Investigation of furo[2,3-*h*]- and pyridazino[3,4-*f*]cinnolin-3-ol scaffolds as substrates for the development of novel HIV-1 integrase inhibitors

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This work is dedicated to Prof. Vito Boido on the occasion of his retirement

Abstract
With the aim to develop novel HIV-1 integrase inhibitors, we obtained a set of condensed ring systems based on the furo[2,3-*h*]cinnolin-3(2*H*)-one and pyridazino[3,4-*f*]cinnolin-3-ol scaffolds bearing a potential chelating pharmacophore, which can be involved in the inhibition mechanism of the enzyme. Herein, we report the design, synthesis, structural investigation and preliminary biological results of these heteroaromatic systems.

Keywords: HIV-1 integrase, IN inhibitors, Furo[2,3-*h*]cinnolin-3(2*H*)-one, Pyridazino[3,4-*f*]cinnolin-3-ol

Introduction

HIV-1 integrase (IN), the viral enzyme that catalyzes the integration of proviral cDNA into the host cell genome, has emerged as an attractive target for novel anti-AIDS agents.[1-3] The first IN inhibitor (e.g. raltegravir, Isentress) was recently approved by the US FDA,[4] and other IN inhibitors are in clinical trials or under clinical investigation.[5,6] These compounds belong to a class of compounds bearing a β-diketo acid (DKA) pharmacophoric motif, which are the most promising lead in anti-IN drug discovery.[7,8] Starting from the simple benzoylpyruvic acid **1** (BPA, Figure 1a), other members of the DKA family, exemplified by L-731,988 **2** have been

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reported and intensively studied.[8,9] Moreover, several DKA bioisosteric analogues have been discovered.[9]

DKAs are comprised of three structural components (Figure 1b): a β-diketo moiety (a), an aromatic or heteroaromatic portion (b), and a carboxylic functionality, which can be replaced with a variety of bioisosteric functions (c).[9] Although the mechanism by which they bind IN has not been well understood, it is believed that the β-diketo acid pharmacophoric motif could be involved in a functional sequestration of one or both divalent metal ions in the enzyme catalytic site, to form a tertiary ligand-M^{2+}-IN complex (Figure 1c).[1] DKAs represented a good starting point for medicinal chemistry discovery and optimization programs, and it has been well suited to explore this chemical space by considering structurally related prototypes. In this context, it is of great interest to develop a new generation of novel and selective IN inhibitors as well as to investigate their mechanism of action.

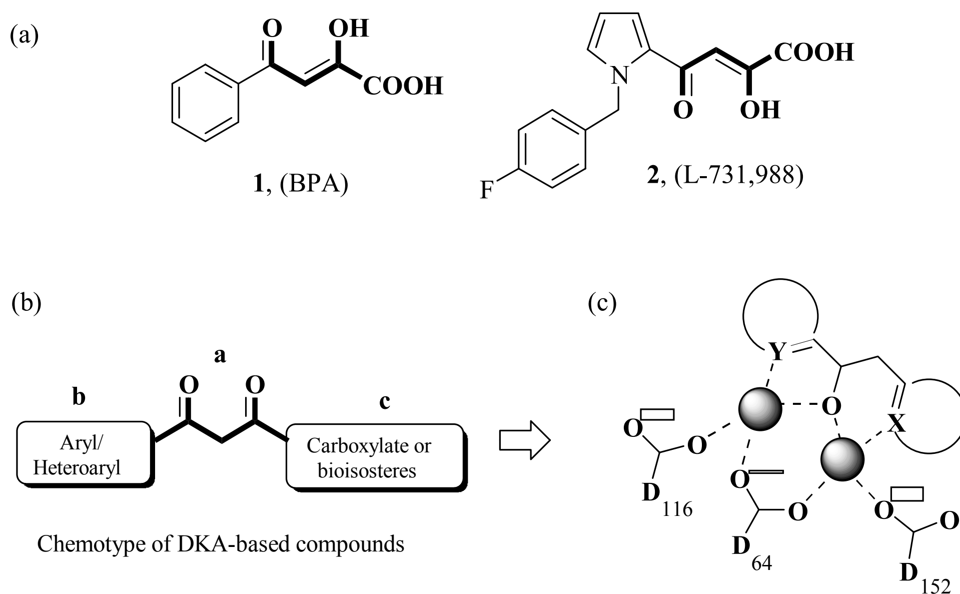

Figure 1. (a) Representative DKAs inhibitors. (b) Chemotype of DKA β-diketo-based inhibitors. (c) The two-metals binding model previously proposed for selective strand-transfer chelating inhibitors. The atoms 'X' and 'Y' represent possible hetero-atoms that serve a lone-pair and the semicircles indicate heteroaromatic rings, optionally including the 'C═X' or 'C═Y' bond. Metals are depicted as grey spheres.

In the course of our drug discovery program focused on developing original IN inhibitors,[10-15] a series of polycyclic templates carrying a novel potential chelating pharmacophore has been designed and synthesized. Our attention was addressed to the furo[2,3-*h*]cinnolin-3(2*H*)-one scaffold **I** (Figure 2), as analogue of the previoulsy reported 6-aryl-5-methyl-4,5-dihydro-3-(2*H*)pyridazinones, 4,4a-dihydro-5*H*-indeno[1,2-*c*]pyridazinones **II**, **III**,[16-19] and more strictly to 5,6-dihydrobenzo[*h*]-, 5,6-dihydrothieno[2,3(3,2)-*h*]cinnolin-3(2*H*)-ones **IV**, **V**.[20-22]

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These condensed ring systems demonstrated a versatile platform to incorporate a pyridazinone ring, which has shown several pharmaceutical properties.[23] In this context, the **N-NH-CO-C-R** (R = OH, NH_2) motif could be considered as a potential chelating fragment, eventually suitable of bioisosteric replacement of the β-diketo enol pharmacophore. Moreover, furan oxygen of the heteroaromatic backbone, can be involved in potential hydrogen bonding with amino acid residues on the active site.

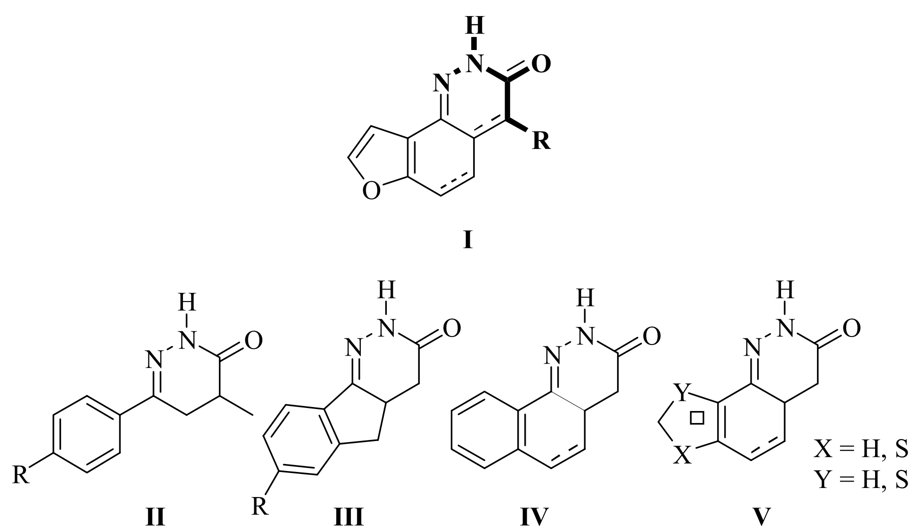

Figure 2. Design of furo[2,3-*h*]cinnolin-3(2*H*)-one scaffold **I**, and previously reported pyridazinones **II**, **III** and cinnolinones **IV**, **V**.

On this basis, the preparation of a first set of furo[2,3-*h*]cinnolin-3(2*H*)-one **3-5** has been performed (Figure 3).

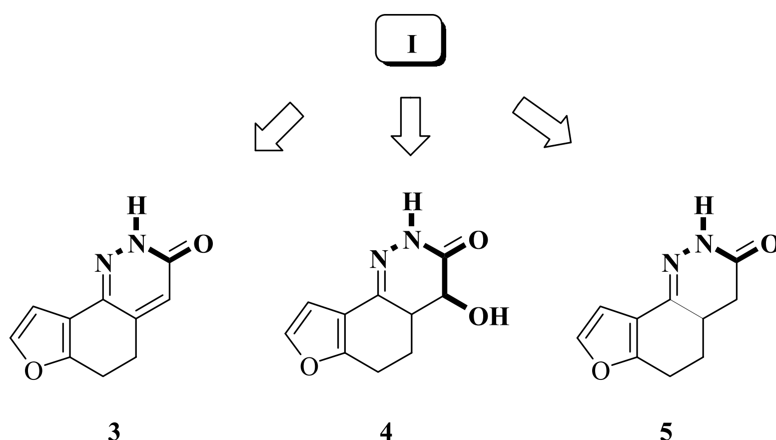

Figure 3. Potential chelating fragments of the designed furo[2,3-*h*]cinnolin-3(2*H*)-ones.

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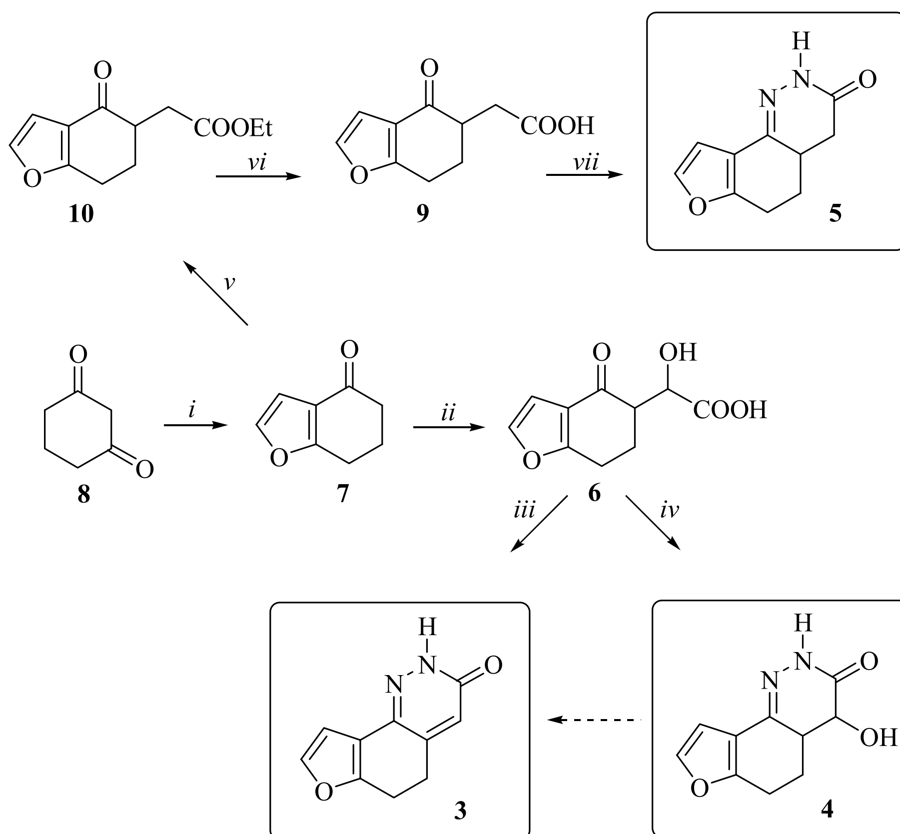
Results and Discussion

The synthetic routes for the preparation of cinnolinones **3-5** are depicted in Scheme 1. 5,6-dihydrofuro[2,3-*h*]cinnolin-3(2*H*)-one **3** was synthesized (62% yield) by refluxing the hydroxy(4-oxo-4,5,6,7-tetrahydro-1-benzofuran-5-yl)acetic acid **6** in an excess of hydrazine hydrate for 30 minutes. The intermediate α-hydroxy acid **6** was obtained by reacting the 6,7-dihydro-1-benzofuran-4(5*H*)-one **7** with equimolar amount of glyoxylic acid in an aqueous solution of sodium hydroxide at room temperature. Addition of chloroacetaldehyde to 1,3-cyclohexanedione **8**, following a previously reported procedure,[24] afforded the ketone **7** in high yield (Scheme 1). 4-hydroxy-4,4a,5,6-tetrahydrofuro[2,3-*h*]cinnolin-3(2*H*)-one **4** was obtained (32% yield) by condensation of the key intermediate **6** with an equimolar amount of hydrazine hydrate in refluxing ethanol for 1 hour. It is noteworthy that if **4** is treated with hydrazine hydrate in acidic condition and refluxed for several hours, dehydration of **4** to **3** is expected, according to the previously reported method.[22]



Scheme 1. Reagents and conditions: *i*: chloroacetaldehyde, aq. $NaHCO_3$, rt, 15 hours; *ii*: glyoxylic acid monohydrate, aq. NaOH, 0 °C then rt; *iii*: hydrazine monohydrate, reflux, 30 min; *iv*: hydrazine monohydrate, ethanol, reflux, 1 hour; *v*: LiHMDS -78 °C , ethyl bromoacetate, -70 °C for 1 hour, then rt for 18 hours; *vi*: 10% aq. NaOH, MeOH, reflux, 2 hours; *vii*: hydrazine monohydrate, ethanol, reflux, 3 hours.

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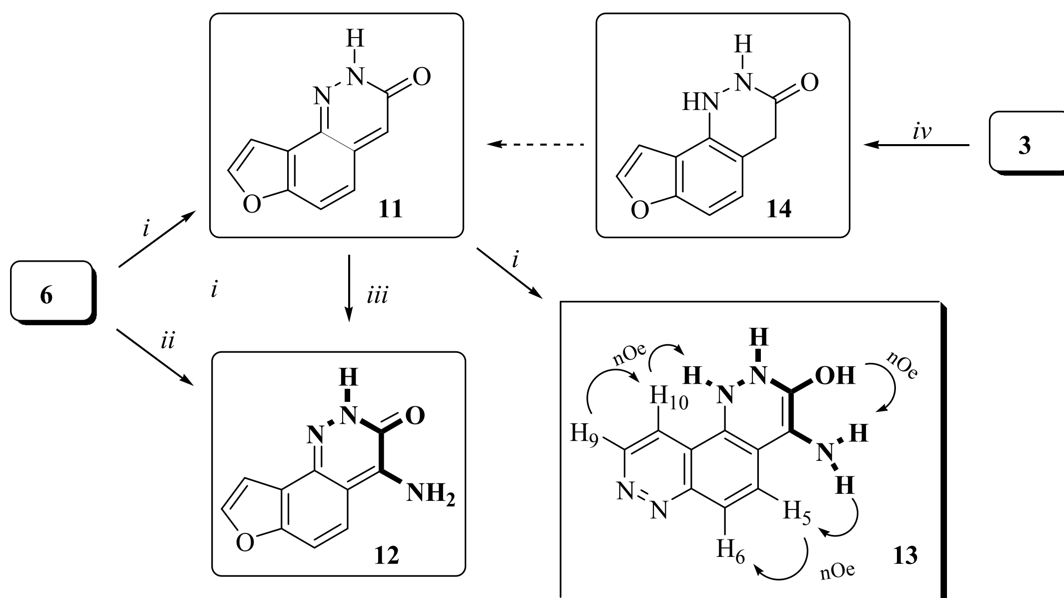
4,4a,5,6-Tetrahydrofuro[2,3-*h*]cinnolin-3(2*H*)-one **5** was obtained (38% yield) by reaction with hydrazine hydrate in refluxing ethanol for 3 hours of the (4-Oxo-4,5,6,7-tetrahydro-1-benzofuran-5-yl)acetic acid **9**, which was obtained in good yield by alkaline hydrolysis of the corresponding ester **10**. The latter was prepared by alkylation of the ketone **7** with ethyl bromoacetate in THF solution of LiHMDS at -78 °C (Scheme 1).

Compounds **3-5** were fully characterized by means of NMR spectroscopy, mass spectrometry and elemental analysis. In particular, these systems presented a common pattern of signals, constituted by broad singlets for the exchangeable NH protons in the range of 10-13 ppm, and two doublets at 7.69-7.63 and 6.75-6.61, for H_8 and H_9, respectively, of the furan ring. In addition to the other aromatic H_4 proton, compound **3** revealed a multiplet in the range of 3.02-2.93 ppm, attributable to H_5 and H_6, whereas a more complicated signal pattern ranging from 3.94 to 1.73 ppm was observed for compound **4**. The latter also displayed a deuterium oxide exchangeable proton of the OH function located in position 4. As for compound **5** H_4 proton appeared upfield as a multiplet centered at 2.84 ppm.

Further exploration of this chemistry resulted in the synthesis of cinnolinones **11-14**. When ketone **6** was refluxed in excess of hydrazine hydrate for 48 hours, furo[2,3-*h*] cinnolin-3(2*H*)-one **11** was isolated in 35% yield (Scheme 2). The structure of **11** has been confirmed by means of the above-mentioned analytical methods. As far as ^{1}H-NMR results are concerned, the five aromatic protons are detected at 7.75 (singlet, H_4), 7.68 (AB system, H_6 and H_5), 7.62 (doublet, H_8) and 6.61 (doublet, H_9) ppm resonance.



Scheme 2. Reagents and conditions: *i*: hydrazine monohydrate reflux, 48 hours; *ii*: 72 hours; *iii*: 24 hours; *iv*: 15-30 min. NOE correlations are indicated for **13**.

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According to the above-mentioned behavior,[22,25] the 4-amino-furo[2,3-*h*]cinnolin-3(2*H*)-one **12** (40%) was synthesized by refluxing **6** in hydrazine hydrate for about 72 hours. The formation of **12** (45%) also occurred starting from **11** under the same conditions and refluxing for 24 hours. When the reaction time between **11** and hydrazine hydrate was increased to 72 hours, a new heterocycle, namely 4-amino-1,2-dihydropyridazino[3,4-*f*]cinnolin-3-ol **13**, was obtained in 25% yield (slightly impure of **11**). The formation of this compound, in which the furan ring was converted to an additional pyridazine ring, is discussed below (Figure 5).

Focusing on **13**, in addition to the two expected doublets detected at 8.05 and 7.57 ppm for H_5 and H_6, respectively, the ^{1}H-NMR spectrum exhibited two other doublets at downfield centered at 9.34 and 8.47 ppm, attributed to the H_9 and H_{10}, respectively. The coupling constant values (J = 5.5 Hz) of these signals are in accordance with similar patterns in the cinnoline ring, further supporting its formation. Also, exchangeable broad singlets detected at 13.50, 7.64, and 6.20 were assigned to the enolic OH in position 3, the NH protons in position 1 and 2, and the NH_2 group in position 4, respectively.

Then, when cinnolinone **3** was kept in refluxing hydrazine hydrate for 15-30 minutes, it was converted into 55% of its tautomer 1,2-dihydrofuro[2,3-*h*]cinnolin-3(4*H*)-one **14**. In particular, the ^{1}H-NMR spectrum of **14** was characterized by a singlet at 3.59 ppm (H_4), a singlet at 7.18 ppm (overlapping H_8 and H_9), two doublets centered at 7.94 and 7.20 ppm, for H_6 and H_5, respectively, and by an exchangeable broad singlet at 5.33 ppm, corresponding to the two NH in positions 1 and 2. Further prolonging of reaction time of **14**, can reasonably give **11** according to a previously observed behavior for similar reaction[22] (data not shown). Further magnetic resonance techniques such as DEPT/APT, COSY and NOESY (i.e. compound **13**, Scheme 2) support the assigned structures for title compounds. A mechanistic hypothesis for the formation of the above-mentioned compounds (**11** and/or **14**) is displayed in Figure 4.

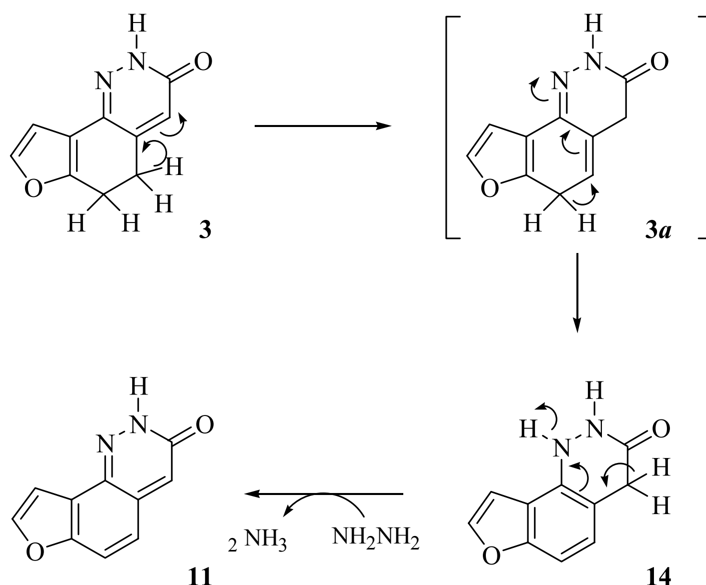

Figure 4. Mechanistic hypothesis for the formation of cinnolinones **11** and **14**.

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The reaction process can start through an initial tautomerization of **3** to [**3*a***] and **14**, which can evolve to give **11** by dehydrogenation in an oxidative step mediated by hydrazine according to the different experimental conditions, whose N-N bond is cleaved to give two ammonia molecules.

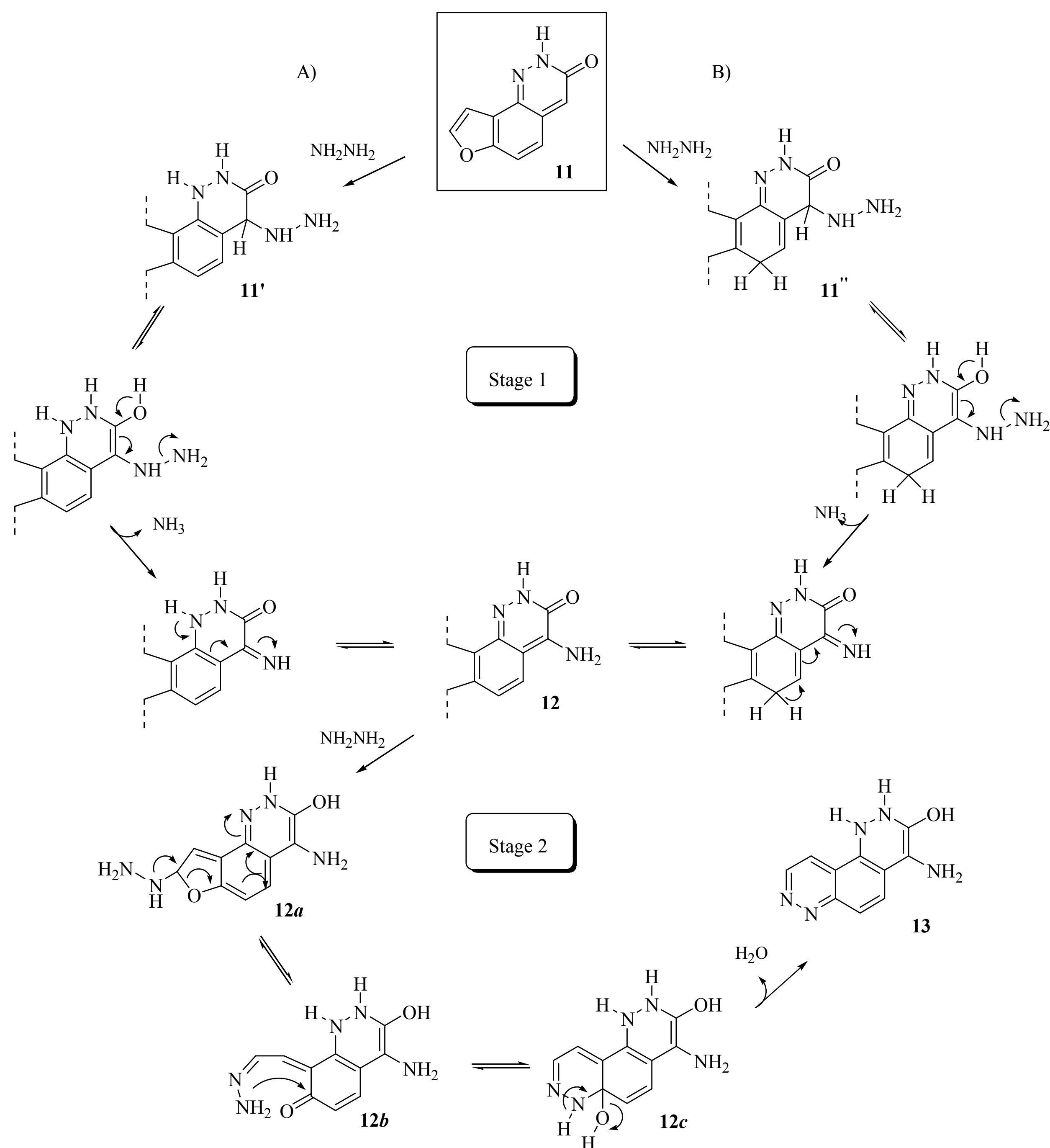

Figure 5. Mechanistic hypothesis for the formation of cinnolinones **12** and **13.**

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Amination at the 4-position of the pyridazinone moiety of **11** to give compounds **12** and **13** was explained according with the mechanism (A, Figure 5) proposed by Singh[26] and Cignarella *et al*.[25] Briefly, this reaction occurs through an initial 1,4 addition of hydrazine to the pyridazinone ring, to form the intermediate **11'**, which, by dehydrogenation and final amination to the 4,4a-5,6 conjugated system, leads to **12** (and stage one of **13**, Figure 5). However, the alternative mechanism (B, Figure 5), with the initial formation of the intermediate **11"**, according with previously reported by Shemyakin *et al.*[27] and Cignarella *et al.*[28] can also be considered. Although these compounds can directly be obtained from **6**, we can reasonably hypothesize that this reaction may proceed *via* compound **11**. The complete formation of **13** can occur by a second-stage mechanism which involves another hydrazine addition to the furan ring of **12** (at position 8 of the furocinnolinone scaffold) to give the intermediate **12*a***, following by a ring-opening to give **12*b*** (Figure 5). The formation of the intermediate **12*a*** can also involve a starting furan epoxydation, which is well-documented in the literature,[29] and that can facilitate the Michael addition of hydrazine. Intramolecular nucleophilic attack to the carbonyl group would be carried out by the N1-amino group of the hydrazine coupled to give a six-membered ring **12*c***, which led to **13** by a final prototropic rearrangement with loss of a molecule of water.

The cinnolin-3(2*H*)-ones **3-5, 11, 12**, the cinnolin-3-ol **13**, and the intermediate **6**, were tested for their ability to inhibit IN catalytic activities in *in vitro* assays employing purified enzyme (Table 1). Inhibitors **1** and **2** were used as reference compounds.[15] With the exception of **12** and **13**, all tested cinnolinone-derivatives, as well as the intermediate **6**, did not show any anti-IN activities. Conversely, the 4-amino-derivatives **12** and **13**, shared a certain inhibitory activity, thus demonstrating some inhibitory properties of this novel chemical scaffold. With a IC_{50} of 60 ± 13 μM against strand transfer reaction, **13** proved to be the most active compound of the series.

Table 1. Inhibition of HIV-1 integrase catalytic activities

Cpds	3'-Processing IC_{50} (μM)	Strand Transfer IC_{50} (μM)	SI[a]
1	>333	69 ± 4	>5
2	15 ± 2	0.54 ± 0.08	28
3	>100	>100	-
4	>100	>100	-
5	>100	>100	-
6	>100	>100	-
11	>100	>100	-
12	>100	96 ± 6	>1
13	>100	60 ± 13	>1.7
14	ND[b]	ND[b]	ND[b]

[a]SI: Selectivity Index. Values are from average of two or three independent experiments. [b]ND: not determined.

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Interestingly, when compared with reference compound **1**, the derivative **13** demonstrated approximately the same inhibitory activities (IC_{50} values of 60 ± 13 and 69 ± 4 μM for **13** and **1**, respectively), thus confirming that several features of these systems could be considered for a structural development. Furthermore, as expected, **13** proved to be more of 100-fold less active of **2**, a well studied and validated DKA inhibitor. From a structural point of view, an amino functionality in position 4 (both for **12** and **13**) and the enol OH in position 3 (only for **13**) of the pyridazinone ring can be predicted as an additional point of chelation on this pharmacophoric fragment, and are important for the anti-IN activity.

Conclusions

In this work, a series of novel heterocycles have been designed and synthesized, to be used as versatile platform in drug design of IN inhibitors. The inhibition of IN enzyme as well as several different viral processes have been targeted via metal chelation. Since the central role of divalent metal ions in these transformations, inhibitors of such processes can be designed on pharmacophores that bind and/or interact to these divalent metal ions. This work has mainly focused in the designing and synthesis of novel chemical scaffold containing a chelating motif addressed toward metal-containing enzymatic sites, such as IN as virological target. Based on the data presented here, these novel prototypes might affect metal affinity in the context of the active site binding. These results prompted us to propose that these types of chromophore are suitable for extensive modifications and will be undertaken in future studies. Therefore, further synthetic and biological investigation for some related congeners are currently in progress and will be reported elsewhere.

Experimental Section

General. Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Merck or Carlo Erba. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Melting points (mp) were determined using an Electrothermal melting point or a Kofler apparatus. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, and NOESY) spectra were determined in $CDCl_3$, DMSO-d_6 or $CDCl_3$/DMSO-d_6 (in 3/1 ratio) and were recorded at 200 MHz and 500 MHz on a Varian XL-200 and a Bruker Avance 500, respectively. Chemical shifts (δ scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Electron ionization and MALDI-TOF mass spectra (70 eV) were recorded on a Hewlett-Packard 5989 Mass Engine

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Spectrometer and on a MALDI micro MX (Waters, micromass) equipped with a reflectron analyser, respectively. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase. Elemental analyses were performed on a Perkin-Elmer 2400 spectrometer at Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari (Italy), and were within ±0.4% of the theoretical values.

5,6-Dihydrofuro[2,3-*h*]cinnolin-3(2*H*)-one (3). A solution of α-hydroxy acid **6** (0.22 g, 0.0011 mol) and hydrazine hydrate (5 mL, 0.10 mol) was refluxed for 30 min. After cooling, the product was filtered and washed with ethanol to give beige crystals. Yield: 62%; mp 219-220 ºC. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 12.65 (brs, 1H, NH), 7.69 (d, 1H, J = 2.0 Hz, H_8), 6.75 (d+s, 2H, J = 2.0 Hz, H_9 and H_4), 3.02-2.93 (m, 4H, H_5 and H_6). MS (EI): m/z [188, M$^+$], 159, 132, 131, 104, 103. Anal. Calc. for $C_{10}H_8N_2O_2$: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.70; H, 4.11; N, 15.15.

4-Hydroxy-4,4a,5,6-tetrahydrofuro[2,3-*h*]cinnolin-3(2*H*)-one (4). A solution of α-hydroxy acid **6** (0.105 g, 0.0005 mol) and hydrazine monohydrate (0.025 g, 0.0005 mol) in ethanol (5 mL) was refluxed for 1 hour. After cooling, the product was filtered, washed with ethanol to give the titled compound as yellow-beige crystals. Yield: 32%; mp 273-274 ºC. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 10.76 (s, 1H, NH), 7.63 (d, 1H, J = 1.8 Hz, H_8), 6.61 (d, 1H, J = 1.8 Hz, H_9), 5.61 (brs, 1H, OH), 3.94-3.85 (m, 1H, H_4), 2.8-2.65 (m, 4H, H_5, H_6), 1.73 (m, 1H, H_{4a}). ^{13}C-NMR (DMSO-d_6): 169.0, 157.7, 146.5, 143.3, 116.4, 105.8, 67.4, 27.4, 21.7. MS (EI): m/z [206, M$^+$], 178, 177, 150, 132, 106. Anal. Calc. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.59. Found: C, 58.43; H, 4.99; N, 13.77.

4,4a,5,6-Tetrahydrofuro[2,3-*h*]cinnolin-3(2*H*)-one (5). A solution of acid **9** (0.097 g, 0.0005 mol) and hydrazine hydrate (0.025 g, 0.0005 mol) in ethanol (5 mL) was refluxed for 3 hours. After cooling, the product was filtered and washed with ethanol to give a beige solid. Yield: 38%; mp 224-225. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 10.73 (s, 1H, NH), 7.64 (d, 1H, J = 1.8 Hz, H_8), 6.64 (d, 1H, J = 1.8 Hz, H_9), 2.82 (m, 2H, H_4), 2.52-2.14 (m, 2H, H_5), 2.25-2.18 (m, 2H, H_6), 1.67(m, 1H, H_{4a}). ^{13}C-NMR (200 MHz, DMSO-d_6): 191.2, 166.8, 157.3, 143.2, 116.2, 105.81, 33.1, 31.7, 28.9, 22.0. MS (EI): m/z [190, M$^+$], 162, 133, 119, 106. Anal. Calc. for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.27; H, 5.22; N, 14.88.

Hydroxy(4-oxo-4,5,6,7-tetrahydro-1-benzofuran-5-yl)acetic acid (6). To a vigorously stirred solution of the ketone **7** (0.73 g, 0.0054 mol) and glyoxylic acid monohydrate (0.50 g, 0.0064 mol) in H_2O (10 mL) at 0 ^{o}C, a solution of sodium hydroxide (0.75 g, 0.0188 mol) in H_2O (40 mL) was then added in small portions. After stirring for 40 min. at room temperature, the alkaline solution was washed with ethyl acetate (10 mL) and then was acidified at 0 ^{o}C with concentrated HCl. After stirring at room temperature for 1 hour, the solution was extracted with ethyl acetate (6 times), dried over Na_2SO_4 and concentrated to give yellow heavy oil, which solidified to give a white solid. Yield: 81%; mp 135-136 ºC. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 12.58 (brs, 1H, COOH), 7.69 (d, 1H, H_8), 6.68 (d, 1H, H_9), 5.32 (brs, 1H, OH), 4.72-4.28 (2d, 1H, Cα-H), 2.96-2.84 (m, 3H, H_2, H_4), 2.18-2-1.97 (m, 2H, H_3). ^{13}C-NMR (200 MHz DMSO-

d_6): 192.4, 175.5, 167.3, 143.8, 120.6, 106.5, 69.6, 68.3, 49.8, 22.3. MS (EI): *m/z* [210, M^+]. Anal. Calc. for $C_{10}H_{10}O_5$: C, 57.14; H, 4.80. Found: C, 57.02; H, 4.98.

6,7-Dihydro-1-benzofuran-4(5*H*)-one (7). To an ice-bath cooled solution of 1,3-cyclohexadione **8** (2 g, 0.0178 mol) and H_2O (16 mL), a solution of $NaHCO_3$ (1.79 g) in H_2O (16 mL) was added dropwise, followed by a 45% aqueous chloroacetaldehyde solution (3.57 mL, 0.056 mol), under vigorous stirring. The reaction mixture was stirred at room temperature for 15 hours (the pH of the solution should be in the range of 6-9). Then ethyl acetate (25 mL) was added and the solution was acidified to pH 1 with 1 M H_2SO_4. After extraction with ethyl acetate the organic layers were washed with an aqueous solution of K_2CO_3, dried over Na_2SO_4 and evaporated under vacuum, to give yellow oil. Yield: 70%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.31 (d, 1H, H_9), 6.69 (d, 1H, H_9), 2.91-2.85 (m, 2H, CH_2), 2.53-2.47 (t, 2H, CH_2), 2.23-2.11 (m, 2H, CH_2). The ^{1}H-NMR spectrum was in accordance with that reported in literature.[24] ^{13}C-NMR (200 MHz, $CDCl_3$): 194.7, 167.2, 142.5, 120.9, 106.3, 37.5, 23.2, 22.5. MS (EI): *m/z* [136, M^+].

(4-Oxo-4,5,6,7-tetrahydro-1-benzofuran-5-yl)acetic acid (9). To a solution of ester **10** (2.0 g, 0.009 mol) in methanol (10 mL), a 10% solution of NaOH in methanol (10.8 mL, 0.027 mol) was added, and the mixture was stirred at reflux for 2 hours. After this time, the solvent was removed under reduced pressure, the residue was dissolved in water, washed with CH_2Cl_2, and acidified with concentrated HCl. The precipitate formed was filtered and the solid was recrystallized from H_2O to give the acid **9** as white crystal. Yield 92%. mp 100°C (Lit. 103-104 ºC).[30] ^{1}H-NMR (200 MHz, DMSO-d_6): δ 12.12 (s, 1H, OH), 7.66 (d, 1H, H_2), 6.64 (d, 1H, H_3), 2.74-2.93 (m, 3H, H_5 and $2H_7$), 2.66 (dd, 1H, HCH-COOH), 2.34 (dd, 1H, HCH-COOH), 2.18-2.20 (m, 1H, $1H_6$), 1.97 (dq, 1H, $1H_6$). MS (EI): (*m/z*): [194, M^+].

Ethyl (4-oxo-4,5,6,7-tetrahydro-1-benzofuran-5-yl)acetate (10). A solution of **7** (1.6 g, 0.012 mol) in anhydrous THF (15 mL) was added dropwise under a nitrogen atmosphere at -70 ^{o}C to a 1 M solution in THF of lithium bis(trimethylsilyl)amide (12 mL, 0.012 mol) and the reaction mixture was stirred for 1 hour at -70 ºC Then ethyl bromoacetate (1.33 mL, 0.012 mol) was added, and the reaction mixture was stirred at -70 ºC for 30 min., and then at room temperature for 18 hours. The solvent was removed *in vacuo*, and the residue was dissolved in AcOEt. The organic phase was washed with water, 5% aqueous solution of $NaHCO_3$, and 5% HCl, dried over Na_2SO_4 and concentrated to give yellow oil, which was purified by column chromatography (petroleum ether/ethyl acetate 2/8) to give a pale yellow oil. Yield 65%. MS (EI): *m/z* [222, M^+]. The ^{1}H-NMR spectra was in accordance with that reported in literature.[30]

Furo[2,3-*h*]cinnolin-3(2*H*)-one (11). A solution of α-hydroxy acid **6** (0.21 g, 0.0010 mol) in hydrazine hydrate (5 mL, 0.10 mol) was refluxed for 48 hours. After cooling the solid was filtered and washed with ethanol. The solid was then triturated with acetone, filtered to give a beige solid. Yield: 35%; mp 300 ºC dec. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 14.00 (brs, 1H, NH), 7.75 (s, 1H, Ar-H_4), 7.68 (AB system, 2H, J = 9.0 Hz, Ar-H_6 and Ar-H_5), 7.62 (d, 1H, J = 1.8 Hz, H_8), 6.61 (d, 1H, J = 1.8 Hz, H_9). MS (MALDI-TOF): [187, M^+ +1]. Anal. Calc. for $C_{10}H_6N_2O_2$: C, 64.52; H, 3.25; N, 15.05. Found: C, 64.34; H, 3.55; N, 14.91.

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4-Amino-furo[2,3-*h*]cinnolin-3(2*H*)-one (12). From **6**. A suspension of **6** (0.23 g, 0.0011 mol) in hydrazine hydrate (5 mL, 0.10 mol) was refluxed for 72 hours. After cooling the excess of hydrazine hydrate was evaporated and the residue was triturated with acetone. The solid formed was purified by flash chromatography eluting with dichloromethane/methanol 9.5/0.5 to give a brown solid. Yield: 40%.

From **11**. A suspension of **11** (0.20 g, 0.0011 mol) and hydrazine hydrate (5 mL, 0.10 mol) was refluxed for 24 hours. After cooling the excess of hydrazine hydrate was evaporated and the residue was triturated with acetone. The solid formed was purified by flash chromatography eluting with dichloromethane/methanol 9.5/0.5 to give a brown solid. Yield: 45%, mp 300 ºC dec. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.58 (d, 1H, *J* = 9.3 Hz, Ar-H_6), 7.07 (d, 1H, *J* = 9.3 Hz, Ar-H_5), 7.21 (d, 1H, *J* = 1.9 Hz, Ar-H_8), 6.69 (d, 1H, *J* = 1.9 Hz, Ar-H_9). MS (MALDI-TOF): [202, M^+ +1]. Anal. Calc. for $C_{10}H_7N_3O_2$: C, 59.70; H, 3.51; N, 20.89. Found: C, 59.75; H, 3.42; N, 21.09.

4-Amino-1,2-dihydropyridazino[3,4-*f*]cinnolin-3-ol (13). A solution of **11** (0.22 g, 0.0011 mol) in hydrazine hydrate (5 mL) was refluxed for 72 hours. After cooling the excess of hydrazine hydrate was evaporated. The residue was triturated with acetone, the solid filtrated and purified by flash chromatography eluting with dichloromethane/methanol 9.5/0.5 to give a green solid. Yield: 25%; mp 300 ºC dec. ^{1}H-NMR (500 MHz, DMSO): δ 13.50 (brs, 1H, OH), 9.34 (d, 1H, *J* = 5.6 Hz, Ar-H_9), 8.47 (d, 1H, *J* = 5.6 Hz, Ar-H_{10}), 8.06 (d, 1H, *J* = 9.0 Hz, Ar-H_6), 7.64 (brs, 2H, NH), 7.58 (d, 1H, *J* = 9.0 Hz, Ar-H_5), 6.20 (s, 2H, NH_2). MS (EI): (*m*/*z*): [216, M^+ +1], 198, 183, 157. Anal. Calc. for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.60; H, 4.02; N, 32.65.

1,2-Dihydrofuro[2,3-*h*]cinnolin-3(4*H*)-one (14). A mixture of **3** (0.19 g, 0.0010 mol) (in hydrazine hydrate (5 mL, 0.10 mol) was refluxed for 15-30 minutes. After cooling the product was filtered, washed with water to give a beige solid. Yield: 55%; mp 300 ºC dec. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 7.94 (d, 1H, *J* = 8.8 Hz, H_6); 7.20 (d, 1H, *J* = 8.8 Hz, H_5); 7.18 (s, 2H, H_8, H_9); 5.33 (bs, 2H, NH); 5.59 (s, 2H, H_4). MS (EI): (*m*/*z*): [188, M^+, 100%], 158, 131, 129, 117, 105. Anal. Calc. for $C_{10}H_8N_2O_4$: C, 63.82; H, 4.28; N, 14.89. Found: C, 63.98; H, 4.03; N, 15.22.

Biological materials, chemicals, and enzymes

All compounds were dissolved in DMSO and the stock solutions were stored at -20 °C. The γ[^{32}P]-ATP was purchased from PerkinElmer. The expression system for wild-type IN was a generous gift of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

Integrase assays. Inhibition of IN catalytic activities, 3'-processing (3'-proc) and strand transfer (ST), were evaluated by oligonucleotide-based assays in *in vitro* assays employing purified enzyme as previously described.[15]

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