Electronic supplementary information

Content

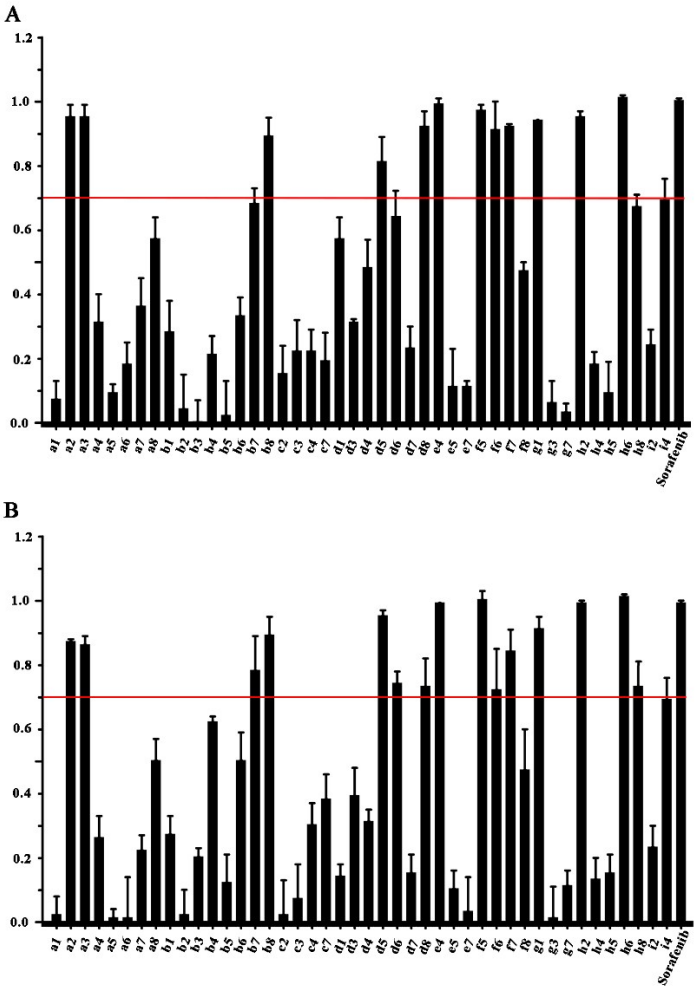


Fig. S1 Single point inhibition ratios of 44 compounds in HeLa and MCF-7 cells at100 μM.

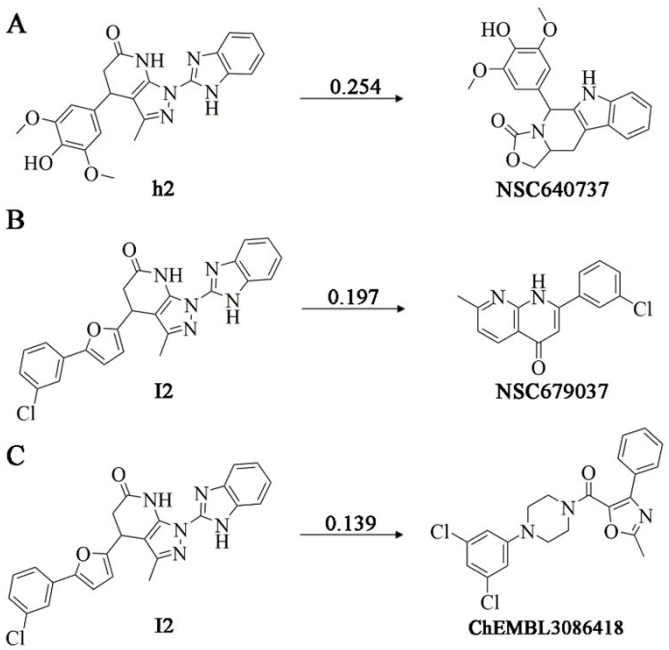


Fig. S2 Similarity analysis of the confirmed anticancer agent hits in this study and their nearest neighbors in the NCI-60 actives set (A and B) and a tubulin inhibitor from the ChEMBL database (C). All calculations were conducted in Discovery Studio 3.5.

Table S1 The 44 virtual hits purchased from ChemDiv for experimental validation.

Compound name	Compound ID	NB_LCFP_6#EstPGood score	NB_FCFP_8#EstPGood score	Cscore
a1	0806-0330	0.632	0.544	0.586
a2	0806-0348	0.733	0.563	0.643
a3	0851-0574	0.747	0.545	0.638
a4	0927-0061	0.675	0.559	0.615
a5	0927-0170	0.661	0.614	0.637
a6	0927-0171	0.682	0.573	0.626
a7	0927-0175	0.666	0.628	0.647
a8	1013-0084	0.670	0.593	0.630
b1	1071-0067	0.688	0.632	0.659
b2	1326-1810	0.705	0.704	0.704
b3	1809-0060	0.699	0.591	0.643
b4	1995-0023	0.691	0.586	0.636
b5	3078-0586	0.831	0.686	0.755
b6	3170-5253	0.707	0.577	0.639
b7	3229-0932	0.678	0.622	0.649
b8	3229-0935	0.717	0.628	0.671
c2	3229-1041	0.677	0.604	0.639
c3	3229-1043	0.622	0.540	0.579
c4	3229-1089	0.602	0.551	0.576
c7	3229-2575	0.640	0.579	0.609
d1	4380-0074	0.663	0.627	0.645
d3	6539-2712	0.672	0.571	0.620
d4	6969-0995	0.786	0.626	0.702
d5	8001-4431	0.665	0.555	0.607
d6	8004-1720	0.729	0.645	0.686
d7	8004-1864	0.625	0.548	0.585
d8	8006-1659	0.660	0.543	0.599
e4	8006-9147	0.749	0.613	0.678
e5	8007-0268	0.676	0.568	0.620
e7	8008-5344	0.785	0.641	0.710
f5	8011-9990	0.762	0.630	0.693
f6	8012-3656	0.768	0.624	0.692
f7	8012-3660	0.740	0.624	0.679
f8	8012-4328	0.768	0.623	0.692
g1	8012-4943	0.718	0.615	0.664
g3	8012-6677	0.658	0.551	0.602
g7	8015-3908	0.633	0.556	0.593
h2	8019-6627	0.641	0.696	0.668
h4	8525-0874	0.746	0.719	0.732
h5	8561-08556	0.735	0.685	0.710
h6	8640-0511	0.680	0.591	0.634
h8	E859-0669	0.610	0.546	0.577

i2	L036-0394	0.677	0.741	0.708
i4	L971-0108	0.601	0.534	0.567

Table S2 *In vitro* cytotoxicity assay results for 61 compounds in HeLa and MDA-MB-231 cells at

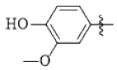
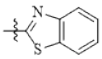
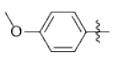
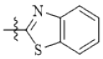
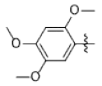
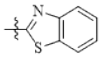
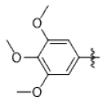
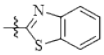
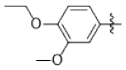
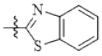
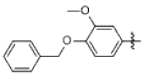
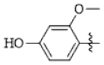
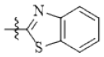
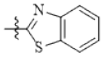
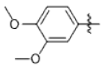
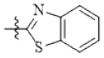
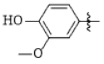
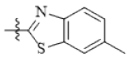
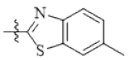
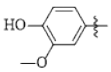
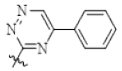
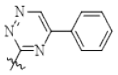
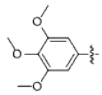
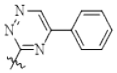
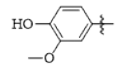
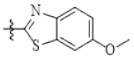
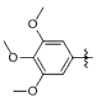
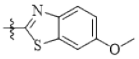
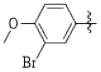
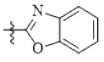
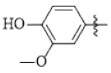
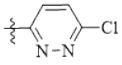
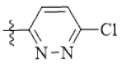
100 μM.

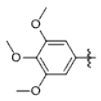
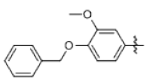
Compound	Compound ID	Inhibition ratio [a]	
		MDA-MB-231	HeLa
A3	8019-5763	0.01 ± 0.21	0.16 ± 0.08
A4	8019-6102	0.31 ± 0.16	0.3 ± 0.08
A5	8019-6103	0.27 ± 0.00	0.38 ± 0.00
A6	8019-6491	0.36 ± 0.02	0.65 ± 0.05
A7	8019-6608	0.98 ± 0.02	0.93 ± 0.05
A8	8019-6609	1.00 ± 0.00	0.92 ± 0.06
B1	8019-6610	0.96 ± 0.05	0.77 ± 0.24
B2	8019-6612	0.99 ± 0.01	0.96 ± 0.03
B3	8019-6613	0.94 ± 0.05	0.88 ± 0.00
B4	8019-6614	0.96 ± 0.01	0.85 ± 0.10
B5	8019-6615	1.00 ± 0.01	1.00 ± 0.00
B6	8019-6628	1.00 ± 0.01	0.99 ± 0.01
B7	8019-6629	0.99 ± 0.00	0.88 ± 0.00
B8	8019-6630	1.00 ± 0.01	0.95 ± 0.07
C1	8019-7263	0.34 ± 0.00	0.53 ± 0.14
C3	D292-0021	0.39 ± 0.08	0.43 ± 0.23
C4	D292-0022	0.38 ± 0.03	0.43 ± 0.01
C5	D292-0024	0.33 ± 0.01	0.43 ± 0.03
C6	D292-0268	0.58 ± 0.11	0.39 ± 0.02
C7	D292-0269	0.57 ± 0.05	0.53 ± 0.10
C8	D292-0271	0.30 ± 0.09	0.76 ± 0.25
D1	D292-0278	0.38 ± 0.00	0.58 ± 0.00
D2	D292-0283	0.37 ± 0.06	0.49 ± 0.08
D3	D292-0284	0.11 ± 0.00	0.30 ± 0.11
D4	D292-0287	0.03 ± 0.05	0.28 ± 0.04
D5	D292-0303	0.47 ± 0.00	0.63 ± 0.04
D6	D302-0061	0.77 ± 0.10	0.67 ± 0.23
D7	D302-0437	0.23 ± 0.05	0.35 ± 0.01
D8	D361-0331	0.27 ± 0.03	0.44 ± 0.06
E1	D361-0859	ND	ND
E2	D361-0922	0.18 ± 0.22	0.32 ± 0.27
E3	D361-0925	0.88 ± 0.06	0.60 ± 0.10
E4	D361-0940	0.33 ± 0.02	0.43 ± 0.03
E5	D361-0943	0.29 ± 0.07	0.43 ± 0.05
E6	D361-1027	0.59 ± 0.16	0.38 ± 0.07
F1	J081-0074	0.58 ± 0.07	0.28 ± 0.37
F2	J081-0182	0.37 ± 0.23	0.58 ± 0.04
F3	J081-0207	0.60 ± 0.14	0.01 ± 0.25
F4	J081-0945	ND	ND
F5	J081-2194	0.55 ± 0.08	0.10 ± 0.10
F6	J081-2207	0.72 ± 0.16	0.62 ± 0.02

F7	J081-2208	0.45 ± 0.12	0.46 ± 0.08
F8	J081-2439	0.65 ± 0.10	0.51 ± 0.13
G1	J081-2452	0.45 ± 0.08	0.53 ± 0.08
G2	J081-2453	0.53 ± 0.05	0.62 ± 0.11
G3	J081-2538	0.41 ± 0.02	0.46 ± 0.12
G4	J094-0187	0.92 ± 0.00	0.68 ± 0.25
G5	J094-0188	1.00 ± 0.01	0.91 ± 0.07
G6	J094-0190	1.01 ± 0.02	0.95 ± 0.05
G7	J094-0252	0.18 ± 0.12	0.04 ± 0.17
G8	J094-0625	0.88 ± 0.09	0.82 ± 0.11
H1	J094-0626	0.99 ± 0.01	0.94 ± 0.06
H2	J094-0633	0.92 ± 0.11	0.93 ± 0.05
H3	J094-0640	0.99 ± 0.01	0.87 ± 0.15
H4	J094-0642	1.02 ± 0.01	0.87 ± 0.16
H5	J094-0643	0.99 ± 0.01	0.9 ± 0.08
H6	J094-0644	1.01 ± 0.03	0.86 ± 0.18
H7	J094-0645	1.03 ± 0.02	0.91 ± 0.15
H8	J094-0652	0.99 ± 0.01	0.88 ± 0.13
I1	J094-0653	0.98 ± 0.02	0.94 ± 0.06
I2	J094-0897	1.00 ± 0.01	0.98 ± 0.00
Sorafenib [b]		1.00 ± 0.03	1.00 ± 0.02

[a] Data are presented as the mean values ± SD from experiments conducted in triplicate at three independent times. [b] Positive control drug. ND: not determined.

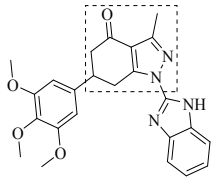
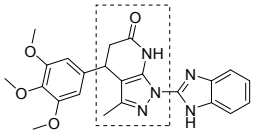
Table S3 SAR analysis of 21 class III analogues obtained by simultaneously changing the R_1 and R_2 groups.

Compound	R_1	R_2	IC_{50} [a] mean ± SD (μM)					
			MDA-MB-231	HeLa	MCF-7	HepG2	CNE2	HCT116
C6			ND	ND	ND	ND	ND	ND
C8			ND	ND	ND	ND	ND	ND
D1			ND	ND	ND	ND	ND	ND
D2			ND	ND	ND	ND	ND	ND
D3			ND	ND	ND	ND	ND	ND
D4			ND	ND	ND	ND	ND	ND
D5			ND	ND	ND	ND	ND	ND
F3			ND	ND	ND	ND	ND	ND
F2			ND	ND	ND	ND	ND	ND
F5			ND	ND	ND	ND	ND	ND
F7			ND	ND	ND	ND	ND	ND
E2			ND	ND	ND	ND	ND	ND
E3			ND	ND	ND	50.20 ± 6.43	56.23 ± 2.22	54.32 ± 1.29
E4			ND	ND	ND	ND	ND	ND
F8			ND	ND	ND	ND	ND	ND
G2			ND	ND	ND	ND	ND	ND
G7			ND	ND	ND	ND	ND	ND
A3			ND	ND	ND	ND	ND	ND
A5			ND	ND	ND	ND	ND	ND

C4			ND	ND	ND	ND	ND	ND
C5			ND	ND	ND	ND	ND	ND

[a] Data are presented as the mean values ± SD from experiments conducted in triplicate at three independent times. ND: not determined.

Table S4 Changes in anticancer activity resulting from scaffold hopping from pyrazolo[3,4-b]pyridin-6-one.

Compound	Scaffold	IC50 [a] mean ± SD (μM)					
		MDA-MB-231	HeLa	MCF-7	HepG2	CNE2	HCT116
A6		ND	ND	ND	ND	ND	ND
B5		11.27 ± 1.18	8.04 ± 1.12	6.83 ± 0.40	9.12 ± 0.48	9.22 ± 1.53	9.04 ± 1.58

[a] Data are presented as the mean values ± SD from experiments conducted in triplicate at three independent times. ND: not determined.

Table S5 Binding free energies of **I2** and tubulin calculated using MM-GBSA method.

Energy terms	Binding free energy (Kcal/mol) (SEM)
ΔE_{vdw} [a]	–54.23 (3.07)
ΔE_{ele} [b]	–19.21 (4.35)
$\Delta E_{pol,solv}$ [c]	39.21 (3.83)
$\Delta E_{nonpol,solv}$ [d]	–5.85 (0.25)
ΔG_{gas} [e]	–73.45 (6.05)
ΔG_{solv} [f]	–33.37 (3.66)
ΔG_{bind} [g]	–40.08 (3.41)

[a] Non-bonded van der Waals; [b] Non-bonded electrostatics; [c] Polar component to solvation; [d] Non-polar component to solvation; [e] Total gas phase energy; [f] Sum of nonpolar and polar contributions to solvation; [g] Final estimated binding free energy calculated from the terms above. Standard errors of the mean are given in parentheses.