Phenethylthiazolethiourea (PETT) Compounds, a New Class of HIV-1 Reverse Transcriptase Inhibitors. 1. Synthesis and Basic Structure–Activity Relationship Studies of PETT Analogs

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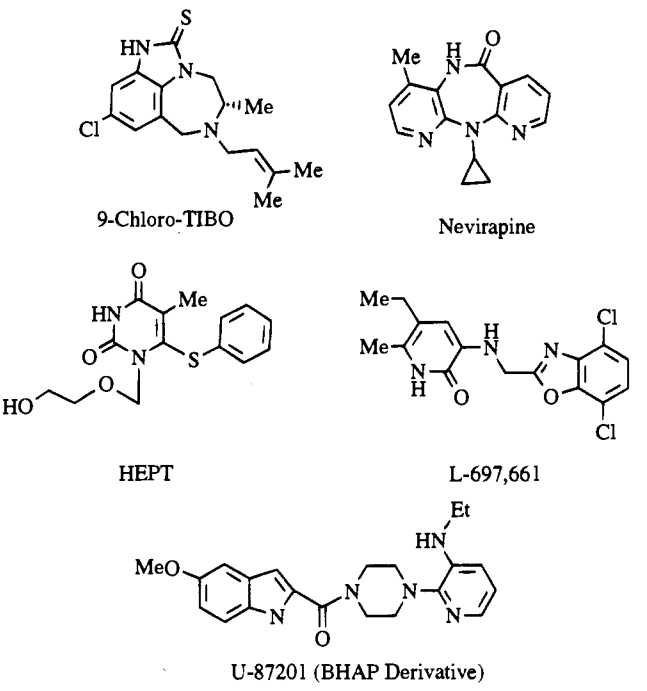
A novel series of potent specific HIV-1 inhibitory compounds is described. The lead compound in the series, *N*-(2-phenethyl)-*N'*-(2-thiazolyl)thiourea (**1**), inhibits HIV-1 RT using rCdG as the template with an IC_{50} of 0.9 μM. In MT-4 cells, compound **1** inhibits HIV-1 with an ED_{50} of 1.3 μM. The 50% cytotoxic dose in cell culture is >380 μM. The chemical structure–activity relationship (SAR) was developed by notionally dividing the lead compound in four quadrants. The SAR strategy had two phases. The first phase involved optimization of antiviral activity through independent variation of quadrants 1–4. The second phase involved the preparation of hybrid structures combining the best of these substituents. Further SAR studies and pharmacokinetic considerations led to the identification of *N*-(2-pyridyl)-*N'*-(5-bromo-2-pyridyl)-thiourea (**62**; LY300046·HCl) as a candidate for clinical evaluation. LY300046·HCl inhibits HIV-1 RT with an IC_{50} of 15 nM and in cell culture has an ED_{50} of 20 nM.

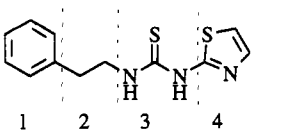
The discovery of the human immunodeficiency virus (HIV) as the causative agent of AIDS has led to enormous efforts to unravel the basic action of the virus at a molecular level. From these efforts, a variety of targets for potential intervention of HIV multiplication have been identified.[1] An essential step for the multiplication and spread of the virus involves reverse transcription of the retroviral RNA to proviral DNA by the enzyme reverse transcriptase (RT).[2] All of the currently approved agents for AIDS are nucleoside analogs inhibiting this vital enzyme.[3] As nucleoside derivatives, these agents must first be transformed by cellular kinases into their corresponding triphosphates in order to exhibit antiviral activity. Mechanistically, these derivatives act as chain terminators and/or competitive inhibitors of HIV-RT, having activity against both HIV-1 and HIV-2.[4] Unfortunately, like many other antiviral agents, resistance development has been problematic with these derivatives.[5] Other severe drawbacks include limitations in efficacy and toxicity.[3] Obviously there is a great need for improved chemotherapy against HIV/AIDS.

Recently, a number of new types of nonnucleoside inhibitors of HIV-1 RT have been reported. In general, these analogs are selective inhibitors of HIV-1 with no activity against either HIV-2 or any other nucleic acid polymerase. Mechanistically, these derivatives appear to be noncompetitive inhibitors of HIV-1 RT and most likely bind to the same allosteric site of the enzyme. Examples of such nonnucleoside inhibitors of HIV-1 RT include the TIBO compounds,[6] HEPT derivatives,[7] nevirapine,[8] L-697,661,[9] and BHAP analogs[10] (Chart 1).

The lead compound in a newly discovered group of HIV-1 RT inhibitors was *N*-(2-phenethyl)-*N'*-(2-thiazolyl)thiourea, LY73497 (**1**).[11] This paper describes the basic structure–activity relationship (SAR) studies within this new PETT series of anti-HIV compounds. Other aspects, such as the discovery,[12] molecular modeling, resistance development, and pharmacokinetic properties of this new class of compounds will be presented in additional publications.

Chart 1





LY73497 (1)
ED_{50} 1.3 μM
IC_{50} 0.9 μM

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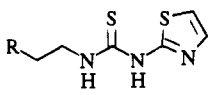
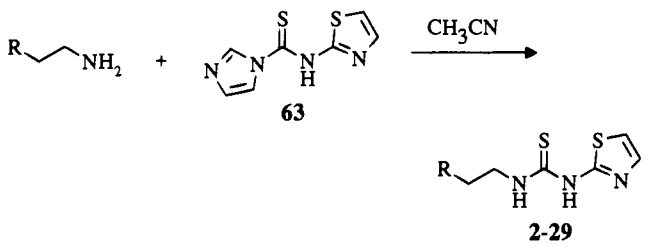
Table 1. Inhibition of HIV-1: Modification of Quadrant 1



no.	R	prep method	anti-HIV-1 activity IC_{50} (μM)[a]	ED^{50} (μM)[b]	CD_{50} (μM)[c]
1	phenyl	II	0.9	1.3	>380
2	2-fluorophenyl	ID	0.06	0.1	75
3	3-fluorophenyl	IA	0.15	0.25	78
4	4-fluorophenyl	IA	1.0	3.3	78
5	2-methoxyphenyl	IA	0.04	0.4	102
6	3-methoxyphenyl	IA	0.15	0.6	143
7	4-methoxyphenyl	IA	0.35	5.5	68
8	2-methylphenyl	ID	0.08	0.95	72
9	2-azidophenyl	ID	0.03	0.1	92
10	2-nitrophenyl	ID	0.15	1.1	123
11	2-hydroxyphenyl	IA	1.1	4.0	100
12	2-chlorophenyl	IA	0.6	0.4	100
13	3-ethoxyphenyl	IB	0.06	0.15	91
14	3-propoxyphenyl	IB	0.2	2.2	75
15	3-isopropoxyphenyl	IB	0.4	0.4	16
16	3-(hexyloxy)phenyl	IB	>10	>10	8
17	3-phenoxyphenyl	IB	1.1	2.8	14
18	2,6-dimethoxyphenyl	IB	0.09	0.09	37
19	2,5-dimethoxyphenyl	IA	0.2	0.4	86
20	3-bromo-6-methoxyphenyl	IB	0.03	0.05	94
21	2-fluoro-6-methoxyphenyl	IB	0.01	0.3	51
22	2-ethoxy-6-fluorophenyl	IB	0.01	0.2	37
23	2,6-difluorophenyl	IC	0.01	0.02	33
24	2-chloro-6-fluorophenyl	IC	0.006	0.05	111
25	2-pyridyl	IA	0.2	1.3	76
26	3-pyridyl	IC	>10	6.4	83
27	4-pyridyl	IC	>10	>10	113
28	1-methylpyrrol-2-yl	IA	1.9	>10	143
29	2-furyl	ID	0.65	5.2	89

[a] The assay used rCdG as the template and dGTP as the substrate as described in ref 17. The concentration producing 50% inhibition (IC_{50}) is stated as the mean of at least two experiments. [b] The cell culture assay used MT4 cells infected with HIV-1, IIIB; for details, see the Experimental Section. The concentration which reduced the cytopathic effect caused by the virus (ED_{50}) is stated as the mean of at least two experiments. [c] The cytotoxic dose (CD_{50}) was defined as the concentration of the compound that reduced the viability of the cells to 50% compared to untreated cells.

Scheme 1. PETT Compounds from Thiocarbonylimidazole Thiazole **63**

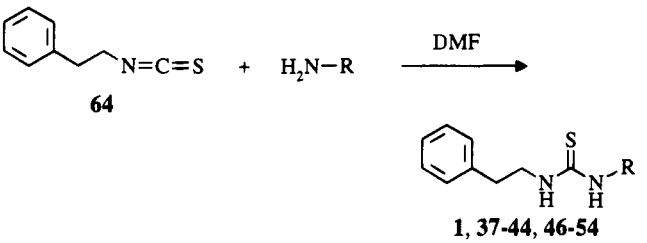


Chemistry

The SAR optimization of LY73497 was developed by notionally dividing the lead compound into four quadrants indicated with broken lines on the lead compound illustrated above. The SAR strategy was divided into two phases. The first phase involved optimization of antiviral activity via independent variation of quadrants 1–4. The second phase involved the preparation of hybrid structures containing combinations of the best substituents within these quadrants.

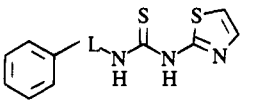
The majority of the PETT derivatives with variations in quadrant 1 (Table 1) were synthesized according to the general method depicted in Scheme 1. Thiourea analogs **2–29** were prepared by condensation of the appropriate amine with the thiocarbonyl reagent (**63**) derived from 2-aminothiazole and thiocarbonyldiimidazole (**68**). The amines used in this study were either commercially available (method IA) or prepared according to three standard methods: (i) condensation of an appropriate aldehyde with nitromethane followed by reduction (method IB), (ii) reduction of an appropriate nitrile (method IC), or (iii) reaction of an appropriate triflate with ammonia (method ID).

Scheme 2. PETT Compounds from Phenethyl Isothiocyanate **64**



Scheme 2 outlines an alternative route to the thiourea analogs in which phenethyl isothiocyanate (**64**) is reacted with a heterocyclic amine (method II). LY73497 (**1**) was prepared via this method where 2-aminothiazole was used as the heterocyclic amine. Compounds containing modifications of quadrant 2 are listed in Table 2. The benzyl and phenylpropyl analogs of LY73497, compounds **30** and **31**, respectively, were prepared analogously to the reaction in Scheme 2. The other quadrant II analogs (**32** and **33**) were prepared in a manner similar to the reaction in Scheme 1, see the Experimental Section.

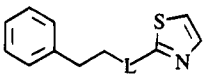
Table 2. Inhibition of HIV-1: Modification of Quadrant 2



no.	L	prep method	anti-HIV-1 activity IC_{50} (μM)[a]	ED_{50} (μM)[b]	CD_{50} (μM)[c]
30	CH_2	II	1.2	>10	120
1	CH_2CH_2	II	0.9	1.3	>380
31	$CH_2CH_2CH_2$	II	4.5	>10	>360
32	$CH_2CH(CH_3)$	cf. ID	>10	>10	87
33	$CH(CH_3)CH_2$	cf. IA	0.45	1.2	108

[a] See footnote *a*, Table 1. [b] See footnote *b*, Table 1. [c] See footnote *c*, Table 1.

Table 3. Inhibition of HIV-1: Modification of Quadrant 3

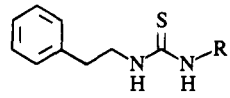


no.	L	prep method	anti-HIV-1 activity IC_{50} (μM)[a]	ED_{50} (μM)[b]	CD_{50} (μM)[c]
1	NHC(S)NH	II	0.9	2.3	>380
34	NHC(O)NH	cf. II	>10	>10	113
35	$N(CH_3)C(S)NH$	IA	>10	>10	29
36	$NHC(S)N(CH_3)$	cf. I	1.5	10	65

[a] See footnote *a*, Table 1. [b] See footnote *b*, Table 1. [c] See footnote *c*, Table 1.

Analogs with modifications in quadrant 3 are depicted in Table 3. Compound **34** was made in accordance with Scheme 2 using a phenyl isocyanate. The methyl-substituted compound **35** was prepared via method 1A using *N*-methylphenethylamine. The isomeric derivative **36** was made from *N*-(methylamino)thiazole and

Table 4. Inhibition of HIV-1: Modification of Quadrant 4[a]



no.	R	anti-HIV-1 activity IC_{50} (μM)[b]	ED_{50} (μM)[c]	CD_{50} (μM)[d]
1	2-thiazolyl	0.9	1.3	>380
37	4-methylthiazol-2-yl	0.1	0.4	>360
38	4-ethylthiazol-2-yl	0.6	0.7	>340
39	4-propylthiazol-2-yl	0.35	1.6	>330
40	4-isopropylthiazol-2-yl	0.2	1.3	>330
41	4-butylisothiazol-2-yl	2.5	1.3	313
42	4-cyanothiazol-2-yl	0.2	0.2	ND[e]
43	4-(trifluoromethyl)thiazol-2-yl	0.55	0.5	9
44	4-carboxythiazol-2-yl	>10	>10	>325
45	4-(ethoxycarbonyl)thiazol-2-yl[f]	0.2	0.5	60
46	5-chlorothiazol-2-yl	2.4	2.7	15
47	3-pyrazolyl	>10	>10	122
48	1,3,4-thiadiazol-2-yl	1.9	5.3	ND
49	2-pyrazinyl	3.9	>10	>390
59	2-pyridyl	0.02	0.2	ND
51	3-bromo-2-pyridyl	>10	>10	297
52	5-bromo-2-pyridyl	0.015	0.05	>300
53	5-methyl-2-pyridyl	0.03	0.15	>370
54	2-benzothiazolyl	0.2	1.1	ND

[a] Prepared by method II. [b] See footnote *a*, Table 1. [c] See footnote *b*, Table 1. [d] See footnote c, Table 1. [e] Not determined. [f] Prepared according to method IA.

(phenethylimidazolyl)thiocarbonyl in a reaction similar to the one described in Scheme 1.

The compounds in Table 4 were prepared in good yields by condensation of phenyl isocyanate with an appropriate heterocyclic amine in DMF at about 100 °C (Scheme 2). For details see the Experimental Section.

Hybrid derivatives **55–58** were prepared as shown in Scheme 3. Nitrile **65** was reduced by sodium borohydride/cobalt chloride in methanol or preferably by borane in THF to give the amine **66**. Compound **66** was then reacted with the thiocarbonyl reagent (**67**) to give compounds **55** and **56**. For compounds **57** and **58**, derivative **66** was converted to an activated thiocarbonyl reagent (**69**) which was reacted with the pyridylamines **70** in DMF according to Scheme 1.

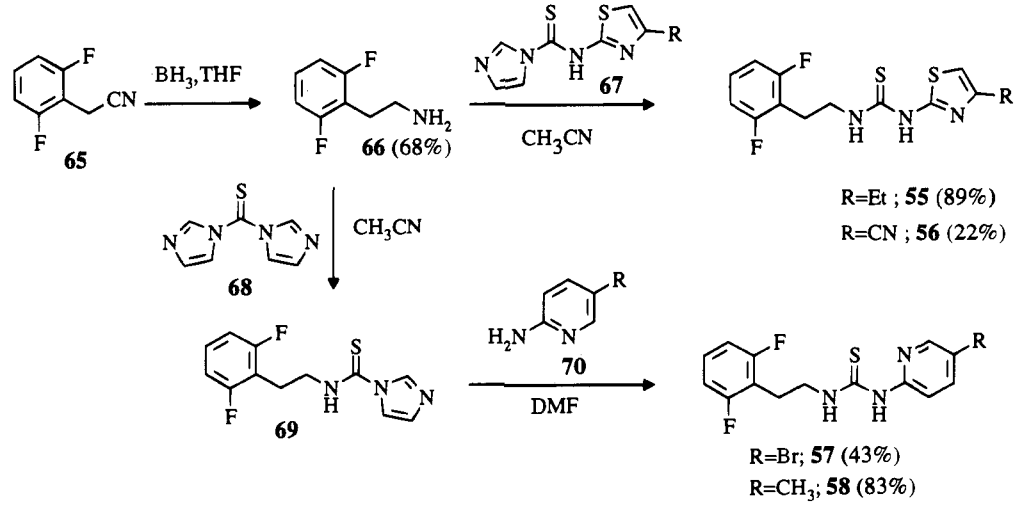
Scheme 4 shows the synthesis of optimized compounds **59–62**. The substituted phenethylamine **73** was prepared according to method IB. Reaction with the thiocarbonyl reagents **74** afforded compounds **59** and **60**. Compounds **61** and **62** were synthesized according to method IA.

Biological Results and Discussion

The anti-HIV-1 activity of the compounds described in the chemistry section were assessed both on recombinant HIV-1 RT and in cell culture. The enzyme assay utilized rCdG as the template/primer and tritiated dGTP as the substrate and the cell assay used MT-4 cells. The IC_{50} and ED_{50} values from these experiments are shown in Tables 1–5. The cytotoxic effect of the present compounds were studied in MT-4 cells and the CD_{50} values are shown in Tables 1–5, and these results indicate that the antiviral effect is well separated from the cytotoxic effect. As mentioned previously, the first phase of the basic SAR studies was performed by notionally dividing LY73497 (**1**) into four quadrants and independently varying each quadrant. The antiviral activities of compounds containing modifications of the phenyl ring in quadrant 1 are shown in Table 1. In general, *meta* and, particularly, *ortho* substitution were preferred over *para* substitution. This trend is clearly apparent with the fluoro- and methoxy-substituted compounds **2–4**, and **5–7**, respectively. The influence of the electronic nature of the *ortho* substituent on antiviral activity was also studied. The data obtained from compounds **2**, **5**, and **7–12** showed that both small electron-donating and small electron-withdrawing groups were comparable with good activity. The preferred groups were fluoro, chloro, azido, and methoxy. The effect of the chain length in a series of *m*-alkoxy substituents was investigated. Maximal activity was observed for the ethoxy derivatives **13** and activity decreased for more sterically demanding propoxy and isopropoxy derivatives, **14** and **15**, respectively. The hexyloxy compound **16** was essentially devoid of activity. A combination of alkoxy and halogen substitution, as well as disubstitution with alkoxy and halogen substituents, resulted in compounds with improved activity. Compounds with a 2,6- or 2,5-substitution pattern were preferred.

Some analogs with heterocyclic replacement of the phenyl ring of LY73497 were also prepared. The

Scheme 3. Synthesis of Compounds **55–58**



Scheme 4. Synthesis of Compounds **59–62**

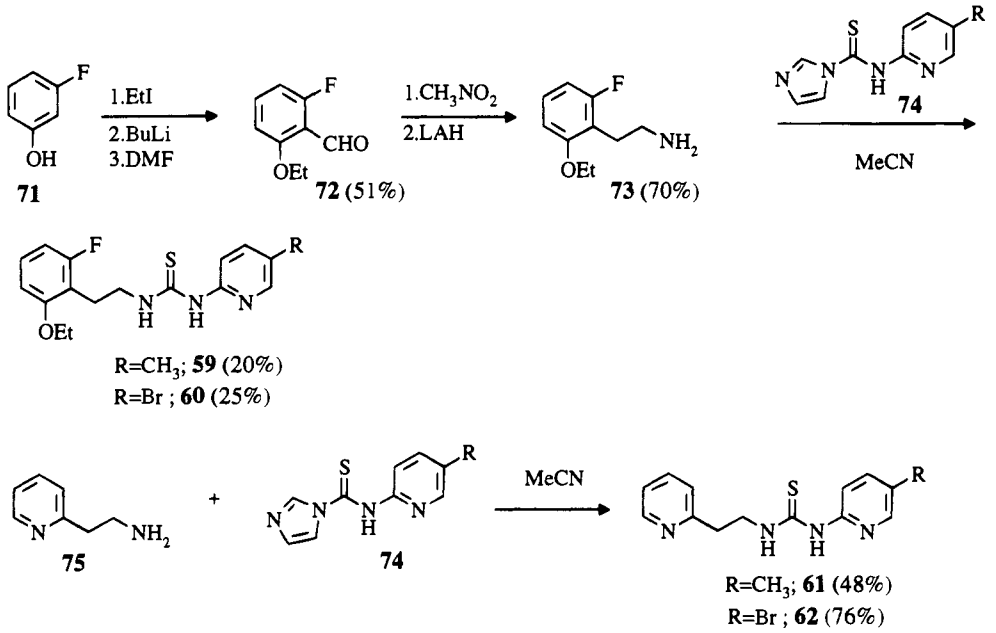
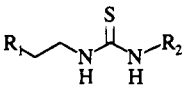


Table 5. Inhibition of HIV-1: Reference Compounds and a Selection of Optimized Compounds



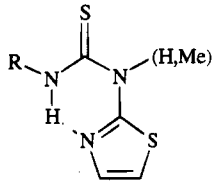
no.	R_1	R_2	anti-HIV-1 activity IC_{50} (μM)[a]	ED_{50} (μM)[b]	CD_{50} (μM)[c]
55	2,6-difluorophenyl	4-ethylthiazol-2-yl	0.0055	0.08	>305
56	2,6-difluorophenyl	4-cyanothiazol-2-yl	0.006	0.03	10
57	2,6-difluorophenyl	5-bromo-2-pyridyl	0.001	0.01	67
58	2,6-difluorophenyl	5-methyl-2-pyridyl	0.003	0.01	>325
59	2-ethoxy-6-fluorophenyl	5-methyl-2-pyridyl	0.0045	0.2	60
60	2-ethoxy-6-fluorophenyl	5-bromo-2-pyridyl	0.006	0.02	50
61	2-pyridyl	5-methyl-2-pyridyl	0.05	0.3	73
62	2-pyridyl	5-bromo-2-pyridyl	0.015	0.02	87
9-chloro-TIBO			0.2	0.25	93
nevirapine			0.2	0.15	188
L-697,661			0.1	0.065	114
AZT				0.007	70

[a] See footnote *a*, Table 1. [b] See footnote *b*, Table 1. [c] See footnote *c*, Table 1.

2-pyridyl compound **25** had the best activity among these types of derivatives. Activity decreased as the pyridine nitrogen was moved to the 3- and 4-position compounds **26** and **27**.

Table 2 displays the activity of compounds with modifications of the ethyl linker in quadrant 2. The results in Table II clearly indicate that an ethyl linker is optimal for activity. Interestingly, a methyl substituent in the benzylic position enhanced activity, whereas a methyl group in the phenethyl position diminished activity. Conformational analyses offer a possible explanation of this since compound **33** adapts a "high-activity" conformation more easily than compound **32**. In this particular case, a difference about 2 kcal could be observed between the two *gauche* conformers having their aromatic rings in close vicinity to each other. In general, according to conformational analyses using PC model,[13,14] the present compounds can exist in three low-energy conformations, one *anti* (a) and two *gauche* (b and c) conformers of which one has the ring systems in a more close vicinity than the other one, i.e. conformation b. There is a correlation between the most active compounds and the preference for that low-energy *gauche* conformation b. This information was used as an interactive tool in guiding the SAR process.

Table 3 shows the effect of variation in the quadrant 3 thiourea function. For the compounds tested, maximum activity was obtained for the parent N,N-unsubstituted thiourea **1**. The urea analog **34** was inactive at the concentrations tested. Other isosters such as a cyanoguanidine derivative met with considerably less success, e.g. a cyanoguanidine with a phenethyl left part and a 5-chloropyridine right part was essentially devoid of any activity when tested in cellculture (data not shown). Methyl substitution on the nitrogen adjacent the phenethyl side chain, as demonstrated for compound **35**, completely eliminated activity. Methyl substitution on the other nitrogen adjacent the thiazole ring (compound **36**) resulted in a slight decrease in activity. The reason for these differences in activity may be due to the presence of an internal hydrogen bond in derivatives **1** and **36** resulting in a "high-activity" rigid conformation, as depicted in structure **76**. Hydrogen bond stabilization of this nature is not possible for compound **35**. According to PC model, this internal hydrogen bonding lowered the energy by about 5 kcal.[13]



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Preliminary studies indicated that the quadrant 4 should be some kind of heterocycle, preferably a thiazole, as a phenyl gave almost inactive compounds (data not shown).

Modifications of the thiazole heterocycle in quadrant 4 are summarized in Table 4. Several 4-substituted thiazole derivatives were quite potent inhibitors, including small alkyl (**37** and **38**), cyano (**42**), trifluoromethyl (**43**) and ethoxycarbonyl (**45**). In the alkyl series, the size of the substituent seems to be critical, since the activity fell off with substituents larger than ethyl (compounds **39**, **40**, and **41**). A dramatic decrease in activity was observed for the carboxylic acid **44**, indicating that the allosteric site of the enzyme does not accept such a polar group. Chloro substitution at the 5-position of the thiazole ring led to the less active derivative **46**. Bicyclic substitution with benzothiazole also resulted in a less potent derivative **54**.

Among the derivatives prepared with another heterocycle in place of the thiazole ring, the 2-pyridyl analog **50** was the most active. Substitution at the 5-position of this heterocycle resulted in even more potent compounds. The highest activity was found for the 5-bromo analog **52**. Interestingly, the 3-bromo analog **51** was much less active, perhaps due to disruption of the coplanarity of the thiourea and pyridyl heterocycle by the *ortho* substituent and subsequent destabilization of the internal hydrogen bond.

Results of the second phase of the SAR, the anti HIV-1 activities of a selection of hybrid compounds combining optimal substituents in quadrants 1–4 are shown in Table 5 compared to reference compounds. The activity parameters from individual quadrants were found to be remarkably additive with combinations of the best substituents providing derivatives with optimal activity. The results in Table 5 show examples of some very potent compounds, e.g. **57**, **58**, **60**, and **62**. These derivatives compare very favorably in anti-HIV-1 activity with previously reported nonnucleoside reference compounds.

As depicted in the tables, a reasonable correlation between the activity against RT and in cell culture was observed, indicating that mechanistically the PETT compounds appear to act as inhibitors of HIV-RT. In some cases, fairly large differences between effect in the RT assay and in cell culture were observed (compounds **21**, **24**, **33**, **45**, **50**, and **59**), perhaps due to differential penetration of these derivatives into MT-4 cells. Similar, but more pronounced differences between enzymatic and cell culture activity have been observed for HIV-protease inhibitors.[15]

On the basis of their excellent antiviral activity, a number of PETT derivatives were further evaluated for their activity against mutant strains of HIV-**1**, potential to develop resistance, pharmacokinetic properties, and toxicology. Out of these studies, to be reported elsewhere, the hydrochloride salt of compound **62** (LY300046·HCl) was selected for clinical studies to assess its potential utility in the treatment of AIDS.[12] Briefly, the activities (ED_{50}) of LY300046·HCl in cells infected with resistant mutants were for clone T3-16 (Cys-181 mutant), 0.7 μM; for clone 118 (Ile-100 mutant), 0.8 μM; and for clone 22 (Ile-100 and His-188 mutant), 7.0 μM. The absolute oral bioavailability in rat was 20%; the levels in the rat brain were comparable with the plasma levels, and the *in vitro* plasma binding was 88.7% in rat plasma and 95.5% in human plasma. The development of other analogs from this series is currently under investigation.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes and are uncorrected. Analytical results are indicated by atomic symbols and are within 0.4% of the theoretical values except where indicated. ^{1}H NMR spectra were recorded on Bruker AC-250 (250 MHz) and General Electric QE-300 (300 MHz) spectrometers using TMS as the internal standard. Intermediates were prepared according to standard methods, and illustrative examples are given. The yields were not optimized. Preparative column chromatography was performed with Merck silica gel (230–400 mesh) or Merck aluminum oxide 90 (70–230 mesh). 9-Chloro-TIBO was purchased from Pharma Tech International. Nevirapine and L-697,661 were prepared as described in refs 8 and 9b, respectively.

Method I. General Procedure. Thiocarbonyldiimidazole (8.90 g, 50 mmol) and 2-aminothiazole (5.0 g, 50 mmol) were added to 50 mL of acetonitrile at 20 °C. The reaction mixture was then warmed to 40 °C and kept at that temperature for a period of 2 h. The reaction mixture was cooled to 0 °C, and the resultant solid was filtered off and washed with 300 mL of cold acetonitrile to afford, after drying, 9.7 g (92%) of compound **63**. To a suspension of **63** (1.1 equiv) in DMF or acetonitrile was added an appropriate amine (1 equiv). The reaction mixture was heated to about 100 °C for 1 h and then cooled to room temperature. Dichloromethane was added and the organic phase washed with 0.5 M HCl, brine, and water. After drying, the organic solvent was removed and the residue purified either by recrystallization or column chromatography.

Method IA. *N*-(2-(3-Fluorophenethyl))-*N*'-(2-thiazolyl)-thiourea (3). A commercially available amine, i.e. 3-fluorophenethylamine was reacted with compound **63** as described in the general procedure for method I. Mp: 126.8–127.3 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.9 (t, 2H, 3.8 (q, 2H), 7.0–7.4 (m, 6H). Anal. ($C_{12}H_{12}FN_3S_2$) C, H, N.

The following compounds were prepared according to method IA:

***N*-(2-(4-Fluorophenethyl))-*N*'-(2-thiazolyl)thiourea (4).** Mp: 124.5–126.0 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.0 (t, 3H), 4.0 (q, 3H), 6.86 (d, 1H), 7.0–7.3 (m, 5H). Anal. ($C_{12}H_{12}FN_3S_2$) C, H, N.

***N*-(2-(2-Methoxyphenethyl))-*N*'-(2-thiazolyl)thiourea (5).** Mp: 126.0–127.5 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.03 (t, 2H), 3.82 (s, 3H), 3.96 (q, 2H), 6.79–6.93 (m, 3H), 7.20–7.26 (m, 3H), 10.35 (broad s, 1H), 10.73 (broad s, 1H). Anal. ($C_{13}H_{15}N_3OS_2$) H; C: calc, 53.2; found, 52.6; N: calc, 14.3; found, 13.7.

***N*-(2-(3-Methoxyphenethyl))-*N*'-(2-thiazolyl)thiourea (6).** Mp: 123.5–124.7 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.9 (t, 2H), 3.75 (s, 3H), 3.9 (q, 2H), 6.8 (m, 4H), 7.1 (d, 1H), 7.2 (t, 1H), 7.4 (d, 1H). Anal. ($C_{13}H_{15}N_3OS_2$) C, H, N.

***N*-(2-(4-Methoxyphenethyl))-*N*'-(2-thiazolyl)thiourea (7).** Mp: 134–136 °C. ^{1}H NMR ((250 MHz, $CDCl_3$): 2.96 (t, 2H), 3.79 (s, 3H), 3.94 (q, 2H), 6.81 (d, 1H), 6.85 (d, 2H), 7.18–7.22 (t, 3H). Anal. ($C_{13}H_{15}N_3OS_2$) C, H; N: calc, 14.3; found, 13.4.

***N*-(2-(2-Hydroxyphenethyl))-*N*'-(2-thiazolyl)thiourea (11).** Mp: 151.2–152 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.85 (t, 2H), 3.5 (broad s, 1H), 3.75 (q, 2H), 6.7–6.9 (m, 2H), 7.0–7.2 (m, 3H), 7.35 (d, 1H). Anal. ($C_{12}H_{13}N_3OS_2$) C, H, N.

***N*-(2-(2-Chlorophenethyl))-*N*'-(2-thiazolyl)thiourea (12).** Mp: 135–136 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.17 (t, 2H), 4.02 (q, 2H), 6.81 (d, 1H), 7.17–7.38 (m, 5H). Anal. ($C_{12}H_{12}ClN_3S_2$) C, H; N: calc, 14.1; found, 13.3.

N-(2-(2,5-Dimethoxyphenethyl))-N'-(2-thiazolyl)thiourea (19). Mp: 149 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.00 (t, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 3.97 (m, 2H), 6.70–6.85 (m, 4H), 7.24 (d, 1H), 10.80 (s, 1H). Anal. ($C_{14}H_{12}N_3O_2S_2$) C, H, N.

N-(2-(2-Pyridylethyl))-N'-(2-thiazolyl)thiourea (25). Mp: 136.5 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 3.1 (t, 2H), 4.0 (m, 2H), 7.1 (d, 1H), 7.2–7.4 (m, 2H), 7.7 (m, 2H), 8.5 (d, 1H), 9.8 (s, 1H), 11.7 (s, 1H). Anal. ($C_{11}H_{12}N_4S_2$) C, H, N.

N-(2-(1-Methyl-2-pyrrolyl)ethyl)-N'-(2-thiazolyl)thiourea (28). Mp: 183–184 °C dec. ^{1}H NMR (250 MHz, DMSO-d_6): 2.86 (t, 2H), 3.55 (s, 3H), 3.75 (q, 2H), 5.85–5.90 (m, 2H), 6.62 (s, 1H), 7.09 (d, 1H), 7.36 (d, 1H), 9.74 (broad s, 1H), 11.65 (broad s, 1H). Anal. ($C_{11}H_{14}N_4S_2$) H; C: calc, 49.6; found, 50.8; N: calc, 21.0; found, 19.8.

N-Methyl-N-(2-phenethyl)-N'-(2-thiazolyl)thiourea (35). Mp: 133.5–134.1 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.9 (t, 2H), 3.2 (s, 3H), 4.0 (t, 2H), 6.8 (d, 1H), 7.2 (m, 1H), 7.3 (m, 5H). Anal. ($C_{13}H_{15}N_3S_2$) C, H, N.

Method IB. N-(2-(3-Ethoxyphenethyl))-N'-(2-thiazolyl)thiourea (13). 3-Ethoxybenzaldehyde (2.9 g, 19.3 mmol) (prepared from 3-hydroxybenzaldehyde, ethyl iodide, and K_2CO_3 in acetone) was reacted with nitromethane (1.04 mL, 19.3 mmol) as described in ref 16. The resulting nitrostyrene (3.22 g, 16.6 mmol) was reduced with LAH (2.5 g, 66.4 mmol) in 100 mL of THF to give 2.6 g (96%) of the crude amine which was reacted with compound **64** according to method I. Mp: 169–170 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.42 (t, 3H), 2.96 (t, 2H), 3.87 (q, 2H), 4.08 (q, 2H), 6.93–7.30 (m, 4H), 7.60 (d, 1H). Anal. ($C_{14}H_{17}N_3OS_2$) C, H, N.

The following compounds were prepared according to method IB:

N-(2-(3-Propoxyphenethyl))-N'-(2-thiazolyl)thiourea (14). Mp: 99 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 1.02 (t, 3H), 1.79 (m, 2H), 3.00 (t, 2H), 3.88 (t, 2H), 3.98 (q, 2H), 6.79–7.23 (m, 6H). Anal. ($C_{15}H_{19}N_3OS_2$) C, H, N.

N-(2-(3-Isopropoxyphenethyl))-N'-(2-thiazolyl)thiourea (15). Mp: 106 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.32–1.36 (m, 6H), 2.96 (t, 2H), 3.87 (q, 2H), 4.64–4.69 (m, 1H), 6.84–7.44 (m, 6H). Anal. ($C_{15}H_{19}N_3OS_2$) C, H, N.

N-(2-(3-(Hexyloxy)phenethyl))-N'-(2-thiazolyl)thiourea (16). Mp: 96 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 0.89–1.83 (m, 11H), 3.02 (t, 2H), 3.91–4.03 (m, 4H), 6.76–7.28 (m, 6H). Anal. ($C_{18}H_{25}N_3OS_2$) C, H, N.

N-(2-(3-Phenoxyphenethyl))-N'-(2-thiazolyl)thiourea (17). Mp: 102.5–104.0 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 2.99 (t, 2H), 3.96 (q, 2H), 6.77 (d, 1H), 6.80–6.94 (m, 1H), 6.95–7.09 (m, 5H), 7.20–7.34 (m, 4H), 10.92 (broad s, 1H), 11.21 (broad s, 1H). Anal. ($C_{18}H_{17}N_3OS_2$) C, H, N.

N-(2-(2,6-Dimethoxyphenethyl))-N'-(2-thiazolyl)thiourea (18). Mp: 153.9–155.0 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.9 (t, 2H), 3.7 (q, 2H), 3.8 (s, 6H), 6.7 (d, 2H), 7.1 (d, 1H), 7.2 (t, 1H), 7.3 (d, 1H). Anal. ($C_{14}H_{18}N_3O_2S_2$) H, N; C: calc, 52.0; found, 51.5.

N-(2-(3-Bromo-6-methoxyphenethyl))-N'-(2-thiazolyl)thiourea (20). Mp: 154–156 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.98 (t, 2H), 3.95 (q, 2H), 6.72 (d, 1H), 6.81 (d, 1H), 7.35 (m, 3H). Anal. ($C_{13}H_{14}N_3OS_2$) C, H, N.

N-(2-(2-Fluoro-6-methoxyphenethyl))-N'-(2-thiazolyl)thiourea (21). Mp: 132.7–133.2 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.95 (t, 2H), 3.8 (t, 2H), 6.7–6.9 (m, 2H), 7.05 (d, 1H), 7.15–7.30 (m, 1H), 7.4 (d, 1H). Anal. ($C_{13}H_{14}FN_3OS_2$) C, H, N.

N-(2-(2-Ethoxy-6-fluorophenethyl))-N'-(2-thiazolyl)thiourea (22). Mp: 146–147 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.33 (t, 3H), 2.92 (t, 2H), 3.78 (q, 2H), 4.00 (q, 2H), 6.72–7.32 (m, 5H). Anal. ($C_{14}H_{16}FN_3OS_2$) C, H, N.

Method IC. N-(2-(2,6-Difluorophenethyl))-N'-(2-thiazolyl)thiourea (23). 2,6-Difluorobenzyl cyanide (5 g, 33 mmol) and cobalt chloride hexahydrate (11.6 g, 49 mmol) were dissolved in 500 mL of methanol. To this solution was added $NaBH_4$ (9.3 g, 244 mmol) in portions. The reaction mixture was filtered after 3 h and the filtrate washed twice with methanol. Evaporation of the combined filtrates gave a residue which was dissolved in 1 M HCl and washed with dichloromethane. The aqueous layer was basified with ammonia and extracted with dichloromethane. The extracts were washed with water and evaporated to afford 2.9 g of 2,6-difluorophenethylamine. This amine was then reacted with compound **63** according to method I. Mp: 148–149 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.11 (t, 2H), 3.96 (q, 2H), 6.81 (d, 1H), 6.86 (m, 2H), 7.12–7.26 (m, 1H), 7.23 (d, 1H). Anal. ($C_{12}H_{11}F_2N_3S_2$) H; C: calc, 48.2; found, 48.8; N: calc, 14.0; found, 13.5.

The following compounds were prepared according to method IC:

N-(2-(2-Chloro-6-fluorophenethyl))-N'-(2-thiazolyl)thiourea (24). Mp: 151 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 3.1 (t, 2H), 3.85 (m, 2H), 7.1 (d, 1H), 7.15–7.30 (m, 3H), 7.40 (d, 1H). Anal. ($C_{12}H_{11}ClFN_3S_2$) C, H, N.

N-(2-(3-Pyridyl)ethyl)-N'-(2-thiazolyl)thiourea (26). Mp: 175.3–176.0 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.93 (t, 2H), 3.79 (q, 2H), 7.10 (d, 1H), 7.32–7.37 (m, 2H), 7.68–7.72 (m, 1H), 8.43–8.45 (1H), 8.48–8.49 (m, 1H). Anal. ($C_{11}H_{12}N_4S_2$) C, H, N.

N-(2-(4-Pyridyl)ethyl)-N'-(2-thiazolyl)thiourea (27). Mp: 151.5–152.5 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 3.04 (t, 2H), 3.93 (q, 2H), 7.18 (d, 1H), 7.38–7.44 (m, 3H), 8.58 (d, 2H), 9.69 (broad s, 1H), 11.70 (broad s, 1H). Anal. ($C_{11}H_{12}N_4S_2$) C, H, N.

Method ID. N-(2-(2-Fluorophenethyl))-N'-(2-thiazolyl)thiourea (2). To a solution of 1-fluoro-2-ethanolbenzene (1.34 mL, 10 mmol) and 4-(dimethylamino)pyridine (1.28 mL, 10.5 mmol) in 30 mL of dichloromethane at about −30 °C was added 1.77 mL (11 mmol) of triflic anhydride. The reaction mixture was allowed to slowly reach room temperature, and the resulting salt was filtered off. The filtrate was added dropwise to 40 mL of a 1:1 mixture of liquid ammonia and THF at about −30 °C. The ammonia was evaporated while the reaction mixture was allowed to reach room temperature. The mixture was diluted with ether (100 mL), washed with water, dried over sodium sulfate, filtered, and concentrated to give 0.56 g (40%) of crude 2-fluorophenethylamine which was reacted with compound **63** according to method I. Mp: 153 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 3.08 (t, 2H), 3.99 (q, 2H), 6.81 (d, 1H), 7.03–7.28 (m, 5H). Anal. ($C_{12}H_{12}FN_3S_2$) H, N; C: calc, 51.2; found, 50.7.

The following compounds were prepared according to method ID:

N-(2-(2-Methylphenethyl))-N'-(2-thiazolyl)thiourea (8). Mp: 143–144 °C. ^{1}H NMR (250 MHz, $CDCl_3$): 2.40 (s, 3H), 3.04 (t, 2H), 3.96 (q, 2H), 6.81 (d, 1H), 7.13–7.16 (m, 4H), 7.25 (d, 1H). Anal. ($C_{13}H_{15}N_3S_2$) C, H, N.

N-(2-(2-Azidophenethyl))-N'-(2-thiazolyl)thiourea (9). Mp: 143.6–144.8 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.9 (t, 2H), 3.8 (q, 2H), 7.0–7.4 (m, 6H). Anal. ($C_{12}H_{12}N_6S_2$) C, H; N: calc, 27.6; found, 26.85.

N-(2-(2-Nitrophenethyl))-N'-(2-thiazolyl)thiourea (10). Mp: 164.1–165.0 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 3.15 (t, 2H), 3.9 (broad s, 2H), 7.0 (d, 2H), 7.35 (d, 1H), 7.4–7.6 (m, 2H), 7.6 (d, 1H). Anal. ($C_{12}H_{12}N_4O_2S_2$) C, H, N.

N-(2-(2-Thienyl)ethyl)-N'-(2-thiazolyl)thiourea (29). Mp: 196 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 3.48 (t, 2H), 3.91 (t, 2H), 7.06–7.10 (m, 2H), 7.21 (d, 1H), 7.48 (d, 2H), 9.8 (s, 1H), 11.8 (s, 1H). Anal. ($C_{10}H_{11}NS$) C, H, N.

Method II. N-(2-Phenethyl)-N'-(2-thiazolyl)thiourea (1). A solution of 2-phenethyl isothiocyanate (7.5 g, 45.9 mmol) and 2-aminothiazole (4.6 g, 45.9 mmol) in DMF (100 mL) was heated at 115 °C for 12 h. The reaction mixture was cooled, poured into EtOAc, and washed with water, 1 M HCl, saturated $NaHCO_3$, and brine. After concentration, the residue was recrystallized twice from EtOAc to give 5.7 g (42%) of the title product. Mp: 169.5–170.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.9 (t, 2H), 3.8 (m, 2H), 7.1 (d, 1H), 7.2–7.4 (m, 6H), 9.7 (broad s, 1H), 11.6 (broad s, 1H). Anal. ($C_{12}H_{12}N_3S_2$) C, H, N.

The following compounds were prepared according to method II:

N-(2-Benzyl)-N'-(2-thiazolyl)thiourea (30). Mp: 165–167 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 4.8 (m, 2H), 7.05 (d, 1H), 7.2–7.4 (m, 6H), 9.9 (broad s, 1H), 11.7 (broad s, 1H). Anal. ($C_{11}H_{13}N_3S_2$) C, H, N.

N-(3-Phenylpropyl)-N'-(2-thiazolyl)thiourea (31). Mp: 126.5–127.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.01–2.12 (m, 2H), 2.74 (t, 2H), 3.71–3.82 (m, 2H), 6.82 (d, 1H),

7.15–7.37 (m, 6H), 10.42 (s, 1H), 10.88 (s, 1H). Anal. ($C_{13}H_{15}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-methylthiazolyl))thiourea (37).** Mp: 190–192 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 2.17 (s, 3H), 3.01 (t, 2H), 3.98–4.04 (m, 2H), 6.31 (s, 1H), 7.20–7.33 (m, 5H), 10.08 (s, 1H), 10.92 (s, 1H). Anal. ($C_{13}H_{15}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-ethylthiazolyl))thiourea (38).** Mp: 145–146 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.1 (t, 3H), 2.45 (q, 2H), 2.9 (t, 2H), 3.8 (m, 2H), 6.6 (s, 1H), 7.2–7.4 (m, 5H), 9.8 (broad s, 1H), 11.5 (broad s, 1H). Anal. ($C_{14}H_{17}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-propylthiazolyl))thiourea (39).** Mp: 135–137 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 0.82 (t, 3H), 1.41–1.53 (m, 2H), 2.40 (t, 2H), 2.85 (t, 2H), 3.73–3.79 (m, 2H), 6.60 (s, 1H), 7.15–7.29 (m, 5H), 9.93 (broad s, 1H), 11.50 (broad s, 1H). Anal. ($C_{15}H_{19}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-isopropylthiazolyl))thiourea (40).** Mp: 155–156 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.07 (d, 6H), 2.71–2.76 (m, 1H), 2.87 (t, 2H), 3.74–3.80 (m, 2H), 6.58 (s, 1H), 7.14–7.29 (m, 5H), 9.89 (broad s, 1H), 11.52 (broad s, 1H). Anal. ($C_{15}H_{19}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-butylthiazolyl))thiourea (41).** Mp: 100–102 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 0.84 (t, 3H), 1.19–1.29 (m, 2H), 1.40–1.50 (m, 2H), 2.40–2.45 (m, 2H), 2.86 (t, 2H), 3.73–3.79 (m, 2H), 6.59 (s, 1H), 7.15–7.29 (m, 5H), 9.89 (broad s, 1H), 11.52 (broad s, 1H). Anal. ($C_{16}H_{21}N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-cyanothiazolyl))thiourea (42).** Mp: 169–170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.02 (t, 2H), 3.93–4.00 (m, 2H), 7.23–7.39 (m, 5H), 7.50 (s, 1H), 10.09 (s, 1H), 10.88 (s, 1H). Anal. ($C_{13}H_{12}N_4S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-(trifluoromethyl)thiazolyl))thiourea (43).** Mp: 162–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.02 (t, 2H), 3.95–4.01 (m, 2H), 7.19–7.33 (m, 6H), 10.31 (s, 1H), 10.49 (s, 1H). Anal. ($C_{13}H_{12}F_3N_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(5-chlorothiazolyl))thiourea (46).** Mp: 163–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.82 (t, 2H), 3.63–3.70 (m, 2H), 7.15–7.30 (m, 5H), 7.39 (s, 1H), 8.41 (s, 1H), 11.60 (broad s, 1H). Anal. ($C_{12}H_{12}ClN_3S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(3-pyrazolyl)thiourea (47).** Mp: 142–144 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.85 (t, 2H), 3.75 (m, 2H), 5.83 (s, 1H), 7.2–7.4 (m, 5H), 7.6 (s, 1H), 9.85 (broad s, 1H), 10.35 (broad s, 1H), 12.4 (broad s, 1H). Anal. ($C_{12}H_{14}N_4S$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(1,3,4-thiadiazolyl))thiourea (48).** Mp: 210–211.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.92 (t, 2H), 3.72–3.84 (m, 2H), 7.18–7.38 (m, 5H), 8.78 (broad s, 1H), 8.92 (s, 1H), 12.35 (broad s, 1H). Anal. ($C_{11}H_{12}N_4S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-pyrazinyl)thiourea (49).** Mp: 142–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.9 (t, 2H), 3.75–3.85 (m, 2H), 7.2–7.4 (m, 5H), 8.05 (d, 1H), 8.5 (s, 1H), 10.95 (broad s, 1H), 11.02 (broad s, 1H). Anal. ($C_{13}H_{14}N_4S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-pyridyl)thiourea (50).** Mp: 142–143 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 3.04 (t, 2H), 4.06 (m, 2H), 6.74 (d, 1H), 6.92 (dd, 1H), 7.26–7.37 (m, 5H), 7.64 (dt, 1H), 7.97 (d, 1H), 8.59 (broad s, 1H), 11.72 (broad s, 1H). Anal. ($C_{14}H_{15}N_3S$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(3-bromopyridyl))thiourea (51).** Mp: 95–96 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.91 (t, 2H), 3.8–3.86 (m, 2H), 7.0–7.4 (m, 1H), 7.18–7.29 (m, 5H), 8.06–8.13 (m, 2H), 8.45 (s, 1H), 11.2 (s, 1H). Anal. ($C_{14}H_{14}BrN_3S$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(5-bromopyridyl))thiourea (52).** Mp: 160–162 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.88 (t, 2H), 3.77–3.82 (m, 2H), 7.05 (d, 1H), 7.18–7.29 (m, 5H), 7.90–7.93 (m, 1H), 8.11 (d, 1H), 10.65 (s, 1H), 11.16 (s, 1H). Anal. ($C_{14}H_{14}BrN_3S$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(5-methylpyridyl))thiourea (53).** Mp: 153–154 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.16 (s, 3H), 2.87 (t, 2H), 3.75–3.82 (m, 2H), 6.99 (d, 1H), 7.16–7.31 (m, 5H), 7.52 (dd, 1H), 7.84 (d, 1H), 10.42 (s, 1H), 11.56 (broad s, 1H). Anal. ($C_{15}H_{17}N_3S$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-benzothiazolyl)thiourea (54).** Mp: 203–207 °C. ^{1}H NMR (300 MHz, $CDCl_3$/DMSO-d_6): 3.05 (t, 2H), 3.95 (m, 2H), 7.2–7.8 (m, 9H), 10.6 (broad s, 1H), 11.7 (broad s, 1H). Anal. ($C_{16}H_{14}N_3S_2$) C, H, N.

***N*-(2-(3-Phenylpropyl)-*N'*-(2-thiazolyl)thiourea (32).** Method ID was followed using the mesylate of 1-phenyl-2-propanol. Mp: 118.5 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.27 (d, 3H), 2.80 (dd, 1H), 3.18 (dd, 1H), 4.75 (m, 1H), 6.79 (d, 1H), 7.23–7.29 (m, 6H). Anal. ($C_{13}H_{15}N_3S_2$) C, H, N.

***N*-(1-(2-Phenylpropyl))-*N'*-(2-thiazolyl)thiourea (33).** Method IA was followed using 1-amino-2-phenylpropane. Mp: 114–116 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.26 (d, 3H), 3.12 (q, 1H), 3.68–3.76 (m, 2H), 7.08 (d, 1H), 7.20–7.37 (m, 6H), 9.70 (broad s, 1H), 11.57 (broad s, 1H). Anal. ($C_{13}H_{15}N_3S_2$) H, N; C: calc, 56.3; found, 55.5.

***N*-(2-Phenethyl)-*N'*-(2-thiazolyl)urea (34).** Method II was followed using 2-phenethyl isocyanate and 2-aminothiazole. Mp: 136.0–136.3 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.75 (t, 2H), 3.37 (q, 2H), 6.53 (t, 1H), 6.99 (d, 1H), 7.18–7.34 (m, 6H), 10.39 (broad s, 1H). Anal. ($C_{12}H_{13}N_3OS$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-(ethoxycarbonyl)thiazolyl))thiourea (45).** Method IA was followed using 1-((2-(4-(ethoxycarbonyl)thiazolyl))thiocarbamoyl)imidazole and 2-(1-phenyl)ethylamine. Mp: 174–175 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.3 (t, 3H), 2.9 (t, 2H), 3.8 (m, 2H), 4.3 (q, 2H), 7.3 (m, 5H), 7.9 s, 1H), 8.7 (broad s, 1H), 12.0 (broad s, 1H). Anal. ($C_{15}H_{17}N_3O_2S_2$) C, H, N.

***N*-(2-Phenethyl)-*N'*-(2-(4-carboxythiazolyl))thiourea (44).** Compound **42** was refluxed for 16 h in 5 M HCl and acetic acid. Standard workup and crystallization from methanol/ethyl acetate afforded **44** in 18% yield. Mp: >230 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.87 (t, 2H), 3.65–3.71 (m, 2H), 7.14–7.26 (m, 6H). HRMS (FAB): *m/e* (M + 1) calcd 309.0527, obs 309.0528.

***N*-(2-(2,6-Difluorophenyl)ethyl)-*N'*-(2-(4-ethylthiazolyl))thiourea (55).** Method IC was followed using 1-((2-(4-ethylthiazolyl))thiocarbamoyl)imidazole and 2-(2,6-difluorophenyl)ethylamine. Crystallization from EtOAc provided the titled compound in a yield of 89%. Mp: 157–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.05 (t, 3H), 2.45 (q, 2H), 2.92 (t, 2H), 3.77 (m, 2H), 6.58 (s, 1H), 7.01 (m, 2H), 7.29 (m, 1H), 9.75 (broad s, 1H), 11.54 (broad s, 1H). Anal. ($C_{14}H_{15}F_2N_3S_2$) C, H, N.

***N*-(2-(2,6-Difluorophenyl)ethyl)-*N'*-(2-(4-cyanothiazolyl))thiourea (56).** Method IC was followed using 1-((2-amino-4-cyanothiazolyl)thiocarbamoyl)imidazole[11] and 2-(2,6-difluorophenyl)ethylamine. Crystallization was from dichloromethane. Mp: 178–179 °C. ^{1}H NMR (250 MHz, $CDCl_3$/CD_3OD): 3.08 (s, 2H), 3.93 (t, 2H), 6.90 (t, 2H), 7.22 (m, 1H), 7.51 (s, 1H). Anal. ($C_{13}H_{10}F_2N_4S_2$) C, H; N: calcd, 17.2; found 16.4.

***N*-(2-(2,6-Difluorophenyl)ethyl)-*N'*-(2-(5-bromopyridyl))thiourea (57).** A modified method I was followed using *N*-(thioimidazolyl)-2-(2,6-difluorophenyl)ethylamine and 2-amino-5-bromopyridine. Crystallization was from a 1:1 mixture of EtOAc and hexanes. Mp: 174–175 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.94–2.98 (m, 2H), 3.77–3.83 (m, 2H), 7.01–7.09 (m, 3H), 7.91–7.95 (m, 1H), 8.11 (s, 1H), 10.68 (s, 1H), 11.20 (s, 1H). Anal. ($C_{14}H_{12}BrF_2N_3S$) C, H, N.

***N*-(2-(2,6-Difluorophenyl)ethyl)-*N'*-(2-(5-methylpyridyl))thiourea (58).** A modified method I was followed using *N*-(thioimidazolyl)-2-(2,6-difluorophenyl)ethylamine and 2-amino-5-methylpyridine. Crystallization was from EtOAc. Mp: 195–196 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 2.16 (s, 3H), 2.96 (t, 2H), 3.80 (m, H), 7.02 (m, 3H), 7.30 (m, 1H), 7.53 (d, 1H), 7.83 (broad s, 1H), 10.44 (broad s, 1H), 11.59 (broad s, 1H). Anal. ($C_{15}H_{15}F_2N_3S$) C, H, N.

***N*-(2-(2-Ethoxy-6-fluorophenethyl))-*N'*-(2-(5-methylpyridyl))thiourea (59).** A modified method I was followed using *N*-(thioimidazolyl)-2-(2-ethoxy-6-fluorophenyl)ethylamine and 2-amino-5-methylpyridine. Mp: 180–181 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.40 (t, 3H), 2.25 (s, 3H), 3.07 (t, 2H), 3.98 (q, 1H), 6.58–6.73 (m, 3H), 7.05–7.22 (q, 1H), 7.41 (d, 1H), 7.83 (s, 1H), 8.65 (broad s, 1H). Anal. ($C_{17}H_{20}FN_3OS$) C, H, N.

***N*-(2-(2-Ethoxy-6-fluorophenethyl))-*N'*-(2-(5-bromopyridyl))thiourea (60).** Method IB was followed using 2-(2-ethoxy-6-fluorophenyl)ethylamine and 1-((2-amino-5-bromopyridyl)thiocarbamoyl)imidazole. Mp: 166 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 1.41 (t, 3H), 3.03 (t, 2H), 3.91 (q, 2H), 4.07 (q, 2H), 6.87 (t, 1H), 7.21 (d, 1H), 7.33 (q, 1H), 8.06 (dd, 1H),

8.18 (d, 1H), 10.80 (s, 1H), 11.23 (s, 1H). Anal. ($C_{16}H_{17}BrFN_3OS$) H, N; C: calc, 48.2; found, 47.7.

***N*-(2-(2-Pyridylethyl))-*N'*-(2-(5-methylpyridyl))thiourea (61).** Thiocarbonyldiimidazole (1.78 g, 10 mmol) and 2-amino-5-methylpyridine (1.58 g, 10 mmol) were dissolved in 15 mL of acetonitrile and stirred at 20 °C for 1 h. 2-(2-Aminoethyl)pyridine (1.22 g, 10 mmol) was added, and the reaction mixture was stirred at 20 °C for 1 h and then at 50 °C for 17 h. After cooling, the formed crystals were collected by filtration and recrystallized from acetonitrile to give 1.30 g (48%) of compound **61**. Mp: 123–125 °C. ^{1}H NMR (250 MHz, DMSO-d_6): 2.2 (s, 3H), 3.1 (t, 2H), 4.0 (m, 2H), 7.0 (d, 1H), 7.2 (m, 1H), 7.3 (d, 1H), 7.6 (m, 1H), 7.7 (m, 1H), 7.8 (m, 1H), 8.6 (m, 1H). Anal. ($C_{14}H_{16}N_4S$) C, H, N.

***N*-(2-(2-Pyridylethyl))-*N'*-(2-(5-bromopyridyl))thiourea (62).** Method IA was followed using 2-(2-aminoethyl)-pyridine and 1-((2-amino-5-bromopyridyl)thiocarbamoyl)imidazole. The base was converted to the HCl salt. Mp: 215–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 3.35 (t, 2H), 4.10 (q, 2H), 7.10 (d, 1H), 7.80 (t, 1H), 7.82–7.90 (d, 1H), 7.97–8.00 (q, 1H), 8.22 (s, 1H), 8.40 (t, 1H), 8.80 (d, 1H), 10.75 (s, 1H), 11.30 (s, 1H). Anal. ($C_{13}H_{14}N_4BrCIS$) C, H, N.

HIV-1 RT Enzyme Assay. The assay has been described.[17] Briefly, the assay used $(rC)_n \cdot (dG)_{12-18}$ as template–primer at saturated concentration (25 μg/mL) and contained 100 mM Tris HCl (pH 7.8), 100 mM KCl, 4 mM dithiothreitol, 4 mM $MgCl_2$, and 250 μg/mL bovine serum albumin. The substrate concentration of tritium-labeled dGTP (35 Ci/mmol) was used close to the K_m value. The reactions were started by addition of enzyme to a final concentration of 300 ng/mL. After 30 min of incubation at 37 °C, 45 μL of the reaction mixture was spotted onto filter disks. The disks were washed with 5% trichloroacetic acid and ethanol. The incorporation of the substrate into template was linear over this time and determined by liquid scintillation counting.

HIV-1 Cell Culture Assay. MT4 cells were adjusted to 2 × 10^5 cells/mL of medium (RPMI 1640 supplemented with 10% FCS, penicillin, and streptomycin) and seeded into 96-well microplates (2 × 10^4 cells/well). The compounds were dissolved in DMSO to 10 mg/mL stock solutions, further diluted in medium, and added in desired concentrations to the cell containing plates. The DMSO concentration in the cell culture assay never exceeded 1%. Finally, the cells were infected with HIV-1, IIIB (10 $TCID_{50}$/well). After 6 days of incubation in a CO_2 atmosphere, the surviving cells were determined by the XTT method.[18] The cytotoxic effect was measured in uninfected cells by the XTT method.

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References

(1) Therapeutic Approaches to HIV. In *Perspectives in Drug Discovery and Design* **1993**, *1*, 1–250.
(2) DeClercq, E. HIV Inhibitors Targeted at the Reverse Transcriptase. *AIDS Res. Hum. Retrovirus* **1992**, *8*, 119–134.
(3) (a) Conolly, K. J.; Hammer, S. M. Antiretroviral Therapy: Reverse Transcriptase Inhibition. *Antimicrob. Agents Chemother.* **1992**, *36*, 245–254. (b) Sandström, E.; Öberg, B. Antiviral Therapy in Human Immunodeficiency Virus Infections. *Drugs* **1993**, *45*, 488–508 and 637–653.
(4) (a) Kedar, P. S.; Abbotts, J.; Kovacs, T.; Lesiak, K.; Torrence, P.; Wilson, S. H. Mechanism of HIV Reverse Transcriptase: Enzyme-Primer Interaction As Revealed through Studies of a dNTP Analogue, 3'-Azido-dTTP. *Biochemistry* **1990**, *29*, 3903–3611. (b) Bridges, E. G.; LeBoeuf, R. B.; Weidner, D. A.; Sommadossi, J.-P. Influence of template primary structure on 3'-azido-3'-deoxythymidine triphosphate incorporation into DNA. *Antiviral Res.* **1993**, *21*, 93–102.
(5) Richman, D. D. Resistance of Clinical Isolates of Human Immunodeficiency Virus to Antiretroviral Drugs. *Antimicrob. Agents Chemother.* **1993**, *37*, 1207–1213.
(6) (a) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Gelder, J. V.; Wostenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Selective Inhibition of HIV-1 Replication In Vitro by a Novel Series of TIBO Derivatives. *Nature* **1990**, *343*, 470–474. (b) Kukla, M. J.; Breslin, H. J.; Diamond, C. J.; Grous, P. P.; Ho, C. Y.; Miranda, M.; Rodgers, J. D.; Sherill, R. G.; De Clercq, E.; Pauwels, R.; Andries, K.; Moens, L. J.; Janssen, M. A. C.; Janssen, P. A. J. Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo-[4,5,1-*jk*][1,4]benzodiazepin-2(1H)-one (TIBO) Derivatives. *J. Med. Chem.* **1991**, *34*, 3187–3197.
(7) Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. A Novel Lead for Specific Anti-HIV-1 Agents: 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-thymine. *J. Med. Chem.* **1989**, *32*, 2507–2509.
(8) Hargrave, K. H.; Proudfoot, J. R.; Grozinger, K. G.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Mauldin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Pal, K.; Skiles, J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Cambell, S. J.; Rosenthal, A. S.; Adams, J. Novel Non-Nucleoside Inhibitors of HIV-1 Reverse Transcriptase. 1. Tricyclic Pyridobenzo- and Dipyridodiazepinones. *J. Med. Chem.* **1991**, *34*, 2231–2241.
(9) (a) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. Pyridinone derivatives: Specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6863–6867. (b) Saari, W. S.; Hoffman, J. M.; Wai, J. S.; Fisher, T. E.; Rooney, C. S.; Smith, A. M.; Thomas, C. M.; Goldman, M. E.; O'Brien, J. A.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Emini, E. A.; Stern, A. M.; Anderson, P. S. 2-Pyridinone Derivatives: A New Class of Nonnucleoside, HIV-1 Specific Reverse Transcriptase Inhibitors. *J. Med. Chem.* **1991**, *34*, 2922–2925.
(10) Romero, D. L.; Busso, M.; Tan, C.-K.; Reusser, F.; Palmer, J. R.; Poppe, S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8806–8810.
(11) Lind, P. T.; Morin, J. M.; Noreen, R.; Ternansky, R. J. New thiourea derivatives inhibit HIV replication – for treating infections caused by HIV and related viruses, and AIDS. Medivir AB. Patent application WO9303022-A1.
(12) Ågren, C.; Backbro, K.; Bell, F. W.; Cantrell, A. S.; Clemens, M.; Colacino, J.; Deeter, J. B.; Engelhardt, J. A.; Högberg, M.; Jaskunas, S. R.; Johansson, N.-G.; Jordan, C. L.; Kasher, J. S.; Kinnick, M. D.; Lind, P.; Lopez, C.; Morin, J. M., Jr.; Muesing, M. A.; Noreen, R.; Öberg, B.; Paget, C. J.; Parrish, C.; Pranc, P.; Rippy, M.; Rydergård, C.; Sahlberg, C.; Swanson, S.; Tang, J.; Ternansky, R. J.; Unge, T.; Vasileff, R. T.; West, S. J.; Vrang, L.; Zhang, H.; Zhou, X.-X. The PETT Series: A New Class of Potent Nonnucleoside Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. *Antimicrob. Agents Chemother.* **1995**, *39*, 1329–1335.
(13) Lind, P.; Noreén, R.; Deeter, J. B. Conformational Studies on a New Class of Potent Non-Nucleoside Reverse Transcriptase Inhibitors. VIth International Conference on Antiviral Research, Venice, Italy, April 25–30, 1993; Abstract 70.
(14) PCMODEL; molecular modeling software available from Serena Software, Bloomington, IN.
(15) For example: Holmes, D. S.; Bethell, R. C.; Cammack, N.; Clemens, I. R.; Kitchin, J.; McMeekin, P.; Mo, C. L.; Orr, D. C.; Patel, B.; Paternoster, I. L.; Storer, R. Synthesis and Structure-Activity Relationships of a Series of Penicillin-Derived HIV Proteinase Inhibitors Containing a Stereochemically Unique Peptide Isostere. *J. Med. Chem.* **1993**, *36*, 3129–3136.
(16) In *Vogel's Textbook of Practical Organic Chemistry*, 4th ed.; Longman: London, 1978; p 796.
(17) Zhang, H.; Vrang, L.; Unge, T.; Öberg, B. Characterization of HIV reverse transcriptase with Tyr181→Cys and Leu100→Ile mutations. *Antiviral Chem. Chemother.* **1993**, *4*, 301–308.
(18) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-Formazan Assay for HIV-1 Cytopathic Effects: Application to High-Flux Screening of Synthetic and Natural Products for AIDS-Antiviral Activity. *J. Nat. Cancer Inst.* **1989**, *81*, 577–586.

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