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Original article

Parallel synthesis, molecular modelling and further structure–activity relationship studies of new acylthiocarbamates as potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

The structure–activity relationships (SARs) of acylthiocarbamates (ATCs), a new class of non-nucleoside HIV-1 reverse transcriptase inhibitors, have been expanded. Sixty-six new analogues were prepared by parallel solution-phase synthesis. In general, the potency of new ATCs was better than that of the first series and *O*-[2-phthalimidoethyl] 4-chlorophenyl(3-nitrobenzoyl) thiocarbamate turned out to be the most potent ATC so far synthesized ($EC_{50} = 1.5$ nM). Several ATCs were active at micromolar concentrations against HIV-1 strains carrying the RT Y181C mutation and one of them was also moderately active against the K103R variant. Docking simulations were carried out to rationalize the most relevant SARs.

1. Introduction

HIV-1 reverse transcriptase (RT) catalyses the conversion of a single-stranded RNA into a double-stranded DNA that is then integrated into the host cells' genome. Due to its essential role in the HIV-1 life-cycle, RT is a primary target for the highly active antiretroviral therapy (HAART). Non-nucleoside inhibitors (NNRTIs) [1–9] are chemically diverse and selective RT targeting agents that lock this enzyme in an inactive form [4] by binding to an allosteric hydrophobic pocket (namely, non–nucleoside inhibitor binding site, NNBS) located about 10 Å far from the polymerase active site.

O-(2-Phthalimidoethyl)-*N*-aryl-*N*-acylthiocarbamates (ATCs) have been recently identified [10] as a new class of HIV-1 NNRTIs, structurally related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) [11–19] and thiocarbamate (TC) [20–23] derivatives (Fig. 1). ATCs can be retrosynthetically fragmented into three portions (namely, **a**–**c**. Fig. 1B). Previous structure–activity relationship (SAR) studies [10], mainly based on modifications of the **b** substructure of lead **I**, had identified the *N*-phenyl *para* substitution as a key feature to gain potent inhibitors (Fig. 1B).

With the aim at acquiring additional key elements to maximize the activity against wild-type RT and some clinically relevant RT mutations, a new series of ATCs was designed. Initially, the SAR strategy was focused on the variation of the substructure **c** [mono- (**1**–**16**), di-substituted (hetero)aroyl (**19**–**47**) and bicyclic (hetero)aroyl (**48**–**50**) groups] by keeping constant portion **a** (2-phthalimidoethyl) and the *para*-substitution pattern (halide, methyl) of portion **b**. Analogues **17** and **18** were synthesized to evaluate the effect of portion **c** polysubstitution on the activity of *N*-unsubstituted phenyl ATCs. Then, some of the most favourable *N*-phenyl(4-chlorophenyl, 4-nitrophenyl) and acyl(3-nitrobenzoyl, 4-chlorobenzoyl, 2-thenoyl, 2-chloronicotinoyl) substructures were assembled with portion **a** carrying modifications on the ethyl linker (**51**–**54**) and on the phthalimide phenyl ring (**55**–**60**). Finally, we evaluated the influence of the *N*-phenyl ring di- and tri-chlorosubstitution on the anti-HIV-1 activity by keeping constant portion **a** (2-phthalimidoethyl) and embedding two of the most promising acyl moieties (2-furoyl, 3-nitrobenzoyl) (**61**–**66**).

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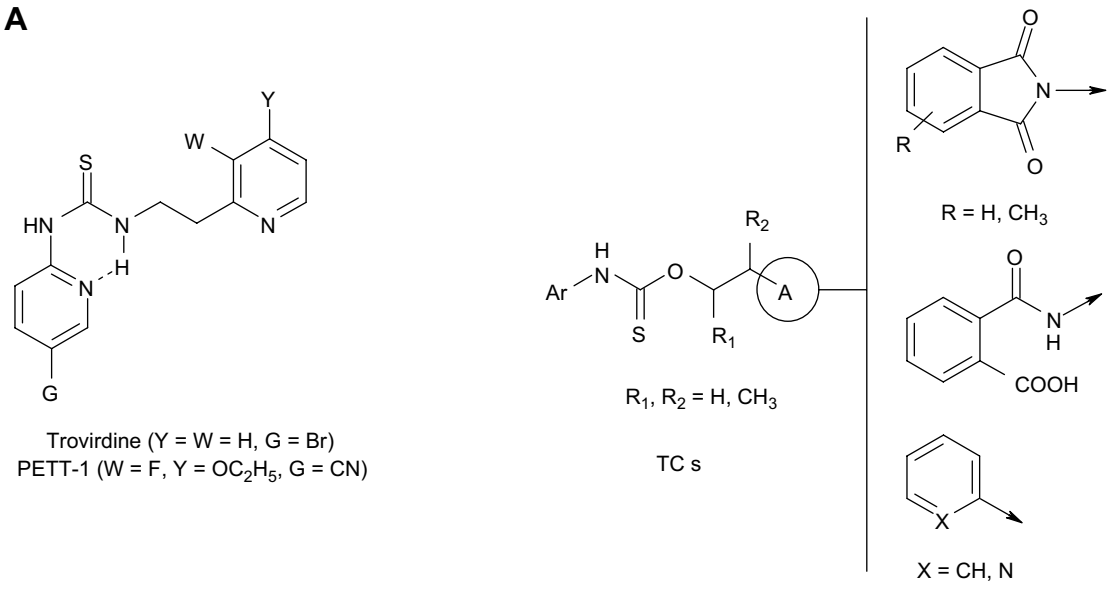


	X	(Het)Ar-CO	EC_{50} (μM)
I	H	benzoyl	0.4
II	Cl	benzoyl	0.1
III	Cl	4-chlorobenzoyl	0.025
IV	Cl	2-furoyl	0.007
V	Cl	2-thenoyl	0.008
VI	Cl	2-chloronicotinoyl	0.005
VII	Cl	6-chloronicotinoyl	0.006

Fig. 1. A] PETT and TC [20–23] derivatives structurally related to ATCs. B] Molecular portions, chemical structure and antiretroviral activity of previously prepared ATCs **I–VII** [10].

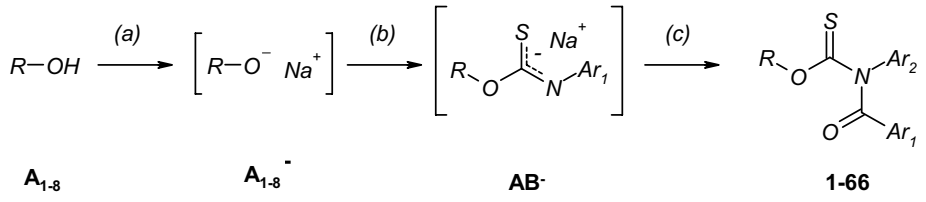
2. Chemistry

The synthesis of ATCs **1–66** was carried out by the parallelization of the previously reported highly convergent three-step one-pot solution-phase protocol [10]. As shown in Scheme 1, starting alcohols $\mathbf{A_{1-8}}$ (Fig. 2A) were transformed into the corresponding alcoholates $\mathbf{A}^-$ in the presence of sodium hydride in anhydrous aprotic solvents (DMF or pyridine) and condensed *in situ* with the proper isothiocyanate **B** (Fig. 2B). The sodium thiocarbamate adducts $\mathbf{AB}^-$ were subsequently coupled with acyl chlorides **C** (Fig. 2C) in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) to afford the desired products. Two synthetic variants (namely, procedures P_1 and P_2, see Section 6) were adopted to obviate the different reactivity of key intermediates $\mathbf{A}^-$ and $\mathbf{AB}^-$ vs building blocks **B** and **C**. Thus, ATCs **1–49**, **52–57** and **59** were prepared in anhydrous pyridine stirring the mixtures overnight at rt (procedure P_1). For derivatives **50**, **51**, **58**, **60–66** (procedure P_2), dry DMF was used as reaction medium and different temperatures and times were employed to increase the product yields. The overall yields for ATCs **1–66** ranged from 12 to 88% (see Section 6).

The most relevant features of this parallel methodology are: i) no intermediate needs to be isolated; ii) high atom economy is realized, as only one molecule of HCl is formally lost in the whole process (this allows the incorporation into the products of all the chemical features of the commercially available or readily accessible building blocks used); iii) minimal sample handling is required during the purification protocol; iv) the modular character of the synthesis allows the independent variation of portions **a–c**.

3. Biological results and discussion

ATCs **1–66** were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells using **Trovirdine** as the reference molecule (Tables 1–5). Selected analogues were screened in enzymatic assays against HIV-1 wt RT, using **Efavirenz** as control compound (Table 6). The most potent derivatives were also tested against the clinically



Scheme 1. General procedure for the preparation of ATCs **1–66** by parallel, convergent, one-pot solution-phase synthesis. *Reaction conditions*: (a) NaH, dry DMF or dry pyridine, 0 °C or rt; (b) Ar_1–NCS ($\mathbf{B_{1-12}}$), 0 °C or rt; (c) TMEDA then Ar_2COCl ($\mathbf{C_{1-33}}$), rt or heating. The structures of alcohols $\mathbf{A_{1-8}}$, isothiocyanates $\mathbf{B_{1-12}}$ and acyl chlorides $\mathbf{C_{1-33}}$ are listed in Fig. 2.