Insight into the structural requirements of thiophene-3-carbonitriles based MurF inhibitors by 3D QSAR, molecular docking and molecular dynamics study

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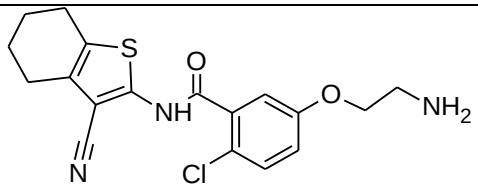
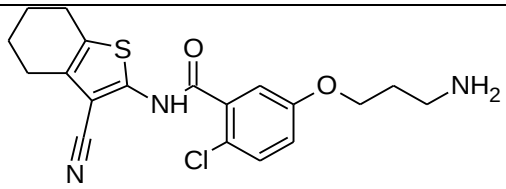
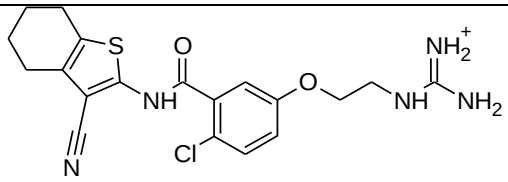
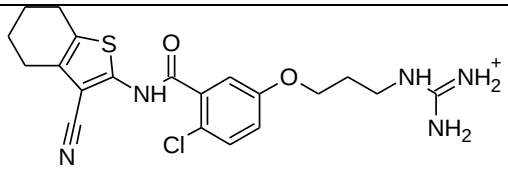
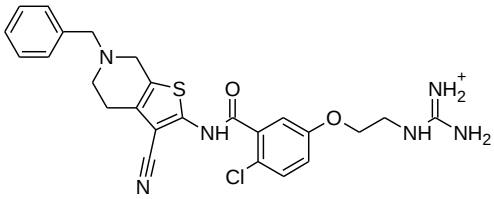
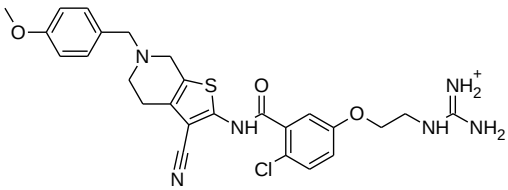
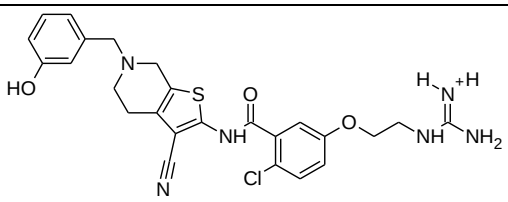
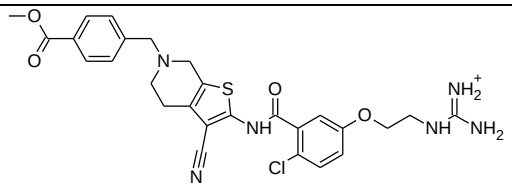
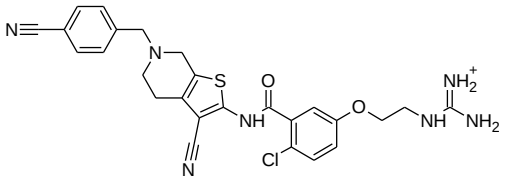
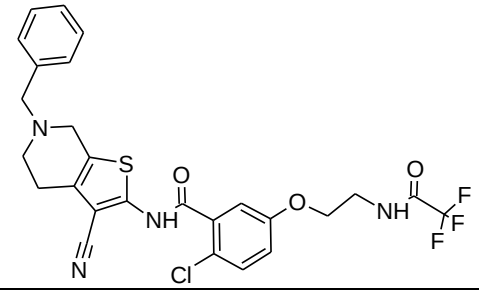
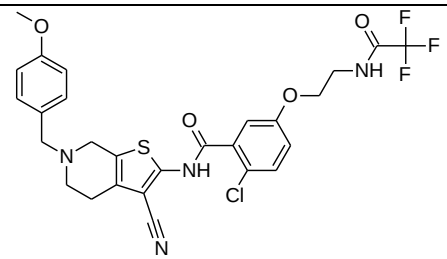
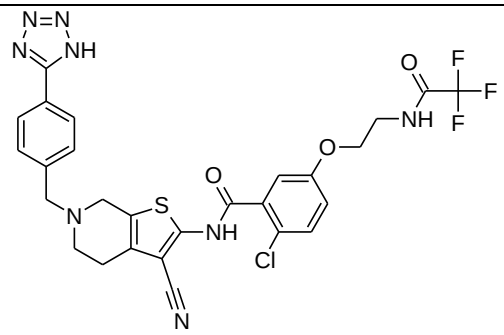
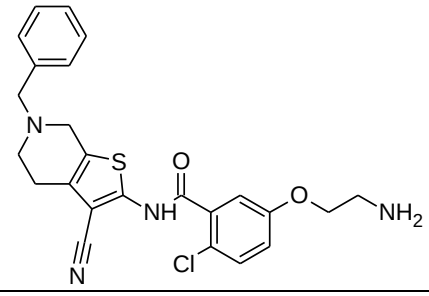
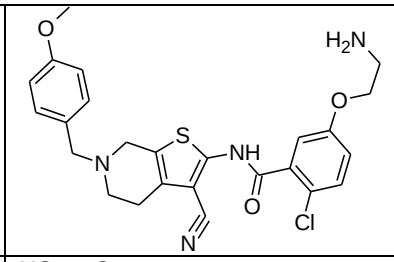
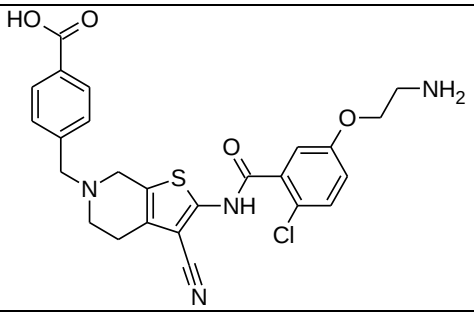
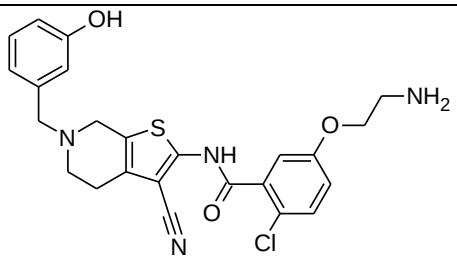
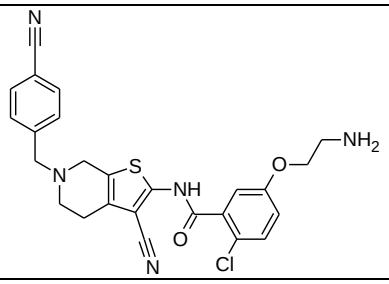
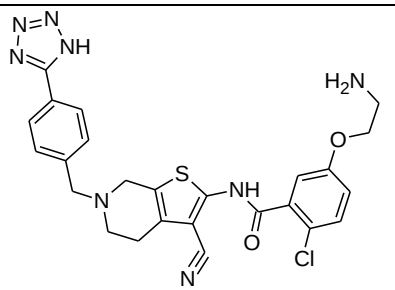
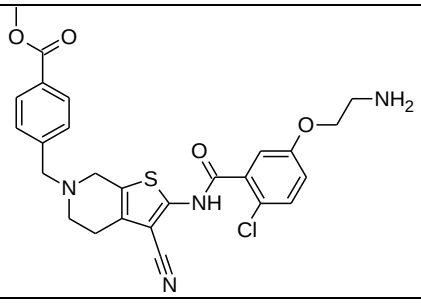
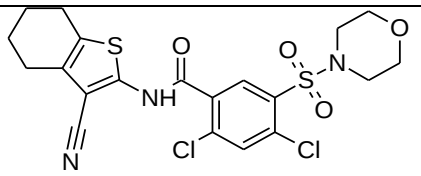
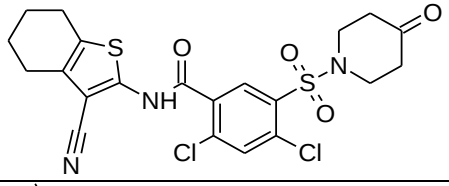
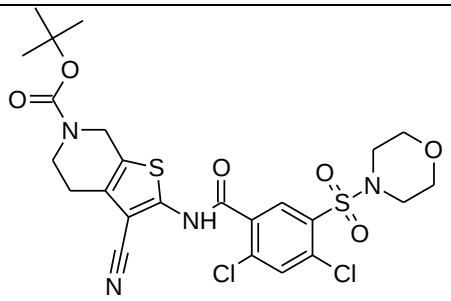
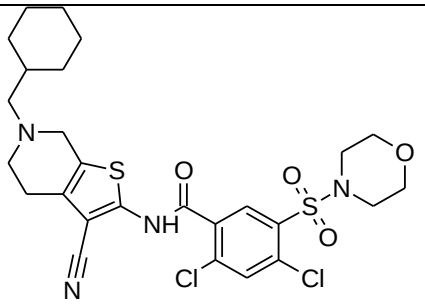
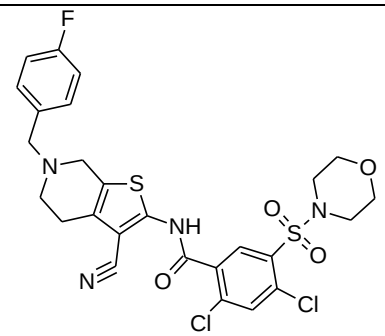
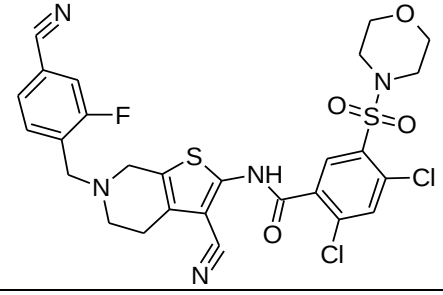
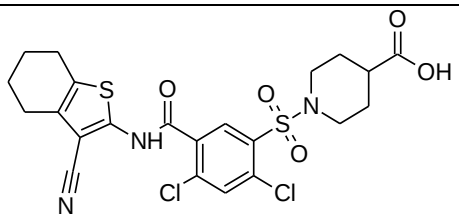
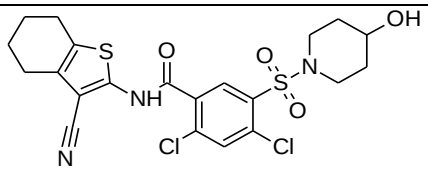
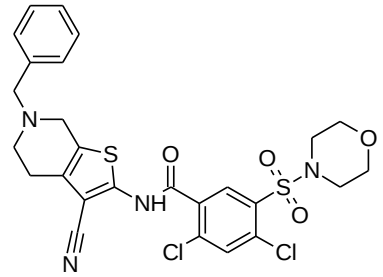
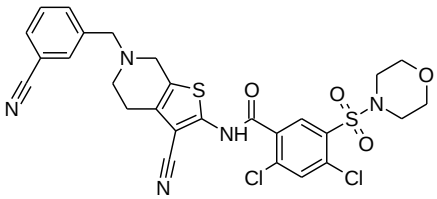
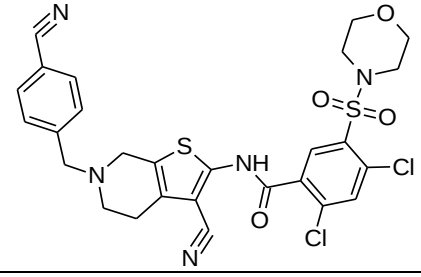
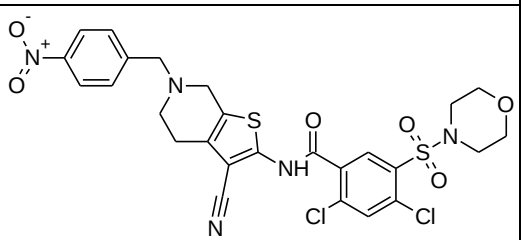
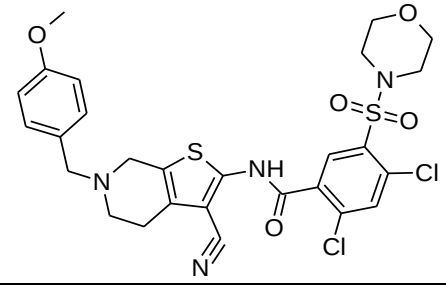
Table S1. Chemical structures of the data set compounds **1-48** with corresponding pIC_{50} values.

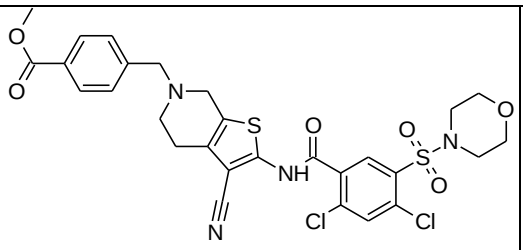
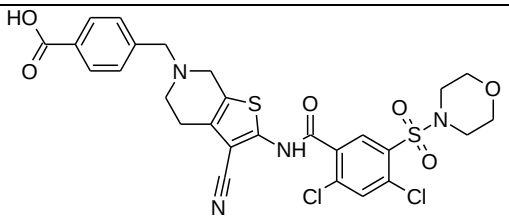
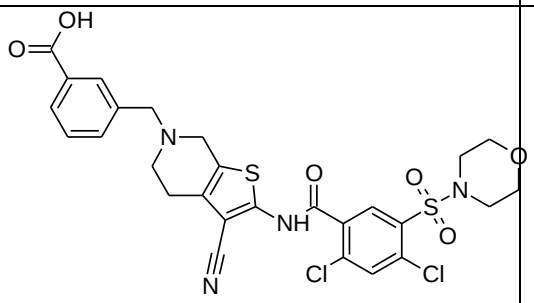
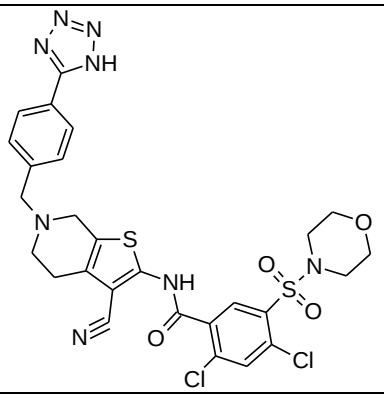
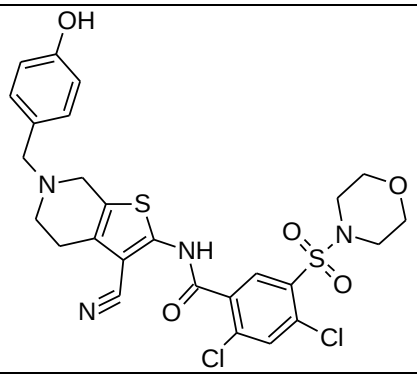
Compd.	Structure	IC_{50} (μM)	Exp. Activity (pIC_{50})	Predicted activity (pIC_{50})	Residual activity	Fitness
1[t]		170	3.769	3.93	-0.161	1.14
2		128	3.892	3.89	0.002	1.19
3		75	4.124	4.360	-0.236	1.55
4		78	4.107	4.378	-0.271	1.29
5		55	4.259	4.304	-0.045	1.01
6		61	4.214	4.331	-0.117	1.09
7		117	3.931	4.319	-0.388	0.95

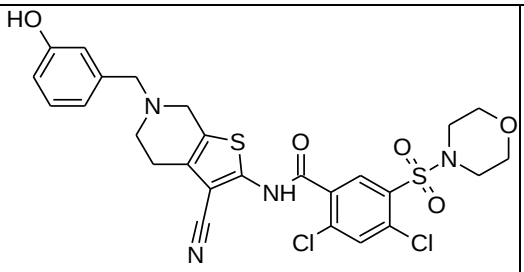
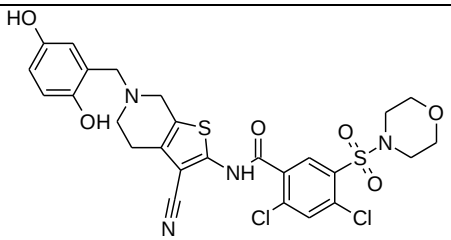
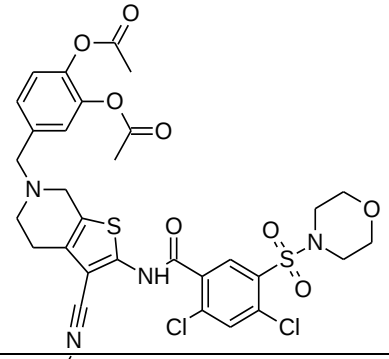
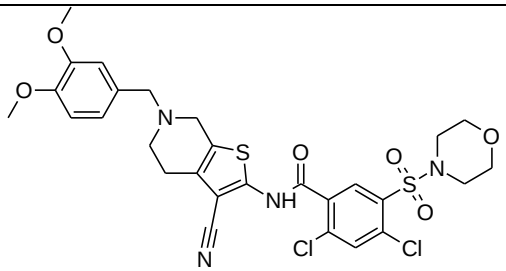
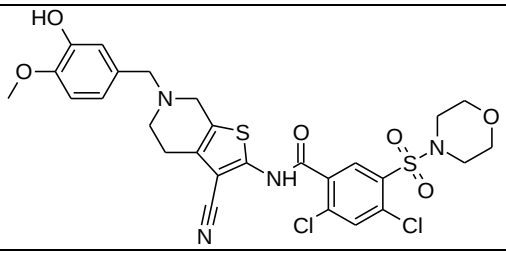
8		37	4.431	4.39	0.041	1.32
9		61	4.214	3.89	0.324	1.55
10		172	3.764	3.81	-0.046	1.16
11		154	3.812	3.86	-0.048	0.97
12		103	3.987	3.95	0.037	0.76
13		51	4.292	4.27	0.022	1.04

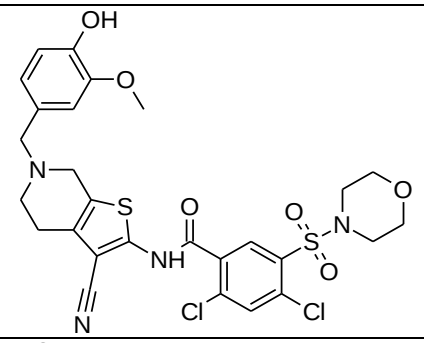
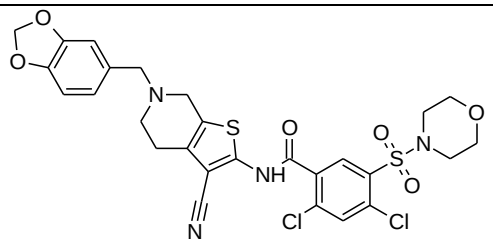
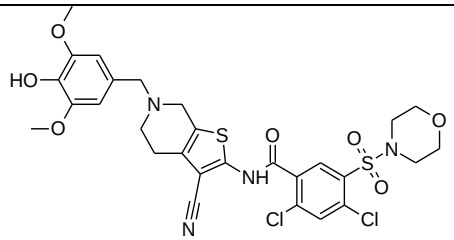
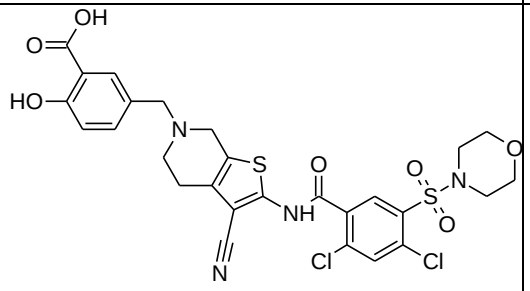
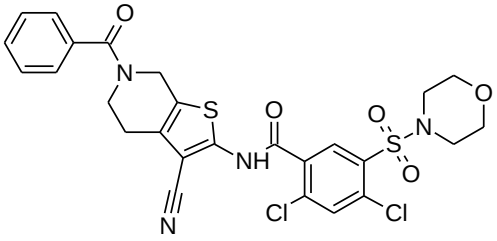
14		125	3.903	3.83	0.073	1.08
15		663	3.178	3.24	-0.062	1.04
16		119	3.924	4.03	-0.106	0.92
17		107	3.970	3.99	-0.02	1.03
18		454	3.342	3.39	-0.048	0.99
19		109	3.962	3.86	0.102	1.03

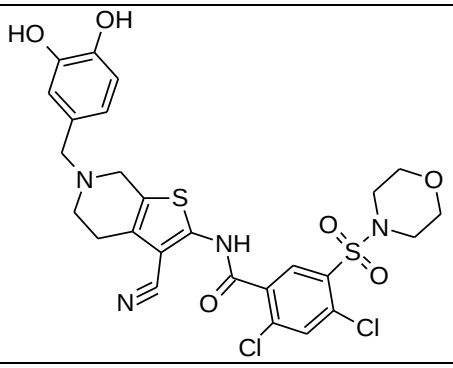
20[t]		4.0	5.397	5.25	0.14	2.43
21		3.37	5.472	5.50	-0.028	1.82
22		19.0	4.721	4.53	0.191	2.43
23[t]		0.46	6.337	6.12	0.21	2.9
24		0.98	6.008	6.10	-0.092	2.58
25		1.92	5.716	5.85	-0.134	2.45

26		17.9	4.747	4.68	0.067	0.98
27		10.45	4.980	4.80	0.18	2.04
28		0.64	6.193	5.98	0.213	2.7
29[t]		1.17	5.931	5.63	0.301	2.94
30[t]		1.36	5.866	6.14	-0.274	2.97
31[t]		1.09	5.962	5.99	-0.028	2.96
32		0.60	6.221	6.29	-0.069	2.5

33		0.72	6.142	6.26	-0.118	2.94
34		0.86	6.065	6.26	-0.195	2.96
35[t]		2.55	5.593	5.88	-0.287	2.96
36		0.30	6.522	6.50	0.022	2.91
37		0.18	6.744	6.78	-0.036	2.7

38		0.42	6.376	6.06	0.316	2.99
39[t]		2.75	5.560	6.01	-0.45	2.61
40[t]		3.03	5.518	5.47	0.048	2.6
41		10.40	4.982	5.47	-0.488	2.65
42[t]		0.60	6.221	6.08	0.141	2.99

43[t]		4.15	5.381	5.35	0.031	2.99
44[t]		0.99	6.004	5.92	0.084	2.98
45		48.0	4.318	4.45	-0.132	2.96
46[t]		1.41	5.850	5.87	-0.02	2.97
47		27.0	4.568	4.53	0.038	2.49

48		0.43	6.366	6.02	0.346	3

[t]Test set

Table S2. Distances between different sites of model AAAHR.1049.

Site1	Site2	Distance Å
A1	A4	6.749
A1	A5	6.404
A1	H13	6.473
A1	R17	3.326
A4	A5	5.105
A4	H13	2.926
A4	R17	4.000
A5	H13	4.954
A5	R17	4.690
H13	R17	3.148

Table S3. Angles between different sites of model AAAHR.1049.

Site1	Site2	Site3	Angle (A^o)
A4	A1	A5	45.6
A4	A1	H13	25.5
A4	A1	R17	25.2
A5	A1	H13	45.3
A5	A1	R17	45.1
H13	A1	R17	0.3
A1	A4	A5	63.6
A1	A4	H13	72.0
A1	A4	R17	20.7
A5	A4	H13	70.3
A5	A4	R17	60.6
H13	A4	R17	51.2
A1	A5	A4	70.8
A1	A5	H13	68.1
A1	A5	R17	30.1
A4	A5	H13	33.8
A4	A5	R17	48.0
H13	A5	R17	38.0
A1	H13	A4	82.5
A1	H13	A5	66.6
A1	H13	R17	0.3
A4	H13	A5	75.9

A4	H13	R17	82.3
A5	H13	R17	66.4
A1	R17	A4	134.0
A1	R17	A5	104.8
A1	R17	H13	179.4
A4	R17	A5	71.5
A4	R17	H13	46.5
A5	R17	H13	75.6

Table S4. Count and percentage of actives in top N% of decoy results.

%Decoys	1%	2%	5%	10%	20%
#Actives	12	12	12	12	12
%Actives	100.0	100.0	100.0	100.0	100.0

Table S5. Count and percentage of actives in top N% of results.

%Results	1%	2%	5%	10%	20%
#Actives	1	2	6	12	12
%Actives	8.3	16.7	50.0	100.0	100.0

Table S6. Enrichment Factors with respect to N% sample size.

% Sample	1%	2%	5%	10%	20%
EF	10	10	10	10	5
EF*	1.1e+02	54	22	9.8	4.9
EF’	1.1e+02	72	36	18	9.4
DEF	n/a	5.5	8.4	10	5
DEF*	1.1e+02	54	22	9.8	4.9
DEF’	1.1e+02	72	36	18	9.4
EFF	0.98	0.961	0.905	0.818	0.667

EF: Enrichment factor (The number of ranked results for which to calculate the enrichment factor); EF*: Enrichment factor (The number of ranked decoys for which to calculate the enrichment factor); EF’: Enrichment factor Prime; DEF: Diverse Enrichment factor; DEF’: Diverse Enrichment factor Prime; EFF: Active recovery Efficiency

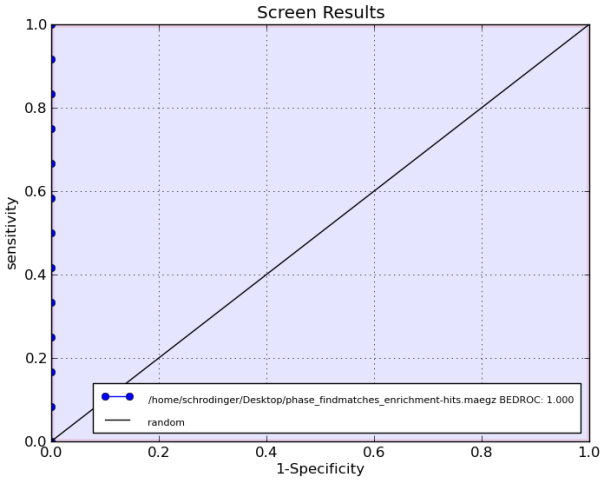
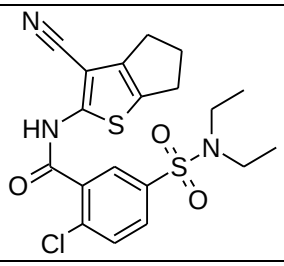
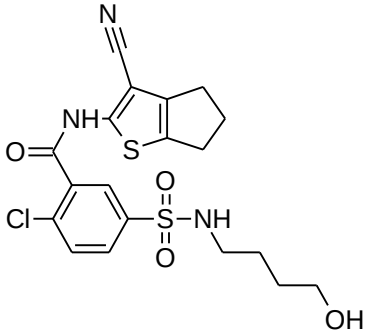
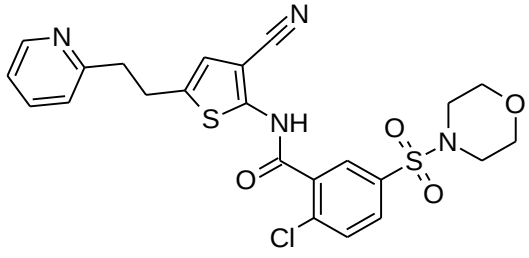
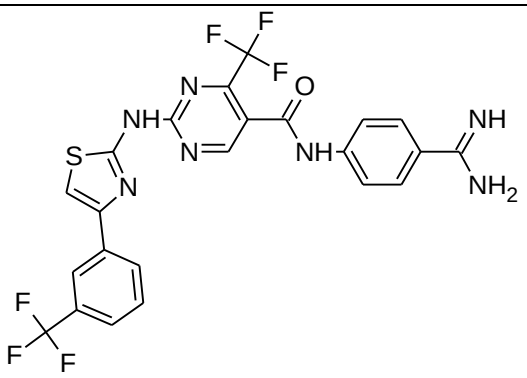
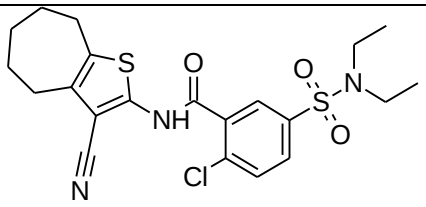
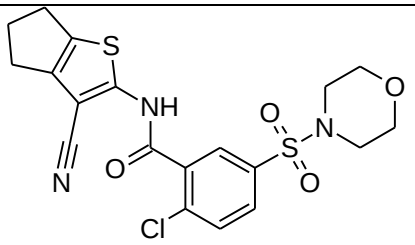
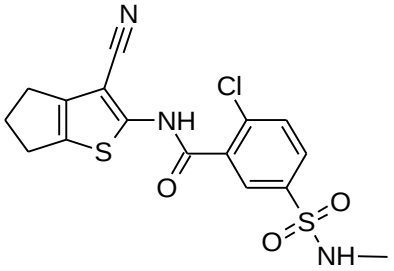
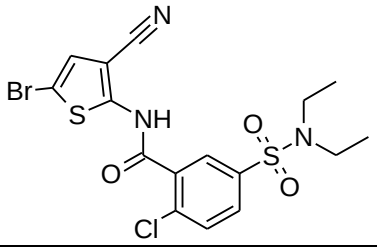
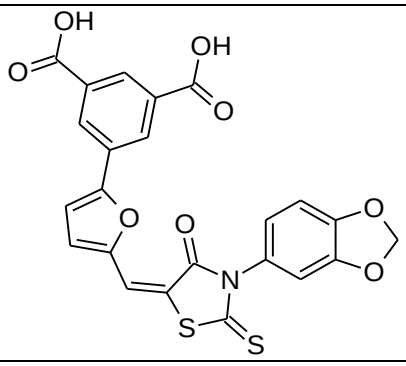
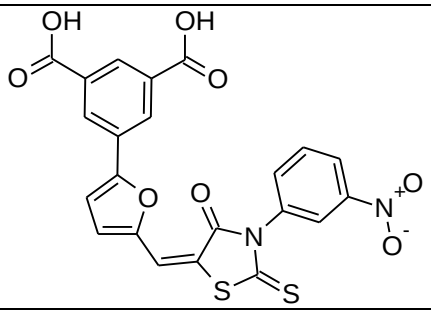
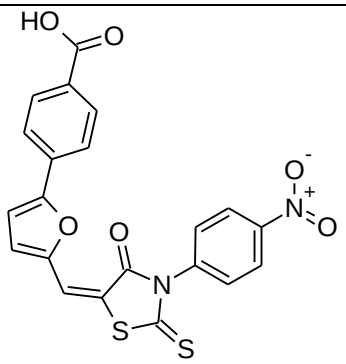
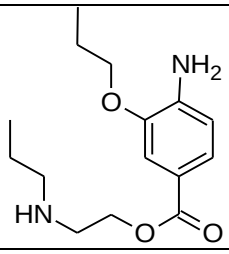
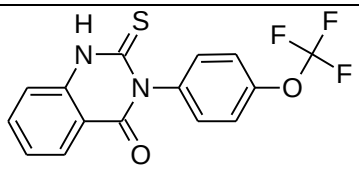


Figure S1. ROC curve obtained by AAAHR.1049 model against randomly curve.

Table S7. Calculated pIC_{50} for compounds in the external test set.

S. No	Structure of compound	Exp. (pIC_{50})	Predicted activity	Residual activity
1		5.09	4.808	0.282
2		4.65	4.244	0.406
3		5.221	4.883	0.338

4		5.602	5.055	0.547
5		5.468	4.794	0.674
6		5.045	4.748	0.297
7		4.823	4.626	0.197
8		4.899	4.304	0.595

9		4.92	4.453	0.467
10		4.721	4.489	0.232
11		4.92	4.556	0.364
12		4.522	4.425	0.097
13		4.721	4.658	0.063

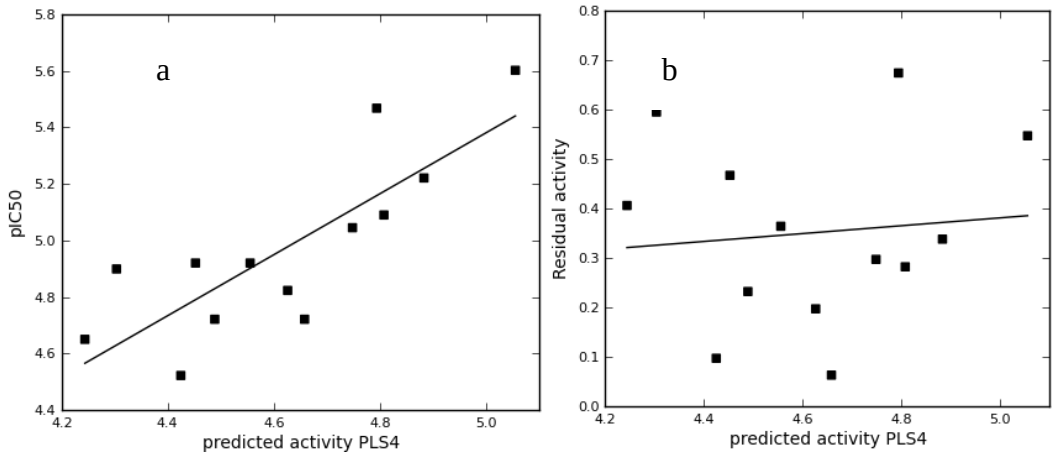


Figure S2. Plot of (a) Experimental pIC_{50} *vs* predicted activity of external training set y = 0.61x + 1.59 (R^2 = 0.70) (b) Plot of residual activity *vs* predicted activity of external training set.

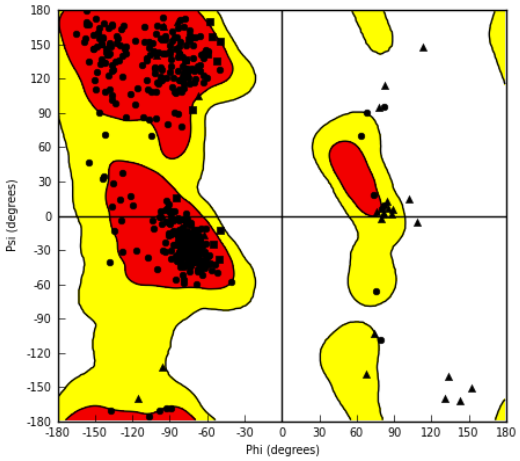


Figure S3. Ramachandran plot of *Streptococcus pneumoniae* MurF protein (PDB ID: 3ZM6).

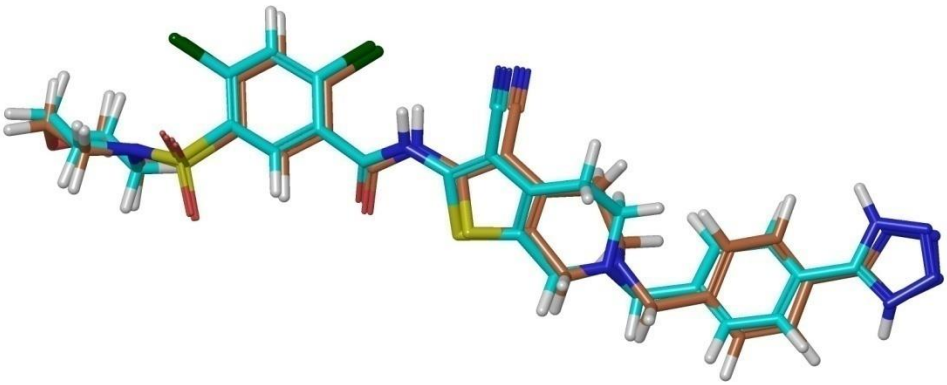


Figure S4. Overlay of co-crystallized ligand (blue) of 3ZM6 with its XP-docked pose (orange) [RMSD: 1.21Å]

Table S8. Molecular docking results (kcal/mol) of phase data set ligands **1-48** showing interacting amino acids

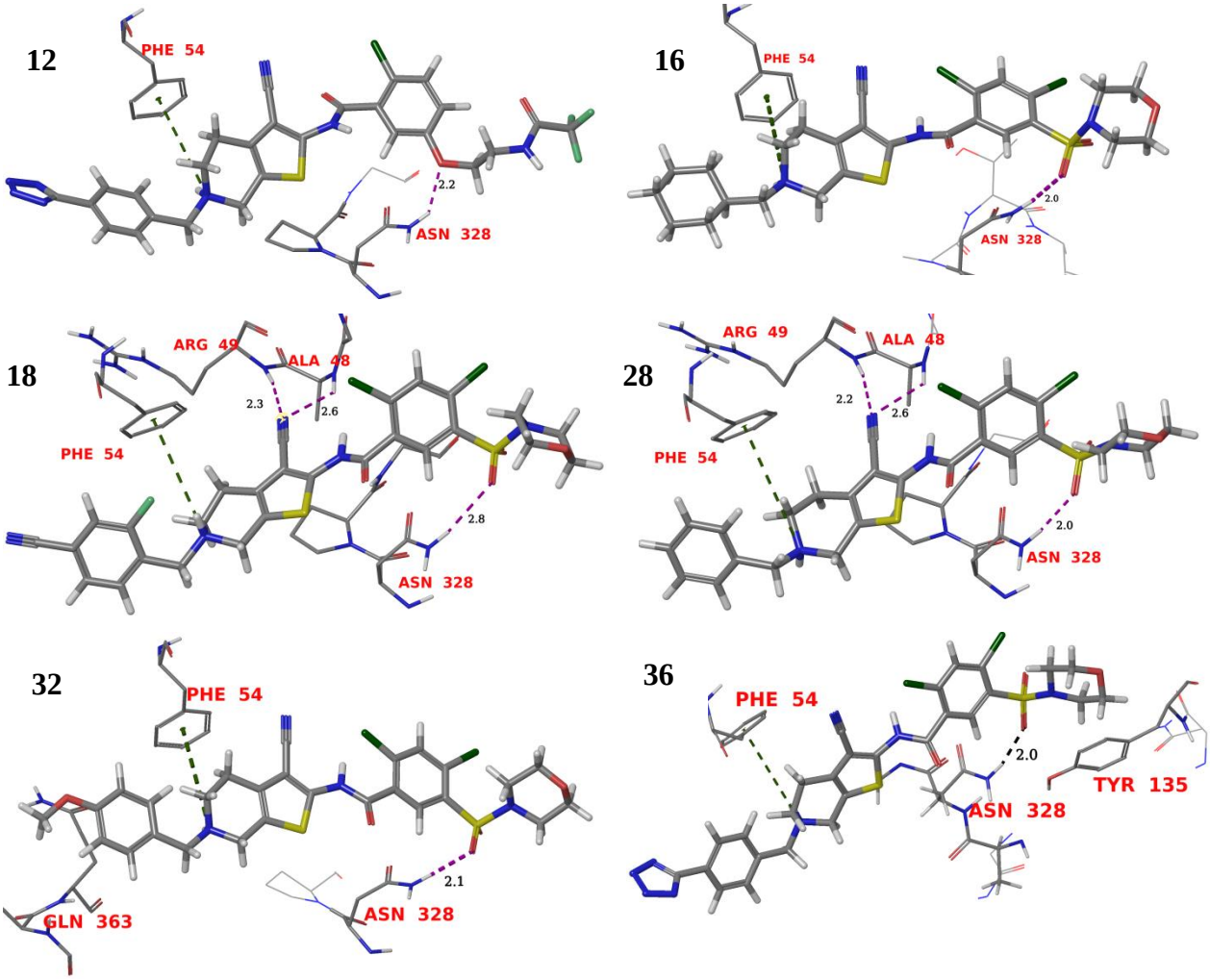
S. No	Glide gscore	Glide emodel	Glide energy	XP lipophilic Evdw	XP RotPenal	Amino acids showing H-bond interactions
1	-8.63	-70.42	-49.97	-6.18	0.29	Ala48, Arg49, Tyr135, Asn137
2	-8.32	-74.20	-52.97	-6.73	0.32	Ala48, Arg49, Tyur135
3	-8.48	-86.84	-58.07	-6.68	0.38	Ala48, Arg49, Asn134,Asn137
4	-8.47	-83.96	-55.81	-6.28	0.41	Asp32, Ser33, Asn328
5	-10.12	-109.86	-65.29	-8.40	0.33	Ala48, Arg49, Asn134,Asn137
6	-8.72	-99.67	-60.34	-7.88	0.30	Asn134, Asn137, Asn328
7	-8.27	-114.34	-66.96	-8.47	0.31	Asn134, Asn137
8	-8.91	-104.26	-63.62	-7.29	0.26	Leu360, Asn326
9	-5.18	-104.55	-68.18	-8.30	0.30	Asn134, Asn137
10	-9.79	-118.65	-67.68	-8.21	0.24	Ala48, Arg49, Phe54, Asn137
11	-9.76	-114.90	-71.69	-8.06	0.20	Ala48, Arg49, Phe54, Asn326
12	-8.81	-119.83	-62.80	-8.26	0.19	Phe54, Asn328
13	-9.10	-93.11	-59.90	-7.48	0.27	Phe31, Asn326, Asp323, Ala331
14	-9.03	-93.07	-58.37	-7.64	0.24	Ala48, Arg49, Phe54, Tyr135, Asn137
15	-8.82	-92.63	-58.54	-7.71	0.23	Phe54, Asn328
16	-9.48	-93.17	-60.17	-7.90	0.26	Asn134, Thr330, Leu360
17	-9.42	-96.57	-60.52	-8.02	0.25	Asp32, Ser33, Thr330
18	-9.13	-99.78	-62.10	-7.87	0.24	Ala48, Arg49, Phe54, Asn328
19	-9.13	-96.99	-62.67	-7.86	0.25	Ala48, Arg49, Phe54, Tyr135
20	-10.07	-105.31	-70.46	-7.57	0.17	Ala48, Arg49, Asn328
21	-9.60	-98.77	-64.89	-7.22	0.16	Ala48, Arg49, Asn328
22	-10.93	-119.35	-82.88	-8.20	0.12	Ala48, Arg49, Asn328
23	-8.80	-116.21	-66.12	-8.66	0.12	Phe54, Asn328
24	-6.36	-119.01	-73.14	-8.83	0.11	Phe328, Asn328
25	-10.03	-130.13	-76.89	-8.80	0.09	Ala48, Arg49, Phe54, Asn328
26	-8.65	-92.07	-63.68	-6.99	0.15	Arg49, Arg49, Asn328
27	-9.54	-96.91	-67.72	-7.60	0.16	Arg49, Asn137, Asn328
28	-8.80	-119.46	-76.94	-8.90	0.13	Ala48, Arg49, Phe54, Asn328
29	-9.75	-130.53	-79.60	-8.70	0.11	Ala48, Arg49, Phe54, Asn328
30	-8.87	-121.90	-71.28	-8.66	0.11	Phe54, Asn328
31	-6.23	-124.15	-75.97	-8.83	0.09	Phe54, Asn328
32	-9.27	-122.17	-72.26	-8.91	0.10	Phe54, Asn328
33	-9.39	-132.40	-74.18	-8.40	0.10	Phe54, Asn328
34	-9.47	-117.54	-71.13	-8.31	0.09	Phe54, Asn328
35	-10.59	-131.47	-78.27	-8.60	0.09	Ala48, Arg49, Phe54, Asn328
36	-10.23	-127.33	-69.86	-8.77	0.10	Phe54, Asn328
37	-10.98	-123.71	-76.41	-8.89	0.11	Ala48, Arg49, Phe54, Thr57, Asn61, Asn137, Asn328, Thr330
38	-6.44	-120.29	-73.88	-8.86	0.11	Phe54, Asn328
39	-9.40	-120.45	-76.84	-8.80	0.10	Arg49, Phe54, Asn328
40	-8.75	-143.15	-84.14	-8.71	0.08	Ala48,Arg49,Phe54, Asn328
41	-8.94	-127.97	-72.29	-8.56	0.08	Phe54, Asn326
42	-10.14	-125.05	-77.32	-8.74	0.09	Ala48, Arg49, Phe54, Thr57

43	-10.13	-131.18	-78.34	-8.77	0.09	Ala48,Arg49,Phe54, Asn328
44	-10.47	-132.44	-78.77	-8.74	0.09	Ala48,Arg49,Phe54, Asn328
45	-9.39	-122.40	-74.18	-8.79	0.07	Phe54, Asn328
46	-9.87	-127.83	-78.78	-8.69	0.08	Ala48,Arg49, Phe54, Asn328, Asp362, Asp363
47	-10.20	-124.16	-82.74	-8.40	0.12	Ala48, Arg49, Asn328
48	-11.16	-123.93	-76.66	-8.81	0.10	Ala48,Arg49,Phe54, Asn328

Table S9. Binding free energy (MM/GB-SA) calculation (kcal/mol) of data set ligands **1-48**.

S. No	ΔGBind	ΔGbind Lipo	ΔGbind Solv GB	ΔGbind Covalent	ΔGBind vdW	ΔBind Coulumb	ΔGbind H-bond
1	-80.97	-28.59	74.00	4.14	-42.75	-67.44	-1.606
2	-85.74	-29.73	77.23	0.79	-72.59	-69.20	-1.44
3	-65.10	-39.87	45.91	-10.74	-47.11	-22.54	9.04
4	-102.13	-61.55	119.43	2.27	-45.55	-120.10	-2.92
5	-105.87	-61.42	81.57	-3.67	-65.61	-55.87	0.35
6	-103.18	-74.82	133.20	15.12	-67.03	-136.00	-2.43
7	-101.82	-80.26	120.35	7.48	-71.78	-117.29	-1.69
8	-101.56	-65.27	120.51	8.93	-78.89	-90.77	0.92
9	-103.14	-75.18	84.24	12.96	-67.63	-75.33	2.48
10	-91.97	-35.12	89.61	2.90	-66.85	-77.70	-1.55
11	-98.02	-35.11	91.76	6.99	-96.53	-83.72	-1.78
12	-74.07	-33.58	-14.26	12.01	-76.38	38.88	-1.61
13	-91.55	-34.34	143.60	10.34	-69.67	-135.37	-2.79
14	-99.19	-38.31	142.17	5.14	-55.04	-136.36	-1.55
15	-88.80	-35.59	2.82	5.00	-71.29	11.91	-1.60
16	-82.70	-31.98	64.13	4.04	-56.24	-41.51	-1.81
17	-98.45	-38.09	139.56	6.51	-67.00	-132.81	-1.60
18	-89.05	-36.67	49.54	7.40	-67.29	-31.38	-1.74
19	-100.0	-38.15	143.81	6.17	-53.80	-136.16	-1.60
20	-92.02	-30.25	10.67	6.06	-68.65	-4.09	-1.89
21	-89.51	-31.11	12.94	3.87	-81.48	-6.47	-2.05
22	-99.03	-31.88	16.61	9.95	-68.17	-7.44	-1.89
23	-97.52	-37.71	73.05	6.74	-66.45	-64.12	-0.56
24	-103.54	-38.29	74.41	7.77	-75.78	-63.97	-0.59
25	-110.95	-39.65	75.46	5.39	-76.48	-70.07	-1.63
26	-68.99	-28.01	-52.96	6.74	-82.93	181.22	-1.99
27	-88.32	-31.81	15.49	6.42	-55.58	-10.58	-2.38
28	-104.05	-40.38	76.72	4.29	-68.01	-69.58	-1.84
29	-99.34	-40.50	75.18	3.30	-71.46	-70.35	-2.33
30	-98.08	-39.57	70.71	8.30	-83.59	-60.67	-0.68
31	-102.21	-38.48	73.24	11.67	-83.48	-62.55	-0.65
32	-104.03	-38.24	78.98	7.76	-69.26	-67.26	-0.60
33	-107.88	-41.56	76.32	4.30	-70.92	-69.64	-1.90
34	-108.52	-38.23	-62.66	4.48	-67.02	78.42	-2.01
35	-103.88	-38.09	-60.68	4.80	-73.50	76.40	-2.49
36	-103.08	-38.47	-26.52	8.78	-78.86	52.08	-0.83
37	-108.81	-39.54	76.85	7.31	-75.86	-63.34	-2.17
38	-84.84	-33.01	-1.91	7.86	-71.05	26.73	-0.62

39	-89.86	-35.26	0.06	6.64	-85.83	17.01	-2.29
40	-103.44	-40.45	84.85	5.08	-84.38	-76.21	-2.38
41	-98.12	-38.71	78.613	7.58	-78.42	-68.40	-0.79
42	-106.40	-38.35	77.385	10.55	-78.26	-74.48	-2.14
43	-101.07	-41.71	78.877	3.85	-62.57	-73.10	-2.37
44	-112.37	-41.01	77.577	3.21	-74.73	-71.69	-1.97
45	-95.27	-41.40	80.207	13.41	-75.50	-62.37	-0.60
46	-103.20	-36.16	-59.24	5.20	-79.29	73.27	-2.82
47	-104.52	-35.97	17.859	9.63	-92.23	-10.07	-1.86
48	-94.80	-36.54	-1.666	3.42	-76.96	20.99	-2.31



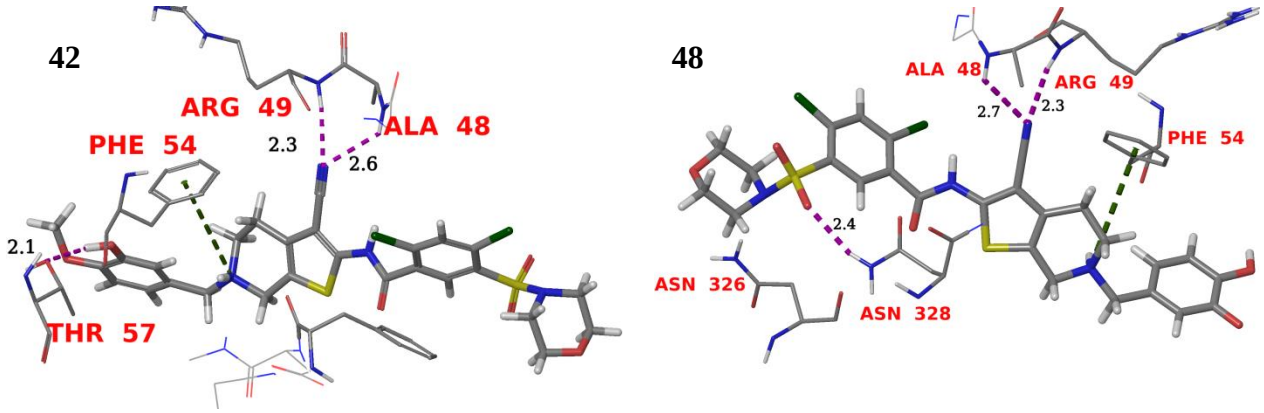


Figure S5. Binding mode of selected inhibitors in the catalytic pocket of MurF enzyme (PDB ID: 3ZM6).

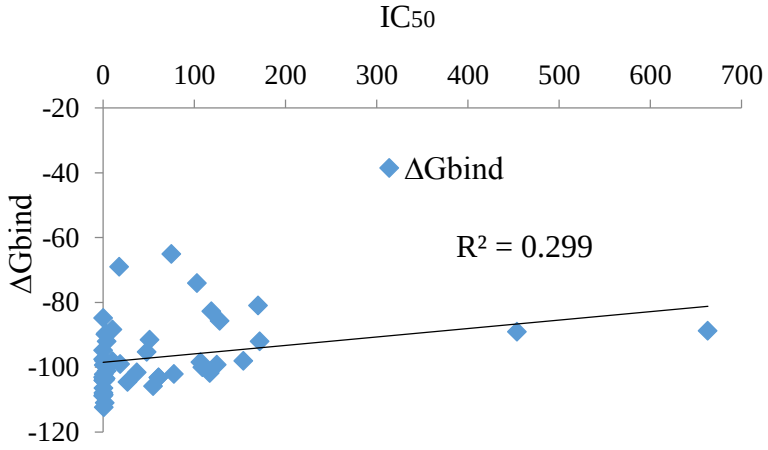


Figure S6. Plot represents correlation between experimental IC_{50} values and prime ΔGbind.

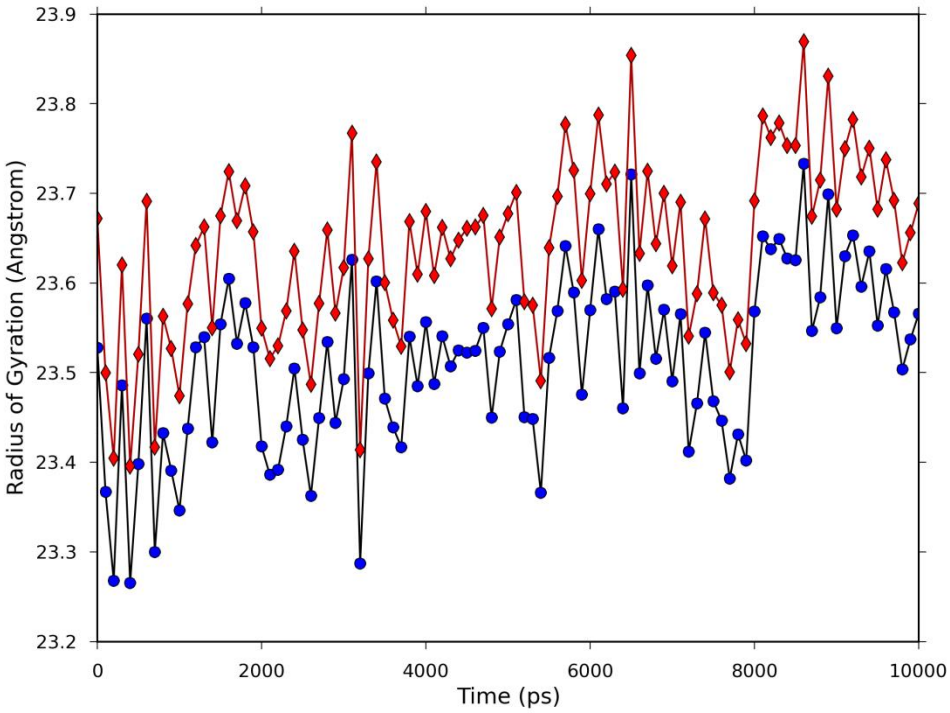


Figure S7. Radius of gyration of backbone (blue circle) and Cα atoms (red thin diamond) of **37**/3ZM6 complex during MD simulation.

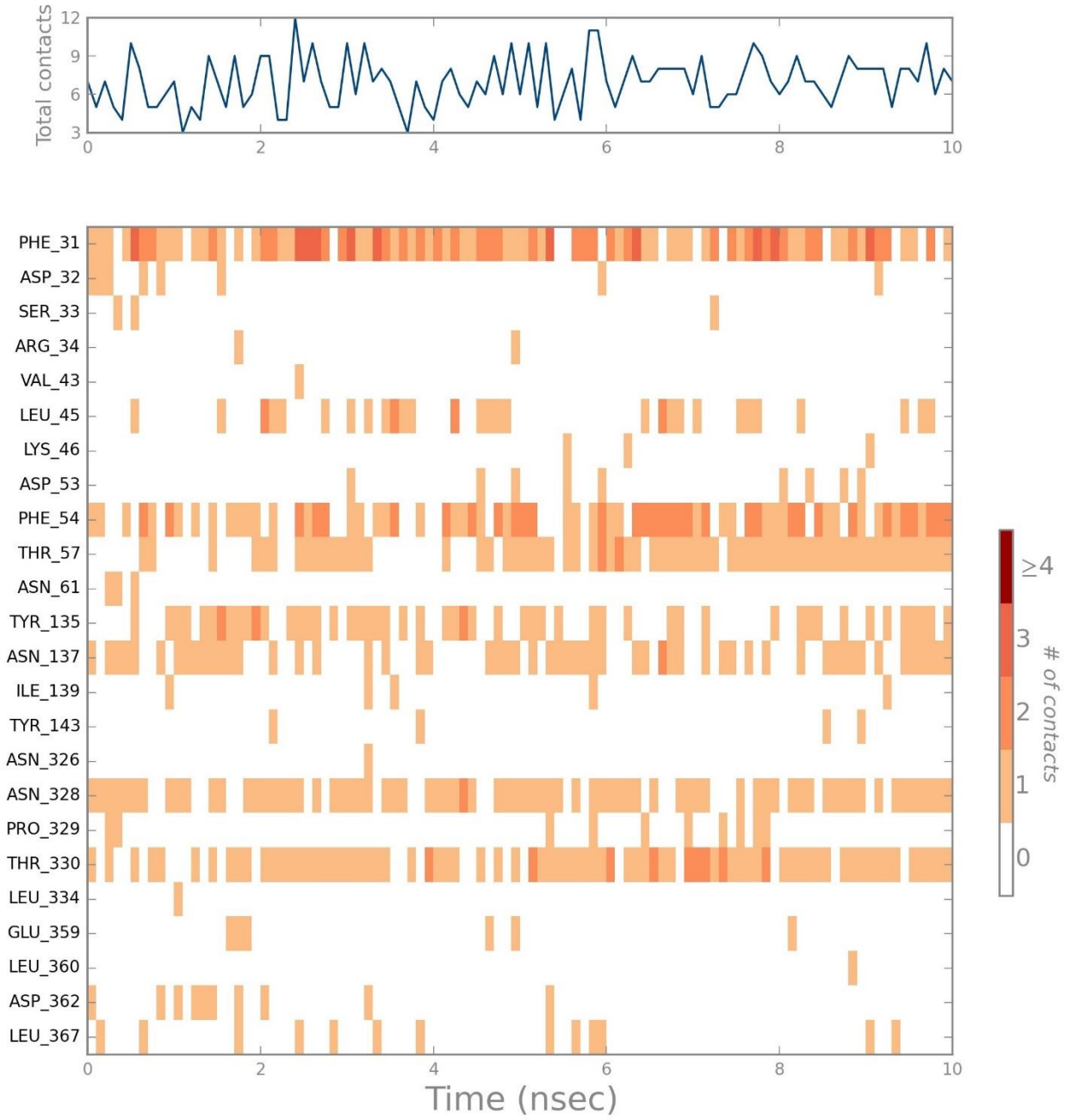


Figure S8. Time line representation of interactions and contacts of ligand **37** in the active site of 3ZM6.

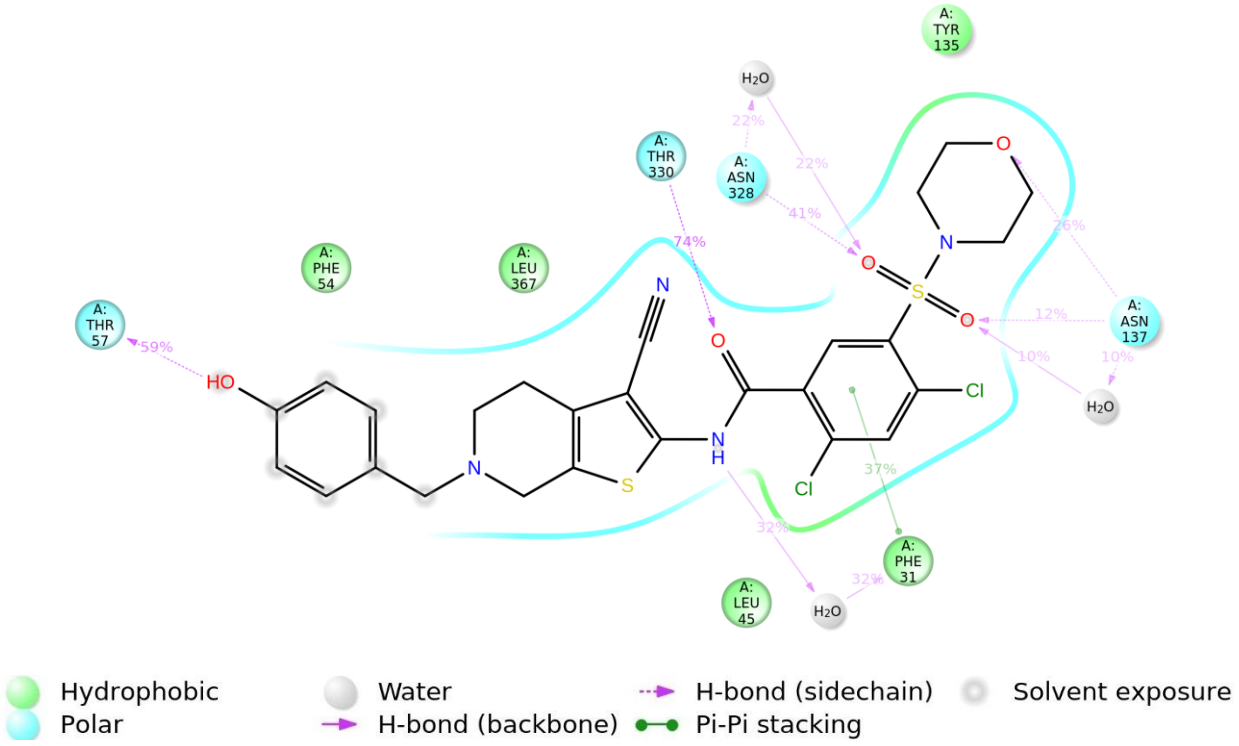


Figure S9. Two dimensional view of the binding interaction of compound **37** into active site of *Streptococcus pneumoniae* MurF ligase enzyme (PDB ID 3ZM6) throughout the simulation trajectory (0.00 through 10.00 nsec).

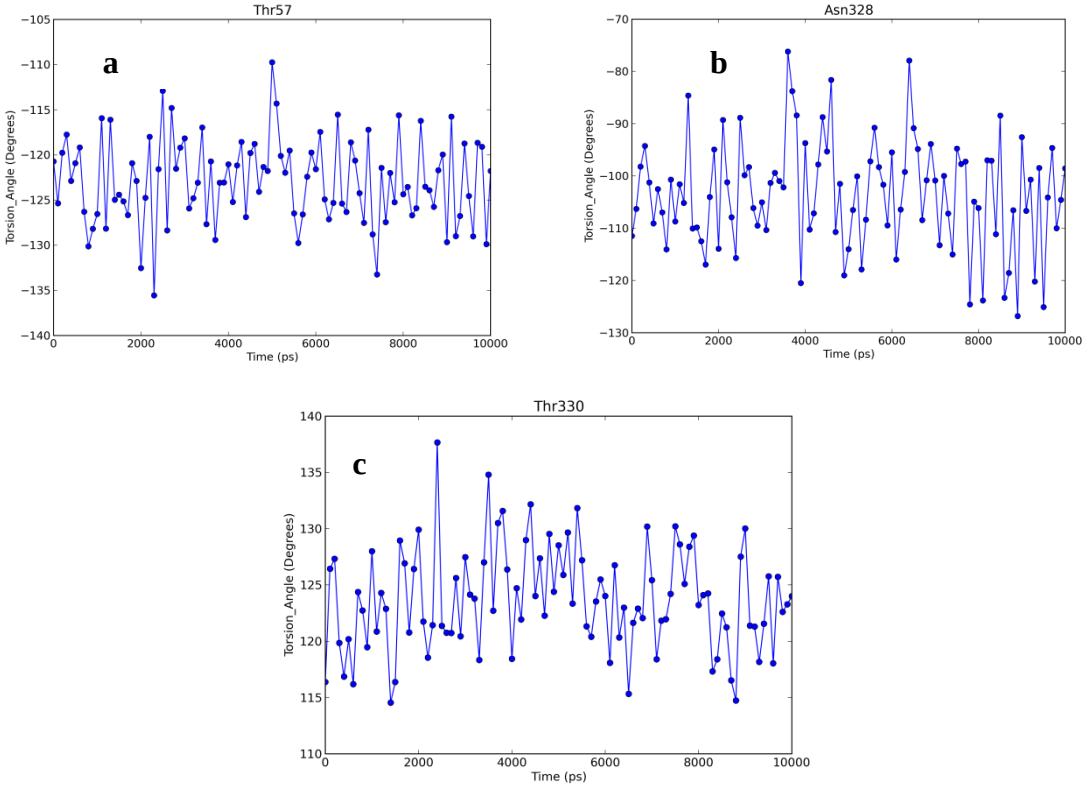


Figure S10. Torsion angles of (a) Thr57 (b) Asn328 and (c) Thr330 MurF binding residues during MD simulation of **37**/3ZM6 complex.

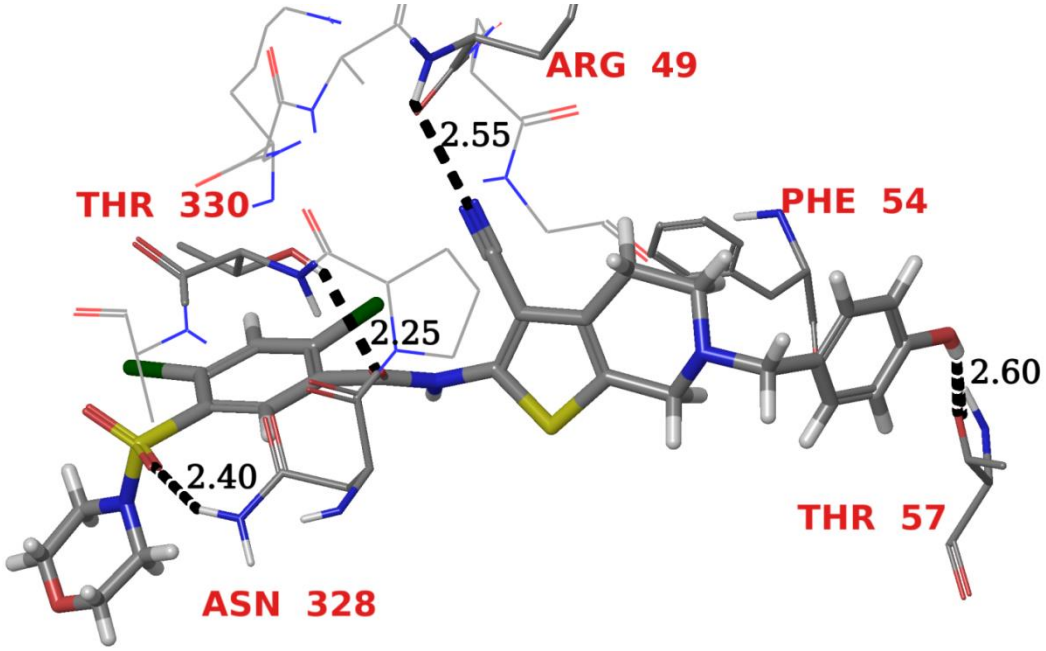


Figure S11. Represents interaction of average structure of lignad **37**/3ZM6 complex extracted from 100 frame structures of trajectory within the catalytic pocket.

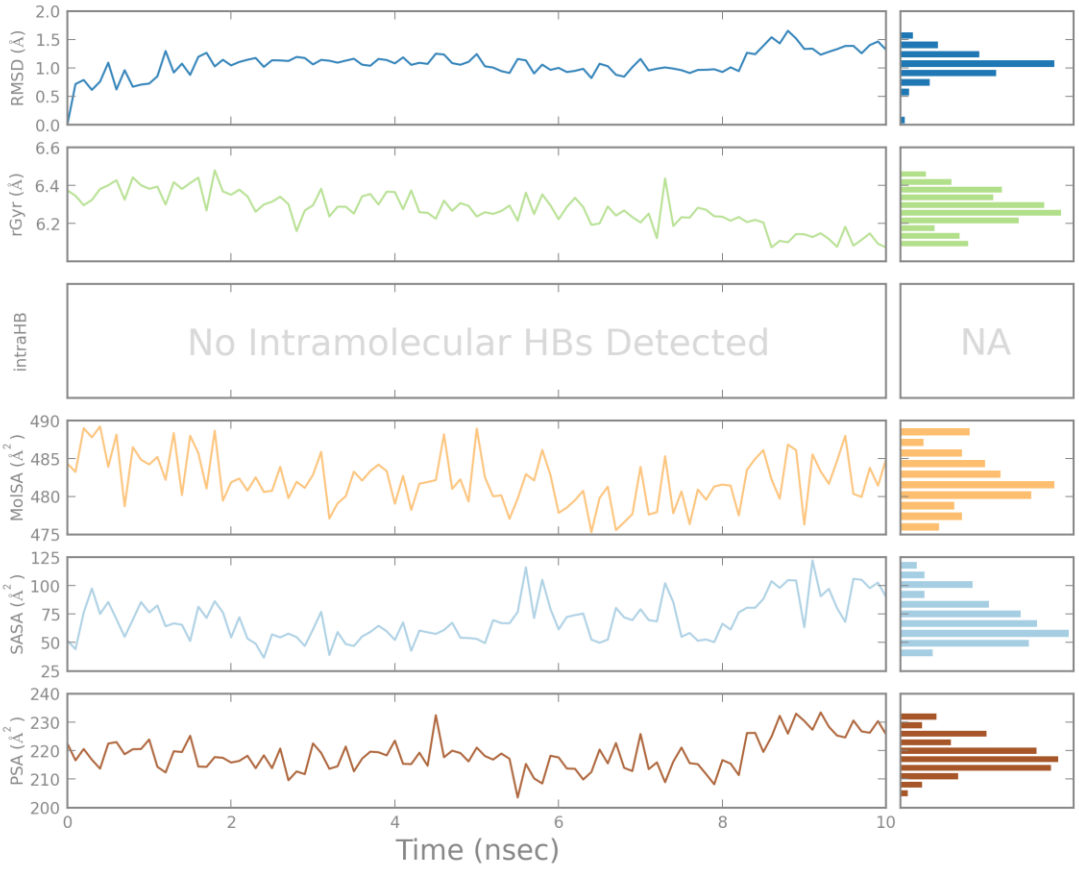


Figure S12. Ligand **37** property during MD simulation. RMSD: Root mean square deviation of a ligand with respect to the reference conformation; rGyr: Radius of Gyraion which measures the 'extendedness' of a ligand; intraHB: Intramolecular Hydrogen Bonds; MolSA: Molecular Surface Area; SASA: Solvent Accessible Surface Area; PSA: Polar Surface Area.

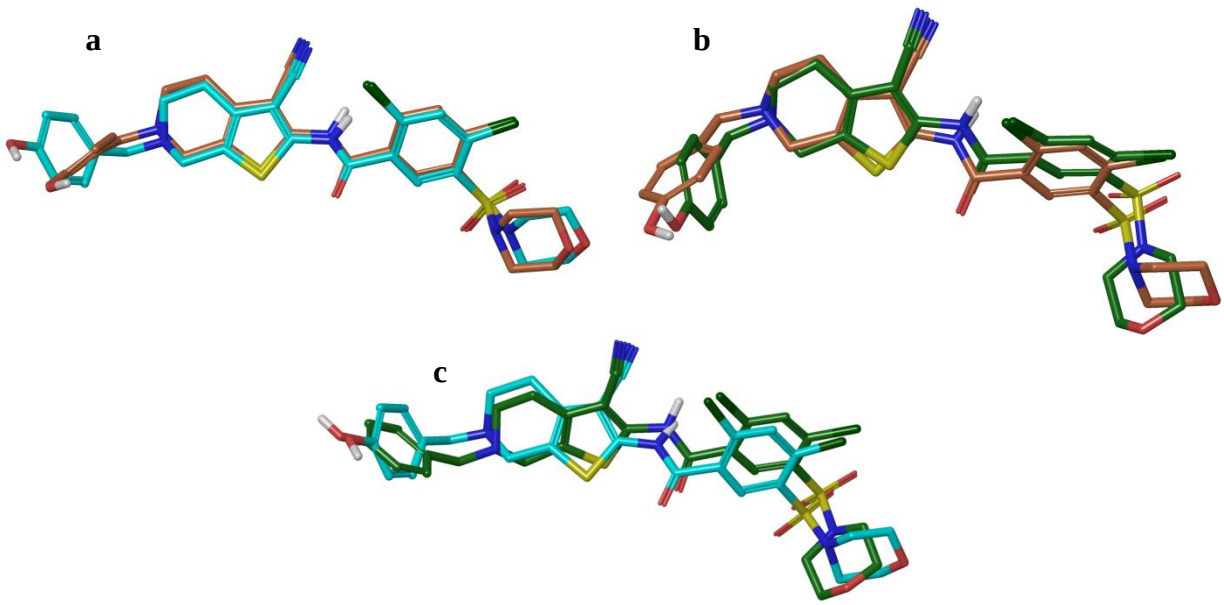
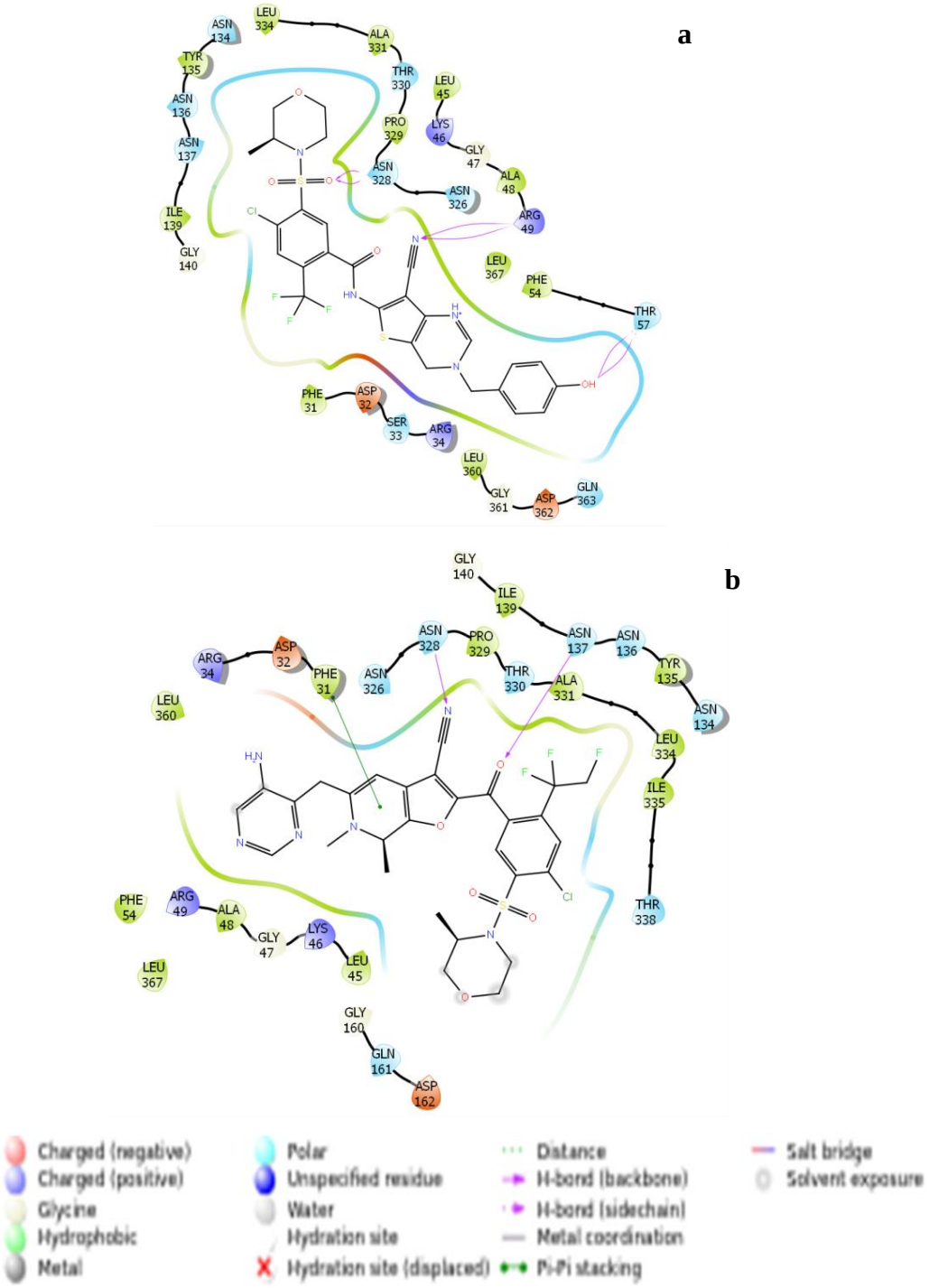


Figure S13. Represents superposition of conformations of inhibitor **37** (a) after MD simulation (blue) and best XP-docking pose (orange) [RMSD: 1.13 Å] (b) best XP-docking pose (orange) and pose of the pharmacophore model (green) [RMSD: 1.32 Å] (c) after MD simulation (blue) and pose of the pharmacophore model (green) [RMSD: 1.47 Å].


a
b
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond (backbone)
H-bond (sidechain)
Metal coordination
Pi-Pi stacking
Salt bridge
Solvent exposure

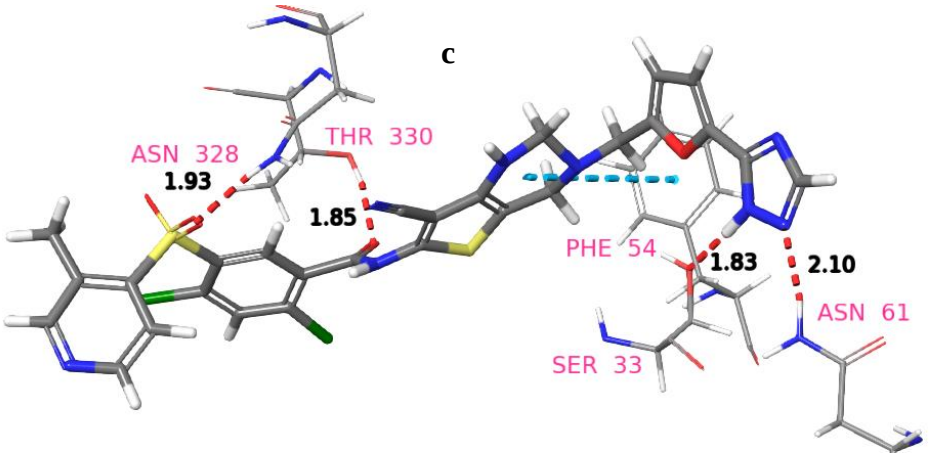


Figure S14. Represents interaction of designed molecules (a) **D1** and (b) **D3** (c) **D4** in the active site of *Streptococcus pneumonaie* MurF ligase (PDB ID: 3ZM6).

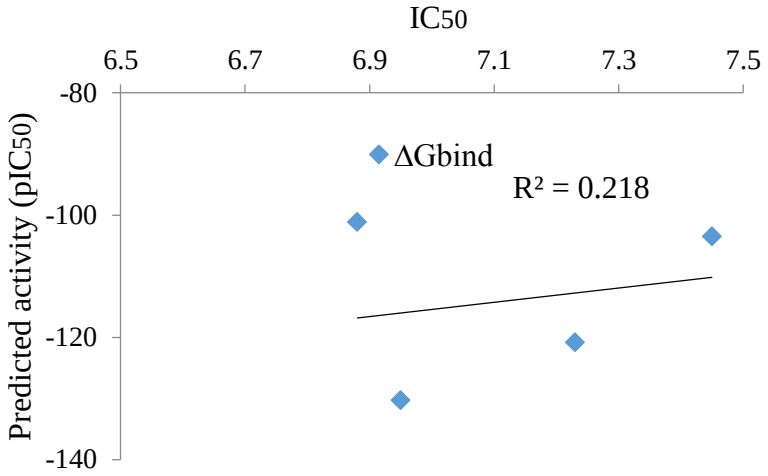
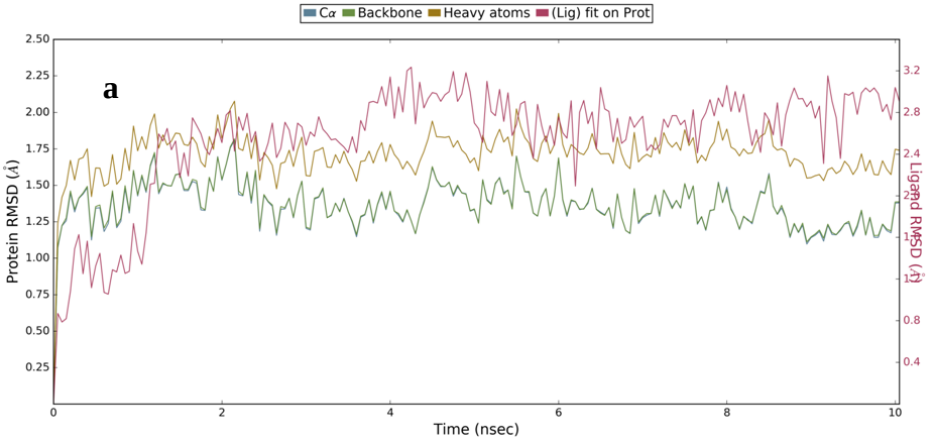
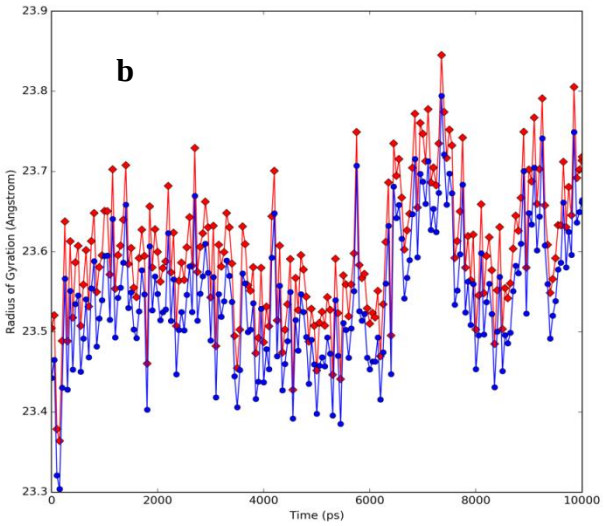
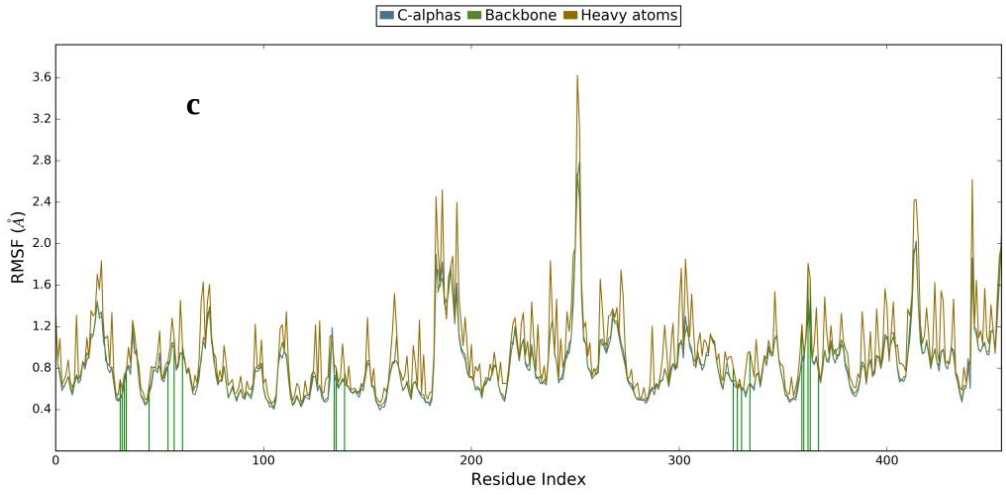
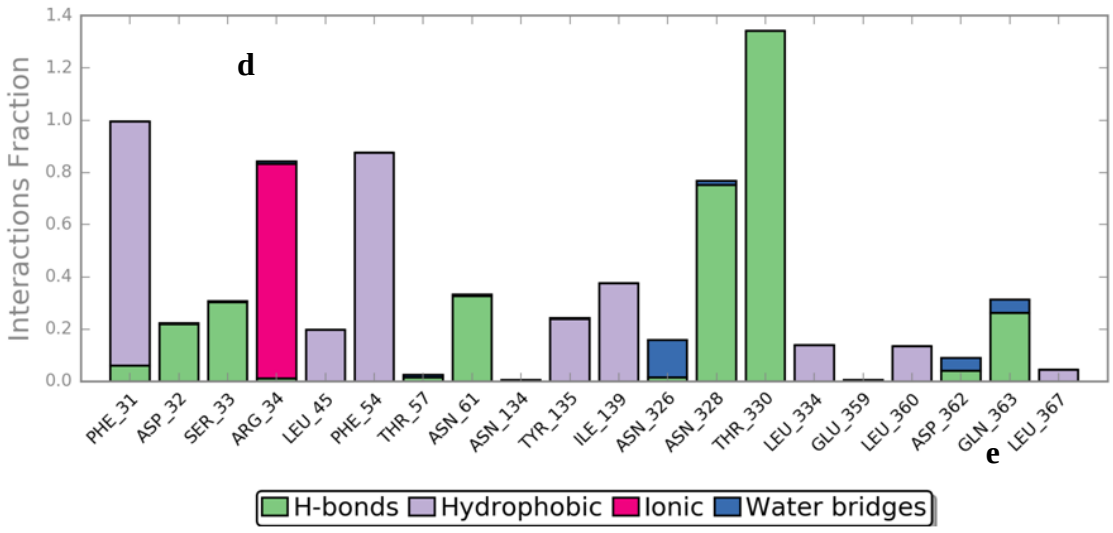


Figure S15. Plot represents correlation between predicted pIC_{50} values and prime ΔGbind.




b
Radius of Gyration (Angstrom)
Time (ps)


C-alphas
Backbone
Heavy atoms
c
RMSF (Å)
Residue Index


d
Interactions Fraction
PHE_31
ASP_32
SER_33
ARG_34
LEU_45
PHE_54
THR_57
ASN_61
ASN_134
TYR_135
ILE_139
ASN_326
ASN_328
THR_330
LEU_334
GLU_359
LEU_360
ASP_362
GLN_363
LEU_367
H-bonds
Hydrophobic
Ionic
Water bridges

e

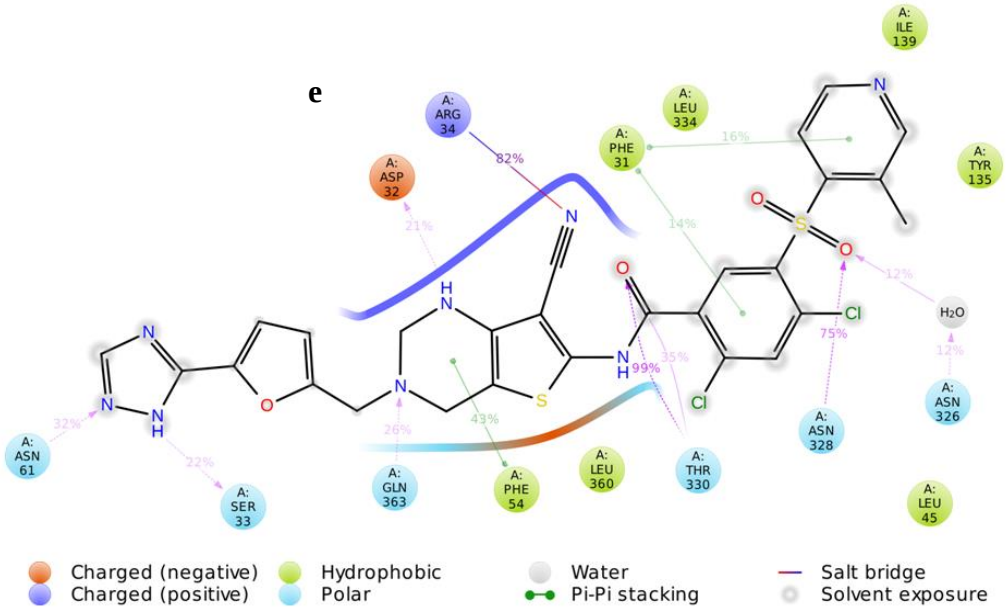


Figure S16. (a) RMSD (Å) of the simulated positions of 3ZM6, C-α and backbone atoms from those in the initial structure (b) Radius of gyration of backbone (blue circle) and Cα atoms (red thin diamond) of **D4**/3ZM6 complex during MD simulation (c) Protein RMSF values of designed molecule **D4**/3ZM6 complex during MD simulation (d) Represents the interaction of designed molecule **D4** with different residues in the catalytic pocket of 3ZM6 during MD simulation (e) Two dimensional view of the binding interaction of designed molecule **D4** into active site of 3ZM6.