Molecular Docking and Dynamics Simulation Study of *Hyrtios erectus* Isolated Scalarane Sesterterpenes As Potential SARS-CoV-2 Dual Target Inhibitors

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Additional Experimental Detail

1) **Table S1.** Descriptive ligand/M-pro binding interactions through directed docking protocol.
2) **Table S2.** Descriptive ligand/Nsp15 NendoU binding interactions through directed docking protocol.
3) **Table S3.** Estimated RMSD and Rg parameter of investigated ligands and reference compound, lopinavir, complexed with M-pro proteins throughout the triplicate all-atom MD simulation.
4) **Table S4:** Estimated ΔRMSF[a] parameter of ligands/M-pro proteins across stable structure trajectories (last 20 ns).
5) **Table S5.** Estimated RMSD and Rg parameter of investigated ligands and reference compound, Benzopurpurin 4B, complexed with Nsp15 NendoU proteins throughout the triplicate all-atom MD simulation.
6) **Table S6:** Estimated ΔRMSF[a] parameter of ligands/Nsp15 NendoU proteins across stable structure trajectories (last 20 ns).
7) **Table S7.** Total binding-free energies and individual energy term ($\Delta G \pm$ SD) of the promising scalarane-based compounds and reference ligands within Nsp15 NendoU and/or M-pro protein binding site, across the three MD simulation runs.
8) **Figure S1.** Superimposing the crystallized (green sticks) and redocked (yellow sticks) ligands at (a) M-pro and (b) Nsp15 NendoU for validating the adopted directed docking protocol.
9) **Figure S2.** The 3D structural representation of the promising scalarane-based hits (sticks) and reference ligands showing respective pharmacophoric features (mesh spheres) as well as projected virtual points (arrows). Features and directionality of H-bond donor, H-bond acceptor, or aromaticity are colored in violet, cyan, or orange, respectively.

Table S1. Descriptive ligand/M-pro binding interactions through directed docking protocol

Compound	Ligand-target interaction description [Type; Length (Å); Angle (°); Binding Residues]
2	H-bond ; 2.6 **Å** ; 132 ° ; Glu166 (main chain N**H** with 12β-**O**H) H-bond ; 3.1 **Å** ; 131 ° ; Thr190 (main chain N**H** with 19α-OC(**O**)CH_3) H-bond ; 3.0 **Å** ; 121 ° ; Gln192 (side chain N**H**$_2$ with 19α-OC(**O**)CH_3) Sulphur-Dipole ; 3.3 **Å** ; Thr165 (side chain **S**CH_3 with 19α-**C**OOCH_3)
4	H-bond ; 3.2 **Å** ; 157 ° ; Glu166 (main chain N**H** with 12β-**O**C(O)CH_3)
6	H-bond ; 2.6 **Å** ; 126 ° ; Cys145 (side chain S**H** with 19-C=**O**) H-bond ; 2.7 **Å** ; 137 ° ; Glu166 (main chain N**H** with 12β-**O**H) π-H interaction ; 3.4 **Å** ; His41 (side chain)
8	H-bond ; 4.0 **Å** ; 106 ° ; His41 (side chain N**H** with 20-CH**O**) H-bond ; 1.9 **Å** ; 159 ° ; Glu166 (main chain N**H** with 12β-**O**H) Sulphur-Dipole ; 3.4 **Å** ; Thr165 (side chain **S**CH_3 with 20-CH**O**)
11	H-bond ; 4.0 **Å** ; 106 ° ; Glu166 (side chain N**H**$_2$ with 19-OH) H-bond ; 1.9 **Å** ; 159 ° ; Thr190 (main chain N**H** with 12β-**O**H) H-bond ; 1.9 **Å** ; 159 ° ; Gln192 (main chain N**H** with 12β-**O**H) Sulphur-Dipole ; 3.4 **Å** ; Thr165 (side chain **S**CH_3 with 20-CH**O**)
12	H-bond ; 3.5 **Å** ; 101 ° ; Thr190 (main chain N**H** with 19-C=**O**) H-bond ; 4.1 **Å** ; 93 ° ; Ala191 (main chain N**H** with 19-C=**O**) H-bond ; 3.6 **Å** ; 105 ° ; Gln192 (side chain N**H** with 12β-OC(**O**)CH_3)
15	H-bond ; 3.5 **Å** ; 117 ° ; Arg188 (main chain N**H** with 16β-**O**H) H-bond ; 2.4 **Å** ; 126 ° ; Arg188 (main chain C**O** with 16β-O**H**) H-bond ; 2.3 **Å** ; 101 ° ; Gln192 (side chain N**H**$_2$ with 20-C=**O**)
Lopinavir	H-bond ; 1.9 **Å** ; 120 ° ; Glu166 (main chain N**H** with amide linker C=**O**) H-bond ; 2.1 **Å** ; 108 ° ; Glu166 (main chain C**O** with pyrimidine N**H**) H-bond ; 4.1 **Å** ; 132 ° ; Gln189 (side chain C**O** with amide linker N**H**)

Table S2. Descriptive ligand/Nsp15 NendoU binding interactions through directed docking protocol

Compound	Ligand-target interaction description [Type; Length (Å); Angle (°); Binding Residues]
1	H-bond ; 2.3 **Å** ; 111 ° ; His235 (side chain N**H** with *δ*-lactone **O**) H-bond ; 2.4 **Å** ; 135 ° ; Gly248 (main chain N**H** with *δ*-lactone **O**) H-bond ; 1.8 **Å** ; 130 ° ; Lys290 (side chain N**H**$_2$ with 19-C=**O**) H-bond ; 3.2 **Å** ; 101 ° ; Lys290 (side chain N**H**$_2$ with 12*β*-**O**H) H-bond ; 1.9 **Å** ; 152 ° ; Try343 (side chain O**H** with 16*β*-OC(**O**)CH$_3$)
2	H-bond ; 2.3 **Å** ; 148 ° ; Leu246 (main chain C**O** with 12*β*-**O**H) H-bond ; 1.7 **Å** ; 161 ° ; Gly248 (main chain N**H** with 12*β*-**O**H) H-bond ; 2.4 **Å** ; 148 ° ; Lys290 (side chain N**H**H with 12*β*-**O**H)
4	H-bond ; 2.4 **Å** ; 111 ° ; Gly248 (side chain N**H** with 12*β*-**O**C(O)CH$_3$) H-bond ; 3.6 **Å** ; 99 ° ; His250 (main chain N**H** with *δ*-lactone **O**) H-bond ; 2.9 **Å** ; 153 ° ; Lys290 (side chain N**H**$_2$ with 12*β*-**OC(O)**CH$_3$) H-bond ; 2.8 **Å** ; 174 ° ; Ser294 (main chain N**H** with *δ*-lactone **O**) *π*-H interaction ; 3.8 **Å** ; Tyr343 (side chain)
6	H-bond ; 1.7 **Å** ; 141 ° ; Gln245 (side chain N**H**$_2$ with 19-C=**O**) H-bond ; 2.7 **Å** ; 104 ° ; Leu246 (main chain C**O** with 12*β*-O**H**) H-bond ; 2.9 **Å** ; 156 ° ; Gly248 (main chain N**H** with 12*β*-**O**H) H-bond ; 2.9 **Å** ; 136 ° ; Gly248 (main chain N**H** with 19-C=**O**) H-bond ; 1.9 **Å** ; 116 ° ; Lys290 (side chain N**H**$_2$ with 12*β*-**O**H)
10	H-bond ; 2.3 **Å** ; 113 ° ; Gln245 (side chain N**H**$_2$ with 19-C=**O**) H-bond ; 2.1 **Å** ; 138 ° ; Gly248 (main chain N**H** with 19-C=**O**) H-bond ; 2.8 **Å** ; 132 ° ; His250 (side chain N**H** with *δ*-lactone **O**) H-bond ; 2.8 **Å** ; 148 ° ; Lys290 (side chain N**H**$_2$ with *δ*-lactone **O**) H-bond ; 1.7 **Å** ; 144 ° ; Thr341 (side chain O**H** with 20-**O**H)
11	H-bond ; 2.1 **Å** ; 98 ° ; Gln245 (side chain N**H** with 16*α*-**O**CH$_3$) H-bond ; 2.3 **Å** ; 161 ° ; Gly248 (main chain N**H** with *δ*-lactone **O**) H-bond ; 2.4 **Å** ; 114 ° ; Lys290 (side chain N**H**$_2$ with 20-C=**O**) H-bond ; 1.7 **Å** ; 132 ° ; Tyr343 (side chain O**H** with 12*β*-OC(**O**)CH$_3$)
12	H-bond ; 2.3 **Å** ; 135 ° ; Gly248 (main chain N**H** with 12*β*-OC(**O**)CH$_3$) H-bond ; 2.5 **Å** ; 97 ° ; Lys290 (side chain N**H**$_2$ with 12*β*-OC(**O**)CH$_3$) H-bond ; 2.3 **Å** ; 170 ° ; Ser294 (side chain N**H** with 19-C=**O**) H-bond ; 2.6 **Å** ; 123 ° ; Ser294 (side chain N**H** with *δ*-lactone **O**) *π*-H interaction ; 3.2 **Å** ; Tyr343 (side chain)
15	H-bond ; 3.4 **Å** ; 137 ° ; Gln245 (side chain C**O** with 19*α*-**O**H) H-bond ; 1.8 **Å** ; 147 ° ; Gln245 (side chain N**H**H with 19*α*-**O**H) H-bond ; 2.3 **Å** ; 163 ° ; Gly248 (main chain N**H** with 12*β*-OC(**O**)CH$_3$) H-bond ; 2.6 **Å** ; 144 ° ; Lys290 (side chain N**H**H with 12*β*-OC(**O**)CH$_3$) *π*-H interaction ; 3.2 **Å** ; Tyr343 (side chain)
Benzopurpurin 4B	H-bond ; 2.1 **Å** ; 152 ° ; Asp240 (side chain CO**O**H with SO$_2$O**H**) H-bond ; 2.5 **Å** ; 164 ° ; Asn278 (side chain N**H**H with S**O**$_2$OH) H-bond ; 2.6 **Å** ; 159 ° ; Glu340 (side chain CO**O**H with Ar-N**H**$_2$) H-bond ; 2.6 **Å** ; 164 ° ; Leu346 (main chain N**H** with S**O**$_2$OH) *π*-H interaction ; 3.1 **Å** ; Tyr343 (side chain)

Table S3. Estimated RMSD and Rg parameter of investigated ligands and reference compound, lopinavir, complexed with M-pro proteins throughout the triplicate all-atom MD simulation

		Compound 6 / M-pro complex				Compound 15 / M-pro complex				Lopinavir / M-pro complex			
		RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)	RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)	RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)
1st MD run	**Max.**	3.91	3.96	4.48	23.66	5.98	6.02	4.57	23.13	4.18	4.91	2.98	22.80
	Min.	1.52	1.59	0.53	21.71	1.45	1.48	0.69	21.61	1.39	1.50	0.52	21.87
	Mean[a]	2.58 ±0.24	2.63 ±0.24	2.94 ±0.46	22.41 ±0.27	4.30 ±0.55	4.30 ±0.49	2.87 ±0.42	22.20 ±0.25	3.10 ±0.38	3.41 ±0.42	2.21 ±0.30	22.31 ±0.14
2nd MD run	**Max.**	3.95	4.56	3.72	23.07	4.36	4.37	3.97	23.69	3.70	3.97	5.60	23.11
	Min.	1.40	1.43	0.95	21.98	1.80	1.81	1.15	22.39	1.31	1.34	0.88	22.02
	Mean[a]	2.51 ±0.34	3.09 ±0.50	2.52 ±0.39	22.40 ±0.16	3.14 ±0.35	3.64 ±0.34	2.74 ±0.3	22.95 ±0.16	2.70 ±0.41	3.14 ±0.32	3.80 ±0.60	22.54 ±0.14
3rd MD run	**Max.**	4.72	6.72	4.55	23.28	5.09	6.55	3.77	23.69	4.82	6.23	3.99	22.84
	Min.	1.43	1.48	0.49	21.91	1.54	1.56	0.86	21.93	1.48	1.46	0.43	21.93
	Mean[a]	3.42 ±0.59	4.70 ±0.94	2.79 ±0.77	22.43 ±0.30	4.22 ±0.46	5.19 ±0.53	2.69 ±0.50	22.78 ±0.24	3.74 ±0.56	4.17 ±0.76	2.74 ±0.44	22.41 ±0.13
		Average ± SD/SE											
	Max.[a]	4.19 ±0.45	5.08 ±1.45	4.25 ±0.46	23.34 ±0.30	5.15 ±0.81	5.65 ±1.14	4.10 ±0.42	23.50 ±0.32	4.23 ±0.56	5.04 ±1.14	4.19 ±1.32	22.92 ±0.17
	Min.[a]	1.45 ±0.06	1.50 ±0.08	0.66 ±0.25	21.87 ±0.14	1.60 ±0.18	1.62 ±0.18	0.90 ±0.23	21.98 ±0.40	1.39 ±0.09	1.44 ±0.08	0.61 ±0.24	21.94 ±0.08
	Mean[b]	2.83 ±0.18	3.47 ±0.63	2.75 ±0.12	22.41 ±0.01	3.86 ±0.02	4.37 ±0.26	2.77 ± 0.05	22.65 ±0.23	3.18 ±0.18	3.57 ±0.32	2.91 ±0.47	22.42 ±0.07

[a] Standard deviation of the mean (SD); [b] Standard error of the mean (SE).

Table S4: Estimated ΔRMSF[a] parameter of ligands/M-pro proteins across stable structure trajectories (last 20 ns).

Sub-pocket	Residue	Compound 6 / M-pro protein residues				Compound 15 / M-pro protein residues				Lopinavir / M-pro protein residues			
		1st MD run	2nd MD run	3rd MD run	Mean ± SD	1st MD run	2nd MD run	3rd MD run	Mean ± SD	1st MD run	2nd MD run	3rd MD run	Mean ± SD
S1′	His41	**0.36**	**0.31**	**0.39**	**0.35 ±0.04**	-0.70	-0.69	-2.09	-1.16 ±0.81	**0.23**	**0.39**	**0.35**	**0.32 ±0.09**
	Gly143	-0.48	-0.35	-0.29	-0.37 ±0.10	0.06	-0.05	-0.14	-0.04 ±0.10	-0.37	-0.32	-0.91	-0.53 ±0.33
	Ser144	-0.19	-0.19	-0.12	-0.17 ±0.04	-0.21	-0.26	-0.27	-0.24 ±0.03	-0.18	-0.11	-0.24	-0.18 ±0.06
	Cys145	-0.07	-0.08	-0.01	-0.05 ±0.03	-0.33	-0.37	-0.37	-0.36 ±0.03	-0.09	-0.02	-0.16	-0.09 ±0.07
S1	Phe140	0.12	0.25	0.26	0.21 ±0.08	**0.52**	**0.44**	**0.50**	**0.49 ±0.04**	-0.06	0.04	-0.06	-0.03 ±0.06
	Leu141	-0.50	-0.20	-0.23	-0.31 ±0.16	0.28	0.14	0.21	0.21 ±0.07	-0.69	-0.53	-0.39	-0.53 ±0.15
	Asn142	-0.69	-0.44	-0.42	-0.52 ±0.15	0.11	-0.10	-0.02	0.001 ±0.10	-0.75	-0.74	-0.71	-0.73 ±0.02
	His163	**0.43**	**0.42**	**0.48**	**0.44 ±0.03**	-0.33	-0.84	-0.29	-0.48 ±0.31	**0.45**	**0.46**	**0.45**	**0.45 ±0.01**
	Glu166	**0.74**	**0.65**	**0.86**	**0.75 ±0.11**	**0.74**	**0.34**	**0.65**	**0.58 ±0.21**	**0.78**	**0.86**	**0.84**	**0.83 ±0.04**
S2	Met49	**1.24**	**1.45**	**1.96**	**1.55 ±0.37**	**1.71**	**1.83**	**1.64**	**1.73 ±0.09**	**1.33**	**1.30**	**1.00**	**1.21 ±0.18**
	Tyr54	**0.48**	**0.68**	**0.70**	**0.62 ±0.12**	0.23	**0.34**	0.29	0.29 ±0.05	**0.66**	**0.59**	**0.51**	**0.59 ±0.08**
	His164	**0.33**	**0.36**	**0.42**	**0.37 ±0.05**	-0.29	-0.91	-0.38	-0.53 ±0.34	**0.42**	**0.44**	**0.43**	**0.43 ±0.01**
	Asp187	**0.91**	**0.94**	**1.01**	**0.95 ±0.05**	**0.51**	**0.54**	**0.59**	**0.55 ±0.04**	**0.75**	**0.82**	**0.93**	**0.84 ±0.09**
	Arg188	**0.78**	**0.84**	**0.96**	**0.86 ±0.09**	**0.63**	**0.43**	**0.45**	**0.50 ±0.11**	**0.76**	**0.36**	**0.68**	**0.60 ±0.22**
S3	Met165	**0.31**	0.09	**0.42**	0.27 ±0.17	**0.01**	**-0.60**	**-0.13**	-0.24 ±0.32	**0.46**	**0.47**	**0.35**	**0.43 ±0.07**
	Leu167	**0.70**	**0.45**	**0.91**	**0.69 ±0.23**	**1.01**	**0.74**	**0.92**	**0.89 ±0.14**	**0.73**	**0.79**	**0.71**	**0.74 ±0.04**
	Gln189	**0.49**	**0.40**	**0.77**	**0.55 ±0.20**	**0.71**	**0.46**	**0.53**	**0.56 ±0.13**	-0.40	-0.03	**0.36**	-0.02 ±0.38
	Thr190	0.15	0.01	**0.47**	0.21 ±0.24	**0.35**	0.20	0.21	0.25 ±0.09	-1.46	-0.24	0.05	-0.55 ±0.80
	Gln192	0.02	0.00	0.32	0.11 ±0.18	-0.34	-0.06	0.06	-0.11 ±0.21	-1.84	-0.09	0.15	-0.59 ±1.09
Vicinal residues	Pro39	**0.49**	**0.57**	**0.60**	**0.55 ±0.06**	-0.48	0.01	-0.23	-0.23 ±0.25	**0.51**	**0.60**	**0.49**	**0.53 ±0.06**
	Val42	**1.14**	**1.09**	**1.19**	**1.14 ±0.05**	-0.06	0.01	-1.19	-0.41 ±0.67	**1.06**	**1.18**	**1.09**	**1.11 ±0.06**
	Ile43	**1.71**	**1.73**	**1.80**	**1.75 ±0.04**	**0.89**	**0.85**	**0.73**	**0.82 ±0.08**	**1.72**	**1.76**	**1.66**	**1.71 ±0.05**

Cys44	**2.14**	**2.15**	**2.42**	**2.24 ±0.16**	**1.68**	**2.13**	**1.36**	**1.72 ±0.38**	**2.22**	**2.37**	**2.18**	**2.25 ±0.10**
Thr45	**1.89**	**1.51**	**2.27**	**1.89 ±0.38**	**1.43**	**2.30**	**1.34**	**1.69 ±0.53**	**1.79**	**2.30**	**1.59**	**1.89 ±0.36**
Ser46	**1.52**	**1.20**	**2.10**	**1.60 ±0.46**	**0.79**	**2.29**	**1.38**	**1.49 ±0.76**	**1.84**	**1.68**	**1.36**	**1.62 ±0.24**
Glu47	**2.04**	**2.36**	**2.76**	**2.39 ±0.36**	**1.69**	**3.12**	**1.33**	**2.05 ±0.95**	**1.71**	**1.99**	**1.98**	**1.89 ±0.16**
Asp48	**1.80**	**1.44**	**2.41**	**1.89 ±0.49**	**1.81**	**2.12**	**1.86**	**1.93 ±0.17**	**0.71**	**1.63**	**1.17**	**1.17 ±0.46**
Leu50	**0.69**	**1.09**	**1.66**	**1.15 ±0.49**	**1.34**	**1.39**	**1.18**	**1.30 ±0.11**	**1.28**	**0.51**	**0.13**	**0.64 ±0.59**
Asn53	-0.03	0.53	0.06	0.18 ±0.30	**0.61**	**0.47**	**0.43**	**0.51 ±0.09**	**0.42**	0.00	-0.03	0.13 ±0.25
Pro168	0.05	-0.24	**0.45**	0.09 ±0.34	**0.75**	**0.68**	**0.68**	**0.70 ±0.04**	0.24	0.16	-0.03	0.13 ±0.14
Phe185	**0.36**	**0.40**	**0.52**	**0.43 ±0.08**	-0.51	0.05	-0.34	-0.27 ±0.29	0.27	**0.35**	**0.49**	**0.37 ±0.11**
Val186	**0.66**	**0.70**	**0.84**	**0.73 ±0.10**	-0.24	0.12	0.11	0.001 ±0.20	**0.55**	**0.49**	**0.74**	**0.59 ±0.13**

[a] Relative difference root-mean-square fluctuation (ΔRMSF) estimated for each ligand-bound protein relative to the apo state of COVID-19 M-pro. Only values with significant mobility changes (ΔRMSF > 0.3 Å) are represented in bold.

Table S5. Estimated RMSD and Rg parameter of investigated ligands and reference compound, Benzopurpurin 4B, complexed with Nsp15 NendoU proteins throughout the triplicate all-atom MD simulation

		Compound 6 / Nsp15 NendoU complex				Compound 15 / Nsp15 NendoU complex				Benzopurpurin 4B / Nsp15 NendoU complex			
		RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)	RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)	RMSD protein (Å)	RMSD complex (Å)	RMSD ligand (Å)	Rg complex (Å)
1st MD run	Max.	4.16	4.77	4.80	24.89	4.64	5.07	4.52	24.75	4.57	5.40	4.26	25.00
	Min.	1.45	1.72	1.83	23.39	2.02	2.01	1.54	23.21	1.85	1.86	0.98	23.40
	Mean[a]	2.61 ±0.32	3.55 ±0.41	3.59 ±0.32	23.931 ±0.20	2.79 ±0.30	4.01 ±0.37	3.55 ±0.36	23.80 ±0.20	2.69 ±0.31	4.00 ±0.42	2.98 ±0.47	23.96 ±0.19
2nd MD run	Max.	4.03	5.11	4.92	24.58	4.82	6.10	4.85	25.44	4.57	4.77	4.80	24.82
	Min.	1.69	1.79	1.83	23.40	1.64	1.89	2.11	23.41	1.67	1.67	0.96	23.36
	Mean[a]	2.55 ±0.27	3.00 ±0.42	4.07 ±0.45	23.87 ±0.19	2.82 ±0.36	4.29 ±0.51	3.32 ±0.41	24.02 ±0.31	2.63 ±0.36	2.83 ±0.36	2.94 ±0.32	23.84 ±0.18
3rd MD run	Max.	4.23	5.07	4.80	25.12	4.81	4.87	5.44	25.17	4.12	4.55	4.26	25.26
	Min.	1.79	2.01	1.76	23.32	1.89	2.01	1.59	23.35	1.66	1.82	1.00	23.42
	Mean[a]	2.72 ±0.37	4.01 ±0.37	3.59 ±0.32	23.89 ±0.25	2.65 ±0.41	3.95 ±0.31	4.12 ±0.44	24.01 ±0.30	2.70 ±0.35	3.41 ±0.28	2.98 ±0.47	23.92 ±0.24
		Average ± SD/SE											
	Max.[a]	4.14 ±0.10	4.98 ±0.18	4.84 ±0.07	24.86 ±0.27	4.76 ±0.10	5.34 ±0.66	4.94 ±0.46	25.12 ±0.35	4.42 ±0.26	4.91 ±0.44	4.44 ±0.31	25.03 ±0.22
	Min.[a]	1.64 ±0.17	1.84 ±0.15	1.81 ±0.04	23.37 ±0.04	1.85 ±0.19	1.97 ±0.07	1.75 ±0.32	23.32 ±0.10	1.73 ±0.11	1.87 ±0.10	0.50 ±0.03	23.39 ±0.03
	Mean[b]	2.62 ±0.19	3.52 ±0.23	3.75 ±0.21	23.90 ±0.12	2.75 ±0.21	4.08 ±0.23	3.66 ± 0.23	23.95 ±0.16	2.67 ±0.20	3.41 ±0.20	2.97 ±0.24	23.91 ±0.12

[a] Standard deviation of the mean (SD); [b] Standard error of the mean (SE).

Table S6: Estimated ΔRMSF[a] parameter of ligands/Nsp15 NendoU bound proteins across stable structure trajectories (last 20 ns).

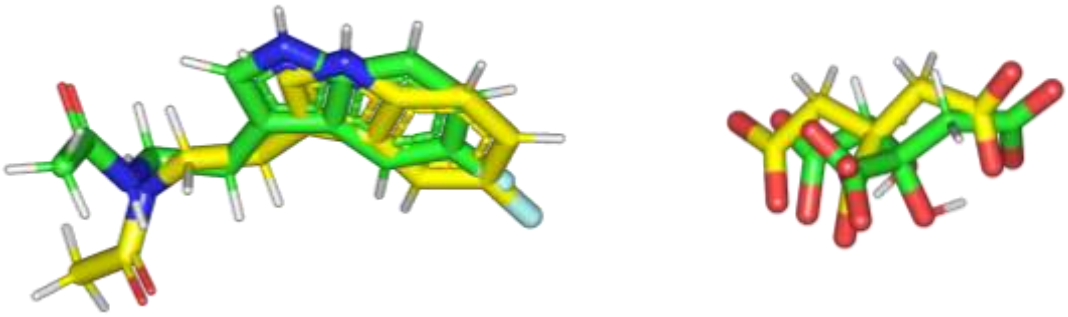
Sub-pocket	Residue	Compound 6 / Nsp15 NendoU protein residues				Compound 15 / M-pro protein residues				Lopinavir / M-pro protein residues			
		1st MD run	2nd MD run	3rd MD run	Mean ± SD	1st MD run	2nd MD run	3rd MD run	Mean ± SD	1st MD run	2nd MD run	3rd MD run	Mean ± SD
B0	Asn278	0.09	0.12	-0.98	-0.26 ±0.62	-0.09	0.11	0.10	0.04 ±0.11	-0.02	-0.06	-0.03	-0.04 ±0.02
	Lys345	**1.53**	**0.74**	**0.88**	**1.05 ±0.42**	**1.21**	**1.19**	**1.58**	**1.32 ±0.22**	**0.71**	-0.03	**1.14**	**0.61 ±0.59**
	Leu346	**1.42**	**0.10**	**0.34**	**0.62 ±0.70**	**0.48**	**0.30**	**1.02**	**0.66 ±0.59**	**1.02**	**1.02**	**1.03**	**1.02 ±0.006**
B1	Gln245	-0.28	-0.18	-1.40	-0.62 ±0.68	-0.38	-0.21	-0.03	-0.21 ±0.18	0.11	-0.13	0.07	0.01 ±0.13
	Gly248	-0.06	-0.03	-0.53	-0.21 ±0.28	-0.07	-0.06	0.13	0.15 ±0.11	0.23	0.08	0.05	0.12 ±0.10
	His250	0.01	-0.01	-0.35	-0.12 ±0.20	-0.06	0.10	0.06	0.01 ±0.09	0.07	0.00	0.06	0.04 ±0.04
	Lys290	**0.44**	**0.31**	0.17	**0.31 ±0.14**	**0.37**	0.20	**0.39**	**0.32 ±0.10**	-0.01	-0.13	-0.03	-0.06 ±0.07
	Ser294	-0.09	0.02	-0.73	-0.27 ±0.40	-0.22	0.02	-0.05	-0.09 ±0.13	0.07	-0.05	0.01	0.01 ±0.06
	Tyr343	0.11	-0.09	-0.19	-0.06 ±0.15	0.02	-0.08	0.17	0.04 ±0.12	-0.04	-0.04	0.10	0.002 ±0.08
B2	His235	-0.17	-0.18	-0.62	-0.33 ±0.26	-0.28	-0.05	-0.14	-0.16 ±0.12	0.16	-0.01	0.01	0.05 ±0.09
	Gly247	-0.14	-0.07	-0.75	-0.32 ±0.38	-0.21	-0.03	0.16	-0.03 ±0.18	0.21	0.02	0.02	0.08 ±0.11
	Trp333	0.25	-0.04	-0.10	0.04 ±0.19	**0.33**	0.27	**0.38**	**0.33 ±0.06**	0.19	-0.03	0.22	0.12 ±0.14
	Thr341	-0.06	-0.20	-0.49	-0.25 ±0.22	-0.04	-0.05	0.08	-0.01 ±0.07	0.15	-0.04	0.11	0.07 ±0.10
B3	Asp240	**0.65**	**0.71**	0.13	**0.50 ±0.32**	**0.68**	**0.77**	**0.89**	**0.78 ±0.11**	**0.72**	**0.53**	**0.65**	**0.63 ±0.10**
	His243	**0.52**	**0.62**	0.24	**0.46 ±0.20**	**0.52**	**0.62**	**0.79**	**0.64 ±0.13**	**0.72**	**0.49**	**0.71**	**0.64 ±0.13**
	Glu340	-0.09	-0.18	-0.64	-0.03 ±0.30	-0.02	-0.05	0.12	0.01 ±0.09	0.19	0.00	0.18	0.13 ±0.11
Vicinal residues	Leu215	**0.39**	**0.48**	**0.36**	**0.41 ±0.06**	0.28	**0.42**	**0.45**	**0.38 ±0.09**	0.21	0.13	0.20	0.18 ±0.05
	Ile223	**0.47**	**0.61**	0.21	**0.43 ±0.21**	**0.40**	**0.51**	**0.52**	**0.48 ±0.07**	0.23	0.18	0.19	0.20 ±0.03
	Tyr226	0.28	**0.40**	0.05	0.24 ±0.18	0.27	**0.33**	**0.35**	**0.31 ±0.04**	0.17	0.16	0.17	0.17 ±0.01
	Leu228	0.23	**0.43**	0.06	0.24 ±0.18	0.29	**0.38**	**0.38**	**0.35 ±0.05**	0.22	0.17	0.11	0.17 ±0.05
	Gly239	**0.34**	**0.31**	-0.09	0.19 ±0.24	0.29	**0.41**	**0.48**	**0.39 ±0.10**	**0.36**	0.16	0.26	0.26 ±0.10

Phe241	**1.07**	**1.17**	0.21	**0.82 ±0.53**	**1.14**	**1.20**	**1.36**	**1.23 ±0.11**	**1.14**	**0.91**	**1.05**	**1.03 ±0.12**
Ser242	**0.77**	**0.89**	-0.28	**0.46 ±0.64**	**0.83**	**0.86**	**1.04**	**0.91 ±0.11**	**0.94**	**0.74**	**0.94**	**0.87 ±0.11**
Leu332	0.20	-0.01	0.11	0.10 ±0.10	**0.32**	**0.30**	**0.36**	**0.33 ±0.03**	0.21	-0.06	0.22	0.13 ±0.16
Cys334	**0.50**	0.24	0.01	0.25 ±0.24	**0.58**	**0.41**	**0.64**	**0.54 ±0.12**	0.24	-0.05	0.27	0.15 ±0.18
Lys335	0.23	0.01	-0.61	-0.12 ±0.43	0.29	0.05	**0.34**	0.23 ±0.16	0.26	-0.03	0.36	0.19 ±0.20

[a] Relative difference root-mean-square fluctuation (ΔRMSF) estimated for each ligand-bound protein relative to the apo state of COVID-19 Nsp15 NendoU. Only values with significant mobility changes (ΔRMSF > 0.3 Å) are represented in bold.

Table S7. Total binding-free energies and individual energy term (ΔG ± SD) of the promising scalarane-based compounds and reference ligands within Nsp15 NendoU and/or M-pro protein binding site, across the three MD simulation runs.

	Energy (kJ/mol ± SD)	M-pro complex			Nsp15 NendoU complex		
		Comp. 6	**Comp. 15**	**Lopinavir**	**Comp. 6**	**Comp. 15**	**Benzopurpurin 4B**
1st MD run	**van der Waal**	-110.60 ± 18.86	-119.83 ± 11.25	-215.43 ± 19.69	-102.33 ± 14.27	-80.21 ± 11.17	-117.31 ± 20.19
	Electrostatic	-13.65 ± 26.693	-13.82 ± 18.22	-26.62 ± 20.49	-46.560 ± 13.84	-8.39 ± 17.01	-82.22 ± 33.47
	Solvation; Polar	70.06 ± 32.18	85.87 ± 23.47	160.01 ± 35.13	115.28 ± 24.61	26.98 ± 35.32	143.16 ± 42.38
	Solvation; SASA	-13.50 ± 1.49	-14.76 ± 1.44	-21.76 ± 1.83	-12.78 ± 1.20	-11.80 ± 1.80	-17.89 ± 2.87
	Binding energy	-67.69 ± 16.45	-62.53 ± 12.37	-103.81 ± 31.62	-46.43 ± 27.21	-73.42 ± 25.24	-74.26 ± 34.28
2nd MD run	**van der Waal**	-149.98 ± 21.32	-105.14 ± 22.53	-178.95 ± 33.26	-95.76 ± 15.75	-75.83 ± 32.75	-105.49 ± 21.29
	Electrostatic	-6.94 ± 10.30	-20.54 ± 19.49	-32.07 ± 14.69	-56.13 ± 8.54	-10.13 ± 4.11	-112.62 ± 4.91
	Solvation; Polar	87.58 ± 11.60	62.37 ± 35.58	146.35 ± 28.35	105.52 ± 14.81	16.20 ± 51.13	153.72 ± 44.05
	Solvation; SASA	-16.28 ± 1.04	-11.12 ± 3.01	-19.69 ± 2.84	-12.75 ± 1.71	-6.65 ± 3.33	-14.05 ± 2.22
	Binding energy	-85.63 ± 16.05	-74.44 ± 19.91	-84.36 ± 21.68	-59.12 ± 14.68	-76.41 ± 43.76	-78.44 ± 22.75
3rd MD run	**van der Waal**	-138.19 ± 14.81	-95.14 ± 22.06	-174.33 ± 12.35	-98.08 ± 10.69	-99.74 ± 18.93	-110.28 ± 34.65
	Electrostatic	-7.46 ± 12.43	-8.81 ± 10.97	-35.98 ± 14.71	-53.16 ± 18.98	-4.63 ± 6.50	-112.53 ± 28.68
	Solvation; Polar	86.31 ± 10.14	43.24 ± 35.17	140.20 ± 20.91	120.34 ± 27.70	32.90 ± 50.22	173.66 ± 42.55
	Solvation; SASA	-16.46 ± 1.46	-7.40 ± 3.19	-20.51 ± 1.09	-12.74 ± 1.25	-12.31 ± 1.88	-16.62 ± 3.53
	Binding energy	-75.79 ± 17.85	-68.11 ± 37.80	-90.62 ± 21.23	-43.65 ± 18.04	-83.78 ± 32.28	-65.78 ± 35. 29



(A) **(B)**

Figure S1. Superimposing the crystallized (green sticks) and redocked (yellow sticks) ligands at **(A)** M-pro and (**B)** Nsp15 NendoU for validating the adopted directed docking protocol.

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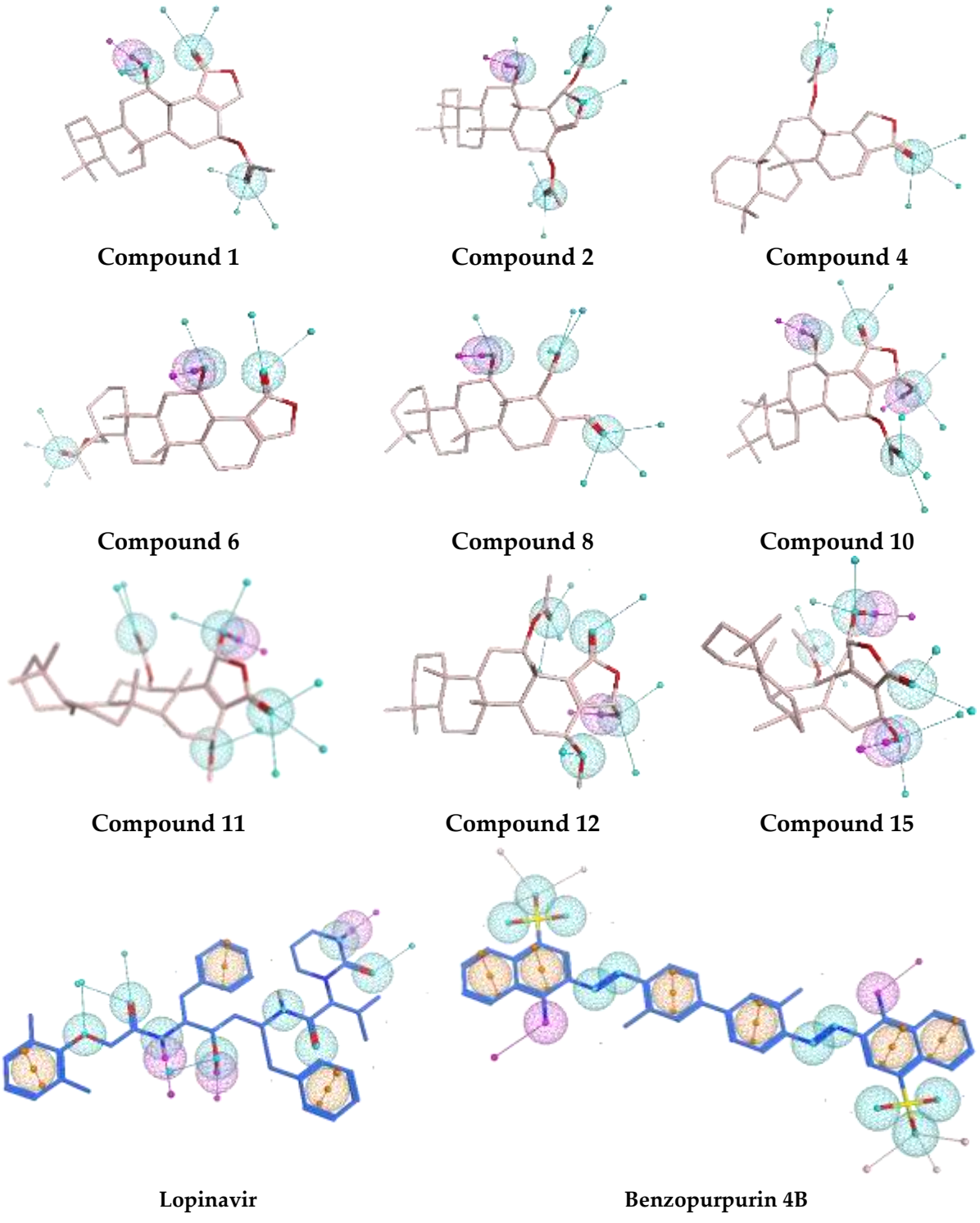

Figure S2. The 3D structural representation of the promising scalarane-based hits (sticks) and reference ligands showing respective pharmacophoric features (mesh spheres) as well as projected virtual points (arrows). Features and directionality of H-bond donor, H-bond acceptor, or aromaticity are colored in violet, cyan, or orange, respectively.