International Journal of
Molecular Sciences

MDPI

Supplementary figures & tables

Low molecular weight inhibitors targeting the RNA-binding protein HuR

Benjamin Philipp Joseph [1,2,#], Verena Weber [1,2,#], Lisa Knüpfer[3], Alejandro Giorgetti[1,4], Mercedes Alfonso-Prieto[1], Sybille Krauß[3,*], Paolo Carloni[1,2,*] and Giulia Rossetti[1,5,6,*]

[1] Institute for Neuroscience and Medicine and Institute for Advanced Simulations (INM-9/IAS-5), Computational Biomedicine, Forschungszentrum Jülich, 52425 Jülich, Germany
[2] Faculty of Mathematics, Computer Science and Natural Sciences, RWTH Aachen, 52062 Aachen, Germany
[3] Institute of Biology, University of Siegen, 57076 Siegen, Germany
[4] Department of Biotechnology, University of Verona, 37134 Verona, Italy
[5] Jülich Supercomputing Center (JSC), Forschungszentrum Jülich, 52425 Jülich, Germany
[6] Department of Neurology, RWTH Aachen University, 44517 Aachen, Germany
Shared first authorship
* Correspondence: sybille.krauss@uni-siegen.de, p.carloni@fz-juelich.de, g.rossetti@fz-juelich.de

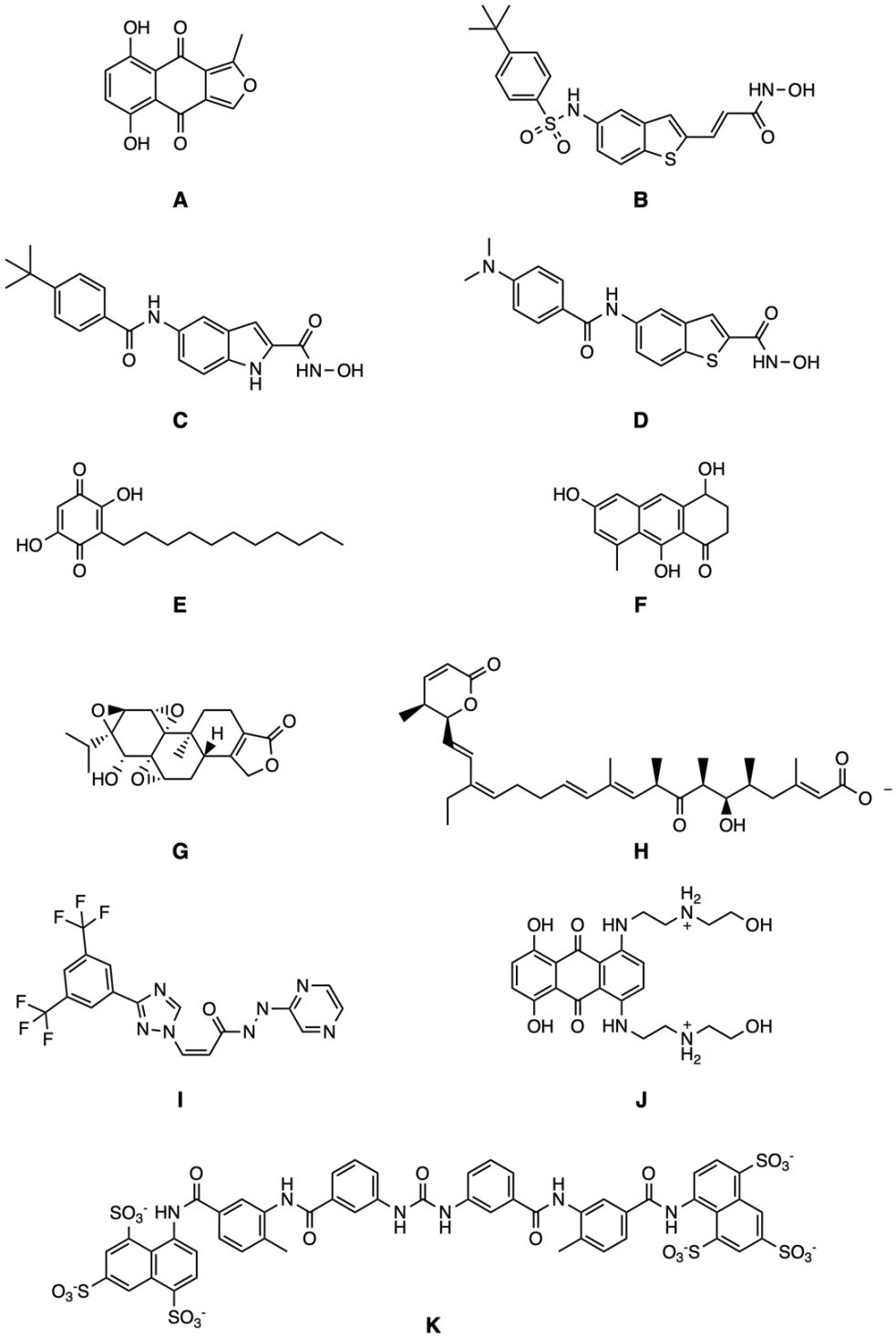


Figure S1. Molecular structures of the HuR inhibitors tested *in vivo*. **(A)** MS-444 (also under preclinical trials). **(B)** KH-3, **(C)** KH-3 derivative 1, **(D)** KH-3 derivative 2, **(E)** Embelin, **(F)** Okicenone, **(G)** Triptolide, **(H)** Leptomycin B, **(I)** Selinexor, **(J)** Mitoxantrone and **(K)** Suramin.

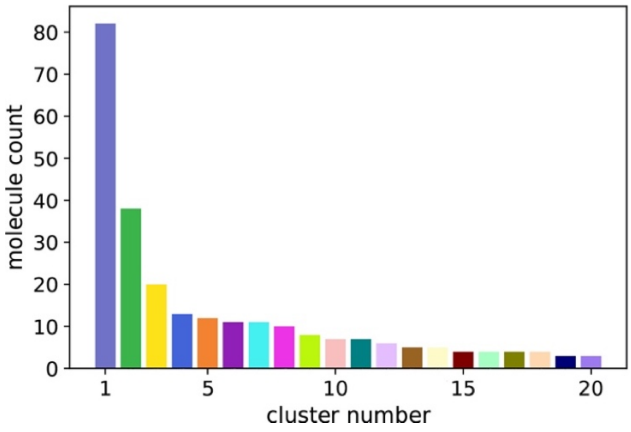


Figure S2. Bar plot representing the number of molecules of the first 20 most populated clusters from the HTVS experiment.

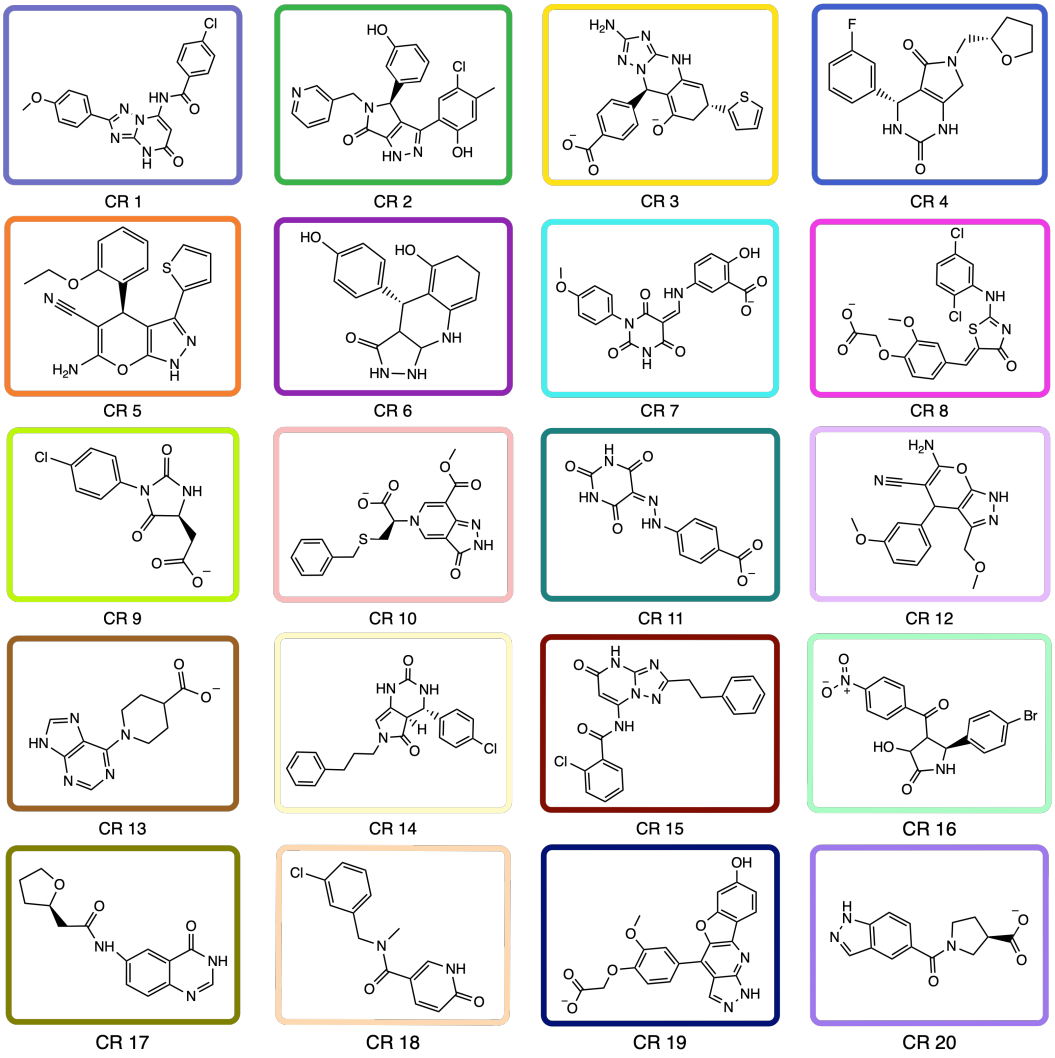
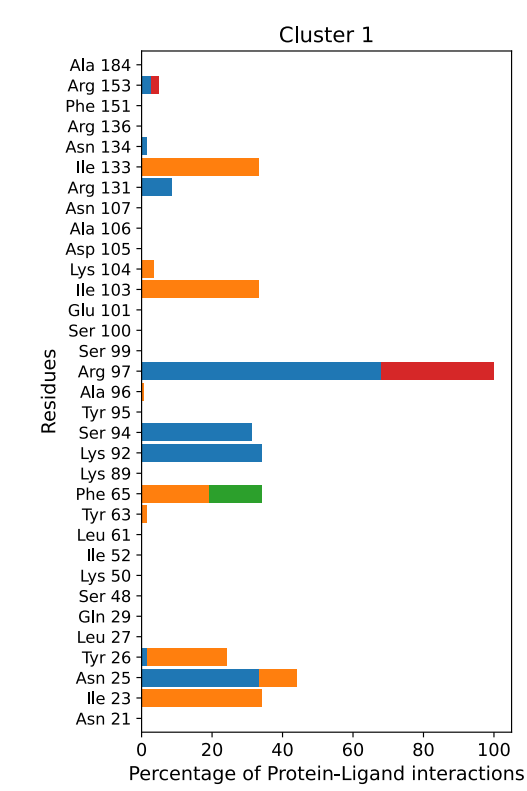
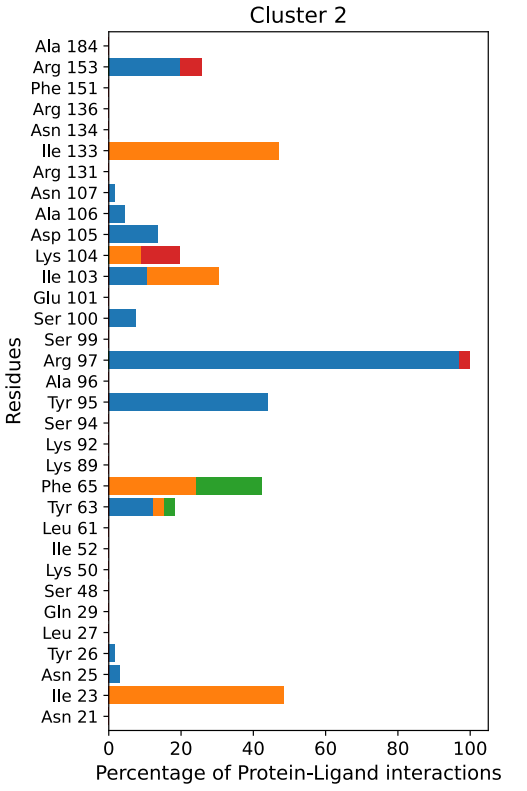
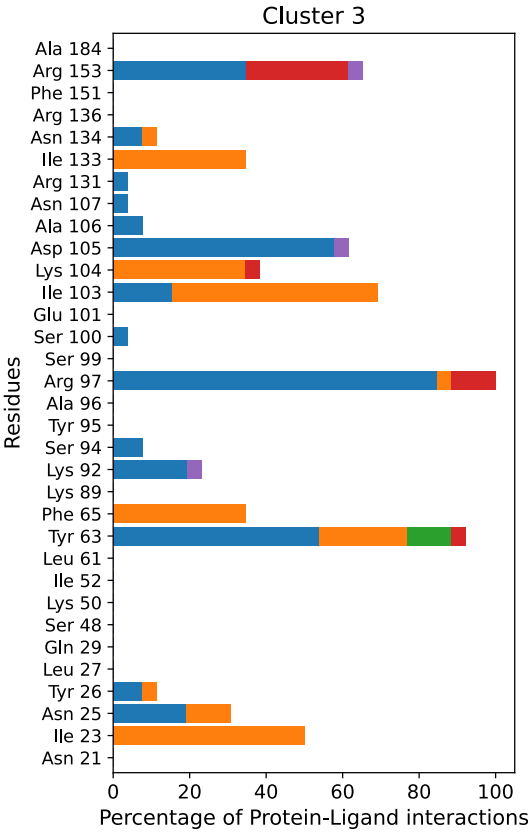


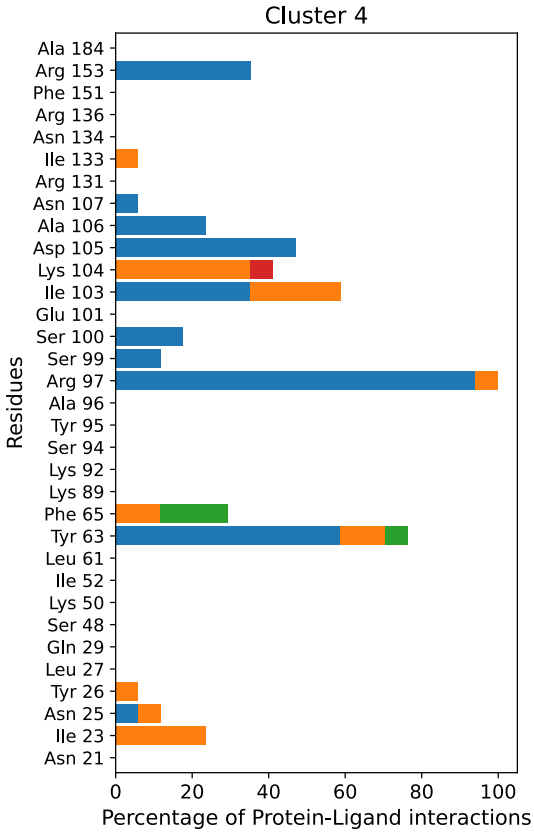
Figure S3. Cluster representatives for the 20 most populated clusters.


Cluster 1
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

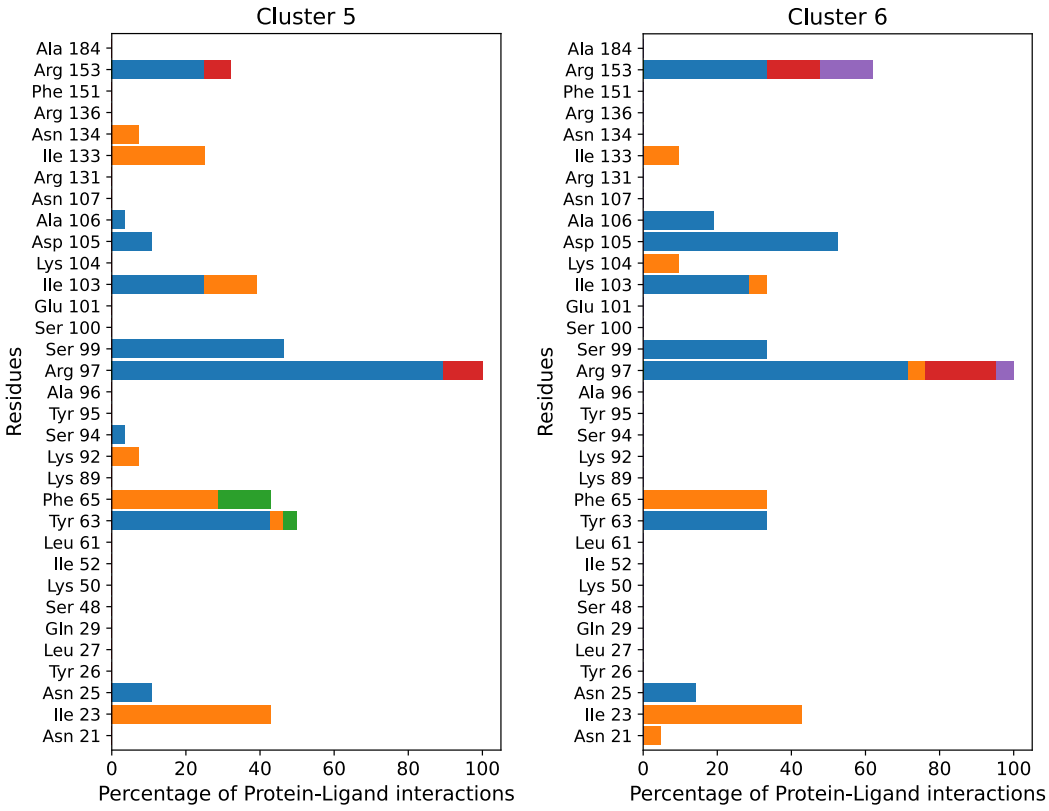

Cluster 2
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

69

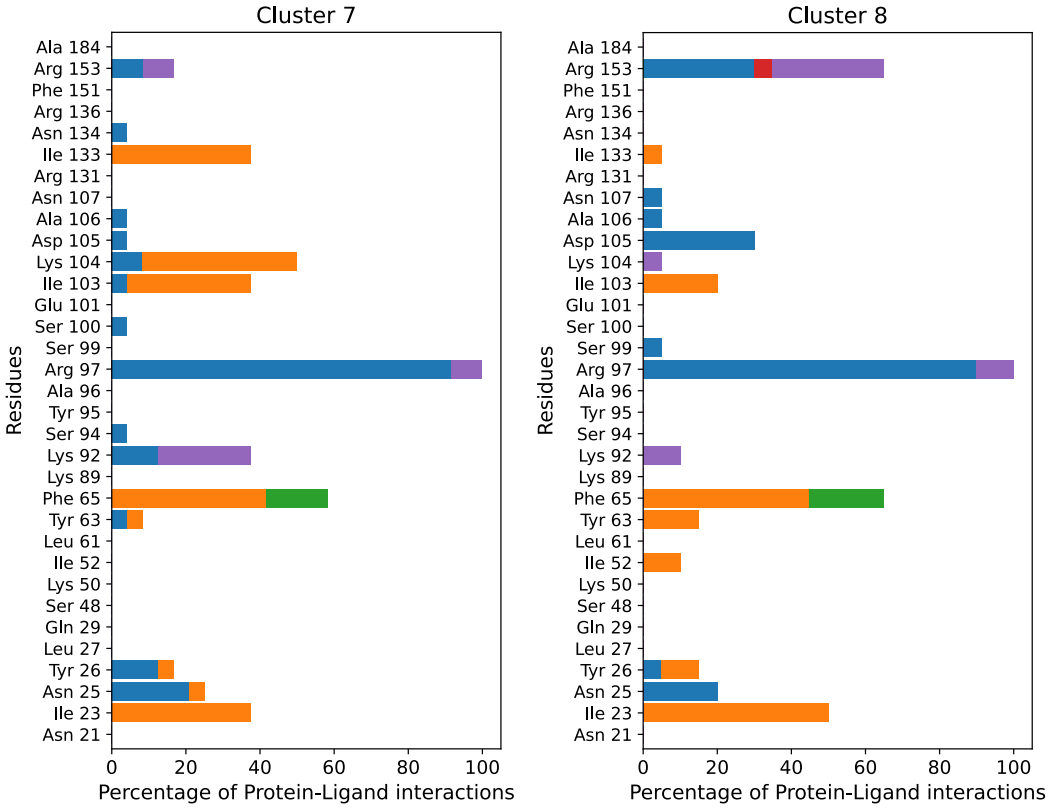

Cluster 3
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions


Cluster 4
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

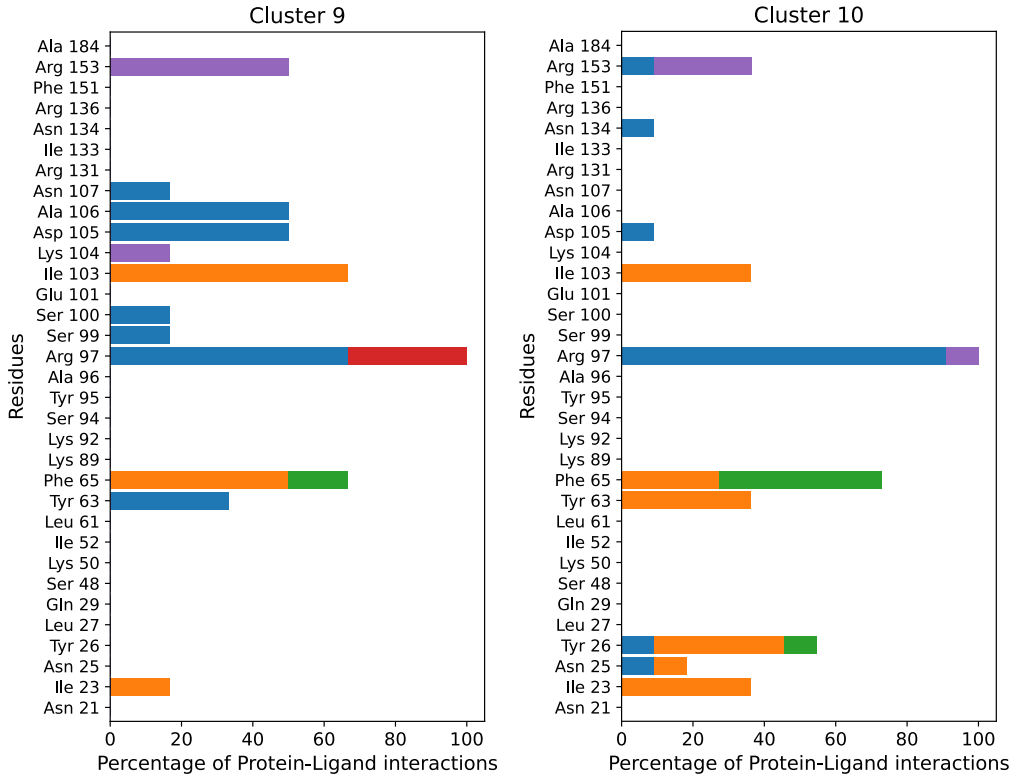
70


Cluster 5
Cluster 6
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

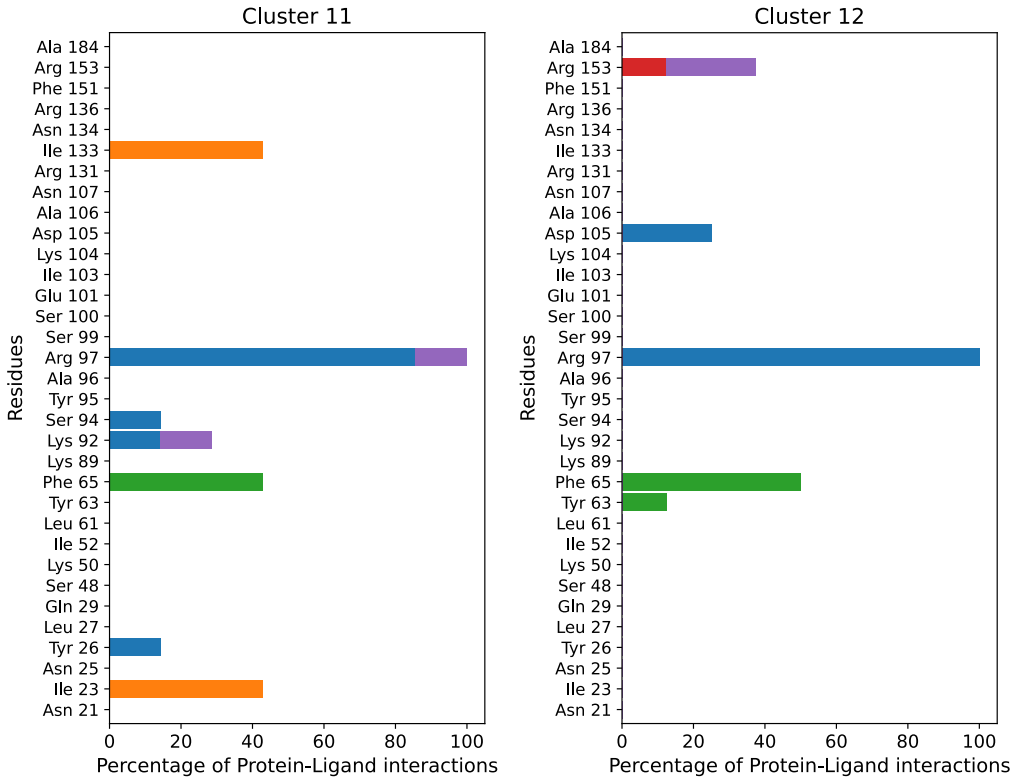
71


Cluster 7
Cluster 8
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

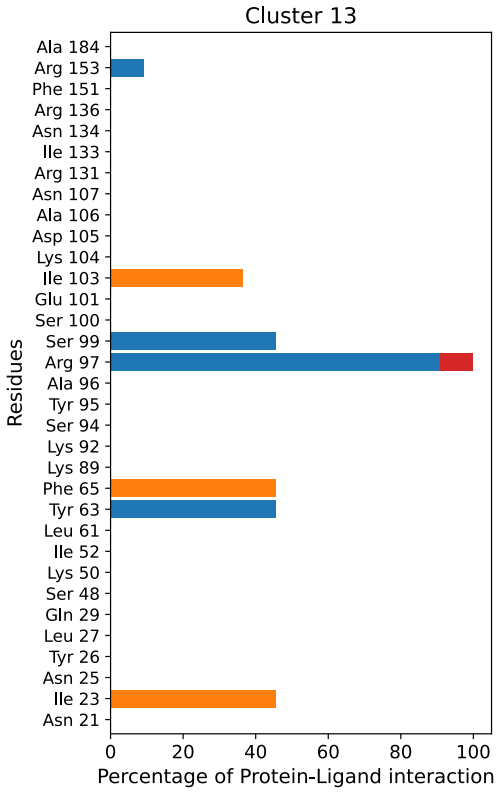
72

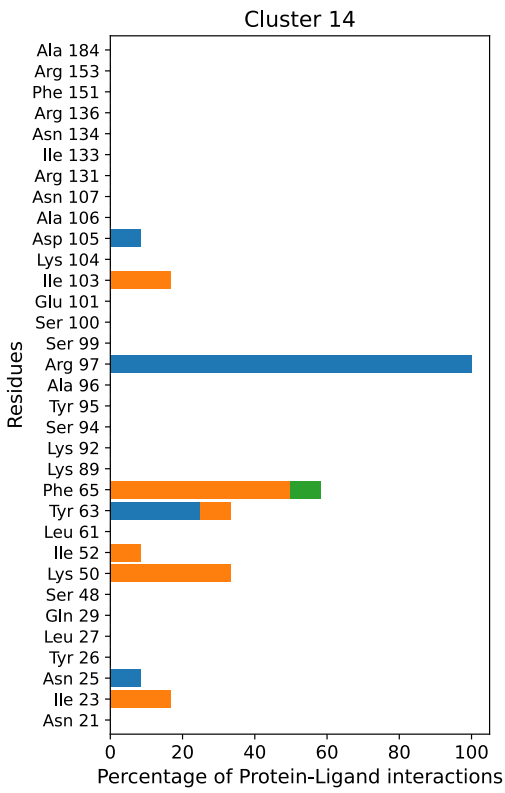

Cluster 9
Cluster 10
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions

73

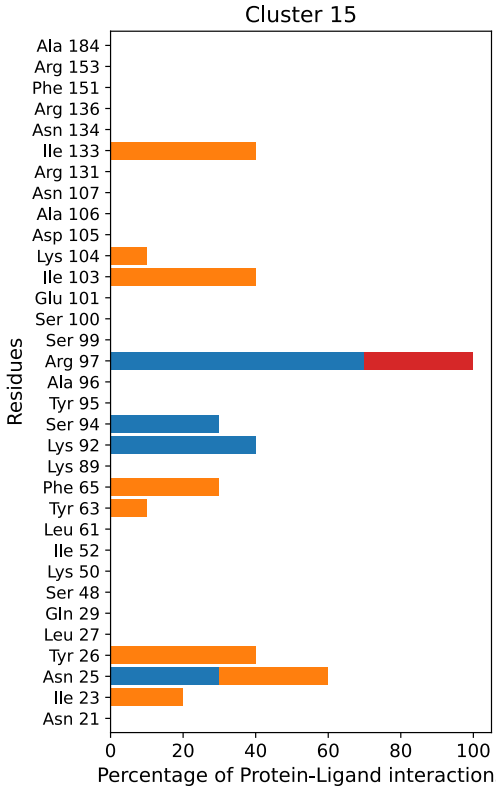

Cluster 11
Cluster 12
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
20
40
60
80
100
Percentage of Protein-Ligand interactions

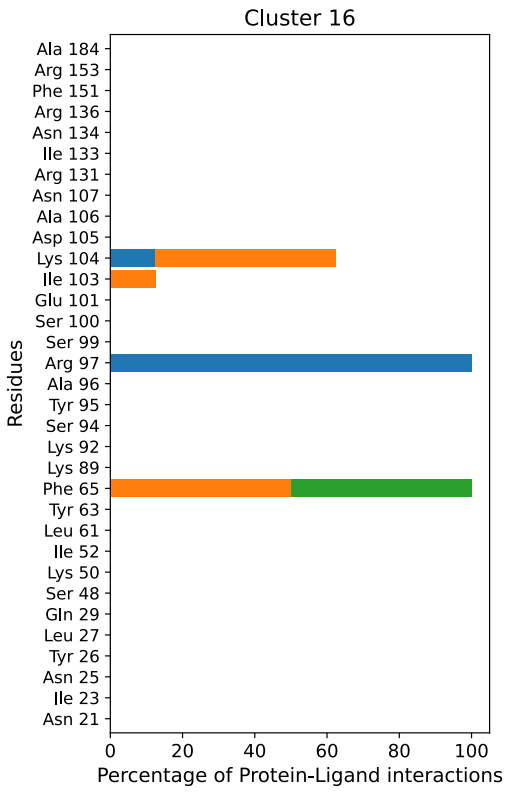
74


Cluster 13
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions


Cluster 14
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

75


Cluster 15
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions


Cluster 16
Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0 20 40 60 80 100
Percentage of Protein-Ligand interactions

76

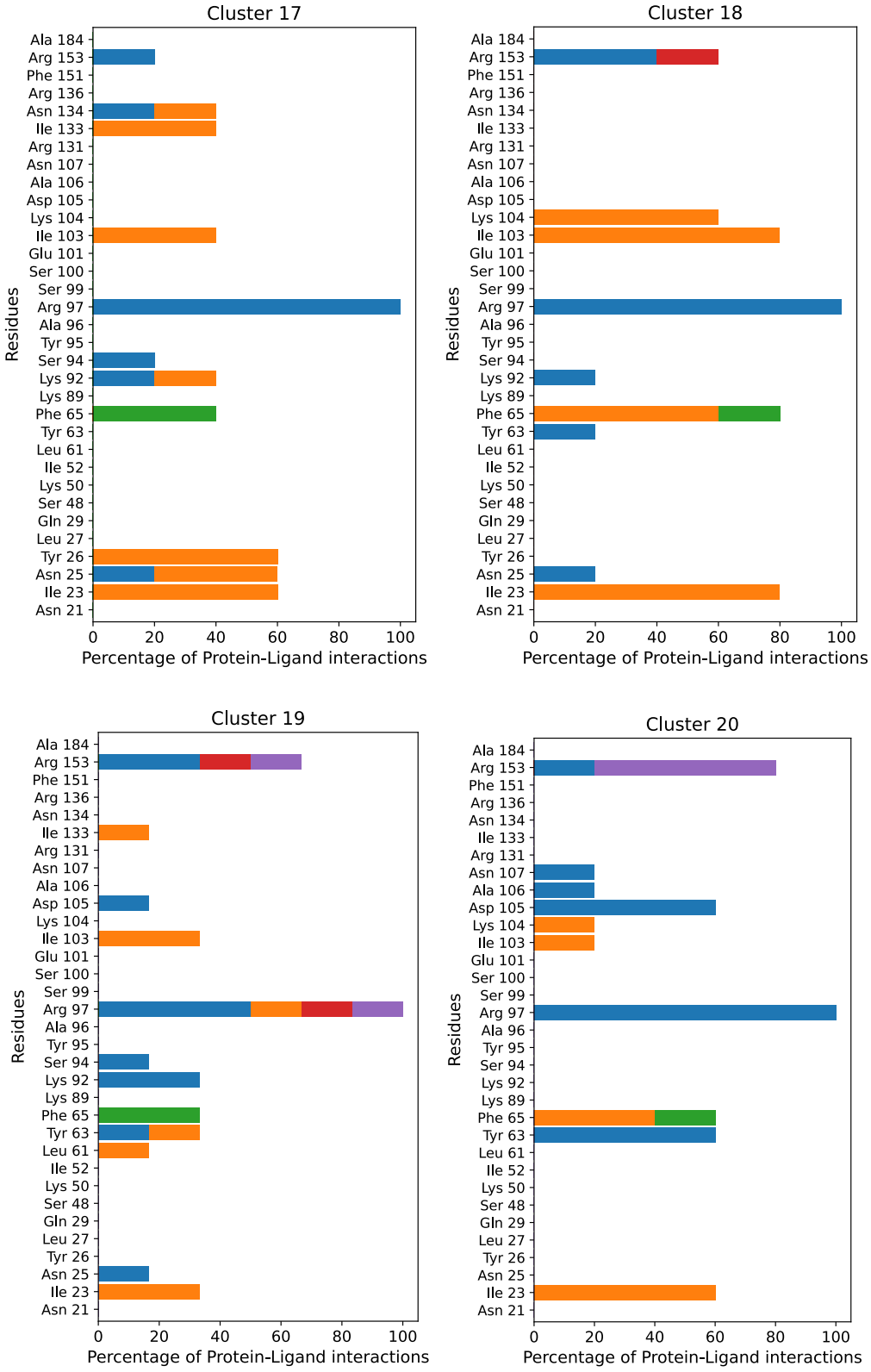
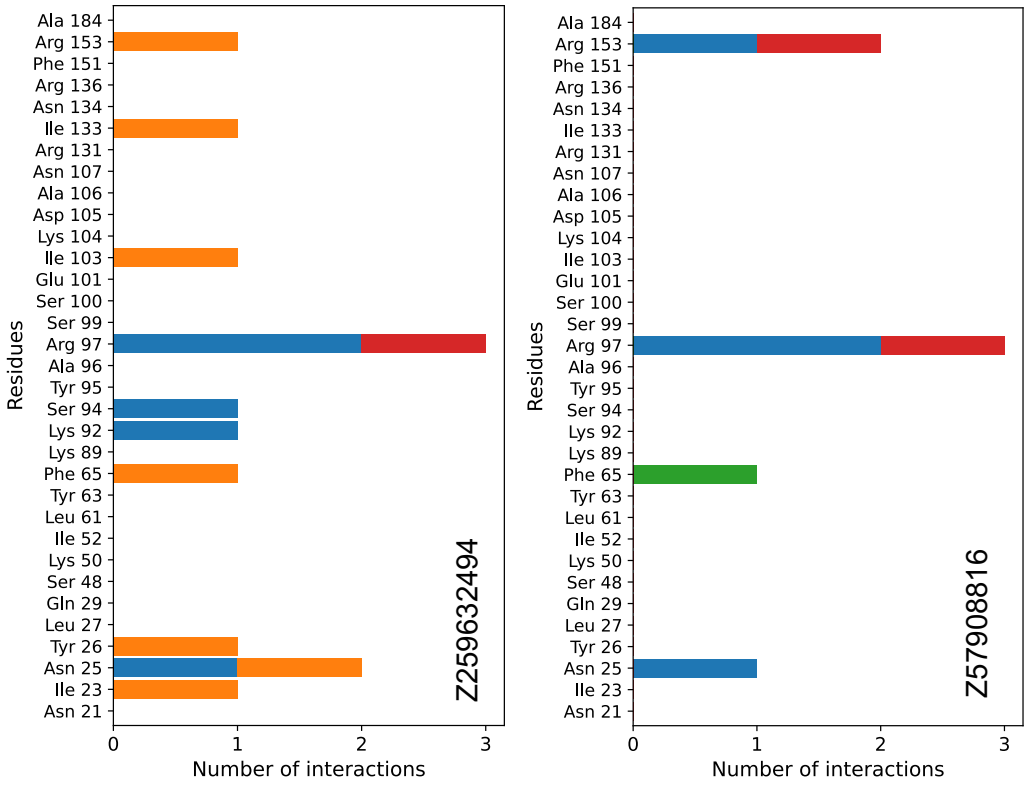
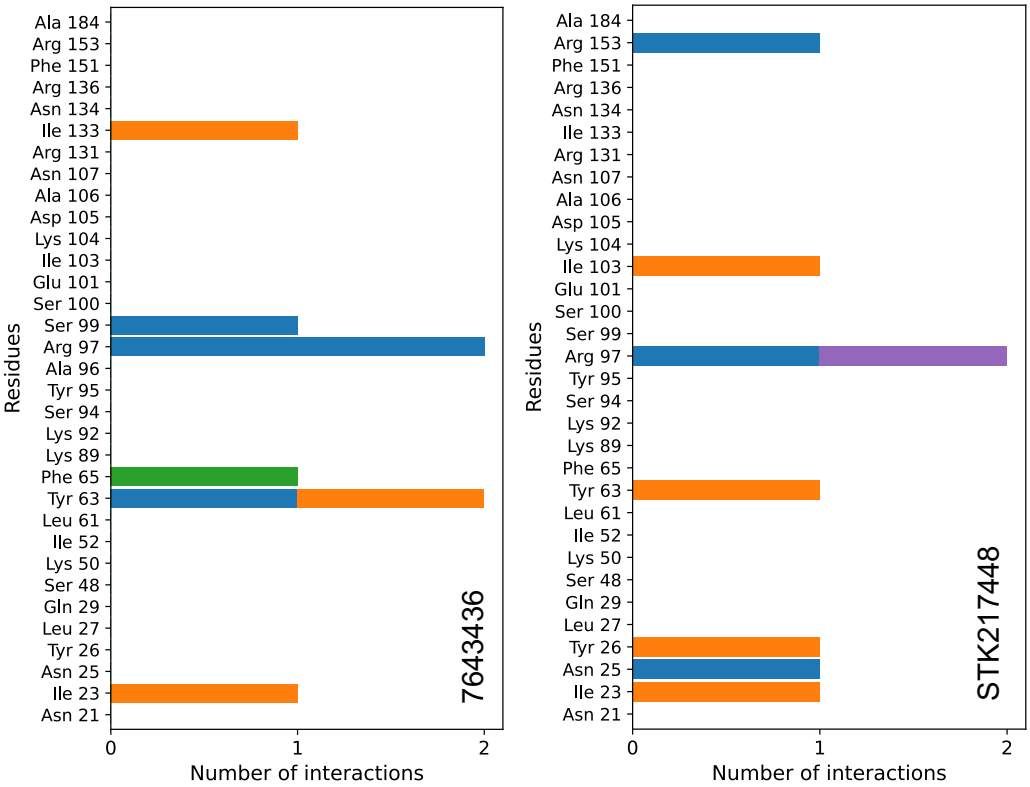


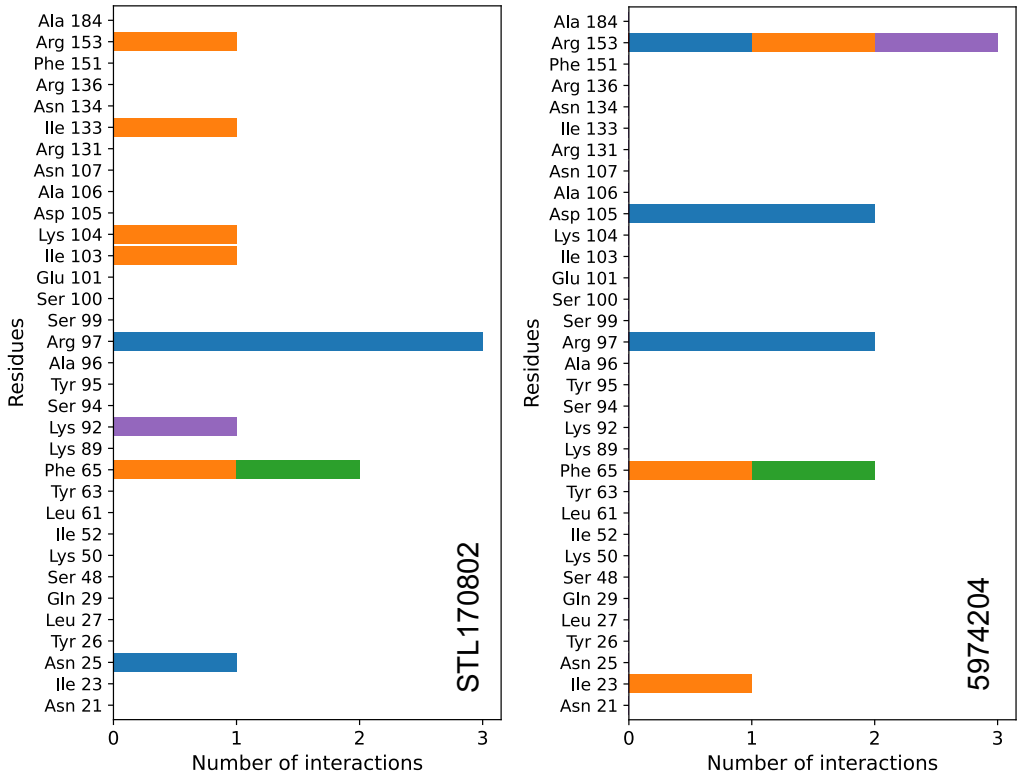
Figure S4. Protein-ligand interaction fingerprints for the 20 most populated clusters. Different interaction types are discriminated using the following color code: blue: hydrogen bonds, orange: hydrophobic interactions, green: π-π-stacking, red: π-cation interaction, purple: salt bridges


Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
1
2
3
Number of interactions
Z259632494
Z57908816

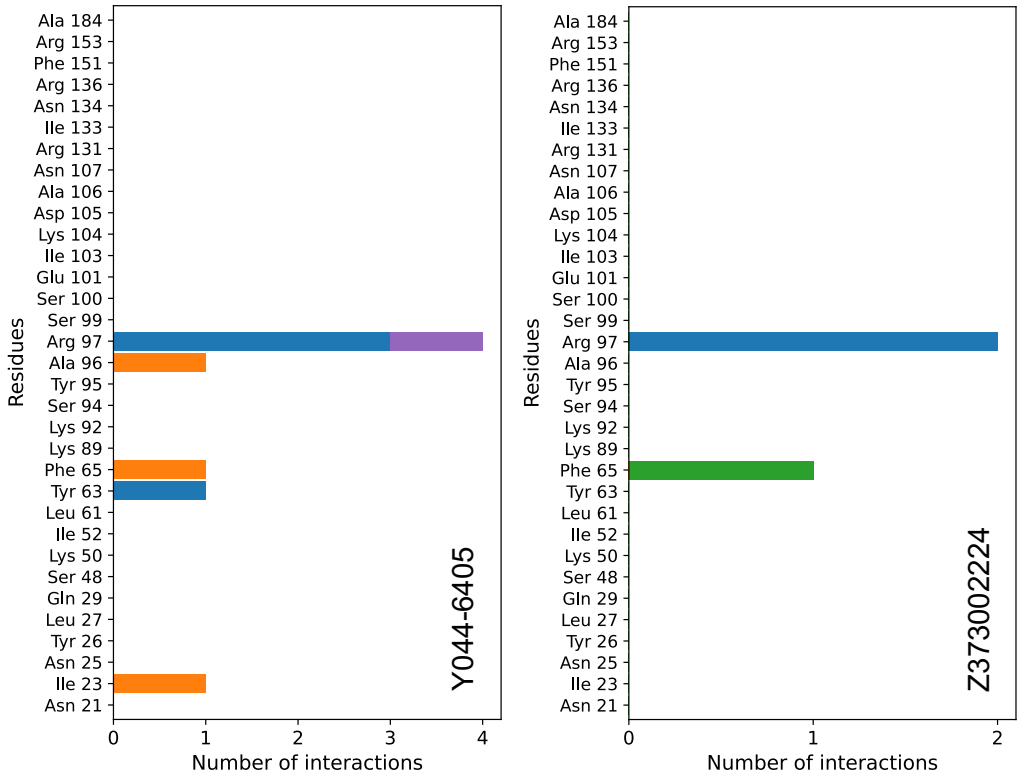
82


Residues
Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
0
1
2
Number of interactions
7643436
STK217448

83


Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
Residues
0
1
2
3
Number of interactions
STL170802
5974204

84


Ala 184
Arg 153
Phe 151
Arg 136
Asn 134
Ile 133
Arg 131
Asn 107
Ala 106
Asp 105
Lys 104
Ile 103
Glu 101
Ser 100
Ser 99
Arg 97
Ala 96
Tyr 95
Ser 94
Lys 92
Lys 89
Phe 65
Tyr 63
Leu 61
Ile 52
Lys 50
Ser 48
Gln 29
Leu 27
Tyr 26
Asn 25
Ile 23
Asn 21
Residues
0
1
2
3
4
Number of interactions
Y044-6405
Z373002224

85

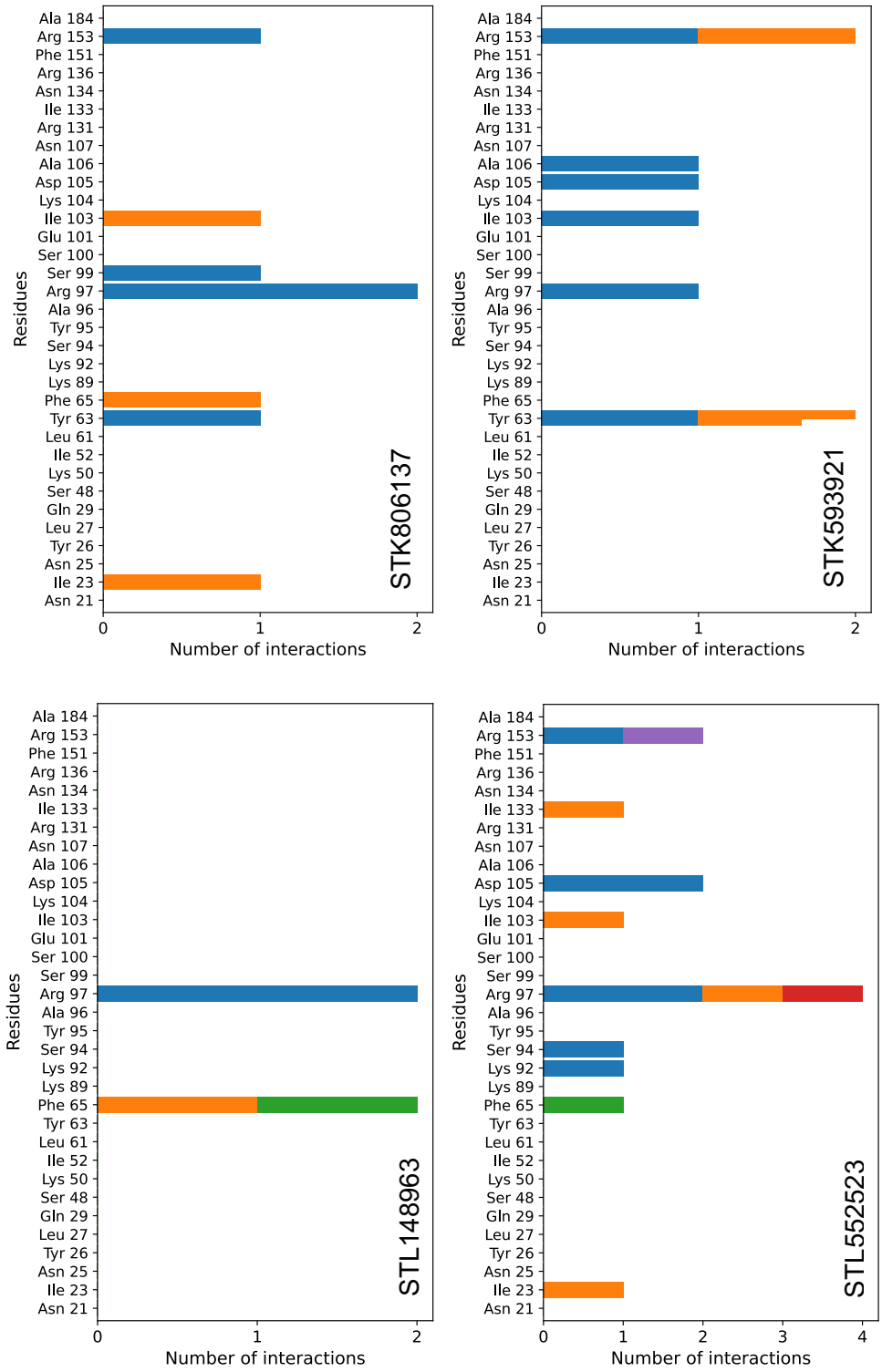


Figure S5: PLIFs of the other 12 bought compounds (C1) derivative, Z259632494; (C2) derivative, Z57908816; (C5) representative, 7643436; (C6) derivative, STK217448; (C7) member, STL170802; (C8) derivative, 5974204; (C9) member, Y044-6405; (C11) representative, STK018404; (C12) derivative, Z373002224; (C13) derivative, STK806137; (C15) derivative, STK593921; (C16) derivative, STL148963; (C19) representative, STL522523. Different interaction types are discriminated using the following color code: blue: hydrogen bonds, orange: hydrophobic interactions, green: π-π-stacking, red: π-cation interaction, purple: salt bridges

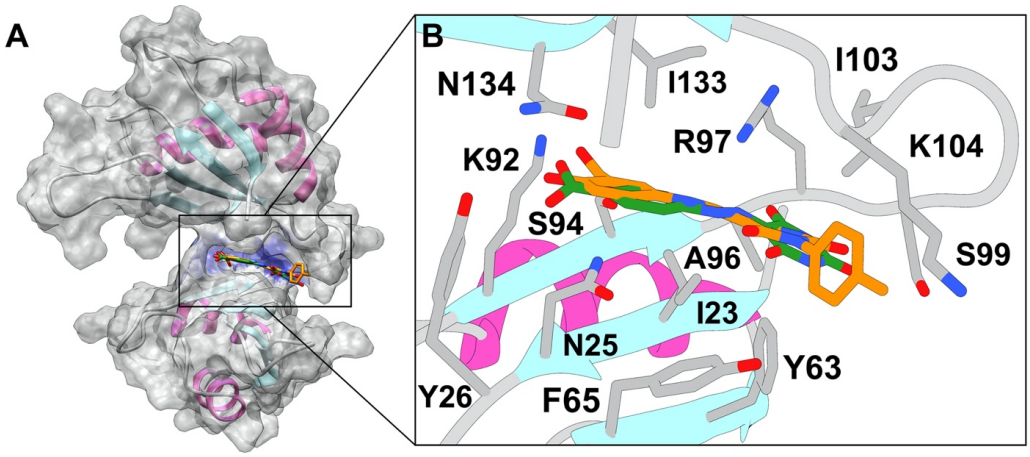


Figure S6: (**A**) Surface representation of HuR RRM1/2 (PDB code 4ED5) with the two docked ligands STK018404 (green carbons) and STL170802 (orange carbons) and the residues Lys92 (left) and Arg97 (right) as purple surface. (**B**) Docking pose in licorice fashion of compound STK018404 (green carbons) and STL170802 (cyan carbons) with labeled residues within 4 Å of the ligands with the protein backbone as cartoon representation with alpha-helices in magenta, beta-sheets in cyan and coils in gray.

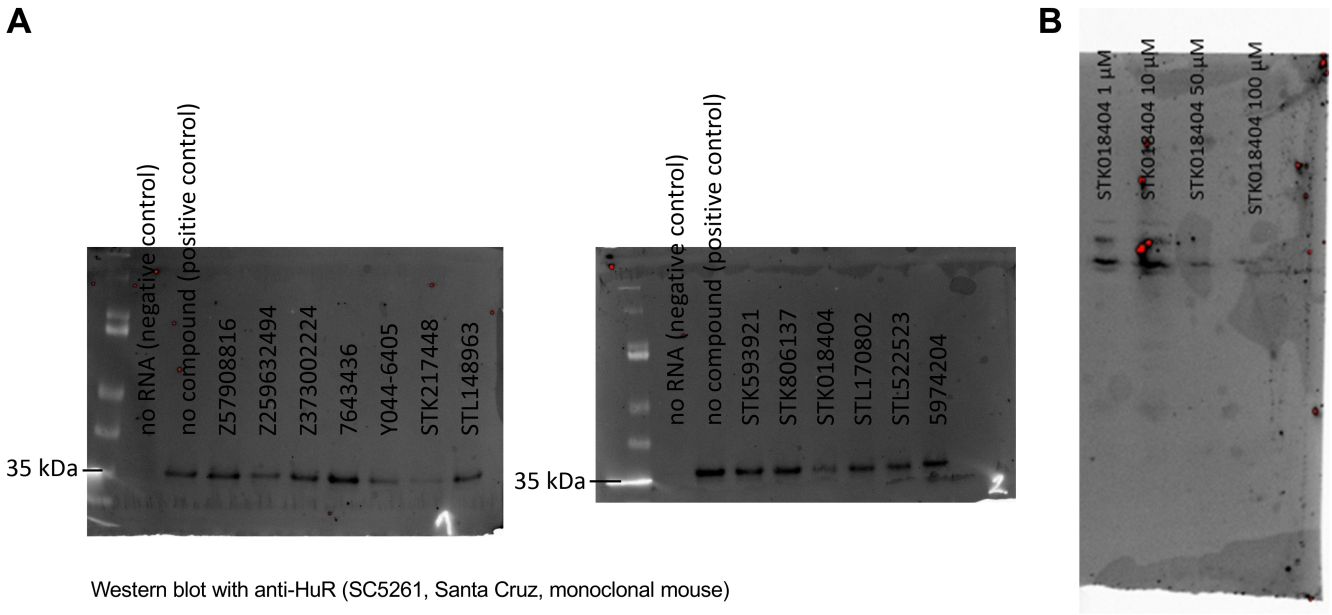


Figure S7: Uncropped western blot gels corresponding to the RNA pull-down assays shown in Figure 4 in the main text. (**A**) RNA–protein pull-down of HuR with its target RNA motif (AUUUUUAUUUU) in the absence (positive control) or presence of the respective compounds (final concentration 100µM). RNA-bound proteins were analyzed on western blots detecting HuR. A negative control that does contain RNA and a positive control with no compound were included. (**B**) RNA–protein pull-down of HuR as described in (A) with different concentrations of the compound STK018404.

Table S1. List of previously reported small molecule inhibitors of the HuR protein as of January 2023.

#	Compound name	Status	IC_{50} [μM]	PubChem ID	Ref.
1	MS-444	*In vivo*	2.1-40.7	132904	[29]
2	Dehydromutactin	*In vitro*	39.0-130.0	54685140	[29]
3	Okicenone	*In vitro*	0.53-2.9	132015	[29]
4	Quercetin	*In cell*	5.78-31.04	5280343	[26]
5	6n	*In cell*	0.015	137652621	[23]
6	24	*In vitro*	-	-	[38]
7	CMLD-1	*In vitro*	5.98	16060621	[27]
8	CMLD-4	*In vitro*	5.22	24868003	[27]
9	CMLD-5	*In vitro*	9.13	16746465	[27]
10	CMLD-6	*In vitro*	8.88	16746321	[27]
11	CMLD-3	*In vitro*	7.11	16746461	[27]
12	31	*In vitro*	44.4	265580	[28]
13	32	*In vitro*	21.7	137651476	[28]
14	23	*In vitro*	41.0	66672	[28]
15	30	*In vitro*	41.9	54677920	[28]
16	MPT0B098	*In vitro*	0.08-0.51	50898338	[62]
17	SP600125	*In vitro*	0.12-16.0	8515	[9]
18	Trichostatin A	*In vitro*	-	444732	[9]
19	5-aza-2-deoxycitidine	*In vivo*	0.01-0.05	460487	[9]
20	N-Benzylcantharidinamide	*In vitro*	50.0-100.0	-	[63]
21	Triptolide	*In vivo*	0.01-0.03	107985	[33]
22	Leptomycin B	*In vivo*	0.01	6917907	[34]
23	Selinexor	*In vivo*	0.12-0.22	71481097	[35]

24	Latrunculin A	*In vitro*	0.33-0.76	445420	[64]
25	Blebbistatin	*In cell*	0.43-22.8	3476986	[64]
26	Mitoxantrone	*In vivo*	0.01-1.13	4212	[38]
27	Suramin	*In vivo*	732	5361	[37]
28	NSC 5836	*In vitro*	14.7	3034178	[28]
29	NSC 7572	*In vitro*	41.0	66672	[28]
30	NSC 44750	*In vitro*	26.9	239577	[28]
31	NSC 50648	*In vitro*	97.4	242249	[28]
32	NSC 62685	*In vitro*	16.7	247668	[28]
33	NSC 123418	*In vitro*	69.9	276150	[28]
34	NSC 143491	*In vitro*	21.7	9575999	[28]
35	NSC 227186	*In vitro*	41.9	54706138	[28]
36	NSC 651084	*In vitro*	17.4	6713252	[28]
37	Myricetin	*In vitro*	1.0	5281672	[26]
38	b-40	*In cell*	0.38	1806245	[65]
39	Ellagic acid	*In vitro*	0.6	5281855	[26]
40	(-)-Epicatechin gallate	*In vitro*	0.2	107905	[26]
41	Rhamnetin	*In vitro*	1.2	5281691	[26]
42	Cryptotanshinone	*In cell*	6.0	160254	[39]
43	(-)-Epigallocatechin gallate	*In vitro*	0.2	65064	[26]
44	Dihydrotanshinone (DHTS)	*In vivo*	0.84-1.2	5316743	[39]
45	6a	*In cell*	0.0128	132251303	[23]
46	b-41	*In cell*	6.21	58739563	[65]
47	b-68	*In cell*	-	1801337	[65]
48	a-63	*In cell*	-	6161395	[65]
49	Azaphilone-9	*In vitro*	51.9-67.6	57326411	[49]

50	CMLD-2	*In vitro*	4.44	16746438	[27]
51	Tanshinone I	*In cell*	-	114917	[39]
52	HuR inhibitor 5	*In cell*	15-27	3634762	[47]
53	1	*In vitro*	-	156010691	[21]
54	2	*In vitro*	-	155560371	[21]
55	3	*In vitro*	-	155517914	[21]
56	4	*In vitro*	-	155540762	[21]
57	Hyperoside	*In vitro*	-	5281643	[55]
58	Rutin	*In vitro*	-	5280805	[55]
59	Isovitexin	*In vitro*	-	162350	[55]
60	Vitexin-2-O-rhamnoside	*In vitro*	-	5282151	[55]
61	Aesculin	*In vitro*	-	5281417	[55]
62	Novobiocin	*In vitro*	-	54675769	[55]
63	Chlorogenic acid	*In vitro*	-	1794427	[55]
64	7-hydroxymatairesinol	*In vitro*	-	44147426	[55]
65	Colchicoside	*In vitro*	-	92763	[55]
66	Asiaticoside	*In vitro*	-	11954171	[55]
67	Embelin	*In cell*	-	3218	[32]
68	KH-3	*In cell*	3.34	45138018	[36]
69	KH-3 derivative 1 (1c)	*In cell*	4.74	155343380	[36]
70	KH-3 derivative 2 (7c)	*In cell*	1.31	11187381	[36]