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ORIGINAL ARTICLE

Pyrimidinylacetamide-based 2-pyridylureas as Angiogenesis Inhibitors: Design, Synthesis and Biological Evaluation

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Background. VEGFR-2 inhibitors have been widely used in the treatment of cancer. In our continued efforts to search for potent and novel VEGFR-2 inhibitors as antitumor agents, we have identified a series of ureas and amides bearing a oxazolopyrimidine scaffold.

Aim of the study. To discover more potent VEGFR-2 inhibitors with stronger binding affinity and better physical and chemical properties.

Methods. 23 pyrimidinylacetamide-based ureas were designed and synthesized. Replacement of oxazolopyrimidine with a pyrimidinylacetamide generated a series of novel VEGFR-2 inhibitors.

Results and Conclusions. In HUVEC inhibition assay, the most potent compound (compound 16) possessed an IC_{50} value of 0.43 μM. Compound **16** also inhibited the migration and capillary like tube formation of HUVECs with inhibition rate at 22% (1 μM) and 17.5% (0.8 μM) respectively. These results support the further investigation of compound 16 as a potential anti-cancer agent.

Key Words: Pyrimidinylacetamide, Ureas, VEGFR-2, Anti-proliferative activity, Anti-angiogenesis.

Introduction

Angiogenesis has been an attractive therapeutic target in the treatment of cancer in the past decades. It is a critical process in solid tumor progression because tumors of a critical size cannot grow until they develop new blood vessels to provide oxygen and nutrients (1). Active research in inhibition of angiogenesis and subsequent clinical trials eventually resulted in US Food and Drug Administration (FDA)approval of bevacizumab for colorectal cancer in 2004 (2). Since then, angiogenesis targeted drugs such as sorafenib, sunitinib, pazopanib and axitinib have been demonstrated as potent cancer treatment methods (3−6). Up to now, various derivatives of quinazolines (7), quinolones (8), phthalazines (9), anthranilamides (10), 2-oxindoles (11), pyrimidines (12) and pyridines (13) have been disclosed as potent inhibitors targeting angiogenesis.

We have recently disclosed the synthesis and the evaluation of oxazolopyrimidines, exemplified by the general structure **1**, as potent VEGFR2 kinase inhibitors (14) (Figure 1). When working further into the compounds, we found that among them the best acting compound 1-(3-chloro-4-fluorophenyl)-3-(4-([5-methyl-2-[4-[3-[4-methylpiperazin-1-yl] propoxy] phenyl] oxazolo [5,4-*d*] pyrimidin-7-yl] amino] phenyl) urea (previously noted as compound 22) possessed a molecular weight of 645.13 and a cLog *p* value of 6.47 (Molinspiration Cheminformatics). This was undesirable since statistics suggest that more than 80% of all traded drugs have a molecular weight below 450 (openmolecules.org) and low hydrophilicities cause poor absorption or permeation.

With the aim to improve the physical and chemical properties of the kinase inhibitors and enhance their affinity with VEGFR-2, we envisioned the replacement of oxazolopyrimidine with pyrimidinylacetamide to provide the general structure **2** (Figure 1). Pyrimidines have been

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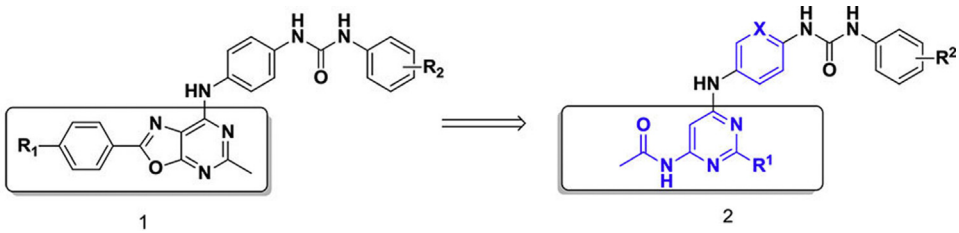

Figure 1. Replacement of the oxazolopyrimidine scaffold in the oxazolopyrimidine- based VEGFR2 kinase inhibitors 1 by a pyrimidinylacetamide moiety.

reported as 'kinase-privileged structure' (15). We hypothesized that the pyrimidine ring would bind to the hinge region of the kinase and the acetyl amino group would serve as a hydrogen bond donor. The urea moiety was kept as a key recognition element to form addition interaction with the back pocket of the kinase.

Hence, in this study we disclose our efforts towards the design, synthesis and *in vitro* evaluation of 23 pyrimidinylacetamide-based ureas as potent angiogenesis inhibitors.

Materials and Methods

General

Melting points (MP) were determined with XT-4 apparatus and are reported without correction. Infrared spectra were obtained with Nicolet Impact 410 spectrophotometer. ^{1}H NMR spectra were collected on a Brucker AMX 300 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent with TMS as the internal reference. Mass spectra (EI)were obtained on SHIMADZU GCMS-QP2010 system. Elemental analyses were performed with Elementar Vario EL III elemental analysis apparatus. All reactions were monitored by HPLC using UV light for visualization. Column chromatography was performed on silica gel (100−200 mesh) made in Qingdao Haiyang Chemical Co. Ltd.

4,6-dichloro-2-methylpyrimidine (1a)

To 2-methylpyrimidine-4,6-diol (6.42 g, 50.9 mmol) was added phosphorus oxy- chloride (20 mL). N,N-diethylaniline (11.8 g, 101 mmol) was then slowly added to the solution. The mixture was heated at reflux for 3 h and then cooled to room temperature. Two thirds of phosphorus oxychloride were removed in vacuo and the residue was poured onto cracked ice, and extracted with dichloromethane (3 × 50 mL). The extracts were then dried and concentrated, and the residue obtained was purified by flash chromatography on silica gel (ethyl acetate/petroleum ether 1:40) to yield the title compound as a pale-yellow solid (3.23 g, 38.9%). ESI-MS: m/z 163.0 $[M+H]^+$. When the same procedure for preparing compound 1a was used, pyrimidine-4,6-diol was obtained.

6-chloro-2-methylpyrimidin-4-amine(3a)

To a solution of 4,6-dichloro-2-methylpyrimidine (900 mg, 5.5 mmol) in 2-propanol (3 mL) was added ammonium hydroxide (3 mL). The tube was sealed and heated at 110°C for 4 h. After cooling, the precipitate was collected by filtration, washed with 2-propanol, and extracted with hot ethyl acetate to give the title compound (600 mg, 75.9%).

N-(6-chloro-2-methylpyrimidin-4-yl) acetamide (4a)

To the mixture of 6-chloro-2-methylpyrimidin-4-amine (573 mg, 4 mmol) in 2-propanol toluene (3 mL) was added acetic anhydride (4.08 g, 40 mmol). The mixture was heated at 115°C for 1 h and oncentrated, and the residue obtained was purified by flash chromatography on silica gel (ethyl acetate/petroleum ether 1:4) to yield the title compound as a white solid (677 mg, 91.2%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ ppm 2.10 (s, 3H), 2.48 (s, 3H), 7.89 (s, 1H), 11.14 (s, 1H).

When the same procedure for preparing compound 4a was used, N-(6-chloropyrimidin-4-yl) acetamide was obtained.

5-nitropyridin-2-amine(31a)

5-nitropyridin-2-amine was prepared as reported by Kokars V. (Chemistry of Heterocyclic Compounds, Vol. 38, No. 7, 2002). 2-Aminopyridine (2.55 g, 26.8 mmol) was dissolved in conc. H_2SO_4 (10 mL) with constant stirring below 20°C. The mixture was cooled to 5−10°C and a mixture of conc. H_2SO_4 (2 mL) and 72% HNO_3 (d = 1.42, 2 mL) was added gradually dropwise with the temperature not exceeding 20°C. The reaction mixture obtained was placed on a water bath thermostatted to 45°C overnight. The mixture obtained was poured onto ice and carefully neutralized using ammonia (200 mL). At pH 6 there appeared a dark-brown precipitate. The filtered precipitate was a mixture of two isomers. It was purified by flash chromatography on silica gel (ethyl acetate/petroleum ether 1:2−1:1) to yield the title compound as a yellow solid (1.2 g, 32.4%). m.p 189~194°C. ESI-MS 138.0$[M-H]^-$.