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Discovery of novel 2-piperidinol-3-(arylsulfonyl)quinoxalines as phosphoinositide 3-kinase α (PI3Kα) inhibitors

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ABSTRACT

A series of novel 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines was synthesized based on the structural features of a previously identified lead, WR1. The 2-piperidinol-3-(arylsulfonyl)quinoxalines, which showed excellent antitumor activities against five human cell lines, with inhibitory activities ranging from 0.34 to 2.32 μM, proved to be a promising class of novel PI3Kα inhibitors. The most potent compound **10d** (WR23) showed an inhibitory IC_{50} value of 0.025 μM against PI3Kα and significant pAkt suppression effect. Molecular docking analysis was performed to determine possible binding modes between PI3Kα and target compounds.

1. Introduction

As a family of lipid kinases, phosphoinositide 3-kinases (PI3Ks) are critical regulators in a wide range of cellular activities such as growth, transformation, proliferation, motility and differentiation.[1,2] PI3Ks are classified into I, II, and III based on sequence homology and substrate preferences.[3] Among class I PI3Ks, PI3Kα has been well established as a promising target for cancer therapy due to the prevalent mutation and amplification of *PIK3CA*, the gene encoding the catalytic subunit of PI3Kα, in a diverse set of human cancers, and the fact that PI3K signaling pathway is one of the most commonly mutated pathways contributing to tumorigenesis and progression.[4–8]

We have previously reported 2-(3,5-dimethoxyphenylamino)-3-(phenylsulfonyl)quinoxaline **1** (WQ2) as a potent PI3Kα inhibitor,[9] while unfavorable pharmacokinetic properties such as poor solubility discouraged its further progression. In screening our chemical library using a pharmacophore-based approach, 2-morpholino-3-phenylsulfonylquinoxaline **2** (WR1) was identified as a promising lead because it shares the morpholinoaryl motif with **3** (LY294002), an extensively studied PI3K inhibitor with an IC_{50} value of 0.63 μM (Fig. 1).[10–12] Herein, we reported the discovery of a series of novel 2-piperidinol-3-(arylsulfonyl)quinoxalines as PI3Kα inhibitors as a continuation of our work in developing PI3K inhibitors for cancer therapy. Different aliphatic cyclic amine substituents including pyrrolidine, 1,2,3,4-tetrahydroisoquinoline, piperidin-3-ol, and piperidin-4-ol were introduced at the 2-position of the quinoxaline ring in this study to investigate corresponding impacts on potency. Molecular parameter based virtual analysis of druglikeness was conducted, and the most potent PI3Kα inhibitor, 2-(piperidin-4-ol)-3-(4-bromophenylsulfonyl)quinoxaline **10d** (WR23) was tested for its suppression against levels of phosphorylated Akt (pAkt). Besides, molecular docking analysis was performed to determine possible binding modes between PI3Kα and target compounds.

2. Chemistry

Based on the reported approach for the synthesis of arylsulfonylquinoxalines,[9] compounds **7a–e** with a pyrrolidine group, **8a–e** with a tetrahydroisoquinoline group, **9a–e** with a piperidin-3-ol

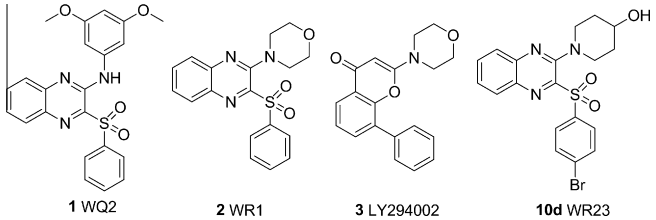

Figure 1. Structures of quinoxaline PI3K inhibitors and LY294002.

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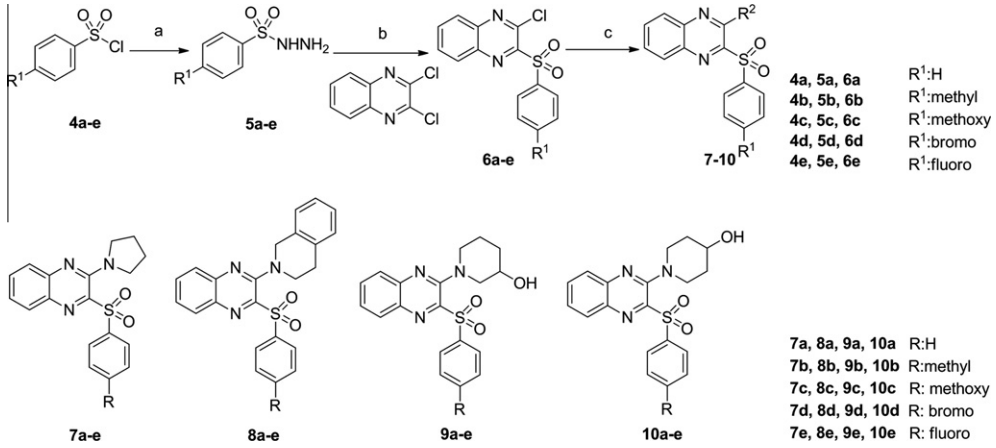
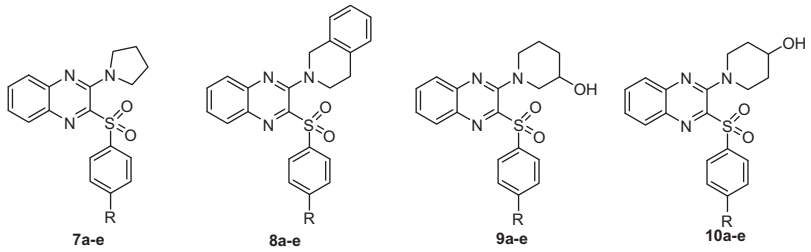


Scheme 1. The synthetic route for target compounds **7**–**10**. Reagents and conditions: (a) hydrazine hydrate, H_2O, 0 °C to rt; (b) ethanol, reflux; (c) *iso*-propanol, microwave irradiation, 80 °C, pyrrolidine (for **7a**–**e**), 1,2,3,4-tetrahydroisoquinoline (for **8a**–**e**), 3-hydroxypiperidine (for **9a**–**e**), or 4-hydroxypiperidine (for **10a**–**e**).

Table 1
In vitro cytotoxicity of 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines against five cancer cell lines



Index	Compound	R	Cytotoxicity IC_{50}[a] (μM)				
			PC3	A549	HCT116	HL60	KB
1	**2**	—	18.88	12.55	5.35	4.47	NT[b]
2	**10a**	H	4.98	2.06	1.87	2.90	5.36
3	**10b**	Methyl	9.52	4.02	5.44	4.05	6.14
4	**10c**	Methoxy	9.73	5.11	4.69	3.23	8.06
5	**10d**	Bromo	2.02	0.95	1.65	0.34	2.32
6	**10e**	Fluoro	2.01	20.23	1.34	1.45	9.58
7	**9a**	H	2.10	0.31	1.39	3.02	3.59
8	**9b**	Methyl	5.33	1.35	3.27	6.08	6.08
9	**9c**	Methoxy	3.41	2.18	3.52	3.05	2.12
10	**9d**	Bromo	3.69	2.10	2.11	3.43	3.72
11	**9e**	Fluoro	2.13	8.17	5.50	2.62	18.30
12	**8a**	H	5.01	19.83	18.73	0.88	26.26
13	**8b**	Methyl	22.39	>50	38.95	3.61	32.06
14	**8c**	Methoxy	>50	>50	22.13	3.69	>50
15	**8d**	Bromo	>50	>50	>50	2.61	>50
16	**8e**	Fluoro	9.23	22.85	16.60	4.08	35.36
17	**7a**	H	20.81	34.82	24.17	3.46	35.75
18	**7b**	Methyl	>50	>50	>50	9.72	>50
19	**7c**	Methoxy	>50	>50	>50	16.03	>50
20	**7d**	Bromo	>50	>50	37.68	6.07	>50
21	**7e**	Fluoro	12.42	31.06	20.56	12.78	30.06
22	LY294002	—	61.35	89.65	56.01	9.94	44.76

[a] IC_{50}, the mean value of at least two separate determinations.
[b] NT, not tested.

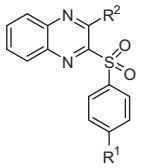
group, and **10a**–**e** with a piperidin-4-ol group were prepared using the synthetic route as shown in Scheme 1. Treatment of arylsulfonyl chloride (**4a–e**) with 85% hydrazine hydrate solution gave phenylsulfonylhydrazines (**5a–e**), whose reaction with 2,3-dicholoroquinoxaline in refluxing ethanol yielded 2-chloro-3-(arylsulfonyl)quinoxalines (**6a**–**e**). Reaction of **6a**–**e** with pyrrolidine, 1,2,3,4-tetrahydroisoquinoline, 3-hydroxypiperidine, and 4-hydroxypiperidine in *iso*-propanol under microwave irradiation afforded compounds **7a–e**, **8a–e**, **9a–e**, and **10a–e**, respectively.

3. Results and discussion

3.1. Cytotoxicity

The synthesized 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines **7**–**10** were tested for their cytotoxicities in vitro against five human cancer cell lines including human prostate cancer cell (PC3), human lung adenocarcinoma epithelial cell (A549), human colon cancer cell (HCT116), human promyelocytic leukemia cell

Table 2
Molecular parameters of 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines



Compound	R^1	R^2	Molecular parameters				
			MW	*C*log*P*[a]	HBA[b]	HBD[c]	tPSA (Å^2)[a]
1	H	3,5-Dimethoxy-phenylamine	421	5.73	7	1	89.35
2	H	Morpholine	355	3.19	6	0	71.33
7a	H	Pyrrolidine	339	4.02	5	0	62.1
7b	Methyl	Pyrrolidine	384	4.52	5	0	62.1
7c	Methoxy	Pyrrolidine	400	4.03	6	0	71.33
7d	Bromo	Pyrrolidine	448	4.90	5	0	62.1
7e	Fluoro	Pyrrolidine	388	4.18	5	0	62.1
8a	H	1,2,3,4-Tetrahydro-isoquinoline	402	5.24	5	0	62.1
8b	Methyl	1,2,3,4-Tetrahydro-isoquinoline	416	5.74	5	0	62.1
8c	Methoxy	1,2,3,4-Tetrahydro-isoquinoline	462	5.25	6	0	71.33
8d	Bromo	1,2,3,4-Tetrahydro-isoquinoline	480	6.12	5	0	62.1
8e	Fluoro	1,2,3,4-Tetrahydro-isoquinoline	419	5.40	5	0	62.1
9a	H	3-Hydroxypiperidine	369	3.24	6	1	82.33
9b	Methyl	3-Hydroxypiperidine	383	3.74	6	1	82.33
9c	Methoxy	3-Hydroxypiperidine	399	3.26	7	1	91.56
9d	Bromo	3-Hydroxypiperidine	448	4.12	6	1	82.33
9e	Fluoro	3-Hydroxypiperidine	387	3.40	6	1	82.33
10a	H	4-Hydroxypiperidine	369	2.49	6	1	82.33
10b	Methyl	4-Hydroxypiperidine	383	2.99	6	1	82.33
10c	Methoxy	4-Hydroxypiperidine	399	2.50	7	1	91.56
10d	Bromo	4-Hydroxypiperidine	448	3.37	6	1	82.33
10e	Fluoro	4-Hydroxypiperidine	387	2.65	6	1	82.33

[a] Calculated by ChemBioDraw Ultra 12.0.
[b] Counted as the sum of Ns and Os.
[c] Counted as the sum of NHs and OHs.

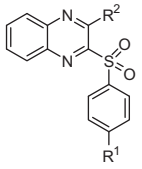
(HL60), and human nasopharyngeal carcinoma cell (KB). LY294002 and compound **2** that share the morpholinoaryl scaffold were used as the positive controls.[10,13] The results are summarized in Table 1.

As shown in Table 1, replacing the 2-morpholine group of compound **2** with pyrrolidine (**7a**–**e**) or tetrahydroisoquinoline (**8a**–**e**) resulted in decreased cytotoxicities against most cell lines in comparison with that of **2**, for example, **7a** with a 3-pyrrolidine group, **8a** with a 3-tetrahydroisoquinoline group, and compound **2** showed IC_{50} values of 24.17, 18.73 and 5.35 μM against HCT116, respectively. While the presence of either a 3-hydroxypiperidine (compounds **9a**–**e**) or a 4-hydroxypiperidine (compounds **10a**–**e**) at the 2-position of the quinoxaline scaffold led to significant increase in cytotoxicities in low micromolar level against the five tested human cancer cell lines, which are much better than that of compound **2** and LY294002, especially against the PI3K-activated PC3 cell lines. For example, **9a** with a 3-hydroxypiperidine and **10a** with a 4-hydroxypiperidine on the 3-position of the quinoxaline scaffold showed IC_{50} values of 2.10 and 4.98 μM against PC3, respectively, and the corresponding IC_{50} values for compound **2** and LY294002 were 18.88 and 61.35 μM, respectively.

3.2. Druglikeness analysis

Analysis of molecular parameters that connected with druglikeness, including molecular weight (MW), calculated partition coefficient for *n*-octanol/water (*C*log*P*), number of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), and total polar surface area (tPSA),[14,15] was conducted to indicate whether the potent piperidinol derivatives bear improved solubility and overall pharmacokinetic property as compared with the previously identified quinoxaline PI3K inhibitor **1**. *C*log*P* and tPSA values were calculated by using ChemDrawUltra 12.0.

Table 3
Inhibition of PI3Kα by 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines



Compound	R^1	R^2	PI3Kα IC_{50}[a] (μM)
2	H	Morpholine	0.44
7a	H	Pyrrolidine	21.83
8a	H	Tetrahydroisoquinoline	>50
10a	H	4-Hydroxypiperidine	0.079
10d	Bromo	4-Hydroxypiperidine	0.025
LY294002[b]	—		0.63

[a] IC_{50}, the mean value of at least two separate determinations.
[b] Report value, Ref. 13

As summarized in Table 2, compounds with a 3-hydroxypiperidine (**9a**–**e**) or a 4-hydroxypiperidine (**10a**–**e**) showed favorable values for the involved molecular parameters that fit well with the Lipinski's Rule of Five.[14] It is noteworthy to mention that the presence of the piperidinol group significantly reduced *C*log*P* values compared with that of compound **2** with a 3,5-dimethoxylphenylamine, that is, **2** had a *C*log*P* value of 5.73, while **9a** with a 3-hydroxypiperidine and **10a** with a 4-hydroxypiperidine had *C*log*P* values of 3.24 and 2.49, respectively.

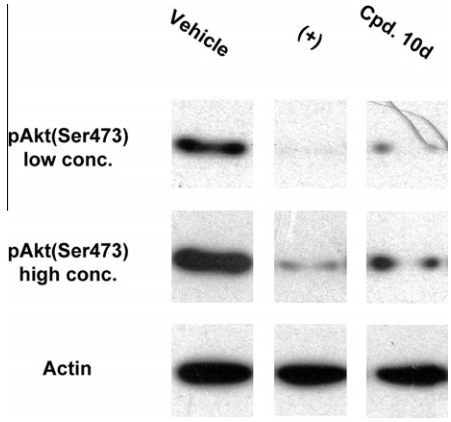


Figure 2. pAkt^{Ser473} inhibition of **9a** and **10d** in PC3 cells.PC3 cells were treated with 10 μM of either **9a** or **10d** for 3 h. After treatment, the cell lysate was subjected to SDS–PAGE and Western blot analysis. Actin was used as the internal control.

3.3. PI3Kα enzymatic activity

2-Piperidinol-3-(arylsulfonyl)quinoxalines **7a**, **8a**, **10a**, and **10d** were then selected to test their PI3Kα inhibition using a competitive fluorescence polarization (CFP) assay.[16] LY294002 was used as the positive control. As shown in Table 3, compounds **7a** with a pyrrolidine group showed poor PI3Kα inhibitory potency with an IC_{50} value of 21.83 μM, compound **8a** with a tetrahydroisoquinoline group did not inhibit PI3Kα with an IC_{50} value >50 μM, while compound **10a** with a 4-hydroxypiperidine exhibited a value of 79 nM against PI3Kα, which was an eightfold improvement than that of LY294002 (IC_{50} value of 0.63 μM against PI3Kα), and a sixfold improvement than that of compound **2** (IC_{50} value of 0.44 μM against PI3Kα). More encouragingly, 2-(piperidin-4-ol)-3-(4-bromophenylsulfonyl)quinoxaline **10d**, the most potent compound in this series in both the PI3K enzymatic assay and cellular assay, showed an IC_{50} value of 25 nM against PI3Kα.

3.4. pAkt suppression

Akt, or Protein kinase B (PKB), is a key downstream signaling effector of PI3K.[17] Inhibition of pAkt would be strong evidence to support the PI3K inhibitory activity of target compounds. Thus, compound **10d** with a 4-hydroxypiperidine was further tested for its suppression against pAkt. GDC-0941, one of the most potent and advanced PI3K inhibitors revealed so far [18,19] was used as the positive control to indicate the suppressive potency of compound **10d**. As shown in Figure 2, the result of pAkt^{Ser473} inhibition in PC3 cells revealed that the most potent compound **10d** led to significant pAkt suppression at both low and high concentrations, the result of which was consistent with cellular PI3Kα inhibition.

3.5. Docking study

To determine possible binding modes and give better guidance for further SAR studies, molecular docking study using co-crystal structure of mutant PI3Kα (PDB ID: 3HHM) (Fig. 3A and B) was conducted using the C-Dock protocol within DiscoveryStudio 2.1.[20] The binding modes of PI3Kα with LY294002, **7a**, or **10d**, are illustrated in Figure 3. As shown in the simplified docking graphics with selected amino acid residues around the ATP binding site, LY294002 forms a hydrogen bond interaction (length: 2.4 Å) with the hinge residue of Val851 (Fig. 3C), which corresponds to the widely existed hydrogen bonds formed between Val882 and PI3K inhibitors in most PI3Kγ co-crystal structures,[5,11,21] and a second interaction with Lys802 in the affinity pocket (length: 1.8 Å); compound **7a** retains the hydrogen bond interaction involving Lys802 (length: 2.3 Å) while fails to form hydrogen bond with hinge Val 851 (Fig. 3E); compound **10d** forms two hydrogen bond interactions with Val 851 (length: 2.0 Å) and Ser854 (length: 3.1 Å), respectively, through its hydroxy group on the piperidine ring (Fig. 3G). The lack of the hydrogen bond interaction with Val851, which is believed to be of crucial importance for PI3K inhibition,[5,11,21] might partly explain the poor activity of **7a**. Comparison of the surface shows of the binding modes between PI3Kα and LY294002, **7a**, or **10d** (Fig. 3D, F, and H, respectively) revealed that **10d** has a better fit into the ATP binding site than that of LY294002 and **7a**, especially in the ribose binding pocket which accommodates the 4-bromophenylsulfonyl moiety of **10d**. Binding-mode study of compounds with similar arylsulfonylquinoxaline scaffold revealed that the sulfonyl oxygen might be potential hydrogen-bond acceptor to interact with residues of PI3Ks.[9] Overall, the molecular docking analysis suggested the potential of potent inhibition of compound **10d** against PI3Kα.

4. Conclusions

A series of novel 2-aliphatic cyclic amine-3-(arylsulfonyl)quinoxalines was designed and synthesized as PI3Kα inhibitors based on a morpholinoaryl lead WR1, which was identified through pharmacophore-based virtual screening. Structural modification revealed that replacement of the morpholine group with either a smaller pyrrolidine group or a larger tetrahydroisoquinoline group reduced in vitro cytotoxicities against five human tumor cell lines, while incorporation of a peridinol group significantly improved in vitro cytotoxicities. From the 2-piperidinol-3-(arylsulfonyl)quinoxaline series, compound **10d** (WR23) that showed significant pAkt^{Ser473} inhibition was discovered as the most potent compound with an IC_{50} value of 0.025 μM against PI3Kα. Molecular docking analysis further suggested PI3Kα inhibitory activity of **10d**. This study might be useful in future development of novel PI3Kα inhibitors.

5. Experimental

5.1. 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 at room temperature (chemical shifts are given in ppm(δ) relative to TMS as internal standard, coupling constants (J) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet, etc.) Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer. Thin layer chromatography was carried out using plate silica gel F254 Merck, Damstadt, Germany. All yields are unoptimized and generally represent the result of a single experiment.

5.1.1. General procedure for the synthesis of 2-pyrrolidinyl-3-(arylsulfonyl)quinoxalines (7)

To a microwave vial (2–5 mL) were added 2-choloro-3-(arylsulfonyl)quinoxaline **6** (0.1 mmol), pyrrolidine (0.05 mL, 0.6 mmol), and isopropyl alcohol (2 mL). The sealed vial was 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 (4:1, v/v) to afford target compounds **7** as yellow solid.

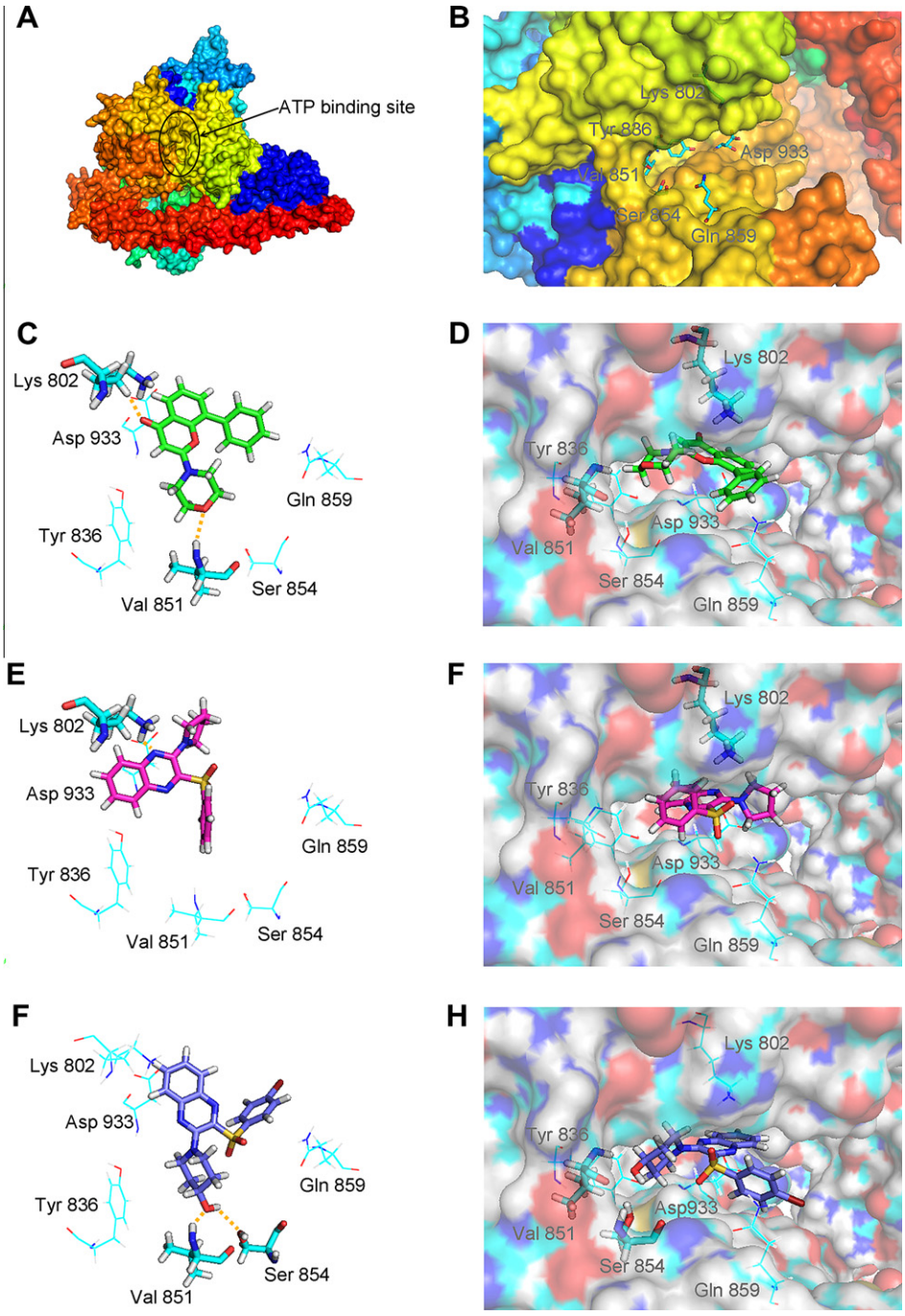


Figure 3. Molecular docking analysis of LY294002, **7a**, and **10d** with PI3Kα. (A) An overall view of the crystal structure of PI3Kα. The kinase catalytic domain which accommodates the ATP binding site is shown in yellow to light orange. (B) A close-up view of the ATP binding site of PI3Kα. Selected amino acid residues around the ATP binding site are shown in cyan sticks. (C) LY294002 (green backbone) bound to PI3Kα, orange dashed lines stand for hydrogen bonds. (D) Surface show of LY294002 (green backbone) bound to PI3Kα. (E) **7a** (pink backbone) bound to PI3Kα, orange dashed line stands for hydrogen bond. (F) Surface show of **7a** (pink backbone) bound to PI3Kα. (G) **10d** (blue backbone) bound to PI3Kα, orange dashed lines stand for hydrogen bonds. (H) Surface show of **10d** (blue backbone) bound to PI3Kα. (PDB ID: 3HHM).

5.1.1.1. 2-Pyrrolidinyl-3-(phenylsulfonyl)quinoxaline (7a). Yield: 91%, mp: 130–133 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (d, 2H, J = 7.0 Hz, aromatic H), 7.66 (d, 1H, J = 8.5 Hz, aromatic H), 7.57–7.54 ()m, 3H, aromatic H), 7.35 (d, 2H, J = 8.0 Hz, aromatic H), 7.23–7.20 (m, 1H, aromatic H), 4.03 (t, 4H, J = 6.5 Hz, pyrrolidine H), 2.08 (t, 4H, J = 6.5 Hz, pyrrolidine H). ESI-MS (m/z): 340 $[M+1]^+$.

5.1.1.2. 2-Pyrrolidinyl-3-(4-methylphenylsulfonyl)quinoxaline (7b). Yield: 96%, mp: 152–154 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (d, 2H, J = 8.0 Hz, aromatic H), 7.65 (d, 1H, J = 8.5 Hz, aromatic H), 7.59 (t, 1H, J = 7.5 Hz, aromatic H), 7.40 (d, 1H, J = 8.0 Hz, aromatic H), 7.35 (d, 2H, J = 8.0 Hz, aromatic H), 7.24 (t, 1H, J = 7.5 Hz, aromatic H), 4.02 (t, 4H, J = 6.5 Hz, pyrrolidine H), 2.48 (s, 3H, CH_3), 2.07 (t, 4H, J = 6.5 Hz, pyrrolidine H). ESI-MS (m/z): 354 $[M+1]^+$.

5.1.1.3. 2-Pyrrolidinyl-3-(4-methoxyphenylsulfonyl)quinoxaline (7c). Yield: 92%, mp: 177–179 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (d, 2H, J = 8.5 Hz, aromatic H), 7.65 (d, 1H, J = 8.5 Hz, aromatic H), 7.59–7.53 (m, 1H, aromatic H), 7.42 (d, 1H, J = 8.5 Hz, aromatic H), 7.25 (t, 1H, J = 7.5 Hz, aromatic H),

7.03 (d, 2H, J = 8.5 Hz, aromatic H), 4.02 (t, 4H, J = 6.5 Hz, pyrrolidine H), 3.91 (s, 3H, OCH_3), 2.06 (t, 4H, J = 6.5 Hz, pyrrolidine H). ESI-MS (m/z): 370 $[M+1]^+$.

5.1.1.4. 2-Pyrrolidinyl-3-(4-bromophenylsulfonyl)quinoxaline (7d). Yield: 76%, mp: 134–136 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (d, 2H, J = 8.5 Hz, aromatic H), 7.70 (d, 2H, J = 8.5 Hz, aromatic H), 7.66 (d, 1H, J = 8.5 Hz, aromatic H), 7.59–7.55 (m, 1H, aromatic H), 7.40 (d, 1H, J = 8.0 Hz, aromatic H), 7.25 (d, 1H, J = 8.0 Hz, aromatic H), 4.01 (t, 4H, J = 6.5 Hz, pyrrolidine H), 2.07 (t, 4H, J = 6.5 Hz, pyrrolidine H). ESI-MS (m/z): 420 $[M+1]^+$.

5.1.1.5. 2-Pyrrolidinyl-3-(4-fluorophenylsulfonyl)quinoxaline (7e). Yield: 71%, mp: 94–97 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98–7.97 (m, 2H, aromatic H), 7.65 (d, 1H, J = 7.5 Hz, aromatic H), 7.58 (d, 1H, J = 6.5 Hz, aromatic H), 7.38 (d, 1H, J = 7.5 Hz, aromatic H), 7.24–7.23 (m, 3H, aromatic H), 4.00 (m, 4H, pyrrolidine H), 2.06 (m, 4H, pyrrolidine H). ESI-MS (m/z): 358 $[M+1]^+$.

5.1.2. General procedure for the synthesis of 2-(3,4-dihydroisoquinolin-2(1*H*)-yl)-3-(arylsulfonyl)quinoxalines (8)

To a microwave vial (2–5 mL) were added 2-choloro-3-(arylsulfonyl)quinoxaline **6** (0.1 mmol), 1,2,3,4-tetrahydroisoquinoline (40 mg, 0.3 mmol), and isopropyl alcohol (2 mL). The sealed vial was 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 (8:1, v/v) to afford target compounds **8** as yellow solid.

5.1.2.1. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(phenylsulfonyl)quinoxaline (8a). Yield: 69%, mp: 149–150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, 2H, J = 7.5 Hz, aromatic H), 7.79 (d, 1H, J = 8.5 Hz, aromatic H), 7.67 (t, 1H, J = 6.0 Hz, aromatic H), 7.62 (t, 2H, J = 8.0 Hz, aromatic H), 7.52 (t, 2H, J = 8.0 Hz, aromatic H), 7.42 (t, 1H, J = 7.5 Hz, aromatic H), 7.23–7.21 (m, 4H, aromatic H), 4.92 (s, 2H, hydroisoquinoline H), 4.13 (t, 2H, J = 5.5 Hz, hydroisoquinoline H), 3.25 (t, 2H, J = 5.5 Hz, hydroisoquinoline H). ESI-MS (m/z): 402 $[M+1]^+$.

5.1.2.2. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(4-methylphenylsulfonyl)quinoxaline (8b). Yield: 63%, mp: 170–172 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (d, 2H, J = 8.0 Hz, aromatic H), 7.79 (d, 1H, J = 8.5 Hz, aromatic H), 7.66–7.62 (m, 2H, aromatic H), 7.42 (t, 1H, J = 8.5 Hz, aromatic H), 7.29 (d, 2H, J = 8.0 Hz, aromatic H), 7.23–7.20 (m, 4H, aromatic H), 4.91 (s, 2H, hydroisoquinoline H), 4.12 (t, 2H, J = 6.0 Hz, hydroisoquinoline H), 3.25 (t, 2H, J = 6.0 Hz, hydroisoquinoline H), 2.43 (s, 3H, CH_3). ESI-MS (m/z): 416 $[M+1]^+$.

5.1.2.3. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(4-methoxyphenylsulfonyl)quinoxaline (8c). Yield: 58%, mp: 148–150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.93 (d, 2H, J = 9.0 Hz, aromatic H), 7.79 (d, 1H, J = 9.0 Hz, aromatic H), 7.65 (d, 1H, J = 8.0 Hz, aromatic H), 7.43 (dt, 1H, J = 8.0 and 1.5 Hz, aromatic H), 7.28–7.18 (m, 4H, aromatic H), 6.96 (d, 2H, J = 9.0 Hz, aromatic H), 4.91 (s, 2H, hydroisoquinoline), 4.11 (t, 2H, J = 6.0 Hz, hydroisoquinoline H), 3.87 (s, 3H, methoxy H), 3.25 (t, 2H, J = 6.0 Hz, hydroisoquinoline H). ESI-MS (m/z): 432 $[M+1]^+$.

5.1.2.4. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(4-bromophenylsulfonyl)quinoxaline (8d). Yield: 59%, mp: 184–185 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (d, 2H, J = 8.5 Hz, aromatic H), 7.79 (d, 1H, J = 8.0 Hz, aromatic H), 7.69–7.60 (m, 4H, aromatic H), 7.44 (t, 1H, J = 8.0 Hz, aromatic H), 7.22–7.20 (m, 4H, aromatic H), 4.92 (s, 2H, hydroisoquinoline H), 4.12 (t, 2H, J = 6.0 Hz, hydroisoquinoline H), 3.24 (t, 2H, J = 6.0 Hz, hydroisoquinoline H). ESI-MS (m/z): 482 $[M+1]^+$.

5.1.2.5. 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-3-(4-fluorophenylsulfonyl)quinoxaline (8e). Yield: 79%, mp: 171–175 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02–7.99 (m, 2H, aromatic H), 7.80 (d, 1H, J = 8.5 Hz, aromatic H), 7.68 (t, 1H, J = 7.5 Hz, aromatic H), 7.61 (d, 1H, J = 8.0 Hz, aromatic H), 7.44 (t, 1H, J = 7.5 Hz, aromatic H), 7.71–7.16 (m, 6H, aromatic H), 4.92 (s, 2H, hydroisoquinoline H), 4.12 (t, 2H, J = 5.5 Hz, hydroisoquinoline H), 3.25 (t, 2H, J = 5.5 Hz, hydroisoquinoline H). ESI-MS (m/z): 420 $[M+1]^+$.

5.1.3. General procedure for the synthesis of 2-(piperidin-3-ol)-3-(arylsulfonyl)quinoxalines (9)

To a microwave vial (2–5 mL) were added 2-choloro-3-(arylsulfonyl)quinoxaline **6** (0.1 mmol), piperidin-3-ol (30 mg, 0.3 mmol), and isopropyl alcohol (2 mL). The sealed vial was 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 (3:1, v/v) to afford target compounds **9** as yellow solid.

5.1.3.1. 2-(Piperidin-3-ol)-3-(phenylsulfonyl)quinoxaline (9a). Yield: 42%, mp: 118–120 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01 (d, 2H, J = 7.5 Hz, aromatic H), 7.79 (d, 1H, J = 7.5 Hz, aromatic H), 7.70–7.64 (m, 3H, aromatic H), 7.56–7.55 (m, 2H, aromatic H), 7.48–7.46 (m, 1H, aromatic H), 4.14 (m, 1H, piperidine H), 3.90–3.89 (m, 2H, piperidine H), 3.68 (br s, 1H, hydroxy H), 3.61–3.58 (d, 1H, J = 13.0 Hz, piperidine H), 3.52 (t, 1H, J = 10.0 Hz, piperidine H), 2.16–2.15 (m, 1H, piperidine H), 1.87–1.82 (m, 2H, piperidine H), 1.73–1.71 (m, 1H, piperidine H). ESI-MS (m/z): 370 $[M+1]^+$.

5.1.3.2. 2-(Piperidin-3-ol)-3-(4-methylphenylsulfonyl)quinoxaline (9b). Yield: 45%, mp: 139–142 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (d, 2H, J = 8.5 Hz, aromatic H), 7.79 (d, 1H, J = 8.5 Hz, aromatic H), 7.70–7.67 (m, 2H, aromatic H), 7.49 (t, 1H, J = 8.0 Hz, aromatic H), 7.34 (d, 2H, J = 8.5 Hz, aromatic H), 4.14–4.13 (m, 1H, piperidine H), 3.93–3.91 (m, 2H, piperidine H), 3.69 (br s, 1H, hydroxy H), 3.57 (d, 1H, J = 13.5 Hz, aromatic H), 3.49 (dt, 1H, J = 10.0 and 2.5 Hz, piperidine H), 2.46 (s, 3H, CH_3), 2.19–2.12 (m, 1H, piperidine H), 1.91–1.87 (m, 1H, piperidine H), 1.83–1.78 (m, 1H, piperidine H), 1.72–1.69 (m, 1H, piperidine H). ESI-MS (m/z): 384 $[M+1]^+$.

5.1.3.3. 2-(Piperidin-3-ol)-3-(4-methoxyphenylsulfonyl)quinoxaline (9c). Yield: 50%, mp: 91–94 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 2H, J = 8.5 Hz, aromatic H), 7.78 (dd, 1H, J = 9.0 and 1.5 Hz, aromatic H), 7.69–7.66 (m, 2H, aromatic H), 7.49 (dt, 1H, J = 8.5 and 1.5 Hz, aromatic H), 7.00 (d, 2H, J = 8.5 Hz, aromatic H), 4.14–4.13 (m, 1H, piperidine H), 3.92 -3.90 (m, 2H, piperidine H), 3.88 (s, 1H, OCH_3), 3.79 (br s, 1H, hydroxy H), 3.56 (dd, 1H, J = 13.0 and 1.5 Hz, aromatic H), 3.48 (dt, 1H, J = 10.0 and 2.5 Hz, piperidine H), 2.17–2.11 (m, 1H, piperidine H), 1.90–1.87 (m, 1H, piperidine H), 1.83–1.76 (m, 1H, piperidine H), 1.72–1.68 (m, 1H, piperidine H). ESI-MS (m/z): 400 $[M+1]^+$.

5.1.3.4. 2-(Piperidin-3-ol)-3-(4-bromophenylsulfonyl)quinoxaline (9d). Yield: 57%, mp: 108–112 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (d, 2H, J = 8.5 Hz, aromatic H), 7.76 (d, 1H, J = 8.5 Hz, aromatic H), 7.69–7.66 (m, 3H, aromatic H), 7.64 (d, 1H, J = 8.5 Hz, aromatic H), 7.46 (t, 1H, J = 8.0 Hz, aromatic H), 4.11–4.08 (m, 2H, piperidine H), 4.03–4.00 (m, 1H, piperidine H),

3.46 (dt, 2H, J = 13.0 and 3.0 Hz, piperidine H), 2.16–2.12 (m, 2H, piperidine H), 1.88–1.81 (m, 2H, piperidine H). ESI-MS (m/z): 450 $[M+1]^+$.

5.1.3.5 2-(Piperidin-3-ol)-3-(4-fluorophenylsulfonyl)quinoxaline (**9e**).

Yield: 68%, mp: 121–124 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04–8.01 (dd, 2H, J = 8.5 and 2.0 Hz, aromatic H), 7.79 (d, 1H, J = 8.0 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), 7.22 (t, 2H, J = 8.5 Hz, aromatic H), 4.14–4.12 (m, 1H, piperidine H), 3.92–3.89 (m, 2H, piperidine H), 3.61–3.59 (m, 2H, piperidine H), 3.52 (dt, 1H, J = 10.0 and 3.0 Hz, piperidine H), 2.19–2.12 (m, 1H, piperidine H), 1.90–1.79 (m, 2H, piperidine H). ESI-MS (m/z): 388 $[M+1]^+$.

5.1.4. General procedure for the synthesis of 2-(piperidin-4-ol)-3-(arylsulfonyl)quinoxalines (10)

To a microwave vial (2–5 mL) were added 2-choloro-3-(arylsulfonyl)quinoxaline **6** (0.1 mmol), piperidin-4-ol (30 mg, 0.3 mmol), and isopropyl alcohol (2 mL). The sealed vial was 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 (3:1, v/v) to afford target compounds **10** as yellow solids.

5.1.4.1. 2-(Piperidin-4-ol)-3-(phenylsulfonyl)quinoxaline (10a). Yield: 69%, mp: 164–167 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (d, 2H, J = 8.0 Hz, aromatic H), 7.76 (d, 1H, J = 8.5 Hz, aromatic H), 7.68–7.62 (m, 3H, aromatic H), 7.54 (t, 2H, J = 8.0 Hz, aromatic H), 7.45 (t, 1H, J = 7.5 Hz, aromatic H), 4.13–4.08 (m, 2H, piperidine H), 4.02–3.99 (m, 1H, piperidine H), 3.45 (dt, 2H, J = 10.0 and 2.5 Hz, aromatic H), 2.15–2.13 (m, 2H, piperidine H), 1.88–1.81 (m, 2H, piperidine H). ESI-MS (m/z): 370 $[M+1]^+$.

5.1.4.2. 2-(Piperidin-4-ol)-3-(4-methylphenylsulfonyl)quinoxaline (10b). Yield: 78%, mp: 130–133 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (d, 2H, J = 8.0 Hz, aromatic H), 7.76 (d, 1H, J = 8.5 Hz, aromatic H), 7.68–7.64 (m, 2H, aromatic H), 7.45 (t, 1H, J = 7.0 Hz, aromatic H), 7.32 (d, 2H, J = 8.0 Hz, aromatic H), 4.10–4.08 (m, 2H, piperidine H), 4.01–3.99 (m, 1H, piperidine H), 3.43 (t, 2H, J = 10.0 Hz, piperidine H), 2.44 (s, 3H, CH_3), 2.15–2.13 (m, 2H, piperidine H), 1.86–1.84 (m, 2H, piperidine H). ESI-MS (m/z): 384 $[M+1]^+$.

5.1.4.3. 2-(Piperidin-4-ol)-3-(4-methoxyphenylsulfonyl)quinoxaline (10c). Yield: 53%, mp: 148–150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (d, 2H, J = 7.5 Hz, aromatic H), 7.75 (d, 1H, J = 7.5 Hz, aromatic H), 7.67 (m, 2H, aromatic H), 7.44 (m, 1H, aromatic H), 6.99 (d, 2H, J = 7.0 Hz, aromatic H), 4.11 (d, 2H, J = 12.0 Hz, piperidine H), 4.00 (m, 1H, piperidine H), 3.88 (s, 3H, OCH_3), 3.43 (t, 2H, J = 10.0 Hz, morpholine H), 2.13 (m, 2H, piperidine H), 1.86–1.85(m, 2H, piperidine H). ESI-MS (m/z): 400 $[M+1]^+$.

5.1.4.4. 2-(Piperidin-4-ol)-3-(4-bromophenylsulfonyl)quinoxaline (10d). Yield: 74%, mp: 129–133 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86 (d, 2H, J = 8.5 Hz, aromatic H), 7.76 (d, 1H, J = 8.5 Hz, aromatic H), 7.67–7.66 (m, 3H, aromatic H), 7.64 (d, 1H, J = 9.0 Hz, aromatic H), 7.46 (t, 1H, J = 7.5 Hz, aromatic H), 4.12–4.09 (m, 2H, piperidine H), 4.02–4.00 (m, 1H, piperidine), 3.46 (dt, 2H, J = 12.5 and 2.5 Hz, piperidine H), 2.15–2.13 (m, 2H, piperidine H), 1.88–1.82 (m, 2H, piperidine H). ESI-MS (m/z): 450 $[M+1]^+$.

5.1.4.5. 2-(Piperidin-4-ol)-3-(4-fluorophenylsulfonyl)quinoxaline (10e). Yield: 67%, mp: 89–93 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03–8.00 (dd, 2H, J = 9.0 and 2.0 Hz, aromatic H), 7.75 (dd, 1H, J = 8.0 and 1.0 Hz, aromatic H), 7.67 (dt, 1H, J = 8.5 and 1.5 Hz, aromatic H), 7.63 (dd, 1H, J = 8.0 and 1.0 Hz, aromatic H), 7.45 (dt, 1H, J = 8.0 and 1.0 Hz, aromatic H), 7.22 (dt, 2H, J = 9.0 and 2.0 Hz, aromatic H), 4.13–4.08 (m, 2H, piperidine H), 4.03–3.98 (m, 1H, piperidine H), 3.45 (dt, 2H, J = 10.0 and 3.5 Hz, piperidine H), 2.16–2.11 (m, 2H, piperidine H), 1.88–1.82 (m, 2H, piperidine H). ESI-MS (m/z): 388 $[M+1]^+$.

5.2. Pharmacology

Five human cancer cell lines, PC3, A549, HCT116, HL60, and KB, were purchased from the cell bank of Chinese Academy of Sciences, Shanghai, China. PI3-Kinase fluorescence polarization activity assay kit (catalog No. K-1100) and recombinant human PI3Kα (catalog No. E-2000) were commercially available from Echelon Biosciences (Salt Lake City, UT, USA).

5.2.1. Cytotoxicity assay

The cytotoxic activity in vitro was measured using the MTT assay. PC3, A549, HCT116, HL60, and KB cell lines were cultured in RPMI-1640 (Invitrogen Corp., Carlsbad, CA) medium with heat-inactivated 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) and incubated in normoxic atmosphere with 20% O_2, 5% CO_2 at 37 °C. All tested compounds were dissolved in DMSO at concentrations of 10.0 mg/mL and diluted to appropriate concentrations. Cells were plated in 96-well plates for 24 h and subsequently treated with different concentrations of all 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 operation instructions provided by the manufacturer. 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.

5.2.2. PI3Kα kinase assay

The PI3Kα inhibitory test was determined using a competitive fluorescence polarization kinase activity assay based on the fact that PI3K phosphorylates $PI(3,4)P_2$ and converts it to $PI(3,4,5)P_3$.[16] 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 the concentrations of inhibitors ranging from 3.2 nM to 50 μM. After incubating for 3 h at room temperature, reactions were quenched by adding chelators. 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 environment 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.

5.2.3. Docking study

X-ray co-crystal structure of PI3Kα-wortmannin downloaded from the RCSB Protein Data Bank (PDB ID: 3HHM) was used as the template to perform molecular docking of LY294002, compounds **7a** and **10d** using C-Dock protocol within DiscoveryStudio 2.1.[20] For ligand preparation, the 3D structures of LY294002, **7a**, and **10d** were generated and minimized using DiscoveryStudio 2.1. For protein preparation, the hydrogen atoms were added, water was removed, and the CHARMm force field was employed. The whole PI3Kα enzyme was defined as a receptor and the site

sphere was selected based on the ligand binding location of wortmannin. LY294002, **7a**, or **10d** was placed in the binding site during the docking procedure. All graphical pictures were made using Pymol.

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References and notes

1. Auger, K. R.; Serunian, L. A.; Soltoff, S. P.; Libby, P.; Cantley, L. C. *Cell* **1989**, *57*, 167.
2. Cantley, L. C. *Science* **2002**, *296*, 1655.
3. Engelman, J. A.; Luo, J.; Cantley, L. C. *Nat. Rev. Genet.* **2006**, *7*, 606.
4. Hennessy, B. T.; Smith, D. L.; Ram, P. T.; Lu, Y.; Mills, G. B. *Nat. Rev. Drug Discov.* **2005**, *4*, 988.
5. Marone, R.; Cmiljanovic, V.; Giese, B.; Wymann, M. P. *BBA-Proteins Proteomics* **2008**, *1784*, 159.
6. Kawauchi, K.; Ogasawara, T.; Yasuyama, M.; Otsuka, K.; Yamada, O. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 1024.
7. Wu, P.; Liu, T.; Hu, Y. Z. *Curr. Med. Chem.* **2009**, *16*, 916.
8. Wu, P.; Hu, Y. Z. *Curr. Med. Chem.* **2010**, *17*, 4326.
9. Wu, P.; Su, Y.; Liu, X. W.; Zhang, L.; Ye, Y.; Xu, J. C.; Weng, S. Y.; Li, Y. N.; Liu, T.; Huang, S. F.; Yang, B.; He, Q. J.; Hu, Y. Z. *Eur. J. Med. Chem.* **2011**, *46*, 5540.
10. Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F. *J. Biol. Chem.* **1994**, *269*, 5241.
11. Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Wymann, M. P.; Williams, R. L. *Mol. Cell* **2000**, *6*, 909.
12. Wu, P.; Zhang, L.; He, Q. J.; Yang, B.; Hu, Y. Z. AAPS J. **2009**, 11(S2). Abstract of Papers, 2009 American Association of Pharmaceutical Scientists Annual Meeting, Los Angeles, USA, 2009; Abstract 3179.
13. Hayakawa, M.; Kaizawa, H.; Moritomo, H.; Koizumi, T.; Ohishi, T.; Okada, M.; Ohta, M.; Tsukamoto, S.-i.; Parker, P.; Workman, P.; Waterfield, M. *Bioorg. Med. Chem.* **2006**, *14*, 6847.
14. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.
15. Vistoli, G.; Pedretti, A.; Testa, B. *Drug Discov. Today* **2008**, *13*, 285.
16. Drees, B.; Weipert, A.; Hudson, H.; Ferguson, C.; Chakravarty, L.; Prestwich, G. D. *Comb. Chem. High Throughput Screen.* **2003**, *6*, 321.
17. Manning, B. D.; Cantley, L. C. *Cell* **2007**, *129*, 1261.
18. Raynaud, F. I.; Eccles, S. A.; Patel, S.; Alix, S.; Box, G.; Chuckowree, I.; Folkes, A.; Gowan, S.; De Haven Brandon, A.; Di Stefano, F.; Hayes, A.; Henley, A. T.; Lensun, L.; Pergl-Wilson, G.; Robson, A.; Saghir, N.; Zhyvoloup, A.; McDonald, E.; Sheldrake, P.; Shuttleworth, S.; Valenti, M.; Wan, N. C.; Clarke, P. A.; Workman, P. *Mol. Cancer Ther.* **2009**, *8*, 1725.
19. Folkes, A. J.; Ahmadi, K.; Alderton, W. K.; Alix, S.; Baker, S. J.; Box, G.; Chuckowree, I. S.; Clarke, P. A.; Depledge, P.; Eccles, S. A.; Friedman, L. S.; Hayes, A.; Hancox, T. C.; Kugendradas, A.; Lensun, L.; Moore, P.; Olivero, A. G.; Pang, J.; Patel, S.; Pergl-Wilson, G. H.; Raynaud, F. I.; Robson, A.; Saghir, N.; Salphati, L.; Sohal, S.; Ultsch, M. H.; Valenti, M.; Wallweber, H. J. A.; Wan, N. C.; Wiesmann, C.; Workman, P.; Zhyvoloup, A.; Zvelebil, M. J.; Shuttleworth, S. J. *J. Med. Chem.* **2008**, *51*, 5522.
20. Mandelker, D.; Gabelli, S. B.; Schmidt-Kittler, O.; Zhu, J. X.; Cheong, I.; Huang, C. H.; Kinzler, K. W.; Vogelstein, B. V.; Amzel, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 16996.
21. Knight, Z. A.; Gonzalez, B.; Feldman, M. E.; Zunder, E. R.; Goldenberg, D. D.; Williams, O.; Loewith, R.; Stokoe, D.; Balla, A.; Toth, B.; Balla, T.; Weiss, W. A.; Williams, R. L.; Shokat, K. M. *Cell* **2006**, *125*, 733.