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N-[2-(1-CYCLOHEXENYL)ETHYL]-N'-[2-(5-BROMOPYRIDYL)]-THIOUREA AND N'-[2-(1-CYCLOHEXENYL)ETHYL]-N'-[2-(5-CHLOROPYRIDYL)]- THIOUREA AS POTENT INHIBITORS OF MULTIDRUG-RESISTANT HUMAN IMMUNODEFICIENCY VIRUS-1

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Abstract: We have replaced the pyridyl ring of trovirdine with an alicyclic cyclohexenyl, adamantyl or *cis*-myrtanyl ring. Only the cyclohexenyl-containing thiourea compound N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-bromopyridyl)]- thiourea (HI-346) (as well as its chlorine-substituted derivative N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-chloropyridyl)]- thiourea/HI-445) showed RT inhibitory activity. HI-346 and HI-445 effectively inhibited recombinant RT with better IC_{50} values than other anti-HIV agents tested. The ranking order of efficacy in cell-free RT inhibition assays was: HI-346 (IC_{50} = 0.4 μM) > HI-445 (IC_{50} = 0.5 μM) > trovirdine (IC_{50} = 0.8 μM) > MKC-442 (IC_{50} = 0.8 μM) = delavirdine (IC_{50} = 1.5 μM) > nevirapine (IC_{50} = 23 μM). In accord with this data, both compounds inhibited the replication of the drug-sensitive HIV-1 strain $HTLV_{IIIB}$ with better IC_{50} values than other anti-HIV agents tested. The ranking order of efficacy in cellular HIV-1 inhibition assays was: HI-445 = HI-346 (IC_{50} = 3 nM) > MKC-442 (IC_{50} = 4 nM) = AZT (IC_{50} = 4 nM) > trovirdine (IC_{50} = 7 nM) > delavirdine (IC_{50} = 9 nM) > nevirapine (IC_{50} = 34 nM). Surprisingly, the lead compounds HI-346 and HI-445 were 3-times more effective 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 they were against $HTLV_{IIIB}$ with wild-type RT. HI-346 and HI-445 were 20-times more potent than trovirdine, 200-times more potent than AZT, 300-times more potent than MKC-442, 400-times more potent than delavirdine, and 5000-times more potent than nevirapine against the multidrug resistant HIV-1 strain RT-MDR. HI-445 was also tested against the RT Y181C mutant A17 strain of HIV-1 and found to be >7-fold more effective than trovirdine and >1,400-fold more effective than nevirapine or delavirdine. Similarly, both HI-346 and HI-445 were more effective than trovirdine, nevirapine, and delavirdine against the problematic NNI-resistant HIV-1 strain A17-variant with both Y181C and K103N mutations in RT, although their activity was markedly reduced against this strain. Neither compound exhibited significant cytotoxicity at effective concentrations (CC_{50} >100 μM). These findings establish the lead compounds HI-346 and HI-445 as potent inhibitors of drug-sensitive as well as multidrug-resistant stains of HIV-1.

Multidrug resistance against anti-retroviral agents has emerged as the major challenge to a more successful outcome of contemporary treatment programs for individuals infected with human immunodeficiency virus (HIV)-1.[1-3] Therefore, the development of novel antiviral agents that are effective against multidrug-resistant HIV-1 has become a focal point of translational AIDS research. Recently, we developed a computer model for the nonnucleoside inhibitor (NNI) binding pocket of HIV-1 reverse transcriptase (RT) as an effective tool for rational structure-based design of potent NNI.[4-6] Our modeling studies revealed several details regarding the RT mutations associated with NNI resistance: The spacious Wing 2 region of the butterfly-shaped NNI-binding pocket contains multiple aromatic residues, including Y181 and Y188, which occupy a substantial volume. Y181C, Y188C, and Y188H mutations in drug-resistant HIV strains result in a larger unoccupied volume in the binding pocket. Preferred inhibitors should maximize the occupancy in the Wing 2 region of the NNI binding site of RT and inhibitors that lack a compatible functional group to interact with the mutated residues and/or a sufficiently large group to provide surface contact with the altered Wing 2 region would not be effective against NNI-resistant HIV-

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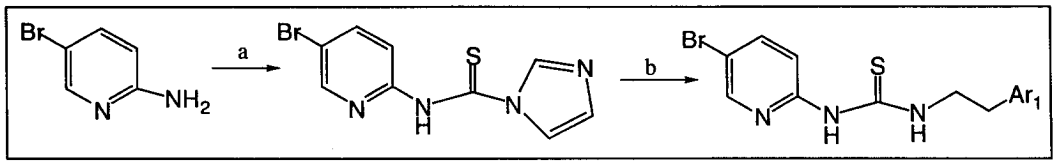
1 strains with these mutations.[3] In the present study, we replaced the pyridyl ring of trovirdine with a cyclohexenyl group that fits well with the Wing 2 region of the NNI binding pocket. The title compounds were synthesized as described in Scheme 1, in which a thiocarbonyl reagent was prepared from phenethylamine or pyridylethylamine and 1,1'-thiocarbonyl-diimidazole in acetonitrile solvent at room temperature for 12 h, and condensed with the appropriate 2-amino compounds in dimethyl formamide (DMF) at 100 °C for 15 h. After work up, the derivatives were purified by column chromatography. Trovirdine was synthesized according to the literature procedure.[2]

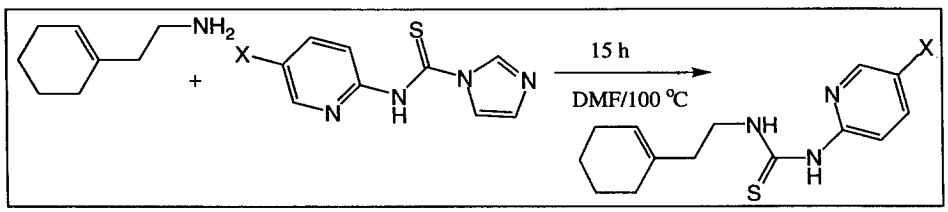
Scheme 1



Reagents and conditions: (a) 1, 1-thiocarbonyl diimidazole, Acetonitrile, 12 h

(b) DMF, 100 °C, 15 h Ar_1—NH$_2$

Scheme 2



X = CF_3, Br, Cl

A computer simulation of the binding of the designed thiourea inhibitors to the NNI binding pocket was accomplished using a molecular docking procedure.[3-6] Once the final energetically favored docked position of the inhibitor in the NNI binding pocket was identified, the inhibitor was assigned an interaction score, from which the inhibition constant (K_i) was estimated. The docking results indicated that the cyclohexenyl group of the designed compounds N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-bromopyridyl)]- thiourea (HI-346), and N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-chloropyridyl)]- thiourea (HI-445) is situated in the Wing 2 region of the NNI binding pocket, providing contact with RT residues including Y181 (Figure 1). The cyclohexenyl group is slightly better than the pyridyl group of trovirdine relative to its hydrophobic interactions with the RT residues. According to our modeling studies, the cyclohexenyl group of the designed lead compound N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-bromopyridyl)]- thiourea (HI-346) makes 94 hydrophobic contacts with the surrounding RT residues including P95, Y181, L100, V179, and Y188 and translates into a 3.0 log unit gain in the final interaction score. The pyridyl group of the reference compound trovirdine bound to the same region of RT would make 81 contacts with surrounding residues, resulting in a 2.7 log unit gain in the final interaction score. The alicyclic cyclohexenyl group contains more ring hydrogens than the heterocyclic pyridyl ring and therefore has more hydrogen atom-mediated contacts and fewer carbon atom-mediated contacts with RT residues than the latter. Our composite binding pocket also indicated a region in Wing 1 that would be compatible with polar atoms; this region corresponds to the predicted location of the bound halogen atoms of HI-346 and HI-445. The bromine atom of

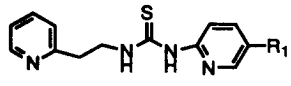
HI-346 makes 21 contacts with 7 RT residues including H235, L234, and V106 because of its large van der Waal radius. The estimated values for the hydrophobic score function in log units were 10.2 for HI-346, and 9.7 for HI-445, whereas the estimated values for the polar score function in log units were 1.7 for HI-346 as well as HI-445. The estimated K_i values were 0.16 μM for HI-346 and 0.50 μM for HI-445, which are better than the K_i value of 0.63 μM for trovirdine. As shown in Table 1, both HI-346 and HI-445 were more effective than trovirdine, as well as the control NNI compounds nevirapine and delavirdine, in inhibiting recombinant RT. Furthermore, both compounds were slightly more effective than trovirdine, as well as the control anti-HIV compounds nevirapine, delavirdine, MKC-442, and AZT in inhibiting the replication of the NNI-sensitive/AZT-sensitive HIV-1 strain $HTLV_{IIIB}$ (Table 1). The ranking order of efficacy in cellular HIV-1 inhibition assays was: HI-445 = HI-346 (IC_{50} = 3 nM) > MKC-442 (IC_{50} = 4 nM) = AZT (IC_{50} = 4 nM) > trovirdine (IC_{50} = 7 nM) > delavirdine (IC_{50} = 9 nM) > nevirapine (IC_{50} = 34 nM).

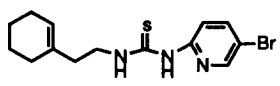
Unlike HI-346 and HI-445, the control cyclohexenyl containing thiourea compound N-[2-(1-cyclohexenyl)ethyl]-N'-[2-(5-trifluoromethylpyridyl)]- thiourea (HI-347), for which we had estimated a relatively poor K_i value of 63 μM, was significantly less potent than trovirdine in both RT inhibition assays and HIV replication assays. Replacement of the pyridyl ring of trovirdine with the alicyclic substituents adamantyl or cis-myrtanyl (instead of cyclohexenyl) resulted in complete loss of RT inhibitory function (Table 2). Thus, both the cyclohexenyl moiety as well as the R2 substituent play critical roles for the anti-HIV activity of HI-346 and HI-445.

Surprisingly, the lead compounds HI-346 and HI-445 were 3-times more effective 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 they were against $HTLV_{IIIB}$ with wild-type RT. The ranking order of potency against RT-MDR was: HI-346 (IC_{50}=1 nM) = HI-445 (IC_{50}=1 nM) > trovirdine (IC_{50}=20 nM) >HI-347(IC_{50}=38 nM)> AZT (IC_{50}=200 nM) > MKC-442 (IC_{50} = 300 nM) > delavirdine (IC_{50} = 400 nM) >nevirapine (IC_{50} = 5000 nM). HI-346 and HI-445 were 20-times more potent than trovirdine, 200-times more potent than AZT, 300-times more potent than MKC-442, 400-times more potent than delavirdine, and 5000-times more potent than nevirapine against the multidrug resistant HIV-1 strain RT-MDR. HI-445 was also tested against the RT Y181C mutant A17 strain of HIV-1 and found to be >7-fold more effective than trovirdine and >1,400-fold more effective than nevirapine or delavirdine. Similarly, both HI-346 and HI-445 were more effective than trovirdine, nevirapine, and delavirdine against the problematic NNI-resistant HIV-1 strain A17-variant with both Y181C and K103N mutations in RT, although their activity was markedly reduced against this strain. Neither compound exhibited significant cytotoxicity at effective concentrations (CC_{50} >100 μM). These findings establish the lead compounds HI-346 and HI-445 as potent NNI of drug-sensitive as well as multidrug-resistant stains of HIV-1.

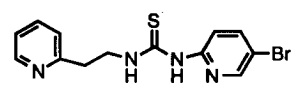
Figure 1. (right) Composite binding pocket of NNI active site of HIV-1 RT illustrated as grid lines representing the collective van der Waals surface. The surface model was constructed as previously described.[2-4] The stick model of compounds **HI-346**, which was docked into the NNI binding site, is illustrated with the composite NNI binding pocket. Blue represents the hydrophobic region, red hydrogen-bond region and yellow polar region. The bromo atom is compatible with the polar region, but not all three fluoro atoms of CF_3 group in **HI-347** would be located within the polar region. (left) Connolly surface representation of compound **HI-346** in the NNI binding site. The molecular surface area associated with hydrogen atoms on cyclohexenyl ring are colored in red. Other surface colors: nitrogen is blue, bromine is brown, sulfur is yellow, carbon is gray and other hydrogens are white, respectively. The residues in contact with Br atom (or CF_3 group in **HI-347** compound) and cyclohexenyl group are labeled and are shown in stick model (pink for sidechains and steel-blue for mainchains), prepared using INSIGHTII.[10]

Table 1. Interaction scores and calculated K_i values of cyclohexenyl containing thiourea compounds.[11] Molecular modeling and docking studies were done as previously described.[2-6]





HI-346



Trovirdine

Compound	R_1	Hydrophobic Score[a]	Polar Score[b]	Solvent[c]	Entropic[d]	K_i (μM)
HI-346	Br	10.2	1.7	-1.8	-3.3	0.16
HI-445	Cl	9.7	1.7	-1.9	-3.2	0.50
HI-347	CF_3	8.8	0.2	-1.6	-3.3	63
Trovirdine		10.2	0.5	-1.2	-3.3	0.63

(a) hydrophobic term of Jain' score function;[7] (b) polar term (hydrogen bond);
(c) solvent effect term; (d) entropic term

Table 2. Potent Anti-HIV activity of cyclohexenyl containing thiourea compounds HI-346 and HI-445. The RT inhibitory activity of the compounds was tested using purified recombinant RT and cell-free Quan-T-RT assay system (Amersham, Arlington Heights, IL), which utilizes the scintillation proximity assay principle, as previously described in detail.[3-6] The results are presented as the IC_{50} values (i.e., concentration at which the compound inhibits recombinant RT by 50%). The anti-HIV activity of the compounds was measured by determining their ability to inhibit the replication of the HIV-1 strains $HTLV_{IIIB}$, RT-MDR, A17, and A17 variant in peripheral blood mononuclear cells (PBMC) from healthy volunteer donors, as described.[8,9] The results are presented as the IC_{50} values for inhibition of HIV p24 antigen production in PBMC (i.e., concentration at which the compound inhibits p24 production by 50%). A Microculture Tetrazolium Assay (MTA), using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium hydroxide (XTT), was performed to evaluate the cytotoxicity of the compounds, as previously reported.[8,9]

Compound	R_1	R_2	IC_{50} rRT (μM)	IC_{50} $HTLV_{IIIB}$ (μM)	IC_{50} RT-MDR (V106A) (μM)	IC_{50} A17 (Y181C) (μM)	IC_{50} A17 variant (Y181C, K103N) (μM)	CC_{50} MTA (μM)
Trovirdine	Pyridyl	Br	0.8	0.007	0.020	0.500	>100	>100
HI-346	Cyclo-hexenyl	Br	0.4	0.003	0.001	N.D.	18.7	>100
HI-445	Cyclo-hexenyl	Cl	0.5	0.003	0.001	0.068	30.0	>100
HI-347	Cyclo-hexenyl	CF_3	4.0	0.079	0.038	0.300	>100	>100
HI-504	Adamentyl*	Br	>100	N.D.	N.D.	N.D.	N.D.	N.D.
HI-444	Myrtanyl*	Br	>100	N.D.	N.D.	N.D.	N.D.	N.D.
Nevirapine	—	—	23	0.034	5.0	>100	>100	10.5
Delavirdine	—	—	1.5	0.009	0.4	50.0	>100	3.6
MKC-442	—	—	0.8	0.004	0.3	N.D.	N.D.	>100
AZT	—	—	—	0.004	0.2	0.006	0.004	>100

* methylene group instead of ethyl linker

References and Notes

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11. Selected physical data for the lead compounds:
N-[2-(5-Bromopyridinyl)]-N'-[2-(1-Cyclohexenyl)ethyl] thiourea (HI-346) yield 45%; mp: 72-173 °C; UV(MeOH) λ_{max}: 208, 275, 306 nm; IR(KBr) ν 3214, 3156, 3085, 3039, 2925, 2831, 1594, 1560, 1531, 1473, 1309, 1267, 1226, 1180, 1135, 1078, 1002, 918. 862, 821, 700, 505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.21 (bs, 1H), 9.42 (bs, 1H), 8.16–8.15 (d, 1H), 7.73–7.69 (dd, 1H), 6.88–6.85 (d, 1H), 5.59 (s, 1H), 3.84–3.78 (q, 2H), 2.35–2.31 (t, 2H), 2.05–1.99 (m, 4H), 1.67–1.55 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 178.6, 151.8, 146.3, 141.1, 134.5, 124.0, 113.6, 112.7, 44.1, 36.8, 27.7, 25.4, 22.8, 22.4.
N-[2-(5-Chloropyridinyl)]-N'-[2-(1-Cyclohexenyl)ethyl] thiourea (HI-445) yield 51%; mp: 165 °C; UV(MeOH) λ_{max}: 206, 273, 305 nm; IR(KBr) ν 3216, 3158, 3087, 3033, 2923, 2831, 1598, 1562, 1533, 1475, 1340, 1307, 1228, 1166, 1107, 1018, 910, 862, 823, 692, 586, 505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.24 (bs, 1H), 9.67 (bs, 1H), 8.07–8.06 (d, 1H), 7.61–7.57 (dt, 1H), 6.99–6.96 (d, 1H), 5.60 (s, 1H), 3.85–3.79 (q, 2H), 2.35–2.31 (t, 2H), 2.02–2.00 (d, 4H), 1.67–1.57 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 178.5, 151.6, 143.9, 138.4, 134.4, 125.0, 124.0, 113.2, 44.0, 36.8, 27.7, 25.4, 22.8, 22.3.