Assays To Detect and Characterize Human Immunodeficiency Virus Type 1 (HIV-1) Receptor Antagonists, Compounds That Inhibit Binding of the HIV-1 Surface Glycoprotein, gp120, to the CD4 Receptor on Human T Lymphocytes

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Human immunodeficiency virus type 1 infects human helper T lymphocytes by an interaction between gp120, the viral coat protein, and the T-cell receptor CD4. Two microtitre-based immunoassays, an enzyme-linked immunosorbent assay (ELISA) and a particle concentration fluorescence assay, were developed to measure gp120-CD4 binding and were then used to screen a variety of compounds for the inhibition of this interaction. Additional protocols, called “consumption assays,” were defined to distinguish inhibitors which functioned by sequestering either gp120 or CD4 to prevent the final effective bimolecular interaction. Monoclonal antibodies of defined specificity and compounds known from other published studies to inhibit gp120-CD4 binding were tested in an attempt to validate the assays used in the study. Once the capacity of these assays to detect known gp120-CD4 inhibitors was confirmed, they were used to screen synthetic agents and fermentation broths for novel compounds that might be used as human immunodeficiency virus receptor antagonists. A 2,4-diaminoquinazoline, CP-101,816-1, was found to inhibit this interaction (50% inhibitory concentration in ELISA, 32.5 μg/ml) and to interact more strongly with CD4 than with gp120 in the consumption assays. The identification of a novel inhibitor, a 2,4-diaminoquinazoline, confirmed that such assays are useful for the detection of human immunodeficiency virus type 1 receptor antagonists.

A major target of human immunodeficiency virus type 1 (HIV-1) is the helper T lymphocyte, with HIV-1 entering the cell via the CD4 receptor that normally recognizes major histocompatibility complex class II antigens on antigen-presenting cells (7, 8). The viral surface glycoprotein gp120 interacts with this receptor, leading to virus-cell fusion and infection (9). In addition, infected cells express gp120 on their surfaces. These cells can fuse in turn with uninfected CD4-expressing T lymphocytes to form giant cells or syncytia. This is believed to contribute to the cytopathogenicity of HIV-1 in vitro and perhaps in vivo (2, 16). Thus, a potential target for the chemotherapy of HIV-1 infection is the gp120-CD4 interaction.

The availability of recombinant, soluble versions of gp120 and CD4 made possible the development of several acellular assays to model the gp120-CD4 interaction and of variations of these assays which predict the mechanism of action of low-molecular-weight inhibitors. By using such assays, a synthetic agent, a 2,4-diaminoquinazoline, was identified as a novel inhibitor of gp120-CD4 binding, and its mechanism of action was elucidated.

MATERIALS AND METHODS

Antiviral compounds and natural products. The L,L and D,D stereoisomers of N-carbethoxymethylcarbonyl-phenylalanylbenzyl ester [CPF(L)] and CPF(0), respectively, CP-101,816-1, and UK-22,233 were synthesized by conventional methods. Poly(sodium 4-styrenesulfonate) was purchased from Aldrich.

Bovine serum albumin (BSA; fraction V, fatty acid ultra free) was from Boehringer Mannheim. Pradimicin A was fermented and purified by the methods of Oki et al. (12), with some modifications. The sample used in the assays was >90% pure. Other compounds were from Sigma.

PCFIA to detect inhibitors of gp120-CD4 binding. A rapid and simple particle concentration fluorescence immunoassay (PCFIA) similar to that of McQuade et al. (10) was developed in which anti-gp120 monoclonal antibody (Mab) was bound to submicron-size polystyrene particles. This served as a solid phase to capture the gp120-CD4 receptor complex in the presence and absence of possible inhibitors. The complex was quantitated with fluorescein isothiocyanate (FITC)-labeled Okt4 (anti-CD4 Mab) in a fluorescence concentration analyzer.

Capture particles were prepared by binding an anti-gp120 mouse Mab (NEA-9284; NEN-DuPont) to polystyrene particles. NEA-9284 (NEN-DuPont) was dissolved in 0.1 M sodium phosphate buffer (pH 5.0) at 100 μg/ml. To 1.0 ml of antibody, 100 μl of 5.0% (wt/vol) polystyrene particle suspension (0.85-μm polystyrene particles; IDEXX) was added. The mixture was vortexed and incubated for 1 h at room temperature. The suspension was spun for 15 min at maximum speed in a microcentrifuge. The supernatant solution was removed, and the antibody-coated particles were washed with 2.0 ml of 0.1 M phosphate buffer. The particles were resuspended in 2.0 ml of phosphate-buffered saline (PBS) plus 1.0% BSA. Twenty microliters of test compound in PBS plus initial solvents was pipetted into a filter-bottomed microtiter plate (Pandex). Twenty microliters of PBS plus 1.0% BSA containing 0.05 pmol of recombinant gp120 (Celltech) was added. A total of 0.2 pmol of sCD4 (American BioTechnologies) in 20 μl of 1% BSA in PBS was also added. After incubating for 15 min...
at room temperature, 2.25 μl of a suspension containing ~0.75 pmol of anti-gp120 coupled to beads mixed with 7.75 μl of BSA-coated beads and 10 μl of BSA-BSA-PBS was added. The test mixture was incubated for 15 min, and 0.6 pmol of OKT4 conjugated with FITC (Ortho Diagnostics) diluted with 20 μl of PBS was added. The mixture was incubated for another 15 min. The protein-antibody conjugates bound to polystyrene beads were collected by vacuum filtration onto a cellulose acetate membrane in a fluorescence concentration analyzer (Pandex). Each well was washed twice with 50.0 μl of 0.05% Nonidet P-40 in PBS and aspirated to dryness. The contents of each well were irradiated at 485 nm, and the fluorescence yield was read automatically at 535 nm as arbitrary fluorescence units (AFU).

Percent inhibition was calculated by using the following formula:

\[
\text{Percent inhibition} = \frac{\text{AFU without compound} - \text{AFU with background}}{\text{AFU without compound} - \text{AFU with background}} \times 100
\]

Inhibition of gp120 binding to CD4 in that assay was shown by a decrease in fluorescence relative to that in control wells without inhibitors. Initial experiments established that the amount of bound gp120-CD4 complex was directly proportional to the fluorescence yield (data not shown).

ELISA to detect inhibitors of gp120-CD4 binding. An antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect inhibitors of gp120-CD4 binding was also developed. In that assay, mouse anti-CD4 MAbs (OKT4) was used to capture CD4-gp120 complexes formed in the presence and absence of inhibitors. Bound complexes were detected with a combination of sheep polyclonal anti-gp120 and rabbit anti-sheep immunoglobulin G (IgG) conjugated to alkaline phosphatase. Mouse anti-CD4 MAb OKT4 (Ortho Diagnostics) was dissolved in 0.05 M Tris (pH 7.4) at a final concentration of 1.0 μg/ml. A total of 100 μl of this solution was added to each well of a microtiter plate, and the plate was incubated with shaking at 4°C. After overnight incubation, each well was washed four times with 300 μl of 0.05 M Tris–0.15 M NaCl (pH 7.5; TBS). Each well was then blocked with 300 μl of 5% BSA (fraction V; fatty acid ultra free) in 0.05 M Tris (pH 7.4) and incubated at 37°C for 1 h. Each well was again washed four times with TBS containing 0.05% Tween 20.

gp120 (0.05 pmol) in 25 μl of TBS (1% BSA, 0.05% Tween 20, 0.05 M Tris [pH 7.4], 0.15 M NaCl) was mixed with 50 μl of putative inhibitor in each well. To this was added 0.2 pmol of scCD4 in 25 μl of TBS. This was incubated for 1 h at 37°C. The plate was washed four times with TBS-Tween.

Human anti-HIV-1 IgG (Epitope) was diluted 1:100 with TBS, and 100 μl was added to each well and the plate was incubated for 1 h at 37°C. The plate was washed as described above. Goat anti-human IgG conjugated to alkaline phosphatase (AP; Sigma) was diluted 1:2,000 with TBS, and 100 μl was added to each well. The plate was incubated for 1 h at room temperature and was washed as described above. AP activity was detected by using an immunoselect alkaline phosphatase amplification kit (BRL) according to the manufacturer's directions. Enzyme activity was measured by reading the A_{405} on a BioTek EL320 microplate reader.

Percent inhibition was calculated by using the following formula:

\[
\text{Percent inhibition} = \frac{\text{A}_{405} \text{without compound} - \text{A}_{405} \text{with background}}{\text{A}_{405} \text{without compound} - \text{A}_{405} \text{with background}} \times 100
\]

Increasing amounts of bound gp120-CD4 complex were visualized in the assay in direct proportion to an increase in the optical density at 490 nm (data not shown). Inhibition of gp120-CD4 binding was measured by a decrease in AP activity; in comparison, complete binding (and maximal AP activity) was found in microtiter wells that contained no inhibitor.

Consumption assays: ELISAs to distinguish gp120-CD4 antagonists that bind gp120 from those that bind CD4. The immunoassays shown schematically in Fig. 1 were developed to distinguish HIV-1 receptor antagonists that bind gp120 from those that bind CD4. To test gp120 consumption, gp120 was preincubated with inhibitor, unbound inhibitor was washed away, and the capacity of the putative inhibitor-gp120 complex to bind CD4 was quantitated. CD4 consumption was determined simultaneously as the capacity of this inhibitor to combine with CD4 and thereby block the CD4-gp120 interaction. Anti-CD4 or anti-gp120 MABs were used to "capture" putative inhibitor-CD4 and inhibitor-gp120 complexes, respectively. The wells were washed to remove unbound inhibitor, and the complementary ligand (gp120 to captured CD4 and CD4 to captured gp120) was added. The bound complexes were visualized by standard methods. The inhibitors that interacted with gp120 or CD4 in these assays so that they could not interact with their complementary ligand caused a dose-related decrease in the signal.

CD4 consumption assay. The mouse anti-CD4 MAB OKT4 was diluted to 3.0 μg/ml in 0.05 M Tris coating buffer (pH 7.4), and 100 μl of this was added to each well of a microtiter plate. After overnight incubation at 4°C, each well was washed four times with 300 μl of TBS and was blocked with 300 μl of 3% BSA at 37°C for 1 h. Each well was again washed four times with TBS containing 0.05% Tween 20.

To each well, 0.6 pmol of scCD4 in 50 μl of TBB mixed with either 50 μl of test compound or buffer was added. The plate was incubated at 37°C for 1 h. The plate was washed four times with TBS-Tween, and 0.3 pmol of gp120 was added to each well along with 100 μl of TBB. The plate was incubated for another hour at 37°C and was again washed four times.

Human anti-HIV-1 IgG (Epitope) was diluted 1:100 with TTB; 100 μl was added to each well, and the plate was incubated for 1 h at 37°C. The plate was washed as described above. Goat anti-human IgG conjugated to AP was diluted 1:2,000 with TTB, and 100 μl was added to each well. The plate was incubated for 1 h at room temperature and was then washed as described above. AP activity was detected and measured as described above. Percent inhibition was calculated as described above. Compounds that bound to and sequestered scCD4 caused inhibition of the signal when they were preincubated with CD4 but not with gp120 or either capture antibody.

gp120 consumption assay. The mouse anti-gp120 MAB NEA-9284 was diluted to 3.0 mg/ml in 0.05 M Tris coating buffer (pH 7.4), and 100 μl was added to each well of a microtiter plate. After overnight incubation at 4°C, each well was washed four times with 300 μl of TBS and was blocked with 300 μl of 3% BSA at 37°C for 1 h. Each well was again washed four times with TBS containing 0.05% Tween 20.

To each well, 0.3 pmol of gp120 in 50 μl of TBB mixed with 50 μl of test compound or buffer was added. The plate was incubated at 37°C for 1 h and was washed four times with TBS-Tween. A total of 0.6 pmol of scCD4 was added to each well along with 100 μl of TBB. The plate was incubated for another hour at 37°C and was washed four times.

Rabbit anti-CD4 polyclonal antisera (American BioTechnologies) was diluted 1:6,000 with TBB, 100 μl was added to each well, and the microtiter plate was incubated for 1 h at 37°C. The plate was washed as described above. Goat anti-
rabbit IgG conjugated to AP was diluted 1:16,000 with TTB, and 100 µl was added to each well. The plate was incubated for 1 h at room temperature and the optical density of each well was measured as described above. Percent inhibition was calculated as described above. The compounds that sequestered gp120 caused inhibition of the signal when they were preincubated with gp120, but not sCD4 or either capture antibody.

CCSAs. Cocultivation syncytium assays (CCSAs) were performed by using the CHO (pIII env3) cell line expressing gp120 and the human CD4-expressing lymphocytic cell line Sup T1 and by the procedures of Walker et al. (21).

RESULTS

Effect of blocking and non-specific MABs in gp120-CD4 ELISA and PCFIA. Mouse MAb OKT4A is directed at an epitope within the gp120-binding domain of the CD4 receptor. Binding of OKT4A to CD4 prevents gp120-CD4 interactions (9, 14). The capacity of OKT4A to act as a specific inhibitor in the PCFIA and ELISA demonstrates that these assays measure a specific interaction between CD4 and gp120. Conversely, the addition of LyT2, a mouse MAB with anti-CD8a specificity (6), produced no inhibition in the ELISA and only a slight inhibition of binding in the PCFIA (Fig. 2).

Activity of known inhibitors in gp120-CD4 binding assays. A number of polycarboxylic acid-like compounds, such as dextran sulfate (DS) and pentosan polysulfate (PPS), are known to be inhibitors of gp120-CD4 interactions (5). As expected, they were also potent inhibitors in the gp120-CD4 binding assays (Table 1). UK-22.233, a polymer of the chemical entity shown in Fig. 3A, was also potent in these assays. DS 5,000 and PPS were also inhibitory in the PCFIA assay and ELISA (Table 1). CPF(90) and CPF(11) were synthesized as potent anti-HIV agents whose mechanism of action was to sequester gp120 on the surface of the HIV-1 virion (3). These derivatives of the dipeptide prolylphenylalanine were tested in the gp120-CD4 ELISA and were shown to be relatively inactive, with 50% inhibitory concentrations (IC50s) of greater than 400 µg/ml (Table 1).

Consumption assays. The ability of the gp120 and CD4 consumption assays to distinguish inhibitors that sequester gp120 from those that sequester CD4 was demonstrated in experiments with two neutralizing MABs, OKT4A and NEA-9305 (4, 9, 14) (data not shown). OKT4A, an anti-CD4 MAB, produced up to 85% inhibition when it was preincubated with sCD4, but it was not inhibitory when it was preincubated with gp120 at comparable concentrations. NEA-9305, an anti-gp120 MAB, inhibited sCD4-gp120 binding in the consumption assays up to 80% when it was preincubated with gp120, but produced no inhibition when it was preincubated with CD4 at these same concentrations.

The dipeptide inhibitor CPF(11) has been reported to bind to gp120 and block its interaction with CD4, leading to a block of HIV-1 infectivity (3). We have shown that it is more active when it is preincubated with gp120 than with CD4 (Table 2), in agreement with the data of Finberg et al. (3).

Some anionic polysaccharides such as DS 500,000 seem to bind to CD4 (13), while others such as PPS bind more strongly to gp120 (15). In our assays, PPS interacted with gp120 but not sCD4 at doses as high as 50 µg/ml (Table 2).
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of compounds

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A

discovered.

of compounds

automated

(IC50,

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[2-(3,4-dimethoxy-phenyl)-ethyl]-amine[Fig. 3B)

FIG.

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IC50s

(Table 1).

The gpl20-CD4 ELISA and PCFIA were used as high-throughput screens to identify inhibitors of gpl20-CD4 binding. In general, compounds assayed for their inhibitory activities in the two assays had comparable IC50s (Table 1). However, those compounds that produced fluorescence when irradiated at 485 nm could not be assayed effectively in the PCFIA. One such compound was CP-101,816-1, which has aromatic ring components. In general, fluorescent compounds had to be evaluated in the ELISA, which has a colorimetric endpoint.

So-called consumption assays were developed to elucidate the mechanism of action of chemical moieties that inhibit the binding of gpl20 to its eucaryotic receptor. When specific MAb s with affinities for gpl20 or CD4 are used in the assays, they correctly differentiate the MAb s which sequester gpl20 from those that sequester CD4. Furthermore, LyT2, a MAb with binding affinity for neither ligand, was inactive in both consumption assays (data not shown). The behavior of the CD4 mimic CPF(1,1) in the consumption assays suggests that it interacts primarily with gpl20 (Table 2), as was shown previously (3). Studies with these known inhibitors confirm that the consumption ELISAs appear to be able to differentiate those inhibitors which function primarily by binding to the viral ligand gpl20 from those that inhibit by binding primarily to the eucaryotic receptor CD4.

Recent studies have demonstrated that sulfated polyanions are potent inhibitors of HIV-1 infection in vitro (1, 20). The mechanism of this inhibition has been analyzed in several laboratories. Schols et al. (15) studied the inhibition of binding of a neutralizing anti-gpl20 MAb to the surface of HIV-1.

FIG. 2. MAb s as inhibitors in the gpl20-CD4 ELISA and PCFIA. MAb OkT4A is specific for an epitope within CD4 (7, 11), and MAb LyT2 is specific for one within CD8a (6).

Pradimicin A is a natural product with anti-HIV properties (12, 17, 18). It was fermented and isolated from a culture of Actinomadura hibiscus and was shown to be an inhibitor of gpl20-sCD4 interactions in the ELISA (IC50, 27.5 μg/ml) and the gpl20 consumption ELISA, binding primarily to gpl20 (IC50, 9 μg/ml) (Tables 1 and 2).

Novel gpl20-CD4 antagonists. In the screening novel classes of compounds that have activity as gpl20-CD4 antagonists were discovered. A synthetic agent, CP-101,816-1 [2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-6,7-dimethoxy-quinoxalin-4-yl]-[2-(3,4-dimethoxy-phenyl)-ethyl]-amine (Fig. 3B) was found to inhibit the interaction of gpl20 with CD4 in the ELISA (IC50, 32.5 μg/ml; Table 1) and to interact more strongly with sCD4 than with gpl20 in the consumption assays (IC50, 110.5 μg/ml; Table 2).

DISCUSSION

Cell-free assays (gpl20-CD4 ELISA and PCFIA) with which to identify inhibitors of the interactions of HIV-1 gpl20 with its cellular receptor CD4 were developed. These assays are specific, reproducible, and sensitive. In addition, they are easily automated to allow for the rapid screening of many potential inhibitors. Known inhibitors such as MAb s, sulfated polymers, and CD4 mimics are active in these assays (Table 1).

The gpl20-CD4 ELISA and PCFIA were used as high-throughput screens to identify inhibitors of gpl20-CD4 binding. In general, compounds assayed for their inhibitory activities in the two assays had comparable IC50s (Table 1). However, those compounds that produced fluorescence when irradiated at 485 nm could not be assayed effectively in the PCFIA. One such compound was CP-101,816-1, which has aromatic ring components. In general, fluorescent compounds had to be evaluated in the ELISA, which has a colorimetric endpoint.

So-called consumption assays were developed to elucidate the mechanism of action of chemical moieties that inhibit the binding of gpl20 to its eucaryotic receptor. When specific MAb s with affinities for gpl20 or CD4 are used in the assays, they correctly differentiate the MAb s which sequester gpl20 from those that sequester CD4. Furthermore, LyT2, a MAb with binding affinity for neither ligand, was inactive in both consumption assays (data not shown). The behavior of the CD4 mimic CPF(1,1) in the consumption assays suggests that it interacts primarily with gpl20 (Table 2), as was shown previously (3). Studies with these known inhibitors confirm that the consumption ELISAs appear to be able to differentiate those inhibitors which function primarily by binding to the viral ligand gpl20 from those that inhibit by binding primarily to the eucaryotic receptor CD4.

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TABLE 1. Potencies of compounds in the gpl20-CD4 ELISA, PCFIA, and CCSA

<table>
<thead>
<tr>
<th>Compound</th>
<th>gpl20-CD4 ELISA (IC50, μg/ml)</th>
<th>PCFIA</th>
<th>CCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(sodium 4-styrenesulfonate)</td>
<td>9.2</td>
<td>11</td>
<td>22.5 (3.5)</td>
</tr>
<tr>
<td>DS 5,000</td>
<td>6.0</td>
<td>5.0</td>
<td>4.7 (2.2)</td>
</tr>
<tr>
<td>DS 500,000</td>
<td>1.5 (0.7)</td>
<td>0.75</td>
<td>2.3 (1.1)</td>
</tr>
<tr>
<td>PPS</td>
<td>4.0 (1.4)</td>
<td>2.0</td>
<td>3.2 (1.0)</td>
</tr>
<tr>
<td>UK-22,233</td>
<td>8.0</td>
<td>2.0</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>CFF(10)</td>
<td>&gt;400</td>
<td>ND</td>
<td>625 (35.4)</td>
</tr>
<tr>
<td>CFF(1L)</td>
<td>&gt;400</td>
<td>ND</td>
<td>450 (70.7)</td>
</tr>
<tr>
<td>Pradimicin A</td>
<td>27.5 (3.5)</td>
<td>ND</td>
<td>2.0 (1.4)</td>
</tr>
<tr>
<td>CP-101,816-1</td>
<td>32.5 (3.5)</td>
<td>ND</td>
<td>1.17 (0.55)</td>
</tr>
</tbody>
</table>

* Where indicated, values are means (standard deviation) of two determinations.
* Values are means (standard deviations) of two determinations.
* ND, not determined.

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TABLE 2. Activities of compounds in gpl20 and CD4 consumption assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>gpl20 consumption assay IC50 (μg/ml)</th>
<th>CD4 consumption assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPS</td>
<td>16 (5.65)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>CPF(1L)</td>
<td>400</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Pradimicin A</td>
<td>9 (1.42)</td>
<td>&gt;125</td>
</tr>
<tr>
<td>CP-101,816-1</td>
<td>&gt;200</td>
<td>110.5 (85.5)</td>
</tr>
</tbody>
</table>

* Values are means (standard deviations) of two determinations.
infected HUT-78 cells and observed dose-related inhibition of MAb binding with DS 5,000, PPS, and aurintricarboxylic acid. Their data support the hypothesis that the DS 5,000 and PPS bind gp120, but not CD4, in their assays. The studies of Thiele et al. (19) extend those findings. Thiele et al. (19) concluded that the antiviral effects of DS 8,000 and PPS at lower concentrations are not due to binding of CD4, while DS 500,000 at 100 and 1,000 nM binds CD4 efficiently. Finally, Parish and Warren (13) examined a collection of sulfated polysaccharides for their abilities to block anti-CD4 MAb binding to human peripheral blood lymphocytes and to bind sCD4 and sgp120 in vitro. In those experiments, PPS failed to block anti-CD4 MAb binding and reacted weakly with sCD4, although it exhibited potent anti-HIV activity. By contrast, DS 500,000 and polyvinyl sulfate appeared to bind a polyanion recognition site on CD4. In summary, some anionic polysaccharides, such as DS 500,000, seem to bind tightly to CD4, while others, such as PPS, bind with the most affinity to gp120.

This is consistent with our results with PPS in the consumption assays: PPS interacts with gp120 at doses of up to 50 μg/ml and demonstrates no consumption of sCD4 (Table 2).

Tanabe-Tochikura et al. (18) have shown that pradimicin A is a potent inhibitor of HIV-1 replication and syncytium formation in vitro. Pradimicin A is also a potent inhibitor in the cell-free gp120-sCD4 ELISA, with an IC_{50} of 27.5 μg/ml (Table 1). In the consumption assays it binds exclusively to gp120 at doses of up to 125 μg/ml (Table 2). In addition, it inhibits gp120-CD4-mediated cell fusion in the CCSA, with an IC_{50} of 2.0 μg/ml (Table 1).

The gp120-CD4 ELISA and PCFIA were used to screen for novel compounds that inhibit the interaction of gp120 and CD4. The 2,4-diamoquinazoline analog CP-101,816-1 was detected as an inhibitor in the ELISA (Table 1) and was the only compound tested that bound significantly to CD4 in the consumption assays (Table 2). Fluorescence-activated cell sorter analysis with human T lymphocytes confirmed that this compound has the capacity to inhibit the binding of CD4-specific MAbs to T cells (data not shown).

Active compounds were tested in cell-based cytotoxicity assays and other assays by cocultivation of gp120- and CD4-expressing cell lines (CCSA) (21). In the absence of inhibitors these cells fuse and produce giant, multinucleate syncytia. In general, when toxicity did not preclude accurate testing, potency in the acellular assays was correlated with activity in syncytial assays, although pradimicin A and CP-101,816-1 were 14- and 28-fold more potent, respectively, in the cell-based assay (Table 1). This may have been due to the increased level of solubilization of these compounds in the CCSA over the 24 to 48 h of incubation at 37°C.

ACKNOWLEDGMENTS

We thank Wayne Barth of the Department of Medicinal Chemistry, Pfizer Central Research, for the synthesis of CPF(L) and G. M. Peterman, ICOS Corporation, Bothell, Wash., for the fluorescence-activated cell sorter analysis.

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