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Original article

Inhibition of HIV-1 integrase-catalysed reaction by new DNA minor groove ligands: the oligo-1,3-thiazolecarboxamide derivatives

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Abstract – Human immunodeficiency virus type 1 (HIV-1) integrase (IN) is an essential enzyme in the life cycle of the retrovirus, responsible for catalysing the insertion of the viral genome into the host cell chromosome. For this reason it provides an attractive target for antiviral drug design. We synthesized a series of novel thiazole (Tz)-containing oligopeptides (TCOs; oligo-1,3-thiazolecarboxamides), specifically interacting within the minor groove of DNA. The oligocarboxamide derivatives contained 1–4 Tz rings and different N- and C-terminal groups. The effect of these oligocarboxamides on the HIV-1 IN-catalysed reaction was investigated. Some of the compounds were able to inhibit the reaction. The inhibitory effect of the TCOs increased with the number of Tz units. The structure of various additional positively and/or negatively charged groups attached to the N- and C-termini of TCOs had a pronounced effect on their interaction with the DNA substrate complexed to IN. Modified TCOs having a better affinity for this complex should provide a rationale for the design of drugs targeting the integration step. © 2000 Éditions scientifiques et médicales Elsevier SAS

HIV-1 / integrase / thiazole-containing oligopeptides / inhibition

1. Introduction

Replication of retroviruses depends on the integration of a double stranded DNA copy of the retroviral genome into the host cell nuclear genome [1–3]. Integration requires the virus-encoded protein, integrase (IN), and DNA sequences located in the U3 and U5 regions at the ends of the viral long terminal repeats (reviewed in [4]). The integration reaction has been analysed in vivo and in vitro and it has been shown that it proceeds in three steps [5–7]. The first step is 3′ processing: two nucleotides (GT) from the 3′-ends of each strand of linear viral DNA are removed by IN leaving at the viral 3′-ends the conserved CA dinucleotide. The second step or strand transfer, is a concerted cleavage ligation reaction during which IN makes staggered cuts in the target DNA and ligates the recessed 3′-OH ends of the viral DNA to the overhanging 5′-phosphate ends of the target DNA at the cleavage site. The product of this reaction is a gapped intermediate. The last step is 5′-end joining, in

Abbreviations: HIV-1, human immunodeficiency virus type 1; IN, integrase; RT, reverse transcriptase; LTR, long terminal repeat; TCO, thiazole-containing oligopeptides; MGL, minor groove ligand; Tz, thiazole; IC_{50}, the concentration giving 50% inhibition of the enzyme activity.

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which the integration process is completed by removal of the two unpaired nucleotides at the 5′-ends of the viral DNA and repair of the gaps between the viral and target DNA sequences. Since IN is essential for productive retroviral infection, the enzyme is a potential target for antiviral chemotherapeutic intervention. In contrast to human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase, for which an extensive number of chemical inhibitors have been described, there are few reports about selective inhibitors of IN.

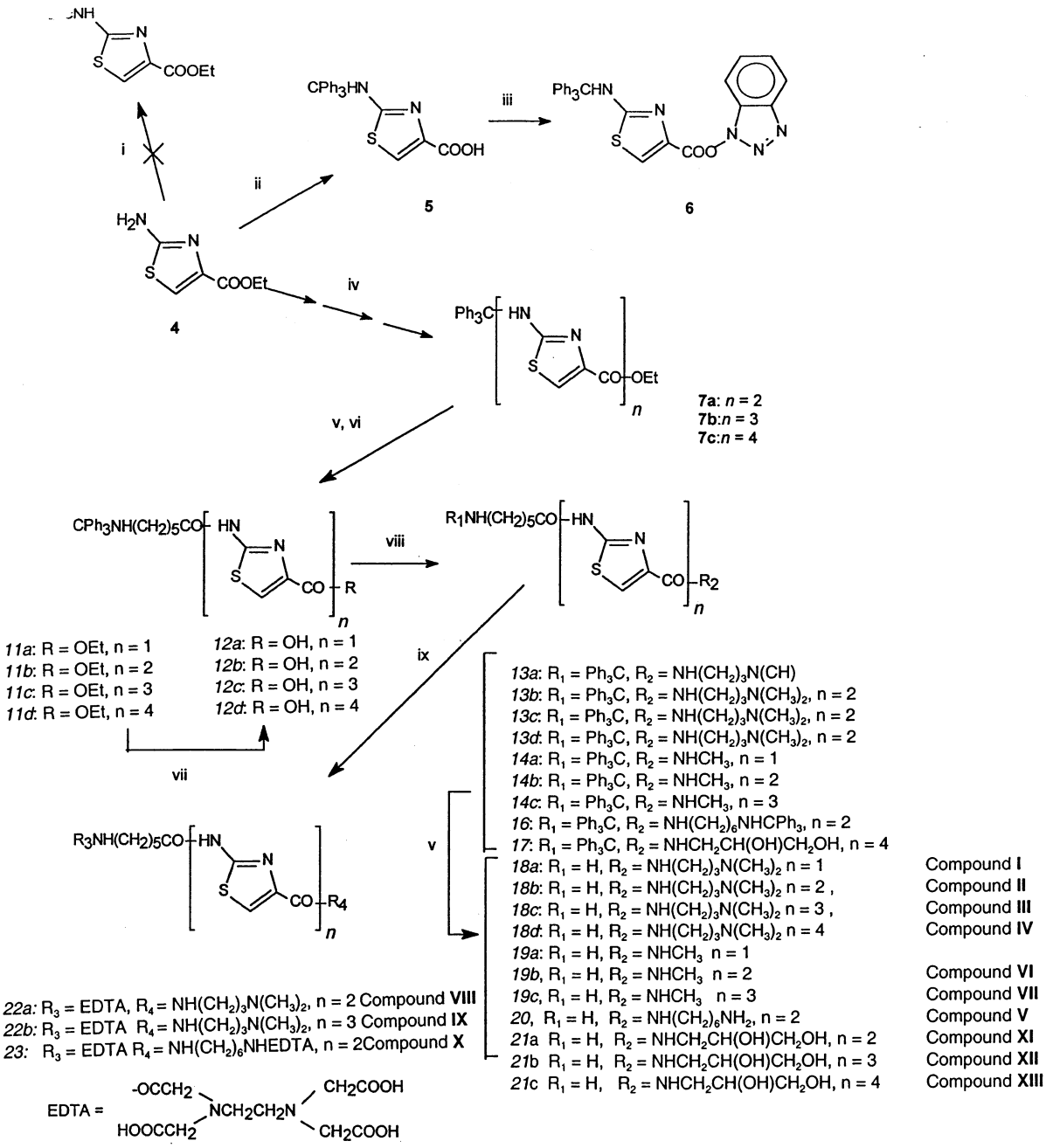
Most of the therapeutic agents used against HIV-1 induce the emergence of resistance strains. That is why one of the most important lines in the pharmacology concerning HIV-1 is focused on the development of new, or on the modification of already existing drugs. Among the new and promising antivirals tools are compounds which interact reversibly with nucleic acids. Minor groove binding molecules are capable of binding within the minor groove of double-stranded B-DNA. Such molecules contain several small aromatic rings such as pyrrole, furan or benzene connected by bonds with torsional freedom.

The prototype minor groove binders are the naturally occurring oligopeptide antibiotics netropsin and distamycin. Distamycin was first isolated from *Streptomyces distallicus* [8] and later synthesized [9–11]. It inhibits both Gram-positive and Gram-negative bacteria and replication of viral DNA (vaccinia, herpes simplex virus) [12]. The synthesis of certain analogues of distamycin, containing either additional pyrrole units or derivatives in which the pyrrole rings were replaced by benzene, pyridine, thiophene, thiazole (Tz), imidazole, pyrazole, or triazole has been reported [13–18]. These compounds were used as antitumour or antiviral drugs [12, 19] or as tools to study the modulation of enzymes [20–22].

The development of methods to synthesize minor groove molecules capable of binding with virtually any DNA sequence increased the interest for such compounds [23, 24]. The heteroanalogues of distamycin and netropsin that have been mostly synthesized are those in which one of the methylpyrrole units was substituted with one Tz. A dipeptide composed exclusively of thiazolecarboxamide fragments has been described [25]. Sulfonated and phosphonated distamycin derivatives capable of suppressing HIV-1 replication are of potential therapeutic importance [26]. Of obvious interest are the analogues of distamycin and netropsin in which the *N*-methylpyrrole fragment is substituted with a Tz cycle, since these compounds are likely to possess different site-specificity compared to oligopyrrolecarboxamides. Due to the low reactivity of the key oligomer 2-amino-4-carbonic acid-1,3-Tz, there are few studies concerning the minor groove binders containing the Tz fragment in their structure [27–30]. Studies related to the site-specificity of a Tz-containing oligopeptide (TCO) analogue of netropsin demonstrated that this compound bound to 4–5 base pairs of the minor groove. There are also reports showing the influence of the methyl substituents of a Tz-containing lexitropsin on the mode of binding to DNA [28].

We recently synthesized a series of new lexitropsins, the TCOs. These compounds are derivatives of oligocarboxamides containing 1–4 Tz rings and different N- and C-terminal groups. We previously showed that TCOs were able to inhibit the polymerization reaction catalysed by HIV-1 RT [31]. Similar to distamycin, the TCOs were good inhibitors when reverse transcription was done in the presence of a DNA–DNA template-primer. But in contrast to distamycin, the TCOs were also able to inhibit the reaction with RNA·DNA or DNA·RNA template-primers. The inhibition obtained with the TCOs showed that these compounds were more powerful and versatile inhibitors of the RT-dependent polymerization than the natural distamycin.

IN binds to specific sequences located on both extremities of the DNA on the HIV-1 LTRs. The retroviral LTR that recognizes a DNA binding site on IN contains an AT-rich sequence. This sequence has been exploited as a possible target for DNA minor groove binders, since the DNA minor groove may fit aromatic molecules better at AT-rich regions. Here we addressed the question whether TCOs can interact with the proviral DNA complexed to HIV-1 IN. If this was the case, the newly synthesized drugs will be targeting different sites of the HIV-1 life cycle: reverse transcription and integration. Thus, attacking the virus on these sites together can prove effective for combination therapy. Therefore, we analysed the effect of a series of oligothiazolecarboxamides on the reaction catalysed by HIV-1 IN and compared their effect with that of distamycin, a natural oligopeptide, containing three pyrrole rings, which is a DNA minor groove ligand.



[a] (i) Boc_2O/i-PrOH/K_2CO_3/DMAP; (ii) Ph_3CCl/Py; (iii) Hobt/DCC/CH_2Cl_2/4 h; (iv) **6**/DMF/NEt_3/50°C/17 h; (v) TFA/$CHCl_3$/1 h; (vi) **10**/NEt_3/DMF/60°C/10 h; (vii) 2M NaOH/70% EtOH/50°C/1 h; (viii) $NH_2(CH_2)N(CH_3)_2$ or $NH_2CH_2CH(OH)CH_2OH$ or NH_2CH_3 or **15** /Hobt/DCC/DMF/60°C /3-10 h; (ix) bisanhydrid EDTA/DMF/Py

Figure 1. Scheme of the synthesis.

2. Chemistry

All thiazolecarboxamide analogues were synthesized by chain extension with 2-triphenylmethylamino-4-carboxy-1,3-Tz (see *figure 1*). The structures of the initial monomers and oligomers synthesized were confirmed chromatographically and by ^{1}H-NMR spectra. The concentrations of TCOs were determined spectrophotometrically using the following extinction coefficients: Tz_1-derivatives = 12.0/mM cm at $\lambda_{max} = 252$ nm; Tz_2-derivatives = 21.1/mM cm at $\lambda_{max} = 264$ nm; Tz_3-derivatives = 32.0/mM cm at $\lambda_{max} = 287$ nm; Tz_4-derivatives = 37.0/mM cm at $\lambda_{max} = 292$ nm.

TLC analysis was carried out on precoated plates of silica gel 60F254 (Merck). Purification was performed on silica gel (40–60 μm). ^{1}H-NMR spectra were recorded with a Bruker AM-200 apparatus. Reverse phase HPLC was performed with Altex dual-pump system and 2.5 × 25 cm with Diasorb 130 C16T (BioChemMack). All the chemicals used were obtained from Fluka. EDTA-bisanhydride was prepared according to [32].

Matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) was performed at the Institute of Molecular Biology (Moscow).

2.1. Ethyl 2-amino-1,3-Tz-4-carboxylate (4)

This compound was prepared as described in reference [33]. After recrystallization from EtOH the yield of pure compound was 62%. ^{1}H-NMR ($CDCl_3$) δ 7.42 (s, 1H), 7.02 (br s, 2H), 4.26 (q, 2H, $J = 7.0$ Hz), 1.29 (t, 3H, $J = 7.0$ Hz).

2.2. 2-Triphenylmethylamino-4-carboxy-1,3-Tz (5)

A mixture of compound **4** (7.4 g, 43 mmol) and triphenylmethyl chloride (12.0 g, 43 mmol) was dissolved in 60 mL of dry pyridine and stirred for 16 h at room temperature. The precipitate was filtered off, washed with pyridine (2 × 10 mL), and the filtrate was evaporated under reduced pressure up to 40–60 mL. Ten grams of NaOH in 100 mL of 60% EtOH was added to the residue. The mixture was stirred until precipitate formation and left at room temperature for 20 h. The mixture was cautiously acidified with acetic acid to pH 5–6. The precipitate was collected by filtration, washed with water (10 × 50 mL) and acetone (2 × 25 mL). The yield of product **5** was 12.6 g (72%). ^{1}H-NMR ($CDCl_3$) δ 9.46 (bs, 1H), 7.27–7.40 (m, 15H), 7.27 (s, 1H).

2.3. 1,2,3-Benzotriazol-1-yl-2-triphenylmethylamino-1,3-thiazol-4-carboxylate (6)

Compound **5** (7.20 g, 18.6 mmol) was rapidly stirred with 1-hydroxy-1,2,3-benzotriazole (2.52 g, 18.6 mmol) in dry dichloromethane (200 mL), and DCC (3.90 g, 19.0 mmol) was added to the mixture in small portions at room temperature. After stirring the mixture for 4 h, the precipitated urea was filtered off and the solution was poured into hexane (200 mL). The precipitate was collected and dried in vacuo over SiO_2. The resulting crude product **6** (9.0 g, 96%) was sufficiently pure and could be used directly for synthesis. ^{1}H-NMR ($CDCl_3$) δ 7.65 (s, 1H), 7.28–7.65 (m, 4H), 7.32 (s, 15H), 7.08 (s, 1H).

2.4. Ethyl-2-[2-(triphenylmethylamino)-1,3-thiazol-4-carboxamido]-1,3-Tz-4-carboxylate (7a)

A mixture of compound **4** (1.83 g, 10.6 mmol), compound **6** (5.35 g, 10.6 mmol) and NEt_3 (2.6 mL, 18 mmol) in dry DMF (25 mL) was stirred for 1 h at 50 °C. The greenish mixture was allowed to react at 50 °C without stirring for 16 h. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography and the resulting homogeneous product **7a** was eluted by chloroform/hexane (1:1) (5.0 g, 88%). ^{1}H-NMR ($CDCl_3$) δ 10.40 (bs, 2H), 7.81 (s, 1H), 7.31 (s, 15H), 7.26 (s, 1H), 6.60 (s, 1H), 4.38 (q, 2H, $J = 7.0$ Hz), 1.37 (t, 3H, $J = 7.0$ Hz).

2.5. Ethyl-2-[2-[2-(triphenylmethylamino)-1,3-thiazol-4-carboxamido]-1,3-thiazol-4-carboxamido]-1,3-Tz-4-carboxylate (7b)

Compound **7a** (2.6 g, 4.8 mmol) dissolved in $CHCl_3$ (30 mL) was treated with TFA (15 mL) for 1 h at room temperature. The mixture was evaporated under reduced pressure and 20 mL of water was added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (20 mL), rinsed with water (4 × 20 mL), dried on filter, washed with ether and dried in vacuo over SiO_2. The resulting amine (1.03 g), compound **6** (1.82 g, 3.5 mmol) and NEt_3 (0.7 mL, 5.0 mmol) in dry DMF (5 mL) was stirred for 1 h at 50 °C. The greenish mixture was allowed to react at 50 °C without stirring for 16 h. The mixture was evaporated under reduced pressure. The

residue was purified by column chromatography using chloroform as eluent giving the homogeneous product **7b** (1.76 g, 55%). ^{1}H-NMR ($(CD_3)_2CO$) δ 10.90 (bs, 1H), 9.80 (bs, 1H), 8.14 (s, 1H), 8.21 (s, 1H), 8.04 (s, 1H), 7.25–7.56 (m, 16H), 4.32 (q, 2H, $J = 7.0$ Hz), 1.34 (t, 3H, $J = 7.0$ Hz).

2.6. Ethyl-2-[2-[2-[2-(triphenylmethylamino)-1,3-thiazol-4 carboxamido]-1,3-thiazol-4-carboxamido]-1,3-thiazol-4-carboxamido]-1,3-Tz-4-carboxylate (7c)

Compound **7b** (0.88 g, 1.3 mmol) in $CHCl_3$ (15 mL) was treated with TFA (7 mL) for 1 h at room temperature. The mixture was evaporated under reduced pressure. Ten millilitres of water were added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (5 mL), rinsed with plenty of water, dried on filter, washed with ether (4 × 5 mL) and dried in vacuo over SiO_2. The resulting amine (0.51 g), compound **6** (1.55 g, 3.1 mmol) and NEt_3 (0.6 mL, 4.2 mmol) in dry DMF (6 mL) were stirred for 1 h at 50 °C. The greenish mixture was allowed to react without stirring at 50 °C for 16 h. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography using chloroform/DMF (9:1) as eluent giving the homogenous product **7c** (0.49 g, 48%). ^{1}H-NMR ($CDCl_3$-$(CD_3)_2SO = 1:1$) δ 7.84 (s, 1H), 7.81 (s, 1H), 7.68 (s, 1H), 7.62 (s, 1H), 7.05–7.25 (m, 16H), 4.17 (q, 2H, $J = 7.0$ Hz), 1.20 (t, 3H, $J = 7.0$ Hz).

2.7. Ethyl ester-6-aminohexanoic acid hydrochloride (8)

Thionyl chloride was added dropwise to the solution of 6-aminohexanoic acid (13.1 g, 100 mmol) in dry ethanol (120 mL) at temperature 0–5 °C. The mixture was stirred for 1 h at room temperature, filtered off and the filtrate was evaporated. The crude product was recrystallized from EtOH. It was homogeneous according to chromatography. The yield of pure compound **8** was 18.6 g (95%). ^{1}H-NMR ($(CD_3)_2CO$) δ 7.05–7.43 (m, 15H), 4.02 (q, 2H, $J = 7.0$ Hz), 2.19 (t, 2H, $J = 7.0$ Hz), 2.08 (t, 2H, $J = 7.0$ Hz), 1.28–1.60 (m, 6H), 1.20 (t, 3H, $J = 7.0$ Hz).

2.8. 6-Triphenylmethylaminohexanoic acid (9)

Triphenylmethyl chloride (26.8 g, 97 mmol) was added to a solution of compound **8** (18.5 g, 95 mmol) and NEt_3 (30 mL) in dry CH_2Cl_2 at 0 °C. The mixture was stirred for 3 h at room temperature. The precipitate was filtered off, the filtrate was washed with water (2 × 50 mL) and then evaporated. The residue was dissolved in ethanol (150 mL), and a mixture of NaOH (27 g) and H_2O (30 mL) was added dropwise at room temperature. The mixture was stirred for 4 h at room temperature, evaporated to 100 mL, water (200 mL) was added to the residue and the mixture was cautiously acidified with acetic acid to pH 5–6. The product was extracted with $CHCl_3$ (2 × 100 mL), the organic layer was washed with water (3 × 200 mL) and dried over Na_2SO_4. After evaporation, practically pure **9** was obtained (24.8 g, 65%). ^{1}H-NMR ($(CD_3)_2CO$) δ 7.12–7.52 (m, 15H), 2.26 (t, 2H, $J = 7.0$ Hz), 2.10 (t, 2H, $J = 7.0$ Hz), 1.28–1.67 (m, 6H).

2.9. 1,2,3-Benzotriazol-1-yl-6-triphenylmethylaminohexylcarboxylate (10)

DCC (3.70 g, 17.9 mmol) in small portions was added to compound **9** (6.36 g, 17.0 mmol) and 1-hydroxy-1,2,3-benzotriazole (2.42 g, 17.9 mmol) in dry dichloromethane (100 mL) at room temperature. After the mixture was stirred for 3 h, the precipitated urea was filtered off. Filtrate was evaporated, dried in vacuo over SiO_2. The resulting crude product **10** (8.27 g, 99%) was sufficiently pure and could be used directly for synthesis. ^{1}H-NMR ($(CD_3)_2CO$) δ 7.12–7.91 (m, 19H), 2.25 (t, 2H, $J = 7.0$ Hz), 2.16 (t, 2H, $J = 7.0$ Hz), 1.28–1.67 (m, 6H).

2.10. 6-Triphenylmethylaminohexylcarboxy derivatives 11a–d

Compound **7a–c** (1.0 mmol) was dissolved in the mixture of $CHCl_3$ (15 mL) and TFA (7 mL). After 1 h at room temperature the mixture was evaporated under reduced pressure, and water (10 mL) was added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (5 mL), rinsed with plenty of water, dried on filter, washed with ether (4 × 10 mL) and dried in vacuo over SiO_2. The resulting amine, 1.0 mmol compound **4** or compound **10** (1.02 g, 2.1 mmol) and NEt_3 (0.3 mL) in dry DMF (6 mL) was stirred for 10 h at 60 °C. The brownish mixture was evaporated under reduced pressure. The residue was purified by column chromatography using chloroform as eluent. The yield of pure compounds **11a**, **11b**, **11c**, **11d** was 85, 76, 58 and 63%, respectively.

^{1}H-NMR of compound **11a** ($(CD_3)_2CO$) δ 11.07 (bs, 1H), 8.02 (s, 1H), 7.1–7.5 (m, 15H), 4.30 (q, 2H, $J = 7.0$ Hz), 2.91 (bs, 2H), 2.57 (t, 2H, $J = 7.0$ Hz), 1.46–1.80 (m, 6H), 1.32 (t, 3H, $J = 7.0$ Hz).

^{1}H-NMR of compound **11b** ($(CD_3)_2CO$) δ 11.29 (bs, 1H), 10.77 (bs, 1H), 8.09 (s, 1H), 8.01 (s, 1H), 7.1–7.5 (m, 16H), 4.29 (q, 2H, $J = 7.0$ Hz), 2.93 (bs, 2H), 2.58 (t, 2H, $J = 7.0$ Hz), 1.49–1.79 (m, 6H), 1.33 (t, 3H, $J = 7.0$ Hz).

^{1}H-NMR of compound **11c** ($(CD_3)_2CO$) δ 11.44 (bs, 1H), 10.85 (bs, 2H), 8.19 (s, 1H), 8.10 (s, 1H), 8.03 (s, 1H), 7.1–7.5 (m, 16H), 4.30 (q, 2H, $J = 7.0$ Hz), 2.90 (bs, 2H), 2.60 (t, 2H, $J = 7.0$ Hz), 1.30–1.84 (m, 6H), 1.33 (t, 3H, $J = 7.0$ Hz).

^{1}H-NMR of compound **11d** (DMF-d_7) δ 11.30 (bs, 1H), 10.60 (bs, 1H), 8.44 (s, 1H), 8.39 (s, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.1–7.5 (m, 16H),4.35 (q, 2H, $J = 7.0$ Hz), 2.90 (bs, 2H), 2.60 (t, 2H, $J = 7.0$ Hz), 1.20–1.90 (m, 6H), 1.36 (t, 3H, $J = 7.0$ Hz).

*2.11. Preparation of acids **12a–d***

4 M NaOH (10 mL) was added to the solution of ethyl ester (**11a–d**) (3 mmol) in ethanol (20 mL). The mixture was stirred for 12 h at 20 °C, diluted with water (30 mL) and cautiously acidified with HCl to pH 5–6. The precipitated acid was collected, washed with water (6×10 mL) and dried in vacuo over SiO_2. The resulting acids (90–95%) were sufficiently pure and could be used directly for synthesis.

*2.12. N,N-dimethyl-1,3-propylendiamine derivatives (**13a–d**) and methylamine derivatives*

DCC (0.4 g, 2.0 mmol) in small portions were added to the mixture of acid **12a–c** (1.0 mmol), 1-hydroxy-1,2,3-benzotriazole (0.3 g, 2.0 mmol) in dry DMF (3 mL) at room temperature. After stirring for 2 h, *N,N*-dimethyl-1,3-diaminopropane (0.2 mL) or methylamine hydrochloride (0.4 g, 6 mmol) and NEt_3 (5 mL) were added and the reaction mixture was left for 12 h. The precipitate was filtered off, washed with CH_2Cl_2 (5 mL). Combined filtrates diluted with $CHCl_3$ (50 mL), were washed with 5% $NaHCO_3$ (2×15 mL), water (3×25 mL), dried over Na_2SO_4 and evaporated. The yield of products **13a, 13b, 13c, 13d** and **14a, 14b, 14c** after column chromatography (0–20% gradient of EtOH in $CHCl_3$/NEt_3 (95:5)) was 60, 49, 55, 35, 65, 52 and 40%, respectively.

^{1}H-NMR of compound **13a** ($(CD_3)_2CO$) δ 8.10 (bs, 1H), 7.87 (s, 1H), 7.15–7.55 (m, 15H), 3.54 (bt, 2H), 2.64 (bs, 2H), 2.49 (bt, 2H), 2.17 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H-NMR of compound **13b** ($(CD_3)_2CO$) δ 8.17 (bs, 1H), 7.96 (s, 1H), 7.86 (s, 1H), 7.15–7.55 (m, 15H), 3.54 (bt, 2H), 2.64 (bs, 2H), 2.49 (bt, 2H), 2.17 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H-NMR of compound **13c** ($(CD_3)_2CO$) δ 8.00 (s, 1H), 7.90 (s, 1H), 7.85 (s, 1H), 7.15–7.55 (m, 15H), 3.58 (b.quin, 2H), 2.60 (bm, 2H), 2.33 (bm, 2H), 2.07 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H-NMR of compound **13d** (DMF-d_7) δ 8.40 (s, 1H), 8.31 (s, 1H), 8.28 (s, 1H), 8.10 (s, 1H), 7.1–7.5 (m, 15H), 3.83 (bs, 2H), 2.55 (bs, 2H), 2.30 (bs, 2H), 2.13 (s, 6H), 1.1–2.0 (m, 10H).

^{1}H-NMR of compound **14a** (CCl_4) δ 10.78 (bs, 1H), 7.82 (bs, 1H), 7.74 (s, 1H), 7.20–7.50 (m, 15H), 3.27 (s, 3H), 2.80 (bt, 2H), 2.35 (bt, 2H), 2.01 (bm, 2H), 1.77 (bm, 4H).

^{1}H-NMR of compound **14b** ($(CD_3)_2CO$) δ 11.49 (bs, 1H), 10.62 (bs, 1H), 8.35 (bs, 1H), 7.78 (bs, 1H), 7.75 (s, 1H), 7.15–7.50 (m, 15H), 3.25 (s, 3H), 2.75 (t, 2H), 2.33 (t, 2H), 1.90–2.10 (m, 2H), 1.70–1.80 (m, 4H).

^{1}H-NMR of compound **14c** (DMF-d_7) δ 8.38 (s, 1H), 8.03 (bs, 1H), 7.83 (s, 1H), 7.15–7.55 (m, 15H), 2.61 (t, 2H), 2.11 (s, 3H), 2.09 (t, 2H), 1.3–1.8 (m, 6H).

*2.13. 1-Triphenylmethyl-1,6-diaminohexane (**15**)*

Triphenylmethyl chloride (6.6 g, 24 mmol) in 50 mL dry CH_2Cl_2 was added to a solution of 1,6-diaminohexane (16.6 mg, 140 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred for 4 h at 20°C. The product was purified by column chromatography using chloroform/EtOH/NEt_3 (94:5:1) as eluent. The yield of homogeneous compound **15** was 2.73 g (7.7 mmol, 32%).

^{1}H-NMR ($(CD_3)_2CO$) δ 7.10–7.60 (m, 15H), 3.09 (t, 7.84, 2H, $J = 7.2$ Hz), 2.10 (t, 7.84, 2H, $J = 7.2$ Hz), 1.52 (bm, 4H), 1.32 (bm, 4H).

*2.14. Preparation of compound **16***

To the mixture of acid **12c** (145 mg, 0.23 mmol), 1-hydroxy-1,2,3-benzotriazole (40 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL) was added DCC (54 g, 0.26 mmol) at room temperature. After stirring for 4 h, the amine **15** (93 mg, 0.26 mmol) and 70 μL NEt_3 were added and the reaction mixture was boiled for 4 h. The yield of pure compound **16** after column chromatography (0–5% gradient of ethanol in chloroform) was 187 mg (0.19 mmol, 84%).

^{1}H-NMR ($(CD_3)_2CO$) δ 11.46 (bs, 1H), 10.50 (bs, 1H), 7.84 (s, 1H), 7.10–7.50 (m, 30H), 7.98 (s, 1H), 3.40 (q, 2H, $J = 6.5$ Hz), 2.84 (t, 2H, $J = 7.0$ Hz), 2.64 (t, 2H,

$J = 7.0$ Hz), 2.14 (t, 2H, $J = 7.0$ Hz), 0.94–1.90 (m, 14H).

2.15. Preparation of compound **17**

DCC (93 g, 0.45 mmol) was added to the mixture of acid **12d** (260 mg, 0.30 mmol), 1-hydroxy-1,2,3-benzotriazole (69 mg, 0.45 mmol) in dry DMF (5 mL) at room temperature. After stirring for 4 h, $NH_2CH_2CH(OH)CH_2OH$ (120 mg, 1.30 mmol) was added and the reaction mixture was left for 4 h at 60 °C. The yield of pure compound **17** after column chromatography (0–15% gradient of EtOH in $CHCl_3/NEt_3$ (95:5)) was 110 mg (0.12 mmol, 40%).

^{1}H-NMR ($(CD_3)_2CO$) δ 11.48 (bs, 1H), 10.75 (bs, 2H), 10.30 (bs, 1H), 8.18 (s, 1H), 8.09 (bs, 1H), 8.02 (s, 1H), 8.01 (s, 1H), 7.82 (s, 1H), 7.10–7.50 (m, 15H), 4.51 (bs, 1H), 3.88 (bs, 1H), 3.60 (m, 4H), 2.60 (t, 2H, $J = 7.0$ Hz), 2.15 (t, 2H, $J = 7.0$ Hz), 1.70 (m, 4H), 1.45 (m, 2H).

2.16. Preparation of compounds **I–IX**

Compounds **13, 14, 16** or **17** (100 mg) were dissolved in the mixture of $CHCl_3$ (2 mL) and TFA (1 mL) and after 1 h at room temperature, the mixture was evaporated under reduced pressure and washed with ether (4×5 mL), dissolved in 0.1% TFA and purified by HPLC (0–15% gradient CH_3CN, 0.1% TFA 50–80%). The yield of homogeneous products **I, II, III, IV, V, VI, VII** after column chromatography (0–20% gradient of EtOH in $CHCl_3/NEt_3$ (95:5)) was 80, 75, 71, 50, 51, 60 and 55%, respectively.

^{1}H-NMR of compound **I** ($(CD_3)_2SO$) δ 11.48 (bs, 1H), 7.84 (s, 1H), 3.56 (t, 2H, $J = 7.0$ Hz), 2.64 (t, 2H, $J = 7.0$ Hz), 2.53 (t, 2H, $J = 7.0$ Hz), 2.20 (s, 6H), 2.10 (t, 2H, $J = 7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H-NMR of compound **II** ($(CD_3)_2SO$) δ 12.30 (bs, 1H), 11.48 (bs, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.54 (t, 2H, $J = 7.0$ Hz), 2.65 (t, 2H, $J = 7.0$ Hz), 2.53 (t, 2H, $J = 7.0$ Hz), 2.17 (s, 6H), 2.11 (t, 2H, $J = 7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H-NMR of compound **III** ($(CD_3)_2SO$) δ 12.40 (bs, 2H), 11.48 (bs, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.55 (t, 2H, $J = 7.0$ Hz), 2.65 (t, 2H, $J = 7.0$ Hz), 2.52 (t, 2H, $J = 7.0$ Hz), 2.18 (s, 6H), 2.10 (t, 2H, $J = 7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H-NMR of compound **IV** ($(CD_3)_2SO$) δ 12.40 (bs, 2H), 12.20 (bs, 1H), 11.48 (bs, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.56 (t, 2H, $J = 7.0$ Hz), 2.63 (t, 2H, $J = 7.0$ Hz), 2.54 (t, 2H, $J = 7.0$ Hz), 2.18 (s, 6H), 2.11 (t, 2H, $J = 7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H-NMR of compound **V** ($(CD_3)_2SO$) δ 12.40 (bs, 1H), 12.00 (bs, 1H), 8.19 (s, 1H), 7.88 (s, 1H), 3.48 (t, 2H, $J = 7.0$ Hz), 3.02 (t, 2H, $J = 7.0$ Hz), 2.52 (t, 2H, $J = 7.0$ Hz), 2.14 (t, 2H, $J = 7.0$ Hz), 1.20–1.80 (m, 14H).

^{1}H-NMR of compound **VI** ($(CD_3)_2SO$) δ 12.43 (bs, 1H), 11.95 (bs, 1H), 8.33 (s, 1H), 8.00 (bs, 1H), 7.85 (s, 1H), 4.30 (bs, 2H), 2.81 (s, 3H), 2.16 (t, 2H, $J = 7.0$ Hz), 1.68 (m, 4H, $J = 7.0$ Hz), 1.39 (m, 4H, $J = 7.0$ Hz).

^{1}H-NMR of compound **VII** ($(CD_3)_2SO$) δ 12.57 (bs, 1H), 12.43 (bs, 1H), 11.95 (bs, 1H), 8.34 (s, 1H), 8.33 (s, 1H), 8.00 (bs, 1H), 7.85 (s, 1H), 4.40 (bs, 2H), 2.79 (s, 3H), 2.12 (t, 2H, $J = 7.0$ Hz), 1.61 (m, 4H, $J = 7.0$ Hz), 1.35 (m, 4H, $J = 7.0$ Hz).

^{1}H-NMR of compound **19a** (D_2O) δ 7.69 (s, 1H), 3.00 (t, 2H, $J = 7.0$ Hz), 2.86 (s, 3H), 2.51 (t, 2H, $J = 7.0$ Hz), 1.69 (bruin, 4H, $J = 7.0$ Hz), 1.43 (bruin, 4H, $J = 7.0$ Hz).

2.17. EDTA derivatives **VIII**, **IX**, **X**

Compounds **18b** or **18c** or **20** (0.2 mmol), after evaporation with pyridine (2×5 mL), were mixed with dry DMF (10 mL), EDTA-anhydride (2 mmol) and pyridine (0.8 mL). The mixture was stirred for 5 h at room temperature and evaporated under reduced pressure. The residue was purified by reverse phase HPLC (0–15% gradient of CH_3CN, 0.1% TFA). The yield of compounds **VIII**, **IX**, **X** was 33, 39 and 40%, respectively.

^{1}H-NMR of compound **VIII** ($(CD_3)_2CO$) δ 8.10 (s, 1H), 7.84 (s, 1H), 3.90 (bm, 14H), 3.50 (t, 2H, $J = 7.0$ Hz), 3.10 (t, 2H, $J = 7.0$ Hz), 3.07 (s, 6H), 2.60 (t, 2H, $J = 7.0$ Hz), 2.10 (bm, 2H), 1.60–1.80 (m, 4H), 1.40–1.50 (m, 2H).

^{1}H-NMR of compound **IX** ($(CD_3)_2CO$) δ 8.12 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 3.91 (bm, 14H), 3.47 (t, 2H, $J = 7.0$ Hz), 3.07 (t, 2H, $J = 7.0$ Hz), 3.04 (s, 6H), 2.57 (t, 2H, $J = 7.0$ Hz), 2.11 (bm, 2H), 1.61–1.85 (bm, 4H), 1.41–1.55 (bm, 2H).

^{1}H-NMR of compound **X** ($(CD_3)_2CO$) δ 8.14 (s, 1H), 7.93 (s, 1H), 3.90 (bm, 24H), 3.50 (bt, 2H, $J = 7.0$ Hz), 3.18 (t, 2H, $J = 7.0$ Hz), 2.83 (t, 2H, $J = 7.0$ Hz), 2.67 (t, 2H, $J = 7.0$ Hz), 1.20–1.80 (m, 14H).

2.18. Preparation of compounds **XI–XIII**

DCC (93 mg, 0.45 mmol) was added to the mixture of acids **12b–d** (0.30 mmol), 1-hydroxy-1,2,3-benzotriazole (69 mg, 0.45 mmol) in dry DMF (5 mL) at room

temperature. After stirring for 4 h, 3-amino-1,2-propanediol (120 mg, 1.30 mmol) was added and the reaction mixture was left for 4 h at 60 °C. Reaction mixtures were purified by column chromatography (0–15% gradient of EtOH in $CHCl_3/NEt_3$ (95:5)). The triphenylmethyl derivatives obtained were dissolved in a mixture of CH_2Cl_2 (2 mL) and TFA (1 mL) and after 1 h at room temperature, the mixture was evaporated under reduced pressure and washed with ether (4 × 5 mL), dissolved in 0.1% TFA and purified by HPLC.

The yield of pure homogeneous compounds **XI**, **XII** and **XIII** after HPLC (0–90% gradient of CH_3CN, 0.1% TFA) was 55, 50 and 40%, respectively.

^{1}H-NMR of compound **XI** ($(CD_3)_2SO$) δ 12.45 (bs, 1H), 11.95 (bs, 1H), 8.30 (s, 1H), 8.05 (bs, 1H), 7.88 (s, 1H), 4.43 (bm, 1H), 4.30 (bs, 2H), 3.34 (m, 3H), 3.07 (m, 1H), 2.15 (t, 2H, $J = 7.0$ Hz), 1.68 (m, 4H, $J = 7.0$ Hz), 1.39 (m, 4H, $J = 7.0$ Hz).

^{1}H-NMR of compound **XII** ($(CD_3)_2SO$) δ 12.62 (bs, 1H), 12.41 (bs, 1H), 11.95 (bs, 1H), 8.32 (s, 1H), 8.30 (s, 1H), 8.02 (bs, 1H), 7.87 (s, 1H), 4.41 (bs, 3H), 4.30 (bs, 2H), 3.32 (m, 3H), 3.05 (m, 1H), 2.13 (t, 2H, $J = 7.0$ Hz), 1.65 (m, 4H, $J = 7.0$ Hz), 1.36 (m, 4H, $J = 7.0$ Hz).

^{1}H-NMR of compound **XIII** ($(CD_3)_2CO$) δ 11.48 (bs, 1H), 10.75 (bs, 2H), 10.30 (bs, 1H), 8.18 (s, 1H), 8.09 (bs, 1H), 8.02 (s, 1H), 8.01 (s, 1H), 7.82 (s, 1H), 4.51 (bs, 1H), 3.88 (bs, 1H), 3.60 (m, 4H), 2.60 (t, 2H, $J = 7.0$ Hz), 2.15 (t, 2H, $J = 7.0$ Hz), 1.70 (m, 4H), 1.45 (m, 2H).

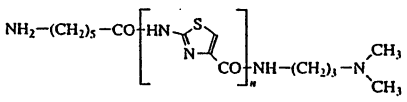
MALDI TOF-MS for the different compounds was the following:

I MALDI TOF-MS (monoisotopic) 342.3 (342.2 calcd for M + H).
II MALDI TOF-MS (monoisotopic) 468.3 (468.2 calcd for M + H).
III MALDI TOF-MS (monoisotopic) 594.3 (594.2 calcd for M + H).
IV MALDI TOF-MS (monoisotopic) 720.2 (720.2 calcd for M + H).
V MALDI TOF-MS (monoisotopic) 482.1 (482.2 calcd for M + H).
VI MALDI TOF-MS (monoisotopic) 397.1 (397.1 calcd for M + H).
VII MALDI TOF-MS (monoisotopic) 523.2 (523.1 calcd for M + H).
VIII MALDI TOF-MS (monoisotopic) 742.2 (742.3 calcd for M + H).
IX MALDI TOF-MS (monoisotopic) 868.3 (868.2 calcd for M + H).
X MALDI TOF-MS (monoisotopic) 1030.7 (1030.4 calcd for M + H).
XI MALDI TOF-MS (monoisotopic) 457.2 (457.1 calcd for M + H).
XII MALDI TOF-MS (monoisotopic) 583.2 (583.1 calcd for M + H).
XIII MALDI TOF-MS (monoisotopic) 709.2 (709.1 calcd for M + H).

3. Results and discussion

A series of oligo-1,3-thiazolcarboxamide derivatives with different numbers of thiazolcarboxamide units in the peptide and substituents in the side chain was synthesized. The structures of the Tz containing oligopeptides are given in *figure 2*. All the compounds synthesized (**I**–**XIII**) were homogeneous according to the data of reverse phase chromatography, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.

To analyse the interaction of the TCOs with HIV-1 IN we used the 3′-end processing reaction catalysed by the enzyme. The reaction was assayed with a double stranded oligonucleotide derived from the HIV-1 U5 end of the LTR, containing [^{32}P]-labeled GT nucleotides in the 3′-end. Sequence-specific removal from the 3′-end generated a radiolabeled dinucleotide product detected following electrophoresis (*figure 3A*). In parallel, the reaction products were analysed by counting the remaining labeled material in the acid-insoluble fraction after dinucleotide cleavage (*figure 3B*). As the measurements using both determinations matched very well, the second method was used to estimate the effect of TCOs on the IN-catalysed reaction. *Figure 3* illustrates the results obtained with one of the TCOs, compound **III**. With increasing concentrations of this compound, a dose-dependent inhibition of the 3′-processing reaction was obtained with an IC_{50} value of 2.3 μM. All TCOs present similar patterns of inhibition, although with different efficiencies. The IC_{50} values for all compounds analysed are given in *table I*. Distamycin, the natural oligopeptide containing three pyrrole rings, was used as a reference of DNA minor groove binder. The IC_{50} value for distamycin, obtained under the same experimental conditions, is also reported in *table I*. TCOs containing 3 or 4 Tz units (compounds **III** and **XIII)** were able to suppress the IN activity as efficiently as distamycin. A very efficient inhibitor of

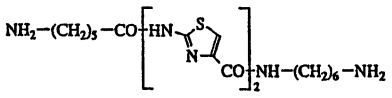


n = 1 (I)

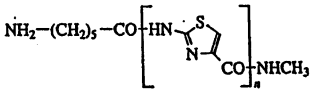
n = 2 (II)

n = 3 (III)

n = 4 (IV)

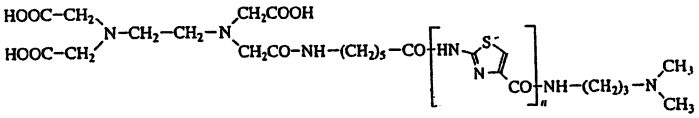


(V)



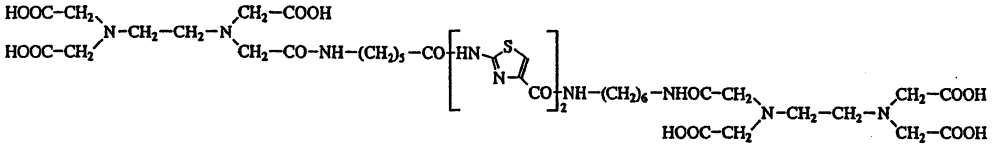
n = 2 (VI)

n = 3 (VII)

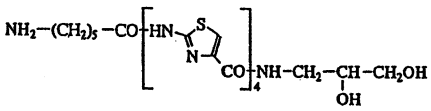


n = 2 (VIII)

n = 3 (IX)



(X)



n = 2 (XI)

n = 3 (XII)

n = 4 (XIII)

Figure 2. Structures of the TCOs **I**–**XIII**.

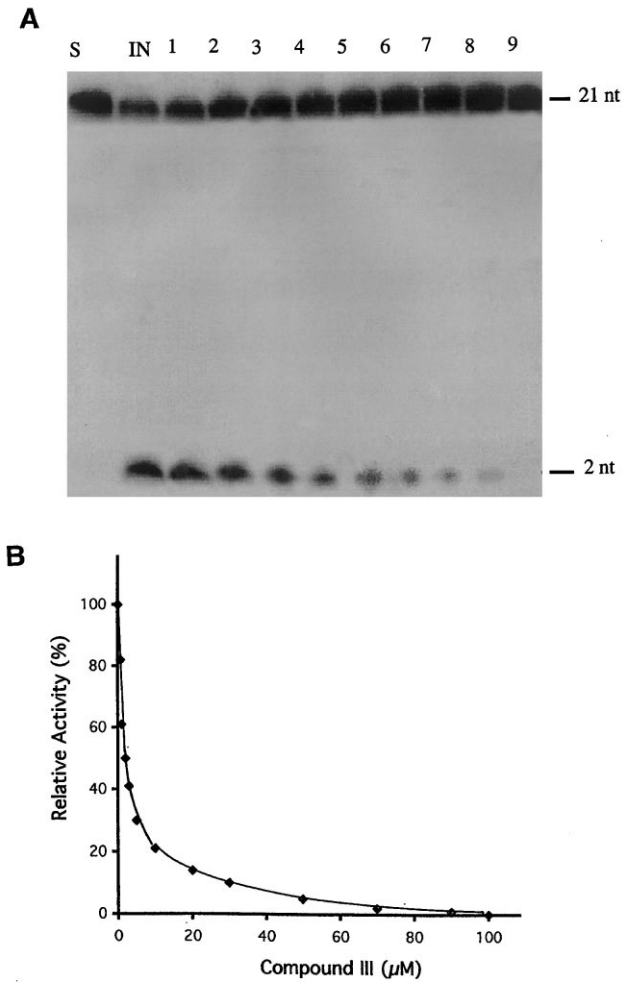


Figure 3. Inhibition on the IN-catalysed 3′-processing reaction by oligopeptide **III**. **A**: Reaction products were analysed by urea-polyacrylamide gel electrophoresis. The complete reaction mixture containing the labeled substrate was incubated for 30 min at 30 °C in the absence of IN (lane S) or in the presence of 40 nM IN (lane IN). Lanes 1–9: same as lane IN, but in the presence of 0.7, 1, 3, 10, 30, 50, 70, 90 and 100 μM of compound **III**, respectively. Arrows indicate the migration of the labeled oligonucleotides containing 21 and 2 nucleotides. **B**: Reaction products were determined by measuring the TCA-precipitable radioactivity remaining after IN-dependent processing. Results are expressed as relative activity, 100% corresponding to IN activity in the absence of the inhibitor.

the IN-catalysed reaction was compound **IV** with an IC_{50} value about one order of magnitude lower than that of distamycin.

Next we analysed the relationship between the inhibitory efficiency and the length of the TCO derivatives. As shown in *figure 4*, the IC_{50} values for TCOs of different length correlated with the number of Tz units in the oligopeptides. The logarithmic dependences of the IC_{50} values on the number of Tz units were close to linear (*figure 4*), suggesting that each monomer of the TCO molecule contributed in an additive form to the interaction with the nucleotide units of DNA.

An interesting point is the influence of the amines, that are positively charged at physiological pH, on the inhibitory activity of the molecules. TCOs having the same number of Tz units, but additional positively charged groups attached to the N- and C-termini were more efficient inhibitors of the IN-dependent reaction. In contrast, the introduction of EDTA (compounds **VIII, IX** and **X**) or diol-groups (compounds **XI, XII** and **XIII**) into the oligopeptides led to higher IC_{50} values. Such decrease on the affinity of TCOs towards DNA complexed with IN may be explained by the negative charges of EDTA- and the diol-groups, and/or by the electrostatic repulsion between TCOs and the internucleoside phosphate groups of DNA.

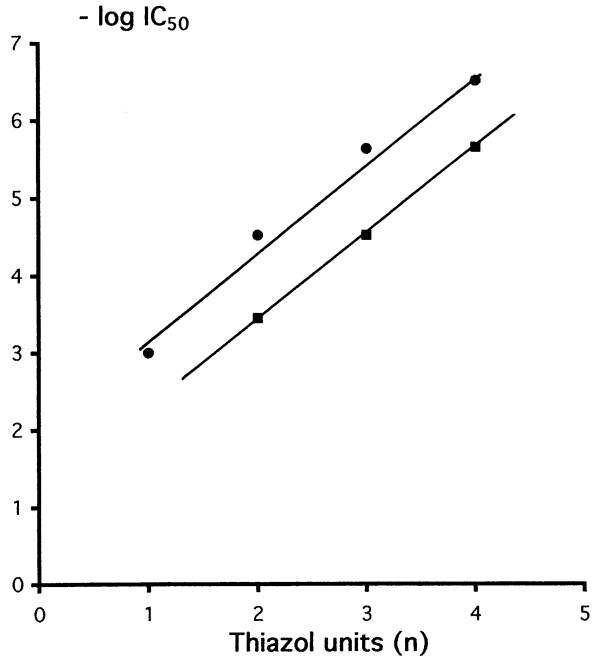


Figure 4. Logarithmic dependence of the IC_{50} values upon the number of Tz units in the structure of TCOs: compounds **I, II, III** and **IV** (●) and **XI, XII** and **XII** (■).

Table I. Concentration of TCOs inhibiting the IN 3′-processing activity by 50% (IC_{50}).

Compound	Structures of TCOs	IC_{50} (μM)
I	$NH_2(CH_2)_5CO$-[Tz]-$NH(CH_2)_3N(CH_3)_2$	1000 ± 200
II	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_3N(CH_3)_2$	30 ± 7
III	$NH_2(CH_2)_5CO$-$[Tz]_3$-$NH(CH_2)_3N(CH_3)_2$	2 ± 0.3
IV	$NH_2(CH_2)_5CO$-$[Tz]_4$-$NH(CH_2)_3N(CH_3)_2$	0.3 ± 0.05
V	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NH(CH_2)_6NH_2$	38 ± 8
VI	$NH_2(CH_2)_5CO$-$[Tz]_2$-$NHCH_3$	70 ± 15
VII	$NH_2(CH_2)_5CO$-$[Tz]_3$-$NHCH_3$	9 ± 2
VIII	EDTA-$NH(CH_2)_5$-CO-$[Tz]_2$-$NH(CH_2)_3$-$N(CH_3)_2$	860 ± 150
IX	EDTA-$NH(CH_2)_5$-CO-$[Tz]_3$-$NH(CH_2)_3$-$N(CH_3)_2$	10 ± 30
X	EDTA-$NH(CH_2)_5$-CO-$[Tz]_2$-NH-EDTA	300 ± 70
XI	$NH_2(CH_2)_5CO$-$[Tz]_2$-NH $CH_2CH(OH)CH_2OH$	350 ± 50
XII	$NH_2(CH_2)_5CO$-$[Tz]_3$-NH $CH_2CH(OH)CH_2OH$	30 ± 8
XIII	$NH_2(CH_2)_5CO$-$[Tz]_4$-NH $CH_2CH(OH)CH_2OH$	2.2 ± 0.4
	Distamycin A	3.5 ± 0.5

Since the affinities of TCOs depended most on their length, it can be hypothesized that DNA complexed with IN interacted mainly with the Tz monomers, and that such interactions were responsible for the efficiency of the inhibition by oligo-1,3-thiazolecarboxamide derivatives. At the same time, it cannot be excluded that the additional groups attached to the N- and C-termini of TCOs may influence the interactions either with the enzyme or *via* the enzyme on their interaction with DNA.

4. Experimental protocol

4.1. Reagents

Reagents were purchased from Merck, Sigma, Pharmacia and Euromedex. All oligodeoxynucleotides (ODNs) were synthesized as described in [34] and their concentration was determined according to [35].

4.2. Enzymes

Recombinant HIV-1 IN, expressed in yeast, was obtained and purified as previously described [36].

4.3. Enzyme assays

Double stranded DNA substrate for 3′-end processing was prepared by annealing the 19-mer ODN (5′-GTGTGGAAAATCTCTAGCA) with the 21-mer complementary strand (5′-ACTGCTAGAGATTTTCCA) by heating for 2 min at 90 °C and slow cooling. This ds-DNA was labeled at the 3′-end with [α-^{32}P]dGTP and [α-^{32}P]TTP in the presence of the exonuclease-free Klenow fragment of *E. coli* DNA polymerase.

IN activity was measured by the 3′-end processing reaction. The standard reaction mixture (20 μL) contained 20 mM Hepes (pH 7.5), 10 mM DTT, 0.1 mM EDTA, 4 mM NaCl, 7.5 mM $MnCl_2$, 0.05% NP 40, 3.5 nM ds [^{32}P]-substrate. The reaction mixture was incubated for different times (2–60 min) at 30 °C in the presence of 10–40 nM IN. The reaction products of IN-dependent [^{32}P](GT) dinucleotide removal were estimated using two methods: a) separation on 12% polyacrylamide gel electrophoresis in the presence of 7 M urea, or b) by measuring the label remaining in the acid-insoluble material by the utilization of dry Whatman 3MM filters (15×15 mm) presoaked in 5% trichloroacetic acid (TCA). Further treatment of the filters was carried out as described in [37]. All measurements were carried out within linear ranges of the time dependence of product accumulation and IN concentration. The IC_{50} values were determined at substrate concentration around 3 times the K_m. The data of 3–4 experiments were averaged. The error was within 10–30%.

5. Conclusions

A set of thiazolecarboxamide derivatives able to interact with the minor groove of DNA was synthesized and its effect was evaluated on the HIV-1 IN-

catalysed reaction. Some TCOs were efficient inhibitors of this reaction: compound **IV**, for example, with an IC_{50} of 0.3 μM. These first generation of TCOs will serve as leads provided that additional chemical groups allow to increase their inhibitory power.

In addition, as we previously showed that short oligonucleotides of specific sequence were efficient inhibitors of HIV-1 IN [36], we can suppose that such oligonucleotides conjugated to the TCOs described in this work could offer a unique opportunity for rational design of new IN-targeting drugs. Further work is being performed to synthesize such derivatives.

Acknowledgements

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