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Effect of stereochemistry on the anti-HIV activity of chiral thiourea compounds

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Abstract—Chiral derivatives of several substituted halopyridyl and thiazolyl PETT compounds were synthesized as non-nucleoside inhibitors of the reverse transcriptase (RT) enzyme (NNRTI) of the human immunodeficiency virus (HIV-1). Molecular modeling studies indicated that because of the asymmetric geometry of the NNRTI binding pocket, the *R* stereoisomers would fit the NNRTI binding pocket of the HIV-1 RT much better than the corresponding *S* stereoisomers, as reflected by their 10^4-fold lower K_i values. The *R* stereoisomers of several PETT derivatives inhibited recombinant RT in vitro with lower IC_{50} values than their enantiomers. The active compounds were further evaluated for their ability to inhibit HIV-1 replication in human peripheral blood mononuclear cells (PBMC). All the *R* isomers once again showed potent anti-HIV activity and inhibited the replication of the HIV-1 strain HTLVIIIB in peripheral blood mononuclear cells (PBMC) at nanomolar concentrations whereas their enantiomers were substantially less potent. The lead compounds in the respective groups were further tested against the NNRTI-resistant HIV strains, A17 (Y181C mutant), and A17Var (Y181C+K103N mutant) and RT MDR (V106N). The results showed that the lead compounds were several logs more potent than the standard NNRTI nevirapine. Structure–activity relationship studies also revealed a preference for the pyridyl unit with halo substitutions primarily at 5-position demonstrating the importance of regiochemistry. Our data provides experimental evidence that the stereochemistry as well as regiochemistry of NNRTI can profoundly affect their anti-HIV activity.

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) is the causative agent for the transmission and development of the acquired immunodeficiency syndrome (AIDS).[1] The main targets in contemporary drug discovery efforts against HIV-1 is RT, a vital enzyme that is responsible for the reverse transcription of retroviral RNA to proviral DNA.[2–9] Non-nucleoside reverse transcriptase inhibitors (NNRTI's) inhibit HIV RT by altering either of the conformation or mobility of RT by binding to a specific allosteric site near the polymerase site, thereby resulting in noncompetitive inhibition of the enzyme.[10,11] In a systematic search for potent anti-AIDS drugs, Bell et al.[12,13] discovered phenethylthiazolylthiourea (PETT) compounds as potent inhibitors of HIV-1 and disclosed certain structure–activity relationships among various substituents in their structure. However, there is no published report on the role of stereochemistry for the anti-HIV activity of PETT derivatives. Recently, we have identified by rational drug design several structurally distinct thiourea compounds as potent NNRTIs for HIV-RT[14–18] and examined their structure–activity relationships. Here, we report the previously unknown impact of stereochemistry on the anti-HIV activity of thiourea compounds with special emphasis on the anti-HIV potency of several lead chiral thiourea compounds.

2. Results and discussion

For the synthesis of the chiral thiourea compounds, we followed the general procedure shown in Scheme 1 as previously reported.[13,14] The chiral phenethyl thiazolyl as well as halopyridyl thioureas in the current study

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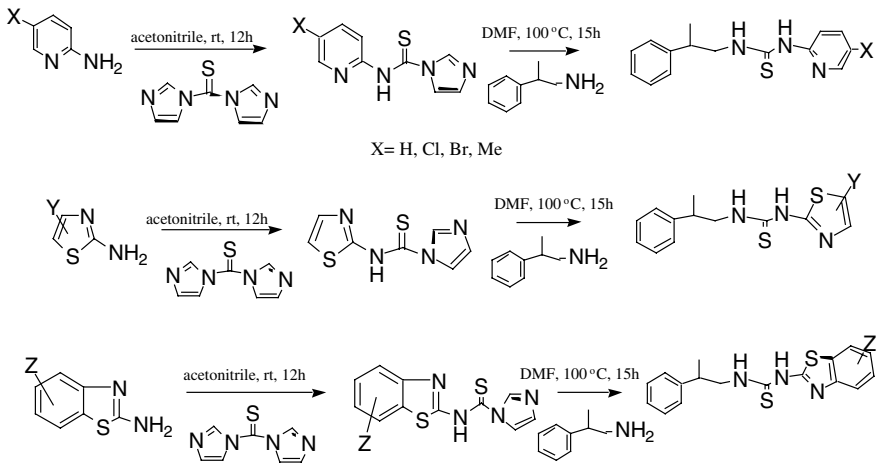


Scheme 1.

were prepared by treating an appropriately substituted aminopyridine or aminothiazole/benzothiazole and thiocarbonyldiimidazole in acetonitrile medium. The resultant intermediate was condensed with various chiral amines in anhydrous dimethyl formamide and heated to 100 °C for 18 h over an oil bath and worked up to yield thioureas. The purity and identity of these compounds were confirmed by standard analytical techniques.

Overall, the *R* stereoisomers of all compounds inhibited recombinant RT in cell free assays with lower IC_{50} values than their enantiomers. Among the **10** β-methyl phenylethyl pyridyl thiourea (β-MPT) compounds, the *R* stereoisomers of compounds **1**, **3**, and **5** with halogen (Br, Cl) or methyl substitutions, respectively, at the 5-position of their pyridyl unit were the most potent RT inhibitors (Table 1). These compounds displayed nanomolar IC_{50} values against recombinant RT in vitro. In contrast, the *S* stereoisomers showed significantly lower potency with micromolar IC_{50} values. Similarly, only the *R* stereoisomers of the β-methyl phenylethyl thiazolyl thioureas (compounds **11** and **15**) exhibited anti-HIV activity (Table 1).

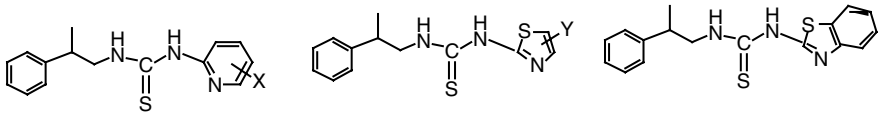
Of the 19 β-MPT compounds whose 'R' stereoisomers exhibited nanomolar IC_{50} values against recombinant RT, five were further evaluated for their ability to inhibit HIV-1 replication in human peripheral blood mononuclear cells (PBMC). All five *R* stereoisomers were active anti-HIV agents and inhibited the replication of the HIV-1 strain $HTLV_{IIIB}$ (NNRTI-sensitive) at nanomolar concentrations. When examined for their ability to inhibit the replication of the NNRTI-resistant HIV-1 strain, A17 (Y181C mutant) and A17Var (Y181C+K103N mutant), the majority of the *R* isomers showed activity at nanomolar to micromolar concentrations. The *R* stereoisomers of β-MPT compounds were less potent against NNRTI-resistant HIV-strains than they were against $HTLV_{IIIB}$. Nevertheless, all compounds were markedly more potent against the NNRTI-resistant HIV-1 strains than the standard NNRTI nevirapine. In addition, the lead β-MPT compound **3**, the 5-chloropyridyl *R* isomer, was 380-fold more active than nevirapine, 2-fold more active than trovirdine and 190-fold more potent than delavirdine, against A17. Compound **3** was >200-fold more potent than nevirapine, trovirdine or delavirdine against A17V. We also confirmed the superior activity of compound **3** against the multi-drug resistant RT-MDR strain (Table 1). Based on the above experimental results, we postulate that β-MPT compounds may be useful candidates for further development as anti-HIV agents especially since they show remarkable activity against mutant strains of HIV-1.

We next focused our attention towards the synthesis of chiral α-methyl benzyl thioureas (α-MBT) and accordingly we followed the general procedure shown in Scheme 2.

The purity and identity of these compounds was confirmed by usual analytical techniques and are reported under physical constant section. Table 2 shows the anti-HIV activity observed for all the isomers of chiral α-MBT derivatives towards recombinant RT. The alpha methyl groups were maintained constant and the pyridyl group was replaced by thiozolyl and benzothiazolyl groups. In addition, several substituents were introduced in those rings in order to extend our study and have some understanding about structure–activity relationships among these derivatives. The results in Table 2 show that all the *R* isomers were active where as their enantiomers (*S* isomers) were inactive.

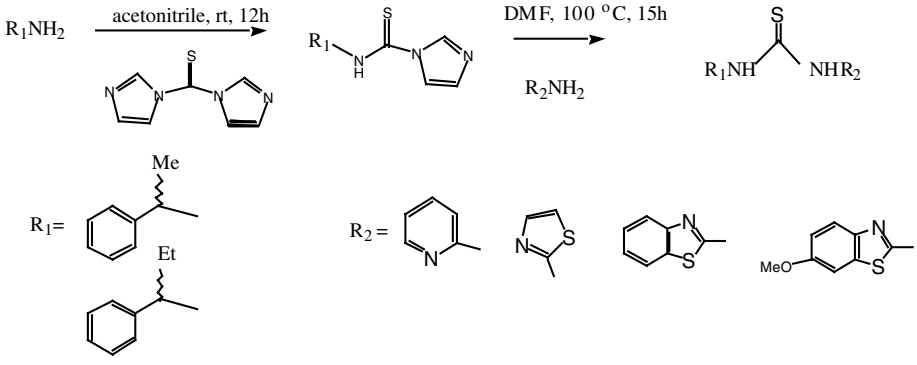
This is in accordance with the results obtained with chiral β-MPT compounds as discussed previously (see Table 1). Substitution on the pyridyl ring at 5-position was essential to retain the activity of the compounds as evidenced from the table values. Among the substituents at 5-position, halo as well as alkyl substitutions seem to yield active derivatives. Among the methyl substituted derivatives, the 5-methyl compound was more active than the 6-methyl substituted compound in these assays. It has been found that changing the pyridyl ring to thiazole ring did not affect the activity of the compound. Also methyl substitution at 4-position of the thiazolyl ring was found to retain the activity of the compound in

Table 1. Effect of stereochemistry on anti-HIV activity of β-methyl phenylethyl substituted thiourea compounds



Compd	Isomer	X	Y	rRT (μM)	$HTLV_{IIIB}$ (μM)	A17 (Y181C) (μM)	A17V (Y181C) (K103N) (μM)	RT-MDR (V106N) (μM)
1	R	5Br	NA	0.4	0.003	0.7	1.1	0.02
2	S	5Br	NA	1.9	0.030	1.2	1.2	0.2
3	R	5Cl	NA	0.1	<0.001	0.3	0.5	0.02
4	S	5Cl	NA	1.4	0.04	0.5	>100	0.2
5	R	5Me	NA	0.5	0.003	1.1	1.8	0.05
6	S	5Me	NA	7.7	ND	ND	ND	ND
7	R	6Me	NA	16.7	0.04	1.4	1.2	0.1
8	S	6Me	NA	>100	ND	ND	ND	ND
9	R	H	NA	8.4	0.03	2.3	0.5	0.2
10	S	H	NA	3.5	ND	ND	ND	ND
11	R	NA	4-Me	5.1	0.06	6.9	1.1	ND
12	S	NA	4-Me	>100	ND	ND	ND	ND
13	R	NA	4-Methylene-carbo	>100	ND	ND	ND	ND
14	S	NA	4-Methylene-carbo	>100	ND	ND	ND	ND
15	R	NA	H	10.0	ND	ND	ND	ND
16	S	NA	H	>100	ND	ND	ND	ND
17	R	4-Methylbenzo		>100	ND	ND	ND	ND
18	R	Benzothiazolyl		>100	ND	ND	ND	ND
19	R	4,6 Me	NA	38.8	ND	ND	ND	ND
Nevirapine	—	—	—	23.0	0.03	>100.0	>100.0	1.4
Trovirdine	—	—	—	0.8	0.007	0.5	>100.0	ND
Delavirdine	—	—	—	1.5	0.009	50.0	>100.0	0.4

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.[19,20] The cell-free RT inhibition assays using recombinant RT(rRT) and the Quan-RT assay kit (Amersham, Arlington heights-IL) were performed as reported.[20,21] Results are presented as the mean IC_{50} values.



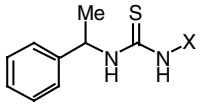
Scheme 2.

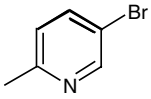
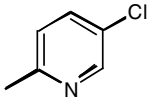
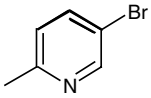
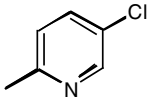
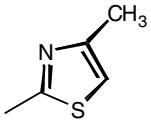
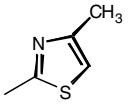
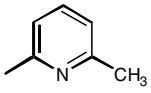
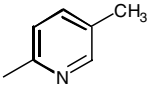
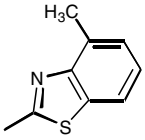
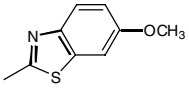
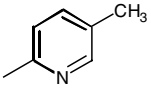
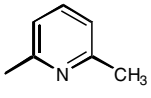
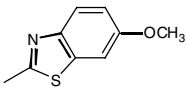
our assay. On the other hand, benzothiazolyl compounds, showed a considerable decrease in activity implying that this substitution is not beneficial among the thioureas. In order to circumvent this problem we introduced a few groups on the benzothiazolyl structure of the compounds **28** and **32**, however we observed no improvement in their anti-HIV activity.

We next examined the ability of these novel thiourea compounds to inhibit the replication of the HIV-1 strain $HTLV_{IIIB}$ in human peripheral blood mononuclear cells (PBMCs). As shown in Table 2, severalof the MBT derivatives inhibited HIV-1 replication at nanomolar concentrations. Once again the *R* isomers were more active compared to their enantiomers. The lead compounds **20**, **21**, and **24** exhibited potent activity against the NNRTI resistant HIV-1 strains A17, 17-Variant as well as the multi-drug resistant HIV strain RT-MDR (Table 2).

Our molecular modeling studies indicated that the *R* stereoisomers of chiral halopyrdiyl as well as chiral

Table 2. Effect of stereochemistry on anti-HIV activity of alpha-methyl benzyl substituted thiourea compounds



Compd	Isomer	X	rRT (μM)	HTLVIII$_B$ (μM)	A17 (μM)	A17V (μM)	RTMDR (μM)
20	*R*		1.6	<0.01	0.010	2.7	0.005
21	*R*		1.2	<0.01	0.200	10.20	0.010
22	*S*		>100	>1	ND	ND	ND
23	*S*		>100	ND	ND	ND	ND
24	*R*		0.8	<0.001	0.164	0.196	0.001
25	*S*		>100	ND	ND	ND	ND
26	*S*		>100	ND	ND	ND	ND
27	*R*		1.5	ND	ND	ND	ND
28	*R*		8.7	ND	ND	ND	ND
29	*S*		>100	ND	ND	ND	ND
30	*S*		>100	ND	ND	ND	ND
31	*R*		1.7	0.025	ND	ND	ND
32	*R*		>100	ND	ND	ND	ND
Nevirapine	NA	NA	23	0.034	100	100	1.4
Trovirdine	NA	NA	0.8	0.007	0.50	100	ND
Delavirdine	NA	NA	1.5	0.009	50	100	0.40
HI-240	NA	NA	0.6	0.001	0.20	41.0	ND

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.[20] The cell-free RT inhibition assays using recombinant RT (rRT) and the Quan-RT assay kit (Amersham, Arlington heights-Il) were performed as reported.[20]

thiazolyl thiourea compounds would fit the target NNRTI binding pocket on HIV-RT much better than their enantiomers. Unfavorable interactions with the NNRTI binding pocket residues near the Y181 side chain would impair the binding of the *S* stereoisomers in their lower energy 'staggered' conformation. This steric hindrance could be relieved if an *S* stereoisomer would adopt an energetically unfavorable 'eclipsed' conformation. In either case, the estimated binding energies would be significantly higher for *S* compounds compared to *R* isomers. Figure 1 shows a representative picture of a phenylethyl pyridylthiourea molecule docked into the NNRTI binding site. Substitutions at the *R* and *S* positions would lead to different conformations of the phenethyl group in the Wing 2 region. The *R* stereoisomer is energetically more favored than the *S* stereoisomer, consistent with the better-fit and thus stronger binding with the NNRTI binding pocket. However, the two-carbon linker between the phenyl group and the thiourea group is adjustable and more forgiving. The energetic difference is minimized by adopting alternative conformation to maintain the snugly fit with the binding pocket for both *R* and *S* stereoisomers, in comparison with those of the compounds with one carbon spacing. The compounds with one-carbon spacing are more rigid and their *S* stereoisomers have to overcome greater energetic barrier to be accommodated by the binding pocket. Based on this rationale we expect that *R* stereoisomers of β-methyl phenylethyl halopyridylthioureas may show much more potency compared to *S* stereoisomers against reverse transcriptase, which is consistent with our experimental results. It is interesting to note that aza analogs of chiral 2-phosphonomethoxy (propyl]guanines of *R* stereoisomeric configuration were found to show potent anti-HIV activity as compared to the *S* isomers[19] a result, which is consistent with our experimental data on chiral thiourea compounds.

Based on the results obtained in the case of β-MPT compounds from molecular modeling studies, we rationalize that a similar trend is anticipated for α-MBT compounds, which differ only by a carbon atom, which can fit into the NNRTI binding pocket as discussed in the previous paragraphs. The experimental data also provide evidence that *R* stereoisomers of α-MBT compounds were active as compared to their enantiomers.

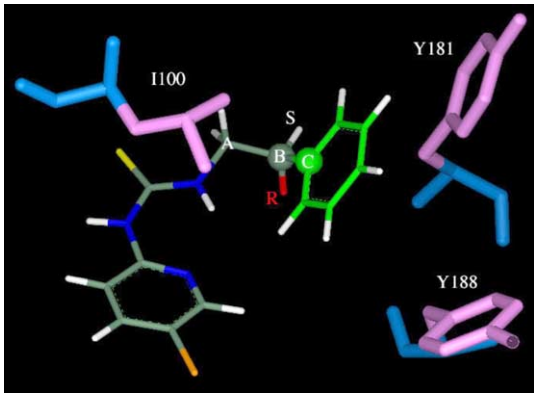


Figure 1. Molecular model of a phenethyl pyridyl thiourea molecule that is docked into the NNRTI binding pocket. Green background indicates the phenyl group attached through a two carbon linker marked as B and A in the figure. The chiral center in the molecule is labeled as 'B'. The pyridyl group is represented on the left hand side of the figure and is connected through thiourea unit.

3. Experimental

All chemicals were purchased from Aldrich (Milwaukee, WI) and were used without further purification. Unless otherwise noted, each reaction vessel was secured with a rubber septa, and the reaction was performed under nitrogen atmosphere. ^{1}H, ^{13}C NMR were obtained on a Varian Mercury 300 instrument at ambient temperature in DMSO-d_6 Chemical shifts are reported as δ values in parts per million down field from tetramethylsilane ($\delta = 0$ ppm) as an internal standard or from the residual dimethylsulfoxide signal ($\delta = 2.49$ ppm for ^{1}H NMR or $\delta = 39.7$ ppm for ^{13}C NMR). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. FT-IR spectra were recorded on a Nicolet Protege 460 spectrometer. Mass spectra were performed on a Hewlett Packard MALDI-TOF spectrometer (Model G2025A LD-TOF). Melting points were determined using a Melt John's apparatus and are uncorrected. HPLC's were done using a Hewlett Packard 1100 series instrument consisting of a automatic sampler, a electronic degasser, a thermostatic control unit, a diode array detector in conjunction with a chemstation software assembly.[22,23] The column used was an analytical RP-18 Lichrosphere column, 5u (4.6×150 mm) and eluent was 35:65, H_2O (0.1% ACOH): ACN. The flow rate was maintained at 1.0 mL/min and the detection wavelength was set at 275 nm. The column was maintained at room temperature throughout the analysis. Column chromatography was performed using silica gel obtained from J.T. Baker Company. The solvents used for elution varied depending on the compound and included either one or a combination of the following: ethylacetate, methanol, chloroform, hexane, methylene chloride, THF, and ether. The optical rotation of the thioureas were determined using a polarimeter. However no effort was made to determine the optical purity of these chiral thiourea derivatives. Elemental analysis was performed at Atlanta Micro Labs, for all the novel thiourea compounds.

Compounds were made by condensing the respective chiral amines and thiocarbonylimidazole derivatives of 5 and 6′-substituted amino pyridines in anhydrous dimethylformamide (Scheme 1). The chiral amines were purchased from Aldrich Chemical Company and were used without further purification. Thiazolyl and benzothiazolyl substituted thioureas were prepared in a similar fashion using thiocarbaimidazole derivatives of either substituted amino thiazoles or benzothiazoles, respectively.

In brief, Scheme 1 involved the addition of thiocarbonyldimidazole and 2-amino-5-halo pyridine to

100 mL of dry acetonitrile under nitrogen atmosphere. The contents were stirred at room temperature for 12–15 h. The precipitate obtained was filtered, washed with cold acetonitrile, and dried thoroughly under vacuum to yield the thiocarbonyl intermediate. In the subsequent step, the intermediates was taken up in a dry flask under nitrogen, 50 mL of anhydrous dimethylformamide was added, and the contents were stirred for 30 min at room temperature. To this mixture, appropriate chiral amines dissolved in 10 mL of dry dimethylformamide was added and the reaction mixture was heated to 110 °C over an oil bath for 15 h. After this period, the reaction mixture was cooled to room temperature and poured into crushed ice/water mixture and the contents were stirred for an additional hour. The precipitate was filtered, washed with cold water, and dried under vacuum. The dried precipitate was dissolved in chloroform and washed with brine, water, and the organic layer was dried over anhydrous magnesium sulfate. Filtration and evaporation of the solvent yielded the target thiourea compound. Compounds were further purified using silica gel column chromatography and finally recrystallized using ethanol as solvent. A similar procedure was followed for other substituted compounds using either thiazolyl or benzothiazolyl precursors.

Stock $HTLV_{IIIB}$ virus: The HIV-1 strain $HTLV_{IIIB}$, which was propagated in CCRF-CEM cells, was used in in vitro assays of the anti-HIV-1 activity of the synthesized thiourea derivatives. Cell-free supernatants of $HTLV_{IIIB}$-infected CCRF-CEM cells were harvested, dispensed into 1 mL aliquots, and frozen at −70 °C. Periodic titration of stock virus was performed by examining its cytopathic effects in MT-2 cells.[20,21] The HIV-1 strains HTLVIIIB (wild-type RT, NRTI-sensitive, NNRTI-sensitive), A17 (Y181C mutant, NNRTI-resistant), A17-variant (Y181C+K103N mutant, NNRTI-resistant), and RT-MDR (M41L, V106N, Y181C, T215Y; NRTI-resistant, NNRTI-resistant) were also obtained from the AIDS Research and Reference Reagent Program, NIAID.

3.1. In vitro assays of anti-HIV-1 activity

Normal human peripheral blood mononuclear cells (PBMC) from HIV-negative donors were cultured 72 h in RPMI 1640 supplemented with 20% (v/v) heat-inactivated fetal bovine serum (FBS), 3% interleukin-2, 2 mM L-glutamine, 25 mM HEPES, 2 g/L $NaHCO_3$, 50 μg/mL gentamicin, and 4 μg/mL phytohemagglutinin prior to exposure to HIV-1 at a multiplicity of infection (MOI) of 0.1 during a 1 h adsorption period at 37 °C in a humidified 5% CO_2 atmosphere. Subsequently, cells were cultured in 96-well microtiter plates (100 μL/well; 2×10^6 cells/mL) in the presence of various concentrations of thiourea analogues or nevirapine, trovirdine or AZT and aliquots of culture supernatants were removed from the wells on the seventh day after infection for p24 antigen assays, as previously described.[20,21] The applied p24 enzyme immunoassay (EIA) was the unmodified kinetic assay commercially available from Coulter Corporation/Immunotech, Inc. (Westbrooke, ME), which utilizes a murine mAb to HIV core protein coated onto microwell strips to which the antigen present in the test culture supernatant samples binds. Percent viral inhibition was calculated by comparing the p24 values from untreated infected cells (i.e., virus controls). In addition, the IC_{50} values measuring the activity of compounds against recombinant HIV-1 RT (rRT) were determined using the Quant-RT assay system (Amersham, Arlington Heights, Il, USA), which utilizes the scintillation proximity assay principle.[20,21]

3.2. Physical constants of compounds

3.2.1. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(5-bromopyridyl)]thiourea (1). Melting point 152–153 °C; ^{1}H NMR (DMSO-d_6) δ 11.14 (s, 1H), 10.68 (s, 1H), 7.97 (s, 1H), 7.91 (m, 1H), 7.32 (s, 4H), 7.23 (m, 1H), 7.07 (d, 1H, $J = 8.7$ Hz), 3.87 (m, 1H), 3.67 (m, 1H), 3.12 (m, 1H), 1.26 (d, 3H, $J = 5.1$ Hz); ^{13}C NMR (DMSO-d_6) δ 179.6, 152.9, 146.1, 144.7, 141.9, 129.1, 127.9, 127.2, 115.1, 112.4, 52.7, 20.1; IR ν 3519, 3222, 3027, 2962, 1592, 1548, 1475, 1309, 1176, 823 cm^{-1}; $[\alpha]_D^t$ +4.5 ($CHCl_3$); MALDI-TOF m/z 352.1 ($C_{15}H_{16}BrN_3S$+2H$^+$); HPLC R_t: 3.64 min. Anal. Calcd for $C_{15}H_{16}BrN_3S$: C, 51.44; H, 4.60; N, 12.00. Found: C, 49.62; H, 4.30; N, 12.16.

3.2.2. *N*-[(2*S*)-β-Methylphenylethyl]-*N′*-[2-(5-bromopyridyl)thiourea (2). Melting point 157–158 °C; ^{1}H NMR (DMSO-d_6) δ 11.13 (t, 1H), 10.67 (s, 1H), 7.98 (d, 1H, $J = 2.7$ Hz), 7.92 (dd, 1H, $J = 2.4$, 8.7 Hz), 7.32 (s, 4H), 7.24 (m, 1H), 7.07 (d, 1H, $J = 8.7$ Hz), 3.85 (m, 1H), 3.68 (m, 1H), 3.11 (m, 1H), 1.26 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR (DMSO-d_6) δ 179.0, 152.3, 145.5, 144.1, 141.4, 128.5, 127.3, 126.2, 114.5, 111.7, 52.1, 19.5; IR ν 3441, 3226, 3024, 2968, 1597, 1541, 1472, 1304, 1175, 827 cm^{-1}; $[\alpha]_D^t$ −4.4 ($CHCl_3$); MALDI-TOF m/z 352.7 ($C_{15}H_{16}BrN_3S$+2H$^+$); HPLC R_t: 3.64 min. Anal. Calcd for $C_{15}H_{16}BrN_3S$: C, 51.44; H, 4.60; N, 12.00. Found: C, 51.67; H, 4.63; N, 11.86.

3.2.3. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(5-chloropyridyl)]thiourea (3). Melting point 167–169 °C; ^{1}H NMR (DMSO-d_6) δ 11.14 (s. 1H), 10.67 (s, 1H), 7.90 (s, 1H), 7.80 (m, 1H), 7.32 (s, 4H), 7.23 (m, 1H), 7.12 (m, 1H), 3.86 (m, 1H), 3.67 (m, 1H), 3.10 (m, 1H), 1.23 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 179.1, 152.1, 144.2, 143.3, 138.9, 128.6, 127.3, 126.6, 123.7, 114.1, 52.1, 38.5, 19.5; IR ν 3507, 3220, 3027, 2966, 1600, 1533, 1477, 1311, 1230, 1178, 1109, 825, 698 cm^{-1}; $[\alpha]_D^t$ +3.1 ($CHCl_3$); MALDI-TOF m/z 312.4 ($C_{15}H_{16}ClN_3S$+4H$^+$); HPLC R_t: 11.20 min. Anal. Calcd for $C_{15}H_{16}ClN_3S$: C, 58.91; H, 5,27; N, 13.74. Found: C, 56.81; H, 4.90; N, 13.74.

3.2.4. *N*-[(2*S*)-β-Methylphenylethyl]-*N′*-[2-(5-chloropyridyl)]thiourea (4). Melting point 171–173 °C; ^{1}H NMR (DMSO-d_6) δ 11.14 (s. 1H), 10.67 (s, 1H), 7.91 (d, 1H, $J = 2.4$ Hz), 7.81 (dd, 1H, $J = 2.4$, 9 Hz), 7.32 (s, 4H), 7.23 (m, 1H), 7.12 (d, 1H, $J = 9.3$ Hz), 3.85 (m, 1H), 3.67 (m, 1H), 3.11 (q, 1H), 1.26 (d, 3H, $J = 6.9$ Hz); ^{13}C

NMR (DMSO-d_6) δ 179.1, 152.1, 144.2, 143.3, 138.9, 128.6, 127.3, 126.6, 123.7, 114.1, 52.1, 38.5, 19.5; IR ν 3500, 3220, 3027, 2966, 1598, 1556, 1477, 1311, 1230, 1178 cm^{-1}; $[\alpha]_D^t$ −3.1 ($CHCl_3$); MALDI-TOF m/z 307.4 ($C_{15}H_{16}ClN_3S$); HPLC R_t: 11.20 min. Anal. Calcd for $C_{15}H_{16}ClN_3S$: C, 58.91; H, 5.27; N, 13.74. Found: C, 59.07; H, 5.28; N, 13.74.

3.2.5. *N*-[(2*R*)-β-Methylphenylethyl]-*N*′-[2-(5-methylpyridyl)]thiourea (5). Melting point 131–132 °C; ^{1}H NMR (DMSO-d_6) δ 11.56 (s, 1H), 10.45 (s, 1H), 7.75 (s, 1H), 7.55 (m, 1H), 7.33 (s, 4H), 7.23 (m, 1H), 7.01 (m, 1H), 3.85 (m, 1H), 3.70 (m, 1 h), 3.10 (m, 1H), 2.17 (s, 3H), 1.27 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 179.1, 151.7, 144.4, 144.2, 139.6, 128.5, 127.2, 126.5, 1112.0, 52.0, 38.6, 19.5, 17.4; IR ν 3519, 3228, 3022, 2968, 1610, 1556, 1533, 1492, 1303, 1188, 702 cm^{-1}; $[\alpha]_D^t$ +2.9 ($CHCl_3$); MALDI-TOF m/z 287.2 ($C_{16}H_{19}N_3S$+2H$^+$); HPLC R_t: 9.72 min. Anal. Calcd for $C_{16}H_{19}N_3S$: C, 67.33; H, 6.71; N, 14.72. Found: C, 67.39; H, 6.64; N, 14.78.

3.2.6. *N*-[(2*S*)-β-Methylphenylethyl]-*N*′-[2-(5-methylpyridyl)]thiourea (6). Melting point 130–131 °C; ^{1}H NMR (DMSO-d_6) δ 11.57 (t, 1H), 10.44 (s, 1H), 7.73 (s, 1H), 7.53 (dd, 1H, J = 2.4, 8.4 Hz), 7.32 (s, 4H), 7.23 (m, 1H), 7.00 (m, 1H), 3.84 (m, 1H), 3.69 (m, 1H), 3.10 (q, 1H), 2.16 (s, 3H), 1.26 (d, 3H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 179.1, 151.7, 144.4, 144.2, 139.6, 128.5, 127.2, 126.5, 112.0, 52.0, 38.6, 19.5, 17.4; IR ν 3506, 3228, 3022, 2968, 1610, 1533, 1492, 1303, 1234, 703 cm^{-1}; $[\alpha]_D^t$ −3.4 ($CHCl_3$); MALDI-TOF m/z 287.1 ($C_{16}H_{19}N_3S$+2H$^+$); HPLC R_t: 9.74 min. Anal. Calcd for $C_{16}H_{19}N_3S$: C, 67.33; H, 6.71; N, 14.72. Found: C, 67.37; H, 6.69; N, 14.87.

3.2.7. *N*-[(2*R*)-β-Methylphenylethyl]-*N*′-[2-(6-methylpyridyl)]thiourea (7). Melting point 125–126 °C; ^{1}H NMR (DMSO-d_6) δ 11.77 (t, 1H), 10.46 (s, 1H), 7.58 (t, 1H), 7.30 (d, 4H, J = 4.2 Hz), 7.21 (m, 1H), 6.89 (d, 1H, J = 8.4 Hz), 6.79 (d, 1H, J = 7.2 Hz), 3.90 (m, 1H), 3.79 (m, 1H), 3.11 (q, 1H), 2.11 (s, 3H), 1.26 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 179.5, 154.3, 153.2, 144.4, 139.1, 128.6, 127.2, 126.1, 116.8, 109.3, 51.4, 38.7, 23.5, 20.2; IR ν 3500, 3228, 3027, 2962, 1608, 1537, 1450, 1236, 788 cm^{-1}; $[\alpha]_D^t$ +2.9 ($CHCl_3$); MALDI-TOF m/z 286.9 ($C_{16}H_{19}N_3S$+2H$^+$); HPLC R_t: 10.13 min.

3.2.8. *N*-[(2*S*)-β-Methylphenylethyl]-*N*′-[2-(6-methylpyridyl)]thiourea (8). Melting point 123–124 °C; ^{1}H NMR (DMSO-d_6) δ 11.76 (s, 1H), 10.46 (s, 1H), 7.56 (t, 1H), 7.29 (d, 4H, J = 4.2 Hz), 7.19 (m, 1H), 6.88 (d, 1H, J = 8.1 Hz), 6.78 (d, 1H, J = 8.1 Hz), 3.89 (m, 1H), 3.78 (m, 1H), 3.11 (q, 1H), 2.10 (s, 3H), 1.25 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 179.5, 154.3, 153.2, 144.4, 139.1, 128.6, 127.1, 126.5, 116.8, 109.3, 51.4, 38.7, 23.5, 20.2; IR ν 3480, 3228, 3027, 29634, 1606, 1548, 1450, 1236, 810 cm^{-1}; $[\alpha]_D^t$ −2.7 ($CHCl_3$); MALDI-TOF m/z 287.1 ($C_{16}H_{19}N_3S$+2H$^+$); HPLC R_t: 10.05 min.

3.2.9. *N*-[(2*R*)-β-Methylphenylethyl]-*N*′-[2-(pyridyl)]thiourea (9). Melting point 130–131 °C; ^{1}H NMR (DMSO-d_6) δ 11.65 (s, 1H), 10.53 (d, 1H, J = 8.7 Hz), 7.90 (q, 1H), 7.70 (q, 1H), 7.32 (m, 4H), 7.22 (m, 1H), 7.09 (t, 1H), 6.95 (m, 1H), 3.88 (m, 1 h), 3.69 (m, 1H), 3.12 (m, 1H), 1.28 (q, 3H); ^{13}C NMR (DMSO-d_6) δ 179.3, 153.7, 145.2, 144.3, 138.9, 128.5, 127.3, 126.6, 117.7, 112.5, 52.1, 38.6, 19.5; IR ν 3463, 3228, 3023, 2966, 1602, 1560, 1540, 1481, 1317, 769 cm^{-1}; $[\alpha]_D^t$ +2.4 ($CHCl_3$); MALDI-TOF m/z 272.9 ($C_{15}H_{17}N_3S$+2H$^+$); HPLC R_t: 7.26 min. Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.13; H, 6.20; N, 15.57.

3.2.10. *N*-[(2*S*)-β-Methylphenylethyl]-*N*′-[2-(pyridyl)]thiourea (10). Melting point 118–119 °C; ^{1}H NMR (DMSO-d_6) δ 11.63 (t, 1H), 10.51 (s, 1H), 7.88 (dd, 1H, J = 1.8, 5.1 Hz), 7.68 (m, 1H), 7.32 (d, 3H, J = 4.5 Hz), 7.21 (m, 2H), 7.07 (d, 1H, J = 8.7 Hz), 6.93 (dd, 1H, J = 5.4, 7.5 Hz), 3.87 (m, 1H), 3.68 (m, 1H), 3.10 (q, 1H), 1.26 (d, 3H, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 179.3, 153.6, 145.1, 144.2, 138.9, 128.5, 128.4, 127.3, 127.1, 126.5, 117.6, 112.4, 52.0, 38.16, 19.5; IR ν 3228, 3170, 3023, 2966, 1602, 1560, 1540, 1450, 1317, 769 cm^{-1}; $[\alpha]_D^t$ −3.1 ($CHCl_3$); MALDI-TOF m/z 292.2 ($C_{15}H_{17}N_3S$+Na-2H$^+$); HPLC R_t: 7.21 min. Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.79; H, 6.34; N, 15.43.

3.2.11. *N*-[(2*R*)-β-Methylphenylethyl]-*N*′-[2-(4-methylthiazolyl)]thiourea (11). Melting point 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 11.49 (s, 1H), 10.04 (s, 1H), 7.31 9s, 4H), 7.22 (s, 1H), 6.61 (s, 1H), 3.79 (m, 1H), 3.69 (m, 1H), 3.09 (t, 1H), 2.09 (s, 3H), 1.25 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 177.8, 161.1, 144.1, 128.5, 127.2, 126.5, 106.2, 51.4, 38.5, 19.7, 16.9; IR ν 3500, 3170, 3025, 2977, 1567, 1531, 1504, 1209, 754, 713 cm^{-1}; $[\alpha]_D^t$ +3.4 ($CHCl_3$); MALDI-TOF m/z 292.9 ($C_{14}H_{17}N_3S_2$+2H$^+$); HPLC R_t: 8.84 min. Anal. Calcd for $C_{14}H_{17}N_3S_2$: C, 57.70; H, 5.88; N, 14.42. Found: C, 57.72; H, 5.84; N, 14.46.

3.2.12. *N*-[(2*S*)-β-Methylphenylethyl]-*N*′-[2-(4-methylthiazolyl)]thiourea (12). Melting point 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 11.49 (s, 1H), 10.03 (s, 1H), 7.30 (s, 4H), 7.22 (m, 1H), 6.60 (s, 1H), 3.79 (m, 1H), 3.68 (m, 1H), 3.08 (q, 1H), 2.09 (s, 3H), 1.24 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 177.6, 160.9, 146.5, 144.1, 128.5, 127.2, 126.5, 106.1, 51.4, 38.5, 19.7, 16.9; IR ν 3430, 3170, 3025, 2919, 1567, 1531, 1504, 1209 cm^{-1}; $[\alpha]_D^t$ −2.5 ($CHCl_3$); MALDI-TOF m/z 293.0 ($C_{14}H_{17}N_3S_2$+2H$^+$); HPLC R_t: 8.87 min.

3.2.13. *N*-[(2*R*)-β-Methylphenylethyl]-*N*′-[2-(4-methylenecarboethoxy thioazolyl)]thiourea (DDE 13). Melting point 106–108 °C; ^{1}H NMR (DMSO-d_6) δ 11.56 (s, 1H), 9.56 (s, 1H), 7.28 (s, 4H), 7.20 (m, 1H), 6.84 (s, 1H), 4.07 (q, 2H), 3.70 (q, 2H), 3.56 (s, 2H), 3.07 (q, 2H), 1.23 (d, 3H, J = 6.9 Hz), 1.17 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 177.5, 169.7, 160.8, 144.1, 143.3, 128.5, 127.1, 126.5,

109.2, 60.5, 51.3, 38.5, 36.5, 19.6, 14.3; IR ν 3448, 3170, 3025, 2977, 1733, 1562, 1506, 1182, 696 cm^{-1}; $[\alpha]_D^t$ +2.5 ($CHCl_3$); MALDI-TOF m/z 365.3 ($C_{17}H_{21}N_3O_2S_2$+ $2H^+$); HPLC R_t: 7.09 min.

3.2.14. *N*-[(2*S*)-β-Methylphenylethyl]-*N′*-[2-(4-methylenecarboethoxy thioazolyl)]thiourea (DDE 14). Melting point 104–106 °C; ^1H NMR (DMSO-d_6) δ 11.57 (s, 1H), 9.56 (s, 1H), 7.28 (m, 4H), 7.21 (m, 1H), 6.84 (s, 1H), 4.07 (q, 2H), 3.71 (q, 2H), 3.56 (s, 2H), 3.07 (q, 1H), 1.24 (d, 3H, J = 6.9 Hz), 1.17 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 177.5, 169.7, 160.8, 144.1, 143.3, 128.5, 127.1, 126.5, 109.2, 60.5, 51.3, 38.5, 36.5, 19.6, 14.3; IR ν 3448, 3172, 3025, 2977, 1733, 1562, 1506, 1182, 696 cm^{-1}; $[\alpha]_D^t$ −2.5 ($CHCl_3$); MALDI-TOF m/z 365.1 ($C_{17}H_{21}N_3O_2S_2$+ $2H^+$); HPLC R_t: 7.10 min.

3.2.15. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(thioazolyl)]-thiourea (15). ^1H NMR (DMSO-d_6) δ 11.55 (s, 1H), 9.65 (s, 1H), 7.29 (t, 5H), 7.21 (m, 1H), 7.06 (t, 1H), 3.71 (m, 2), 3.11 (q, 1H), 1.23 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 177.9, 161.7, 144.1, 136.8, 128.5, 127.2, 126.5, 112.1, 51.4, 38.4, 19.5; IR ν 3178, 3023, 2950, 1558, 1508, 1467, 1178, 698 cm^{-1}; $[\alpha]_D^t$ +3.8 ($CHCl_3$); MALDI-TOF m/z 280.1 ($C_{13}H_{15}N_3S_2$+$2H^+$); HPLC R_t: 6.23 min.

3.2.16. *N*-[(2*S*)-β-Methylphenylethyl]-*N′*-[2-(thioazolyl)]-thiourea (16). Melting point 128–130 °C; ^1H NMR (DMSO-d_6) δ 11.56 (s, 1H), 9.66 (s, 1H), 7.27 (t, 4H), 7.21 (m, 2H), 7.04 (d, 1H, J = 3.6 Hz), 3.71 (m, 2H), 3.10 (q, 1H), 1.23 (d, 3H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 177.9, 161.7, 144.0, 136.8, 128.5, 127.2, 126.5, 112.1, 51.4, 38.4, 19.4; IR ν 3444, 3174, 3025, 1576, 1515, 1176, 678 cm^{-1}; $[\alpha]_D^t$ −4.0 ($CHCl_3$); MALDI-TOF m/z 280.3 ($C_{13}H_{15}N_3S_2$+$2H^+$); HPLC R_t: 6.52 min.

3.2.17. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(4-methyl benzothiazolyl)]thiourea (17). Melting point 203–205 °C; ^1H NMR (DMSO-d_6) δ 11.87 (s, 1H), 10.16 (s, 1H), 7.67 (d, 1H, J = 7.2 Hz), 7.27 (d, 4H, J = 4.2 Hz), 7.18 (dd, 1H, J = 3.9, 12.6 Hz), 7.13 (s, 1H), 7.11 (s, 1H), 3.81 (t, 2H), 3.14 (q, 1H), 2.34 (s, 3H), 1.26 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 177.9, 159.8, 147.8, 144.0, 129.7, 129.2, 128.5, 127.1, 126.7, 126.5, 123.7, 118.9, 51.2, 38.4, 19.9, 17.9; IR ν 3443, 3172, 3027, 2966, 1556, 1521, 1216, 696 cm^{-1}; MALDI-TOF m/z 343.3 ($C_{18}H_{19}N_3S_2$+$2H^+$); HPLC R_t: 18.99 min.

3.2.18. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(benzothiazolyl)]thiourea (18). Melting point 181–183 °C; ^1H NMR (DMSO-d_6) δ 11.86 (s, 1H), 10.14 (s, 1H), 7.85 (d, 1H, J = 7.8 Hz), 7.47 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 7.2 Hz), 7.32 (d, 4H, J = 4.5 Hz), 7.22 (m, 2H), 3.86 (m, 1H), 3.72 (m, 1H), 3.15 (q, 1H), 1.26 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 178.6, 161.1, 148.2, 129.7, 128.6, 127.2, 126.6, 126.2, 123.7, 121.7, 119.5, 51.6, 38.4, 19.7; IR ν 3444, 3160, 3025, 2958, 1556, 1525, 1195, 754 cm^{-1}; $[\alpha]_D^t$ +2.5 ($CHCl_3$); MALDI-TOF m/z 329.2 ($C_{17}H_{17}N_3S_2$+$2H^+$); HPLC R_t: 12.66 min.

3.2.19. *N*-[(2*R*)-β-Methylphenylethyl]-*N′*-[2-(4,6-dimethylpyridyl)]thiourea (19). Melting point 123–124 °C; ^1H NMR (DMSO-d_6) δ 12.60 (d, 1H, J = 8.1 Hz), 10.46 (s, 1H), 7.36 (m, 4H), 7.28 (m, 1H), 6.76 (d, 2H, J = 5.1 Hz), 5.47 (m, 1H), 2.33 (s, 3H), 2.21 (s, 3H), 1.53 (d, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 178.6, 153.8, 153.5, 150.1, 143.3, 128.6, 127.2, 126.2, 118.2, 109.5,. 54.2, 23.4, 22.9, 21.0; IR ν 3484, 3224, 3029, 2964, 1623, 1592, 1540, 1211, 698 cm^{-1}; HPLC R_t: 13.46 min.

3.2.20. *N*-[1-(1*R*)-α-Methylbenzyl]-*N′*-[2-(5-bromopyridyl)]thiourea (20). Melting point 170–172 °C; ^1H NMR (DMSO-d_6) δ 11.79 (m, 1H), 10.76 (s, 1H), 8.39 (d, 1H, J = 2.1 Hz), 7.98 (dd, 1H, J = 9.0, 9.0 Hz), 7.35 (d, 4H, J = 3.9 Hz), 7.28–7.23 (m, 1H), 7.15 (d, 1H, J = 9 Hz), 5.55 (q, 1H), 1.51 (m, 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; IR ν 3245, 3027, 2979, 2925, 1594, 1525, 1475, 1188, 704 cm^{-1}; UV (MeOH) λ_{max} 211, 257, 275, 278 nm; $[\alpha]_D^t$ −21.5 ($CHCl_3$); MALDI-TOF MS m/z 337.7 ($C_{14}H_{14}BrN_3S$+H^+). Anal. Calcd for $C_{14}H_{14}BrN_3S$: C, 50.01; H, 4.20; N, 12.50. Found: C, 50.27; H, 4.16; N, 12.53.

3.2.21. *N*-[1-(1-(1*R*)-α-Methylbenzyl]-*N′*-[2-(5-chloropyridiyl)]thiourea (21). Melting point 185–187.5 °C; ^1H NMR ($CDCl_3$) δ 11.77 (m, 1H), 9.47 (s, 1H), 8.12 (d, 1H, J = 2.1 Hz), 7.53 (d, 1H, J = 8.7 Hz), 7.40–7.26 (m, H), 6.85 (d, 1H, J = 8.7 Hz), 5.73–5.64 (m, 1H), 1.65 (d, 3H, J = 6.9 Hz); ^{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; IR ν 3247, 3169, 3087, 2978, 1600, 1529, 1483, 1189, 1034, 822, 761, 694 cm^{-1}; $[\alpha]_D^t$ −20.9 ($CHCl_3$); UV (MeOH) λ_{max} 204, 255, 275, 305 nm; MALDI-TOF MS m/z 293.7 ($C_{14}H_{14}ClN_3S$+$2H^+$). Anal. Calcd for $C_{14}H_{14}ClN_3S$: C, 57.63; H, 4.84; N, 14.40. Found: C, 57.71; H, 4.84; N, 14.38.

3.2.22. *N*-[1-(1*S*)-α-Methylbenzyl]-*N′*-[2-(5-bromopyridyl)]thiourea (22). Melting point 170–172 °C; ^1H NMR ($CDCl_3$) δ 11.73 (m, 1H), 8.95 (s, 1H), 8.23 (d, 1H, J = 2.1 Hz), 7.69 (d, 1H, J = 8.7 Hz), 7.40–7.28 (m, 5H), 6.73 (d, 1H, J = 8.7 Hz), 5.72–5.63 (m, 1H), 1.65 (d, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 178.8, 151.9, 146.8, 142.8, 141.5, 128.9, 127.5, 126.4, 113.8, 113.0, 55.4, 22.6; IR ν 3461, 3243, 3089, 2979, 1594, 1525, 1473, 1186, 825, 703 cm^{-1}; $[\alpha]_D^t$ +21.3 ($CHCl_3$); UV (MeOH) λ_{max} 209, 257, 276, 306 nm; MALDI-TOF MS m/z 337.0 ($C_{14}H_{14}BrN_3S$+H^+). Anal. Calcd for $C_{14}H_{14}ClN_3S$: C, 57.63; H, 4.84; N, 14.40. Found: C, 57.69; H, 4.80; N, 14.24.

3.2.23. *N*-[1-(1*S*)-α-Methylbenzyl]-*N′*-[2-(5-chloropyridyl)]thiourea (23). Melting point 185–187 °C; ^1H NMR ($CDCl_3$) δ 11.75 (m, 1H), 9.12 (s, 1H), 8.13 (d,

1H, $J = 2.4$), 7.55 (d, 1H, $J = 8.7$ Hz), 7.41–7.25 (m, 6H), 6.79 (d, 1H, $J = 8.7$ Hz), 5.73–5.63 (m, 1H), 1.65 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR ($CDCl_3$) 178.5, 151.7, 144.5, 142.8, 138.9, 128.9, 127.5, 126.4, 125.4, 113.4, 55.3, 22.6; IR ν 3236, 2973, 1601, 1524, 1483, 1189, 828, 761 cm^{-1}; IR ν 3461, 3243, 3089, 2979, 1594, 1525, 1473, 1186, 825, 703 cm^{-1}; $[\alpha]_D^t$ +21.6 ($CHCl_3$); UV (MeOH) λ_{max} 207, 256, 276 nm; MALDI-TOF MS m/z 293.8 ($C_{14}H_{14}ClN_3S$+2H$^+$).

3.2.24. *N*-[1-(1*R*)-α-Methylbenzyl]-*N*′-[2-(4-methylthiazolyl)]thiourea (24). Melting point 120–121 °C; ^{1}H NMR (DMSO-d_6) δ 11.50 (s, 1H), 10.24 (s, 1H), 7.35 (m, 4H), 7.26 (m, 1H), 6.63 (s, 1H), 5.44 (m, 1H), 2.21 (s, 3H), 1.49 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 177.3, 143.2, 128.6, 127.1, 126.1, 106.2, 53.6, 22.5, 16.7; IR ν 3170, 3014, 2961, 1579, 1504, 1207, 700 cm^{-1}; $[\alpha]_D^t$ −8.4 ($CHCl_3$); UV (MeOH) λ_{max} 210, 259, 293 nm; MALDI-TOF MS m/z 279.1 ($C_{13}H_{15}N_3S_2$+2H$^+$). Anal. Calcd for $C_{13}H_{15}BrN_3S_2$: C, 56.29; H, 5.45; N, 15.15. Found: C, 56.33; H, 5.41; N, 15.24.

3.2.25. *N*-[1-(1*S*)-α-Methylbenzyl]-*N*′-[2-(4-methylthiazolyl)]thiourea (25). Melting point 120–121 °C; ^{1}H NMR (DMSO-d_6) δ 11.50 (s, 1H), 10.23 (s, 1H), 7.34 (m, 4H), 7.26 (m, 1H), 6.63 (s, 1H), 5.44 (m, 1H), 2.21 (s, 3H), 1.48 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 177.6, 157.5, 144.9, 143.2, 128.6, 127.1, 126.1, 106.2, 53.7, 22.5, 16.7; IR ν 3170, 3014, 2919, 1589, 1502, 1207, 700 cm^{-1}; $[\alpha]_D^t$ +8.8 ($CHCl_3$); UV (MeOH) λ_{max} 207, 212, 260, 294 nm; MALDI-TOF MS m/z 279.4 ($C_{13}H_{15}N_3S_2$+2H$^+$).

3.2.26. *N*-[1-(1*S*)-α-Methylbenzyl]-*N*′-[2-(6-methylpyridyl)]thiourea (26). Melting point 134–135 °C; ^{1}H NMR (DMSO-d_6) δ 12.52 (d, 1H, $J = 7.8$ Hz), 10.52 (s, 1H), 7.61 (t, 1H), 7.40–7.31 (m, 4H), 7.27–7.25 (m, 1H), 6.93 (d, 1H, $J = 8.4$ Hz), 6.84 (d, 1H, $J = 7.2$ Hz), 5.51–5.42 (m, 1H), 2.34 (s, 3H), 1.52 (d, 3H, $J = 6.6$ Hz); ^{13}C NMR (DMSO-d_6) δ 178.6, 154.3, 153.5, 143.4, 139.4, 128.8, 127.3, 126.3, 117.1, 109.6, 54.4, 23.7, 22.9; IR ν 3193, 3031, 2923, 1604, 1535, 1450, 1226, 1195, 786 cm^{-1}; $[\alpha]_D^t$ +14.6 ($CHCl_3$); MALDI-TOF m/z 272.9 ($C_{15}H_{17}N_3S$+2H$^+$). Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.39, 66.27; H, 6.25, 6.36; N, 15.55, 15.44.

3.2.27. *N*-[1-(1*R*)-α-Methylbenzyl]-*N*′-[2-(5-methylpyridyl)]thiourea (27). Melting point 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 12.21 (d, 1H, $J = 7.8$ Hz), 10.55 (s, 1H), 8.04 (s, 1H), 7.57 (dd, 1H, $J = 8.4$, 8.7 Hz), 7.34 (t, 4H), 7.26–7.22 (m, 1H), 7.09 (d, 1H, $J = 8.4$ Hz), 5.62–5.52 (m, 1H), 2.18 (s, 3H), 1.51 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR (DMSO-d_6) δ 179.1, 152.6, 145.5, 144.0, 140.4, 129.2, 127.6, 127.4, 126.7, 112.9, 54.4, 23.4, 18.0; IR ν 3317, 3139, 2954, 1558, 1488, 1180, 640 cm^{-1}; $[\alpha]_D^t$ −19.6 ($CHCl_3$); MALDI-TOF m/z 273.2 ($C_{15}H_{17}N_3S$+2H$^+$). Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.38; H, 6.30; N, 15.48.

3.2.28. *N*-[1-(1*R*)-α-Methylbenzyl]-*N*′-[2-(4-methylbenzothiazolyl)]thiourea (28). Melting point 166–171 °C; ^{1}H NMR (DMSO-d_6) δ 11.88 (s, 1H), 10.92 (s, 1H), 7.70 (m, 1H), 7.42–7.34 (m, 5H), 7.30–7.26 (m, 1H), 7.18–7.14 (m, 1H), 5.51–5.39 (m, 1H), 2.45 (m, 3H), 1.56 (m, 3H); ^{13}C NMR (DMSO-d_6) 177.1, 160.4, 147.8, 142.8, 129.6, 129.2, 128.8, 127.5, 127.0, 126.3, 124.0, 119.3, 54.2, 22.5, 18.1; IR ν 3170, 3023, 1565, 1527, 1203, 740, 702 cm^{-1}; MALDI-TOF m/z 329.4 ($C_{17}H_{17}N_3S_2$+2H$^+$).

3.2.29. *N*-[1-(1*S*)-α-Methylbenzyl]-*N*′-[2-(6-methoxybenzothiazolyl)]thiourea (29). Melting point 181–182 °C; ^{1}H NMR (DMSO-d_6) δ 11.74 (s, 1H), 10.42 (s, 1H), 7.55 (d, 1H, $J = 9$ Hz), 7.50 (s, 1H), 7.40–7.33 (m, 4H), 7.25 (t, 1H), 6.98 (dd, 1H, $J = 1.5$, 8.7 Hz), 5.54–5.46 (m, 1H), 1.54 (m, 3H); ^{13}C NMR (DMSO-d_6) 177.5, 159.6, 156.2, 149.0, 131.1, 128.6, 128.3, 127.2, 126.2, 120.1, 114.6, 105.2, 55.8, 53.8, 22.4; IR ν 3170, 3031, 1558, 1527, 1465, 1218, 694 cm^{-1}; $[\alpha]_D^t$ +18.9 ($CHCl_3$); MALDI-TOF m/z 344.8 ($C_{17}H_{17}N_3OS_2$+2H$^+$). Anal. Calcd for $C_{17}H_{17}N_3OS_2$: C, 59.45; H, 4.99; N, 12.23. Found: C, 59.42; H, 4.95; N, 12.20.

3.2.30. *N*-[1-(1*S*)-α-Methylbenzyl]-*N*′-[2-(5-methylpyridyl)]thiourea (30). Melting point 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 12.21 (d, 1H), 10.54 (s, 1H), 8.04 (t, 1H), 7.58 (dd, 1H, $J = 2.1$, 8.4 Hz), 7.35 (d, 4H, $J = 3.6$ Hz), 7.27–7.23 (m, 1H), 7.08 (d, 1H, $J = 8.7$ Hz), 5.61–5.52 (m, 1H), 2.19 (s, 3H), 1.51 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 178.5, 152.0, 144.9, 143.4, 139.8, 128.6, 127.0, 126.8, 126.0, 112.3, 53.8, 22.8, 17.4; IR ν 3317, 3139, 2977, 1589, 1558, 1403, 1180, 825 cm^{-1}; $[\alpha]_D^t$ +19.7 ($CHCl_3$); MALDI-TOF m/z 273.4 ($C_{15}H_{17}N_3S$+2H$^+$). Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.56; H, 6.27; N, 15.49.

3.2.31. *N*-[1-(1*R*)-α-Methylbenzyl]-*N*′-[2-(6-methylpyridyl)]thiourea (31). Melting point 136–137 °C; ^{1}H NMR (DMSO-d_6) δ 12.52 (m, 1H), 10.55 (s, 1H), 4.61 (t, 1H), 7.41–7.33 (m, 4H), 7.28–7.25 (m, 1H), 6.95 (d, 1H, $J = 8.1$ Hz), 6.84 (d, 1H, $J = 7.2$ Hz), 5.53–5.44 (m, 1H), 2.35 (s, 3H), 1.54 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR (DMSO-d_6) δ 178.5, 154.2, 153.4, 143.3, 139.3, 128.6, 127.2, 126.2, 116.9, 109.5, 54.2, 23.6, 22.8; IR ν 3193, 3031, 2923, 1604, 1565, 1535, 1450, 1226, 1195, 786 cm^{-1}; $[\alpha]_D^t$ −16.1 ($CHCl_3$); MALDI-TOF m/z 273.1 ($C_{15}H_{17}N_3S$+2H$^+$). Anal. Calcd for $C_{15}H_{17}N_3S$: C, 66.39; H, 6.31; N, 15.48. Found: C, 66.41; H, 6.27; N, 15.52.

3.2.32. *N*-[1-(1*R*)-α-Methylbenzyl]-*N*′-[2-(6-methoxybenzothiazolyl)]thiourea (32). Melting point 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 11.71 (s, 1H), 10.41 (s, 1H), 7.55 (d, 1H, $J = 8.7$ Hz), 7.50 (s, 1H), 7.38 (s, 4H), 7.27 (d, 1H, $J = 5.7$ Hz), 6.79 (d, 1H, $J = 8.7$ Hz), 5.49 (t, 1H), 3.77 (s, 3H), 1.54 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 177.4, 159.6, 156.2, 143.0, 131.1, 128.6, 127.2, 126.2, 120.2, 114.6, 105.3, 55.8, 53.8, 22.4; IR ν 3170, 3031, 1565, 1527, 1465, 1218, 694 cm^{-1}; $[\alpha]_D^t$ −16.4 ($CHCl_3$);

MALDI-TOF m/z 345.4 ($C_{17}H_{17}N_3OS_2+H^+$). Anal. Calcd for $C_{17}H_{17}N_3OS_2$: C, 59.45; H, 4.99; N, 12.23. Found: C, 59.51; H, 4.95; N, 12.24.

4. Conclusion

In summary, our data provide unprecedented evidence that the stereochemistry is a key determinant of thiourea compounds as NNRTI. Molecular modeling studies indicated that the two carbon linker between the phenyl group and the thiourea groups is adjustable and more forgiving. In addition, the energetic difference is minimized by adopting alternative conformation to maintain the snugly fit with the binding pocket. Furthermore, we have identified β-methyl phenyl ethyl thioureas as a promising new class of NNRTI with potent anti-HIV activity.

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