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Communications to the Editor

3-Alkoxybenzo[*b*]thiophene-2-carboxamides as Inhibitors of Neutrophil–Endothelial Cell Adhesion

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The adherence of leukocytes to vascular endothelium is essential to the pathogenesis of inflammation.[1] This adhesion process precedes the transendothelial migration of leukocytes into surrounding tissue and ensuing tissue damage. The various adhesion molecules present on leukocytes are recognized by complimentary molecules on endothelial cells. Two of the key adhesion molecules found on the endothelium, E-selectin (ELAM-1, endothelial leukocyte adhesion molecule-1)[2] and ICAM-1 (intercellular adhesion molecule-1),[3] are upregulated by inflammatory stimuli. A compound with the ability to inhibit this upregulation would hinder leukocyte adhesion and transmigration, thereby providing an antiinflammatory drug with a novel mechanism of action. Current therapies for the treatment of arthritis such as nonsteroidal antiinflammatory drugs (NSAIDs) and disease modifying antirheumatic drugs (DMARDs) are only palliative, providing symptomatic relief to patients. Inhibitors of cell adhesion may actually inhibit the initiation of the disease.

An *in vitro* assay has been used to identify compounds that prevent the upregulation of adhesion molecules. Human umbilical vein endothelial cells (HUVECs) are stimulated with a cytokine such as tumor necrosis factor-α (TNF-α) in the presence of the compounds of interest. After a 4-h incubation, ^{51}Cr-labeled human neutrophils are added to the HUVECs and allowed to adhere. At this timepoint E-selectin expression is maximal and significant levels of ICAM-1 are also present on the surface of control cells.[4]

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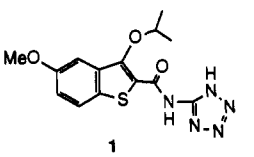
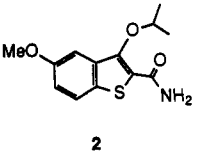
Initial screening in our laboratories identified that 5-methoxy-3-(1-methylethoxy)-*N*-(1*H*-tetrazol-5-yl)benzo[*b*]thiophene-2-carboxamide (CI-959, 1) blocked the adhesion of neutrophils to HUVECs (IC_{50} = 49 μM).[5] After investigating several analogs of 1, it was determined that the primary carboxamide derivative 2 was 1 log order more potent than 1 (IC_{50} = 3.8 μM) in inhibiting cell adhesion (see Table 1). Using 2 as a prototype, additional amides



1
CI-959



2

were prepared as depicted in Scheme 1. The previously reported benzo[*b*]thiophene-2-carboxylic acids[6] were converted to the corresponding imidazolides. Subsequent reaction with aqueous ammonium hydroxide gave the desired primary carboxamides **2–7**, **10**, and **12–14** while reaction with a primary amine resulted in formation of the secondary amides **16–18**. Two exceptions are amide **15**, which was prepared from the corresponding acid chloride, and compound **11**, which resulted from reaction of the analogous methyl ester with lithium amide. The 5-amino compound **8** was obtained by reduction of the 5-nitro analog **6**; while the 5-hydroxy compound **9** was obtained by hydrogenolysis of the 5-benzyloxy analog **7**.

In compounds **3–9**, the nature of the substituent at C-5 was varied to include hydrogen, chloro, methyl, nitro, benzyloxy, amino, and hydroxy. The most active compound was **9** which contains a 5-hydroxy group. Interestingly, **7**, the 5-benzyloxy precursor to **9**, was not active. Analogs of **2** where the aromatic methoxy group is now at position 7 or 6, compounds **10** and **11**, respectively, had weak activity. Compound **13**, where the C-3 isopropoxy group of **2** is replaced by *tert*-butoxy, showed good activity, while the C-3 methoxy, benzyloxy, and phenoxy analogs **12**, **14**, and **15** were equipotent and less effective than **2**. Substitution on the amide nitrogen decreased the activity in relation to the size of the substituent. The methyl-substituted amide **16** was more potent than the ethyl analog **17**, and the phenyl analog **18** was the least active.

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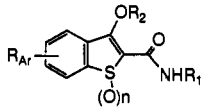
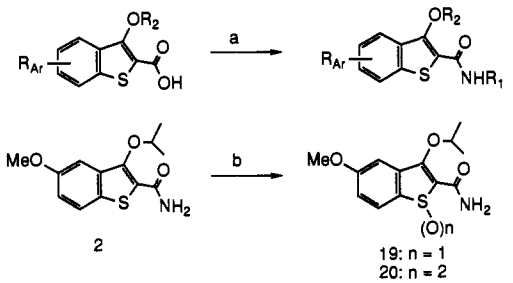
Table 1. Inhibition of Adhesion by 3-Alkoxybenzo[*b*]thiophene-2-carboxamides



compd	*n*	R_1	R_2	R_{Ar}	adhesion[a]	no.[b]
1	0	tetrazole	*i*-Pr	5-OMe	49	3
2	0	H	*i*-Pr	5-OMe	3.8	4
3	0	H	*i*-Pr	5-H	60%	1
4	0	H	*i*-Pr	5-Cl	72%	1
5	0	H	*i*-Pr	5-Me	61%	1
6	0	H	*i*-Pr	5-NO_2	40%	1
7	0	H	*i*-Pr	5-OCH_2Ph	NA[c]	2
8	0	H	*i*-Pr	5-NH_2	49%	2
9	0	H	*i*-Pr	5-OH	6.4	2
10	0	H	*i*-Pr	7-OMe	35%	1
11	0	H	*i*-Pr	6-OMe	24%	1
12	0	H	Me	5-OMe	60%	1
13	0	H	*t*-Bu	5-OMe	97%	2
14	0	H	CH_2Ph	5-OMe	57%	1
15	0	H	Ph	5-OMe	59%	1
16	0	Me	*i*-Pr	5-OMe	15	3
17	0	Et	*i*-Pr	5-OMe	64%	2
18	0	Ph	*i*-Pr	5-OMe	12%	1
19	1	H	*i*-Pr	5-OMe	8.7	4
20	2	H	*i*-Pr	5-OMe	NA[c]	1

[a] Data reported as IC_{50} (μM) or as the percent inhibition at a dose of 100 μM. Each percentage value is from a single assay that was run in triplicate. The mean standard error is <5%. [b] No. = number of experiments. [c] NA = less than 4% inhibition at 100 μM.

Scheme 1[a]



[a] (a) *N,N*-Carbonyldiimidazole; aqueous ammonium hydroxide or a primary amine (b) **19**: 30% H_2O_2/HOAc/room temperature/8 h; **20**: 30% H_2O_2/HOAc/reflux/6 h.

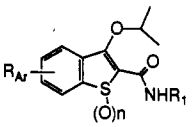
By varying the reaction conditions, it was possible to oxidize **2** with hydrogen peroxide in acetic acid to obtain either the sulfoxide **19** or the sulfone **20** derivative as the major product. While the sulfone **20** was not active, the sulfoxide **19** inhibited adhesion with an IC_{50} of 8.7 μM.

To evaluate potential adverse effects, morphological evaluation of compound treated HUVECs was performed by light microscopy. No effect on cell structure or monolayer integrity was noted. In addition, **1**, **2**, and **19** did not induce release of lactate dehydrogenase, a cytoplasmic enzyme, demonstrating that the compounds lack cytotoxic activity under the conditions employed.

To determine which adhesion molecules were being effected in the functional assay, several analogs were evaluated in an ELISA-based detection system for the expression of E-selectin and ICAM-1 on the surface of TNF-α-activated HUVECs. Table 2 shows that those compounds active in the functional assay, namely **2**, **9**, **16**, and **19**, inhibited the expression of both E-selectin and ICAM-1. The results of the ELISA assay for compounds **10** and **20** also reflect the findings of the functional assay.

The reverse passive Arthus reaction (RPA) is an immune complex induced model of inflammation that is characterized by neutrophil accumulation.[7] RPA has been utilized in the rat to demonstrate *in vivo* activity of drugs that block neutrophil migration.[8] In this model, an oral dose of 10 mg/kg of **19** inhibited the accumulation of both neutrophils (93%) and exudate (68%) into the pleural cavity. We are currently evaluating additional analogs in RPA and continuing to investigate **19** in other models of inflammation.

Table 2. Inhibition of E-selectin and ICAM-1 Expression via ELISA



compd	*n*	R_1	R_{Ar}	ESEL[a]	ICAM[b]	no.[c]
2	0	H	5-OMe	0.70 μM	0.39 μM	2
9	0	H	5-OH	3.3 μM	3.5 μM	2
10	0	H	7-OMe	NA[d]	NA[d]	2
16	0	Me	5-OMe	1.4 μM	6.6 μM	2
19	1	H	5-OMe	6.6 μM	4.0 μM	2
20	2	H	5-OMe	NA[d]	NA[d]	2

[a] ELISA for E-selectin, data reported as IC_{50} (μM) or as the percent inhibition at a dose of 30 μM. [b] ELISA for ICAM-1, data reported as IC_{50} (μM) or as the percent inhibition at a dose of 30 μM. [c] No. = number of experiments. [d] NA = less than 30% inhibition at a dose of 30 μM.

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Supplementary Material Available: Experimental procedures, including, yields, melting points, and combustion data, the protocols for the adhesion assay, the ELISA assays, and the reversed passive Arthus reaction (12 pages). Ordering information is given on any current masthead page.

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