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Bioorganic & Medicinal Chemistry 12 (2004) 4459–4466
Bioorganic & Medicinal Chemistry

Synthesis of novel thiazolothiazepine based HIV-1 integrase inhibitors

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Received 14 April 2004; revised 26 May 2004; accepted 28 May 2004
Available online 26 June 2004

Abstract—Thiazolothiazepines are among the smallest and most constrained inhibitors of human immunodeficiency virus type-1 integrase (HIV-1 IN) inhibitors (*J. Med. Chem.* **1999**, *42*, 3334). Previously, we identified two thiazolothiazepines lead IN inhibitors with antiviral activity in cell-based assays. Structural optimization of these molecules necessitated the design of easily synthesizable analogs. In order to design similar molecules with least number of substituent, herein we report the synthesis of 10 novel analogs. One of the new compounds (**1**) exhibited similar potency as the reference compounds, confirming that a thiazepinedione fused to a naphthalene ring system is the best combination for the molecule to accommodate into the IN active site. Thus, the replacement of sulfur in the thiazole ring with an oxygen does not seem considerably affect potency. On the other hand, the introduction of an extra methyl group at position 1 of the polycyclic system or the shift from a thiazepine to an oxazepine skeleton decreased potency. In order to understand their mode of interactions with IN active site, we docked all the compounds onto the previously reported X-ray crystal structure of IN. We observed that compounds **7**–**9** occupied an area close to D64 and Mg^{2+} and surrounded by amino acid residues K159, K156, N155, E152, D116, H67, and T66. The oxygen atom of the oxazolo ring of **7** and **8** could chelate Mg^{2+}. These results indicate that the new analogs potentially interact with the highly conserved residues important for IN catalytic activities.

1. Introduction

Among the three viral enzymes encoded by the *pol* gene of HIV-1, namely reverse transcriptase (RT), protease (PR), and integrase (IN), only the latter still lacks FDA approved drugs. Currently adopted cocktails of RT and PR inhibitors although highly effective, suffer from several unwanted effects like toxicity, patient adherence, and emergence of drug-resistant viral phenotypes.[1] Therefore, IN represents an attractive and validated target because it is essential for viral replication cycle and has no cellular homologue. IN mediates the integration of retroviral DNA into host chromosomes by two distinct processes referred to as 3′-processing and strand transfer, respectively.[2,3]

The availability of in vitro assays using recombinant IN has allowed the discovery of numerous compounds belonging to different chemical classes. The two most predominant classes of inhibitors are the hydroxylated aromatics and the β-diketoacid containing compounds (for recent reviews, see Refs. 4–6). Currently, two examples of latter class are under clinical investigations.[7,8]

Previously, we discovered several novel thiazolothiazepines as IN inhibitors with antiviral activity in cell-based assays.[9] Moreover, unlike many other classes of inhibitors, they retain activity in the presence of Mg^{2+} as a cofactor. Due to their low cytotoxicity, low molecular weight, drug-like properties, and structural novelty we embarked on a study to perform structural optimization. In order to perform an extensive SAR studies, we decided to design simpler molecules based on the parent

Keywords: HIV-1 integrase inhibitors; Structure-based drug design; Docking; Thiazolothiazepines; Oxazepines.
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doi:10.1016/j.bmc.2004.05.037

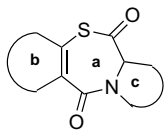


Figure 1. Common structural motif of novel compounds presented in this study.

compounds that are easier to synthesize than the previously reported compounds.

The structural motif common to these compounds consists of a central thiazepinedione moiety (a) fused to a carbocyclic aromatic system (benzo or naphtho) (b), and to a 1,3-thiazolidine ring (c), as depicted in Figure 1. Several structural modifications on the reference compounds have been planned in an attempt to identify novel compounds with similar potency. Herein, we report the preparation and preliminary biological evaluation of derivatives obtained by: (1) the replacement of sulfur atom(s) by oxygen or (2) the introduction of a methyl group on position 1 of the polycyclic system, and (3) a combination of both features, while maintaining a fused benzo or naphtho moiety with a various geometry (Table 1). Such alterations should cause negligible variation in both lipophilicity and overall topology of rings, with potential to coordinate a metal cofactor on the IN active site.

2. Results and discussion

2.1. Chemistry

The synthesis of thiazepinediones **1**–**3** has been accomplished following the general method outlined in Scheme 1 and as previously described using 2,2′-dithiodibenzoic acid **11** or 3,3′-dithio-2,2′-dinaphthoic acid **12** and an appropriate cyclic amino acid **15** or **16** (Chart 1), as starting materials. A modification of the above procedure was developed for the synthesis of oxazepinedione derivatives.[9–15] 2-Acetoxybenzoic acid **17** or the proper acetoxynaphthoic acid **18**–**20** was converted to the corresponding acid chloride,[10] which was in turn added to a cyclic amino acids **13**–**16** (Chart 1) giving amides of general formula **21**, in good yields. (*R*)-(−)-Thiazolidine-4-carboxylic acid **13** and (±)-thiazolidine-2-carboxylic acid **14** are commercially available, while oxazolidine-4-carboxylic acid **15** and (4*S*,5*R*)-oxazolidine-5-methyl-4-carboxylic acid **16** were prepared starting from formaldehyde and L-serine or L-threonine, respectively, and in situ *N*-acylation as described.[11] The hydroxyacids **22**, in turn obtained by controlled hydrolysis of **21**, were subjected to lactonization using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/4-(dimethylamino)pyridine (DMAP)[13] (method A) or 2-chloro-1-methylpyridinium iodide/triethylamine (TEA)[14] (method B), or acetic anhydride (method C) as dehydrating agents, leading to polycyclic compounds **4**–**10** (Scheme 2). It is worth noting that racemization occurred during synthetic elaboration when optically active starting aminoacids were used. Only in the case of L-threonine, optically active final compounds were obtained, because racemization was prevented by the presence of the methyl group adjacent to the α-carbon of the amino acid.

2.2. Inhibition of IN by thiazepinedione and oxazepinediones

Previously, we identified thiazolothiazepines (**Tz-19** and **Tz-20**, Table 1) as representatives of a novel class of HIV-1 IN inhibitors with moderate activities in cell-based assays.[9] In an attempt to establish a coherent structure–activity relationship amongst thiazolothiazepines we wanted to know whether the sulfur atom could be replaced by an oxygen and whether a naphthalene ring could be replaced by fused rings. We also wanted to employ structure-based optimization methods by determining all the amino acid residues on the active site of IN that are important for ligand binding. Our results presented in Table 1 show that (1) sulfur is preferred over oxygen, (2) addition of a methyl group at position 1 lowers potency, and (3) fused ring structures can be tolerated without a significant loss of potency. However, additional compounds must be tested to establish a quantitative structure–activity relationship.

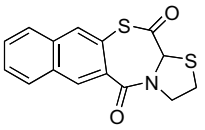
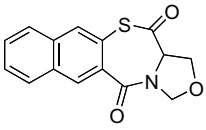
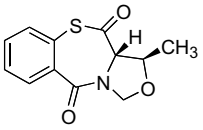
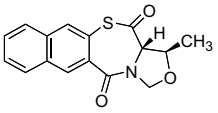
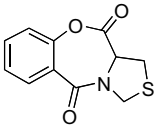
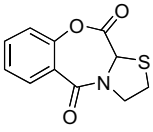
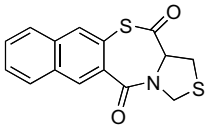
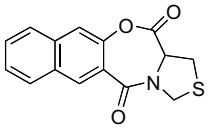
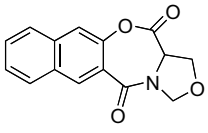
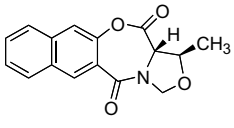
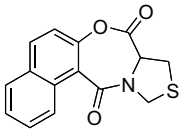
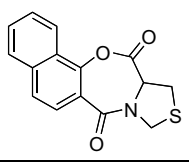
2.3. Docking studies

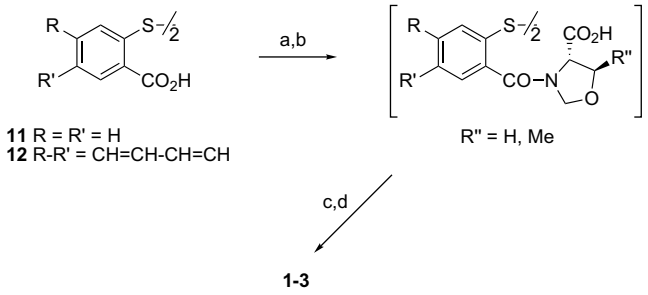
In order to identify the biologically active conformation of the compounds **1**–**10**, we docked all the compounds onto the active site of IN using GOLD. The GOLD fitness scores along with structures are given in Table 1 while the H-bonding interactions and the active site amino acid residues that interact with compounds are given in Table 2. Compounds **1**–**6** and **10** occupied a wide cavity surrounded by the active site amino acid residues E152, I151, P142, I141, G140, F139, N117, D116, H114, D64, and Q62. The bound conformation of the compounds **1** and **6** inside the active site of IN is shown in Figure 2. Compounds **7**–**9** occupied an area close to D64 and Mg^{2+} surrounded by amino acid residues K159, K156, N155, E152, D116, H67, and T66. The oxygen atom of the oxazolidine ring of **7** and **8** formed a coordination bond with Mg^{2+}. An H-bonding interaction is observed between H67 and compounds **8** and **9** (Table 2).

3. Conclusions

We designed and synthesized novel and simplified analogs of previously reported thiazolothiazepines. Some of the new analogs showed similar potency as reference compounds and amenable to further structural modifications for enhanced potency and selectivity. Furthermore, our docking studies demonstrate that these compounds interact with key residues as well as divalent metal ion important for the catalytic activities of IN. Further structural modifications will be reported in due course.

Table 1. Inhibition of HIV-1 integrase catalytic activities and docking scores of thiazepinediones and oxazepinediones

No	Structure	IC_{50} (μM)		GOLD score
		3′-Processing	Strand transfer	
Tz-19		40	47	34.18
1		95	90	31.60
2		910	900	36.24
3		800	700	37.78
4		904	873	33.85
5		450	400	32.21
Tz-20		92	100	35.28
6		100	90	37.17
7		150	100	35.30
8		200	200	34.07
9		350	300	32.23
10		300	300	34.42



Scheme 1. Reagents and conditions: (a) $SOCl_2$/reflux; (b) **15** or **16**/Na_2CO_3/THF/H_2O/rt; (c) $NaBH_4$/EtOH/reflux; (d) CDI/THF/reflux.

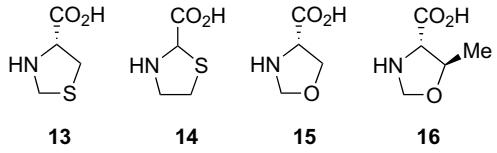


Chart 1.

4. Experimental

4.1. Chemistry

All reactions were carried out under a nitrogen atmosphere. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Organic solutions were dried over $MgSO_4$ and evaporated on a rotary evaporator under reduced pressure. Melting points were measured using an Electrothermal 8103 apparatus and are uncorrected. Specific rotations $[\alpha]_D$ were determined to the stated temperature and conditions by a Perkin–Elmer 343 polarimeter. IR spectra were recorded as thin films on Perkin–Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Brüker 200-MHz spectrometer with TMS as an internal standard: chemical shifts are expressed in δ values (ppm) and coupling constants (J) in Hz. Mass spectral data were determined at 70 eV with a VG70 spectrometer. Merck silica gel (Kieselgel 60/230–400 mesh) was used for flash chromatography columns. Elemental analyses were performed on a Perkin–Elmer 240C elemental analyzer, and the results are within ±0.4% of the theoretical values. Yields refer to purified products and are not optimized.

4.2. (±)-1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho[2,3-*f*]oxazolo[4,3-*c*][1,4]thiazepine-5,13-dione (1)

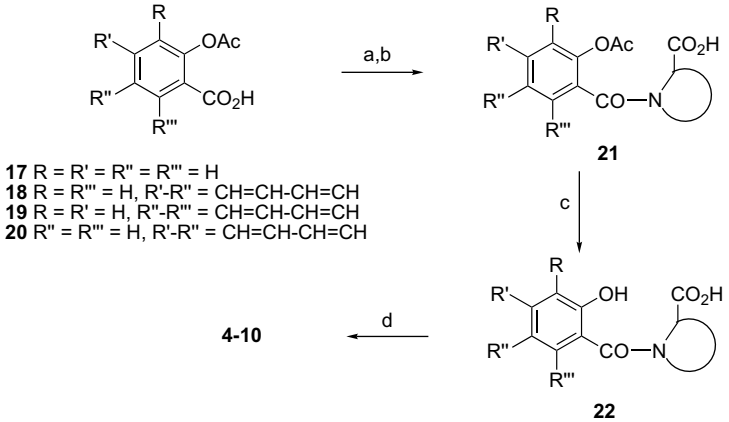
This compound was prepared starting from oxazolidine-4-carboxylic acid **15** and 3,3′-dithio-2,2′-dinaphthoic acid **12**, essentially as described.[9] Compound **1** was obtained as a colorless solid (21% for the entire process from **15**): mp 154–156 °C (dichloromethane/petroleum ether); IR (KBr) 1695, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.57 (1H, s), 8.02 (1H, s), 7.97 (1H, m), 7.85 (1H, m), 7.63 (2H, m), 5.34 (1H, 0.5 of ABq, $J = 5.4$ Hz), 5.23 (1H, 0.5 of ABq, $J = 5.4$ Hz), 4.85 (1H, d, $J = 8.6$ Hz), 4.34 (1H, d, $J = 5.8$ Hz), 3.98 (1H, dd, $J = 8.6$ and 5.8 Hz); MS (EI) m/z 285 (M^+). Anal. ($C_{15}H_{11}NO_3S$) C, H, N.

4.3. (1*R*,11a*S*)-1,11a-Dihydro-1-methyl-3*H*,5*H*,11*H*-oxazolo[4,3-*c*][1,4]benzothiazepin-5,11-dione (2)

Starting from (4*S*,5*R*)-oxazolidine-5-methyl-4-carboxylic acid **16** and 2,2′-dithiodibenzoic acid **11**, compound **2** was obtained as colorless crystals (32% for the entire process from **11**): mp 94–96 °C (diethyl ether/light petroleum); $[\alpha]_D^{21}$ −95.5 (*c* 1, $CHCl_3$); IR (KBr) 1705, 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.01 (1H, m), 7.47 (3H, m), 5.51 (1H, 0.5 of ABq, $J = 5.8$ Hz), 5.02 (1H, 0.5 of ABq, $J = 5.8$ Hz), 4.95 (1H, m), 3.83 (1H, d, $J = 3.7$), 1.31 (3H, d, $J = 6.2$); MS (EI) m/z 249 (M^+). Anal. ($C_{12}H_{11}NO_3S$) C, H, N.

4.4. (1*R*,13a*S*)-1,13a-Dihydro-1-methyl-3*H*,5*H*,13*H*-naphtho[2,3-*f*]oxazolo[4,3-*c*][1,4]thiazepine-5,13-dione (3)

Starting from (4*S*,5*R*)-oxazolidine-5-methyl-4-carboxylic acid **16** and 3,3′-dithio-2,2′-dinaphthoic acid **12**,



Scheme 2. Reagents and conditions: (a) $(COCl)_2$/toluene/DMF; (b) cyclic aminoacid/THF–H_2O/pH 8/rt; (c) Na_2CO_3/H_2O/rt; (d) cyclization (see Experimental).

Table 2. H-bonding interactions and the active site amino acids residues interact with compounds **1**–**10**

Compound	H-bonding interactions (Å)	Interacting amino acid residues
1	—	E152, I151, P142, D116, H114, D64, Q62
2	—	E152, I151, P142, D116, H114, D64, Q62
3	—	I151, P142, I141, F139, N117, D116, H114, Q62
4	N–C=O···HN Q62 (3.13)	E152, I151, P142, F139, H114, D116
5	—	E152, I151, Q148, P142, P141, H114, D116, D64
6	—	E152, I151, P142, D116, D64, Q62
7[a]	—	K159, K156, N155, T66, D64
8[a]	O–C=O···HN H67 (2.66)	K159, K156, N155, E152, D116, T66, D64
9	O–C=O···HN H67 (2.08)[b]	K159, K156, N155, E152, T66, D64
10	—	E152, I151, P142, G140, D116, H114, D64, Q62

[a] The oxygen atoms of oxazolidine ring of **7** and **8** coordinate with active site Mg^{2+} ion; O···Mg^{2+} (2.06 Å), O···Mg^{2+} (1.94 Å).
[b] Backbone NH of H67.

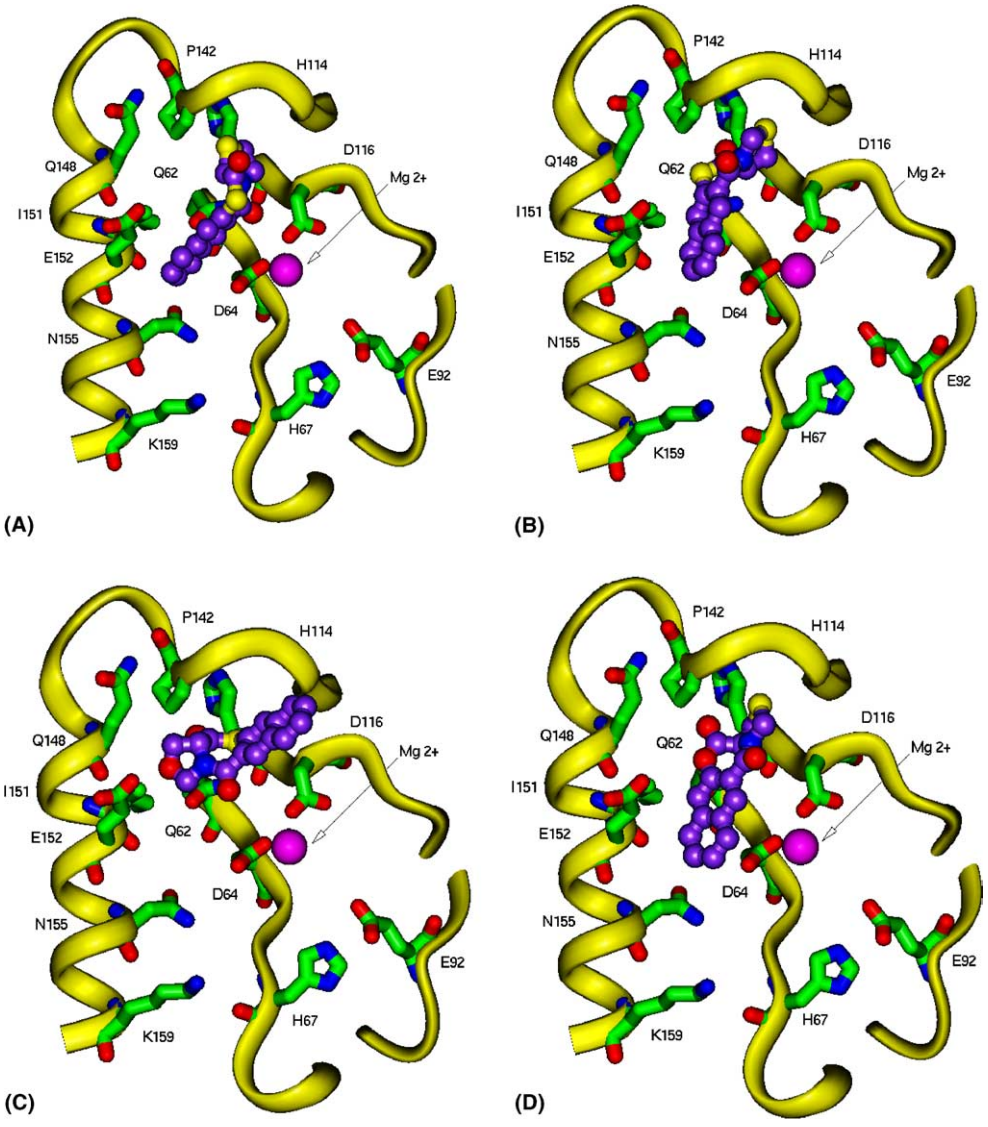


Figure 2. The bound conformation of molecules **Tz-19** (A), **Tz-20** (B), **1** (C), and **6** (D) inside the active site of IN.

compound **3** was obtained as colorless needles (37% for the entire process from **12**): mp 163–165 °C (diethyl ether); $[\alpha]_D^{21}$ −121.0 (*c* 1, $CHCl_3$); IR (KBr) 1700, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60 (1H, s), 8.12 (1H, s), 8.00 (1H, m), 7.80 (1H, m), 7.60 (2H, m), 5.56 and 5.09 (2H, ABq, $J = 5.5$ Hz), 4.99 (1H, dq, $J = 6.5$ and 3.4 Hz), 3.93 (1H, d, $J = 3.4$), 1.29 (3H, d, $J = 6.5$); MS (CI) m/z 300 (MH^+). Anal. ($C_{16}H_{13}NO_3S$) C, H, N.

4.5. General procedure for the synthesis of compounds 21

The preparation of *N*-(3-acetoxy-2-naphthoyl)oxazolidin-4-carboxylic acid is described as a representative example. A mixture of formaldehyde (37% solution in water, 14.0 mmol, 1.0 mL), L-serine (1.4 g, 13.3 mmol), and 2 N NaOH (6.5 mL) was stirred overnight at 0 °C. Sodium carbonate (0.7 g, 6.6 mmol) was added in one portion to the cold solution. A solution of 3-acetoxy-2-naphthoic acid chloride (3.3 g, 13.3 mmol) in dry THF (20 mL) was then added dropwise, while a weakly alkaline pH was maintained by the addition of solid sodium carbonate. The mixture was stirred overnight, then concentrated and made acidic (pH 3–4) by adding concentrated HCl. The solid formed was extracted into ethyl acetate, and the resulting solution was washed with water, dried, and evaporated. The resulting residue was purified by column chromatography on silica gel ($CHCl_3/CH_3OH/HCO_2H$, 86/14/1) to give a waxy yellow solid (3.0 g, 70%): IR (KBr) 2700 (br), 1715, 1690, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.57 (1H, br, exch. with D_2O), 7.78–7.65 (3H, m), 7.48 (1H, m), 7.32 (2H, m), 5.30 (2H, s), 5.01 (1H, t, $J = 6.7$ Hz), 4.40 (1H, m), 4.09 (1H, m), 2.31 (3H, s); MS (EI) m/z 329 (M^+). Anal. ($C_{17}H_{15}NO_6$) C, H, N.

4.6. General procedure for the synthesis of compounds 22

The preparation of *N*-(3-hydroxy-2-naphthoyl)oxazolidin-4-carboxylic acid is described as a representative example. *N*-(3-Acetoxy-2-naphthoyl)oxazolidin-4-carboxylic acid (2.0 g, 6.0 mmol) was dissolved in a solution of sodium carbonate (0.8 g, 7.5 mmol) in water (20 mL) and stirred overnight at room temperature, then made acidic (pH 3–4) by adding concentrated HCl at 0 °C. The solid formed was extracted with ethyl acetate, and the resulting solution was washed with water, dried, and evaporated. The resulting residue was purified by crystallization to give a solid (1.5 g, 85%): mp 182–184 °C (acetone/diethyl ether); IR (KBr) 2800 (br), 1695, 1635 cm^{-1}; ^1H NMR (CD_3OD) δ 9.00 (2H, br), 7.82 (1H, s), 7.76 (1H, m), 7.64 (1H, m), 7.40 (1H, m), 7.28 (1H, m), 7.20 (1H, s), 5.21 (2H, s), 4.99 (1H, t, $J = 6.7$ Hz), 4.47 (1H, m), 4.11 (1H, m); MS (EI) m/z 287 (M^+). Anal. ($C_{15}H_{13}NO_5$) C, H, N.

4.7. Cyclization reaction: method A

A mixture of the appropriate hydroxyacid **22** (0.4 mmol), EDC (0.085 g, 0.44 mmol), and DMAP (cat.) in dry dichloromethane (20 mL) was stirred at room temperature for 40 h. The solution was then shaken with a saturated solution of NH_4Cl and water, successively. After drying and solvent evaporation, a pale yellow oil was obtained and purified by column chromatography on a silica gel (15% ethyl acetate in hexanes).

4.8. Method B

2-Chloro-1-methylpyridinium iodide (0.12 g, 0.48 mmol) and TEA (110 μL, 0.8 mmol) were added to a suspension of the appropriate hydroxyacid **22** (0.4 mmol) in dry dichloromethane (15 mL). After heating overnight under reflux, the mixture was worked up as described for method A.

4.9. Method C

A suspension of the appropriate compound **22** (0.4 mmol) in acetic anhydride (2 mL) was stirred overnight at room temperature. The resulting solution was then diluted with chilled water and left for several hours at 4 °C. The aqueous solution was extracted with chloroform and the organic layer was shaken successively with a 5% sodium bicarbonate solution and brine. A pure solid was obtained after evaporation of the solvent.

4.10. (±)-1,11a-Dihydro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*]-[1,4]benzoxazepin-5,11-dione (4)

Compound **4** was prepared following the method A (33 mg, 35%); mp 143–145 °C (diethyl ether/light petroleum); IR (KBr) 1695, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.94–7.20 (4H, m), 4.86 and 4.72 (2H, ABq, $J = 10.6$ Hz), 4.46 (1H, dd, $J = 6.8$ and 3.3 Hz), 3.76 (1H, dd, $J = 12.3$ and 3.3 Hz), 3.32 (1H, dd, $J = 12.3$ and 6.8 Hz); MS (CI) m/z 236 (MH^+). Anal. ($C_{11}H_9NO_3S$) C, H, N.

4.11. (±)-2,3-Dihydro-5*H*,11a*H*-thiazolo[2,3-*c*][1,4]benzoxazepin-5,11-dione (5)

Compound **5** was prepared following the method B (13 mg, 14%); mp 154–156 °C (benzene); IR (KBr) 1700, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95–7.22 (4H, m), 5.27 (1H, s), 4.20 (1H, m), 3.88 (1H, m), 3.37 (1H, m), 3.16 (1H, m); MS (CI) m/z 236 (MH^+). Anal. ($C_{11}H_9NO_3S$) C, H, N.

4.12. (±)-1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho[2,3-*f*]thiazolo[4,3-*c*][1,4]oxazepine-5,13-dione (6)

This compound was prepared following the method C (46 mg, 38%); mp 217 °C (dichloromethane/petroleum ether); IR (KBr) 1705, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55 (1H, m), 7.98 (1H, d, $J = 8.3$ Hz), 7.86 (1H, d, $J = 8.3$ Hz), 7.60 (3H, m), 4.91 (1H, 0.5 of ABq, $J = 10.7$ Hz), 4.56 (1H, 0.5 of ABq, $J = 10.7$ Hz), 4.55 (1H, dd, $J = 6.8$ and 3.4 Hz), 3.80 (1H, dd, $J = 12.5$ and 3.4 Hz), 3.34 (1H, dd, $J = 12.5$ and 6.8 Hz); MS (CI) m/z 286 (MH^+). Anal. ($C_{15}H_{11}NO_3S$) C, H, N.

4.13. (±)-1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho[2,3-*f*]oxazolo[4,3-*c*][1,4]oxazepine-5,13-dione (7)

This compound was prepared following the method A (56 mg, 52%); mp 205 °C (ethyl ether); IR (KBr) 1695, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.52 (1H, s), 7.95 (1H, d, $J = 7.9$ Hz), 7.83 (1H, d, $J = 7.9$ Hz), 7.60 (3H, m),

5.28 and 5.21 (2H, ABq, $J = 5.3$ Hz), 4.82 (1H, dd, $J = 8.8$ and 2.4 Hz), 4.28 (2H, m); MS (EI) m/z 269 (M^+). Anal. ($C_{15}H_{11}NO_4$) C, H, N.

4.14. (1*R*,13a*S*)-1,13a-Dihydro-1-methyl-3*H*,5*H*,13*H*-naphtho[2,3-*f*]oxazolo[4,3-*c*][1,4]oxazepine-5,13-dione (8)

Method C was followed for the preparation of compound **8** (49 mg, 43%): mp 197–198 °C (benzene); $[\alpha]_D^{20}$ −197.0 (*c* 1, $CHCl_3$); IR (KBr) 1700, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60 (1H, s), 7.98 (1H, d, $J = 7.5$ Hz), 7.86 (1H, d, $J = 7.9$ Hz), 7.70 (1H, s), 7.65 (2H, m), 5.62 and 5.03 (2H, ABq, $J = 5.5$ Hz), 4.88 (1H, m), 3.82 (1H, d, $J = 6.9$), 1.45 (3H, d, $J = 6.0$); MS (CI) m/z 284 (MH^+). Anal. ($C_{16}H_{13}NO_4$) C, H, N.

4.15. (±)-1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho[1,2-*f*]thiazolo[4,3-*c*][1,4]oxazepine-5,13-dione (9)

This compound was prepared following the method C (52 mg, 43%); mp 237 °C (dichloromethane/light petroleum); IR (KBr) 1725, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.64 (1H, d, $J = 8.1$ Hz), 8.03 (1H, d, $J = 8.6$ Hz), 7.91 (1H, d, $J = 7.2$ Hz), 7.65 (2H, m), 7.33 (1H, d, $J = 8.6$ Hz), 5.00 (1H, 0.5 of ABq, $J = 10.5$ Hz), 4.90 (1H, 0.5 of ABq, $J = 10.5$ Hz), 4.64 (1H, dd, $J = 6.5$ and 1.7 Hz), 3.79 (1H, dd, $J = 12.0$ and 1.7 Hz), 3.39 (1H, dd, $J = 12.0$ and 6.5 Hz); MS (CI) m/z 286 (MH^+). Anal. ($C_{15}H_{11}NO_3S$) C, H, N.

4.16. (±)-1,13a-Dihydro-3*H*,5*H*,13*H*-naphtho[2,1-*f*]thiazolo[4,3-*c*][1,4]oxazepine-5,13-dione (10)

This compound was prepared following the method C (26 mg, 23%); mp 200 °C (dichloromethane/light petroleum); IR (KBr) 1740, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (1H, m), 7.90 (2H, m), 7.82 (1H, d, $J = 8.4$ Hz), 7.70 (2H, m), 4.95 (1H, 0.5 of ABq, $J = 10.6$ Hz), 4.85 (1H, 0.5 of ABq, $J = 10.6$ Hz), 4.55 (1H, dd, $J = 6.5$ and 1.8 Hz), 3.82 (1H, dd, $J = 11.8$ and 1.8 Hz), 3.37 (1H, dd, $J = 11.8$ and 6.5 Hz); MS (CI) m/z 286 (MH^+). Anal. ($C_{15}H_{11}NO_3S$) C, H, N.

4.17. Molecular modeling

The structures of all the compounds (Table 1) were built and minimized using Catalyst (Accelrys, Inc.)[16] running on a multiprocessor linux PC and a 24-processor Silicon Graphics Onyx workstation as described.[17] The poling algorithm implemented within Catalyst was used to generate conformations for all the compounds. For each compound all feasible unique conformations were generated over a 20 kcal/mol range of energies using the best flexible conformation generation method in Catalyst. The subunit B of the core domain X-ray structure of IN (PDB 1BIS) in which all the active site amino acid residues were resolved was chosen for docking purpose. A Mg^{2+} ion was placed in the active site between carboxylate oxygen atoms of amino acid residues D64 and D116 considering the geometry of the Mg^{2+} ion that was present in the subunit A of the IN in PDB 1BIS and subunit A in IN-5CITEP complex X-ray structure (PDB 1SQ4) as recently described.[17] All the water molecules present in protein were removed and hydrogen atoms were added to the protein considering appropriate ionization states for both the acidic and basic amino acid residues. Docking was performed using version 1.2 of the GOLD: Genetic Optimization for Ligand Docking (Cambridge Crystallographic Data Centre) software package. A 12 Å radius active site was defined considering the carboxylate oxygen atom (OD1) of amino acid residue D64 as the center of the active site. All conformers of the compounds were docked into the active site of the IN. Based on the GOLD fitness score, for each molecule a bound conformation with high fitness score was considered as the best-bound conformation. All docking runs were carried out using standard default settings with a population size of 100, a maximum number of 100,000 operations, a mutation and crossover rate of 95.[18–20] The fitness function that was implemented in GOLD consisted basically of H-bonding, complex energy, and ligand internal energy terms.

4.18. Biological materials, chemicals, and enzymes

All compounds were dissolved in DMSO and the stock solutions were stored at −20 °C. The $\gamma[^{32}P]$-ATP was purchased from either Amersham Biosciences or ICN. The expression systems for the wild-type IN and soluble mutant $IN^{F185KC280S}$ were generous gifts of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

4.19. Preparation of oligonucleotide substrates

The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′ and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′ were purchased from Norris Cancer Center Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3′-processing and strand transfer using 5′-end labeled substrates, 21top was 5′-end labeled using T4 polynucleotide kinase (Epicentre, Madison, WI) and $\gamma[^{32}P]$-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated and 21bot was added in 1.5 M excess. The mixture was heated at 95 °C, allowed to cool slowly to room temperature, and run through a spin 25 mini-column (USA Scientific) to separate annealed double-stranded oligonucleotide from unincorporated material.

4.20. Integrase assays

To determine the extent of 3′-processing and strand transfer, wild-type IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin,

10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. Then, 20 nM of the 5′-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional one hour. Reactions were quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea).

Gels were dried, exposed in a PhosphorImager cassette, and analyzed using a Typhoon 8610 Variable Mode Imager (Amersham Biosciences) and quantitated using ImageQuant 5.2. Percent inhibition (% I) was calculated using the following equation:

$$\% I = 100 \times [1 - (D - C)/(N - C)],$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC_{50} values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain concentration that produced 50% inhibition.

References and notes

1. Richman, D. D. HIV chemotherapy. *Nature* **2001**, *410*, 995–1001.
2. Brown, P. O. *Integration*; Cold Spring Harbor Press: Cold Spring Harbor, 1999.
3. Asante-Appiah, E.; Skalka, A. M. HIV-1 integrase: structural organization, conformational changes, and catalysis. *Adv. Virus Res.* **1999**, *52*, 351–369.
4. Neamati, N. Structure-based HIV-1 integrase inhibitor design: a future perspective. *Expert Opin. Invest. Drugs* **2001**, *10*, 281–296.
5. Neamati, N. Patented small molecule inhibitors of HIV-1 integrase: a ten-year saga. *Expert Opin. Ther. Pat.* **2002**, *12*, 709–724.
6. Dayam, R.; Neamati, N. Small-molecule HIV-1 integrase inhibitors: the 2001–2002 update. *Curr. Pharm. Des.* **2003**, *9*, 1789–1802.
7. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **2000**, *287*, 646–650.
8. Yoshinaga, T.; Sato, A.; Fujishita, T.; Fujiwara, T. In vitro activity of a new HIV-1 integrase inhibitor in clinical development. *9th Conference on Retroviruses and Opportunistic Infections*; Seattle, 2002.
9. Neamati, N.; Turpin, J. A.; Winslow, H. E.; Christensen, J. L.; Williamson, K.; Orr, A.; Rice, W. G.; Pommier, Y.; Garofalo, A.; Brizzi, A.; Campiani, G.; Fiorini, I.; Nacci, V. Thiazolothiazepine inhibitors of HIV-1 integrase. *J. Med. Chem.* **1999**, *42*, 3334–3341.
10. Bergeron, R. J.; Wiegand, J.; Wollenweber, M.; McManis, J. S.; Algee, S. E.; Ratliff-Thompson, K. Synthesis and biological evaluation of naphthyldesferrithiocin iron chelators. *J. Med. Chem.* **1996**, *39*, 1575–1581.
11. Wolfe, S.; Militello, G.; Ferrari, C.; Hasan, S. K.; Lee, S. L. Conversion of amino hydroxyacids to oxazolidine-4-carboxylic acids, oxazolidine-5-carboxylic acids and tetrahydrooxazine-4-carboxylic acids. *Tetrahedron Lett.* **1979**, *20*, 3913–3916.
12. Falorni, M.; Conti, S.; Giacomelli, G.; Cossu, S.; Soccolini, F. Optically active 4-oxaproline derivatives: new useful chiral synthons derived from serine and threonine. *Tetrahedron: Asymmetry* **1995**, *6*, 287–294.
13. Magnus, P.; Kreisberg, J. D. Synthesis of benzofuranones related to diazonamide via an intramolecular Pummerer reaction. *Tetrahedron Lett.* **1999**, *40*, 451–454.
14. Saigo, K.; Usui, M.; Kikuchi, K.; Shimada, E.; Mukaiyama, T. New method for the preparation of carboxylic esters. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1863–1866.
15. Narasaka, K.; Masui, T.; Mukaiyama, T. The facile synthesis of macrocyclic lactones by the use of 2-chloro-3-methoxymethyl-1-methylpyridinium iodide. *Chem. Lett.* **1977**, 763–766.
16. Accelrys, I. Catalyst; V.4.8: San Diego.
17. Long, Y. Q.; Jiang, X. H.; Dayam, R.; Sanchez, T.; Shoemaker, R.; Sei, S.; Neamati, N. Rational design and synthesis of novel dimeric diketoacid-containing inhibitors of HIV-1 integrase: implication for binding to two metal ions on the active site of integrase. *J. Med. Chem.* **2004**, *47*, 2561–2573.
18. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727–748.
19. Nissink, J. W.; Murray, C.; Hartshorn, M.; Verdonk, M. L.; Cole, J. C.; Taylor, R. A new test set for validating predictions of protein–ligand interaction. *Proteins* **2002**, *49*, 457–471.
20. GOLD Cambridge Crystallographic Data Centre (CCDC), V1.2, UK, 2001.