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Pyridylmethylthio derivatives as VEGF inhibitors: Part 2

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ABSTRACT

Optimization of compounds **5** and **6** led to the discovery of VEGF inhibitor **10g** which reduced CYP inhibition. It was highly active in vitro (VEGF induced HUVEC proliferation assay) and showed efficacies in three disease models in vivo (cancer, RA, and AMD).

Vascular endothelial growth factor (VEGF) is widely known as a key molecule in angiogenesis, and related to the pathogenesis and/or progression of several diseases such as cancer, rheumatoid arthritis (RA) and age-related macular degeneration (AMD).[1] In recent years, various types of VEGF inhibitors have been launched on the market. For example, Pegaptanib (Macugen)[2] is a VEGF trap, and has been applied for the treatment of AMD. Ranivizumab (Lucentis)[3] and Bevacizumab (Avastin)[4] are monoclonal antibodies. The former is also for AMD, the latter is for cancer. Sunitinib (Sutent)[5] and Pazopanib (Armala)[6] are small molecule VEGF inhibitors, and have been marketed for cancer.

Like these compounds, a lot of small molecule VEGF inhibitors have been also reported. PTK-787 (**1**)[7] has a phthalazine ring and has been developed in clinical study. AAL-993 (**2**)[8] and AMG-706 (**3**),[9] which are anthranil amide derivatives, showed antitumor efficacy and it was reported that compound **2** has the similar conformation to **1** by their intramolecular hydrogen bonds (Fig. 1).

In the past we reported compounds **4**, **5** and **6** were discovered as VEGF inhibitors.[10] The conformation of them was controlled by S–O intramolecular nonbonded interaction, and they also have similar conformation to compound **1**.

They showed potent activities in vitro and efficacies in vivo. Another report found that 4-pyridyl moiety causes the inhibition of cytochrome P450 (CYP)[11] activity. Then we evaluated the CYP inhibition activity of compound **5**, and found it showed the activity. This meant compound **5** could cause a drug–drug interaction.[12] Therefore, to reduce the CYP inhibition activity we planed

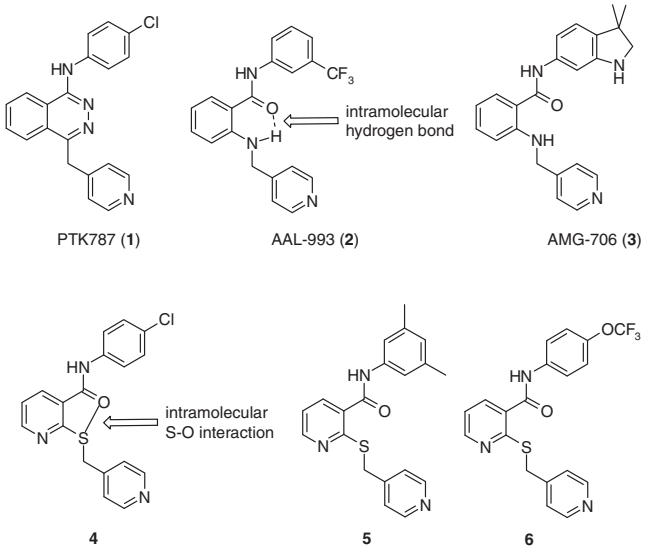

Figure 1. Chemical structures of compounds **1**–**6**.

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the oxidation of pyridine and the introduction of the substituent at 2-position of pyridine ring according to the reported method.[13] This strategy is to prevent a direct interaction between the pyridine nitrogen atom and the heme of CYP.

We utilized the cell based assay (VEGF induced human umbilical vein endothelial cell [HUVEC] proliferation assay)[14] as evaluation of in vitro growth inhibitory activity for structural development. At first, we modified the nitrogen atom of the pyridine ring like compounds **7** and **8**. As a result, though compound **8** did not show activity in HUVEC assay, compound **7** showed the potent activity (Table 1). And the CYP inhibition activity of compound **7** was diminished.

We also synthesized the derivatives **9a–l** which had various substituents on pyridine 2-position. A lot of derivatives showed efficacy in the HUVEC assay, meaning there was a space toward 2-position (Table 2). Even a bulky morpholine ring in compound **9i** was tolerated. Among these derivatives compound **9g** was the most potent compound in HUVEC assay and its CYP inhibition activity was reduced. So, further optimization was focused on the derivatives of compound **9g**.

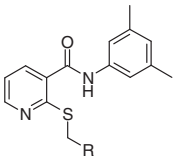
In optimization of 2-acetylamino group from compound **9g** we designed its derivatives while considering its polarity, because it has less solubility. Moreover, its acetyl group was found to be easily hydrolyzed in acidic condition into compound **9e**, so we tried to introduce hydroxyacetyl or aminoacetyl group. The oxygen atom of hydroxyacetyl and the nitrogen atom of aminoacetyl were electron donating groups, and they would prevent the hydrolysis, we considered. Most derivatives showed potent activities in HUVEC assay, reduced CYP inhibitions (Table 3), and were hydrophilic. Especially compounds **10g**, **10h**, **11f**, and **11g** had two hetero atoms in R group, and their hydrophilicities thought to be very high.

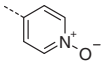
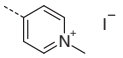
These derivatives were generally synthesized along with Scheme 1. Commercially available starting material **12** was brominated, and compound **13** was obtained without purification. Intermediate **16** was furnished by a condensation between compounds **14** and **15** with CDI. Successive acylation, deacetylation and chlorination of compound **17** led to intermediate **18**, and next N-alkylation gave compound **10g**.

Another way to introduce an amino group into 2-position of the pyridine ring is shown in Schemes 2 and 3. In Scheme 2, microwave irradiation promoted the reaction and compound **9i** was obtained from fluorinated compound **9a**.

N-Methylurea was introduced by palladium coupling to give urea derivative **10d** (Scheme 3).

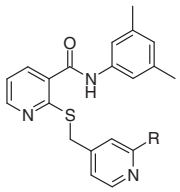
Table 1
Activity of novel 4-pyridylmethylthio derivatives



Compd	A	IC_{50}, nM[a]	Inhibition %, CYP3A4[b]
1		33	na[c]
5		6.3	96
7		24	16
8		>2000	na[c]

[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.
[b] Inhibition % of CYP3A4 at 10 μM (testosterone).
[c] na: not available.

Table 2
Activity of novel 4-pyridylmethylthio derivatives



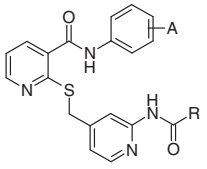
Compd	R	IC_{50}, nM[a]	Inhibition %, 3A4[b]
5	H	6.3	96
9a	F	13	40
9b	Cl	12	60
9c	Me	68	na[c]
9d	CN	3.5	48
9e	NH_2	5.8	21
9f	NHMe	21	na[c]
9g	NHAc	<0.91	20
9h	NMeAc	65	na[c]
9i	Morpholino	84	na[c]
9j	OMe	100	na[c]
9k	SMe	100	69
9l	CONHMe	18	na[c]

[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.
[b] Inhibition % of CYP3A4 at 10 μM (testosterone).
[c] na: not available.

Then we evaluated these derivatives in a mouse xenograft model,[15] and the results of representative compounds were shown in Table 4. Most derivatives inhibited tumor growth at 100 mg/kg/day (po). Among them we selected compound **10g** as a candidate for development, because it was highly hydrophilic. We also investigated to evaluate compound **10g** in the other disease models, such as rat adjuvant arthritis model for RA and rat laser induced choroidal neovascularization model (CNV model) for AMD.

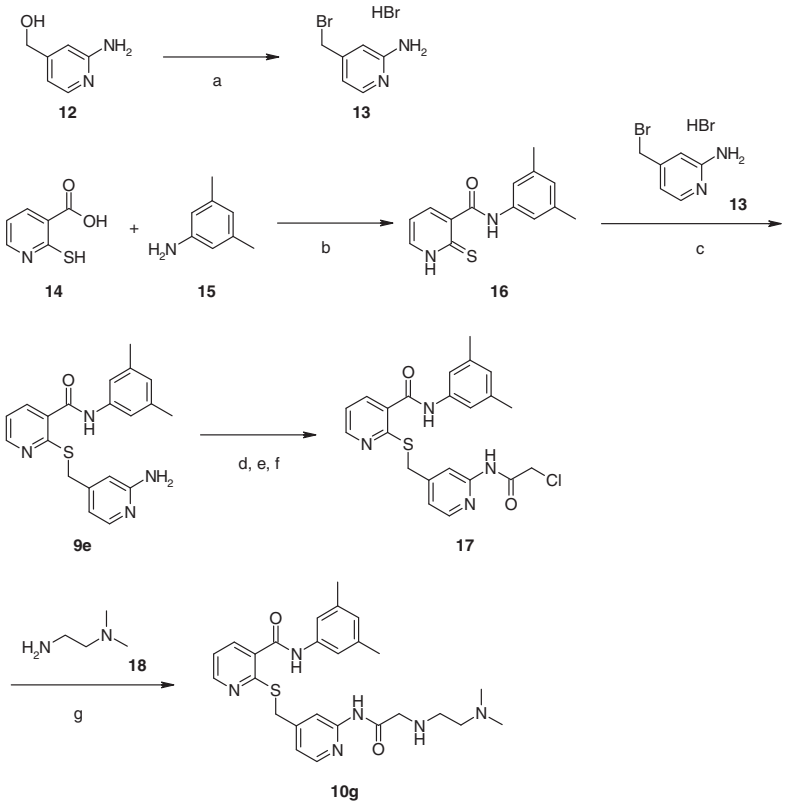
Compound **10g** also showed more potent efficacy at 10 mg/kg/day (po) in rat adjuvant arthritis model[16] than compounds **5** and **6** (Table 5).

Table 3
Activity of novel 4-pyridylmethylthio derivatives

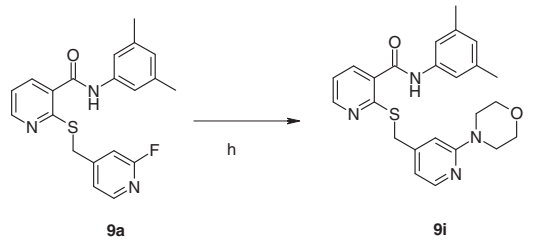


Compd	A	R	IC_{50}, nM[a]	CYP3A4[b]
9g	3,5-Di-Me	Me	<0.91	20
10a	3,5-Di-Me	Et	1.8	na[c]
10b	3,5-Di-Me	OMe	15	na[c]
10c	3,5-Di-Me	CH_2OH	6.6	na[c]
10d	3,5-Di-Me	NHMe	8.8	24
10e	3,5-Di-Me	CH_2NH_2	48	10
10f	3,5-Di-Me	CH_2NMe_2	22	na[c]
10g	3,5-Di-Me	$CH_2NHCH_2CH_2NMe_2$	5.9	14
10h	3,5-Di-Me	$CH_2NHCH_2CH_2CH_2OH$	18	23
11a	4-OCF_3	Me	3.9	38
11b	4-OCF_3	CH_2OH	7.4	17
11c	4-OCF_3	NHMe	20	na[c]
11d	4-OCF_3	CH_2NH_2	49	na[c]
11e	4-OCF_3	CH_2NMe_2	35	19
11f	4-OCF_3	$CH_2NHCH_2CH_2NMe_2$	18	29
11g	4-OCF_3	$CH_2NHCH_2CH_2CH_2OH$	26	33

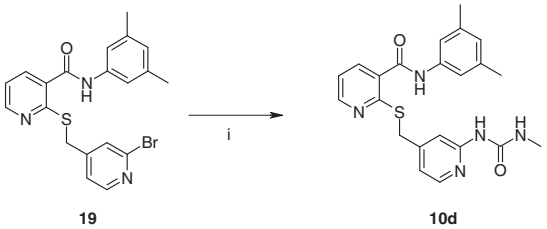
[a] IC_{50} values are the half inhibition concentrations (nM) of VEGF induced HUVEC proliferation.
[b] Inhibition % of CYP3A4 at 10 μM (testosterone).
[c] na: not available.



Scheme 1. Reagents and conditions: (a) 47% HBr aq, 150 °C, 6 h, 71%; (b) CDI, DMF, rt, 16 h quant.; (c) **13**, Et_3N, DMF, rt, 6 h, quant.; (d) $AcOCH_2CO_2H$, $AcOCH_2COCl$, pyridine, rt, 18 h, 30%; (e) NaOH aq, THF, MeOH, rt, 15 min, quant.; (f) $SOCl_2$, CH_2Cl_2, rt, 5 h, quant.; (g) **18**, sealed tube, rt, 5 h, 37%.



Scheme 2. Reagents and conditions: morpholine, DMF, microwave, 150 °C, 30 min, 35%.



Scheme 3. Reagents and conditions: *N*-methylurea, $Pd_2(dba)_3$, xanthophos, 1,4-dioxane, 100 °C, 5 h, 22%.

In rat laser induced CNV model,[17] whereas compounds **5** and **6** were effective at 30 mg/kg/day (po), compound **10g** showed the efficacy at the lower dose (3 mg/kg/day, po) (Table 6). Although the structures and activities in vitro of compounds **5** and **6** were very similar, considering these results, there were the differences in their activities in vivo. We suggested that the solubility of compound **10g** was increased because of its two alkylamine groups and it contributed to improve the absorption of the drug.

Table 4
Efficacy of tumor growth inhibition in mouse xenograft model (100 mg/kg/day (po))

Compd	Inhibition % of tumor tissue weight
5	71
6	69
7	76
10d	43
10g	83
10h	77
11b	85
11f	79
11g	79

Table 5
Anti-inflammation efficacy in rat adjuvant arthritis model (10 mg/kg/day (po))

Compd	Inhibition % of the paw edema
5	46
6	60
10g	70
1	23

Table 6
Anti-angiogenic efficacy in rat laser induced choroidal neovascularization model (po)

Compd	Inhibition % of neovascularization incidence rate
5 (30 mg/kg/day)	22
6 (30 mg/kg/day)	51
10g (3 mg/kg/day)	55
BP* (1 mg/kg/day)	96

* BP: betamethasone phosphate.

In conclusion, we designed, synthesized, and evaluated the novel 4-pyridylmethylthio derivatives from compounds **5** and **6** to discover effective VEGF inhibitors and reduce CYP inhibition activity. As a result, we found *N*-oxide derivative **7** and 2-substituted pyridine derivatives (like **10g**) showed activities in the cell based assay (VEGF induced HUVEC proliferation assay), and the CYP inhibition activities were reduced. Among these compounds, **10g** was highly active and hydrophilic. Moreover it showed efficacies in all three animal models: xenograft model reflecting cancer, rat adjuvant arthritis model revealing the RA, and rat laser induced CNV model known as the indicating model of AMD. These results suggested that compound **10g** had favorable potency for drugs against the above mentioned diseases.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.071.

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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. Paw edema inhibition rate was calculated and compared with control; see also Supplementary data.
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