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CONCISE ARTICLE

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

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A pharmacophore model of PI3Kα inhibitors was built using the DiscoveryStudio 2.0 package. Pharmacophore-based screening (PBS) retrieved a series of novel morpholino–quinoxalines as PI3Kα inhibitors, as exemplified by **1a** (PI3Kα IC_{50}: 0.44 μM). All target compounds showed good *in vitro* cytotoxicity against tested human cell lines. A pharmacophore mapping analysis and docking study indicated that both the morpholino group and the sulfonyl group contributed significantly to the potent PI3Kα inhibitory activity and cytotoxicity of the compounds.

The phosphoinositide 3-kinase (PI3K) pathway is one of the most highly activated and mutated signaling cascades for a wide spectrum of cancers.[1,2] The PI3Ks are lipid kinases which differ in substrate preference and regulation mechanism.[3] Among these, PI3Kα has emerged as a promising target for cancer therapy due to prevalent gain-of-function mutations which have been observed in *Pik3ca*—the gene which encodes the p110α catalytic subunit of PI3Kα.[4] In addition, loss-of-function mutations in the phosphotase and tensin homologue (PTEN) contribute indirectly to the activation of the PI3K pathway.[5] Thus, considerable interests are currently being focused on developing inhibitors targeting PI3K, especially PI3Kα, for the treatment of cancer.[6–9]

As a part of our group's continued work on developing small-molecule inhibitors targeting the PI3K signaling pathway,[10] a pharmacophore model was built in an attempt to retrieve novel PI3Kα inhibitors. Pharmacophore-based screening against an in-house database revealed a series of morpholino–quinoxalines as potential PI3Kα inhibitors. Synthesized target compounds showed good to excellent *in vitro* cytotoxicity against tested human cell lines. Further pharmacophore mapping and docking studies indicated that the morpholino group and the sulfonyl group were important pharmacophores for PI3K inhibition. The identified leads, as exemplified by **1a**, provided starting points for further modificaiton and optimization.

The pharmacophore model was built based on 75 reported PI3K inhibitors with varied potency and chemical structures, among which 25 were selected to be a training set based on the principle of structural diversity and wide coverage of activity range (ESI, Fig. S1†), the remaining 50 molecules with maximal 3D diversity and continuous bioactivity magnitude constituted a test set (ESI, Fig. S2†).[11] Ten pharmacophore models of PI3Kα inhibitors were generated by using the Catalyst package 4.11 (ESI, Table S1 and S2†). The best pharmacophore model (Hypo1), evaluated in terms of cost functions and other statistical parameters such as correlation coefficient and RMSD value, featured four pharmacophore elements: two hydrogen bond acceptors (HBA), one hydrophobic element (HY) and one ring aromatic element (RA) (Fig. 1). Hypo1 was then validated using the test set containing 50 reported PI3K inhibitors (see ESI, Fig. S3 and Table S3†) and CatScarmble randomization method (see ESI, Table S4†).

DiscoveryStudio 2.0/Ligand Pharmacophore mapping protocol was used with Hypo1 as the pharmacophore model to screen an in-house database consisting of approximately three-thousand compounds that were synthetically available in our laboratory to retrieve molecules as novel PI3K inhibitors.

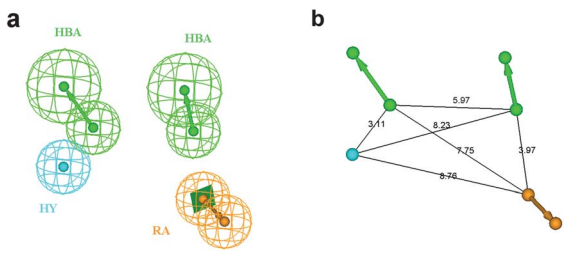

Fig. 1 (a) The best pharmacophore model (Hypo1) with four elements; and (b) 3D spatial relationship and distance constrains of Hypo1.

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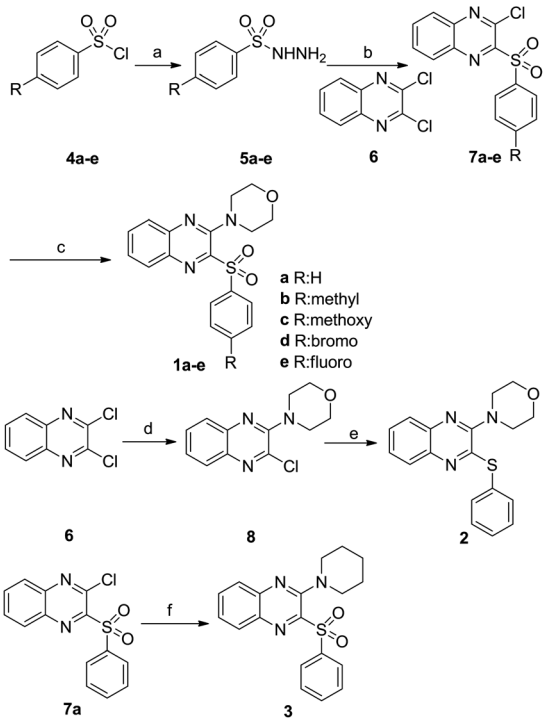
‡ The authors have declared no conflict of interest.

Among the molecules with high fit-values and potent predictive activity, the interesting lead, 2-morpholino-3-phenylsulfonylquinoxaline (**1a**, predictive PI3Kα IC_{50} of 1.0 μM) which contains a morpholinoaryl scaffold—as shown in the widely studied PI3K inhibitor LY294002, which was believed to be one of the privileged structures for PI3K inhibition[12]—was selected for further synthesis and biological evaluation. 2-Morpholino-3-phenylthioquinoxaline (**2**, predictive PI3Kα IC_{50} of 38.0 μM), the predictively inactive PI3K compound that shares the morpholinoaryl scaffold with **1a** but lacks the sulfonyl group, was also synthesized to test the importance of the sulfonyl group for PI3K inhibition. In addition, 2-piperidinyl-3-phenylsulfonylquinoxaline **3**, which has a piperidinyl group rather than a morpholino group at the 2-position of the quinoxaline ring, was synthesized to evaluate the impact of the morpholino group on PI3K inhibition (Fig. 2).

The 2-chloro-3-arylsulfonylquinoxaline intermediates **7a–e** were synthesized *via* the method reported previously by our group starting from the arylsulfonylchlorides **4a–e** to give the arylsulfonylhydrazides **5a–e**, whose reaction with 2,3-dichloroquinoxaline **6** afforded **7a–e**.[10] A final step of **7a–e** with morpholine in isopropyl alcohol under microwave irradiation yielded the target compounds **1a–e**. Substitution of 2,3-dichloroquinoxaline **6** with morpholine gave 2-morpholino-3-chloroquinoxaline **8**, which was further reacted by thiophenol to give compound **2**. Reaction of 2-chloro-3-phenylsulfonylquinoxaline **7a** with piperidine in isopropyl alcohol under microwave irradiation yielded compound **3** (Scheme 1).

Compounds **1a–e**, **2**, and **3** were tested for their PI3Kα enzymatic inhibitory activity using a competitive fluorescence polarization kinase activity assay.[13] LY294002 was used as the positive control. The results are presented in Table 1. Compound **1a** showed a PI3Kα IC_{50} value of 0.44 μM, which is comparable to that of LY294002 and close to the predictive value of 1.0 μM. Derivatives of **1a** with electro-donating methyl (**1b**) and methoxy (**1c**) groups and electro-withdrawing fluoro (**1d**) and bromo (**1e**) groups at the 4-position of the sulfonylphenyl ring also exhibited favorable PI3Kα inhibitory activities ranging from 1.58 to 2.89 μM. While compound **2** with a thio group at the 3-position of the quinoxaline ring showed an IC_{50} value bigger than 50 μM, and compound **3** with a piperidinyl group at the 2-position of the quinoxlaine ring showed an IC_{50} value of 21.63 μM. This result suggested that both the sulfonyl group and the morpholino group are important for the PI3K inhibitory activity in this series of compounds.



Scheme 1 Synthesis of morpholino–quinoxaline derivatives **1a–e**, **2**, and piperidinyl–quinoxaline **3**. Conditions and reagents: (a) hydrazine hydrate, H_2O, 0 °C–r.t., 90–99%; (b) ethanol, reflux, 14–32%; (c) morpholine, microwave irradiation, isopropyl alcohol, 80 °C, 72–97%; (d) morpholine, isopropyl alcohol, reflux, 93%; (e) thiophenol, DMF, NaH, r.t., 70%; and (f) piperidine, microwave irradiation, isopropyl alcohol, 80 °C, 92%.

Compounds **1a–e**, **2**, and **3** were then tested for their *in vitro* cytotoxicity against four human cancer cell lines, prostate cancer cells PC3, lung adenocarcinoma epithelial cells A549, colon cancer cells HCT116, and promyelocytic leukemia cells HL60. LY294002 was used as the reference compound. The results are summarized in Table 2. All morpholino–quinoxaline derivatives **1a–e** showed an obviously improved cytotoxicity compared with that of LY294002, and **1a–e** were generally more active than **2**

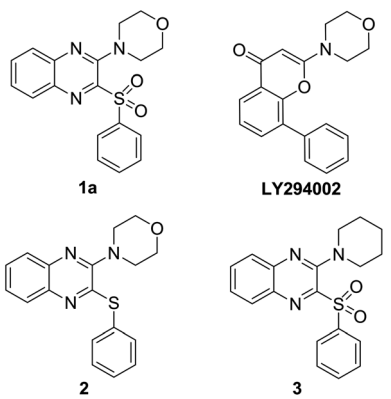


Fig. 2 Structures of **1a**, **2**, **3** and LY294002.

Table 1 Inhibition of PI3Kα by target compounds

	IC_{50} (μM)[a]	
Cpd.	Tested value	Predictive value
1a	0.44	1.0
1b	1.58	1.0
1c	1.40	0.7
1d	2.89	0.5
1e	2.49	1.9
2	>50	38.0
3	21.63	16.0
LY294002[b]	0.63	2.6

[a] The mean value of at least two separate determinations. [b] Reported value, ref. 12.

Table 2 *In vitro* cytotoxicities of target compounds

	IC_{50} (μM)[a]			
Cpd.	PC3	A549	HCT116	HL60
1a	18.88	12.55	5.35	4.47
1b	9.34	3.3	10.48	0.95
1c	0.88	19.35	>50	4.80
1d	25.44	6.65	35.02	3.41
1e	3.40	5.64	3.61	1.74
2	>50	7.25	45.58	5.18
3	26.94	>50	6.48	27.08
LY294002	61.35	89.65	56.01	9.94

[a] The mean value of at least two separate determinations.

and **3**, especially against PC3, the PI3K activated cell line. For example, compounds **1c**, **2**, **3** and LY294002 showed PC3 IC_{50} values of 0.88, bigger than 50, 26.94 and 61.35 μM, respectively.

Further pharmacophore mapping and docking analyses were performed to better explain the potency gap between **1a** and **2**, and understand the binding mode of the target compounds.

Catalyst software package 4.11 was used with Hypo1 as the pharmacophore model to compare the mapping mode between **1a** and **2**. As shown in Fig. 3, **1a** was mapped well with three of the four pharmacophore elements with the oxygen atom of the morpholin ring and one of the sulfonyl oxygens functioning as hydrogen bond acceptors (Fig. 3a and c). Although the morpholino oxygen was also indicated as a potential hydrogen bond acceptor in **2**, the other hydrogen bond acceptor element was absent due to the lack of the sulfonyl group in **2**. The overall mapping result also showed that the quinoxaline scaffold of **2** was outside of the pharmacophore elements due to a change in conformation compared with that of **1a** (Fig. 3b and d).

Docking analysis using the C-DOCKER program within DiscoveryStudio 2.0 package was performed to further explore the binding mode of the target compounds. The co-crystal structure of PI3Kγ with small-molecule inhibitor was used as a surrogate due to its amenability and the fact that PI3Kα-small molecule inhibitor has not been resolved yet.[14] As shown in Fig. 4, both **1a** and **2** form a hydrogen bond through the morpholino oxygen with the hinge residue of Val 882. Disparities in docking modes were in two facets: an additional hydrogen bond was formed involving one of the sulfonyl oxygens with Thr887, and the quinoxaline scaffold was oriented toward the affinity pocket for the PI3Kγ-**1a** complex and close to the ribose binding pocket for the PI3Kγ-**2** complex.

In summary, a series of novel morpholino–quinoxalines **1a–e** were discovered to be good PI3Kα inhibitors *via* pharmacophore-based screening (PBS). All target compounds showed good *in vitro* cytotoxicity against four tested human cell lines. Mapping analysis and docking study indicated that both the morpholino group and the sulfonyl group were important pharmacophores for PI3Kα inhibition in this series of compounds. The morpholino–quinoxaline lead compounds described in this study provide promising starting points for further development of novel quinoxalines as PI3K inhibitors.

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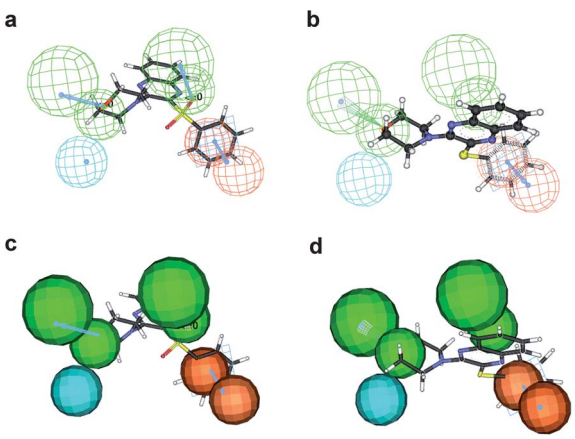

Fig. 3 Pharmacophore mapping using Hypo1: (a) Hypo1 mapped with **1a** in transparent show; (b) Hypo1 mapped with **2** in transparent show; (c) Hypo1 mapped with **1a** in solid show; and (d) Hypo1 mapped with **2** in solid show. Hydrogen bond acceptor (HBA) in green, hydrophobic (HY) in blue, and ring aromatic (RA) in orange.

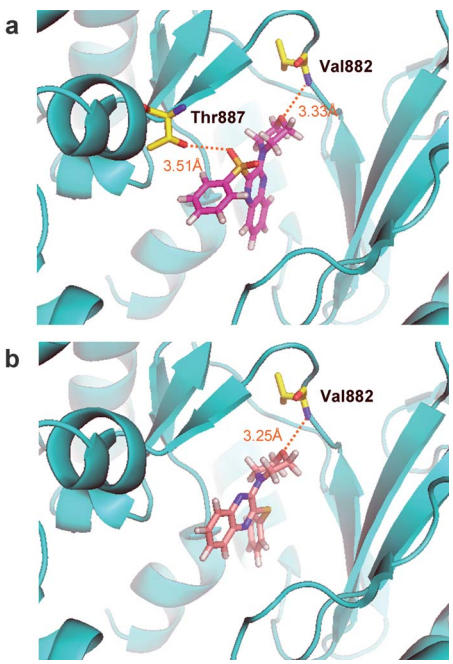

Fig. 4 Docking analysis using PI3Kγ co-crystal structure as a template (PDB ID: 3L54). (a) Ribbon show of **1a** docked into the ATP binding pocket of PI3Kγ; and (b) ribbon show of **2** docked into the ATP binding pocket of PI3Kγ. Selected residues are shown in yellow backbones, **1a** in pink backbone, **2** in wheat backbone, orange dashed lines indicate hydrogen bonds, prepared using PyMOL.

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