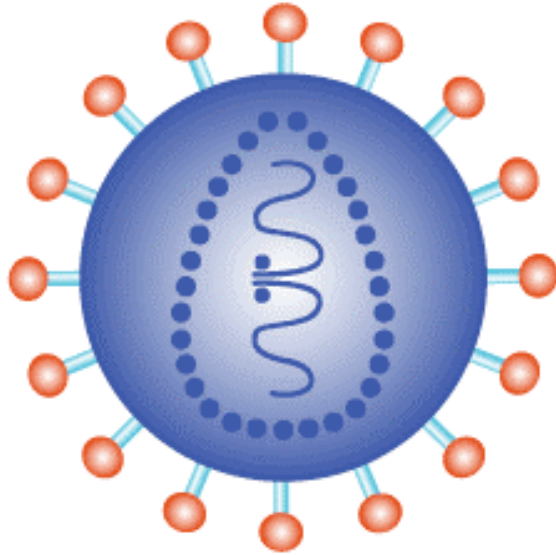


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*on Retroviruses and
Opportunistic Infections*

NOVEL LOW MOLECULAR WEIGHT SPIRODIKETOPIPERAZINE DERIVATIVES POTENTLY INHIBIT R5 HIV-1 INFECTION THROUGH THEIR ANTAGONISTIC EFFECTS ON CCR5



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February 24-28, 2002

Washington State Convention & Trade Center
Seattle, WA

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9th Conference

Background: HAART has brought about a significant impact on the AIDS epidemic, however, the eradication of HIV-1 is currently impossible. The emergence of drug resistant HIV-1 variants and a number of inherent adverse effects exacerbate the limited efficacy of antiviral therapy of AIDS. Approximately 1% of Caucasians have a gene encoding a mutant form of CCR5 termed $\Delta 32$, known to contribute to their resistance to HIV-1 infection, which possibly renders CCR5 an attractive target for possible intervention of HIV-1 infection.

Methods: We employed two methods in the search of lead compounds: a chemokine binding assay and assays for inhibition of cytosolic Ca^{2+} mobilization. Anti-HIV-1 activity of the compounds was determined using the MAGI assay employing CCR5⁺ MAGI cells and p24 assay using PBM (See Chart).

Chart

SEARCH FOR CCR5 INHIBITORS:

Chemokine Binding Assay

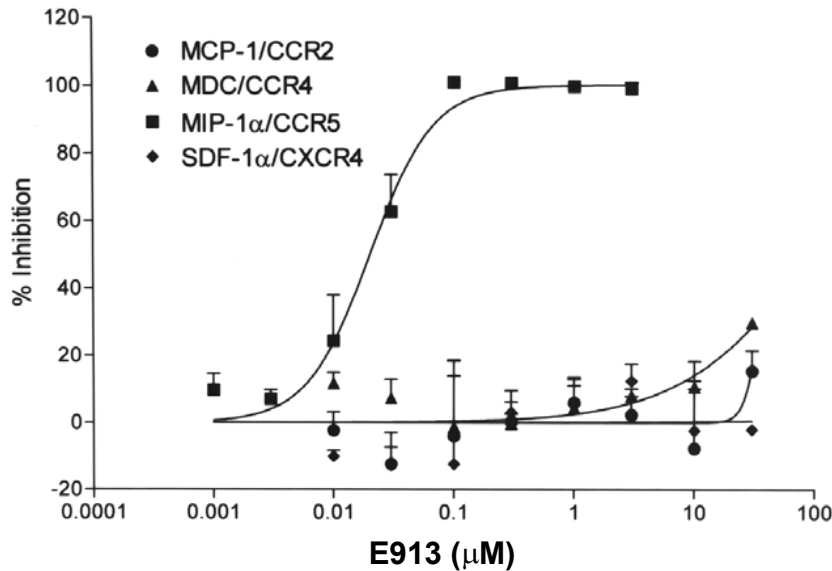
The inhibition by test compounds of the [125 I] -labeled MIP-1 α binding to CCR5-CHO cells was determined.

Assays for Inhibition of Cytosolic Ca $^{2+}$ Mobilization

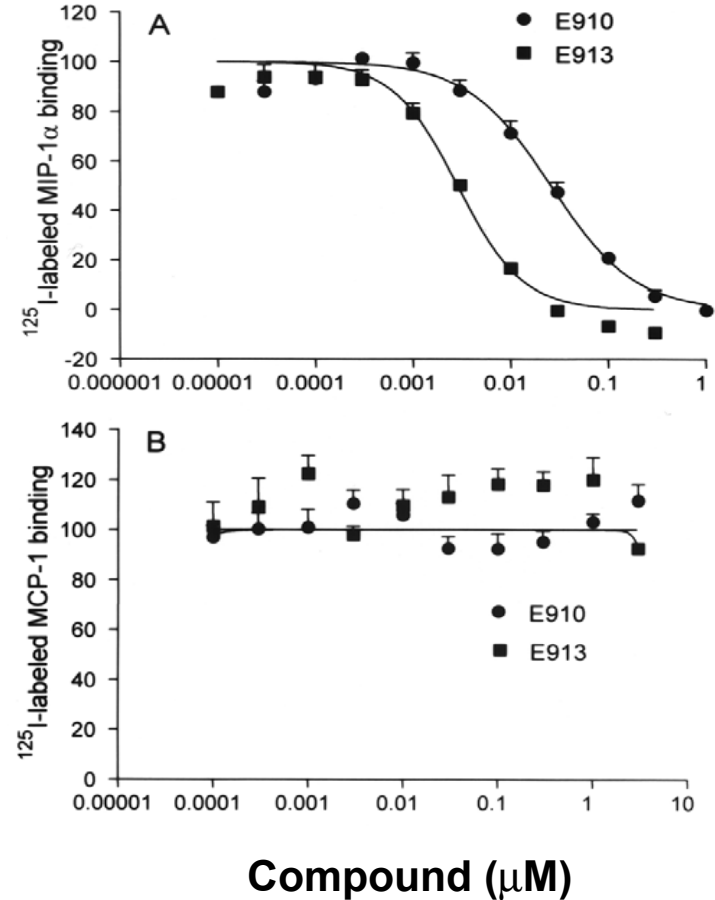
The inhibition by test compounds of the cytosolic Ca $^{2+}$ mobilization elicited by various chemokines was determined.

Agents which induce chemotaxis or Ca $^{2+}$ mobilization (agonists) were excluded.

1. Agents inhibiting MIP-1 α -driven cytosolic Ca²⁺ flux



2. Agents blocking MIP-1 binding



1. E913 blocks MIP-1 α -induced intracellular Ca²⁺ mobilization but fails to block MCP-1, MDC and SDF-1-induced intracellular Ca²⁺ mobilization. E913 blocked MIP-1 α -induced intracellular Ca²⁺ mobilization in CCR5-CHO cells, but failed to block Ca²⁺ mobilization induced by MCP-1, MDC, or SDF-1 in CCR2-CHO, CCR4-CHO and CXCR4-CHO cells.

2. Inhibition of MIP-1 α binding to CCR5 and MCP-1 binding to CCR2 by E910 and E913. CCR5-CHO cells and CCR2-CHO cells were exposed to 0.1 nM [¹²⁵I]-labeled MIP-1 α (Panel A) and 0.1 nM [¹²⁵I]-labeled MCP-1 (Panel B), respectively, and incubated for 40 min in the presence of increasing concentrations of E910 or E913. The results shown are the mean values (\pm S.D.) from three independent assays.

Table 1.

• *Activity to Block Chemokine Binding and Chemokine-Elicited Ca²⁺ Mobilization*

| • • • • • | • Binding assay | | Ca ²⁺ assay | | | |
|-----------|--------------------------|---------|--------------------------|---------|--------|------|
| | (I C ₅₀ , μM) | | (I C ₅₀ , μM) | | | |
| | Compound | M IP-1α | M CP-1 | M IP-1α | M CP-1 | M DC |
| E 910 | 0 .032 | > 30 | 0 .120 | 17 | 17 | 2 4 |
| E 913 | 0 .002 | > 30 | 0 .024 | > 30 | > 30 | > 30 |
| E 916 | 0 .007 | > 30 | 0 .070 | > 30 | > 30 | > 30 |
| E 917 | 0 .009 | > 30 | 0 .079 | > 30 | > 30 | > 30 |

Table 2.

Activity of SDP derivatives against Laboratory HIV-1 in CCR5⁺, CXCR4⁺ MAGI Cells

| Compound | IC ₅₀ (μM) | | |
|-------------|----------------------------|---------------------------|-------------|
| | HIV-1 _{Ba-L} (R5) | HIV-1 _{LAI} (X4) | |
| E910 | >1 | >1 | 20 |
| E913 | 0.033 | >1 | 1200 |
| E916 | 0.073 | >1 | 50 |
| E917 | 0.059 | >1 | 70 |
| AMD-3100 | >1 | 0.001 | • >10000 |
| AZT | • 0.058 | 0.076 | >10000 |

MAGI assay, SI: selectivity index

Table 3.

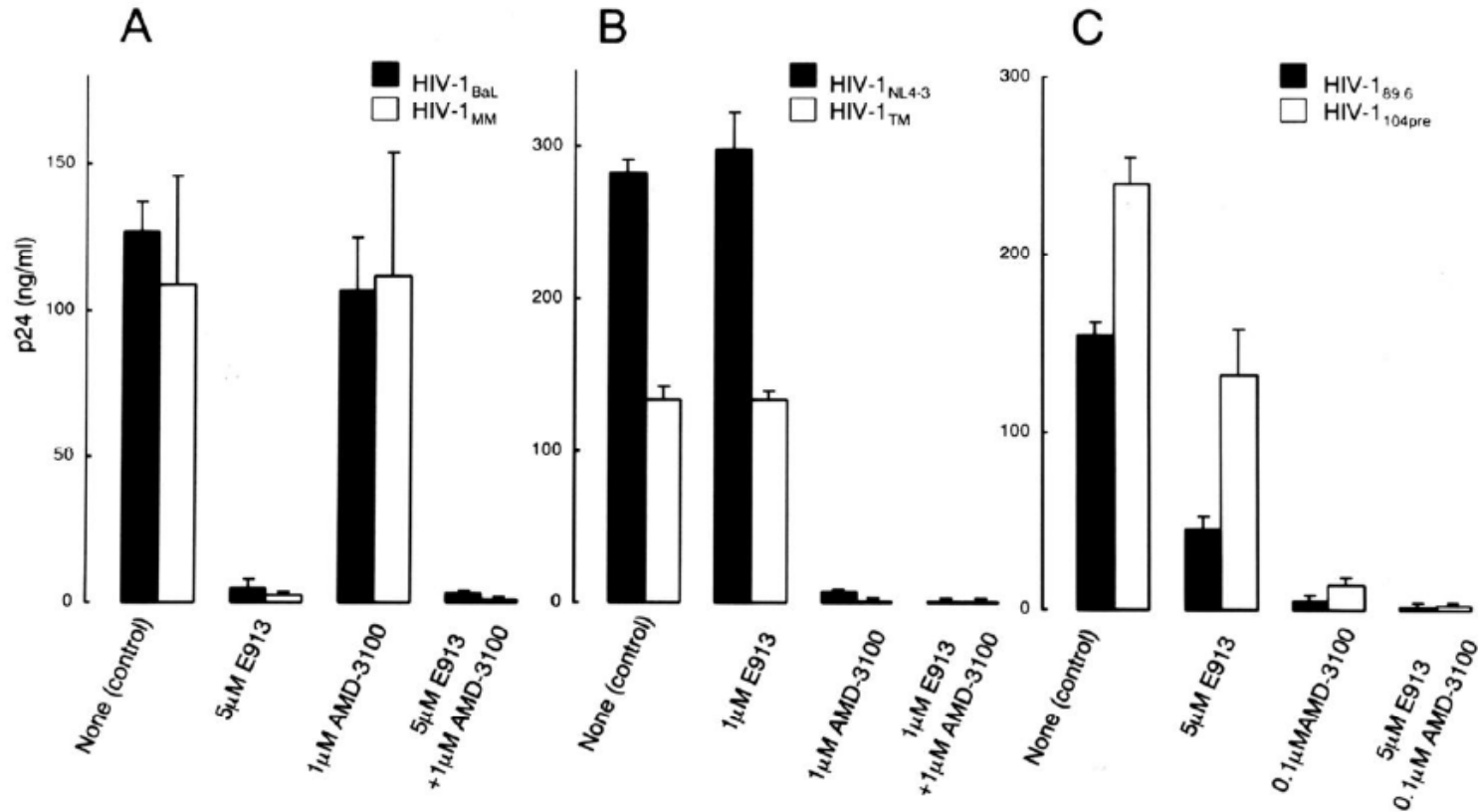
Activity of E913 against Clinical HIV-1 Isolates in PHA-PBM

| | IC ₅₀ (μM) | | |
|-------------|-------------------------|-------------------|-------------------|
| | R5 _{wild type} | R5 _{MDR} | X4 _{MDR} |
| AZT | 0.001 (1x) | 0.041 (41x) | 0.098 (98x) |
| ddl | 0.482 (1x) | 3.9 (8x) | 2.5 (5x) |
| NFV | 0.014 (1x) | >1 (>71x) | >1 (>71x) |
| E913 | 0.040 (1x) | 0.048 (1x) | >1 |
| AMD-3100 | >1 | >1 | 0.008 |

p24 assay, MDR: multi-drug resistance

Figure 2.

NO ANTAGONISTIC EFFECTS WHEN E913 WAS COMBINED WITH AMD-3100



Effects of E913 combined with AMD-3100 on the replication of R5, X4, and dualtropic HIV-1.

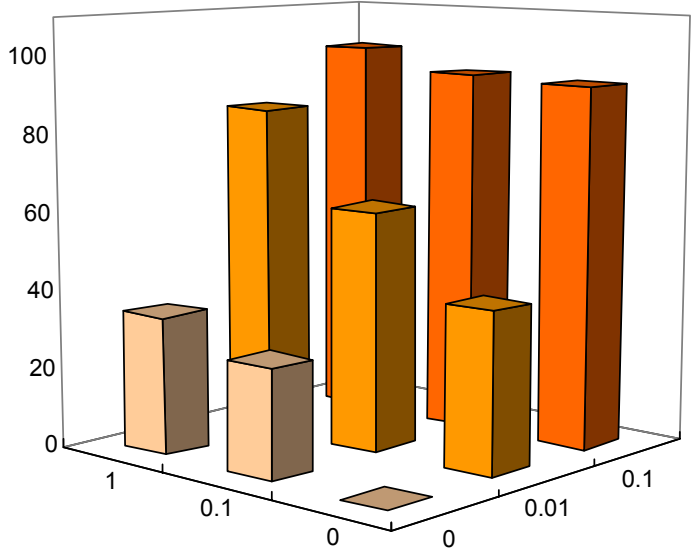
Panel A: E913 (5 μM) completely blocked R5 HIV-1 replication but AMD-3100 (1 μM) totally failed, and no obvious antagonistic effect was seen. Panel B: AMD-3100 (1 μM) completely blocked X4 HIV-1 replication while E913 (1 μM) totally failed, and no obvious antagonistic effect was seen. Panel C: E913 (5 μM) and AMD-3100 (0.1 μM) partially blocked the replication of dualtropic HIV-1, while the combination of E913 and AMD-3100 completely suppressed its replication.

Figure 3.

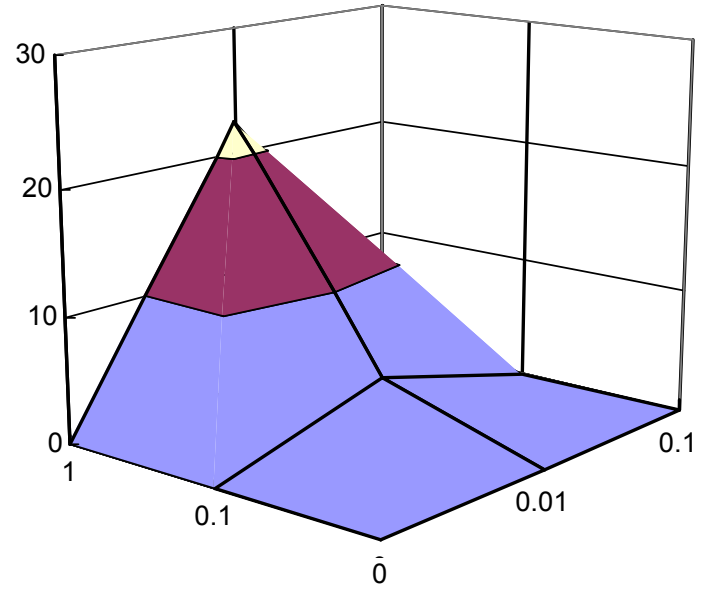
SYNERGISM WITH E913 AND AMD-3100

A. HIV-1_{89.6} (dual-tropic)

Anti-HIV-1 activities
in p24 assay



Combination Effects



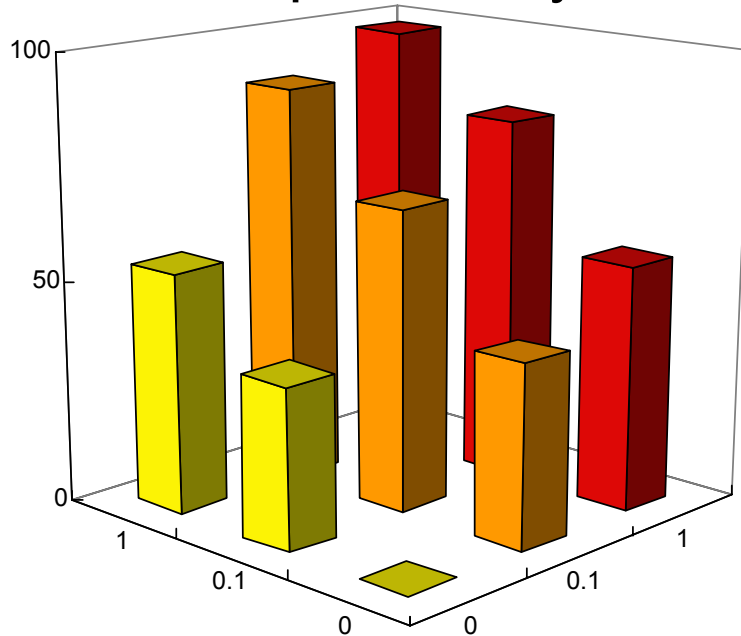
Concentration (μM)

Effects of E913 combined with AMD-3100 on the replication of dualtropic HIV-1 and mixed HIV-1 populations. Panel A: E913, combined with AMD-3100, effectively blocked the replication of dualtropic HIV-1_{89.6} (50 TCID₅₀)(left). The antiviral activity of the combined drugs was analyzed using the method by Prichard *et al.* (right) and found to be synergistic.

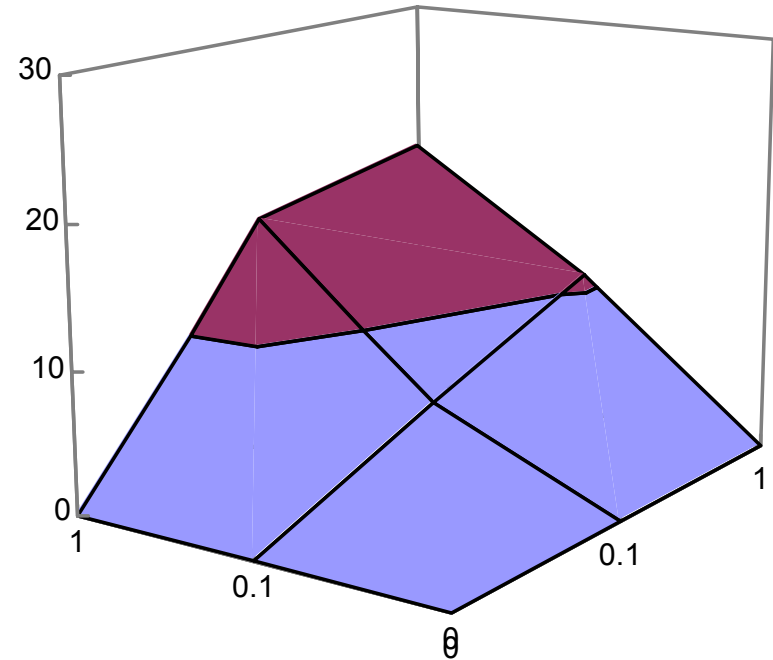
SYNERGISM WITH E913 AND AMD-3100

B. HIV-1_{Ba-L} : HIV-1_{NL4-3} = 1 : 1

Anti-HIV-1 activities
in p24 assay



Combination Effects



Concentration (μM)

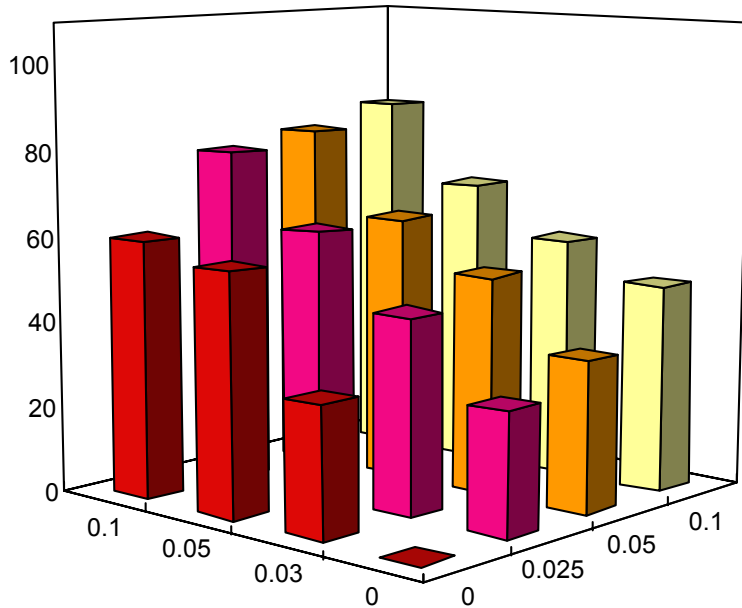
Panel B: E913, combined with AMD-3100, completely blocked the replication of the 50:50 mixture of R5 HIV-1_{BaL} (25 TCID₅₀) and X4 HIV-1_{NL4-3} (25 TCID₅₀) (left). The antiviral activity of the combination was also synergistic (right).

Figure 4.

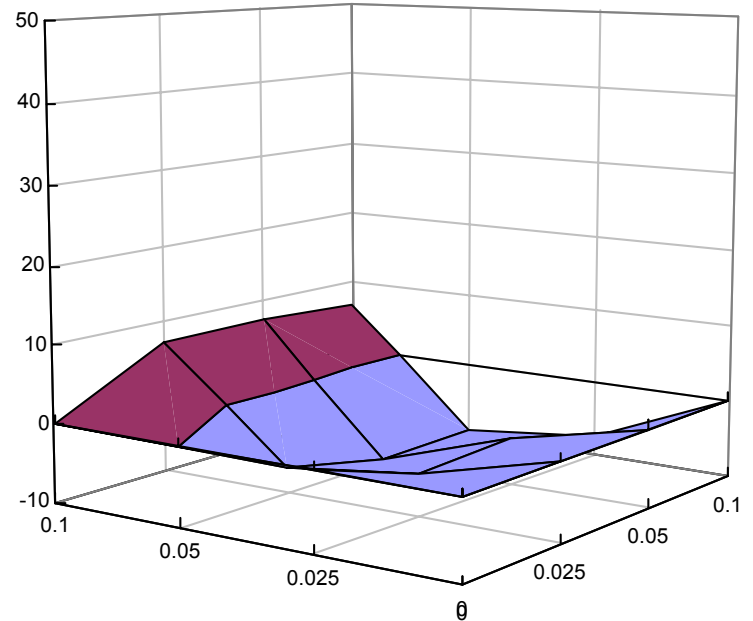
ADDITIVISM WITH E913 & AZT

HIV-1 BaL

Anti-HIV-1 activities
in MAGI assay



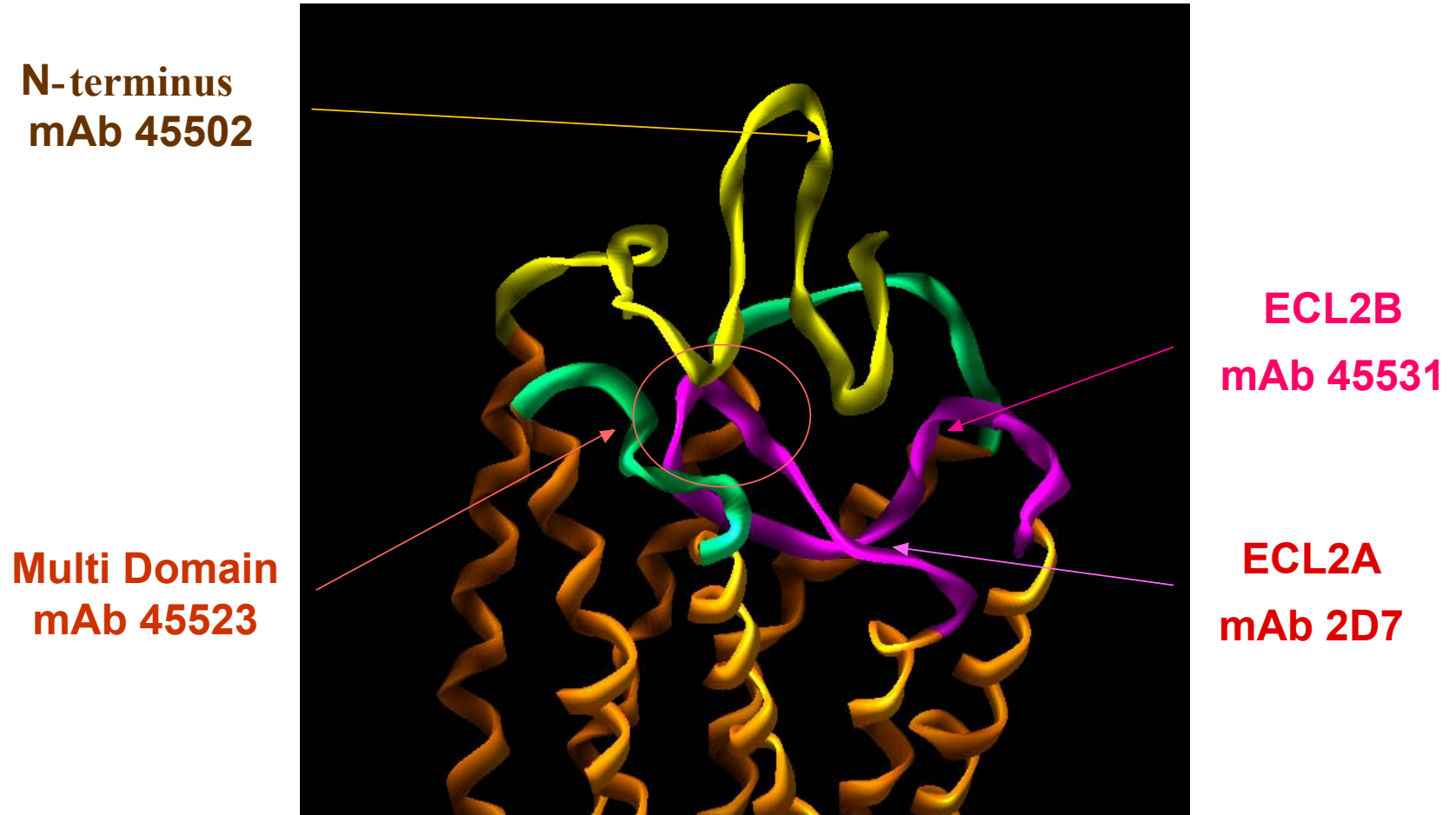
Combination Effects



Concentration (μM)

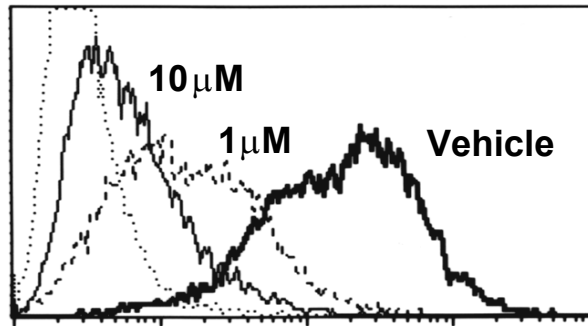
Figure 5.

Anti-CCR5 mAb Binding Sites



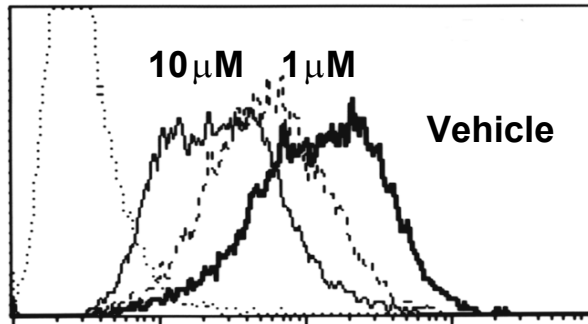
**CCR5 Model Based on the
Bovine Rhodopsin Structure**

E913 Inhibits mAb Binding to CCR5



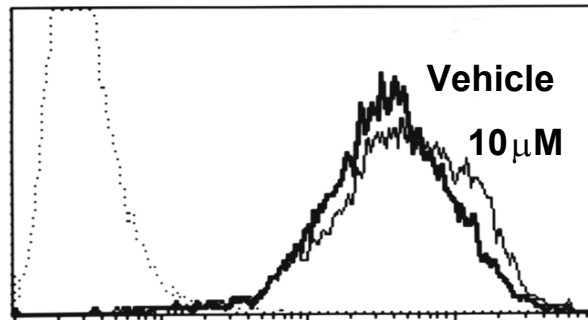
45523

multi domain



45531

ECL2B



2D7

ECL2A

E913 binds to the domain B of the second extracellular loop of CCR5 (ECL2B).

E913 competitively blocked the binding of two monoclonal antibodies, 45523 reactive against multidomain epitopes of CCR5 and 45531 specific for ECL2B of CCR5. Note that there was no E913 inhibition of the binding of a monoclonal antibody 2D7 which binds to the domain A of the second extracellular loop (ECL2A) of CCR5.

Results: Low molecular weight spirodiketopiperazine derivatives (Figure 1) which potently inhibit R5 HIV-1 were identified. • One such compound E913 (*Mr.* 484) specifically blocked the binding of MIP-1 α to CCR5 (IC₅₀: 0.002 μ M) and MIP-1 α -elicited cellular Ca²⁺ mobilization (IC₅₀: ~0.02 μ M) (Table 1). E913 potently inhibited the replication of laboratory and primary R5 HIV-1 strains as well as various multi-drug resistant monocyte/macrophage-tropic (R5) HIV-1 at IC₅₀ values of 0.03 to 0.05 μ M (Tables 2 & 3). E913 was inactive against T cell-tropic (X4) HIV-1 (Figure 2); however, when combined with a CXCR4 antagonist AMD-3100, E913 potently and synergistically inhibited the replication of dualtropic HIV-1 (Figure 3-A) and a 50:50 mixture of R5 and X4 HIV-1 (Figure 3-B). Antagonism in anti-HIV-1 activity was not seen when E913 was combined with the reverse transcriptase inhibitor zidovudine (Figure 4). E913 competed with the binding of antibodies to CCR5 that recognize the C-terminal half of the second extracellular loop (ECL2B) of CCR5 (Figure 5).

Conclusion: These data warrant that spirodiketopiperazine derivatives should be further developed as potential therapeutics for HIV-1 infection.

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