Med Chem Res
DOI 10.1007/s00044-016-1731-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Anti-human immunodeficiency activity of novel 2-arylpyrrolidine analogs

Ashot H. Martirosyan[1] · Sahak P. Gasparyan[1] · Marina V. Alexanyan[1] · Gohar K. Harutyunyan[1] · Henry A. Panosyan[2] · Raymond F. Schinazi[3]

Received: 2 July 2016 / Accepted: 7 October 2016

Abstract A series of 26 new compounds were synthesized and screened for their anti-human immunodeficiency virus-1 and cytotoxicity activity. Of these, 14 were found to be inhibitors of human immunodeficiency virus replications in primary human lymphocytes with 50 % effective concentration values <20 μM. Moreover, most of the compounds were cytotoxic to human lymphocytes, CEM, and Vero cells. Our structure activity relationship study identified different patterns. Compounds **2g–j** and **4** (whose structure is closer to the loviride structure) were very potent. Comparing the activity of the compounds containing the 2-aryl substituents, we observed that compounds with benzyloxyphenyl groups were more potent. Compounds in which the 1-aryl moiety contained methyl group in 4- or 3,5-positions also showed high activity. In the series of compounds containing the nitrile, amine, and amide groups, we observed a decrease in activity with $CN > NH_2 > C(O)NH_2$. The difference of activity between the 5-membered and 4-membered rings compounds was not significant. This initial information could be used to design improved anti-human immunodeficiency virus compounds in this class.

Keywords Pyrrolidinecarbonitriles · Aminomethylpyrrolidines · Anti-HIV-1 · Cytotoxicity activity

Introduction

Acquired immunodeficiency syndrome (AIDS) has become the leading pandemic disease, and the cause of death worldwide. Human immunodeficiency virus (HIV) is the etiological agent of AIDS. Following infection, this retrovirus uses three key enzymes to complete its replication cycle: the reverse transcriptase (RT), the integrase, and the protease (Sarafianos et al. 2004).

The first RT inhibitors approved were nucleoside derivatives (NRTIs), which compete as 5′-triphosphates with normal nucleoside substrates for incorporation into the viral genome, thus behaving as chain terminators. Unlike nucleoside analogs, non-nucleoside RT inhibitors (NNRTIs) bind in a non-competitive manner to a specific pocket of the HIV-1 RT, which is closely associated with, but distinct from the substrate binding site, altering its ability to function. NNRTIs gained the greatest importance because of their specificity and low cytotoxicity. All NNRTIs bind to a hydrophobic pocket near the polymerase active site. NNRTIs were found to be a more potent class of

Electronic supplementary material The online version of this article (doi:10.1007/s00044-016-1731-7) contains supplementary material, which is available to authorized users.

✉ Sahak P. Gasparyan
g_sahak@yahoo.com
Raymond F. Schinazi
rschina@emory.edu

[1] Scientific and Technological Centre of Organic and Pharmaceutical Chemistry, Institute of Fine Organic Chemistry, National Academy of Sciences of RA, Azatutyan Avenue 26, Yerevan 0014, Armenia

[2] Scientific and Technological Centre of Organic and Pharmaceutical Chemistry, Molecule Structure Research Centre, National Academy of Sciences of RA, Azatutyan Avenue 26, Yerevan 0014, Armenia

[3] Center for AIDS Research, Laboratory of Biochemical Pharmacology, Emory University School of Medicine, 1760 Haygood Drive, Atlanta, GA 30322, USA

Published online: 17 October 2016

compounds than the NRTIs and nucleotide RT inhibitors because they differ structurally from the nucleoside analogs. NNRTIs do not interfere with the human cell cycle and are specific inhibitors of HIV-1 RT (Prajapati et al. 2009).

The era of NNRTIs began two decades ago, with the discovery of HEPT and TIBO, two specific inhibitors of the HIV RT. Shortly thereafter, loviride, a representative of the α-anilinophenylacetamides (α-APA) family, was discovered. With the discovery of α-APAs, the era of flexible derivatives started. These exhibited 50 % effective concentration (EC_{50}) values in the nanomolar range. The most active compound in this series is loviride with an EC_{50} of 13 nM, and an inhibitory concentration 10,000-fold less than the cytotoxic concentration (Fig. 1) (De Corte 2005; Pauwels et al. 1993).

The simplicity of its structure and the relative ease of its synthesis made the α-APA series attractive for lead optimization. The compounds in this series were found to bind to the allosteric pocket of RT (Ding et al. 1995).

The purpose of our studies was to discover new antiviral compounds, identify fragments responsible for biological/antiviral/anti-HIV activity in the synthesized compounds to efficiently design and synthesize compounds of this class with high activity.

Herein, we report the synthesis and the biological study of new derivatives of 1,2-diarylpyrrolidinecarbonitriles, which contain fragments of the known RT inhibitor—loviride. During the course of our efforts toward the development of our drug candidate, a synthesis of pyrrolidine framework was required as a core structure (Fig. 2).

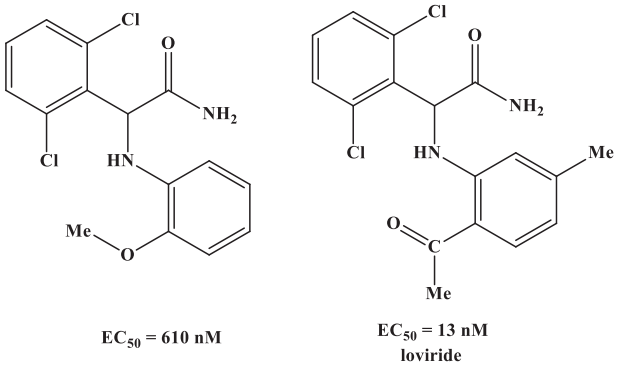


Fig. 1 Prototype α-APA compounds and their inhibition of HIV-1 replication

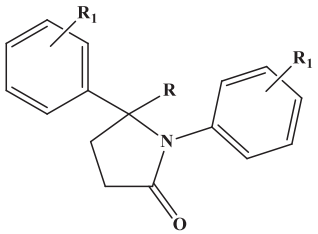


Fig. 2 New derivatives of 1,2-diarylpyrrolidinecarbonitriles

Materials and methods

General

All the chemicals were purchased from Alfa Aesar or Sigma Aldrich and were used without purification. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300Vx instrument at 300 and 75 MHz, respectively. Chemical shifts are reported in ppm referenced to the residual solvent signal. FT-IR spectra were recorded on a Nicolet Avatar 330 spectrometer. Melting points were recorded with a Boetius PHMK 77/1479 (Veb Analytik Dresden, Germany) apparatus and are uncorrected.

Chemistry

*General procedure for the synthesis of acetonitriles (**2a–j**)*

A solution of sodium cyanide (1 equiv, 10 mmol) in water (10 mL) was added to a solution of the appropriate aldehyde **1** (1 equiv, 10 mmol) in EtOH (20 mL) at room temperature. The mixture was stirred for 10 min and then acetic acid (1 equiv, 10 mmol) was added. After 10 min, the corresponding amine (1 equiv, 10 mmol) in EtOH (10 mL) was added and the resulting mixture was stirred for another 2 h at room temperature. After completion of the reaction, ice water (10 mL) was then added to the mixture. The formed crystals were filtered off, washed with water (10 mL), dried, and recrystallized from EtOH.

Compounds **2a**, **2b** were synthesized by us and described in Gasparyan et al. (2012).

Compounds **2c**, **2d** were synthesized by us and described in Gasparyan et al. (2014).

2-(4-Isopropoxyphenyl)-2-(4-toluidino)acetonitrile (**2e**) was prepared in 80 % of yield, white crystalline m.p. 91–92 °C; IR (nujol mull) ν_{max}, 3355 (NH), 2232 (C≡N), 1612 (arom.) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 7.49$–7.44 (2H, m) and 6.92–6.87 (2H, m, 4-*i*PrOC$_6$H$_4$), 6.96–6.91 (2H, m) and 6.69–6.64 (2H, m, 4-MeC$_6$H$_4$), 6.06 (1H, d, $J = 9.1$ Hz, NH), 5.52 (1H, d, $J = 9.1$ Hz, CHCN), 4.59 (1H, sp, $J = 6.0$ Hz, CH-*i*Pr), 2.24 (3H, s, Me), 1.33 (6H, d, $J = 6.0$ Hz, 2×Me-*i*Pr); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 157.5$, 143.2, 128.9 (2×CH), 128.1 (2×CH), 126.5, 126.4, 118.6 (CN), 115.2 (2×CH), 113.7 (2×CH), 68.8 (OCH), 48.1 (CH),

21.5 (Me_2), 20.0 (Me); anal. calcd. for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.23; H, 7.31; N, 9.74.

Compound **2f** was synthesized by us and described in Gasparyan et al. (2014).

Compound **2g** was synthesized by us and described in Gasparyan (2014).

2-[4-(2,4-Dichlorobenzyloxy)phenyl]-2-(4-toluidino)acetonitrile (**2h**) was prepared in 68 % of yield, light yellow crystalline m.p. 142–143 °C; IR (nujol mull) ν_{max} 3340 (NH), 2229 (C≡N), 1612 (arom.), 1590 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta = 7.57$ (1H, d, $J = 8.3$ Hz, H-6 C_6H_3), 7.46 (1H, d, $J = 2.1$ Hz, H-3 C_6H_3), 7.34 (1H, dd, $J = 2.1$, 8.3 Hz, H-5 C_6H_3), 7.55–7.50 (2H, m) and 7.04–6.99 (2H, m, 4-Me$C_6\underline{H}_4$), 6.95–6.90 (2H, m) and 6.68–6.63 (2H, m, C_6H_4O), 6.12 (1H, d, $J = 9.2$ Hz, NH), 5.56 (1H, d, $J = 9.2$ Hz, CH), 5.14 (2H, s, OCH_2), 2.23 (3H, s, Me); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 157.9$, 143.1, 133.4, 132.9, 132.8, 129.9 (CH), 128.9 (2×CH), 128.5 (CH), 128.3 (2×CH), 127.6, 126.8 (CH), 126.5, 118.6 (CN), 114.5 (2×CH), 113.8 (2×CH), 66.0 (OCH_2), 48.1 (CH), 19.9 (Me); anal. calcd. for $C_{22}H_{18}Cl_2N_2O$: C, 66.51; H, 4.57; N, 7.05. Found: C, 66.38; H, 4.69; N, 7.23.

Compound **2i** was synthesized by us and described in Gasparyan et al. (2014).

Compound **2j** was synthesized by us and described in Gasparyan (2014).

*Preparation for 2-aryl-5-oxo-2-pyrrolidinecarbonitriles (**3a–j**)*

3-Chloropropanoyl chloride (1 equiv, 10 mmol) was added to a mixture of dry potassium carbonate (1 equiv, 10 mmol) and the appropriate acetonitrile **2** (1 equiv, 10 mmol) in 1,2-dichloroethane (20 mL) at 10–15 °C. The mixture was stirred at room temperature for 30 min and stirring was continued at 40–45 °C for 2 h. After addition of 1,2-dichloroethane (20 mL), the mixture was washed with water and dried over $CaCl_2$. After distillation of the 1,2-dichloroethane dry potassium carbonate (1 equiv, 10 mmol), triethylbenzylammonium chloride (0.5 equiv, 5 mmol) (TEBA), and acetonitrile (20 mL) were added to the residue and the solution was stirred for 4 h at 45–50 °C. Product was filtered, the filtrate was evaporated, and the residue was dissolved in chloroform, washed with water, and dried over sodium sulfate. Chloroform was distilled off and the compound was recrystallized from EtOH.

Compounds **3a**, **3b** were synthesized by us and described in Gasparyan et al. (2012).

Compounds **3c**, **3d** were synthesized by us and described in Gasparyan et al. (2014).

2-(4-Isopropoxyphenyl)-1-(4-methylphenyl)-5-oxo-2-pyrrolidinecarbonitrile (**3e**) was prepared in 72 % of yield, white crystalline m.p. 107–108 °C; IR (nujol mull) ν_{max} 2229 (C≡N), 1721 (C=O), 1614 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta = 7.40$–7.35 (2H, m, H-2, H-2′ 4-*i*PrO$C_6\underline{H}_4$), 7.08–7.00 (4H, m, 4-Me$C_6\underline{H}_4$), 6.85–6.80 (2H, m, H-3, H-3′ 4-*i*PrO$C_6\underline{H}_4$), 4.56 (1H, sp, $J = 6.0$ Hz, OCH), 2.92–2.67 (3H, m) and 2.62–2.53 (1H, m, CH_2CH_2), 2.29 (3H, s, Me), 1.31 (6H, d, $J = 6.0$ Hz, 2×$C\underline{H}_3$-*i*Pr); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 172.2$, 157.8, 135.7, 133.0, 128.7 (2×CH), 127.3, 126.9 (2×CH), 125.4 (2×CH), 118.8 (CN), 115.2 (2×CH), 68.9 (OCH), 65.5 ($\underline{C}CN$), 36.6 (CH_2), 29.2 (CH_2), 21.4 (Me_2), 20.4 (Me); anal. calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 75.49; H, 6.51; N, 8.28.

Compound **3f** was synthesized by us and described in Gasparyan et al. (2014).

2-(4-Benzyloxyphenyl)-1-(4-methylphenyl)-5-oxo-2-pyrrolidinecarbonitrile (**3g**) was prepared in 61 % of yield, yellow crystalline m.p. 146–147 °C; IR (nujol mull) ν_{max} 2236 (C≡N), 1712 (C=O), 1605 (arom.), 1584 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta =$ 7.44–7.27 (7H, m) and 6.98–6.93 (6H, m, Ar–H), 5.05 (2H, s, OCH_2), 2.93–2.67 (3H, m) and 2.62–2.54 (1H, m, CH_2CH_2), 2.29 (3H, s, Me); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 172.1$ (CO), 158.6, 136.1, 135.7, 132.9, 128.7 (2×CH), 128.0, 127.8 (2×CH), 127.3 (CH), 127.0 (2×CH), 126.9 (2×CH), 125.4 (2×CH), 118.8 (CN), 114.7 (2×CH), 69.2 (OCH_2), 65.5 ($\underline{C}CN$), 36.6 (CH_2), 29.2 (CH_2), 20.4 (Me); anal. calcd. for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32. Found: C, 78.33; H, 5.72; N, 7.45.

2-[4-(2,4-Dichlorobenzyloxy)phenyl]-1-(4-methylphenyl)-5-oxo-2-pyrrolidinecarbonitrile (**3h**) was prepared in 81 % of yield, yellow crystalline m.p. 161–162 °C; IR (nujol mull) ν_{max} 2228 (C≡N), 1712 (C=O), 1611 (arom.), 1587 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta = 7.53$ (1H, d, $J = 8.3$ Hz, H-6 C_6H_3), 7.45 (1H, d, $J = 2.1$ Hz, H-3 C_6H_3), 7.32 (1H, dd, $J = 2.1$, 8.3 Hz, H-5 C_6H_3), 7.07–6.99 (4H, m, 4-Me$C_6\underline{H}_4$), 7.47–7.42 (2H, m) and 6.99–6.94 (2H, m, C_6H_4O), 5.10 (2H, s, OCH_2), 2.94–2.86 (1H, m), 2.84–2.71 (2H, m) and 2.69–2.55 (1H, m, CH_2CH_2), 2.29 (3H, s, Me); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 172.1$ (CO), 158.1, 135.7, 133.5, 132.8, 132.6, 129.9 (CH), 128.7 (2×CH), 128.5, 128.4 (CH), 127.1 (2×CH), 126.8 (CH), 125.4 (2×CH), 118.7 (CN), 114.7 (2×CH), 65.9 (OCH_2), 65.4 ($\underline{C}CN$), 36.6 (CH_2), 29.2 (CH_2), 20.4 (Me); anal. calcd. for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21. Found: C, 66.38; H, 4.32; N, 6.14.

Compounds **3i**, **3j** were synthesized by us and described in Gasparyan et al. (2014).

*2-(2,6-Dichlorophenyl)-2-(3,5-dimethylanilino)acetamide (**4**)*

2-(2,6-Dichlorophenyl)-2-(3,5-dimethylanilino)acetonitrile (**2c**) (1 equiv, 10 mmol) was dissolved in conc. H_2SO_4 (10 mL) at 0–5 °C, left at room temperature for 3 h, and slowly poured into a beaker with ice. The resulting crystals were filtered off, washed with dilute $NaHCO_3$ solution and H_2O, and recrystallized from EtOH. Compound (**4**) was obtained in 94 % yield, white crystalline m.p. 147–148 °C; IR (nujol mull) ν_{max} 3453 (NH_2), 3364 (NH), 1704 (C=O), 1602 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 7.33$ (2H, d, $J = 7.8$ Hz, H-3,5 $C_6H_3Cl_2$), 7.23 (1H, t, $J = 7.8$ Hz, H-4 $C_6H_3Cl_2$), 7.35 (1H, br) and 6.88 (1H, br., NH_2), 6.32 (2H, s, H-2,6 $C_6\underline{H}_3Me_2$), 6.23 (1H, s, H-4 $C_6\underline{H}_3Me_2$), 5.65 (1H, d, $J = 8.7$ Hz, CH), 5.41 (1H, d, $J = 8.7$ Hz, NH), 2.16 (6H, s, 2×Me); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 170.3$ (CO), 146.0, 137.4 (2C), 135.3, 135.0 (br.), 128.8 (CH), 128.4 (CH), 119.4 (CH), 111.1 (C<u>H</u>-2,2′ $C_6H_3Me_2$), 57.7 (CH), 20.9 (2×Me); anal. calcd. for $C_{16}H_{16}Cl_2N_2O$: C, 59.46; H, 4.99; N, 8.67. Found: C, 59.38; H, 4.77; N, 8.54.

*Preparation for 5-aminomethyl-5-aryl-2-pyrrolidinones (**5a–g**)*

To a solution of appropriate 2-aryl-5-oxo-2-pyrrolidine-carbonitrile **3** (1 equiv, 3 mmol) in methylene chloride (10 mL), a mixture of $CoCl_2 \cdot 6H_2O$ (0.2 equiv, 0.6 mmol) and PEG-400 (1 equiv, 3 mmol) was added. The mixture was stirred and sodium borohydride (5 equiv, 15 mmol) was slowly added at −5 to 0 °C. The stirring was continued at −5 to 0 °C for 2 h and then the reaction mixture was slowly warmed up to room temperature. After completion of the reaction (Thin-layer chromatography), ice water (10 mL) was added. After filtration, and extraction with methylene chloride (20 mL), the organic layer was washed with water, dried over $CaCl_2$, and distilled off. Product was purified by crystallization from the appropriate solvent or was prepared by forming the corresponding salt.

5-Aminomethyl-1-(4-methylphenyl)-5-phenyl-2-pyrrolidinone (**5a**) was prepared in 55 % of yield, white crystal m. p. 130 °C; IR (nujol mull) ν_{max} 3400 broad (NH_2), 1650 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta = 7.38–7.23$ (5H, m, C_6H_5), 7.02–6.97 (2H, m) and 6.90–6.85 (2H, m, C_6H_4), 3.27 (1H, d, $J = 13.5$ Hz) and 3.20 (1H, d, $J = 13.5$ Hz, NCH_2), 2.92 (2H, br., NH_2+H_2O), 2.79–2.68 (1H, m, CH_2), 2.53–2.47 (2H, m, CH_2), 2.28 (3H, s, Me), 2.01–1.92 (1H, m, CH_2); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 174.0$ (CO), 143.9, 134.6, 134.0, 128.4 (2×CH), 128.2 (2×CH), 126.8 (CH), 125.3 (2×CH), 125.2 (2×CH), 70.0 (N-<u>C</u>Ph), 44.1 (NCH_2), 31.0 (CH_2), 29.4 (CH_2), 20.4 (Me); anal. calcd. for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.32; H, 7.28; N, 10.15.

Compounds **5b–e** were synthesized by us and described in Gasparyan et al. (2014).

5-Aminomethyl-5-(4-benzyloxyphenyl)-1-(4-methylphenyl)-2-pyrrolidinone (**5f**) was prepared in 52 % yield, which gives the corresponding hydrochloric salt, yellow crystal m.p. 169–170 °C; IR (nujol mull) ν_{max} 3214 broad (NH_2), 1680 (C=O), 1608 (arom.) cm^{-1}; ^1H NMR (DMSO-d_6/CCl_4 1/3, 300 MHz): $\delta = 8.65$ (3H, br., NH_2, and HCl), 7.43–7.25 (5H, m, C_6H_5), 7.25–7.20 (2H, m), 7.05–7.00 (2H, m), 6.98–6.93 (2H, m) and 6.84–6.79 (2H, m, 2×C_6H_4), 5.08 (2H, s, OCH_2), 3.61 (1H, d, $J = 13.6$ Hz) and 3.28 (1H, d, $J = 13.6$ Hz, NCH_2), 2.80–2.53 (3H, m) and 2.33–2.19 (1H, m, CH_2CH_2), 2.29 (3H, s, Me); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 173.6$ (CO), 157.7, 136.4, 135.5, 133.8, 132.8, 128.7 (2×CH), 127.8 (2×CH), 127.2 (CH), 127.0 (2×CH), 126.8 (2×CH), 126.6 (2×CH), 111.6 (2×CH), 69.1 (OCH_2), 68.9 (NC), 41.5 (NCH_2), 30.3 (CH_2), 29.1 (CH_2), 20.4 (Me); anal. calcd. for $C_{25}H_{26}N_2O_2 \cdot HCl$: C, 70.99; H, 6.43; N, 6.62. Found: C, 71.26; H, 6.59; N, 6.45.

Compound **5g** was synthesized by us and described in Gasparyan et al. (2014).

Compounds **6a**, **b** were synthesized by us and described in Gasparyan (2014).

Compound **7** was synthesized by us and described in Martirosyan et al. (2000).

Compounds **8a–c**, **9a–c** were synthesized by us and described in Gasparyan et al. (2012).

2-Aminomethyl-2-phenyltetrahydro-1H-1-pyrrolyl-4-bromophenylmethanone (**10**) was prepared in 44 % yield according to the method described for the preparation of compounds (**5a–g**), which gives the corresponding hydrochloric salt, white crystal m.p. 216–218 °C; IR (nujol mull) ν_{max} 3302 (NH_2), 1645 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): $\delta = 11.28$ (1H, br., HCl), 9.36 (1H, br.) and 9.05 (1H, dd, $J = 5.6$, 7.2 Hz, NH_2), 7.88 (2H, m), 7.56–7.49 (4H, m) and 7.40–7.27 (3H, m, Ar–H), 3.96 (1H, dd, $J = 7.2$, 14.6 Hz) and 3.82 (1H, dd, $J = 5.6$, 14.6 Hz, $NH_2C\underline{H}_2$), 3.58 (1H, m) and 3.35 (1H, m, $NC\underline{H}_2CH_2$), 2.56 (1H, m), 2.32 (1H, m), 2.21 (1H, m) and 2.02 (1H, m, N $CH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-d_6/CCl_4 1/3, 75 MHz): $\delta = 165.9$ (CO), 138.2, 133.3, 130.5, 127.9 (2×CH), 127.5 (CH), 127.5 (2×CH), 127.2 (2×CH), 126.0 (2×CH), 72.6 (<u>C</u>-Ph), 45.0 (NCH_2), 43.3 (NCH_2), 33.4 (CH_2), 21.7 (CH_2); anal. calcd. for $C_{18}H_{19}BrN_2O \cdot HCl$: C, 54.63; H, 5.09; N, 7.08. Found: C, 54.29; H, 5.34; N, 7.13.

Anti-HIV-1 assay

Primary human peripheral blood mononuclear (PBM) cells were stimulated with phytohemagglutinin A for 2–3 days prior to use. HIV-1/LAI obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays (Schinazi et al. 1990). The antiviral EC_{50} and 90 % effective concentration (EC_{90}) were determined from the concentration-response curve using the median effect method (Table 1).

Cytotoxicity assay

Compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD), and Vero (African green monkey kidney) cells. The 50 % inhibition concentration (IC_{50}) was determined from the concentration-response curve using the median effect method (Table 1) (Stuyver et al. 2002).

Results and discussion

Chemistry

We have previously developed a method for the synthesis of various cyclic α-amino acids and their analogs, particularly 2-phenylproline derivatives, by intramolecular cyclization of corresponding *N*-(3-chloro- or 1-oxo-3-chloropropyl)-α-phenylglycines under phase-transfer catalytic conditions in acetonitrile in the presence of potassium carbonate and TEBA as catalyst (Martirosyan et al. 2000).

Table 1 Anti-HIV-1 and cytotoxic activities of synthesized compounds

Compounds	R, R_1	Anti-HIV-1 activity in human PBM cells (μM)		Cytotoxicity (IC_{50}, μM)		
		EC_{50}	EC_{90}	PBM	CEM	Vero
2g	4-$PhCH_2O$, 4-MeC_6H_4	4.7	>100	23.5	20.1	69.6
2h	4-(2,4-$Cl_2C_6H_3CH_2O$), 4-MeC_6H_4	3.1	22.4	15.0	11.7	39.7
2i	4-(2,6-$Cl_2C_6H_3CH_2O$), 4-MeC_6H_4	9.6	30.0	14.0	16.8	80.9
2j	2-$PhCH_2O$, 4-MeC_6H_4	14.4	50.1	15.6	3.8	23.7
3c	3,5-$Me_2C_6H_3$, 2,6-Cl_2	72.2	>100	59.0	20.3	>100
3d	4-*i*PrO, Ph	5.2	29.8	95.8	>100	47.6
3e	4-*i*PrO, 4-MeC_6H_4	37.3	>100	>100	17.0	46.5
3f	4-$PhCH_2O$, $PhCH_2$	9.8	30.7	5.9	7.0	3.0
3g	4-$PhCH_2O$, 4-MeC_6H_4	16.7	>100	>100	30.3	53.9
3h	4-(2,4-$Cl_2C_6H_3CH_2O$), 4-MeC_6H_4	5.3	24.4	9.7	6.6	21.8
3i	4-(2,6-$Cl_2C_6H_3CH_2O$), 4-MeC_6H_4	2.0	51.9	14.1	16.6	52.9
3j	2-$PhCH_2O$, 4-MeC_6H_4	17.3	>100	22.8	16.1	77.9
4	2,6-Cl_2, 3,5-$Me_2C_6H_3$	0.43	3.1	6.1	5.8	68.7
5a	H, 4-MeC_6H_4	32.6	>100	54.1	53.6	>100
5b	H, $PhCH_2$	>100	>100	>100	>100	>100
5c	2,6-Cl_2, 3,5-$Me_2C_6H_3$	>100	>100	>100	≥100	>100
5d	4-*i*PrO, Ph	>100	>100	48.3	32.9	33.0
5e	4-$PhCH_2O$, $PhCH_2$	33.4	>100	15.7	23.6	13.1
5f	4-$PhCH_2O$, 4-MeC_6H_4	10.8	41.5	15.9	33.7	12.3
5g	2-$PhCH_2O$, 4-MeC_6H_4	11.5	>100	45.2	47.9	>100
6a	4-$PhCH_2O$, $PhCH_2$	>100	>100	>100	29.4	6.2
6b	4-$PhCH_2O$, 4-MeC_6H_4	8.9	>100	>100	70.1	38.3
9a	2-Br	50.3	>100	>100	>100	>100
9b	4-Br	>100	>100	>100	>100	>100
9c	4-MeO-3-NO_2	>100	>100	>100	>100	>100
10	4-Br	>100	>100	>100	96.1	>100
AZT		0.0018	0.015	>100	14.3	50.6

α-Aminonitriles **2a–j** were synthesized in high yields by treatment of appropriate aldehydes **1a–g**, amines and NaCN in presence of acetic acid in solution in EtOH/H_2O. By acylation of 2-substituted acetonitriles **2a–j** with 2-chloropropanoyl chloride (in 1,2-dichloroethane and in presence of K_2CO_3) and further intramolecular cyclization under phase-transfer catalytic conditions, in presence of K_2CO_3, TEBA, and acetonitrile afforded the corresponding 1,2-diaryl substituted pyrrolidinecarbonitriles **3a–j** in high yields (Scheme 1) (Gasparyan et al. 2012).

2-(2,6-Dichlorophenyl)-2-(3,5-dimethylanilino)acetamide (**4**) was synthesized for biological evaluation by reacting 2-(2,6-dichlorophenyl)-2-(3,5-dimethylanilino)acetonitrile (**2c**) in conc. H_2SO_4 (Gasparyan et al. 2012).

Substituted pyrrolidinecarbonitriles **3a–d**, **f**, **g**, **j** were reduced to the corresponding aminomethylpyrrolidines **5a–g** in methylene chloride at −5–0 °C, using a $NaBH_4$/PEG-400/$CoCl_2$ system (ratio 1/0.2/1/5) (Gasparyan et al. 2014).

With the aim to ascertain the role of the pyrrolidine ring in the observed inhibitory activity, we have synthesized 1,2-diarylazetidines **6a**, **b**. In the same way, 2-substituted acetonitriles **2f**, **g** were first acylated with 2-chloroethanoyl chloride (instead 2-chloropropanoyl chloride) and subsequently cyclized under phase-transfer catalytic conditions (Gasparyan 2014).

Acylation of 2-((3-chloropropyl)amino)-2-phenylacetonitrile hydrochloride (**7**) (Martirosyan et al. 2000) with 2-bromo-, 4-bromo- and 4-methoxy-3-nitrobenzenecarbonyl chlorides, also under phase-transfer catalysis conditions, followed by intramolecular cyclization produced proline derivatives **8a–c** (Gasparyan et al. 2012), from which were synthesized the corresponding amides **9a–c** and amine **10** following the above mentioned methods (Gasparyan et al. 2012, 2014) (Scheme 2).

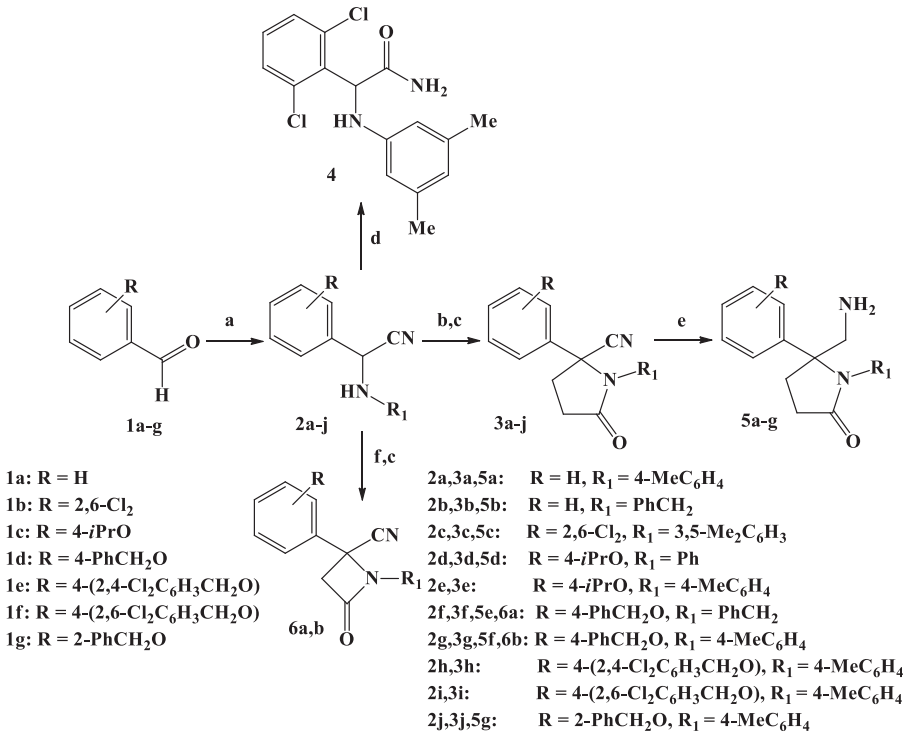
Pharmacology

Anti-HIV activity

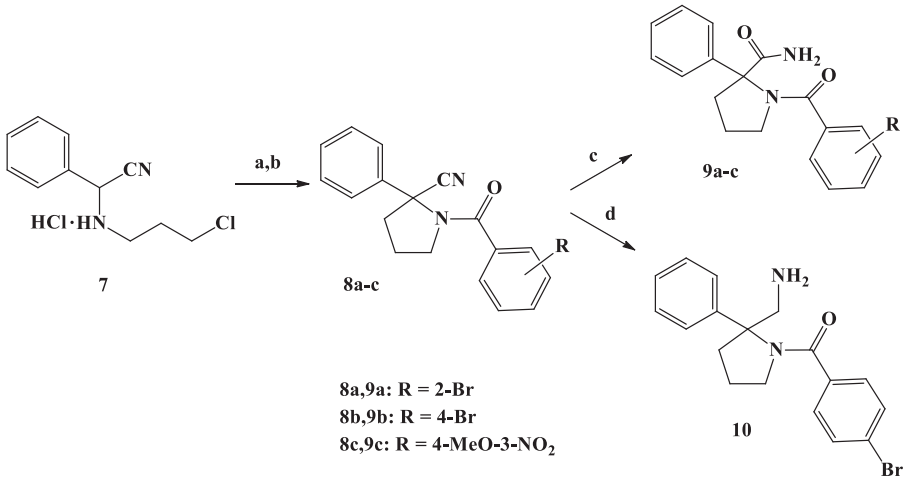
Certain synthesized compounds appeared to be active against HIV, and among the 26 compounds in this series, 14 exhibited good activity with EC_{50} values <20 μM. Compounds **2h**, **3i**, and **4** proved to be the most active compounds with EC_{50} value of 3.1, 2.0, and 0.43 μM, respectively, but they were highly toxic in all the cell lines used. Compound **3d** in particular showed good activity with an EC_{50} value of 5.2 μM without marked cytotoxicity against all the cell lines.

In our structure activity relationship study, we identified some interesting patterns. Compounds **2g–j** and **4** (whose structure is closer to the loviride structure) were very active.

By comparing the activity of the compounds containing the 2-aryl substituents, we noted that compounds with benzyloxyphenyl groups were more active. Compounds in which the 1-aryl moiety contained methyl group in 4- or 3,5-positions also showed high activity.



Scheme 1 Synthesis of 1,2-diaryl substituted 5-oxopyrrolidinecarbonitrile analogs. Reagents and conditions: **a** NaCN/RNH_2/H^+/EtOH/H_2O, room temp., 3 h; **b** $ClCH_2CH_2COCl$/$ClCH_2CH_2Cl$/K_2CO_3, 10–15 °C to 40–45 °C, 3 h; **c** CH_3CN/TEBA/K_2CO_3, 45–50 °C, 4 h; **d** conc. H_2SO_4, 0–5 °C, 3 h; **e** $NaBH_4$/PEG-400/$CoCl_2$/CH_2Cl_2, −5–0 °C, 8 h; **f** $ClCH_2COCl$/$ClCH_2CH_2Cl$/K_2CO_3, 10–15 °C to 40–45 °C, 3 h



Scheme 2 Synthesis of 1,2-diaryl substituted pyrrolidinecarbonitrile analogs. Reagents and conditions: **a** $RCOCl/ClCH_2CH_2Cl/K_2CO_3$, 10–15 °C to 40–45 °C, 3 h; **b** $CH_3CN/TEBA/K_2CO_3$, 45–50 °C, 4 h; **c** conc. H_2SO_4, 0–5 °C, 3 h; **d** $NaBH_4/PEG\text{-}400/CoCl_2/CH_2Cl_2$, −5–0 °C, 8 h

We also observed a decrease in activity with $CN > NH_2 > C(O)NH_2$, however, the difference of activity between 5-membered and 4-membered rings containing compound was not significant.

Based on these findings, we believe that further structural modifications could be made to obtain new potent anti-HIV-1 derivatives (Table 1).

Cytotoxicity studies

In order to determine their spectrum of toxicity, cytotoxicity of the compounds was determined in primary human PBM, human CEM, and Vero cells (Table 1).

Compounds **5b**, **c** and **9a–c** were nontoxic in all the cell systems tested; however, they were inactive against HIV-1. Similarly, compound **10** showed no toxicity in any of the cell system and exhibited no inhibition of HIV replication. However, **6a** and **6b** were not toxic in PBM cells (Table 1).

Conclusions

Herein, we report the anti-HIV-1 activity of new 1,2-diarylpyrrolidine derivatives. Fourteen compounds were active against HIV-1 with EC_{50} values less than 20 μM, which indicates the potential of these compounds as anti-HIV-1 agents. Among these 26 compounds, **2g**, **2h**, **3i**, and **4** were potent anti-HIV-1 agents with EC_{50} values <5.0 μM. They were less potent than the 3'-Azido-2',3'-dideoxythymidine or azidothymidine used as a positive control, however, the activity may be due to the toxicity observed in PBM cells. On these grounds, further structural modifications has to be made to improve potency against HIV and reduce cytotoxicity.

Acknowledgments The authors are grateful to the Civilian Research and Development Foundation (CRDF) (Grant No. ARB2-2701-YE-05). This work was also facilitated in part by the NIH grant P30-AI-050409 (to RFS). This paper is dedicated to our friend and colleague Dr. Ashot H. Martirosyan who died after a long illness on June 25, 2016.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

De Corte BL (2005) From 4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-jk](1,4)benzodiazepin-2(1H)-one (TIBO) to Etravirine (TMC125): Fifteen years of research on non-nucleoside inhibitors of HIV-1 reverse transcriptase. J Med Chem 48:1689–1696

Ding J, Das K, Tantillo C, Zhang W, Clark Jr AD, Jessen S, Lu X, Ysiou Y, Jacobo-Molina A, Andries K, Pauwels R, Moereels H, Koymans L, Janssen PAJ, Smith Jr RH, Kroeger Smith MB, Koepke M, Michejda CJ, Hughes SH, Arnold E (1995) Structure of HIV-1 reverse transcriptase in a complex with the non-nucleoside inhibitor R-APA R 95845 at 2.8 Å resolution. Structure 3:365–379

Gasparyan SP, Alexanyan MV, Harutyunyan GK, Hovhannesyan VE, Martirosyan VV, Paronikyan RV, Stepanyan HM, Martirosyan AH (2012) Synthesis and biological properties of new analogs of 2-arylpyrrolidinecarbonitriles and pyrrolidinecarboxamides. Pharm Chem J 46(6):331–333

Gasparyan SP (2014) Synthesis of new analogs of 2-aryl-2-azetancarbonitriles. Chem J Armenia 67(1):111–118

Gasparyan SP, Alexanyan MV, Harutyunyan GK, Hovhannesyan VE, Martirosyan AH, Panosyan HA (2014) Metal-complex reduction of nitrile groupe in substituted pyrrolidinecarbonitriles. Chem J Armenia 67(2-3):239–246

Martirosyan AH, Gasparyan SP, Hovhannesyan VE, Mnjoyan ShL, Alexanyan MV, Nikishchenko MN, Babayan GSh (2000) New method for the synthesis of 2-phenylproline and its derivatives. Chem Het Comp 36:416–420

Pauwels R, Andries K, Debyser Z, Van Daele P, Schols D, Stoffels P, De Vreese K, Woestenborghs R, Vandamme AM, Janssen CGM, Anne J, Cauwenbergh G, Desmyter J, Heykants J, Janssen MAC, De Clercq E, Janssen PAJ (1993) Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of a-anilinophenylacetamide derivatives targeted

at HIV-1 reverse transcriptase. Proc Natl Acad Sci USA 90:1711–1715

Prajapati DG, Ramajayam R, Yadav MR, Giridhar R (2009) The search for potent, small molecule NNRTIs. Bioorg Med Chem 17:5744–5762

Sarafianos SG, Das K, Hughes SH, Arnold E (2004) Taking aim at a moving target: designing drugs to inhibit drug-resistant HIV-1 reverse transcriptases. Curr Opin Struct Biol 14:716–730

Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie M-W, Hart GC, Smith GA, Hahn EF (1990) Activities of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. Antimicrob Agents Chemother 34(6):1061–1067

Stuyver LJ, Lostia S, Adams M, Mathew J, Pai BS, Grier J, Tharnish P, Choi Y, Chong Y, Choo H, Chu CK, Otto MJ, Schinazi RF (2002) Antiviral activities and cellular toxicities of modified 2′,3′-dideoxy-2′,3′-didehydrocytidine analogues. Antimicrob Agents Chemother 46(12):3854–3860