Supplementary Information

Small-molecule allosteric modulators of the protein kinase PDK1 from structure-based docking

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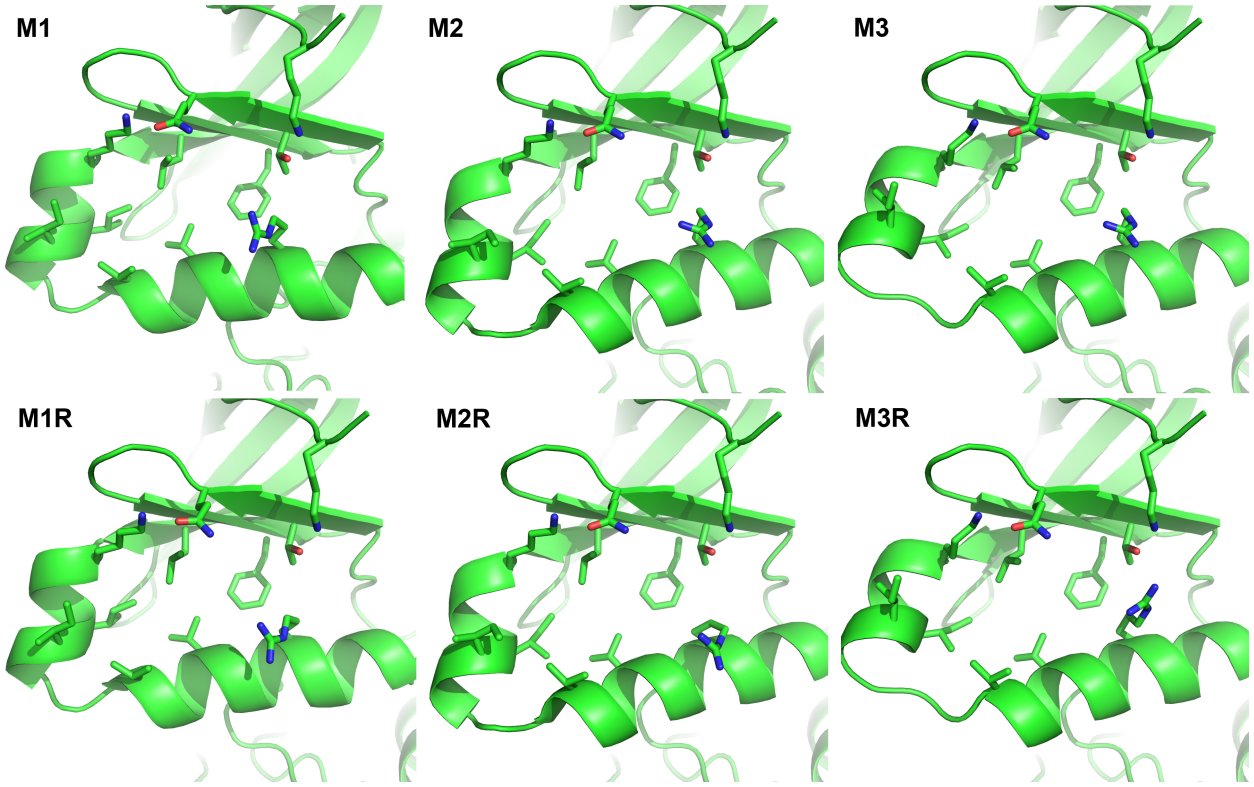
Supplementary Figure 1. Six PIF pocket models used for virtual screening.



Supplementary Figure 2. Experiments to rule out compound aggregation. Dynamic light scattering reveals no formation of colloidal particles by compounds 1, 3, and 4 in the (**A**) FP competitive binding assay buffer or (**B**) kinase activity assay buffer. (**C**) Cruzain enzyme activity assay demonstrates detergent-reversible inhibition by a known small-molecule aggregator, but no effect of compounds **1** and **3**. Error bars are SD (n = 2).

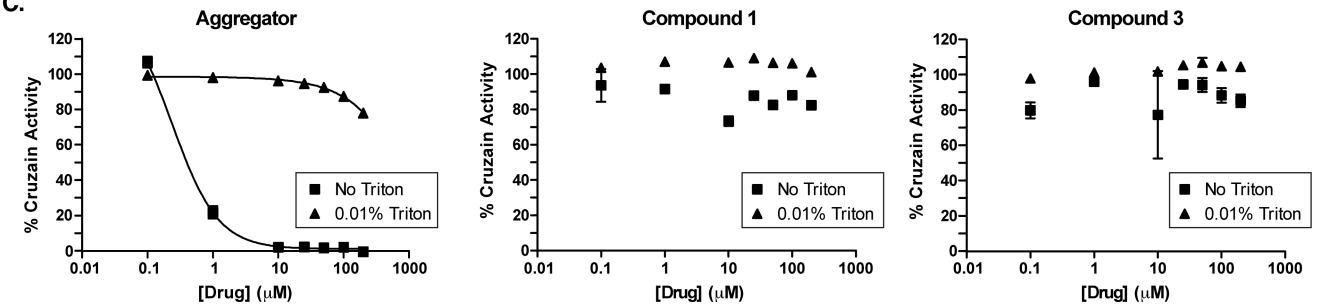
A.

sample	DLS intensity (kcount/s)	Radius (nm)
FP assay buffer	16.2	2.4
aggregator	2066.3	44.0
100 μM compound **1**	20.7	2.8
300 μM compound **1**	27.5	11.2
300 μM compound **3**	12.2	0.6
200 μM compound **4**	21.7	1.4

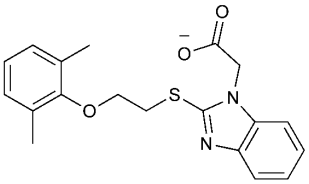
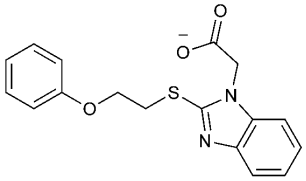
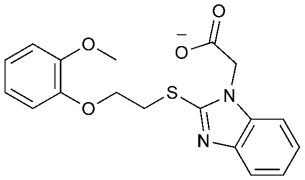
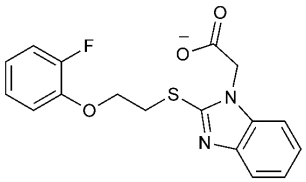
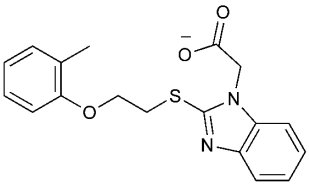
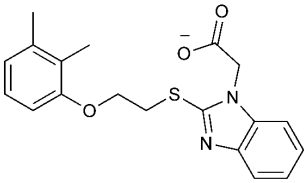
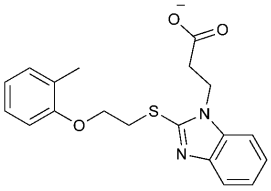
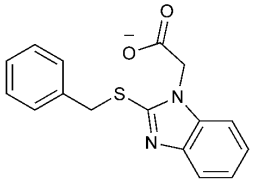
B.

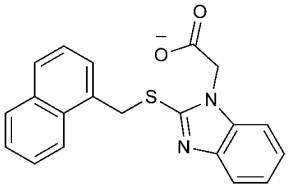
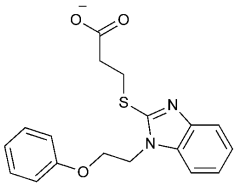
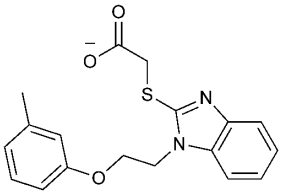
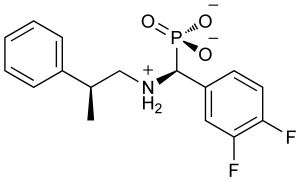
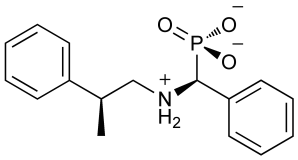
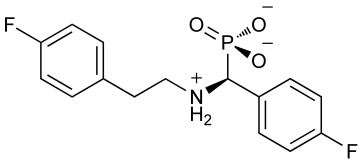
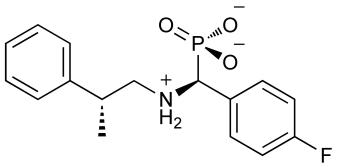
sample	DLS intensity (kcount/s)	Radius (nm)
Activity assay buffer	11.0	2.0
aggregator	3376.5	55.7
200 μM compound **1**	16.2	0.8
200 μM compound **3**	12.2	0.6
200 μM compound **4**	76.1	1.5

C.



Supplementary Table 1. Docking ranks and binding affinities for the 15 PIF pocket ligand analogs.

	Compound structure [a]	Docking rank [b]						K_d μM[c] (95% CI)	LE [d]
		M1	M1R	M2	M2R	M3	M3R		
4		1	9	2	26	15	3	8.4 (7.6-9.1)	0.28
5		9	45	27	198	72	17	>200	-
6		13	389	238	410	194	192	>200	-
7		15	90	24	169	18	34	>200	-
8		18	433	188	437	108	159	50 (41-60)	0.25
9		60	375	95	416	80	117	110 (100-120)	0.22
10		4	1	1	12	29	55	74 (62-87)	0.23
11		68	363	116	408	71	74	>200	-

	Compound structure [a]	Docking rank [b]						K_d mM[c] (95% CI)	LE [d]
		M1	M1R	M2	M2R	M3	M3R		
12		5	89	6	160	1	9	120 (100-140)	0.22
13		73	122	37	170	91	24	120 (110-130)	0.24
14		55	232	31	165	16	6	75 (66-85)	0.24
15		65	83	223	89	249	53	83 (76-90)	0.25
16		63	410	470	2	405	115	150 (140-170)	0.25
17		271	306	407	7	466	59	>200	-
18		412	221	468	164	455	447	>200	-

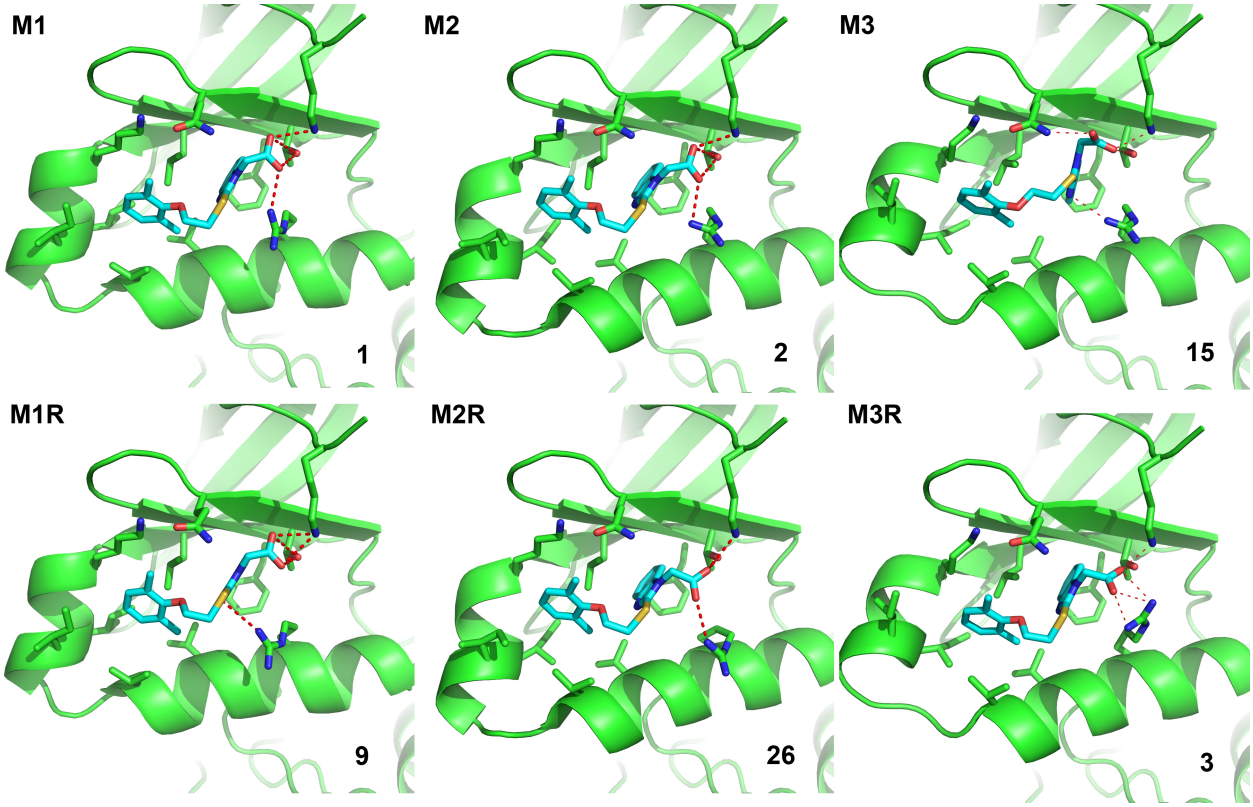
[a] Charged states are depicted assuming a physiological pH of 7.4.

[b] Ranks reported do not consider the molecules discarded by the three geometry filters described in the text.

[c] K_d was calculated from the IC_{50} in the FP assay using an equation that accounts for ligand depletion.[1]

[d] Ligand Efficiency (LE) is calculated as experimental binding energy (ΔG, kcal/mol) per non-hydrogen atom.

Supplementary Figure 3. The docking pose of compound **4** across the 6 PIF pocket models. The docking rank out of 518 analogs is shown at the bottom right of each panel.



Supplementary Table 2. Data collection and refinement statistics (molecular replacement)

	PDK1+ATP+RF4 PDB ID: 4XX9
Data collection[a]	
Space group	C 1 2 1
Cell dimensions	
a, *b*, *c* (Å)	148.3, 44.4, 47.6
α, β, γ (°)	90, 101.1, 90
Resolution (Å)	46.75-1.40 (1.45-1.40)
R_{merge}	0.065 (0.828)
I / σ	18.9 (1.92)
Completeness (%)	99.7 (99.6)
Redundancy	3.8 (3.6)
Refinement	
Resolution (Å)	1.40 (1.45-1.40)
No. reflections	59563 (5875)
R_{work} / R_{free}	12.9 / 16.5
No. atoms	5294
Protein	4880
Ligand/ion	138
Water	276
Average *B* factors (Å^2):	
Protein	21.3
Ligand/ion	30.6
Water	33.6
R.m.s. deviations:	
Bond lengths (Å)	0.012
Bond angles (°)	1.468
Ramachandran statistics[b] (%):	
Favored	98.3
Allowed	1.7
Outliers	0

[a]Values in parentheses are for highest-resolution shell. [b]As calculated by Molprobity.