In vitro susceptibility of HIV isolates with high growth capability to antiretroviral drugs

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Research Article

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Abstract

It has been considered that reduced antiretroviral susceptibility can occur with drug-resistance mutations in the HIV genome. In the present study, we assessed the susceptibility of HIV isolates with high growth capability to antiretroviral drugs using an *in vitro* model. Phytohemagglutinin-activated peripheral blood mononuclear cells (1.5×10^6 cells) were infected with HIV isolates (10^6 copies/mL). The culture was carried out at different concentrations (0.001–20 µM) of 13 synthetic antiretroviral compounds (six nucleoside/nucleotide reverse transcriptase inhibitors, one non-nucleoside reverse transcriptase inhibitor, four integrase inhibitors, and two protease inhibitors), and HIV production was assessed using HIV-RNA copies in culture. The 90% inhibitory concentration (IC_{90}) and pharmacokinetics of an antiretroviral agent were used as parameters to determine the reduced antiretroviral drug susceptibility of HIV isolates with high growth capability to synthetic antiretroviral compounds. The high growth capability of HIV isolates affected their susceptibility to tenofovir (IC_{90} = 2.05 ± 0.40 µM), lamivudine (IC_{90} = 6.83 ± 3.96 µM), emtricitabine (IC_{90} = 0.68 ± 0.37 µM), and efavirenz (IC_{90} = 3.65 ± 0.77 µM). These antiretroviral drugs showed IC_{90} values close to or above the C_{min}-C_{max} range against HIV isolates with a high growth capability without any drug resistance-related mutations. Our results may contribute to the development of effective antiretroviral therapy strategies to tailor and individualize ART in patients harboring HIV isolates with a high growth capability.

Introduction

Despite the decrease in overall HIV-related mortality, recent data have shown that approximately 37.7 million people live with HIV/AIDS, which continues to be the underlying cause of mortality for about 1 million people annually [1]. Considering this challenge, sustained global efforts with the expansion of antiretroviral therapy (ART) have dramatically increased the number of people receiving HIV treatment in recent years, particularly in resource-limited countries. In 2020, 73% of all HIV-infected individuals received treatment, of which 66% were virally suppressed [2]. The primary goal of ART is to suppress viral load to an undetectable level and prevent its transmission to other uninfected individuals [3–5].

Treatment options recommended by the World Health Organization (WHO) provide guidelines on when and what antiretroviral regimens should be administered to patients. According to the current WHO guidelines, the initial antiretroviral regimens used by most national treatment programs in resource-limited settings include the following three antiretroviral agents: one key antiretroviral drug, either a non-nucleotide reverse transcriptase inhibitor (NNRTIs), integrase inhibitor (INIs), protease inhibitor (PIs), and two backbone antiretroviral drugs from among the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) [4]. These combined antiretroviral regimens provide excellent potency, safety, and tolerability, making lifelong viral suppression achievable [6].

However, the above-mentioned treatment options have been impaired frequently due to the reduced antiretroviral drug susceptibility [7]. This condition is typically associated with limited treatment
monitoring, poor drug adherence and tolerance, and the emergence of resistance mutations during antiretroviral therapy [8–12]. Although the exact mechanism by which HIV viral factors compromise virological control remains unclear, it is likely to involve intrinsic replication capability of the virus [11]. Recent studies showed that HIV replication capability also appears to influence the ART response and is postulated to influence clinical outcomes [7, 13–15]. Selhorst et al. demonstrated that a virus with high growth capability during early infection significantly contributed to disease progression, even during antiretroviral drug therapy [11]. Of note, our previous study showed various viral growth capabilities in vitro in clinical samples collected from the surveillance of drug-resistant strains in the Philippines and good relationships between growth capability and plasma viral load in vivo [16]. These results suggest that it may be challenging to suppress the replication of isolates with a high growth capability.

In the present study, we examined the antiviral activity of synthetic antiretroviral compounds against HIV with high growth capability in vitro.

**Materials And Methods**

**HIV isolates**

From the 37 HIV strains previously isolated as part of the surveillance program on drug-resistant isolates in the Philippines [16], seven representative HIV isolates and one laboratory strain of HIVpNL4-3 (originated from HIV-1 infectious molecular clone, pNL4-3 provided by the NIH AIDS Reagent Program) were used in the study. Among these, HIV isolates DR1509-397, DR1605-400, S1506-064 (except zidovudine [ZDV]), and DR1606-559 (except efavirenz [EFV]) did not have any drug-resistance-related mutations against the antiretroviral drugs used in the study. HIV isolates DR1606-479, DR1606-521, and DR1510-726 have drug resistance-related mutations in NRTIs/NNRTIs and PIs according to the HIV Drug Resistance Database at Stanford University (http://hivdb.stanford.edu/). Aliquots of HIV isolates were prepared and stored in a deep freezer (-75°C) until ready for use.

**Growth Capability Assessment**

HIV-seronegative peripheral blood mononuclear cells (PBMCs) were stimulated with phytohemagglutinin-P (PHA-P; 2.0 µg/mL) in RPMI1640 complete medium supplemented with recombinant interleukin 2 (IL-2, 10 ng/mL; Genzyme, Cambridge, Massachusetts, USA) and 10% fetal bovine serum for 48–72 h to promote T cell blast formation. PBMCs (1.5×10^6 cells) were inoculated with 100 µL of HIV epidemic isolates (10^6 HIV-RNA copies/mL) and incubated at 37°C for 1 h. To remove unbound and non-infectious viruses, the cells were washed twice with RPMI1640 medium and resuspended in complete medium to a final volume of 1 mL. Half of the culture medium was replaced on day 3 to maintain the culture conditions of PBMCs. The culture supernatants on days 3, 5, and 7 were subjected to RNA extraction and quantitative one-step RT-PCR to create growth curves for the representative HIV isolates.
Drug-induced cytotoxicity assay

HIV-seronegative PBMCs were incubated with various concentrations of antiretroviral compounds in 96-well plates for 72 hours. Cell toxicity was then evaluated using the Cell Counting Kit-8 (CCK-8) (DOJINDO LABORATORIES, Kumamoto Japan) [17]. The absorbance was measured at 450 nm using a 96 well plate reader (AS ONE MPR-A100; AS ONE Corporation, Tokyo, Japan).

Evaluation Of Drug Response

The activity of 13 synthetic antiretroviral compounds at concentrations ranging from 0.001–20 µM was evaluated against HIV isolates with diverse growth capabilities and without drug resistance-related mutations. The following NRTIs were used: emtricitabine (FTC; Tokyo Chemical Industry, Japan), lamivudine (3TC; Tokyo Chemical Industry, Japan), tenofovir (TFV; Selleck Biotech, Japan), tenofovir alafenamide (TAF; Selleck Biotech, Japan), tenofovir disoproxil fumarate (TDF; Tokyo Chemical Industry, Japan), and zidovudine (FUJIFILM Wako, Japan). As it was reported that TFV and its prodrugs (TDF and TAF) are measurable in plasma, the antiviral activity of these three synthetic drugs were assessed in vitro [18, 19]. In addition, one NRTI, efavirenz (EFV, Tokyo Chemical Industry, Japan); two protease inhibitors, atazanavir (ATZ, Selleck Biotech, Japan) and lopinavir (LPV, Funakoshi Frontiers in Life Science, Japan); and four integrase inhibitors, bictegravir (BIC; Selleck Biotech, Japan), dolutegravir (DTG, Selleck Biotech, Japan), elvitegravir (EVG, Selleck Biotech, Japan), and raltegravir (RAL, Selleck Biotech, Japan) were analyzed. Representative HIV isolates and a laboratory strain of HIVpNL4-3 were screened for antiretroviral drugs. Briefly, PMBCs (1.5×10^6 cells) were pelleted at 1,000 g for 5 min, resuspended with an HIV preparation (10^6 copies) in 100 µL volume, and incubated at 37°C for 1 h. To remove unbound and non-infectious viruses, the cells were washed twice with RPMI1640 medium and then cultured in different concentrations of antiretroviral compounds (1 mL). The culture was carried out for 7 days, as described above, for growth capability assessment. All procedures were performed in triplicate.

Evaluation Of Antiretroviral Susceptibility

To determine the susceptibility of HIV isolates with high growth capability to synthetic antiretroviral compounds, we used the in vitro susceptibility of the virus (90% inhibitory concentration [IC_{90}]) and pharmacokinetics of an antiretroviral agent (compared to reported minimum plasma concentration [C_{min}] and maximum plasma concentration [C_{max}]). The reduced antiretroviral drug susceptibility of HIV isolates is reflected in an increase in the inhibitory concentration to a certain level close to or higher than the C_{min}-C_{max} range [20].

Rna Extraction And Polymerase Chain Reaction
HIV-RNA in the culture medium (140 µL) was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN KK-Japan, Tokyo, Japan) and subjected to quantitative reverse transcription polymerase chain reaction (qRT-PCR). The amount of HIV-RNA was assayed using a Luna Universal One-Step qRT–PCR kit (New England Biolabs Japan, Tokyo, Japan) and forward (GagB-1F, 5'-AGTGGGGGGACATCAAGCAGCCATGCAAAT-3', HXB2 position:1359–1388) and reverse (GagB-1R, 5'-TGCTATGTCACTTCCCCTTGGTTCT-3', 1500 – 1474) primers. The PCR signal was assessed by comparison with a standard curve (encompassing $10^2$–$10^8$ copies/reaction of the in vitro-transcribed RNA transcripts).

Hiv Pol Region Nucleotide Sequences And Genotyping

HIV-RNA in the pol region (HXB2:1827–3528) was reverse-transcribed and amplified in the first round of PCR with primers F1849/R3500 [16, 21] using the SuperScript III One-Step Real-Time RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Tokyo, Japan). Two HIV pol regions were amplified in the second round of PCR with DRPRO5/DRPRO2L [16, 21] and DRRT1L/DRRT4L [16, 21] using AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The annealing temperature and elongation time were 57°C and 90 s for the first PCR, and 55°C and 60 s for the second PCR, respectively [16]. Subtype assignments were compared to those from Stanford-v8.1, COMET (COntext-based modeling for expeditious typing), Luxembourg Institute of Health, and REGA HIV Subtyping Tool – Version 3.0 – Stanford University [22]. The sequences described in this study have been deposited in the International Nucleotide Sequence Database under the accession numbers ON229916-ON229922 (Table 1).
### Table 1
Characteristics of seven representative strains tested for drug response

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Amount of HIV-RNA (×10^6 copies/mL) in Culture sup(^a)</th>
<th>Drug resistance-related mutation(^c)</th>
<th>Genotype</th>
<th>Coreceptor usage(^d)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1509-397</td>
<td>2,000</td>
<td>None</td>
<td>CRF01_AE</td>
<td>CXCR4</td>
<td>ON229916</td>
</tr>
<tr>
<td>DR1606-521</td>
<td>340</td>
<td>EFV, FTC, TDF, 3TC</td>
<td>CRF01_AE</td>
<td>CXCR4</td>
<td>ON229919</td>
</tr>
<tr>
<td>DR1510-726</td>
<td>300</td>
<td>EFV, FTC, TDF, 3TC</td>
<td>CRF01_AE</td>
<td>CXCR4</td>
<td>ON229920</td>
</tr>
<tr>
<td>DR1605-400</td>
<td>230</td>
<td>None</td>
<td>CRF01_AE</td>
<td>CXCR4</td>
<td>ON229917</td>
</tr>
<tr>
<td>DR1606-479</td>
<td>180</td>
<td>EFV, FTC, TDF, 3TC</td>
<td>CRF01_AE</td>
<td>CXCR4</td>
<td>ON229921</td>
</tr>
<tr>
<td>S1506-064</td>
<td>100</td>
<td>Not done</td>
<td>ZDV</td>
<td>Subtype B</td>
<td>ON229918</td>
</tr>
<tr>
<td>DR1606-559</td>
<td>9.7</td>
<td>EFV</td>
<td>CRF01_AE</td>
<td>CCR5</td>
<td>ON229922</td>
</tr>
</tbody>
</table>

\(\): Maximum amount of HIV-RNA detected in culture supernatant of phytohemagglutinin-activated peripheral blood mononuclear cells

\(b\): Patient's plasma viral load

\(c\): Interpreted using Stanford university HIV drug resistance database

\(d\): Interpreted using Geno2pheno [coreceptor] 2.5, Max Planck Institute for Informatics.

Efavirenz (EFV), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF), lamivudine (3TC), zidovudine (ZDV).

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**Hiv V3 Region Nucleotide Sequences And Coreceptor Prediction**

The HIV env region was reverse-transcribed and amplified in the first round of PCR with the forward primer SQV3F1 [23] and reverse primer CO602 [23] using a One Taq One-Step RT-PCR kit (New England Biolabs). The HIV V3 region was amplified in the second round of PCR with the forward primer SQV3F2 [23] and reverse primer CD4R [23] using AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The annealing temperature and elongation time were 57°C and 30 s for the first PCR and 55°C and 60 s for the second PCR, respectively. Coreceptor usage prediction from genotype was
employed using geno2pheno (version 2.5), and sequences were confirmed using the HIV sequence locator tool [24].

Data analysis

The IC$_{90}$ was calculated using a nonlinear regression curve fit of each transformed drug concentration and normalized viral load (copies/mL) using GraphPad Prism software (version 8.0) and an online GraphPad calculator (https://www.graphpad.com/quickcalcs/ECanything1/) [25]. The results are expressed as the mean IC$_{90}$ of three values obtained in triplicate for each isolate.

Results

HIV isolates characteristics

From the 37 clinical samples collected during the surveillance of drug-resistant strains in the Philippines, diverse replication capabilities ($5 \times 10^6$–$3 \times 10^9$ copies/mL) were observed using a primary culture system of PBMCs in vitro (Fig. 1A). Seven representative isolates and one laboratory strain of HIVpNL4-3 were selected for analysis of viral growth kinetics. Among these, six showed a higher virus yield at the end of the virus isolation procedure and one had a lower yield. Three different time points (days 3, 5, and 7) were evaluated to observe the viral growth kinetics using a primary culture system of PBMCs (Fig. 1B, Table 1). After the inoculation of virus ($10^6$ HIV-RNA copies), isolates DR1509-397, DR1606-521, DR1510-726, DR1605-400, DR1606-479 and S1506-064 multiplied to $2.0 \times 10^9$, $3.4 \times 10^8$, $3.0 \times 10^8$, $2.3 \times 10^8$, $1.8 \times 10^8$, and $1.0 \times 10^8$ copies/mL on day 7, respectively. HIVpNL4-3 produces a viral copy of $1.3 \times 10^8$ copies/mL. In contrast, DR1606-559 showed limited growth, 1-fold that of the inoculum size ($9.69 \times 10^6$ copies/mL) (Fig. 1B, Table 1). Genotyping results showed that all isolates were CRF01_AE, except S1506-064, which was Subtype B. Moreover, all isolates were CXCR4 viruses, except DR1606-559, which was CCR5, based on the coreceptor prediction (Table 1).

Screening For Antiretroviral Drugs

Representative HIV isolates with relatively high growth capabilities were subjected to drug screening using 13 synthetic antiretroviral compounds. None of the tested antiretroviral compounds exhibited 50% cytotoxicity at the maximum concentration used in the assay. Remarkably, the IC$_{90}$ values were close to C$_{max}$ and above C$_{min}$ for some isolates with high growth capability and without any drug resistance-related mutations. A typical case was the use of TFV against isolates with a high growth capability. Three isolates (DR1509-397, DR1605-400, and S1506-064) had IC$_{90}$ values of $2.0 \pm 0.40$, $1.4 \pm 0.87$, and $2.8 \pm 0.47$ µM, respectively, which were higher than their C$_{max}$ values (Fig. 2A).

To determine whether host factors contributed to the viral load of isolates with high growth capabilities, DR1509-397 was screened using two additional blood sources, even in the presence of TFV. The results
were still consistent with a mean IC\textsubscript{90} of 1.9 ± 0.04 µM (Fig. 2B). As expected, isolates with drug resistance-related mutations (DR1606-521, DR1510-726, and DR1606-479) had higher IC\textsubscript{90} values for TFV (8.3 ± 0.12, 7.7 ± 0.06, and 8.5 ± 0.10 µM) respectively, which were above the C\textsubscript{max} (Fig. 3). Moreover, the \textit{in vitro} growth behavior of the TFV-sensitive (DR1509-397) and TFV-resistant (DR1606-521) isolates showed the same pattern in the presence of TFV 1 µM (close to C\textsubscript{max}). Surprisingly, the isolate with high growth capability hardly showed decreased virus production, similar to that of the isolate with drug resistance-related mutations. An unreachable concentration \textit{in vivo} (IC\textsubscript{90}:10 µM, above C\textsubscript{max}) was required to fully suppress the number of viral copies in both isolates (Fig. 4).

An NNRTI regimen containing EFV, which is used by most national treatment programs in resource-limited settings, showed IC\textsubscript{90} values above C\textsubscript{min} both with and without any drug resistance-related mutations (Fig. 5). EFV failure could be replaced by the integrase inhibitor DTG and protease inhibitor ATV, which remarkably suppressed the viral yield of HIV isolates \textit{in vitro} (Fig. 5). Among the 13 antiretroviral synthetic compounds, ATV, TAF, ZDV, and DTG showed the lowest IC\textsubscript{90} values of 1.30 ± 1.12, 0.30 ± 0.23, 1.50 ± 1.11, and 1.10 ± 0.72 nM, respectively. All drugs were highly active against HIVpNL4-3.

**Discussion**

In \textit{vitro} viral growth kinetics in PHA-PBMC cultures has been demonstrated for isolates with diverse growth capabilities. HIV isolates with higher growth capability but without any drug resistance-related mutations were screened for antiretroviral drugs, and eight compounds (ATV, BIC, DTG, EVG, LPV, RAL, TAF, and ZDV) retained susceptibility with lower IC\textsubscript{90} values. However, 3TC, FTC, and EFV had IC\textsubscript{90} values that were close to or above C\textsubscript{min}. A compound of TFV, the active form of TDF in the plasma and the backbone drug in the first-line antiretroviral regimen, showed IC\textsubscript{90} values above C\textsubscript{max}.

The extent to which HIV replicates during ART remains controversial [26] and most studies have associated drug resistance with drug-resistance-related mutations [12, 27, 28]. However, as described above, viral copies of HIV isolates can still be high, even when they are not affected by resistance-related mutations in EFV, FTC, TFV, or 3TC. The computed IC\textsubscript{90} values were similar to those of isolates with drug resistance-related mutations. The difficulty in EFV, FTC, TFV, and 3TC inhibiting HIV production in PBMC cultures stands in conflict with a large body of evidence that they are effective in suppressing HIV replication [4, 29–32]. Our results suggest that HIV gains a replicative advantage and produces increased viral copies with drugs such as NRTIs and NNRTIs because of its intrinsic viral growth capability. The reduced antiretroviral susceptibility of HIV isolates with a high growth capability to these antiretroviral drugs is critical because these regimens are the most popular first-line HIV treatment worldwide and are still included in the 2021 updated WHO recommendations [33].

The attained IC\textsubscript{90} of TFV compared to that of TAF presented in this study showed a potential advantage of TAF in suppressing HIV isolates with high growth capability. TAF has also been found to be active against HIV isolates with drug resistance-related mutations in TDF. \textit{Ex vivo} clinical studies have also
indicated that TAF achieves a high intracellular concentration of the active drug tenofovir diphosphate [25, 26] and that most viruses harboring major NRTI drug resistance-related mutations are fully suppressed but not inhibited by TFV, although TFV prodrugs (TDF and TAF) have similar resistance profiles [35, 36]. Similar to what has been observed ex vivo [32, 37], the results of this study demonstrated that TAF maintains its antiviral activity and can be used if TDF failure is observed. Our results also provide evidence that most of the antiretrovirals tested, such as TAF, ZDV, protease, and integrase inhibitors, were highly effective in suppressing viral copies in vitro. Moreover, integrase inhibitors have been shown to exhibit potent in vitro antiviral activity in multiple cell-based assays [4, 38, 39] and in vivo efficacy in clinical studies [40–42].

As described above, the reduced susceptibility of high growth capability isolates to antiretroviral drugs emphasizes the importance of considering high growth capability of epidemic strains. Further analyses are needed to identify the genes responsible for growth capability and invent a high throughput system of screening assay. Moreover, prospective studies are required to evaluate the clinical significance of HIV isolates with high growth capabilities and subsequent virological response to antiretroviral therapy.

Reduced antiretroviral drug susceptibility was observed with tenofovir, lamivudine, emtricitabine, and efavirenz, even in the absence of specific drug-resistance-related mutations in HIV isolates with high growth capability. This evidence, if not all, as a limited number of high growth capability isolates were tested against ART, suggests the need for surveillance to strengthen the concept that the intrinsic viral growth capability is attributable to viral isolates and may influence their susceptibility to antiretroviral drugs. Furthermore, development of high-throughput assays can be applied for an efficient and less time-consuming cell-culture based assays. Finally, this study may aid in the development of diagnostic tools to identify this viral factor as an additional determinant of treatment failure and allow ART to be tailored appropriately.

**Abbreviations**

3TC  
lamivudine  
ART  
antiretroviral therapy  
ATV  
atazanavir:BIC:bictegravir  
$C_{\text{max}}$  
maximum plasma concentration  
$C_{\text{min}}$  
minimum plasma concentration  
DTG  
dolutegravir  
EFV
efavirenz
EVG
elvitegravir
FTC
emtricitabine
IC_{90}
90% inhibitory concentration
IL-2
interleukin 2
INIs
integrase
LPV
lopinavir
NNRTI
non-nucleoside/nucleotide reverse transcriptase inhibitor
NRTI
nucleoside/nucleotide reverse transcriptase inhibitor
PBMC
peripheral blood mononuclear cells
PCR
polymerase chain reaction
PHA-P
phytohemagglutinin-P
PIs
protease inhibitors
RAL
raltegravir
TAF
tenofovir alafenamide
TDF
tenofovir disoproxil fumarate
TFV
tenofovir
WHO
World Health Organization
ZDV
zidovudine.

Declarations
Ethics approval and consent to participate

The present study was conducted with the approval and under the control of the Institutional Review Boards of San Lazaro Hospital, the Philippines (RERU 2014-024) and the Faculty of Medicine, Tottori University, Japan (no. 1982). All procedures performed in this study involving human participants were in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author contributions

All the authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Alfredo Jr. A. Hinay, Seiji Kageyama and Kyosuke Kanai. The laboratory work was assisted by Akeno Tsuneki-Tokunaga, Mizuki Komatsu, and Elizabeth O. Telan. The first draft of the manuscript was written by Alfredo Jr. A. Hinay and all authors commented on the updated versions of the manuscript. All the authors have read and approved the final manuscript.

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Figure 1

Growth capabilities of HIV epidemic isolates.

(A) The wide distribution of growth capabilities was determined for the 37 clinical isolates tested and shown using production levels in culture with phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBMC) for 7 days in vitro (mean ± standard error). (B) In vitro viral growth kinetics in PHA-PBMC culture are shown for seven representative HIV isolates and one laboratory strain of HIV (pNL4-3, ●DR1509-397, ▼DR1605-400, S1506-064, DR1606-521, △DR1510-726, ◊DR1606-479, and DR1606-559). Closed symbols denote drug sensitive isolates and open symbols denote drug-resistant isolates against NRTIs, NNRTIs, and PI inhibitors based on the Stanford university HIV drug resistance database genotype-phenotype comparison scores.
Figure 2

Thirteen synthetic antiretroviral compounds were evaluated: six nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), emtricitabine (FTC), lamivudine (3TC), tenofovir alafenamide (TAF), tenofovir (TFV), and zidovudine (ZDV). One non-nucleoside/nucleotide reverse transcriptase inhibitor (NNRTI), efavirenz (EFV); two protease inhibitors (PIs), atazanavir (ATZ) and lopinavir (LPV); and four integrase inhibitors (INIs), bictegravir (BIC), dolutegravir (DTG), elvitegravir (EVG), and raltegravir (RAL) against HIV with high growth capability (●DR1509-397, ▼DR1605-400, S1506-064), low growth capability (DR1606-559), and laboratory strain of HIV (pNL4-3). Because of the presence of drug-resistance mutations, isolates of S1506-064 and DR1606-559 were not evaluated for ZDV and EFV, respectively. (B) Tenofovir response to isolate (●DR1509-397) with the highest growth capability, was evaluated using three different blood sources.
Figure 3

Influence of growth capability on the therapeutic effects of a TDF-based regimen.

The 90% inhibitory concentrations (IC₉₀) of three isolates with a high growth capability without drug resistance-related mutations (left panel, ●DR1509-397, ▼DR1605-400, and □S1506-064) and three isolates with such mutations ( DR1606-521, ΔDR1510-726, ◇and DR1606-479) were compared.
Figure 4

Anti-HIV activity of TFV against isolates without and with TDF-drug-resistance-related mutations

The tested concentration of 1 µM corresponded to the $C_{\text{max}}$ value of TFV, and 10 µM is the highest IC$_{90}$ expected to significantly suppress the viral load of isolates without TDF drug resistance-related mutations (●DR1509-397) or with mutations (△DR1606-521).
Application of the IC$_{90}$ and its relationship with C$_{\text{min}}$/C$_{\text{max}}$ to WHO recommended regimens

The levels of IC$_{90}$ and C$_{\text{min}}$/C$_{\text{max}}$ are shown for an NNRTI-, INI-based regimen with DTG and TFV (active form of TDF in plasma), INI-based regimen (alternative) with TAF, and PI-based regimens including ATV (●DR1509-397, ▼DR1605-400, and S1506-064, DR1606-521, ΔDR1510-726, ◊DR1606-479). Closed symbols were assigned to all isolates in DTG because integrase resistance mutations were not assessed.