Supporting Information

Novel inhibitors of the main protease enzyme of SARS-CoV-2 identified via molecular dynamics simulation-guided *in vitro* assay

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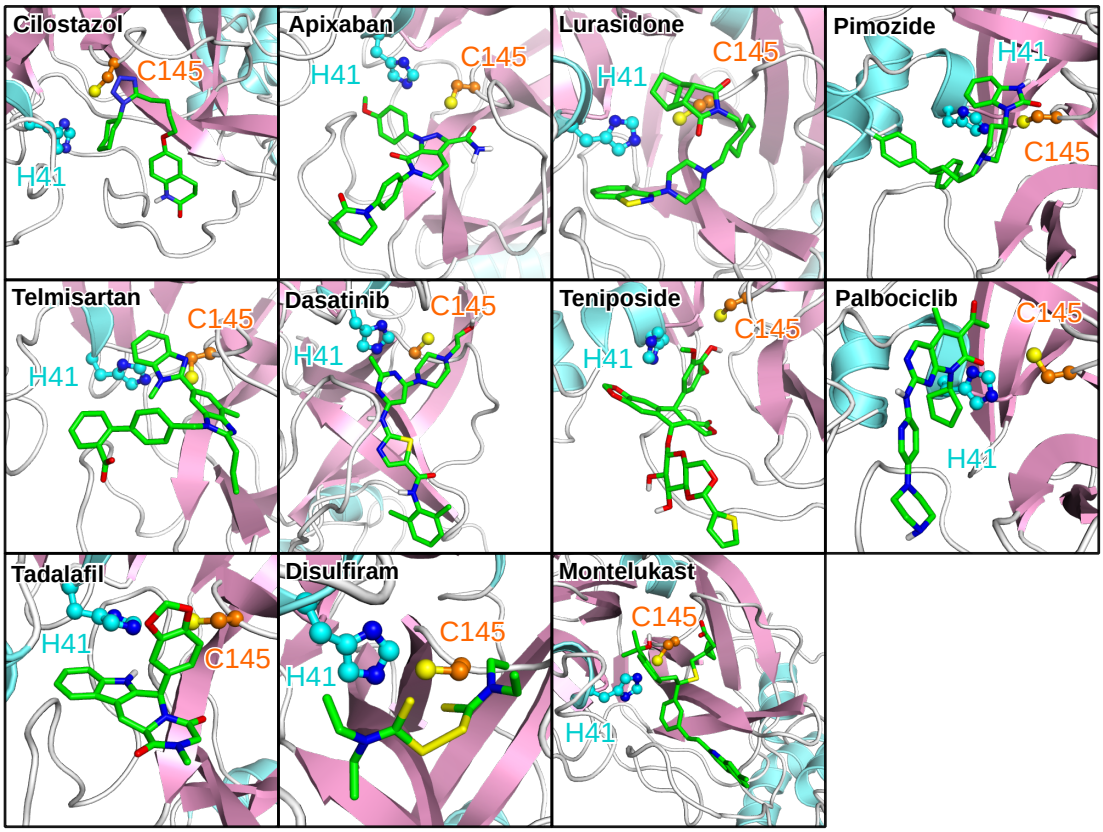

Figure S1.: Binding poses of the eleven FDA-approved drugs belongig to top 35 ligands identified by MD simulations.

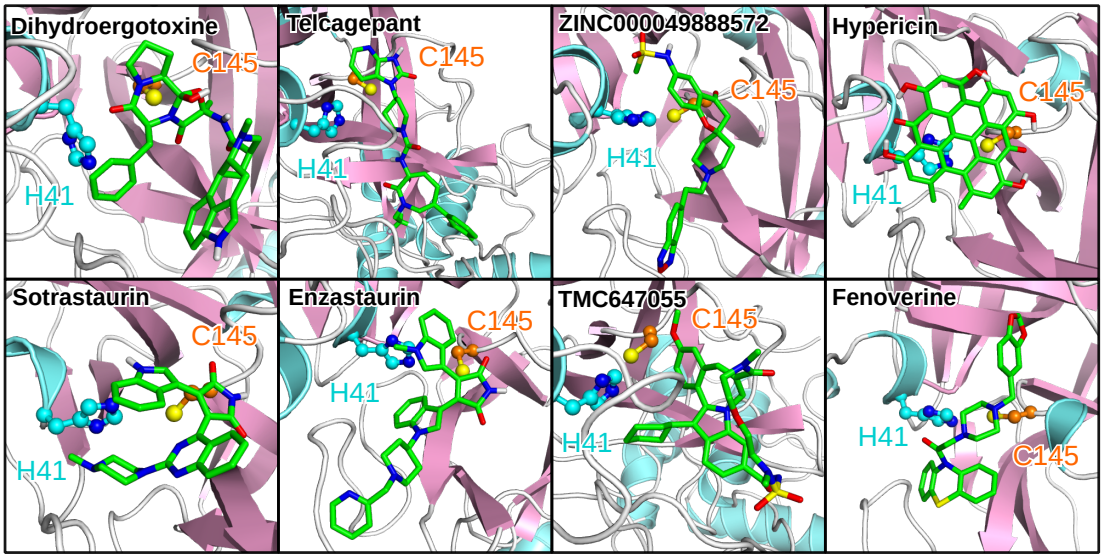

Figure S2.: Binding poses of the eight non-FDA and investigational drugs belongig to top 35 ligands identified by MD simulations.

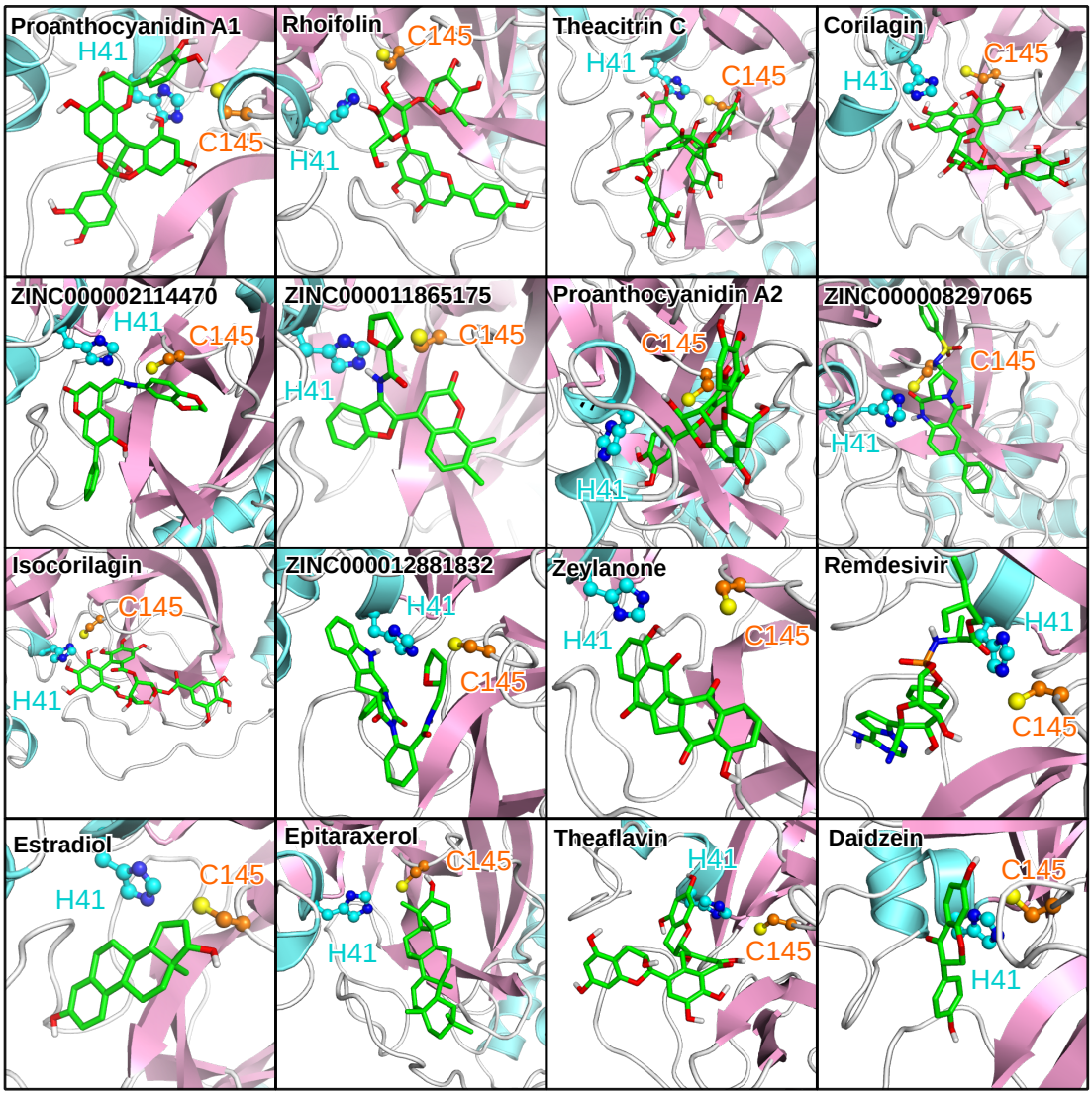

Figure S3.: Binding poses of the sixteen natural products belongig to top 35 ligands identified by MD simulations.

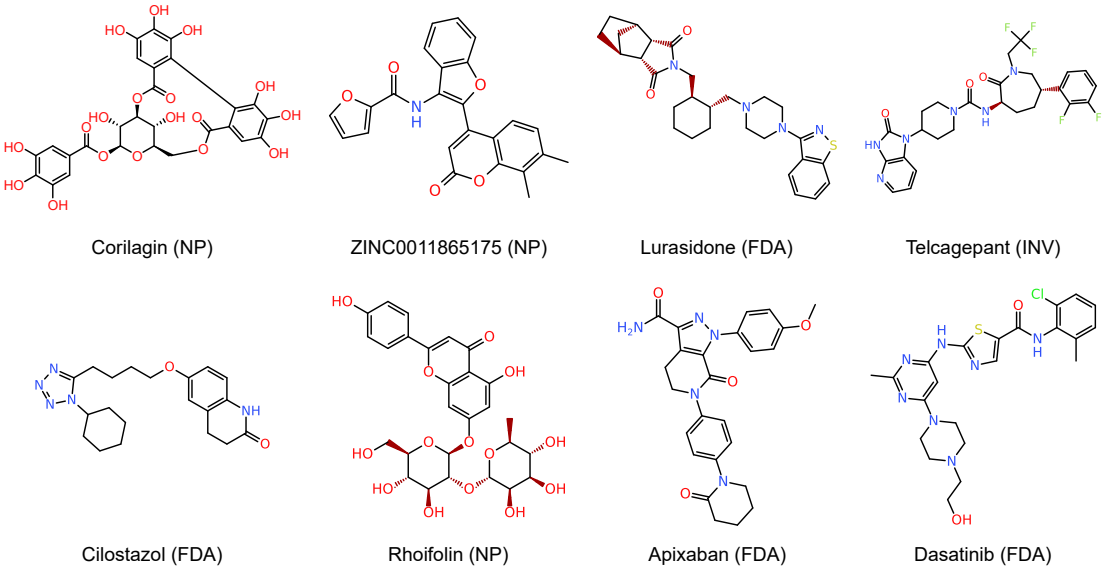

Figure S4.: Chemical structures of the top eight inhibitors.

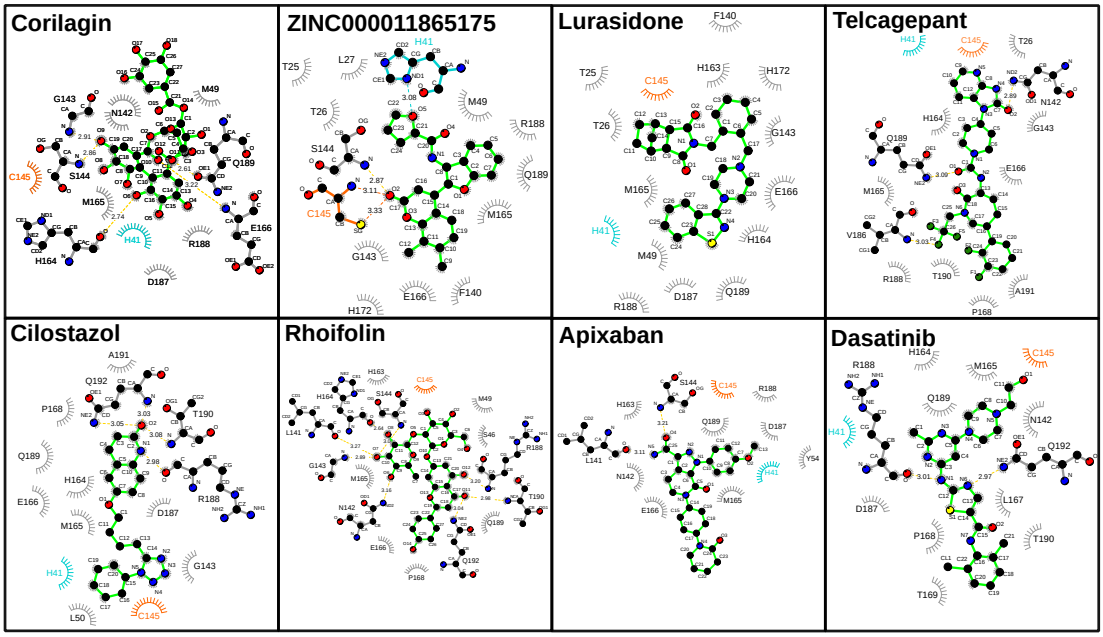

Figure S5.: The 3CL^{pro}-ligand interactions for the top eight inhibitors.

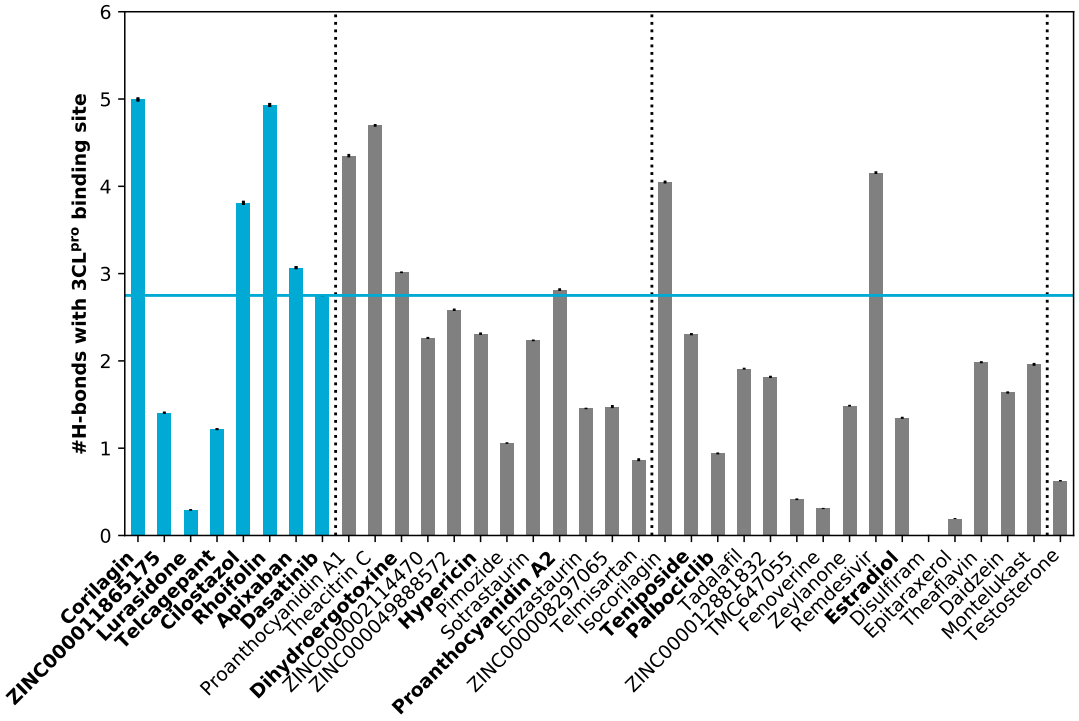

Figure S6.: Average number of hydrogen bonds formed between the 3CL$^{\text{pro}}$ binding site and the 35 top ligands identified by MD simulations. The top eight inhibitors (blue bars) identified by *in vitro* screening are separated by a vertical dashed line from the remaining compounds. Other vertical lines are addeded to separate the ligands interacting with both dyad residues from those interacting with only one of these residues and to separate testosterone which failed almost all *in silico* selection criteria. The ligands are shown in increasing ΔG_{bind} order. At 2.75 hydrogen bonds a horizontal line (blue) is shown to indicate ligands forming many hydrogen bonds with 3CL$^{\text{pro}}$. Labels of the ligands used in the 3CL$^{\text{pro}}$ inhibition assay are written in bold. The standard deviation is displayed as error bars.

Table S1.: Names and properties of the compounds submitted to MD simulation. The $RMSD_{ligand}$ as well as the distances to the catalytic dyad (d_{dyad}) and the binding site (d_{BS}) are values averaged over the last 5 ns of the 20 ns MD simulations and the last 25 ns in the case of the 100 ns MD simulations. The compounds highlighted in bold were identified as the top eight inhibitors.

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
			FDA-approved drugs			
1	ZINC000006716957	Nilotinib	100 ns	9.555	2.722	1.929
2	ZINC000064033452	Lumacaftor	20 ns	8.516	2.879	2.243
3	ZINC000003932831	Dutasteride	20 ns	6.592	2.935	2.149
4	ZINC000003993855	Tadalafil	100 ns	3.643	2.333	1.938
5	ZINC000052955754	Ergotamine	100 ns	5.002	2.574	1.920
6	ZINC000100378061	Naldemedine	20 ns	6.528	2.549	1.982
7	ZINC000003920266	Idarubicin	100 ns	5.082	2.623	2.067
8	ZINC000169289453	Simeprevir	100 ns	5.124	2.262	1.887
9	ZINC000013831130	Raltegravir	100 ns	6.112	2.510	2.012
10	ZINC000014210642	Azilsartan	100 ns	5.026	2.437	1.971
11	ZINC000058581064	Dolutegravir	20 ns	11.002	2.266	2.047
12	**ZINC000003927822**	**Lurasidone**	**100 ns**	**4.044**	**2.153**	**1.963**
13	ZINC000004214700	Paliperidone	20 ns	3.768	2.712	2.020
14	ZINC000222731806	Enasidenib	20 ns	7.767	2.69	2.114
15	ZINC000012503187	Conivaptan	20 ns	19.474	10.233	1.980
16	ZINC000001530886	Telmisartan	100 ns	4.045	2.360	1.908
17	ZINC000003938684	Etoposide	100 ns	3.734	2.175	1.874
18	ZINC000029416466	Saquinavir	100 ns	4.358	2.327	1.871
19	ZINC000072318121	Abemaciclib	100 ns	6.522	2.608	2.050
20	ZINC000008101127	Indocyanine	100 ns	5.747	2.303	1.851
21	ZINC000011617039	Pazopanib	100 ns	4.566	2.307	1.869
22	ZINC000003938686	Palbociclib	100 ns	4.201	2.347	1.942
23	ZINC000004099008	Teniposide	100 ns	3.424	2.232	1.796
24	ZINC000035328014	Ibrutinib	20 ns	6.041	2.396	2.069
25	ZINC000001530788	Cromolyn	20 ns	7.103	2.409	1.996
26	ZINC000043100709	Trametinib	20 ns	9.172	2.841	2.133

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
27	ZINC000003831151	Montelukast	100 ns	3.494	2.186	1.896
28	ZINC000022448696	Indinavir	100 ns	5.657	2.571	1.996
29	ZINC000040430143	Olaparib	100 ns	8.624	2.472	1.957
30	ZINC000049036447	Suvorexant	100 ns	6.313	2.532	2.107
31	ZINC000003976838	Afatinib	100 ns	7.933	2.263	1.960
32	ZINC000003816514	Rolapitant	100 ns	3.556	2.404	2.006
33	ZINC000013986658	Idelalisib	100 ns	5.302	2.673	1.957
34	ZINC000013818943	Regadenoson	20 ns	6.904	2.614	1.819
35	ZINC000100003902	Maraviroc	20 ns	8.464	2.666	2.111
36	ZINC000019632618	Imatinib	20 ns	8.128	2.279	2.015
37	ZINC000003827556	Delafloxacin	20 ns	6.271	2.622	1.933
38	**ZINC000003986735**	**Dasatinib**	**100 ns**	**2.552**	**2.361**	**1.881**
39	ZINC000027990463	Lomitapide	20 ns	7.005	2.688	2.167
40	ZINC000003932831	Candesartan	100 ns	3.817	2.137	1.867
41	ZINC000035902489	Crizotinib	100 ns	27.285	18.416	10.359
42	ZINC000004175630	Pimozide	100 ns	3.805	2.382	1.928
43	ZINC000019796168	Sildenafil	100 ns	7.924	2.563	2.008
44	ZINC000043206370	Niraparib	20 ns	6.142	2.745	1.985
45	ZINC000003918453	Ertapenem	20 ns	4.111	3.177	1.813
46	ZINC000003860453	Fluorescein	100 ns	5.251	2.359	2.059
47	ZINC000001481815	Deferasirox	100 ns	4.190	2.803	1.857
48	ZINC000018324776	Vardenafil	20 ns	5.242	2.508	2.038
49	ZINC000060325170	Cobimetinib	20 ns	5.302	3.078	1.960
50	ZINC000100001976	Glimepiride	100 ns	5.425	2.388	1.971
51	ZINC000001489478	Sitagliptin	20 ns	6.793	2.303	2.179
52	ZINC000003812865	Olsalazine	100 ns	8.607	2.742	2.024
53	**ZINC000011677837**	**Apixaban**	**100 ns**	**3.024**	**2.511**	**1.901**
54	ZINC000005844788	Nebivolol	20 ns	6.078	2.624	2.009
55	ZINC000000897240	Azelastine	100 ns	9.088	2.506	2.056
56	ZINC000100022637	Tipranavir	100 ns	4.377	2.713	2.087

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
57	**ZINC000001552174**	**Cilostazol**	**100 ns**	**4.458**	**2.295**	**1.856**
58	ZINC000030691797	Perampanel	100 ns	5.798	2.642	2.112
59	ZINC000085537017	Cangrelor	100 ns	8.740	2.874	1.817
60	ZINC000003944422	Ritonavir	20 ns	6.620	2.407	2.099
61	ZINC000001530948	Thalidomide	20 ns	6.182	2.556	2.191
		Non-FDA and investigational drugs				
62	ZINC000003780340	Hypericin	100 ns	1.667	2.410	1.865
63	ZINC000003922429	Adozelesin	20 ns	9.524	5.298	2.017
64	ZINC000003975327	Telomestatin	20 ns	11.493	2.487	2.136
65	ZINC000043203371	MK-3207	100 ns	2.822	2.328	1.957
66	ZINC000059749972	Radotinib	20 ns	7.091	2.397	2.114
67	ZINC000003812168	Ruboxistaurin	100 ns	5.198	2.558	1.887
68	ZINC000084726167	TMC647055	100 ns	3.762	2.336	2.083
69	ZINC000095092808	—	100 ns	8.755	2.386	2.048
70	ZINC000049888572	—	100 ns	3.592	2.109	1.930
71	ZINC000095539256	UK-432,097	20 ns	6.997	2.237	1.634
72	ZINC000038576002	R-343	100 ns	5.518	2.373	1.942
73	ZINC000014880002	Dihydroergotoxine	100 ns	1.447	2.456	1.810
74	ZINC000004215648	Dihydroergocornine	100 ns	6.681	2.260	2.042
75	ZINC000003817327	Ly2090314	20 ns	5.324	3.159	2.103
76	ZINC000003781738	Lestaurtinib	20 ns	4.712	2.776	1.833
77	ZINC000254071113	Ciluprevir	100 ns	6.289	2.626	1.997
78	ZINC000063933734	Rebastinib	100 ns	6.521	2.307	2.048
79	ZINC000059185874	GDC-0834	100 ns	4.908	2.177	1.969
80	ZINC000043133316	Tirilazad	20 ns	9.004	2.701	2.010
81	ZINC000098208742	Entospletinib	100 ns	11.974	5.332	2.051
82	ZINC000018710085	—	20 ns	9.355	2.998	2.117
83	ZINC000003930598	—	100 ns	6.666	2.685	2.058
84	ZINC000004215770	Elsamitrucin	20 ns	5.481	3.589	1.803
85	ZINC000003780800	Amrubicin	100 ns	10.287	6.029	1.872

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
86	ZINC000001539348	—	100 ns	11.058	2.994	2.030
87	ZINC000003978083	Tubocurarine	100 ns	3.920	2.261	1.941
88	ZINC000068250462	Tucatinib	20 ns	11.174	2.956	2.081
89	ZINC000001494900	Enzastaurin	100 ns	3.692	2.545	1.910
90	ZINC000003950115	Lonafarnib	100 ns	5.733	2.490	1.857
91	ZINC000019899628	Fenoverine	100 ns	3.677	2.281	2.005
92	ZINC000095535868	Rwj-58259	100 ns	8.472	2.433	1.993
93	ZINC000001490807	—	100 ns	4.913	2.323	1.872
94	ZINC000006717782	BMS-599626	100 ns	6.066	2.047	1.946
95	ZINC000100001820	PF-00477736	100 ns	7.137	2.640	1.922
96	**ZINC000028827350**	**Telcagepant**	**100 ns**	**4.156**	**2.665**	**1.931**
97	ZINC000003973984	Sotrastaurin	100 ns	1.677	2.143	1.911
98	ZINC000021290045	—	20 ns	6.012	2.332	1.748
99	ZINC000100029945	Zosuquidar	20 ns	5.263	3.791	2.066
100	CID121304016	Remdesivir	100 ns	4.163	2.421	1.843
		Natural products				
101	ZINC000150352420	Theacitrin A	100 ns	6.451	2.307	1.717
102	**ZINC000004098612**	**Corilagin**	**100 ns**	**4.102**	**2.134**	**1.667**
103	ZINC000008214976	Theasinensin B	100 ns	9.649	2.376	1.721
104	ZINC000169372863	Theasinensin A	20 ns	4.085	2.918	1.683
105	**ZINC000003978446**	**Theaflavin**	**100 ns**	**3.426**	**2.482**	**1.847**
106	ZINC000004235306	—	100 ns	5.733	2.503	2.114
107	ZINC000230071666	Theacitrin C	100 ns	2.309	2.278	1.660
108	ZINC000003984030	Amentoflavone	100 ns	3.925	2.396	1.701
109	ZINC000169333962	Theasinensin F	100 ns	4.612	2.439	1.851
110	ZINC000001531664	Ginkgetin	20 ns	6.071	3.245	2.114
111	ZINC000044351169	Proanthocyanidin A1	100 ns	4.011	2.151	1.696
112	**ZINC000003978800**	**Rhoifolin**	**100 ns**	**3.114**	**2.101**	**1.745**
113	ZINC000004098619	Proanthocyanidin A2	100 ns	3.335	2.584	1.845
114	ZINC000095619717	Proanthocyanidin A5’	100 ns	7.077	2.685	1.731

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
115	ZINC000003197535	Isoginkgetin	100 ns	5.567	2.396	1.929
116	ZINC000014887561	Zeylanone	100 ns	5.009	2.827	1.954
117	CID10077799	Isocorilagin	100 ns	3.733	2.250	1.683
118	ZINC000003870412	Epigallocatechin gallate (EGCG)	20 ns	5.590	3.688	1.572
119	ZINC000006624329	—	20 ns	6.683	2.681	1.952
120	ZINC000002148919	—	100 ns	4.422	2.743	2.102
121	ZINC000002107922	—	20 ns	9.171	4.921	2.176
122	ZINC000002161217	—	100 ns	11.053	2.278	1.829
123	ZINC000008297065	—	100 ns	3.521	2.030	1.992
124	ZINC000002125422	—	100 ns	6.062	2.640	1.952
125	ZINC000008764269	—	20 ns	25.191	11.891	3.143
126	ZINC000008789992	—	20 ns	2.944	2.311	1.974
127	ZINC000012296408	—	100 ns	6.046	2.043	1.955
128	ZINC000002147804	—	100 ns	7.889	2.999	1.914
129	ZINC000012881832	—	100 ns	3.545	2.153	1.984
130	ZINC000002158857	—	20 ns	8.281	2.733	2.130
131	CID5321811	Bavacoumestan A	20 ns	11.095	2.575	1.919
132	ZINC000100828606	Neodiosmin	100 ns	4.959	2.277	1.814
133	**ZINC000011865175**	**—**	**100 ns**	**1.694**	**2.046**	**2.012**
134	ZINC000002114470	—	100 ns	1.385	2.479	1.817
135	ZINC000100777667	Glabrolide	100 ns	8.081	2.663	1.956
136	CID12443227	Epitaraxerol	100 ns	2.943	2.263	2.049
137	ZINC000004098322	Homoeriodictyol	20 ns	5.386	2.771	1.998
138	ZINC000018847034	Daidzein	100 ns	3.937	2.504	1.760
		Steroids				
139	CID27125	Estetrol	100 ns	10.076	8.798	1.928
140	ZINC000004340309	Cortisol	20 ns	7.689	2.315	1.870
141	CID5757	Estradiol	100 ns	2.676	2.395	1.880
142	ZINC000004428526	Androstenedione	20 ns	11.190	5.440	2.084
143	CID91451	17-α-hydroxypregnenolone	20 ns	8.524	5.911	1.696

No.	Accession ID	Compound name	Simulation time [ns]	$RMSD_{ligand}$ [Å]	d_{dyad} [Å]	d_{BS} [Å]
144	ZINC000004096681	2-Hydroxyestrone	20 ns	8.524	5.911	1.696
145	ZINC000003815419	2-Hydroxyestradiol	20 ns	6.507	2.591	1.862
146	ZINC000003807917	Dehydroepiandrosterone	20 ns	9.917	2.253	2.006
147	ZINC000004081043	Allopregnanolone	20 ns	10.757	2.565	1.939
148	ZINC000118912393	Testosterone	100 ns	7.026	2.430	2.085
		Reference compounds				
149	ZINC000013985228	Tideglusib	20 ns	8.488	2.716	1.998
150	PDB 6LU7	N3	20 ns	9.746	2.652	1.878
151	ZINC000001714738	Cinanserin	100 ns	6.419	2.498	2.123
152	CID3194	Ebselen	100 ns	7.887	2.704	2.090
153	ZINC000001542916	Carmofur	20 ns	8.433	2.544	2.023
154	ZINC000013209429	PX-12	20 ns	6.963	2.706	2.127
155	ZINC000001529266	Disulfiram	100 ns	3.602	2.317	2.102
156	ZINC000002015152	Shikonin	20 ns	6.963	2.706	2.127
157	—	Inhibitor 11r	20 ns	7.280	2.422	1.895
158	—	Inhibitor 13a	20 ns	5.306	2.369	1.960
159	—	Inhibitor 13b	20 ns	8.298	2.362	1.984
160	—	Inhibitor 14b	20 ns	5.642	2.369	1.930