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Research paper

Structural optimization elaborates novel potent Akt inhibitors with promising anticancer activity

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ABSTRACT

Targeting of Akt has been validated as a well rationalized approach to cancer treatment, and represents a promising therapeutic strategy for aggressive hematologic malignancies. We describe herein an exploration of novel Akt inhibitors for cancer therapy through structural optimization of previously described 4-(piperazin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives. Our studies yielded a novel series of pyrrolopyrimidine based phenylpiperidine carboxamides capable of potent inhibition of Akt1. Notably, **10h** exhibited robust antiproliferative effects in both mantle cell lymphoma cell lines and primary patient tumor cells. Low micromolar doses of **10h** induced cell apoptosis and cell cycle arrest in G_2/M phase, and significantly downregulated the phosphorylation of Akt downstream effectors GSK3β and S6 in Jeko-1 cells.

1. Introduction

Akt, also called protein kinase B or PKB, is a serine/threonine kinase that acts as a central junction in the phosphoinositide 3-kinase (PI3K) – Akt signaling pathway [1]. This cascade plays a critical role in regulating cell survival and proliferation, oncogenesis and mechanisms of drug resistance [2,3]. There are three closely related Akt isoforms, Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ), all of which share significant sequence identity in the ATP-binding site [4]. Akt promotes cell proliferation through the phosphorylation of various substrates such as glycogen synthase kinase 3 (GSK-3), TSC1/2, NF-κB, Bcl-2 family proteins and mammalian target of rapamycin complex 1 (mTORC1) [5,6]. Aberrant Akt activation has been confirmed in various cancers, including both hematological neoplasms and solid tumors, often in correlation with resistance to chemotherapy [7,8]. Therefore, targeting Akt is a well rationalized approach to cancer treatment [9].

Mantle cell lymphoma (MCL) is a subtype of Non-Hodgkin's Lymphoma (NHL) with poor prognosis [10]. Although MCL typically responds to conventional chemotherapy, most patients eventually experience disease progression [11]. Aberrant Akt activation has been implicated in MCL pathogenesis and survival [12,13]. While no PI3KCA mutations have been reported as contributors to constitutive Akt activation, loss of tumor suppressor PTEN may be an underlying drive of constitutive Akt signaling in MCL [14]. Such findings support the possibility of Akt inhibition as a novel strategy for the treatment of MCL.

Multiple strategies have been adopted for the discovery of small molecules that target Akt, including ATP- or substrate-competitive inhibitors and allosteric inhibitors [15]. Of these, ATP-competitive inhibitors have yielded the most promising results. Common interactions between the ATP-competitive inhibitors and Akt include a heteroaromatic ring which forms bidentate hydrogen bonds to residues Glu228 and Ala230 in the hinge region, a basic amino group that can form hydrogen binding interactions with the acid hole formed by Glu234 and Glu278, and an aromatic group positioned in a hydrophobic pocket under the P-loop [16,17].

Our laboratory has reported the development of a weak hit **1** from self-library screening into lead **2,** an ATP-competitive

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inhibitor with desirable antiproliferative effects in prostate cancer cell lines [18]. For further modification of **2**, we decided to introduce an amino functionality at the benzylic position, which was inspired by the common structural platform of ATP-competitive inhibitors. In this regard, using a well-established conformational restriction drug design strategy, a piperidyl moiety with more conformational rigidity was deliberately chosen and incorporated, elaborating a novel series of (4-(7*H*-pyrrolo [2,3-d]pyrimidin-4-yl) piperazin-1-yl)(4-phenylpiperidin-4–yl)methanones with enhanced inhibitory potency in both enzyme activity and cancer cell growth (Fig. 1).

2. Results and discussion

2.1. Chemistry

The general synthetic route for the pyrrolopyrimidine derivatives is outlined in Scheme 1. Chlorination or bromination at the 5-position of the readily available **3a** afforded compound **3b** or **3c**, subsequent introduction of the Boc-protected piperazine linker via S_NAr delivered **4a-4c**, followed by removal of the Boc group affording **5a-5c** as dihydrochloride salts. Commercially available **6** reacted with di-*tert*-butyl dicarbonate to produce the Boc-protected **7**, which was further used in the dialkylation of the substituted benzeneacetonitrile to afford intermediate **8a-8g**, basic hydrolysis of the nitrile **8a-8g** gave the carboxylic acid **9a-9g**. Amide coupling between **5a-5c** and **9a-9g** and deprotection of the Boc group achieved the target compounds **10a-10n**.

2.2. Akt inhibition and cell antiproliferative activity

The fourteen newly synthesized pyrrolopyrimidine analogs were evaluated for their activity against Akt1 via a Homogeneous Time-Resolved Fluorescence (HTRF) kinase activity assay. Their effects on proliferation were further assessed in MCL cell lines. The first Akt inhibitor to enter clinical trials, GSK690693, served as a positive control. GSK690693 was previously shown to induce growth inhibition and apoptosis in acute lymphoblastic leukemia [19].

As Table 1 shows, introduction of the piperidyl moiety at the benzylic position represented a substantial improvement in Akt1 inhibition over the initial lead (**2** *vs* **10m**). C5-substitution for chloro and bromo substituents produced an equivalent profile, with more than a 10-fold increase in enzyme inhibitory activity (**10c** *vs* **10g** and **10m**). Examination of substituents on the aromatic ring indicated that the chlorine or bromine substituent (**10k**, **10m** and **10n**) increased potency relative to the unsubstituted phenyl group of **10j**. The small decrease in enzyme potency for **10f** *vs* **10h** indicated that the *meta*-position was potentially less promising for manipulation; however, in the *para*-position, chloro and bromo analogues (**10g**, **10h** and **10m**) displayed similar levels of efficacy against Akt1 and showed the greatest enzyme potency comparable to that of **GSK690693**. A reduction in Akt1 potency was observed in the case of methoxy substitution (**10l**), whereas 3, 4-di-chloro substitution (**10i** and **10n**) was somewhat less active than the p-substitution (**10g** and **10m**).

The promising candidates **10h** and **10m** were further explored in a panel of MCL cell lines. As shown in Table 2, in comparison with the previous lead **2**, both compounds **10h** and **10m** demonstrated improved antiproliferative activities in MCL cell lines compared with GSK690693.**10h** was the most potent inhibitor in MCL cells with an IC_{50} value lower than 1 μM.

2.3. Cell apoptosis and cell cycle assay

Activated Akt influences many factors that mediate apoptosis

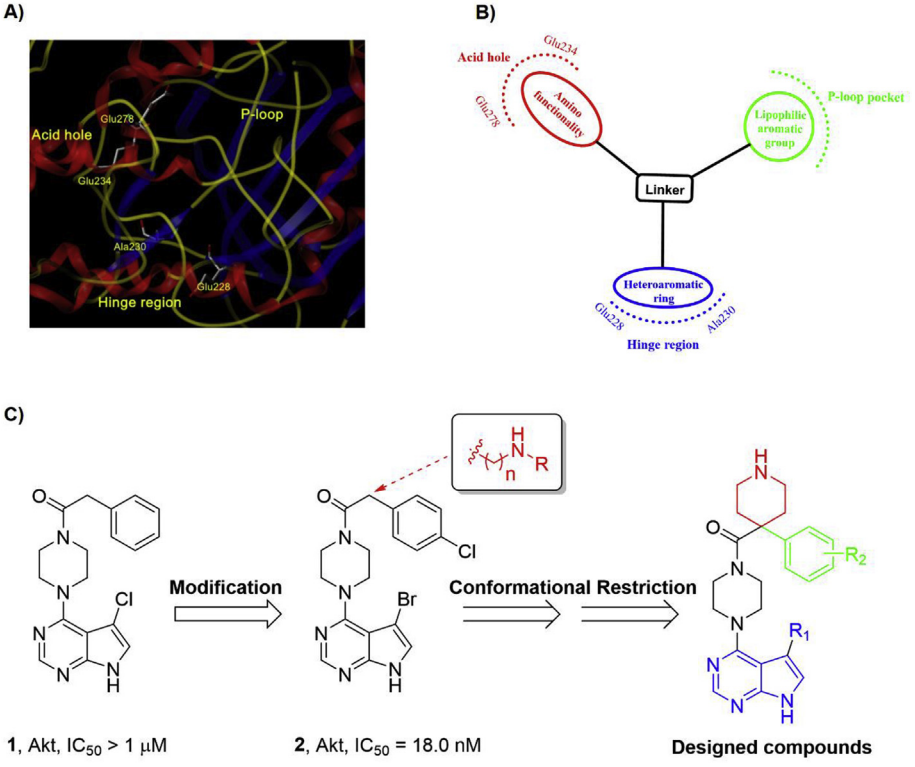

Fig. 1. Design of the targeted compounds. A) Binding site of the ATP-competitive inhibitors; B) common features of ATP-competitive inhibitors; C) Design of the targeted compounds from the initial hit 1.