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STRUCTURE-BASED DESIGN OF N-[2-(1-PIPERIDINYLETHYL)]-N'-[2-(5-BROMOPYRIDYL)]-THIOUREA AND N-[2-(1-PIPERAZINYLETHYL)]-N'-[2-(5-BROMOPYRIDYL)]-THIOUREA AS POTENT NON-NUCLEOSIDE INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

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Abstract: A novel computer model of the HIV reverse transcriptase (RT) non-nucleoside inhibitor (NNI) binding pocket, which was generated using high resolution crystal structure information from 9 individual RT/NNI complexes, revealed previously unrecognized ligand derivatization sites for phenethylthiazolylthiourea (PETT) derivatives. Spatial gaps surrounding the pyridyl ring of the active PETT derivative trovirdine were discovered during modeling procedures. Docking studies using the computer-generated model of the binding pocket (composite binding pocket) suggested that the replacement of the planar pyridyl ring of trovirdine with a nonplanar piperidinyl or piperazinyl ring, which occupy larger volumes, would better fill the spacious Wing 2 region of the butterfly-shaped NNI binding pocket. The anti-HIV activity of the synthesized heterocyclic compounds N-[2-(1-piperidinylethyl)]-N'-[2-(5-bromopyridyl)]-thiourea and N-[2-(1-piperazinylethyl)]-N'-[2-(5-bromopyridyl)]-thiourea was examined in HTLV$_{IIIB}$-infected peripheral blood mononuclear cells. Both compounds were more potent than trovirdine and abrogated HIV replication at nanomolar concentrations without any evidence of cytotoxicity.

Design of potent inhibitors of HIV reverse transcriptase (RT) has been a focal point in translational AIDS research efforts.[1–3] Among the promising inhibitors are the non-nucleoside inhibitors (NNIs), which include tetrahydroimidazobenzodiazepinthione (TIBO) compounds,[4] 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-thymine (HEPT) derivatives,[5–8] dihydroalkoxybenzyloxopyrimidine (DABO),[9–12] bis(heteroaryl)piperazine (BHAP) analogs,[13] 2'-5'-bis-O-(tertbutyldimethylsilyl)-3'-spiro-5"-(4"-amino-1", 2"-oxathiole-2", 2"-di-oxide) pyrimidine (TSAO),[14] and phenethylthiazolylthiourea (PETT) derivatives.[15–21] NNIs have been found to bind to a specific allosteric site of HIV-1 RT[22–28] near the polymerase site and interfere with reverse transcription by altering either the conformation or mobility of RT, thereby leading to a noncompetitive inhibition of the enzyme.[22–27]

The NNI binding site of HIV-1 RT is among the most extensively studied drug binding pockets.[22–30] The high-resolution crystal structures of HIV-1 RT from RT/NNI complexes have shown distinct properties of the NNI binding pocket within the three-dimensional structure of HIV-1 RT, which can be utilized for structure-based rational drug design. However, each reported structure revealed a unique binding pattern indicating that rational drug design efforts should not rely on one particular crystal structure. We have used the NNI binding

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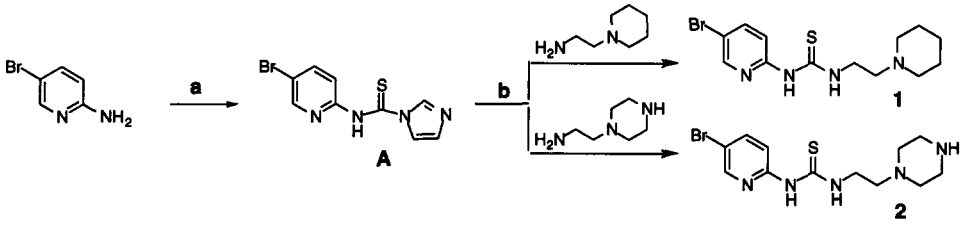
site coordinates of 9 individual RT/NNI structures to generate a composite molecular surface as a new model of the NNI binding pocket.[19] In the present study, we used the composite NNI binding pocket for structure-based design of new PETT analogs as potent NNIs of HIV-1 RT.

Compounds **1** and **2** were prepared as illustrated in **Scheme I**, following the methods used for the synthesis of PETT derivatives.[15] The synthesis involved condensing 2-amino-5-bromopyridine with 1,1-thiocarbonyl diimidazole to furnish the required thiocarbonyl derivative **A**. Further reaction of this thiocarbonyl derivative with the commercially available 1-(2-aminoethyl)piperidine or 1-(2-aminoethyl)piperazine gave **1** and **2**, respectively, in good yields.[31] The expected structure of **1** was consistent with the determined crystal structure.

Scheme 1



Reagents and conditions: (**a**) 1,1-thiocarbonyldiimidazole, acetonitrile, RT, 12 h. (**b**) DMF, 100 °C, 15 h.

Design and Activity of Novel PETT Derivatives with Heterocyclic Substituents

Modeling studies using the composite NNI binding pocket revealed previously unrecognized ligand derivatization sites for PETT derivatives.[19] Importantly, we discovered previously unrecognized spacious regions of approximately 160 Å^3 (molecular volume, see **Table I** note) surrounding the pyridyl ring of the active PETT derivative trovirdine (**Figure IA**). We reasoned that an efficient use of this sterically allowed space by strategically designed functional groups could lead to high affinity binding and yield NNI more potent than trovirdine. Docking studies using the composite binding pocket suggested that the replacement of the planar pyridyl ring of trovirdine with a nonplanar ring such as a piperidinyl or piperazinyl ring, which occupy larger volumes, would better fill the spacious Wing 2 region of the butterfly-shaped NNI binding pocket[26] (**Figure IA** and **B**). The calculated molecular volumes of **1** and **2** were 276 Å^3 and 272 Å^3, respectively. These values are larger than that of trovirdine (261 Å^3) because of the larger volume of the heterocyclic rings and thus were predicted to better fit into the potentially usable space of the binding site. In addition to volume occupied by the functional group, the molecular surface and curvature are other critical factors which contribute to binding. Certain heterocyclic rings possess a favorable curvature to fit into the Wing 2 region of the butterfly-shape binding site. Such heterocyclic rings are also conformationally more flexible than the aromatic ring found in trovirdine and hence are likely to have an added advantage by being able to fit an uncompromising binding pocket more effectively, despite the expense paid for loss of entropy upon binding.

The conformation of **1** in our low-energy model used for docking studies was found to be very similar to that observed in the crystal structure of **1** (data not shown) and would enable the molecule to fit favorably into binding site.

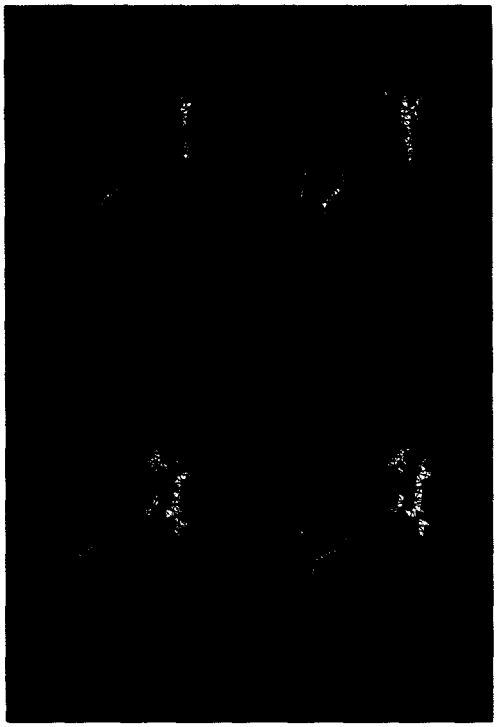
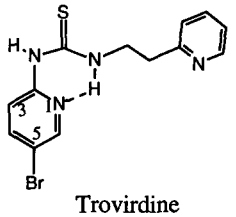


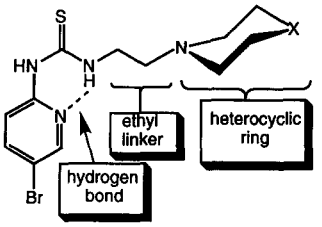
Figure 1. Stereoviews of non-nucleoside RT inhibitors (PETT derivatives, hydrogen atoms not shown) in composite binding pocket constructed from combined coordinates of 9 crystal structures of RT complexed with NNI compounds. Composite binding pocket of NNI active site of HIV-1 RT is illustrated as grid lines representing the collective van der Waals surface encompassing the 9 different inhibitor crystal structures superimposed in the NNI active site and highlight the available space for binding (inhibitor structures include HEPT, MKC, TNK, APA, Nevirapine, N-ethyl Nevirapine derivative, 8-Cl TIBO, and two 9-Cl TIBO compounds, with PDB access codes rti, rt1, rt2, hni, vrt, rth, hnv, rev, and tvr, respectively). The surface is color-coded for hydrogen bonding (orange), hydrophobic (gray), and hydrophilic (blue) regions of the binding pocket. When trovirdine (**A**) is docked into the binding pocket, substantial volume (approximately 160 $Å^3$) surrounds the pyridyl ring and defines potential space that could be more efficiently used by a larger group. (**B**) The larger, nonplanar ring of compound **1** occupies more volume in the pocket, leaving less unoccupied volume (approximately 145 $Å^3$) than for trovirdine, suggesting that **1** can better contact RT residues.

The anti-HIV activities of the synthesized novel heterocylic compounds, N-[2-(1-piperidinylethyl)-N'-[2-(5-bromopyridyl)]-thiourea (**1**) and N-[2-(1-piperazinylethyl)-N'-[2-(5-bromopyridyl)]-thiourea (**2**), were examined in HTLVIIIB-infected peripheral blood mononuclear cells. Both compounds were more potent than trovirdine and abrogated HIV replication at nanomolar concentrations (IC_{50} = 2 nM (**2**) and <1 nM (**1**) for inhibition of p24 production, an indicator of HIV replication) without any evidence of cytotoxicity (no inhibition of cellular proliferation at concentrations as high as 100 μM) (**Table 1**). Various combinations of double substitutions at axial or equatorial positions on these novel heterocyclic rings could yield PETT derivatives with a broader range of curvatures than trovirdine derivatives and would serve to better fit Wing 2 which itself contains some curvature.

Table 1. Inhibitory effects of PETT derivatives on p24 production in HIV-infected peripheral blood mononuclear cells and on viability of peripheral blood mononuclear cells. IC_{50} [p24] values represent inhibition of HIV-1 replication relative to virus control as measured by p24 EIA. IC_{50} [MTA] values measure cellular proliferation by microculture tetrazolium assay (MTA) using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2*H*-tetrazolium hydroxide to quantitate cytotoxicity.[32–34] Compounds **1** and **2** can form an internal hydrogen bond (observed in crystal structure of **1**) which is critical for active binding conformation. The ethyl linker and heterocyclic ring regions of the molecule represent sites near the most spacious regions of the binding pocket based on docking studies.



Trovirdine



Compounds **1** & **2**

Compound	X	Molecular Volume[a] (Å^3)	IC_{50}[p24] (μM)	IC_{50}[MTA] (μM)	SI
1	CH_2	276	< 0.001	>100	>1x10^5
2	NH	272	0.002	>100	>5x10^4
Trovirdine	n.a.	261	0.007	>100	>1x10^4
AZT	n.a.	n.a.	0.006	50	8x10^3

n.a. = not applicable. [a]Molecular volume calculated using GRASP,[35] based on molecular surface defined as boundary of volume within any probe sphere (meant to represent a water molecule) of given radius sharing no volume with hard sphere atoms which make up the molecule.

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31. Selected physical data:

N-[2-(1-piperidinylethyl)]-N'-[2-(5-bromopyridyl)]-thiourea (1): Yield: 74%; mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 11.53 (br s, 1H), 9.72 (br s, 1H), 8.22 (d, 1H), 7.72–7.68 (dd, 1H), 6.95–6.92 (d, 1H), 3.84–3.78 (q, 2H), 2.61–2.57 (t, 2H), 2.45 (br s, 4H), 1.64–1.48 (m, 6H); ^{13}C NMR($CDCl_3$) δ 178.1, 151.8, 146.3, 140.8, 113.5, 112.6, 56.1, 54.0, 43.0, 26.3, and 24.3; Anal. calcd for $C_{13}H_{19}BrN_4S$: C, 45.49; H, 5.58; Br, 23.28; N, 16.32; S, 9.34; Found: C, 45.67; H, 5.59; Br, 23.12; N, 16.20; S, 9.36.

N-[2-(1-piperazinylethyl)]-N'-[2-(5-bromopyridyl)]-thiourea (2): Yield: 75%; mp 178–180 °C; ^{1}H NMR ($CDCl_3$) δ 11.50 (br s, 1H), 9.77 (br s, 1H), 8.19–8.18 (d, 1H), 7.75–7.71 (dd, 1H), 6.97–6.95 (d, 1H), 3.87–3.86 (m, 2H), 3.63–3.60 (t, 2H), 3.45–3.42 (m, 3H), 2.74–2.69 (t, 2H), 2.59–2.52 (m, 4H) ; ^{13}C NMR($CDCl_3$) δ 178.7 , 151.8, 146.1, 141.0 , 113.7, 112.7 , 55.2 , 52.0, 51.9 and 45.8; Anal. calcd for $C_{12}H_{18}BrN_5S$: C, 41.87; H, 5.27; Br, 23.21; N, 20.34; S, 9.31; Found: C, 41.98; H, 4.88; N, 18.74; Br, 21.58; S, 8.52.

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