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Synthesis of a novel biotin-tagged photoaffinity probe for VEGF receptor tyrosine kinases

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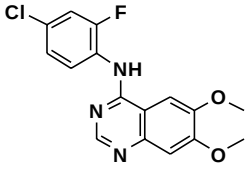
Abstract—A novel biotin-tagged photoaffinity probe was synthesized and evaluated as a vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase inhibitor. The probe (**2**) is a potent VEGFR-2 inhibitor with an IC_{50} value of 7.1 μM, and inhibits VEGF-induced proliferation in human umbilical vein endothelial cells (HUVEC), with an IC_{50} value of 40.3 μM. This probe will be a useful reagent for investigating ligand–protein interactions.

Photoaffinity labeling is a useful method for the identification of ligand-binding sites of target proteins and the investigation of ligand–receptor interactions. Photoactive moieties, such as an azido or a diazirine group, have been used as precursors for the highly reactive intermediate nitrene or carbene, which is generated upon photolysis. Perfluorophenyl azides are the most widely applied photolabeling reagents and belong to a new class of photolabeling reagents with improved C–H insertion efficiency compared with their nonfluorinated analogues.[1,2] Biotin-labeling is a powerful technique for the radioisotope-free detection of photolabeled proteins based on the strong interaction of biotin with either avidin or streptavidin. Therefore, biotinylated photoaffinity probes can be applied to separate photolabeled proteins from complex mixtures as well as to study the interactions of ligand–protein in living cells using advanced imaging techniques.[3] Recently, much attention has been devoted to the application of this method to the investigation of small molecule–target protein interactions.[4]

Vascular endothelial growth factor receptors (VEGFRs) are located on the surfaces of vascular endothelial cells and their activity is crucial for the induction of tumor angiogenesis, which is the process of new blood vessel formation from preexisting blood vessels.[5] For this reason, VEGFRs are attractive therapeutic targets for the development of novel agents to treat diseases such as cancer.[6,7] Several inhibitors of VEGFRs have



CB676475 (1)

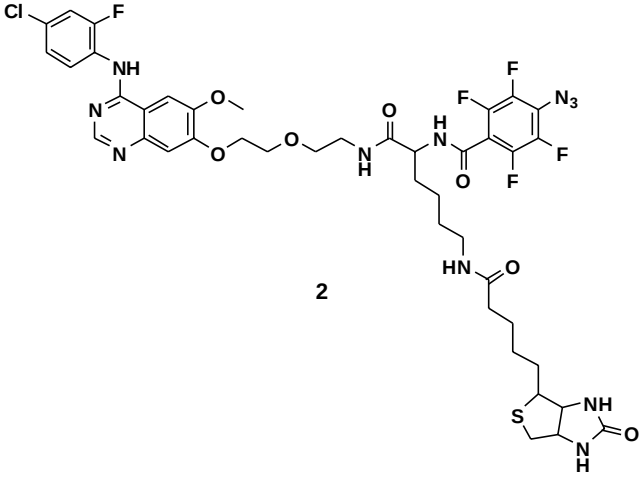


Figure 1. Structures of CB676475 (**1**) and a biotinylated photoactive quinazoline analogue (**2**).

Keywords: Biotin-tagged photoaffinity probe; Vascular endothelial growth factor receptors; Inhibitor; Ligand–protein interactions.
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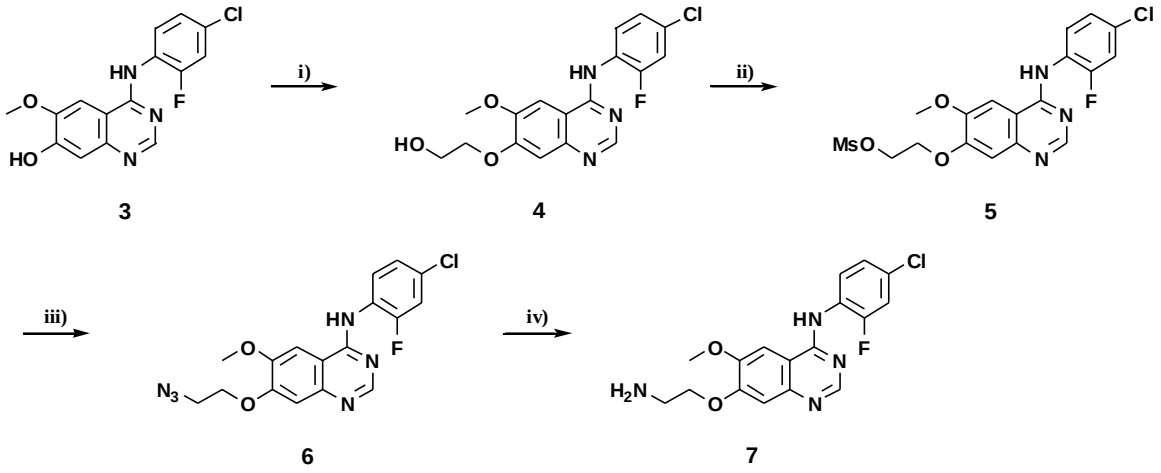
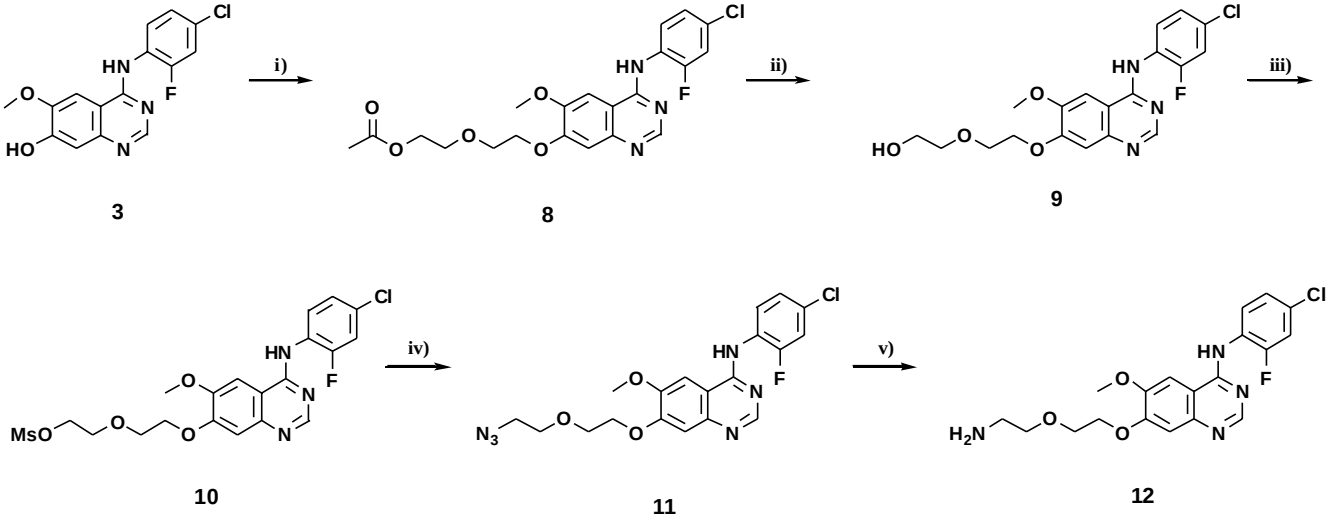


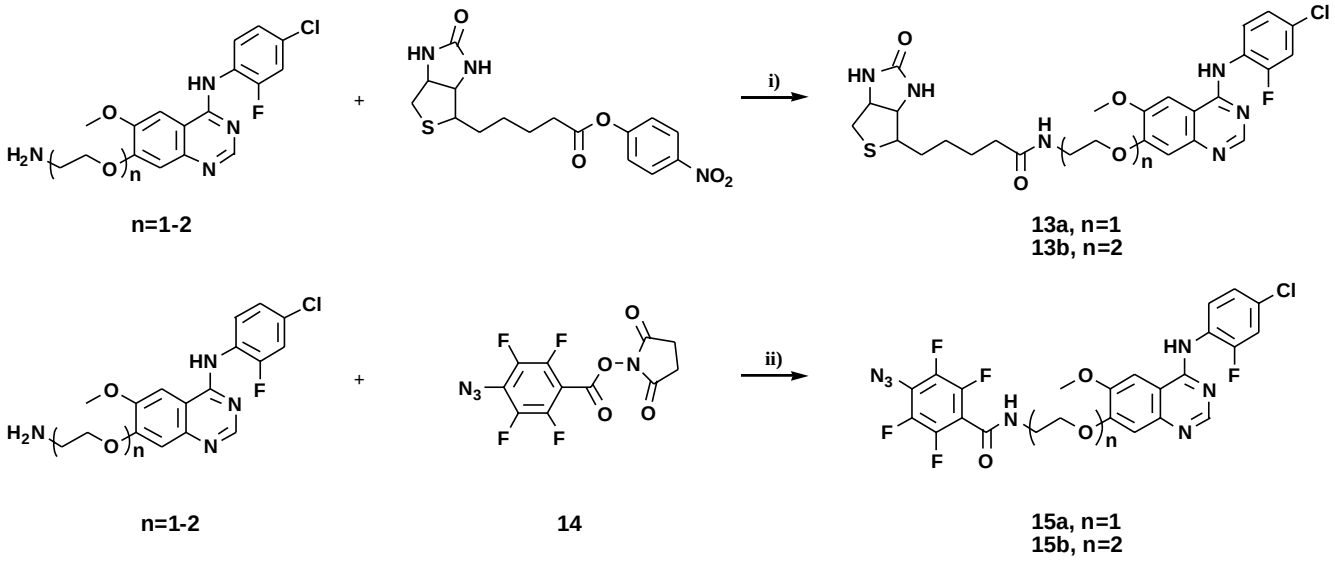
Scheme 1. Synthesis of compound **7**. Reagents and conditions: (i) 2-bromoethanol, K_2CO_3, THF/DMF, reflux, 2 h, 88%; (ii) MsCl, NEt_3, THF/DMF, 0 °C, 1 h, 83%; (iii) NaN_3, EtOH/H_2O, 80 °C, 3 h, 78%; (iv) H_2, $Pd(OH)_2$/C, MeOH, 89%.

been shown to inhibit tumor angiogenesis and the growth of tumors in animal models.[8] Recently, 4-anilinoquinazoline derivatives that selectively inhibit VEGFRs (KDR and Flt) have been reported by Hennequin et al.[6] To design probes that are bioactive in the same range as its parent compound, we analyzed the structure–activity relationships reported in the various synthetic derivatives. Structure–activity studies of 4-anilinoquinazoline inhibitors indicated that the addition of various substituents at the C-7 position of the quinazoline ring can retain good inhibitory activity.[6,7] In order to synthesize small molecule probes that can be applied for the study of VEGFRs, we investigated the introduction of a photoactive moiety and a biotin group at the C-7 position of the quinazoline ring, and synthesized chemical probes with perfluorophenyl azide and/or biotin moiety.

4-[(4′-Chloro-2′-fluoro)phenylamino]-6,7-dimethoxyquinazoline (CB676475, **1**) is a potent inhibitor of VEGFR (KDR and Flt) tyrosine kinases. IC_{50} values for KDR and Flt are 0.1 and 2 μM, respectively (Fig. 1).[6] Based on the structure of CB676475 (**1**), a novel biotinylated photoaffinity probe (**2**) was synthesized and evaluated. As shown in Scheme 1, the key intermediate 4-(4′-chloro-2′-fluorophenylamino)-6-methoxyquinazolin-7-ol (**3**) was prepared from 3,4-dimethoxybenzoic acid, as described previously.[7,9] The side chain at the C-7 position of quinazoline ring of compound **4** was introduced by the reaction of 7-hydroxyanilinoquinazoline **3** with 2-bromoethanol. The azide **6** was synthesized by the mesylation of alcohol **4** and the replacement of the mesyl group using NaN_3. 7-(2′-Aminoethoxy)quinazoline derivative **7** was obtained by the reduction of the azido group. The amine **12** was prepared as illustrated in Scheme 2. The intermediate **9** was synthesized by the O-alkylation of **3** with 2-acetoxyethyl-2′-tosyloxyethyl ether followed by the deprotection of the O-acetyl group.[10] Compound **9** was treated with methansulfonyl chloride to give O-mesylated quinazoline **10**. Finally,



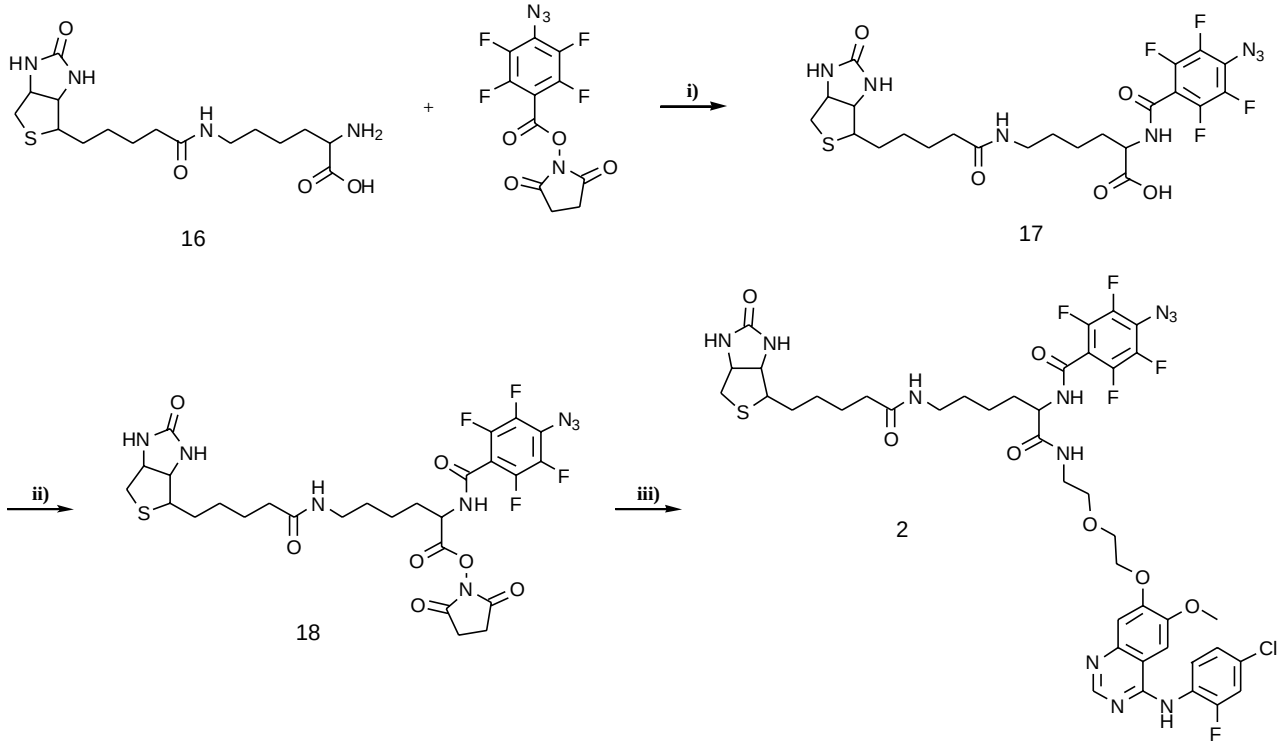
Scheme 2. Synthesis of compound **12**. Reagents and conditions: (i) $CH_3CO_2(CH_2CH_2O)_2Ts$, K_2CO_3, DMF, 95 °C, 81%; (ii) 30% NH_4OH, MeOH, rt, 12 h, 65%; (iii) MsCl, NEt_3, THF/DMF, 0 °C, 1 h, 99%; (iv) NaN_3, EtOH/H_2O, 80 °C, 3 h, 67%; (v) H_2, $Pd(OH)_2$/C, MeOH, 95%.



Scheme 3. Synthesis of compounds **13** and **15**. Reagents and conditions: (i) NEt_3, THF/CH_2Cl_2, rt, 10 h, 94%; (ii) DMF, rt, overnight, 98%.

compound **12** was obtained from **10** in high yield, under the same conditions as those used to prepare the amine **7**. As shown in Scheme 3, the biotinylated quinazoline derivatives (**13a**,**b**) were prepared by the condensation of amines **7** or **12** with (+)-biotin 4-nitrophenyl ester. The 4-azidotetrafluorobenzamide derivatives (**15a**,**b**) were obtained by the condensation of amines **7** or **12** with *N*-succinimidyl 4-azidotetrafluorobenzoate **14**.[1] The synthesis of the novel biotinylated photoreactive derivative **2** is summarized in Scheme 4. The intermediate **18** was synthesized by the condensation of commercially available biocytin **16** with **14** followed by the succinimidyl activation of acid **17** with *N*-hydroxysuccinimide.[11,12] Finally, the target biotin-tagged photoprobe **2** was obtained by the condensation of the NHS ester **18** with **12** in excellent yield.

In order to confirm whether a series of quinazoline derivatives (**2**, **13a**,**b**, **15a**, and **b**) effectively inhibit VEGFR-2, we measured their inhibitory activities against VEGFR-2 tyrosine kinase.[6,13a] All compounds were found to be potent VEGFR-2 inhibitors as shown in



Scheme 4. Synthesis of compound **2**. Reagents and conditions: (i) NaOH, DMF/H_2O (2/1), rt, overnight, 89%; (ii) *N*-hydroxysuccinimide, DCC, DMF, rt, 4 h, 77%; (iii) **12**, DMF, rt, overnight, 98%.

Table 1. Inhibitory activity of 4-anilinoquinazoline derivatives

Compound	IC_{50} (μM)[a]	
	VEGFR-2	HUVEC
1	1.5	1.0
13a	4.8	14.7
13b	1.5	1.9
15a	<1	4.1
15b	<1	3.2
2	7.1	40.3

[a] IC_{50} values are expressed as the average of at least three determinations.

Table 1. The biotin-tagged photoaffinity probe **2** was a potent VEGFR-2 inhibitor (IC_{50} = 7.1 μM) being only 4.7-fold less potent than CB676475 (IC_{50} = 1.5 μM). On the basis of their activities against VEGFR-2, quinazoline derivatives were evaluated in the VEGF-stimulated HUVEC proliferation assay (Table 1).[13b] The biotinylated compounds **13a**,**b** inhibited VEGF-dependent HUVEC proliferation with IC_{50} values of 14.7 and 1.9 μM, respectively. The 4-azidotetrafluoroaryl derivatives **15a**,**b** were found to be more potent inhibitors, with IC_{50} values of 4.1 and 3.2 μM, respectively. The extension of side chain at the C-7 position of quinazoline ring resulted in more potent inhibitors **13b** and **15b** compared to compounds **13a** and **15a**. The probe **2** inhibited HUVEC proliferation with an IC_{50} value of 40.3 μM. The inhibitory ability of probe **2** was 40-fold less potent than that of CB676475, which could be explained by unfavorable steric interactions of the bulky biotinylated photoactive moiety with the target enzymes. Although the inhibition effect was slightly decreased compared to that of CB676475, the probe **2** is potentially useful in selective photolabeling of target proteins.

Photodecomposition of probe **2** was examined by monitoring the UV absorbance change in the range between 200 and 400 nm in quartz cell.[14] The absorption spectra of photolysis are presented in Figure 2. The absorption maxima of **2** were observed at 250 and 330 nm. The photolysis of **2** in methanol was tested by UV irradiation at 254 or 365 nm at a distance of 5 cm from a UV lamp (VL-4LC, 4 W). Upon UV irradiation, the photolysis rate of **2** at 254 nm was faster than that at 365 nm (data not shown), and the decrease in absorption at 250 nm after 254 nm UV irradiation was greater than that at 330 nm, suggesting that the photodecomposition of the azidotetrafluorophenyl group resulted in the decrease in absorption at 250 nm. These results demonstrated that the probe **2** could be a powerful photoaffinity reagent to label VEGFRs involved in various aspects of tumor angiogenesis.

In conclusion, we have designed and synthesized a novel biotin-tagged photoaffinity probe **2** that is a potent VEGFR-2 inhibitor. This compound can be a useful photoaffinity labeling reagent for the identification and purification of target proteins as well as for the investigation of ligand–protein interactions in living cells using advanced imaging techniques. Further studies of VEGFRs using this probe are in progress.

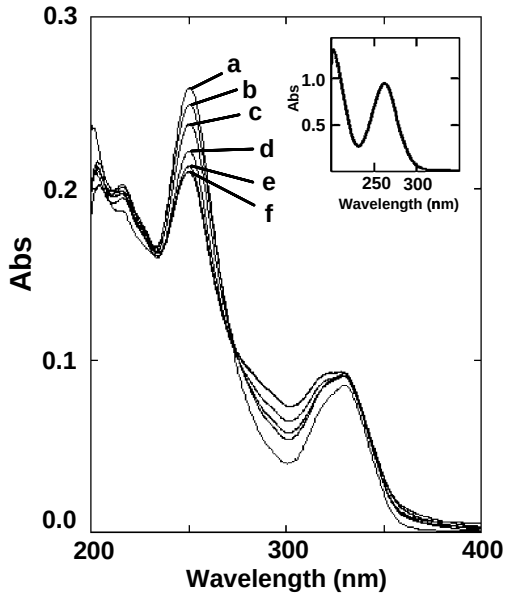


Figure 2. UV spectra for the photolysis of **2** (6 μM) in methanol at (a) 0, (b) 5, (c) 10, (d) 20, (e) 40, and (f) 60 s. The inset shows the UV absorption spectrum of *N*-succinimidyl 4-azidotetrafluorobenzoate **14** (10 μM) in methanol.

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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.2005.09.036.

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