SUPPORTING INFORMATION

In-crystal affinity ranking of fragment hit-compounds revealed a relationship with their inhibitory activities.

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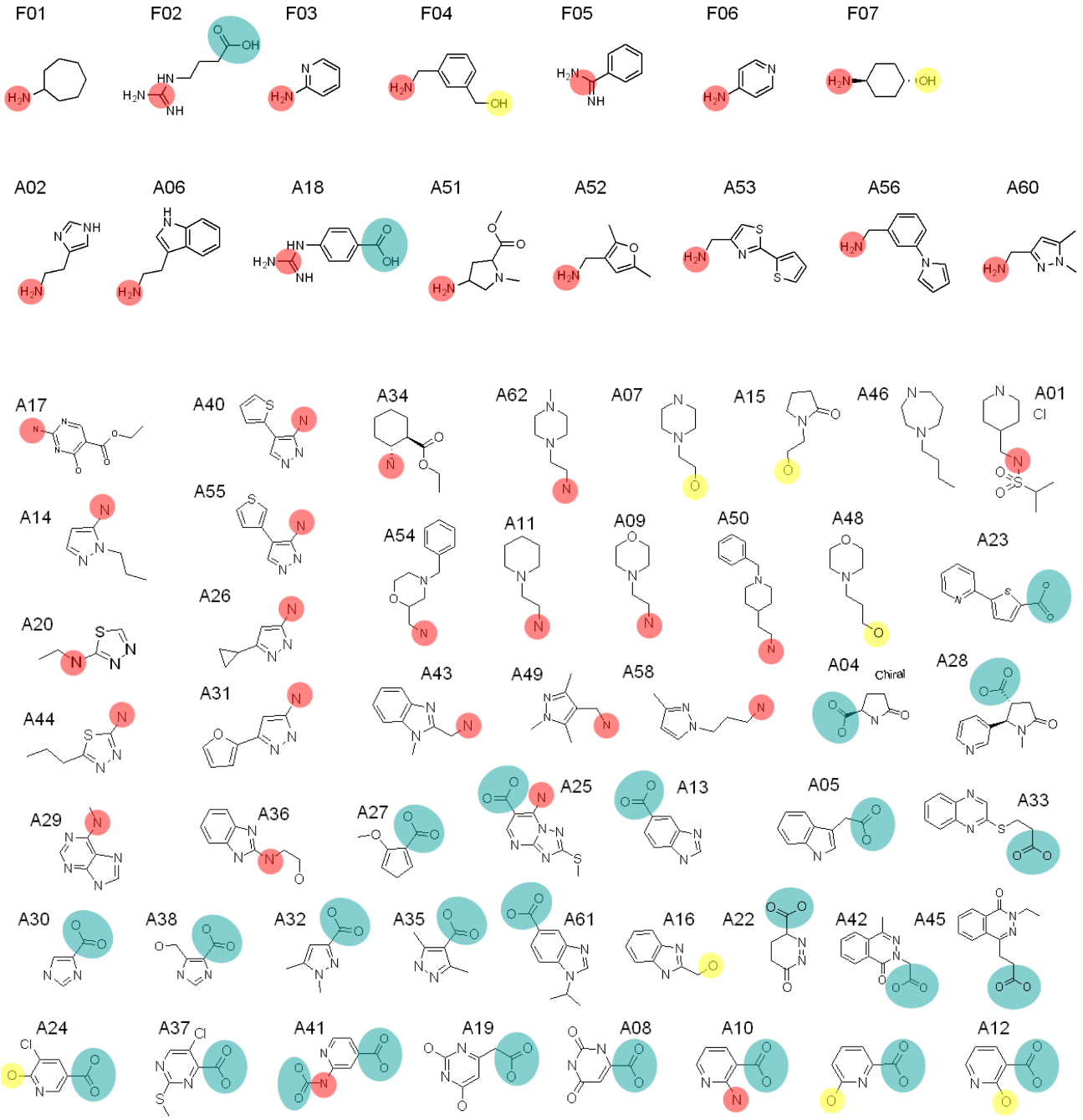

Figure S1 The focused fragment-compound library contained 62 molecules. All compounds were obtained from commercial sources.

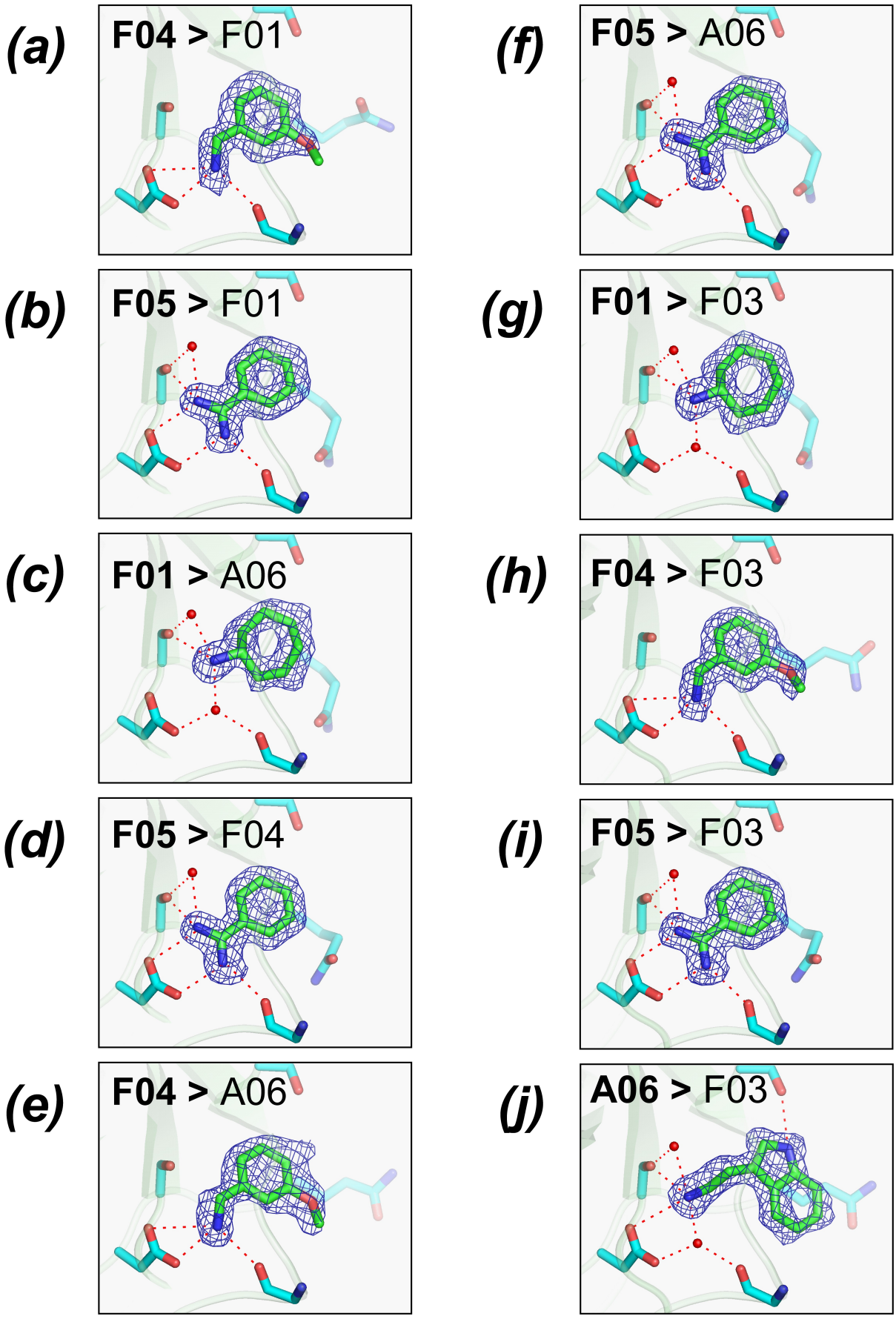

Figure S2 The relative order of binding affinities in the protein crystals (in-crystal affinity ranking) was assessed by crystallographic competition experiment. The sigma-weighted Fo-Fc maps contoured at 2.5 σ (blue mesh) superposed with each fragment-compound (green carbon sticks). Interacting side chains and waters molecules are shown as sticks and red spheres. The hydrogen bond is given by a red line.

Table S1 Data collection and refinement statistics for crystallographic competition experiments.

Fragment-compound	F01	F02	F03	F04	F05
Data collection					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	54.2, 58.4, 66.7	54.6, 58.4, 66.8	54.5, 58.3, 66.6	54.3, 58.2, 66.8	54.3, 58.5, 66.3
Resolution (Å)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)
R^b_{merge}	0.048 (0.196)	0.075 (0.147)	0.081 (0.197)	0.044 (0.176)	0.030 (0.107)
I / σ(I)	60.2 (16.1)	53.6 (20.6)	33.5 (10.2)	43.9 (11.3)	45.6 (16.5)
Completeness (%)	100 (100)	99.5 (98.6)	97.7 (96.0)	98.0 (95.0)	98.2 (95.5)
Redundancy	6.9 (6.8)	5.6 (5.5)	3.7 (3.7)	4.7 (4.7)	3.6 (3.6)
Refinement					
Resolution (Å)	20−1.70	20−1.70	20−1.70	20−1.70	20−1.70
No. reflections	22519	22636	22020	22072	22057
R^c_{work} / R^d_{free} (%)	15.2 / 17.0	15.1 / 17.1	16.6 / 18.4	15.8 / 17.7	15.1 / 18.1
No. atoms					
Protein	1629	1629	1629	1629	1629
Ligand/ion	45	23	24	37	28
Water	332	384	341	315	356
B-factors (Å^2)					
Protein	14.0	10.4	11.1	13.6	12.1
Ligand/ion	28.9	16.6	28.3	21.9	21.2
Water	29.2	25.3	26.2	27.9	28.1
R.m.s. deviations					
Bond lengths (Å)	0.006	0.005	0.006	0.005	0.005
Bond angles (°)	1.1	1.0	1.0	1.1	1.1
Ramachandran plot (%)					
Favored	95.9	96.4	96.4	96.4	96.4
Allowed	4.1	3.6	3.6	3.6	3.6
Generous	0	0	0	0	0

[a]Values in parentheses are for the outermost resolution shell. [b]$R_{merge} = \Sigma_h\Sigma_j | < I >_h - I_{h,j} | / \Sigma_h\Sigma_j I_{h,j}$, where $< I >_h$ is the mean intensity of symmetry-equivalent reflections. [c]$R_{work} = \Sigma | F_{obs} - F_{cal} | / \Sigma F_{obs}$, where F_{obs} and F_{cal} are observed and calculated structure factor amplitudes. [d]R_{free} value was calculated for R factor, using only an unrefined subset of reflections data.

Table S1 continued.

Fragment-compound	F06	F07	A02	A06	A18
Data collection					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	54.5, 58.4, 66.6	54.5, 58.2, 66.7	54.3, 58.3, 66.7	54.3, 58.3, 66.7	54.4, 58.4, 66.6
Resolution (Å)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.60 (1.66−1.60)	50−1.70 (1.76−1.70)
R^b_{merge}	0.050 (0.194)	0.031 (0.090)	0.044 (0.103)	0.046 (0.167)	0.054 (0.209)
$I / \sigma(I)$	36.4 (9.8)	31.7 (13.6)	70.7 (32.3)	38.8 (8.8)	21.5 (6.8)
Completeness (%)	99.3 (98.1)	94.1 (90.5)	96.5 (93.7)	92.7 (96.9)	96.8 (96.4)
Redundancy	4.7 (4.6)	3.7 (3.7)	7.2 (7.3)	3.8 (3.0)	5.0 (4.8)
Refinement					
Resolution (Å)	20−1.70	20−1.70	20−1.70	20−1.60	20−1.70
No. reflections	22395	21302	21904	24915	22291
R^c_{work} / R^d_{free} (%)	15.7 / 17.2	16.1 / 18.4	15.8 / 17.7	15.8 / 17.6	16.9 / 18.8
No. atoms					
Protein	1629	1629	1629	1629	1629
Ligand/ion	28	35	41	39	34
Water	366	325	318	335	294
B-factors (Å^2)					
Protein	11.0	12.4	11.9	12.1	16.7
Ligand/ion	23.5	22.3	24.7	20.9	33.9
Water	27.8	26.9	26.1	27.0	30.2
R.m.s. deviations					
Bond lengths (Å)	0.006	0.006	0.005	0.005	0.006
Bond angles (°)	1.1	1.1	1.0	1.0	1.1
Ramachandran plot (%)					
Favored	95.9	95.9	96.4	95.9	95.9
Allowed	4.1	4.1	3.6	4.1	4.1
Generous	0	0	0	0	0

[a]Values in parentheses are for the outermost resolution shell. [b]$R_{merge} = \Sigma_h\Sigma_j | < I >_h - I_{h,j} | / \Sigma_h\Sigma_j I_{h,j}$, where $< I >_h$ is the mean intensity of symmetry-equivalent reflections. [c]$R_{work} = \Sigma | F_{obs} - F_{cal} | / \Sigma F_{obs}$, where F_{obs} and F_{cal} are observed and calculated structure factor amplitudes. [d]R_{free} value was calculated for *R* factor, using only an unrefined subset of reflections data.

Table S1 continued.

Fragment-compound	A51	A52	A53	A56	A60
Data collection statistics					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	54.4, 58.4, 66.5	54.4, 58.2, 66.6	54.4, 58.2, 66.9	54.4, 58.4, 66.6	54.4, 58.3, 66.7
Resolution (Å)	50−1.60 (1.66−1.60)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.70 (1.76−1.70)	50−1.60 (1.66−1.60)
R^b_{merge}	0.034 (0.107)	0.038 (0.129)	0.034 (0.081)	0.029 (0.092)	0.035 (0.116)
I / σ(I)	62.3 (21.7)	53.9 (17.8)	53.0 (29.1)	51.4 (19.8)	54.5 (17.2)
Completeness (%)	98.3 (93.8)	98.4 (96.4)	98.4 (96.4)	99.2 (98.2)	97.4 (91.7)
Redundancy	5.6 (4.8)	5.7 (5.7)	5.7 (5.7)	3.9 (3.8)	4.7 (4.0)
Refinement					
Resolution (Å)	20−1.60	20−1.70	20−1.70	20−1.70	20−1.60
No. reflections	22519	22222	22517	22695	26382
R^c_{work} / R^d_{free} (%)	15.2 / 17.0	15.9 / 17.2	15.9 / 17.8	16.0 / 17.5	16.5 / 18.4
No. atoms					
Protein	1629	1629	1629	1629	1629
Ligand/ion	38	30	39	40	36
Water	330	324	324	315	320
B-factors (Å^2)					
Protein	12.0	11.8	10.5	12.0	11.6
Ligand/ion	22.0	22.1	19.1	20.5	21.7
Water	26.5	26.1	24.8	26.0	25.4
R.m.s. deviations					
Bond lengths (Å)	0.005	0.005	0.006	0.005	0.005
Bond angles (°)	1.1	1.2	1.4	1.2	1.1
Ramachandran plot (%)					
Favored	96.4	96.4	96.4	95.9	96.4
Allowed	3.6	3.6	3.6	4.1	3.6
Generous	0	0	0	0	0

[a]Values in parentheses are for the outermost resolution shell. [b]$R_{merge} = \Sigma_h\Sigma_j | < I >_h - I_{h,j} | / \Sigma_h\Sigma_j I_{h,j}$, where $< I >_h$ is the mean intensity of symmetry-equivalent reflections. [c]$R_{work} = \Sigma | F_{obs} - F_{cal} | / \Sigma F_{obs}$, where F_{obs} and F_{cal} are observed and calculated structure factor amplitudes. [d]R_{free} value was calculated for R factor, using only an unrefined subset of reflections data.

Table S2 Data collection and refinement statistics for crystallographic competition experiments.

Mixture Solution	F01 and F04	F01 and F05	F01 and A06	F01 and F03	F04 and F05
Data collection statistics					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	54.4, 58.0, 66.8	54.3, 58.2, 66.8	54.5, 58.1, 66.6	54.5, 58.0, 66.7	54.6, 58.1, 66.4
Resolution (Å)	50−1.71 (1.77−1.71)	50−1.68 (1.74−1.68)	50−1.74 (1.80−1.74)	50−1.72 (1.78−1.72)	50−1.74 (1.80−1.74)
R^b_{merge}	0.057 (0.194)	0.036 (0.086)	0.031 (0.110)	0.044 (0.153)	0.087 (0.210)
$I / \sigma(I)$	24.8 (6.2)	43.1 (14.7)	43.8 (12.5)	29.4 (8.6)	15.6 (6.3)
Completeness (%)	96.4 (90.3)	99.6 (96.9)	95.4 (91.1)	96.1 (90.2)	94.4 (90.7)
Redundancy	6.1 (5.2)	7.2 (4.9)	6.5 (6.3)	5.9 (5.4)	5.7 (5.5)
Refinement					
Resolution (Å)	20−1.71	20−1.68	20−1.74	20−1.72	20−1.74
No. reflections	21295	23382	20173	21030	19887
R^c_{work} / R^d_{free} (%)	19.3 / 22.2	15.8 / 18.2	16.1/ 18.4	15.8 / 17.8	15.9 / 18.8
No. atoms					
Protein	1629	1629	1629	1629	1629
Ligand/ion	27	30	21	29	22
Water	289	333	311	320	327
B-factors (Å^2)					
Protein	15.3	11.9	12.4	12.7	14.4
Ligand/ion	27.0	22.2	26.6	23.5	21.6
Water	29.5	28.2	28.4	28.3	30.0
R.m.s. deviations					
Bond lengths (Å)	0.007	0.006	0.006	0.006	0.006
Bond angles (°)	1.1	1.1	1.1	1.1	1.1
Ramachandran plot (%)					
Favored	96.4	96.8	96.8	97.7	96.8
Allowed	3.6	3.2	3.2	2.3	3.2
Generous		0	0	0	0

[a]Values in parentheses are for the outermost resolution shell. [b]$R_{merge} = \Sigma_h\Sigma_j | < I >_h - I_{h,j} | / \Sigma_h\Sigma_j I_{h,j}$, where $< I >_h$ is the mean intensity of symmetry-equivalent reflections. [c]$R_{work} = \Sigma | F_{obs} - F_{cal} | / \Sigma F_{obs}$, where F_{obs} and F_{cal} are observed and calculated structure factor amplitudes. [d]R_{free} value was calculated for *R* factor, using only an unrefined subset of reflections data.

Table S2 continued.

Mixture Solution	F04 and A06	F04 and F03	F05 and A06	F05 and F03	A06 and F03
Data collection statistics					
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	54.4, 58.0, 66.7	54.5, 57.9, 66.6	54.4, 58.1, 66.7	54.5, 58.1, 66.5	54.3, 58.1, 66.7
Resolution (Å)	50−1.74 (1.80−1.74)	50−1.70 (1.76−1.70)	50−1.68 (1.74−1.68)	50−1.70 (1.76−1.70)	50−1.72 (1.78−1.72)
R^b_{merge}	0.068 (0.254)	0.060 (0.157)	0.045 (0.135)	0.048 (0.149)	0.035 (0.077)
I / σ(I)	23.2 (6.6)	21.4 (7.2)	33.9 (9.3)	30.4 (8.4)	40.6 (18.5)
Completeness (%)	95.3 (91.2)	98.7 (94.7)	99.5 (95.0)	99.6 (97.8)	96.4 (90.8)
Redundancy	7.3 (6.9)	6.0 (4.7)	7.5 (5.1)	6.5 (5.0)	6.4 (6.0)
Refinement					
Resolution (Å)	20−1.74	20−1.70	20−1.68	20−1.70	20−1.72
No. reflections	20078	22221	23299	22531	21090
R^c_{work} / R^d_{free} (%)	16.1 / 17.9	17.9 / 20.2	15.7 / 18.5	16.2 / 18.7	15.4 / 16.7
No. atoms					
Protein	1629	1629	1629	1629	1629
Ligand/ion	27	27	26	22	25
Water	279	295	313	366	342
B-factors (Å^2)					
Protein	15.9	14.1	12.7	13.0	11.1
Ligand/ion	25.7	27.6	21.3	19.5	20.1
Water	30.2	28.9	29.0	29.3	28.1
R.m.s. deviations					
Bond lengths (Å)	0.006	0.006	0.006	0.006	0.006
Bond angles (°)	1.1	1.1	1.1	1.0	1.1
Ramachandran plot (%)					
Favored	96.8	97.3	96.8	96.4	97.3
Allowed	3.2	2.7	3.2	3.6	2.7
Generous	0	0	0	0	0

[a]Values in parentheses are for the outermost resolution shell. [b]$R_{merge} = \Sigma_h \Sigma_j | < I >_h - I_{h,j} | / \Sigma_h \Sigma_j I_{h,j}$, where $< I >_h$ is the mean intensity of symmetry-equivalent reflections. [c]$R_{work} = \Sigma | F_{obs} - F_{cal} | / \Sigma F_{obs}$, where F_{obs} and F_{cal} are observed and calculated structure factor amplitudes. [d]R_{free} value was calculated for R factor, using only an unrefined subset of reflections data.

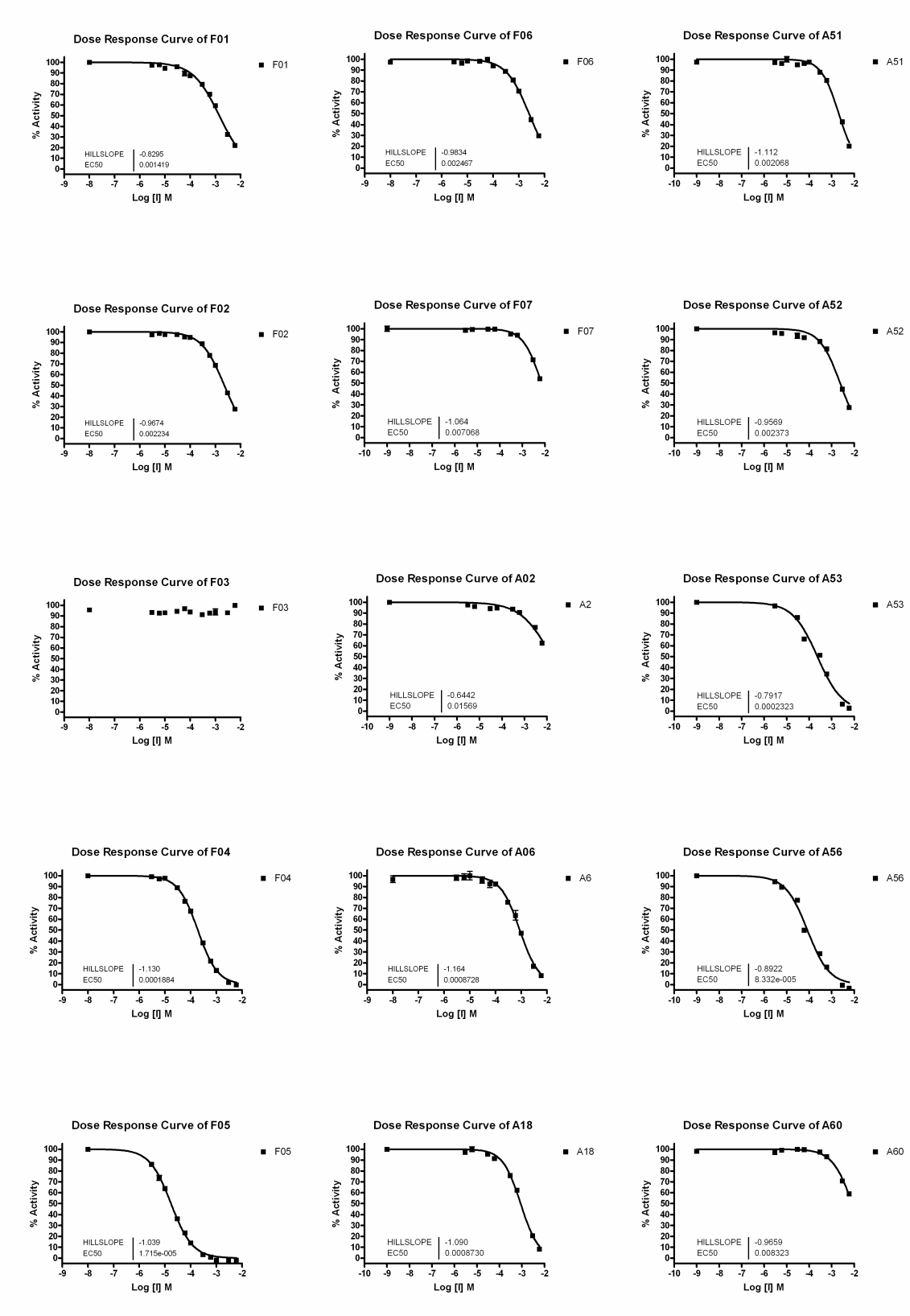

Figure S3. Dose response curves of 15 hit-compounds.