Supporting Information:

How to Deal with Low-Resolution Target Structures: Using SAR, Ensemble Docking, Hydropathic Analysis, and 3D-QSAR to Definitively Map the αβ-Tubulin Colchicine Site

Chenxiao Da,[1] Susan L. Mooberry,[2] John T. Gupton[3] and Glen E. Kellogg[1,*]

[1]*Department of Medicinal Chemistry & Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, Virginia, USA;* [2]*Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA;* [3]*Department of Chemistry, University of Richmond, Richmond, Virginia, USA.*

Table S1. Activity data, as reported, for compounds in study.

Cmpd.	Antiproliferation	Microtubule Depolymerization
1	0.014	0.03 μM
2	0.036±0.002	100% loss at 500 nM
3	0.618±0.07	50% loss at 5 μM, 100% loss at 30 μM
4	0.067±0.002	75% loss at 5 μM
5	0.109±0.008	70% loss at 5 μM
6	1.82±0.3	20% loss at 10 μM
7	1.30±0.04	15% loss at 10 μM
8	3.3±0.3	20% loss at 5 μM, 35% at 10 μM
9	5.3±0.3	No MT effect up to 10 μM
10	4.6±0.2	10% loss at 10 μM
11	5.2	10% loss at 10 μM
12	8.0±0.3	No MT effects up to 10 μM
13	10.7±0.4	No MT effects up to 10 μM
14	18.3±2.7	No MT effects up to 10 μM
15	10.3±1.3	15% loss at 50 μM
16	2.24±0.2	20% loss at 30 μM
17	0.919±0.020	30% loss at 50 μM
18	0.312±0.020	20% loss at 20 μM; 30% loss at 30 and 50 μM
19	0.843	7.0 μM; 65% loss at 5 μM; 80% loss at 10 μM
20	0.633±0.01	2.4 μM; 90% loss at 5 μM
21	12.9±1.9	35% loss at 50 μM
22	2.64±0.30	14 μM; 20% loss at 5 μM; 75% loss at 10 μM
23	3.24±0.20	7.0 μM; 50% loss at 5 μM; 95% loss at 10 μM
24	1.98±0.20	17.8 μM
25	1.70±0.10	27.1 μM; 50% MT loss at 30 μM
26	0.626±0.020	18.5 μM

27	0.806±0.060	9.9 µM
28	0.539±0.040	14.1 µM
29	1.99±0.20	15% MT loss at 10 µM
30	1.80±0.20	20.9 µM; 40% MT loss at 30 µM
31	4.36±0.3	20.9 µM; 50% MT loss at 20 µM
32	5	20% loss at 30 µM; cells dead at 50 µM
33	10	No MT effect up to 50 µM
34	>10	No MT effect up to 50 µM
35	3	25% loss at 50 µM
36	4	No MT effect up to 50 µM
37	100	No MT effect up to 50 µM
38	2	No MT effect up to 50 µM
39	10	No MT effect up to 50 µM
40	0.003±0.0002	0.007 µM
41	0.9	4.5 µM
42	0.35	18.6 µM
43	0.095	5.6 µM
44	0.182	1.8 µM
45	0.183±0.0034	5.8 µM
46	>10	> 40 µM
47	0.0966	1.2 µM
48	0.193	1.4 µM
49	0.0303	0.22 µM
50	0.298	8.4 µM
51	0.0427	0.23 µM
52	0.012±0.0008	0.023 µM
53	>10	> 40 µM
54	0.0945	1.6 µM
55	2.7	> 40 µM
56	0.051±0.004	0.278 µM
57	>10	> 40 µM
58	0.402	6.9 µM
59	2.7	> 40 µM

Table S2. Experimental and predicted activities for all models in internal training and test sets.

Cmpd.	Exptl. pIC_{50}	Exptl. pEC_{50}	Docking Alignment				Semi-ligand Align.		Naïve Align.	
			CoMFA	CoMFA+ HINT	CoMSIA	CoMFA (pEC_{50})	CoMFA	CoMSIA	CoMFA	CoMSIA
1	7.70	7.52	7.82	7.41	8.29	7.36	7.62	8.15	7.96	8.34
2	7.44	6.31	6.82	6.82	6.35	5.77	6.76	6.36	6.55	5.83
3	6.21	5.30	6.49	6.43	6.43	5.64	6.50	6.29	6.34	5.87
4	7.17	5.48	6.37	6.51	6.34	5.47	6.45	6.33	6.06	5.73
5	6.96	5.44	6.93	6.99	6.59	5.90	6.89	6.44	6.62	5.85
6	5.74	4.60	5.97	5.89	5.79	4.70	5.82	5.52	6.21	5.83
7	5.89	4.48	5.95	5.84	5.47	4.57	5.72	5.43	5.87	5.66
8	5.48	4.85	5.68	5.56	5.67	4.95	5.72	5.48	5.56	5.63
9	5.28	4.00	5.32	5.26	5.38	4.16	5.52	5.38	5.15	5.39
10	5.34	4.30	5.20	5.34	5.37	4.09	5.57	5.32	5.23	5.47
11	5.28	4.30	5.35	5.35	5.47	4.26	5.52	5.34	5.20	5.52
12	5.10	4.00	5.21	5.14	4.79	3.86	4.89	5.16	5.21	4.88
13	4.97	4.00	4.73	4.79	4.85	4.02	4.83	5.05	4.94	4.85
14	4.74	4.00	5.08	5.31	5.28	4.09	5.20	5.78	5.26	5.80
15	4.99	3.78	4.62	4.81	4.88	3.23	5.12	5.70	5.66	5.86
16	5.65	4.12	5.58	5.75	5.77	4.32	5.57	5.83	6.03	5.92
17	6.04	4.08	5.78	5.95	5.82	4.16	5.71	5.91	6.11	6.02
18	6.51	4.03	5.91	6.14	5.89	4.25	5.74	5.99	6.16	6.09
19	6.07	5.15	5.75	6.01	6.03	4.56	5.62	5.63	5.95	5.66
20	6.20	5.62	6.16	6.24	6.37	5.13	5.94	6.34	5.84	6.15
21	4.89	4.15	4.88	4.75	5.05	4.35	5.48	5.08	4.84	5.62
22	5.58	4.85	5.65	5.69	5.81	5.27	6.32	6.17	5.98	5.92
23	5.49	5.15	5.88	5.93	5.91	4.89	5.73	5.89	5.36	5.88
24	5.70	4.75	6.05	5.87	5.75	4.99	5.97	5.57	6.00	5.75
25	5.77	4.57	5.84	5.83	5.94	4.52	5.79	6.10	6.11	6.07
26	6.20	4.73	5.96	6.12	6.03	4.66	5.89	5.86	6.23	5.85
27	6.09	5.00	6.23	6.41	5.92	4.84	6.16	6.02	6.39	6.09
28	6.27	4.85	6.00	6.16	5.91	4.59	5.91	5.80	5.92	5.68
29	5.70	4.48	5.63	5.80	5.95	4.83	5.68	5.72	5.56	5.81
30	5.74	4.53	5.78	5.92	6.16	4.52	5.72	5.96	5.78	5.92
31	5.36	4.68	5.49	5.52	5.99	4.71	5.66	5.91	5.74	5.81
32	5.30	4.12	5.74	5.65	6.13	4.81	5.85	5.69	5.49	5.79
33	4.00	3.30	3.87	3.87	3.85	2.80	3.29	4.09	4.46	3.50
34	4.00	3.30	4.00	3.99	4.01	2.79	3.86	4.30	4.20	4.61
35	5.52	4.00	5.70	5.71	5.49	4.15	5.85	5.56	5.46	5.65
36	4.00	3.30	3.90	3.95	4.10	3.38	4.03	4.10	3.91	4.32
37	4.00	3.30	4.12	4.09	4.31	3.78	4.34	4.07	3.99	4.30
38	4.00	3.30	4.66	4.68	4.34	3.80	5.31	5.26	4.96	5.43
39	4.00	3.30	4.32	4.01	4.21	2.97	3.78	3.67	3.70	4.06

40	8.52	8.15	8.45	8.39	7.88	7.75	7.45	8.06	8.71	7.90
41	6.46	4.73	6.59	5.26	6.57	5.97	6.01	7.13	6.48	6.41
42	7.02	5.25	6.62	6.65	6.61	5.19	7.07	7.00	6.40	6.80
43	6.74	5.74	6.85	6.96	6.86	5.36	6.90	6.78	6.94	6.62
44	6.06	5.35	6.74	6.70	7.17	5.64	7.45	7.64	6.84	7.33
45	6.74	5.24	5.76	6.03	5.83	4.91	6.09	6.42	6.23	6.23
46	4.70	4.10	4.64	4.41	4.41	4.13	4.82	4.38	4.26	4.44
47	7.02	5.92	7.19	7.13	6.89	5.94	6.95	7.06	6.97	7.19
48	6.71	5.85	6.87	7.02	6.78	5.64	6.81	6.75	6.65	6.97
49	7.52	6.66	7.35	7.56	7.20	5.98	7.33	7.24	7.17	7.28
50	6.53	5.08	7.16	7.01	6.69	5.89	7.29	5.87	6.95	6.56
51	7.37	6.64	7.00	7.29	7.35	5.77	6.53	6.97	6.49	7.17
52	7.92	7.64	7.44	7.35	7.09	6.69	6.76	6.99	7.41	6.68
53	4.92	4.37	4.78	4.65	4.50	4.15	5.42	4.95	5.07	4.88
54	7.02	5.80	6.98	6.90	6.98	5.89	6.87	6.64	7.27	6.48
55	5.78	4.37	5.41	5.65	5.74	4.15	6.19	6.46	5.71	6.50
56	7.29	6.56	7.08	6.89	6.97	6.31	6.26	6.83	6.87	6.58
57	4.29	3.28	4.56	4.28	4.62	3.99	4.94	4.79	4.59	4.79
58	6.40	5.16	6.43	6.43	6.68	5.47	6.32	6.48	6.70	6.39
59	5.15	3.28	6.01	5.90	6.49	5.16	5.75	6.30	5.32	6.40

Table S3. Experimental and predicted activities for Semi-ligand and Naive models in external test set.

Cmpd.	Experimental		Semi-Ligand Align.		Naïve Align.	
	Activity: IC_{50} or K_d (μM)	pActivity	CoMFA	CoMSIA	CoMFA	CoMSIA
60a	2.13	5.67	5.93	7.24	6.28	7.37
60b	4.76	5.32	5.44	6.66	5.88	7.10
60c	47	4.33	4.93	6.45	5.07	7.03
61a	0.001	9.00	7.31	7.62	6.87	7.24
61b	0.31	6.51	6.88	7.06	6.58	6.90
61c	5	5.30	5.70	6.19	5.57	6.65
62a	0.02	7.70	6.75	7.38	6.69	7.10
62b	0.1	7.00	5.23	6.12	5.32	6.45
62c	2	5.70	4.79	5.74	4.65	6.09
63a	0.007	8.15	6.17	6.98	5.80	6.33
63b	0.59	6.23	6.15	7.22	6.00	6.65
63c*	> 10	4.70	4.62	6.12	4.51	6.30
64a	0.013	7.89	6.39	6.78	5.67	6.70
64b	0.025	7.60	5.98	6.63	4.82	6.76
64c	2.5	5.60	5.06	5.82	4.57	6.63
65a	0.00021	9.68	5.72	6.69	5.32	6.05
65b	1.9	5.72	5.46	6.65	5.21	6.25
65c*	>25	4.30	5.78	6.14	5.46	5.93
66a	0.13	6.89	5.87	7.02	5.72	6.69
66b	6	5.22	5.93	7.00	6.03	6.70
66c*	> 100	3.70	4.41	5.89	3.93	5.56
67a	0.001	9.00	5.36	5.42	5.61	5.70
67b	19	4.72	5.00	6.12	4.68	6.17
67c*	> 50	4.00	4.86	5.58	4.99	5.93
r^2 for correlation			0.38	0.16	0.23	0.03
Ranking performance			6/8	6/8	4/8	3/8

*Literature report was IC_{50} or K_d > x μM. We used IC_{50} or K_d = 2x for modeling.

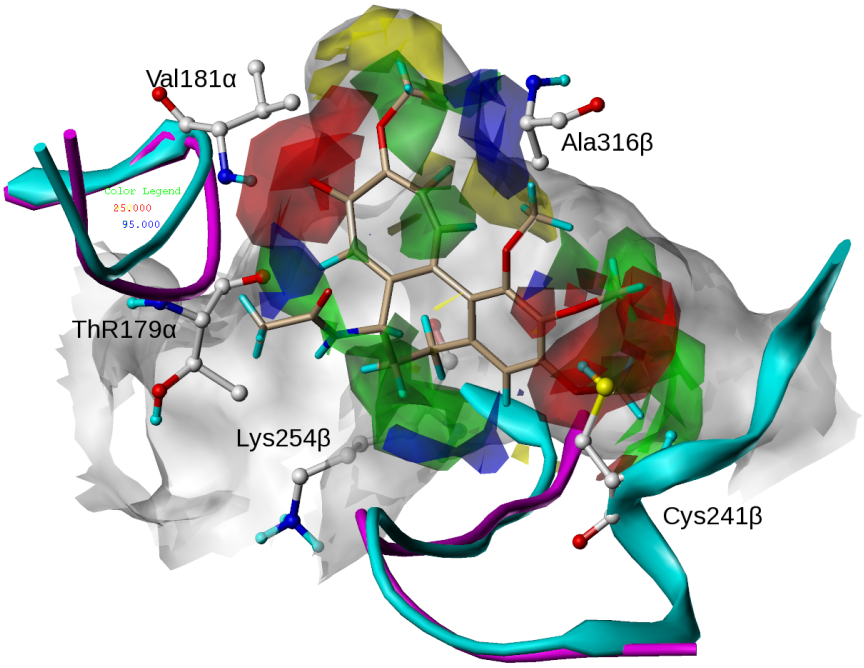

Figure S1. CoMFA model contour maps based on docked poses for EC_{50} target variable. For reference, colchicine is shown in beige. Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups.

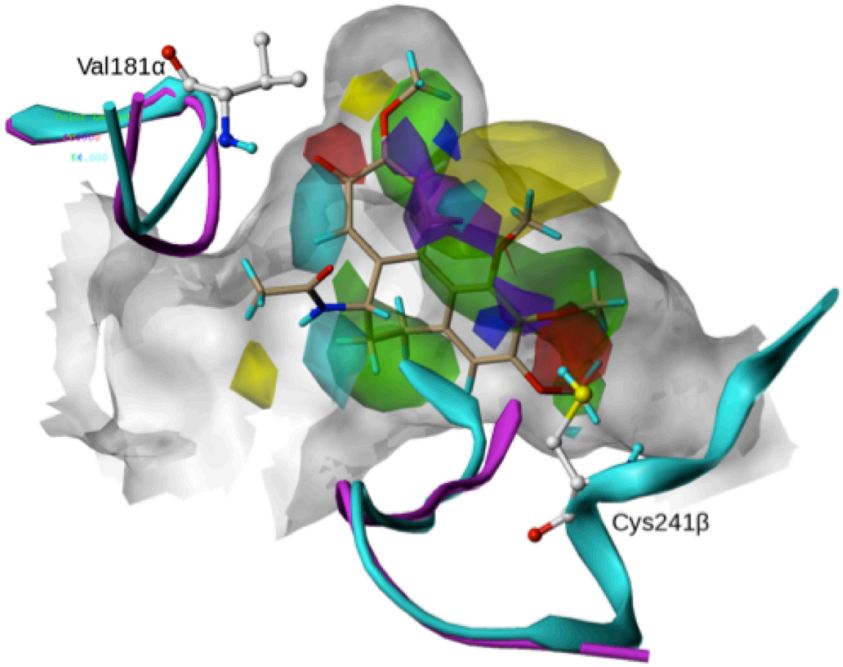

Figure S2. CoMSIA model contour maps based on docked poses for IC_{50} target variable. For reference, colchicine is shown in beige. Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups. Cyan regions favor hydrophobic groups and purple regions favor polar groups.

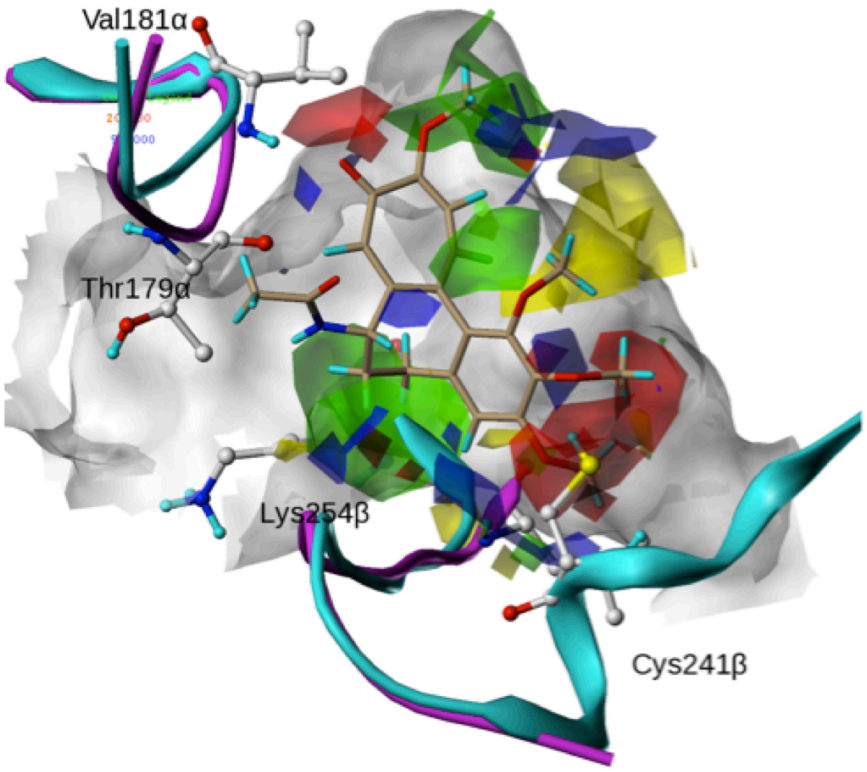

Figure S3. CoMFA model contour maps based on semi-ligand alignment poses for IC_{50} target variable. For reference, colchicine is shown in beige. Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups.

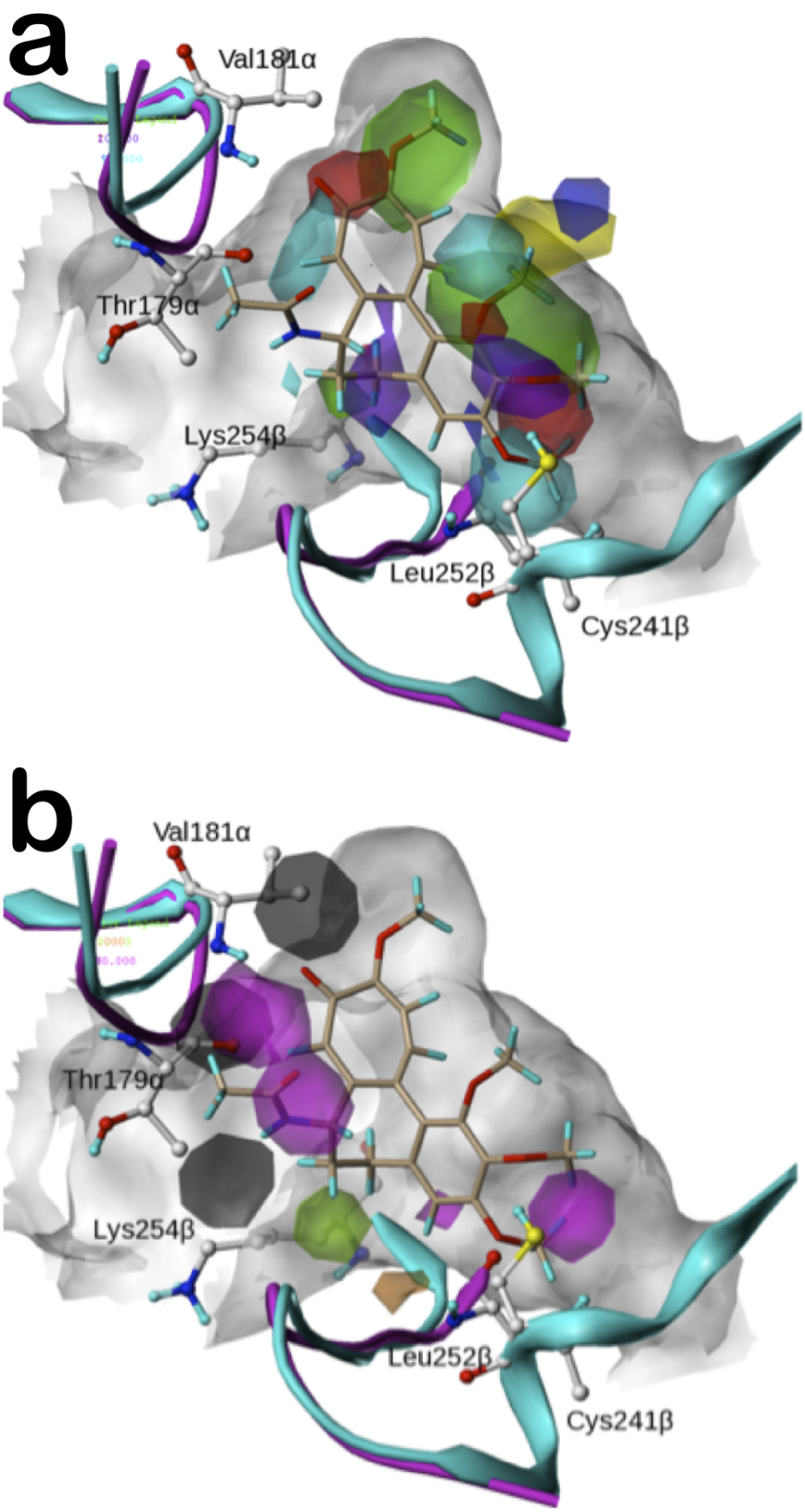

Figure S4. CoMSIA model contour maps based on semi-ligand alignment poses for IC_{50} target variable. For reference, colchicine is shown in beige. a) Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups. Cyan regions favor hydrophobic groups and purple regions favor polar groups. b) Black and light green represent regions that favor and disfavor hydrogen bond donors. Magenta and orange represent regions that favor and disfavor hydrogen bond acceptors.

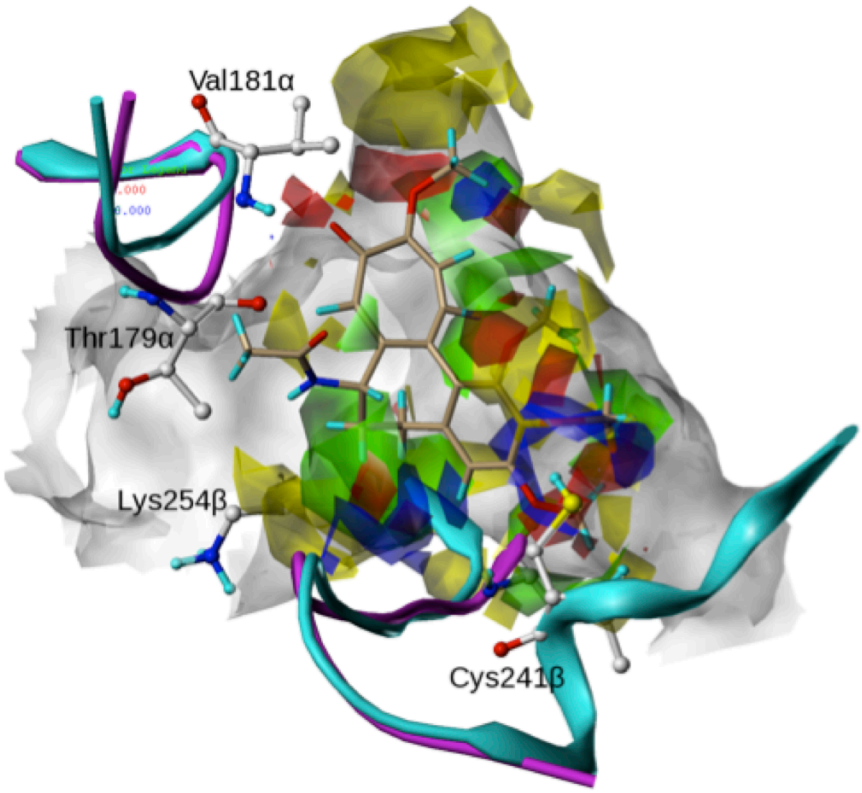

Figure S5. CoMFA model contour maps based on naïve alignment poses for IC_{50} target variable. For reference, colchicine is shown in beige. Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups.

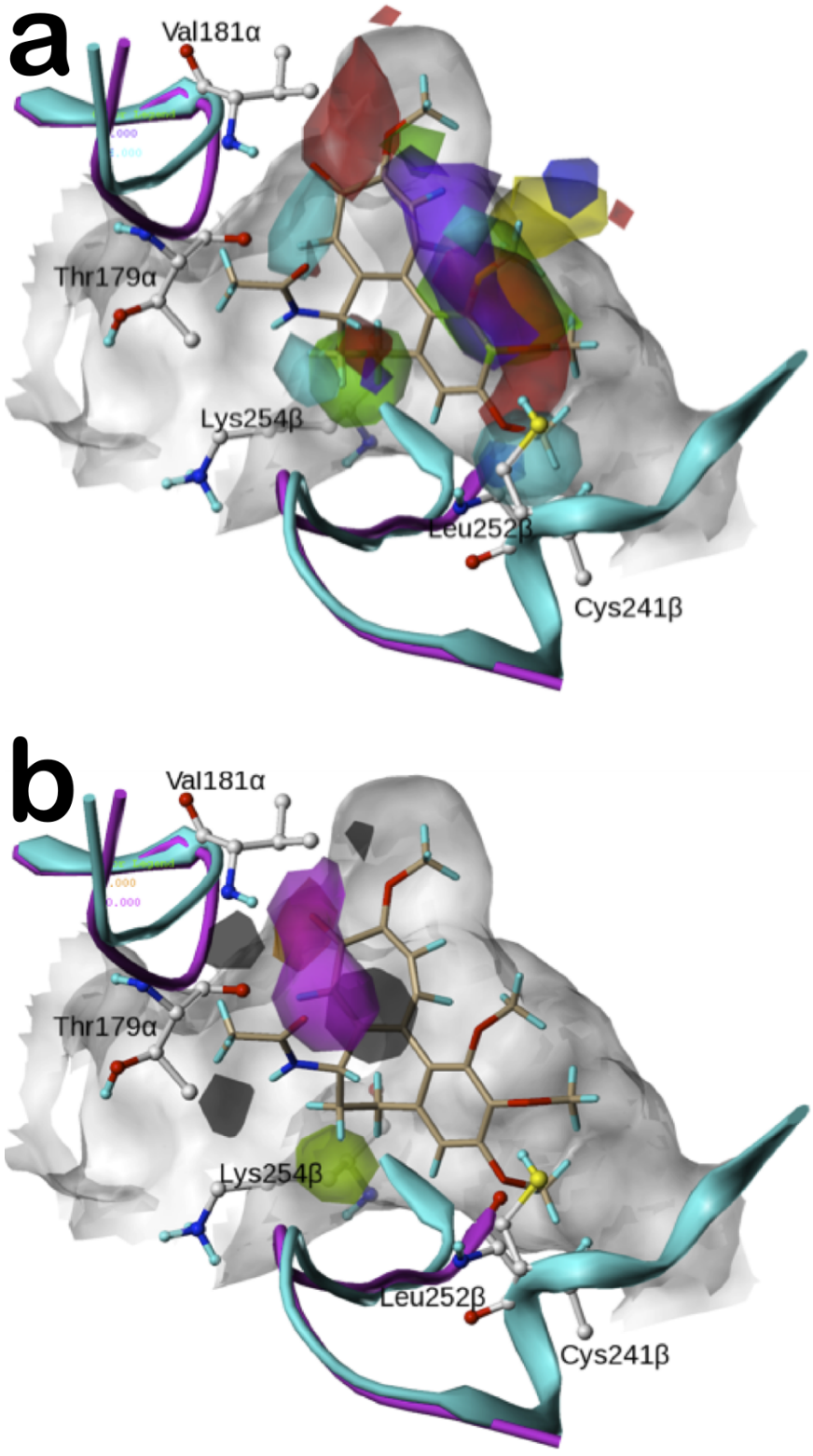

Figure S6. CoMSIA model contour maps based on naïve alignment poses for IC_{50} target variable. For reference, colchicine is shown in beige. a) Green and yellow contours indicate favorable and unfavorable steric interactions, respectively. Blue regions favor electropositive groups and red regions favor electronegative groups. Cyan regions favor hydrophobic groups and purple regions favor polar groups. b) Black and light green represent regions that favor and disfavor hydrogen bond donors. Magenta and orange represent regions that favor and disfavor hydrogen bond acceptors.

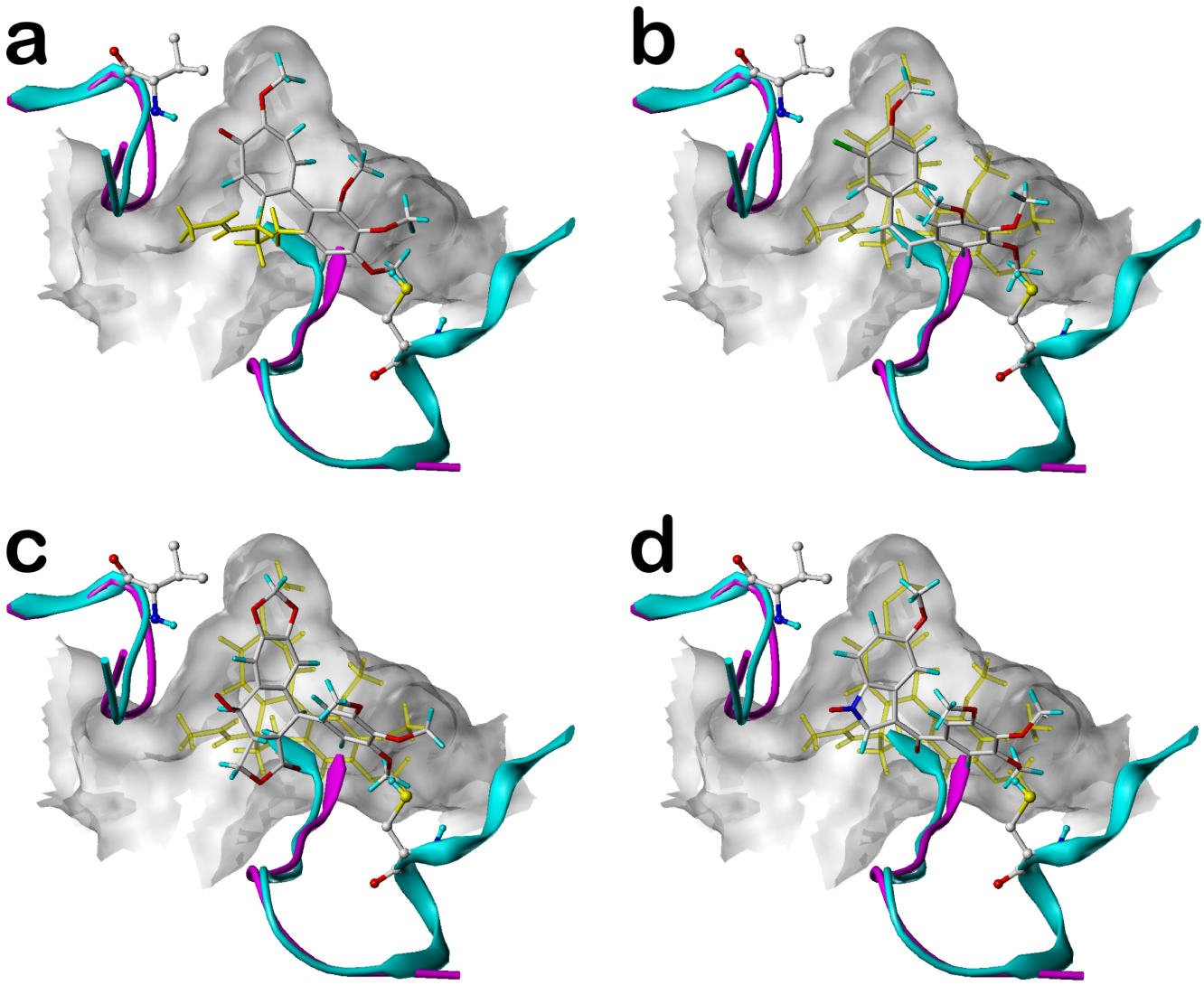

Figure S7. Docking models for external test set representatives (most active compounds). a) Compound **60a**; b) Compound **61a**; c) Compound **62a**; and d) Compound **63a**. Colchicine (yellow) is included for reference.

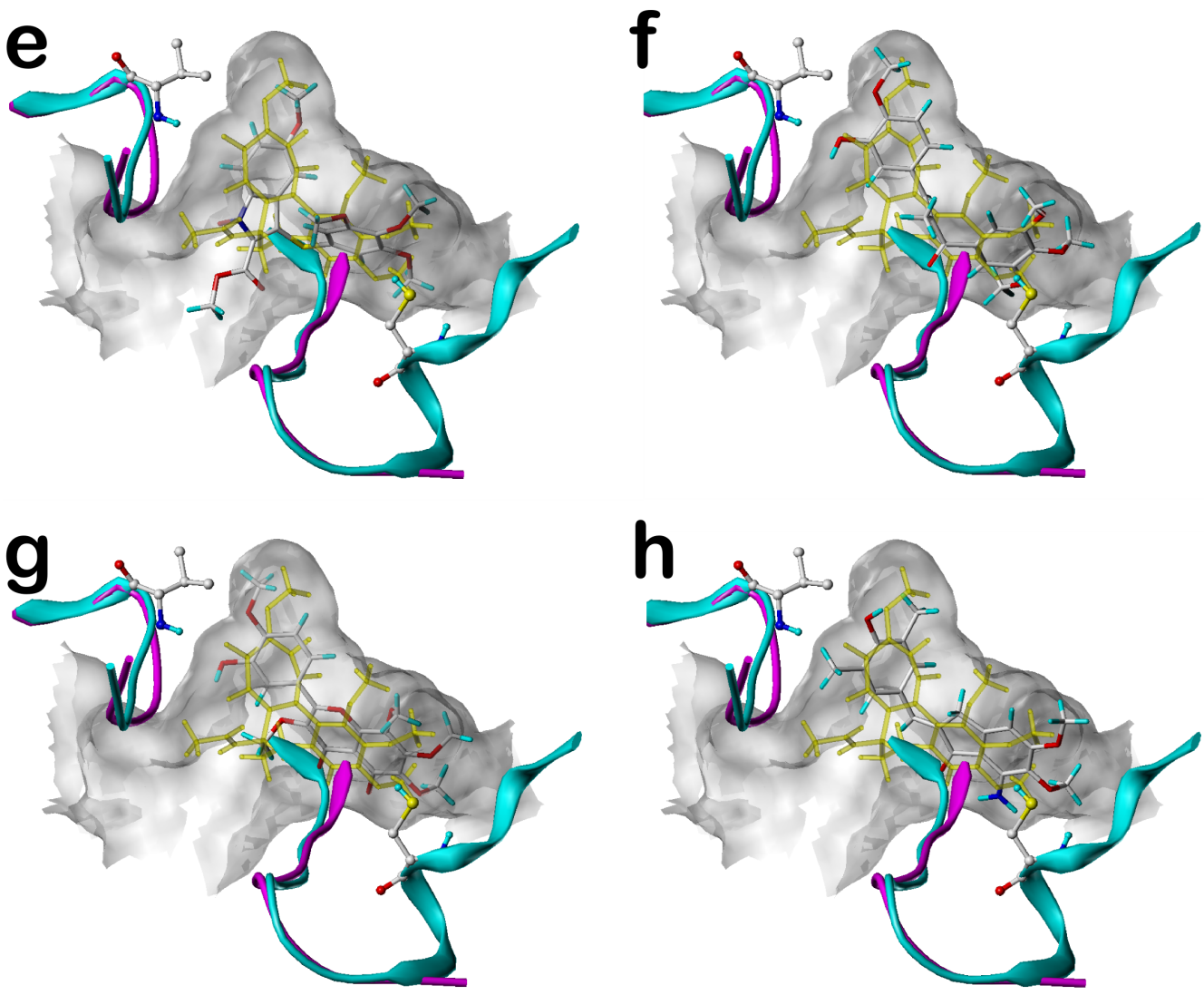

Figure S7 (continued). Docking models for external test set representatives (most active compounds). e) Compound **64a**; f) Compound **65a**; g) Compound **66a**; and h) Compound **67a**. Colchicine (yellow) is included for reference.