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UK-453,061: A NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR FOR THE TREATMENT OF DRUG-RESISTANT HIV INFECTIONS

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Introduction

Non-nucleoside inhibitors of HIV-1 reverse transcriptase (RT) are key components of highly active anti-retroviral therapy (HAART) that can lead to sustained reductions in viral load and slow the rate of disease progression when combined with other anti-retroviral agents. There are three approved non-nucleoside RT inhibitors (NNRTIs) (efavirenz, nevirapine, and delavirdine), but all are associated with point mutations in HIV-1 that can lead to decreased susceptibility and class resistance, as well as significant adverse effects. Therefore, an unmet need exists for a well-tolerated NNRTI with activity against clinically significant drug-resistant HIV-1 mutants.

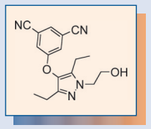
UK-453,061 (Figure 1) was selected as the NNRTI candidate on the basis of its low clearance when unbound (Cl_u; = <8 mL/min/kg) and the desire to minimize the octanol-water distribution coefficient (logD; = 1.8), which reduces metabolism by both Phase I and Phase II enzyme systems.[1] At least 50% of the administered dose of UK-453,061 can be absorbed from the intestinal tract of the rat, and absorption is complete in the dog. UK-453,061 has shown negligible metabolism in dog hepatocytes and in rat and dog liver microsomes, and is relatively stable to metabolism in human liver microsomes and human hepatocytes. Clearance of UK-453,061 is likely to be by a combination of glucuronidation and oxidative metabolism (primarily mediated by the cytochrome P450 isoform CYP3A4).

The data presented below summarize the biochemical and structural optimization of UK-453,061 against a panel of clinically relevant HIV-1 RT mutants and the further preclinical characterization of this emerging NNRTI candidate.

Methods

Isothermal titration calorimetry (ITC)

Figure 1. Structure of UK-453,061.



- Recombinant HIV-1 RT was concentrated to suitable concentrations for ITC (typically 13.2-13.9 µM) using Microcon-10 microconcentrators (Amicon). ITC experiments were performed twice at 25°C in 5% dimethyl sulfoxide using a MicroCal VP-ITC, with 215 µM UK-453,061 titrated into HIV-1 RT over 25 injections of 10 µL, made every 230 sec, into a fixed-reaction cell volume of 1.4272 mL.

X-ray crystallography

- Recombinant *wild-type (wt)* and K103N mutant HIV-1 RT (strain HXB2) were crystallized in the presence of UK-453,061, and X-ray diffraction was used to determine the structures of the resulting complexes to 2.8Å and 3.2Å resolution, respectively.

In vitro selectivity analysis

- *Human DNA polymerase β assay*: The activity of UK-453,061 against human DNA polymerase β, was determined using a primer extension assay. A 5' biotinylated 16mer oligo d(T) primer DNA was annealed to a poly(dA) template and was extended during incubation with human DNA polymerase β. Incorporation of [^{3}H]TTP was detected by scintillation.

In vitro anti-viral activity

- *Reverse transcriptase assays:* The activity of UK-453,061 against *wt* and mutant HIV-1 RT (BH10 strain) was determined as described above for the human DNA polymerase β assay by using a poly(rA) template.
- *HeLaP4 indicator cell line:* The anti-viral activity of UK-453,061 against the HIV-1 strain NL4-3 *wt* and a panel of NL4-3 recombinant viruses with NNRTI-resistance mutations was determined using the indicator cell line, HeLaP4. Virus replication was quantified by measuring levels of HIV-1 Tat-induced β-galactosidase 5 days after virus infection.
- *Peripheral blood lymphocytes (PBLs):* The anti-viral activity and cytotoxicity of UK-453,061 were assessed against HIV-1 strain Ba-L in mitogen-activated human peripheral blood lymphocytes. Viral replication was quantified using an HIV core antigen (p24) enzyme-linked immunosorbent (ELISA) assay.
- *Phenotypic resistance profiling:* The phenotypic resistance profile of UK-453,061 was assessed against:
  - A panel of 191 HIV-1 isolates derived from the plasma of treatment-naïve and anti-retroviral-treatment-experienced individuals using Virco's Antivirogram® assay
  - A panel of 100 HIV-1 isolates derived from NNRTI-treatment-experienced patients failing on a protease inhibitor regimen using Monogram Biosciences PhenoSense™ assay.

Results

ITC

- ITC experiments (one of which is shown in Figure 2) demonstrated that UK-453,061 binds to recombinant HIV-1 RT at 25°C and that the mean geometric dissociation constant (K_D) for this interaction was 624 nM (193.7 ng/mL) with a standard error of the geometric mean of 42.5 nM (13.2 ng/mL). The derived stoichiometry of the interaction was 0.750 ± 0.040 (mean), consistent with a 1:1 binding mechanism.

X-ray crystallography

- Structural analyses revealed that UK-453,061 binds non-covalently to the non-nucleoside binding site of HIV-1 RT, forming interactions with residues L100, V106, Y181, Y188, F227, W229, Y318, L234, and P236 of the p66 subunit (Figure 3a).
- The mode of binding of UK-453,061 to *wt* and K103N mutant HIV-1 RT is very similar (Figure 3a). In both of these structures, residue Y181 of HIV-1 RT is rotated approximately 100° around χ_1 (flipped "down") when compared with its location in complex with other NNRTIs, such as nevirapine (Figure 3b).
- This "tyrosine flip" opens a lipophilic pocket that could be exploited to achieve a 10-fold increase in potency.

In vitro selectivity analysis

Human DNA polymerase β assay

- UK-453,061 showed very weak activity against DNA polymerase β in a primer extension assay, generating two IC_{50} values greater than 100 µM (31.0 ng/mL), with a predicted geometric mean IC_{50} of 19.6 nM. The selectivity index of UK-453,061 for inhibition of HIV-1 RT over human DNA polymerase β was >1000.

In vitro anti-viral activity

Figure 2. Titration of UK-453,061 into recombinant *wt* HIV-1 RT at 25°C in 5% dimethyl sulfoxide.

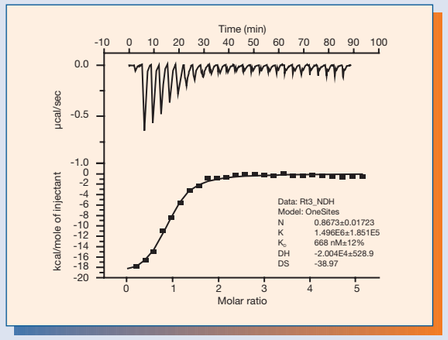
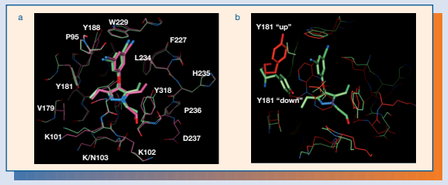


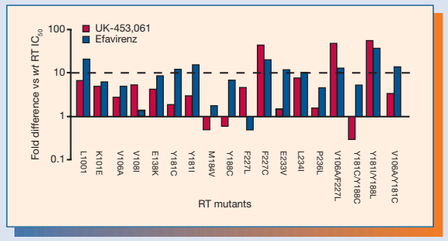
Figure 3. X-ray crystal structures of (a) UK-453,061 bound at the non-nucleoside binding site of recombinant HIV-1 RT (*wt* in pink, K103N mutant in green) and (b) an overlay of the NNRTI binding pocket for UK-453,061 (green) and nevirapine (red), showing the change in conformation of Y181.



Reverse transcriptase assays

- The K103N point mutation in HIV-1 RT is found in 40% of all clinical mutants and is the most common mutation associated with viral resistance to all three available NNRTIs.[2] The potency of UK-453,061 against HIV-1 RT is not markedly affected by the presence of the K103N mutation (geometric mean IC_{50} of 118 nM and 215 nM for *wt* and K103N RT, respectively).
- UK-453,061 retained potency similar (<10-fold increase in IC_{50}) to that observed against the *wt* enzyme in 15 of 18 single- and double-point engineered mutant HIV-1 RT enzymes (Figure 4). The activity of UK-453,061 was significantly reduced relative to that against *wt* HIV-1 RT (>10-fold increase in IC_{50}) for the mutant enzymes F227C, V106A/F227L, and Y181I/Y188L (Figure 4).

Figure 4. Activity profiles of UK-453,061 and efavirenz against a panel of 18 clinically relevant single- and double-point mutants of HIV-1 RT.



HeLaP4 indicator cell line

- 14 of 18 HIV-1 strain NL4-3 viruses harboring mutations associated with resistance to commercially available NNRTIs were susceptible to inhibition by UK-453,061, when compared with HIV-1 strain NL4-3 *wt* (Table 1). The activity of UK-453,061 was significantly reduced relative to that against *wt* HIV-1 (>10-fold increase in IC_{50}) for the mutant viruses F227C, L234I, V106A/F227L, and Y181I/Y188L (Table 1).

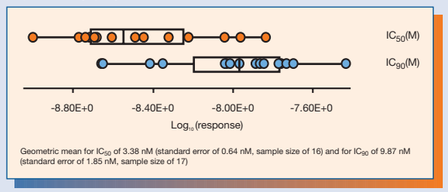
Table 1. Activity of UK-453,061 against a selected panel of HIV-1 RT point mutant viruses.

NL4-3 virus	Geometric mean IC_{50} (nM)	Fold increase over *wt* IC_{50}	Geometric mean IC_{90} (nM)	Fold increase over *wt* IC_{90}
L100I	11.5	2.9	120	4.2
K101E	34.4	8.7	212	7.5
V106A	12.3	3.1	92.6	3.3
V108I	19.4	4.9	139	4.9
E138K	22.9	5.8	120	4.2
Y181C	8.77	2.2	53.9	1.9
Y181I	3.18	0.8	16.1	0.6
M184V	5.95	1.5	33.1	1.2
Y188C	1.31	0.3	6.87	0.2
F227C	223	56.4	>990	>35.1
F227L	25.0	6.3	162	5.8
E233V	5.44	1.4	29.3	1.0
L234I	86.0	21.7	>749	>26.6
P236L	1.37	0.3	5.31	0.2
V106A/Y181C	20.4	5.1	125	4.4
V106A/F227L	>855	>216	>1330	>47.2
Y181C/Y188C	1.74	0.4	10.0	0.4
Y181I/Y188L	265	67.0	>1190	>42.2

Peripheral blood lymphocytes (PBLs)

- UK-453,061 potently inhibited HIV-1 Ba-L in PBLs during a 5-day culture assay, with geometric means for IC_{50} and IC_{90} of 3.38 nM (1.05 ng/mL) and 9.87 nM (3.06 ng/mL), respectively (Figure 5). The ability of UK-453,061 to inhibit HIV-1 replication in this system was not associated with any cytotoxicity.

Figure 5. Box and whisker plots of IC_{50} and IC_{90} data for UK-453,061 against HIV-1 Ba-L in peripheral blood lymphocytes.

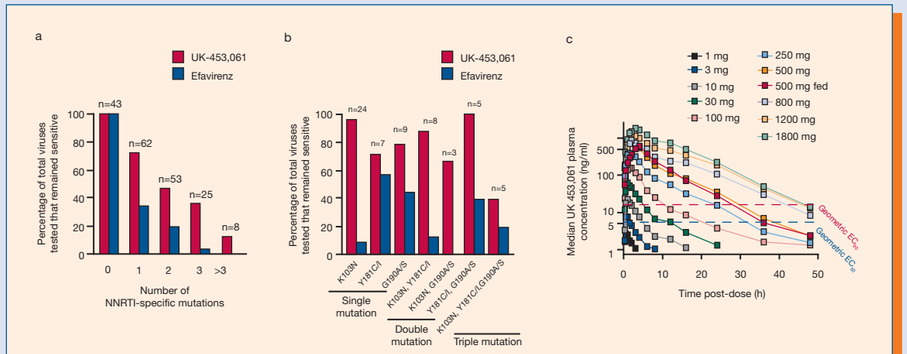


Geometric mean for IC_{50} of 3.38 nM (standard error of 0.64 nM, sample size of 16) and for IC_{90} of 9.87 nM (standard error of 1.85 nM, sample size of 17)

Virco Antivirogram® assay

- UK-453,061 demonstrated anti-viral activity against 45 of 62 isolates containing single NNRTI mutations (including 23 of 24 K103N mutants), 35 of 86 isolates containing multiple NNRTI mutations, and against all 43 isolates with non-NNRTI-specific Virco specifications (the majority of which had phenotypic resistance to nucleoside reverse transcriptase inhibitors and protease inhibitors) (Figure 6a). The sensitivity of clinically relevant HIV-1 NNRTI mutants to UK-453,061 and the commercially available NNRTIs is shown in Figure 6b. The EC_{50} and EC_{90} from the 191 data generated in this study have been plotted against the median UK-453,061 plasma concentration versus time from the Phase I clinical study (Figure 6c).[3]

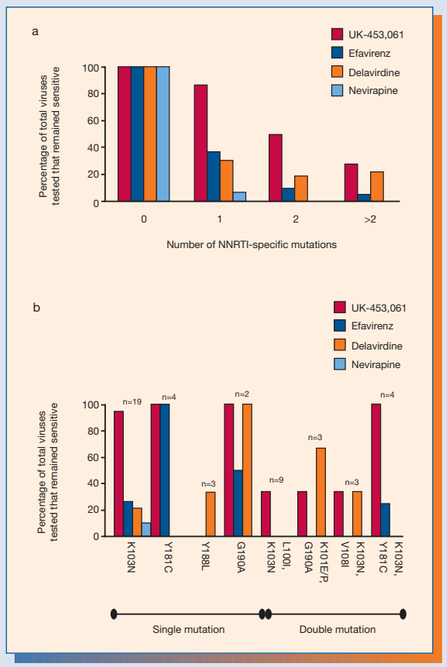
Figure 6. UK-453,061 and efavirenz sensitivity of HIV-1 viruses with (a) single, double, triple, more than three, or no NNRTI mutations and (b) key clinically relevant NNRTI mutations UK-453,061, efavirenz, delavirdine, and nevirapine, determined by Virco's Antivirogram® assay. (c) Plain lines represent the log linear plot of median UK-453,061 plasma concentration versus time for all single-dose cohorts.[3] Dotted lines represent the geometric mean EC_{50} (blue) and EC_{90} (red) derived from the Antivirogram® data.



Monogram Biosciences PhenoSense™ assay

UK-453,061 demonstrated anti-viral activity against 26 of 30 isolates containing single NNRTI mutations (Y188L demonstrated high-level [>100-fold] resistance), 21 of 50 isolates containing multiple NNRTI mutations, and against all 20 isolates with no NNRTI mutations (Figure 7a). The sensitivity of clinically relevant HIV-1 NNRTI mutants to UK-453,061 and the commercially available NNRTIs is shown in Figure 7b.

Figure 7. Sensitivity of HIV-1 viruses with (a) single, double, more than two, or no NNRTI mutations and (b) key clinically relevant NNRTI mutations to UK-453,061, efavirenz, delavirdine, and nevirapine, determined by Monogram Biosciences PhenoSense™ assay.



Conclusions

- UK-453,061 is a novel and selective NNRTI that binds *wt* and K103N HIV-1 RT in a very similar manner and induces conformational changes in tyrosine residues 181 and 188 that are different from those observed with other NNRTIs. The excellent anti-viral profile of UK-453,061 against HIV-1 NNRTI-resistance mutants may be related to the flexibility of this tyrosine sub-pocket and the H-bonds and lipophilic contacts formed within it.
- UK-453,061 demonstrated anti-viral activity in HIV-1-infected PBLs and displayed balanced activity against clinically relevant HIV-1 RT single- and double-point NNRTI mutants in in vitro assays, retaining potency similar to that observed against the *wt* enzyme. When compared with other NNRTIs, UK-453,061 displayed broader anti-viral activity against the key HIV-1 RT NNRTI-resistance mutants K103N, Y181C and G190A.
- Studies in healthy male subjects found that UK-453,061 was well tolerated, was rapidly absorbed, had a mean terminal half-life of 7-11 hours on multiple dosing, and that food had a small but non-clinically relevant effect on systemic exposure.[3]
- A randomized, double-blind, placebo-controlled, multicenter study involving asymptomatic NNRTI-naïve HIV-1-infected patients recently demonstrated that 7-day monotherapy with UK-453,061 was well tolerated and that doses ⩾500 mg/day resulted in a ⩾1.7 log_{10} mean decrease in HIV-1 RNA from baseline to nadir.[4] These doses provide a median UK-453,061 plasma concentration above the geometric mean EC_{90} from 191 clinical isolates for more than 24 hours.
- Altogether, data suggest that UK-453,061 has the pharmacokinetic, pharmaceutical, and drug safety credentials of a potential treatment for HIV-1 infection that merits further evaluation in clinical studies.

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Abstract

Background: NNRTIs are key components of HAART: when combined with other anti-retroviral agents they can lead to sustained reductions in viral load and slow the rate of disease progression. However, NNRTIs are susceptible to mutations in HIV RT, in particular changes that can lead to class resistance. There is a need for a safe and well-tolerated NNRTI with activity against clinically significant drug-resistant viruses, which can be conveniently combined with agents from other classes.

Methods: We utilized biochemical and structural information to optimize a series of pyrazoles against a panel of mutant enzymes encompassing the majority of clinically relevant mutations. Leads were evaluated for efficacy against a panel of 300 recombinant point-mutated and clinical isolates including AntiVirogram™ and PhenoSense™ HIV phenotypic drug susceptibility assays.

Results: The emerging candidate, UK-453,061 inhibits over 60% of viruses bearing key RT mutations with IC_{50} values within 10-fold of *wt* viruses, as opposed to less than 40% for currently available NNRTIs. In vitro-derived UK-453,061-resistant virus is sensitive to licensed NNRTIs. In non-clinical safety pharmacology studies, the candidate was well tolerated and displayed little interaction with physiologically important receptors or enzymes. Preclinical ADME data indicates that UK-453,061 is likely to be cleared in humans by a combination of glucuronidation and cytochrome P450 metabolism.

Conclusions: Altogether preclinical data suggest that UK-453,061 is a highly potent and selective NNRTI, with excellent efficacy against NNRTI-resistant viruses, and the pharmacokinetic, pharmaceutical and drug safety credentials of a convenient and well-tolerated HAART component. Clinical studies with UK-453,061 are in progress.