Table S1 Pharmacophore summary.

Pharmacophore	AUROC	Feature
4WNO-01*	0.805	DDHH
4WNO-02	0.607	ADHH
4WNP	No pharmacophore generated	
5CI7-01	0.784	ADHHP
5CI7-02	0.690	DHHP
5CI7-03	0.801	ADHP
5CI7-04	0.753	ADHP
5CI7-05	0.541	AHHP
5CI7-06	0.667	ADHH
6MNH-01	0,674	AADH
6MNH-02	0.749	AAHH
6QAS-01	0.608	AHHH

* True positive: 43, false negatives: 3, true positive rate: 0.935.

Table S2 Homology model summary.

Model Scores			
Name	**PDF Total Energy**	**PDF Physical Energy**	**DOPE Score***
NUAK1.M0018	2334.8259	1231.28618060	-34203.781250
NUAK1.M0007	2344.2502	1247.03479372	-34237.679688
NUAK1.M0004	2345.6169	1226.63662998	-33729.171875
NUAK1.M0006	2346.4548	1244.79779120	-34100.566406
NUAK1.M0016	2365.7112	1242.31421816	-34300.804688
NUAK1.M0008	2381.8101	1245.26049660	-34237.273438
NUAK1.M0003	2412.6985	1254.21213695	-34010.359375
NUAK1.M0020	2417.3689	1243.48410610	-34306.800781
NUAK1.M0010	2420.5493	1247.51451240	-33814.273438
NUAK1.M0011	2434.2039	1266.74076130	-33925.558594
NUAK1.M0009	2439.0706	1254.21984960	-34229.218750
NUAK1.M0001	2443.0352	1249.76974990	-34137.761719
NUAK1.M0005	2443.9963	1246.00901009	-34062.078125
NUAK1.M0019	2459.6414	1248.45637300	-34291.367188
NUAK1.M0014	2462.1699	1245.18211603	-33909.878906
NUAK1.M0013	2484.7612	1258.08922800	-33993.703125
NUAK1.M0002	2506.2153	1269.86334210	-34078.839844
NUAK1.M0012	2516.3420	1280.44279549	-34188.160156
NUAK1.M0017	2516.9004	1250.93798350	-33832.425781
NUAK1.M0015	2583.1794	1256.70395440	-33826.605469

*DOPE score is a statistical energy score that use to compare different conformations of a protein. A lower score indicates smaller model errors.

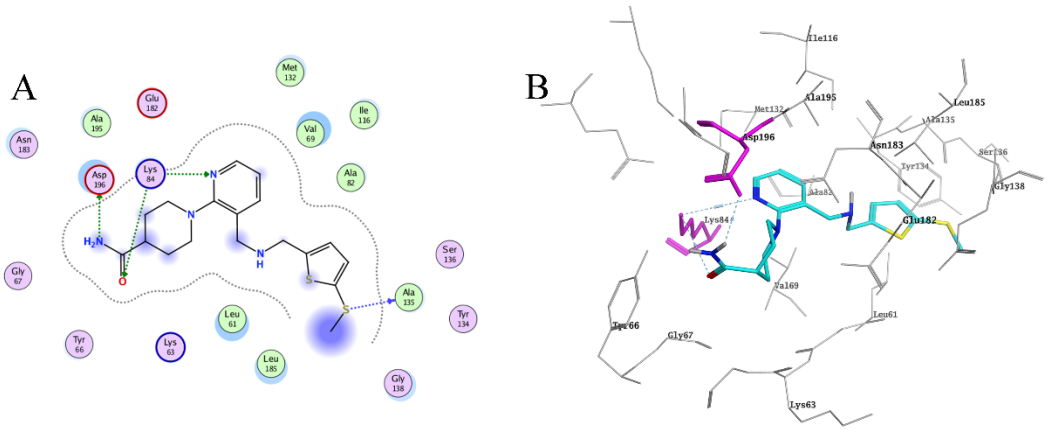
Table S3 Docking results and interaction summary of NUAK1 inhibitors.

Compound	Docking energies (kcal/mol)	RMSD_refine (Å)	Residues	Interactions	Distance (Å)	Interaction energies (kcal/mol)
HTH-01-015	-16.8607	1.0922	GLY 138	H-acceptor	3.25	-0.8
			LYS 84	H-acceptor	3.30	-1.2
			LYS 84	H-acceptor	3.35	-2.5
HTH-02-006	-15.6113	1.7706	GLU 182	H-donor	3.07	-2.3
			LYS 63	H-acceptor	3.13	-3.8
			GLU 139	pi-H	3.74	-0.9
WZ-4003	-15.0512	1.2484	GLU 139	H-donor	3.04	-6.7
			GLU 182	H-donor	3.97	-0.4
			GLY 62	H-acceptor	3.45	-0.9
			LYS 84	H-acceptor	3.53	-2.7
HMD-17-51	-16.8980	2.1356	LYS 84	H-acceptor	3.37	-9.8
			TYR 66	pi-H	4.67	-0.7
HMD-18-42	-15.8107	1.7645	LYS 84	H-donor	3.10	-1.8
			ASN 183	H-acceptor	3.33	-2.6
			GLY 64	H-acceptor	3.53	-1.2

Table S4 Redocking results and interaction summary of ULK1 with 3RF.

Complexes	Docking energies (kcal/mol)	RMSD (Å)[#]	RMSD_refine (Å)	Residues	Interactions	Distance (Å)	Interaction energies (kcal/mol)
4WNO	-14.7096	0.2137	1.1869	ASP 165	H-donor	3.45	-0.9
				CYS 95	H-donor	3.09	-1.5
				GLU 93	H-donor	3.05	-4.0
				CYS 95	H-acceptor	2.94	-4.3

[#] Molecular redocking was used to assess the accuracy of the docking method. It is recommended that RMSD values to be less than 2 Å for the docking process.



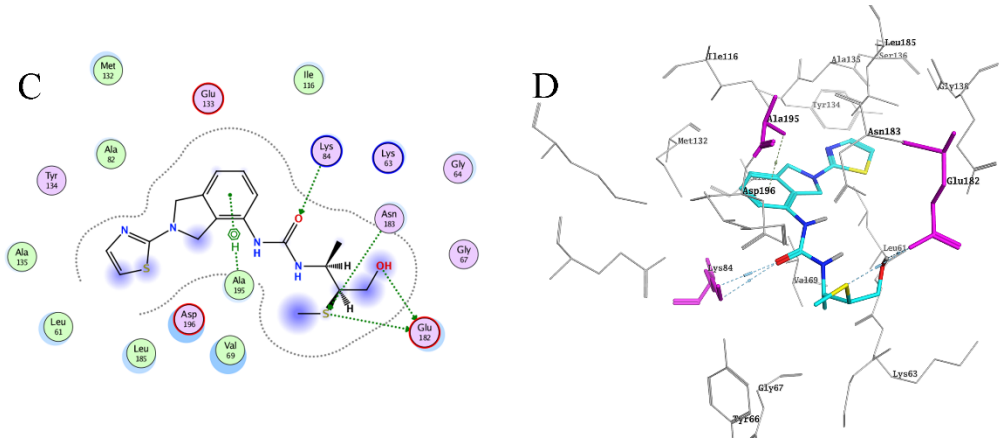


Fig. S1 The molecular docking 2D and 3D interaction diagrams of compound 4 and compound 5 with NUAK1. NUAK1-compound 4 (A, B), NUAK1-compound 5 (C, D). The color scheme is the same as.

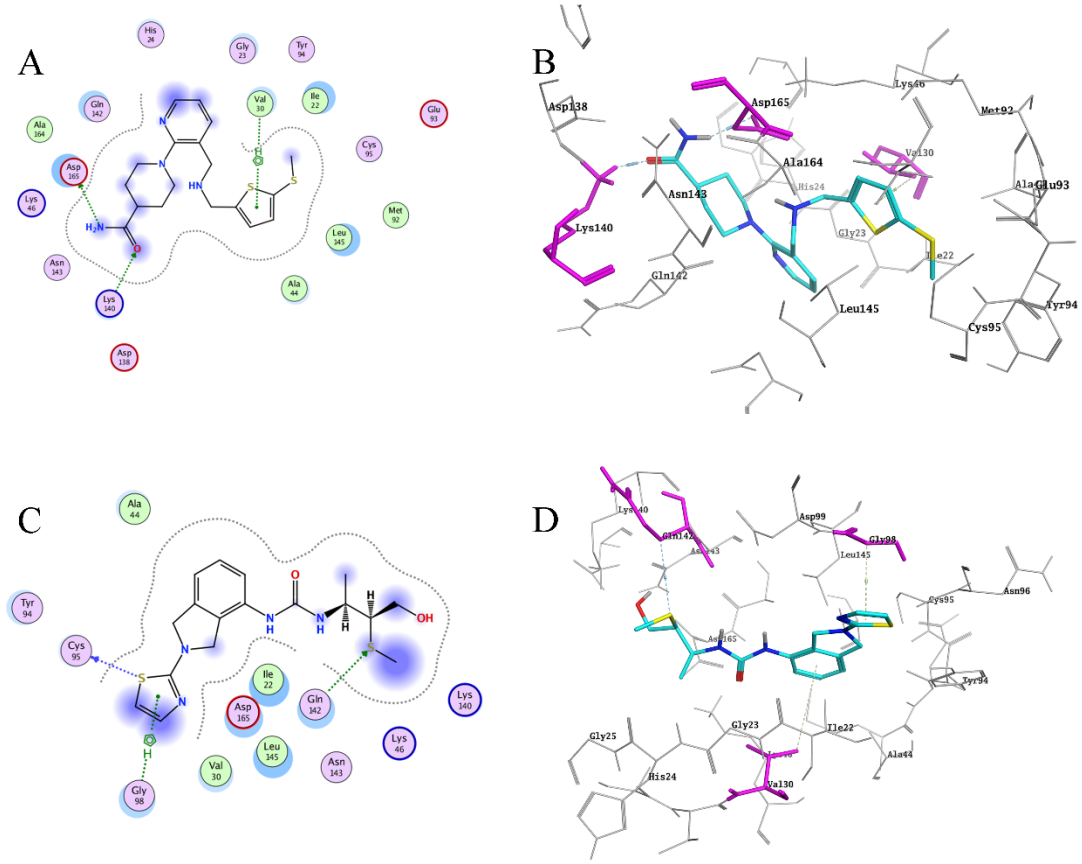


Fig. S2 The molecular docking 2D and 3D interaction diagrams of compound 4 and compound 5 with ULK1. ULK1-compound 4 (A, B), ULK1-compound 5 (C, D). The color scheme is the same as Fig.

Table S5 Interaction summary of each hit compound with NUAK1. Compound, interaction with residues in NUAK, type of interaction, distance in angstroms and interaction energies.

Compound	Interactions	Residues	Distance (Å)	Interaction energies (kcal/mol)
1	H-donor	LYS 63	2.81	-2.4
	H-donor	GLU 133	2.85	-7.4
	H-acceptor	LYS 63	3.72	-1.7
	H-acceptor	ASN 183	3.01	-1.2
	H-acceptor	ALA 135	3.17	-3.4
2	H-acceptor	LYS 84	2.97	-11.0
	H-donor	GLU 139	3.35	-0.6
3	H-donor	GLU 139	3.28	-2.3
	H-donor	LEU 61	2.89	-3.1
	H-acceptor	LYS 63	3.58	-0.6
	pi-H	VAL 69	3.66	-1.0
	pi-H	ASP 196	4.33	-1.7
4	H-donor	ALA 135	3.21	-0.3
	H-donor	ASP 196	3.19	-1.2
	H-acceptor	LYS 84	3.85	-3.0
	H-acceptor	LYS 84	3.61	-0.8
5	H-donor	GLU 182	3.50	-1.9
	H-donor	GLU 182	3.14	-0.9
	H-acceptor	ASN 183	4.32	-1.0
	H-acceptor	LYS 84	3.33	-1.2
	H-acceptor	LYS 84	3.32	-3.0
	pi-H	ALA 195	3.73	-1.0

Table S6 Interaction summary of each hit compound with ULK1. Compound, interaction with residues in MEK, type of interaction, distance in angstroms and interaction energies.

Compound	Interactions	Residues	Distance (Å)	Interaction energies (kcal/mol)
1	H-donor	ASP 165	3.13	-2.3
	H-donor	CYS 95	2.95	-3.0
	pi-H	VAL 30	4.13	-0.7
2	H-donor	MET 92	3.86	-0.4
	H-donor	ASP 165	2.92	-3.1
	H-donor	ASP 138	3.08	-4.6
	H-acceptor	LYS 140	3.24	-4.8
	pi-H	VAL 30	4.45	-0.6
3	H-donor	ASP 165	3.30	-1.0
	H-acceptor	LYS 46	3.40	-0.8
	H-acceptor	CYS 95	2.91	-2.9
4	H-donor	ASP 165	3.06	-3.0
	H-acceptor	LYS 140	3.04	-4.2
	pi-H	ASN183	4.35	-0.8
5	H-donor	CYS 95	3.31	-0.4
	H-acceptor	GLN 142	3.88	-0.8
	pi-H	GLY 98	3.69	-0.7

Table S7 Interaction summary of MRT68921 with NUAK1 and ULK1.

Receptor	Docking energies (kcal/mol)	RMSD_refine (Å)	Interactions	Residues	Distance (Å)	Interaction energies (kcal/mol)
NUAK1	-15.6343	1.3667	H-donor	GLY 139	3.08	-4.3
			H-acceptor	LYS 63	3.28	-7.5
ULK1	-18.8131	1.7720	H-acceptor	CYS 95	2.88	-3.3
			pi-H	GLY 98	3.67	-0.9

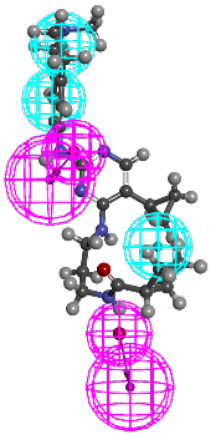


Fig. S3 Common feature pharmacophore generated by the 5 hits. MRT68921 matches the features of the pharmacophore.

Table S8 Fit values of the 5 hits and MRT68921 for common feature pharmacophore.

Compound	Fit value
ZINC000822294216	4.99933
ZINC000826240974	3.24750
ZINC000270620259	2.87123
ZINC000561411090	2.83600
ZINC000378284052	2.28828
MRT68921	2.08435

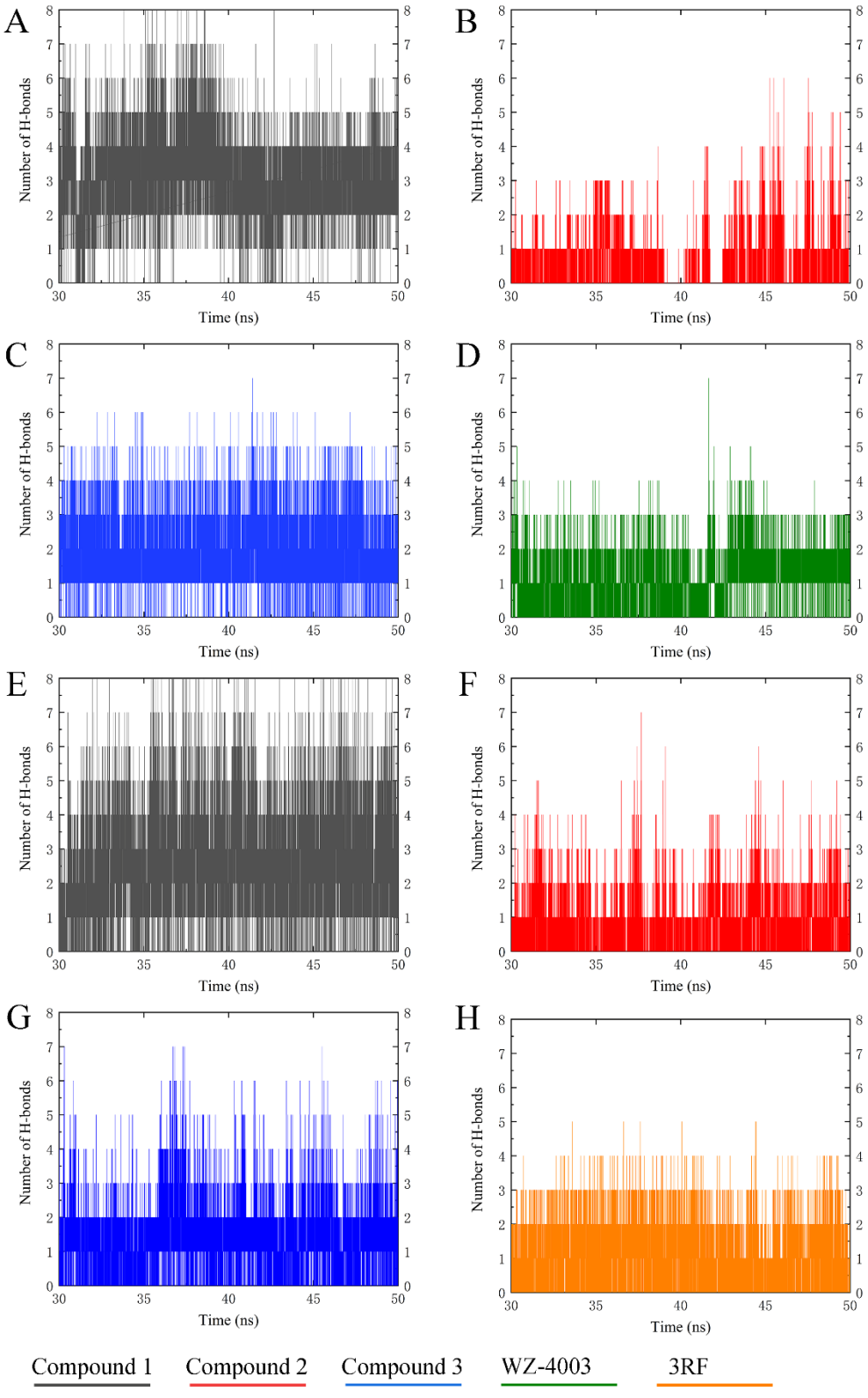


Fig. S4 Hydrogen bond profiles of NUAK1 in complex with (A-C) compound 1-3 and (D) WZ-4003, hydrogen bond profiles of ULK1 in complex with (E-G) compound 1-3 and (H) 3RF.

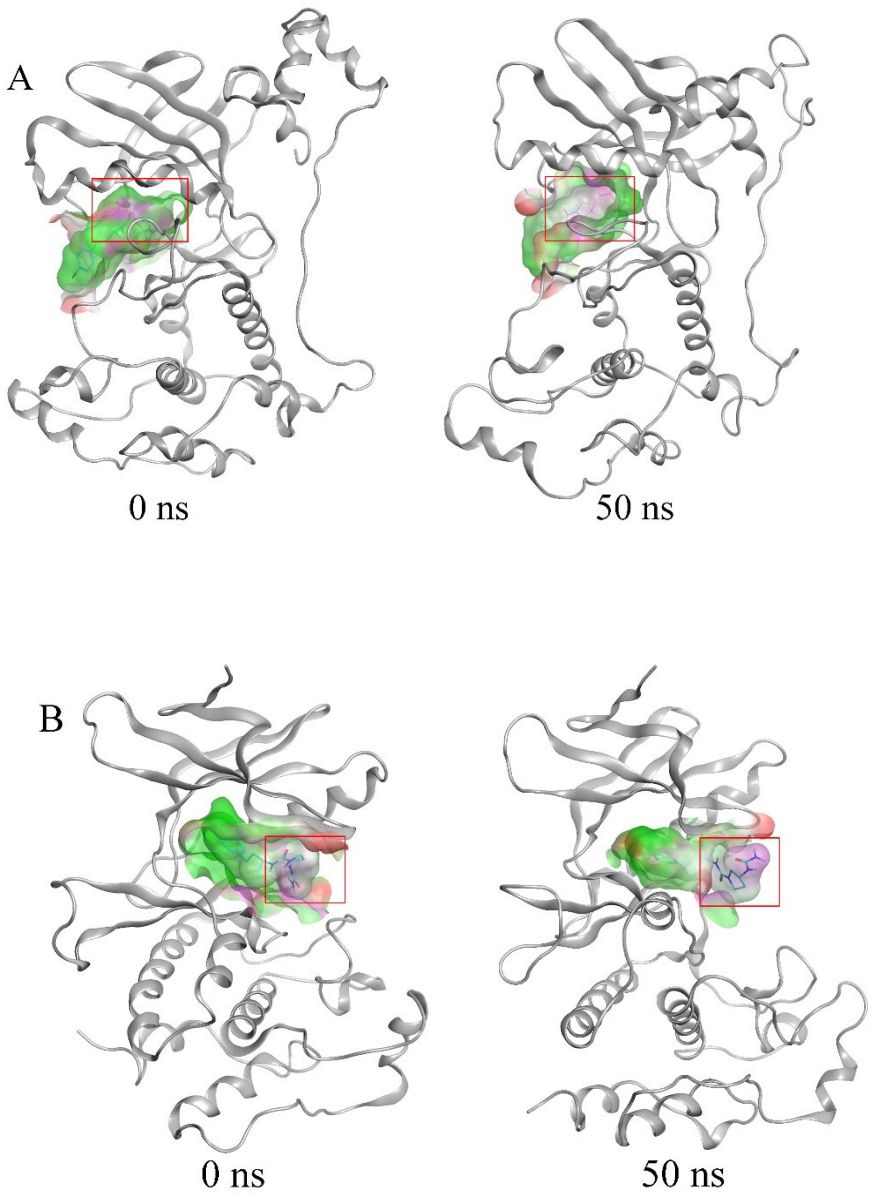


Fig. S5 Snapshots obtained in the initial (0 ns) and final (50 ns) of MD simulations. A (NUAK1-compound 2), B (ULK1-compound 2). Fuchsia: polar hydrophilic area, green: non-polar hydrophobic area.

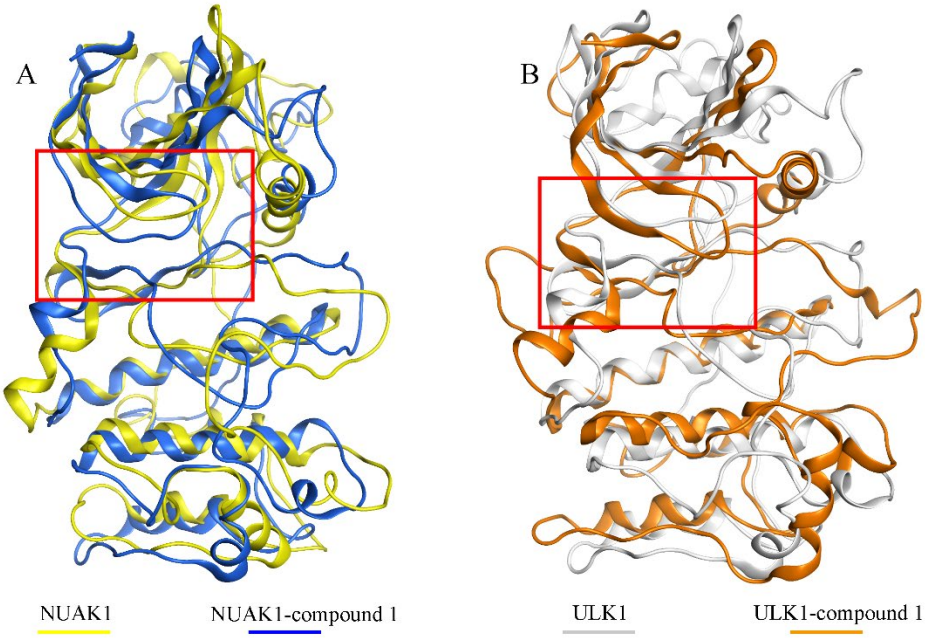


Fig. S6 Structural superimpositions of (A) NUAK1 final conformations and (B) ULK1 final conformations of MD simulations (50ns).

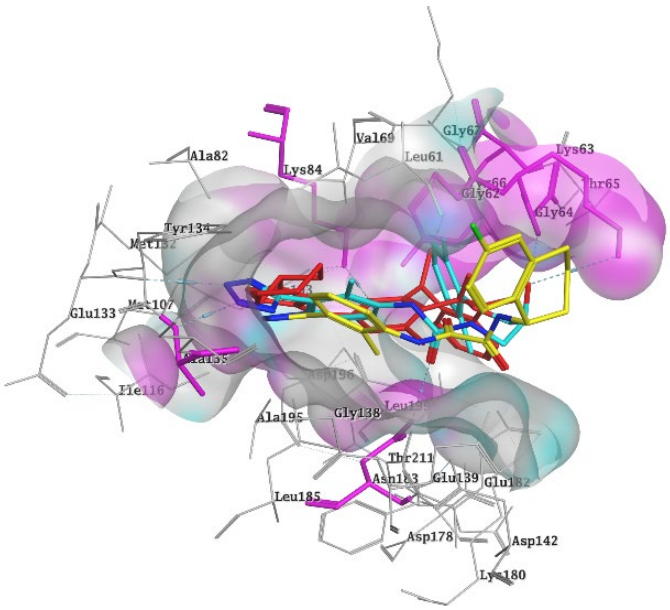


Fig. S7 Binding modes of HMD-17-51 (red), compound 1 (blue) and 3 (yellow) with NUAK1. The three compounds have similar binding poses in the binding site.