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Inhibition of RANTES/CCR1-Mediated Chemotaxis by Cosalane and Related Compounds

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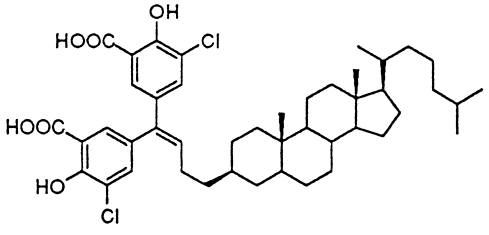
Abstract—The anti-HIV agent cosalane and several of its analogues inhibited RANTES-induced migration of human monocytes, but they did not inhibit migration induced by MIP1α or MIP1β. RANTES-induced migration of single receptor CCR1-HEK transfectants was also inhibited by the cosalanes. Acetylation of the reactive amino groups of RANTES abrogated the inhibitory activity of cosalane. The data suggest that cosalane and its structural analogues may interfere with the RANTES/CCR1 interaction by binding to RANTES.

The anti-HIV agent cosalane (**1**) inhibits both the binding of gp120 to CD4 as well as the fusion of the viral envelope with the cell membrane.[1] Since some chemokine receptors function as co-receptors with CD4 during HIV-1 infection,[2–7] it is possible that the mechanism of action of the cosalanes might involve interaction with the chemokine co-receptors. We therefore evaluated the ability of cosalane (**1**) and several of its derivatives to block chemokine-induced cell migration. The chemokines are 8–15 kDa secreted proteins that induce directional leukocyte migration (chemotaxis) by binding to seven transmembrane G-coupled protein receptors.[8] The chemokines and their receptors have been shown to participate in several disease states including allergy and asthma,[9,10] HIV-1 infection,[2–7] and angiogenesis during solid-tumor development.[11]

The compounds examined in the present study included cosalane (**1**), a series of benzyl ether derivatives **2**–**11**, and a number of amino acid conjugates **12**–**16**. Syntheses of the carboxybenzyl derivatives **2**–**5**, the *m*-nitrobenzyl analogue **7**, and the biphenyl derivative **6** from cosalane (**1**) and protected benzyl bromides were previously reported,[12–14] and the synthesis of the benzyl congener **11** was performed by straightforward modification of the published routes. The sodium salt **8** of the *p*-benzylboronic acid derivative was synthesized by benzylic bromination of 4,4,5,5-tetramethyl-2-*p*-tolyl-1,3,2-dioxaborolane (**17**)[15] with NBS in CCl_4 in the presence of benzoyl peroxide at reflux for 24 h to afford the corresponding benzyl bromide **18**, followed by reaction of **18** with cosalane (**1**) in DMF with potassium carbonate as the base at room temperature for 24 h to yield intermediate **19**. Treatment of **19** with K_2CO_3 in aq EtOH, followed by Na_2CO_3 in aq EtOH, provided the desired product **8**. The corresponding *meta* and *ortho* boronic acid salts **9** and **10** were synthesized similarly. The syntheses of the amino acid conjugates **12**–**14** and **16** have also been reported, and **15** was obtained by similar peptide chemistry.[16]



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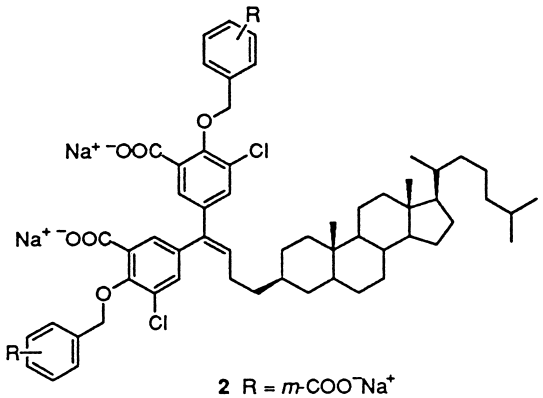
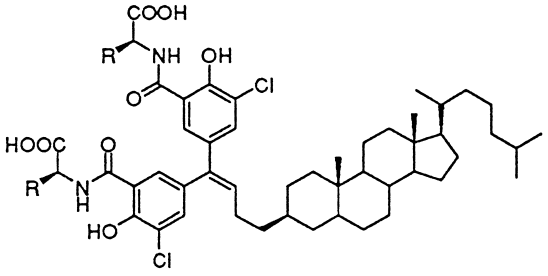
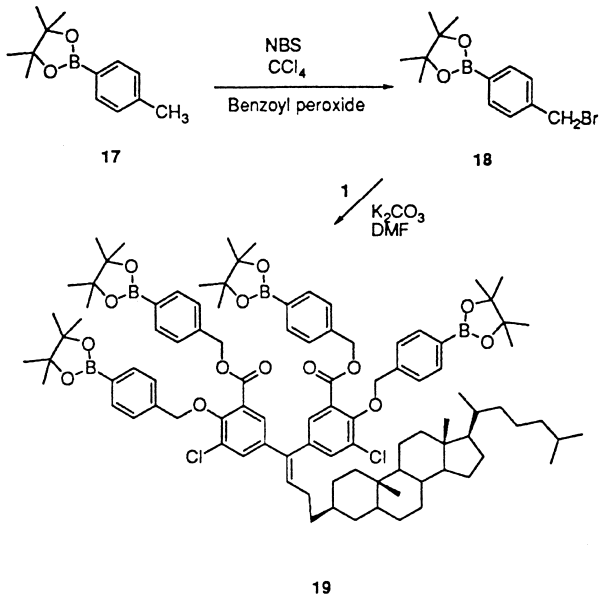


2 R = *m*-COO^-Na^+
3 R = *o*-COO^-Na^+
4 R = *p*-COOH
5 R = *p*-COO^-Na^+
6 R = *p*-(*m*-PhCOOH)
7 R = *m*-NO_2
8 R = *p*-$BO_2H^-Na^+$
9 R = *m*-$BO_2H^-Na^+$
10 R = *o*-$BO_2H^-Na^+$
11 R = *o*-OCH_3, *m*-COOH



12 R = H
13 R = CH_2COOH
14 R = CH_2CH_2COOH
15 R = $CH_2CH_2SCH_3$
16 R = CH_2Ph



Scheme 1.

The initial hypothesis to be tested was that cosalane (**1**) would bind to chemokines and thereby inhibit chemokine-induced cell migration. The ability of cosalane (**1**) to block SDF-1α (a CXCR4 ligand) and RANTES (a CCR1, CCR3, and CCR5 ligand) induced migration of human monocytes or lymphocytes was therefore examined. Monocyte chemotaxis assays were performed by resuspending cells in RPMI-1640 media containing 1% BSA, 25 mM HEPES at pH 8.0, at a concentration of 1–2×10^6 cells/mL. Chemokines, diluted in binding media, were mixed with cosalane (**1**) or one of the cosalane analogues **2**–**16** and placed in the lower well of a microchemotaxis chamber (Neuro Probe, Gaithersburg, MD). Five micrometer polyvinyl-free polycarbonate membranes were placed over the chemokine mixtures. After the micro-chemotaxis chamber was assembled, 50 μL of cells were placed in the upper wells. The filled chemotaxis chambers were incubated in a humidified CO_2 incubator for 90 min. After incubation, the membranes were removed from the chemotaxis chamber assembly followed by gently removing cells from the upper membrane. The cells on the lower side of the membrane were stained, and the number of cells migrating was determined for binding media alone, with added chemokine, and with cosalane-treated and untreated cells. For chemotaxis assays involving HEK-293 cells, the cells were transfected to express human CCR1, CCR3, or CCR5 and were suspended in binding media at 7.5×10^5 cells/mL. Ten micrometer polyvinyl-free polycarbonate membranes (Neuro Probe) were treated with 47 μg/mL of rat tail collagen type I (Collaborative Biomedical Products, Bedford, MA) in RPMI-1640 overnight, dried and placed over the chemoattractants. The assembled chemotaxis chambers were incubated in a humidified CO_2 incubator for 5.5–6.0 h. After incubation, the membranes were removed from the chemotaxis chamber assembly, followed by gently removing the cells from the upper side of the membrane. The stained and dried membrane was mounted and the cells on the underside of the membrane were counted at 200× magnification. The results are reported as the average number of cells per high powered field at a given chemokine concentration ± standard deviation.

We initially evaluated the ability of the cosalane analogues **3** and **4** to block SDF-1α (a CXCR4 ligand) and RANTES (a CCR1, CCR3, and CCR5 ligand) induced migration of human monocytes or lymphocytes (Fig. 1). In these experiments, the cosalane analogues **3** and **4** were mixed with either RANTES of SDF-1α prior to their being placed in the lower wells of the chemotaxis chamber. Both **3** and **4** were screened at a concentration of 1 μM. The results in Figure 1 show that the bis(*o*-carboxybenzyl) compound **3** was an effective inhibitor of both RANTES-induced and SDF-1α-induced monocyte migration, but the bis(*p*-carboxybenzyl) analogue **4** was inactive. These relative potencies are the reverse of what was previously seen in anti-HIV assays in which compound **4** proved to be an inhibitor of fusion and attachment.[13]

This initial observation led us to evaluate the ability of other CCR1, CCR5 ligands to be inhibited by the cosalane analogues **2**, **3**, **4**, and **5**. Surprisingly, neither MIP1α-

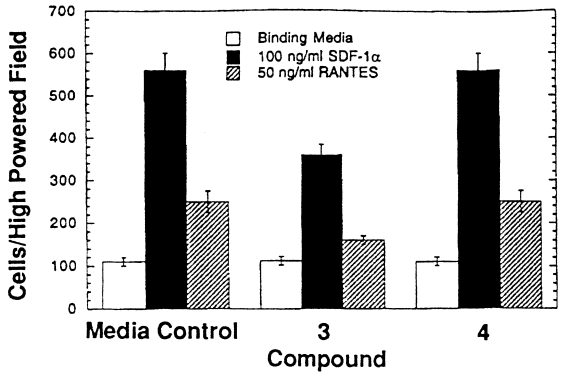


Figure 1. Inhibition of human lymphocyte migration induced by RANTES and SDF1-α.

nor MIP1β-induced monocyte migration was inhibited by compounds **3**, **4**, and **5**, while congener **2** proved to be a very weak inhibitor of MIP1β that induced less than 10% reduction in cell numbers. MIP1α migration was not reduced by compound **2** (data not shown). Both MIP1α and MIP1β are ligands for CCR1, CCR5, and CCR10.[8] This led us to investigate which RANTES-chemokine interaction was being blocked by compound **3**.

We tested the ability of compound **3** to inhibit RANTES-induced migration of human embryonic kidney (HEK) cells transfected to express individual chemokine receptors. The cosalane analogue **3** (1 μM) had no effect in RANTES-induced migration of CCR5 transfectants, but it did inhibit 50% of RANTES-induced CCR1 transfectant migration (Fig. 2). In contrast, the MIP1α- and MIP1β-induced migration of CCR1 transfectants was not inhibited by compounds **1**, **2**, or **3** when tested at concentrations of 1 μM. Overall, these studies suggested that the cosalane analogue **3** was acting as a rather selective inhibitor of the RANTES–CCR1 and SDF-1α–CXCR4 interactions, since experiments with a variety of other chemokines and chemokine receptors were largely negative.

Cosalane (**1**) and a wider range of cosalane analogues **2**–**16** were then evaluated as inhibitors of both RANTES-induced monocyte migration and RANTES-induced CCR1/HEK migration. The results of these studies are

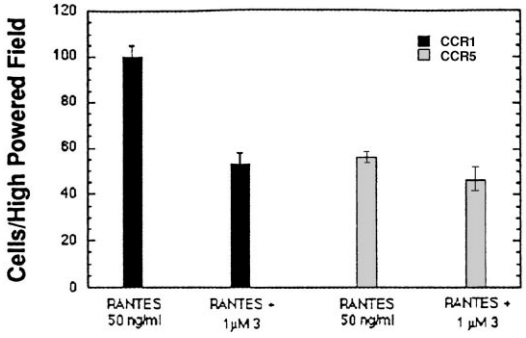


Figure 2. Cosalane analogue **3** inhibits RANTES-induced CCR1/HEK-293 transfectant migration but not RANTES-induced CCR5/HEK-293 transfectant migration.

listed in Table 1. Considering the benzyl-substituted analogues **2**–**11** first, the data show that although some of them (**2**, **3**, **6**, **7**, and **9**) do retain activity as inhibitors of RANTES-induced migration, none of them offer a distinct advantage in potency over cosalane (**1**) itself, and five of the analogues (**4**, **5**, **8**, **10**, and **11**) were inactive. Of the three boronic acid derivatives **8**–**10**, only the *meta*-substituted compound **9** was active, and the corresponding *meta* carboxy analogue **2** and the *meta* nitro compound **7** were also active. Considering the fact that the active analogues all have similar potencies, it seems that the benzyl substituents do not contribute any critical structural elements that are recognized by the receptor, although some of the substituents are simply not tolerated.

Of the amino acid derivatives, only the glycine conjugate **12** and the methionine derivative **15** retained activity, while the aspartic acid analogue **13**, the glutamic acid congener **14**, and the phenylalanine compound **16** were inactive. Although the glycine derivative **12** was slightly more potent than cosalane (**1**) versus RANTES-induced monocyte migration, it was less potent as an inhibitor of CCR1/HEK transfectant migration.

In order to gain some insight into the possible mechanism of action of the cosalanes as inhibitors of the RANTES/CCR1 interaction, RANTES (10 μL of a 1 μg/mL solution) was acetylated with sulfo-*N*-hydroxysuccinimide acetate (8 μL of a 1 μg/μL solution made in 10 mM sodium acetate buffer, pH 4.0, and then diluted with 2 μL of 10 mM sodium acetate buffer, pH 4.0) at room temperature for 30 min. Although the acetylated protein in the concentration range of 50–1000 ng/mL retained the ability to attract monocytes, the activity of the modified RANTES was not inhibited by either 30 μM cosalane (**1**) or by 10 μM **3**. This seems to indicate that cosalane (**1**) and its analogue **3** are binding to RANTES and not to CCR1. Ongoing studies are attempting to

Table 1. Inhibition of RANTES-induced monocyte and CCR1/HEK migration by cosalanes[a]

Compound[b]	IC_{50} (μM) RANTES-induced monocyte migration	IC_{50} (μM) RANTES-induced CCR1/HEK migration
1	0.76	0.26
2	10	10
3	3.9	0.26
4	NA[c]	NT[d]
5	NA[c]	NA[c]
6	3.0	9.1
7	3.5	5.7
8	NA[c]	NT[d]
9	2.2	4.0
10	NA[c]	NT[d]
11	NA[c]	NT[d]
12	0.37	10
13	NA[c]	NT[d]
14	NA[c]	NT[d]
15	0.82	12.7
16	NA[c]	NT[d]

[a]There was ≤5% standard error in the IC_{50} values.
[b]See refs 1, 13, 14, and 16 for anti-HIV activities.
[c]Not active.
[d]Not tested.

map the location of the acetyl group(s) in RANTES, since this might indicate where the cosalanes are binding.

The involvement of CCR1, RANTES, and MIP1α in multiple sclerosis and rheumatoid arthritis has stimulated interest in the design and synthesis of small molecule CCR1 antagonists. Recent studies published by Ng et al. and by Liang et al. have documented a series of CCR1 antagonists that inhibit the binding of MIP1α, MCP-3, and RANTES.[17,18] In contrast, the present series of compounds inhibit the RANTES–CCR1 interaction, but not the MIP1α interaction, and are therefore complementary in activity to the prior series of CCR1 antagonists. Hypothetically, the difference in selectivity between the present series and the prior series could reflect binding to RANTES versus binding to CCR1, respectively.

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