



MK-8591 (4'-Ethynyl-2-Fluoro-2'-Deoxyadenosine) Exhibits Potent Activity against HIV-2 Isolates and Drug-Resistant HIV-2 Mutants in Culture

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ABSTRACT There is a pressing need to identify more effective antiretroviral drugs for HIV-2 treatment. Here, we show that the investigational compound MK-8591 (4'-ethynyl-2-fluoro-2'-deoxyadenosine [EFdA]) is highly active against group A and B isolates of HIV-2; 50% effective concentrations [EC₅₀] for HIV-2 were, on average, 4.8-fold lower than those observed for HIV-1. MK-8591 also retains potent activity against multinucleoside-resistant HIV-2 mutants (EC₅₀ ≤ 11 nM). These data suggest that MK-8591 may have antiviral activity in HIV-2-infected individuals.

KEYWORDS HIV-2, MK-8591, 4'-ethynyl-2-fluoro-2'-deoxyadenosine, EFdA, NRTI, antiretroviral therapy, drug resistance, HIV-1

Antiretroviral therapy (ART) can prolong the life spans of individuals infected with human immunodeficiency virus type 1 (HIV-1) by years or even decades, but these benefits have yet to be formally demonstrated in clinical trials involving human immunodeficiency virus type 2 (HIV-2) and HIV-1/2 dually infected patients (1–3). HIV-2 is endemic in West Africa and is also prevalent in other areas with socioeconomic ties to the region (4, 5). Choices of antiretroviral (ARV) drugs for HIV-2 treatment are constrained by the intrinsic resistance of the virus to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the reduced susceptibility of HIV-2 to most HIV-1-active protease inhibitors (5–8). In addition, ART-experienced HIV-2 patients frequently harbor mutants resistant to both protease inhibitors and NRTIs (9–11), and newer ARV drugs, such as integrase strand transfer inhibitors, are not broadly available in areas where significant numbers of HIV-2-infected individuals reside. These limitations underscore the need to identify new ARV agents that exhibit potent activity against HIV-2, including drug-resistant strains of the virus.

MK-8591 (4'-ethynyl-2-fluoro-2'-deoxyadenosine [EFdA]) (Fig. 1) is an investigational nucleoside analog that inhibits HIV-1 replication with 50% effective concentrations (EC₅₀s) in the low nanomolar-to-picomolar range (12–16). Direct comparisons show that MK-8591 is 10-fold more potent than tenofovir disoproxil fumarate (TDF) (15), 10 to 100 times more potent than zidovudine (AZT) (12, 14), and 1,000 times more potent than emtricitabine (FTC) (16) against HIV-1 in culture. MK-8591 also exhibits favorable pharmacokinetic properties, including a long intracellular half-life compared to those of conventional, FDA-approved NRTIs (16–18). This property is attributable to the fluorine at the 2 position of the adenine base, which renders the compound resistant to degradation by adenosine deaminase (13, 14). MK-8591 suppresses HIV-1 and simian immunodeficiency virus (SIV) replication at clinically achievable concentrations in

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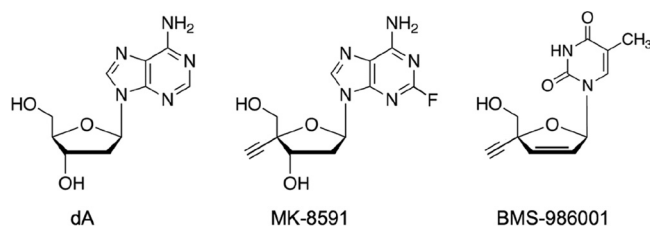


FIG 1 Chemical structures of 2'-deoxyadenosine (dA), MK-8591 (4'-ethynyl-2-fluoro-2'-deoxyadenosine [EFdA]), and BMS-986001 (2',3'-didehydro-3'-deoxy-4'-ethynyl-thymidine; also known as fostinavir, cenvavudine, 4'-ethynyl stavudine, 4'-ethynyl-d4T, and OBP-601).

humanized mice and rhesus macaques, respectively (16, 19–21). In a phase 1b proof-of-concept clinical trial, a single 10-mg dose of MK-8591 demonstrated antiviral activity for 10 days in ART-naive, HIV-1-infected participants (22). The *in vivo* potency, pharmacokinetic profile, and physical properties of MK-8591 open the possibility for once-weekly oral dosing, and studies of long-acting formulations of MK-8591 indicate that a single parenteral administration may provide HIV-1-suppressive levels of the drug for 6 months or longer (23). Thus, MK-8591 potentially represents a new ARV with multiple modalities for preventing and treating HIV-1 infection.

The exceptional antiretroviral activity of MK-8591 is likely related to its unique mode of action. As with FDA-approved NRTIs, MK-8591 is converted to MK-8591-5'-triphosphate (MK-8591-TP) by cellular kinases and serves as an alternative substrate for HIV-1 reverse transcriptase (RT)-catalyzed DNA synthesis (14, 17, 24–26). However, due to its extendable 3'-hydroxyl group, MK-8591-TP is not an obligate chain terminator. Instead, incorporation of the analog results in either immediate stalling of polymerization or delayed termination after incorporation of the next complementary nucleotide (26, 27); the latter mechanism renders the newly synthesized DNA resistant to ATP- or pyrophosphate-mediated drug removal (excision) (27). In pre-steady-state kinetic assays, HIV-1 RT utilized MK-8591-TP with an efficiency comparable to or greater than observed for dATP (depending on the template sequence context), indicating that the enzyme is unable to discriminate between the two substrates (26, 27). When insertion of the analog was favored, the difference between MK-8591-TP and dATP was attributable to a lower K_d (dissociation constant) for the former (26, 27), suggesting that MK-8591-TP bound to RT with a higher affinity than dATP. This inference is supported by surface plasmon resonance data (27) and by crystallographic data from the recently solved structure of HIV-1 RT with MK-8591-TP as the incoming nucleotide (i.e., in the N site of the polymerase) (28). In this structure, the 4'-ethynyl moiety of the analog is embedded within a well-defined hydrophobic pocket in the polymerase active site. This interaction appears to stabilize MK-8591-TP in the N site complex. Additional RT structures in which MK-8591-5'-monophosphate occupies the penultimate position of the nascent strand (i.e., the P site) reveal localized distortions in the phosphate backbone that may affect nucleic acid binding and/or positioning. Taken together, the pre- and postincorporation complexes (28) reveal structural features that are consistent with MK-8591-mediated inhibition of HIV-1 RT translocation and arrest of DNA synthesis (27). To distinguish the compound from conventional chain-terminating NRTIs, MK-8591 is more accurately described as a nucleoside reverse transcriptase translocation inhibitor (NRTTI).

Although MK-8591 has been shown to potently inhibit HIV-1 and SIV in culture (12–16, 19) and in experimentally infected nonhuman primates and humanized mouse models (16, 19–21), efforts to evaluate the activity of the drug against HIV-2 are limited; a single report showed that a group B strain (HIV-2_{EHO}) is sensitive to the drug in spreading infections of MT-4 cells (14). In addition, the ability of MK-8591 to inhibit HIV-2 mutants that are resistant to other NRTIs is unknown. In HIV-1, the M184V replacement in RT confers low-level resistance to the drug (7.5- to 15-fold increase in EC_{50}) (13, 14), whereas the tenofovir resistance-associated change K65R confers 3- to

5-fold hypersusceptibility to MK-8591 (14, 15). However, these findings cannot be extended *a priori* to HIV-2, as important differences exist between the two HIV types with respect to NRTI resistance pathways and mechanisms (29–31).

We recently evaluated the antiviral activity of a 4'-ethynyl thymidine analog bearing a 2',3'-didehydro-3'-deoxy sugar (BMS-986001; also known as festinavir or censavudine) (Fig. 1) and found that the compound had greater activity against HIV-2 than against HIV-1 in culture (32). To our knowledge, this remains the only report of an NRTI that, when tested against a diverse panel of HIV-1 and HIV-2 isolates, exhibits more-potent inhibition of HIV-2 replication. Molecular modeling suggests that the ethynyl group of BMS-986001-5'-triphosphate fits within a hydrophobic pocket in HIV-2 RT that corresponds to the aforementioned 4' pocket of the HIV-1 enzyme (28, 32). In agreement with this model, other 4'-modified nucleosides with 2'-deoxy (14, 33) and 2'-deoxy-2'- β -fluoro (34) sugar configurations also inhibit HIV-2 replication, with EC_{50} s in the nanomolar range. Collectively, these findings suggest that MK-8591 might inhibit a broad range of HIV-2 isolates with a potency comparable to or even better than that observed for HIV-1.

In the present study, we tested the activity of MK-8591 against a panel of HIV-1 and HIV-2 strains that were originally isolated from ART-naive individuals. We also evaluated the ability of MK-8591 to inhibit HIV-2 variants with mutations in RT that emerge in response to ART (9, 10, 35–49) and that confer resistance to various NRTIs in culture (29, 40, 50–52) and in biochemical assays with purified HIV-2 RT (30, 31). Unless otherwise specified, drug susceptibility measurements were performed using MAGIC-5A indicator cells, which are CD4⁺ CXCR4⁺ CCR5⁺ HeLa cells containing an HIV-inducible reporter gene (HIV long terminal repeat [LTR]- β -galactosidase). A detailed description of the assay protocol has been provided elsewhere (32). Importantly, this methodology directly quantifies antiviral activity in a single round of HIV infection (as opposed to measuring virus-induced cell death or viral protein release following multiple rounds of HIV replication), and the resultant EC_{50} s are not affected by strain-specific differences in replication rate, infectivity, cytopathic potential, or cell-to-cell spread (32, 53, 54). The 50% cytotoxic concentration (CC_{50}) for MK-8591 in MAGIC-5A cells was >100 nM, as determined using the CellTiter Glo assay (Promega) (32).

We initially compared the ability of MK-8591 to inhibit two prototypic isolates derived from full-length plasmid molecular clones: HIV-1_{NL4-3} and HIV-2_{ROD9}. We have previously shown that these isolates exhibit comparable susceptibilities to FDA-approved NRTIs in the MAGIC-5A cell line, with EC_{50} s that differ by 2-fold or less between the two strains (53). In the present study, we performed a total of 10 independent assay runs in which the sensitivity of HIV-1_{NL4-3} and HIV-2_{ROD9} to MK-8591 was tested head-to-head (e.g., Fig. 2A). In these experiments, the mean EC_{50} for HIV-1_{NL4-3} was 2.0 ± 0.6 nM. This result agrees with previously published measurements for HIV-1 in single-cycle assays (1.1 nM for HIV-1_{BH10} in MAGI cells [14], 3.2 nM for HIV-1_{NL101} in TZM-bl cells [15]). In contrast, within each assay run, the EC_{50} for HIV-2_{ROD9} was lower than the corresponding value for HIV-1_{NL4-3} by a factor of 2.3 to 9.9 (Fig. 2A and B). Following additional determinations ($n = 14$ total), the mean EC_{50} for HIV-2_{ROD9} was 0.42 ± 0.12 nM (Fig. 3A). Thus, MK-8591 was 4.8-fold more active against HIV-2_{ROD9} than against HIV-1_{NL4-3} in the single-cycle assay. These data are concordant with the results of spreading infection assays with CEMss cells (see reference [32] for the assay protocol), which yielded EC_{50} s of 38 pM for HIV-2_{ROD9} and 120 pM for HIV-1_{NL4-3}.

Other HIV-1 and HIV-2 isolates from ART-naive individuals showed a similar trend with respect to MK-8591 susceptibility in the single-cycle assay (Fig. 3A). The mean EC_{50} for 10 HIV-1 strains from groups M and O was 2.6 ± 0.6 nM (range = 2.0 to 3.8 nM), whereas HIV-2 isolates from groups A and B yielded EC_{50} s of 0.55 ± 0.17 nM and 0.50 ± 0.12 nM, respectively (range = 0.25 to 0.76 nM) (Fig. 3A and C). Overall, HIV-2 was more susceptible to MK-8591 than HIV-1 was by a difference of 4.8-fold (Fig. 3B).

To identify structural features that might explain the increased activity of MK-8591 against HIV-2, we constructed a molecular model of HIV-2 RT with MK-8591-TP bound

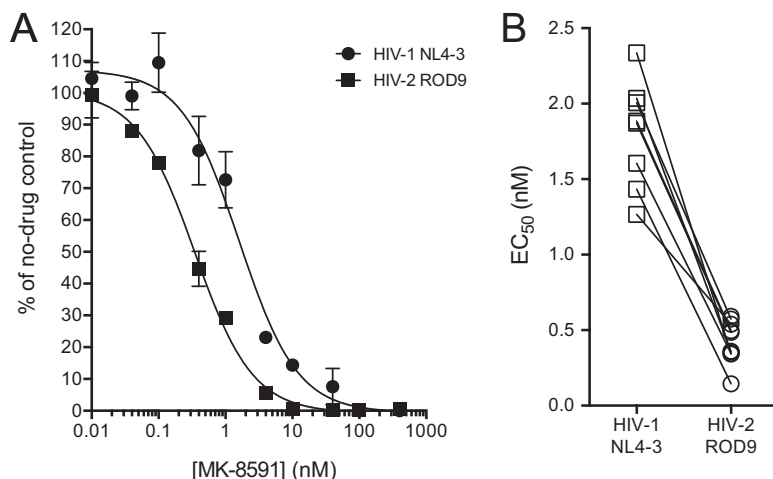


FIG 2 Antiviral activity of MK-8591 against HIV-1_{NL4-3} and HIV-2_{ROD9}. (A) Representative data from a single run of the drug susceptibility assay in MAGIC-5A cells (single-cycle assay) (32). Data points are the percentages of β -galactosidase activity in MK-8591-treated cultures relative to that in solvent-only controls. Each point represents the mean of results from two cultures that were maintained in parallel. Error bars represent ± 1 standard deviations and when not visible are smaller than the symbols. The curves were generated using a sigmoidal regression equation (GraphPad Prism 6.0 software). (B) EC_{50} s from 10 independent runs of the single-cycle assay in which HIV-1_{NL4-3} and HIV-2_{ROD9} were tested head-to-head. Diagonal lines indicate the paired results from a single assay run.

at the polymerase active site and compared the model to the published structure of the HIV-1 RT–MK-8591–TP complex (28) (see Fig. S1 in the supplemental material). This model recapitulates important features of the HIV-1 structure, including base-pairing interactions between MK-8591–TP and the template thymidine, coordination of the triphosphate by magnesium, and the presence of a hydrophobic pocket that accommodates the 4′-ethynyl moiety of the inhibitor. However, closer inspection of the two structures suggests that subtle differences in the positioning of residues A114, Y115, F160, M184, and D185 result in a wider 4′ pocket in HIV-2 RT than in the HIV-1/MK-8591–TP complex (see Fig. S1 in the supplemental material). A similar disparity in the sizes of the 4′ pockets is evident in our models of HIV-1 and HIV-2 RT with BMS-986001–5′-triphosphate as the incoming substrate (32). Whether this variation accounts for the differential sensitivities of HIV-1 and HIV-2 to MK-8591 is unclear and will likely remain uncertain until a crystal structure of the HIV-2/MK-8591–TP complex is obtained.

Mutations that are associated with NRTI treatment can have differing effects on drug susceptibility/resistance in HIV-1 versus HIV-2, as demonstrated for thymidine analog mutations and for the Q151M replacement in RT (29–31). We therefore examined the resistance profile of MK-8591 using a panel of site-directed HIV-2_{ROD9} RT mutants and a recombinant clone of HIV-2 (designated 4.7a) that was derived from a patient receiving AZT, lamivudine (3TC), and ritonavir-boosted lopinavir; this clone encodes five amino acid substitutions that are associated with ART in HIV-2-infected individuals (RT changes K65R, N69S, V111I, Q151M, and M184V; see reference [32] for treatment history, clone construction, and additional details). A site-directed mutant of HIV-1_{NL4-3} encoding the M184V replacement was included in this analysis as an MK-8591-resistant control (14).

Relative to the parental clone, the K65R mutant of HIV-2_{ROD9} was hypersusceptible to MK-8591, with a mean EC_{50} that was 2.5-fold lower than the value for the wild-type virus; the K65R+Q151M HIV-2_{ROD9} mutant was also hypersusceptible to MK-8591 (2.1-fold) (Table 1). MK-8591 was fully active against Q151M HIV-2_{ROD9}, whereas the M184V change conferred 26-fold resistance to the drug. A slightly lower level of resistance (9-fold) was observed for M184V HIV-1_{NL4-3} (Table 1). In contrast, in both HIV-1 and HIV-2, the M184V replacement confers $>1,000$ -fold resistance to FTC and 3TC (50, 51, 55). Overall, the changes in MK-8591 susceptibility that resulted from single

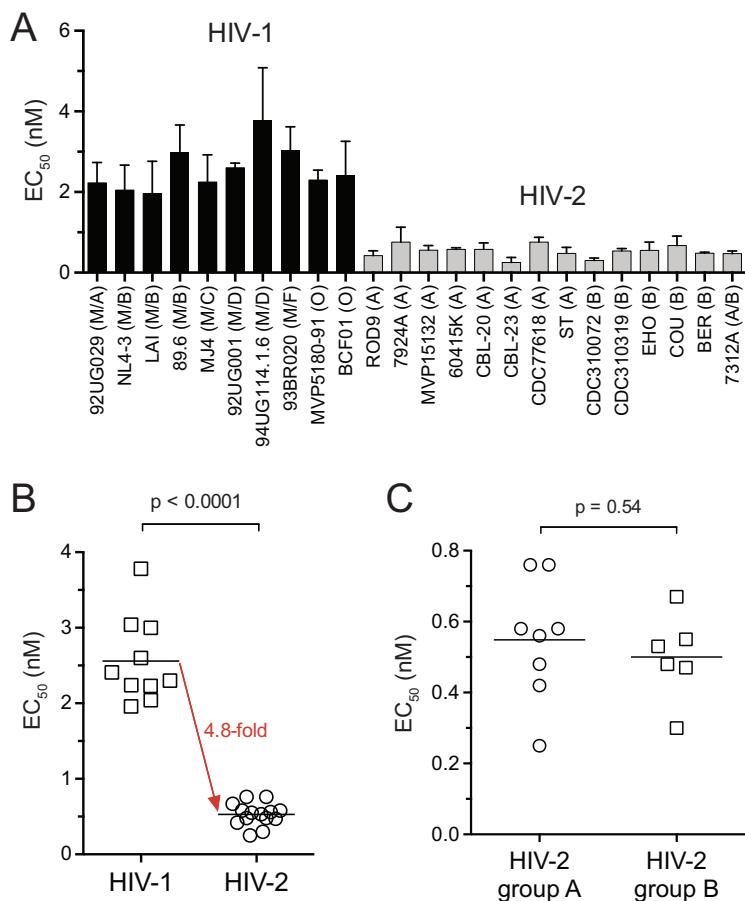


FIG 3 Susceptibility of HIV-1 and HIV-2 isolates from ART-naive individuals to MK-8591. (A) EC₅₀s for 10 HIV-1 and 14 HIV-2 strains. Bars are the means of results from three or more independent single-cycle assays. Error bars are +1 standard deviation. Group/subtype assignments are shown in parentheses. The HIV-2 intergroup recombinant 7312A encodes a group B *pol* gene (57). (B) Summary for the isolates shown in panel A. Each point represents the mean EC₅₀ for a single isolate. (C) Comparison of EC₅₀s for group A and B isolates of HIV-2. In panels B and C, the horizontal lines are the means from the respective data groups; *P* values are the results of Welch's *t* tests.

amino acid changes K65R, Q151M, and M184V in HIV-2_{ROD9} were similar to those reported for HIV-1 (13–15).

We also observed moderate MK-8591 resistance (13- to 19-fold) for HIV-2 mutants that encode M184V in combination with one or more treatment-associated amino acid changes in RT, including those in the patient-derived clone 4.7a (Table 1). In HIV-2_{ROD9}, the addition of up to three changes (K65R, Q151M, and either K70R or Y115F) together with M184V did not lead to higher levels of resistance than with M184V alone. It is important to note that the EC₅₀s for all HIV-2_{ROD9} clones containing M184V (with or without other changes) were only 2- to 4-fold greater than the mean EC₅₀ for HIV-1 isolates from ART-naive individuals (2.6 nM). Although we cannot exclude the possibility that other amino acid replacements in HIV-2 RT confer higher levels of MK-8591 resistance, our analysis demonstrates that MK-8591 retains substantial activity against the types of drug-resistant variants that typically emerge in NRTI-treated HIV-2 patients (9, 10, 35–49).

Lastly, we performed a series of experiments to confirm that the hypersusceptibility and resistance phenotypes observed with MK-8591 (Fig. 1 and 2 and Table 1) were not an artifact of the conditions used in the MAGIC-5A single-cycle assay. The protocol for this analysis was identical in all aspects to the one used in the above-described assays. In addition, for each strain tested in the follow-up study, the multiplicity of infection (MOI) was comparable to the MOI used in previous assay runs with MK-8591 (there was

TABLE 1 Susceptibilities of HIV-1 and HIV-2 RT mutants to MK-8591

HIV clone ^a	Genotype ^b	EC ₅₀ (nM) ^c	No. ^d	Fold change ^e
HIV-1 _{NL4-3}	Wild type	2.0 ± 0.62	10	
	M184V	18 ± 6.8	4	9
HIV-2 _{ROD9}	Wild type	0.42 ± 0.12	14	
	K65R	0.17 ± 0.03	3	0.4
	Q151M	0.38 ± 0.15	3	1
	M184V	11 ± 6.4	6	26
	K65R+Q151M	0.20 ± 0.06	3	0.5
	K65R+M184V	8.0 ± 2.7	3	19
	Q151M+M184V	5.9 ± 3.6	5	14
	K65R+Q151M+M184V	6.2 ± 2.0	3	15
	K65R+K70R+Q151M+M184V	7.0 ± 2.5	3	17
K65R+Y115F+Q151M+M184V	5.3 ± 3.3	5	13	
HIV-2 _{ROD9-4.7a}	K65R+N69S+V111I+Q151M+M184V	5.4 ± 0.7	3	13

^aViruses produced from full-length plasmids pNL4-3, pROD9, and patient-derived clone pROD9-4.7a.

^bAmino acid changes listed for HIV-1_{NL4-3} and HIV-2_{ROD9} were engineered by site-directed mutagenesis. Changes listed for HIV-2_{ROD9-4.7a} were encoded by a *pol* gene segment that was PCR amplified from an HIV-2-infected patient (32).

^cEC₅₀, 50% effective concentrations measured in the MAGIC-5A single-cycle assay. Values shown in bold are significantly different from the corresponding wild-type value ($P < 0.05$; analysis of variance of log₁₀-transformed EC₅₀s with Sidak's posttest).

^dNumber of independent dose-response assays performed for each strain.

^eEC₅₀ for the mutant divided by the EC₅₀ for the corresponding wild-type clone (wild-type HIV-2_{ROD9} for the patient-derived strain).

a <2-fold difference between the two experimental sets); we have previously shown that variations in MOIs of up to 32-fold have no effect on the EC₅₀s obtained in the single-cycle assay (32). As observed in our previously published work (29, 32, 53, 56), HIV-1_{NL4-3} and HIV-2_{ROD9} were equally susceptible to AZT, stavudine [d4T], and the integrase inhibitor raltegravir, with EC₅₀s that differed by <1.2-fold between the two strains (see Table S1 in the supplemental material). HIV-1_{NL4-3} and HIV-2_{ROD9} were also comparable to each other with respect to TDF susceptibility in this analysis (EC₅₀s of 13 ± 1.3 nM and 6.4 ± 1.3 nM, respectively). Furthermore, HIV-2 RT mutants that were either hypersusceptible or resistant to MK-8591 (Table 1) were fully sensitive to raltegravir (maximal change in EC₅₀ of 1.2-fold relative to the EC₅₀ for wild-type HIV-2_{ROD9}) (Table S2). Collectively, these data demonstrate that the MK-8591 hypersusceptibility and resistance phenotypes reported here for HIV-2 stem from intrinsic properties of the virus, rather than a systematic bias in assay setup or performance.

In summary, our findings show that MK-8591 is highly active against a broad range of HIV-2 isolates from ART-naïve individuals (Fig. 3A and B). In conjunction with our earlier study of BMS-986001 (Fig. 1) (32), data from the present work provide an additional example of a nucleoside analog that exhibits greater activity against HIV-2 than against HIV-1 in a single cycle of infection. MK-8591 is also highly active against NRTI-resistant HIV-2 mutants, including variants that harbor the M184V change in RT, with EC₅₀s of ≤11 nM (Table 1). Furthermore, the addition of K65R, Q151M, or both in combination with M184V did not lead to higher levels of MK-8591 resistance than with M184V alone. This result is significant given the frequent appearance of K65R+M184V, Q151M+M184V, and K65R+Q151M+M184V variants in HIV-2-infected patients (9, 36, 44, 48, 49). We speculate that, relative to 3TC/FTC, MK-8591 might delay the appearance of M184V in both HIV-1- and HIV-2-infected patients and, in the event of its emergence, retain substantial activity against the mutant virus. MK-8591 might also be considered for second-line or salvage therapy in patients harboring M184V variants. This could be particularly important for HIV-2 treatment, since second-line options for HIV-2 patients are restricted by both innate and emergent ARV resistance. Collectively, our findings suggest that MK-8591 may have clinical activity in HIV-2 infection.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00744-17>.

SUPPLEMENTAL FILE 1, PDF file, 9.0 MB.

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