Bioorganic & Medicinal Chemistry Letters 27 (2017) 845–849

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of thiazole derivatives as novel USP7 inhibitors

Chao Chen [a,b,1], Jiemei Song [a,b,1], Jinzheng Wang [a,b,1], Chang Xu [a,b], Caiping Chen [a,b], Wei Gu [c], Hongbin Sun [a,b,*], Xiaoan Wen [a,b,*]

[a] Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China
[c] Institute for Cancer Genetics, College of Physicians and Surgeons, Columbia University, New York, NY, USA

ARTICLE INFO

Article history:
Received 9 September 2016
Revised 2 January 2017
Accepted 9 January 2017
Available online 10 January 2017

Keywords:
USP7 inhibitor
Thiazole derivatives
Human colon cancer

ABSTRACT

Herpesvirus-associated Ubiquitin-Specific Protease (HAUSP, also called USP7) interacts with and stabilizes Mdm2, and represents one of the first examples that deubiquitinases oncogenic proteins. USP7 has been regarded as a potential drug target for cancer therapy. Inhibitors of USP7 have been recently shown to suppress tumor cell growth in vitro and in vivo. Based on leading USP7 inhibitors P5091 and P22077, we designed and synthesized a series of thiazole derivatives. The results of in vitro assays showed that the thiazole compounds exhibited low micromolar inhibition activity against both USP7 enzyme and cancer cell lines. The compounds induced cell death in a p53-dependent and p53-independent manner. Taken together, this study may provide thiazole compounds as a new class of USP7 inhibitors.

The p53 tumor suppressor is a short-lived protein that is maintained at low levels in normal cells. Tight regulation of p53 pathway signal is essential for its effect on tumorigenesis as well as maintaining normal cell growth. It is well accepted that Mdm2, a major E3 ubiquitin ligase for p53, is a major negative regulator of p53. Unusually activated Mdm2 could counteract the tumor suppressor function of p53 through promoting both degradation and degradation-independent effects on p53.[1]

Targeting Mdm2 is a validated approach to restoration of p53 function for cancer therapy. Nevertheless, there exist some limitations for Mdm2 inhibitors such as Nutlin-3. For example, although Nutlin-3 can effectively block the interaction of Mdm2 and p53, Nutlin-3 cannot decrease the level of Mdm2 protein. In fact, it increases the expression of Mdm2 mRNA through the p53-Mdm2 positive feedback loop and protects Mdm2 from degradation, thus leading to a rapid rebound of cancer cell growth upon temporary removal of the inhibitor.[2]

Owing to the success of the proteasomal inhibitor Bortezomib in treating multiple myeloma, targeting the ubiquitin-proteasome system (UPS) has become one of the most concerned strategies in anti-cancer therapies in the past decade. Herpesvirus-associated Ubiquitin-Specific Protease (HAUSP, also called USP7) interacting with the p53/Mdm2 complex was one of the first examples that deubiquitinases (DUBs) exhibited a specific role in regulating the stability of oncogene protein Mdm2.[3,4] These studies demonstrate that inactivation of USP7 induces Mdm2 degradation thus allowing for activation of p53 in tumor cells expressing wild-type p53. In fact, several small-molecule inhibitors of USP7 have been recently shown to activate p53 by down-regulating Mdm2 activities without inducing genotoxic stress.[5–8] More importantly, in xenograft mouse models, USP7 inhibitors were well tolerated and effectively suppressed tumor growth in vivo.[6]

Biochemical and tissue culture studies showed that thiophene compounds P5091 and P22077 (Fig. 1) exhibited specific and selective inhibitory activity against USP7.[7] Recently, we found that P22077 significantly suppressed the growth of MYCN-amplified human neuroblastoma cell lines in xenograft mouse models.[9] However, the currently leading small-molecule inhibitors of USP7 such as P22077 have several issues. For example, the solubility of these compounds is poor and the potency of the USP7 inhibition is not very high. Besides, the toxicity of the compounds at relatively higher doses to achieve significant efficacy was observed in in vivo studies. Therefore, novel USP7 inhibitors with higher potency and safety are highly desirable. By analyzing the structure-activity relationship of P5091and P22077 derivatives reported

* Corresponding authors at: State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China.
E-mail addresses: hongbinsun@cpu.edu.cn (H. Sun), wxagj@126.com (X. Wen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2017.01.018
0960-894X/

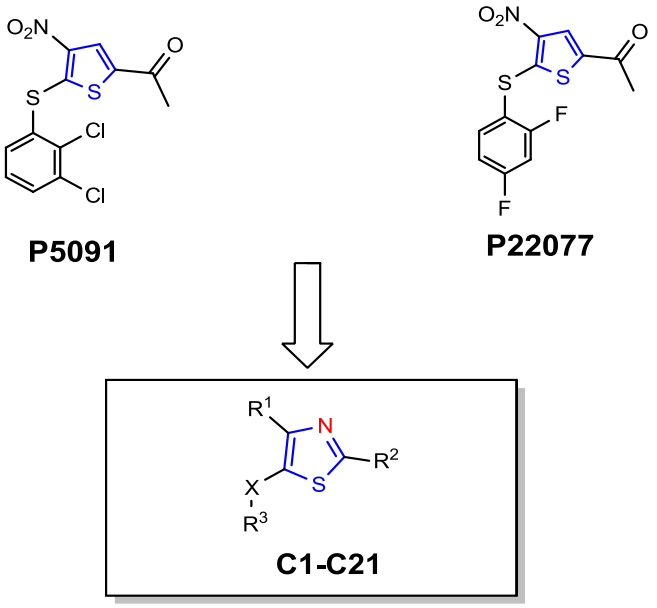

Fig. 1. Design of thiazole derivatives as USP7 inhibitors.

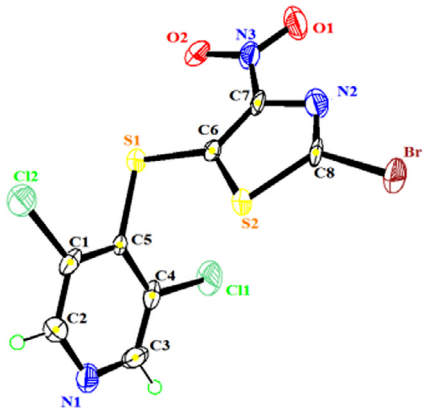

Fig. 2. ORTEP representation of compound **9**.

by Weinstock et al.,[10] it was reasoned that electron-withdrawing substituents on thiophene would beneficial for the anti-USP7 activity. Thiazole could be regarded as a bioisosteric structure of thiophene with decreased electron density. In this study, a series of thiazole derivatives **C1–C21** (Fig. 1) were designed and synthesized based on P5091 and P22077 as the lead compounds. We present the synthesis, USP7 inhibition and anti-proliferation activities of these compounds.

The key intermediate 1-(5-chlorothiazol-2-yl)ethanone **4** was prepared from commercially available 2-acetylthiazole over three steps (acetal protection, chlorination and protecting group removal). Treatment of **4** using corresponding thiophenols in the presence of sodium methoxide provided the 4-nonsubstituted thiazole derivatives **C1** and **C2**. Nitration of compounds **4** was carried out under nitric acid condition to afford nitro thiazole **5**. According to the same procedure for **C1** and **C2**, the target compounds **C3–C10**, **C21** were obtained from the reaction of the corresponding thiophenols with **5**. Subsequently, compounds **C11–C14** were prepared via reduction and subsequent acylation of **C7**. Reductive amination of **C7** afforded compound **C15**. The general route to the thiazole-2-carboxamide derivatives starts with the brominating of 2-bromothiazole **6** to give 2, 5-dibromothiazole **7**. Nitration of **7** with nitric acid provided nitro thiazole **8** which was used for further modification by nucleophilic substitution reaction with 3,5-dichloropyridine-4-thiol to afford compound **9**, whose structure was confirmed by the X-ray crystallography (Fig. 2). Compound **9** was treated with cuprous cyanide at high temperature up to 120 °C to afford the thiazole-2-carboxamide derivative **10**, followed by hydrolysis reaction with nitrous acid to give carboxylic acid **11**, which was extremely unstable due to decarboxylation. However, decarboxylation of **11** could be avoided by hydrolysis reaction at low temperature. The target compounds **C16-C20** were obtained by the amidation reaction of **11** with amines in the presence of phosphorus oxychloride (see Scheme 1).

Enzymatic assay. A summary of the inhibitory effect of compounds **C1–C21** against USP7 in enzyme based assay is listed in Table 1. As a positive control, P22077 showed moderate activity (IC_{50} = 19.33 μM) in this assay. To our delight, the thiazole compounds showed definite improvement on inhibitory activity in comparison with the thiophene class of USP7 inhibitors. It was found that 4-nitro group was indispensable since analogues of these compounds with the 4-nitro group replaced by hydrogen were almost inactive (**C1**, **C2**). The substitution of an electron-withdrawing group (e.g. 2-acetyl) at the 2-position of thiazole seemed to be critical for the potency (**C6**, **C7**), since 2-alkyl substitution resulted in significant loss of potency (**C11**, **C12**). Like 2-acetyl compounds (**C6**, **C7**), *N*-benzyl substituted 2-amide compounds were also potent inhibitors, especially when the phenyl ring was substituted with fluorine atom, (e.g. **C19**, IC_{50} = 1.35 μM). 5-Aryl thio substitution also had a significant impact on potency (**C4** vs **C10**), and 3, 5-dichloropyridin-4-yl was considered as a beneficial group which is consist with the finding of Weinstock et al.[10] Within this series of compounds, **C7** and **C19** are the most potent compounds with IC_{50} 0.67 μM and 1.35 μM, respectively. Compound **C21** (IC_{50} = 6.12 μM) is more potent than its parent compound P22077 (IC_{50} = 19.33 μM).

Cellular assay. All the compounds were tested on human colon cancer HCT-116 cell line. P22077 was used as the positive control. The results are summarized in Table 2. Notably, the inhibitory activity of these compounds on the proliferation of HCT-116 cell is basically correlated with their enzyme inhibitory activity. For example, compounds **C7** and **C19** exhibited good inhibition activity in both enzymatic and cellular assays. Nevertheless, off-target effects of compound **C14**, **C17** could not be ruled out due to its weak enzyme inhibitory activity in comparison with its modest inhibitory potency on HCT-116 cell line. Notably, even though **C21** showed more potent USP7 inhibitory activity than P22077, **C21** was less potent than P22077 on inhibition of HCT-116 cell growth, probably due to the off-target effects.

Next, the effect of these compounds on cell viability was tested on three cancer cell lines with different p53 status, including HCT-116 ($p53^{wt/wt}$), RPM I-8226 ($p53^{mt/mt}$) and H1299 (lack of p53 gene). Similar to the observation by Reverdy et al.,[5] the thiazole compounds showed a similar level of activity on cell viability regardless of the p53 status (Table 3). Notably, P5091 treatment decreased the viability of p53 null ARP-1 MM cells, associated with decreased Mdm2 levels and increased p21 expression.[6] This suggests that although P5091 increases p53 levels, its cytotoxic activity is not solely dependent on p53, indicating that P5091-induced cytotoxicity is mediated only in part via p53. In this regard, the thiazole USP7 inhibitors should also have p53-independent effects on cell viability.

Binding mode of Thiazole USP7 Inhibitors. In order to understand the structural basis of the binding of thiazole compounds to USP7, the potential binding site of the compounds to USP7 was investigated. Also P5091 and P22077 can inhibit USP7 with moderate activity and selectivity, related research on the structural basis for binding to USP7 has not been reported so far.