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Research paper

Design, synthesis and biological evaluation of novel 4-arylaminopyrimidine derivatives possessing a hydrazone moiety as dual inhibitors of L1196M ALK and ROS1

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ABSTRACT

A series of 4-arylaminopyrimidine derivatives possessing a hydrazone moiety were designed, synthesized and evaluated for their biological activity. Most compounds exhibited moderate to excellent cytotoxic activity against ALK-addicted KARPAS299 and ROS1-addicted HCC78, while also showing much less potent activity against A549, H460 and HT-29, whose growth were not dependent on ALK and/or ROS1, as compared with crizotinib and ceritinib. The most promising compound, **7b**, showed high antiproliferative effects on ALK-addicted KARPAS299 and ROS1-addicted HCC78 cell lines with IC_{50} of 20 nM and 28 nM, respectively, but showed no inhibitory activity against A549, H460 and HT-29. The enzymatic assay identified **7b** as a potent and selective ALK and ROS1 dual inhibitor with IC_{50} of 2.5 nM and 2.7 nM, respectively. It also exhibited good inhibitory activity against the L1196M ALK with an IC_{50} value of 67 nM.

1. Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK), structurally belonging to the insulin receptor superfamily. The precise physiological role of full-length wild-type ALK in mammals is enigmatic [1]. By contrast, the constitutively active ALK has been identified to be involved in the initiation and progression of various cancers, such as anaplastic large cell lymphoma (ALCL), inflammatory myofibroblastic tumor (IMT), diffuse large B-cell lymphoma (DLBCL), and non-small cell lung cancer (NSCLC), among others [2]. It is noteworthy that the echinoderm microtubule-associated protein-like-4(EML4)-ALK fusion gene has been identified to be the driving force in approximately 5% of NSCLC patients, making it a promising molecular target for the treatment of NSCLC [1]. Crizotinib, the first-generation ALK inhibitor, was approved by FDA in 2011 for the treatment of patients with locally advanced or metastatic ALK-positive NSCLC [3,4]. Despite the remarkable clinical benefit achieved by crizotinib, clinically acquired resistance remains a serious challenge. In this case, the leucine^{1196} → methionine^{1196} (L1196M), identified as the "gate-keeper" mutation, is most frequently detected [5]. Ceritinib and lectinib, two second-generation ALK inhibitors which could overcome crizotinib-resistant issues, were approved in 2014.

Proto-oncogene tyrosine-protein kinase ROS (ROS1) is one of the last two orphan RTKs and the sole member of the ROS1 RTK family [6]. Among human tissues, the highest level of *ROS1* is expressed in the lungs, and its normal functions have not yet been fully identified [7]. However, dysregulation of ROS1, as a result of *ROS1* gene fusion, has been identified as another clinically actionable oncogenic driver mutation in approximately 1.4% of NSCLCs [8–10]. Although rare, the evidence supports ROS1 fusions as a promising therapeutic target in a subset of NSCLC patients [11]. Currently, only a series of pyrazole derivatives have been developed by S. H. Lee as selective and potent ROS kinase inhibitors [12–14].

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ALK and ROS1 share a 49% amino acid sequence homology in the kinase domains and a 77% identity at the adenosine triphosphate (ATP)-binding site [15]. Given the high homology of ROS1 and ALK, some ALK inhibitors, including crizotinib, AP26113 and TAE-684, were tested efficacious against ROS1-positive cell lines and tumors [16–18]. In a phase I trial (NCT00585195), crizotinib demonstrated marked antitumor activity in patients with advanced NSCLC, harboring ROS1 rearrangements with a response rate of 57% and a disease control rate of 79% at 8 weeks [19]. The success of crizotinib as an ALK and ROS1 dual inhibitor validates the pursuit of novel L1196M ALK and ROS1 dual inhibitors.

Recently, 4-arylaminopyrimidine derivatives have become an important class of L1196M ALK inhibitors, such as ceritinib, A and NVP-TAE684 (Fig. 1) [20–22]. The co-crystal structure of the ALK catalytic domain in complex with ceritinib reveals that the Cl moiety of ceritinib can interact with the Met in L1196M. This interaction may make up for the loss of the contact between Cl and the Leu side chain in wild-type ALK [23]. From this molecular model and SAR analysis, ceritinib forms hydrogen bond interactions onto the backbone of Met_{1199} *via* the pyrimidine [11]. The isopropylsulfonyl group in the pyrimidin-2-yl aminobenzamide moiety significantly increased ALK potency and repositioned the selectivity from c-Met to ALK [21]. This structure analysis indicates that 4-(2-(isopropylsulfonyl)aryl)aminopyrimidine nuclei are critical for potent activity and selectivity.

The hydrazone moiety has been widely applied in drug design due to its ability to act as a hydrogen bond donator and acceptor. Additionally, this functional group can impart a degree of flexibility to a chemical structure [24,25]. The beneficial properties of hydrazones prompted us to insert this moiety into ceritinib in order to design L1196M ALK and ROS1 dual kinase inhibitors (Fig. 2). Several kinds of aromatic groups, indicated by Ar (**6a-e**), were introduced to identify a better scaffold. In addition, various substituents at the R_1 position (**6f-x**) were added to the terminal phenyl ring to explore the electronic and steric effects. Further modifications were performed by introducing methyl groups at the R_2 position (**7a-h**) of the hydrazone moiety.

In the current study, a series of 4-arylaminopyrimidine derivatives possessing a hydrazone moiety were designed and synthesized. Compounds were then assayed for their anti-proliferative activity *in vitro* against five cancer cell lines: KARPAS299, HCC78, A549, H460 and HT-29. Based on the results anti-proliferative results, eight compounds were selected for further *in vitro* enzymatic, inhibitory studies.

2. Chemistry

Compounds were designed by a synthetic route illustrated in Scheme 1. Substitution of commercially available 2-fluoronitrobenzene with isopropyl mercaptan provided intermediate **1**, which was oxidized by hydrogen peroxide to afford **2** as a white solid. Intermediate **2** was then subjected to reduction by hydrazine hydrate in the presence of activated carbon and ferric chloride in EtOH to yield **3**. A regioselective condensation of **3** with the 4-position of 2,4,5-trichloropyrimidine in the presence of NaH in DMF provided intermediate **4**, which was substituted with hydrazine hydrate to lead to the key intermediate **5**. **5** was condensed with an appropriate aromatic aldehyde or ketone to afford target compounds with good yields.

The chemical structures of the target compounds were confirmed by infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis. The configuration of the imino double bonds in the target compounds was determined by nuclear overhauser spectroscopy (NOESY). Taking compound **6f** as an example, a clear NOESY signal was observed between the proton of =N–N**H**– (δ 11.37 ppm, singlet) and the proton of –C**H**=N– (δ 8.14 ppm, singlet), which existed only in the *E* isomer due to the appropriate intramolecular H–H distance (see Supporting Information). Thus, the target compounds were confirmed as the *E* isomer.

3. Results and discussion

3.1. In vitro cytotoxic activities and structure-activity relationships

The MTT colorimetric assay was used to measure the cytotoxic activities of compounds **6a-x** and **7a-h** in KARPAS299, HCC78, A549, H460 and HT-29 cell lines. Since our goal was to discover novel dual inhibitors of ALK and ROS1, ALK and ROS1 inhibition were evaluated in a cellular context by measuring the proliferation of the KARPAS299 cell line expressing the NPM-ALK fusion protein [19] and the HCC78 cell line which expresses the SLC34A2-ROS1 fusion protein [14]. Tumor cell lines A549 (an EGFR-positive human NSCL cell line), H460 (a large cell lung cancer cell line) and HT-29 (a human colon cancer cell line), whose growth was not dependent on ALK and/or ROS1, were used to test the potential off-target effects. Crizotinib and ceritinib were used as the positive controls. These cytotoxic experiments performed in triplicate and are presented as IC_{50} values in Tables 1 and 2.

As a general trend, most of the compounds exhibited moderate to excellent cytotoxic activities against ALK-addicted KARPAS299 cells. Additionally, a similar trend was observed with the ROS1-addicted HCC78 cell lines. Furthermore, these compounds showed little potency against A549, H460 and HT-29 cells, which suggests that these compounds are selective for ALK and ROS1. The most promising compound, **7b**, displayed significant activity against KARPAS299 and HCC78, with respective IC_{50} values of 20 nM and IC_{50} = 28 nM. **7b** was approximately 2.4- and 11.1-fold more active than that of crizotinib (IC_{50} = 68 nM against KARPAS299, IC_{50} = 340 nM against HCC78), respectively, and was approximately as potent as ceritinib (IC_{50} = 27 nM against KARPAS299, IC_{50} = 18 nM against HCC78). Meanwhile, **7b** showed no inhibitory activity against A549, H460 and HT-29.

In order to preliminarily define a structure affinity relationship, a small set of compounds (**6a-e**) with different aryl groups were synthesized and evaluated for their cytotoxic activity. The results

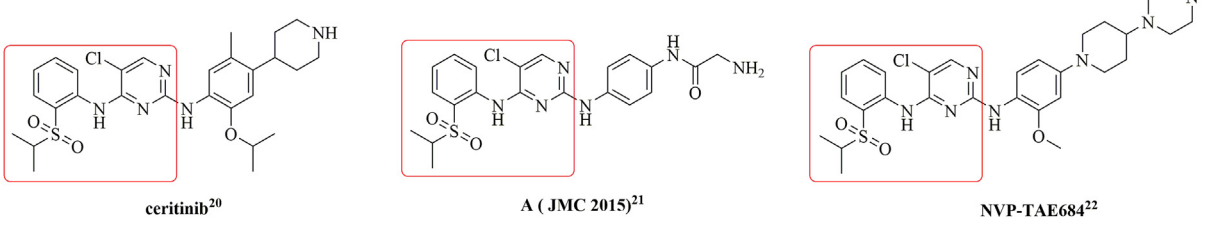

Fig. 1. Structures of representative L1196M ALK inhibitors containing 4-arylaminopyrimidine nuclei.