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Pyridylmethylthio derivatives as VEGF inhibitors. Part 1

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ABSTRACT

Optimization from compound **4a**, having intramolecular S–O nonbonded interaction, led to discover compounds **4m** and **4n**. They were highly active in vitro (VEGF induced HUVEC proliferation assay) and showed efficacies in three disease models in vivo (cancer, RA, AMD).

Angiogenesis is closely related to tumor proliferation or metastasis,[1] and also pathogenesis and the progression of rheumatoid arthritis (RA)[2] and age-related macular degeneration (AMD).[3] The pathway of signal transduction through vascular endothelial growth factor (VEGF) plays a very important role in pathological angiogenesis. It is considered that VEGF receptor 2 (VEGFR2) inhibitors are likely to be useful agents for these diseases. And recently, the anti-VEGF antibody, bevacizumab (Avastin), was filed by the FDA as a therapeutic agent for cancer and AMD.[4]

Several small molecule VEGFR2 inhibitors have also been reported as the orally available drugs or candidates for the treatment of these diseases. They inhibit VEGFR2 kinase (KDR) selectively or non-selectively and have various scaffolds, such as indolinones,[5] quinazolines,[6] quinolines,[7] and phthalazines. PTK-787 (**1**), which has a phthalazine ring, has been developed in clinical studies[8] (Fig. 1). AAL-993 (**2**)[9] and AMG-706 (**3**),[10] categorized anthranil amide derivatives, demonstrated efficacy in vitro and in vivo in preclinical studies. Furthermore, it was reported that compound **2** has similar conformation to **1** by its intramolecular hydrogen bond.[11]

Recently we reported that 4-pyridylmethylthio derivative **4a** was discovered, its conformation was controlled by S–O intramolecular nonbonded interaction, and has similar conformation to compounds **1** and **2**.[12]

In this Letter, we report the syntheses, in vitro assay of the various derivatives of compound **4a**, and in vivo biological evaluations of the selected compounds. We utilized the cell based assay (VEGF induced human umbilical vein endothelial cell (HUVEC) proliferation assay)[13] as evaluation of in vitro growth inhibitory activity

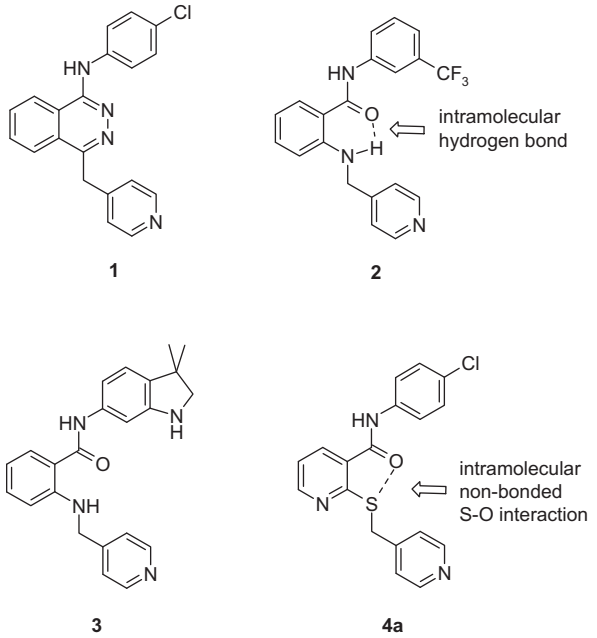

Figure 1. Chemical structures of compounds **1**–**3** and **4a**.

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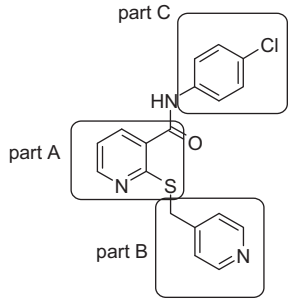


Figure 2. Strategy of optimization from **4a**.

for structural development. In our strategy these derivatives were divided into three parts of compound **4a** as shown in Figure 2.

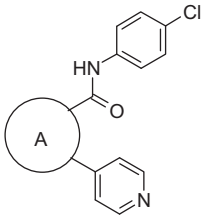
At first we investigated nicotinic acid moiety defined as part A (Table 1). Compound **5a**, which had a benzene ring as a replacement of the pyridine ring, showed moderate potency. However, compounds **6–8**, on which substituents were introduced, showed diminished activity. These results indicated that the introduction of substituents at each position on the benzene ring might be not preferable. Compounds **9** and **10** did not show activity at all, though these compounds had pyridine rings like **4a**. In the same way, though compounds **11a**, **12**, and **13** had thiophene rings, only **13** did not show the activity. It is difficult to explain these differences of activity only with the angle by two substituents on the rings. Compound **14** did not show activity, and its sulfoxide group was considered to prevent from conforming S–O intramolecular interaction like **4a**.

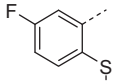
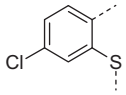
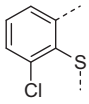
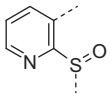
Next we optimized part B and assay results are shown in Table 2. Changing from 4-pyridyl group to 2-pyridyl and 3-pyridyl group, like compounds **15** and **16**, respectively, resulted in diminishing potency. The other modifications, compounds **17**–**19**, also led to reduce activity. These results indicated the 4-pyridylmethylthio group was the most suitable to show the activity.

On the other hand, the investigation of the substitution on the benzene ring defined as part C led to interesting results (Table 3). The activity of *N*-(3-chlorophenyl)nicotinamide **4b** got better, however *N*-(2-chlorophenyl)nicotinamide **4c** did not show activity at all. In the same way, compound **4f** showed much less potency than compounds **4d** and **4e**. The substitution on 2-position seemed to affect the conformation of the derivatives. The activity of unsubstituted compound **4g** was not so potent, so functional groups were considered to be required on 3 or 4 position. Then we designed and synthesized several 3 and/or 4-substituted derivatives. As a result, we found compounds, such as **4i, 4k, 4l** and **4m**, have the most potent activity. Interestingly, not only electron withdrawing groups but also electron donating groups seemed to be acceptable as substituents. And these results indicated 3-position was more preferable than 4-position as the point of substitution. As a further investigation, the combination of favorable groups was explored in each part, and highly potent compounds **5k** and **5m** were found (Table 4).

These derivatives can be synthesized generally in three routes (Scheme 1). In the first route, S-alkylation of 2-mercaptonicotinoic acid (**20**) with 4-(bromomethyl)pyridine hydrochloride (**21**) under basic condition gave desirable carboxylic acids **22** without purification. Compound **4a** was obtained by the condensation of intermediate **22** with 4-chloroaniline (**23**) in the presence of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetrauronium hexafluorophosphate (HATU).[14] However, in some cases the yields of the condensation step were low because of the less nucleophilicity of aniline with the electron withdrawing groups such as trifluoromethyl group and chlorine atom. At that time the second route was used.

Table 1
Activity of novel 4-pyridylmethylthio derivatives

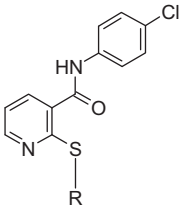


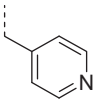
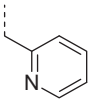
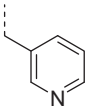
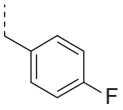
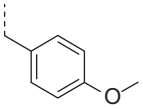
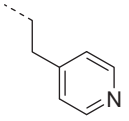
Compd	A	IC_{50}[a] (nM)
1		33
4a		230
5a		480
6		1500
7		>2000
8		>2000
9		>2000
10		>2000
11a		240
12		510
13		>2000
14		>2000

[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.

In this route, for the amide bond formation of compound **25**, 4-chloroaniline (**23**) was carried out at first by treating with 2-chloronicotinic acid chloride (**24**) under basic condition. By the following reaction with 4-(mercaptomethyl)pyridine hydrochloride (**26**) in the presence of potassium carbonate, compound **4a** could be furnished via the sulfide bond formation. On the other hand, in the syntheses of benzanilide like **5k** the second route could not be applied. So another route was explored, and palladium coupling was adopted to introduce 4-(mercaptomethyl)pyridine hydrochloride (**26**) as shown in the third route.

Table 2
Activity of various 4-arylmethylthio derivatives



Compd	R	IC_{50}[a] (nM)
4a		230
15		>2000
16		>2000
17		>2000
18		>2000
19		>2000

[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.

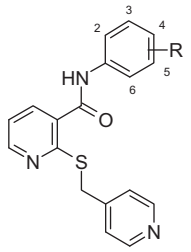
Thiophene-2-carboxamide derivatives **11a**, **11k**, and **11m** were also synthesized with this palladium catalyzed coupling.

Several derivatives were evaluated in mouse xenograft model[15], and among them compound **4m** showed a potent effect at 100 mg/kg/day (Table 5). On the other hand, compounds **4i**, **4k** and **5m** showed only moderate efficacy in spite of its potent activity in cell based assay. Especially, **5k** did not show efficacy. Conversely compound **4n** showed potent efficacy in comparison with its activity in vitro.

Then compounds **4m** and **4n** showed efficacy in rat adjuvant arthritis model[16] at 10 mg/kg/day, po as the model of RA (Table 6). Moreover these compounds also showed efficacy in rat laser induced choroidal neovascularization (CNV) model[17] at 30 mg/kg/day, po, which is the disease model of AMD (Table 7). These results indicated that compounds **4m** and **4n** have potential as a therapeutic drug for cancer, RA and AMD clinically with oral administration.

In conclusion we designed, synthesized and evaluated the novel 4-pyridylmethylthio derivatives from **4a** to discover effective VEGFR2 inhibitors. As a result, several compounds showed activity in the cell based assay (VEGF induced HUVEC proliferation assay). Among them compounds **4i**, **4k**, **4m**, **5k** and **5m** showed highly potent activity. And compounds **4m** and **4n** showed efficacy in all three animal models; one is xenograft model reflecting cancer, another is rat adjuvant arthritis model revealing the RA, and the other is rat laser induced CNV model known as the indicating model of AMD. These results suggested that compounds **4m** and **4n** had favorable properties for drugs against above mentioned diseases.

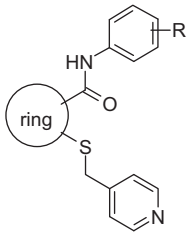
Table 3
Activity of R-group variants of novel 4-pyridylmethylthio derivatives



Compd	R	IC_{50}[a] (nM)
4a	4-Cl	230
4b	3-Cl	29
4c	2-Cl	>2000
4d	4-OMe	230
4e	3-OMe	200
4f	2-OMe	1800
4g	H	370
4h	4-Me	140
4i	3-Me	<2.7
4j	4-CF_3	48
4k	3-CF_3	11
4l	3,4-di-Me	22
4m	3,5-di-Me	6.2
4n	4-OCF_3	54
4o	3-OCF_3	27

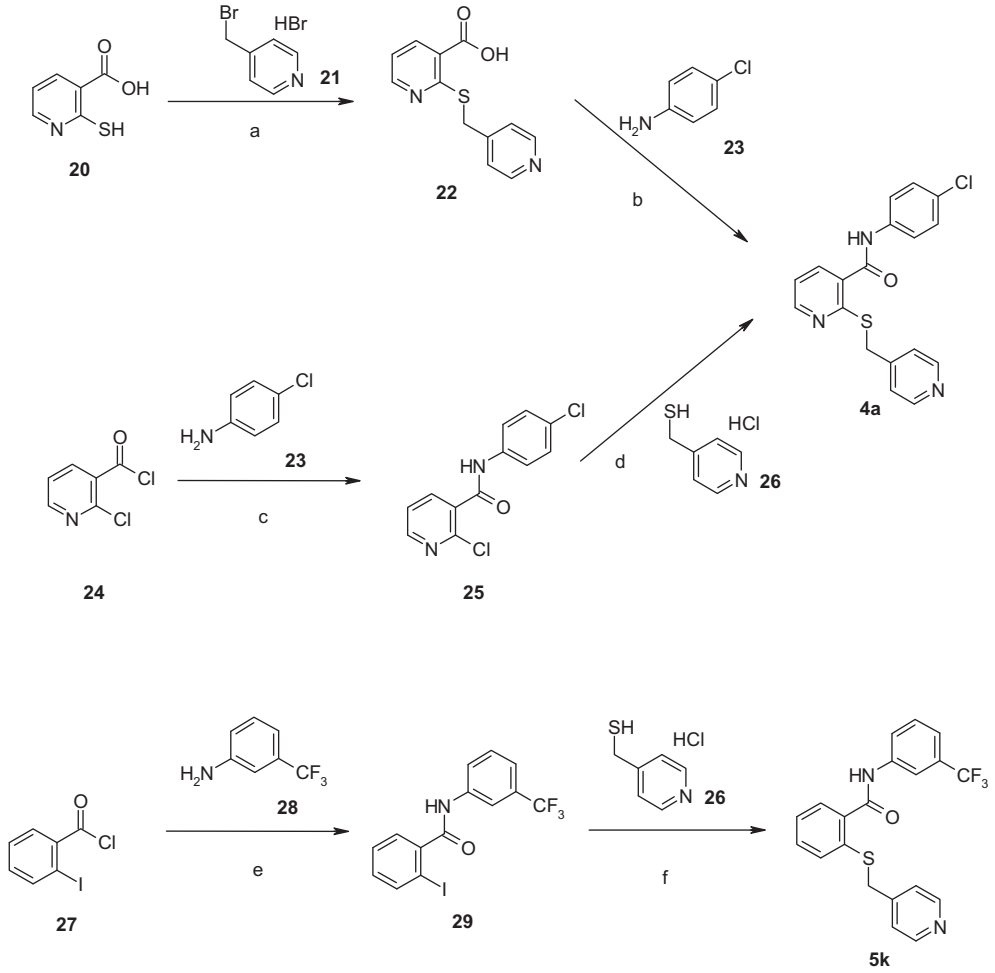
[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.

Table 4
Activity of centered ring variants of novel 4-pyridylmethylthio derivatives



Compd	Ring	R	IC_{50}[a] (nM)
4k		3-CF_3	11
5k		3-CF_3	3.3
11k		3-CF_3	400
4m		3,5-di-Me	6.2
5m		3,5-di-Me	8.7
11m		3,5-di-Me	240

[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.



Scheme 1. Reagents and conditions: (a) triethylamine, DMF, rt, 6 h, 61%; (b) HATU, DIEA, DMF, rt, 3 h, 91%; (c) DIEA, THF, rt, 3 h, 82%; (d) K_2CO_3, EtOH, 65 °C, 8 h, 37%; (e) DIEA, THF, rt, 24 h, quant.; (f) $Pd_2(dba)_3$, dppf, DIEA, DMA, 60 °C, 21 h, 76%.

Table 5
Efficacy on mouse xenograft model (100 mg/kg/day, po)

Compd	Inhibition% of tumor tissue weight
4i	40
4k	35
4m	71
4n	69
5k	9
5m	49

Table 6
Efficacy on rat adjuvant arthritis model (10 mg/kg/day, po)

Compd	Inhibition% of the paw edema
4m	46
4n	60

Table 7
Efficacy on rat laser induced choroidal neovascularization model (30 mg/kg/day, po)

Compd	Inhibition% of neovascularization incidence rate
4m	22
4n	51

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.096.

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16. The adjuvant was injected subcutaneously into a left hind paw sole of Lewis rats (male, nine weeks old) to induce arthritis. The suspension of a test compound was administrated orally once a day every day. Twenty one days after induction, the paw volume was measured. Compare with control, paw edema inhibition rate was calculated; see also Supplementary data.
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