European Journal of Medicinal Chemistry 100 (2015) 151–161

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Identification of a potent 5-phenyl-thiazol-2-ylamine-based inhibitor of FLT3 with activity against drug resistance-conferring point mutations

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ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form
30 April 2015
Accepted 5 May 2015
Available online 9 May 2015

Keywords:
FLT3 inhibitor
Acute myeloid leukemia
Receptor tyrosine kinase
ITD/D835Y
Xenograft model

ABSTRACT

Numerous FLT3 inhibitors have been explored as a viable therapy for the treatment of acute myeloid leukemia (AML). However, clinical data have been underwhelming due to incomplete inhibition of FLT3 or the emergence of resistant mutations treated with these older agents. We previously developed a series of 3-phenyl-1*H*-5-pyrazolylamine derivatives as highly potent and selective FLT3 inhibitors with good *in vivo* efficacy using an intravenous (IV) route. However, the poor bioavailability of these pyrazole compounds limits the development of these promising antileukemic compounds for clinical use. Herein, we describe a novel class of 5-phenyl-thiazol-2-ylamine compounds that are multi-targeted FLT3 inhibitors. From this class of compounds, compound **7h** was very potent against AML cell lines and exhibited excellent oral efficacy in AML xenograft models. In addition, further studies demonstrated that compound **7h** exhibited potent *in vitro* and *in vivo* activities against clinically relevant AC220 (**3**)-resistant kinase domain mutants of FLT3-ITD.

1. Introduction

Acute myeloid leukemia (AML) is an aggressive disease in which the rapid growth of abnormal leukemic cells in bone marrow inhibits the production of normal blood cells. Despite the success of conventional chemotherapy, the rate of relapse among AML patients is relatively high. The failure of the initial chemotherapy treatment and the refractory nature of AML have been associated with mutations that activate signal transduction pathways, resulting in enhanced proliferation and survival of leukemia cells [1,2]. The FMS-like tyrosine kinase-3 (FLT3) is a member of the class III membrane-bound receptor tyrosine kinase (RTK) family, along with c-KIT, FMS, and PDGFR [3,4]. The most frequent mutations among AML are FLT3 mutations, which account for approximately 30% of the genetic mutations that are predictive markers for a poor prognosis [4–6]. These mutations are typically internal tandem duplications (ITDs) in the juxtamembrane domain of the receptor and the missense point, or short-length mutations in the activation loop (AL) of the tyrosine kinase domain (KD) [7–9]. In addition to mutant forms of FLT3, wt-FLT3 is highly expressed in most cases of acute leukemia, and FLT3 over-expression is an unfavorable prognostic factor for overall survival with AML. In addition, over-expressed wt-FLT3 has the same sensitivity to FLT3 inhibitors as

Abbreviations: FLT3, FMS-like Tyrosine Kinase-3; AML, acute myeloid leukemia; IV, intravenous; RTK, receptor tyrosine kinase; ITDs, internal tandem duplications; AL, activation loop; KD, kinase domain; TKIs, tyrosine-kinase inhibitors; SAR, structure–activity relationships; VEGFR2, vascular endothelial growth factor receptor 2; CSF1R, macrophage-colony stimulating factor receptor; PDGFR, platelet-derived growth factor receptor; RET, proto-oncogene tyrosine-protein kinase receptor; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; CR, complete regression; SC, subcutaneous; PO, per oral.

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http://dx.doi.org/10.1016/j.ejmech.2015.05.008
0223-5234/

the FLT3/ITD mutants [10,11].

Due to the prevalence and refractory nature of the FLT3 mutations as well as the poor prognosis for those affected by it, numerous agents have been developed to directly inhibit wild-type and mutant FLT3. Several of these agents, such as midostaurin (PKC412, **1**) [12], sorafenib (**2**) [13] and quizartinib (AC220, **3**) [14] (Fig. 1), have been investigated as potential agents for the treatment of AML [15]. However, clinical responses to several of the early multi-targeted agents, such as lestaurtinib and **1**, have been underwhelming with limited reduction in bone marrow blasts and weak short-term responses. These underwhelming results may be primarily due to low potency and/or inadequate toleration of the inhibitors at effective doses, which result in failure to achieve complete and sustained inhibition of FLT3 in the patients' leukemic blast cells [15]. Newer, more potent and selective FLT3 inhibitors, such as **2** and **3**, possess the ability to achieve more sustained *in vivo* inhibition of FLT3 and have exhibited highly promising activity in early clinical studies [16]. Nevertheless, K. W. Pratz et al. reported that the inhibition of FLT3 autophosphorylation in a FLT3/ITD specimen does not always induce cell death, suggesting that some FLT3/ITD AML may not be addicted to FLT3 signaling. In addition, the response rate for diagnostic specimens is clearly higher for the least selective of the drugs due to the cytotoxic effect. These results indicate the potential therapeutic use of FLT3 inhibitors in patients with newly diagnosed FLT3-mutant AML might be less likely to respond clinically to highly selective FLT3inhibition [17].

Another significant result was that patients achieving a response to **3** often developed resistance-conferring point mutations (i.e., most commonly at D835 and less frequently at a "gatekeeper" residue, phenylalanine 691 (F691)) [18,19]. In addition, the emergence of resistant mutations has been reported in relapsed AML patients with FLT3-ITD treated with **1** [20] and **2** [21,22]. These clinical results suggest that secondary mutations conferring resistance were clinically problematic, which has spurred the development of new tyrosine-kinase inhibitors (TKIs) with activity against drug-resistant FLT3-ITD mutations [23].

Using structure-based design, we previously developed a novel class of 3-phenyl-1*H*-5-pyrazolylamine derivatives (Fig. 2) as a versatile template for the development of specific kinase inhibitors and successfully identified a sulfonamide-substituted series of benzamides (Structure **I**) that inhibit both FLT3 and cellular proliferation [24,25]. Using rational design, we identified a second

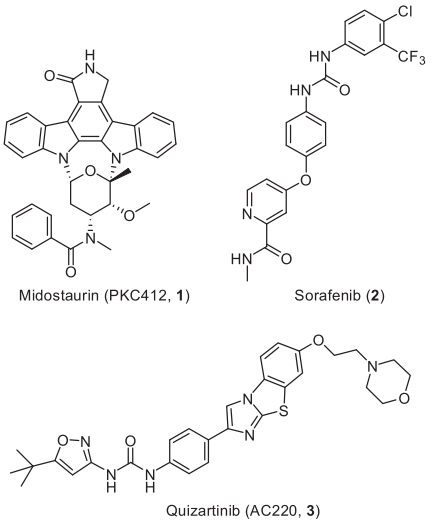

Fig. 1. Structures of FLT3 inhibitors in the clinic.

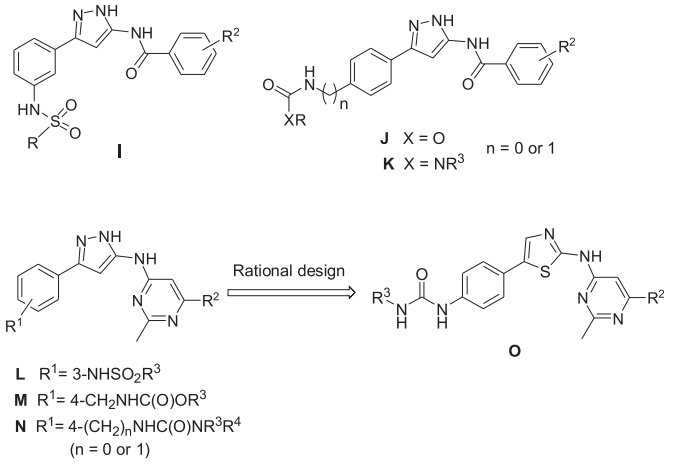

Fig. 2. Identification of 5-phenyl-thiazol-2-ylamine derivatives as FLT3 inhibitors.

sulfonamide-substituted series of pyrimidines (Structure **L**) [25] and two carbamate-substituted series of benzamides and pyrimidines (Structures **J** and **M**, respectively) [26] as potent inhibitors of FLT3. Recently, we developed another series of 3-phenyl-1*H*-5-pyrazolylamine-based inhibitors capable of greater inhibition of *in vitro* AML MOLM-13 cell growth and with a prolonged duration of *in vivo* action. In the previous study, two urea-substituted series of benzamides (Structure **K**) and pyrimidines (Structure **N**) were reported (Fig. 2) [27,28]. However, these pyrazolylamine-based inhibitors were not orally active and exhibited moderate inhibitory activity toward drug-resistant FLT3-ITD mutations [24,28]. Both drawbacks could limit the future development of this pyrazole class of inhibitors for the treatment of AML patients in clinical trials.

We supposed that little or no oral absorption for this class of pyrazole compounds may be due to the presence of more hydrogen-bond donors in the molecules. To continue to develop potent and orally active FLT3 inhibitors, we rationally designed a second class of 5-phenyl-thiazol-2-ylamine-based inhibitors (Structure **O**, Fig. 2). The approach can remove a hydrogen-bond donor in the pyrazole ring. In this study, the structure–activity relationships (SAR), *in vitro* properties and pharmacokinetics of a series of pyrimidine derivatives are reported. This study has led to the discovery of urea **7h**, which is a multi-targeted kinase inhibitor with significant *in vitro* potency and *in vivo* efficacy against FLT3-ITD–expressing MOLM-13 and MV4; 11 cell lines and FLT3-ITD/D835Y–expressing 32D cell line [29]. These promising results demonstrate the potential of **7h** as a drug candidate for further preclinical and clinical development.

2. Results and discussion

2.1. Chemistry

The general synthetic route to 5-phenyl-thiazol-2-ylamine pyrimidines **7**–**10** (Table 1) is shown in Scheme 1. The synthesis began with the preparation of 5-phenyl-thiazol-2-ylamine **4** according to a previously published protocol [30]. Treatment of 5-phenyl-thiazol-2-ylamine **4** with 4,6-dichloropyrimidine in the presence of NaH and THF at 0 °C afforded 4-monosubstituted pyrimidine derivatives **5**. The treatment of these derivatives with 2° amines in pyridine at 80 °C yielded 4,6-disubstituted pyrimidines **6**, which were coupled with phenyl isocyanates (or heteroaryl carbamic acid 4-nitro-phenyl esters), benzenesulfonyl chloride, benzyl chloroformate, or benzoyl chloride to afford the final pyrimidine ureas **7** (except for Boc-**7b**, -**7d** and -**7i**), sulfonamide **8**, carbamate **9** or