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Design, synthesis and preclinical evaluation of 5-methyl-N^4-aryl-furo[2,3-*d*] pyrimidines as single agents with combination chemotherapy potential

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ABSTRACT

The design, synthesis and biological evaluation of 4-substituted 5-methyl-furo[2,3-*d*]pyrimidines is described. The Ullmann coupling of 5-methyl-furo[2,3-*d*]pyrimidine with aryl iodides was successfully optimized to synthesize these analogs. Compounds **6–10** showed single-digit nanomolar inhibition of EGFR kinase. Compounds **1** and **6–10** inhibited VEGFR-2 kinase better than or equal to sunitinib. Compounds **1** and **3–10** were more potent inhibitors of PDGFR-β kinase than sunitinib. In addition, compounds **4–11** had higher potency in the CAM angiogenesis assay than sunitinib. Compound **1** showed in vivo efficacy in an A498 renal xenograft model in mice. Multiple RTK and tubulin inhibitory attributes of **1**, **4**, **6** and **8** indicates that these compounds may be valuable preclinical single agents targeting multiple intracellular targets.

Angiogenesis, the formation of new blood vessels from the existing vasculature, plays a vital role in tumor growth, invasion, and metastasis.[1] To grow beyond 2–3 mm^3 in size, solid tumors depend on angiogenesis to meet their demand for nutrients, oxygen, and proteolytic enzymes. Angiogenesis is tightly regulated by a balance between proangiogenic and antiangiogenic factors.[2] Tumor growth and metastasis occur by activation of an "angiogenic switch," which results in the increased expression of proangiogenic factors including vascular endothelial growth factor (VEGF). Proangiogenic factors activate receptor tyrosine kinases (RTKs), facilitating tumor growth, invasion and metastasis.[3] RTKs such as vascular endothelial growth factor receptor-2 (VEGFR-2), platelet-derived growth factor receptor-β (PDGFR-β) and epidermal growth factor receptor (EGFR) are key mediators of angiogenesis.

Several anti-angiogenic agents such as sorafenib, sunitinib and erlotinib are currently approved for use in cancer patients (Fig. 1). Sorafenib is a multi-RTK inhibitor (VEGFR-2, VEGFR-3, PDGFR-β and RAF) used in the treatment of renal,[4] hepatocellular[5] and thyroid[6] cancers. Sunitinib is a multi-RTK inhibitor (VEGFRs, PDGFRs and c-kit) important in the treatment of renal,[7] pancreatic,[8] and gastrointestinal[9] cancers. Erlotinib is an EGFR inhibitor used to treat pancreatic[10] and non-small cell lung[11] cancers.

However, antiangiogenic treatments do not directly kill cancer cells and are usually cytostatic. Thus, antiangiogenic agents need to be combined with radiotherapy or chemotherapy to provide effective anticancer treatment to achieve additive or synergistic effects.[12] Since microtubules play a crucial role in mitosis, cellular transport, and cellular trafficking,[13] microtubule targeting agents (MTAs) represent an important class of anticancer agents. Paclitaxel and vinca alkaloids are widely used MTAs for the treatment of solid tumors and hematological malignancies.[13–15]

Combination chemotherapy with antiangiogenic and cytotoxic drugs is generally more effective in cancer treatment than either class of agent used alone.[12,16] Gangjee et al.[17,18] reported that single agents with dual antiangiogenic and cytotoxic activities significantly inhibited tumor growth, tumor metastasis and angiogenesis and are superior to

Abbreviations: RTKs, Receptor tyrosine kinases; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; PDGFR-β, platelet-derived growth factor receptor-β; EGFR, epidermal growth factor receptor; ATP, adenosine triphosphate; NCI, National Cancer Institute; CA-1, combretastatin A-1; CA-4, combretastatin A-4; PDB, Protein Data Bank; CAM, chorioallantoic membrane; Pgp, P-glycoprotein

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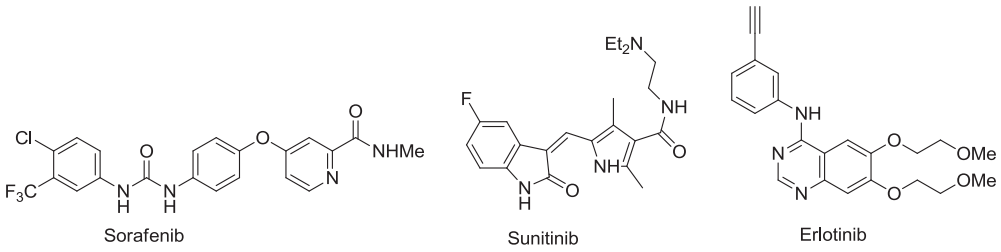

Fig. 1. Representative RTK inhibitors.

docetaxel and sunitinib in xenograft mouse models, remarkably without any toxicity. Such single agents could potentially avoid drug-drug interactions and pharmacokinetic problems associated with two or more agents administered separately.[19,20] In addition, they could prevent or delay the emergence of resistance and not cause overlapping toxicities.[20,21] Most significantly, single agents with dual cytotoxic and antiangiogenic activities simultaneously target proliferating cancer cells and the tumor vasculature. Such single agents could afford potentiated effects because they can exert their cytotoxic effects as soon as or even during transient tumor vasculature normalization caused by their antiangiogenic attributes.[2] These single agents offer other advantages, such as decreased cost and increased patient compliance,[19] which can play a major part in the clinical success of a therapy. The remarkable success and clinical trials[22] of combination chemotherapy using antitubulin agents such as paclitaxel and docetaxel and antiangiogenic agents targeting VEGFR-2 and/or PDGFR-β (e.g., sunitinib, lapatinib, erlotinib), along with our previous reports, prompted our design and development of novel single agents with both antitubulin activity and VEGFR-2 and/or PDGFR-β inhibitory activity.

Development of resistance to chemotherapeutic agents is one of the major hallmarks of cancer.[23] The clinical activity of MTAs, especially taxanes and vinca alkaloids, is limited by mechanisms of drug resistance: the overexpression of the multidrug resistance protein P-glycoprotein (Pgp) and the βIII isotype of tubulin.[24] With RTK inhibitors, resistance to EGFR inhibition can lead to VEGFR-2 upregulation in tumors, and this promotes tumor growth signaling independent of EGFR and contributes to tumor resistance of EGFR inhibitors.[25,26] The effect of EGFR inhibition can also be partially overcome by activation of PDGFR-β.[25,27] Similarly, VEGFR-2 inhibition is associated with increased PDGFR-β expression in tumor endothelial cells, increased recruitment of pericytes to tumor vasculature, and increases in other proangiogenic factors.[28] Due to the complexity of angiogenic pathways and the activation of multiple RTKs, disrupting a single pathway of angiogenesis may not result in significant clinical success. Hence, multiple RTKs such as EGFR, VEGFR-2 and PDGFR-β need to be inhibited to maximize the proportion of angiogenic signaling that is effectively targeted and to minimize resistance via redundant pathways.

We recently reported[29] the 5-methylfuro[2,3-*d*]pyrimidine **1** (Fig. 2) as a potent microtubule depolymerizer (EC_{50} = 24 nM in A-10 cells). We also[30] reported the 2,6-dimethylfuro[2,3-*d*]pyrimidine **2** with potent microtubule depolymerizing activity and multiple RTK inhibition. The similarity of the core scaffolds for **1** and **2** prompted us to evaluate **1** as an RTK inhibitor in this study using molecular docking studies.

Docking of compounds **1** and **2** was carried out in the published X-ray crystal structures of EGFR (PDB: 4JQ7,[31] 2.73 Å) and VEGFR-2 (PDB: 4AG8,[32] 1.95 Å) and in a homology model of PDGFR-β[17] using Molecular Operating Environment (MOE 2016.10).[33]

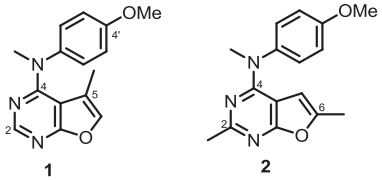

Fig. 2. Lead compounds **1** and **2**.

Fig. 3 shows the docked conformation of **1** and **2** in the binding site of EGFR. The furo[2,3-*d*]pyrimidine scaffold of **1** binds at the hinge region of the adenine binding pocket of ATP via a hydrogen bond of N1 with the backbone NH of Met769 and a hydrogen bonding interaction of N3 with HOH2015. In addition, the scaffold of **1** forms hydrophobic interactions with Ala719 and Leu820 and the 5-Me is oriented towards hydrophobic Leu694. The N^4-Me group forms hydrophobic interaction with the side chain carbon atoms of Lys721. The 4′-OMe-phenyl lies in a hydrophobic pocket lined by residues Phe699, Val702, Leu820, Thr830, and carbon atoms of Arg817. Due to the presence of a 2-position methyl moiety, compound **2** is unable to maintain the hinge binding interaction of N1 with Met769 but maintains all the other interactions as indicated for **1** in the binding site. The best docked pose of **1** had a score of −6.08 kcal/mol, comparable to that of **2** (−5.94 kcal/mol), which suggests that **1** should be an inhibitor of EGFR.

Fig. 4 shows the docked conformation of **1** in the binding site of VEGFR-2. The furo[2,3-*d*]pyrimidine scaffold binds at the hinge region of the adenine binding pocket of ATP via a hydrogen bond of N1 with the backbone NH of Cys919. Additional hydrophobic interactions of the scaffold are formed with Leu840, Ala866 and Leu1035 with the 5-Me group oriented towards Phe1047. The N^4-Me group forms hydrophobic interactions with Val848 and Phe1047. The 4′-OMe-phenyl lies in a hydrophobic pocket lined by residues Leu889, Val914, Val916, Cys1045 and the carbon atoms of Lys868. Compound **2** binds in the active site in an alternate mode as compared to **1** due to steric hindrance afforded by the 2-Me group. In this mode, the N1 undergoes H-bonding with HOH2085 rather than the hinge region binding interaction with Cys919 as for **1**. The best docked pose of **1** had a score of −8.17 kcal/mol, comparable to that of **2** (–7.84 kcal/mol), which suggests that **1** should be a VEGFR-2 inhibitor.

In the absence of a crystal structure for PDGFR-β, a homology model of PDGFR-β was used for docking compounds **1** and **2**. Fig. 5 shows the docked conformation of **1** in the binding site of the homology model of PDGFR-β. The furo[2,3-*d*]pyrimidine scaffold of **1** binds at the hinge region of the adenine binding pocket of ATP via the H-bonding of N1 and O7 with the backbone NH of Cys684. Additional stabilization of the furo[2,3-*d*]pyrimidine scaffold of **1** is provided by hydrophobic interactions of the scaffold with Ala632, Tyr683 and Leu833. The N^4-Me group forms hydrophobic interactions with Phe845 and Ala848. The 4′-OMe-Ph group lies in a hydrophobic pocket lined by residues Leu606, Val614, Val615 and Tyr683. Compound **2** binds to the site in a different mode where the scaffold flips to accommodate the bulk of the methyl group at the 2-position. The docked score for the best docked pose of **1** was −6.13 kcal/mol, which is comparable to that of **2** (−6.55 kcal/mol), which suggests that **1** should be an inhibitor of PDGFR-β.

Based on the molecular modeling studies, compound **1** was evaluated for inhibition against EGFR, VEGFR-2 and PDGFR-β kinases using a high-throughput phosphotyrosine ELISA assay (Table 1). Compound **1** was more potent than **2** in the EGFR, VEGFR-2 and PDGFR-β cellular kinase assays. Compound **1** was 2-fold and 7-fold more active than sunitinib in the VEGFR-2 and PDGFR-β kinase assays, respectively. In