Discovery of Inhibitors of Cell Adhesion Molecule Expression in Human Endothelial Cells. 1. Selective Inhibition of ICAM-1 and E-Selectin Expression

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A critical early event in the inflammatory cascade is the induced expression of cell adhesion molecules on the lumenal surface of vascular endothelial cells. These adhesion molecules include E-selectin, ICAM-1, and VCAM-1, which serve to recruit circulating leukocytes to the site of the inflammation. These adhesive interactions allow the leukocytes to firmly adhere to and cross the vascular endothelium and migrate to the site of tissue injury. Pharmaceutical agents which would prevent the induced expression of one or more of the cell adhesion molecules on the endothelium might be expected to provide a novel mechanism to attenuate the inflammatory responses associated with chronic inflammatory diseases. A thieno[2,3-*d*]pyrimidine, A-155918, was identified from a whole-cell high-throughput assay for compounds which inhibited the tumor necrosis factor-α (TNFα)-induced expression of E-selectin, ICAM-1, or VCAM-1 on human vascular endothelial cells. Traditional medicinal chemistry methods were applied to this low-micromolar inhibitor, resulting in the 2,4-disubstituted thieno[2,3-*c*]pyridine A-205804, a potent and selective lead inhibitor of E-selectin and ICAM-1 expression (IC_{50} = 20 and 25 nM, respectively). The relative position of the nitrogen atom in the thienopyridine isomer was shown to be critical for activity, as was a small amide 2-substituent.

Introduction

Inflammation results from a complex series of events, including vasodilation, increased vascular permeability, and exudation of fluid and plasma protein. Inflammatory mediators including cytokines, complement fragments, and lipid mediators are generated at the site of the initial lesion and serve to recruit inflammatory cells. A critical early event in the inflammatory cascade is the induced expression of cell adhesion molecules on the lumenal surface of vascular endothelial cells.[1−3] These adhesion molecules, which include E-selectin, ICAM-1, and VCAM-1, serve to recruit circulating leukocytes to the site of the inflammation. E-selectin binding to its counter-receptor, sialylated Lewis-X antigens on leukocytes, causes the cells to roll on the vascular endothelium.[4] The rolling leukocytes then become firmly attached to the vessel wall through interactions of the leukocyte integrins $\alpha_L\beta_2$ (lymphocyte function-associated antigen 1, LFA-1, CD11a/CD18), $\alpha_M\beta_2$ (Mac-1, CD11b/CD18, CR3), and $\alpha_4\beta_7$ (very late after-activation antigen 4, VLA-4) with their corresponding counter-receptors on the endothelial cells. LFA-1 and Mac-1 interact with the immunoglobulin superfamily member intercellular adhesion molecule 1 (ICAM-1) on activated endothelium, whereas VLA-4 interacts with vascular cell adhesion molecule 1 (VCAM-1).[5] The arrested leukocytes then transmigrate the vascular wall and move toward the lesion along a chemotactic gradient.

The elevated expression of these adhesion molecules is due primarily to upregulation of the genes encoding them, resulting in the de novo synthesis of these proteins. As has been recently described,[6−8] diverse signals act on endothelial cells to activate members of the nuclear factor of kappa B (NF-κB) transcription factor family. NF-κB family members are homo- or heterodimeric proteins which are normally present in the cytoplasm as an inactive complex with members of a family of inhibitor proteins, IκB (inhibitor of NF-κB). A signal transduction kinase cascade results in the phosphorylation of IκB in the complex of NF-κB/IκB, which in turn allows for polyubiquination of IκB. The ubiquinated IκB is then degraded by the 26S proteosome, thereby releasing the active NF-κB and allowing it to translocate to the nucleus and initiate transcription of its target genes.

Adhesion molecules have been implicated in a variety of pathological conditions, including rheumatoid arthritis, Crohn's disease, radiation pneumonitis,[9] and inflammatory bowel disease. An ICAM-1 antisense oligonucleotide from Isis Pharmaceuticals (ISIS 2302) has been tested in human clinical trials for Crohn's disease and showed beneficial effects in early studies.[10,11] In a pivotal efficacy study, ISIS 2302 provided a statistically significant remission rate of 38% for Crohn's disease patients who received the higher exposure levels. Further studies at higher doses of ISIS 2302 are reportedly underway, as are studies on a topical formulation for

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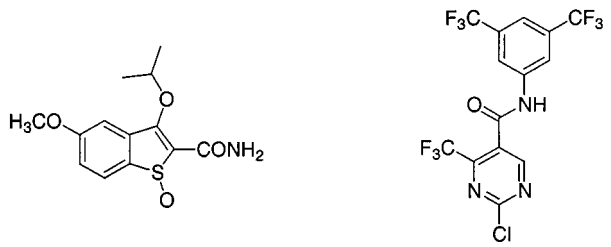
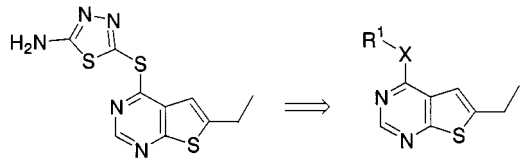


1 (PD 144795) **2** (SP100030)

Figure 1. Structures of known small-molecule cell adhesion molecule expression inhibitors.



3 **4**

Figure 2. Structures of A-155918 (**3**) and proposed 4-substituted analogues.

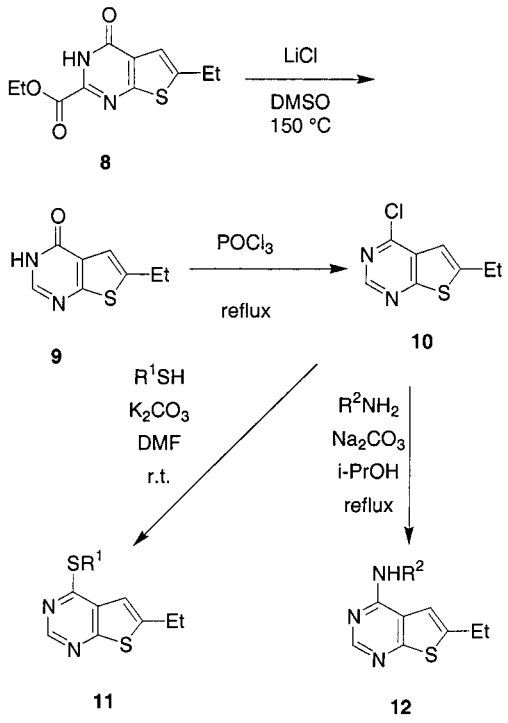
treatment of psoriasis.[12] Studies with a monoclonal antibody to ICAM-1 have indicated that repeated treatments induced an immunogenic response in human patients, precluding the therapeutic use of such an antibody.[13] Previously, workers at Parke-Davis reported on a series of small molecules, such as benzothiophene **1** (Figure 1), which inhibited the expression of adhesion molecules on human endothelial cells.[14,15] A series of dual inhibitors of NF-κB and activator protein 1 (AP-1) transcription factor activation have been disclosed by workers at Signal Pharmaceuticals, exemplified by 2-chloropyrimidine arylamide **2**.[16]

As mentioned above, many of the signal transduction pathways have been elucidated in greater detail very recently, but much of this information was not known to us at the outset of our efforts described in this paper. Therefore, we initiated a program to discover compounds which inhibited the TNFα-induced expression of E-selectin, ICAM-1, or VCAM-1 on human vascular endothelial cells in a whole-cell assay. A set of high-throughput screening assays were developed to identify potential hit compounds with the desired activity, using a cellular toxicity assay to eliminate false positives. The E-selectin, ICAM-1, and VCAM-1 promoter regions were individually cloned and linked to luciferase reporter genes. Stable HUVEC cell lines were constructed expressing these reporter gene constructs, and the Abbott compound library was screened for expression inhibitors using these cell lines. By this process, we identified A-155918 (**3**) (Figure 2) as a low-micromolar inhibitor of expression of all three adhesion molecules of interest. In this paper, we describe the medicinal chemistry efforts on this hit and its evolution into a potent and selective inhibitor of ICAM-1 and E-selectin expression.

Synthetic Chemistry

Our initial chemistry strategy was to briefly investigate the 4-substituent of A-155918 thieno[2,3-*d*]pyrimidine analogues, in an effort to improve the in vitro potencies. The synthesis of these thieno[2,3-*d*]pyrimidines is illustrated in Scheme 1. The known thieno[2,3-*d*]pyrimidin-4-one **8**[17] was decarbethoxylated using

Scheme 1



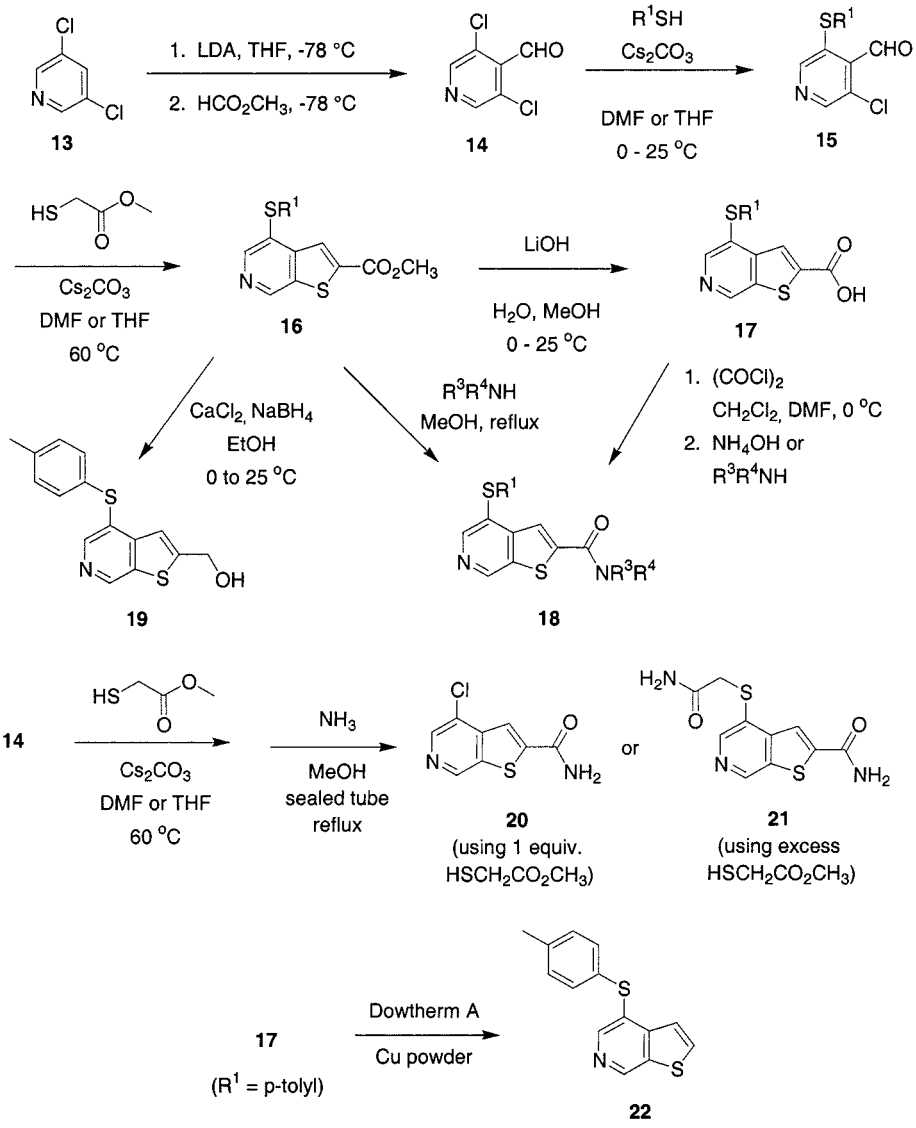
8 **9** **10** **11** **12**

standard conditions,[18] and the resulting pyrimidinone **9** was converted to the 4-chlorothieno[2,3-*d*]pyrimidine **10**. The chloro derivative **10** was substituted with thiols to provide 4-thioethers **11**, or alternatively substituted with primary amines to provide 4-aminothieno[2,3-*d*]-pyrimidines **12**.

As will be discussed below, our efforts turned toward alternative heterocyclic core structures related to A-155918. The syntheses of thieno[2,3-*c*]pyridines were accomplished as shown in Scheme 2. In a method analogous to that of Ito,[19] deprotonation of 3,5-dichloropyridine (**13**) with LDA at −78 °C, followed by an inverse quench into a solution of methyl formate in THF, provided the known dichloropyridinecarboxaldehyde **14**. Substitution of one of the two chlorides with 1 equiv of aryl-, alkyl-, or heterocyclic-thiols under mild basic conditions proceeded smoothly to yield sulfides **15**. These compounds were generally not purified but rather were reacted with methyl thioglycollate to form thieno-[2,3-*c*]pyridine esters **16** by a displacement followed by cyclization reaction. Standard methods were utilized to produce the corresponding acids **17** and amides **18**. Esters **16** could be directly converted to amides **18** by simply heating in a methanolic solution of the desired amine. The alcohol **19** was produced by calcium borohydride reduction of ester **16**. The 4-chloro derivative **20** was produced by condensation and cyclization of **14** directly with methyl thioglycollate. We also were able to use an excess of methyl thioglycollate in this reaction, which leads to a double substitution of **14**. Cyclization of the symmetrical intermediate, followed by amide formation, led to acetamide derivative **21**.

A number of other alternative ring structures were pursued as well. Scheme 3 shows the preparation of thieno[2,3-*b*]pyridine-2-carboxamide **28**. The 4-chloro group of **23**[20] was substituted with 4-methylthiophenol using potassium carbonate as basic catalyst, which produced diaryl sulfide **24**. The ester group of **24** was

Scheme 2



removed by a two-step process, proceeding through acid **25** which was thermally decarboxylated to give the monosubstituted thieno[2,3-*b*]pyridine **26**. A carboxyl group was then introduced into the 2-position by deprotonation using *n*-butyllithium in tetrahydrofuran at low temperature, followed by quench with solid carbon dioxide. The derived acid **27** was then converted to primary amide **28**, via its acid chloride.

The thieno[3,2-*c*]pyridine isomer was synthesized from a known thieno[2,3-*b*]pyridin-4-one **29**,[21] as shown in Scheme 4. The cyano pyridone **29** was converted to 4-chlorothieno[3,2-*c*]pyridine **30** with phosphoryl chloride in 73% yield. Chloride **30** was substituted with 4-methylthiophenol, using potassium *tert*-butoxide as base, to provide sulfide **31** in 97% yield. The final partial hydrolysis was carried out using polyphosphoric acid at 110 °C for 3 h to return amide **32** in 75% yield.

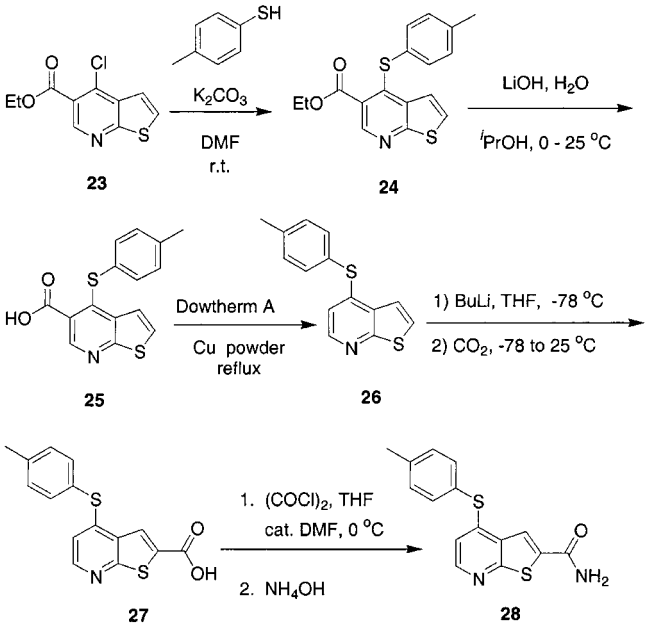
Benzo[*b*]thiophene derivatives were produced in a method analogous to that reported by Bridges et al.[22] (Scheme 5). The nitro group of 5-chloro-2-nitrobenzaldehyde (**33**) was substituted with *p*-thiocresol under basic catalysis to produce sulfide **34**. The chlorobenzaldehyde **34** was condensed with methyl thioglycollate, using sodium methoxide as base, which led to benzothiophene **35** in 32% yield. Hydrolysis of the methyl ester provided acid **36**, which was coupled to ammonia using carbodiimide conditions to produce primary amide **37**.

A series of 2-substituents were prepared from thieno[2,3-*c*]pyridine ester **17** utilizing straightforward methodologies, which are summarized in Scheme 6. Carboxylic acid **17** (R^1 = *p*-tolyl) was converted to the corresponding *N*-methyl-*N*-methoxylamide **38**. Amide **38** served as intermediate in the synthesis of aldehyde **39**, as well as for the synthesis of ketones **41**, utilizing standard methodologies. Oximes **40** and **42** were formed from **39** and **41** and the corresponding hydroxylamine derivatives. Methyl ketone **51** (**41**, $R^6 = CH_3$) was subjected to Wolff−Kishner reduction to produce the 2-ethyl derivative **43**. Thioamide **44** was formed from amide **7** using Lawesson's reagent in toluene, as shown in Scheme 7. The potential sulfoxide metabolite **45** was prepared from sulfide ester **55** (**16**, R^1 = *p*-tolyl), using *m*-CPBA as the oxidant.

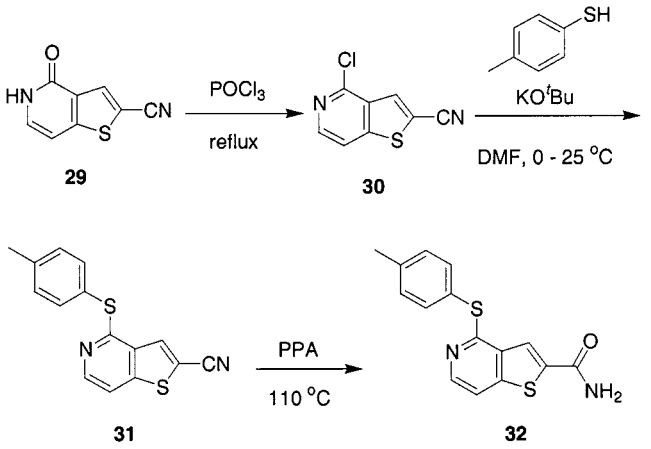
Results and Discussion

Our strategy for finding a novel inhibitor of the induced expression of E-selectin, ICAM-1, or VCAM-1

Scheme 3



Scheme 4



was to use a set of high-throughput transcriptional screening assays in human umbilical vein endothelial cells to identify potential hits. The hits were simultaneously evaluated for toxicity in the same cell line, to remove false positives. By this process, we identified compound **3** as a single-digit micromolar inhibitor of the

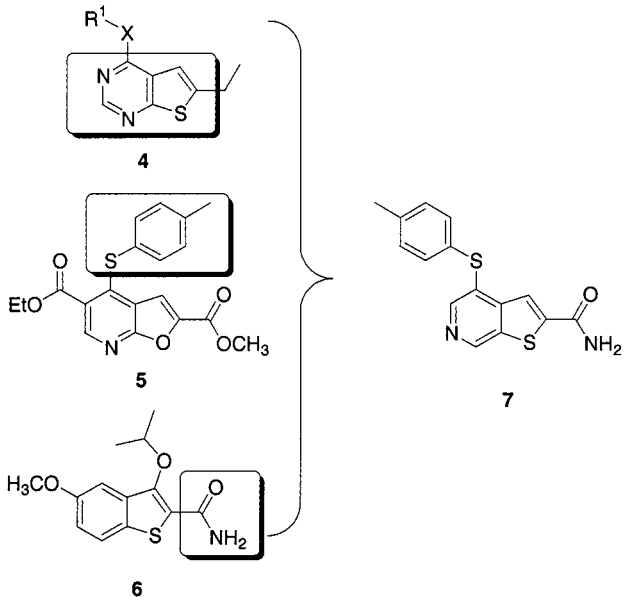
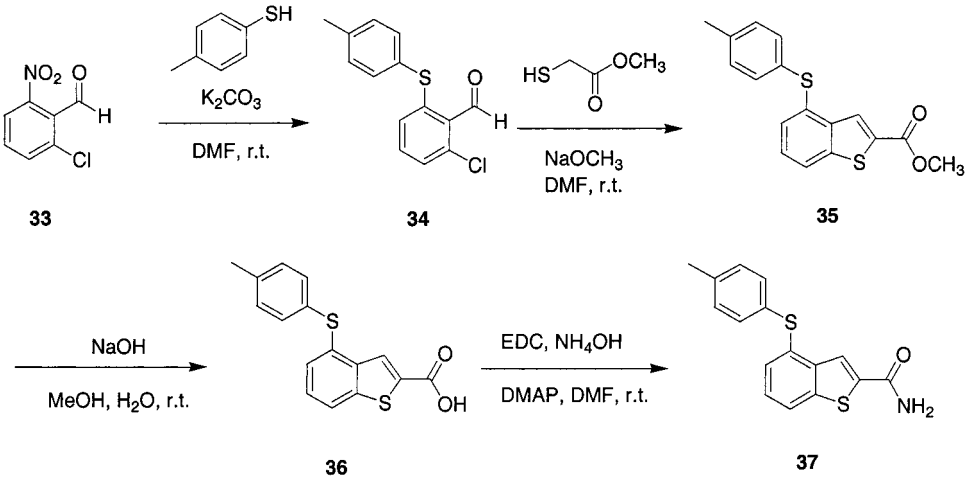


Figure 3. Derivation of thieno[2,3-*c*]pyridines.

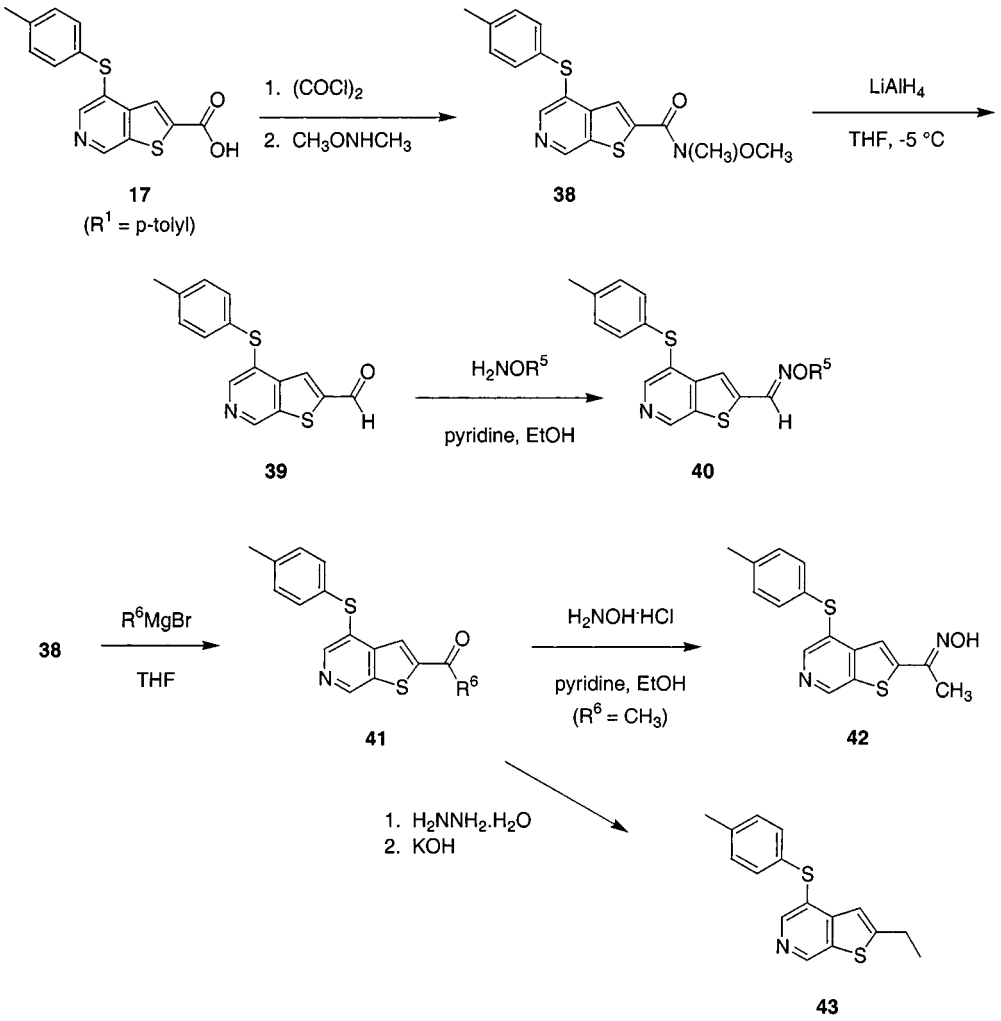
expression of all three adhesion molecules of interest, with >10-fold higher value for toxicity in HUVECs. The transcriptional screen was replaced by a whole-cell ELISA assay for each of the three cell adhesion molecules for the subsequent routine structure−activity studies (SARs), which provided a direct measure of protein expression on the surface of the endothelial cells. A study of the role of the (5-amino-1,3,4-thiadiazol-1-yl)thio group was undertaken initially, and the potency results are summarized in Table 1. The synthetic intermediates **9** and **10** were devoid of activity. Heterocyclic sulfides such as 1,3,4-thiadiazole **46** and pyridine **49**, as well as *p*-tolyl sulfide **48**, had similar activity to the original hit **3**. However, benzyl sulfide **47** had little activity beyond cellular toxicity. Significantly, replacing the sulfide linkage with an amino group (**50** and **51**) abolished the weak activity seen for **3**. None of these replacements led to enhanced activity over our starting point.

We also tested individual compounds in the Abbott compound collection with structural similarity to **3**, derived by substructure searches of the database. Compound **5** (Figure 3) was one such compound, with a different core (furo[3,2-*b*]pyridine). We also were aware

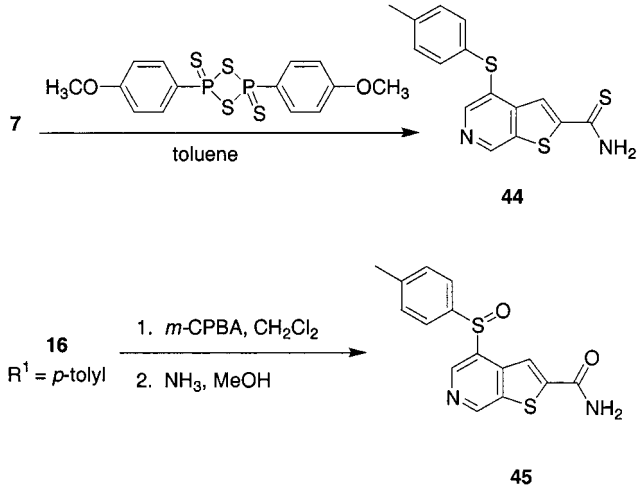
Scheme 5



Scheme 6



Scheme 7

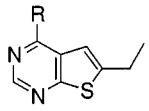


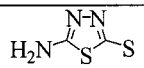
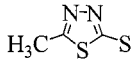
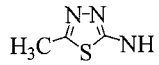
of the structure of PD144795 (**1**; Figure 1), and despite uncertainty regarding its biological target we felt there might be some relationship to our hit series. Various structural elements of compounds **1**, **3**, and **5** were incorporated into differing heterocyclic core structures in an effort to improve the potency of the inhibitors, and through this intuitive approach the thieno[2,3-*c*]pyridine **7** was synthesized. The thieno[2,3-*c*]pyridine isomer was selected because we anticipated better chemical stability with the arylthio group in the unactivated 3-position of the pyridine ring. As shown in Table 2, this compound provided a remarkable boost in potencies, down to an IC_{50} of 20−25 nM for inhibition of E-selectin and ICAM-1 expression. Also notable was the selectivity of **7** for E-selectin and ICAM-1 inhibition over that of VCAM-1. The IC_{50} for cellular toxicity was determined to be 3 orders of magnitude higher than that for adhesion molecule expression inhibition.

We next explored the positioning of the nitrogen in the core of **7**. Three thienopyridine isomers, as well as the benzothiophene analogue, were prepared and tested in the whole-cell ELISA assays, and the data are shown in Table 2. Somewhat unexpectedly, thieno[3,2-*c*]pyridine **32**, thieno[2,3-*d*]pyridine **28**, and benzothiophene **37** were all inactive in the ELISA assays. A comparison with the unoxidized, parent structure of PD144795 (**6**) showed that **6** possessed roughly equivalent sub-micromolar potencies for inhibition of expression of all three adhesion molecules in our assays.

With the finding that the thieno[2,3-*c*]pyridine isomer was critical for activity, we embarked on a multipronged SAR study to further explore this class of molecules to further improve in vitro activity. A series of 2-substituted analogues were screened, and the data are shown in Table 3. The unsubstituted analogue **22** was not active at concentrations below its toxic concentration,

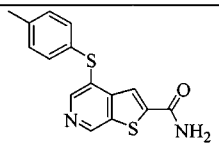
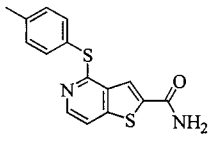
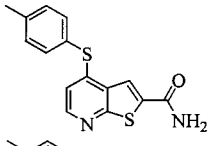
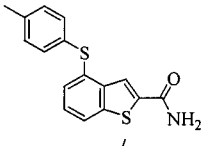
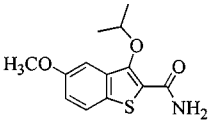
Table 1. Comparison of in Vitro Potencies and Cellular Toxicities for 4-Substituted Thieno[2,3-*d*]pyrimidines



Compound	R	CAM ELISA IC_{50} ± sem,[a] μM			Toxicity IC_{50} ± sem,[a] μM
		E-Selectin	ICAM-1	VCAM-1	(HUVEC, MTS)
3 A-155918.0		3.0±0.5	6.3±3.2	2.5±0.5	95
9	OH	>100	>100	>100	>100
10	Cl	27	>100	18	>100
46		4	5	3.5	51
47	$PhCH_2S$	95	99	17	>100
48	(*p*-CH_3)PhS	22±19	6.6±3.4	20±3.3	85±15
49		2.2±0.8	3.6±1.2	39±4.9	>100
50		42±9	92±15	19±2	48±3.5
51	BnNH	49	56	59	72

[a] Values for triplicate assay; SEM for multiple triplicate determinations.

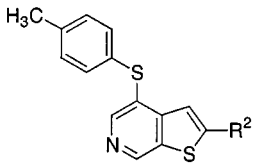
Table 2. Comparison of in Vitro Potencies and Cellular Toxicities for 4-Substituted Thienopyridine- and Benzothiophene-2-carboxamides

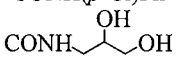
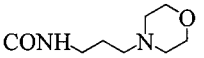
Compound	Structure	CAM ELISA IC_{50} ± sem,[a] μM			Toxicity IC_{50} ± sem,[a] μM
		E-Selectin	ICAM-1	VCAM-1	(HUVEC, MTS)
7		0.02±0.01	0.025±0.01	>1	152
32		>4	>4	>4	95
28		16±6.7	39±6	15±2.3	44±12
37		17	19	10	20
6		0.050	0.14±0.005	0.175±0.1	16±9

[a] Values for triplicate assay; SEM for multiple triplicate determinations.

and the same was true of the 2-ethyl analogue **43**. The fact that our original hit possessed an ethyl group in this relative position gave further credence to the notion that the thieno[2,3-*c*]pyridines were biologically distinct from the thieno[3,2-*d*]pyrimidines. A functional group series of alcohol **19**, aldehyde **39**, ketones **52** and **53**, acid **54**, and ester **55** were all less active than the primary amide **7**. With the exception of acid **54**, one would expect similar cellular penetration properties for the compounds − a confounding issue for interpreting the cellular data. Other carboxamides were tolerated, so long as the substituent on nitrogen remained small. Hydrazide **56** was nearly equivalent in potency to **7**, but the somewhat larger methoxylamides **38** and **57** and glycol amide **59** were somewhat less potent. Anilide **58** and 3-(4-morpholino)propylamide **60** apparently were too large, with complete loss of activity. The very polar acylguanidine **61** also was devoid of potency. Oximes **62**−**66** bearing various additional functionalities were examined, and only the methyl ketone oxime **42** pos-

Table 3. Comparison of in Vitro Potencies and Cellular Toxicities for 2-Substituted Thieno[2,3-*c*]pyridines



Compound	R^2	CAM ELISA IC_{50} ± sem,[a] μM			Toxicity IC_{50} ± sem,[a] μM
		E-Selectin	ICAM-1	VCAM-1	(HUVEC, MTS)
22	H	1.7±0.5	1.8±0.14	2.1±0.28	5.8±2.3
43	CH_2CH_3	1.8±0.2	2.3±0	2.3±0.3	3.2±0.2
19	CH_2OH	0.20±0.06	0.54±0.01	>4	68±2.2
39	CHO	0.69±0.03	1.9±0.3	>4	13±1.3
52	$COCH_3$	0.14±0.01	0.25±0.02	>4	>100
53	COPh	0.50±0.04	1.4±0.7	2.7±0.2	15
54	CO_2H	3.2±0.05	3.7±0.24	15±2.8	68±8
55	CO_2CH_3	8.4±5.6	11±7.4	20	67±7
56	$CONHNH_2$	0.032	0.032	46±7	82±0.5
38	$CONCH_3(OCH_3)$	0.06±0.019	0.099±0.01	>4	44±0.3
57	$CONH(OCH_3)$	0.088±0.02	0.17±0.07	21±18	52±6.5
44	$CSNH_2$	0.15±0.04	0.16±0.06	19±17	51±3.2
58	CONH(*p*-Cl)Ph	>100	>100	>100	>100
59		0.62±0.09	0.60±0.04	>4	74±4
60		>4	>4	>4	68±4
61	$CONHC(NH)NH_2$	>4	>4	>4	47±1.5
62	CHNOH	1.5±0.9	1.9±0.2	>4	63±3
63	CHNOPh	>4	>4	3.5	>100
64	$CHNOCH_2Ph$	>4	>4	>4	>100
65	$CHNOCH_2CO_2H$	>4	>4	>4	84
66	$CHNOCH_2CONH_2$	0.2±0.1	1.4±0.7	>1	20±2.3
42	$C(CH_3)NOH$	0.055±0.005	0.085±0.005	>1	9.5±1

[a] Values for triplicate assay; SEM for multiple triplicate determinations.

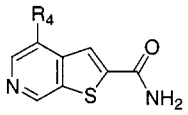
sessed potency in the same range as amide **7**. From these and related analogues it became clear that simple amides were the preferred 2-position substituents.

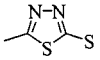
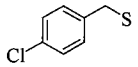
The next area of investigation was the 4-position sulfide group. A series of aryl, heterocyclic, and aliphatic sulfides were synthesized, maintaining the primary amide at the 2-position. A listing of these compounds and their in vitro data is presented in Table 4. Compound **20**, with only a 4-chloro group, possessed modest potency against E-selectin and ICAM-1 expression. The *S*-phenyl analogue **67** was equipotent with the lead, but placing substituents in the *ortho*- or *meta*-position of the *S*-phenyl produced somewhat less potent inhibitors (e.g. **68** and **69**). Analogue **71**, bearing two *meta*-methyl groups, was inactive in the ELISA assays, illustrating the rather restrictive nature of the binding pocket for this region of the inhibitors. However, there appeared to be space to accommodate *para*-substituents, with trifluoromethyl (**73**) and chloro (**74**) having similar activity to lead **7**. *para*-Bromophenyl analogue **76** possessed significantly improved activity relative to **7**. The *para*-methoxyphenyl analogue **77** had reduced potency, and the biphenyl analogue **78** was essentially inactive, again leading to the supposition that this region of the binding pocket was also bounded. Inhibitor **70**, which possessed a 3,4-dimethylphenylthio group, has slightly improved activity, despite having a *meta*-methyl substituent. By replacing the *S*-phenyl with selected *S*-heteroaryl groups (**79**−**81**), a loss of potency was observed. The cyclohexyl version **82** was also much less potent, as was the benzyl derivative **83** and acetamide **21**. Interestingly, compound **45**, the sulfoxide derivative of **7**, was completely devoid of adhesion molecule expression inhibitory activity. It was clear that *S*-aryl groups, with *para*-substituents, were the preferred 4-position groups.

We undertook a more detailed biological evaluation of compound **7**. The ability of **7** to inhibit interleukin-1 β (IL-1β)- or phorbol myristate acetate (PMA)-induced ICAM-1 and E-selectin expression on HUVEC was determined, using the same protocol as described for TNFα-induced expression. Compound **7** inhibited the IL-1β-induced expression of E-selectin (IC_{50} = 20 nM) and ICAM-1 (IC_{50} = 10 nM) with essentially the same level of potency as that for TNFα-induced expression. For reasons which are unclear, the ability of **7** to inhibit PMA-induced E-selectin expression was significantly reduced (IC_{50} > 1000 nM), but **7** was a potent inhibitor of ICAM-1 expression (IC_{50} = 40 nM) using this protein-kinase C-activating agent. These findings are consistent with a mechanism of action of **7** which lies downstream of cell surface receptors and is not a TNFα-specific pathway.[1] Similar results were obtained using related compounds in this series, and these data will be reported in due course.

We examined the ability of **7** to inhibit expression of ICAM-2, a constitutively expressed adhesion molecule gene product. Inhibition of ICAM-2 expression on HU-

Table 4. Comparison of in Vitro Potencies and Cellular Toxicities for 4-Substituted Thieno[2,3-*c*]pyridine-2-carboxamides



Compound	R^4	CAM ELISA IC_{50} ± sem,[a] μM			Toxicity IC_{50} ± sem,[a] μM
		E-Selectin	ICAM-1	VCAM-1	(HUVEC, MTS)
20	Cl	0.25±0.09	0.36±0.04	1.3±0.4	62±38
67	PhS	0.020±0.0	0.025±0.005	>4	>100
68	(2-CH_3)PhS	0.055±0.04	0.040±0.02	>1	68±2
69	(3-CH_3)PhS	0.045±0.02	0.14±0.03	>4	96±2
70	3,4-di(CH_3)PhS	0.013±0.01	0.008±0.003	>1	>100
71	3,5-di(CH_3)PhS	>4	>4	>4	23±5
72	2,4-di(CH_3)PhS	0.043±0.017	0.054±0.017	0.77	>10
73	(4-CF_3)PhS	0.044±0.017	0.039±0.018	>0.1	95±1
74	(4-Cl)PhS	0.012±0.002	0.048±0.019	>4	>100
75	3,4-$(Cl)_2$PhS	0.10±0.001	0.037±0.005	>1	nd[b]
76	(4-Br)PhS	0.005±0.001	0.01±0.004	>4	>100
77	(4-CH_3O)PhS	0.13±0.005	0.14±0.005	>0.2	nd
78	(4-Ph)PhS	>1	>1	>4	>100
79		0.66±0.01	2.3±0.5	65±12	>100
80		15±0.5	12±3.5	10±0	>100
81		0.60±0.005	0.57±0.005	>1	nd
82		>1	0.84±0.025	>1	nd
83		0.34±0.12	0.69±0.09	>1	nd
21	H_2NCOCH_2S	>4	>4	>4	>100
45	(4-CH_3)PhS=O	>4	>4	>4	nd

[a] Values for triplicate assay; SEM for multiple triplicate determinations. [b] Value not determined.

VEC (by ELISA assay) was only seen at high doses (>10 μM), indicating that **7** did not generally inhibit protein synthesis or gene transcription. Compound **7** did not inhibit human T-cell proliferation in either an antigen-specific (tetanus toxoid) or super-antigen (*Staphylococcus* enterotoxin-A) stimulated proliferation assays at concentrations below 5−20 μM. Finally, compound **7** was effective in reducing in vitro cell−cell adhesion under flow conditions. A monolayer of HUVEC pretreated with 0.1 μM of **7** exhibited markedly reduced adhesion of flowing human leukocytic cells (HL60) by 60% as monitored by video microscopy (Figure 4).[23−25]

We evaluated the pharmacokinetic behavior of compound **7** in rats, as a prelude to further in vivo experiments. The poor aqueous solubility of 0.4 mg/mL (pH 7.4, 0.05 M NaH_2PO_4 buffer) precluded intravenous dosing; hence the compound was dosed orally at 5 mg/kg. Figure 5 shows the drug concentration of **7** as a function of time. Two major metabolites were detected in the HPLC analysis of the plasma samples, and their structures were determined by mass spectral analysis and subsequent comparison to synthetic standards. Not unexpectedly, the two major routes of metabolism were amide hydrolysis to acid **54**, as well as sulfide oxidation to sulfoxide **45**. The concentrations of these two metabolites as a function of time are also included in the graph in Figure 5. Sulfoxide **45** is rapidly produced and cleared over the 8-h time course of the experiment. Acid

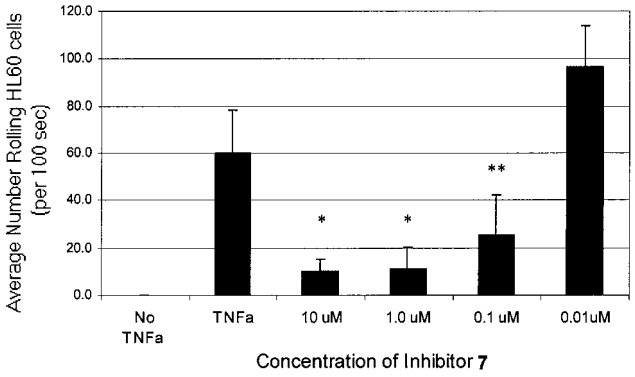
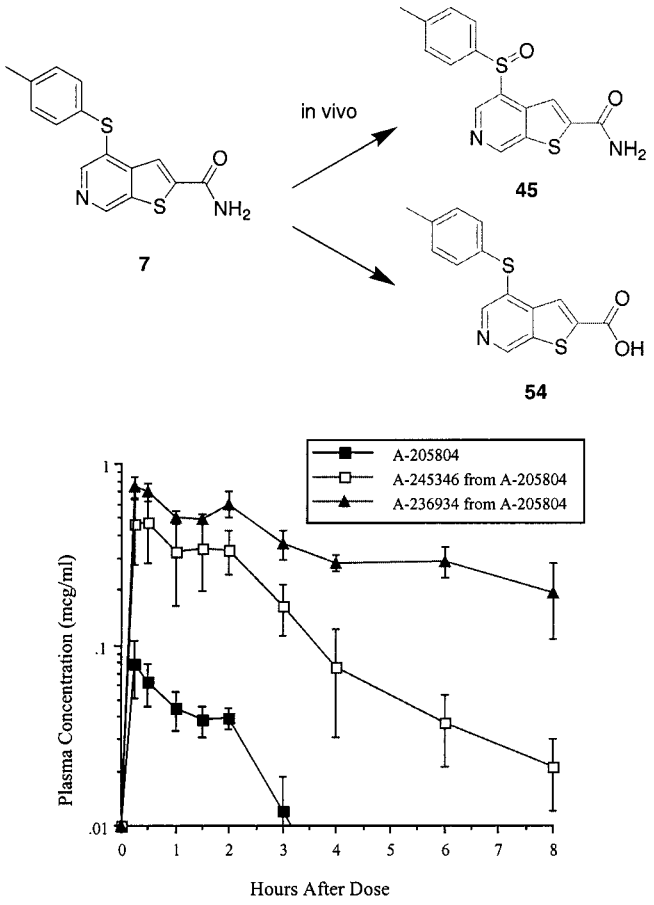


Figure 4. Effect of inhibitor **7** on HL60 cell rolling on a TNFα-activated monolayer of HUVECs.

54 is also rapidly produced but has a longer half-life in the plasma. The potential metabolite resulting from oxidation and hydrolysis may have been produced, but it was not detected in our experiments. Parent compound **7** therefore achieved low peak concentration (80 ng/mL) and area under the curve (0.13 μg·h/mL). Given that the metabolites were both inactive as adhesion molecule expression inhibitors, the poor pharmacokinetic performance of **7** limited our ability to use it for proof-of-principle studies in animal models of inflammatory diseases. Such studies awaited the discovery of more metabolically stable analogues.



Compound	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}8h}$ (μg•hr/ml)	P/M
A-205804 (**7**)	0.078 *(0.028)*	0.25 *(0.0)*	1.0	0.131 *(0.030)*	--
A-245346 (**45**)	0.476 *(0.195)*	0.42 *(0.08*		1.245 *(0.464)*	0.11 *(0.02)*
A-236934 (**54**)	0.751 *(0.102)*	0.33 *(0.08)*	nd	2.947 *(0.161)*	0.05 *(0.01)*

P/M - parent to metabolite AUC ratio; mean (±*SEM*, n=3)

Figure 5. Mean plasma concentrations of parent drug and potential metabolites following a 5 mg/kg oral dose of **7** in rat.

Conclusions

We have identified a series of thieno[2,3-*c*]pyridine amides, exemplified by compound **7**, which have the ability to penetrate cells and selectively inhibit the surface expression of the cell adhesion molecules ICAM-1 and E-selectin in human endothelial cells. Using a transcriptional inhibition high-throughput cellular assay, the micromolar hit compound **3** was identified. A substructure search of the Abbott compound library identified a set of similar structures, including furopyridine **5**. Using a chemical combination of various structural features of these compounds along with previously described expression inhibitor **1**, a new series of thieno[2,3-*c*]pyridines were synthesized, which led to the identification of **7** (IC_{50} = 25 nM vs ICAM-1 expression). Using traditional approaches, the potency of this series was optimized and resulted in the 4-((4-bromophenyl)thio)thieno[2,3-*c*]pyridine-2-carboxamide (**76**), with an IC_{50} of 5 nM vs ICAM-1 expression. The position of the nitrogen in the core ring system was critical for activity. Preliminary biological characterization of this class of inhibitors indicated that the compounds were effective regardless of method of activation of the endothelial cells, that they were not general inhibitors of protein synthesis or gene transcription, and that they had little effect on T-cell function. Compound **7** was an effective inhibitor of cell−cell adhesion in an in vitro flow experiment, demonstrating relevance in a model physiological system. Initial pharmacokinetic evaluation of **7** was disappointing, with low drug concentrations in plasma following oral dosing in rats. Two major metabolites were identified: the products of amide hydrolysis and sulfide oxidation. Further studies which address these issues will be reported in due course.

Experimental Section

Chemistry. 6-Ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**9**) and 4-chloro-6-ethylthieno[2,3-*d*]pyrimidine (**10**) were prepared according to the method of Ram et al.[26] Ethyl 4-chlorothieno[2,3-*b*]pyridine-5-carboxylate was prepared as described by Khan and Guarçoni.[20] 4-Oxo-4,5-dihydrothieno[3,2-*c*]pyridine-2-nitrile (**29**) was prepared by the method of Eloy et al.[21] Other reagents and solvents were used as obtained from the suppliers. All reactions were run under a nitrogen atmosphere. Melting points were obtained on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a General Electric QE300 or QE300 plus spectrometer, and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer. Electrospray ionization (ESI) mass

spectra were recorded on a Finnegan TSQ700 instrument. Direct chemical ionization (DCI/NH_3) and atmospheric pressure chemical ionization (APCI) mass spectra were recorded on a Finnigan DCI/MS SSQ700. Infrared spectra were recorded on a Nicolet 5SXC FT-IR spectrometer. Combustion analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ.

6-Ethyl-4-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]thieno-[2,3-*d*]pyrimidine (46). Chloropyrimidine **10** (0.250 g, 1.26 mmol) was dissolved in DMF (1.5 mL), the solution was treated sequentially with 5-methyl-1,3,4-thiadiazole-2-thiol (0.167 g, 1.26 mmol) and potassium carbonate (0.174 g, 1.26 mmol), and the mixture was stirred at room temperature for 18 h. The mixture was partitioned between water and EtOAc, the extract was concentrated in vacuo, and the residue was recrystallized from EtOH−H_2O to provide 0.175 g (47%) of thieno[2,3-*d*]-pyrimidine **46** as a white solid: mp 132−135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3H), 2.82, (s, 3H), 3.05 (q, J = 7 Hz, 2H), 7.42 (s, 1H), 8.88 (s, 1H); MS (DCI/NH_3) m/z 295 $(M + H)^+$. Anal. ($C_{11}H_{10}N_4S_3$) C, H, N.

Compounds **47**−**49** were prepared from compound **10** by a procedure analogous to that for **46**.

6-Ethyl-4-[(phenylmethyl)thio]thieno[2,3-*d*]pyrimidine (47): mp 54−60 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 2.96 (q, J = 7 Hz, 2H), 4.65 (s, 2H), 7.16 (s, 1H), 7.21−7.36 (m, 3H), 7.46 (m, 2H), 8.83 (s, 1H); MS (DCI/NH_3) m/z 287 $(M + H)^+$. Anal. ($C_{15}H_{14}N_2S_2$) C, H, N.

6-Ethyl-4-[(4-methylphenyl)thio]thieno[2,3-*d*]pyrimidine (48): mp 56−58 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (t, J = 7 Hz, 3H), 2.38 (s, 3H), 2.99 (q, J = 7 Hz, 2H), 7.20 (s, 1H), 7.33 (m, 2H), 7.52 (m, 2H), 8.63 (s, 1H); MS (DCI/NH_3) m/z 286 $(M + H)^+$. Anal. ($C_{15}H_{14}N_2S_2$) C, H, N.

6-Ethyl-4-(2-pyridinylthio)thieno[2,3-*d*]pyrimidine (49): mp 76.5−79 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (t, J = 8 Hz, 3H), 2.99 (q, J = 8 Hz, 2H), 7.18 (s, 1H), 7.46 (dt, 1H), 7.81 (d, 1H), 7.90 (dt, 1H), 8.60 (m, 1H), 8.74 (s, 1H); MS (DCI/NH_3) m/z 274 $(M + H)^+$. Anal. ($C_{13}H_{11}N_3S_2$) C; H: calcd, 4.06; found, 4.94; N: calcd, 15.37; found, 9.71.

6-Ethyl-*N*-(phenylmethyl)thieno[2,3-*d*]pyrimidin-4-amine (50). Chloropyrimidine **10** (0.27 g, 1.37 mmol) in 2-propanol (1.5 mL) was treated with benzylamine (0.19 mL, 1.71 mmol) and sodium carbonate (0.24 g, 2.3 mmol), and the mixture was stirred at room temperature overnight. The mixture was filtered to remove the inorganic materials, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 25% EtOAc/hexanes to provide (0.275 g, 75% yield) of 4-benzylamino analogue **50**: mp 128−131 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 2.88 (q, J = 7 Hz, 2H), 4.72 (d, J = 6 Hz, 2H), 7.20−7.40 (m, 6 H), 8.26 (s, 1H), 8.34 (t, J = 6 Hz, 2H); MS (DCI/NH_3) m/z 270 $(M + H)^+$. Anal. ($C_{15}H_{15}N_3S_2$) C, H, N.

6-Ethyl-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)thieno[2,3-*d*]-pyrimidin-4-amine (51). A solution of chloropyrimidine **10** (0.20 g, 1.01 mmol) in 2-propanol (2 mL) was treated with 2-amino-5-methyl-1,3,4-thiadiazole (0.15 g, 1.26 mmol) and sodium carbonate (0.18 g, 1.7 mmol), stirred at room temperature for 48 h. To drive reaction to completion, cesium carbonate (0.55 g, 1.7 mmol) was added, and the resulting mixture was stirred at reflux for 24 h. The mixture was concentrated in vacuo, the residue was partitioned between water and dichloromethane. The dichloromethane extract was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was recrystallized with ethanol/water to provide 92.1 mg (33% yield) of **51**: mp 277−280 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (t, J = 7 Hz, 3H), 2.63 (s, 3H), 2.96 (q, J = 7 Hz, 1H), 7.81 (br s, 1H), 8.65 (s, 1H); MS (DCI/NH_3) m/z 278 $(M + H)^+$. Anal. ($C_{11}H_{11}N_5S_2$) C, H, N.

3,5-Dichloropyridine-4-carboxaldehyde (14). The compound was prepared in a manner similar to that of Ito.[19] Diisopropylamine (15.6 mL, 0.111 mol) in dry THF (25 mL) at 0 °C was treated with *n*-BuLi (44.6 mL, 2.5 M in hexane, 0.111 mol) over 35 min, the solution was stirred for 30 min at 0 °C, then cooled to −78 °C and diluted with THF (100 mL). A solution of 3,5-dichloropyridine (**13**; 15.0 g, 0.101 mol) in THF (175 mL) was added slowly over 3.5 h to the LDA solution, maintaining an internal temperature below −74 °C. The solution was stirred at −78 °C for 30 min, then treated dropwise over 35 min with a solution of methyl formate (12.5 mL, 0.203 mmol) in THF (50 mL), again maintaining an internal temperature below −74 °C. The resulting solution was stirred at −78 °C for 1.4 h, then it was cannulated rapidly into a 0 °C solution of saturated $NaHCO_3$, with vigorous stirring. The quenched mixture was partitioned with EtOAc (500 mL), then extracted sequentially with saturated $NaHCO_3$ (2 × 100 mL), brine (3 × 150 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography on silica gel with 10% acetone/hexane to provide 16.0 g (90% yield) of aldehyde **14** as a pale yellow waxy solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 2H), 10.31 (s, 1H); MS (DCI/NH_3) m/z 176, 178, 180 $(M + H)^+$.

3-[(4-Methylphenyl)thio]-5-chloro-4-pyridinecarboxyaldehyde (15, R^1 = *p*-tolyl). Aldehyde **14** (5.05 g, 28.7 mmol) in DMF (70 mL) was treated with *p*-thiocresol (3.56 g, 28.7 mmol) and potassium carbonate (4.36 g, 31.6 mmol), and the mixture was stirred for 0.5 h at 0 °C then for 1 h at room temperature. The reaction mixture was poured into water, diluted with brine, and extracted with dichloromethane. The extract was washed sequentially with water and brine, dried ($MgSO_4$), filtered, and concentrated to provide the crude monosubstituted product **15** (R^1 = *p*-tolyl), which was used in the next step without further purification.

Methyl 4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxylate (55). Crude **15** (28.7 mmol maximum), methyl thioglycollate (3.04 g, 28.7 mmol) and potassium carbonate (4.0 g, 31.0 mmol) were combined in 100 mL of DMF, and the mixture was heated to 60 °C for 2 h with stirring. The reaction was cooled and poured into ice−water, giving a viscous semisolid. The aqueous mixture was diluted with brine and extracted with methylene chloride (5 × 400 mL). The combined organic extract was washed sequentially with water and brine, dried ($MgSO_4$), and concentrated in vacuo to a semisolid residue. The crude product was purified by flash chromatography on silica gel, eluting with 10% EtOAc−hexane to provide 2.97 g (33% over two steps): mp 116−119 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28 (s, 3H), 3.91 (s, 3H), 7.20 (m, 2H), 7.29 (m, 2H), 8.00 (s, 1H), 8.44 (s, 1H), 9.36 (s, 1H); MS (DCI/NH_3) m/z 316 $(M + H)^+$. Anal. ($C_{16}H_{13}NO_2S_2 \cdot 0.25H_2O$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxylic Acid (54). A suspension of ester **16** (2.0 g, 6.35 mmol) and $LiOH \cdot H_2O$ (1.4 g, 32 mmol) in 2-propanol (25 mL) and water (15 mL) was heated to 75 °C for 1 h, then cooled and partitioned between water and diethyl ether. The aqueous layer was cooled in an ice bath and adjusted to pH 2 with 10% aqueous HCl. The resulting solid was collected by suction filtration, the solid was washed with water, then dried in vacuo. The crude product was recrystallized from ethanol/water to provide 1.77 g (93% yield) of acid **54** as a white solid: mp 272−274 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.20 (m, 2H), 7.28 (m, 2H), 7.92 (s, 1H), 8.44 (s, 1H), 9.34 (s, 1H); MS (DCI/NH_3) m/z 302 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (7). A suspension of acid **54** (0.535 g, 1.78 mmol) in dichloromethane (25 mL) at 0 °C was treated sequentially with oxalyl chloride (0.34 g, 2.67 mmol) and DMF (1 drop). The solution was stirred at room temperature for 0.5 h, then concentrated in vacuo to provide the acid chloride derivative. The residue was suspended in THF, treated with NH_4OH (30 mL) dissolved in THF (60 mL) and water (30 mL), and the resulting mixture was stirred at room temperature for 0.5 h. The THF layer was separated, washed with brine, partially dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was partially purified by flash chromatography on silica gel with 5% methanol−dichloromethane, then further purified by recrystallization from 95% ethanol to provide 0.30 g of amide **7** (56% yield) as white needles: mp 198−199 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.20 (m, 2H), 7.30 (m, 2H),

7.89 (br s, 1H), 8.26 (s, 1H), 8.35 (s, 1H), 8.54 (br s, 1H) 9.16 (s, 1H); MS (DCI/NH_3) *m/z* 301 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

Compounds **38**, **57**, and **58** were prepared from acid **54** in analogy to the procedure for compound **7**.

***N*-Methoxy-*N*-methyl-4-[(4-methylphenyl)thio]thieno-[2,3-*c*]pyridine-2-carboxamide (38):** mp 103−107 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H), 3.34 (s, 3H), 3.74 (s, 3H), 7.19 (m, 2H), 7.27 (m, 2H), 8.02 (s, 1H), 8.46 (s, 1H); MS (DCI/NH_3) *m/z* 345 $(M + H)^+$. Anal. ($C_{17}H_{16}N_2O_2S_2$) C, H, N.

***N*-Methoxy-4-[(4-methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (57):** mp 200−203 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 3.76 (s, 3H), 7.20 (m, 2H), 7.30 (m, 2H), 7.89 (br s, 1H), 8.15 (s, 1H), 8.4 (s, 1H), 9.3 (s, 1H); MS (DCI/NH_3) *m/z* 331 $(M + H)^+$. Anal. ($C_{16}H_{14}N_2O_2S_2 \cdot 0.25H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-4-[(4-methylphenyl)thio]thieno-[2,3-c]pyridine-2-carboxamide (58):** mp 208−211 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.23 (m, 2H), 7.33 (m, 2H), 7.47 (m, 2H), 7.81 (m, 2H), 8.34 (s, 1H), 8.57 (s, 1H), 9.31 (s, 1H), 10.90 (br s, 1H); MS (DCI/NH_3) *m/z* 411 $(M + H)^+$. Anal. ($C_{12}H_{15}ClN_2OS_2$) C, H, N.

4-Chlorothieno[2,3-*c*]pyridine-2-carboxamide (20). Aldehyde **14** was treated with 1 equiv of methyl thioglycollate as described for ester **55** to provide 4-chlorothienopyridine **20**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.93 (br s, 1H, NH), 8.28 (s, 1H), 8.55 (br s, 1H, NH), 8.58 (s, 1H), 9.28 (s, 1H); MS (DCI/NH_3) *m/z* 213 $(M + H)^+$. Anal. ($C_8H_5ClN_2OS$) C, H, N.

4-[(2-Amino-2-oxoethyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (21). Reaction of **14** with an excess of methyl thioglycollate, in analogy to that described for compound **55**, provided methyl 4-[(2-methoxy-2-oxoethyl)thio]thieno[2,3-*c*]-pyridine-2-carboxylate: ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.59 (s, 3H), 3.94 (s, 3H), 4.04 (s, 2H), 8.14 (s, 1H), 8.55 (s, 1H), 9.27 (s, 1H); MS (DCI/NH_3) *m/z* 298 $(M + H)^+$. The derived ester was dissolved in 2 M methanolic ammonia 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 provide a 36% yield of diamide **21**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 2H), 7.17 (br s, 1H), 7.59 (br s, 1H), 7.82 (br s, 1H), 8.29 (br s, 1H), 8.46 (s, 1H), 8.52 (br s, 1H), 9.14 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 36.5 (S-*CH*$_2$), 122.5 (CH), 127.7 (3-C), 136.2 (7a−C), 142.5 (5-CH), 143.2 (7-CH), 144.0 (Ar-C), 146.5 (Ar-C), 162.6 (*CO*NH_2), 169.4 (*CO*NH_2); MS (APCI) *m/z* 268 $(M + H)^+$; HRMS calcd for $C_{10}H_9N_3O_2S_2$ (M^+), 267.0138; found, 267.0136. Anal. ($C_{10}H_9N_3O_2S_2 \cdot H_2O$) C, H; N: calcd, 14.73; found, 13.93.

Compounds **67**−**83** were prepared from dichloropyridine-carboxaldehyde **14** in analogy to that described for compound **7**, using the appropriate aryl- or alkylthiol.

4-(Phenylthio)thieno[2,3-*c*]pyridine-2-carboxamide (67): ^{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. ($C_{14}H_{10}N_2OS_2$) C, H, N.

4-[(2-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (68): mp 170−172 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.41 (s, 3H), 7.04 (dd, J = 1, 8 Hz, 1H), 7.15 (dt, J = 1.5, 7 Hz, 1H), 7.27 (dt, J = 1.5, 7.5 Hz, 1H), 7.38 (br d, J = 7.5 Hz, 1H), 7.86 (br s, 1H), 8.20 (s, 1H), 8.23 (s, 1H), 8.53 (br s, 1H), 9.28 (s, 1H); MS (DCI/NH_3) *m/z* 301 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

4-[(3-Methylphenyl)thio]thieno[2,3-c]pyridine-2-carboxamide (69): mp 171−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H), 7.06−713 (m, 2H), 7.21−7.27 (m, 2H), 7.89 (br s, 1H), 8.26 (s, 1H), 8.42 (s, 1H), 8.55 (br s, 1H), 9.30 (s, 1H); MS (DCI/NH_3) *m/z* 301 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2 \cdot 0.25H_2O$) C, H, N.

4-[(3,4-Dimethylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (70): mp 192−194 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.09 (s, 3H), 2.11 (s, 3H), 7.05 (m, 2H), 7.19 (s, 1H) 7.81 (br s, 1H), 8.12 (d, J = 6 Hz, 2H), 8.49 (br s, 1H), 9.15 (s, 1H); MS (APCI) *m/z* 315 $(M + H)^+$. Anal. ($C_{16}H_{14}N_2OS_2 \cdot 0.25H_2O$) C, H, N.

4-[(3,5-Dimethylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (71): mp 177−179 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.13 (s, 6H), 6.83 (s, 1H), 6.92 (s, 1H), 7.81 (br s, 1H), 8.21 (s, 1H), 8.30 (s, 1H), 8.50 (br s, 1H), 9.19 (s, 1H); MS (DCI/NH_3) *m/z* 315 $(M + H)^+$. Anal. ($C_{16}H_{14}N_2OS_2$) C, H, N.

4-[(2,4-Dimethylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (72): mp 193−195 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28 (s, 3H), 2.38 (s, 3H), 7.02 (d, J = 6 Hz, 1H), 7.13 (d, J = 6 Hz, 1H), 7.20 (s, 1H), 7.91 (br s, 1H), 8.05 (s, 1H), 8.58 (br s, 1H), 9.22 (s, 1H); MS (APCI) *m/z* 315 $(M + H)^+$. Anal. ($C_{16}H_{14}N_2OS_2 \cdot 0.25H_2O$) C, H, N.

4-[[4-(Trifluoromethyl)phenyl]thio]thieno[2,3-*c*]pyridine-2-carboxamide (73): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.31 (d, J = 8 Hz, 2H), 7.65 (d, J = 8 Hz, 2H), 7.85 (br s, 1H), 8.19 (s, 1H), 8.50 (br s, 1H), 8.68 (s, 1H), 9.44 (s 1H); MS (DCI/NH_3) *m/z* 355 $(M + H)^+$. Anal. ($C_{15}H_9F_3N_2OS_2$) C, H, N.

4-[(4-Chlorophenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (74): mp 239−241 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.31 (m, 2H), 7.43 (m, 2H), 7.89 (br s, 1H), 8.24 (s, 1H), 8.54 (br s, 1H), 8.56 (s, 1H), 9.38 (s, 1H); MS (DCI/NH_3) *m/z* 321 $(M + H)^+$. Anal. ($C_{14}H_9ClN_2OS_2$) C, H, N.

4-[(3,4-Dichlorophenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (75): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.10 (dd, J = 2, 8 Hz, 1H), 7.55 (d, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.91 (br s, 1H), 8.21 (s, 1H), 8.53 (br s, 1H), 8.62 (s, 1H), 9.41 (s, 1H); MS (ESI) *m/z* 355 $(M + H)^+$. Anal. ($C_{14}H_8Cl_2N_2OS_2$) C, H, N.

4-[(4-Bromophenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (76): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.20 (dt, J = 2, 8 Hz, 2H), 7.53 (dt, J = 2, 8 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. ($C_{14}H_9BrN_2OS_2$) C, H, N.

4-[(4-Methoxyphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (77): mp 219−221 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.76 (s, 3H), 6.99 (d, J = 8 Hz, 2H), 7.46 (d, J = 8 Hz, 2H), 7.89 (br s, 1H), 8.17 (s, 1H), 8.30 (s, 1H), 8.54 (br s, 1H), 9.18 (s, 1H); MS (ESI) *m/z* 317 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2O_2S_2$) C, H, N.

4-(4-Biphenylylthio)thieno[2,3-*c*]pyridine-2-carboxamide (78): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33−7.42 (m, 2H), 7.46 (td, J = 1.5, 7 Hz, 2H), 7.5−7.7 (br m, 4H), 7.91 (s, 1H), 8.30 (d, J = 0.8 Hz, 1H), 8.54 (s, 1H), 8.57 (s, 1H), 9.37 (s, 1H); MS (DCI/NH_3) *m/z* 363 $(M + H)^+$. Anal. ($C_{20}H_{14}N_2OS_2$) C, H, N.

4-(2-Pyridinylthio)thieno[2,3-*c*]pyridine-2-carboxamide (79): mp 239−242 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.99 (d, 1H), 7.17 (dd, 1H), 7.65 (dt, 1H), 7.85 (br s, 1H), 8.18 (s, 1H), 8.36 (m, 1H), 8.49 (br s, 1H), 8.69 (s, 1H), 9.23 (s, 1H); MS (DCI/NH_3) *m/z* 305 $(M + NH_4)^+$. Anal. ($C_{13}H_9N_3OS_2$) C, H, N.

4-[(5-Methyl-1,3,4-thiadiazol-2-yl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (80): mp 240−243 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.60 (s, 3H), 7.92 (br s, 1H), 8.32 (s, 1H), 8.56 (br s, 1H), 8.82 (s, 1H), 9.50 (s, 1H); MS (DCI/NH_3) *m/z* 309 $(M + H)^+$, 326 $(M + NH_4)^+$. Anal. ($C_{11}H_8N_4OS_3$) H; C: calcd, 42.84; found, 43.43; N: calcd, 18.17; found, 17.27.

4-[(2-Methyl-3-furanyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (81): mp 236−239 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.41 (s, 3H), 6.68 (d, J = 1.5 Hz, 1H), 7.74 (d, J = 1.5 Hz, 1H), 7.93 (br s, 1H), 8.19 (s, 1H), 8.38 (s, 1H), 8.60 (br s, 1H), 9.15 (s, 1H); MS (ESI) *m/z* 291 $(M + H)^+$. Anal. ($C_{13}H_{10}N_2O_2S_2 \cdot 0.25H_2O$) C, H, N.

4-(Cyclohexylthio)thieno[2,3-*c*]pyridine-2-carboxamide (82): mp 205−207 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.14−1.43 (br m, 6H), 1.51−1.61 (br m, 1H), 1.66−1.78 (br m, 2H), 1.83−1.98 (br m, 2H), 7.90 (br s, 1H), 8.33 (s, 1H), 8.52 (s, 1H), 8.57 (br s, 1H), 9.22 (s, 1H); MS (ESI) *m/z* 293 $(M + H)^+$. Anal. ($C_{14}H_{16}N_2OS_2$) C, H, N.

4-[[(4-Chlorophenyl)methyl]thio]thieno[2,3-*c*]pyridine-2-carboxamide (83): mp 198−199 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.40 (s, 2H), 7.31 (s, 4H), 7.86 (br s, 1H), 8.26 (s,

1H), 8.41 (s, 1H), 8.52 (br s, 1H), 9.15 (s, 1H); MS (APCI) *m/z* 335 $(M + H)^+$. Anal. ($C_{15}H_{11}ClN_2OS_2$) C, H, N.

***N*-(2,3-Dihydroxypropyl)-4-[(4-methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (59).** A solution of acid **54** (2.5 g, 8.3 mmol) and *N*-hydroxysuccinimide (0.95 g, 8.3 mmol) in dichloromethane (35 mL) was treated with EDC (1.882 g, 9.13 mmol) in methylene chloride (15 mL), stirred at room temperature for 18 h, and concentrated in vacuo. The residue was dissolved in EtOAc, washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was added to a solution of 3-amino-1,2-propanediol (0.144 g, 1.6 mmol) in 3:1 dioxane/methanol (20 mL), was stirred at room temperature for 18 h, then the mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 6% methanol/dichloromethane to provide 0.22 g (56% yield) of amide **59**: mp 120−122 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3 H), 3.19 (m, 1H), 3.4 (m, 1 H), 3.65 (m, 1 H), 4.62, (t, 1 H), 4.88 (d, 1 H) 7.20 (m, 2 H), 7.30 (m, 2 H), 8.38 (s, 1 H), 9.1 (s, 1 H), 9.28 (s, 1H); MS (DCI/NH_3) *m/z* 375 $(M + H)^+$. Anal. ($C_{18}H_{18}N_2O_3S_2 \cdot 0.75H_2O$) C, H, N, S.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxylic Acid, Hydrazide (56). Hydrazide **56** was prepared analogous to amide **59**, using hydrazine hydrate: mp 176−178 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3 H), 4.68 (br s, 2H), 7.20 (m, 2 H), 7.30 (m, 2 H), 8.2 (s, 1 H), 8.4 (s, 1 H), 9.28 (s, 1 H) 10.4 (br s, 1 H); MS (DCI/NH_3) *m/z* 316 $(M + H)^+$. Anal. ($C_{15}H_{13}N_3OS_2 \cdot 0.25H_2O$) C, H, N.

4-[(4-Methylphenyl)thio]-*N*-[3-(4-morpholinyl)propyl]thieno[2,3-*c*]pyridine-2-carboxamide, Trifluoroacetate (60). Ester **55** (200 mg, 0.635 mmol) in a 9:1 (v/v) mixture of 4-(3-aminopropyl)morpholine and acetic acid (2 mL total) was warmed at 70 °C for 4 h, diluted with acetonitrile (6 mL), and purified by C-18 reverse-phase HPLC with a gradient of 20% acetonitrile/water to 100% CH_3CN containing 0.1% trifluoroacetic acid to provide 46.0 mg (17% yield) of morpholinopropylamide **60** as its trifluoroacetic acid salt: HPLC (C-18 reverse-phase, gradient elution with 0−100% MeCN/H_2O with 0.1% TFA, 20 min elution) t_R 9.19 min (98 area %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95 (m, 2H), 3.08 (m, 2H), 3.18 (m, 2H), 3.36 (m, 2H), 3.43 (m, 2H), 3.68 (m, 4H), 7.20 (d, J = 7.5 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 8.0 (br s, 1H), 8.27 (s, 1H), 8.34 (m, 1H), 9.27 (m, 1H); ^{13}C NMR ($CDCl_3$, 400 Hz) δ 23.4 (CH_2, CONHCH$_2$*CH$_2$*CH$_2$), 36.8 (CONHCH$_2$CH$_2$*CH$_2$*), 51.1 (NCH$_2$), 54.0 (CONH*CH$_2$*CH$_2$CH$_2$), 63.4 (OCH$_2$), 121.9 (CH), 129.7 (C), 130.4 (Ar-CH), 130.8 (Ar-CH), 137.7 (C), 144.6 (CH), 145.1 (CH), 145.4 (C), 146.9 (C), 147.9 (C), 150.8 (C), 158.0, 158.3, 160.8 (CO); MS (APCI) *m/z* 428 $(M + H)^+$.

***N*-(Aminoiminomethyl)-4-[(4-methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxamide (61).** A solution of guanidine hydrochloride (0.095 g, 1 mmol) in methanol was treated with potassium *tert*-butoxide (0.112 g, 1 mmol), stirred at room temperature for 30 min, then treated with ester **55** (0.1 g, 0.3 mmol). The reaction was warmed to room temperature for 16 h and then concentrated in vacuo. The residue was dissolved in EtOAc (100 mL), washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 6% methanol/dichloromethane to provide 0.080 g (82% yield) of acylguanidine **61**: mp 202−205 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 6.90 (br s, 2H), 7.20 (m, 4H), 7.80 (s, 1H), 8.00 (br s, 2H), 8.20 (s, 1H), 8.40 (s, 1H), 9.24 (s, 1H); MS (DCI/NH_3) *m/z* 343 $(M + H)^+$.

Ethyl 4-[(4-Methylphenyl)thio]thieno[2,3-*b*]pyridine-5-carboxylate (24). Ethyl 4-chlorothieno[2,3-*b*]pyridine-5-carboxylate (**23**) and thiocresol were processed as described for compound **46**: mp 60−63 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 1.30 (t, J = 6 Hz, 3H), 2.28 (s, 3H), 4.26 (q, J = 6 Hz, 2H), 7.00 (d, J = 6 Hz, 1H), 7.17 (m, 2H), 7.24 (m, 2H), 7.91 (d, J = 6 Hz, 1H), 8.81 (s, 1H); MS (DCI/NH_3) *m/z* 347 $(M + NH_4)^+$, 330 $(M + H)^+$. Anal. ($C_{17}H_{15}NO_2S_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*b*]pyridine (26). Ester **24** was hydrolyzed to provide acid **25** according to the procedure described for acid **54**. Acid **25** was decarboxylated using the procedure described for compound **22** to produce thieno[2,3-*b*]pyridine **26**: mp 90−92 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3 H), 6.66 (d, J = 6 Hz, 1 H), 7.38 (m, 2 H), 7.46 (d, J = 6 Hz, 1 H), 7.53 (m, 2 H), 7.46 (d, J = 6 Hz, 1 H), 7.53 (m, 2 H), 7.95 (d, J = 6 Hz, 1 H), 8.12 (d, J = 6 Hz, 1 H); MS (DCI/NH_3) *m/z* 275 $(M + NH_4)^+$ and 258 $(M + H)^+$. Anal. ($C_{14}H_{11}NS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*b*]pyridine-2-carboxamide (28). Diisopropylamine (0.056 g, 0.56 mmol) in THF (10 mL) at −78 °C was treated with *n*-butyllithium (0.22 mL, 0.56 mmol, 2.5 M in hexanes), stirred for 15 min, treated with thieno[2,3-*b*]pyridine **26** (0.13 g, 0.51 mmol) in THF (5 mL). The solution was stirred for 0.5 h, warmed to 0 °C for 1 min, recooled to −78 °C, then transferred into a flask containing excess solid CO_2. The resulting mixture was stirred for 0.5 h, then diluted with saturated NH_4Cl and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and concentrated to provide 0.127 g (83% yield) of acid **27** as a white solid. Crude acid was treated as described for compound **7** to provide amide **28**: mp 280−282 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.41 (s, 3 H), 6.62 (d, J = 6 Hz, 1 H), 7.40 (m, 2 H), 7.57 (m, 2 H), 7.77 (br s, 1 H), 8.26 (s, 1 H), 8.36 (d, J = 6 Hz, 1 H), 8.43 (br s, 1 H); MS (DCI/NH_3) *m/z* 318 $(M + NH_4)^+$ and 301 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine (22). Boiling Dowtherm A (2 mL) was treated sequentially with acid **27** (0.6 g, 1.99 mmol) and copper powder (0.3 g), stirred for 5 min, cooled, diluted with hexane, and partially purified by flash chromatography on silica gel with 15% EtOAc/hexane. The product was then recrystallized from hexanes to provide 0.306 g (90% yield) of thieno[2,3-*b*]pyridine **22** as a crystalline solid: mp 94−95 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H), 7.16 (m, 2H), 7.23 (m, 2H), 7.44 (d, J = 6 Hz, 1H), 8.20 (d, J = 6 Hz, 1H), 8.40 (s, 1H), 9.27 (s, 1H); MS (DCI/NH_3) *m/z* 258 $(M + H)^+$. Anal. ($C_{14}H_{11}NS_2$) C, H, N.

1-[4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridin-2-yl]ethanone (52). A solution of *N*-methyl-*N*-methoxylamide **38** (0.89 g, 2.59 mmol) in THF (25 mL) at 0 °C was treated with methylmagnesium bromide (1.4 M in toluene/THF, 1.85 mL, 2.6 mmol), warmed to room temperature and stirred overnight. The reaction was treated with an additional portion of methylmagnesium bromide (1.4 M in toluene/THF, 0.4 mL, 0.56 mmol) and stirred for 20 h. A third portion of methylmagnesium bromide (1.4 M in toluene/THF, 0.3 mL, 0.42 mmol) was added, and the reaction was stirred for 1 h, at which time TLC (25% EtOAc−hexane) indicated completed consumption of starting amide. The mixture was poured with constant swirling onto ice and saturated aqueous NH_4Cl. The mixture was extracted with EtOAc, and the extract was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 20% EtOAc−hexanes to provide 0.62 g (80% yield) of methyl ketone **52**: mp 134−138 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 (s, 3H), 2.71 (s, 3H), 7.24 (m, 2H), 7.38 (m, 2H), 8.28 (s, 1H), 8.31 (s, 1H), 9.29 (s, 1H); MS (DCI/NH_3) *m/z* 317 $(M + NH_4)^+$. Anal. ($C_{16}H_{13}NOS_2$) C, H, N.

2-Benzoyl-4-[(4-methylphenyl)thio]thieno[2,3-*c*]pyridine (53). The phenyl ketone **53** was prepared in a manner similar to methyl ketone **52**, using phenyllithium: mp 103−107 °C; ^{1}H NMR (300 MHz, DMSO d_6) δ 2.33 (s, 3H), 7.26 (m, 4H), 7.57 (m, 2H), 7.71 (m, 4H), 8.49 (s, 1H), 9.40 (s, 1H); MS (DCI/NH_3) *m/z* 362 $(M + H)^+$. Anal. ($C_{21}H_{15}NOS_2 \cdot 1.25H_2O$) C, H, N.

2-Ethyl-4-[(4-methylphenyl)thio]thieno[2,3-*c*]pyridine (43). A solution of ketone **52** (0.311 g, 1.04 mmol) in ethylene glycol (10 mL) was treated with hydrazine hydrate (0.18 mL, 5.8 mmol), stirred at 160 °C for 30 min and then cooled to room temperature. The resulting solution was treated with potassium hydroxide (0.21 g, 3.7 mmol), stirred at 150 °C for 45 min, cooled to room temperature, treated with water, and extracted with EtOAc. The extract was washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel with 10%

EtOAc−hexanes to provide 0.34 g, (100% yield) of 2-ethyl analogue **43**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (t, J = 7 Hz, 3H), 2.26 (s, 3H), 2.99 (q, J = 7 Hz, 2H), 7.14−7.27 (m, 5H), 8.34 (s, 1H), 9.11 (s, 1H); MS (DCI/NH_3) m/z 286 (M + H)$^+$. Anal. ($C_{16}H_{15}NS_2$·0.25H_2O) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxaldehyde (39). A solution of *N*-methyl-*N*-methoxylamide **38** (3.33 g, 9.6 mmol) in THF at −5 °C was treated dropwise with 1 M DIBAl-H in THF (14.5 mL, 14.5 mmol) and stirred for 45 min. The reaction was poured into a mixture of ice and 10% aqueous HCl with constant stirring, then extracted with dichloromethane. The extract was dried ($MgSO_4$), filtered, and concentrated to provide 2.87 g (100% yield) of aldehyde **39** as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.22 (m, 2H), 7.34 (m, 2H), 8.40 (s, 1H), 8.48 (s, 1H), 9.38 (s, 1H), 10.23 (s, 1H); MS (DCI/NH_3) m/z 303 (M + H)$^+$. Anal. ($C_{15}H_{11}NOS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carbothioamide (44). A solution of amide **7** (190 mg, 0.63 mmol) and Lawsesson's reagent (383 mg, 9.48 mmol) in toluene (15 mL) was heated to room temperature for 5 h, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 4% methanol/dichloromethane to provide 0.16 g (80% yield) of thioamide **44**: mp 181−183 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 3H), 7.20 (m, 2H), 7.30 (m, 2H), 8.18 (br s, 1H), 8.32 (s, 1H), 9.2 (s, 1H) 10.1 (br s, 1H) 10.2 (br s, 1H); MS (DCI/NH_3) m/z 317 (M + H)$^+$. Anal. ($C_{15}H_{12}N_2S_3$·0.25H_2O) C, H, N.

4-[(4-Methylphenyl)sulfinyl]thieno[2,3-*c*]pyridine-2-carboxamide (45). A solution of ester **52** (144 mg, 0.46 mmol) in dichloromethane (10 mL) at 0 °C was treated with 3-chloroperoxybenzoic acid (57−86%, 82 mg), warmed to room temperature over 4 h, treated with dichloromethane (50 mL), washed sequentially with 1 N NaOH, water, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 50% EtOAc/hexane to provide 130 mg (86% yield) of methyl 4-[(4-methylphenyl)sulfinyl]thieno[2,3-*c*]pyridine-2-carboxylate: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, 3H), 3.84 (s, 3H), 7.38 (d, J = 8 Hz, 2H), 7.65 (d, J = 8 Hz, 2H), 8.41 (s, 1H), 9.0 (s, 1H), 9.58 (s, 1H); MS (DCI/NH_3) m/z 332 (M + H)$^+$. The ester (50 mg, 0.15 mmol) was dissolved in 2 M methanolic ammonia and warmed to 40 °C in a sealed tube for 18 h. The reaction was cooled in an ice bath, causing a precipitate to form. The precipitate was filtered, washed with methanol−diethyl ether (1:1) and dried under vacuum to provide 40 mg (84% yield) of amide **45**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.31 (s, 3H), 7.38 (d, J = 8 Hz, 2H), 7.79 (d, J = 8 Hz, 2H), 7.94 (br s, 1H), 8.43 (s, 1H), 8.62 (br s, 1H), 8.85 (s, 1H), 9.43 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8, 120.2, 124.8, 130.0, 135.7, 137.8, 139.2. 139.6, 141.0, 141.8, 148.2, 149.0, 162.0; MS (DCI/NH_3) m/z 317 (M + H)$^+$.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-methanol (19). A suspension of sodium borohydride (28 mg, 0.743 mmol) in a mixture of THF and ethanol (2:3 ratio, 2 mL) under nitrogen atmosphere was stirred at 0 °C for 10 min and treated with $CaCl_2$ (41.2 mg, 0.37 mmol). After 15 min a solution of ester **55** (117 mg, 0.37 mmol) in a mixture of THF and ethanol (2:3 ratio, 3 mL) was added dropwise and stirred at 0 °C for 4 h. The reaction mixture was quenched with 5 mL of 20% aqueous acetic acid and concentrated under reduced pressure. The aqueous layer obtained was adjusted to pH 7 with saturated aqueous $NaHCO_3$ solution and partitioned with EtOAc (3 × 20 mL). The organic layer obtained was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude alcohol (140 mg). Purified alcohol **19** was obtained in 42% yield (45 mg) by flash chromatography on silica gel eluting with 15% acetone−hexane: ^{1}H NMR (500 Hz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 4.80 (s, 2H, CH_2OH), 5.90 (br s, 1H, OH), 7.14 (d, J = 7.5 Hz, 2H, Ar-CH), 7.18 (d, J = 7.5 Hz, 2H, Ar-CH), 7.32 (s, 1H, 3-CH), 8.36 (s, 1H, 5-CH), 9.15 (s, 1H, 7-CH); ^{13}C NMR (500 Hz, DMSO-d_6) δ 20.5 (CH_3), 58.9 (CH_2OH), 117.1 (3-CH), 123.7 (4-C), 129.8 (Ar-CH), 130.2 (Ar-CH), 130.7 (Ar-C), 135.6 (7a-C), 136.9 (Ar-C), 144.4 (7-CH), 146.1 (3a-C and 5-CH), 156.6 (2-C); MS (DCI/NH_3) m/z 288 (M + H)$^+$. Anal. ($C_{15}H_{13}NOS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxaldehyde, Oxime (62). A solution of aldehyde **39** (0.19 g, 0.65 mmol) in 1:1 pyridine:ethanol (8 mL) was treated with hydroxylamine hydrochloride (0.091 g, 1.31 mmol), stirred at room temperature for 18 h, concentrated, and the residue was stirred with water and dichloromethane. The insoluble solid was isolated by suction filtration and washed with water, then dried under high vacuum to provide 0.059 g (30% yield) of oxime **62**: mp 209−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28 (s, 3H), 7.18 (m, 2H), 7.70 (s, 0.3H), 7.87 (s, 0.7H), 8.19 (s, 0.7H), 8.35 (s, 0.3H), 8.38, (s, 0.7H), 8.56 (s, 0.3H), 9.17 (s, 0.3H), 9.27 (s, 0.7H); MS (DCI/NH_3) m/z 301 (M + H)$^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

Compounds **63**−**65** were made by a procedure analogous to that for oxime **62**.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxaldehyde, *O*-phenyloxime (63): mp 94−97 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H), 7.09−7.50 (m, 9H), 7.98 (s, 0.5H), 8.16 (s, 0.5H), 8.39 (s, 0.5H), 8.42 (s, 0.5H), 8.71 (s, 0.5H), 9.16 (s, 0.5H), 9.27 (s, 0.5H), 9.37 (s, 0.5); MS (DCI/NH_3) m/z 377 (M + H)$^+$. Anal. ($C_{21}H_{16}N_2OS_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridine-2-carboxaldehyde, *O*-(phenylmethyl)oxime (64): mp 127−133 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 2.27 (s, 3H), 5.22 (s, 1.2H), 5.38 (s, 0.8H), 7.15−7.26 (m, 4H), 7.31−7.47 (m, 5H), 7.78 (s, 0.6H), 7.96 (s, 0.4H), 8.31 (s, 0.4H), 8.36 (s, 0.6H), 8.39 (s, 0.4H), 8.74 (s, 0.6H), 9.20 (s, 0.6H), 9.30 (s, 0.4H); MS (DCI/NH_3) m/z 391 (M + H)$^+$. Anal. ($C_{22}H_{18}N_2OS_2$) C, H, N.

2-[[[4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridin-2-yl-methylene]amino]oxy]acetic acid (65): mp 227−230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.28 (s, 3H), 4.71 (s, 2H), 7.19 (m, 2H), 7.25 (m, 2H), 7.84 (s, 1H), 8.36 (s, 1H), 8.79 (s, 1H), 9.20 (s, 1H); MS (DCI/NH_3) m/z 359 (M + H)$^+$. Anal. ($C_{17}H_{14}N_2O_3S_2$) C, H, N.

2-[[[4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridin-2-yl-methylene]amino]oxy]acetamide (66). Acid **63** was converted to primary amide **66**, using the procedure described for amide **7**: mp 152−156 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H), 4.52 (s, 0.6H), 4.66 (s, 0.4H), 7.19 (m, 2H), 7.25 (m, 2H), 7.32 (br s, 1H), 7.40 (br s, 1H), 7.84 (s, 0.6H), 7.97 (s, 0.4H), 8.32 (s, 0.4H), 8.37 (s, 0.6H), 8.40 (s, 0.4H), 8.75 (s, 0.6H), 9.21 (s, 0.6H), 9.32 (s, 0.4H); MS (DCI/NH_3) m/z 358 (M + H)$^+$. Anal. ($C_{17}H_{15}N_3O_2S_2$·1.25H_2O) C, H, N.

1-[4-[(4-Methylphenyl)thio]thieno[2,3-*c*]pyridin-2-yl]-ethanone, Oxime (42). Ketone **52** was converted to oxime **42** by the procedure described for oxime **62**: mp 209−213 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.22 (s, 1.5H), 2.28 (s, 3H), 2.32 (s, 1.5H), 7.20 (m, 2H), 7.30 (m, 2H), 7.62 (s, 0.5H), 7.70 (s, 0.5H), 8.30 (s, 0.5H), 8.34 (s, 0.5H), 9.12 (s, 0.5H), 9.24 (s, 0.5H); MS (DCI/NH_3) m/z 315 (M + H)$^+$. Anal. ($C_{16}H_{14}N_2OS_2$) C, H, N.

4-Chlorothieno[3,2-*c*]pyridine-2-carbonitrile (30). A solution of 4-oxo-4,5-dihydrothieno[3,2-*c*]pyridine-2-nitrile (**29**; 500 mg, 2.84 mmol) and phosphoryl chloride (5 mL) was heated at reflux for 1 h. The red solution was poured onto ice and extracted with methylene chloride (2 × 150 mL). The dichloromethane solution was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography with 1:10 EtOAc/hexanes to provide 404 mg (73% yield) of nitrile **30**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 6 Hz, 1H), 8.10 (s, 1H), 8.41 (d, J = 6 Hz, 1H); MS (DCI/NH_3) m/z 195 (M + H)$^+$.

4-[(4-Methylphenyl)thio]thieno[3,2-*c*]pyridine-2-carbonitrile (31). *p*-Thiocresol (192 mg, 1.54 mmol) in DMF (5 mL) at room temperature was treated with potassium *tert*-butoxide (173 mg, 1.54 mmol), stirred for 15 min, cooled to 0 °C, treated with chloropyridine **30** (200 mg, 1.03 mmol), stirred 4 h at 0 °C and then at room temperature for 48 h, treated with water, and extracted with dicholomethane. The extract was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 1:7 EtOAc−hexane to provide 281 mg (97% yield) of sulfide

31: IR (KBr, cm^{-1}) 2200 (w), 1550 (s), 1520 (s); ^1H NMR (300 MHz, $CDCl_3$) δ 2.40 (s, 3H), 7.25 (d, J = 8 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 7.49 (d, J = 6 Hz, 1H), 8.07 (s, 1H), 8.33 (d, J = 6 Hz, 1H); MS (DCI/NH_3) m/z 283 $(M + H)^+$. Anal. ($C_{15}H_{10}N_2S_2$) C, H, N.

4-[(4-Methylphenyl)thio]thieno[3,2-*c*]pyridine-2-carboxamide (32). Nitrile **31** (198 mg, 0.7 mmol) in polyphosphoric acid (5 mL) was heated at 110 °C for 3 h, cooled, treated with water, and extracted with dichloromethane. The extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel with 4:5 EtOAc−hexane to provide 158 mg (75% yield) of amide **32**: IR (KBr, cm^{-1}) 3300 (m), 3130 (s), 1660 (s), 1600 (s); ^1H NMR (300 MHz, DMSO-d_6) δ 2.36 (s, 3H), 7.28 (d, J = 8 Hz, 2H), 7.47 (d, J = 8 Hz, 2H), 7.78 (br s, 1H), 7.84 (d, J = 5 Hz, 1H), 8.19 (d, J = 5 Hz, 1H), 8.34 (s, 1H), 8.46 (br s, 1H); MS (DCI/NH_3) m/z 301 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

4-[(4-Methylphenyl)thio]benzothiophene-2-carboxamide (37). A solution of 2-chloro-6-nitrobenzaldehyde (4.60 g, 24.9 mmol) and *p*-thiocresol (3.10 g, 25.0 mmol) in 50 mL of DMF was treated with solid K_2CO_3 (3.45 g, 25.0 mmol) and the mixture was stirred at room temperature for 18 h. The reaction was poured into ice−water, forming a yellow precipitate, which was collected, washed with water and dried to provide 6.19 g of 2-[(4-methylphenyl)thio]-6-chlorobenzaldehyde (**34**) in crude form. The crude was purified by column chromatography on silica gel, eluting with 15% EtOAc−hexane to give 2.94 g (11.2 mmol, 45% yield) of benzaldehyde **34**: ^1H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 6.71 (m, 1H), 7.13−7.19 (m, 2H), 7.24−7.30 (m, 2H), 7.44 (dt, J = 2, 8 Hz, 2H), 10.68 (s, 1H); MS (DCI/NH_3) m/z 280, 282 $(M + NH_4)^+$.

Chlorobenzaldehyde **34** (2.80 g, 11.0 mmol), methyl thioglycollate (1.17 g, 11.0 mmol) and sodium methoxide (0.60 g, 11.0 mmol) were combined in 30 mL of DMF and the reaction solution was stirred at ambient temperature for 72 h. The reaction mixture was poured into ice−water and then extracted with EtOAc. The organic phase was washed with brine, dried ($MgSO_4$), filtered and rotoevaporated to an oil. The crude benzothiophene was purified by column chromatography on silica gel, eluting with 7.5% EtOAc−hexane to provide 1.1 g (3.5 mmol, 32% yield) of methyl 4-[(4-methyphenyl)thio]benzo[*b*]thiophene-2-carboxylate (**35**) as an oil, which solidified upon standing. Methyl ester **35** (1.1 g, 3.5 mmol) was hydrolyzed with 10 mL of 1 N NaOH in 25 mL of methanol at reflux for 2 h. The reaction was cooled, diluted with water, and then acidified with 10% aqueous citric acid. The solid which precipitated was collected by suction filtration, and dried under high vacuum to give 0.90 g (3.0 mmol, 86%) of carboxylic acid **36** as an off-white solid: ^1H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H), 3.7−4.4 (v br s, approximately 2H including H_2O), 7.17 (s, 4H), 7.24 (dd, J = 1, 8 Hz, 1H), 7.35 (t, J = 8 Hz, 1H), 7.69 (s, 1H), 7.90 (dt, J = 1, 8 Hz, 1H); MS (DCI/NH_3) m/z 318 $(M + NH_4)^+$.

Acid **36** (150 mg, 0.50 mmol) was combined with EDC (95 mg, 0.50 mmol), concentrated aqueous ammonium hydroxide (0.30 mL, 4.5 mmol) and 4-(dimethylamino)pyridine (7 mg, 0.05 mmol) in 15 mL of CH_2Cl_2 at room temperature, and the solution was stirred 18 h. The solution was concentrated in vacuo, and the residue was purified by column chromatography on silica gel, eluting with 3% MeOH−CH_2Cl_2 to provide amide **37** as a pale yellow solid: mp 182−183 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 2.30 (s, 3H), 7.20 (m, 3H), 7.30 (m, 2H), 7.41 (t, J = 8 Hz, 1H), 7.56 (br s, 1 H), 7.97 (d, J = 8 Hz, 1H), 8.26 (d, J = 0.5 Hz, 1H), 8.37 (br s, 1H); MS (DCI/NH_3) m/z 300 $(M + H)^+$, 317 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2OS_2$) C, H, N.

Biology. 1. Cell ELISA Assay. 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 plates incubated 24 h at 37 °C. TNFα (Gibco/BRL) then was added to a final concentration of 5 ng/mL and plates were incubated an additional 6 h at 37 °C. Plates were washed once with D-PBS (Gibco/BRL) and primary antibody (Becton Dickinson) 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. Plates were stored overnight at 4 °C then washed 3 times with D-PBS. Secondary antibody, HRP-conjugated donkey anti-mouse IgG (Jackson Labs) in D-PBS/2%BSA, was added, the plates were incubated 1−2 h at room temperature and then washed 3 times with D-PBS. OPD solution (Abbott) was added to the wells, plates were developed for 15−20 min, neutralized with 1 N sulfuric acid, and the absorbance read at 490 nm.

For assays using alternative inducing agents, IL-1, PMA, or LPS was substituted for TNFα in the above protocol.

2. Toxicity Assays. Primary HUVECs were plated and treated with test compounds and TNFα as described for the ELISA assays. Approximately 24 h after compound addition, Promega MTS reagent was added to the culture wells. After incubating cultures an additional 2 h at 37 °C, absorbance was read at 490 nm.

3. Flow Adhesion Assay. The apparatus for determining the ability of compound **7** to inhibit the rolling adhesion of HL60 cells on a monolayer of human umbilical vein endothelial cells was performed with a Glycotech flow cell according to the method of Patten.[22]

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