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Preclinical Evaluation of HBY 097, a New Nonnucleoside Reverse Transcriptase Inhibitor of Human Immunodeficiency Virus Type 1 Replication

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HBY 097 [(*S*)-4-isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihydroquinoxaline-2(1*H*)-thione] was selected from a series of quinoxalines as a nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (NNRTI). HBY 097 was shown to be a highly potent inhibitor of HIV-1-induced cell killing and HIV-1 replication in a variety of human cell lines as well as in fresh human peripheral blood lymphocytes and macrophages. The compound was also active against a variety of clinical isolates of HIV-1 including different HIV-1 subtypes and viruses resistant to 3′-deoxy-3′-azidothymidine. Mutant reverse transcriptases which arise as a consequence of treatment with other nonnucleoside inhibitors of HIV-1 reverse transcriptase were still inhibited by HBY 097 at relatively low concentrations. An HIV-1_{MN} variant resistant to inhibition by HBY 097 displayed in the reverse transcriptase gene a mutation causing a substitution at position 190 of a glutamic acid for a glycine residue (G190→E), which is characteristic for quinoxaline derivatives. The drug was demonstrated to possess a favorable toxicity profile and to show good oral bioavailability in both mice and dogs. As a consequence of its outstanding properties, HBY 097 was selected for further development and is at present undergoing clinical trials.

Nonnucleoside inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), referred to as NNRTIs, have been shown to be potent suppressors of in vitro HIV-1 replication (see reference 7 for a review). Belonging to different chemical classes, including tetrahydroimidazo (4,5,1-jk)(1,4)-benzodiazepin-2(1*H*)-one and -thione (TIBO), dipyridodiazepinone, pyridinone, bis(heteroaryl)piperazine, and α-anilinophenylacetamide derivatives (10, 21, 24, 25, 32), all NNRTIs are believed to bind to a lipophilic pocket within the p66 subunit of the RT specified by HIV-1 (12, 17). Although no data describing the structure of the corresponding enzyme of HIV-2 are available, the lack of inhibitory potency of any NNRTI against both in vitro HIV-2 RT activity and replication of HIV-2 in cell cultures may serve as proof of the absence of a comparable tertiary structure of the protein in this molecule.

The use of NNRTIs as therapeutic agents for the treatment of HIV-1 infection has been limited so far by the rapid emergence of drug-resistant viral variants in patients (30, 35, 39). The most common RT amino acid change, which appeared with most NNRTIs either as a result of the administration of these drugs to HIV-1-infected individuals or during in vitro selection experiments, is cysteine for a tyrosine at position 181 (Y181→C) (3, 18, 22, 28, 30, 35, 39).

We have previously reported the synthesis and in vitro anti-HIV-1 activities of 6-chloro-3,3-dimethyl-4-(isopropenyloxycarbonyl)-3,4-dihydroquinoxaline-2(1H)-thione (S-2720), which arose from a screening program and which served as a lead structure (14). S-2720 and other quinoxaline NNRTIs share the favorable property of an improved resistance profile, selecting for a characteristic G190→E mutant HIV-1 RT in vitro, which possesses clearly reduced enzymatic activity (2, 14, 15).

Structure-activity studies led to (*S*)-4-isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihydro-quinoxaline-2(1*H*)-thione (HBY 097) (23, 31), which was selected for further development and is at present undergoing clinical trials. We describe here the preclinical evaluation of HBY 097.

MATERIALS AND METHODS

Compounds. The quinoxaline derivative S-2720 was synthesized as described previously (14). HBY 097 was obtained by refluxing 2-chloro-4-methoxynitrobenzene with *S*-methylcysteine in 2-methoxyethanol by using 2 N NaOH as the base. Reduction with Raney nickel in methanol-acetic acid was followed by spontaneous cyclization to afford the quinoxaline ring system. Acylation at the 4 position is achieved with isopropyl chloroformate-pyridine in methylene chloride. Thiation was performed with phosphorous pentasulfide in toluene to yield HBY 097. L-697661 and nevirapine were synthesized in-house by published methods, and 3′-deoxy-3′-azidothymidine (AZT) was obtained from Burroughs Wellcome (Research Triangle Park, N.C.).

Cells and viruses. H9 cells (26, 27) were used to propagate HIV-1_{MN} (9, 37), and these reagents were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Peripheral blood lymphocytes (PBLs) were prepared to grow HIV-1_{D34} (33), HIV-$1_{19/34\text{-}TI}$ (14), HIV-$1_{D\ 117\ III}$, HIV-$1_{MVP5180}$ (subtype O) (11), HIV-1_{I757} (subtype C) (8), HIV-$1_{TH93051}$ (subtype E), HIV-1_{1420}, HIV-$1_{D148/89}$ (34), and HIV-2_{ROD} (5), as well as all primary clinical isolates. HIV-$1_{D148/89}$ is an AZT-resistant strain and displays RT amino acid substitutions M41→L, D67→N, K70→R, and T215→Y (13, 20). HIV-1_{1420} harbors the same genotypic AZT resistance pattern as HIV-$1_{D148/89}$ and was obtained from a male

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patient with hemophilia A who underwent 26 months of AZT monotherapy at the time of virus isolation. HIV-$1_{19/34\text{-TI}}$ is a mutant virus generated via in vitro selection for resistance against TIBO R82150, specifying A98→G and L100→I RT amino acid changes.

Cells were maintained in RPMI 1640 medium containing 10% fetal calf serum and 40 IU of interleukin-2 per ml (for PBLs) in a humid 5% CO_2 atmosphere. For propagation of HIV-$1_{D\ 117\ III}$, fresh human mononuclear cells were prepared from the blood of healthy donors by Ficoll gradient centrifugation. The cells were suspended in RPMI 1640 medium containing 5% human type AB^+ serum and were cultivated on hydrophobic membranes (Teflon bags; 3×10^6 cells per ml). After 24 h, the MNCs were infected by the addition of HIV-$1_{D\ 117\ III}$-infected lymphocytes (5×10^4 infected PBLs per ml of MNCs). Forty-eight hours after infection, the cells were transferred to 24-well plates (Falcon), and the nonadhering cells were quantitatively washed off. The adherent cells were further grown in a culture volume of 1.3 ml, and fresh medium was added every week (38).

Determination of IC_{50}s and 50% cytotoxic concentrations. A total of 10^5 cells were infected with virus stocks at 0.01 PFU per cell, seeded onto 24-well plates, and incubated for 72 to 96 hours in the presence of compounds (initially dissolved in dimethyl sulfoxide [DMSO] and diluted in cell culture medium). For experiments with macrophages, compounds were added either at the beginning of the experiment (day 0) or 2 h prior to infection (day 1). Virus replication was evaluated either by light microscopy for syncytium formation or by testing 1:1 to 1:1,000 dilutions of the culture supernatants by a p24 antigen capture assay (Innotest HIV-1/HIV-2 antigen; Medipro, Ketsch, Germany). The concentrations of compounds that inhibited virus replication by 50% (IC_{50}) were calculated by using data from the antigen assay. In general, the presence of syncytium formation was examined by light microscopy, and syncytium formation was used to confirm the results of the p24 antigen assay.

For determination of the 50% cytotoxic concentrations, media containing dilutions of compounds dissolved in DMSO were added to wells containing 2.5×10^5 cells and the mixtures were incubated. The number of cells was determined after 96 h of cultivation by using an automated cell counter (Sysmex CC-130) to determine the drug concentration causing a 50% decrease in cell proliferation.

Inhibitory effects of drugs on the oxidative metabolism of cells. Test compounds were dissolved in DMSO and were further diluted in medium. The human T-lymphocyte cell lines H9 and J.Jhan were incubated with the test compounds for 40 h. After the addition of MTT (Promega 96), the cells were incubated for another 4 h and were finally solubilized for 1 h. The A_{540} was determined.

Selection of an HIV-1 variant resistant to inhibition by HBY 097 and genotypic analysis of the RT gene. HIV-1_{MN} was passaged on H9 cells in the presence of increasing amounts of HBY 097 through a total of 11 passages (0.5, 1, 2, 4, 8, 16, 64, 128, and 500 nM and 2 and 10 μM HBY 097, respectively) to obtain HIV-$1_{097/1}$. For determination of proviral HIV-1 *pol* gene sequences, purified cellular DNA was used in a nested PCR protocol. The first reaction mixture contained 0.5 μg of DNA and 10 pmol of primers JA99 and RTRIT137 per 50 μl (1). An aliquot of the first PCR mixture was subjected to a second amplification with primers JA100 (1) and RTRIT138 (5′-biotin-CTGTCTTTTTCTG GTAGCACTATAGG). The biotinylated DNA strand was separated from the unlabeled strand by using streptavidin-coated magnetic beads (Dynal) and served as a template for dideoxy chain termination sequencing with primers RT1SEQ2F (5′-fluorescein-CAATGGCCATTGACAGAAG) and RT8KF (5′-fluorescein-CTGCATTTACCATACCTAG). Analysis of the reaction products on an A.L.F. automated DNA sequencer (Pharmacia) allowed determination of the sequences corresponding to HIV-1 RT amino acids 35 to 242.

For specific detection of the G190→E mutation by selective PCR (19, 29), lysates of HIV-1_{MN}-infected cells were obtained at different drug concentrations (passages). The 1.7-kbp RT gene was amplified with primers RT101 (5′-ACTT TAAATTTTCCCATTAG) and RT102 (5′GGGCCTTATCTATTCCATC). A 1-μl aliquot of the first reaction mixture was used for either wt 190 PCR with oligonucleotides RT101 and RTGLUG (5′CCTATTTCTAAGTCAGATC) or for 190E PCR with primers RT101 and RTGLUA (5′CCTATTTCTAAGTC AGATT). Products of the heminested amplifications of 599 bp were detected on a 2% agarose gel.

Preparation of recombinant RTs and in vitro RT assays. Total DNA was prepared from cells infected with HIV-1 strains. RT-coding regions were amplified by PCR with primers RT1 and RT2B for HIV-1 and primers HIV2RT1 and HIV2RT2 for HIV-2 (14, 15). RT mutants were generated by site-specific mutagenesis as described previously (14, 15). PCR products of 1.7 kbp were inserted into the *Bam*HI-*Xba*I cloning sites (the *Sac*I-*Hin*dIII cloning sites for the HIV-2 RT gene) of the *Escherichia coli* expression vector pMalcRI. The P_{tac} promoter was induced with 0.3 mM isopropyl-β-D-thiogalactopyranoside to produce recombinant proteins in bacteria transformed with plasmid DNA encoding RT genes. Extraction of enzymes fused to the *E. coli* maltose binding protein and purification of enzymes were performed as described previously (14, 15). The IC_{50}s of the test compounds were obtained by using a heteropolymeric primer-template and scintillation proximity assay reagents essentially as described previously (14, 15).

Pharmacokinetics. Female NMRI mice weighing 22 to 25 g were used for the experiments. The animals had free access to food and drinking water before and during the experiment. The compounds were dissolved in a mixture of Epicholin 75–Cremophor EL–Glucofurol 75 (1:1:8.5) and were further diluted in water (1:10). A total of 0.1 or 0.2 to 0.3 ml of these formulations was administered either intravenously or orally, respectively. Blood samples obtained by heart puncture after urethane narcosis were pooled (two animals per pool) for each individual time point. Appropriate galenic formulations of S-2720 or HBY 097 were administered intravenously (only HBY 097) or orally to beagle dogs which were fasted for 12 h with free access to water. Food was allowed again approximately 5 h after drug administration. Blood samples were collected by venipuncture and serum was obtained by coagulation for 30 to 60 min at 4°C. Within 1 h the samples were centrifuged at 3,000 rpm for 10 min and 4°C. All samples were stored at −20°C until further analysis. Serum samples were precipitated in 70% (vol/vol) acetonitrile at 0°C. After centrifugation for 15 min at $16{,}000 \times g$ the supernatant was analyzed by high-pressure liquid chromatography. A reversed-phase column (C_{18}, 4 by 250 mm) was eluted with acetonitrile-water-trifluoroacetic acid (60:40:0.1) at a flow rate of 1 ml/min at 25°C. The compounds were detected by UV absorption at 335 nm. The detection limit was 10 ng/ml of serum for both HBY 097 and S-2720. All analytical determinations were done in duplicate. The pharmacokinetic parameters were calculated by fitting the drug concentration in serum to a series of exponential equations by nonlinear least-squares regression.

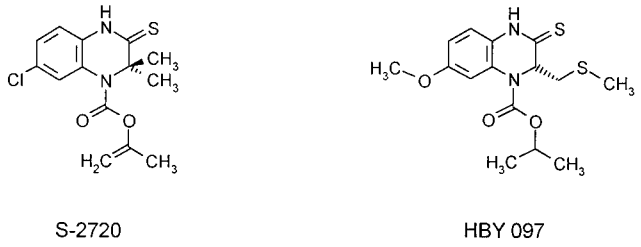

FIG. 1. Structural formulas of S-2720 and HBY 097.

RESULTS

Inhibition of virus replication by HBY 097. HBY 097 is a derivative of the quinoxaline lead compound S-2720 (Fig. 1). We first determined the activity of HBY 097 against different strains of HIV-1 commonly used in our laboratories for the in vitro evaluation of anti-HIV compounds, including viruses resistant to inhibition by AZT and TIBO R82150 (Table 1). Antiviral activity at very low concentrations was seen against all of the strains with the exception of HIV-$1_{097/1}$. However, like other NNRTIs, HBY 097 did not prevent the replication of HIV-2. When compared with S-2720, HBY 097 was only slightly more active against the viruses tested, but it proved to be at least 10 times more potent than two other NNRTIs (L-697661 and nevirapine) which were evaluated. The phosphorylation-independent inhibitory activity of HBY 097 was seen against HIV-$1_{D\ 117\ III}$ in mononuclear cell cultures, as expected with a NNRTI.

The compound was also demonstrated to be highly active against a series of fresh clinical HIV-1 isolates. Forty-one HIV-1 isolates were tested, including 17 viruses from which the RT gene sequences were determined: the median IC_{50}s and IC_{90}s of HBY 097 for all strains were 2.0 and 7.1 nM, respectively. AZT resistance and the different cytopathogenicities of the isolates investigated did not affect their susceptibilities to the quinoxaline derivative (Table 2).

No antiviral activity was found against HIV-related animal retroviruses such as the simian immunodeficiency virus or visna virus in vitro. The compound was also devoid of activity against adenovirus type 5, herpes simplex virus types 1 and 2, influenza virus type A2 (A/Aichi), and rhinovirus type 2 in vitro (data not shown).

Determination of possible cytotoxic properties of HBY 097. A 50% cytotoxic concentration of 25 μM was found with HBY

TABLE 1. Antiviral activities of HBY 097 and different RT inhibitors against laboratory strains of HIV

Virus	IC_{50} (μM)[a]				
	HBY 097	S-2720	L-697661	Nevirapine	AZT
$HIV\text{-}1_{MN}$	0.006 ± 0.0005	0.007 ± 0.001	0.067 ± 0.013	0.208 ± 0.030	0.021 ± 0.006
$HIV\text{-}1_{D34}$	0.002 ± 0.0004	0.003 ± 0.0008	0.030 ± 0.007	0.030 ± 0.004	0.007 ± 0.0009
$HIV\text{-}1_{1420}$	0.001 ± 0.0007	0.003 ± 0.001	0.046 ± 0.011	0.032 ± 0.009	>30
$HIV\text{-}1_{D148/89}$	<0.010				>1.00
$HIV\text{-}1_{19/34\text{-}TI}$	0.05 ± 0.01				
$HIV\text{-}1_{097/1}$	>10	>10	>10	>10	
$HIV\text{-}1_{D117III}$	0.006 ± 0.001	0.008 ± 0.002			0.025 ± 0.007
$HIV\text{-}1_{MVP5180}$	0.012 ± 0.003				
$HIV\text{-}1_{I757}$	0.001 ± 0.0002				
$HIV\text{-}1_{TH93051}$	0.001 ± 0.0002				
$HIV\text{-}2_{ROD}$	>10	>10	>10	>10	0.150 ± 0.020

[a] Values are means ± standard deviations.

097 on PBLs. In addition, we measured the toxicities of HBY 097 and S-2720 in cell culture using the MTT assay. Neither S-2720 nor HBY 097 affected the oxidative pathway in H9 cells, as measured by formazan formation (50% effective concentrations, >290 μM). Likewise, HBY 097 and S-2720 displayed low cytotoxic potentials when incubated with J.Jhan cells (50% effective concentrations, 200 and >290 μM, respectively).

Characterization of HBY 097-resistant HIV-1 and in vitro activity of HBY 097 against drug-resistant HIV-1 RT mutants. $HIV\text{-}1_{MN}$ was passaged on H9 cells in the presence of increasing concentrations of HBY 097. At passage 11 (10 μM HBY 097, $HIV\text{-}1_{097/1}$) the proviral RT gene sequence was analyzed: a point mutation in codon 190 of the RT gene was detected (GGA→GAA), leading to a G190→E RT mutant. No mutations other than the latter transition were found. By using a selective PCR method, it was demonstrated that the proportion of G190→E RT-specifying genomes increased during the course of the selection experiment (Fig. 2). The gradual rise of RT G190→E-mutated genomes was confirmed by sequence analysis of PCR products obtained from different passages. Selection for the resistance of $HIV\text{-}1_{MN}$ against the pyridinone L-697661 resulted in the appearance of the RT Y181→C amino acid change, as expected.

Using recombinant RTs expressed in *E. coli*, we showed that the G190→E RT is the only single mutant causing high-level resistance to inhibition by HBY 097. Other mutations known to confer viral resistance against NNRTIs and the [1-[2′,5′-bis-*O*-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]pyrimidine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (TSAO)-specific E→138K RT substitution (4) either did not affect the inhibitor's potency or mediated only moderate decreases in enzyme susceptibility to HBY 097 activity. Multiple RT mutants carrying the G190→E substitution and a triple mutant with L100→I, V108→I, and Y181→I amino acid changes were highly resistant to inhibition by HBY 097 (Table 3).

Pharmacokinetic properties of HBY 097 upon oral and intravenous administration to mice and dogs. HBY 097 was rapidly absorbed in mice after administration of an oral dose (100 mg/kg of body weight), achieving maximum levels in plasma approximately 30 min after dosing (3 μg/ml). In contrast, only low drug levels were achieved in plasma with S-2720 (0.14 μg/ml). Mean elimination half-life values for intravenous doses were comparable for both drugs (35 min for S-2720 and 24 min for HBY 097) (Table 4). Assuming dose proportionalities, the oral bioavailabilities for HBY 097 and S-2720 were calculated from the area under the data values for intravenous and oral administration. When compared with S-2720, HBY

TABLE 2. Inhibition of in vitro replication of primary clinical isolates of HIV-1 by HBY 097

HIV-1 strain characterization[a]	Mean (range) IC_{50} (nM)	Mean (range) IC_{90} (nM)
AZT resistant (n = 14)	3.3 (0.1–11)	5.1 (0.9–21)
AZT susceptible (n = 17)	1.7 (0.1–3.9)	6.2 (0.4–19)
SI (n = 38)	2.0 (0.1–16)	7.4 (0.4–57)
NSI (n = 3)	1.8 (0.6–4.0)	3.3 (2.0–4.0)
Total (n = 41)	2.0 (0.1–16)	7.1 (0.4–57)

[a] AZT-resistant strains either had at least one of the five known resistance-conferring mutations (13, 20) in the RT gene or the IC_{90} of AZT for the strains was >1.5 μM. Differentiation of SI and NSI viruses was done as described previously (36).

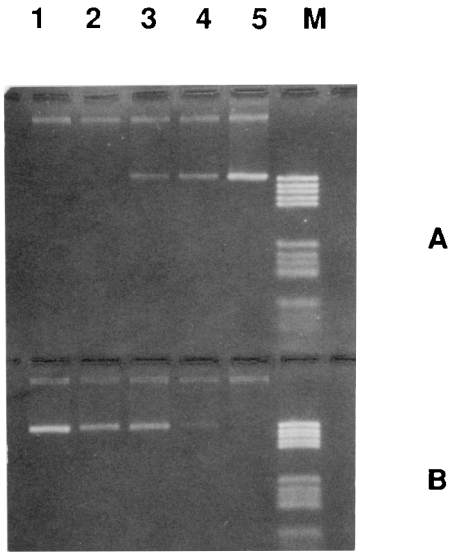

FIG. 2. Increasing resistance of $HIV\text{-}1_{MN}$ against HBY 097 as monitored by selective PCR. Lanes 1 to 5: 2 nM, 16 nM, 64 nM, 128 nM, and 10 μM HBY 097, respectively. (A) PCR for G190→E (GAA) mutant codon; (B) PCR for G190 (GGA) wild-type codon.

TABLE 3. In vitro inhibition of mutant recombinant RTs by HBY 097

Enzyme	Amino acid substitution	IC_{50} of HBY 097 (μM)[a]	Fold increase in IC_{50} compared with that for wild type
RT-1 MN	Wild type	0.08 ± 0.0025	1.0
RT-1 E	G190→E	>29	>363
RT-1 A	G190→A	0.71 ± 0.0068	8.9
RT-1 S	G190→S	1.0 ± 0.031	12.5
RT-1 I3	Y181→I	3.9 ± 0.049	49
RT-1 C	Y181→C	0.56 ± 0.069	7.0
RT-1 L3	Y188→L	2.0 ± 0.043	25
RT-1 I2	L100→I	0.59 ± 0.01	7.4
RT-1 A2	V106→A	0.58 ± 0.01	7.3
RT-1 L4	M230→L	1.0 ± 0.031	12.5
RT-1 L2	P236→L	0.11 ± 0.0043	1.4
RT-1 K	E138→K	0.22 ± 0.0063	2.8
RT-1 R2	K104→R	0.08 ± 0.0029	1
RT-1 EC	Y181→C, G190→E	>29	>363
RT-1 EI3	Y181→I, G190→E	>29	>363
RT-1 II3	L100→I, V108→I, Y181→I	>29	>363
RT-1 IERA	K103→R, V106→A, Y181→I, G190→E	>29	>363
RT-1 VE	M184→V, G190→E	>29	>363
RT-1 EY	G190→E, T215→Y	>29	>363

[a] Values are means ± standard deviations.

097 achieved a considerably higher degree of oral absorption (48 versus 10%).

The excellent oral bioavailability of HBY 097 compared with that of S-2720 was also confirmed in dogs. Oral administration of HBY 097 resulted in maximum levels in serum of more than 2 μg of drug per ml with both 34- and 83-mg/kg doses, whereas with a dose as high as 100 mg of S-2720 per kg, the amount of drug in serum was found to be less than 0.1 μg/ml. With the administration of 5.3-mg/kg intravenous doses, maximum levels of about 6 μg of HBY 097 per ml were achieved. The mean elimination half-life of HBY 097 for the intravenous administration route was 1.6 h. The oral bioavailability of HBY 097 in dogs was calculated to be 54%, assuming dose proportionality (Table 4).

DISCUSSION

We have described here the preclinical investigation of HBY 097, a member of the quinoxaline class of NNRTIs. These specifically substituted quinoxalines were previously shown to be highly potent and specific inhibitors of both in vitro HIV-1 replication and HIV-1 RT activity (2, 14, 23, 31). Structure-activity relationship studies resulted in the lead compound S-2720 (14). Since all of the known RT inhibitors cause the appearance of resistant viruses both in vitro (cell culture) and in vivo (patients), S-2720 was used to investigate its resistance profile in tissue cultures infected with HIV-1. It was found, that, unlike other NNRTIs, S-2720 selected for a novel RT G190→E substitution, which caused high-level viral resistance to the drug and contemporarily reduced the enzymatic activity of the resistant RT.

These observations prompted us to select a quinoxaline derivative for further development. HBY 097 fulfilled a number of essential preconditions. The most important difference between HBY 097 and S-2720 is the favorable bioavailability of HBY 097 in both mice and dogs (Table 4). Furthermore, the cytotoxic potential of the new drug was demonstrated to be relatively low, resulting in a selectivity index in the range of 10^4. The antiviral activity of the compound was studied intensively and was shown to be outstanding against different subtypes of HIV-1 and also against a panel of 41 clinical isolates (Tables 1 and 2). The IC_{50}s of HBY 097 against a number of NNRTI-resistant recombinant RTs demonstrate the dominant role of the G190→E substitution. This result was confirmed by in vitro selection for HBY 097-resistant HIV-1 variants (16; this study). Assuming that drug-resistant viruses specific for any RT inhibitor will occur, the appearance of a viral variant with decreased RT activity and delayed growth properties should indicate that a new compound has favorable attributes (6). However, it still must be demonstrated that the in vitro selection for an attenuating mutation reflects what might happen in HIV-1-infected patients treated with HBY 097.

We conclude that the properties of HBY 097 summarized above may help to address the main problem seen with other NNRTIs: the prevention of a more prolonged clinical benefit of treated patients caused by the rapid selection of drug-resistant viruses (30, 35, 39).

Since AZT is still the only first-line treatment for patients infected with HIV-1, it is of particular interest how the mutations selected for by AZT and the mutants found with quinoxalines might interact. However, a viral variant displaying the RT G190→E amino acid change together with the AZT-specific substitutions has so far not been able to be generated by in vitro selection procedures.

Preliminary results from a phase I single-dose study in healthy volunteers have confirmed the good bioavailability and good tolerability of HBY 097 in humans (data not shown). HBY 097 is undergoing further clinical trials in patients infected with HIV-1.

TABLE 4. Pharmacokinetic properties of HBY 097 and S-2720 in mice and dogs[a]

Species	Compound	Dose (mg/kg)	Route of administration	AUD (μg · min/ml)	$t_{1/2}$ (min)	C_{max} (μg/ml)	T_{max} (min)	CL (ml/min)	Bioavailability (%)
Mouse	S-2720	10	i.v.	16.7	34.7	0.7	5	4.3	
Mouse	S-2720	100	p.o.	17.1		0.1	30		10
Mouse	HBY 097	7.4	i.v.	35.7	24.1	1.6	5	5.0	
Mouse	HBY 097	100	p.o.	229.9		3.0	20		48
Dog	HBY 097	2	i.v.	50.7	99.0	1.2	5	597.8	
Dog	HBY 097	5.3	i.v.	292.8	95.2	5.8	5	317.4	
Dog	HBY 097	33.8	p.o.	873.6		2.1	120	732.8	54
Dog	HBY 097	82.5	p.o.	1,434.4		2.5	90	888.6	

[a] i.v., intravenous; p.o., oral; AUD, area under the data curve; $t_{1/2}$, half-life; C_{max}, maximum concentration of drug in serum; T_{max}, time to maximum concentration of drug in serum; CL, clearance.

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