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KDR inhibitor with the intramolecular non-bonded interaction: Conformation–activity relationships of novel indole-3-carboxamide derivatives

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ABSTRACT

We previously reported that compound **1**, having a similar conformation to PTK787 (**2**) by forming a pseudo ring structure with an intramolecular non-bonded S–O interaction, exhibited a potent inhibitory activity against VEGFR2 tyrosine kinase (KDR).[1] Applying the ideas of pseudo ring formations, we have designed three types of novel indole carboxamide derivatives **5–7** with an intramolecular hydrogen bonding or non-bonded S–O interaction. We describe the design and synthesis of **5–7**, and also discuss the relationships of their KDR inhibitory activity and conformations that were stabilized by their intramolecular non-bonded interactions.

Angiogenesis is closely related to the proliferation or metastasis of cancer cells.[2] Vascular endothelial growth factor (VEGF) plays a very important role in the pathological angiogenesis. Thus, the compounds which hamper this pathway show promise as drugs for treating cancer. Stimuli to the VEGF/VEGF-receptor signaling pathway can result in angiogenesis, which is also closely linked to rheumatoid arthritis (RA) and age-related macular degeneration (AMD). 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.[3] Macugen^{R} is a 28-base PEGylated aptamer which has been approved as a remedy for AMD.[4] Numerous small molecules for inhibiting VEGF receptor tyrosine kinase (RTK) have been developed in clinical trials and some of them have been already marketed for various cancer therapies. These VEGF-RTK inhibitors can be classified by their chemical structures into types, such as: indolinones, quinazolines, phthalazines and others.[5] Vatalanib (**2**) is one of the phthalazines under PIII clinical development for the treatment of cancer.[6] An anthranilamide derivative AAL993 (**3**)[7] is a selective VEGF-RTK inhibitor of this chemotype. This compound was reported to have been identified by a database search with a substructure query for **4** which listed phthalazines with an intramolecular hydrogen bond.[8] We previously reported a synthesis of 4-pyridylmethylthio derivative **1**, as a member of this chemotype,[9] 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).[1] The X-ray crystallography of **1** (monohydrate), possessing 2-mercaptonicotinamide framework, suggested an intramolecular non-bonded S–O interaction[10] with the shorter S–O distance (2.82 Å) than the sum (3.32 Å) of van der Waals radii of sulfur and oxygen atoms,[11] and then revealed to have similar conformation to **2** and **3**.

Meanwhile, X-ray co-crystallography analysis of **3** and KDR uncovered that the amide NH of **3** interacted with the Glu885, which formed a salt bridge with Lys868 residue, the carbonyl moiety interacted with the Asp1046 of the DFG-loop and the 4-pyridylmethyl moiety engaged Cys919 of the hinge region (Fig. 2A).[3] The intramolecular hydrogen bonding in **3** was effective to keep the favorable conformation for interacting at the ATP binding site of KDR in the so-called 'DFG-out' conformation,[12] to show the inhibitory activity. On the basis of these results, we considered that the anthranilamide in **3** or 2-mercaptonicotinamide framework in **1** could be replacable as a scaffold of this type KDR inhibitor, with the indole derivatives (**5–7** in Fig. 1) that can form larger size pseudo rings (Fig. 2B). Then we designed three types of scaffolds (Fig. 3), two of which (**5**, **6**) could have similar conformations and be inhibitory active with an internal hydrogen bond or S–O interaction, and the rest (**7**) having a different mode of hydrogen bond that might cause less or no active. We synthesized them and investigated the conformation–activity relationships between the three kind derivatives **5–7**.

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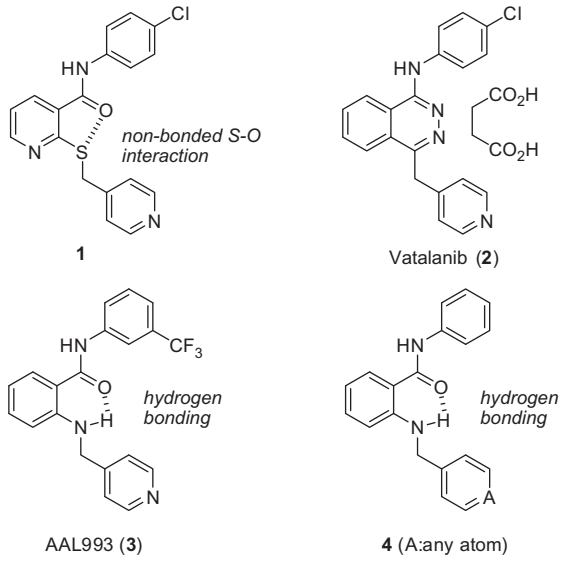
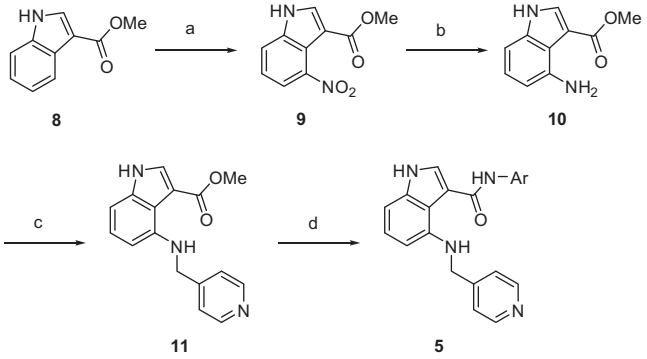


Figure 1. Structures of 4-pyridylmethylthio derivative **1**, Vatalanib (**2**), AAL993 (**3**) and substructure (**4**).

Syntheses of novel 4-substituted indole-3-carboxamides **5**–**7** are illustrated in Schemes 1–3. (4-Pyridylmethylamino)indoles **5** were synthesized by the reaction of methyl (4-pyridylmethylamino)indole-3-carboxylate **11** with various $ArNH_2$ using $AlMe_3$.[13] The methyl ester **11** was furnished by the reductive amination of 4-pyridinecarboxaldehyde with **10** which was prepared from commercially available methyl indole-3-carboxylate **8** via nitration[14] and reduction.



Scheme 1. Reagents and conditions: (a) HNO_3, AcOH, DMF, 85 °C, y. 33%; (b) H_2, 10% Pd/C, MeOH, rt y. 65%; (c) 4-pyridinecarboxaldehyde, $NaBH_3CN$, MeOH, rt y. 28%; (d) $ArNH_2$, $AlMe_3$, toluene, reflux, y. 11–42%.

(4-Pyridylmethylthio)indoles **6** were synthesized by the reaction of methyl (4-pyridylmethylthio)indole-3-carboxylate **13** with various amines ($ArNH_2$) in a manner similar with (4-pyridylmethylamino)indoles **5**. The methyl ester **13** was furnished from methyl 4-iodoindole-3-carboxylate **12** by the coupling reaction with 4-pyridinemethanethiol hydrochloride using the Pd-Xantphos catalyst system reported by Itoh and Mase.[15] In the preparation of iodide **12** (39% yield) from methyl indole-3-carboxylate **8** by the reaction using $Tl(OCOCF_3)_3$ and KI,[16] the diiodide **14** was also produced in 4% yield.[17]

After the amide **17** was synthesized from commercially available 4-benzyloxyindole **15** by the Friedel–Crafts type acylation[18] with trichloroacetic acid anhydride and dehydrative condensation with various amines ($ArNH_2$), (4-pyridylmethoxy)indoles **7** were

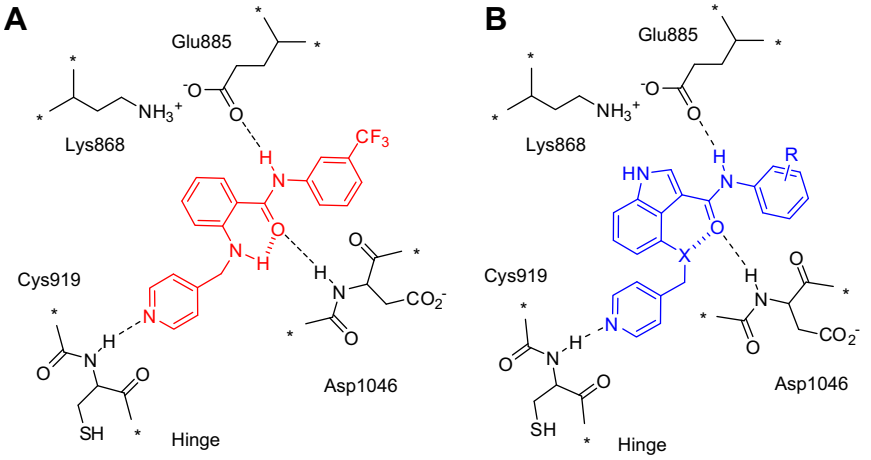


Figure 2. Schematic drawing of X-ray co-crystallography of **3** and KDR (**A**). Plausible binding mode of indole derivative **5**–**7** (**B**).

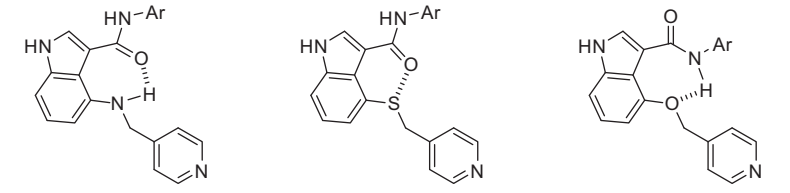
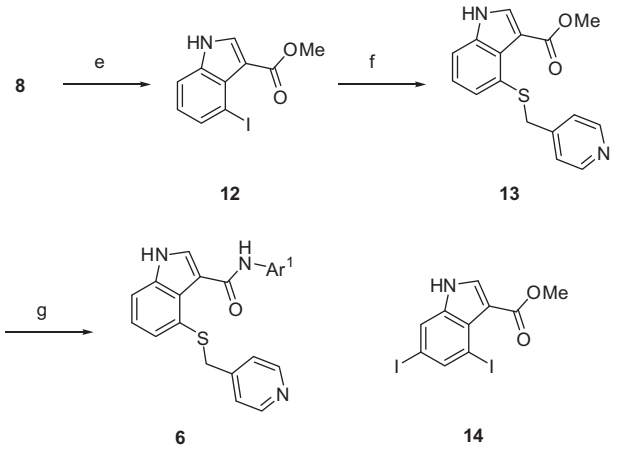
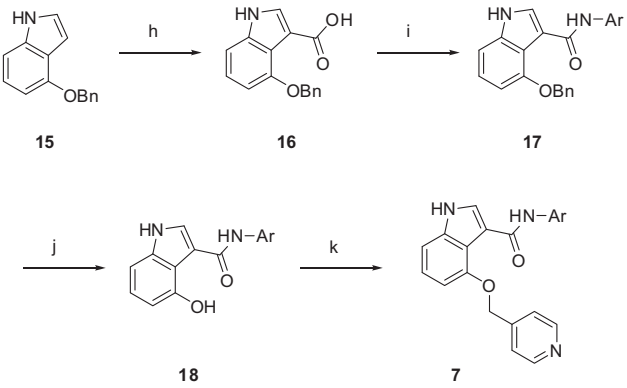


Figure 3. Possible interaction in indole derivatives **5**–**7**.



Scheme 2. Reagents and conditions: (e) $Tl(OCOCF_3)_3$, CF_3CO_2H, rt, then KI, H_2O, rt y. 41%; (f) 4-pyridinemethanethiol hydrochloride, cat. Xantphos, cat. $Pd_2(dba)_3$, DIEA, 1,4-dioxane, 90 °C, 85%; (g) $ArNH_2$, $AlMe_3$, toluene, reflux, y. 33–71%.



Scheme 3. Reagents and conditions: (h) trichloroacetic acid anhydride, DMF, rt then 4 M NaOH aq, THF, rt y. 17%; (i) $ArNH_2$, HATU, DIEA, DMF, rt y. 17–76%; (j) 10% Pd/C, MeOH, rt y. 62–91%; (k) 4-chloromethylpyridine hydrochloride, K_2CO_3, DMF, rt y. 19–31%.

prepared from 4-hydroxyindole-3-carboxamide **18**, which was obtained from the hydrogenation of 4-benzyloxyindole-3-carboxamide **17**.

The results of X-ray crystallographic analyses of selected compounds (**5d** and **6c**) are shown as ORTEP drawings in Figures 4 and 5.[19] Compound **5d** had the distance between the hydrogen on nitrogen (N3) and the oxygen (O1) of 1.97 Å, which would be intramolecular hydrogen bonding. The S–O distance (3.01 Å) of **6c** was shorter than the sum of van der Waals radii of sulfur and oxygen atoms (3.32 Å), thus suggesting intramolecular S–O non-bonded interaction.[20] The two terminal aromatic rings were apart distant in both of **5d** and **6c**; these were similar to the conformation of **1**, **2**, **3**. Thus, the compounds (**5d** and **6c**) were found to form seven-membered or six-membered pseudo ring as we expected. The X-ray crystallography of **7d** (Fig. 6) revealed that the distance between the hydrogen on nitrogen (N3) and the oxygen (O1) was 1.88 Å, thus intramolecular hydrogen bonding was also made in **7d**. The two terminal aromatic rings, however were close (4.33 Å) and nearly parallel (22°), which suggests π–π stacking interaction.

KDR inhibitory activities of indole derivatives **5**–**7** were evaluated by the reported ELISA assay method,[21] and are summarized in Table 1. While 4-pyridylmethylamino derivative **5a–d** inhibited KDR weakly (23–25% inhibition at 10 μM), 4-pyridylmethylthio derivatives **6a–d** showed more or potent inhibitory activities (34–92% inhibition at 10 μM), in which **6b** was most potent with an IC_{50} value of 309 nM. 4-Pyridylmethoxy derivatives **7a–d** did not show the activity (4–19% inhibition at 10 μM). The activities of the compounds are in order of **7** < **5** < **6** with the identical Ar group and appear limited to a certain range by every scaffold. This implies that the other compounds than observed ones (**5d**, **6c**, **7d**) by X-ray crystallography take similar conformations according to their scaffolds, in their binding to KDR. These results suggested that the inhibitory activities are driven with their stable conformations introduced with the non-bonded interactions which were observed in the X-ray crystallographic analyses mentioned above.

From these results, a rationale to the KDR binding of **5**–**7** will be given as follows (Figure 2B). In compound **5** or **6** taking the pseudo ring by intramolecular hydrogen bonding or S–O interaction that involves the carbonyl oxygen as a member of the pseudo ring, the amide NH to the Glu885 of KDR, the carbonyl oxygen to Asp1046 and the pyridine N to Cys919 would effectively work for the inhibitory activity. Their conformations will be similar to **1**, **2** and **3**. Though **7** is able to form intramolecular hydrogen bond and pseudo ring, its carbonyl oxygen being outside the ring would not be preferable to Glu885 side chain.

In summary, we investigate their conformation–activity relationships of novel indole-3-carboxamide derivatives **5**–**7** against KDR by X-ray crystallography under the pseudo ring idea.[22] Compounds **5** and **6** taking the pseudo seven- or six-membered ring by the intramolecular interaction, whose conformations are similar to active **1**, **2**, and **3**, showed KDR inhibitory activities, in particular, the IC_{50} of **6b** was 309 nM. Compound **7** having also a pseudo seven-membered ring did not show the inhibitory activity as its pseudo ring was deficient of carbonyl oxygen that would bind to KDR. This study led to novel KDR inhibitor **6b**, which was thought to be a promising lead for potent KDR inhibitor. Further studies of **6b** and its derivatives will be presented in due course.

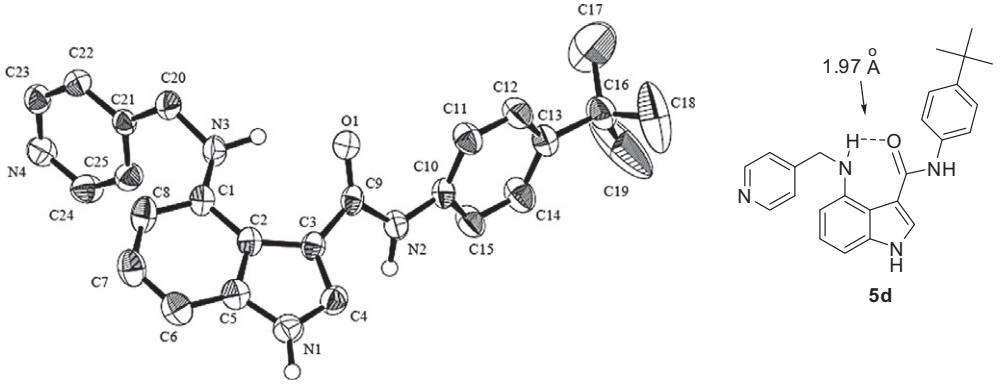


Figure 4. ORTEP drawing of compound **5d**. Hydrogen atoms on carbon atoms are omitted for the sake of clarity.

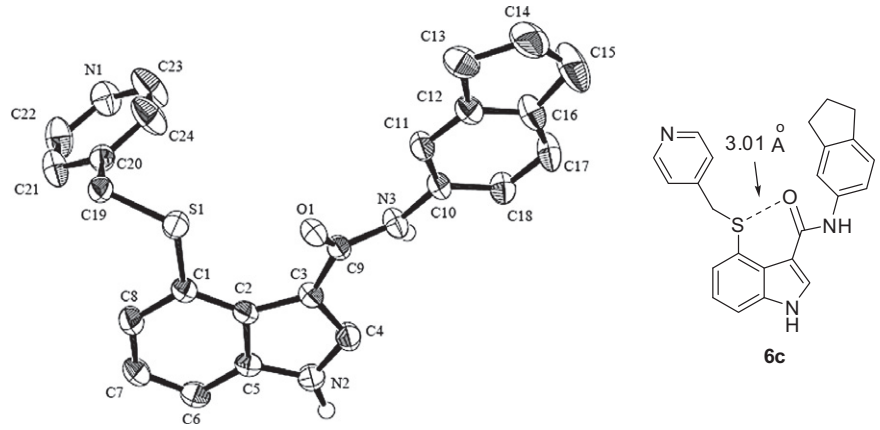


Figure 5. ORTEP drawing of compound **6c**. Hydrogen atoms on carbon atoms are omitted for the sake of clarity.

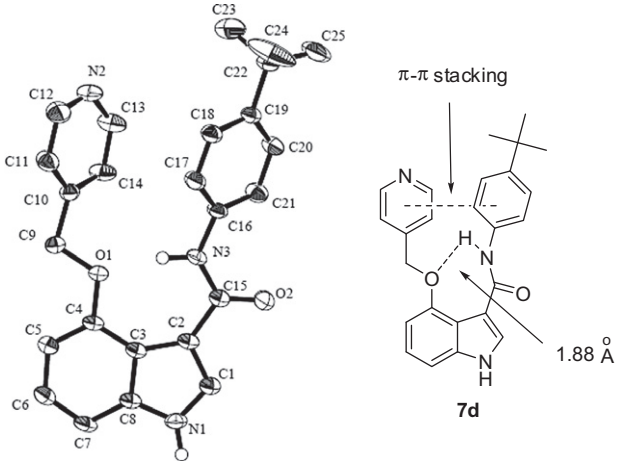


Figure 6. ORTEP drawing of compound **7d**. Hydrogen atoms on carbon atoms are omitted for the sake of clarity.

Table 1
KDR inhibitory activity[a] at 10 μM of compounds **5**–**7**

Compounds	Inhibition (%)	Compounds	Inhibition (%)
5a (Ar = 4-Cl-Ph)	23	**6c** (Ar = 5-indan)	59
5b (Ar = 3-CF_3-Ph)	25	**6d** (Ar = 4-*t*-Bu-Ph)	51
5c (Ar = 5-indan)	27	**6e** (Ar = 3-Cl-Ph)	35
5d (Ar = 4-*t*-Bu-Ph)	25	**6f** (Ar = 3-Me-Ph)	52
5e (Ar = 3-Cl-Ph)	18	**6g** (Ar = 3,5-Me-Ph)	35
5f (Ar = 3-Me-Ph)	18	**6h** (Ar = 4-OCF_3-Ph)	35
5g (Ar = 3,5-Me-Ph)	19	**6i** (Ar = 3-isoquinolin)	74
5h (Ar = 4-OCF_3-Ph)	14	**7a** (Ar = 4-Cl-Ph)	9
5i (Ar = 3-isoquinolin)	55	**7b** (Ar = 3-CF_3-Ph)	4
6a (Ar = 4-Cl-Ph)	34	**7c** (Ar = 5-indan)	19
6b (Ar = 3-CF_3-Ph)	92 (309 nM)[b]	**7d** (Ar = 4-*t*-Bu-Ph)	6

[a] KDR inhibitory activity was measured with a kinase aasay development kit purchased from CARNA BIOSCIENCE Co. Ltd.
[b] Parenthesis is a value of IC_{50}.

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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.2011.01.063.

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