SUPPORTING INFORMATION

Novel Aromatase Inhibitors By Structure-Guided Design

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Table S1. Detailed inhibition data and individual dose response curves

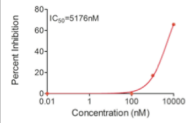
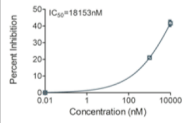
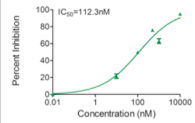
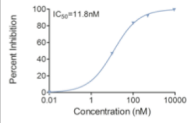
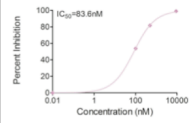
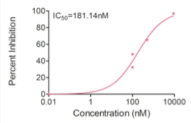
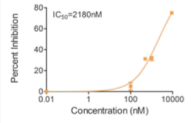
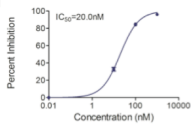
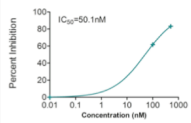
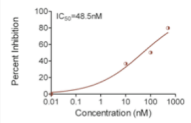
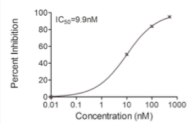
Compound	Percent Inhibition						Dose-Response Curve	IC_{50} (nM)	95% Confidence Interval (nM)
	10μM	9.1μM	1μM	500nM	100nM	10nM			
	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)			
2	65.7 (2.4)		17.2 (0.2)					5200	4700 to 5500
3	41.6 (5.3)		20.9 (1.3)					18100	13100 to 25200
4	95.0 (0.3)		63.0 (5.5)	76.0 (1.7)	49.9 (2.7)	21.6 (5.3)		112.3	78.2 to 161.3
5		99.1 (0.1)		91.9 (0.2)	83.1 (4.9)	46.4 (0.4)		11.8	9.3 to 14.9
6		99.2 (0.1)		83.1 (4.9)	53.9 (3.6)			83	74.5 to 93.9
7		97.0 (0.1)		65.3 (2.3)	40.2 (3.1)			181.1	164.0 to 200.0
8		75.0 (1.6)	31.5 (3.7)	31.2 (0.8)	5.0 (7.4)			2180	1750 to 2710
9			96.0 (0.4)		84.5 (1.7)	32.8 (4.2)		20	18.1 to 22.0
Exemestane				83.2 (1.3)	61.8 (5.2)			50.1	40.9 to 61.4
Formestane				79.9 (1.5)	50.3 (2.9)			48.6	33.6 to 71.1
Letrozole				95.1 (0.02)	83.9 (0.6)	50.5 (1.3)		9.9	9.3 to 10.5

Table S2. Detailed anti-proliferation data and individual dose response curves

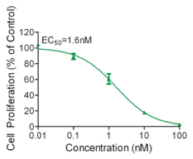
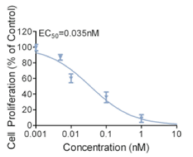
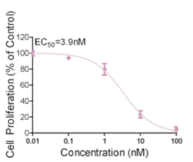
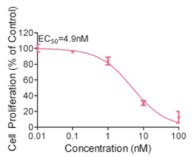
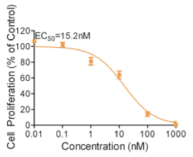
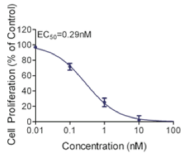
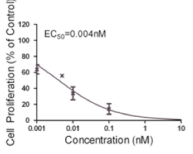
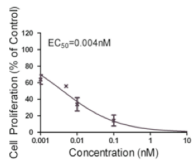
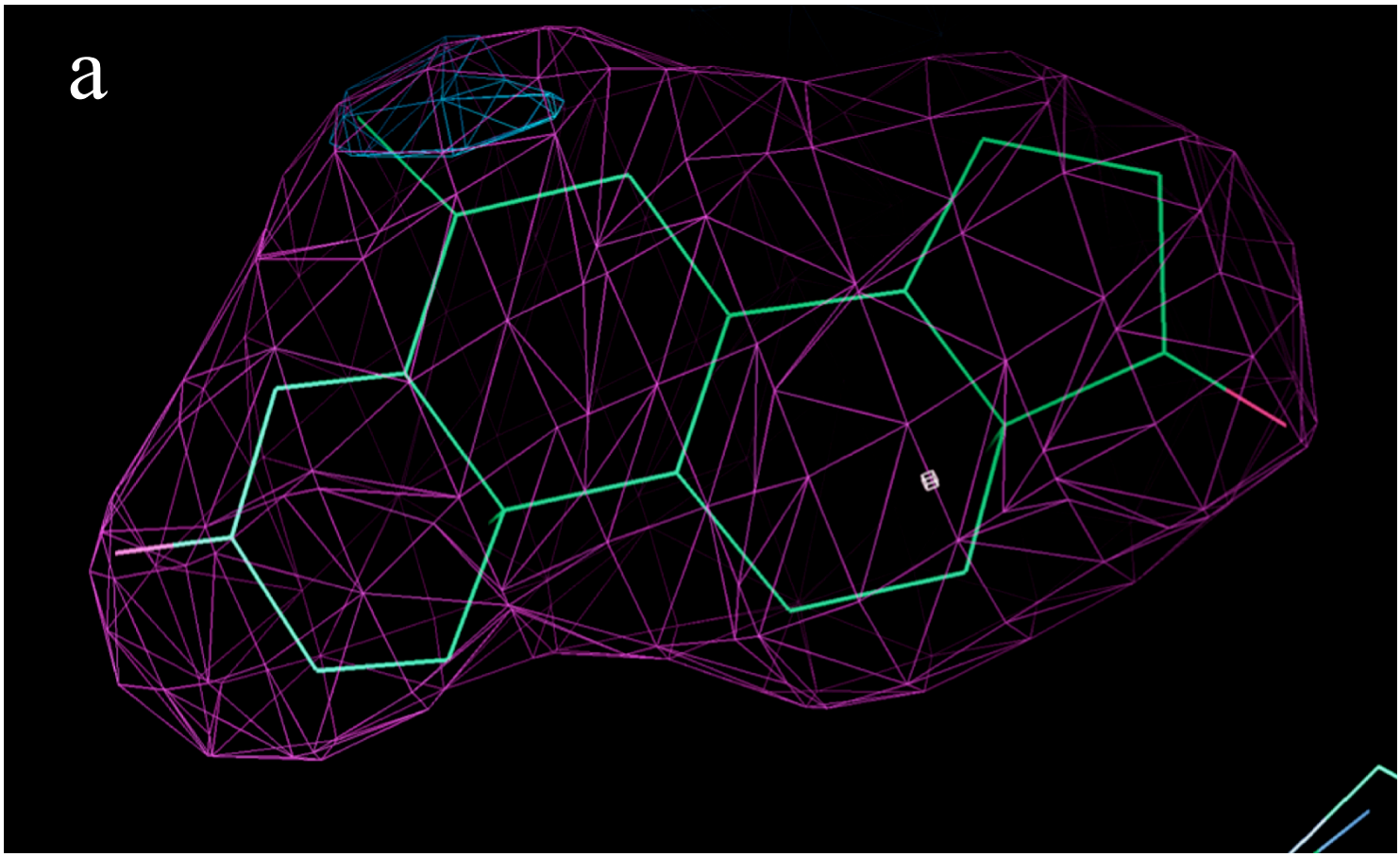
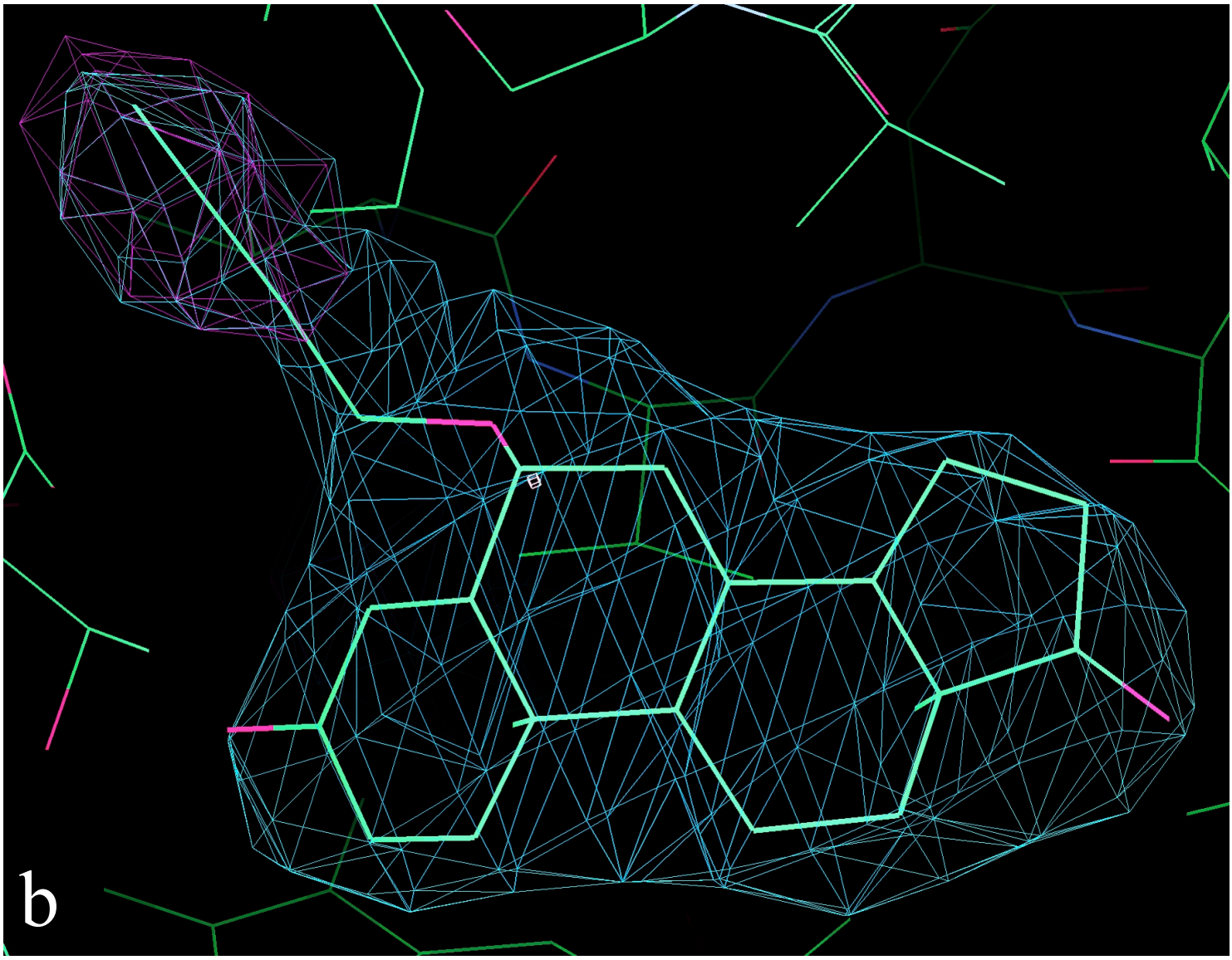
Compound	Cell Proliferation (% of Control)								Dose-Response Curve	EC_{50} (nM)	95% Confidence Interval(nM)
	1000nM	100nM	10nM	1nM	0.1nM	0.01nM	0.005nM	0.001nM			
	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)	Avg (SD)			
4		2.9 (2.6)	18.1 (1.7)	60.6 (11.3)	89.1 (6.5)	104.5 (2.7)				1.7	1.2 to 2.2
5			0.0 (5.83)	8.7 (8.6)	35.8 (12.0)	60.1 (9.7)	87.0 (6.3)	99.4 (7.1)		0.03	0.02 to 0.06
6		4.3 (5.9)	23.3 (7.8)	79.6 (12.3)	93.9 (3.5)	99.7 (5.2)				3.4	2.4 to 4.7
7		12.5 (12.9)	30.6 (5.4)	84.1 (8.5)	95.7 (4.2)	100.4 (8.3)				5.4	3.2 to 7.3
8	0.15 (7.2)	13.9 (4.7)	64.0 (8.4)	81.2 (8.1)	102.0 (5.5)	106.6 (2.4)				15.7	10.1 to 22.9
9		0.0 (1.7)	2.0 (10.0)	25.2 (9.6)	76.0 (1.7)	83.1 (4.9)				0.3	0.2 to 0.4
Exemestane		9.4 (6.0)	29.5 (15.9)	78.5 (4.0)	103.8 (1.0)	108.3 (1.6)				5.6	2.7 to 6.5
Letrozole			0.0 (3.2)	0.0 (2.2)	14.2 (11.5)	33.8 (14.1)	55.8 (3.4)	63.8 (9.8)		0.004	0.003 to 0.007

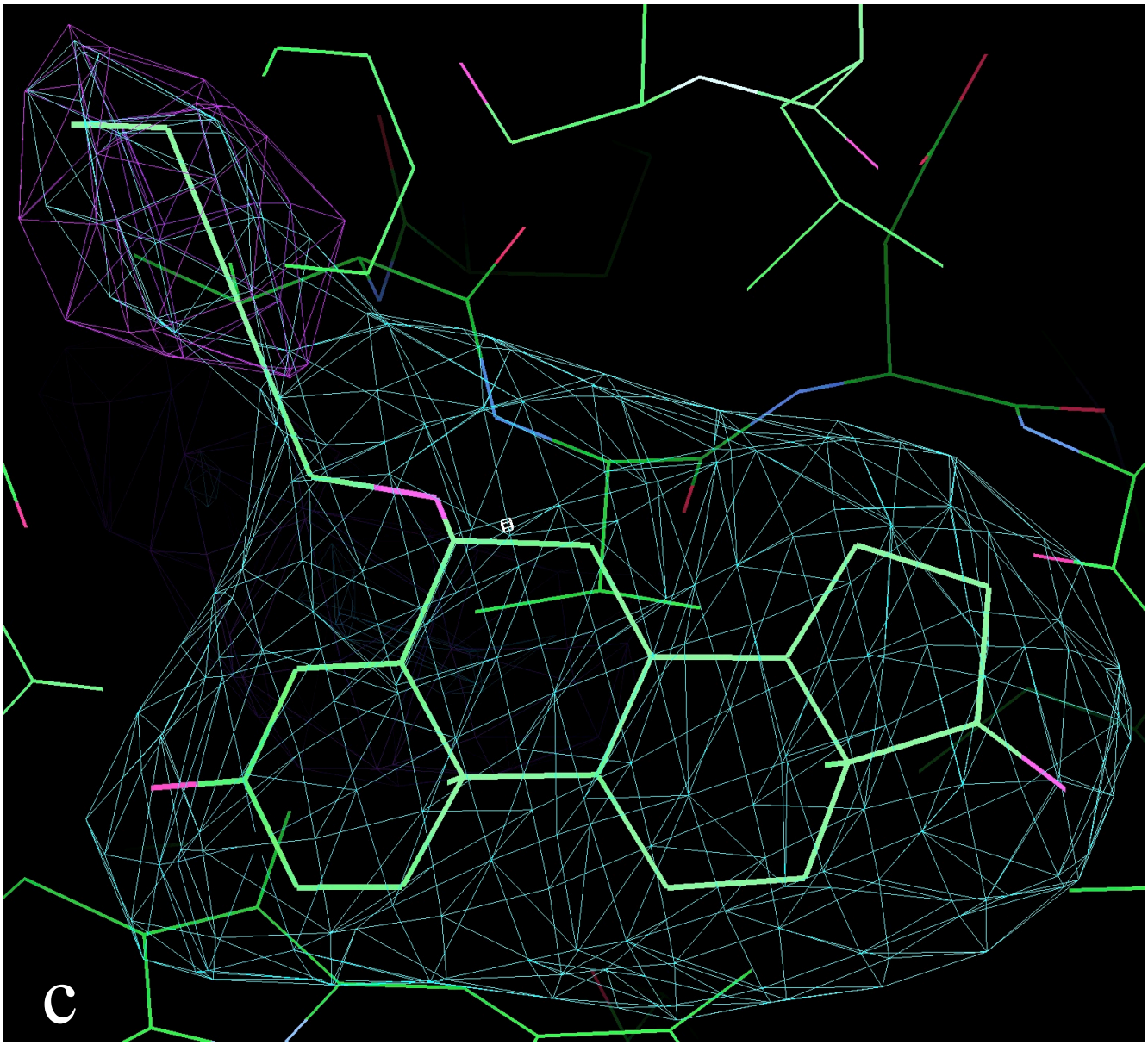
Table S3. Summary of X-ray data collection and refinement results				
Protein	Androstenedione (ASD) complex	Exemestane (EXM) complex	Compound **4** complex	Compound **5** complex
PDB ID code	3S79	3S7S	4GL5	4GL7
Data collection				
Space group	$P3_221$	$P3_221$	$P3_221$	$P3_221$
Cell dimensions *a*, *b*, *c* (Å)	140.22, 140.22, 119.27	140.63, 140.63, 119.02	140.33, 140.33, 118.73	141.45, 141.45, 118.85
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution range (Å)	50.0 - 2.747	50.0 - 3.208	50.0 - 3.481	50.0 - 3.900
R_{sym} or R_{merge}	0.077 (0.790)*	0.110 (0.850)	0.154 (0.816)	0.114 (0.793)
$I / \sigma I$	25.42 (1.73)	23.93 (2.07)	14.10 (2.34)	16.72 (1.25)
Completeness (%)	99.5 (100.0)	100.0 (100.0)	91.5 (95.0)	99.5 (100.0)
Redundancy	6.0 (6.1)	11.1 (10.9)	3.8 (3.9)	4.6 (4.6)
Number of crystals used	1	1	1	1
Refinement				
Resolution (Å)	2.747	3.208	3.481	3.900
Unique reflections	33618	21353	17289	12126
R_{work} / R_{free}	0.219/0.237	0.221/0.256	0.210/0.260	0.214/0.254
No. atoms				
Protein	3668	3668	3658	3658
Heme/Steroid	43/21	43/22	43/26	43/27
Water/ion	37/15	0	0	0
B-factors (Å^2)				
Wilson (overall)	83.2	106.4	-	-
Protein	69.1	96.4	91.2	147.5
Heme/Steroid	46/54	62/74	60/75	97/116
Water/ion	76.9	-	-	-
R.m.s. deviations				
Bond lengths (Å)	0.008	0.009	0.009	0.012
Bond angles (°)	1.261	1.227	1.311	1.474
Overall coordinate error				
From R_{free} (Å)	0.22	0.35	0.47	0.56
max likelihood (Å)	0.18	0.27	0.33	0.41
Structure validation Φ/Ψ plot[1]				
Favored region	430 (95.6%)	419 (93.1%)	390 (86.7%)	391 (86.9%)
Allowed region	20 (4.4%)	27 (6.0%)	53 (11.8%)	52 (11.6%)
Outlier region	0 (0.0%)	4 (0.9%)	7 (1.6%)	7 (1.6%)

*Numbers in parenthesis are for the highest resolution shells

Figure S1. Unbiased difference electron density (Fobs-Fcal) maps, calculated before inclusion of the inhibitors in the models. Shown are the refined atomic models of the aromatase complexes with (a) EXM: 3.21Å resolution contoured at 4.5σ. Also shown in blue is the difference electron density in the absence of C6 methylidene carbon, contoured at 2.0σ, representing the missing atom. (b) 2-butynyloxy derivative **4**: 3.48Å at 4.0σ. Shown in magenta is the difference electron density in the absence of the side chain atoms, contoured at 3.5σ, representing the missing atoms. (c) 2-pentynyloxy derivative **5**: 3.90Å at 2.7σ. Shown in magenta is the difference electron density in the absence of the side chain atoms, contoured at 2.5σ, representing the missing atoms.




b


C

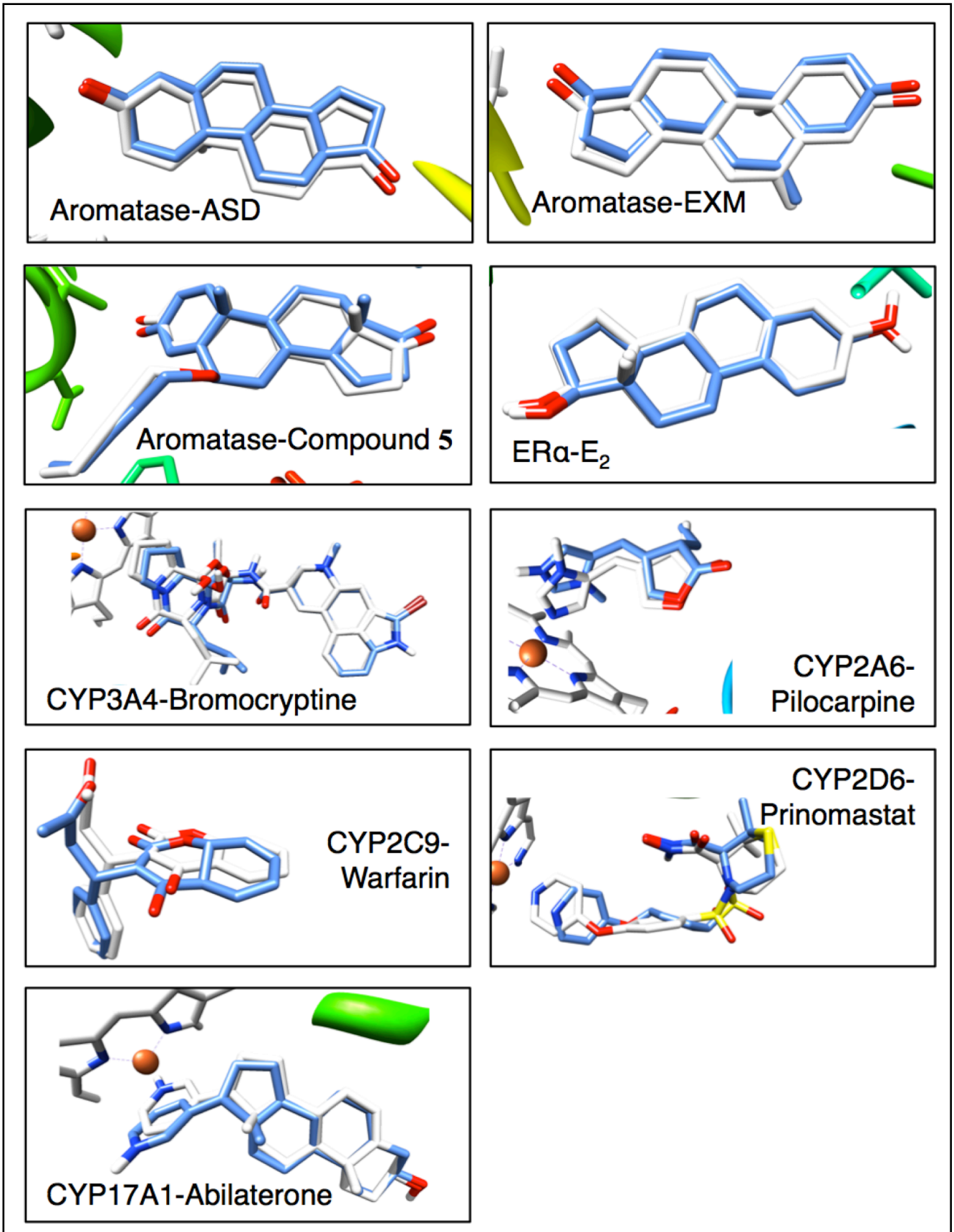


Figure S2. Docking validation results. Ligands of selected receptor-ligand complexes were docked onto their respective crystal structures. Each panel shows the superposition of the docked ligand (light blue) to the ligand (white) from the crystal structure.

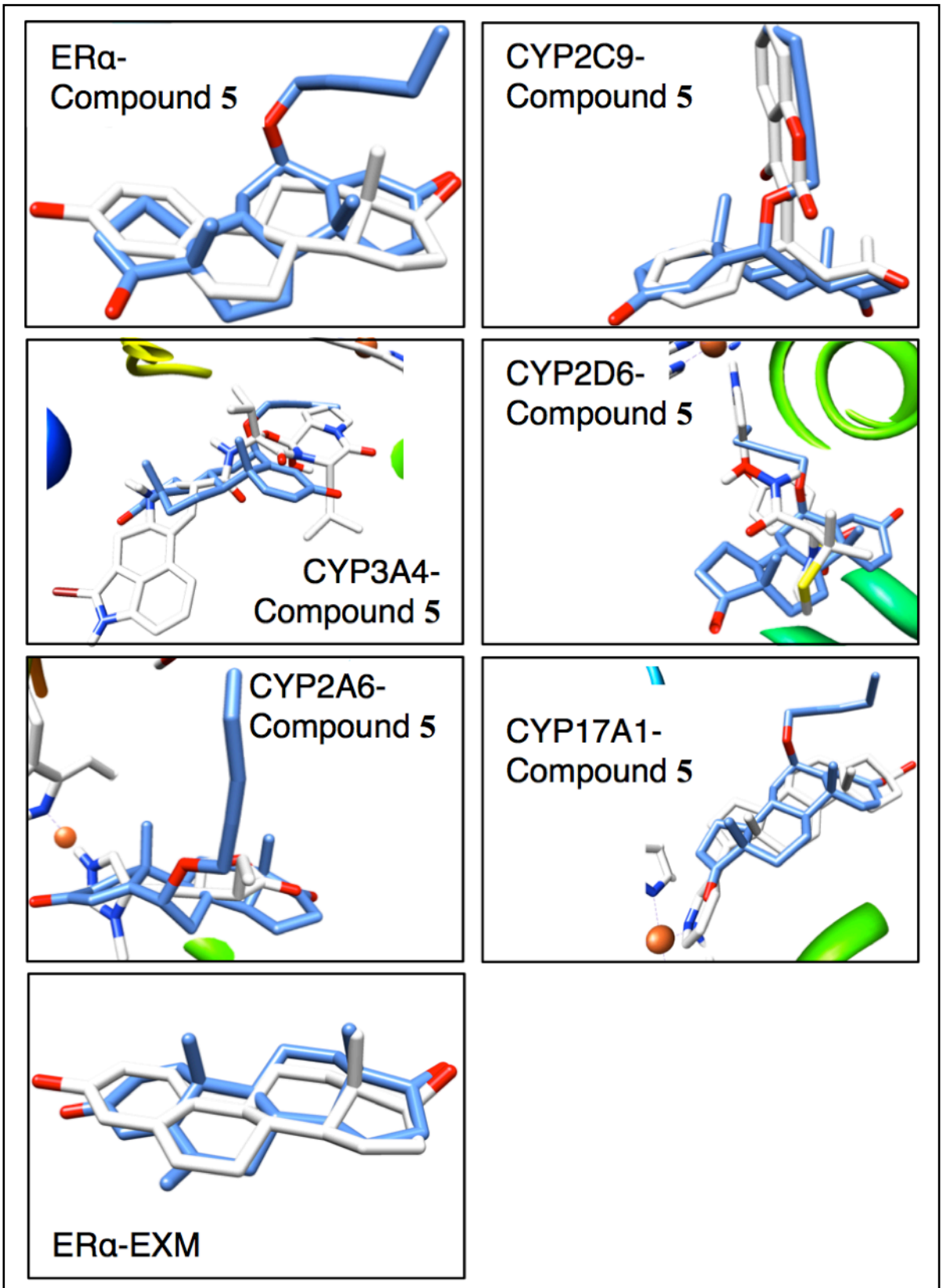


Figure S3. Docking of compound 5 into the binding sites of potential targets. The docking of compound **5** (light blue) to each target was compared to the crystal structure of actual ligand (white)-receptor complexes. The panel on the bottom left shows the docking of EXM (light blue) to ERα.

Table S4. Summary of results from docking experiments: root mean square deviation (RMSD) of each ligand from the crystal structure and free-energy of ligand-binding

Receptor	Ligand	RMSD of docked native ligand Å	Scoring function (Free energy of ligand binding, kcal/mole)	Docking to receptor with pharmacophore (receptor features specific for ligand recognition) query
Aromatase CYP19A1	ASD	0.5	-21.1	Best poses replicate the crystal structures.
	EXM	0.6	-16.0	
	5	0.5	-16.1	
ERα	E2	0.4	-25.2	
	EXM	0.7	7.2	No conformations passed pharmacophore filters. No poses generated.
	5	N/A	58.0	
CYP3A4	Bromocryptine	1.0	-34.0	
	5	N/A	-19.3	
CYP2A6	Pilocarpine	1.0	10.9	
	5	N/A	186.4	
CYP2C9	S-Warfarin	1.1	-11.6	
	5	N/A	64.9	
CYP2D6	Prinomastat	1.0	-10.8	
	5	N/A	-0.1	
CP17A1	Abilaterone	0.7	-11.5	
	5	N/A	7.1	

Reference:

1. Lovell, S. C.; Davis, I. W.; Arendall, W. B., 3rd; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C., Structure validation by Calpha geometry: phi,psi and Cbeta deviation. *Proteins* **2003,** *50*, 437-450.