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Conformation–activity relationship on novel 4-pyridylmethylthio derivatives with antiangiogenic activity

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Abstract—We found 4-pyridylmethylthio derivative **1** to be very effective in using antiangiogenesis activity to prevent proliferation of HUVECs (Human Umbilical Vein Endothelial Cells), which was induced by vascular endothelial growth factor (VEGF). Compound **1** was equally effective in inhibiting VEGF receptor2 tyrosine kinase (KDR, IC_{50} = 26 nM). We deduced that the inhibition was the result of binding the catalytic domain of VEGF receptor2 tyrosine kinase in a similar fashion to both phthalazine derivative PTK787 **2** and anthranylamide derivative AAL993 **3**. In this report, we will describe the conformational analyses, from ab initio MO calculation and X-ray crystallographic analyses, of compound **1** and the analogs, which include non-active **9**, all in comparison with **2** and **3**. The conformation–activity relationships suggest that a nonbonded intramolecular interaction between the sulfur and the carbonyl oxygen of **1** was very important in inhibiting KDR.

Stimuli to the VEGF/VEGF-receptor signaling pathway can result in angiogenesis, which is closely linked to the development of cancer, rheumatoid arthritis and aged macular degeneration. The clinical studies of Avastin™, a humanized anti-VEGF monoclonal antibody, revealed that the VEGF/VEGF-receptor signaling inhibitor could be used in treating cancer.[1] A variety of VEGF receptor tyrosine kinase (RTK) inhibitors of low molecular weight have been developed in clinical trials. These VEGF-RTK inhibitors can be classified into chemical structure classes such as indolinones,[2] quinazolines,[3] and phthalazines.[4] PTK787 **2** is a phthalazine, which has been under PIII clinical development for the treatment of cancer.[5] An anthranylamide derivative AAL993 **3**[6] is a selective VEGF-RTK inhibitor of the same chemical class as phthalazines.[7] This compound was identified by a database search with a substructure query for **4** which listed phthalazines with an intramolecular hydrogen bond.[8] Although 4-pyridylmethylthio derivative **1** does not have an intramolecular hydrogen bond, it has a structure that is similar, it is a member of the same chemical class as phthalazines and may be effective.[9] Therefore, we synthesized it and found it to have potent inhibitory activities in a cell-based angiogenesis assay (IC_{50} = 250 nM) and in a KDR assay (IC_{50} = 26 nM) (Table 1 and Fig. 1).

We have previously reported the syntheses and the biological activities of compounds **1** and **5**–**7** elsewhere.[9] This time, in addition to compounds **1** and **5**–**7** reported previously, compound **9** was obtained from **8** by reversing the positions of sulfur in **7** (Scheme 1).[10] Their biological activities are described in Table 1. The 4-pyridylmethylamino derivative **5** had potent inhibitory activities with KDR (IC_{50} = 20 nM) as did the 4-pyridylethyl derivative **6b** (IC_{50} = 200 nM).[11] Compounds **1** and **7**, which are also members of the 4-pyridylmethylthio group, showed similar potency against KDR. On the other hand, neither compound **8** nor **9** displayed such potent activity (Table 1). From these data, we believed that the stable conformations by the intramolecular nonbonded interaction of **1**, **3**, and compounds **5**–**7** were similar to those of phthalazine PTK787 **2** (Fig. 2).

Compounds **V**–**IX** in Figure 3 are the models of **5**–**9**, respectively, used for the MO calculation at the RHF/

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Table 1. Inhibitory activities (IC_{50} value, nM)

Compound	KDR[a]	HUVECs proliferation[b]
1	26	250
2	37 (Ref. 5)	6 (Ref. 5)
3	23 (Ref. 6)	N.D.
5	20 (Ref. 8)	N.D.
6b	200 (Ref. 11)	N.D.
7	46	530
8	>1000 (54% inhibit. at 10μM)	>2000
9	>1000 (0% inhibit. at 10μM)	N.D.

[a] IC_{50} of a KDR assay was measured with a kinase assay development kit purchased from CARNA BIOSCIENCE Co. Ltd.[12]

[b] IC_{50} of HUVECs proliferation inhibitory assay was determined by the method described in Ref. 13.

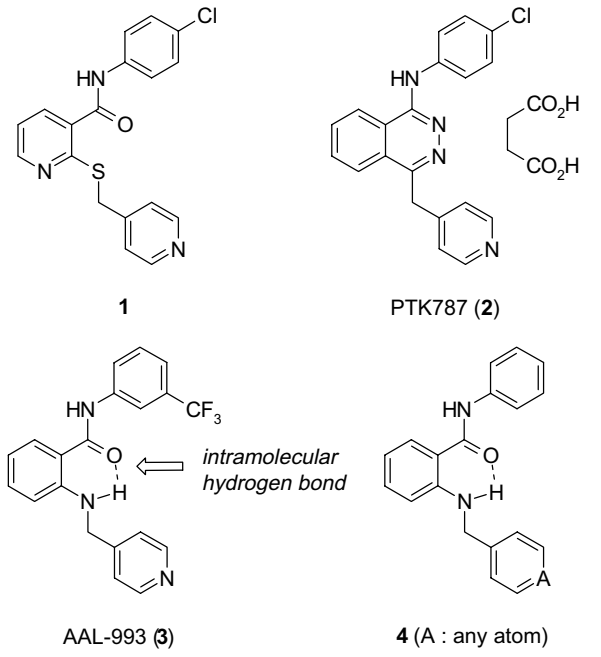
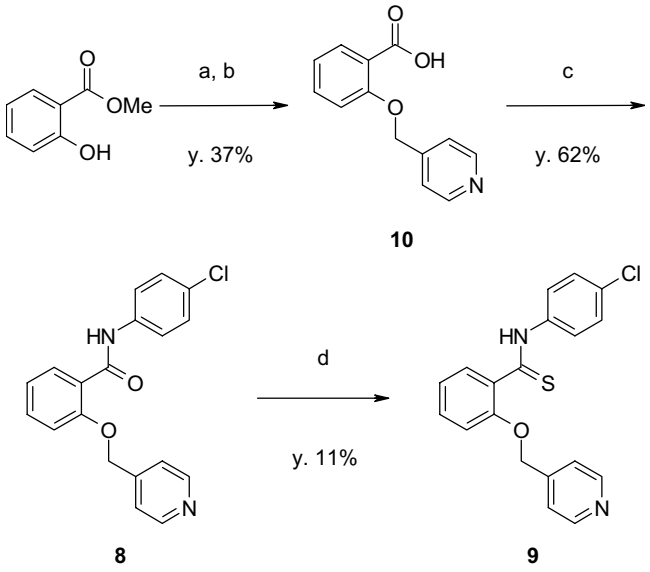


Figure 1. Structures of 4-pyridylmethylthio derivative **1**, PTK787 **2**, and anthranylamides **3**, **4**.

6-31G* level. Relative potential energies (ΔE) to the conformation ($\chi = 0°$) of the compound were obtained for structures whose torsional angle, C_1–C_2–C_3–Y was rotated at +30° intervals from 0° to 180° (Fig. 3). Figure 4 shows the energy profiles.[14] The most stable conformations of compounds **V** and **VI** were conformers **C** ($\chi = 30°$), being structurally similar to PTK787 (**2**), which would be caused by an intramolecular hydrogen bond between either NH or CH_2 and carbonyl oxygen. In compound **VII**, although conformer **C** ($\chi = 30°$) was again a major component in the conformation, conformer **B** ($\chi = 180°$) was slightly more stable (5 kJ/mol). In compounds **VIII** and **IX**, conformer **B** ($\chi = 180°$) and conformer **D** ($\chi = 150°$) were found to be the most stable conformations. These results suggest that the stable conformations of compounds **5** and **6** are different from those of **8** and **9**, and that compound **7** has two stable conformers.



Scheme 1. Reagents and conditions: (a) 4-chloromethylpyridine hydrochloride, K_2CO_3, DMF, rt; (b) 1 M NaOH aq, THF, rt; (c) *p*-chloroaniline, HCTU, DIEA, DMF, rt (d) P_2S_5, chlorobenzene, reflux.

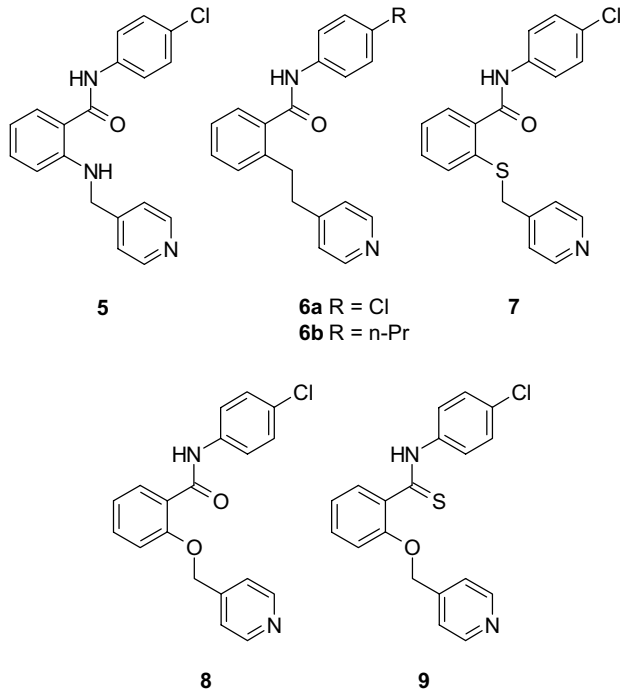


Figure 2. Structures of compounds **5**–**9**.

We assumed a relevance between the stable conformation and the inhibition of KDR among compounds **1**–**3** and **5**–**9**, and this was consistent with the results of ab initio MO calculations. We, then, carried out X-ray crystallographic analyses of compounds **1** (monohydrate) and **9**.[15] Analysis of compound **1** (monohydrate) revealed that the torsion angle of C1–C2–C3–O moiety (χ) was 35°, and that the S–O distance (2.82 Å) was shorter than the sum (3.32 Å) of van der Waals radii of sulfur and oxygen,[16] thus suggest-

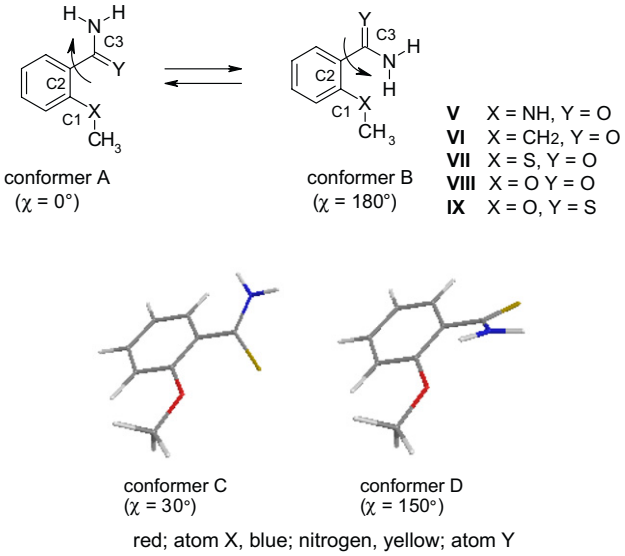


Figure 3. Various conformers of compounds **V**–**IX**.

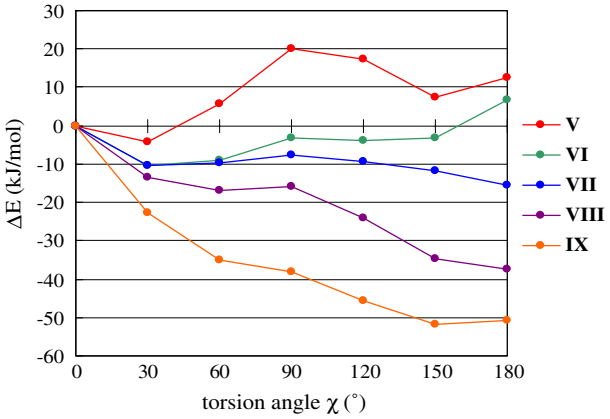


Figure 4. Relative potential energy (Δ*E*) versus torsion angle (χ) of C1–C2–C3–Y moiety.

Table 2. Summary of X-ray crystallographic analyses of compounds **1** (monohydrate) and **9**

	1 (monohydrate)	**9**
Formula	$C_{18}H_{16}ClN_3O_2S$	$C_{19}H_{15}ClN_2OS$
Formula weight	373.86	354.85
Crystal color, habit	Brown, platelet	Yellow, prism
Crystal system	Triclinic	Monoclinic
Space group	*P*−1 (#2)	$P2_1/c$ (#14)
Lattice constants		
a (Å)	7.561(5)	9.655(5)
b (Å)	9.586(6)	12.530(4)
c (Å)	13.363(10)	14.799(7)
α (°)	98.73(3)	
β (°)	95.74(3)	102.832(20)
γ (°)	110.10(2)	
Volume (Å^3)	886.8(10)	1745.6(13)
Z	2	4
Density (calcd) (g/cm^3)	1.400	1.350
Residual *R*, R_w	0.0425, 0.0499	0.0360, 0.1123

ing an intramolecular nonbonded S–O interaction.[17] Compound **1** had a conformation similar to PTK787 **2** and AAL993 **3** with the same interaction. On the other hand, such S–O close contact was not observed in compound **9** (the torsion angle of the C1–C2–C3–S: 108°, S–O distance 3.79 Å). Thus the most stable conformation of **9** would be different from that of **1**. These results suggest that a conformation, which forms a pseudobicyclic ring through intramolecular nonbonded interaction,[18] such as hydrogen bonding or nonbonded S–O interaction, is necessary to exert inhibition of KDR (Fig. 5).

In summary, we analyzed novel 4-pyridylmethyl derivatives **1**–**9** and evaluated their KDR inhibitory activities to investigate the relationship with conformation–activity. The IC_{50} of **1** and **7** was approximately 10^{-7} nM, which is comparable to anthranylamide derivatives **2** or **5**. The conformational analysis of the model

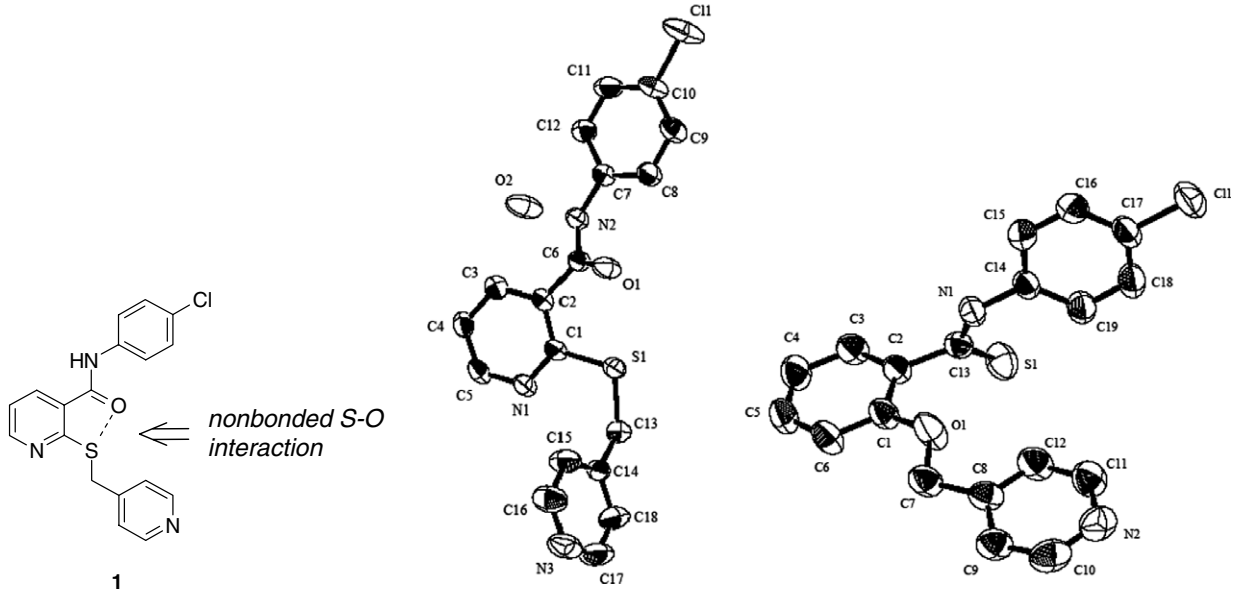


Figure 5. Left, structure of compound **1**; middle, ORTEP drawing of compound **1** (monohydrate) (S–O; 2.82 Å, χ = 35°); right, ORTEP drawing of compound **9** (S–O; 3.79 Å, χ = 108°).

compounds **V**–**IX** of 4-pyridylmethyl derivatives **5**–**9** suggests that the major conformations of the active derivatives **5**–**7**, apparently shaped pseudobicyclic rings through intramolecular hydrogen bonding/S–O nonbonded interaction, were similar to each other, and thus to PTK787 **2**. This conformation was found in the crystal of **1** monohydrate, in which an intramolecular nonbonded S–O interaction was indicated. X-ray crystallography of an inactive **9** crystal revealed that the conformation was very different from that of **1**. An intramolecular nonbonded interaction, such as hydrogen bonding or S–O interaction, is a critical structural property for compounds in this class for the inhibition of KDR (Table 2).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.068.

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10. Synthesis of **10**: To the mixture of methyl salicylate (9.48 g, 62.2 mmol), 4-chloropyridine hydrochloride (10.2 g, 62.2 mmol), and *N*,*N*-dimethylformamide (100 ml), potassium carbonate (17.3 g, 125 mmol) was added at 4 °C. The reaction mixture was stirred at rt over night and was poured into ice water. The extract with AcOEt (250 ml) was washed with saturated NaCl aq and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (Hexane–AcOEt) to give the desired alkylated product **11** (7.34 g) as a colorless solid in 49% yield. To the solution of the above product (8.75 g, 36.0 mmol) in THF (60 ml), 1.0 M NaOH aq (108 ml, 108 mmol) was added at 4 °C and the mixture was stirred at rt for 2 h. After the addition of 1.0 M HCl aq (100 ml, 100 mmol) to the mixture, the insoluble material was filtered out and the filtrate was concentrated in vacuo. Carboxylic acid **10** (6.87 g) was obtained as a colorless solid in 83% yield.
Synthesis of **8**: To the mixture of **10** (1.00 g, 4.36 mmol), *p*-chloroaniline (612 mg, 4.80 mmol), *N*,*N*-diisopropylethylamine (1.69 g, 13.1 mmol), and *N*,*N*-dimethylformamide (30 ml), 1-[bis(dimethylamino)methylene]-5-chloro-1H-benzotriazolium-3-oxidehexafluorophosphate (HCTU, 3.61 g, 8.72 mmol) was added at 4 °C. The reaction mixture was stirred at rt over night. The mixture was diluted with AcOEt (40 ml), and then 0.1 M NaOH aq (60 ml, 6.0 mmol) was added to it. The resulting colorless solid was filtered and dried in vacuo. Anilide **8** (963 mg) was obtained as a colorless powder in 65% yield.
Synthesis of **9**: After refluxing the mixture of **8** (501 mg, 1.48 mmol), phosphorous pentasulfide (660 mg, 1.48 mmol), and chlorobenzene (40 ml) for 2 h, the mixture was cooled to rt and diluted with *N*,*N*-dimethylformamide (40 ml). Saturated $NaHSO_4$ aq (100 ml) was added to the mixture. The AcOEt layer of the mixture was dried over anhydrous Na_2SO_4, and then the solvent was removed by evaporation. The residue was purified by NH silica gel column chromatography (Hexane–AcOEt) to give thioamide **9** (57.5 mg) as a yellow solid in 11% yield.
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