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Original article

Thiazolyl *N*-benzyl-substituted acetamide derivatives: Synthesis, Src kinase inhibitory and anticancer activities

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ABSTRACT

KX2-391 (KX-01/Kinex Pharmaceuticals), *N*-benzyl-2-(5-(4-(2-morpholinoethoxy)phenyl)pyridin-2-yl) acetamide, is a highly selective Src substrate binding site inhibitor. To understand better the role of pyridine ring and *N*-benzylsubstitution in KX2-391 and establish the structure–activity relationship, a number of *N*-benzyl substituted (((2-morpholinoethoxy)phenyl)thiazol-4-yl)acetamide derivatives containing thiazole instead of pyridine were synthesized and evaluated for Src kinase inhibitory activities. The unsubstituted *N*-benzyl derivative (**8a**) showed the inhibition of c-Src kinase with GI_{50} values of 1.34 μM and 2.30 μM in NIH3T3/c-Src527F and SYF/c-Src527F cells, respectively. All the synthesized compounds were evaluated for inhibition of cell proliferation of human colon carcinoma (HT-29), breast carcinoma (BT-20), and leukemia (CCRF-CEM) cells. 4-Fluorobenzylthiazolyl derivative **8b** exhibited 64–71% inhibition in the cell proliferation of BT-20 and CCRF cells at concentration of 50 μM.

1. Introduction

Src is the prototype and most widely studied member of one of the largest family of non-receptor protein tyrosine kinases (PTKs), known as the Src family kinases (SFKs) [1], which are key regulators of cellular proliferation, survival, motility and invasiveness [2–4]. Src was first discovered in viral sarcoma and thus was pronounced as “sarc”. Src offers a promising molecular target for anticancer therapy, as increased Src activity upregulates a number of signaling cascades associated with tumor development and progression leading to increased cell growth, migration and invasion. Moreover, Src has been shown to play a critical role in other pathologic disorders, such as myocardial infarction [5], stroke [6], osteoporosis [7], and neurodegeneration [1].

In the last two decades, synthesis of Src kinase inhibitors has been based on designing ATP binding site inhibitors and substrate binding site inhibitors. Despite of the large variety in PTKs structural organization, their ATP binding site is mostly conserved. The ATP binding site competitive inhibitors of Src that mimic the binding of ATP are potent, but often lack selectivity in a panel of isolated kinase assays [8–10]. In contrary, the substrate binding site sequences of PTKs are less conserved, which results in improved selectivity and less toxicity of designed substrate binding site inhibitors when compared with those of ATP mimics targeting ATP binding site.

KX2-391 (KX-01/Kinex Pharmaceuticals) (Fig. 1) is a novel class and highly selective non-ATP Src kinase inhibitor that targets the substrate binding site of Src, has tubulin polymerization inhibition as a second mechanism of action, and is currently in Phase-2 testing for solid tumors [11]. KX2-391 was found to inhibit certain leukemia cells that are resistant to current commercially available drugs, such as those derived from chronic leukemia cells with the T3151 mutation. In pre-clinical animal models of cancer, orally administered KX2-391 was shown to inhibit primary tumor growth and to suppress metastasis. In combination with certain chemotherapeutic agents, KX2-391 was synergistic, thereby, offering the potential to prescribe lower doses of some current cytotoxic agents that have undesirable side effects.

In addition, previous structural studies [12–14] have proven that occurrence of heterocyclic scaffolds such as thiazole may result in generating effective kinase inhibitors, including potent Src kinase inhibitors. Dasatinib (Fig. 1) with amino-thiazole moiety, is one of

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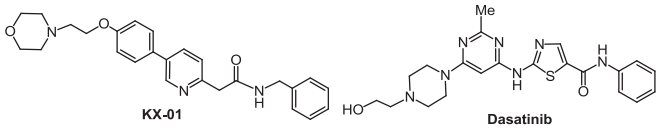


Fig. 1. KX2-391 (KX-01) a potent and highly selective non-ATP Src kinase inhibitor; Dasatinib a potent *pan*-Src kinase inhibitors.

the potent *pan*-Src kinase inhibitors, which has been approved by FDA for the treatment of Gleevec-resistant CML [15,16].

Since the crystal structure of substrate binding site with Src inhibitors is not available yet, the designing strategy for discovering selective Src substrate binding site inhibitors has been mostly based on screening rather than rational designing [17]. Considering these facts, and in continuation of our efforts to design small molecules as Src kinase inhibitor or anticancer agents [18], we herein report the synthesis a series of substrate binding site inhibitors by substituting pyridine ring in KX2-391 molecule with a thiazole group and introducing substitutions on the benzyl ring. Src kinase inhibitory and anticancer activities of the compounds were evaluated in cell-based assays.

2. Results and discussions

2.1. Chemistry

Scheme 1 outlines the procedure for the synthesis of thiazolyl benzyl acetamides **8a**–**e**. Commercially available 4-(2-chloroethyl) morpholine hydrochloride (**1**) was reacted with 4-hydroxybenzo nitrile (**2**) in presence of K_2CO_3 in refluxing DMF for 24 h to yield 4-(2-morpholinoethoxy)benzonitrile (**3**). Subsequent reaction of **3** with ammonium sulfide at room temperature afforded 4-(2-morpholinoethoxy)benzothioamide **4**. Treatment of **4** with ethyl 4-chloroacetoacetate resulted in the formation of thiazolyl derivative **5**, which underwent basic ester hydrolysis to generate acetic acid derivative **6**. Finally, the reaction of **6** with the corresponding benzylamines **7a**–**e** in the presence of 1-hydroxybenzotriazole (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro chloride (EDCI) in acetonitrile afforded desired thiazolyl benzyl acetamides **8a**–**e**.

2.2. Biological activity

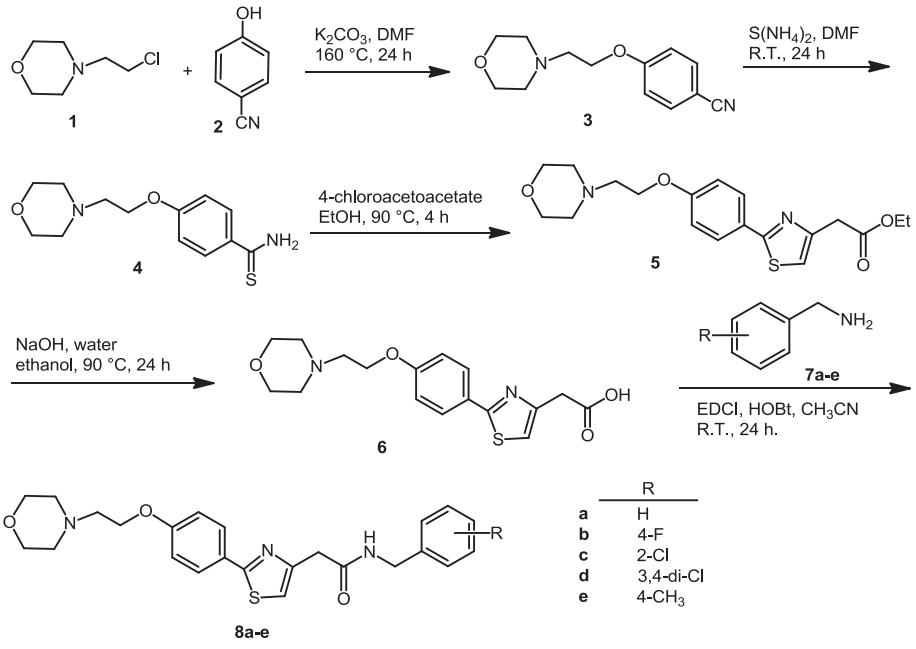
2.2.1. Src kinase inhibitory activity

The compounds were evaluated in engineered Src driven cell growth assays in NIH3T3/c-Src527F and SYF/c-Src527F cells. NIH3T3/c-Src527F cell line has constitutively active human Src driving growth. SYF/c-Src527F has Src, Yes, and Fyn mouse knockouts with c-Src527F added back. The results of Src kinase inhibitory activity of compounds **8a**–**e** are shown in Table 1 and Fig. 2. Compound **8a** with no substitution on *N*-benzyl group showed GI_{50} values of 1.34 μM and 2.30 μM in NIH3T3/c-Src527F and SYF/c-Src527F cells, respectively, and was found to be the most potent compound of this series. Introducing of a fluoro group at position 4 of benzyl group led to a slight decrease of inhibitory activity in compound **8b** (GI_{50} = 1.49–2.51 μM) versus **8a**. Introducing of a methyl group at position 4 of benzyl group in **8e** led to almost 4-fold decrease of potency in comparison with **8a**. Similarly 2-chlorobenzyl and 3,4-dichlorobenzyl substituted analogs (**8c** and **8d**) showed significantly less inhibitory activities when compared to other compounds (GI_{50} = 7.93–13.02 μM). The data suggest that incorporation of bulky groups, such as chlorine and methyl in compounds **8c**–**e** with higher lipophilicity (Log *P*), results in decreased potency.

All the tested compounds were significantly less potent than KX2-391 (KX-01), suggesting that introducing thiazole replacement of pyridine has led to decreased activity. In other words, pyridine is possibly a pharmacophore of KX2-391 that has major interactions with Src substrate binding site. Nevertheless, none of the compounds were active in vitro assay against Src kinase, which confirm earlier results that the peptide binding site is not well formed outside of cells and these compounds indeed inhibit Src in cellular environment when the substrate binding site is deeper for binding interactions.

2.2.2. Anticancer activities

The effect of the inhibitors at the concentration of 50 μM on the cell proliferation of human colon adenocarcinoma (HT-29) cancer



Scheme 1. Synthesis of thiazolyl benzyl acetamides **8a**–**e**.