

Supporting Information

Receptor-ligand interaction based virtual screening for novel Eg5/KSP (Kinesin Spindle Protein) inhibitors

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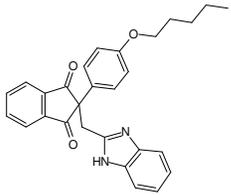
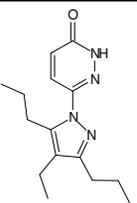
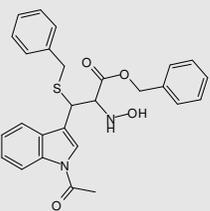
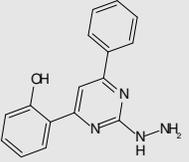
² School of Science, Korea University of Science and Technology, 52 Eoeun dong, Yuseong-gu, Daejeon 305-333, Republic of Korea.

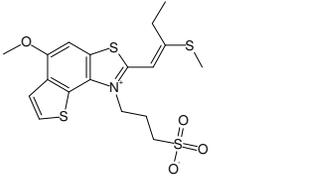
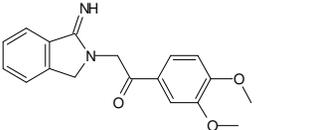
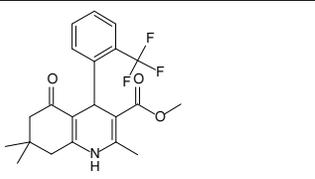
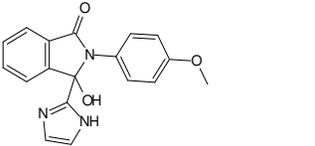
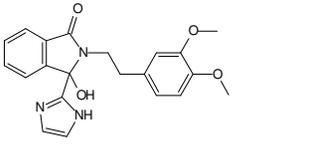
³ Institute for Structural Biology (CEA-CNRS-UJF), 41 rue Jules Horowitz, 38027 Grenoble Cedex 1, France,

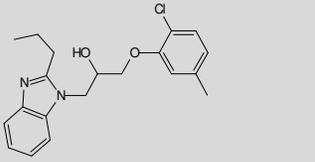
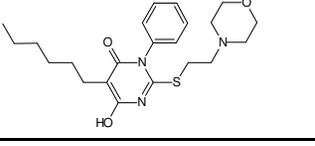
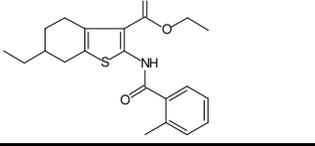
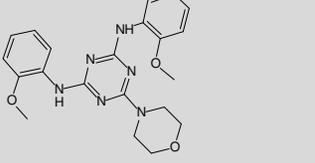
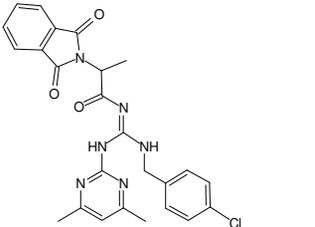
⁴ The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, Scotland, UK.

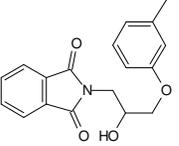
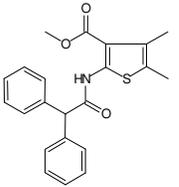
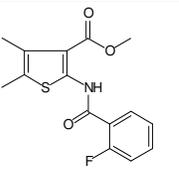
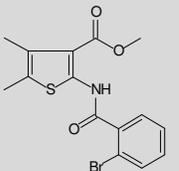
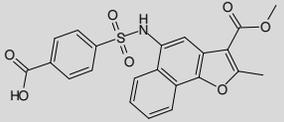
Table S1: 54 hits obtained by virtual screening investigated in proliferation assays using DU145 and HT29 tumor cell lines. Compounds were first tested at three concentrations between 1 μ M and 100 μ M. For potent compounds the GI₅₀ values were determined in triplicate. Compounds shaded in light gray are active in proliferation assays or inhibit the Eg5 ATPase activity. Compounds shaded in dark gray inhibit Eg5 ATPase activity and induce monoastral spindles in HeLa cells. Compound numbers coloured in violet, blue or green belong to the quinazoline-, thioxoimidazolidin- or purin/triazolepyrimidine- scaffolds.

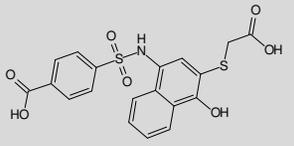
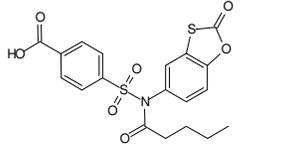
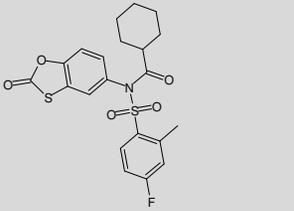
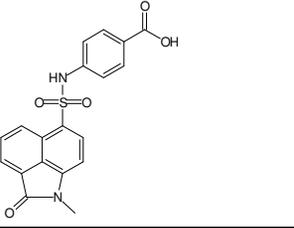
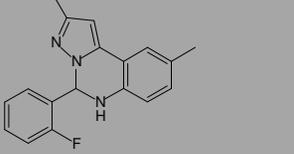
Cmpd No	Chemical structure	Purity % (UV254) LC/MS	DU145				HT29				Inhibition Eg5 ATPase activity IC ₅₀ (μ M) MIA (%)	Monoastral spindle phenotype at 25 μ M inhibitor
			100 μ M	10 μ M	1 μ M	GI ₅₀ (μ M)	100 μ M	10 μ M	1 μ M	GI ₅₀ (μ M)		

1		100	55.8	18.5	11.7	n.d.	48.9	12.5	7.5	n.d.	n.i.	n.d.
2		n.s.	15.6	12.3	11.9	n.d.	12.2	3.8	4.6	n.d.	n.i.	n.d.
3		92.2	49.5	2.5	8.9	36.5 ± 2.9	82.5	18.4	4.8	11.3 ± 2.6	n.i.	n.d.
4		97.7	79.3	24.0	5.0	36.8 ± 2.7	89.6	84.7	5.4	10.7 ± 1.7	n.i.	n.d.

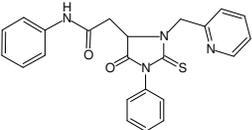
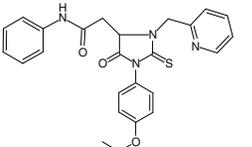
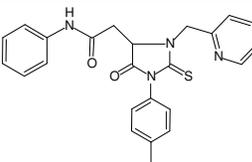
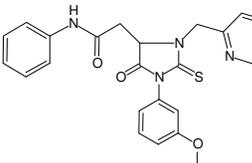
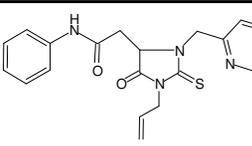
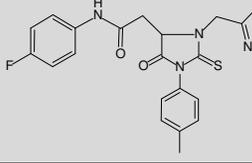
5		100	37.9	14.9	12.7	n.d.	22.9	13.3	7.4	n.d.	n.i.	n.d.
6		100	27.2	14.2	10.8	n.d.	62.4	10.6	2.7	n.d.	n.i.	n.d.
7		n.s.	43.7	7.8	9.6	n.d.	52.0	1.9	3.6	n.d.	n.i.	n.d.
8		99	19.5	13.3	8.7	n.d.	13.6	-0.7	0.6	n.d.	n.i.	n.d.
9		100	20.1	16.9	13.9	n.d.	8.5	4.4	1.7	n.d.	n.i.	n.d.

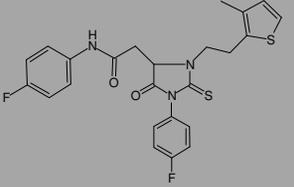
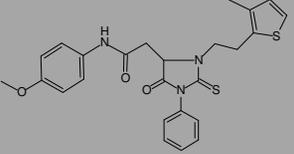
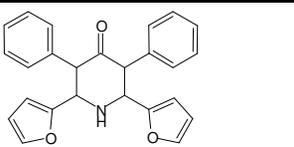
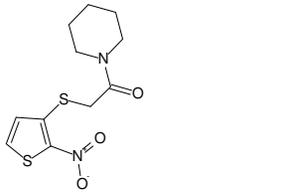
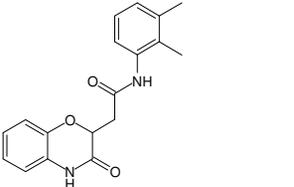
10		92.1	53.9	21.6	8.7	41.5 ± 3.1	91.4	5.3	4.1	26.1 ± 1.4	n.i.	n.d.
11		n.s.	19.7	11.3	13.5	n.d.	15.1	3.5	3.7	n.d.	n.i.	n.d.
12		100	7.1	9.4	13.5	n.d.	69.3	5.5	3.2	n.d.	n.i.	n.d.
13		97.2	40.3	17.7	20.8	>100	91.6	88.7	25.8	0.7 ± 0.1	n.i.	n.d.
14		90	-8.6	9.8	12.5	n.d.	12.1	18.3	6.7	n.d.	n.i.	n.d.

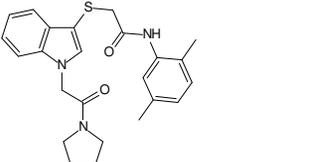
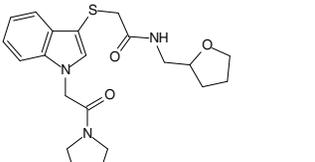
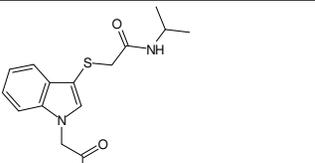
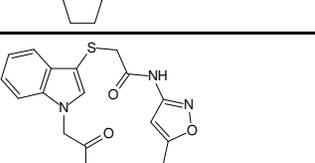
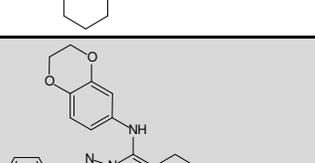
15		n.s.	20.9	13.1	13.9	n.d.	16.3	8.1	3.0	n.d.	n.i.	n.d.
16		n.s.	0.9	11.3	14.8	n.d.	41.2	13.1	8.2	n.d.	n.i.	n.d.
17		91.7	42.3	3.5	7.6	n.d.	68.7	25.1	4.5	n.d.	n.i.	n.d.
18		92.3	48.2	-0.8	9.3	>100	87.8	4.6	2.6	33.7 ± 2.7	n.i.	Multiple cells with misaligned chromosomes
19		n.s.	-9.9	12.2	12.1	n.d.	10.2	-1.2	7.3	n.d.	79.1 ± 22.4 (80)	n.m.s.o.

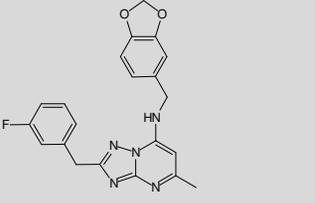
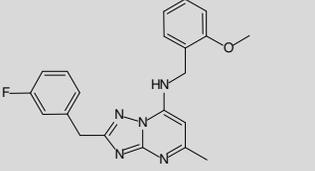
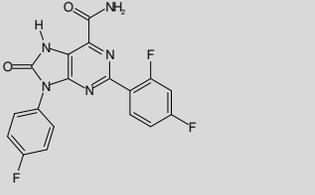
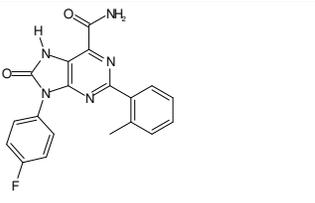
20		n.s.	89.5	26.6	10.5	21.0 ± 2.4	91.9	90.1	8.4	4.4 ± 0.3	n.i.	Interphase cells affected; MT bundles; no mitotic cells
21		91.5	-8.5	1.1	13.4	n.d.	61.7	-2.8	6.8	n.d.	n.i.	n.d.
22		96	87.9	-0.4	13.4	41.2 ± 2.2	90.3	14.5	7.1	19.4 ± 4.0	n.d.	n.m.s.o.
23		n.s.	21.7	12.9	14.8	n.d.	18.9	4.3	10.3	n.d.	n.i.	n.d.
24		96.4	46.5	16.3	13.6	n.d.	78.8	3.9	4.1	n.d.	6.3 ± 1.3 (60)	n.m.s.o. Monoastral spindles at 50 μM

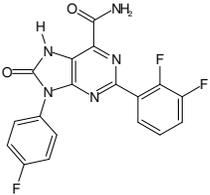
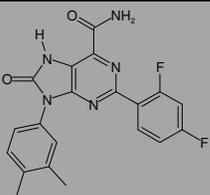
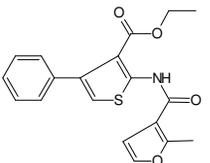
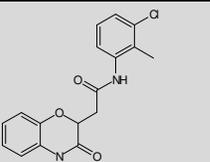
25		99.1	55.0	10.4	13.7	n.d.	46.3	3.7	6.2	n.d.	n.i.	n.d.
26		92.5	91.8	14.1	15.3	31.0 ± 1.5	93.7	8.9	10.0	20.5 ± 2.0	n.i.	n.d.
27		n.s.	63.1	21.6	17.3	38.8 ± 2.9	85.8	17.3	13.3	23.1 ± 1.2	n.i.	n.d.
28		91.3	51.3	-3.0	9.0	41.6 ± 0.7	81.9	3.6	8.8	30.4 ± 2.8	n.i.	n.d.
29		99.3	79.5	3.7	6.8	39.4 ± 2.2	83.6	16.0	4.6	27.5 ± 1.9	n.i.	n.d.
30		98	75.1	5.3	8.3	34.3 ± 1.9	85.4	17.3	6.0	17.7 ± 2.8	1.0 ± 0.1 (60)	~ 50% monoastral spindles

31		98.1	53.9	8.8	6.5	n.d.	32.9	5.1	8.4	n.d.	n.i.	n.d.
32		97.8	59.7	36.6	12.8	n.d.	78.6	38.3	10.6	n.d.	n.i.	n.d.
33		99.6	59.8	10.3	12.9	n.d.	74.2	10.7	9.3	n.d.	n.i.	n.d.
34		97.8	61.6	8.2	12.2	n.d.	53.7	-2.8	0.9	n.d.	n.i.	n.d.
35		100	53.9	12.2	14.4	n.d.	52.3	-10.6	-0.7	n.d.	n.i.	n.d.
36		98.3	79.0	10.2	12.7	46.3 ± 1.8	82.9	4.5	1.1	37.7 ± 1.3	n.i.	n.d.

37		91.5	84.6	-4.2	7.7	31.0 ± 0.8	92.7	11.4	6.7	16.7 ± 1.1	0.6 ± 0.2 (80)	Most cells show monoastal spindles
38		97	71.3	-7.3	12.7	42.3 ± 1.3	86.7	10.1	2.1	19.6 ± 3.9	2.4 ± 0.4 (60)	Very few monoastal spindles
39		96.0	-2.4	4.3	9.1	n.d.	57.1	6.7	2.2	n.d.	n.i.	n.d.
40		n.s.	39.7	15.9	12.5	n.d.	71.6	9.0	3.3	n.d.	n.i.	n.d.
41		n.s.	24.0	24.6	10.5	n.d.	51.0	54.2	5.7	n.d.	n.i.	n.d.

42		95.0	14.1	-3.8	13.0	n.d.	56.3	0.6	0.7	n.d.	n.i.	n.d.
43		93.8	26.1	9.3	10.1	n.d.	20.6	6.8	0.8	n.d.	n.i.	n.d.
44		96.3	28.2	9.4	10.0	n.d.	24.5	5.7	2.0	n.d.	n.i.	n.d.
45		99.1	28.1	10.4	9.4	n.d.	37.0	7.0	1.3	n.d.	n.i.	n.d.
46		100	91.3	11.4	14.1	33.7 ± 0.7	95.5	7.4	1.9	24.4 ± 0.7	n.i.	n.d.

47		n.s.	91.9	12.0	11.3	32.1 ± 1.1	96.5	5.5	1.3	18.7 ± 2.5	n.i.	n.d.
48		n.s.	95.3	6.5	12.3	40.7 ± 4.8	96.1	4.7	4.1	28.6 ± 2.8	n.i.	n.d.
49		100	39.5	21.6	33.6	60.7 ± 10.7	55.1	57.8	16.8	7.9 ± 1.1	n.i.	n.d.
50		97.9	41.3	38.3	6.7	n.d.	59.5	35.5	2.2	n.d.	n.i.	n.d.

51		100	24.4	18.0	13.4	n.d.	65.1	32.2	0.9	n.d.	n.i.	n.d.
52		95	90.9	54.2	27.2	8.7 ± 2.1	93.7	58.2	-2.4	9.3 ± 1.1	41.5 ± 4.9 (60)	n.m.s.o.
53		n.s.	43.4	30.2	17.1	n.d.	57.9	41.1	6.5	n.d.	n.i.	n.d.
54		n.s.	23.8	26.6	17.3	>100	58.4	56.8	17.6	10.0 ± 2.6	n.i.	n.d.

n.s.: no sample to assess purity level; n.d.: not determined; n.i.: no inhibition; MIA: Maximum inhibition attained; n.m.s.o.: no monoastal spindles observed.

Figure S1. Concentration dependence of induction of monastral spindles by the indicated compounds. After 8 h exposure to drugs HeLa cells were fixed and stained for immunofluorescence microscopy as described in figure 3. Mitotic cells were then counted by microscopy and the cells with monastral spindles were scored as a percentage of total mitotic cells.

