Structural insights into plasticity and discovery of remdesivir metabolite GS-441524 binding in SARS-CoV-2 macrodomain

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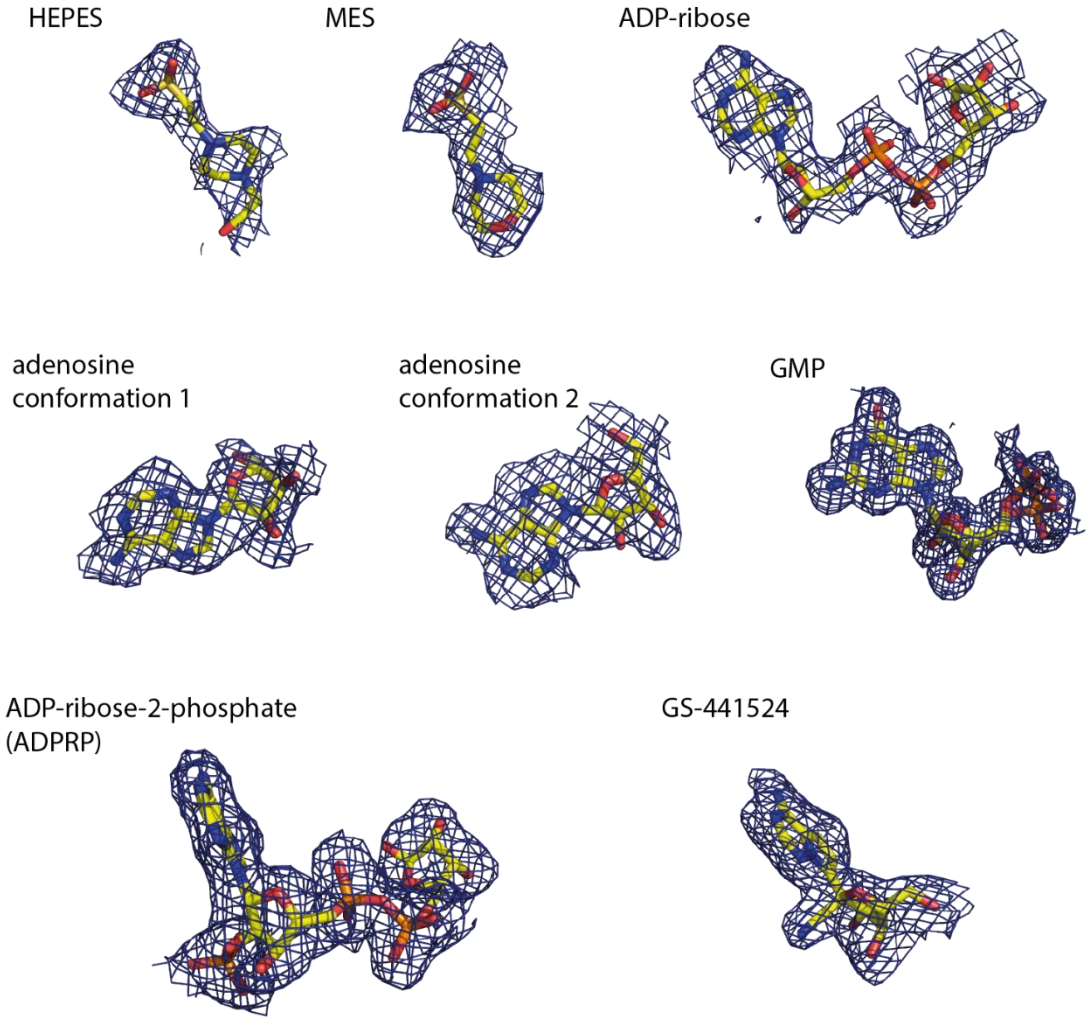
Supplementary information



Supplementary Figure s1. $|F_O|-|F_C|$ omitted electron density map contoured at 3σ for the bound ligands.

Supplementary table s1. Details of recombinant SARS-CoV-2 macrodomain.

	Vector	Recombinant protein sequence
SAR-CoV-2 macrodomain	**pET-28a(+)**	**MGSSHHHHHHSSGENLYFQGHMVNSFSGYLKLTDNVYIKNADIVEEAK KVKPTVVVNAANVYLKHGGGVAGALNKATNNAMQVESDDYIATNGP LKVGGSCVLSGHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLL APLLSAGIFGADPIHSLRVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMK**

Supplementary Table s2. Data collection and refinement statistics.

Complex	**apo/HEPES**	**apo/MES**	**ADP-ribose**
PDB codes	**6ywk**	**6ywm**	**6ywl**
Beamline	SLS X06SA	SLS X06SA	SLS X06SA
Data Collection			
Resolution[a] (Å)	49.09-2.20 (2.28-2.20)	49.22-2.16 (2.24-2.16)	48.83-2.50 (2.64-2.50)
Space group	$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_12_12_1$
Cell dimensions	a=39.2, b=111.8, c=196.4 Å α=β=γ=90.0	a=37.8, b=109.1, c=114.4 Å α=β=γ=90.0	a=38.4, b=111.9, c=195.3 Å α=β=γ=90.0°
Number of unique reflections[a]	45,087 (4,348)	26,281 (2,558)	30,002 (4,288)
Completeness[a] (%)	100.0 (99.9)	100.0 (100.0)	99.4 (99.3)
I/σI[a]	10.7 (2.0)	8.3 (2.0)	6.9 (1.9)
R_{merge}[a] (%)	0.138 (0.925)	0.162 (0.873)	0.199 (0.930)
CC (1/2) [a]	0.998 (0.762)	0.995 (0.787)	0.990 (0.736)
Redundancy[a]	8.5 (7.9)	6.7 (6.9)	5.6 (5.9)
Refinement			
Number atoms in refinement (P/L/O)[b]	6,496/ 15/ 424	3,885/ 24/ 287	6,472/ 180/ 248
B factor (P/L/O)[b] (Å^2)	39/ 76/ 48	34/ 57/ 39	40/ 31/ 38
R_{fact} (%)	17.6	17.5	18.9
R_{free} (%)	21.4	22.9	22.3
rmsd bond[c] (Å)	0.013	0.013	0.010
rmsd angle[c] (°)	1.4	1.3	1.1
Molprobity Ramachandran			
Favor (%)	99.65	99.01	98.11
Outlier (%)	0	0	0
Crystallization condition	33% broad-molecular-weight PEG smears, 0.1 M $MgCl_2$, 0.1 M HEPES, pH 7.0	23% PEG 6000, 0.1 M $MgCl_2$, 5% ethylene glycol, 0.1 M MES, pH 6.0	27% PEG 4000, 0.2 M sodium acetate, 0.05 M $MgCl_2$, 0.1 M tris, pH 8.0

[a] Value in brackets indicates high-resolution shell statistics.

[b] P/L/O indicates protein, ligands and others.

[c] rmsd indicates root-mean-square deviation.

Supplementary Table s2. ***(continued)*** Data collection and refinement statistics.

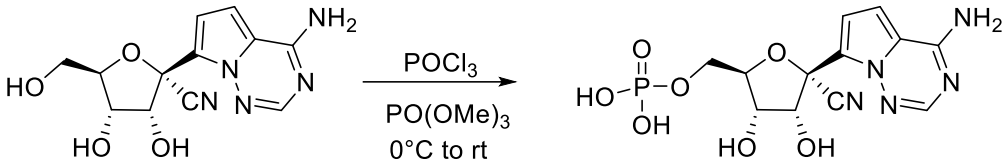
Complex	Adenosine	GMP	ADPRP	GS-441524
PDB codes	**7bf3**	**7bf4**	**7bf5**	**7bf6**
Beamline	SLS X06SA	SLS X06DA	SLS X06SA	SLS X06SA
Data Collection				
Resolution[a] (Å)	48.99-2.00 (2.07-2.00)	36.26-1.55 (1.60-1.55)	48.80-2.05 (2.12–2.05)	48.43-2.15 (2.23–2.15)
Space group	*P* $2_12_12_1$	*P* 4_1	*P* $2_12_12_1$	*C* 2
Cell dimensions	a=39.2, b=111.4, c=196.0 Å α=β=γ=90.0	a=b=72.5, c=33.4 Å α=β=γ=90.0	a=38.6, b=111.3, c=195,2 Å α=β=γ=90.0°	a=157.2, b=30.5, c=111,7 Å α= γ=90.0°, β=119.9°
Number of unique reflections[a]	59,412 (5,774)	25,315 (2,287)	53,878 (5,196)	25,440 (2,459)
Completeness[a] (%)	100.0 (100.0)	99.2 (92.8)	99.7 (99.8)	99.1 (99.2)
I/σI[a]	10.9 (2.0)	13.8 (2.6)	8.3 (1.9)	10.7 (1.9)
R_{merge}[a] (%)	0.127 (0.885)	0.064 (0.349)	0.141 (0.839)	0.090 (0.755)
CC (1/2) [a]	0.998 (0.735)	0.998 (0.807)	0.995 (0.695)	0.998 (0.677)
Redundancy[a]	7.5 (7.5)	6.1 (3.3)	6.2 (6.3)	5.3 (5.2)
Refinement				
Number atoms in refinement (P/L/O)[b]	6,538/ 38/ 628	1,329/ 48/ 241	6,500/ 160/ 569	3,838/ 63/ 168
B factor (P/L/O)[b] (Å^2)	29/ 60/ 37	14/ 13/ 30	28/ 41/ 35	49/ 39/ 43
R_{fact} (%)	17.5	13.8	17.7	18.0
R_{free} (%)	21.7	17.4	21.7	22.6
rmsd bond[c] (Å)	0.013	0.018	0.014	0.012
rmsd angle[c] (°)	1.4	1.7	1.4	1.4
Molprobity Ramachandran				
Favor (%)	98.94	99.40	97.64	99.00
Outlier (%)	0	0	0	0
Crystallization condition	33% broad-molecular-weight PEG smears, 0.1 M $MgCl_2$, 0.1 M tris, pH 7.0	30% PEG 4000, 0.2 M sodium acetate, 0.1 $MgCl_2$, 0.1 M tris, pH 8.3	30% broad-molecular-weight PEG smears, 0.1 M $MgCl_2$, 0.1 M tris, pH 7.0	30% PEG 4000, 0.2 M sodium acetate, 0.1 M tris, pH 8.3

[a] Value in brackets indicates high-resolution shell statistics.

[b] P/L/O indicates protein, ligands and others.

[c] rmsd indicates root-mean-square deviation.

Supplementary method. Synthesis of GS-441524 monophosphate



GS-441524 monophosphate

A solution of GS-441524 (43.7 mg, 0.15 mmol) in trimethyl phosphate (1.5 mL) was stirred in a sealed tube under Ar at rt for 15 min. The solution was then cooled to 0°C and freshly distilled phosphorous oxychloride (21.2 µL, 0.225 mmol) was added dropwise. The resulting solution was stirred at rt for 1h. Further 100 µL of phosphorous oxychloride were added at rt and the resulting solution was stirred at rt for 1h (full conversion by HPLC). The reaction mixture was quenched with water at 0°C and directly purified by preparative HPLC to obtain 43.6 mg (78%) of the expected product as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 7.98 (s, 1H), 7.27 (d, *J* = 4.9 Hz, 1H), 7.05 (d, *J* = 4.9 Hz, 1H), 4.83 (d, *J* = 5.2 Hz, 1H), 4.43-4.39 (m, 1H), 4.31 (t, *J* = 4.7 Hz, 1H), 4.04-3.92 (m, 2H); R_f HPLC: 3.4 Min (13 Min from 10 to 95% MeCN in water (0.1 % formic acid), then 7 min 95% MeCN). 95.7 % purity; HRMS (MALDI): m/z found. 372.0705 $[M+H]^+$ (cal. $C_{12}H_{15}N_5O_7P$ 372.0704).

To record NMR-spectra, the compound was dissolved in D_2O and measured on Avance 300 from Bruker Corporation (Massachusetts, USA). All chemical shift values are reported in ppm, the multiplicity of the signals assigned as follows: s (singlet), d (duplet), t (triplet) and m (multiplet). Mass spectrometry analysis was performed in positive ion mode by electrospray-ionization (ESI) on a LCMS-2020 single quadrupole MS from Shimadzu (Duisburg, Deutschland). Precision mass was measured using MALDI Orbitrap XL from Life Technologies GmbH (Darmstadt, Germany). For purity estimation of the synthesized compounds, a reverse phase high-performance liquid chromatography (RP-HPLC) was performed using the Luna 10 µm C18(2) 100 Å, LC Column 250 x 4.6 mm from Phenomenex LTD (Aschaffenburg, Germany) and the analysis was conducted using the Shimadzu prominence module from Shimadzu. Acetonitrile and aqueous formic acid 0.1% were used as eluents. The established method for purity determination was initiated with 90% water (0.1% formic acid), then a linear gradient from 90% to 5% water (0.1% formic acid) for 13 min was chosen, finally additional 7 min 5% water (0.1% formic acid). The flow rate was adjusted to 1.0 mL/min and the UV–vis detection occurred at 254 nm and 280 nm, respectively.