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Discovery of novel picolinamide-based derivatives as novel VEGFR-2 kinase inhibitors: synthesis, *in vitro* biological evaluation and molecular docking†

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Vascular endothelial growth factor receptor-2 (VEGFR-2) plays a crucial role in tumor angiogenesis, and inhibition of the VEGFR-2 signaling pathway has emerged as an attractive target for cancer therapy. In our effort, a novel series of picolinamide-based derivatives were designed and synthesized as potent and effective VEGFR-2 inhibitors. All the newly prepared compounds were evaluated *in vitro* for their antiproliferative activity against A549 and HepG2 cell lines. Among the new compounds, **8j** and **8l** exhibited better activity against both A549 and HepG2 cell lines. Molecular docking was performed to investigate the binding capacity and binding mode with VEGFR-2 (PDB code: 4ASD).

Introduction

Cancer is the second leading cause of death worldwide. Cancer management is an extremely challenging field for medicinal chemists to discover effective and safer chemotherapeutic agents targeting various biochemical processes involved in the progression of different kinds of cancers.¹ Among these targets, angiogenesis, the generation and growth of new blood vessels from the endothelium of an existing vascular network, is one of the critical processes that affect the growth and development of cancerous cells.² Angiogenesis is also a complex process regulated by multiple growth factors that involves the migration, growth and differentiation of endothelial cells.^{3,4} Among these factors, vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1/Flt-1, VEGFR-2/KDR/FLK-1 and VEGFR-3/Flt-4) are recognized as the major mediators of tumor angiogenesis.^{5–7} In particular, VEGFR-2, a type of receptor tyrosine kinase, mediates the major angiogenic function of VEGF,⁸ which is expressed primarily in endothelial cells and secreted by endothelial cells and various tumor cells. Over-activation of VEGFR-2 is correlated with poor prognosis and metastasis in the majority of solid tumor patients.^{9,10} Due to the vital roles of VEGFR-2 in tumor angiogenesis, inhibition of VEGFR-2 activation has become a compelling approach for de-

veloping targeted cancer therapies.¹¹ Hence, several kinase inhibitors targeting VEGFR-2 are acknowledged as promising therapeutic drugs for treatment against a wide variety of human cancers. At present, many structurally diverse VEGFR-2 kinase inhibitors have been approved or in clinical trials for the treatment of cancers, such as Sorafenib,¹² Regorafenib,¹³ Axitinib¹⁴ and ENMD-2076¹⁵ (Fig. 1).

As is well known, Sorafenib and Axitinib, known as tyrosine kinase inhibitors, could significantly inhibit the activity of VEGFR-2 and then cause the reduction of angiogenesis.^{12,16} The structure–activity relationships (SARs) demonstrate that Sorafenib binds to the ATP binding site of VEGFR-2. It presents a typical pharmacophore model where the central aryl moiety, occupying the linker region, is bound to the pyridine core occupying the adenine pocket *via* an ether linker, while the terminal aryl ring in the hydrophobic pocket is bound to the central aryl moiety *via* the urea group in the

Fig. 1 VEGFR-2 inhibitors currently approved or in clinical trials.

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Fig. 2 Design of picolinamide-based derivatives **8**.

para position. A similar model between Axitinib and VEGFR-2 can also be discovered, where the central indazole moiety holds the linker region, while the aryl ring and the terminal pyridine ring are linked to the indazole.

In this paper, we report the design, synthesis and SARs of a novel series of picolinamide-based derivatives as potential VEGFR-2 inhibitors. In an attempt to develop new scaffolds for VEGFR-2 inhibitors, we hypothesized that a hybrid scaffold could be created by grafting the 2-ethenylpyridine fragment of Axitinib onto the 4-phenoxy picolinamide fragment of Sorafenib (Fig. 2). In order to further explore the SARs, a series of picolinamide-based derivatives **8** were designed and synthesized as VEGFR-2 inhibitors. As expected, these picolinamide derivatives displayed effective antiproliferative activity and could be considered as promising VEGFR-2 inhibitors.

Results and discussion

Chemistry

The synthetic route to compounds **8a–8v** is depicted in Scheme 1. Specifically, the synthesis was initiated by reacting

Fig. 3 ORTEP diagram of the crystal structure of **8b** (thermal ellipsoids drawn at the 50% probability level).

Fig. 4 ORTEP diagram of the crystal structure of **8l** (thermal ellipsoids drawn at the 50% probability level).

picolines **1a** and **1b** with 4-hydroxybenzaldehyde **2** in refluxing acetic anhydride/acetic acid to furnish compounds **3a** and **3b** in 75% and 72% yield. Next, hydrolysis of compounds **3a** and **3b** was achieved *via* potassium hydroxide in ethanol to afford the corresponding compounds **4a** and **4b** in 92% and 94% yield, respectively. Furthermore, the starting material 2-picolinic acid **5** was treated with thionyl chloride to prepare 4-chloropicolinoyl chloride **6**. Subsequently, 4-chloropicolinoyl chloride **6** was treated with the corresponding amine to form 4-chloropicolinamide derivatives **7a–7k** in 71–81% yield. Finally, the target compounds **8a–8v** were prepared through the reaction of 4-chloropicolinamide derivatives **7a–7k** and compounds **4a** and **4b** in 54–70% yield. The final products were purified by column chromatography and their structures were characterized by ^1H NMR, ^{13}C NMR and HRMS. The crystal structures of **8b** (CCDC number 1588889) and **8l** (CCDC number 1588886) were confirmed by single crystal X-ray diffraction (Fig. 3 and 4).

Scheme 1 Synthetic route of target compounds **8a–8v**. Reagents and conditions: a) acetic anhydride/acetic acid, reflux, 72–75%; b) KOH, EtOH, reflux, 92–94%; c) SOCl_2/DMF , reflux, 100%; d) amines, dichloromethane, rt, 71–81%; e) Cu, Cs_2CO_3 , DMF, N_2 , 110 °C, 54–70%.

Biological evaluation

The *in vitro* antiproliferative activity of the 22 picolinamide derivatives was evaluated against A549 and HepG2 cell lines by the CCK assay using Sorafenib and Axitinib as controls. Both cell lines were stimulated by VEGF and usually used as the tested cells in the discovery of VEGFR-2 kinase inhibitors.

As shown in Table 1, most target compounds exhibited effective inhibitory activities against both cell lines with IC_{50} = 12.5–25.6 μ M and 18.2–37.2 μ M compared to Sorafenib (IC_{50} = 19.3 μ M and 29.0 μ M) and Axitinib (IC_{50} = 22.4 μ M and 38.7 μ M). The results indicated that the hybrid scaffold created by grafting the 2-ethenylpyridine fragment onto the 4-phenoxypicolinamide structure could maintain and even

Table 1 The structures and IC_{50} values of the target compounds against cancer cell lines

Entry	Substituent			IC_{50}^a (μ M)		Selectivity ^b
	R	X	Y	A549	HepG2	
8a		N	CH	17.4 ± 1.1	21.6 ± 1.3	1.2
8b		N	CH	19.1 ± 0.9	30.2 ± 1.4	1.6
8c		N	CH	20.0 ± 1.5	30.0 ± 2.0	1.5
8d		N	CH	16.2 ± 1.7	31.3 ± 1.3	1.9
8e		N	CH	18.8 ± 0.8	27.9 ± 1.5	1.5
8f		N	CH	63.6 ± 2.1	26.0 ± 1.0	0.4
8g		N	CH	>100	45.1 ± 2.6	<0.5
8h		N	CH	21.4 ± 0.9	>100	>4.7
8i		N	CH	17.3 ± 1.3	31.0 ± 2.4	1.8
8j		N	CH	12.5 ± 1.0	20.6 ± 1.9	1.6
8k		N	CH	15.6 ± 2.2	27.0 ± 2.1	1.7
8l		CH	N	13.2 ± 1.6	18.2 ± 0.8	1.4
8m		CH	N	79.7 ± 0.8	>100	>1.2
8n		CH	N	25.6 ± 2.2	>100	>3.9
8o		CH	N	62.1 ± 2.8	56.2 ± 1.4	0.9
8p		CH	N	17.0 ± 0.7	28.9 ± 2.5	1.7
8q		CH	N	19.3 ± 1.5	>100	>5.2
8r		CH	N	18.2 ± 1.7	37.2 ± 1.6	2.0
8s		CH	N	20.6 ± 2.3	33.7 ± 0.6	1.6
8t		CH	N	35.5 ± 0.9	77.5 ± 2.7	2.2
8u		CH	N	16.8 ± 1.2	22.4 ± 2.2	1.3
8v		CH	N	18.6 ± 1.7	41.6 ± 1.5	2.2
Sorafenib	—	—	—	19.3 ± 0.9	29.0 ± 2.0	1.5
Axitinib	—	—	—	22.4 ± 1.4	38.7 ± 1.9	1.7

^a IC_{50} values are the mean ± S.D. of three separate experiments. ^b Selectivity = IC_{50} value (HepG2)/ IC_{50} value (A549).

enhance the antiproliferative activity. Among them, **8d** and **8k** demonstrated better antiproliferative activities against A549 cells with $IC_{50} = 16.2 \mu M$ and $15.6 \mu M$, and **8a** and **8u** demonstrated better inhibitory activities against HepG2 cells with $IC_{50} = 21.6 \mu M$ and $22.4 \mu M$ compared to other compounds. Moreover, **8j** showed effective inhibitory activities against A549 and HepG2 cell lines with $IC_{50} = 12.5 \mu M$ and $20.6 \mu M$, and **8l** showed similar inhibitory activities against both cell lines with $IC_{50} = 13.2 \mu M$ and $18.2 \mu M$, respectively.

In the following, the impacts of the substituent R on the carbonyl group and the substituents X/Y on the terminal pyridine were investigated, which may influence the selectivity on A549 and HepG2 cell lines. As shown in Table 1, compound **8g** bearing a 4-fluorobenzylamino group provided a significant reduction of inhibition potency on A549 ($IC_{50} > 100 \mu M$) and selectivity (< 0.5 -fold vs. HepG2). This trait was also obvious in compound **8f** containing 4-methoxybenzylamino. In contrast, compound **8q** containing a 4-methoxybenzylamino group showed good antiproliferative activity to A549 with $IC_{50} = 19.3 \mu M$ and > 5.2 -fold selectivity against HepG2. Moreover, it was notable that compound **8h** bearing an isopropylamino group and compound **8n** bearing a 4-methoxyanilino group showed good inhibitory activities against A549 ($IC_{50} = 21.4 \mu M$ and $25.6 \mu M$) and selectivity (> 3.9 -fold and > 5.2 -fold vs. HepG2).

To assess the inhibition for VEGFR-2 of the target compounds, the most active compounds **8a**, **8j**, **8l** and **8u** were selected to be further evaluated for their inhibitory activity against VEGFR-2 kinase with Sorafenib as the positive control. The results indicated that these compounds displayed good inhibitory activity on VEGFR-2 kinase with IC_{50} values ranging from 0.29 to $1.22 \mu M$ (Table 2). Among them, compound **8l** potently inhibited VEGFR-2 at a low IC_{50} value of $0.29 \mu M$. Moreover, compounds **8a** and **8j** possessed IC_{50} values of 0.87 and $0.53 \mu M$, respectively. The result of the VEGFR-2 kinase assay corresponded to that of the *in vitro* inhibitory activity on A549 and HepG2 cell lines, which might indicate that the target compounds acted as VEGFR-2 kinase inhibitors.

Molecular docking

To understand the SARs between inhibitors and VEGFR-2, molecular docking simulations were performed using AutoDock 4.2 software.¹⁷ The VEGFR-2 kinase domain crystal structure of Sorafenib (PDB ID: 4ASD) was selected as the re-

Table 3 The docking results of the target compounds

Entry	ΔG^a	K_i^b	Entry	ΔG^a	K_i^b
8a	-9.53	0.104	8l	-10.52	0.019
8b	-8.53	0.561	8m	-9.65	0.084
8c	-9.00	0.253	8n	-9.20	0.179
8d	-8.95	0.274	8o	-9.56	0.098
8e	-9.66	0.082	8p	-9.10	0.214
8f	-9.14	0.198	8q	-8.59	0.503
8g	-9.32	0.147	8r	-9.76	0.069
8h	-9.37	0.135	8s	-8.59	0.505
8i	-9.61	0.090	8t	-8.31	0.815
8j	-10.28	0.029	8u	-10.27	0.030
8k	-9.42	0.125	8v	-9.45	0.119
Sorafenib	-11.03	0.008			
Axitinib	-9.15	0.195			

^a Binding free energy (kcal mol⁻¹). ^b Inhibition constant (μM).

ceptor and Sorafenib and Axitinib would be considered as the reference compounds in the docking study.¹⁸ All the target compounds were docked into the binding site, and the binding affinities were reported as the binding free energy (ΔG) and inhibition constant (K_i). The docking results are presented in Table 3. Compound **8** fitted well into the binding site of VEGFR-2, with ΔG ranging from -10.52 to -8.31 kcal mol⁻¹ and K_i ranging from 0.019 to $0.815 \mu M$. Among the target compounds, **8l** ($\Delta G = -10.52$ kcal mol⁻¹, $K_i = 0.019 \mu M$) exhibited a better binding capacity with VEGFR-2 owing to the introduction of methylamino in suitable positions. The introduction of cyclohexylamino in **8j** ($\Delta G = -10.28$ kcal mol⁻¹, $K_i = 0.029 \mu M$) and **8u** ($\Delta G = -10.27$ kcal mol⁻¹, $K_i = 0.030 \mu M$) also resulted in better binding capacity than other compounds. It was obvious that the docking results of **8j**, **8l** and **8u** were consistent with their better antiproliferative activities.

To further evaluate the SARs, compound **8l** was chosen as a representative compound and docked into the binding site of VEGFR-2 in comparison with Sorafenib and Axitinib. As displayed in Fig. 5 and 6, compound **8l** almost totally overlapped with Sorafenib and Axitinib, and showed a good binding mode with VEGFR-2. On the moiety of the pyridine core, there were two important hydrogen bonds formed with

Fig. 5 Comparison of the binding site between **8l** (magenta), Sorafenib (yellow) and Axitinib (blue).

Table 2 The VEGFR-2 inhibitory activity of the target compounds

Entry	VEGFR-2 IC_{50}^a (μM)
8a	0.87 ± 0.06
8j	0.53 ± 0.09
8l	0.29 ± 0.02
8u	1.22 ± 0.12
Sorafenib	0.95 ± 0.09

^a IC_{50} values are the mean $\pm$ S.D. of three separate experiments.

Fig. 6 Docking simulations for **8l** in the active site of VEGFR-2.

Cys919. The N1-nitrogen of the pyridine core was hydrogen bonded to the backbone NH of hinge residue Cys919, and the NH group of picolinamide was hydrogen bonded to the backbone carbonyl of hinge residue Cys919. Furthermore, the pyridine core had some hydrophobic interactions and van der Waals contacts with Leu840, Leu1035, Ala866 and Glu917. The central aryl moiety is located in the linker region and in contact with Ala866, Cys1045, Lys868, Val848, Val916 and Phe1047. The terminal pyridine ring that connected the central aryl moiety *via* ethenyl penetrated into the hydrophobic pocket created by the hinge residues Asp1046, Glu885, Leu889, Leu1019 and Val914.

Conclusions

In summary, a novel series of picolinamide-based derivatives were designed and synthesized as potential VEGFR-2 inhibitors, and their structures were characterized by ^1H NMR, ^{13}C NMR, HRMS and single crystal X-ray diffraction. The antiproliferative activities were evaluated against A549 and HepG2 cell lines with Sorafenib ($\text{IC}_{50} = 19.3 \mu\text{M}$ and $29.0 \mu\text{M}$) and Axitinib ($\text{IC}_{50} = 22.4 \mu\text{M}$ and $38.7 \mu\text{M}$) as references. Of these derivatives, **8j** ($\text{IC}_{50} = 12.5 \mu\text{M}$ and $20.6 \mu\text{M}$) and **8l** ($\text{IC}_{50} = 13.2 \mu\text{M}$ and $18.2 \mu\text{M}$) emerged as the most active members against both cell lines. Also, **8d** and **8k** displayed good inhibitory activities against A549 with $\text{IC}_{50} = 16.2 \mu\text{M}$ and $15.6 \mu\text{M}$, and **8a** and **8u** exhibited effective inhibitory activities against HepG2 with $\text{IC}_{50} = 21.6 \mu\text{M}$ and $22.4 \mu\text{M}$. The inhibitory activities of the most active compounds **8a**, **8j**, **8l** and **8u** against VEGFR-2 were further evaluated. Compound **8l** was found to be the most active compound against VEGFR-2 with an IC_{50} value of $0.29 \mu\text{M}$. Molecular docking was performed to investigate the binding capacity and binding mode with VEGFR-2. The docking results indicated that **8j** ($\Delta G = -10.28 \text{ kcal mol}^{-1}$, $K_i = 0.029 \mu\text{M}$) and **8l** ($\Delta G = -10.52 \text{ kcal mol}^{-1}$, $K_i = 0.019 \mu\text{M}$) exhibited better binding capacities, corresponding to their antiproliferative activities. The representative compound **8l** showed a good binding mode with VEGFR-2. The N1-nitrogen of pyridine and the NH group of picolinamide formed two essential hydrogen bonds with Cys919. The central aryl moiety and the terminal pyridine ring were involved in hydrophobic interactions with residues. In conclusion, these compounds have strong poten-

tial to be further developed as novel VEGFR-2 inhibitors. Further structural optimization of the most active candidates may lead to the discovery of better inhibitors, and the studies are currently in progress.

Conflicts of interest

All the authors declare that there is no conflict of interest.

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