Novel Thiazole Based Heterocycles as Inhibitors of LFA-1/ICAM-1 Mediated Cell Adhesion

Pauline J. Sanfilippo,* Michele C. Jetter, Richard Cordova, Robert A. Noe, Erika Chourmouzis,† Catherine Y. Lau,† and Elizabeth Wang†

Drug Discovery Division, The R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477, and Don Mills, Ontario, Canada M3C 1L9

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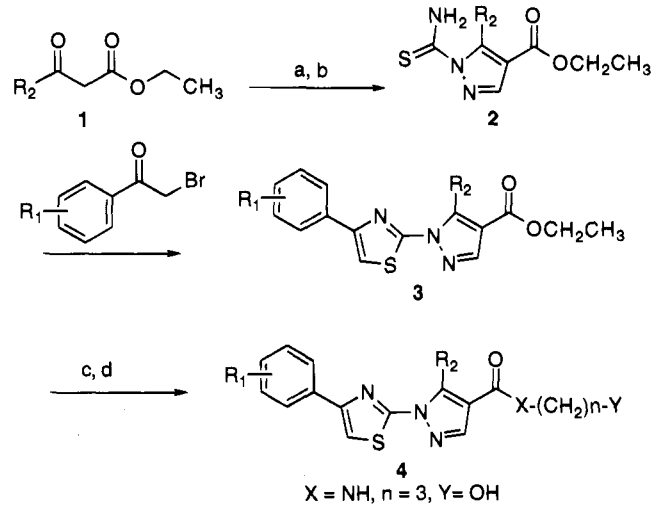
Cell adhesion molecules (CAMs) are important in the regulation of the immune response and inflammation.[1] The extracellular interactions between specific CAMs that are expressed on the endothelium and/or leukocytes mediate leukocyte entry into tissues, T-cell proliferation, and antigen presentation.[2-5] The key event in autoimmune disease is the migration of leukocytes to the disease site. However, the continuous recruitment of leukocytes from blood vessels into inflamed tissues that occurs in chronic inflammation actually perpetuates tissue injury. An agent that inhibits leukocyte adhesion and transmigration represents a novel mechanism of action as an immunosuppressive and/or antiinflammatory drug. The major adhesive force for lymphocyte extravasation from the blood stream into tissue sites is the protein–protein interaction of the adhesion molecules lymphocyte function-associated molecule 1 (LFA-1, CD11a/CD18, β_2 integrin) and its endothelial counterreceptor intercellular adhesion molecule 1 (ICAM-1, CD54, immunoglobulin).[6-9] Monoclonal antibodies to ICAM-1 have been shown to inhibit lymphocyte transendothelial migration and have yielded very promising results in clinical trials for rheumatoid arthritis and organ transplantation.[10,11] Recently a series of naturally-occurring secoliminoids, isolated from the root of *Trichilia rubra*, were reported as potent inhibitors of cellular LFA-1/ICAM-1-mediated adhesion.[12] However, the major active component from this root extact was isolated in only 0.005% overall yield. We initiated a drug discovery program to identify small molecule inhibitors of the LFA-1/ICAM-1 interaction for the potential treatment of rheumatoid arthritis and organ transplantation as well as other inflammatory disorders.

An *in vitro* cell/protein adhesion assay was established as a screening assay for small molecules that inhibit the LFA-1/ICAM-1 interaction. The myelomonocytic cell line HL60 was chosen as the source of LFA-1. Flow cytometry analyses (FACS) indicate HL60 cells express the β_2 integrin LFA-1. HL60 cells, by FACs analysis, do not express the β_2 integrin Mac-1 (macrophage antigen-1, CD11b/CD18), another counterreceptor for ICAM-1.[13] Fluorochrome-labeled LFA-1 bearing HL60 cells are allowed to adhere to recombinant soluble ICAM-1 (sICAM-1) that is immobilized onto plastic. The sICAM-1 utilized in this assay is obtained in milligram quantities after cDNA insertion into a baculovirus expression system, isolation, and purification to homogeneity by immunoaffinity chromatography.[14] The adherence levels are quantified using a fluorescence concentration analyzer. After a 40 min incubation period at 37 °C, the effect of test compounds or antibodies on adhesion can be monitored by washing away nonadherent cells. Blocking antibodies to either LFA-1 or ICAM-1 exert >90% inhibition in this assay.

Scheme 1[a]



[a] (a) DMF–dimethyl acetal, benzene, reflux; (b) thiosemicarbazide, HOAc; (c) NaOH, EtOH; (d) CDI, CH_3CN, a primary amine or 1,3-propanediol.

Utilizing this assay, we identified 2-[4-[(3-hydroxypropyl)carbamoyl]-5-methylpyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole (RWJ-50271, **4**) as an inhibitor of lymphocyte adhesion to sICAM. Compound **4** inhibits the adhesion of LFA-1 bearing HL60 cells to immobilized ICAM-1 with an IC_{50} = 5.0 μM. Several analogues of RWJ 50271 were prepared as depicted in Scheme 1. An appropriately substituted ethyl acetoacetate derivative **1** is converted to the pyrazolothioamide **2** via the dienaminone. Thioamide **2** is condensed with an appropriately substituted phenacyl bromide to give the 4-(substituted phenyl)-2-(substituted pyrazol-1-yl)thiazole **3**. Saponification of the ester, followed by activation of the resulting acid with *N,N*-carbonyldiimidazole and coupling with an appropriate primary amine or 1,3-propanediol affords the compounds in Table 1.

Compounds in Table 1 were evaluated for inhibitory activity in the cellular LFA-1/purified ICAM-1 (cell/protein) adhesion assay. Lengthening the side chain to a four methylene unit (**5**) results in a 2-fold decrease in inhibitory activity. A five methylene spacer (**6**) is completeliy devoid of activity. The ester derivative **7** shows greatly diminished inhibitory activity in the cell/protein assay. Activity is exquisitely sensitive to aromatic substitution as indicated by the severely attenuated potency with the unsubstituted (**8**), 3-chloro (**9**), 4-methyl (10), and 3,4,5-trimethoxy (**11**) analogues. The best aromatic substituent is a 3-trifluoromethyl group.

When the methyl moiety on the pyrazole ring is substituted with an ethyl (**12**), a modest increase in inhibitory activity is observed. Compound **12** has an IC_{50} = 3 μM. However, homologation to the propyl derivative **13** results in 4–5-fold decrease in potency. Replacing the methyl group with a trifluoromethyl substituent (**14**) also significantly attenuates activity.

* Address correspondence to this author at the R. W. Johnson Pharmaceutical Research Institute, Spring House, PA.
† Don Mills, Ontario, Canada.

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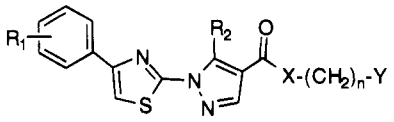
Table 1. Inhibition of LFA-1/ICAM-1-Mediated Adhesion



compd	R_1	R_2	X-$(CH_2)_n$-Y	mp, °C[a]	cell/ protein[b]
4	3-CF_3	CH_3	$NH(CH_2)_3OH$	166–167	5.0 μM
5	3-CF_3	CH_3	$NH(CH_2)_4OH$	169–171	51%
6	3-CF_3	CH_3	$NH(CH_2)_5OH$	210–211	0%
7	3-CF_3	CH_3	$O(CH_2)_3OH$	110–111	25%
8	H	CH_3	$NH(CH_2)_3OH$	154–155	5%
9	3-Cl	CH_3	$NH(CH_2)_3OH$	181–183	20%
10	4-CH_3	CH_3	$NH(CH_2)_3OH$	170–171	7%
11	tri-OCH_3	CH_3	$NH(CH_2)_3OH$	185–186	0%
12	3-CF_3	CH_2CH_3	$NH(CH_2)_3OH$	210–211	3.0 μM
13	3-CF_3	$(CH_2)_2CH_3$	$NH(CH_2)_3OH$	158–159	26%
14	3-CF_3	CF_3	$NH(CH_2)_3OH$	184–187	5%
15	3-CF_3	CH_3	$NH(CH_2)_3OCH_3$	162–163	48%
16	3-CF_3	CH_3	$NH(CH_2)_3CO_2CH_2$-CH_3	167–168	0%
17	3-CF_3	CH_2CH_3	$NH(CH_2)_3$imidazole	202–203	34%
18	3-CF_3	CH_2CH_3	$NH(CH_2)_3$pyrrolidinone	181–183	10%

[a] All compounds exhibited satisfactory (±0.4%) elemental analyses for C, H, and N. [b] Data reported as IC_{50} (μM) or as percent inhibition at a dose of 10 μM. Each percentage value is from a single assay that was run in five replicates. The mean standard error is <5%.

Table 2. Specificity Profile of **4**

adherence assay	test system	inhibitory activity
LFA-1/ICAM-1[a]	HL60/U937[17]	IC_{50} = 5 μM
LFA-1/ICAM-1[b]	HL60/rICAM-1	IC_{50} = 5 μM
Mac-1/ICAM-1[b]	fMLP-PMN/rICAM-1	no effect up to 20 μM
sLe^X/E-selectin[a]	HL60/IL-1β HUVEC	no effect up to 20 μM
VLA-4/VCAM-1[a]	Ramos/IL-1β HUVEC	no effect up to 20 μM

[a] cell/cell adherence assay. [b] Cell/protein adherence assay.

The hydroxy terminus is a key requisite for good inhibitory activity in the cell/protein assay. The methoxy analogue, **15**, resulted in a 3-fold decrease in potency. Oxidation to the ester **16** completely eliminated activity. The imidazole **17** has weak inhibitory activity, and the pyrrolidinone **18** has only slight activity. The data from our preliminary SAR studies indicate **4** and **12** as the most interesting small molecule inhibitors of LFA-1/ICAM-1-mediated cell adhesion.

The specificity of our novel series of adhesion molecule antagonists was determined through additional adherence assays which are governed by other leukocyte–endothelium adhesion molecules as summarized in Table 2. Fluorescence technology was used to quantify the adherence of ligand-bearing cells. In each assay system, adherence was inhibited by specific antibodies and not by antibodies irrelevant to the adhesion molecules in the study. Mac-1/ICAM-1-mediated cell adhesion was determined using human neutrophils which are stimulated with f-MetLeuPhe to specifically activate Mac-1. Treated neutrophils were allowed to interact with plastic-immobilized ICAM-1 for 30 min at 37 °C. To evaluate the interaction between E-selectin and its carbohydrate ligand sialyl Lewis X, E-selectin expression was induced by treating confluent monolayers of human umbilical vein endothelial cells [HUVEC] with IL-1β for 4 h. This interaction was determined by a 20 min incubation of HL60 cells with the stimulated HUVEC at 4 °C. The interaction of another inducible adhesion molecule, VCAM-1 (vascular cell adhesion molecule-1), with its ligand VLA-4 (very late antigen-4, β_1 integrin) was determined using Ramos cells, a B lymphoblastoid cell line which expresses VLA-4, with overnight IL-1β stimulated HUVECs which express VCAM-1. Compound **4** does not inhibit Mac-1/ICAM-1, E-selectin/sialyl Lewis X or VLA-4/VCAM-1-mediated cell adhesion up to 20 μM concentrations.

FACS analyses indicate **4** does not alter the LFA-1 expression levels on HL60 cells. In addition, **4** inhibited adhesion of peripheral blood lymphocytes to plastic immobilized sICAM-1. When studied in an LFA-1/ICAM-1-dependent natural killer [NK] cytotoxicity assay, **4** inhibited both human and murine NK activity with an IC_{50} = 5.0 μM.[15] Membrane integrity was monitored during the natural killer [NK] cytolysis testing by exposing ^{51}Cr-labeled target cells to varying doses of the test compounds for 4 h. Lysis above the spontaneous lysis is attributed to compound toxicity. Compounds **4** and **12** did not exhibit any cell lysis up to 100 μM concentrations. To eliminate cellular toxicity as a possible cause of observed inhibitory activity, the viability of the HL60 cells after treatment with **4** is determined. HL60 cells are treated with various doses of **4** for 1 h at 37 °C before testing for viability by trypan blue exclusion. Compound **4** did not exhibit any toxic activity up to 100 μM concentrations.

The delayed-type hypersensitivity [DTH] reaction is a valuable model for the study of T-cell-mediated immunity which involves the release of lymphocytes and the recruitment of monocytes and macrophages. Antibodies to LFA-1 and ICAM-1, given at the time of challenge to previously immunized mice, have been found effective in reducing swelling and lympocyte infiltration at the site of challenge.[16] In this model, an oral dose of 50 mg/kg of **4**, given 4 h after challenge, significantly reduced foot pad swelling (>50%) 48 h after the challenge. We are currently evaluating additional analogues in DTH and continuing to investigate **4** in other animal models of inflammation.

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Supplementary Material Available: Experimental procedures for compound RWJ 50271 as well as the positive controls (antibodies) for all adhesion assays (2 pages). Ordering information is given on any current masthead page.

References

(1) Springer, T. A. Adhesion Receptors of the immune system. *Nature* **1990**, *346*, 425–434.
(2) Springer, T. A. Traffice signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **1994**, *76*, 301–314.
(3) Adams, D. H.; Shaw, S. Leucocyte-endothelial interactions and regulation of leucocyte migration. *Lancet* **1994**, 831–836.
(4) Carlos, T. M.; Harlan, J. M. Leukocyte-Endothelial Adhesion Molecules. *Blood* **1994**, *84*, 2068–2101.
(5) Hogg, N.; Landis, R. C. Adhesion molecules in cell interactions. *Curr. Opin. Immunol.* **1993**, *5*, 383–390.
(6) Marlin, S. D.; Springer, T. A. Purified Intercellular Adhesion Molecule-1 is a Ligand for Lymphocyte Function-Associated Antigen 1. *Cell* **1987**, *51*, 813–819.
(7) Staunton, D. E.; Dustin, M. L.; Erickson, H. P.; Springer, T. A. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding site for LFA-1 and rhinovirus. *Cell* **1990**, *61*, 243–254.

(8) Hogg, N. *Integrins and ICAM-1 in Immune Responses*; Karger: Basel, 1991.
(9) Hogg, N.; Landis, R. C.; Bates, P. A.; Stanley, P.; Randi, A. M. The sticking point: how integrins bind to their ligands. *Trends Cell Biol.* **1994**, *4*, 379–382.
(10) Kavanaugh, A. F.; Davis, L. S.; Nichols, L. A.; Norris, S. H.; Rothlein, R.; Scharschmidt, L. A.; Lipsky, P. E. Treatment of refractory rheumatoid arthritis with a monoclonal antibody to intercellular adhesion molecule 1. *Arthritis Rheum.* **1994**, *37*, 992–999.
(11) Haug, C. E.; Colvin, R. B.; Delmonico, F. L.; Auchincloss, H.; Tolkoff-Rubin, N.; Preffer, F. I.; Rothlein, R.; Norris, S.; Scharschimdt, L.; Cosimi, A. B. A phase I trial of immunosuppression with anti-ICAM-1 (CD54) mAb in renal allograft recipients. *Transplantation* **1993**, *55*, 766–773.
(12) Musza, L. L.; Killar, L. M.; Speight, P.; McElhiney, S.; Barrow, C. J.; Gillum, A. M.; Cooper, R. Potent new cell adhesion inhibitory compounds from the root of *Trichilia rubra*. *Tetrahedron* **1994**, *50*, 11369–11378.
(13) Diamond, M. S.; Staunton, D. E.; deFougerolles, A. R.; Stacker, S. A.; Garcia-Aguilar, J.; Hibbs, M. L.; Springer, T. A. ICAM-1 (CD54)-a counter-receptor for Mac-1 (CD11b/CD18). *J. Cell Biol.* **1990**, *111*, 3129–3139.
(14) Cobb, R. R.; Dubins, J. S.; Warner, J.; Molony, L. Functional expression of soluble ICAM-1 by baculovirus-infected sf9 cells. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 1022–1033.
(15) Boyd, A. W.; Wawryk, S. O.; Burns, G. F.; Fecondo, J. V. Intercellular adhesion molecule (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3095–3099.
(16) Unpublished results.
(17) U937, a histocytic cell line, expresses low level of ICAM-1 on its surface. Upon PMA stimulation (3 days), the cells form a monolayer and increase their ICAM-1 expression by 1–2 log.

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