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Nitrobenzene Compounds Inhibit Expression of VCAM-1

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Abstract—A series of nitrobenzene compounds has been discovered as potent inhibitors of VCAM-1 expression and, therefore, potential drug candidates for autoimmune and allergic inflammatory diseases. Structure–activity relationship (SAR) studies showed that a nitro group and two other electron-withdrawing groups are essential for these compounds to be potent inhibitors of VCAM-1 expression.

Leukocyte recruitment into inflamed tissue is an essential physiologic process to remove the inflammatory stimulus, such as during wound repair or invasion by infectious microorganisms. However, this beneficial response can lead to a chronic and detrimental inflammatory process if the stimulus is not properly eliminated. Therefore, leukocyte recruitment is also a key factor in the pathogenic process of inflammation. At sites of inflammation, the recruitment of leukocytes is mediated, at least in part, by expression of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, on the endothelial cells that are induced in response to various cytokines. Adhesion of leukocytes to an endothelial cell surface is a multistep process involving primary adhesion and rolling, secondary firm adhesion, and finally transmigration. E-selectin mediates early and reversible events while VCAM-1 and ICAM-1 regulate later and irreversible steps leading to firm attachment and subsequent diapedesis of leukocytes.[1]

Although steroids and other antiinflammatory drugs with broad-spectrum activities are certainly effective in treating numerous diseases and inflammatory conditions, long-term usage of these drugs often leads to unacceptable side effects, such as greater risk of infection caused by impairment of phagocytic leukocyte migration and function. For similar reasons, there is concern that ICAM-1 might not be a proper target for drug discovery since interference with lymphocyte function-associated antigen-1 (LFA-1), a leukocyte receptor for ICAM-1, may be associated with an increased susceptibility to severe infections.[2]

A major counter-ligand for VCAM-1 is very late antigen-4 (VLA-4), also known as integrin $\alpha 4\beta 1$. Circulating neutrophils do not express VLA-4 and some of the leukocytes that constitutively express VLA-4 (T cells, monocytes, eosinopils) are key effecter cells in various inflammatory disorders. Autoreactive effecter T cells appear to play a pivotal role in rheumatoid arthritis,[3] diabetes,[4] and multiple sclerosis.[5] Adhesion of monocytes and lymphocytes to the arterial endothelial lining is one of the earliest detectable events in human atherosclerosis and these are the principal leukocyte subsets populating atherosclerotic lesions.[6] Eosinophil infiltration and the deposition of tissue-damaging products from activated eosinophils are prominent features of asthma and other allergic respiratory diseases.[7] Tissue samples from patients have also indicated that VCAM-1 is highly expressed in many diseases.[8] Selective inhibitors of VCAM-1/VLA-4 ligand interaction are likely to have potential as therapeutic agents without directly blocking neutrophil migration, even after chronic administration.[2]

A variety of agents have been reported as potent inhibitors of VCAM-1 expression. A soluble protein containing the two N-terminal domains of human VCAM-1 gene fused to a human IgG1 constant region binds to cells bearing activated $\alpha 4$-integrin receptors in vitro and delays the onset of adoptively transferred autoimmune diabetes.[9] Antisense oligonucleotides also offer the

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potential for specific inhibitors of VCAM-1 expression.[10] For example, ISIS 3801 selectively inhibits VCAM-1 expression in vitro by 90% at 3 nM without affecting ICAM-1 or E-selectin.[11] Some cyclic peptides have been found to be potent inhibitors of VCAM-1 expression.[12] A cyclopeptolide effectively inhibited TNFα-induced VCAM-1 ($IC_{50} = 2$ nM) relative to ICAM-1 ($IC_{50} = 60$ nM) and exhibited pharmacological activity in animal models of inflammation.[13,14] Some small molecule compounds of different structural types have also been identified as inhibitors of VCAM-1 expression.[15–20]

Table 1. Inhibiting profile of nitrobenzene compounds on VCAM-1 expression[21]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	3	**19**	10
2	NE	**20**	10
3	15	**21**	15
4	15	**22**	9
5	11	**23**	10
6	NE	**24**	10
7	NE	**25**	7
8	7	**26**	4
9	10	**27**	8
10	5	**28**	18
11	NE	**29**	5
12	NE	**30**	19
13	5	**31**	8
14	17	**32**	NE
15	20	**33**	5
16	40	**34**	7
17	11	**35**	6
18	15	**36**	30

In our search for small molecule inhibitors of inducible VCAM-1 expression, we discovered a class of nitrobenzene compounds out of a commercial combinatorial library. The inhibiting activities on VCAM-1 expression of these compounds are shown in Table 1. In this Letter, we report on the SAR studies of these compounds.

Compound **1**[22] was one of our initial hits. It inhibited VCAM-1 expression potently ($IC_{50} = 3$ μM). As shown in Figure 1, we set out to perform SAR studies around this compound. First, we wanted to find out whether the sulfonamide group was essential. Its removal led to an inactive compound (**2**). However, when it was replaced by an aldehyde (**3**), ketone (**4**), or nitrile (**5**) the resulting compound maintained activity, though slightly lower. Therefore, the sulfonamide group was not essential, but an electron-withdrawing group on the phenyl ring might well be. Then we probed the importance of chlorine in compound **1**. Removal of the chlorine resulted in an inactive compound (**6**). Replacement of the chlorine with an amino group also lost the activity (**7**). However, compound **8**, though in a different setting than **1**, was active and showed that the chlorine of **1** could be replaced with a fluorine. Compounds **9** and **10**, both active as inhibitors of VCAM-1 expression, showed that a chlorine or fluorine was not essential. It could be replaced with an ester or nitro group, though at different positions. Finally, we looked at the nitro group of **1**. Its removal from **1** resulted in an inactive compound (**11**). When there is no nitro group in the molecule, even with two chlorines (**12** with **9**) the compound is inactive. Based on these studies, we concluded that a nitro group, together with a hetero double bond (such as a carbonyl) conjugated to the phenyl ring and a third electron-with-

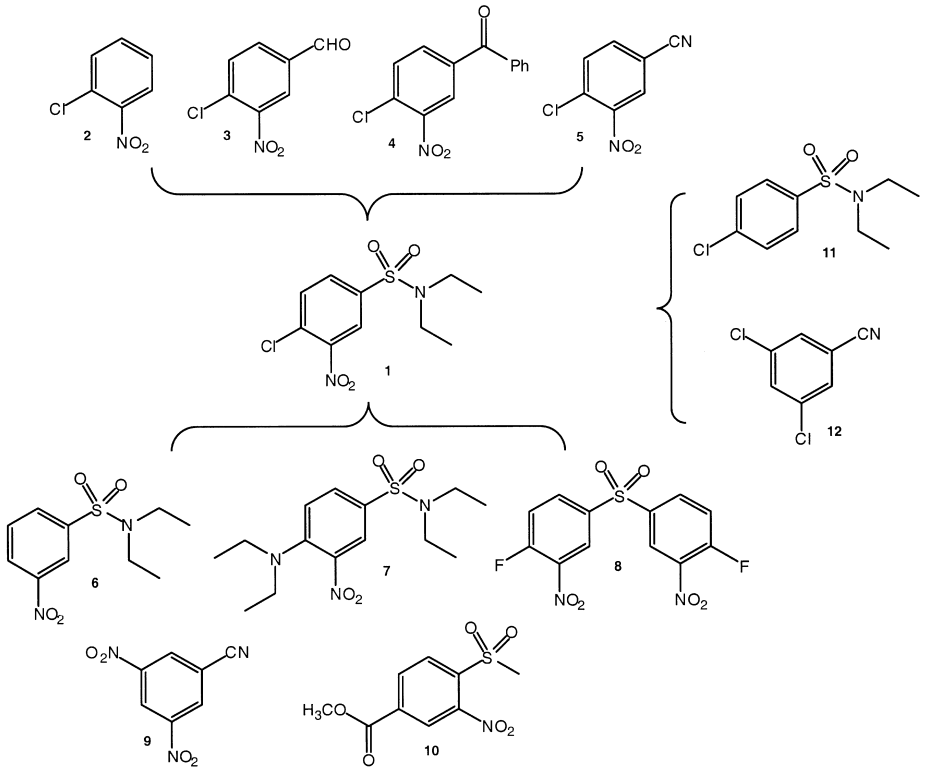

Figure 1.

drawing group were essential for these nitrobenzene compounds to be potent inhibitors of VCAM-1 expression.

We also found out, as shown in Figure 2, that the relative position of the nitro group to the other two groups was not crucial although it does make some compounds more potent than others. Notably, compound **13** with the nitro *ortho* to the nitrile is more potent than the ones with the nitro *meta* to the nitrile (cf. **13** with **5** and **14**). When a fourth electron-withdrawing group is introduced the potency dropped (cf. **9** with **15**). This suggested that a certain range of electrodensity on the phenyl ring is required for a compound to be a potent inhibitor of VCAM-1 expression.

The sulfonamide side chain could tolerate a variety of substitutions. As shown in Figure 3, the unsubstituted sulfonamide (**16**) was almost inactive. However, as long as there was at least one alkyl nitrogen substituent the compound was potent, although it seemed that *N*,*N*-dimethyl was the optimal substitution (**1**). An amino group on the side chain was tolerated (**27**), but a benzyl group lowered the potency (**28**).

A certain range of lipophilicity was required for a compound to be active. As shown in Figure 4, compound **29** was a potent inhibitor ($IC_{50} = 5\,\mu M$), but its corresponding diol, which is less lipophilic than the diether, was much less potent ($IC_{50} = 19\,\mu M$). Similarly, ester **31** was active while the corresponding acid (**32**) was inactive.

Besides compound **8** shown in Figure 1, we have discovered several other symmetrical nitro compounds as inhibitors of VCAM-1 expression. As shown in Figure 5, when the spacer between the two phenyl rings was short, whether it was a ketone (**33**) or disulfonamide (**34** and **35**), the compound was active. However, when the spacer is longer the potency dropped (**36**).

Some of the compounds that showed potent inhibition of VCAM-1 expression have been shown to have no effect on the expression of ICAM-1. Therefore, the compounds reported in this Letter are specific inhibitors of VCAM-1 expression. Special attention was paid to any cytotoxic effect of these compounds in the in vitro assay. We made sure that the observed inhibition of VCAM-1 expression was not due to cytotoxicity. Although a whole cell assay was used in our study we found that the numbers we got were quite tight, especially when the test compounds were compared to compound **1**, which we used as an internal standard. Some more functional assays are in progress for this and other classes of compounds in our laboratory to assess the impact of inhibition of VCAM-1 expression.

The nitro group in some compounds is known to cause marrow depression, cancer, etc.[23] However, it plays an important role in the action of certain drugs and the risk of side effects is small compared to the benefit gained in the treatment of acute illness.[23] Tolcapone, a nitrobenzene compound, was approved for chronic use in 1997.[24] The molecular mechanism of action of the

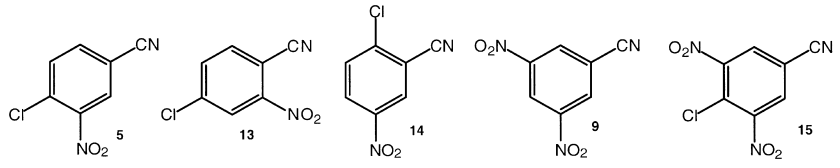

Figure 2.

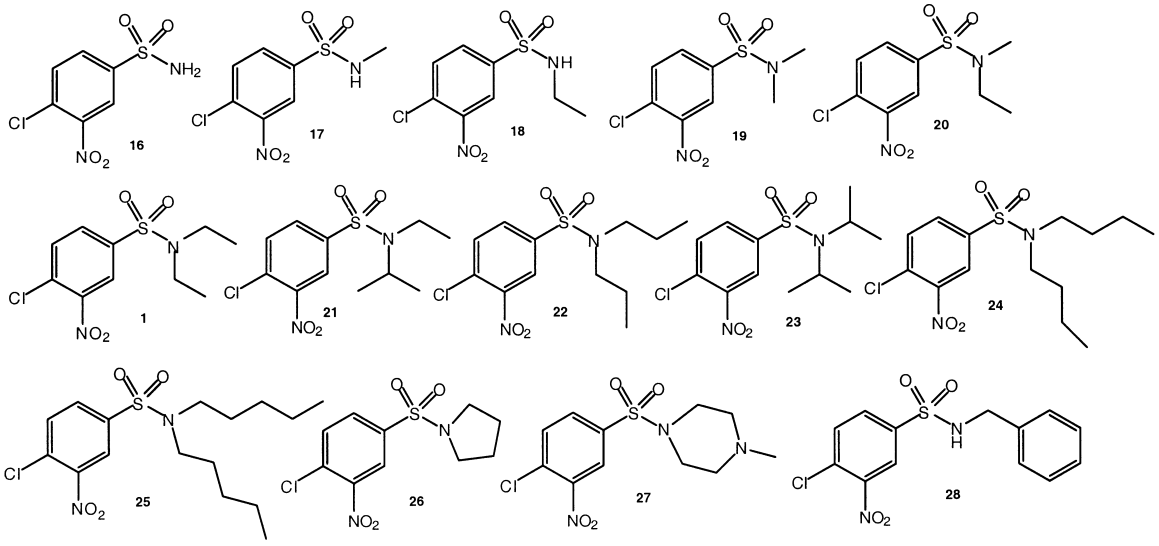

Figure 3.

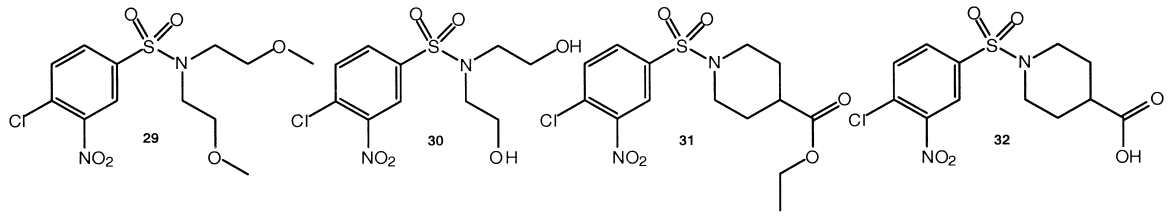

Figure 4.

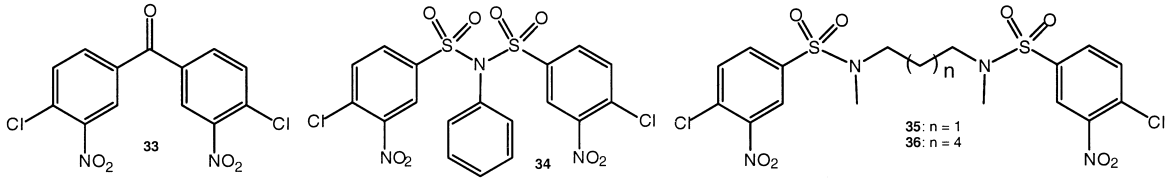

Figure 5.

nitrobenzene compounds we reported here with regard to the inhibition of VCAM-1 expression is not clear, although an electron transfer process involving the nitro group is likely. Also whether these compounds as a group or individually will cause side effects remains to be determined.

In summary, we have discovered a class of nitrobenzene compounds as potent inhibitors of VCAM-1 expression. Although animal studies continue to provide encouraging evidence that selective inhibition of VCAM-1 mediated adhesion is a realistic strategy in the treatment of autoimmune and allergic inflammatory diseases, clinical trials will ultimately determine whether a single anti-adhesion approach by blocking VCAM-1 is effective or proves to be inadequate because of biologic redundancy involving other important pathways.

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21. "NE" stands for "no effect." General protocol used to test compounds for inhibition of inducible VCAM-1 expression: Human aortic endothelial cells (HAEC) ($\leq$passage 9) were plated on gelatin-coated 96-well tissue culture dishes. The cells were incubated at 37 °C overnight in media + 5% FBS until they were 90–95% confluent. Compounds, suspended in DMSO, and 100 U/mL TNF-α were added to the cells in media + 5% FBS and incubated overnight ($\sim$16 h). The cells were then washed with 1:1 HBSS/PBS (wash buffer) and incubated with anti-VCAM-1 monoclonal antibody diluted in 5% FBS wash buffer solution. The antibody solution was aspirated and the cells washed. Wash buffer was replaced with anti-mouse IgG/HRP-conjugate diluted in 5% FBS wash buffer solution and incubated with the cells. The conjugate solution was aspirated and the cells washed. TMB substrate was then applied to the cells. When adequate color had developed the reaction was stopped with 2 N sulfuric acid. Spectrophotometric readings were then taken at wavelength 450 nm.
22. Compounds reported in this Letter were either commercially available or prepared in the following manner: 4-

Chloro-3-nitrobenzenesulfonyl chloride (256 mg, 1 mmol) was dissolved in THF (10 mL). Diethylamine (0.2 mL, 2 mmol) and triethylamine (0.5 mL) were added and the mixture was stirred at room temperature over a weekend. It was poured into water (50 mL) and extracted with dichloromethane (2×50 mL). The organic phase was dried over sodium sulfate and evaporated. Chromatography (hexanes/ethyl acetate, 4:1) gave compound **1** (83 mg) as a yellow solid and compound **7** (177 mg) as a brownish solid. When 1 equiv of diethylamine is used compound **1** was the exclusive product and chromatography could be avoided.

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