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N'-[2-(2-THIOPHENE)ETHYL]-N'-[2-(5-BROMOPYRIDYL)] THIOUREA AS A POTENT INHIBITOR OF NNI-RESISTANT AND MULTIDRUG-RESISTANT HUMAN IMMUNODEFICIENCY VIRUS-1

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Abstract: The thiophene-ethyl thiourea (TET) compound N'-[2-(2-thiophene)ethyl]-N'-[2-(5-bromopyridyl)]-thiourea (compound **HI-443**) was five times more potent than trovirdine, 1250 times more potent than nevirapine, 100 times more potent than delavirdine, 75 times more potent than MKC-442, and 50 times more potent than AZT against the multidrug resistant HIV-1 strain RT-MDR with a V106A mutation. HI-443 was almost as potent against the NNI-resistant HIV-1 strain A17 with a Y181C mutation as it was against $HTLV_{IIIB}$. The activity of HI-443 against A17 was ten times more potent than that of trovirdine, 2083 times more potent than that of nevirapine, and 1042 times more potent than that of delavirdine. HI-443 inhibited the replication of the NNI-resistant HIV-1 strain A17 variant with Y181C plus K103N mutations in RT with an IC_{50} value of 3.3 μM, whereas the IC_{50} values of trovirdine, nevirapine, and delavirdine were all >100 μM. These findings establish the novel thiophene containing thiourea compound HI-443 as a novel NNI with potent antiviral activity against NNI-sensitive, NNI-resistant and multidrug-resistant strains of HIV-1.

Anti-HIV compounds that are currently used to treat the human immunodeficiency virus (HIV) include AZT (nucleoside analog), nelfinavir (protease inhibitor), and delavirdine, efavirenz, and nevirapine (non-nucleoside reverse transcriptase inhibitors [NNI or NNRTI]). However, the effectiveness of these agents can be significantly compromised by the appearance of specific mutations of HIV reverse transcriptase (RT). The high replication rate of the virus leads to drug-resistant genetic variants (mutants), especially when selective pressure is introduced therapeutically .[1] A challenge for future anti-HIV therapies is to find effective agents against new drug-resistant strains of HIV. Recently, we reported a novel computer model for the NNI binding pocket of RT that provides an effective tool for the rational design of NNIs.[2—4] We utilized this novel composite binding pocket, 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 in the NNI binding site of RT and reported the synthesis of a series of potent compounds which abrogated HIV replication in peripheral blood mononuclear cells (PBMC) at nanomolar concentrations without evidence of cytotoxicity.[2—4]

Recently, we reported that the replacement of the planar pyridyl ring of trovirdine with a puckered piperidinyl or piperazinyl ring, which occupy larger volumes, would better fill the spacious Wing 2 region of the butterfly-shaped NNI binding pocket.[4] Such heterocyclic rings are conformationally more flexible than an aromatic ring 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 first two heterocyclic compounds synthesized were N-[2-(1-piperidinylethyl)]-N'[2-(5-bromopyridyl)]-thiourea (compound HI-172)

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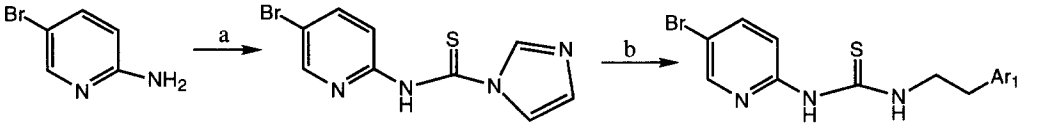
and N-[2-(1-piperazinylethyl)]-N'[2-(5-bromopyridyl)]-thiourea.[4] Both compounds were more potent than trovirdine and abrogated the replication of the NNI-sensitive HIV-1 strain $HTLV_{IIIB}$ in human peripheral blood mononuclear cells (PBMC) at nanomolar concentrations.[4] However, unlike trovirdine, neither compound inhibited the replication of NNI-resistant HIV-1 strains.[5] These initial findings demonstrated that the replacement of the pyridyl ring of trovirdine is feasible, but it may not be as forgiving as originally thought if one wants to retain the ability of trovirdine to inhibit HIV-1 strains with RT mutations.[5]

In the present study, we have replaced the pyridyl ring of trovirdine with one of eight different heterocyclic substituents, including (a) the heterocyclic amines pyrrolidine, 1-methyl- pyrrolidine, morpholine, imidazole, indole; (b) heterocyclic aromatic groups furan and thiophene; and (c) the aromatic acetal piperonyl.[11] The thiourea 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.[6]

Scheme 1



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

(b) DMF, 100 °C, 15 h. Ar_1—⌒—NH_2

Notably, the substitution of its pyridyl ring had a major impact on the RT-inhibitory function of trovirdine. Only the thiophene-ethyl thiourea (TET) compound N'-[2-(2-thiophene)ethyl]-N'-[2-(5-bromopyridyl)]- thiourea (compound HI-443) inhibited recombinant RT in vitro by more than 90% (Table 1). The thiophene-substituted HI-443, piperonyl-substituted HI-257, indolyl-substituted HI-442, furanyl-substituted HI-503, and pyrrolidinyl-substituted HI-230 inhibited recombinant RT with IC_{50} values of 0.8, 0.7, 0.9, 1.2, and 4.9 μM, respectively. Whereas the reference compound trovirdine and the novel lead compound HI-443 inhibited recombinant RT with IC_{90} values of 12 and 15 μM, respectively, none of the other seven compounds inhibited recombinant RT by >90% even at a 100 μM concentration. The thiophene group of HI-443 occupies the same Wing 2 region of the NNI binding pocket of RT as trovirdine, but it has a smaller molecular volume. Furthermore, the docked position of HI-443 in the RT binding site indicated that the 2'-NH atom forms a hydrogen bond to the amide carbonyl of RT residue 101. However, the geometry of the hydrogen bond with HI-443 deviates from the optimum geometry that was observed for trovirdine and HI-172. (A less than optimum hydrogen bonding geometry may contribute to slightly weaker binding.). Therefore, it was not surprising that HI-443 had a lower inhibitory activity against

HTLV IIIB RT (IC_{50} = 0.030 μM) relative to trovirdine (IC_{50} = 0.007 μM) or our previously published lead compound HI-172 (IC_{50} < 0.001 μM)[4] that contains a more bulky heterocyclic piperidinyl group (Table 2).

We next compared the anti-HIV activity of HI-443 with that of trovirdine as well as HI-172. As shown in Table 2, HI-443 effectively inhibited the replication of the HIV-1 strain $HTLV_{IIIB}$ in human peripheral blood mononuclear cells (PBMC) in three of three independent experiments with an average IC_{50} value of 0.03 μM. In accordance with the higher IC_{50} value of HI-443 against recombinant RT, the IC_{50} value of HI-443 for inhibition of $HTLV_{IIIB}$ replication was five times higher than the IC_{50} value of trovirdine and 30 times higher than the IC_{50} value of HI-172 (Table 2).

Table 1. HIV-RT Inhibitory Activity of HI-443. 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.[3,7] The results are presented as the IC_{50} values (i.e., concentration of the compound which inhibits recombinant RT by 50%). Unlike other compounds in the table, HI-503 and HI-257 have a methylene linker group instead of an ethyl linker.

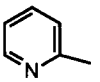
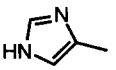
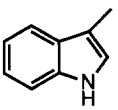
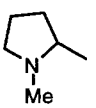
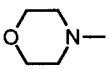
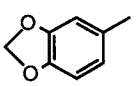
Compounds	R_1		IC_{50} rRT(μM)	IC_{90} rRT(μM)
Trovirdine		Pyridine	0.6	12.0
HI-443		Thiophene	0.8	15.0
HI-230		Pyrrolidine	4.9	>100
HI-436		Imidazole	>100	>100
HI-442		Indole	0.9	>100
HI-206		1-Methyl Pyrrolidine	>100	>100
HI-276		Morpholine	>100	>100
HI-257		Piperonyl	0.7	>100
HI-503		Furan	1.2	>100

Table 2. Anti-HIV activity of HI-443. 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 PBMC from healthy volunteer donors, as described.[2,8] 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.[9,10] The physicochemical data are presented in ref.11.

Compound	Anti-HIV activity					
	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)
HI-443	0.8	0.030	0.004	0.048	3.3	>100
Trovirdine	0.6	0.007	0.020	0.500	>100	>100
Nevirapine	23	0.034	5.000	>100	>100	10.5
Delavirdine	1.5	0.009	0.400	50.0	>100	3.6
MKC-442	0.8	0.004	0.270	N.D	N.D	>100
AZT	>100	0.004	0.200	0.006	0.004	>100
HI-172	0.6	<0.001	>100	>100	>100	>100
HI-240	0.6	<0.001	0.005	0.200	41	>100

We also examined the activity of HI-443 against NNI-resistant as well as multidrug resistant HIV-1 strains. Surprisingly, HI-443 was ten 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 HTLVIIIB. HI-443 was almost as potent against the NNI-resistant HIV-1 strain A17 with a Y181C mutation as it was against $HTLV_{IIIB}$ (IC_{50}: 0.048 μM vs 0.030 μM), and it was capable of inhibiting the trovirdine-resistant A17 variant with Y181C plus K103N mutations in RT (IC_{50}: 3.3 μM), albeit with a 100-fold lower potency than $HTLV_{IIIB}$ (Table 2). HI-443 was five times more potent than trovirdine, 1250 times more potent than nevirapine, 100 times more potent than delavirdine, 75 times more potent than MKC-442, 25,000 times more potent than HI-172, 1.25 times more potent than HI-240 (a recently reported fluorine-substituted PETT derivative with potent anti-HIV activity),[5] and 50 times more potent than AZT against the multidrug resistant HIV-1 strain RT-MDR. Similarly, HI-443 was ten times more potent than trovirdine, 2083 times more potent than nevirapine, 1042 times more potent than delavirdine, 2083 times more potent than HI-172, and 4.2 times more potent than HI-240 against the NNI-resistant HIV-1 strain A17. Finally, HI-443 inhibited the replication of the AZT-sensitive but NNI-resistant HIV-1 stain A17 with an IC_{50} value of 3.3 μM, whereas the IC_{50} values of trovirdine, nevirapine, delavirdine, and HI-172 were all >100 μM and the IC_{50} value of HI-240 was 41 μM[5] (Table 2). When the thiophene-containing thiourea compound HI-443 was modeled into the NNI binding site of RT using a previously reported docking procedure,[2,4] the thiophene group of HI-443 was found to be located in close proximity of the

Y181 residue. In this docked position, the sulfur atom of the thiophene ring is only 4.4 Å away from the C(g) atom of the tyrosine 181 residue which is mutated to a sulfur atom in the RT Y181C mutant strains (A17 or A17 variant) (Figure 1). Therefore, while the thiophene ring of HI-443 loses hydrophobic contact with the sidechain atoms of residue 181 in the Y181C mutant (hence higher IC_{50} values against the Y181C mutant strains A17 and A17 variant), the sulfur atom of its thiophene group may be more compatible with the sulfur-containing cysteine 181 residue than the pyridyl group of trovirdine. These findings establish the TET compound HI-443 as a novel NNI with potent antiviral activity against NNI-resistant as well as multidrug resistant stains of HIV-1.

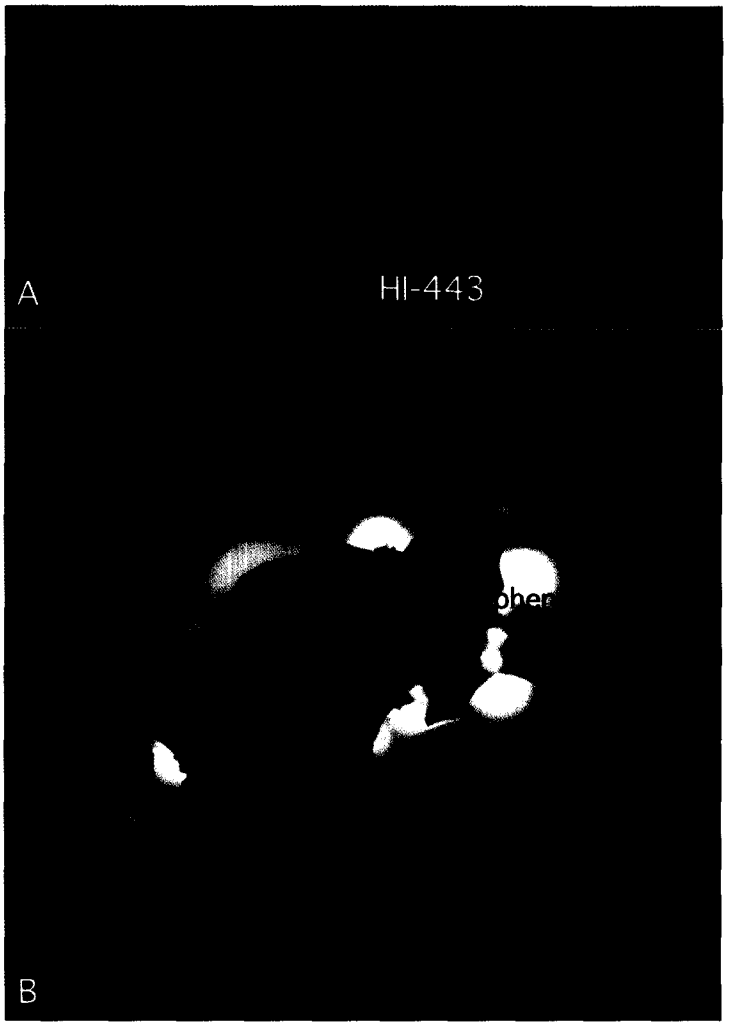


Figure 1. (A) The stick model of compound **HI-443** that was docked into the NNI binding site (B). (B) Connolly surface representation of compound **HI-443** in the NNI binding site. The molecular surface area associated with bromo atom was colored in red. Other surface colors: nitrogen in blue, bromine in red, sulfur in yellow, carbon in gray and other hydrogens in white. The residues in contact with **HI-443** compound are labeled and are shown in stick model (pink for sidechains and steel-blue for mainchains), prepared using INSIGHTII.

References and Notes

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11. Selected physical data for active compounds:

HI-230, N-[2-(1-pyrolidylethyl)]-N'-[2-(5-bromopyridyl)]thiourea: Yield: 72%; mp: 136-138°C ; UV (MeOH) λmax: 203, 206, 252, 277, 306 nm, IR(KBr)ν 3454, 3220, 3159, 3059, 2941, 2787, 1595, 1531, 1475, 1311, 1229, 1182, 1061, 1003, 864, 821, 706 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 11.53 (bs, 1H), 9.17 (bs, 1H), 8.19-8.11 (d, 1H), 7.73-7.69 (d, 1H), 6.82-6.79 (dd, 1H), 3.85-3.83 (q, 2H), 2.79(t, 2H), 2.60 (bm, 4H), 1.81 (bm); ^{13}C($CDCl_3$) δ 178.7 , 151.7 , 146.5 , 141.1, 113.4, 112.7 , 53.8 , 53.6, 44.9 and 23.7; Maldi Tof mass : 329 (M+1), Calculated mass : 328; Anal. ($C_{12}H_{17}Br\ N_4\ S$), Found: C: 42.64, H: 4.80, N:16.71, S: 7.72, Br: 28.04;

HI-257, N-[2-(1-piperonyl)]-N'-[2-(5-bromopyridyl)]thiourea: Yield: 70%; mp: 159-162°C; UV (MeOH) λmax: 209, 276nm, IR(KBr) ν 3450, 3215, 3151, 3082, 3009, 2931, 1591, 1562, 1529, 1500, 1475, 1305, 1238, 1168, 1086, 1041, 933, 858, 825, 794, 688 cm^{-1}; ^{1}HNMR (DMSO-d_6) δ 11.64 (bs, 1H),10.68 (bs, 1H), 8.17-8.16(s, 1H), 7.75-7.72(d, 1H), 7.19-7.16 (d,1H), 6.91-6.90 (s, 1H), 6.84-6.83 (d, 1H), 6.79-6.77(d,1H) , 6.01 (s, 2H), 4.86-4.84 (d, 2H); ^{13}C($CDCl_3$) δ 178.7 , 151.3, 146.4 , 144.7 , 139.7, 130.3 , 119.5, 113.5 ,110.9 , 106.9, 99.7 and 47.3 , Maldi Tof mass : 366 (M+Na), Calculated mass : 345; Anal. ($C_{14}H_{12}Br\ N_3\ O_2\ S$) C, H, N, S, Br;

HI-443: N-[2-(5-Bromopyridinyl)]-N'-[2-(2-Thiophenylethyl)] thiourea: Yield 40%; mp: 160-161°C; UV(MeOH) λ_{max}: 260, 276, 306 nm; IR(KBr) ν 3218, 3151, 3087, 2935, 2873, 1594, 1552, 1531, 1332, 1297, 1265, 1224, 1188, 1134, 1089, 1076, 1006, 833, 811, 784, 742, 688, 582, 503 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.45 (bs, 1H), 10.40 (bs, 1H), 8.03 (s, 1H), 7.68-7.64 (dd, 1H), 7.20-7.19 (d, 1H), 7.08-7.04 (dd, 1H), 6.99-6.95(m, 1H), 6.91 (s, 1H), 4.04-3.97 (q, 2H), 3.24-3.20 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 179.1, 151.7, 145.1, 140.6, 140.1, 126.2, 124.8, 123.3, 113.8, 111.5, 46.1, 28.4;

HI-442, N-[2-(5-Bromopyridinyl)]-N'-[2-(3-Indolylethyl)] thiourea: Yield 44%; mp: 208-209°C; UV(MeOH) λ_{max}: 222, 274, 305 nm; IR(KBr) ν 3351, 3207, 3147, 3079, 3035, 2915, 2869, 2840, 1591, 1556, 1531, 1465, 1421, 1328, 1299, 1230, 1189, 1105, 1004, 950, 906, 860, 831, 752, 644, 588, 509 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.30 (bs, 1H), 10.32 (bs, 1H), 10.20 (bs, 1H), 7.81 (d, 1H), 7.65-7.58 (m, 2H), 7.41-7.39 (d, 1H), 7.16-7.11 (t, 2H), 7.05-7.00 (t, 2H), 4.06-4.00 (q, 2H), 3.15-3.11 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 178.4, 151.6, 144.9, 139.8, 135.7, 126.4, 122.0, 120.6, 117.9, 117.7, 113.5, 111.1, 111.0, 110.7, 45.4, 23.7;

HI-503, N-[2-(5-Bromopyridinyl)]-N'-[2-(2-Furylmethyl)] thiourea: Yield 44%; mp: 187-188°C; UV(MeOH) λ_{max}: 209, 276, 307 nm; IR(KBr) ν 3216, 3155, 3083, 3037, 2921, 1594, 1550, 1529, 1463, 1307, 1228, 1176, 1135, 1093, 1006, 968, 864, 817, 719, 568 cm^{-1}; ^{1}H NMR (DMSO) δ 11.50 (t, 1H), 10.86 (bs, 1H), 8.32-8.31 (d, 1H), 7.99-7.95 (dd, 1H), 7.60 (t, 1H), 7.17-7.14 (d, 1H), 6.42-6.35(m, 2H), 4.87-4.85 (d, 2H); ^{13}C NMR (DMSO) δ 179.8, 152.5, 151.0, 146.3, 142.7, 141.7, 114.8, 112.3, 110.8, 107.8, 41.6.