Synthesis and anti-HIV activity evaluation of new phenyl ethyl thiourea (PET) derivatives

Roberta Ettari,[a]* Andrea Pinto,[b] and Nicola Micale[a]

[a]Dipartimento Farmaco-Chimico, Università degli Studi di Messina, Viale Annunziata 98168, Messina, Italy
[b]Dipartimento di Scienze Farmaceutiche "Pietro Pratesi", via Mangiagalli, 25 – 20133 Milano, Italy
E-mail: rettari@pharma.unime.it

Abstract
This manuscript describes the synthesis of a new series of phenyl ethyl thiourea (PET) derivatives, with the aim to extend the SAR studies of the well known PET molecules endowed with anti-HIV activity. Preliminary results indicated that the synthesized compounds possess low anti-HIV activity.

Keywords: Phenyl ethyl thiourea derivatives, antiviral activity

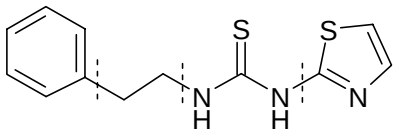
Introduction

Human immunodeficiency virus type-1 (HIV-1) is the causative agent for the transmission and development of the acquired immunodeficiency syndrome (AIDS). AIDS remains one of the most urgent world health problems, being the leading cause of death in Africa and the fourth worldwide.[1]

Even if there is no definitive cure for HIV infection, a number of drugs slow or halt disease progression. However, HIV can rapidly become resistant to any single antiretroviral drug, therefore a combination of three or more drugs are usually required to effectively suppress the virus. The highly active antiretroviral therapy (HAART)[2] consists of the combination of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs) with non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs).[2] In this context, while NRTIs (e.g. zidovudine, lamivudine, abacavir, tenofovir) act competitively at the catalytic site of the RT as DNA chain-terminating analogues of the natural deoxynucleoside triphosphates, NNRTIs (e.g. nevirapine, efavirenz) bind to an allosteric site located about 10 Å from the catalytic site thus leading to a noncompetitive inhibition of the enzyme.[3] The latter ones, despite their lower toxicity with respect to NRTIs, are particularly vulnerable to the development of viral

resistance caused by mutations in RT that can retain viable enzymatic function. Therefore significant efforts in this field have focused on developing new NNRTIs with a favourable profile of resilience to many drug resistant mutations.[4]

Studies that have been made in the Lilly laboratories on compounds of molecular simplification of TIBO (4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one) series, evidenced a new lead the *N*-(2-phenylethyl)-*N*'-(2-thiazolyl)thiourea named LY73497 (Figure 1) which possesses a phenyl ethyl thiourea (PET) motif.[5-6] This compound showed a significant inhibition value against the RT.



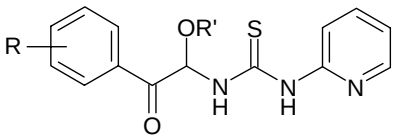
LY73497

Figure 1. Structure of *N*-(2-phenylethyl)-*N*'-(2-thiazolyl)thiourea.

Basic SAR studies were performed by notionally dividing LY73497 into four portions and independently varying each one of them.[5] The results of these SAR studies provided evidence of important features for the interaction with RT: i) small electron withdrawing substituents (F, Cl) can be favorably introduced on the phenyl ring especially in ortho position; ii) the ethyl linker is optimal for the activity; iii) the urea derivatives are less active than the corresponding thiourea derivatives; iv) the thiazolyl nucleus can be replaced successfully with other heterocyclic rings such as pyridine, pyrazine, benzothiazole, imidazole, triazole.

The aim of this work was to synthesize new PET derivatives **1** (Figure 2), in which important requirements for the anti-HIV activity such as two aromatic systems, benzene and pyridine, and the thiourea moiety were maintained, while the ethylene bridge was modified by introducing a carbonyl group and a hydroxyl function. This modification took into account the work of Högberg et al.[7] in which the introduction of a carbonyl group in proximity of the phenyl ring resulted in an enhanced the anti-HIV activity both in enzymatic assays and in cell cultures. The importance of this substitution on the ethyl linker was recently confirmed by QSAR studies.[8]

In compounds of series **1** the introduction of a hydroxyl group on the ethyl linker should reduce the conformational freedom by forming an intramolecular hydrogen bond with the sulfur atom. Thus, a series of ethers (series **2**, Figure 2) were also synthesized to compare them with derivatives of series **1** and confirm our hypothesis. On the aromatic ring various halogens atoms were introduced, such as chlorine and fluorine, in agreement with the SAR studies on PET derivatives.

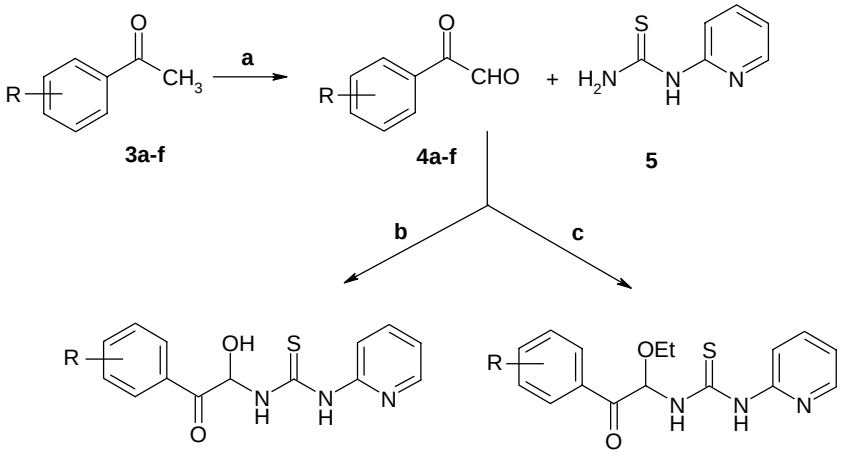


1, R = Cl, F, R' = H
2, R = Cl, F, R' = Et

Figure 2. Structure of PET derivatives **1-2**.

Results and Discussion

The synthesis of N_1-(1-hydroxy-2-oxo-2-phenylethyl)-N_3-pyridin-2-yl-thioureas **1a-f** was realized by nucleophilic addition of 2-pyridylthiourea **5** to the variously substituted 2-oxophenyl-acetaldehydes **4a-f** (Scheme 1), prepared by easy oxidation of the corresponding acetophenones **3a-f** with selenium dioxide according to a previously reported procedure.[9] The reaction between **4a-f** and **5** has been promoted by catalytic amounts of the mixture acetic acid/hydrochloric acid in dioxane. By maintaining unchanged the reaction conditions and switching the solvent from dioxane to ethanol, it was possible to obtain N_1-(1-ethoxy-2-oxo-2-phenylethyl)-N_3-pyridin-2-yl-thioureas **2a-f**, realistically due to the addition of ethanol to the N-aroylmethylene thiourea intermediate.



3a-f **4a-f** **5**

1a-f **2a-f**

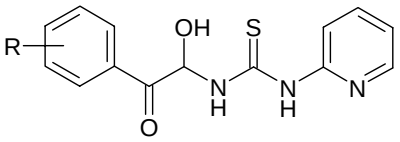
	R		R
a	2-Cl	**d**	2-F
b	3-Cl	**e**	4-F
c	4-Cl	**f**	2,6-F

Scheme 1. (a) SeO_2, H_2O/dioxane, 60°C, 12 h; (b) CH_3COOH/HCl, dioxane, reflux, 6-8 h; (c) CH_3COOH/HCl, ethanol, reflux, 6-8 h.

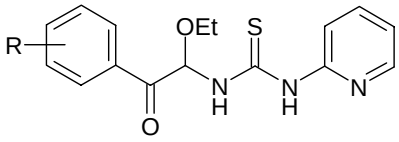
Both series **1a-f** and **2a-f** have been tested to evaluate their anti-HIV activity at National Cancer Institute (NCI) of Bethesda, USA. The assay was carried on MT-4 cell lines exposed to the strain HIV-1 III B in the presence of compounds **1a-f** and **2a-f** to determine the concentration that reduces to the 50% (EC_{50}) the cytopathic effect induced by HIV-1 in MT-4 cells, the cytotoxicity (CC_{50}) and the selectivity index that is the ratio CC_{50}/EC_{50}.

Only the derivatives **1a**, **1c**, **2a** and **2c** showed a moderate anti-HIV activity (Table 1), with an EC_{50} value of 118.08 μM and 166.25 μM for the hydroxyl derivatives **1a** and **1c**, and of 67.05 μM and 69.48 μM for the O-ethyl derivatives **2a** and **2c** respectively. All the other compounds protected the MT-4 cells from the cytopathic effect in a low percentage (8-22%). Generally, all compounds showed a very low cytotoxicity profile >400 μM for the derivatives **1a-f** and >200 μM for O-ethyl analogues **2a-f**.

Table 1. Anti-HIV activity (EC_{50}), cytotoxicity (CC_{50}), and selectivity index in MT-4 cells of compounds **1** and **2**.



1



2

Compd.	R	EC_{50} (μM)[a] or protection %	CC_{50} (μM)[b]	*SI*[c]
1a	2-Cl	118.08	>400	>3
1b	3-Cl	13%	>400	
1c	4-Cl	166.25	>400	>2
1d	2-F	8%	>400	
1e	4-F	12%	>400	
1f	2,6-F	16%	>400	
2a	2-Cl	67.05	>200	
2b	3-Cl	15%	>200	
2c	4-Cl	69.48	210	3
2d	2-F	21%	>200	
2e	4-F	18%	>200	
2f	2,6-F	22%	>200	
LY73497		1.3[5]	>380[5]	

[a]Concentration that reduces to the 50% the cytopathic effect induced by HIV-1 in MT-4 (for details, see *Exper. Part*).

[b]Concentration that reduces to the 50% the growth of the MT-4 cells.

[c] Selectivity index: ratio CC_{50}/EC_{50}.

The obtained results suggested that the presence of a chlorine atom at position 2 or 4 of the phenyl ring positively affected the anti-HIV activity of this new series of PET derivatives. The slightly higher activity of O-ethyl derivatives **2a** and **2c** with respect to the corresponding hydroxyl derivatives **1a** and **1c** indicated that a hydrophobic character of the linker is favourable for antiviral activity. This is confirmed when we compare our results either with the LY73497 analogues or the series of compounds with the carbonyl function next to phenyl ring.[5,7]
In conclusion, this work may offer an additional contribution to extend the SAR studies of the well known PET derivatives endowed with anti-HIV activity, useful for further developments of this series.

Experimental Section

General. Melting points were determined on a *Kofler* hot-stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a *Carlo Erba Model 1106* elemental analyzer; the results were correct within ±0.4% of the calc. values. *Merck* silica-gel *60* F_{254} plates were used for anal. TLC, and column chromatography (CC) was performed on *Merck* silica gel *60* (70-230 mesh). ^{1}H-NMR Spectra were recorded in $CDCl_3$ on a *Varian Gemini-300* spectrometer; chemical shifts δ in ppm rel. to Me_4Si, coupling constants *J* in Hz.

General procedure for the synthesis of substituted 2-oxophenyl-acetaldehydes 4
Selenium dioxide (11 mmol) was solubilized in a mixture water/dioxane (26 mL, 4/96) at 60°C, when the solution was homogenous, it was cooled and the required acetophenone **3** (11 mmol) was added. The reaction mixture was heated at 100°C for 12 h filtered on celite and then evaporated to dryness. The residue was diluted with ethyl acetate, washed with water, then saturated $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated and the residue containing the title compound in quantitative yields (as observed by TLC) was used for the next step without any further purification.

General procedure for the synthesis of N_1-[2-aryl-1-hydroxy-2-oxoethyl]-N_3-pyridin-2-yl-thioureas 1a-f
2-Pyridylthiourea **5** (200 mg, 1.3 mmol)**,** acetic acid (0.6 mL) and hydrochloric acid (0.6 mL) were added to a solution of variously substituted 2-oxophenyl-acetaldehydes **4a-f** [9] (1.5 mmol) in 15 mL of dioxane. The reaction mixture was stirred at 100 °C for 8 h, then cooled and evaporated to dryness. The residue was purified by column chromatography using silica gel as absorbent with 9:1 $CHCl_3$/MeOH as eluent to give the title compounds.
N_1-[2-(2-Chlorophenyl)-1-hydroxy-2-oxoethyl]-N_3-pyridin-2-yl-thiourea 1a. Yield: 53%. Mp 157-159 °C. ^{1}H NMR ($CDCl_3$): 6.40 (d, 1H, *J* = 7.72, CH), 7.04–8.24 (m, 8H, arom. H), 10.86 (s, 1H, H-N(3)), 12.77 (d, 1H, *J* = 7.72, H-N(1)). Anal. calc. for $C_{14}H_{12}ClN_3O_2S$ (321.79): C, 52.26; H, 3.76; N, 13.06. Found: C, 51.90; H, 4.10; N, 12.76.

***N*$_1$-[2-(3-Chlorophenyl)-1-hydroxy-2-oxoethyl]-*N*$_3$-pyridin-2-yl-thiourea 1b.** Yield: 31%. Mp dec. > 193 °C ^{1}H NMR ($CDCl_3$): 5.48 (d, 1H, *J* = 6.20, CH), 7.01–8.28 (m, 8H, arom. H), 10.48 (s, 1H, H-N(3)), 12.33 (d, 1H, *J* = 6.20, H-N(1)). Anal. calc. for $C_{14}H_{12}ClN_3O_2S$ (321.79): C, 52.26; H, 3.76; N, 13.06. Found: C, 52.21; H, 3.59; N, 13.15.

N$_1$-[2-(4-Chlorophenyl)-1-hydroxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 1c. Yield: 37%: Mp 160-162 °C. ^{1}H NMR ($CDCl_3$): 5.94 (d, 1H, *J* = 7.14, CH), 7.05–8.31 (m, 8H, arom.H), 10.85 (s, 1H, H-N(3)), 12.50 (d, 1H, *J* = 7.14, H-N(1)). Anal. calc. for $C_{14}H_{12}ClN_3O_2S$ (321.79): C, 52.26; H, 3.76; N, 13.06. Found: C, 52.60; H, 3.46; N, 13.40.

N$_1$-[2-(2-Fluorophenyl)-1-hydroxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 1d. Yield: 61%. Mp 188-191 °C. ^{1}H NMR ($CDCl_3$): 5.80 (d, 1H, *J* = 6.59, CH), 7.01–8.25 (m, 8H, aromH), 10.47 (s, 1H, H-N(3)), 12.43 (d, 1H, *J* = 6.59, H-N(1)). Anal. calc. for $C_{14}H_{12}FlN_3O_2S$ (305.33): C, 55.07; H, 3.96; N, 13.76. Found: C, 54.87; H, 4.16; N, 13.10.

N$_1$-[2-(4-Fluorophenyl)-1-hydroxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 1e. Yield: 25%. Mp 152-155 °C. ^{1}H NMR ($CDCl_3$): 5.54 (d, 1H, *J* = 6.92, CH), 6.99–8.27 (m, 8H, arom.H), 10.44 (s, 1H, H-N(3)), 12.35 (d, 1H, *J* = 6.92, H-N(1)). Anal. calc. for $C_{14}H_{12}FlN_3O_2S$ (305.33): C, 55.07; H, 3.96; N, 13.76. Found: C, 54.91; H, 4.27; N, 13.45.

N$_1$-[2-(2,6-Difluorophenyl)-1-hydroxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 1f. Yield: 27%. Mp dec. > 215 °C. ^{1}H NMR ($CDCl_3$): 5.83 (d, 1H, *J* = 6.71, CH), 6.90–8.19 (m, 7H, arom.H), 10.37 (s, 1H, H-N(3)), 12.34 (d, 1H, *J* = 6.71, H-N(1)). Anal. calc. for $C_{14}H_{12}FlN_3O_2S$ (323.32): C, 52.01; H, 3.43; N, 13.00. Found: C, 51.66; H, 3.58; N, 13.36.

General Procedure for the Synthesis of the N$_1$-[2-aryl-1-ethoxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thioureas 2a-f. The synthesis of compounds **5a-f** was realized in an analogous manner to that reported for compounds **4a-f,** using ethanol as solvent instead of dioxane. The pure title compound was recovered by recrystallisation from ethanol.

N$_1$-[2-(2-Chlorophenyl)-1-ethoxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 2a. Yield: 27%. Mp 119-121 °C. ^{1}H NMR ($CDCl_3$): 1.23 (t, 3H, *J* = 7.2, Me), 4.24 (m, 2H, CH_2), 6.57 (d, 1H, *J* = 7.69, CH), 6.72–8.25 (m, 8H, arom.H), 8.70 (s, 1H, H-N(3)), 12.93 (d, 1H, *J* = 7.69, H-N(1)). Anal. calc. for $C_{16}H_{16}ClN_3O_2S$ (349.89): C, 54.93; H, 4.61; N, 12.01. Found: C, 54.73; H, 4.96; N, 11.76.

N$_1$-[2-(3-Chlorophenyl)-1-ethoxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 2b. Yield: 29%. Mp 128-130 °C. ^{1}H NMR ($CDCl_3$): 1.15 (t, 3H, J = 7.14, Me), 4.15 (m, 2H, CH_2), 6.02 (d, 1H, *J* = 6.86, CH), 7.07-8.25 (m, 8H, arom.H), 10.93 (s, 1H, H-N(3)), 12.80 (d, 1H, *J* = 6.86, H-N(1)). Anal. calc. for $C_{16}H_{16}ClN_3O_2S$ (349.89): C, 54.93; H, 4.61; N, 12.01. Found: C, 54.67; H, 4.86; N, 12.41.

N$_1$-[2-(4-Chlorophenyl)-1-ethoxy-2-oxoethyl]-N$_3$-pyridin-2-yl-thiourea 2c. Yield: 75%. Mp 139-142 °C. ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, *J* = 6.95, Me), 4.22 (m, 2H, CH_2), 5.74 (d, 1H, *J* = 6.95, CH), 7.31–8.15 (m, 8H, arom.H), 10.42 (s, 1H, H-N(3)), 14.33 (d, 1H, *J* = 6.95, H-N(1)). Anal. calc. for $C_{16}H_{16}ClN_3O_2S$ (349.89): C, 54.93; H, 4.61; N, 12.01. Found: C, 54.83; H, 4.44; N, 12.38.

Synthesis of N_1-[2-(2-Fluorophenyl)-1-ethoxy-2-oxoethyl]-N_3-pyridin-2-yl-thiourea 2d. Yield: 26%. Mp 152-153 °C. ^{1}H NMR ($CDCl_3$): 1.23 (t, 3H, *J* = 6.95, Me), 4.23 (m, 2H, CH_2), 6.42 (d, 1H, *J* = 7.32, CH), 6.64–8.29 (m, 8H, arom.H), 12.80 (d, 1H, *J* = 7.32, H-N(1)). Anal. calc. for $C_{16}H_{16}FN_3O_2S$ (333.39): C, 57.64; H, 4.84; N, 12.60. Found: C, 57.94; H, 4.50; N, 12.20.

N_1-[2-(4-Fluorophenyl)-1-ethoxy-2-oxoethyl]-N_3-pyridin-2-yl-thiourea 2e. Yield: 88% Mp 133-135 °C. ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, *J* = 7.1, Me), 4.24 (m, 2H, CH_2), 6.11 (d, 1H, *J* = 7.0, CH), 6.68–8.29 (m, 8H, arom.H), 8.38 (s, 1H, H-N(3)), 12.76 (d, 1H, *J* = 7.0, H-N(1)). Anal. calc. for $C_{16}H_{16}FN_3O_2S$ (333.39): C, 57.64; H, 4.84; N, 12.60. Found: C, 57.99; H, 4.53; N, 12.35.

N_1-[2-(2,6-Difluorophenyl)-1-ethoxy-2-oxoethyl]-N_3-pyridin-2-yl-thiourea 2f. Yield: 70%. Mp 177-180 °C. ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, *J* = 7.1, Me), 4.24 (m, 2H, CH_2), 6.11 (d, 1H, *J* = 7.0, CH), 6.68–8.29 (m, 8H, arom.H), 8.38 (s, 1H, H-N(3)), 12.76 (d, 1H, *J* = 7.0, H-N(1)). Anal. calc. for $C_{16}H_{15}F_2N_3O_2S$ (351.38): C, 54.69; H, 4.30; N, 11.96. Found: C, 54.91; H, 4.62; N, 11.80.

Pharmacology. The *in vitro* anti-HIV drug testing system was performed in the National Cancer Institute's Developmental Therapeutics Program, AIDS antiviral screening program, according to reported procedures.[10] The assay involves the killing of T_4 lymphocytes by HIV-1. T_4 lymphocytes (CEM-SS cell line) are exposed to HIV-1 (RF strain) at a multiplicity of infection (MOI) of approximately 0.05. Each agent, dissolved in dimethyl sulfoxide, was added at varying concentrations ranging from 10^{-9} to 10^{-5} M. Uninfected cells with the test compound serve as toxicity control, and infected and uninfected cells without the compound serve as basic controls. Activity is expressed as the effective concentration 50% (EC_{50}) which represents the concentration of each compound resulting in a 50% reduction of the viral cytopathic effect. The 50% inhibitory concentration (CC_{50}) represents the toxic concentration of drug resulting in 50% growth inhibition of normal, uninfected cells. The selectivity index (SI) is determined by dividing CC_{50} by EC_{50}.

Acknowledgements

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