Accepted Manuscript

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PII: S1001-8417(15)00302-2
DOI: http://dx.doi.org/doi:10.1016/j.cclet.2015.07.016
Reference: CCLET 3393

To appear in: *Chinese Chemical Letters*

Received date: 19-5-2015
Revised date: 20-6-2015
Accepted date: 6-7-2015

Please cite this article as: <doi>http://dx.doi.org/10.1016/j.cclet.2015.07.016</doi>

Graphical Abstract

Design, synthesis and biological evaluation of *O*-linked indoles as VEGFR-2 kinase inhibitors (I)

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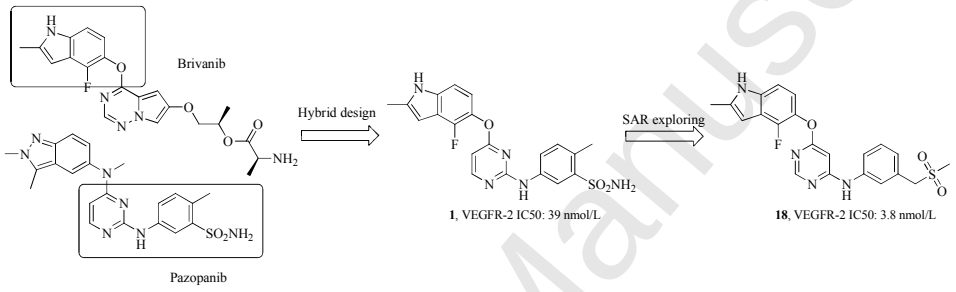

In an effort to discover potent VEGFR-2 inhibitors, a series of 2,4 or 4,6-disubstitued *O*-linked indoles derivatives were designed and synthesized. The structural activity relationships led to identification of a potential VEGFR-2 inhibitor compound **18**.

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Original article

Design, synthesis and biological evaluation of *O*-linked indoles as VEGFR-2 kinase inhibitors (I)

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ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 20 June 2015
Accepted 6 July 2015
Available online

Keywords:
VEGFR-2
Inhibitor
Indole

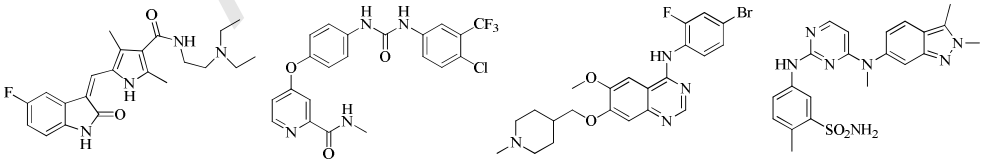
ABSTRACT

Inhibition of VEGFR-2 signaling pathway has already become one of the most promising approaches for the treatment of cancer. In this study, we describe the design, synthesis, and biological evaluation of a series of *O*-linked indoles as potent inhibitors of VEGFR-2. Among these compounds, **18** showed significant anti-angiogenesis activities *via* VEGFR-2 in enzymatic proliferation assays, with IC_{50} value of 3.8 nmol/L. Kinase selectivity profiling revealed that **18** was a multitargeted inhibitor, and it also exhibited good potency against VEGFR-1, PDGFR-α and β.

1. Introduction

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is a normal and vital process in growth and development, as well as in wound healing and female reproductive cycling[1,2]. Pathological angiogenesis has been correlated with a variety of diseases, such as retinopathies, diabetes, rheumatoid arthritis, psoriasis and cancer [3,4]. Tumor angiogenesis is a prerequisite for tumor growth as it is a fundamental step in the growth and metastasis of cancer [5]. This makes angiogenesis inhibition a promising therapeutic strategy against cancer.

Tumor angiogenesis is initiated by many factors, produced by both host and tumor cells, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and other cytokines [6]. VEGFs and receptor (VEGFR-2) is very important in the direct regulation of angiogenesis, mitogenic signaling, and permeability-enhancing effects [7]. VEGFR-2 is expressed at high level in many kinds of cancers [8]. Disruption of VEGF signaling pathway by either specific binding of circulating VEGF or inhibiting receptor tyrosine kinases with small molecules has been found to inhibit angiogenesis, tumor progression and dissemination. Some VEGFR-2 inhibitors have been approved by FDA, such as Sunitinib [9], Sorafenib [10], Vandetanib [11], Pazopanib [12] (Fig. 1).



Sunitinib Sorafenib Vandetanib Pazopanib

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Fig. 1. Some reported VEGFR-2 inhibitors.

Brivanib (Fig. 2) was reported as an ATP competitive VEGFR-2 inhibitor with an IC_{50} of 25 nmol/L [13]. By analyzing the structure of Pazopanib and Brivanib, we speculated that the indole segment in Brivanib and indazole segment in Pazopanib may have similar interaction patterns with VEGFR-2. On the basis of this assumption, compound **1** (Fig.2) was designed and synthesized. As expected, compound **1** potently inhibited VEGFR-2 with enzymatic IC_{50} values of 39 nmol/L. Encouraged by this promising result, we investigated the structure-activity relationship (SAR) of compound **1** by exploring changes on the pyrimidine core structure and the side chains. In this regard, a series of *O*-linked indole analogues were synthesized and tested in enzymatic level.

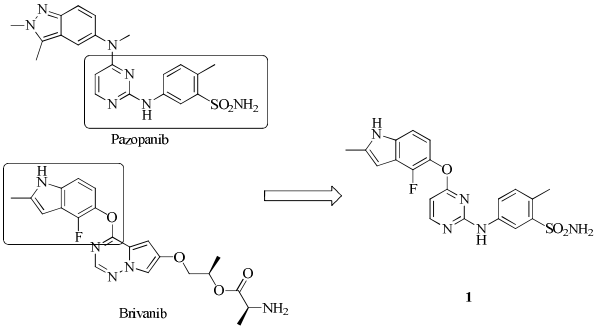
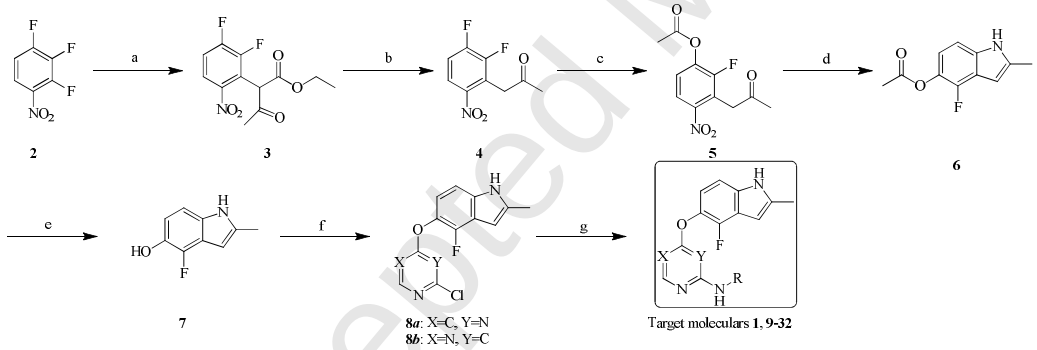


Fig. 2. Design of hybrid compound **1.**

2. Experimental

The synthesis of compounds **1**, and **8-31**was shown in Scheme 1. 2,3,4-Trifluornitrobenzene was chosen as starting material. Key intermediate (compound **7)** was obtained according to the reported method [14]. 2,4-Dicholoropyrimidine or 4,6- dicholoropyrimidine was coupled with compound **7** to give **8*a*** and **8*b***. All the analogues (**1**, **9-32**) were prepared through an acid-catalyzed SNAr reaction of *O*-linked indole intermediates with substituted anilines. The structure of the new analogues was characterized by ^{1}H NMR and MS. All analogues depicted in Tables 1 were evaluated for their enzymatic activities against VEGFR-2.



Scheme 1. Synthesis of compounds **1,** and **9-32**. Reagents and conditions: (*a*) NaH, ethyl acetoacetate, THF, 5 °C –r.t., 12 h; (*b*) HCl, HOAc, reflux, 12 h; (*c*) NaOAc, DMF, 100 °C, 10 h; (*d*) H_2, Pd/C, EtOH-DMF, 40 °C, 10 h; (*e*) aq.$NaHCO_3$, MeOH, r.t., 10 h; (*f*) 2,4-dicholoropyrimidine or 4,6- dicholoropyrimidine, aq. NaOH, acetone, 0-80 °C; (*g*) substituted anilines, 36% HCl, *i*-PrOH, sealed tube, 100 °C.

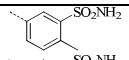
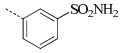
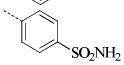
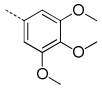
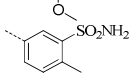
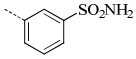
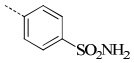
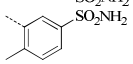
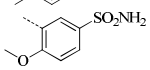
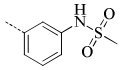
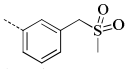
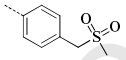
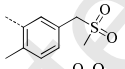
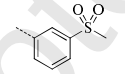
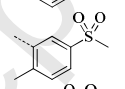
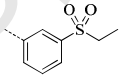
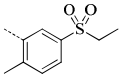
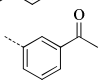
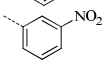
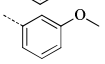
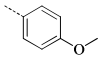
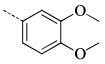
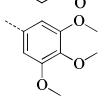
3. Results and discussion

We firstly investigated the effect of substitution of the aniline at pyrimidine ring on the activity. Removal of methyl group led to compound **9**, which exhibited higher potency on VEGFR-2 than compound **1** with an IC_{50} of 9 nmol/L. The result showed that the *p*-methyl group on the ring was not crucial to enzymatic activity. The *para*-sulfonamide analogue (compound **10**) showed a drastically decreased potency by 3.4-fold (IC_{50}=31 nmol/L) than compound **9**, which was indicated that the influence of the position of substituent was important. Compound **11**, with trimethoxy group on the ring, showed similar potency with compound **9** (IC_{50}=31 nmol/L).

Next, 4,6-disubstitued compound **12** was designed and synthesized. To our delight, this compound showed an improved VEGFR-2 inhibitory potency by 6-fold (IC_{50}=6.5 nmol/L) than 2,4-disubstitued compound **1**. Then a series of 4, 6-substitued indole analogues were obtained as new scaffold for further research. Similarly, compound **13** with removal of *p*-methyl group increased activity by 1.3-fold (IC_{50}=5 nmol/L) than compound **12**. By variation of the position of substituent, compound **14** reduced potency with an IC_{50} of 40 nmol/L. compound **15** (IC_{50}=6.8 nmol/L) and **16** (IC_{50}=10.3 nmol/L) indicated that bulky group at the *ortho*-position of ring was not tolerable. Then a set of compounds (**17-32**) was obtained to investigate the effect of substitution pattern of the aniline at pyrimidine ring on the activity. As shown in Table 1, most of analogues showed good VEGFR-2 inhibitory activity. Analogues (compound **17~26**) with electron-withdrawing group displayed potent VEGFR-2 inhibitory activities. Among these analogues, compound **18** exhibited high inhibitory activity with an IC_{50} value of 3.8 nmol/L. The *meta*-nitro substituted compound **26** exhibited weak activity (IC_{50}=21.3 nmol/L) compared with other analogues. Then analogues (compound **27~30**) with methoxy group as electron donor were screened.

Compound **29** exhibited potent activity in enzymatic assay (IC_{50} = 4.9 nmol/L). Compound **31**, with no substituent on the ring also showed good activity with an IC_{50} value of 12.1 nmol/L. Interestingly, compound **32** with alkyl ring could inhibit VEGFR-2 with an IC_{50} value of 35.1 nmol/L. The SARs study revealed that this place can tolerate different substituent on the 4-position of pyrimidine.

Table 1
VEGFR-2 inhibitory activity of compounds**1** and **9**-**32**.

Compound	X	Y	R	VEGFR-2 IC_{50} (μmol/L)
1	C	N		0.039±0.001
9	C	N		0.009±0.004
10	C	N		0.031±0.001
11	C	N		0.009±0.001
12	N	C		0.0065±0.0011
13	N	C		0.005±0.001
14	N	C		0.0404±0.0055
15	N	C		0.0068±0.0004
16	N	C		0.0103±0.0008
17	N	C		0.0077±0.0018
18	N	C		0.0038±0.0033
19	N	C		0.0071±0.0008
20	N	C		0.0838±0.0084
21	N	C		0.0073±0.0007
22	N	C		0.0084±0.0018
23	N	C		0.0060±0.0004
24	N	C		0.0046±0.0023
25	N	C		0.0107±0.0062
26	N	C		0.0213±0.0033
27	N	C		0.0088±0.0019
28	N	C		0.0221±0.0077
29	N	C		0.0049±0.0006
30	N	C		0.0065±0.0011
31	N	C		0.0121±0.0017

32	N	C		0.0351±0.0027
Su11248	-	-	-	0.0022±0.0005

To assess the selectivity of this class of derivatives, compound **18** was further evaluated on a panel of tyrosine kinases. As shown in Table 2, compound **18** demonstrated good potency against VEGFR-1, PDGFR-α, and PDGFR-β, with IC_{50} values of 40.4, 24.1, and 33.6 nmol/L, respectively. However, it showed high selectivity over VEGFR-3 (135-fold, IC_{50} = 515.8 nmol/L) and excellent selectivity (>2530-fold) over ErbB2, ErbB4, EGFR, EGFR (T790M/L858R), ABL, EPH-A2, FGFR-1, FGFR-2 and IGF-1R.

Table 2
Kinase-selectivity profiling of compound **18**.[a]

enzyme	IC_{50} (nmol/L)	enzyme	IC_{50} (nmol/L)
VEGFR-1	40.4	EGFR	>10000
VEGFR-2	3.8	EGFR(T790M/L858R)	>10000
VEGFR-3	515.8	ABL	>10000
PDGFR-α	24.1	EPH-A2	>10000
PDGFR-β	33.6	FGFR-1	>10000
ErbB2	>10000	FGFR-2	>10000
ErbB4	>10000	IGF-1R	>10000

[a] The inhibitory activity of compounds against 14 tyrosine kinases were measured with ELISA at a concentration of 10 μmol/L.

Finally, to further analyze the SAR of this series of analogues with *O*-linked indole scaffold, the docking model of compound **18** with VEGFR-2 was investigated (Fig. 3). Hydrogen bond was formed between the amide NH of indole with Glu885. Backbone amide NH and carbonyl oxygen of Cys919 formed hydrogen bonds with nitrogen of the pyrimidine and adjacent N-H of amine in the hinge region. The model study revealed the binding mode of VEGFR-2 to its inhibitor and helps to interpret the SAR of the *O*-linked indoles analogues.

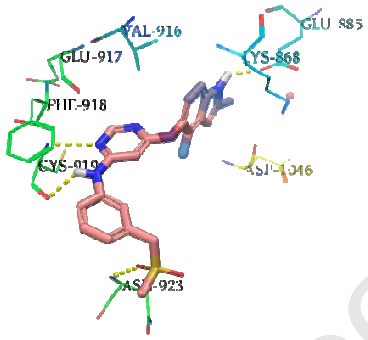


Fig. 3. Docking model of compound **18** with VEGFR-2 (PDB: 4ASD).

4. Conclusion

In summary, a series of *O*-linked indoles analogues were designed and synthesized, and they were identified as potential VEGFR-2 inhibitors. Therefore, our results indicated that this class of compounds could be served as lead compound for development of more selective anticancer medication.

Acknowledgment

We thank the National Natural Science Foundation (Nos. 81273365, 81173080 and 81321092), National Science & Technology Major Project ''Key New Drug Creation and Manufacturing Program'' (Nos. 2012ZX09103101-024 and 2014ZX09304002-008-001), Chinese National Programs for High Technology Research and Development (No. 2012AA020302) and the Shanghai Science and Technology Commission (No.12DZ1930802) for their financial support.

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