European Journal of Medicinal Chemistry 77 (2014) 351–360

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Design, synthesis and evaluation of novel diaryl urea derivatives as potential antitumor agents

Chenshu Lu, Ke Tang, Yan Li, Peng Li, Ziyun Lin, Dali Yin, Xiaoguang Chen*, Haihong Huang**

Beijing Key Laboratory of Active Substances Discovery and Druggability Evaluation, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100050, China

ARTICLE INFO

Article history:
Received 25 August 2013
Received in revised form
4 February 2014
Accepted 8 March 2014
Available online 12 March 2014

Keywords:
Diaryl ureas
Sorafenib
Linker
Antitumor activity

ABSTRACT

A novel series of diaryl ureas containing different linker groups were designed and synthesized. Their *in vitro* antitumor activity against MX-1, A375, HepG2, Ketr3 and HT-29 was evaluated using the standard MTT assay. Compounds having a rigid linker group such as vinyl, ethynyl and phenyl showed significant inhibitory activity against a variety of cancer cell lines. Specifically, compound **23** with a phenyl linker group demonstrated broad-spectrum antitumor activity with IC_{50} values of 5.17–6.46 μM against five tested tumor cell lines. Compound **23** is more potent than reference drug sorafenib (8.27–15.2 μM), representing a promising lead for further optimization.

1. Introduction

Cellular signaling network in tumor cells is complex and redundant, therefore broad-acting and multi-targeted kinase inhibitors may be more advantageous than selective agents because of their ability to block multiple signaling pathways associated with tumor survival [1–3]. A number of multi-targeted kinase inhibitors based on different scaffolds, such as sorafenib [4], linifanib [5,6] and curcumin [7], have reached market or have been in late-stage clinical trials. These agents have demonstrated clinical benefits with manageable side effects (Fig. 1).

The antitumor activity of kinase inhibitors containing a diaryl urea scaffold has been described in literature [8–10]. These molecules possess a unique binding mode and kinase inhibition profile [11]. For example, sorafenib, a diaryl urea multi-targeted inhibitor of several kinases including Raf, VEGFR and PDGFR, was approved by Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma (RCC) in 2005 and unresectable hepatocellular carcinoma (HCC) in 2007 [4,12].

Crystal structure of B-Raf in complex with sorafenib revealed a distinctive mode of protein–ligand interaction [13]. The diaryl urea portion of the molecule is highly conserved and shared by most type II kinase inhibitors. The *N*-methyl-4-picolinamide moiety that binds to the hinge region of the kinase is highly mobile. The pyridyl ring nitrogen atom in sorafenib forms hinge hydrogen bonding, but it is known to be less critical compared with that in other kinase inhibitors [14,15].

The interest in designing novel diaryl ureas with improved antitumor activity has been growing following the successful launch of sorafenib [16–20]. Linifanib is another potent kinase inhibitor in clinical development. As compared to the structure of sorafenib, linifanib has a more rigid structure with a 3-aminoindazole moiety at the hinge region. It has been demonstrated that a large group at the hinge region can be well tolerated [15,21–24] and there is growing interest in finding an optimal hinge binding scaffold.

To develop a new generation of more potent multi-targeted kinase inhibitors, we took advantage of the previous structure–activity relationships (SAR) of sorafenib analogs. The diaryl urea and amide groups, as key pharmacophores, were kept intact. At the same time, we planned to preserve the chloro and trifluoromethyl substituents on the distal phenyl ring, while replacing the *N*-methyl group by α-methylbenzyl and cyclic alkyl groups in order to investigate their effects on antitumor activity. We focused our main modifications on the oxygen atom and pyridyl ring of sorafenib, exploring a variety of linker groups such as vinyl, ethynyl and phenyl to connect the diaryl urea and amide moieties. A total of

* Corresponding author. Tel./fax: +86 010 63165207.
** Corresponding author. Tel./fax: +86 010 63165244.
E-mail addresses: chxg@imm.ac.cn (X. Chen), joyce@imm.ac.cn (H. Huang).

http://dx.doi.org/10.1016/j.ejmech.2014.03.020
0223-5234/

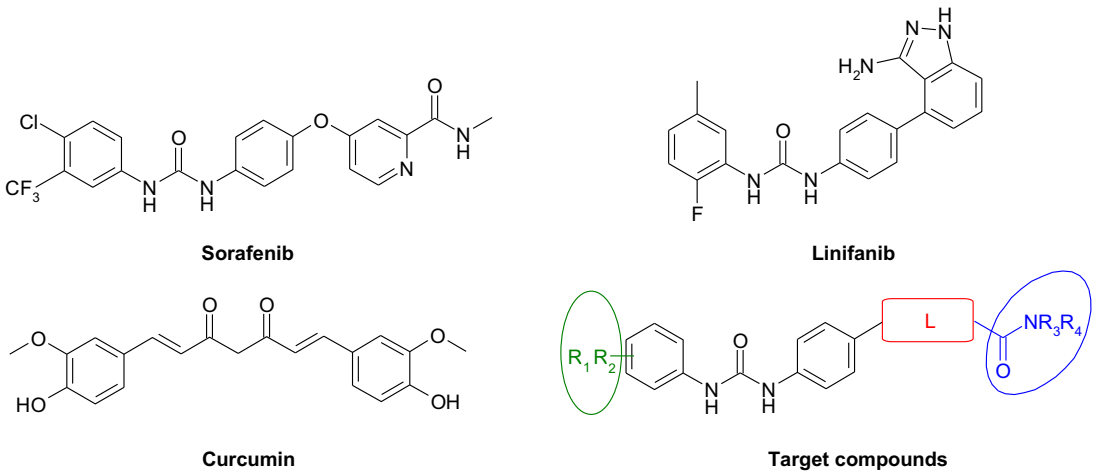

Fig. 1. Structures of known multi-targeted kinase inhibitors and target compounds.

twenty seven novel diaryl ureas were designed and synthesized. The inhibitory activity of these compounds **1**–**27** against five cancer cell lines, including human breast cancer (MX-1), human melanoma (A375), human liver cancer (HepG2), human kidney cancer (Ketr3) and human colon cancer (HT-29) cell lines, was evaluated using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Herein, we wish to report the synthesis and SAR of the target compounds.

2. Results and discussion

2.1. Chemistry

The intermediate amines **A1**–**A8** were synthesized following known synthetic procedures as outlined in Scheme 1. Some improvements were made, especially for **A6**. The general synthetic procedures of target compounds **1**–**27** were illustrated in Scheme 2.

2.1.1. General synthesis of intermediate *A1*–*A3*

4-Nitrobenzaldehyde was submitted to the Knoevenagel reaction with malonic acid or methylmalonic acid [25]. Condensation with corresponding amines, followed by reduction of nitro group with $SnCl_2$ gave intermediates **A1(a–d)** or **A3(a–c)**. The subsequent catalytic hydrogenation of nitro group and double bond simultaneously using 5% Pd/C gave intermediates **A2(a–d)**.

2.1.2. General synthesis of intermediate *A4*

4-Nitroacetophenone was treated with trimethyl phosphonoacetate in the presence of *N*-sodiumhexamethyldisilazane (NaHMDS) [26]. Ester saponification and amidation, followed by reduction of nitro group with $SnCl_2$ provided intermediates **A4(a–c)**.

2.1.3. General synthesis of intermediate *A5*

Treatment of *N*-Boc-4-aminophenol with methyl propiolate in the presence of a catalytic amount of 1,4-diazabicyclo[2.2.2]octane (DABCO) [27], followed by ester saponification and amidation using (benzotriazol-1-yloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) as a condensation reagent [28], and Boc deprotection afforded intermediates **A5(a–b)**.

2.1.4. General synthesis of intermediate *A6*

The conversion of 4-iodonitrobenzene to methyl 3-(4-nitropheny1)propiolate was accomplished according to the reported procedure [29], followed by ester saponification and amidation using BOP reagent gave amide intermediate. Herein, an improved approach for selective reduction of nitro group in compounds containing an ethynyl group was investigated. We reduced the nitro group with Fe powder in ethanol under acidic condition, which gave intermediates **A6(a–b)** in high yield (about 80%).

2.1.5. General synthesis of intermediate *A7*–*A8*

Suzuki coupling between 4-bromonitrobenzene and 3-carboxybenzeneboronic acid with $Pd(PPh_3)_4$ as a catalyst in the presence of K_2CO_3 afforded 4-nitrobiphenyl-3-carboxylic acid quantitatively [30]. 4-Biphenylcarboxylic acid was treated with ice-cold nitric acid to provide 4-nitrobiphenyl-4-carboxylic acid [31]. Condensation the two acids with the corresponding amines, followed by reduction of nitro group with 5% Pd/C gave intermediates **A7(a–b)** and **A8(b–c)**, respectively.

2.1.6. General synthesis of target compounds *1*–*27*

Finally, condensation of **A1**–**A8** with corresponding aryl isocyanates in anhydrous dichloromethane (DCM) afforded the desired diaryl urea derivatives **1**–**27** in 79%–91% yields [32].

2.2. Biological evaluation

The *in vitro* inhibitory activity of target compounds against MX-1, A375, HepG2, Ketr3 and HT-29 cell lines was evaluated using the MTT-based assay. The IC_{50} results of various analogs were summarized in Table 1 with sorafenib as the reference compound.

As illustrated in Table 1, most compounds bearing a rigid linker group such as vinyl (compounds **2**–**4**), ethynyl (compounds **20**–**21**) and phenyl (compounds **23**–**25**) exhibited moderate to potent activities against the tested cancer cell lines, while flexible linker group such as saturated ethyl chain (compounds **6**–**9**) and bulky linker group such as branched vinyl (compounds **10**–**15**) were all detrimental for activities. The results were consistent with several recently published articles [33–35] which suggested that a,β-unsaturated ketone may be the pharmacophore of many antitumor agents like curcumin. It was also noted that a moderate to dramatic decrease in potency was found if the vinyl group was replaced with a more electron-deficient –OCH=CH group (compounds **2** versus **17**, **3** versus **18**). These observations may be due to a combination of electronic and steric effects of the linker groups. Interestingly, most compounds that contain a rigid linker group demonstrated potent activities against both MX-1 and HT-29, especially with an