Supplementary Material

The small molecule tool (*S*)-(-)-Blebbistatin: novel insights of relevance to myosin inhibitor design

Cristina Lucas-Lopez[1]†, John S. Allingham[2]†, Tomas Lebl[1], Christopher P.A.T. Lawson[1], Ruth Brenk[3], James R. Sellers[4], Ivan Rayment[2]*, Nicholas J. Westwood[1]*

Figure S1 – Cyclisation of amidine **11** to give quinolone **16** was observed to be slow compared with the equivalent reaction for the other amidine analogues. A possible reason may be the preferred formation of the lithium enolate **S1b** conformer formed on deprotonation of **11** by LiHMDS as shown below based on the assumption that the N-Li group is the largest group due to formation of an $(NLi)_n$ aggregate.

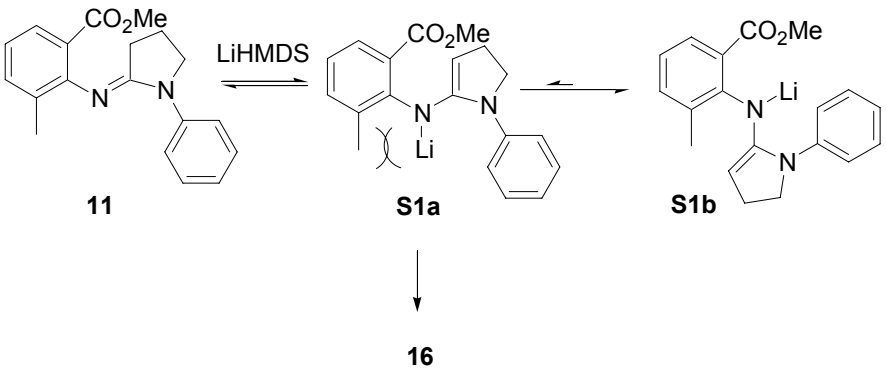

Table S1. Observed percentage of maximal ATPase activity as a function of chemical structure against rabbit skeletal myosin S1 fragment at a concentration of 5 and 50μM and *Dictyostelium* myosin II S1dC fragment at 50μM. No inhibition was observed by any of the analogues at 5μM with the S1dC fragment. [a] number in parentheses is the standard deviation assessed for n = 3.

Analogue	SkS1		S1dC
	5 μM	50 μM	50 μM
1	6.6 (1.2)[a]	3.0 (1.5)	8.5
4	13.9 (0.9)	4.6 (0.1)	12.2
5	14.5 (1.2)	5.9 (1.3)	10
6	69.2 (9.2)	28.2 (2.3)	65
7	16.5 (1.3)	4.8 (0.7)	11.7

Table S2. Data collection and refinement statistics for the co-crystal structures

	4	**5**	**6**	**7**
Data collection				
Space group	$C222_1$	$C222_1$	$C222_1$	$C222_1$
Cell dimensions				
a, *b*, *c* (Å)	88.2, 146.0, 153.1	88, 147.8, 153.4	87.8, 145.4, 152.8	88.7,146.3, 154.1
α, β, γ (°)	90,90,90	90,90,90	90, 90, 90	90,90,90
Resolution (Å)	50-2.0 (2.07-2.0)	50-2.2 (2.28-2.2)	50-2.15 (2.23-2.15)	50-2.1 (2.18-2.1)
R_{merge}	7.1 (23.4)	7.3 (22.4)	7.7 (26.9)	6.7 (27.2)
$I / \sigma I$	24.3 (5.1)	20.1 (4.9)	19.5 (4.4)	23.4 (4.3)
Completeness (%)	99.9 (99.7)	99.1 (94.8)	99.7 (99.6)	98.4 (98.8)
Redundancy	5.3 (4.8)	5.7 (4.5)	4.9 (4.8)	5.6 (5.5)
Refinement				
Resolution (Å)	50-2.0 (2.07-2.0)	50-2.2 (2.28-2.2)	50-2.15 (2.23-2.15)	50-2.1 (2.18-2.1)
No. reflections	353590 (66638)	288277 (50604)	257141 (52873)	322203 (57734)
R_{work} / R_{free}	0.183/0.223	0.192/0.244	0.174/0.219	0.191/0.238
No. atoms				
S1dC	5561	5529	5529	5587
Blebbistatin	22	22		21
MgADP-Vanadate	33	33	33	33
Water	728	597	583	500
B-factors				
S1dC	25.0	34.9	23.6	28.9
Blebbistatin	20.6	35.8		19.3
MgADP-Vanadate	14.1	22.9	12.3	15.7
Water	39.0	41.6	31.8	35.1
R.m.s deviations				
Bond lengths (Å)	0.016	0.022	0.017	0.02
Bond angles (°)	1.39	1.826	1.489	1.605

Data for each S1dC-Blebbistatin Analogue complex was collected from a single crystal. *Highest resolution shell is shown in parenthesis.