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Original article

An approach to the stereo-controlled synthesis of polycyclic derivatives of L-4-thiazolidinecarboxylic acid active against HIV-1 integrase

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Abstract

Herein, we describe a new strategy for the preparation of thiazolothiazepine-based inhibitors of human immunodeficiency virus type-1 integrase (IN). The present method allows facile preparation of the title compounds in a single enantiomeric form starting from L-4-thiazolidinecarboxylic acid. This method could be easily extended to the synthesis of several analogs derived from optically active cyclic aminoacids. We also present a putative model showing the interaction between L- and D-isomers of compound **1** in the IN active site. A sensibly lower IC_{50} value was found for (–)-**1** over racemic-**1** in an anti-IN assay.

Keywords: HIV-1 integrase inhibitors; L-4-thiazolidinecarboxylic acid; Thiazolothiazepines

1. Introduction

Currently, chemotherapeutic intervention for the treatment of HIV infection is based on the administration of drugs targeting reverse transcriptase (RT) and protease (PR) [1]. Combinations of RT and PR inhibitors have made a major impact in the life of many AIDS patients. However, this approach does not eradicate the virus. Because of persistent toxicity, difficulties in patient adherence and development of drug-resistance, there is an urgent need to develop novel therapeutics targeting other viral proteins with better safety profiles [2].

HIV-1 integrase (IN) is such an attractive target and is essential for viral replication. IN mediates the insertion of retroviral DNA into host chromosomes by a complex process consisting of two fundamental reactions known as 3′-processing and strand transfer [3,4]. The in vitro assaying of a large number of compounds was made possible by the availability of recombinant IN and thus far two agents have been tested in the clinic [5,6].

Thiazolothiazepines, previously prepared by us in a racemic mixture, despite of their low potency, represent a class of compounds with interesting characteristics, such as: 1) activity in the presence of Mg^{2+} as a cofactor, 2) activity in cell-based antiviral assays, 3) low cytotoxicity, and 4) good selectivity against IN [7]. Such properties prompted us to optimize these molecules by designing analogs bearing several structural alterations, with the main aim at increasing potency [8]. The structures of the two original leads **1** and **2** are shown in Fig. 3.

As a crucial step for the development of more potent inhibitors, we wanted to know which enantiomers of compound **1** and **2** is responsible for activity. Previously, our most potent thiazolothiazepine, **2**, was obtained as an enantiomeric mixture starting from (±)-thiazolidine-2-carboxylic acid. However, for the preparation of compound **1**, even though we started from commercially available L-4-thiazolidinecarboxylic acid, the optical activity was lost during the synthesis. The different arrangement of the two enantiomers of compound **1** as well

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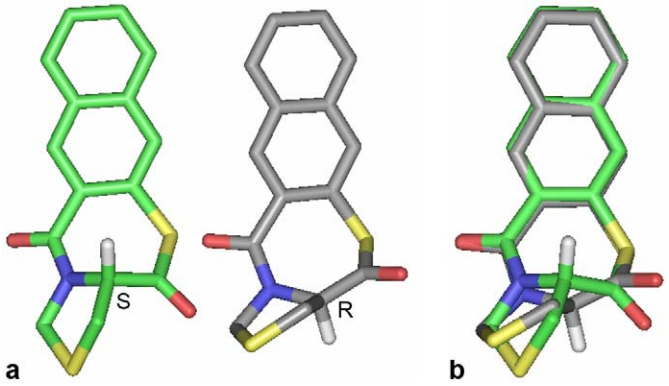


Fig. 1. *S* and *R* enantiomers of compound **1** are shown side by side (a) and superimposed (b).

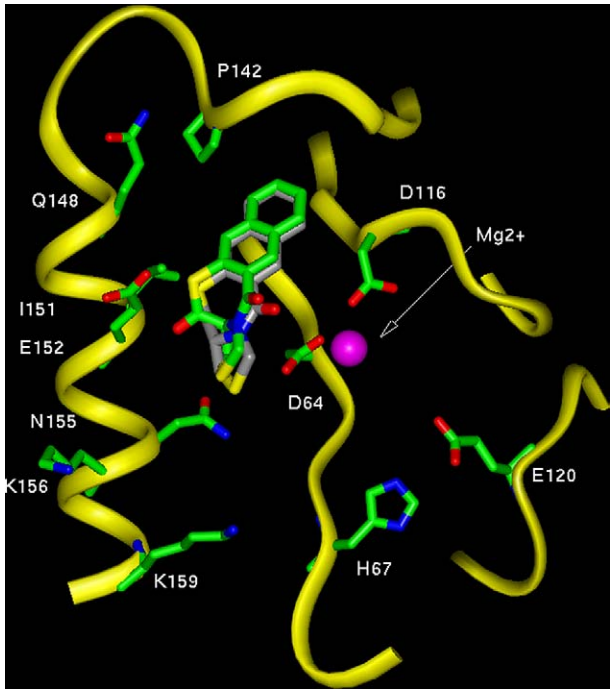


Fig. 2. The predicted bound conformation of *S* and *R* enantiomeric forms of compound **1** inside the HIV-1 IN active site. The two isomers are shown as stick models in green (*S* isomer), gray (*R* isomer). The yellow ribbon model represents active site region of the HIV-1 IN. The prominent active site amino acid residues are shown as stick models while magenta sphere represents Mg^{2+}.

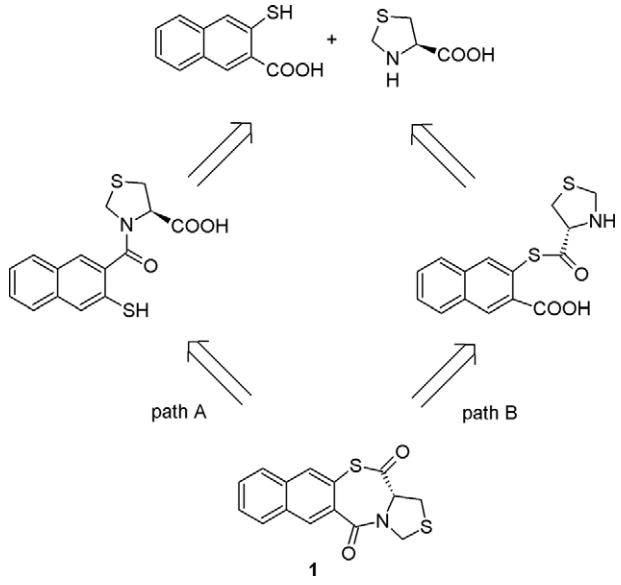
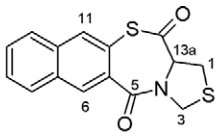


Fig. 4. Retrosynthetic approaches for compound **1**.

as their bound conformation inside the HIV-1 IN active site are shown in Figs. 1 and 2.

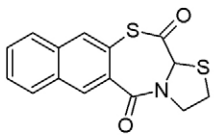
The previous synthesis of **1** consisted of two subsequent condensation steps between L-4-thiazolidinecarboxylic acid and 3-mercapto-2-naphthoic acid (Fig. 4, path A) [7]. The formation of the thiolactone bond by means of *N*,*N*′-carbonyldiimidazole (CDI) required a fairly harsh condition (i.e.: prolonged reaction time and high temperature). Under this condition a racemic mixture was obtained because of the rigidity caused by the preformed amide moiety that hindered the closure of the seven-membered ring. Similar results were obtained during the preparation of other analogs using path A [8]. Previously, when other precursors such as various L-4-oxazolidinecarboxylic acids [9] and aromatic *o*-hydroxy carboxylic acids were condensed, we always obtained racemic mixtures. However, none of the compounds where heterocyclic sulfur was replaced with oxygen(s) showed superior activity over the parent compounds.

Therefore, a new synthetic route aimed at avoiding racemization was undertaken. First we wanted to form the thiophenol ester bond in milder conditions so that the chiral center would not be affected. In this case the seven-membered ring closure reaction by the subsequent formation of the lactam moiety would lead to the desired homochiral compounds (Fig. 4, path B).



(±)-**1**

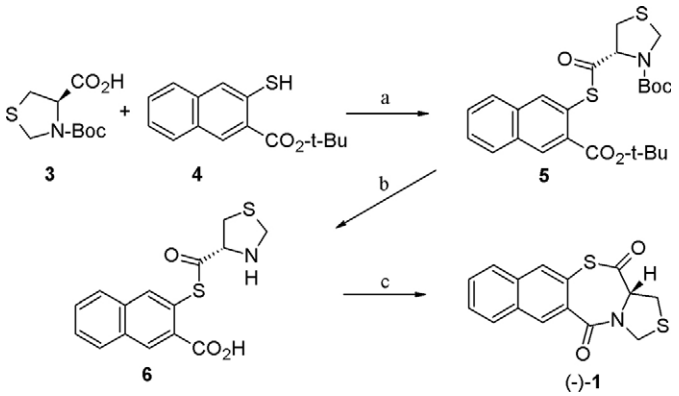
1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho-[2,3-*f*]-thiazolo[4,3-*c*][1,4]thiazepine-5,13-dione



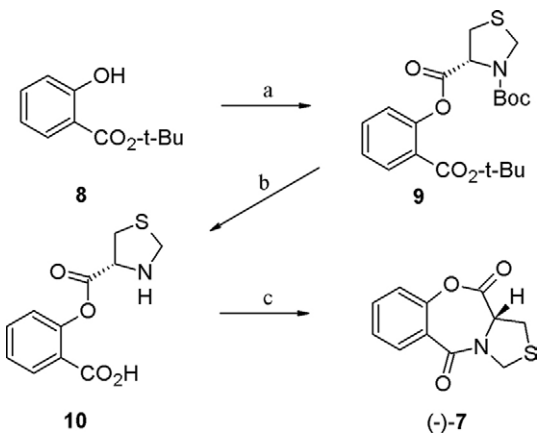
(±)-**2**

2,3-Dihydro-5*H*-naphtho-[2,3-*f*]thiazolo-[2,3-*c*][1,4]thiazepine-5,13(13a*H*)-dione

Fig. 3. Chemical structures of the lead compounds.



Scheme 1. Reagents and conditions: a) BOP/DIEA/DCM/rt/2 h (42%); b) TFA/anisole (excess)/rt; c) PyBOP/DIEA/DMF/1 h (35%).



Scheme 2. Reagents and conditions: a) **3**/BOP/TEA/DCM/rt/3 h (65%); b) TFA/anisole (excess)/rt; c) PyBOP/DIEA/DMF/1 h (75%).

However, several synthetic challenges have to be resolved in this case. For example, appropriate protections for the secondary amino group of the aminoacid as well as the aromatic carboxylic group were required prior to the thiophenol ester bond formation. After such consideration, the synthetic pathway depicted in Scheme 1 was pursued.

2. Chemistry

N-Boc-L-4-thiazolidinecarboxylic acid **3** and the *tert*-butyl 3-mercapto-2-naphthoate **4** both bearing a protective group easily removable in mild hydrolytic conditions were selected as ideal precursors. Their condensation to form the corresponding *N-Boc*-D-4-{[2-(*tert*-butoxycarbonyl)naphthalen-3-ylthio] carbonyl}-1,3-thiazolidine **5** was accomplished with BOP/DIEA in DCM, under nitrogen and in dark condition, in a 42% yield [10]. The fully protected intermediate was purified by column chromatography on silica gel and then amino and carboxylic groups were deprotected simultaneously by trifluoroacetic acid and excess anisole at room temperature. The thiophenol ester bond was not affected under this condition. Because of easy removal of excess reagents no further purification of D-3-[(1,3-thiazolidin-4-ylcarbonyl)thio]-2-naphthoic acid **6** was needed. Compound (–)-**1** was obtained by a cyclization reaction attempted under different experimental conditions. The best result was achieved by using PyBOP/DIEA as an intramolecular coupling reagent in DMF [11]. Optical activity and purity measurements on the final compound confirmed the stereo-controlled synthesis: $[\alpha]^{20}_{D} = -91$ ($CHCl_3$, $c = 0.8$); ee = > 93%. Enantiomeric excesses were determined by ^{1}H NMR spectroscopy using europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate].

To investigate the generality of this method, we prepared a related benzoxazepine. In particular the synthesis of L-(–)-1,11a-dihydro-5*H*,11*H*-[1,3]thiazolo[4,3-*c*] [1,4]benzoxazepine-5,11-dione **7** was undertaken by only slight modifications in the above reaction sequence (Scheme 2). Condensation between *N-Boc*-L-4-thiazolidinecarboxylic **3** and *tert*-butyl salicilate **8**, using the BOP/TEA system in DCM [12], gave the corresponding ester **9**, which was deprotected in the above conditions, to give essentially pure L-2-[(1,3-thiazolidin-4-ylcarbonyl)oxy]benzoic acid **10**. The final cyclization step was carried out with PyBOP/DIEA in DMF to give optically pure L-(–)-**7** in a 75% yield; $[\alpha]^{20}_{D} = -111$ ($CHCl_3$, $c = 1$); ee = > 97%. The present procedure could therefore be used for the synthesis of homochiral compounds, when the starting cyclic amino acid is available in a single enantiomeric form.

3. Results and discussion

Racemic **1** and pure (–)-**1** have been singly subjected to preliminary anti-IN evaluation in order to detect any possible difference of potency. The extent of 3′-processing and strand transfer was essentially determined as described in Ref. [8].

In earlier experiments, (±)-**1** showed IC_{50} (μM) = 92 and 100 for the 3′-processing and strand transfer, respectively, in the inhibition of HIV-1 IN catalytic activity [7]. In the same conditions, pure (–)-**1** showed IC_{50} (μM) = 65 and 88, respectively.

The comparison of such values proves that both enantiomers possess inhibitory activity, though a greater potency has to be ascribed to pure (–)-**1**. Thus (–)-**1** may be considered the eutomer. By merely theoretical conjectures, an eudismic ratio = 2.4 (–/+) could be then calculated in the inhibition of the 3′-processing step, assuming that both stereoisomers would act as inhibitors at the active site, each being independent from the other. More definitive conclusions could be drawn after the synthesis and testing of the remaining stereoisomer (+)-**1**.

In conclusion, these preliminary results would prove that chirality is a crucial feature to be taken into consideration for optimizing interaction of thiazolothiazepine-based IN-inhibitors inside the IN active site. Along with other structural modifications, the development of stereoselective synthetic procedures therefore represents an additional opportunity for the production of even more active compounds.

4. Experimental section

4.1. Chemistry

For general experimental informations see Ref. [8]. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values.

4.2. Synthetic procedure

A typical procedure is described for the synthesis of compound (–)-**7.** *N-Boc*-L-4-thiazolidinecarboxylic **3** (1.40 g, 6 mmol), *tert*-butyl salicilate **8** (1.17 g, 6 mmol), triethylamine (1.21 g, 12 mmol) and BOP reagent (2.65 g, 6 mmol) are dissolved successively in dichloromethane (45 ml). The solution is kept at room temperature for 3 h. Saturated aqueous sodium chloride solution (100 ml) is added and the resultant mixture is extracted with ethyl acetate. The organic phase is separated, washed successively with 1 N hydrochloric acid, saturated sodium hydrogen carbonate solution and sodium chloride solution, dried ($MgSO_4$) and evaporated in vacuo. Pure *N-Boc*-L-[2-(*tert*-butoxycarbonyl)phenyl]-1,3-thiazolidine-4-carboxylate **9** was obtained as a white solid after column chromatography purification on silica gel (diethyl ether/light petroleum, 1:1 as eluent); 1.60 g, 65% yield. M.p. 89.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 1.51 (s, 9 H), 1.62 (s, 9 H), 3.48 (m, 1 H), 3.75 (m, 1 H), 4.55 (t, 1 H, $J = 1.7$), 4.70 (dd, 1 H, $J = 6.5$, 1.7), 5.05 (d, 1 H, $J = 6.5$), 7.2–7.9 (m, 4 H). Anal. $C_{20}H_{27}NO_6S$ (C, H, N).

Compound **9** (0.20 g, 0.49 mmol) is dissolved in a mixture of trifluoroacetic acid/anisole, 1:1 (20 ml) at 0 °C. The mixture is then stirred at room temperature for 50 min. The volatiles are evaporated under vacuum and the residue is azeotropized several times with toluene to give essentially pure 2-[(1,3-thiazolidin-4-ylcarbonyl)oxy]benzoic acid **10** which is subjected to cyclization without further purification.

A solution of the hydroxyacid **10** (0.10 g, 0.39 mmol) in dry DMF (2 ml) is slowly added to a solution of PyBOP (0.20 g, 0.39 mmol) and DIEA (0.05 g, 0.39 mmol) in dry DMF (5 ml). The mixture is stirred for 1 h and then the solvent is evaporated under vacuum. The residue obtained is partitioned between water and ethyl acetate and the organic layer is separated and washed with 1 N hydrochloric acid and water. After evaporation of the solvent, the oily residue is purified by column chromatography (ethyl acetate/hexanes, 1:6) to give pure compound (–)-**7** (70 mg, 75% yield) which shows identical data as reported in Ref. [8], but $[\alpha]^{20}_D = -111$ ($CHCl_3$, $c = 1$), ee = > 97%.

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