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Design and synthesis of novel photoaffinity reagents for labeling VEGF receptor tyrosine kinases

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Abstract—Novel biotin-tagged photoaffinity probes based on a trifunctional tertiary amine scaffold were synthesized and evaluated as vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitors. Probes **3a–c** inhibit VEGF induced proliferation in HUVE cells, with IC_{50} values of 29.7, 33.3, and 37.7 μM, respectively. Moreover, we identified the interaction of **3b** with VEGFR-2 in photoaffinity labeling experiment using HUVE cells.

Angiogenesis is a process of new blood vessel formation from preexisting blood vessels and is essential for the growth of solid tumors and their metastasis.[1] Vascular endothelial growth factor receptors (VEGFRs) stimulate endothelial proliferation and play a critical role in endothelial cell survival/growth and tumor angiogenesis.[2] Recently, Casanovas et al. have demonstrated that the inhibition of VEGFR-2 in endothelial cells is important to prevent tumor growth.[3] Several VEGFR-2 inhibitors have been shown to inhibit tumor angiogenesis.[4]

Photoaffinity labeling is a powerful method in the chemical proteomic approach of protein functions. This method is especially useful for the identification of ligand-binding sites of target proteins and the investigation of ligand–receptor interactions.[5] Biotinylated photoaffinity probes can be applied to separate labeled proteins from complex mixtures as well as to study the interactions of ligand–protein in living cells.[6]

Previously, we have described the preparation of a photoaffinity probe **2** that is a potent VEGFR-2 inhibitor.[7] Moreover, we carried out the photocrosslinking experiment using HUVE cells and identified the interaction of **2** with VEGFR-2. The specific binding of **2** with VEGFR-2 was confirmed by the disappearance (or reduction) of the corresponding band under the condition in the presence of two-fold molar excess of non-labeled mother compound, CB676475 (data not shown). However, in the case of probe **2**, a photolabel tag and a bioactive ligand have been attached to two functional groups of biocytin, which has a number of disadvantages to insert a spacer between biotin and a photoaffinity moiety/ligand and to facilitate interactions between ligand and proteins in cells. This led to the design of a trifunctional scaffold for photoaffinity labeling, which can change the length of the side chain and enhance the inhibitory potency. Here, we developed an efficient synthesis of trifunctional probes that contain a photoreactive group, a biotin tag, and a bioactive ligand (see Fig. 1).

To introduce the side chains of various lengths between a photophore and biotin, we prepared the mono-Boc-protected compounds **4–6** by treatment of diamines with di-*ter*-butyl dicarbonate (Boc_2O) (Scheme 1).[8] Trifunctional tertiary amine derivatives (**22–24**) were prepared as illustrated in Scheme 2. Reductive amination of monoprotected amines (**4–6)** with perfluorobenzaldehyde afforded the pentafluorobenzylamine derivatives **7–9** in good yields.[9] The biotinylated precursors **13–15** were synthesized by deprotection of the Boc group with trifluoroacetic acid (TFA) followed by coupling with biotin in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC).[10] The intermediates (**16–18**) were obtained by the condensation of

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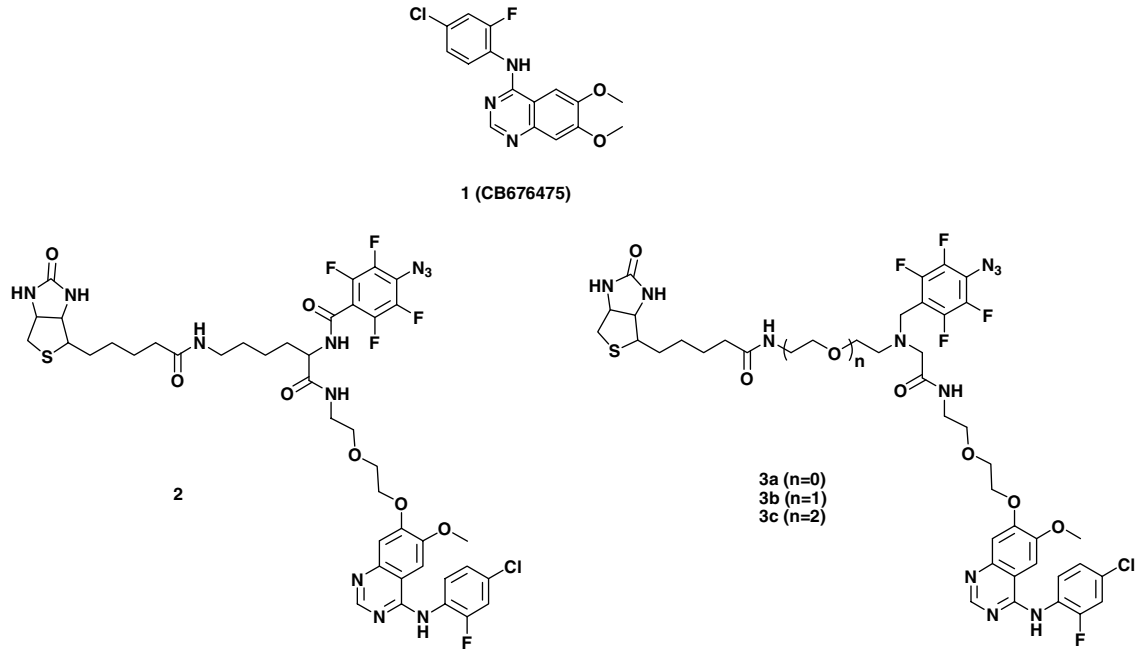
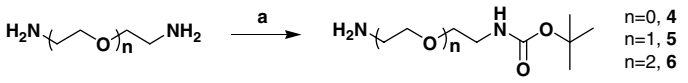
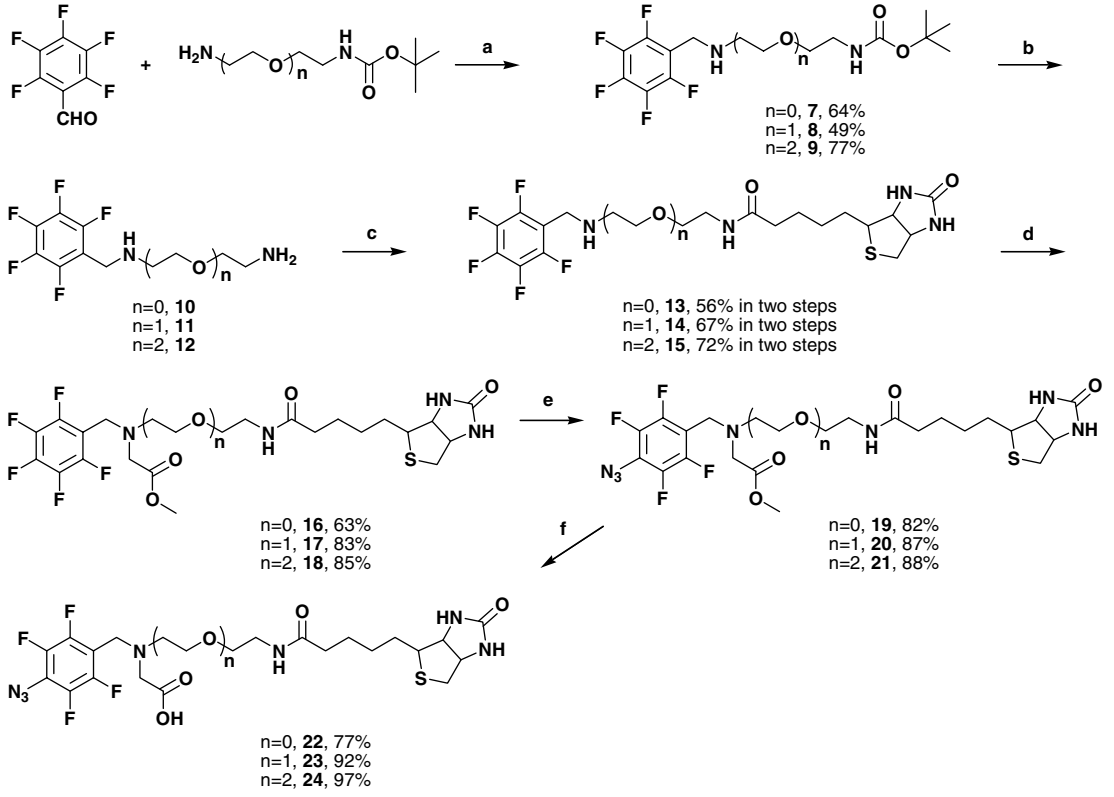


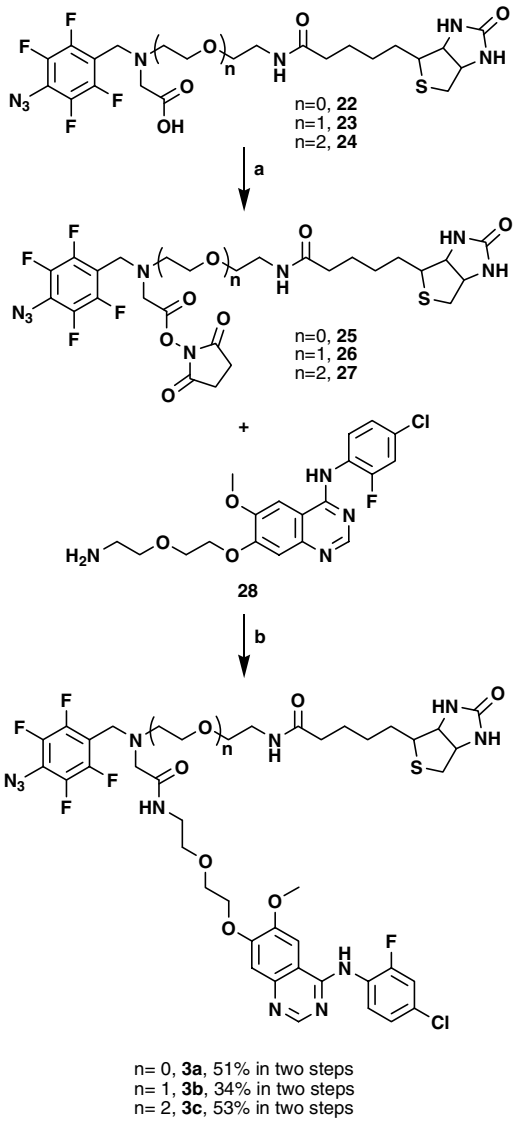
Figure 1. Structures of CB676475 (**1**) and biotin-tagged photoaffinity probes.



Scheme 1. Reagents and conditions: (a) (i) $n = 0$, $(Boc)_2O$, $CHCl_3$, 0 °C to rt, 24 h, 98%; (ii) $n = 1$, NaOH, MeOH/THF, rt, overnight, 97%; (iii) $n = 2$, $(Boc)_2O$, dioxane, rt, 5 h, 97%.



Scheme 2. Reagents and conditions: (a) (i) $MgSO_4$, NEt_3, MeOH, rt, 1.5 h, (ii) $NaBH_4$, 0 °C, 2 h; (b) TFA, CH_2Cl_2, rt, 1 h; (c) EDC, MeOH/CH_3CN (1/3), rt, 5 h; (d) methyl bromoacetate, DIEA, DMF, 5 °C to rt, 12 h; (e) NaN_3, Bu_4NN_3 (cat.), DMF, 80 °C, 12 h; (f) 1 N NaOH, MeOH, rt, 3 h.



Scheme 3. Reagents and conditions: (a) DCC, NHS, DMF, rt, overnight; (b) DMF, rt, overnight.

secondary amines (**13**–**15**) with methyl bromoacetate.[11] Treatment of intermediates (**16**–**18**) with NaN_3 in addition of catalytic amount of Bu_4NN_3 in DMF at 80 °C afforded the desired azides (**19**–**20**), which were confirmed by ^{19}F NMR.[11,12] Finally, intermediates (**22**–**24**) were obtained by hydrolysis of methyl esters (**19**–**21**) under basic conditions. The synthesis of novel trifunctional probes (**3a**–**c**) is summarized in Scheme 3. The *N*-hydroxysuccinimide (NHS) esters (**25**–**27**) were synthesized by the condensation of acid derivatives (**22**–**24**) with *N*-hydroxysuccinimide using dicyclohexylcarbodiimide (DCC). The quinazoline derivative (**28**) was prepared from 3,4-dimethoxybenzoic acid, as described previously.[7] Finally, the target biotin-tagged photoprobes **3a**–**c** were obtained by the condensation of the NHS esters (**25**–**27**) with **28** in moderate yield.[13]

Table 1. Inhibition of HUVEC proliferation by novel biotin-tagged photoaffinity probes

Compound	IC_{50} (μM)[a]
1	1.7 ± 0.3
2	41.7 ± 4.7
3a	29.7 ± 1.5
3b	33.3 ± 4.2
3c	37.7 ± 2.5

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

In order to confirm whether a series of quinazoline derivatives (**3a**–**c**) effectively inhibit HUVEC proliferation, we determined their inhibitory activity against VEGF-stimulated HUVEC proliferation using Cell Counting Kit-8 (CCK-8) assay (Dojindo laboratories) (Table 1).[14] The probe **2** had an IC_{50} of 41.7 μM, which was 25-fold less potent than CB676475 ($IC_{50} = 1.7$ μM). All probes **3a**–**c** were found to be more potent inhibitors compared to compound **2**, with IC_{50} values of 29.7, 33.3, and 37.7 μM, respectively. Activity was dependent on the chain length of the linker between biotin and the photoaffinity moiety/ligand. The decrease of side chain length resulted in more potent inhibitors, which could be explained by the decrease of unfavorable steric interactions of the bulky biotinylated photoactive moiety with the target enzymes. The result suggested that novel probes based on a trifunctional amine scaffold are very useful to probe the structural requirements for optimal biological activity.

The UV absorption maxima of probes **3a**–**c** in MeOH showed at 250 and 330 nm. The decrease in absorption at 250 nm was observed after 254 nm UV irradiation, suggesting the decomposition of the azido group (data not shown). In photoaffinity labeling experiment using HUVE cells, VEGFR-2 was detected in all lines (1) proteins only, (2) UV-irradiated proteins, (3) proteins mixed with **3b**, and (4) after proteins mixed with **3b**, UV-irradiated—at ca. 220-kDa using VEGFR-2 specific antibody (Fig. 2).[15,16] Proteins in the absence or presence of **3b** were irradiated at 254 nm twice for 2 min. When used both streptavidin and biotin antibodies, which can detect **3b**, the immunoreactivities were detected only in the fourth line at similar molecular weight position with that of VEGFR-2. This indicated the interaction of **3b**

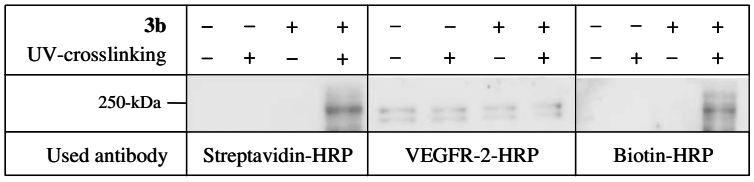


Figure 2. Western blot analysis showing the interaction of **3b** with VEGFR-2.

with VEGFR-2. These results demonstrated that the probes **3a**–**c** could be powerful photoaffinity reagents to label VEGFRs involved in various aspects of tumor angiogenesis.

In conclusion, we have synthesized novel biotin-tagged photoaffinity probes **3a**–**c** and estimated the importance of the side chain length between biotin and a photoactive moiety/ligand. Moreover, we showed that the probes can be used in photoaffinity crosslinking to label VEGFR-2 in HUVE cells. Novel trifunctional reagents for photoaffinity labeling should play a useful rule to identify target proteins and to investigate ligand–protein interactions in living cells. The biochemical application of these probes is currently under investigation.

Acknowledgements

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References and notes

1. (a) Neufeld, G.; Cohen, T.; Gengrinovitch, S.; Poltorak, Z. *FASEB J.* **1999**, *13*, 9–22; (b) Carmeliet, P. *Nat. Med.* **2003**, *9*, 635–660; (c) Shibuya, M.; Claesson-Welsh, L. *Exp. Cell Res.*, in press.
2. Ferrara, N.; Gerber, H.-P.; LeCouter, J. *Nat. Med.* **2003**, *9*, 669–676.
3. Casanovas, O.; Hicklin, D. J.; Bergers, G.; Hanahan, D. *Cancer Cell.* **2005**, *8*, 299–309.
4. (a) Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S. E.; Ple, P. A.; Lohmann, J.-J. M.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Lambert-Van Der Brempt, C. *J. Med. Chem.* **1999**, *42*, 5369–5389; (b) Cross, M. J.; Claesson-Welsh, L. *Trends Pharmacol. Sci.* **2001**, *22*, 201–207; (c) Adams, J.; Huang, P.; Patrick, D. *Curr. Opin. Chem. Biol.* **2002**, *6*, 486–492; (d) Borzilleri, R. M.; Cai, Z.-W.; Ellis, C.; Fargnoli, J.; Fura, A.; Gerhardt, T.; Goyal, B.; Hunt, J. T.; Mortillo, S.; Qian, L.; Tokarski, J.; Vyas, V.; Wautlet, B.; Zheng, X.; Bhide, R. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1429–1433; (e) Sridhar, J.; Akula, N.; Sivanesan, D.; Narasimhan, M.; Rathinavelu, A.; Pattabiraman, N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4125–4129; (f) Kubo, K.; Shimizu, T.; Ohyama, S. I.; Murooka, H.; Iwai, A.; Nakamura, K.; Hasegawa, K.; Kobayashi, Y.; Takahashi, N.; Takahashi, K.; Kato, S.; Izawa, T.; Isoe, T. *J. Med. Chem.* **2005**, *48*, 1359–1366.
5. (a) Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64–77; (b) Hatanaka, Y.; Sadakane, Y. *Curr. Top. Med. Chem.* **2002**, *2*, 271–288.
6. (a) Frick, W.; Bauer-Schafer, A.; Bauer, J.; Girbig, F.; Corsiero, D.; Heuer, H.; Kramer, W. *Bioorg. Med. Chem.* **2003**, *11*, 1639–1642; (b) Hashimoto, M.; Okamoto, S.; Nabeta, K.; Hatanaka, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2447–2450; (c) Kinoshita, T.; Cano-Delgado, A.; Seto, H.; Hiranuma, S.; Fujioka, S.; Yoshida, S.; Chory, J. *Nature* **2005**, *433*, 167–171; (d) Ballell, L.; Alink, K. J.; Slijper, M.; Versluis, C.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2005**, *6*, 291–295; (e) Chen, I.; Howarth, M.; Lin, W.; Ting, A. Y. *Nat. Methods* **2005**, *2*, 99–104; (f) Visintin, C.; Aliev, A. E.; Riddall, D.; Baker, D.; Okuyama, M.; Hoi, P. M.; Hiley, R.; Selwood, D. L. *Org. Lett.* **2005**, *7*, 1699–1702; (g) Fujii, T.; Manabe, Y.; Sugimoto, T.; Ueda, M. *Tetrahedron* **2005**, *61*, 7874–7893.
7. Han, S. Y.; Park, S. S.; Lee, W. G.; Min, Y. K.; Kim, B. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 129–133.
8. (a) Braun, M.; Camps, X.; Vostrowsky, O.; Hirsch, A.; Endreß, E.; Bayerl, T. M.; Birkert, O.; Gauglitz, G. *Eur. J. Org. Chem.* **2000**, 1173–1181; (b) Herges, R.; Dikmans, A.; Jana, U.; Kohler, F.; Jones, P. G.; Dix, I.; Fricke, T.; Konig, B. *Eur. J. Org. Chem.* **2002**, 3004–3014; (c) Eisenfuhr, A.; Arora, P. S.; Sengle, G.; Takaoka, L. R.; Nowick, J. S.; Michael Famulok, M. *Bioorg. Med. Chem.* **2003**, *11*, 235–249.
9. Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. *J. Org. Chem.* **1997**, *62*, 411–416.
10. Eisenfuhr, A.; Arora, P. S.; Sengle, G.; Takaoka, L. R.; Nowick, J. S.; Famulok, M. *Bioorg. Med. Chem.* **2003**, *11*, 235–249.
11. Redman, J. E.; Ghadiri, M. R. *Org. Lett.* **2002**, *25*, 4467–4469.
12. Compound **19**: ^{19}F NMR ($CFCl_3$): δ −142.91 (m, 2F), −151.87 (m, 2F). Compound **20**: ^{19}F NMR ($CFCl_3$): δ −143.09 (m, 2F), −152.27 (m, 2F). Compound **21**: ^{19}F NMR ($CFCl_3$): δ −143.05 (m, 2F), −152.37 (m, 2F).
13. Compound **3a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30–1.70 (m, 6H), 2.06 (t, 2H, $J = 7.5$ Hz), 2.57 (m, 2H), 2.66 (d, 1H, $J = 12.6$ Hz), 2.85 (m, 1H), 3.05 (m, 1H), 3.12 (s, 2H), 3.24 (m, 2H), 3.48 (m, 2H), 3.66 (m, 2H), 3.79 (s. 2H), 3.97 (m, 5H), 4.22 (m, 1H), 4.30 (m, 2H), 4.44 (m, 1H), 5.55 (s, 1H), 6.25 (s, 1H), 6.83 (m, 1H), 7.17–7.27 (m, 3H), 7.34 (s, 1H), 7.45 (m, 1H), 8.13 (t, 1H, $J = 8.7$ Hz), 8.21 (br s, 1H), 8.58 (s, 1H); ^{19}F NMR ($CFCl_3$): δ −122.63 (s, 1F), −142.40 (m, 2F), −151.84 (m, 2F); HRMS-FAB (*m*/*z*) $[M+H]^+$ calcd for $C_{40}H_{44}ClF_5N_{11}O_6S$, 936.2805, found, 936.2805. Compound **3b**: ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.30–1.45 (m, 2H), 1.50–1.78 (m, 4H), 2.19 (t, 2H, $J = 7.3$ Hz), 2.60–2.75 (m, 3H), 2.88 (m, 1H), 3.10–3.20 (m, 1H), 3.20 (s, 2H), 3.28–3.35 (m, 2H), 3.40–3.55 (m, 6H), 3.68 (m, 2H), 3.80 (s. 2H), 3.86–4.0 (m, 2H), 3.97 (s, 3H), 4.28 (m, 3H), 4.46 (m, 1H), 7.11 (s, 1H), 7.27–7.36 (m, 2H), 7.65 (m, 2H), 8.34 (s, 1H); ^{19}F NMR ($CFCl_3$): δ −123.47 (s, 1F), −142.80 (m, 2F), −151.92 (m, 2F); HRMS-FAB (*m*/*z*) $[M+H]^+$ calcd for $C_{42}H_{48}ClF_5N_{11}O_7S$, 980.3068, found, 980.3069. Compound **3c**: ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.25–1.45 (m, 2H), 1.48–1.78 (m, 4H), 2.15 (t, 2H, $J = 7.4$ Hz), 2.60–2.73 (m, 3H), 2.88 (m, 1H), 3.05–3.20 (m, 1H), 3.20 (s, 2H), 3.27–3.35 (m, 2H), 3.40–3.60 (m, 10H), 3.67 (m, 2H), 3.78 (s, 2H), 3.84–4.0 (m, 2H), 3.96 (s, 3H), 4.15–4.30 (m, 3H), 4.45 (m, 1H), 7.08 (m, 1H), 7.26–7.36 (m, 2H), 7.60–7.66 (m, 2H), 8.33 (s, 1H); ^{19}F NMR ($CFCl_3$): δ −117.18 (s, 1F), −142.01 (m, 2F), −152.26 (m, 2F); HRMS -FAB (*m*/*z*) $[M+Na]^+$ calcd for $C_{44}H_{51}ClF_5N_{11}O_8SNa$, 1046.3149, found, 1046.3146.
14. Wood, J. M.; Bold, G.; Buchdunger, E.; Cozens, R.; Ferrari, S.; Frei, J.; Hofmann, F.; Mestan, J.; Mett, H.; O'Reilly, T.; Persohn, E.; Rosel, J.; Schnell, C.; Stover, D.; Theuer, A.; Towbin, H.; Wenger, F.; Woods-Cook, K.; Menrad, A.; Siemeister, G.; Schirner, M.; Thierauch, K. H.; Schneider, M. R.; Drevs, J.; Martiny-Baron, G.; Totzke, F.; Marme, D. *Cancer Res.* **2000**, *60*, 2178–2189.
15. Photoaffinity labeling experiment—HUVEC cells were homogenized in buffer consisting of 10 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.05% (v/v) Tween 20, 1 mM PMSF, and one protease inhibitor cocktail tablet (Roche, Germany) at 4 °C and centrifuged at 10,000*g* for 15 min. The BCA protein assay Kit (Pierce, IL) was used to determine the concentration of protein in the supernatant. Proteins (100 μg in 50 μl) were incubated with **3b** (100 μM) at 4 °C for 2 h, and placed on an ice tray under a UV light source (BIO-LINK with 5×8 W tubes, 254 nm, Vilber

Lourmat, France) twice for 2 min. Compound **3b** was first dissolved in dimethyl sulfoxide (DMSO; Sigma, MO), and then diluted with homogenate buffer, so that the final DMSO concentration was 1% in the samples. Irradiated protein samples (20 μg) were mixed with sample buffer (100 mM Tris–HCl, 2% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 2% glycerol, 0.01% bromophenol blue, pH 7.6), incubated at 50 °C for 10 min, and loaded onto 8% polyacrylamide gels. Electrophoresis was performed using the Mini Protean 3 Cell (Bio-Rad). Proteins separated on the gels were transferred onto nitrocellulose membrane (Scheicher & Schnell BioScience, Germany), and the membrane was incubated in blocking buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20, 3% nonfat dry milk). The membrane was incubated for 2 h at room temperature with 1:1000 diluted primary antibody. Horse radish peroxidase (HRP)-conjugated antibody against VEGFR-2 and antibodies against streptavidin and biotin were purchased from Santa Cruz Biotechnology Inc. (CA) and Calbiochem (Merck Biosciences, Korea), respectively. After washing three times for 15 min with blocking buffer, and developed with SuperSignal West Femto Maximum Sensitivity Substrate (Pierce). The immunoreactivity was detected using LAS-3000 luminescent image analyzer (Fuji Photo Film Co., Ltd, Japan).

16. Takahashi, T.; Shibuya, M. *Oncogene* **1997**, *14*, 2079–2089.