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Original article

Design, synthesis and biological evaluation of acylhydrazone derivatives as PI3K inhibitors

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ABSTRACT

Since the PI3K signaling pathway is the most commonly activated in human cancers, inhibition of PI3K is a promising approach to cancer therapy. In this study, a series of 2-methyl-5-nitrobenzeneacylhydrazones were designed and synthesized. All the new derivatives were tested by p110α enzymatic and Rh30 cellular assays. Further enzyme selectivity profiling proved that **6e** and **7** were potential selective PI3K inhibitors.

1. Introduction

Phosphatidylinositol-3-kinases (PI3Ks) [1] play an important role in signal transduction as a key regulator of cell cycle proliferation, growth, survival and protein synthesis [2], and they catalyze phosphorylation of 3-hydroxyl position of phosphatidylinositides (PIs) [3–5]. Based on their primary structure and mechanism of action [6], PI3Ks are divided into three major classes [7]: class I, II, and III [8]. Class I is further split into class Ia: p110α, p110β, and p110δ, which are activated by tyrosine kinase receptors; and class Ib: p110γ, which is activated by G protein-coupled receptor [9]. The PIK3CA gene that encodes p110α is also frequently mutated in many cancers. Thus class Ia PI3Ks, and particularly p110α, are potential therapeutic targets for cancers [10].

Wortmannin [11] and LY-294002 [12] have been extensively studied, but both of them lack selectivity over the class I PI3K isoforms (Fig. 1). In recent years, many small molecular inhibitors have entered into preclinical or clinical stage [13], such as compound **1** (PIK-75) [7] and **2** (PKI-587) [14]. Compound **1** (PIK-75) was reported to be a sub-nanomolar p110α inhibitor with more than100-fold selectivity over p110β and p110γ, and it also showed activity in human cancer xenograft model.

The work we reported here investigated the structure-activity relationship (SAR) of compound **1** (PIK-75) by exploring major changes on the bicyclic core structure and the side chains. We intended to determine whether the position of substituents or the number of the nitrogen atom on the ring was crucial, and to test the influence of the variants in side chain. In this regard, a series of acylhydrazone analogs were synthesized and tested in enzymatic and cellular levels.

2. Experimental

The synthesis of compounds **6a–m**, **7**, and **9a–b** was shown in Scheme 1. Aldehydes **4a–g** were prepared from **3a–g** by cyclization with bromomalonaldehyde. Compounds **6a–m** and **9a–b** were synthesized by condensation of the aldehydes with substituted benzohydrazide followed by alkylation. Compound **7** was obtained from **6e** by replacement of oxygen with sulfur using Lawesson's reagent. The synthesis of compound **12** was shown in Scheme 2. The structure of the new analogs was characterized by ^{1}H NMR and MS.

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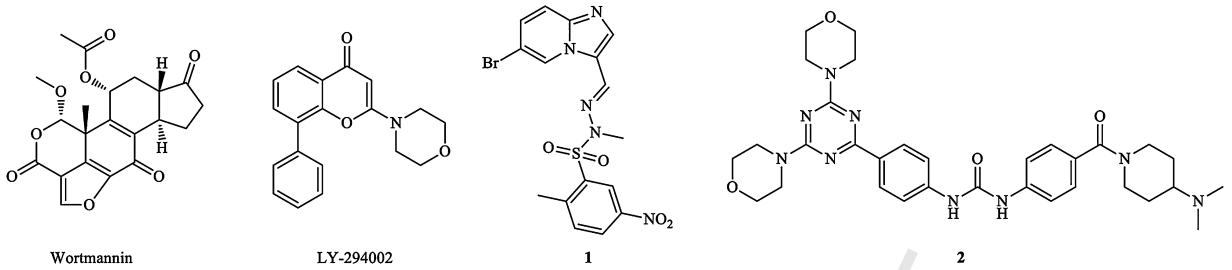


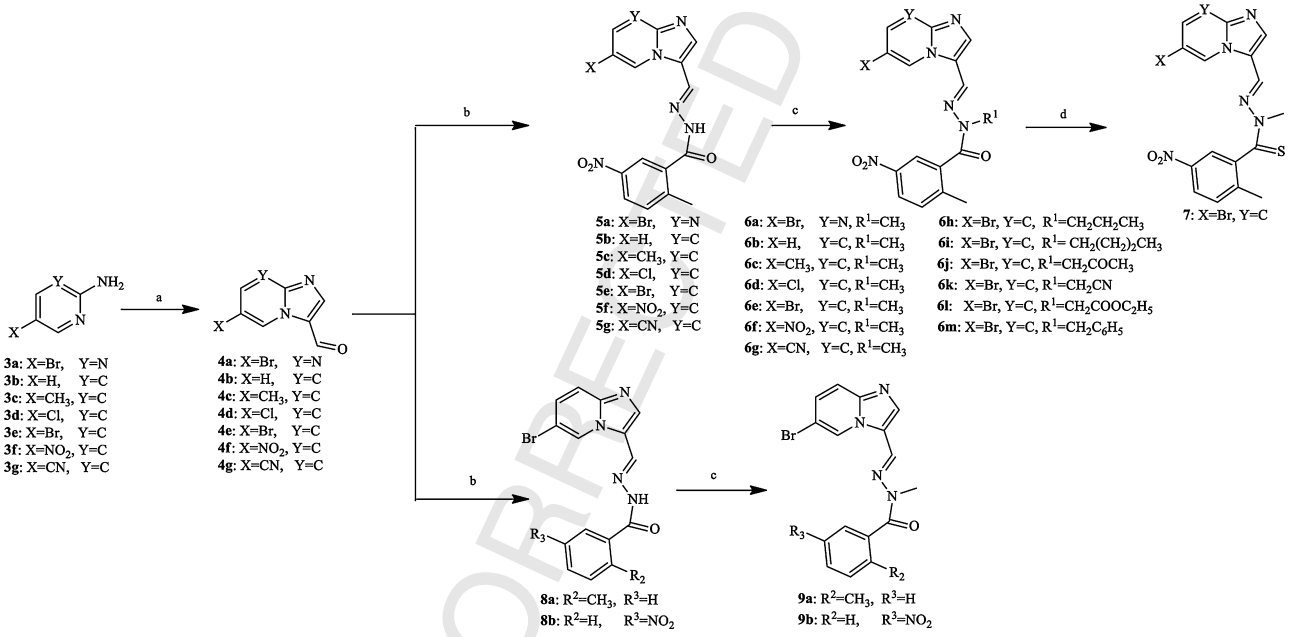
Fig. 1. Some reported PI3K inhibitors.

In addition, the structure of the analogs (represented by compound **6e**) was also confirmed by single crystal X-ray diffraction.

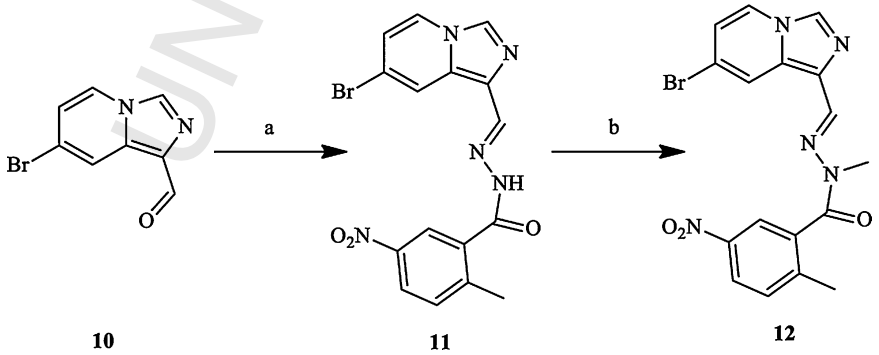
3. Results and discussion

We firstly investigated the influence of the position of substituents and number of nitrogen atoms on the ring in the compounds on their activity. Compounds **6a** and **12** showed poor activity against p110α ($IC_{50} > 10$ μmol/L) and Rh30 ($IC_{50} > 10$ mmol/L) (Table 1). Compound **6e** displayed good activity with an IC_{50} of 11 nmol/L (Table 2). The results indicated that the position of substituents and the number of nitrogen atoms on the bicyclic core structure were crucial to maintain both enzymatic and cellular activities for this series of compounds.

Next, various groups at the C6 position of the imidazo [1,2-α]pyridine moiety were screened (Table 1). Compound **6b**, without any substituent at C6 position, showed poor activity. Introduction of a methyl group as electron donor led to reduced potency. The less bulky chloro-substituted derivative (**6d**) and the derivatives with strong electron-withdrawing groups NO_2 and CN



Scheme 1. Synthesis of compounds **6a**–**m**, **7**, and **9a**–**b**. Reagents and conditions: (a) bromomalonaldehyde, EtOH, reflux, 12 h; (b) benzohydrazide, HOAc (cat.), MeOH, 60 °C, 3 h; (c) NaH, R-X, THF-DMF, rt, 1 h; (d) Lawesson's reagent, dioxane, reflux, 12 h.



Scheme 2. Synthesis of compound **12**. Reagents and conditions: (a) benzohydrazide, HOAc (cat.), MeOH, 60 °C, 3 h; (b) NaH, MeI, THF, rt, 1 h.

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Table 1
Biological activity of compounds **6a**–**m**, **7**, **9a**–**b** and **12**.

Compd.	IC_{50} (μmol/L)		Compd.	IC_{50} (μmol/L)	
	p110α	Rh30		p110α	Rh30
6a	>10	>10	**6j**	0.114	5.01
6b	>10	>10	**6k**	0.098	3.02
6c	>10	9.12	**6l**	>10	>10
6d	0.029	3.26	**6m**	>10	>10
6e	0.011	2.04	**7**	0.014	1.59
6f	>10	>10	**9a**	3.742	8.61
6g	0.034	4.2	**9b**	0.049	4.11
6h	>10	3.93	**12**	>10	>10
6i	0.261	5.30	PIK-75	0.015	0.15

Table 2
Kinase-selectivity profiling of compound **7**.[a]

Compd.	Inhibition rate for tyrosine kinases (%)														
	Flt-1	KDR	c-Kit	PDGFR-α	PDGFR-β	RET	EGFR	ErbB2	EGFR/T790M/L858R	ErbB4	Src	Abl	EPH-A2	RON	FGFR1
7	34.9	37.2	32.8	20.3	41.0	49.5	11.7	50.4	49.3	24.2	5.1	36.6	54.4	22.3	53.1
Su11248	86.7	90.4	87.3	80.9	87.4	87.6									
BIBW2992							86.9	86.1	83.4	83.1					

[a] The inhibitory activity of compounds against 15 tyrosine kinases were measured with ELISA at a concentration of 10 μmol/L.

(**6f** and **6g**) were less potent than **6e**. We envisioned that the bromine atom played a critical role in compound binding with PI3K due to the reason that compound with a bromine substitution may fit well in the active site of PI3K.

With an optimized bromo substitution on bicyclic ring, various R^1 groups were screened. Compounds **6h** and **6i** with longer carbon chain were less potent than **6e**. Compounds with bulky substitution (**6l** and **6m**) had very poor potency (IC_{50} > 10 μmol/L). The results indicated that the length and the size of the substituents had a substantial influence on activity. It was to our delight that compound **7**, with sulfur replacement of carbonyl oxygen, showed good activity against p110α and Rh30 (IC_{50}: 14 nmol/L and 1.59 μmol/L, respectively). Removal of the nitro group on benzene ring (**9a**) led to a sharp drop in p110α activity (IC_{50}: 3.75 μmol/L) compared to **9b** (IC_{50}: 49 nmol/L).

Finally, compound **7**, the representative compound in this series of compounds, was further evaluated on a panel of tyrosine kinases (Table 2), and it was inactive against other kinases, indicating that it was a selective PI3K inhibitor.

4. Conclusion

In summary, a series of acylhydrazone derivatives were synthesized, and they were identified as potential PI3K inhibitors with no apparent inhibition on a panel of other kinases. Therefore, our results indicated that this class of compounds could be served as lead compound for development of more selective anticancer medication.

Acknowledgments

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