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Research paper

Indoline ureas as potential anti-hepatocellular carcinoma agents targeting VEGFR-2: Synthesis, *in vitro* biological evaluation and molecular docking

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ABSTRACT

In our effort to develop potent and effective agents with anti-proliferative activity towards HepG2 hepatocellular carcinoma cells with potential inhibitory activity against VEGFR-2, a novel series of 1-(4-((2-oxoindolin-3-ylidene)amino)phenyl)-3-arylureas was designed and synthesized. All the newly prepared ureas **9a**–**x** were evaluated *in vitro* for their anti-proliferative activity against HepG2 hepatocellular carcinoma cell line. Compounds **9a**–**c**, **9e**, **9f**, **9j**, **9m**–**o**, **9t**–**v** and **9x** exhibited good activity against HepG2 cancer cells (IC_{50} = 1.22 ± 0.11–8.37 ± 0.85 μM) comparable to that of doxorubicin and sorafinib (IC_{50} = 2.90 ± 0.36 and 3.40 ± 0.25 μM, respectively). These thirteen compounds were further evaluated for their inhibitory activity against VEGFR-2. Compound **9x** emerged as the most active counterpart against VEGFR-2 with IC_{50} value of 0.31 ± 0.04 μM. Furthermore, a molecular docking of the tested compounds was carried out in order to investigate their binding pattern with the prospective target, VEGFR-2 (PDB-code: 4ASD).

1. Introduction

The term angiogenesis is generally applied to the process of sprouting or splitting of novel capillary blood vessels from the pre-existing vasculature. This process involves the migration, growth, and differentiation of endothelial cells, which line the inside wall of the blood vessels [1,2]. Normally, angiogenesis occurs during embryogenesis, reproduction, wound healing and ovulation [3]. Angiogenesis is a complex process, in both physiological and pathophysiological conditions, and it is orchestrated *via* the production of a range of pro-angiogenic and anti-angiogenic factors [4]. However, aberrant equilibrium between these factors that regulate the angiogenesis is implicated in the etiology of different pathologies, such as rheumatoid arthritis, age-related macular degeneration, atherosclerosis, diabetic retinopathy and cancer [5]. Indeed, to grow beyond a size of 1–2 mm, tumors demand new blood capillaries to guarantee their own oxygen and nutrient supply, remove metabolic waste and facilitate metastasis formation [6]. These requirements can be achieved by means of the expression of pro-angiogenic growth factors, including members of the vascular endothelial growth factor (VEGF) family of ligands. The subtype VEGFR-2 exists predominately in vascular endothelial cells and hematopoietic stem cells and appears to mediate almost all of the known cellular responses to VEGF, which stimulate multiplication

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of vascular endothelial cells and growth of blood vessels [7]. The discovery of small molecule inhibitors that block the autophosphorylation of VEGFR-2 has represented a major area of therapeutic intervention for the treatment of different cancers [8].

Hepatocellular carcinoma (HCC), one of the vascular solid tumors, accounts for 70%–85% of all malignant neoplasms of the liver burden worldwide [9]. HCC in men is the fifth most frequently diagnosed cancer worldwide but the second-commonest cause of cancer-related death. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death [9]. An estimated 782,000 new liver cancer cases and 746,000 cancer deaths occurred worldwide in 2012 [10]. Pro-angiogenic signaling pathways are rational therapeutic targets for this disease. HCC is characterized by vascular recruitment as well as invasion and high microvessel density predicts early recurrence after potentially curative surgery. The VEGF signaling pathway in particular is involved in HCC angiogenesis and seems to play a crucial role in disease pathogenesis [11]. In addition several HCC studies correlated the high VEGF levels with the biological behavior of the disease and clinical outcomes. Elevated VEGF levels have been detected in the serum and cancerous tissue of patients with HCC [12–14]. While sorafenib is the only current standard of care for HCC, the development of anti-angiogenic agents for HCC treatment has been saddled with higher attrition rate due to narrow therapeutic windows or suboptimal efficacy. Therefore, there is an urgent necessity to pay much attention to update and modify drug leads from the point of view of medicinal chemistry and drug design to fulfill more potent and effective therapies.

Sorafenib, **1** (BAY-439006) (Fig. 1), trade name Nexavar, is a potent diphenylurea VEGFR-2 inhibitor. Moreover, it inhibits c-Kit, PDGFRβ, and Raf kinases [15]. It was approved by U.S. FDA for the treatment of advanced renal cell carcinoma (RCC) and for HCC [16]. Regorafenib, **2** (BAY 73–4506) (Fig. 1), a fluoro derivative of sorafenib developed by Bayer [17], inhibits angiogenic kinases VEGFR-1/3, PDGFRβ, FGFR1, and Tie-2. Furthermore, it showed anti-proliferative activities on different cancer cell lines [18]. On September 27, 2012, the FDA approved regorafenib for the previously treated metastatic colorectal cancer (mCRC) and then in February 2013, FDA expanded the approved use of regorafenib to treat patients with advanced gastrointestinal stromal tumors (GIST) [19]. A phase III study is currently underway to evaluate the efficacy and safety of regorafenib in patients with HCC whose disease has progressed after treatment with sorafenib [10].

Isatin (1*H*-indole-2,3-dione) is a privileged scaffold and one of the most promising class of heterocycles that possesses diverse interesting activity profiles and well-tolerated in humans [20–22]. Sunitinib, **3** (Fig. 1), trade name Sutent, is a small-molecule indol-2-one multikinase inhibitor active on VEGFR, PDGFR, FLT3 and c-Kit. In 2006, it was approved by the FDA for the treatment of advanced renal cell carcinoma RCC and GIST [23–25]. Moreover, the efficacy of sunitinib in patients with advanced HCC was evaluated in four phase II studies [26–29].

Based on the previous findings, we have inspired to design and synthesize a novel series of indolines incorporating a diphenylurea moiety, the main pharmacophoric feature in sorafenib, with the prime aim of developing agents with potential anti-proliferative activity towards HepG2 hepatocellular carcinoma cells targeting VEGFR-2. Of note, many studies reported useful data about the binding pattern of sorafenib with the active site of VEGFR-2 [30–32]. Utilizing these data we will carry out molecular docking to justify the inhibitory action of our target compounds towards VEGFR-2.

2. Results and discussion

2.1. Chemistry

The route adopted for the preparation of compounds **9a**–**x** is depicted in Scheme 1. Synthesis was initiated by reacting appropriate anilines **5a**–**h** with 4-nitrophenyl isocyanate **4** in refluxing acetonitrile to furnish the 1-(4-nitrophenyl)-3-phenylureas **6a**–**h**. Next, reduction of the nitro derivatives **6a**–**h** was achieved *via* hydrogenation with Pd/C in methanol to afford the corresponding 1-(4-aminophenyl)-3-phenylurea derivatives **7a**–**h**. Finally, the condensation of amines **7a**–**h** with indoline-2,3-diones **8a**–**c** in ethanol in the presence of a catalytic amount of glacial acetic acid furnished the target ureas **9a**–**x.**

The IR spectra of the target compounds **9a**–**x** revealed the presence of the two characteristic carbonyl absorption bands of isatin and urea moieties around 1699–1749 cm^{-1}, in addition to absorption bands due to the NH groups of isatin and urea moieties around 3196–3333 cm^{-1}. Also, the IR spectra of **9c**, **9k** and **9s** showed the sharp absorption band of carbonitrile group in the region 2220–2227 cm^{-1}. On the other hand, the ^1H NMR spectra of **9a**–**x** showed D_2O exchangeable singlet signals attributable to NH protons of the isatin and the urea function (NH–CO–NH) in the regions δ 10.85–11.12, 8.30–9.13 and 8.67–9.29 ppm, respectively. Moreover, the ^1H NMR spectra of **9h**, **9p** and **9x** revealed the D_2O exchangeable singlet signals of the sulfonamido group in the range δ 7.19–7.22 ppm. In addition, the ^1H NMR spectra of **9g**, **9o** and **9w** exhibited the signals of the methoxy group protons in the range δ 3.72–3.73 ppm.

2.2. Biological evaluation

2.2.1. In vitro anti-proliferative activity against HepG2

The *in vitro* anti-hepatocellular carcinoma activity of the newly prepared compounds **9a**–**x** was examined against HepG2 cell line

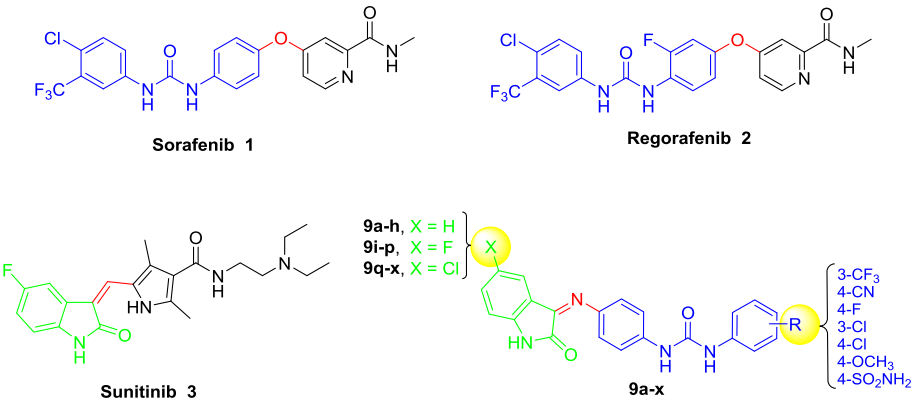

Fig. 1. Structures of VEGFR-2 inhibitors currently approved or in clinical trials for treatment of HCC **1**–**5** and the designed target indoline ureas **9a**–**x**.