Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Discovery of novel morpholino-quinoxalines as PI3Kα inhibitors by pharmacophore-based screening

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PHARMACOPHORE ESTABLISHMENT AND VALIDATION

Materials and methods

A total of 75 PI3K inhibitors were collected from reported literature.[1-10] Among which 25 were selected to be a training set based on the principle of structural diversity and wide coverage of activity range (at least spanning four orders of magnitude and here the IC_{50} values of the training set compounds range from 0.00026 to 200μM, covering seven orders of magnitude). 2D chemical structures of the training set compounds are shown in Fig. S1. The remaining 50 compounds with maximal 3D diversity and continuous bioactivity magnitude constituted a test set, as shown in Fig. S2.

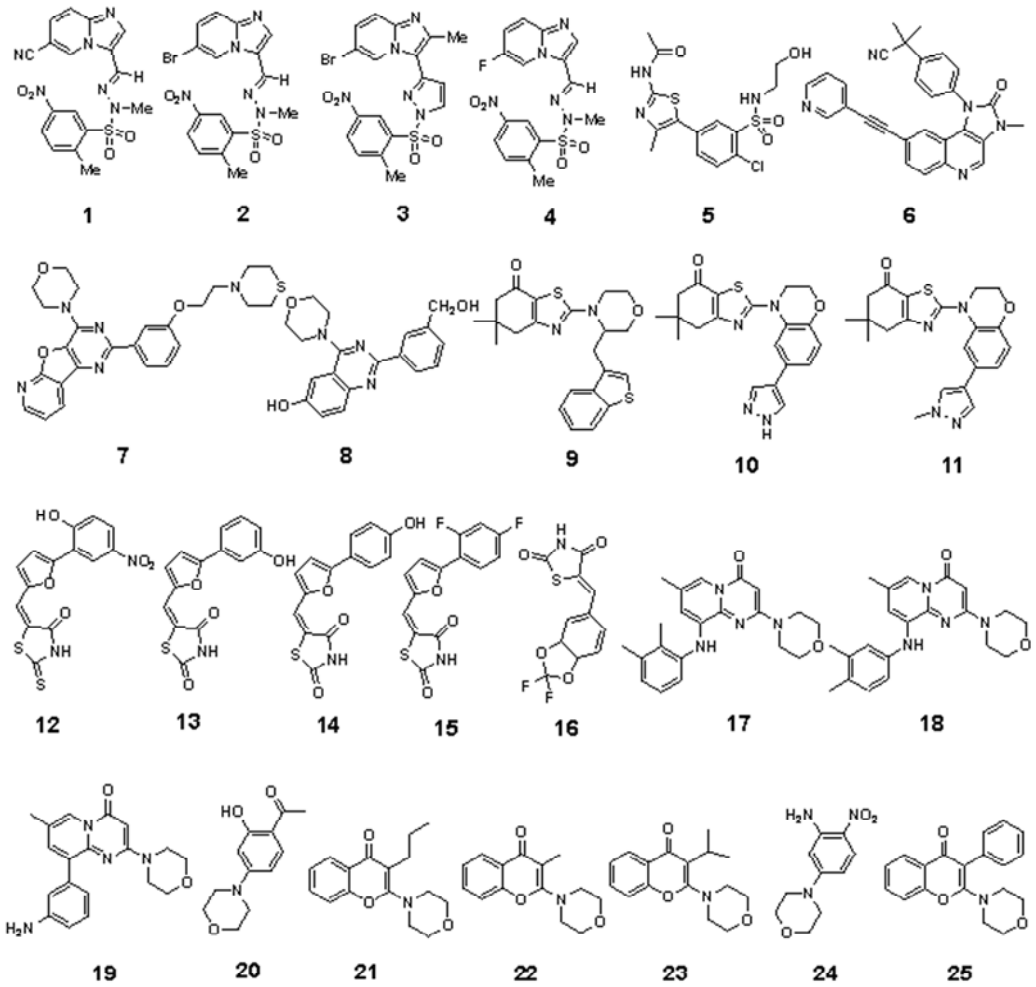

Fig. S1 Chemical structures of the 25 training set compounds.

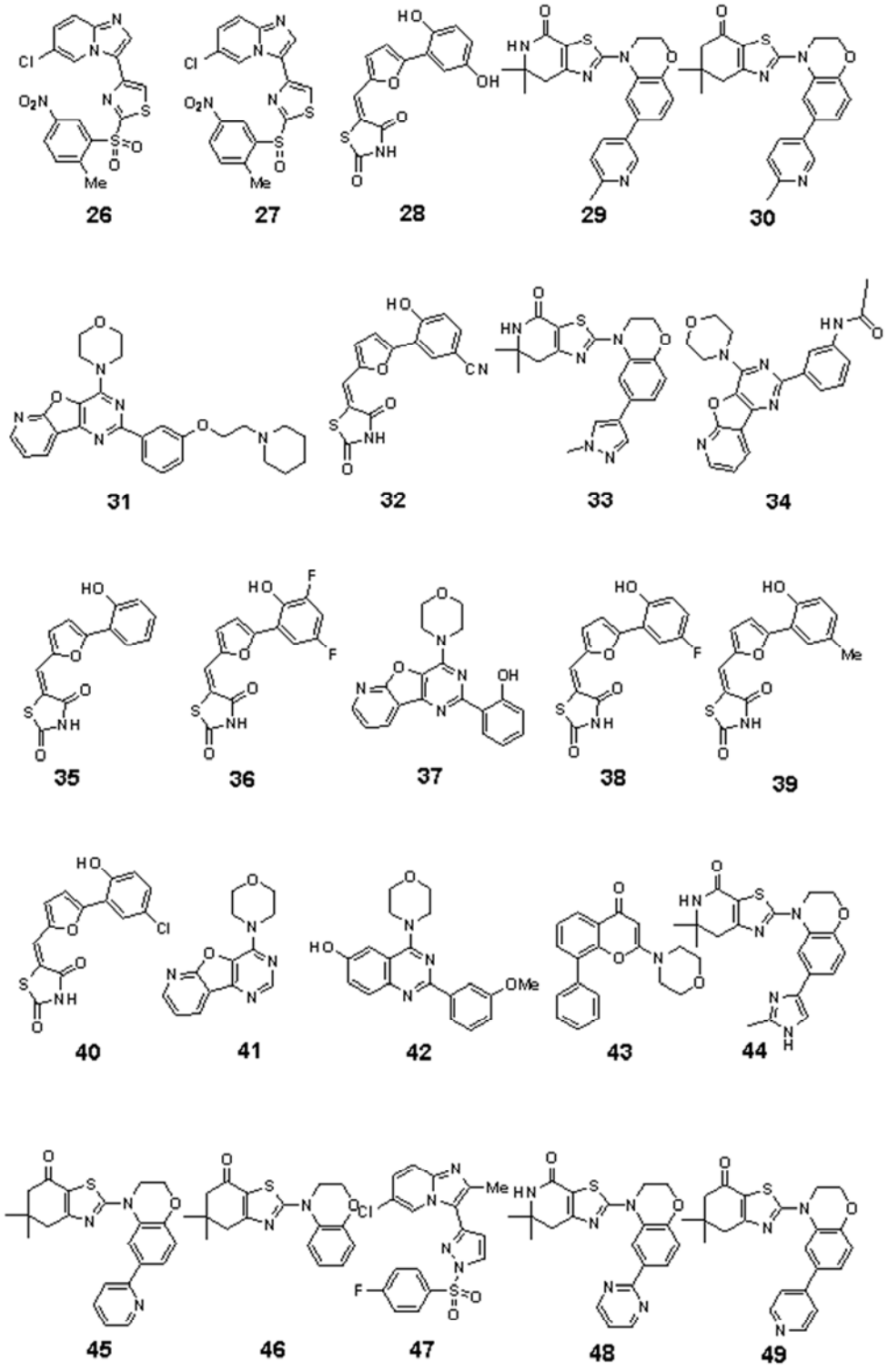

Fig. S2 (Part I) Chemical structures of the 50 test set compounds.

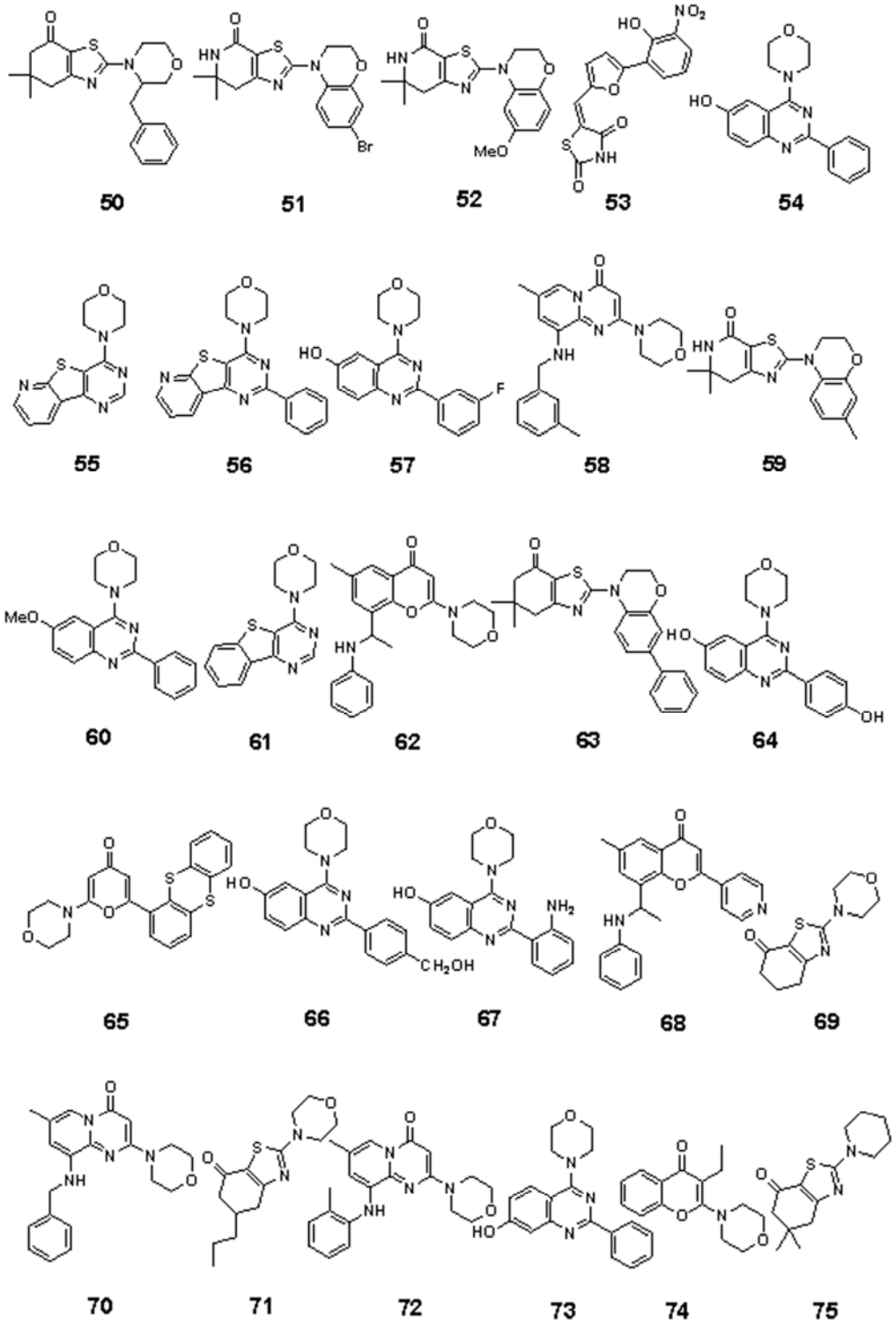

Fig. S2 (Part II) Chemical structures of the 50 test set compounds.

All molecules, either in training set or test set, were built using the 2D and 3D sketcher of Catalyst. A conformational set was generated for each molecule using the "Best-Quality Conformational Analysis" method, based on CHARMm force field. All other parameters used were kept at the default settings. A maximum of 250 conformations within 20kcal/mol as energy threshold from the minimum energy level were saved. The molecules associated with their conformational models were then submitted to catalyst hypothesis generation. Catalyst software package (version 4.11, Accelrys Inc., San Diego, CA) on a Silicon GraphicImage Origin 3800 workstation was used in this study to generate all pharmacophore models.

Generation of pharmacophore hypotheses

The top 10 hypotheses generated are presented in Table S1 together with their statistical parameters. The evaluation of HypoGen pharmacophore models is based on values of cost functions and other statistical parameters calculated by HypoGen module during hypothesis generation. An ideal pharmacophore model should bear a high correlation coefficient, low RMSD value and low total cost which means the total cost should be close to the fixed cost and away from the null cost.[11] The null cost of the 10 top-ranked hypotheses was equal to 228.253 bits, the fixed cost value was 100.84 bits and the configuration cost was 15.629 bits.

Then a classification scheme was used to distinguish compounds based on activity range. The training set compounds were roughly classified into four categories: extremely active ($IC_{50} \leq 0.01\mu M$, ++++), highly active ($0.01\mu M < IC_{50} \leq 1\mu M$, +++), moderately active ($1\mu M < IC_{50} \leq 10\mu M$, ++) and low active ($IC_{50} > 10\mu M$, +). Table S2 shows the experimental and estimated inhibitory activities of the 25 training set compounds. The overall success rate of prediction based on this classification scheme for the training set was 96%. All compounds in the training set were correctly predicted except compound **19**, which originally was low active but was predicted as active.

Validation of pharmacophore model

Test set validation involving 50 compounds was performed to determine Hypo1's capability to successfully identify active compounds. The experimental and estimated IC_{50} values of the test set compounds based on Hypo1 are shown in Table S3. The overall correlation coefficient of the experimental and estimated IC_{50} values of the test set compounds based on Hypo1 is 0.879 (Fig. S3), indicating that Hypo1 has a good predictive ability.

Furthermore, CatScramble randomization test method in the Catalyst software program was implemented to evaluate the statistical correlation of Hypo1. This is achieved by randomizing the activity data associated with the training set compounds, generating pharmacophore hypotheses (here 19 random trials) using the same features and parameters to develop the original pharmacohopore model.[12] The confidence level was set to 95%. The statistical parameters of pharmacophore models obtained in the 19 HypoGen runs as well as the most favorable HypoGen run (Hypo1) are presented in Table S4. The results of CatScramble clearly demonstrate that the original hypothesis was far more superior to the hypotheses generated by randomization test method, which provided confidence on the established pharmacophore model Hypo1.

Table S1 Statistical parameters of the top 10 HypoGen pharmacophore models

Hypo. No.	Total Cost	Cost Diff.	RMSD	Correlation (r)	Features[a]
1	106.864	121.389	0.5316	0.9883	HBA HBA HY RA
2	108.860	119.393	0.6967	0.9792	HBA HBA HY RA
3	109.635	118.618	0.6769	0.9808	HBA HBA HY RA
4	109.819	118.434	0.6610	0.9819	HBA HBA HY RA
5	110.388	117.865	0.7111	0.9788	HBA HBA HY RA
6	110.981	117.272	0.8320	0.9699	HBA HBA HY RA
7	111.499	116.754	0.8408	0.9693	HBA HBA HY RA
8	112.152	116.101	0.8720	0.9669	HBA HBA HY RA
9	112.714	115.539	0.9509	0.9601	HBA HBA HY RA
10	112.720	115.533	0.9234	0.9626	HBA HBA HY RA

[a] Abbreviations stand for: HBA, hydrogen bond acceptor; HY, hydrophobic; RA, ring aromatic

Table S2 Experimental and estimated IC_{50} values of the training set compounds

Cpd.	Exp. IC_{50}(μM)	Est. IC_{50} (μM)	Error[a]	Fit value[b]	Exp. scale[c]	Est. scale[c]
1	0.00026	0.00039	+1.5	10.2	++++	++++
2	0.0003	0.00029	- 1	10.4	++++	++++
3	0.0031	0.0025	- 1.3	9.49	++++	++++
4	0.0053	0.005	- 1.1	9.19	++++	++++
5	0.039	0.041	+1.1	8.27	+++	+++
6	0.072	0.10	+1.4	7.88	+++	+++
7	0.079	0.14	+1.7	7.76	+++	+++
8	0.10	0.24	+2.4	7.50	+++	+++
9	0.13	0.24	+1.9	7.52	+++	+++
10	0.19	0.10	- 1.9	7.89	+++	+++
11	0.29	0.36	+1.2	7.33	+++	+++
12	0.56	0.87	+1.5	6.95	+++	+++
13	1.8	3.1	+1.7	6.40	++	++
14	3.6	3.2	- 1.1	6.39	++	++
15	3.8	3.1	- 1.2	6.39	++	++
16	4.5	2.1	- 2.1	6.57	++	++
17	6.2	10	+1.6	5.88	++	++
18	10	10	+1	5.87	++	++
19	14	7.4	- 1.9	6.02	+	++
20	16	35	+2.2	5.34	+	+
21	31	35	+1.1	5.34	+	+
22	33	36	+1.1	5.34	+	+
23	51	36	- 1.4	5.33	+	+
24	100	42	- 2.4	5.27	+	+
25	200	36	- 5.6	5.33	+	+

[a] Error value of 1 means that the estimated IC_{50} is equal to the experimental IC_{50}; + means that the estimated IC_{50} is higher than the experimental IC_{50}; - means that the estimated IC_{50} is lower than the experimental IC_{50}

[b] Fit value indicates how well the features of pharmacophore models map with the chemical features of

compounds. Big fit value is favorable

[c] Four activity scales: ++++, exremely active ($IC_{50} \leq 0.01$ μM); +++, highly active (0.01 μM< $IC_{50} \leq 1$ μM); ++, moderately active (1 μM < $IC_{50} \leq 10$ μM) and +, low active ($IC_{50} > 10$ μM)

Table S3 Experimental and estimated IC_{50} values of the test set compounds based on Hypo1

Cpd.	Exp. IC_{50} (μM)	Est. IC_{50} (μM)	Error[a]
26	0.0028	0.006	+2.2
27	0.031	0.011	- 2.9
28	0.1	0.23	+2.3
29	0.116	0.095	- 1.2
30	0.164	0.059	- 2.8
31	0.17	0.18	+1
32	0.22	0.49	+2.2
33	0.295	0.37	+1.3
34	0.301	0.5	+1.7
35	0.33	0.38	+1.1
36	0.34	0.31	- 1.1
37	0.4	0.26	- 1.5
38	0.455	0.34	- 1.3
39	0.5	0.21	- 2.4
40	0.545	0.43	- 1.3
41	0.56	1.1	+2
42	0.6	0.64	+1.1
43	0.63	2.6	+4.1
44	0.636	0.3	- 2.1
45	0.677	0.69	+1
46	0.676	1.3	+1.9
47	0.76	0.28	- 2.7
48	0.791	0.63	- 1.3
49	0.825	0.88	+1.1
50	0.833	0.46	- 1.8
51	1.108	1.3	+1.2
52	1.131	0.52	- 2.2
53	1.25	0.48	- 2.6
54	1.3	4.3	+3.3
55	1.4	1.8	+1.3
56	1.7	1.1	- 1.5
57	1.8	4.2	+2.3
58	1.8	2.4	+1.3
59	1.812	1.1	- 1.7
60	2.1	3	+1.4
61	2.4	3.1	+1.3
62	2.6	3.7	+1.4
63	2.666	0.97	- 2.7
64	2.8	2.9	+1

65	3.3	1.2	- 2.7
66	3.7	4.1	+1.1
67	3.9	2.4	-1.6
68	4.5	3.4	- 1.3
69	8.063	10	+1.3
70	8.1	22	+2.7
71	8.393	3.9	- 2.2
72	9.0	14	+1.5
73	9.8	3.7	- 2.7
74	28	36	+1.3
75	40	100	+2.5

[a] Error value of 1 means that the estimated IC_{50} is equal to the experimental IC_{50}; + means that the estimated IC_{50} is higher than the experimental IC_{50}; - means that the estimated IC_{50} is lower than the experimental IC_{50}

Table S4 Statistical parameters of CatScramble randomization trials comparing with that of Hypo1

Trial No.	Total Cost[a]	Fixed Cost[a]	RMSD	Correlation (r)
1	184.958	91.0731	2.7211	0.5996
2	204.676	98.0281	2.9174	0.5119
3	169.907	96.8990	2.4126	0.7038
4	181.565	95.5155	2.6122	0.6394
5	188.981	101.311	2.6343	0.6317
6	170.951	101.561	2.2721	0.7473
7	215.285	92.1220	3.1278	0.3927
8	183.449	96.6565	2.6002	0.6454
9	208.963	96.0063	3.0054	0.4654
10	179.913	100.458	2.4702	0.6891
11	169.085	96.9112	2.3882	0.7113
12[b]	228.196	84.0901	3.3954	1.238e-08
13	183.460	100.806	2.5633	0.6562
14	194.324	100.721	2.6494	0.6358
15	185.992	98.4592	2.6351	0.6312
16	221.968	94.2785	3.1827	0.3500
17	196.622	96.6377	2.8255	0.5547
18	179.746	100.151	2.4791	0.6858
19	206.471	96.7523	2.9638	0.4884
Hypo1	106.864	100.840	0.5316	0.9883

[a] All cost values are in bits

[b] No valid hypothesis could be found because the unsuitable setting of parameters and training set

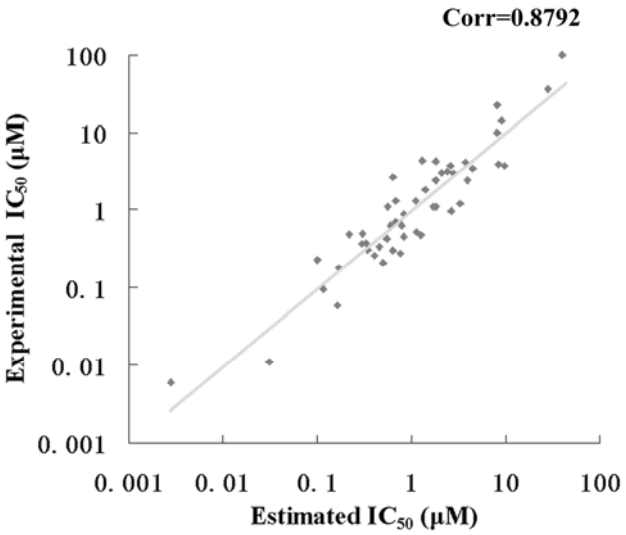

Fig. S3 Correlation between the experimental and predicted activities for the 50 test set molecules against Hypo1.

EXPERIMENTAL

Chemistry

Melting points were determined with a B-540 Büchi apparatus and are uncorrected. NMR spectra were recorded on a Brüker 500 (500 MHz) spectrometer (chemical shifts are given in ppm(δ) relative to TMS as internal standard, coupling constants (*J*) are in hertz (Hz), and signals are using the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet, etc. Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. The purities of compounds **1a-e**, **2**, and **3** were above 95% tested on a Agilent 1100 series HPLC system. All yields are unoptimized and generally represent the result of a single experiment.

*Synthesis of 2-morpholino-3-(phenylsulfonyl)quinoxaline **1a***

To a microwave vial (2-5 mL) were added 2-choloro-3-(phenylsulfonyl)quinoxaline **7a** (30 mg, 0.1 mmol), morpholine (0.05 mL, 0.5 mmol), and isopropyl alcohol (2 mL). The vial was sealed and heated at 80˚C for 10 min by microwave irradiation in a Biotage™ Initiator Synthesizer using a fixed hold time. The mixture was then cooled to room temperature and the residue obtained after evaporating under vacuum was subjected to purification over silica gel chromatography eluting with PE: EtOAc (3:2, v/v) to afford target compound as a bright yellow solid (32 mg, yield: 89%), mp: 152-154˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01 (d, 2H, *J* = 8.5 Hz, aromatic H), 7.79 (d, 1H, *J* = 8.0 Hz, aromatic H), 7.70 (d, 1H, *J* = 8.0 Hz, aromatic H), 7.66-7.63 (m, 2H, aromatic H), 7.55 (t, 2H, *J* = 8.0 Hz, aromatic H), 7.48 (d, 1H, *J* = 8.0 Hz, aromatic H), 3.97 (t, 4H, *J* = 4.5 Hz, morpholine H), 3.77 (t, 4H, *J* = 4.5 Hz, morpholine H). ESI-MS (*m/z*): 356 $[M+1]^+$.

*Synthesis of 2-morpholino-3-(4-methylphenylsulfonyl)quinoxaline **1b***

Same as **1a** above, except with 2-choloro-3-(4-methylphenylsulfonyl)quinoxaline **7b**. Target

compound was obtained as a bright yellow solid (32 mg, yield: 86%), mp: 150-152˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (d, 2H, *J* = 8.5 Hz, aromatic H), 7.79 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.69 (m, 2H, aromatic H), 7.48 (t, 1H, *J* = 8.0 Hz, aromatic H), 7.33 (d, 2H, *J* = 8.0 Hz, aromatic H), 3.97 (t, 4H, *J* = 4.5 Hz, morpholine H), 3.75 (t, 4H, *J* = 4.5 Hz, morpholine H), 2.45 (s, 3H, CH_3). ESI-MS (*m/z*): 370 $[M+1]^+$.

*Synthesis of 2-morpholino-3-(4-methoxyphenylsulfonyl)quinoxaline **1c***

Same as **1a** above, except with 2-choloro-3-(4-methoxyphenylsulfonyl)quinoxaline **7c**. Target compound was obtained as a bright yellow solid (28 mg, yield: 72%), mp: 116-118˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (dd, 2H, *J* = 7.5 and 2.0 Hz, aromatic H), 7.79 (d, 1H, *J* = 9.0 Hz, aromatic H), 7.69 (m, 2H, aromatic H), 7.48 (dt, 1H, *J* = 8.0 and 1.5 Hz, aromatic H), 7.01 (dd, 2H, *J* = 7.0 and 1.5 Hz, aromatic H), 3.98 (t, 4H, *J* = 4.5 Hz, morpholine H), 3.89 (s, 3H, OCH_3), 3.77 (t, 4H, *J* = 4.5 Hz, morpholine H). ESI-MS (*m/z*): 386 $[M+1]^+$.

*Synthesis of 2-morpholino-3-(4-bromophenylsulfonyl)quinoxaline **1d***

Same as **1a** above, except with 2-choloro-3-(4-bromophenylsulfonyl)quinoxaline **7d**. Target compound was obtained as a bright yellow solid (39 mg, yield: 89%), mp: 142-146˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (d, 2H, *J* = 8.5 Hz, aromatic H), 7.79 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.71 (t, 3H, *J* = 8.5 Hz, aromatic H), 7.65 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.49 (t, 1H, *J* = 8.0 Hz, aromatic H), 3.98 (t, 4H, *J* = 4.5 Hz, morpholine H), 3.78 (t, 4H, *J* = 4.5 Hz, morpholine H). ESI-MS (*m/z*): 436 $[M+1]^+$.

*Synthesis of 2-morpholino-3-(4-fluorophenylsulfonyl)quinoxaline **1e***

Same as **1a** above, except with 2-choloro-3-(4-fluorophenylsulfonyl)quinoxaline **7e**. Target compound was obtained as a bright yellow solid (32 mg, yield: 97%), mp: 149-153˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04-8.01 (m, 2H, aromatic H), 7.79 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.71 (dt, 1H, *J* = 8.5 and 1.5 Hz, aromatic H), 7.64 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.49 (dt, 1H, *J* = 8.5 and 1.5 Hz, aromatic H), 7.24 (t, 2H, *J* = 8.5 Hz), 3.98 (t, 4H, *J* = 4.5 Hz, morpholine H), 3.78 (t, 4H, *J* = 4.5 Hz, morpholine H). ESI-MS (*m/z*): 374 $[M+1]^+$.

*Synthesis of 2-morpholino-3-chloroquinoxaline **8***

To a microwave vial (10-20 mL) were added 2,3-dicholoroquinoxaline **6** (1.0 g, 5.1 mmol), morpholine (0.5 mL, 5.1 mmol), and isopropyl alcohol (20 mL). The vial was sealed and heated at 80˚C for 20 min by microwave irradiation in a Biotage™ Initiator Synthesizer using a fixed hold time. The mixture was then cooled to room temperature and the residue obtained after evaporating under vacuum was subjected to purification over silica gel chromatography eluting with PE: EtOAc (10:1, v/v) to afford target compound as a bright yellow solid (1.16 g, yield: 93%), mp: 79-82˚C, (Lit.,[13] 78˚C).

*Synthesis of 2-morpholino-3-phenylthioquinoxaline **2***

A mixture of thiophenol (0.5 mL, 4.8 mmol), NaH (0.4g), 2-morpholino-3-chloroquinoxaline 8 (0.81g, 3.2 mmol) in 10 mL DMF was stirred at room temperature overnight. Upon finishing of the reaction as indicated by TLC, the reaction mixture was diluted with water. The aqueous layer

was extracted with EtOAc twice, and the organic layer was combined and washed with water and brine. The residue obtained after evaporation under vacuum was then purified by silica gel chromatography eluting with PE:EtOAc (8:1, v/v) to afford target compound as a yellow solid (0.96 g, yield: 92%), mp: 121-123˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (dd, 1H, *J* = 8.5 and 1.5 Hz, aromatic H), 7.61-7.57 (m, 3H, aromatic H), 7.54 ((dt, 1H, *J* = 8.5 and 1.5 Hz, aromatic H), 7.47-7.41 (m, 4H, aromatic H), 3.97 (t, 4H, *J* = 5.0 Hz, morpholine H), 3.54 (t, 4H, *J* = 5.5 Hz, morpholine H). ESI-MS (*m/z*): 324 $[M+1]^+$.

*Synthesis of 2-piperidinyl-3-(phenylsulfonyl)quinoxaline **3***

To a microwave vial (2-5 mL) were added 2-choloro-3-(phenylsulfonyl)quinoxaline **7a** (30 mg, 0.1 mmol), piperidine (25 mg, 0.3 mmol), and isopropyl alcohol (2 mL). The vial was sealed and heated at 80˚C for 10 min by microwave irradiation in a Biotage™ Initiator Synthesizer using a fixed hold time. The mixture was then cooled to room temperature and the residue obtained after evaporating under vacuum was subjected to purification over silica gel chromatography eluting with PE: EtOAc (5:1, v/v) to afford target compound as a bright yellow solid (32mg, yield: 92%), mp: 151-152˚C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (d, 2H, *J* = 7.5 Hz, aromatic H), 7.74 (d, 1H, *J* = 8.5 Hz, aromatic H), 7.63-7.61 (m, 3H, aromatic H), 7.53 (t, 2H, *J* = 7.5 Hz, aromatic H), 7.41 (t, 2H, *J* = 7.5 Hz, aromatic H), 3.69 (t, 4H, *J* = 5.5 Hz, piperidine H), 1.86 (dt, 4H, *J* = 5.5 Hz, piperidine H), 1.73 (t, 2H, *J* = 5.5 Hz, piperidine H). ESI-MS (*m/z*): 354 $[M+1]^+$.

Cytotoxicity assay

Four human cancer cell lines (PC3, A549, HCT116, and HL60) were purchased from cell bank of China Science Academy, Shanghai, China. The above cells were cultured in RPMI-1640 (Invitrogen Corp., Carlsbad, CA) medium with heat-inactivated 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100μg/mL) and incubated in atmosphere with 20% O_2, 5% CO_2 at 37˚C. MTT assay was used to measure the *in vitro* cytotoxic activity. All the tested compounds were dissolved in DMSO at the concentrations of 10.0 mg/mL and were then diluted to appropriate concentrations. Cells were plated in 96-well plates for 24 h and subsequently treated with different concentrations of tested compounds for 72 h. Viable cells were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay kit (MTT, Sigma) according to the manufacturer's instructions. The concentration of drug causing 50% inhibition in absorbance compared with control cells (IC_{50}) was calculated using the software of dose–effect analysis with microcomputers.

PI3Kα enzyme assay

The inhibition of PI3Kα activity was determined using a competitive fluorescence polarization kinase activity assay based on the principle that PI3K phosphorylates $PI(3,4)P_2$ and converts it to $PI(3,4,5)P_3$.[14] Both the PI3-Kinase fluorescence polarization activity assay kit (catalogue No. K-1100) and recombinant human PI3Kα (catalogue No. E-2000) were commercial available from Echelon Biosciences (Salt Lake City, UT, USA). PI3K reactions were performed in 5 mM HEPES, pH 7, 2.5 mM $MgCl_2$, 10 mM DTT and 50 μM ATP, using diC_8-$PI(4,5)P_2$ as the substrate, and the final reaction volumes were 10 μl. For evaluation of PI3K inhibitors, 50 ng of enzyme and 10 μM of substrate were used per 10 μl reaction volume with inhibitors concentrations ranging from 3.2 nM to 50 μM. After incubating at room temperature for three hours at room temperature, reactions

were quenched by the addition of a chelator. A mixture of phosphoinositide binding protein was added and mixed, followed by the addition of a fluorophore-labeled phosphoinositide tracer. Samples were then mixed in 384-well black Corning nonbinding plates and incubated in a dark location for 1 h to equilibrate. Finally, polarization values were measured using red fluorophores with appropriate filters to determine the extent of enzyme activity in the reaction.

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