; Ct_i – average Ct value for infected cells without the drug (virus control); Ct_{si} – average Ct value for infected samples after treatment with polysaccharides. Data represent mean ± SD from three independent experiments. * Significance of the differences between the parameters of κ -CRG/Ech complex compared to its components (κ -CRG and Ech) ($p \leq 0.05$).