Synthesis and Mode of Action of ^{125}I- and ^{3}H-Labeled Thieno[2,3-*c*]pyridine Antagonists of Cell Adhesion Molecule Expression

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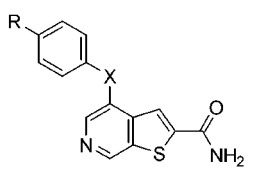
A series of thieno[2,3-*c*]pyridine antagonists of cell adhesion molecule (CAM) expression, such as A-205804 (**1**) and A-249377 (**2**), selectively suppressed the induced expression of E-selectin and ICAM-1 over VCAM-1. In an effort to explore the biological mechanism of action of these inhibitors, we synthesized ^{125}I- and ^{3}H-labeled thieno[2,3-*c*]pyridines **5** and **6**. An isolated diazonium tetrafluoroborate salt efficiently trapped Na^{125}I on very small scale (7.5 μg of Na^{125}I), providing the corresponding ^{125}I-labeled thieno[2,3-*c*]pyridine in modest yield. Preliminary mechanistic investigations using these radiolabeled compounds revealed that, upon incubation with human umbilical vein endothelial cells (HUVECs), these inhibitors of CAM expression translocated to the cell nucleus and were noncovalently associated with macromolecules of molecular weight greater than 650 kDa.

Introduction

Cell adhesion molecules (CAMs) are a group of cell-surface proteins that are involved in mediating adhesion of cells to each other and to extracellular matrix proteins. Binding of CAMs on vascular endothelial cells (e.g., E-selectin, ICAM-1, and VCAM-1) to their counter-receptors on circulating leukocytes (e.g., Lewis-X antigens, β_1 and β_2 integrins) lead to transmigration of leukocytes to the site of injury.[1] Continuous recruitment[2] of leukocytes from blood vessels may result in disease states, such as perpetuation of tissue injury in chronic inflammation.[3] An early and critical step in stimulated leukocyte recruitment is the induced expression of cell adhesion molecules on the lumenal surface of vascular endothelial cells.[4,5] We have discovered a series of thieno-[2,3-*c*]pyridine antagonists such as A-205804 (**1**) and A-249377 (**2**) that selectively suppressed the tumor necrosis factor-α (TNFα)-induced expression of E-selectin and ICAM-1 over VCAM-1 (**1**: $IC_{50} \sim 25$ nM for E-selectin and ICAM-1, $IC_{50} > 1$ μM for VCAM-1; **2**: $IC_{50} \sim 4$ nM for E-selectin and ICAM-1, $IC_{50} = 48$ μM for VCAM-1).[6,7] Significant clinical benefit was demonstrated by **2** in a rat rheumatoid arthritis model and in a mouse asthma model.[7]



1 X= S, R= CH_3 (**A-205804**)
2 X= O, R= Br (**A-249377**)
3 X= O, R= I
4 X= S, R= H
5 X= O, R= ^{125}I
6 X= S, R= ^{3}H

Preliminary biological characterization using a series of structurally diverse inhibitors indicated that the compounds were effective regardless of method of activation of the endothelial cells [e.g., interleukin-1 β (IL-1β)]. These compounds were not general inhibitors of protein synthesis or gene transcription and had little effect on T-cell function. However, the mode of action of our lead compounds on the molecular level has not been elucidated. To explore the biomolecular target of our thienopyridine inhibitors, a tritium (^{3}H), or preferably the higher energy emitting ^{125}I-labeled thienopyridine inhibitor, would be required. Since compound **3** and **4** were very similar to our lead **2** and **1** in all aspects of biological characterizations (**3**: $IC_{50} = 25$ nM for ICAM-1, **4**: $IC_{50} = 4$ nM for ICAM-1) and represented two series of thieno-[2,3-*c*]pyridine antagonists of cell adhesion molecule expression, herein we report the synthesis of their ^{125}I- and ^{3}H-labeled compound **5** and **6** and preliminary investigation toward elucidation of their mechanism of action.

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(1) (a) Carter, W.; Robinson, J. In *Phagocyte Function*; Robinson, J.; Babcock, G., Eds.; Wiley-Liss: New York, 1998; pp 253−276. (b) Forlow, S. B.; Ley, K. *Am. J. Physiol. Heart Circ. Physiol.* **2001**, *280*, H634−H641.

(2) *Leukocyte Recruitment, Endothelial Cell Adhesion Molecules, and Transcriptional Control: Insight for Drug Discovery*,; Collins, T., Ed.; Kluwer: Boston, MA, 2001; pp 1−327.

(3) Winn, R.; Vedder, N.; Ramamoorthy, C.; Sharar, S.; Harlan, J. *Blood Coagulation Fibrinolysis* **1998**, *9*(2), S17−S22

(4) Kubes, P.; Ward, P. A. *Brain Pathol.* **2000**, *10*, 127−135.

(5) (a) Newman, W.; Mirabelli, C. *Expert Opin. Invest. Drugs* **1998**, *7*, 19−25. (b) Panes, J.; Granger, D. *Gastroenterology* **1998**, *114*, 1066−1090.

(6) Stewart, A.; Bhatia, P.; McCarty, C.; Patel, M.; Staeger, M.; Arendsen, D.; Gunawardana, I.; Melcher, L.; Zhu, G.-D.; Boyd, S.; Fry, D.; Cool, B.; Kifle, L.; Lartey, K.; Marsh, K.; Kempf-Grote, A.; Kilgannon, P.; Wisdom, W.; Meyer, J.; Gallatin, M.; Okasinski, G. *J. Med. Chem.* **2001**, *44*, 988−1002.

(7) Zhu, G.-D.; Arendsen, D.; Gunawardana, I.; Boyd, S.; Stewart, A.; Fry, D.; Cool, B.; Kifle, L.; Schaefer, V.; Meuth, J.; Marsh, K.; Kempf-Grote, A.; Kilgannon, P.; Gallatin, M.; Okasinski, G. *J. Med. Chem.* **2001**, *44*, 3469−3487.

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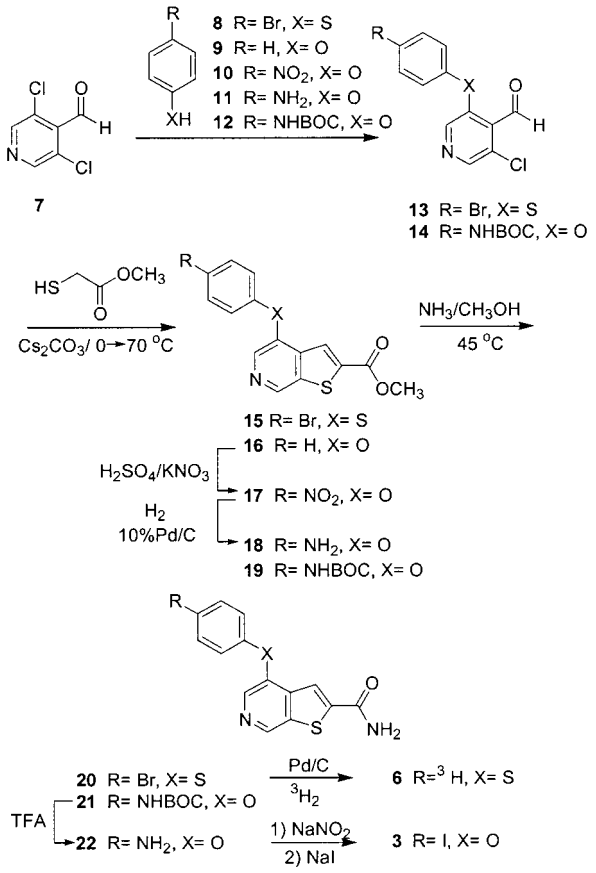
Scheme 1



Results and Discussion

To save the expensive isotopically enriched reagents and reduce the number of steps involving radioactive intermediates, an ideal process of radiolabeling introduces the radioactive reagent at the end of synthesis.

In our case, Sandmeyer reaction of aniline **22**, followed by $Na^{125}I$ trapping, and hydrogenation of **20** under tritium would serve as the ideal labeling steps for the synthesis of **5** and **6**. We envisioned that substitution of one chloride of 3,5-dichloropyridinecarboxaldehyde (**7**) with a functionalized thio- or regular phenol would install the requisite bromide and amino groups. Displacement of the second chloride of **7** by a methyl thioglycolate, followed by a base-catalyzed Aldol type cyclization of the glycolate with the 4-carbonyl, would construct the thieno[2,3-*c*]pyridine core of **20** and **22**. The detailed syntheses are outlined in Scheme 1. Treatment of dichloropyridinecarboxaldehyde **7** with 1 equiv of 4-bromothiophenol (**8**) afforded a monosubstituted diaryl sulfide **13**. The diaryl sulfide **13** was not purified but rather was reacted with methyl thioglycolate in the same pot. The Aldol type cyclization needed assistance of a weak base such as potassium carbonate, giving the 4-substituted thieno[2,3-*c*]pyridine-2-carboxylate **15** in 50% yield over two steps. Heating of the methyl ester **15** in a methanolic ammonia solution at 45 °C furnished **20** in 95% yield. Hydrogenation of **20** in ethanol under tritium (10% Pd/C, 0.125 atm $^3H_2$) afforded the desired tritium labeled thieno[2,3-*c*]-pyridine **6**. This tritium-labeled compound was not characterized spectroscopically, but was identical by HPLC and TLC with protio compound **4** that was prepared by the same protocol.

Since phenols are less nucleophilic than thiophenols, and because additional challenges were expected for a one-pot synthesis of the 4-phenoxythieno[2,3-*c*]pyridine carboxylate (e.g., **18**), we first used parent phenol to explore the reaction and avoid the complicating functional groups. Indeed, displacement of the chloride in **7** with a phenol was much slower and required assistance of a base (Scheme 1, X = O). Under conditions similar to the synthesis of **15** (see Experimental Section, method A of **16**), the reaction of **7** with phenol afforded only 20% of desired compound **16.** Using potassium phenoxide as the nucleophile gave a cleaner reaction, and generated **16** in 40% yield (method B). Interestingly, when **7** was heated with two equiv of potassium phenoxide at 70 °C for 3 h, both chlorides were displaced. Substitution of one of the phenoxides by methyl thioglycolate proceeded smoothly at room temperature, followed by cyclization, to give **16** in a respectable 75% yield (method C).

With an efficient protocol to construct the 4-phenoxythieno[2,3-*c*]pyridine-2-carboxylate core in hand, we next explored the reaction of **7** with 4-aminophenol **11** and 4-nitrophenol **10**. The former phenol would provide the requisite amino compound **18** for the ^{125}I-labeling, and the nitro-product **17** could be easily converted to **18** via hydrogenation (Scheme 1). Unfortunately, no desired product (**17** and **18**) was detected for both reactions under all three conditions described for the unsubstituted phenol.

To circumvent this problem, we explored a direct nitration of **16**. There are several electron-rich sites in **16**; therefore, a traditional H_2SO_4/HNO_3 protocol would not be desired because of the challenges in preventing over-nitration. We envisioned that addition of 1 equiv of solid potassium nitrate to concentrated sulfuric acid would generate 1 equiv of fuming nitric acid that could be directly used for nitration. Indeed, nitration of **16** in sulfuric acid with 1 equiv of solid KNO_3 gave a spot-to-spot conversion to **17**, specifically at the *para*-position of the phenoxide. The major advantage of this nitration strategy included easy operation for smaller scale reactions. Hydrogenation of the nitrophenyl ether **17** in the presence of 10% Pd/C afforded **18**.

When working on the nitration strategy to access aniline **18**, we also screened the potential protecting groups for the troublesome amino functionality in **11**. Under the best conditions for synthesis of **16** (method C of Experimental Section), the reaction of 4-*tert*-butoxycarbonylaminophenol (**12**) with **7** did not provide any detectable desired product **19**. By employing the Cs_2CO_3/DMF protocol (method A), only 10% desired **19** was obtained. When we first isolated intermediate **14** in pure form by column chromatography (67%) and resubjected it to methyl thioglycolate substitution/cyclization, the same reaction furnished **19** in 37% overall yield. Heating ester **19** in a methanolic ammonia solution afforded amide **21**. The BOC protecting group of **21** was removed by treating with trifluoroacetic acid (TFA) to provide aniline **22**.

The results of Sandmeyer reactions of **22** are summarized in Table 1. The Sandmeyer reaction of **22** on a 30 mg scale with 3 equiv of sodium iodide afforded 82% of desired iodide **3**. A major side reaction was reduction of the diazonium salt **24** to give the unsubstituted phenyl ether **23**. There was still about 5% diazonium salt **24** that was not trapped by NaI, as determined by HPLC.

Since $Na^{125}I$ was much more expensive and less available than **22**, our goal was to increase the yield of

Table 1. Sandmeyer Reactions[a] of 22

22 (mg)	**22**/NaI mol/mol	yield (**3**) (from **22**), %	yield (**3**) (from NaI), %	yield (**23**), %
30	1/3	82	27	10
5	2/1	25	50	20
3	5/1	3	17	6
1	5/1	1	5	6
0.5	5/1	<1	2	2
0.5	35/1	0	0	0

[a] Aniline **22** was treated with 1 equiv of KNO_2 in 3 M H_2SO_4.

Scheme 2

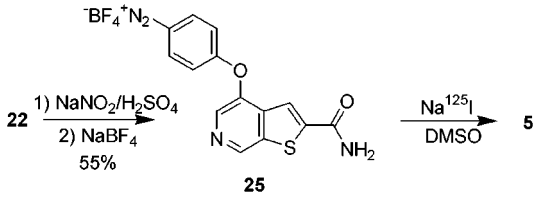
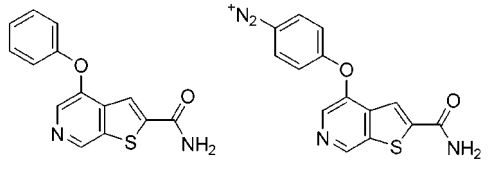


Table 2. Trapping NaI with 25

25 (mg)	**25**/$Na^{125}I$ mol/mol	Yield (**5**) (from **25**), %	Yield (**5**) (from NaI), %	Yield (**23**), %
2	1/1[a]	44	44	35
1	4/1[a]	11	42	40
0.5	2/1[a]	23	42	40
0.5	18/1	2.5	38	42
0.5	35/1	1	35	45

[a] Naturally occurring NaI was used in the reaction. The product was **3** (instead of **5**).

radiolabeled compound **5** based on consumed $Na^{125}I$. When less NaI (0.5 equiv) was used in the reaction, a higher 50% yield was achieved for NaI, even though the yield based on **22** was much lower (25%) on 5 mg scale. However, when the reaction scale was reduced from 3 mg to 0.5 mg, the yield for iodine incorporation to **3** dropped dramatically from 17% to 2%. Other than small amount of **23** (2%), the majority of the derived diazonium salt **24** remained unreacted in the 0.5 mg reaction. On a practical scale of ^{125}I labeling in a regular biological laboratory, we attempted to trap 7.5 μg of $Na^{125}I$ with the diazonium salt **24** from 0.5 mg of **22**. In this reaction, none of the desired product **5** was detected by HPLC.



We suspected that the strongly acidic conditions employed (3 M H_2SO_4) could be a major factor in the NaI trapping. The diazonium tetrafluoroborate **25** was then prepared by reaction of **24** with sodium tetrafluoroborate. Indeed, when treating **25** with NaI in DMSO, desired product **5** was isolated consistently in modest yield. The amount of NaI employed had no significant affect on the yield (Scheme 2 and Table 2). The reaction of 0.5 mg of **25** with 7.5 μg of $Na^{125}I$ essentially gave the same yield of **5** (35%) compared to a reaction on a much larger scale (44% for 1 mg of NaI).

With the two radiolabeled compounds **5** and **6** in hand, we first performed a direct binding experiment with HUVEC lysates to evaluate if our thienopyridine leads

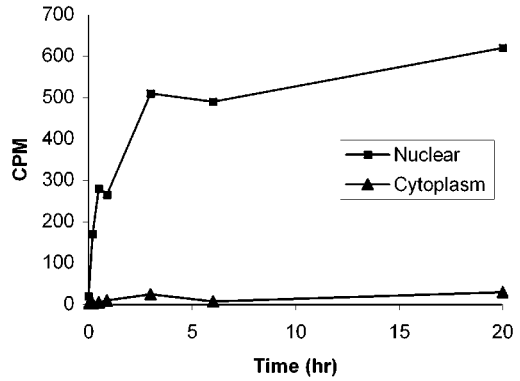


Figure 1. Kinetics of **6** in HUVECs. Essentially all of the radioactivity which bound to the cell was translocated to the nucleus. The nucleus was saturated about 5 h after treatment of the cells with radiolabeled compound. Less than 1% of the radioactivity counts were detected in the cytoplasmic fraction.

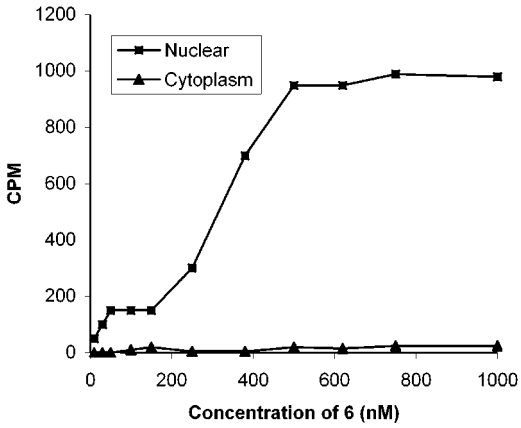


Figure 2. Binding saturation of **6**. With increasing concentration of **6**, the total CPM increased nearly linear to the concentration (not shown in chart). The radioactivity in the nucleus increased and saturated at about 500 nM of **6**. Less than 1% of the counts was found in the cytoplasm, and the percentage was not dependent on the concentration of **6**.

bound to any enzymes that involved in the signaling pathway leading to activation of adhesion molecule expression. Thus, the more readily available **6** was incubated with HUVEC lysates at 37 °C for 2 and 18 h and subjected to a BioRad P30 spin column assay. The void volume containing molecules with molecular weight >40 kDa was collected after centrifugation at 600 g for 4 min. Less than 0.1% of the total radioactivity was detected in the void volume, demonstrating lack of interaction of **6** with any molecule with a molecular weight of 40 kDa or greater in the HUVEC cell lysates.

We then incubated **6** with HUVECs in log phase growth at 37 °C, with samples taken over 20 h. The cells were fractionated according to a modified protocol by Goldstein et al.[8] Figure 1 shows the distribution of radiolabeled compounds in HUVECs.

As illustrated in Figure 1, with increasing time of incubation (~5 h), the thienopyridine **6** became localized to the cell nucleus. The cytoplasm contained less than 1% of the total radioactivity. Figure 2 shows that the translocation of **6** to nucleus increased with increasing concentration of radiolabeled drug (total CPM was linear

(8) Wang, X.; Sato, R.; Brown, M.; Hua, X.; Goldstein, J. *Cell* **1994**, *77*, 53−62.

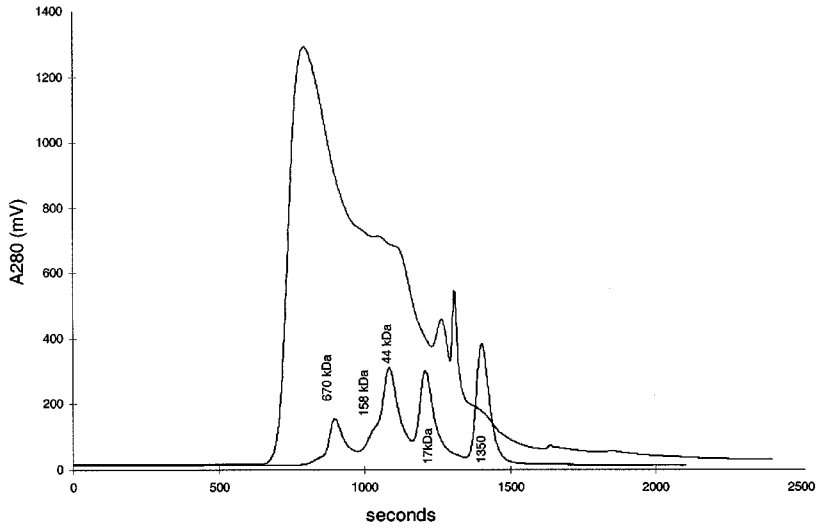


Figure 3. Gel filtration of the nuclear fraction of **5** treated HUVECs. The major peak associated with **5** had a molecular weight greater than 650 kDa as deduced from the markers of 670, 158, 44, 17 kDa and 1350 Da. The gel filtration was performed on a Beckman Gold HPLC chromatography system with a SynChropak GPC-300 column (250 × 4.6 mm). Mobile phase: 20 mM sodium phosphate (pH 7.4) with 135 mM NaCl. Flow rate: 100 μL/min.

with the concentration of **6** and is not shown in Figure 2). The radioactivity counts, however, saturated at about 500 nM of **6**. Only a very small amount of thienopyridine (<1%) was found in the cytoplasm, and the percentage was not dependent on the concentration of **6**.

These two experiments clearly showed that **6** did not bind to any macromolecules in the cytoplasm of HUVECs with a molecular weight >40 kDa. Since tritium has relative low energy, further exploration by the tritium-labeled **6** on the biomolecular target was not conclusive. When the higher energy emitting ^{125}I-labeled **5** was employed, the tris glycine native gel of the nuclear fraction revealed that **5** was associated with very large molecules in the cell nucleus. When treated with SDS detergent, **5** was released from the nuclear fraction, indicating a noncovalent binding of **5** with the macromolecule. Gel filtration of the nuclear lysates confirmed that the macromolecule had a molecular weight greater than 650 kDa (Figure 3).

In summary, the reaction of the diazonium salt **25** with Na^{125}I in DMSO represented a practical and efficient method for ^{125}I-labeling of our thienopyridine antagonist of cell adhesion molecule expression. Preliminary investigation using our ^{3}H- and ^{125}I-labeled compounds showed that our thienopyridine inhibitors noncovalently bound to a macromolecule or complex in the cell nucleus with a molecular weight greater than 650 kDa.

Experimental Section

General Spectroscopic and Experimental Data. The NMR spectra were obtained on Varian UP-300, Varian M-300, Bruker AMX-400, and Varian U-400 magnetic resonance spectrometer (300/400 MHz for ^{1}H and 75/100 MHz for ^{13}C) with deuteriochloroform as solvent and internal standard unless otherwise indicated. The chemical shifts are given in delta (δ) values and the coupling constants (J) in hertz (Hz). Infrared spectra were recorded on Nicolet 5SX and Nicolet Magna-IR 750 spectrometer. Mass spectra were acquired on a JEOL JMS-SX-102 spectrometer. Elemental analysis was performed by Robertson Microlit Laboratories, Inc., Madison, NJ. All manipulations were performed under nitrogen atmosphere unless otherwise mentioned. All solvents and reagents were purified when necessary using standard procedures. Flash column chromatography was performed on silica gel 60 (Merck, 230−400 mesh) using the indicated solvent. For routine aqueous workup, the reaction mixture was partitioned between brine and EtOAc, and the organic layer was washed with brine and dried over $MgSO_4$.

Methyl 4-[(4-Bromophenyl)thio]thieno[2,3-*c*]pyridine-2-carboxylate (15). To a solution of pyridine aldehyde **7**[6] (400 mg, 2.27 mmol) in DMF (10 mL) were added 4-bromobenzenethiol **8** (429 mg, 2.27 mmol) and powered K_2CO_3 (627 mg, 4.54 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and at rt for 3 h. After the reaction mixture was cooled to 0 °C again, methyl thioglycolate (241 mg, 2.27 mmol) and K_2CO_3 (314 mg, 2.27 mmol) were added. The mixture was stirred at rt for 0.5 h and at 70 °C for 2 h. After being cooled to rt, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water, dried, and concentrated. The residual material was purified by flash chromatography (15% EtOAc in hexane) to provide **15** (432 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (s, 3 H), 7.14 (d, J = 8.5 Hz, 2 H), 7.41 (d, J = 8.5 Hz, 2 H), 8.13 (s, 1 H), 8.50 (s, 1H), 9.13 (s, 1 H). MS (DCI/NH_3) m/z 380, 382 $(M + H)^+$. Anal. Calcd for $C_{15}H_{10}BrNO_2S_2$: C, 47.38; H, 2.65; N, 3.68. Found: C, 47.45; H, 2.60; N, 3.71.

4-[(4-Bromophenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (20). Ester **15** (381 mg, 1 mmol) was dissolved in 2 M methanolic ammonia (10 mL) and warmed to 45 °C in a sealed tube for 18 h. The precipitate was filtered, washed with methanol−diethyl ether (1:1), and dried under vacuum to give amide **20** (250 mg). The mother liquor was concentrated, and the residual material was purified by flash chromatography to provide additional **20** (97 mg). Combined yield: 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.20 (dt, J = 8.5, 2.0 Hz, 2H), 7.53 (dt, J = 8.6, 2.0 Hz, 2H), 7.87 (br s, 1H), 8.21 (s, 1H), 8.51 (br s, 1H), 8.54 (s, 1H), 9.36 (s, 1H); MS (DCI/NH_3) m/z 365 $(M + H)^+$. Anal. Calcd for $C_{14}H_9BrN_2OS_2$: C, 46.03; H, 2.48; N, 7.67. Found: C, 46.21; H, 2.57; N, 7.77.

4-(Phenylthio)thieno[2,3-*c*]pyridine-2-carboxamide (4). To a solution of **20** (20 mg) in a 1:1 mixture of ethanol and DMF (5 mL) were added triethylamine (20 mg) and 10% Pd/C (20 mg) under nitrogen. This suspension was purged with hydrogen and was stirred under hydrogen (1 atm) for 72 h. The solid material was filtered off and washed with ethanol. The filtrate was concentrated, and the residue was purified by flash chromatography (50% EtOAc in hexane) to provide **4** (13 mg, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.29−7.40 (m, 5H), 7.86 (br s, 1H), 8.25 (s, 1H), 8.46 (s, 1H), 8.52 (br s, 1H), 9.31 (s, 1H); MS (DCI/NH_3) m/z 287 $(M + H)^+$. Anal. Calcd for

$C_{14}H_{10}N_2OS_2$: C, 58.72; H, 3.52; N, 9.78. Found: C, 58.90; H, 3.50; N, 9.89.

4-(4-^{3}H-Phenylthio)thieno[2,3-*c*]pyridine-2-carboxamide (6). To a solution of **20** (5 mg, 0.014 mmol) in a 1:1 mixture of ethanol and DMF (2 mL) were added triethylamine (5 mg) and 10% Pd/C (5 mg) under nitrogen. This suspension was degassed under vacuum. Tritium (0.125 atm) was then introduced, and the suspension was stirred at room temperature under tritium for 72 h. The solid material was filtered off and washed with ethanol. The filtrate was concentrated and the residue was purified by HPLC (C-18, CH_3CN/H_2O containing 0.1% TFA) to provide **6** (3.2 mg, 80%). The radiochemical yield was 70%, and the specific activity of **6** was 29 Ci/mmol. **6** was identical by HPLC and TLC with protio compound **4** and had an IC_{50} of 25 nM against E-selectin and 20 nM against ICAM-1 in a cell ELISA assay.

Methyl 4-Phenoxythieno[2, 3-c]pyridine-2-carboxylate (16). Method A: To a solution of phenol (213 mg, 2.27 mmol) and pyridinealdehyde **7** (400 mg, 2.27 mmol) in DMF (8 mL) was added cesium carbonate (740 mg, 2.27 mmol) at rt. The reaction mixture was stirred at 70 °C for 1 h and was cooled to 0 °C. Methyl thioglycolate (241 mg, 2.27 mmol) was added. After the reaction mixture was stirred at rt for 1 h, more cesium carbonate (740 mg, 2.27 mmol) was added. The reaction mixture was stirred at rt for 1 h and at 70 °C for 0.5 h. After being cooled to rt, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water, dried, and concentrated. The residual material was purified by flash chromatography (20% EtOAc in hexane) to provide **16** (129 mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95 (s, 3 H), 7.07 (d, J = 7.7 Hz, 2 H), 7.17 (t, J = 7.3 Hz, 1 H), 7.37 (t, J = 8.0 Hz, 2 H), 8.10 (s, 1 H), 8.12 (s, 1 H), 8.92 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.8, 118.5, 124.2, 125.8, 129.9, 132.3, 136.5, 138.0, 139.2, 139.9, 149.0, 156.2, 162.2; IR (KBr) ν 1717 (s) cm^{-1}; MS (DCI) m/z 286 (M + H)$^+$. Anal. Calcd for $C_{15}H_{11}NO_3S$: C, 63.14; H, 3.89; N, 4.91. Found: C, 63.25; H, 3.87; N, 4.97.

Method B: To a solution of phenol (213 mg, 2.27 mmol) in THF (8 mL) was added potassium *tert*-butoxide (1 M solution in THF, 2.27 mL, 2.27 mmol) at 0 °C. The solution was kept at rt for 1 h, and was then cooled to 0 °C. Pyridinecarboxaldehyde **7** (400 mg, 2.27 mmol) was added as solid. The reaction mixture was stirred at rt for 0.5 h, and at 70 °C for 1 h. After the reaction mixture was cooled to 0 °C again, methyl thioglycolate (241 mg, 2.27 mmol) was added and stirred for 1 h. Cesium carbonate (740 mg, 2.27 mmol) was added. The reaction mixture was stirred at rt for 1 h and at 70 °C for 0.5 h. After being cooled to rt, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water, dried, and concentrated. The residual material was purified as above to give **16** (258 mg, 40%).

Method C: A solution of phenol (0.94 g, 10 mmol) in THF (10 mL) was treated with a solution of potassium *tert*-butoxide (1.0 M solution in THF, 10 mL, 10 mmol) at 0 °C. This solution was stirred at 25 °C for 1 h and was cooled to 0 °C. A solution of 3,5-dichloropyridine-4-carboxaldehyde (**7**) (0.88 g, 5 mmol) in THF (5 mL) was added, and the reaction mixture was heated at 70 °C for 3 h. After cooled to 0 °C, methylthioglycolate (0.53 g, 5 mmol) and Cs_2CO_3 (1.63 g, 5 mmol) were added. The mixture was heated at 70 °C for 0.5 h and was cooled to room temperature and filtered. The filtrate was diluted with ethyl acetate, washed sequentially with water and brine, dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by flash chromatography afforded **16** (1.06 g, 75%).

Methyl 4-(4-Nitrophenoxy)thieno[2, 3-c]pyridine-2-carboxylate (17). Compound **16** (50 mg, 0.175 mmol) was dissolved in concentrated H_2SO_4 (3 mL) at rt, and the solution was cooled to 0 °C. Potassium nitrate (18 mg, 0.175 mmol) was then added as solid in several portions. The solution was kept at 0 °C for 5 min and was then poured into ice. The mixture was basified with sodium bicarbonate to pH 8 and was extracted with ethyl acetate. The combined ethyl acetate solution was washed with water, dried over $MgSO_4$, and filtered. The filtrate was concentrated, and the residue was purified by flash chromatography (20% EtOAc in hexane) to give **17** (36.4 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (s, 3 H), 7.10 (d, J = 9.2 Hz, 2 H), 7.97 (s, 1 H), 8.26 (d, J = 9.2 Hz, 2 H), 8.36 (s, 1 H), 9.12 (s, 1 H); MS (DCI) m/z 331 (M + H)$^+$. Anal. Calcd for $C_{15}H_{10}N_2O_5S$: C, 54.54; H, 3.05; N, 8.48. Found: C, 54.65; H, 3.15; N, 8.40.

Methyl 4-(4-Aminophenoxy)thieno[2, 3-c]pyridine-2-carboxylate (18). To a solution of **17** (40 mg) in ethyl acetate (5 mL) was added 10% Pd/C (30 mg) under nitrogen. This suspension was purged with hydrogen and was stirred at rt under hydrogen (1 atm) for 42 h. The solid material was filtered off and washed with ethyl acetate. The filtrate was concentrated, and the residue was purified by flash chromatography (70% EtOAc in hexane) to provide **18** (27 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (s, 3 H), 6.71 (d, J = 8.6 Hz, 2 H), 6.94 (d, J = 8.7 Hz, 2 H), 8.00 (s, 1 H), 8.21 (s, 1 H), 8.87 (s, 1 H); MS (DCI) m/z 301 (M + H)$^+$. Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 60.06; H, 4.10; N, 9.21.

[4-(5-Chloro-4-formylpyridin-3-yloxy)phenyl]carbamic Acid *tert*-Butyl Ester (14). To a solution of pyridinecarboxaldehyde **7** (2.0 g, 11.4 mmol) and phenol **12** (2.38 g, 11.4 mmol), which was prepared according to literature method,[9] in DMF (30 mL) was added cesium carbonate (3.70 g, 11.4 mmol) at rt. The reaction mixture was stirred at rt for 1 h and at 70 °C for 0.5 h. After being cooled to rt, the reaction mixture was partitioned between ethyl acetate and brine. The organic layer was washed with water, dried, and concentrated. The residual material was purified by flash chromatography (15% EtOAc in hexane) to provide **14** (2.66 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (s, 9 H), 6.52 (s, 1 H), 7.03 (d, J = 9.2 Hz, 2 H), 7.43 (d, J = 9.2 Hz, 2 H), 8.17 (s, 1 H), 8.43 (s, 1 H), 10.54 (s, 1 H); MS (DCI) m/z 349 (M + H)$^+$. Anal. Calcd for $C_{17}H_{17}ClN_2O_4$: C, 58.54; H, 4.91; N, 8.03. Found: C, 58.55; H, 4.97; N, 8.10.

Methyl 4-(4-*tert*-Butoxycarbonylaminophenoxy)thieno-[2,3-*c*]pyridine-2-carboxylate (19). To a solution of **14** (2.64 g, 7.58 mmol) in THF (30 mL) was added methyl thioglycolate (804 mg, 7.58 mmol) at 0 °C. The reaction mixture was stirred at rt for 1 h after which cesium carbonate (2.47 g, 7.58 mmol) was added. The mixture was stirred at rt for 1 h and at 70 °C for 0.5 h. After cooled to rt, the reaction mixture was partitioned between ethyl acetate and brine. The organic layer was washed with water, dried, and concentrated. The residual material was purified by flash chromatography (20% EtOAc in hexane) to provide **19** (1.66 g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48 (s, 9 H), 3.91 (s, 3 H), 7.09 (d, J = 8.8 Hz, 2 H), 7.51 (d, J = 9.2 Hz, 2 H), 7.98 (s, 1 H), 8.06 (s, 1 H), 9.14 (s, 1 H), 9.40 (s, 1 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 28.06, 53.1, 79.0, 119.1, 119.7, 124.9, 131.6, 135.4, 136.2, 137.9, 138.6, 140.4, 148.9, 150.4, 152.7, 161.7; IR (KBr) ν 3371 (m), 1731 (s), 1701 (s) cm^{-1}; MS (DCI) m/z 401 (M + H)$^+$. Anal. Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99; H, 5.03; N, 7.00. Found: C, 60.08; H, 5.15; N, 7.10.

4-(4-Aminophenoxy)thieno[2, 3-*c*]pyridine-2-carboxamide (22). A solution of ester **19** (800 mg, 2 mmol) in 2 M methanolic ammonia (100 mL) was heated at 55 °C in a sealed tube for 24 h. Concentration of the reaction mixture gave essentially pure 4-[(4-*tert*-Butyloxycarbonylamino)phenoxy]-thieno[2,3-*c*]pyridine-2-carboxamide (**21**). This material was not purified further and was dissolved in trifluoroacetic acid (20 mL). The formed yellow solution was kept at room temperature for 1 h before TFA was distilled off under reduced pressure. The residual oil was triturated with a mixture of ethyl acetate and aqueous $NaHCO_3$ solution. The resultant solid was collected by filtration, washed successively with ethyl acetate, aqueous $NaHCO_3$ solution, water, methanol, and ethyl acetate, and dried to provide **22** (492 mg, 86% over 2 steps) as a yellow solid. mp > 250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.62 (br s, 2H), 6.65 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 7.86 (s, 1H), 8.30 (s, 1H), 8.44 (s, 1H), 9.00 (br s, 1H); IR (KBr) ν 3347 (m), 3136 (m), 1676 (s) cm^{-1}; MS (DCI/NH_3) m/e

(9) Vigroux, A.; Bergon, M.; Zedde, C.; *J. Med. Chem.* **1995**, *38*, 3983.

286 $(M + H)^+$. Anal. Calcd for $C_{14}H_{11}N_3O_2S \cdot 0.5\ CH_3OH$: C, 57.79; H, 3.85; N, 13.94. Found: C, 57.69; H, 3.95; N, 13.77.

4-[4-Iodophenoxy]thieno[2,3-*c*]pyridine-2-carboxamide (3) and 4-Phenoxythieno[2,3-c]pyridine-2-carboxamide (23). Compound **22** (30 mg, 0.105 mmol) was dissolved in 30% H_2SO_4 (2 mL), and the solution was cooled to 0 °C with an ice bath. A solution of sodium nitrite (8.0 mg, 0.116 mmol) in H_2O (80 μL) was then added, and the reaction mixture was stirred at 0 °C for 1 h. Sodium iodide (48 mg, 0.315 mmol) as a solution in H_2O (0.3 mL) was added. The reaction mixture was stirred at 0 °C for 1 h and was allowed to warm to rt for 3 h. Ethyl acetate (30 mL) was added, and the mixture was washed with saturate aqueous sodium bicarbonate solution, 5% aq sodium bisulfite solution, and water. The EtOAc solution was dried over $MgSO_4$, filtered, and concentrated. The residual material was separated by flash chromatography to give 34 mg of **3** (82%) and 2.8 mg of **23** (10%). Data for **3**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.94 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.86 (br s, 1H), 8.13 (s, 1H), 8.17 (s, 1H), 8.44 (br s, 1H), 9.16 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 87.5, 119.9, 120.2, 133.1, 137.5, 138.2, 138.8, 141.3, 146.5, 147.0, 156.7, 162.3; IR (KBr) ν 3351 (m), 3199 (m), 1663 (s) cm^{-1}; MS (DCI/NH_3) m/z 397 $(M + H)^+$. Anal. Calcd for $C_{14}H_9IN_2O_2S$: C, 42.44; H, 2.29; N, 7.07. Found: C, 42.35; H, 2.19; N, 6.99. Data for **23**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15 (d, J = 8.0 Hz, 2H), 7.20 (t, J = 7.8 Hz, 1H), 7.45 (t, J = 8.0 Hz, 2H), 7.85 (br s, 1H), 8.10 (s, 1H), 8.20 (s, 1H), 8.45 (br s, 1H), 9.15 (s, 1H); MS (DCI/NH_3) m/z 271 $(M + H)^+$. Anal. Calcd for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36. Found: C, 62.35; H, 3.79; N, 10.45.

4-(2-Carbamoylthieno[2,3-*c*]pyridin-4-yloxy)benzenediazonium Tetrafluoroborate (25). To a solution of **22** (30 mg, 0.105 mmol) in 30% H_2SO_4 (2 mL) was added sodium nitrite (10 mg, 0.145 mmol) in 100 μL of H_2O at 0 °C. The reaction mixture was stirred at the same temperature for 2 h, and the solid material was removed by filtration. $NaBF_4$ (32 mg, 2.1 mmol) in 0.25 mL of H_2O was added, and the solution was stirred at 0 °C for 30 min. The formed slightly yellow solid was collected by filtration and triturated with 100 μL of H_2O. After filtration, the solid was washed with ether (200 μL) and methanol (200 μL) and dried to give **25** (23 mg, 55%). This material was not characterized spectroscopically and was directly used for the following iodination reaction.

4-[4-Iodophenoxy]thieno[2,3-c]pyridine-2-carboxamide (3) from 25. To a solution of diazonium salt **25** (500 μg, 1.3 μmol) in DMSO (25 μL) was added a solution of sodium iodide (100 μg, 0.65 μmol) in H_2O (5 μL) at rt. The solution was stirred at rt for 2 h, and was then separated by HPLC (C-18, CH_3CN/H_2O containing 0.1% TFA) to give **3** (113 μg as determined by analytical HPLC, 42%).

(^{125}I)-(4-Iodophenoxy)thieno[2,3-*c*]pyridine-2-carboxamide (5). A solution of diazonium salt **25** (500 μg, 1.3 μmol) in DMSO (25 μL) was added to a 1.5 mL polypropylene vial that contained 2 mCi of Na^{125}I (Amersham Corporation, ~15 μg) in H_2O (20 μL). The solution was stirred at rt for 2 h and was then separated by HPLC (C-18, CH_3CN/H_2O containing 0.1% TFA) to give **5** (15 μg as determined by analytical HPLC using **3** as standard). The radiochemical yield was 35−40% in several trials. The obtain material had a same retention time with **3** on HPLC by coinjection and showed similar activity as compared with **3** on inhibition of cell adhesion molecule expression (IC_{50} = 5 nM for ICAM-1 versus 4 nM for **3**) in a cell ELISA assay.

Direct Binding Studies of 6 with HUVEC Nuclei. Primary HUVECs (total 9×10^7 cells) were washed with PBS, scraped, and pelleted. The cell pellet was resuspended in 1 mL of imidazole HCl buffer (pH7.4) that contained 1 mM EGTA and Boehringer Mannheim complete protease inhibitor cocktail (imidazole cocktail). After douncing, the cytosol was separated from nuclear pellet. The nuclear pellet was sonicated in 0.5 mL of imidazole cocktail and was then clarified by centrifigation. The nuclear lysate was incubated with **6** at 37 °C for 2 and 18 h separately. BioRad P30 spin columns were preequilibrated with imidazole cocktail by gravity and then centrifugated at 300 g for 2 min prior to loading 50 μL of the nuclear lysates. The radiocounts were evaluated before and after the BioRad P30 spin column fractionation. Void volume containing molecules with a molecular weight >40 kDa was collected after centrifigation at 600 g for 4 min.

Gel Filtration Experiment of the Nuclear Fraction of 5 Treated HUVECs. Primary HUVECs (total 9×10^7 cells) were incubated in EBM/2% FBS/human epidermal growth factor/bovine brain extract/gentamicin (Clonetics/BioWhitaker). The following day labeled compound **5** was added, and the plate was incubated at 37 °C for 18 h. The plate was washed once with D-PBS (Gibco/BRL), and the cells were scraped and pelleted. The cell pellet was resuspended in 1 mL of imidazole HCl buffer (pH7.4) that contained 1 mM EGTA and Boehringer Mannheim complete protease inhibitor cocktail (imidazole cocktail). After douncing, the cytosol was separated from the nuclear pellet. The nuclear pellet was sonicated in 0.5 mL imidazole cocktail and was then subjected to a gel filtration that was performed on a Beckman Gold HPLC chromatography system with a SynChropak GPC-300 column (250 × 4.6 mm). Mobile phase: 20 mM sodium phosphate (pH 7.4) with 135 mM NaCl. Flow rate: 100 μL/min.

General Cell ELISA Assay for Compounds 1−6. Primary HUVECs were plated in 96 well plates at 5×10^4 cells/mL in EBM/2% FBS/human epidermal growth factor/bovine brain extract/gentamicin (Clonetics/BioWhitaker). The following day test compounds were added and the plates incubated 24 h at 37 °C. TNFα (Gibco/BRL) then was added to a final concentration of 5 ng/mL, and the plates were incubated an additional 6 h at 37 °C. The plates were washed once with D-PBS (Gibco/BRL), and primary antibody (Becton Dickinson) was added in D-PBS/2% BSA (Sigma)/0.01% NaN_3. Antibodies used were mouse monoclonal anti-ELAM-1, anti-ICAM-1, and anti-VCAM-1. The plates were stored overnight at 4 °C and then washed three times with D-PBS. Secondary antibody, HRP-conjugated donkey anti-mouse IgG (Jackson Labs) in D-PBS/2%BSA, was added, and the plates were incubated 1−2 h at room temperature and then washed three times with D-PBS. OPD solution (Abbott) was added to the wells, the plates were developed for 15−20 min and neutralized with 1 N sulfuric acid, and the absorbance was read at 490 nm.

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