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Stereochemistry of Halopyridyl and Thiazolyl Thiourea Compounds is a Major Determinant of their Potency as Nonnucleoside Inhibitors of HIV-1 Reverse Transcriptase

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Abstract—Chiral derivatives of two cyclohexylethyl halopyridyl thiourea compounds (HI-509 and HI-510), two α-methyl benzyl halopyridyl compounds (HI-511 and HI-512), and a cyclohexyl ethyl thiazolyl thiourea compound (HI-513) were synthesized as nonnucleoside inhibitors (NNI) of human immunodeficiency virus (HIV) reverse transcriptase (RT). The *R* stereoisomers of all five compounds inhibited the recombinant RT in vitro with 100-fold lower IC_{50} values. HI-509^R, HI-510^R, HI-511^R, HI-512^R and HI-513^R were active anti-HIV agents and inhibited HIV-1 replication in human peripheral blood mononuclear cells at nanomolar concentrations, whereas their enantiomers were inactive. Each of these five compounds was also active against NNI-resistant HIV-1 strains, with HI-511^R being the most active agent. When tested against the NNI-resistant HIV-1 strain A17 with a Y181C mutation in RT, HI-511^R was found to be 10,000-times more active than nevirapine, 5000-times more active than delavirdine, and 50-times more active than trovirdine. HI-511^R inhibited the HIV-strain A17 variant, containing RT mutations Y181C plus K103N, with an IC_{50} value of 2.7 μM, whereas the IC_{50} values of nevirapine, delavirdine, and trovirdine against this highly NNI-resistant HIV-1 strain were >100 μM.

We previously reported the construction of a novel computer model of the nonnucleoside inhibitor (NNI) binding pocket of the HIV reverse transcriptase (RT).[1–11] We use this model together with a computer docking procedure and a structure-based semi-empirical score function as a guide to predict energetically favorable positions of new NNIs.[1–11] In the present study, we synthesized *R* and *S* stereoisomers of two cyclohexyl methyl halopyridyl thiourea compounds (HI-509 and HI-510), two α-methyl benzylhalopyridyl thiourea compounds (HI-511 and HI-512) and one cyclohexyl ethyl thiazolyl thiourea compound (HI-513) that were rationally designed as NNI inhibitors of HIV-RT. For the synthesis of the thiourea compounds, we followed the general procedure shown in Scheme 1 as previously reported.[2] The physicochemical properties were determined using standard analytical procedures.[12] The structures of the chiral bromopyridyl thiourea compounds N-[1-(1-(1*R*)-cyclohexylethyl)]-N-[2-(5-bromopyridyl)] thiourea (HI-509^R) and N-[1-(1-(1*S*)-cyclohexylethyl)]-N-[2-(5-bromopyridyl)] thiourea (HI-509^S) were resolved by X-ray crystallography[13] (Fig. 1). Both compounds adopt lower energy conformations in the crystalline state relative to the C14–C7–C8–C13 torsion angle [62.0(5)°] in HI-509^R and the C14–C7–C8–C9 torsion angle [−62.4(5)°] in HI-509^S, with staggering of the cyclohexyl and methyl groups on the chiral carbon.

Molecular modeling studies indicated that the *R* stereoisomer (HI-509^R) would fit the target NNI binding pocket on HIV-RT much better than its enantiomer (HI-509^S) (Fig. 2). Unfavorable steric interactions with the NNI binding pocket residues near the Y181 side chain would impair the binding of HI-509^S in a lower energy 'staggered' conformation. This steric hindrance would be relieved if HI-509^S adopts an energetically unfavorable 'eclipsed' conformation. In either case, the estimated binding energy would be significantly higher for HI-509^S, as reflected by the higher estimated K_i value (Table 1). Similar assumptions could be made in favor of the *R* stereoisomer in modeling studies of the chiral chloropyridyl thiourea compounds N-[1-(1-(1*R*)-cyclohexylethyl)]-N-[2-(5-chloropyridyl)] thiourea (HI-510^R) and N-[1-(1-(1*S*)-cyclohexylethyl)]-N-[2-(5-chloro-

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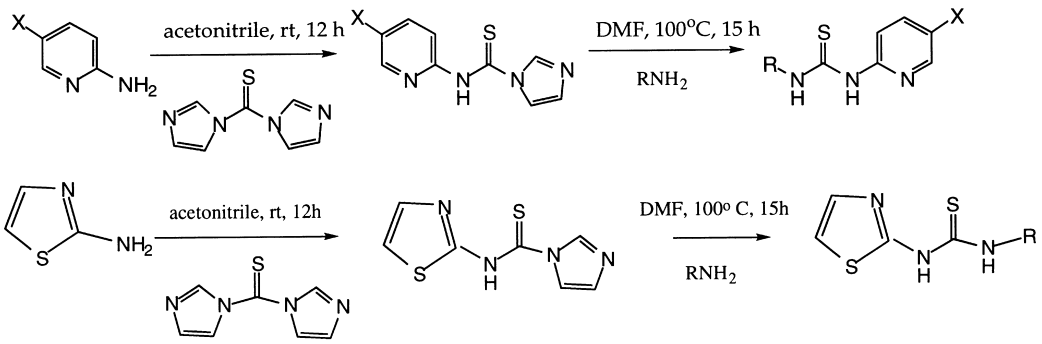


Scheme 1. [2]

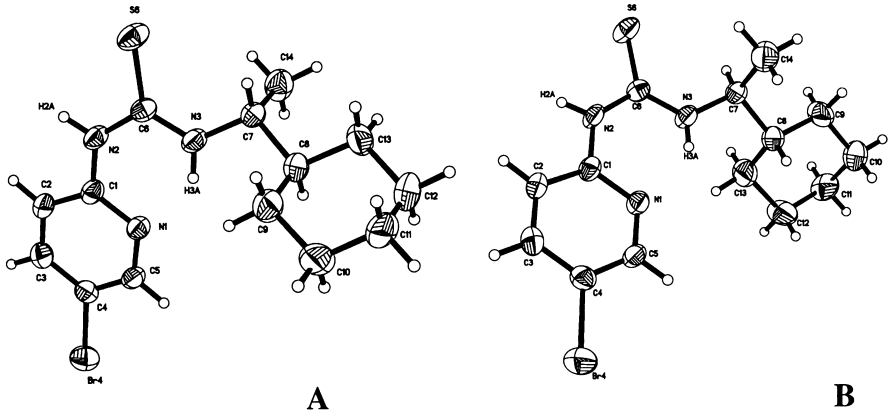


Figure 1. X-ray crystal structures of compounds HI-509^{R} and HI-509^{S}.[13] 30% probability ellipsoids, T = 22 °C. **[A]** HI-509^{R}: Space group: $P3_22_1$, unit cell: a = 9.2322(4) Å, b = 9.2322(4) Å, c = 32.9810(18) Å, α = 90°, β = 90°, γ = 120°, volume = 2434.5(2) Å^3, Z = 6, θ range for data collection = 1.85 to 28.17° (λ = 0.71073 Å), total reflections collected = 14,953, independent reflections = 3775 (R_{int} = 0.030), data/restraints/parameters = 3775/0/173, R1 [I > 2σ(I)] = 0.041, wR2 = 0.11, Goodness of Fit on F^2 = 0.986. Absolute structure parameter: 0.019(12). **[B]** HI-509^{S}: Space group: $P3_12_1$, unit cell: a = 9.2290(4) Å, b = 9.2290(4) Å, c = 32.979(2) Å, α = 90°, β = 90°, γ = 120°, volume = 2432.6(2) Å^3, Z = 6, θ range for data collection = 1.85 to 28.20° (λ = 0.71073 Å), total reflections collected = 14,952, independent reflections = 3792 (R_{int} = 0.038), data/restraints/parameters = 3792/0/181, R1 [I > 2σ(I)] = 0.041, wR2 = 0.099, Goodness of Fit on F^2 = 0.913. Absolute structure parameter: 0.014(12).

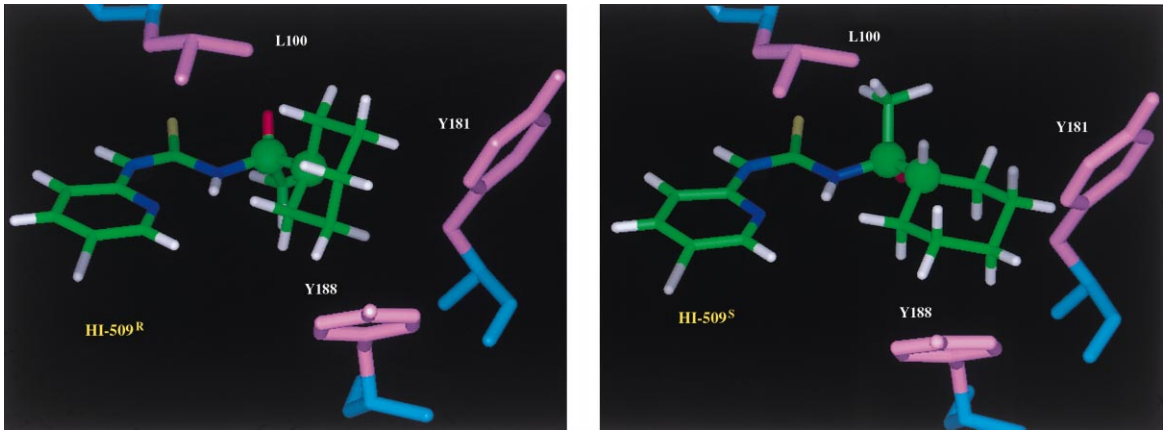
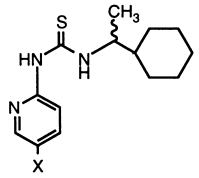


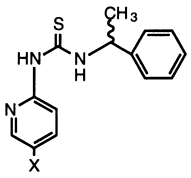
Figure 2. Binding of thiourea compounds to NNI binding pocket of HIV-RT. HI-509^{R} and HI-509^{S} were docked into the NNI binding site of HIV reverse transcriptase. The stick models (green) of HI-509^{R} (*R* isomer, left) and HI-509^{S} (*S* isomer, right) were docked into the NNI binding site as lower energy *staggered* conformations with respect to the cyclohexane and methyl groups on the chiral carbon. Only the *R* isomer (HI-509^{R}) provided a suitable fit into the NNI binding pocket. The *S* isomer (HI-509^{S}) formed unfavorable steric interactions with binding pocket residues near

pyridyl)] thiourea (HI-510^{S}). Modeling studies indicated that the methyl group on the chiral carbon of HI-509^{R}/HI-510^{R} likely promotes its strong binding to the NNI binding pocket via van der Waals contacts with residue V179. Since this methyl group is 7 Å away from Y181 (as measured from the carbon atom of the methyl group to the Cγ position of the protein residue), 9 Å from Y188, 8.5 Å from V106, and 6.5 Å from K103, its

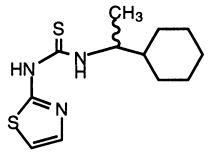
Table 1. Effect of stereochemistry on anti-HIV activity of thiourea compounds[a]



X = Br, HI-509
X = Cl, HI-510



X = Br, HI-511
X = Cl, HI-512



HI-513

Compound no.	Isomer	X	Estimated K_i (μM)[b]	IC_{50} (μM)				
				rRT	$HTLV_{IIIB}$	RT-MDR (V106A)	A17 (Y181C)	A17 (Y181C, K103N)
HI-509^R	R	Br	1.1	1.2	0.001	0.2	0.4	10.0
HI-509^S	S	Br	>100	>100	>1	ND	ND	ND
HI-510^R	R	Cl	1.2	1.4	0.025	0.06	0.07	8.2
HI-510^S	S	Cl	>100	>100	>1	ND	ND	ND
HI-542	S	H	>100	>100	>1	ND	ND	ND
HI-543	R	H	>100	>100	>1	ND	ND	ND
HI-511^R	R	Br	ND	1.6	0.01	0.005	0.01	2.7
HI-511^S	S	Br	ND	>100	>1	ND	ND	ND
HI-512^R	R	Cl	ND	1.2	0.010	0.010	0.2	10.2
HI-512^S	S	Cl	ND	>100	ND	ND	ND	ND
HI-513^R	R	NA	12.0	13.0	0.001	5.6	0.9	5.8
HI-513^S	S	NA	>100	>100	>1	ND	ND	ND
Nevirapine	NA	NA	ND	23	0.034	5.0	>100	>100
Trovirdine	NA	NA	0.6	0.8	0.007	0.02	0.5	>100
Delavirdine	NA	NA	ND	1.5	0.009	0.4	50.0	>100
HI-240	NA	NA	0.6	0.6	<0.001	0.005	0.2	41.0

[a]The anti-HIV activity was measured by determining the inhibition of the HIV-1 strain $HTLV_{IIIB}$ in human PBMC as previously described in detail.[5] H9 cells instead of PBMC were used for the RT-MDR experiments. The cell-free RT inhibition assays using recombinant RT (rRT) and the Quan-RT assay kit (Amersham, Arlington Heights, IL) were performed as reported.[5]
[b]K_i values were estimated based on our previously published procedures.[2–7] ND, not determined. NA, not applicable.

favorable impact on the binding of HI-509^R/HI-510^R to RT should not be affected by frequently encountered mutations involving these residues. Control compounds with unsubstituted pyridyl rings were predicted to fit poorly into the NNI binding pocket (Table 1). Modeling studies indicated that the Wing 2 group influences the orientation of the Wing 1 group and a local change may be translated to overall positional rearrangement. We found that the unsubstituted thiazole can be better accommodated by the binding site than unsubstituted pyridine (in combination with the bulky cyclohexylethyl group) as a whole molecule. On the other hand, halogen substitution on pyridine adds a considerable number of favorable interactions at the Wing 1 region, which improved the final interaction score for the substituted pyridine thiourea compounds. Along these same lines, a proper substitution on the thiazole group may produce an even better compound in our future designs.

The accuracy of the predictions of the modeling studies was evaluated in cell free RT inhibition assays. As evidenced in Table 1, HI-509^R and HI-510^R, with estimated K_i values 100-fold lower than those of the *S* stereoisomers, inhibited recombinant RT in vitro with 100-fold lower IC_{50} values. The control compounds with unsubstituted pyridyl rings did not exhibit detectable RT inhibitory activity (Table 1). We next examined the ability of *R* stereoisomers HI-509^R and HI-510^R to inhibit the replication of the HIV-1 strain $HTLV_{IIIB}$ in human peripheral blood mononuclear cells (PBMC). Both HI-509^R and HI-510^R inhibited HIV-1 replication with IC_{50} values of 0.001 μM and 0.025 μM, respectively. In contrast, the IC_{50} values of the *S* stereoisomers (HI-509^S and HI-510^S) and the control compounds with unsubstituted pyridyl rings (HI-542 and HI-543) were >1 μM (Table 1). Similarly the *R* stereoisomers (but not *S* stereoisomers) of the α-methyl benzyl halopyridyl compounds HI-511 and HI-512 exhibited potent activity both in cell-free RT inhibition assays and cellular HIV-1 replication assays (Table 1). The substitution of the pyridyl ring of HI-509^R and HI-510^R with a thiazolyl ring (compound HI-513^R) resulted in 10-fold higher K_i values and 10-fold higher IC_{50} values in cell free RT inhibition assays. The *S* stereoisomer with an estimated K_i value of >100 μM did not exhibit any RT inhibitory activity even at 100 μM. Taken together, these results provide unprecedented evidence that the stereochemistry of a thiourea compound can profoundly affect its ability to fit into the NNI binding pocket of RT and therefore its anti-HIV activity.

We also examined the activity of the lead compounds HI-509^R, HI-510^R, HI-511^R, HI-512^R, and HI-513^R against three NNI-resistant HIV-1 strains. Each of these five compounds was more active than nevirapine or delavirdine against these three drug resistant HIV-1 strains. The most active agent against drug-resistant HIV-1 strains was HI-511^R. This compound was as active against the NNI-resistant HIV-1 strain A17 with a Y181C mutation as it was against $HTLV_{IIIB}$ (Table 1)

and it was twice as active against the multidrug resistant HIV-1 strain RT-MDR with a V106A mutation as well as additional mutations involving the RT residues 74V, 41L, and 215Y than $HTLV_{IIIB}$ (Table 1). When tested against RT-MDR, HI-511^R was found to be 1000-times more active than nevirapine, 80-times more active than delavirdine, 4-times more active than trovirdine, and as active as our previously reported fluorine-substituted thiourea compound HI-240 (Table 1). When tested against A17, HI-511^R was found to be 10,000-times more active than nevirapine, 5000-times more active than delavirdine, 50-times more active than trovirdine, and 20-times more active than HI-240 (Table 1). HI-511^R was also capable of inhibiting the highly NNI-resistant HIV-1 strain A17 variant with Y181C plus K103N mutations in RT with an IC_{50} value of 2.7 μM. In contrast, the IC_{50} values of nevirapine, delavirdine, as well as trovirdine against A17 variant were >100 μM and the IC_{50} value of HI-240 was 41 μM. It is noteworthy that besides HI-511^R, compounds HI-509^R, HI-510^R, HI-512^R, and HI-513^R were also more active than nevirapine, delavirdine, trovirdine, and HI-240 against the A17 variant (Table 1). These findings demonstrate that the α-methyl benzyl halopyridyl compound HI-511^R has potent antiviral activity against NNI-resistant and multidrug resistant strains of HIV-1.

References and Notes

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12. Physical data: ***N*-[1-(1-(1*R*)-Cyclohexylethyl)]-*N*-[2-(5-bromopyridyl)] thiourea (HI-509^R).** Yield 32%, mp: 176–177 °C; UV (MeOH) λ_{max} 206, 210, 275 nm; IR: 3207, 3151, 3078, 3026, 2979, 2925, 2850, 1593, 1556, 1467, 1353, 1305, 1270, 1226, 1176, 1134, 1095, 1006, 960, 925, 862, 827, 731 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.32 (d, 1H, J = 8.7), 9.70 (s, 1H), 8.16 (d, 1H, J = 9), 4.40–4.33 (m, 1H), 1.85–1.48 (m, 7H), 1.18–1.16 (d, 3H, J = 6.6), 1.21–0.98 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 177.6, 152.0, 146.1, 140.9, 113.8, 112.4, 56.1, 42.7, 29.1, 29.0, 26.5, 26.3, 26.2, 17.1; MS (MALDI-TOF) 344.4 ($C_{14}H_{20}BrN_3S$ + 2).

***N*-[1-(1-(1*R*)-Cyclohexylethyl)]-*N*-[2-(5-chloropyridyl)]thiourea (HI-510^R).** Yield 29%, mp: 144–145 °C; UV (MeOH) λ_{max} 202, 207, 211, 256, 273, 304 nm; IR: 3210, 3024, 2931, 2850, 1601, 1549, 1474, 1230, 1108, 823 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.28 (d, 1H, J = 8.4), 10.62 (s, 1H), 8.25 (d, 1H, J = 2.7), 7.86–7.82 (dd, 1H, J = 8.7), 7.18 (t, 1H), 4.32–4.24 (m, 1H), 1.70–1.56 (m, 7H), 1.21–1.10 (d, 3H, J = 6.6), 1.18–0.99 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 178.8, 152.9, 144.4, 139.4, 124.2, 114.7, 55.5, 42.8, 29.5, 29.4, 26.8, 26.6, 26.5, 17.7; MS (MALDI-TOF) 298.6 ($C_{14}H_{20}ClN_3S$ + 1).

***N*-[1-(1*R*)-(1-α-methylbenzyl]-*N'*-[2-(5-bromopyridyl)]thiourea (HI-511^R).** Yield 43%, mp: 170–172 °C; UV λ_{max} 211, 257, 275, 278 nm; IR ν_{max} 3245, 3027, 2979, 2925, 1594, 1525, 1475, 1188, 704 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.79 (d, 1H), 10.76 (s, 1H), 8.39 (d, 1H, J = 2.1), 7.98 (dd, 1H, J = 9), 7.35 (d, 4H, J = 3.9), 7.28–7.23 (m, 1H), 7.15 (d, 1H, J = 9), 5.55 (q, 1H), 1.51 (d, 3H); ^{13}C NMR (DMSO-d_6) δ 178.4, 152.5, 146.2, 143.2, 141.5, 128.6, 127.0, 126.1, 114.7, 112.0, 54.0, 22.7; MS (MALDI-TOF) 337.7.

***N*-[1-(1-(1*R*)-α-methylbenzyl]-*N'*-[2-(5-chloropyridiyl)]thiourea (HI-512^R).** Yield 44%, mp: 185–187.5 °C; UV λ_{max} 204, 255, 275, 305 nm; IR ν_{max} 3247, 3169, 3087, 2978, 1600, 1529, 1483, 1189, 1034, 822, 761, 694 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.77 (d, 1H), 9.47 (s, 1H), 8.12 (d, 1H, J = 2.1), 7.53 (dt, 1H, J = 8.7), 7.40–7.26 (m, H), 6.85 (d, 1H, J = 8.7), 5.73–5.64 (m, 1H), 1.65 (d, 3H, J = 6.9); ^{13}C NMR ($CDCl_3$) δ 178.4, 151.8, 142.8, 144.4, 138.8, 128.9, 127.5, 126.4, 125.4, 113.6, 55.3, 22.7; MS (MALDI-TOF) 293.7.

***N*-[1-(1-(1*R*)-Cyclohexyl)ethyl]-*N'*-[2-(thiazolyl)]thiourea (HI-513^R).** Yield 45%; mp: 118–119 °C; UV (MeOH) λ_{max} 204, 259, 288 nm; IR ν_{max} 3170, 3040, 2968, 2927, 2849, 1656, 1514 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.87 (s, 2H), 7.31 (d, 1H, J = 3.6), 6.81 (d, 1H, J = 3.6), 4.47–4.36 (m, 1H), 1.87–1.53 (m, 4H), 1.24 (d, 3H, J = 6.6), 1.40–1.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 176.3, 162.2, 137.9, 111.2, 56.4, 43.1, 29.5, 29.3, 26.8, 26.6, 26.5, 17.5; MS (MALDI-TOF) 271.2.

***N*-[1-(1-(1*R*)-Cyclohexylethyl]-*N'*-[2-(pyridyl)]thiourea HI-543).** Yield 28%, mp: 125–127 °C; UV (MeOH) λ_{max} 206, 211, 249, 266, 294 nm; IR ν_{max} 3212, 3169, 3031, 2925, 2850, 1600, 1556, 1531, 1493, 775 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.98 (d, 1H), 9.12 (s, 1H), 8.14 (dd, 1H, J = 4.8), 7.64–7.58 (m, 1H), 6.96–6.86 (m, 2H), 4.49–4.37 (m, 1H), 1.86–1.54 (m, 5H), 1.24 (d, 3H), 1.30–1.06 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 178.4, 153.8, 145.8, 138.7, 117.9, 112.4, 56.4, 43.2, 29.5, 29.4, 26.9, 26.7, 26.6, 17.5; MS (MALDI-TOF) 264.8.

13. Atomic coordinates will be deposited in the Cambridge Structural Database, Cambridge Crystallographic Data Centre, Cambridge, UK.