Most non-nucleoside reverse transcriptase (RT) inhibitors are specific for HIV-1 RT and demonstrate minimal inhibition of HIV-2 RT. However, we report that members of the phenylethylthiazolylthiourea (PETT) series of non-nucleoside reverse transcriptase inhibitors showing high potency against HIV-1 RT have varying abilities to inhibit HIV-2 RT. Thus, PETT-1 inhibits HIV-1 RT with an IC_{50} of 6 nM but shows only weak inhibition of HIV-2 RT, whereas PETT-2 retains similar potency against HIV-1 RT (IC_{50} of 5 nM) and also inhibits HIV-2 RT (IC_{50} of 2.2 μM). X-ray crystallographic structure determinations of PETT-1 and PETT-2 in complexes with HIV-1 RT reveal the compounds bind in an overall similar conformation albeit with some differences in their interactions with the protein. To investigate whether PETT-2 could be acting at a different site on HIV-2 RT (e.g. the dNTP or template primer binding site), we compared modes of inhibition for PETT-2 against HIV-1 and HIV-2 RT. PETT-2 was a noncompetitive inhibitor with respect to the dGTP substrate for both HIV-1 and HIV-2 RTs. PETT-2 was also a noncompetitive inhibitor with respect to a poly(C)(dG) template primer for HIV-2 RT. These results are consistent with PETT-2 binding in corresponding pockets in both HIV-1 and HIV-2 RT with amino acid sequence differences in HIV-2 RT affecting the binding of PETT-2 compared with PETT-1.

The use of multidrug combination therapy has resulted in a significant improvement in the survival rate of HIV^{1}-infected individuals (1, 2). The regimens are based on inhibitors of HIV reverse transcriptase (RT), together with protease inhibitors. RT catalyzes the conversion of genomic RNA to proviral DNA, by RNA-dependent DNA polymerase, RNase H, and DNA-dependent DNA polymerase reactions. The well-established nucleoside analogue reverse transcriptase inhibitor-based drugs (e.g. 3′-azido-2′,3′-dideoxythymidine, 2′,3′-dideoxyinosine, and 2′,3′-dideoxy-3′-thiacytidine) act as terminators of RT catalyzed DNA synthesis in their activated triphosphate forms. Nucleoside analogue reverse transcriptase inhibitors are relatively broad spectrum drugs that inhibit both HIV-1 and HIV-2 serotypes with comparable potency (3). More recently non-nucleoside inhibitors (NNRTIs) nevirapine, delavirdine, and efavirenz have established an important role in combination therapy of HIV infection. NNRTIs include a wide range of chemical series and are usually specific for HIV-1 RT, showing minimal inhibition of HIV-2 RT. A series of biaryl acids has been reported that inhibit both HIV-1 and HIV-2 RT, although these appear not to bind at the NNRTI site (4). NNRTIs such as nevirapine generally act as noncompetitive inhibitors of HIV-1 RT with respect to substrates (5), binding in a pocket some 10 Å from the polymerase active site (6–8). The NNRTI binding site is contained largely within the p66 subunit of the RT heterodimer with only a few residues at the periphery of the site being contributed by the p51 subunit. The mechanism of inhibition for NNRTIs has been shown to be via a distortion of the key catalytic active site aspartyl residues (9).

One series of NNRTIs previously described is the phenylethylthiazolylthiourea (PETT) series that have been shown to have potent activity against both HIV-1 virus and it’s RT (10–13). Further PETT analogues have been designed using information from the three-dimensional structure of HIV-1 RT (14–16). In this work we report structural and biochemical studies for two members of this series referred to as PETT-1 and PETT-2 (Scheme 1).

Most NNRTIs rapidly select for drug-resistant HIV-1 strains, both in tissue culture and in clinical studies, which has largely precluded their use as monotherapy (17). In contrast to the “first-generation” drugs, nevirapine and delavirdine, the so-called “second-generation” NNRTI drug, efavirenz, demonstrates resilience to the effects of certain common resistance mutations (18). However resistance to such compounds is
emerging, meaning that there is a continued need for the development of new drugs to combat such mutant viruses.

The HIV-2 serotype was isolated three years after the identification of HIV-1 (19, 20) and was shown to be closely related to SIVmac. HIV-2 RT has approximately 60% overall amino acid sequence identity with HIV-1 RT and has comparable polymerase activity (21). There are, however, significant sequence changes for amino acids lining the equivalent region to the HIV-1 NNRTI binding pocket within HIV-2 RT, which presumably explains the poor binding of NNRTIs for this latter enzyme. Thus, the tyrosine residues at 181 and 188 in HIV-1 RT, whose side chains are important in aromatic ring-stacking interactions with many NNRTIs (6, 7, 22–27) are replaced in HIV-2 RT by aliphatic leucine and isoleucine residues, respectively. The direct role of amino acid sequence changes is confirmed by studies of chimeric HIV-1/HIV-2 RTs where several residues from HIV-1 have to be incorporated into an HIV-2 chimera to give sensitivity to NNRTIs approaching that for wild-type HIV-1 RT (28, 29).

We report studies on two NNRTIs of the PETT series and show that they vary in their ability to inhibit HIV-2 RT. Additionally we have determined the crystal structures of these inhibitors complexed with HIV-1 RT. We also describe kinetic studies comparing the modes of inhibition for the PETT compounds with HIV-1 and HIV-2 RTs to address the possibility of alternative binding sites on HIV-2 RT for PETT-2.

EXPERIMENTAL PROCEDURES

Crystallization and Data Collection—Crystals of the complex of HIV-1 RT with PETT-2 were grown as described previously (30). Crystals of RT with PETT-1 were obtained by inhibitor exchange with a weak binding inhibitor, HEPT, in the crystal (7, 9). Crystals were equilibrated in 50% polyethylene glycol 3400 prior to data collection as described previously (25, 30). X-ray data were collected at either beamline PX7.2 (SRS Daresbury Laboratory UK) using an oscillation camera or at beamline BL-6A (KEK, Photon Factory, Japan) using a Weissenberg camera (31, 32) (see Table I for further details). Crystals were either frozen in liquid propane and maintained at 100 K during data collection (RT/PETT-1) or maintained at 288 K (RT/PETT-2). Data frames of 1.5° oscillations were collected on a MAR image plate with exposure times of 90 s for RT/PETT-1. For RT/PETT-2, 3.5° frames with a coupling constant 1.5°/mm and an exposure time of 112 s were recorded on pairs of 200 × 400 mm Fuji Bas-IIIB imaging plates positioned 429.7 mm from the crystal on a cylindrical cassette. To reduce background noise, the collimator, crystal enclosure, and camera cassette were flooded with helium. The imaging plates were scanned off-line using Fuji BA 100 IP scanners. Indexing and integration of data images were carried out with DENZO, and the data were merged with SCALPACK (33). Details of the x-ray data statistics are given in Table I.

Structure Solution and Refinement—The orientation and position of RT in the unit cell were determined using rigid body refinement with X-PLOR (34). Coordinates from RT/9-Cl-TIBO (22) and RT/1051U91 (7) complexes were used as initial models for RT/PETT-1 and RT/PETT-2, respectively. The structures were first refined with X-PLOR (34) and then with CNS (35) using positional, simulated annealing and individual B-factor refinements with bulk solvent correction together with anisotropic B-factor scaling. Model rebuilding was carried out with FRODO (36) on an Evans and Sutherland ESV workstation.

The structure of RT/PETT-1 has been refined to an R factor of 0.224 ($R_{	ext{free}}$ of 0.295) for all data in the range of 30.0–2.8 Å resolution. The r.m.s. deviations of bond lengths and bond angles from ideality were 0.008 Å and 1.4°, respectively, for a model containing 7637 protein atoms, the inhibitor, and 26 water molecules. The current model of the RT/PETT-2 complex containing 7827 protein atoms, the inhibitor, and no water molecules, has an R factor of 0.199 ($R_{	ext{free}}$ of 0.276) for all data in the range 30.0–3.0 Å resolution with r.m.s. deviations of 0.008 Å and 1.4° from ideal bond lengths and bond angles, respectively. Table I summarizes these refinement statistics. The coordinates and structure

![Fig. 1. Simulated annealing omit electron density maps showing the bound inhibitors at the NNRTI pocket of HIV-1 RT. a, PETT-1; b, PETT-2. The maps are contoured at 4σ.](http://www.jbc.org/)

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TABLE I

| Data collection details | RT/PETT-1 | RT/PETT-2 |
|-------------------------|-----------|-----------|
| Data set                | SRS PX7.2 | KEK BL-6A2 |
| Wavelength (Å)          | 1.488     | 1.000     |
| Collimation (mm)        | 0.20      | 0.10      |
| Unit cell dimensions    | 137.1, 115.0, 65.6 | 140.7, 110.8, 73.4 |
| Outer resolution shell  | (cell form F) | (cell form C) |
| Resolution range (Å)   | 30.0–2.8  | 30.0–3.0  |
| Observations            | 89165     | 86903     |
| Unique reflections      | 25847     | 20940     |
| Completeness (%)        | 95.9      | 98.6      |
| Reflections with F(s)/F(u) > 3 | 18622 | 16702 |
| $R_{merge}$ (%)         | 9.8       | 9.5       |
| Mean B-factor for main chain, side chain, inhibitor and water atoms, respectively | 2.1 | 2.1 |

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* $R_{merge} = 2[1 - <I^2-<I>]/<I^2-<I>$.  
* $R$ factor = $\sum F_{o} - F_{c}/2F_{c}$.  
* Mean B factor for main chain, side chain, inhibitor and water atoms, respectively.
factors for the RT/PETT-1 and RT/PETT-2 complexes have been deposited with the Protein Data Bank.

Preparation of HIV-2 Reverse Transcriptase—HIV-2 RT (pROD) was expressed and purified using methods described previously for HIV-1 RT (37, 38).

Assay for Reverse Transcriptase Activity—Assays for both HIV-1 and HIV-2 RTs were carried out using the methods described previously (39), but with poly(rC)z(dG)12–18 as the template primer and with the incorporation of [3H]dGTP into DNA to follow the reaction.

Kinetic Studies of Inhibition Modes—IC50 values for PETT-1 and PETT-2 were determined at template-primer and substrate concentrations of 50 μg/ml poly(rC)z(dG) and 5 μM dGTP, respectively, with variation of inhibitor concentrations on a log scale. Inhibition curves were fitted by nonlinear regression methods using ORIGIN (Microcal Software Inc.). The mode of inhibition studies and Ki determinations were carried out using a protocol involving a 4 × 5 matrix of varying substrate and inhibitor concentrations over ranges of [S]/[Km] from 0.5 to 7 and [I]/[Ki] from 0.5 to 10 (40). In each case when either dGTP or template primer was not being varied in concentration, then it was held at saturating levels (i.e. 30 μM dGTP or 50 μg/ml poly(rC)z(dG)). Duplicate measurements were made for each experimental point. Between 2 and 4 replicate experiments were performed, and these were scaled together prior to curve fitting. Data were fitted to standard enzyme inhibition models (i.e. competitive, noncompetitive, uncompetitive, and mixed) using GRAFIT (Erithacus Software). The best fit was judged from the residual χ2 values, the run of signs of the residuals, and percentage errors in the fitted parameters.

RESULTS

A summary of the refinement statistics for the RT/PETT complexes is shown in Table I. Both structures have excellent stereochemistry. The electron density for each structure is of good quality particularly in the region of the well ordered NNRTI binding site. Omit electron density maps for the inhibitors are very clear (Fig. 1).

Bound PETT Conformations—The orientation of PETT-1 and PETT-2 within the NNRTI binding site could be determined unambiguously due to the presence of clear electron density for the thioether and the two pyridinyl rings. The ether substituent of ring A, which is common to both compounds, could be clearly distinguished from the less bulky nitrile or chlorine group of the B rings (Fig. 1). Both compounds adopt similar conformations when bound to RT such that their aromatic rings are positioned approximately at right angles, whereas the linking group is folded in a somewhat constrained nonextended conformation (Fig. 2). There is an intramolecular hydrogen bond between the ring B pyridinyl nitrogen and one of the nitrogen atoms of the thioether group. Comparison of PETT inhibitors with other examples of NNRTIs, viz. nevirapine and TIBO, reveals that these inhibitors adopt conformations that occupy similar volumes of space within the drug
pocket despite their diverse chemical structures (Fig. 3).

**Interactions of Inhibitors with HIV-1 RT**—The interactions of PETT-1 and PETT-2 with HIV-1 RT are shown in Fig. 4. These are predominantly hydrophobic in nature but with some polar interactions also present. The larger nitrile substituent on ring B of PETT-1 compared with the chloro of PETT-2 alters the contacts in several places. Thus although ring A of both compounds is positioned at the “top” of the NNRTI pocket forming ring-stacking interactions with the side chains of both Tyr-181 and Tyr-188, the latter has more contacts with PETT-2 than with PETT-1. PETT-2 also makes a number of contacts with the side chain of Leu-100. The nitrile group of PETT-1 has extensive contacts with residues Leu-234, His-235, Pro-236, and Tyr-318, whereas the equivalent chlorine group of PETT-2 has few contacts with this region. The side chain of Glu-138 from the p51 subunit is positioned closer to the NNRTI pocket than for other inhibitor complexes (7), allowing contacts with ring A for both PETT-1 and PETT-2. The sulfur atom of the thiourea group makes a number of van der Waals contacts with the backbone of Lys-101. There is a single hydrogen bond to the main chain, from one of the thiourea nitrogens to the carbonyl of Lys-101.

**Kinetics of PETT-2 Inhibition of HIV-1 and HIV-2 RT**—The results of the IC_{50} determinations for PETT inhibition of the RTs are shown in Fig. 5. Both PETT-1 and PETT-2 inhibit HIV-1 RT with similar IC_{50} values in the low nM range (6 and 5 nM, respectively) (Fig. 5). For HIV-2 RT, PETT-2 gave an IC_{50} of 2.2 μM, whereas for PETT-1 a maximum of 20% inhibition at 50 μM was observed, meaning that it was not possible to determine an IC_{50} value.

Results of studies to determine the mode of inhibition of PETT-2 against HIV-1 and HIV-2 RTs are shown in Tables II and III and in Figs. 6 and 7. A comparison of χ^2 values for different models shows that with dGTP as the varying substrate, the noncompetitive model was strongly preferred over the competitive model for both HIV-1 and HIV-2 RT (Table II).
HIV-2 RT was incubated for 30 min at 25 °C with the various concentrations of PETT-2 and poly(rC)-(dG)12–18 as varying substrate. Previously published resistance data for a PETT compound trovirdine (11), show that mutations of Leu-100→Ile, Glu-138→Arg, and Tyr-188→His give 10–20-fold resistance when compared with wild-type HIV-1 RT. These three residues all make contacts with structural features of PETT-1 and PETT-2 that are also common to trovirdine. As outlined previously mutations 100 and 188 make van der Waals contact with ring A. From modeling studies it is clear that these interactions would be disrupted by mutations to Ile and His, respectively. The side chain of Glu-138 from the p51 subunit forms contacts with ring A, which would be disrupted by the mutation to a bulkier side chain such as arginine.

What are the particular features of a compound such as PETT-2 compared with most NNRTIs that allows it to bind to HIV-2 RT? One possibility we considered, given the significant amino acid sequence changes both within the NNRTI site and more generally, was whether PETT-2 could be binding at an entirely different site. The kinetic studies reported here attempt to address this question. The mode of inhibition experiments show that PETT-2 is noncompetitive with respect to dGTP for both HIV-1 and HIV-2 RT, thus indicating it is not binding at the dNTP site (Table II). For the equivalent experiments with template primer and HIV-2 RT, the level of discrimination for the different inhibition models is not so marked, nevertheless noncompetitive inhibition is the preferred model in each experiment (Table II). For HIV-1 RT with template primer and PETT-2 varied there was a large discrimination against the competitive model, whereas mixed and uncompetitive modes were difficult to distinguish (Table II).

Our data indicate that PETT-2 is neither competing for template primer nor for dNTP substrate sites on HIV-2 RT but are consistent with PETT-2 interacting at an HIV-2 equivalent of the HIV-1 NNRTI site. In considering this possibility and in attempting to explain the varying potency of PETT-1 and PETT-2, we examined both differences in the compounds and the sequence differences between HIV-1 and HIV-2 RTs, particularly in the region where the PETT B ring interacts. First we considered whether the pK_a of the B ring pyridinyl nitrogen varied due to differing electron withdrawing/donating properties of nitride and chloride substituents, which could potentially modulate the hydrogen bonding capability to the main chain of Lys-101. In fact, the measured pK_a of these nitrogens is very similar (4.25 and 4.32 for PETT-1 and PETT-2, respectively), making it unlikely that this is the reason for the different potencies observed.

For poly(rC)-(dG) as the varying substrate the discrimination between the different models was lower, although noncompetitive inhibition was preferred for HIV-2 RT in all experiments performed, whereas for HIV-1 RT mixed or uncompetitive modes were preferred (Table II). The $K_i$ values (Table III) generally agree with the corresponding IC_{50} values (Fig. 5).

### DISCUSSION

The work reported here shows that, in line with reports for other members of this series, PETT-1 and PETT-2 are potent inhibitors of HIV-1 RT (10–12). PETT-1 and PETT-2 differ only at the para position of the B ring (nitirile or chloride, respectively), yet they show differing ability to inhibit HIV-2 RT. Although PETT-2 is a much weaker inhibitor of HIV-2 RT than it is of HIV-1 RT, such a relatively high level of inhibition of HIV-2 RT by an NNRTI is unusual. Recently the inhibitory activity of the NNRTIs delavirdine and emivirine, with IC_{50} values in the low micromolar range, have been reported against HIV-2 (EHO strain) in MT-4 cell cultures, although their activities against HIV-2 RT appeared minimal (41).

Crystallographic structure determinations of complexes of PETT-1 and PETT-2 with HIV-1 RT reveal that the overall mode of binding of both inhibitors adheres to the “two ring” conformation observed for a range of other chemically diverse NNRTI series (7, 22–27). There are some differences between the interactions of the two PETT compounds with the NNRTI site. These appear to be related to the larger nitride substituent on the B ring of PETT-1, which results in closer contacts with the main chain of residues 234 to 236, whereas PETT-2 makes slightly closer contacts with Tyr-188.

The PETT conformations described here are generally in agreement with modeling studies, where the hydrogen bonding to the main chain of Lys-101 was correctly predicted (15, 16). Previously published resistance data for a PETT compound trovirdine (11), show that mutations of Leu-100→Ile, Glu-138→Arg, and Tyr-188→His give 10–20-fold resistance when compared with wild-type HIV-1 RT. These three residues all make contacts with structural features of PETT-1 and PETT-2 that are also common to trovirdine. As outlined previously mutations 100 and 188 make van der Waals contact with ring A. From modeling studies it is clear that these interactions would be disrupted by mutations to Ile and His, respectively. The side chain of Glu-138 from the p51 subunit forms contacts with ring A, which would be disrupted by the mutation to a bulkier side chain such as arginine.

PETT-2 is noncompetitive with respect to dGTP for both HIV-1 and HIV-2 RT, thus indicating it is not binding at the dNTP site (Table II). For the equivalent experiments with template primer and HIV-2 RT, the level of discrimination for the different inhibition models is not so marked, nevertheless noncompetitive inhibition is the preferred model in each experiment (Table II). For HIV-1 RT with template primer and PETT-2 varied there was a large discrimination against the competitive model, whereas mixed and uncompetitive modes were difficult to distinguish (Table II).

Our data indicate that PETT-2 is neither competing for template primer nor for dNTP substrate sites on HIV-2 RT but are consistent with PETT-2 interacting at an HIV-2 equivalent of the HIV-1 NNRTI site. In considering this possibility and in attempting to explain the varying potency of PETT-1 and PETT-2, we examined both differences in the compounds and the sequence differences between HIV-1 and HIV-2 RTs, particularly in the region where the PETT B ring interacts. First we considered whether the pK_a of the B ring pyridinyl nitrogen varied due to differing electron withdrawing/donating properties of nitride and chloride substituents, which could potentially modulate the hydrogen bonding capability to the main chain of Lys-101. In fact, the measured pK_a of these nitrogens is very similar (4.25 and 4.32 for PETT-1 and PETT-2, respectively), and this means there will be minimal protonation of the pyridinyl nitrogen at physiological pH in either case. Hence, this cannot account for the different binding strength of the two compounds to HIV-2 RT.

The nitride group of PETT-1 makes van der Waals interactions with the main chain of Leu-234 and His-235 and with the
side chain of Tyr-318. In HIV-2 RT, Leu-234 and Tyr-318 are conserved, but His-235 is replaced by a Trp residue. It is conceivable that the larger side chain of this Trp residue causes an alteration in the main chain position resulting in a slight reduction in the volume of the NNRTI site in this region, although still allowing accommodation of the smaller chlorines of PETT-2 but not the bulkier nitrile group of PETT-1.

Certain members of the PETT series of NNRTIs may be better able to bind to HIV-2 RT due to a greater flexibility compared with the fused ring systems found in other NNRTIs such as nevirapine and TIBO. The linker region joining the two PETT pyridine rings could have enough flexibility to allow significant rearrangement and thereby be better accommodated within the HIV-2 RT "NNRTI" binding site. If this is the case then there are still clearly significant constraints on PETT binding to HIV-2 RT as exemplified by the effect of different B-ring substituents on potency of inhibition for this enzyme.

To further define the binding of PETT-2 to HIV-2 RT in detail, a crystal structure of this complex will be required. Given the success of using NNRTIs to produce crystals of HIV-1 RT differing from medium to high resolution (6, 30), it is hoped that NNRTIs that bind to HIV-2 RT could provide a similar means for obtaining a high resolution structure of this latter enzyme. A high resolution HIV-2 RT structure might provide insights into ways of inhibiting widely variant RTs including different serotypes as well as drug-resistant forms. The rational design of potent non-nucleoside inhibitors with broad spectrum activity against a wide range of RTs should be of importance in the continued fight against AIDS.

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REFERENCES

1. Brettle, R. P., Wilson, A., Povey, S., Morris, S., Morgan, R., Leen, C. L., Houghton, S. Lewis, S., and Gere, S. (1996) Int. J. STD AIDS 8, 80–87.

2. Morecroft, A., Vella, S., Benfield, T. L., Chiesi, A., Miller, V., Gargalalinos, P., d’Arnimono-Fortte, A., Yust, I., Braun, J. N., Phillips, A. N., and Lundgren, J. D. (1996) Lancet 348, 1725–1730.

3. De Clercq, E. (1993) Med. Res. Rev. 13, 229–258.

4. Milton, J., Slater, M. J., Bird, A. J., Spinks, D., Scott, G., Price, C. E., Downing, S., Green, D. V., Madar, S., Bethell, R., and Stammers, D. K. (1996) Bioorg. Med. Chem. Lett. 6, 2623–2628.

5. Merluzzi, V. J., Hargrave, K. D., Labadia, M., Grozinger, K., Skoog, M., Wu, J. C., Shih, C.-K., Eckner, K., Hattex, S., Adams, J., Rosenhal, A. S., Faanes, R., Eckner, R. J., Koup, R. A., and Sullivan, J. L. (1996) Science 250, 1411–1413.

6. Kohlscheidt, L. A., Wang, J., Friedman, J. M., Rice, P. A., and Steitz, T. A. (1992) Science 256, 1783–1790.

7. Rej, J., Esnouf, R., Garman, E., Somers, D., Ross, C., Kirby, L., Keeling, J., Darby, G., Jones, Y., Stuatt, D., and Stammers, D. (1995) Nat. Struct. Biol. 2, 293–302.

8. Ding, J., Das, K., Moereels, H., Koymans, L., Andries, K., Janssen, P. A. J., Hughes, S. H., and Arnold, F. (1996) Nat. Struct. Biol. 2, 407–415.

9. Esnouf, R., Ren, J., Ross, C., Jones, Y., Stuatt, D., and Stammers, D. (1995) Nat. Struct. Biol. 2, 303–308.

10. Agprega, C., Backro, K., Bell, F. W., Cantrell, A. S., Clemens, M., Colacino, J. M., Bieger, J. F., Engels, J. A., Jaskunas, S. R., Johansson, N. G., Jordan, C. L., Kascher, J. S., Kinnick, M. D., Lind, P., Lopez, C., Morin, J. M., Jr., Muesing, M. A., Noreen, R., Oberg, B., Palet, C. J., Falkowitz, J. A., Parish, C. A., Pran, P., Rich, M. K., Ryderogard, C. S., Sahilberg, C., Swanson, T., Ternansky, R. J., Uge, T., Vasilefe, R. T., Vrang, L., West, S. J., Zhang, H., and Zhou, X. X. (1995) Antimicrob. Agents Chemother. 39, 1329–1335.

11. Zhang, H., Vrang, L., Backro, K., Lind, P., Sahilberg, C., Uge, T., and Oberg, B. (1995) Antiviral Res. 28, 331–342.

12. Cantrell, A. S., Engels, P., Holberg, M., Jaskunas, S. R., Johansson, N. G., Jordan, C. L., Jaskunas, S. R., Lind, P., Morin, J. M., Jr., Muesing, M. A., Noreen, R., Oberg, B., Pran, P., Sahilberg, C., Ternansky, R. J., Ufielet, R. T., Vrang, L., West, S. J., and Zhang, H. (1996) J. Med. Chem. 39, 4261–4274.

13. Sahilberg, C., Noreen, R., Engels, P., Holberg, M., Jaskunas, S. R., Jang, L., and Zhang, H. (1998) Bioorg. Med. Chem. Lett. 8, 1511–1516.

14. Vig, P., Mao, C., Venkatakathalas, T. K., Tuel-Ahlgren, L., Sudbeck, E. A., and Uckun, F. M. (1998) Bioorg. Med. Chem. 6, 1789–1797.

15. Mao, C., Venkatakatlas, T. K., Sudbeck, E. A., and Uckun, F. M. (1998) Bioorg. Med. Chem. Lett. 8, 2213–2218.

16. Mao, C., Sudbeck, E. A., Venkatakathalas, T. K., and Uckun, F. M. (1999) Bioorg. Med. Chem. Lett. 9, 1593–1599.

17. Richman, D. D., Havlir, D., Looney, D., Ignacio, C., Spector, S. A., Rich, M. K., and Griffin, J. (1994) J. Med. Chem. 37, 1660–1666.

18. Young, S. D., Birtcher, S. F., Tran, L. O., Payne, L. S., Lumma, W. C., Lyle, T. A., Huff, R., Anderson, P. S., Olsen, D. B., Carroll, S. S., Pettibone, D. J., O’Brien, J. A., Ball, R. G., Balani, S. K., Lin, J. H., Chen, I.-W., Schleif, D., Sahlberg, C., and Stammers, D. K. (1999) J. Med. Chem. 42, 4500–4505.

19. Hopkins, A. L., Ren, J., Esnouf, R., Willis, B. E., Jones, E. Y., Ross, C., Miyakawa, T., Walker, R. T., Tanaka, H., Stammers, D. K., and Stammers, D. I. (1999) Biochem. J. 343, 1439–1440.

20. Hopkins, A. L., Ren, J., Tanaka, H., Baba, M., Okamoto, M., Stuatt, D. I., and Stammers, D. K. (1999) J. Med. Chem. 42, 4500–4505.

21. Ren, J., Esnouf, R., Hopkins, A. L., Stuatt, D. I., and Stammers, D. K. (1999) J. Med. Chem. 42, 4500–4505.

22. Ren, J., Esnouf, R., Hopkins, A. L., Stuatt, D. I., and Stammers, D. K. (1999) J. Med. Chem. 42, 4500–4505.
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28. Shih, C.-O., Rose, J. M., Hansen, G. L., Wu, J. C., Bacolla, A., and Griffin, J. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8678–8682
29. Yang, G., Song, Q., Charles, M., Drossopoulos, W. C., Arnold, E., and Prasad, V. R. (1996) J. Acquired Immune Defic. Syndr. Hum. Retrovirol. 11, 326–333
30. Stammers, D. K., Somers, D. O. N., Ross, C. K., Kirby, L., Ray, P. H., Wilson, J. E., Norman, M., Ren, J. S., Esnouf, R. M., Garman, E. F., Jones, E. Y., and Stuart, D. I. (1994) J. Mol. Biol. 242, 586–588
31. Sakabe, N. (1991) Nucl. Instr. Methods Phys. Res. Sect. A 303, 448–463
32. Stuart, D. I., and Jones, E. Y. (1993) Curr. Opin. Struct. Biol. 3, 737–740
33. Otwinowski, Z., and Minor, W. (1996) Methods Enzymol. 276, 307–326
34. Brunger, A. T. (1992) X-PLOR Manual, Version 3.1 Ed., Yale University Press, New Haven, CT
35. Brunger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Grosse, K. R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sec. D 54, 965–921
36. Jones, T. A. (1985) Methods Enzymol. 115, 157–171
37. Stammers, D. K., Tisdale, M., Court, S., Parmar, V., Bradley, C., and Ross, C. K. (1991) FEBS Lett. 283, 298–302
38. Stammers, D. K., Ross, C. K., Idriss, H., and Lowe, D. M. (1992) Eur. J. Biochem. 206, 437–440
39. Lowe, D. M., Aitken, A., Bradley, C., Darby, G. K., Larder, B. A., Powell, K. L., Purifoy, D. J. M., Tisdale, M., and Stammers, D. K. (1988) Biochemistry 27, 8884–8889
40. Stammers, D. K., Dann, J. G., Harris, C. J., and Smith, D. R. (1987) Arch. Biochem. Biophys. 258, 413–420
41. Witvrouw, M., Pannecoque, C., Van Laethem, K., Desmyter, J., De Clercq, E., and Vandamme, A. M. (1999) AIDS 13, 1477–1483
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