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Synthesis and biological evaluation of novel pazopanib derivatives as antitumor agents

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ABSTRACT

A series of novel pazopanib derivatives, **7a**–**m**, were designed and synthesized by modification of terminal benzene and indazole rings in pazopanib. The structures of all the synthesized compounds were confirmed by ^{1}H NMR and MS. Their inhibitory activity against VEGFR-2, PDGFR-α and c-kit tyrosine kinases were evaluated. All the compounds exhibited definite kinase inhibition, in which compound **7l** was most potent with IC_{50} values of 12 nM against VEGFR-2. Furthermore, compounds **7c**, **7d** and **7m** demonstrated comparable inhibitory activity against three tyrosine kinases to pazopanib, and compound **7f** showed superior inhibitory effects than that of pazopanib.

Carcinogenesis in numerous cases is based on a pathological intracellular signal transduction, in which the activation of specific tyrosine kinases plays a major role including regulation of cell growth, differentiation, adhesion, motility, death as well as other processes. Mutations in tyrosine kinases and aberrant activation of their intracellular signaling pathways have been causally linked to cancers. Based on the connection between tyrosine kinases and cancers, scientists have developed a new generation of drugs that block or attenuate tyrosine kinases activity, providing a broader therapeutic window with less toxicity and high efficiency.[1–4] Pazopanib (Votrient®, GW786034) is a novel multi-targeted receptor tyrosine kinase inhibitor, with both direct anti-proliferative effects and anti-angiogenic properties, targeting the vascular endothelial growth factor receptor (VEGFR-1, -2, and -3), platelet-derived growth factor receptor (PDGFR-α and -β), and c-kit. It was first approved by Food Drug Administration (FDA) as an agent to treat metastatic renal cell carcinoma in 2009, then approved by FDA to treat soft tissue sarcoma in 2012 again.[5–7] Clinical experience with pazopanib demonstrates the advantages of broad-spectrum anticancer potency and less prone to resistance. On the other hand, it may inevitably cause diarrhea, hypertension, hair discoloration, nausea, anorexia and other symptoms. Pazopanib has a black box warning against liver transaminase elevation.[8–11] Therefore, more and more attentions are given to the decoration of pazopanib.[12–14]

As shown in Figure 1, the chemical structure of pazopanib was sectioned into three main parts, indazole, pyrimidine and terminal benzene ring. Harris et al. reported that substituents in these three parts of the molecule were closely related with the kinase inhibition.[15] Their encouraging results intensified our interest. To explore the electronic and steric effect of substituents in the molecule, with pazopanib as a lead compound, we designed and synthesized a new series of compounds (**7a**–**m**) based on biological isostere principle. The inhibitory effects of 13 compounds against VEGFR-2, PDGFR-α and c-kit tyrosine kinases were evaluated using pazopanib as a positive control.

Target compounds were prepared as outlined in Scheme 1. Methylation of compounds **1a**–**b** to the 2-methylindazole analogues **2a**–**b** were carried in the presence of dimethyl carbonate (DMC) and triethylenediamine (DABCO). Subsequent hydrogenation of **2a**–**b** in the presence of Pd/C and H_2 afforded the aminoindazole derivatives **3a**–**b**, which were condensed with 2,4-dichloropyrimidine to yield the pyrimidinylaminoindazole **4a**–**b**. After methylation of **4a**–**b** with CH_3I and Cs_2CO_3, the resulting **5a**–**b** were condensed with anilines **6a**–**h** to give target compounds **7a**–**m**.[12,15–17] The structures of all the compounds synthesized were confirmed by ^{1}H NMR and MS.

In our experiments, the key intermediates **4a**–**b** were synthesized from **3a**–**b** and 2,4-dichloropyrimidine. However, there are two active sites (C2 and C4) in 2,4-dichloropyrimidine, and the chlorines in the two positions could be replaced by the amino group of **3a**–**b**. In order to verify the substitution reaction taking

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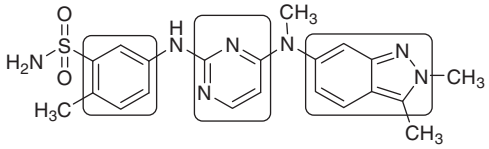


Figure 1. Chemical structure of pazopanib.

place at C4 rather than C2, we were successful in preparing X-ray quality single crystals of intermediate **4b** obtained by slow evaporation of its ethyl acetate solution.[18] As shown in Figure 2, the substitution reaction indeed occurs at C4 in 2,4-dichloropyrimidine. Similarly, the result is also suitable for intermediate **4a**. Therefore, it can be seen that 2,4-dichloropyrimidine displayed high regioselectivity in this reaction, however, the mechanism of this phenomenon is not very clear until now.[19–21]

To explore the electronic effect of the substituent in the pazopanib, the structure–activity relationship (SAR) study was focused on the terminal benzene skeleton. Keeping the indazole ring constant, replaced 5-amino-2-methylbenzenesulfonamide with other arylamines (**6a**–**h**) at the 2-position of the pyrimidine, a series of new pazopanib derivatives **7a**–**h** were designed and synthesized, and their inhibitory effects against VEGFR-2, PDGFR-α and c-kit tyrosine kinases were evaluated. As shown in Table 1, all the eight compounds exhibited potent inhibitory activity against all three tyrosine kinases. Among them, compounds **7c** and **7d** demonstrated competitive inhibitory activity against VEGFR-2 and c-kit to pazopanib, and compound **7f** showed superior activity against all three tyrosine kinases compared with pazopanib. Additionally, the compound **7g** also exhibited excellent inhibitory activity for c-kit kinase with IC_{50} values of 71 nM. However, the inhibitory effects to all three kinases of compounds **7a** and **7b** were not as active as pazopanib. In short, it can be seen from the above discussion that, when the pazopanib was decorated by substituents with different electronic effects at the terminal benzene ring, the kinase inhibition of these synthesized compounds were changed. However, the clear specific mechanism of this phenomenon is not established until now, further investigation is in progress.

On the other hand, to study the steric effect of substituent group in the pazopanib, our attention was transfer to the indazole ring. Using 6-nitro-1*H*-indazole (**1b**) instead of 3-methyl-6-nitro-1*H*-indazole (**1a**) as starting material, the other series of pazopanib

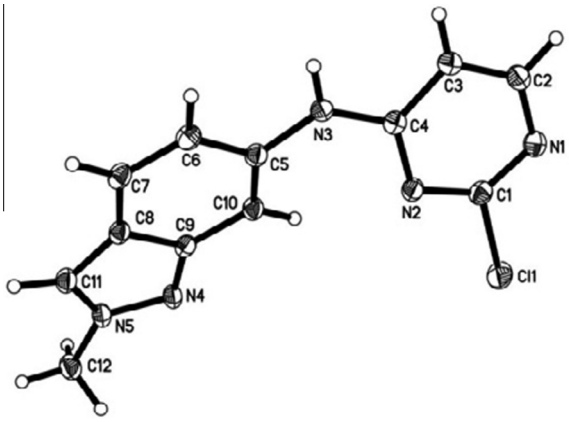
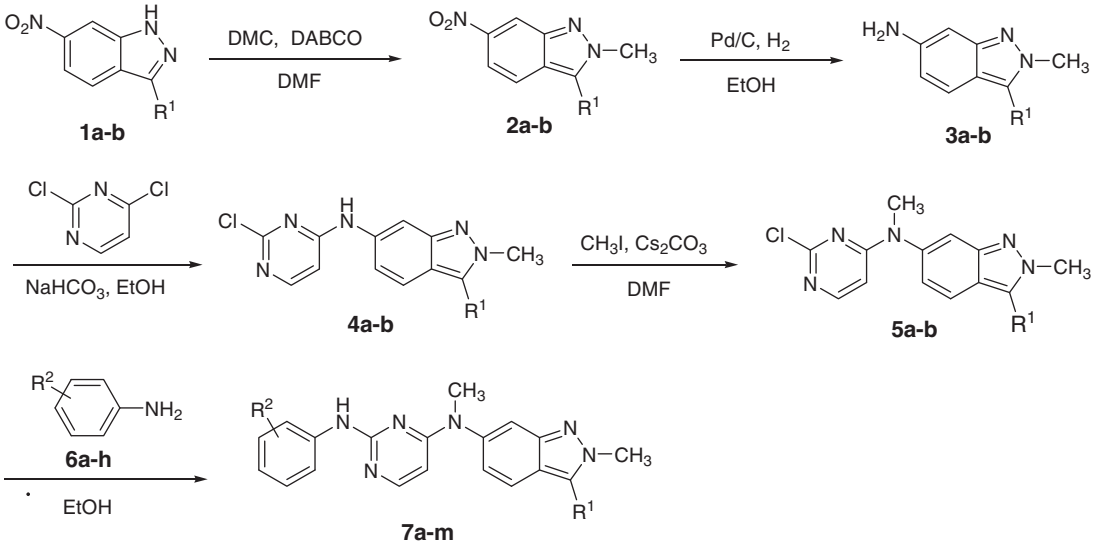


Figure 2. X-ray monocrystal diffraction of intermediate **4b**.

Table 1
VEGFR-2, PDGFR-α and c-kit kinases activity for the target compounds **7a**–**m**

Compound	Substituent		Kinase inhibition (IC_{50}, nM)		
	R^2	R^1	VEGFR-2	PDGFR-α	c-Kit
7a	3-F	CH_3	78	130	102
7b	3-Br	CH_3	64	97	98
7c	3-Cl	CH_3	25	85	80
7d	3-OCH_3	CH_3	38	96	72
7e	3-CH_3	CH_3	42	80	87
7f	3, 5-$DiCH_3$	CH_3	21	52	40
7g	4-SCH_3	CH_3	51	94	71
7h	4-OCF_3	CH_3	72	104	89
7i	3-F	H	93	140	96
7j	3-Cl	H	72	95	72
7k	3-OCH_3	H	108	86	77
7l	4-OCF_3	H	12	72	83
7m	3,5-$DiCH_3$	H	28	75	61
Pazopanib	—	—	30	71	74

derivatives compounds **7i**–**m** were designed and synthesized. As shown in Table 1, it is easy to notice that compound **7l** exhibited the best inhibitory activity against VEGFR-2 kinase and much better than its counterpart **7h** (the IC_{50} value: 12 vs 72 nM). However, the inhibitory effects for VEGFR-2 of compounds **7j** and **7k** were obviously less than their counterparts (**7j** vs **7c**, **7k** vs **7d**).



Scheme 1. Synthetic route of compounds **7a**–**m**.

In addition, compound **7m** demonstrated excellent inhibitory activity against three tyrosine kinases, but it was not as active as its corresponding compound **7f**. On the other side, there are some similarities between these two series of compounds in kinase inhibition, for example, with the comparisons of compounds **7i** and **7a**, compounds **7j** and **7c**, compounds **7k** and **7d**, it can be seen that they exhibited the similar inhibitory activity against PDGFR-α and c-kit tyrosine kinases in each group. Through comparing these two series of compounds, the results revealed that the steric hindrance of the indazole heterocycle played an important and complicated role in the interaction with kinase inhibition.

In summary, 13 novel pazopanib derivatives were designed and synthesized by varying the substituents on the terminal benzene and indazole rings of pazopanib, and these structures were characterized by ^{1}H NMR and MS. All compounds have potential inhibitory activity against VEGFR-2, PDGFR-α and c-kit tyrosine kinases.

The electronic and steric effects of substituents in the synthesized compounds were closely related to the kinase inhibition. Among these compounds, **7l** was the most activity compound against VEGFR-2 kinase, compounds **7c**, **7d**, **7m** and **7f** exhibited significant inhibition for the three kinases. Thus, these five compounds will be under further study as drug candidates.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.003. These data include MOL files and InChiKeys of the most important compounds described in this article.

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