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Short communication

Diketoacid chelating ligands as dual inhibitors of HIV-1 integration process

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ABSTRACT

HIV-1 Integrase (IN) represents a very attractive pharmacological target for the development of new and more efficient drugs. Recently, an allosteric inhibitory approach also emerged, that targets the interaction between IN and cellular cofactors, such as LEDGF/p75. Small molecules based on the diketoacid pharmachophore were studied for their ability to inhibit at the same time integration and IN-LEDGF/p75 interaction (dual inhibitors): in this study, we evaluated three indole diketoacid derivatives and their magnesium(II) complexes for their ability to act as dual inhibitors.

Effectively, the metal complexes exhibited IN inhibition potency in low nanomolar/micromolar concentration range; both the complexes and the free ligands are also able to inhibit the IN-LEDGF/p75 interaction at low μM values. Moreover, these magnesium compounds showed good antiviral activity, suggesting the possibility to exploit metal coordination for the design of new antivirals.

1. Introduction

Human Immunodeficiency Virus (HIV) is the etiological agent of the acquired immunodeficiency syndrome (AIDS), which has become a major epidemic [1]. Highly Active Antiretroviral Therapy (HAART) currently in use provides good results [2,3] but, as usual, problems related to drug toxicity and to the emergence of drug resistant phenotypes urge for the identification of novel pharmacological targets. In recent years, big efforts have been made to develop efficient inhibitors of HIV Integrase (IN), the enzyme that catalyzes the integration of proviral cDNA into the host cell genome through two different steps, 3′-processing (3-P) and strand-transfer (ST) [4–7]. By 3-P, the enzyme recesses the 3′-terminal ends of the viral DNA to generate two CA-3′-hydroxyl ends, which are the reactive intermediates required for the next step. IN, still bound to the 3′-processed viral DNA, translocates into the nucleus of the infected cell, wherein the terminal 3′-OH of the viral DNA attacks the host DNA in the ST step. IN contains a catalytic core domain that presents an amino acidic triad (the so-called "D,D(35)E" motif), that coordinates two divalent Mg^{2+} cofactors [8,9]. These two ions are essential to the catalytic process, according to the 'two-metal-ion' mechanism [10]. Chelation of the metal cofactors within the active site has emerged as a successful strategy in the drug design of IN inhibitors and, in general, in the development of inhibitors of viral enzymes containing magnesium, as HIV reverse transcriptase, Hepatitis C polymerase and Influenza virus endonuclease [11]. A milestone in this sense was the approval, in late 2007, of the chelating inhibitor Raltegravir (Isentress®) as the first drug against HIV-1 IN [12–15]; other chelating IN inhibitors were recently approved or are under clinical trials [16–20]. In recent years a great

Abbreviations: 3-P, 3′-processing; AIDS, acquired immunodeficiency syndrome; DKAs, α,β-diketoacids; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IN, integrase; lens epithelium derived growth factor, LEDGF/p75; ST, strand-transfer.

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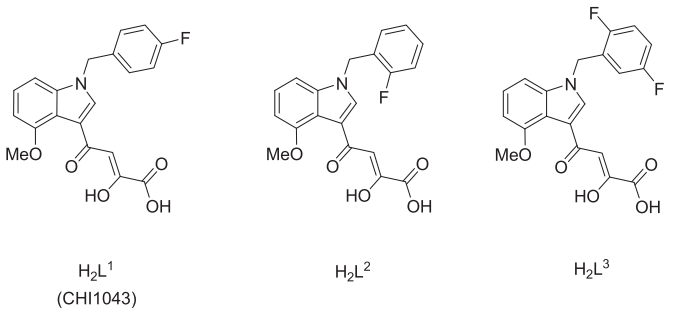
number of compounds have been studied as HIV-1 IN inhibitors [5,21–28]. Among them, one of the most important classes is represented by the α,β-diketoacids (DKAs) [29], that selectively inhibit the ST reaction and exhibit antiviral activity against HIV-1 infected cells. They comprise a β-diketo moiety, an aromatic or heteroaromatic portion, and a carboxylic functionality able to chelate divalent metal ions and therefore to block the interaction of the enzyme with the DNA substrate. Some of us have synthesized a series of indole derivatives belonging to the DKAs that are active IN inhibitors [30–34]; in particular **H_2L^1** (CHI-1043, Fig. 1) presents very good activity both in enzymatic and in cellular assays with low toxicity (IC_{50} = 0.04 μM; EC_{50} = 0.59 μM; SI = 70) [31]. Docking studies [35] highlighted that **H_2L^1** has a binding mode similar to that observed for other IN ST inhibitors crystallized within the active site of the enzyme [9], with the diketo acid moiety coordinating the two metal cofactors. The diketo acid functionality chelates divalent metal ions in solution, forming metal complexes with different stoichiometric ratios [36–39]. We also isolated metal complexes with DKAs ligands, and we tested them for their ability to inhibit IN in enzymatic assays, finding, quite surprisingly, that also some preformed complexes are active at a high nanomolar to low micromolar range [37–39]. It is generally recognized that the study of the coordinating ability of the DKA pharmachophore is of paramount importance, since it can lead to the design of more efficient IN inhibitors [40].

Indole DKAs are also inhibitors of the interaction between HIV-1 IN and the cellular cofactor LEDGF/p75 (Lens Epithelium Derived Growth Factor) [35–42]. Problems related to drug-resistant strains observed with the use of Raltegravir has highlighted the necessity to identify molecules able to target different steps in the integration process [43]. LEDGF/p75 is a cellular protein that has been identified as a cellular cofactor of HIV integration and replication [44,45]. It binds HIV-1 IN *via* a small IN-binding domain ($LEDGF_{IBD}$) within its C-terminal region and several studies pointed out the essential role of LEDGF/p75 in viral replication and fitness [46–51]. Therefore, the development of protein–protein disrupting therapeutics is currently a very important pharmacological target to improve available HAART. In particular, "dual inhibitors" able to interfere with distinct steps of the integration process are very attractive, since a multimode mechanism of action could result in cooperative inhibition of DNA integration and HIV-1 replication in infected cells.

It is also worth noting that data regarding the biological activity of isolated metal complexes towards HIV IN, in particular in cellular assays, are surprisingly scarce [52–56].

With this in mind, we focused our attention on the potent IN ST inhibitor **H_2L^1** and its analogues **H_2L^2** and **H_2L^3** (Fig. 1) [33] in order to isolate and characterized the corresponding magnesium complexes and to test their ability to inhibit HIV-1 IN in enzymatic assays and to evaluate their activity against HIV-1 infected cells. Finally, both the ligands and the magnesium complexes have been tested as IN-LEDGF/p75 inhibitors.



H_2L^1 (CHI1043) H_2L^2 H_2L^3

Fig. 1. Chemical structures of the ligands **H_2L^1**–**H_2L^3**.

2. Experimental

2.1. Material and methods

All reagents of commercial quality were used without further purification. Purity of compounds was determined by elemental analysis and verified to be ≥95% for all synthesised molecules. NMR spectra were recorded at 27 °C on a Bruker Avance 400 FT spectrophotometer; IR spectra were obtained with a Nicolet 5PCFT-IR spectrophotometer in the 4000–400 cm^{-1} range, in reflectance mode on the powder. Elemental analyses were performed by using a Carlo Erba Model EA 1108 apparatus. Electrospray mass spectral analyses (ESI-MS) were performed with an electrospray ionization (ESI) time-of-flight Micromass 4LCZ spectrometer.

2.2. Synthesis

Ligands **H_2L^1**–**H_2L^3** were synthesized as previously reported [31,33].

Synthesis of the complexes **1**, **2**, **3**, general procedure: A suspension of magnesium hydroxide (0.27 mmol) in water (2 ml) was added to a methanolic solution (13 ml) of the ligand (0.27 mmol). The reaction mixture was stirred at room temperature overnight. On concentrating the solution, a powder was obtained, which was filtered, washed with cool water and dried on vacuum.

$Mg_2(L^1)_2 \cdot 7H_2O$, ***1***. Yellow powder (45%). m.p. >350 °C. ^{1}H NMR ($CDCl_3$): δ = 4.06 (s, 3H, OCH_3), 5.32 (s, 2H, CH_2), 6.77 (br, 1H, Ar–H), 6.95 (br, 1H, Ar–H), 7.05 (br, 2H, Ar–H), 7.16 (br, 2H, Ar–H), 7.24 (br, 1H, Ar–H), 7.94 (s, br, 1H, =CH), 8.02 (s, br, 1H, =CH) ppm. ^{19}F{^{1}H} NMR ($CDCl_3$): δ = −113.18 ppm. IR (ATR): ν = 1592, 1508 cm^{-1}. ESI/MS (+) *m/z* 821.3 $[M + K]^+$; 805.4 $[M + Na]^+$. Anal. Calcd. for $C_{40}H_{28}F_2Mg_2N_2O_{10} \cdot 7H_2O$: C 52.83, H 4.66, N 3.08. Found: C 52.76, H 4.25 N 2.96. *$Mg_2(L^2)_2 \cdot 5H_2O$*, **2**. Yellow powder (48%). m.p. >350 °C. ^{1}H NMR ($CDCl_3$): δ = 4.05 (br, 3H, OCH_3), 5.39 (br, 2H, CH_2), 6.76–7.28 (m, br, 7H, Ar–H), 7.93 (s, br, 1H, =CH), 8.05 (s, br, 1H, =CH) ppm. ^{19}F{^{1}H} NMR ($CDCl_3$): δ = −117.6 ppm. IR (ATR): ν = 1592, 1501 cm^{-1}. ESI/MS (+) *m/z* 805.4 $[M + Na]^+$. Anal. Calcd. for $C_{40}H_{28}F_2Mg_2N_2O_{10} \cdot 5H_2O$: C 55.01, H 4.39, N 3.21. Found: C 54.94, H 4.28, N 3.07.

$Mg_2(L^3)_2 \cdot 7H_2O$, ***3***. Yellow powder (45%). m.p. > 350 °C. ^{1}H NMR ($CDCl_3$): δ = 4.01 (br, 3H, OCH_3), 5.33 (br, 2H, CH_2), 6.52–7.21 (m, br, 7H, Ar–H), 7.92 (s, br, 1H, =CH), 8.01 (s, br, 1H, =CH) ppm. ^{19}F{^{1}H} NMR ($CDCl_3$): δ = −123.6, −117.0 ppm. IR (ATR): ν = 1594, 1493 cm^{-1}. ESI/MS (+) *m/z* 841.3 $[M + Na]^+$. Anal. Calcd. for $C_{40}H_{26}F_4Mg_2N_2O_{10} \cdot 7H_2O$: C 50.82, H 4.26, N 2.96. Found: C 51.07, H 3.71, N 2.84.

2.3. Potentiometric measurements

Equilibrium constants for protonation and complexation reactions were determined by means of potentiometric measurements (pH = −log[H^+]), carried out in methanol/water = 9:1 v/v solution at ionic strength 0.1 M KCl and 25 ± 0.1 °C, in the pH range 2.5–11 under N_2. Temperature was controlled to ±0.1 °C by using a thermostatic circulating water bath (ISCO GTR 2000 IIx). Appropriate aliquots of ligand solution, prepared by weight, were titrated with standard KOH (solvent: methanol/water = 9:1 v/v, *I* = 0.1 M KCl) with and without metal ions. Constant-speed magnetic stirring was applied throughout. Freshly boiled methanol and bidistilled water, kept under N_2, were used throughout. The experimental procedure in order to reach very high accuracy in the determination of the equilibrium constants in this mixed solvent has been

described in detail elsewhere [57]. The protonation constants of $\mathbf{H_2L^1}$ were obtained by titrating 20–50 ml samples of the ligand (2×10^{-3}–7×10^{-3} M). For the complex formation constants, the metal ion stock solution was prepared from $MgCl_2 \cdot 6H_2O$ (Carlo Erba) and the concentration was determined by using EDTA as titrant and Eriochrome black T as indicator. The titrations were performed with different ligand/metal ratios (1 up to 4). At least two measurements (about 60 experimental points in each) were performed for each system. Potentiometric titrations were carried out by a fully automated apparatus equipped with a CRISON GLP 21-22 digital voltmeter (resolution 0.1 mV) and a 5 ml Metrohm Dosimat 655 autoburette, both controlled by a home-made software, written in BASIC, working on an IBM computer. The electrodic chain (Crison 5250 glass electrode and KCl 0.1 M in methanol/water = 9:1 v/v calomel electrode, Radiometer 401) was calibrated in terms of $[H^+]$ by means of a strong acid-strong base titration, by the Gran's method [58], allowing the determination of the standard potential, E_o (366.7 ± 0.1 mV) and of the ionic product of water, K_w (pK_w = 14.38 ± 0.01) in the experimental conditions used. The software HYPERQUAD [59] was used to evaluate the protonation and complexation constants from *e.m.f.* data.

2.4. 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 Perkin-Elmer. The expression system for wild-type IN was a generous gift of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

2.5. 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 analyse the extent of 3′-P and ST using 5′-end labelled substrates, 21top was 5′-end labelled using T_4 polynucleotide kinase (Epicentre, Madison, WI) and γ [^{32}P]-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated and 21bot was added in 1.5 molar 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.

2.6. Integrase assays

To determine the extent of 3-P and ST, wild-type IN was pre-incubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, 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% DMSO, and 25 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.2) at 30 °C for 30 min. Then, 20 nM of the 5′-end ^{32}P-labelled linear oligonucleotide substrate was added, and incubation was continued for an additional 1 h. 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, analysed 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′-proc product) or ST products for DNA alone, DNA plus IN, and DNA plus 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.

2.7. LEDGF/p75-IN AlphaScreen proximity luminescent assay

The AlphaScreen assay was performed according to the manufacturer's protocol (Perkin Elmer, Waltham, MA). Reactions were performed in 25 μl final volume in 384-well Optiwell™ microtiter plates (Perkin Elmer). The reaction buffer contained 25 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM MgCl2, 0.01% (v/v) Tween-20 and 0.1% (w/v) bovine serum albumin. Wild type IN with a His6-tag (300 nM final concentration) was pre-incubated with each inhibitor for 30 min at 4 °C. Next, 100 nM Flag peptide tagged-LEDGF/p75 was added to the reaction and incubated for an additional hour at 4 °C. Subsequently 5 μl of Ni-chelate-coated donor beads and 5 μl anti-Flag acceptor beads were added to a final concentration of 20 μg/ml for each beads. Proteins and beads were incubated for 1 h at 30 °C in order to allow association to occur. Exposure of the reaction to direct light was omitted as much as possible and the emission of light from the acceptor beads was measured in the EnVision plate reader (Perkin Elmer).

2.8. In vitro anti-HIV and drug susceptibility assays

The inhibitory effect of antiviral drugs on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay [60]. This assay is based on the reduction of the yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan derivative, which can be measured spectrophotometrically. The 50% cell culture infective dose ($CCID_{50}$) of the HIV(IIIB) strain was determined by titration of the virus stock using MT-4 cells. For the drug-susceptibility assays, MT-4 cells were infected with 100–300 $CCID_{50}$ of the virus stock in the presence of fivefold serial dilutions of the antiviral drugs. The concentration of the various compounds that achieved 50% protection against the CPE of the different HIV strains, which is defined as the EC_{50}, was determined. In parallel, the 50% cytotoxic concentration (CC_{50}) was determined.

3. Results and discussion

3.1. Chemistry

Firstly, DKAs $\mathbf{H_2L^1}$–$\mathbf{H_2L^3}$ were studied for their ability to coordinate divalent magnesium ions, in order to isolate the corresponding complexes. The α,β-diketo-acid pharmachophore is able to chelate divalent metals in different ways: the hydroxy-carboxylate form [61], or the acetyl-acetonate coordinating mode [62]. If both of them are used, dimers Mg_2L_2 are obtained (Fig. 2). In all the cases the coordination sphere of the metals can be completed by solvent molecules.

Reaction of magnesium hydroxide with $\mathbf{H_2L^1}$–$\mathbf{H_2L^3}$ in a 1:1 stoichiometric *ratio* gives the corresponding complexes **1**–**3** (Fig. 2). Upon coordination, the ligand is completely deprotonated, as can be inferred by spectroscopic data. In particular, in the IR spectra of the complexes the OH absorption disappears (2800-3100 cm^{-1} in the free ligands), and the C=O bands shift from

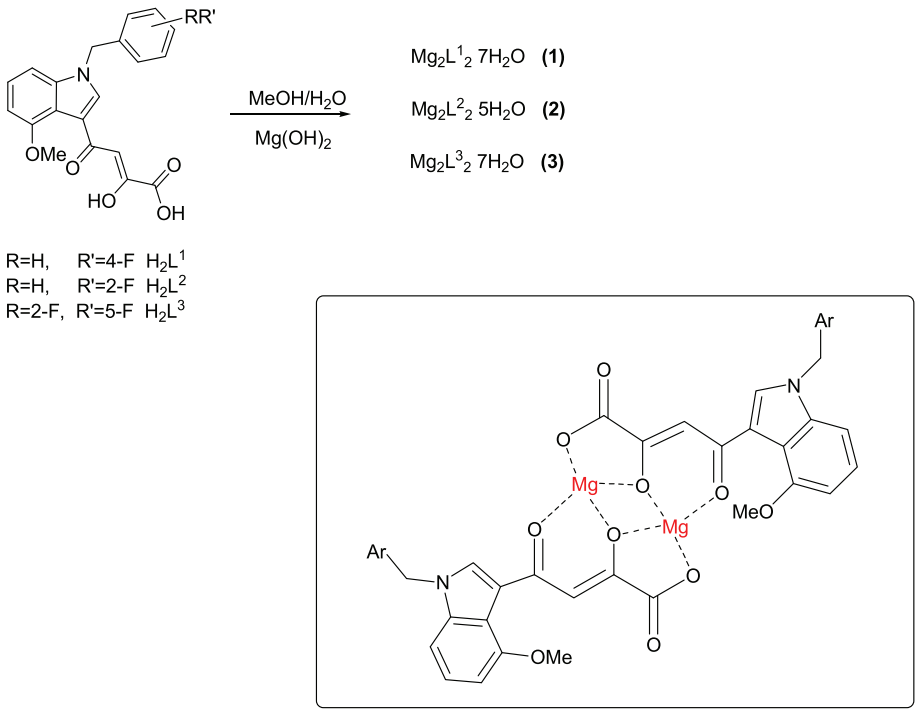


Fig. 2. Synthesis of the complexes **1**–**3** and scheme of their structure (inset).

1700-1720 cm^{-1} in the free ligands to 1592-1594 cm^{-1}. A broad absorption band at about 3400 cm^{-1} indicates the presence of water molecules in the complexes. The ^{1}H-NMR spectra confirm the deprotonation of the ligands: the signals of the acidic protons are in fact absent in all the cases. Coordination to the metal implies also a broadening of the ^{1}H and ^{19}F NMR signals. Elemental analysis and mass data confirm the formula $Mg_2L_2 \cdot nH_2O$ (complex **(1)** $n = 7$, **(2)** $n = 5$, **(3)** $n = 7$). Experimental data are in accord with a structural hypothesis that considers the acetyl, the hydroxy and the carboxylate groups coordinated to the metal (Fig. 2, inset), as we already observed with analogous DKA complexes [37–39]. In order to gain insight on the complexing ability of these ligands towards Mg(II) ions in solution and to confirm the proposed stoichiometry, potentiometric measurements have been carried out with ligand **H_2L^1**.

To avoid solubility problems, all the titrations were carried out in methanol/water 9/1 (v/v) and ionic strength 0.1 M KCl, where both the reactants and the complex species are soluble. The software Hyperquad has been employed for the refinement of the trial equilibrium constants. The best fit of the experimental titration curves was obtained by the set of species reported in Table 1.

H_2L^1 is a diprotic acid with protonation constants $\log \beta_1 = 11.75(0.01)$ and $\log \beta_2 = 16.34\,(0.02)$. The values are in good agreement with the protonation constant of the DKA analogue (2*Z*)-4-[1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic acid ($\log \beta_1 = 11.66(0.03)$ and $\log \beta_2 = 15.91\,(0.06)$), we have studied before [37]. The stoichiometry of the magnesium(II) complexes with **H_2L^1** and their formation constants have been determined. The best statistical parameters for the fit are obtained by using the set of species MgL_2^{2-}, Mg_2L_2, and, in alkaline environment, $Mg_2L_2(OH)$ (Table 1). In particular, at physiological pH the species Mg_2L_2 is predominant. The species M_2L^{2+} [37] was always rejected by the software. This species is very sensitive to the steric hindrance of the ligand [37], therefore probably the presence of a bulky 4-methoxy indol group disfavours the simultaneous interaction of two metal ions with the same ligand molecule. The distribution diagram for the M/L = 1/4 is shown in Fig. 3.

The formation constants of the monomeric species, ML and of the dimeric one, M_2L_2 cannot be refined together, probably because of their high mathematical correlation. By means of potentiometry only, we cannot surely reject the monomeric species, because the model with ML instead of M_2L_2 is only a little worse. However, the model here reported shows better statistical values and it is in accord with the chemical data (in particular, mass spectra) and with molecular modelling calculations with analogous diketo acid ligands [36].

Table 1
Logarithms of formation constants ($\beta_{pqr} = [M_pL_qH_r]/[M]^p\,[L]^q\,[H]^r$) in methanol/water = 9:1 v/v, I = 0.1 M KCl at 25 °C for the ligand under study with Mg(II). Standard deviations are given in parentheses. Charges are omitted for simplicity.

	p	q	r	Log β_{pqr}
$M + 2L \rightleftharpoons ML_2$	1	2	0	11.56 (0.15)
$2M + 2L \rightleftharpoons M_2L_2$	2	2	0	17.09 (0.20)
$2M + 2L + OH \rightleftharpoons M_2L_2(OH)$	2	2	−1	6.81 (0.63)
$L + H \rightleftharpoons LH$	0	1	1	11.75 (0.01)
$L + 2H \rightleftharpoons LH_2$	0	1	2	16.34 (0.02)

3.2. Biological activity

Indole DKA derivatives have been identified as good prototypes of small molecules able to inhibit two different stages of the integration process: the IN ST and the IN-LEDGF/75 interaction [35,63]. **H_2L^1**–**H_2L^3** were tested for their ability to inhibit 3′-P and ST catalytic activities by employing purified enzyme (Table 2). They are effectively selective IN inhibitors, with good Selectivity Index for ST (SI, Table 2).

H_2L^1–**H_2L^3** differ for the position or the numbers of the fluorine atoms in the aromatic ring. This structural modification could be important, since it is known that the fluorophenyl ring is involved in the displacement of the 3′-viral adenosine from the active site,

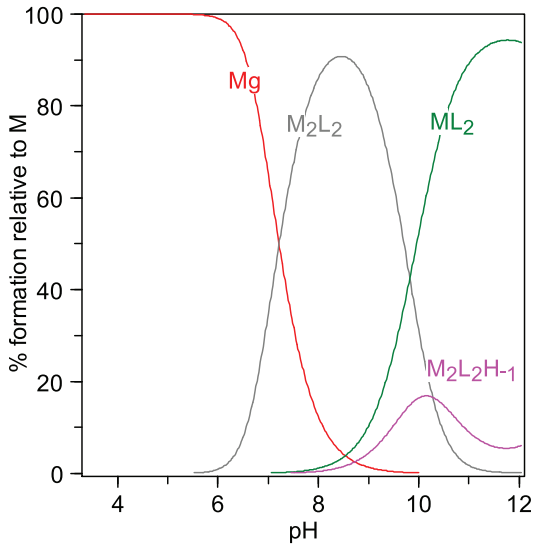


Fig. 3. Distribution diagram for the system under investigation at L:M = 4:1 (the concentration of the ligand $\mathbf{H_2L^1}$ is 4 mM).

leading to deactivation of the intasome [9,64]; however, in this case, the different benzyl substituents do not influence significantly the activity.

Since some metal complexes have shown a good activity against IN in enzymatic assays [37–39,65,66], we tried to verify if also the magnesium complexes of $\mathbf{H_2L^1}$–$\mathbf{H_2L^3}$ are active. **1**–**3** were tested for their ability to inhibit 3′-P and ST catalytic activities by employing purified enzyme (Table 2). All the compounds showed inhibition potency in low nanomolar/micromolar concentration range, but their specificity for ST diminished; in fact, their SI is similar (compound **2**) or worse than that of the corresponding ligand.

Both the complexes and the free ligands are also able to inhibit the IN-LEDGF/p75 interaction at low μM values (Table 2). Modification of the position of the fluorine substituent in the aromatic ring resulted, in this case, in a variation of the activity, with $\mathbf{H_2L^2}$ and $\mathbf{H_2L^3}$ five-fold more potent than $\mathbf{H_2L^1}$. The interactions that the metal complexes engage at the IN-LEDGF interface are quite different from that of the free ligands: upon complexation, for instance, substantial modification of the hydrogen bonding donor-acceptor capability occurs. In this sense, it is quite normal to observe IC_{50} values that are different in $\mathbf{H_2L^1}$–$\mathbf{H_2L^3}$ and in their complexes. Anyway, these are the first data about metal complexes able to inhibit the IN-LEDGF/p75 interaction and any generalization risks to be only speculative.

Finally, the ligands and the metal complexes were tested for their antiviral activity in HIV-infected MT-4 cells, revealing good capability of inhibiting viral infection (Table 2). The EC_{50} values, in fact, ranges from 0.14 to 0.53 μM; the magnesium complexes **(1)** and **(2)** substantially retain the activity of the corresponding free ligands, while **(3)** has a better EC_{50} than $\mathbf{H_2L^3}$ (0.188 *versus* 0.53). Unfortunately, the metal complexes are more cytotoxic of the corresponding free ligands, thus resulting in a worse therapeutic index.

Table 2
Biological evaluation of the ligands $\mathbf{H_2L^1}$–$\mathbf{H_2L^3}$ and of the corresponding magnesium complexes **1**–**3**.

Compd.	IN enzymatic activity IC_{50} (μM)		Selectivity index	LEDGF/p75-IN IC_{50} (μM)	Activity in MT-4 cells		
	3′-P	ST	SI[a]		HIV-1 EC_{50} (μM)[b]	Cytotoxicity CC_{50} (μM)[c]	TI[d]
$\mathbf{H_2L^1}$	2.10	0.04	52.5	14	0.238	47.5 ± 0.5	200
$\mathbf{H_2L^2}$	1.80	0.06	30.0	0.14	0.141	33.5 ± 5.5	238
$\mathbf{H_2L^3}$	2.00	0.03	66.7	0.53	0.534	24 ± 5	45
1	1.00	0.08	12.5	4.80	0.310	24.5 ± 2.5	79
2	0.94	0.02	47.0	8.50	0.165	15.5 ± 0.5	94
3	0.85	0.05	17.0	17.00	0.188	15.00 ± 3.0	80

[a] Selectivity Index.
[b] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[c] Cytotoxic concentration to reduce MT-4 cell viability by 50%.
[d] Therapeutic index: CC_{50}/EC_{50}.

4. Conclusions

Coordination of metal cofactors represents a key aspect in the development of new and efficient antivirals and it is at the basis of on-going research efforts [11,67]. Here we present the synthesis and characterisation of magnesium complexes of HIV-1 IN inhibitors based on the DKA pharmachophore, confirming the capability of these ligands to effectively coordinate the metals. In a scenario of few available data on the activity of isolated metal complexes [52–56], we showed that the preformed magnesium complexes of indole DKA inhibitors have both enzymatic and antiviral activity on infected cells. Moreover, **1**–**3** revealed significant IN-LEDGF/p75 interaction inhibition, behaving as dual inhibitors. These are, to the best of our knowledge, the first data regarding the activity of metal complexes as allosteric inhibitors. The capability of magnesium coordination compounds to block the IN-LEDGF/p75 protein–protein interactions, behaving as dual inhibitors with encouraging antiviral activity, offers a promising approach to prevent viral replication and underlines the possibility to use coordination chemistry to obtain unconventional scaffold to target enzymes.

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