RESEARCH ARTICLE

Synthesis, Molecular Modelling and Biological Studies of 3-hydroxy-pyran-4-one and 3-hydroxy-pyridine-4-one Derivatives as HIV-1 Integrase Inhibitors

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Abstract: Background: Despite the progress in the discovery of antiretroviral compounds for treating HIV-1 infection by targeting HIV integrase (IN), a promising and well-known drug target against HIV-1, there is a growing need to increase the armamentarium against HIV, for avoiding the drug resistance issue.

Objective: To develop novel HIV-1 IN inhibitors, a series of 3-hydroxy-pyran-4-one (HP) and 3-hydroxy-pyridine-4-one (HPO) derivatives have been rationally designed and synthesized.

Method: To provide a significant characterization of the novel compounds, in-depth computational analysis was performed using a novel HIV-1 IN/DNA binary 3D-model for investigating the binding mode of the newly conceived molecules in complex with IN. The 3D-model was generated using the proto-type foamy virus (PFV) DNA as a structural template, positioning the viral polydesoxyribonucleic chain into the HIV-1 IN homology model. Moreover, a series of *in vitro* tests were performed including HIV-1 activity inhibition, HIV-1 IN activity inhibition, HIV-1 IN strand transfer activity inhibition and cellular toxicity.

Results: Bioassay results indicated that most of HP analogues including **HPa**, **HPb**, **HPc**, **HPd**, **HPe** and **HPg**, showed favorable inhibitory activities against HIV-1-IN in the low micromolar range. Particularly halogenated derivatives (**HPb** and **HPd**) offered the best biological activities in terms of reduced toxicity and optimum inhibitory activities against HIV-1 IN and HIV-1 in cell culture.

Conclusion: Halogenated derivatives, **HPb** and **HPd**, displayed the most promising anti-HIV profile, paving the way to the optimization of the presented scaffolds for developing new effective antiviral agents.

Keywords: 3-hydroxy-pyran-4-one; 3-hydroxy-pyridine-4-one; HIV-1 IN inhibitors (HIV-1 INIs); Molecular modelling; anti-HIV agents.

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1. INTRODUCTION

According to the WHO *Causes of death 2016 update*, it was estimated that Acquired Immune Deficiency Syndrome (AIDS), ranked 6 among the top 12 deadliest diseases, representing the cause of 3.1% of all reported deaths. Over the last three decades, more than 35 million people were suffer-

Fig. (1). (A) Graphical depiction of the metal binding pharmacophore model for HIV-1 INIs. (B) Chemical structure of three FDA-approved INIs.

ing from human immunodeficiency virus (HIV)/AIDS worldwide. At the end of 2015, about 36.7 million people have been infected with HIV and 1.1 million people worldwide have been the victims of AIDS [1].

Entry, fusion, reverse transcription, integration, and protein maturation are critical steps in the viral life cycle. Each of the thirty-seven FDA-approved anti-HIV drugs **inhibits** one or a combination of 2-3 of these steps [2]. Among the enzymes involved in these phases, HIV-1 integrase (IN) has emerged as a notable drug target for the design of novel anti-HIV agents since it does not have a functional counterpart in the host cells. IN plays a vital role in establishing proper viral replication, producing stable infection by catalyzing the incorporation of viral DNA (vDNA) into the host cellular chromosome [3-5]. The integration process is performed via two coordinated and consecutive reactions, both accomplished in the same active site. The first catalytic function of IN, known as 3'-processing, involves the specific endonucleolytic cleavage from each 3'-ends of vDNA creating two reactive 3'-CA hydroxyl recessed termini. This is followed by a strand transfer (ST) step in which IN catalyzes a concerted nucleophilic attack by the two reactive 3'-OH recessed ends of vDNA to 5'-phosphodiester bond of the host DNA. This attack results in the covalent joining of the two DNA molecules [6, 7]. The catalytic core of HIV-1 IN is characterized by a highly conserved triad of carboxylate residues Asp₆₄, Asp₁₁₆, and Glu₁₅₂ (DDE triad). These acidic residues bind a pair of divalent metal ions within the active site which are critical cofactors for the catalytic reactions of IN [8, 9].

Among IN inhibitors (INIs), diketo acids (DKAs) and their heterocyclic bioisosteres represent the most promising and highly active INIs [10, 11]. DKAs selectively bind to the DNA binding site of the enzyme and act by sequestering the metal ions in the IN active site forming a ligand- Mg^{2+} -IN complex. This mechanism of inhibition stabilizes the macromolecular IN/DNA complex at the 3'-processing step of integration, preventing the subsequent ST step [12].

In the last decade, a metal binding pharmacophore model has been exploited for the design of several INIs. This model comprises: (1) a metal binding group, (MBG), able to interact with Mg^{2+} cofactors within the IN active site, (2) a pendent aromatic or heteroaromatic hydrophobic moiety close to the MBG [13-15]. The major breakthrough in developing INIs based on this pharmacophore model was achieved by the FDA approval of three INIs as effective anti-HIV drugs, Raltegravir (RLT), Elvitegravir (EVG) and Dolutegravir (DTG) in 2007, 2012 and 2013, respectively (Fig. 1) [16-20].

More than two decades ago, hydroxy-pyranones (HP) and hydroxy-pyridinones (HPO) have been described as representative examples of heterocyclic compounds with strong metal-chelating capacity. These scaffolds have been employed to develop inhibitors of some metalloproteins [21]. Accordingly, we have recently developed several 5-hydroxy-pyridine-4-ones derivatives as potential INIs [22, 23].

As a continuation of our efforts in developing INIs, we describe herein the synthesis, a molecular modelling approach consisting in the development of a novel and

Fig. (2). (A) Schematic representation of the rational design of HP derivatives based on RLT structure. (B) Schematic representation of the rational design of HPO derivatives based on DTG structure and a recently reported chemotype as HIV-1 INI. MBGs are highlighted in light grey, while hydrophobic aromatic moieties are highlighted in the boxes. The designed chemotypes satisfy minimal pharmacophore requirements for HIV-1 IN inhibition.

proach consisting in the development of a novel and reliable model of full-length HIV-1 IN in complex with vDNA coupled to molecular docking protocols, and the biological evaluations of two series of HP/HPO derivatives as HIV-1 INIs. The synthesis of compounds was based on the minimal structural requirements (MBG and a hydrophobic aromatic moiety) to bind the HIV-1 IN active site (Fig. 2).

The first series of compounds, **HP(a-i)**, was characterized by a carboxamide moiety at C-2 position of the HP ring. It was postulated that α -hydroxyketone moiety of 3-hydroxypyran-4-one scaffold in combination with an amide carbonyl oxygen atom provides three donor oxygen atoms to bind the two Mg^{2+} cofactors in the IN active site as observed in RAL.

The 3-hydroxy-pyridine-4-one core was used for generating a second series of derivatives, **HPO(a-h)**. This chelating scaffold has been reported as INIs and it is also included in the structure of DTG [24]. This set of analogues consists of two oxygen donor atoms as MBG.

Given the crucial role of hydrophobic aromatic moiety of INIs in binding affinity against the IN active site [15, 25], the influence of the substituted benzyl- or phenethyl-moieties on the anti-IN activity was investigated in both se-

ries of derivatives, keeping unmodified the chelating moiety in each series of compounds (Fig. 2).

2. MATERIALS AND METHODS

2.1. Chemistry

Experimental details regarding the synthesis of the compounds presented in the manuscript are provided in the Supplementary Material.

2.2. Computational Details

2.2.1. Generation of HIV-1 IN/DNA Model

The modelling of the HIV-1 IN bound to DNA was performed based on previous reports exploiting multiple templates-based homology modelling approach [26]. The use of multiple templates significantly improves the quality of the generated homology models [27-30].

2.2.2. Molecular Docking Simulation

To investigate the binding modes of the synthesized compounds within the IN active site, molecular docking studies were conducted using Glide software [31, 32]. Moreover, with the aim of further increasing the accuracy of

the docking results, the best-docked poses obtained from Glide docking studies were subjected to Quantum Polarized Ligand docking (QPLD) protocol implemented in Schrödinger suite [33, 34]. The ligands and the proteins were prepared as previously reported [35-37].

2.2.3. Ligand Binding Energy Calculations

In order to determine the ligand binding energies, Prime/MM-GBSA method implemented in Prime software was applied to the best IN-ligand complexes obtained from docking calculations [38, 39].

2.2.4. In silico Prediction of Physicochemical Properties and Cytochrome P450 (CYP)-mediated Sites of Metabolism

Physicochemical properties of the studied compounds were evaluated by QikProp [40], while the sites of metabolism were predicted by P450 site of metabolism (P450 SOM) workflow implemented in Schrödinger suite [41, 42].

Full details of the computational procedures are provided in the Supplementary Material.

2.3. Methods for the Biological Evaluation

2.3.1. Integrase Assays

The enzymatic integration reactions were carried out with minor modifications as described previously [43, 44]. To determine the susceptibility of the HIV-1 IN enzyme to different compounds, an enzyme-linked immunosorbent assay (ELISA) adapted from Hwang *et al* was used [45]. The overall integration assay uses an oligonucleotide substrate for which one oligonucleotide (5'-ACTGCTAGAGATTTCC AACTGACTAAAAGGGTC-3') is labeled with biotin at the 3' end and the other oligonucleotide (5'-GACCCTTTT AGTCAGTGTGGAAAATCTCTAGCAGT-3') is labeled with digoxigenin at the 5' end. For the ST assay, a pre-cleaved oligonucleotide substrate (the second oligonucleotide lacks GT [underlined] at the 3' end) was used. The IN enzyme was diluted in 750 mM NaCl, 10 mM Tris (pH 7.6), 10% glycerol, and 1 mM β -mercaptoethanol. To perform the reaction, 4 μ L of diluted IN (corresponding to a concentration of 1.6 μ M) and 4 μ L of annealed oligonucleotides (7 nM) were added in a final reaction volume of 40 μ L containing 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 20 mM HEPES (pH 7.5), 5% polyethylene glycol, and 15% DMSO. As such, the final concentration of IN in this assay was 160 nM. The reaction was carried out for 1 h at 37 °C. Reaction products were denatured with 30 mM NaOH and detected by ELISA on avidin-coated plates. For determining the effect of compounds on the 3'-processing activity a classical cleavage assay with detection of products by denaturing gel electrophoresis was performed as described [43, 44]. Briefly, 0.2 pmol of the radioactively labeled oligonucleotide substrate (INT1, ^{32}P -5'-TGTGGAAAATCTCTAGCAGT-3'; INT2, 5'-ACTGCTAGAGATTTTCCACA-3') and 10 nmol IN in a final volume of 10 μ L was incubated for 1 h at 37 °C. The final reaction mixture contained 20 mM HEPES (pH 7.5), 5 mM DTT, 10 mM $MgCl_2$, 0.5% (v/v) polyethylene glycol 8000, 15% DMSO. IN was diluted previously in 750 mM NaCl, 10 mM Tris (pH 7.6), 10% glycerol and 1 mM β -mercaptoethanol. The reactions were stopped by the addition of formamide loading buffer (95% formamide, 0.1% xylene

cyanol, 0.1% bromophenol blue and 0.1% sodium dodecyl sulfate). Samples were loaded on a 15% denaturing polyacrylamide/ureum gel. The extent of 3'-processing or DNA ST was based on measuring the respective amounts of -2 bands or ST products relative to the intensity of the total radioactivity present in the lane. These data were determined using the OptiQuant Acquisition and Analysis software (Perkin Elmer Corporate, Fremont, CA).

2.3.2. 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 [46]. 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 of the HIV strains was determined by titration of the virus stock using MT-4 cells. For the drug susceptibility assays, MT-4 cells were infected with 100 to 300 50% cell culture infective doses of the HIV strains in the presence of five-fold serial dilutions of the antiviral drugs. The concentration of the compound achieving 50% protection against the CPE of HIV, which is defined as the 50% effective concentration (EC_{50}), was determined. In parallel, the concentration of the compound destroying 50% of the MT-4 cells, which is defined as the 50% cytotoxic concentration (CC_{50}), was determined as well.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The general structures and the structural details of the two series of developed compounds are provided in Table 1.

3.1.1. Synthesis of the 3-hydroxy-pyran-4-one derivatives, HP(a-i)

The general procedure employed for the synthesis of this series of compounds is summarized in Scheme 1. This set of derivatives was prepared in nine steps starting from commercially available Kojic acid 1. The synthesis of intermediates 2, 3, 4 and 5 has been reported previously [47, 48]. The direct conversion of the primary alcohol at the C-2 position of the protected pyranone 5 to carboxylic acid was initially attempted by the Jones reagent. However, the pyranone moiety was found to be too unstable under the reaction condition, leading to structural degradation. Thus in an alternative way, the hydroxyl group was converted to the corresponding aldehyde group before further oxidation to the carboxylic acid. The selective oxidation of the alcohol derivative 5 to the aldehyde product 6 proceeded efficiently (Yield: 81%) using manganese dioxide as described by Becker [49]. The 3-(benzyloxy)-6-methyl-4-oxo-4H-pyran-2-carboxylic acid 7 was achieved by subsequent oxidation of aldehyde 6 using sodium chlorite and sulfamic acid under Pinnick oxidation reaction in excellent yield (85%) [50]. Subsequently, the carboxylic acid group of compound 7 was activated with carbodiimide reagent EDCI and NHS. The activated ester 8 was used *in situ* for the amide coupling reaction with the desired primary amines, resulting in 2-amido substituted

Table 1. General structures and structural details of the compounds investigated in this study.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>HP(a-i)</p> </div> <div style="text-align: center;"> <p>HPO(a-h)</p> </div> </div>				
Cmpd	R	n	Molecular Formula	Molecular Weight
HPa	H	1	C ₁₄ H ₁₃ NO ₄	259.26
HPb	F	1	C ₁₄ H ₁₂ FNO ₄	277.25
HPc	CF ₃	1	C ₁₅ H ₁₂ F ₃ NO ₄	327.26
HPd	Cl	1	C ₁₄ H ₁₂ ClNO ₄	293.70
HPe	CH ₃	1	C ₁₅ H ₁₅ NO ₄	273.28
HPf	OCH ₃	1	C ₁₅ H ₁₅ NO ₅	289.28
HPg	H	2	C ₁₅ H ₁₅ NO ₄	273.28
HPH	OH	2	C ₁₅ H ₁₅ NO ₅	289.28
HPi	Cl	2	C ₁₅ H ₁₄ ClNO ₄	307.73
HPOa	H	1	C ₁₃ H ₁₃ NO ₂	215.25
HPOb	F	1	C ₁₃ H ₁₂ FNO ₂	233.24
HPOc	Cl	1	C ₁₃ H ₁₂ ClNO ₂	249.69
HPOd	CH ₃	1	C ₁₄ H ₁₅ NO ₂	229.27
HPOe	OCH ₃	1	C ₁₄ H ₁₅ NO ₃	245.27
HPOf	H	2	C ₁₄ H ₁₅ NO ₂	229.27
HPOg	OH	2	C ₁₄ H ₁₅ NO ₃	245.27
HPOh	Cl	2	C ₁₄ H ₁₄ ClNO ₂	263.72

pyranone analogues 9a-i [51, 52]. Finally, the removal of the benzyl protecting group of the obtained amide derivatives was accomplished through two different methods to yield the corresponding bidentate chelators, HP(a-i), as final products. So, the benzylic linkage cleavage of the majority of compounds was conducted using boron tribromide reagent (BBr₃) under mild conditions.

Thus, the second method was applied only on compound **9f** which had methoxy substituent at the *para*-position of the aryl backbone. The methoxy group of this compound was susceptible to the reaction with boron tribromide reagent. As a result, an acid mediated deprotection with a 1:1 mixture of CH₃CO₂H/HCl (v/v 1:1) was adopted for selective elimination of benzyl group for this derivative. This procedure does not influence the methoxy group which can readily be removed by BBr₃ [52].

3.1.2. Synthesis of 3-hydroxy-pyridine-4-one Derivatives, HPO(a-h)

The general procedure employed for the synthesis of this series of compounds is summarized in Scheme 2.

All the HPO derivatives were prepared from commercially available maltol **10** through a three-step synthetic pathway, applying the methodology described by Harris [53]. In the first step, the 3-hydroxyl group of maltol was benzylated to give product **11**. N-substituted pyridine-4-one derivatives, **12a-h**, were then obtained by reflux of protected maltol **11** with desired primary amines under basic conditions in moderate yields (between 28.6% and 55.6%). Finally, the benzyl-protected 3-hydroxy-pyridine-4-ones were subjected to the deprotection reaction using BBr₃ [54]. In case of the methoxy-bearing compound at the *para*-position

Scheme 1. General procedure applied for the synthesis of the compounds based on HP core. Reagents and conditions: a): SOCl_2 , RT; b) Zn powder, HCl, H_2O , 65-70 °C; c) HCHO , NaOH, H_2O , RT; d) Benzyl bromide, NaOH, $\text{CH}_3\text{OH}/\text{H}_2\text{O}$, 75 °C, 24 h reflux; e) MnO_2 , dioxane, 5 h reflux; f) NaClO_2 , $\text{NH}_2\text{SO}_3\text{H}$, $\text{H}_2\text{O}/\text{acetone}$, RT; g) NHS, EDCI, CH_2Cl_2 (dry), RT; 3 h; h) RNH_2 , RT, 3 days; i) method A: BBr_3 , dry CH_2Cl_2 , 0 °C to RT Method B: $\text{HCl}/\text{CH}_3\text{COOH}$ (v/v 1:1), RT.

of aryl backbone, **12e**, 1:1 mixture of $\text{CH}_3\text{CO}_2\text{H}/\text{HCl}$ was employed for selective elimination of benzyl group. The final bidentate N-substituted HPOs were achieved in good yields.

3.2. Computational Studies

To elucidate the possible binding mode of the synthesized compounds in the IN active site, molecular modelling studies were conducted. We generated a novel three-dimensional (3D) model of HIV-1 IN in complex with the vDNA by homology modelling. Taking the advantage of the high level of conservation among the active sites of retroviral INs [55-57], we used the crystal structures of proto-type foamy virus (PFV) intasome containing vDNA as a conven-

ient proxy for developing a 3D-model of HIV-1 IN in complex with viral (PFV) DNA [26, 58, 59]. Thus, the PFV DNA was positioned into the IN model by superimposing the HIV-1 IN homology model with PFV-IN/DNA complex. Successively, we developed a docking protocol using the developed model to establish the binding features of the compounds into the binary HIV-1 IN complex.

Accordingly, the main obstacle in molecular modelling studies of HIV-1 INs (design and structure-activity relationship rationalization) is the absence of a complete DNA-bound HIV-1 IN crystal structure along with the two metal ions in the active site. It is noteworthy that the binding site of INs is located at the interface between the catalytic core domain (CCD) and the C-terminal domain (CTD). This site

Scheme 2. General procedure applied for the synthesis of the compounds based on HPO core. Reagents and conditions: a) Benzyl Bromide, NaOH, CH₃OH, 24 h reflux; b) substituted benzyl amine or phenyl-ethyl amine, ethanol/H₂O, 80 °C, 48 h reflux; c) method A: BBr₃, dry CH₂Cl₂, 0 °C to RT; Method B: HCl/CH₃COOH (v/v 1:1), RT.

establishes contacts with two Mg²⁺ ions and the 3'-terminus of the vDNA. Therefore, clinical inhibitors of HIV-1 IN preferentially target the DNA-bound form of IN (binary complex) rather than the apoprotein [60-64]. Moreover, in **previously** published studies the molecules were docked using fragments of the enzyme, so the vDNA contribution in the binding of certain inhibitors was not taken into consideration. Recently, a number of crystal structures of full-length PFV-IN/DNA binary complexes with various HIV-1 INIs such as RLT, DTG and EVG have been determined [65-67]. None of the approved drugs was co-crystallized with the HIV-1 IN/DNA complex.

3.2.1. Generation of HIV-1 IN/DNA Model

Considering the lack of experimental structures for HIV-1 IN/vDNA, the preparation of a reliable HIV-1 IN/vDNA model is important to gain detailed molecular insights into the correct binding mode of HIV-1 INIs. Accordingly, a new homology model from full-length HIV-1 IN was built featuring a critical IN/DNA interface using one of the crystal structures of PFV-IN as a template (PDB ID: 3OYA) [26]. As a result, the modelled HIV-1 IN presented the best overlap between the catalytic triad (DDE motif) of the PFV and HIV enzymes (Fig. S1). The quality of our HIV-1 IN model was assessed by the Ramachandran plot generated by RAMPAGE [68]. The plot (Fig. S2) revealed that 261 amino acids (91.3% of residues) were in the favored regions, 25 amino acids (8.7% of the residues) lay in an additional allowed region and no residues were found in the disallowed region. These results showed that all the residues of the modelled HIV-1 IN sat in the allowed regions of Ramachandran plot [69]. This value is in agreement with the defined cut-off value (96.1%) for the most reliable models [70]. Consequently, the stereochemical quality of our homology model was found to be satisfactory.

The theoretical HIV-1 IN 3D-model was used in docking studies. In this way, further validation of the model was done by docking RLT, EVG and DTG into the active site of the modelled HIV IN/vDNA complex and by the comparison with the X-ray structures of PFV-IN bound to INSTIs.

3.2.2. Molecular Docking Simulations

The modelled HIV-1 IN was used to build HIV-1 IN/vDNA complexes with three reference INSTIs (RLT,

EVG and DTG) applying a QPLD docking procedure. The binding modes of these inhibitors within modelled HIV-1 IN are presented in Fig. (S3). Furthermore, detailed results for each compound are provided in Table S1.

The crystallographic data suggest an interfacial mechanism of inhibition by INIs as they can establish interactions with three receptor key elements: (1) enzyme pocket, by hydrophobic contacts, hydrogen bonds and π - π stackings (2) metals, by chelation and cationic- π interactions and (3) vDNA, by π - π stackings. The docking results of reference ligands into HIV-1 IN model supported this mechanism of inhibition. The chelating triad of these inhibitors was involved in the coordination of two metal ion centers between catalytic residues, Asp₁₁₆, Asp₆₄ and Glu₁₅₂. The terminal halobenzyl groups of inhibitors were embedded in a hydrophobic cavity between two catalytic loop residues Pro₁₄₅ and Gln₁₄₆ at the DNA-protein interface. Interestingly these two residues, which correspond to Pro₂₁₄ and Gln₂₁₅ in PFV-IN, are directly involved in the separation of the vDNA strands upon the ST reaction. This can lead to the reduction of the catalytic loop mobility and physically hampers the binding of the host DNA [60, 64]. The phenyl rings of the three inhibitors participated in π - π interactions with deoxycytosine DC₁₆ of the vDNA. In each case, the 5-hydroxy-pyrimidin-4(3H)-one of RLT, quinolin-4(1H)-one of EVG and tricyclic system of DTG established π - π interactions with the terminal adenosine of the vDNA strand. The sidechain of RLT containing *gem*-dimethyl oxadiazole carboxamide moiety was located into the cleft formed by Asn₁₁₇, Gly₁₁₈, and loop residues Pro₁₄₂ and Tyr₁₄₃. The oxygen of oxadiazole ring of RLT was involved in a hydrogen bond with the amino group of Tyr₁₄₃. The methyl on the oxadiazole established van der Waals interactions with the sidechain methylene of Asn₁₁₇. In the modelled HIV-1 IN, the sidechain of EVG at the N-1 position and 1,3-oxazine ring of DTG were located in the above-mentioned cleft. The two methyl groups of the isopropyl substituent of EVG established hydrophobic contacts with Tyr₁₄₃ and Pro₁₄₂. Tyr₁₄₃ is a conserved residue among all the classes of polynucleotidyl transferases and is considered important for vDNA binding [55]. Another key residue is Asn₁₁₇, which is implicated in the binding with the host DNA [71] and should be targeted for obtaining compounds possessing relevant ST inhibitory activity. In general, the interaction pattern of these inhibitors in the active site of the

modelled HIV-1 IN was in accordance with binding modes reported in their original co-crystallized structures with PFV-IN.

This early computational study was aimed at performing the molecular docking simulations of compounds into the PFV-IN and the modelled HIV-1 IN active sites. Glide docking and QPLD procedures were used. The docking procedure, using PFV-IN enzyme, was validated by removing the co-crystallized ligands (RLT, EVG and DTG) from the binding site and re-docking them into the same site. RMSD between the predicted and the X-ray conformation for the RLT (PDB ID: 3OYA), EVG (PDB ID: 3L2U) and DTG (PDB ID: 3S3M) were 0.75 Å, 0.25 Å and 0.015 Å, respectively [22, 23].

Accordingly, due to the results obtained in the mentioned validation experiments, QPLD method was chosen for providing reliable docking outcomes [72, 73]. PFV-IN and the modelled HIV-1 IN were used to perform the docking calculations for investigating the binding modes of the developed compounds. In QPLD procedure, ligand partial charges were calculated with reasonable accuracy based on the polarization effects induced by the surrounding atoms of receptor within the binding site. Then, the ligands with improved charges were employed for re-docking (XP mode) into the modelled binary HIV-1 IN and PFV-IN.

The initial docking results for each compound are presented in Table S2. The studied compounds showed reasonable IN binding modes with docking emodel energy values ranging from -73.837 to -97.123 kcal/mol for HP derivatives, and from -52.48 to -74.695 kcal/mol for HPO compounds. On the other hand, re-docking of the best poses obtained from Glide calculation into PFV-IN/DNA using QPLD method resulted in a decrease of corresponding energy values, confirming the potential of the compounds as INIs (Table S3).

The results confirmed that most of the derivatives based on the HP scaffold adopt an interfacial binding mode comparable to that found for RLT. These compounds are characterized by a unique carboxamide moiety at the C-2 position of the HP ring. All the HP compounds have the same MBG but differ in the benzyl- and phenethyl-substituents attached to the amide portion. These compounds occupied the DNA/IN interface with the oxygen donor triad of MBG and were involved in a bis-bidentate chelation with both Mg^{2+} ions as observed for RLT. Interestingly, the formation of the 5- and 6-membered chelating rings appears to be one of the most critical features of highly effective INSTIs, as recently reported [74]. The superimposition of the best-docked poses of this series of compounds in the active site of PFV-IN is represented in Fig. (S4). The interaction details of these compounds with three key elements of PFV-IN, including amino acids, nucleotide residues and metal ions as found by molecular docking are reported in Tables S2 and S3, respectively.

Based on the computational studies, among HP derivatives, **HPb** and **HPg** were predicted as the best performing compounds of the series. A comparison of the best docked poses of these compounds demonstrated that the chelating oxygen triad is positioned in a suitable distance with both

Mg^{2+} ions. The *para*-fluorobenzyl and phenethyl groups perfectly fitted inside the hydrophobic cavity close to the active site formed by the vDNA bases (DA₁₇, DC₁₆, DG₄), and two residues (Pro₂₁₄, Gln₂₁₅). This space is originally occupied by the terminal adenosine on the 3'-end of the vDNA. Significantly, this insertion allowed the phenyl rings of *para*-fluorobenzyl and phenethyl moieties to establish a π - π stacking with the pyrimidinone ring of cytosine base, C₁₆, of vDNA and thus hinders the positioning of the host DNA in the active site. Moreover, regarding **HPb**, it seems that a favorable van der Waals interaction occurs between the fluorine atom on the benzyl ring and methylene units of Gln₂₁₅ residue. In this orientation, hydrophobic benzyl/phenethyl moieties displaced the terminal adenosine belonging to the vDNA and its reactive 3-OH functionality from the active site, leading to the deactivation of the DNA-IN complex in the ST step. In both cases, further binding stabilization was mediated by hydrogen bonds and π - π stackings of displaced 3'-adenosine terminal base (A₁₇) with a hydroxyl group at the C-3 position of the scaffold and 4-pyranone ring, respectively.

The role of HIV-1 IN residues associated with resistance to INIs has been reported previously. One of the effective and key residues involved in the development of resistance to INIs is Tyr₁₄₃ of HIV-IN corresponding to Tyr₂₁₂ in PFV-IN [75]. As previously mentioned, RLT established interactions with this residue in both binary PFV-IN and modelled HIV-1 IN complexes. Thus, Tyr₁₄₃ mutation confers resistance to RLT. Interestingly, some HP derivatives lacking the oxadiazole moiety (**HPb**, **HPe** and **HPg**), do not interact with Tyr₁₄₃. Consequently, it was assumed that the Tyr₁₄₃ mutation could not influence the binding of the subset of HP derivatives to IN. On the other hand, in the HP derivatives, only a substituent (methyl) was considered at the C-6 position instead of the sidechain containing oxadiazole as in RLT. The orientation of the methyl group of HP derivatives such as **HPb** and **HPg** in PFV-IN and in the modelled HIV-1 IN binary complexes does not significantly interact with the protein.

Remarkably, through QPLD studies, the same interaction pattern is observed for HP compounds in the catalytic site of HIV-1 IN (Table S4). In general, the benzyl and phenethyl-groups point towards the hydrophobic pocket created by the rearrangement of the terminal adenosine and the enzyme surface. These groups are stabilized by favorable hydrophobic contacts with catalytic loop residues, Pro₁₄₅, Gln₁₄₆, and Gly₁₄₉ as well as π - π stacking interaction with DC₁₆ of vDNA. In these docking models, methyl substituent at C-6 position of scaffold weakly participates in hydrophobic interaction with Tyr₁₄₃. The molecular interactions of **HPb** and **HPg** with PFV-IN and with HIV-1 IN binary complex are illustrated in Figs. (3 and 4), respectively.

In the structure of HPO derivatives, hydrophobic aromatic moiety was incorporated into the nitrogen atom of the main scaffold and connected to the chelating core through methylene or ethylene linkers. These compounds only comprise two oxygen binding moieties. They lack a third donor oxygen atom and so are not fully able to coordinate both metal ions within the enzyme active site. A comparison of the best docked poses of these derivatives within HIV-1 IN

Fig. (3). (Left) Best docked pose of the **HPb** in the active site of PFV-IN (panel A) and in the modelled HIV-1 IN (panel B) as found by QPLD method. (Right) Interaction diagram between **HPb** and PFV-IN (panel A) and between **HPb** and the modelled HIV-1 IN (panel B).

and PFV-IN binary complexes revealed that the terminal benzyl- or phenethyl-group is skewed away from the hydrophobic pocket at the vDNA/IN interface. Indeed, these compounds adopt a conformation in which the lipophilic sidechain at the N-1 position of the main scaffold is pointed to the direction of a cavity surrounded by residues Asp₁₈₅, Gln₁₈₆, Pro₂₁₁, Tyr₂₁₂, His₂₁₃ and Pro₂₁₄. With this orientation, MBG of compounds **15** is involved in bidentate chelation of one Mg²⁺ ion in the active site and monodentate coordination to the other one. Interactions of these compounds have been displayed in more details in Tables S2, S3 and S4. Based on docking experiments, HPO compounds were not able to strongly bind the HIV-1 IN and PFV-IN active sites. The binding mode of one of these derivatives, **HPOe** into PFV-IN and modelled HIV-1 IN is depicted in Fig. (S5).

In general, the docking results showed a better IN binding affinity of HP derivatives with respect to the HPO derivatives. These results indicated that the presence of MBG as a heteroatom triad in HP compounds can be one of the important determinants for the improvement of binding affinity. Moreover, one intriguing feature of docking models based on HP derivatives is the orientation of hydrophobic aromatic moieties (benzyl or phenethyl) toward a hydrophobic cavity at the IN/DNA interface, like the binding mode adopted by INSTIs, RLT, EVG and DTG. This could be a possible reason for the stronger and more effective *in silico*

interactions of HP derivatives with protein-metal-DNA complex in comparison with HPO derivatives. Accordingly, the designed compounds based on HP scaffold could hamper host DNA binding in the ST integration step.

3.2.3. Ligand Binding Energy Calculations

To provide more reliable results, we evaluated the ligand binding energies (ΔG_{bind}) of compounds since docking calculations often fail to correctly rank compounds on the basis of their binding affinity and thereby biological activity [76, 77]. The best-docked poses obtained from QPLD studies were rescored using MM-GBSA implemented in Prime [78, 79]. The calculated ΔG_{bind} of the ligands for PFV-IN and HIV-1 IN **15** provided in Table S5. Analysis of the MM-GBSA results revealed a completely different ranking of the studied compounds in comparison with QPLD calculations. As shown in Table S5, the lowest ΔG_{bind} energy value was obtained for **HPb** within the binding site of both the PFV-IN (ΔG_{bind} : -61.539 kcal/mol) and the HIV-1 IN binary complex (ΔG_{bind} : -36.647 kcal/mol). Moreover, the less potent HPO derivatives displayed higher ΔG_{bind} than HP compounds, suggesting a lower affinity for HIV-1 IN.

3.2.4. In silico Prediction of Physicochemical Properties

The drug-like profile of the compounds was evaluated by QikProp [40], employing RLT, EVG and DTG as reference

Fig. (4). (Left) Best docked pose of the **HPg** in the active site of PFV-IN (panel A) and in the modelled HIV-1 IN (panel B) as found by QPLD method. (Right) Interaction diagram between **HPg** and PFV-IN (panel A) and between **HPg** and the modelled HIV-1 IN (panel B).

compounds (Table S6). Almost all the predicted pharmacokinetic properties of the compounds were within the permissible ranges as defined by QikProp. Only HPO compounds exhibited potential cardiac toxicity (QLogHERG) slightly out of the allowed limit. All the studied compounds also satisfy the Lipinski's rule of five. Overall, the designed compounds represent a good starting point for developing HIV-1 INIs.

3.2.5. *In silico* Structure-based Prediction of Cytochrome P450 Sites of Metabolism

An *in silico* analysis of the potential sites of CYP450-mediated metabolism (soft spots) for HP compounds was performed using the P450 site of metabolism (SOM) prediction workflow of Schrödinger software [41, 42]. The prediction for isoforms CYP2C9 and CYP2D6 combined Induced-Fit Docking (IFD) protocol, for the determination of accessibility of the compounds to the heme iron within CYP450 catalytic center, with a rule-based approach for the estimation of intrinsic reactivity. In the case of isoform CYP3A4, which has a highly flexible binding site, only intrinsic reac-

tivity was used to predict soft spots. The results are presented in Table S7. The data indicate that the developed compounds contain potential soft spots for three isoforms CYP3A4, CYP2C9 and CYP2D6, especially at the benzyl- and phenethyl-moieties. It seems that the phenethyl-sidechains are more susceptible to metabolism than benzyl-ones because they displayed an additional soft spot in an extra methylene unit. Furthermore, the predicted soft spots are highly remarkable in aromatic rings bearing *para*-methyl, *para*-methoxy and *para*-hydroxyl groups. It was observed that in compounds **HPe**, **HPf**, methoxy- and methyl-substituents reduced the potential metabolic sites at both aromatic ring and methyl position. The presence of the hydroxyl group in compounds, **HPb** also strongly affected the intrinsic reactivity and accessibility of *ortho*-positions of aromatic ring to the reactive center of CYP450 isoforms. The predicted soft spots revealed that best metabolic stability was achieved by introducing halogen substituents, Cl, F and CF₃ on the phenyl ring of **HP** derivatives. In particular, the insertion of the F atom in **HPb** was predicted to reduce CYP3A4-mediated metabolism, and no soft spots were found for the

Fig. (5). Representative binding poses of **HPf** (panel A) and **HPb** (panel B) in the binding site of CYP2C9. The distance between one of the carbon atoms on phenyl ring and iron center is reported. The compounds and heme group are represented by sticks.

phenyl ring of this compound. Moreover, the phenyl ring of the compound **HPc**, bearing a trifluoromethyl group, was the most stable analogue in the prediction of CYP2C9- and CYP2D6-mediated metabolisms. Representations of **HPb** and **HPf** inside CYP2C9 (Fig. 5) depicted how the F atom of **HPb** at the *para*-position reduced the accessibility of the phenyl ring to the iron center of heme group.

3.3. Biological Evaluation

In order to gain a correct perception of SAR, the inhibitory activity of the investigated compounds against HIV-1 IN catalytic activity was determined based on an *in vitro* enzymatic assay. Given that several HIV-1 IN inhibitors such as RLT target the ST step of the integration reaction, the inhibition of the ST activity of HIV-1 IN was determined in addition to overall HIV-1 IN inhibition of the compounds. Moreover, a screening of all the synthesized compounds for their anti-HIV-1 potential in MT-4 cells was performed in a multiple round cell-based antiviral assay. Cytotoxicity of these compounds for the host cells was also evaluated and their therapeutic indices were calculated. In these experiments, RLT was employed as a reference HIV-1 INI. The *in vitro* results (cytotoxicity (CC₅₀), antiviral activity (EC₅₀) and inhibitory activities of overall HIV-1 IN and ST step (IC₅₀)) for each compound are reported in Table 2.

3.3.1. IN Inhibitory Activity

As shown in Table 2, the most of the HP derivatives showed considerable HIV-1 IN inhibitory activity with IC₅₀ values in the low micromolar range, whereas HPO compounds were approximately devoid of any inhibitory activity against HIV-1 IN (IC₅₀ > 250 μM). The docking results were in a good agreement with superior inhibitory activities of HP derivatives compared to HPO compounds against HIV-1 IN. This noteworthy difference in potency between HP and HPO compounds can be attributed to different structural features.

The most important feature is associated with the presence of amide backbone attached at the C-2 position of the hydroxypyranone ring in HP derivatives. Amide oxygen of these compounds provides the third donor atom as part of MBG that enables HP derivatives to interact with both Mg²⁺ ions in a bidentate chelation manner. HPO compounds lacking a third donor atom can establish a full bidentate chelation to only one Mg²⁺ ion, leading to a sharp decline in potency. Moreover, to obtain optimal binding of INIs to the Mg²⁺ ions, the central bridging donor atom in chelating triad should be deprotonated. The introduction of electron-withdrawing groups such as the carboxamide at the C-2 position of the HP compounds makes the 3-hydroxyl group more acidic, reducing the pKa value, thereby facilitating deprotonation [80]. The anionic hydroxyl group is a potent Lewis base donor and serves as a stronger bridging donor atom between Mg²⁺ ions. Accordingly, it seems that this difference in pKa values of the 3-hydroxyl group of HP derivatives in comparison with HPO can influence the inhibitory activity against HIV-1 IN. Another possible explanation which reflects the profound effect of amide backbone in the IC₅₀ values of HP compounds is an intramolecular hydrogen bond between the exocyclic amide hydrogen and the oxygen at position 3. Some studies have pointed out the importance of this intramolecular hydrogen bonding [81]. The replacement of the amide proton by a methyl group led to a strong decrease of the HIV-1 IN inhibition. In fact, this intramolecular hydrogen bond allows a convenient orientation of the lipophilic aromatic sidechain of HP compounds towards the desired hydrophobic pocket close to the IN catalytic site at the DNA-protein interface. This argument was supported by the different orientation of hydrophobic aromatic moieties of HP and HPO compounds in docking studies. Accordingly, HPO compounds adopted a conformation in which terminal hydrophobic aromatic groups at the N-1 position is pointed towards the opposite direction of the protein-DNA interfacial hydrophobic pocket.

Table 2. Results for anti-HIV activity, inhibitory potential of HIV-1 IN and cytotoxicity of the designed compounds and RLT.

Cmpd	HIV-1 Activity Inhibition EC ₅₀ (μM) ^a	Overall HIV-1 IN Activity Inhibition IC ₅₀ (μM) ^b	HIV-1 IN Strand Transfer Activity Inhibition IC ₅₀ (μM) ^c	Cellular Toxicity CC ₅₀ (μM) ^d	TI ^e
HPa	15.33±4.88	1.66±0.84	7.16±5.13	>250	>16
HPb	1.72±0.99	0.37±0.17	1.405±0.01	>250	>145
HPc	23.74±1.63	1.915±0.23	5.0±3.95	>250	>10
HPd	1.95±0.93	0.7±0.31	1.54±0.2	195.5±4.4	100.3
HPe	1.29±0.45	1.64±0.34	3.87±1.69	>250	>193
HPf	152.38±1.83	34.28±0.43	>250	>250	>1.64
HPg	>189	0.69±0.42	2.22±0.61	189	-
HPb	>250	>250	>250	>250	-
HPi	>178	40.99±2.26	>250	178	-
HPOa	>41	>250	>250	41	-
HPOb	>107	>250	>250	107	-
HPOc	>24	>250	>250	24	-
HPOd	>250	>250	>250	>250	-
HPOe	29	>250	>250	>250	>8
HPOf	>26	>250	>250	26	-
HPOg	>132	>250	>250	132	-
HPOh	25	>250	>250	>250	>10
RLT	0.013±0.008	0.01	0.04	>18.329	>141

^aEffective concentration in which 50% inhibition is observed; ^{b,c}Concentration required to inhibit 50% of the *in vitro* overall and ST integrase activities, respectively; ^dCytotoxic concentration in which 50% of the cells are killed; ^eTherapeutic index: defined by CC₅₀/EC₅₀ ratio.

Among HP compounds, six derivatives strongly inhibited HIV-1 IN with IC₅₀ values ranging from 0.37 to 1.91 μM. These compounds were **HPa**, **HPb**, **HPc**, **HPd**, **HPe** and **HPg**. The results clearly demonstrate that these compounds also inhibited ST step of the HIV-1 IN in the low micromolar range, showing IC₅₀ values in the range of 1.40-7.16 μM (Table 2). Among the remaining derivatives, **HPf** and **HPi** displayed weak potencies against HIV-1 IN with IC₅₀ values of 40.99 and 34.28 μM, respectively. Moreover, these compounds were inactive in ST step inhibition. Finally, no activity was found for **HPb** at concentrations > 250 μM in both overall HIV-1 IN and ST assays. These results are in perfect agreement with ligand binding energies evaluation since the mentioned weakly active compounds showed the worst computational score (Table S5). Examination of SAR of HP compounds at the benzyl amide portion demonstrated that halogen substitution of the aromatic benzyl ring significantly benefits the potency. The positive influence of the electron withdrawing fluorine atom on the inhibitory activity was observed in compound **HPb** with respect to unsubstituted compound, **HPa**. This *para*-fluorine derivative emerged as the most potent compound within this series with 4-fold and 5-fold improvement in potency against HIV-1 IN (IC₅₀ = 0.37 μM) and ST step of integration (IC₅₀ = 1.40 μM), re-

spectively. Although **HPd** bearing *para*-chlorine substituent on the benzyl ring was 2-fold less active than **HPb** in HIV-1 IN inhibition (IC₅₀ = 0.7 μM) but it showed remarkable potency against ST function of IN (IC₅₀ = 1.54 μM) similarly to the *para*-fluorine analogue **HPb**. The importance of *para*-halogen substitution on benzyl ring of HP compounds was also proved by derivatives bearing other groups (**HPc**, **HPe** and **HPf**) which were less active than halogenated analogues (**HPb** and **HPd**). For example, although **HPc** possesses a strong electron withdrawing *para*-trifluoromethyl group, it displayed slightly stronger activity than **HPa**. The IC₅₀ values of **HPc** were improved for overall HIV-1 IN and ST step inhibition only by 1.2 and 1.4 orders. Conversely, the introduction of a methyl group at the *para*-position of the benzene ring led to **HPe**. It showed a comparable HIV-1 IN inhibitory activity (IC₅₀ = 1.64 μM) with respect to the unsubstituted analogs, **HPa** (IC₅₀ = 1.66 μM). This compound also exhibited a 2-fold increase in potency against ST function inhibition (IC₅₀ = 3.87 μM). Moreover, *para*-substitution with higher electron rich methoxy group sharply diminished the HIV-1 IN inhibitory activity (**HPf**, IC₅₀ = 34.28 μM) and completely abrogates its potency against ST step. Analysis of the results of HP compounds bearing phenethyl moieties (**HPg**, **HPb** and **HPi**) indicated that

linker's length between the amide function and the aromatic ring has a drastic influence on the activity. Insertion of an additional methylene unit as in **HPg** provided a 2.5-fold and 3-fold enhancement in HIV-1 IN ($IC_{50} = 0.69 \mu M$) and ST function inhibition ($IC_{50} = 2.22 \mu M$) compared to the benzyl analog, **HPa**. On the other hand, *para*-substitution of phenyl ring of phenethyl moiety with either electron-donor or withdrawing groups was not well tolerated. In this regard, the addition of a chlorine atom at the *para*-position of the phenethyl ring in compound **HPi** did not lead to an improvement in enzymatic inhibitory activities. Unlike corresponding benzyl derivative (**HPd**), caused a sharp decline in inhibition potency against HIV-1 IN ($IC_{50} = 40.99 \mu M$) along with the loss of ST inhibitory activity. It was also found that the *para*-hydroxyl atom on the phenyl ring of phenethyl moiety, compound **HPb**, tends to abolish activity in both HIV-1 IN and ST assays ($IC_{50} > 250 \mu M$). These results can be correlated with the better fitting of unsubstituted phenethyl sidechain (**HPg**) within the hydrophobic cavity at the IN/DNA interface in comparison with substituted ones (**HPb** and **HPi**).

3.3.2. Antiviral and Cytotoxicity Properties

Results of anti-HIV-1 activity of compounds based on cell-based assay indicated that HIV-1 IN inhibition activities and antiviral potencies can be reasonably correlated (Table 2). Compounds **HPb** and **HPd** showed the best IN inhibitory activity demonstrating also a considerable anti-HIV-1 activity against HIV-1 infected cells with EC_{50} values of 1.72 and 1.95 μM , respectively. The cytotoxicity assay showed that all HP compounds, including **HPb** and **HPd**, are generally safe and characterized by low cytotoxicity (CC_{50} values in the range of 178 to $> 250 \mu M$). This led to robust therapeutic indices for **HPb** (TI > 145) and **HPd** (TI = 100), clearly presenting significant anti-HIV-1 activity at non-cytotoxic concentrations. It was also found that the **HPe** considerably improved the anti-HIV-1 activity appearing to be the most active derivative against HIV-1 with the highest therapeutic index (TI > 193). By contrast, *para*-methoxy group was not favorable for both IN inhibitory and anti-HIV activities. Some of HP compounds with good potency against HIV-1 IN (**HPa** and **HPc**) were moderately active against HIV-1 replication in a cellular context. Interestingly, the results also indicated that replacement of the benzyl group of carboxamide portion with phenethyl moiety in compounds **HPg**, **HPb** and **HPi** was not tolerated for inhibition of HIV-1 replication. These findings strongly suggested that benzyl group at the carboxamide sidechain is more crucial than the phenylethyl group for achieving both optimal HIV-1 IN and HIV-1 replication inhibitory activities in enzymatic and cellular assays.

Examination of the cell-based anti-HIV-1 data of HPO series of compounds revealed that HPO compounds exhibited reduced inhibitory potencies than HP derivatives. The weak activity of HPO compounds in the cellular assays was thought to be due to suboptimal physicochemical properties that could affect the cell permeability of these inhibitors. In this regard, **HPOe** and **HPOh** were moderately active against HIV-1-infected cells (EC_{50} values of 29 and 25 μM , respectively) with no significant cytotoxicity to the host cells ($CC_{50} > 250 \mu M$). Unfortunately, all the residual HPO com-

pounds exhibited relatively high cellular cytotoxicity. In other words, most of HPO derivatives showed anti-HIV-1 potential at concentration values (EC_{50}) higher than cytotoxicity values (CC_{50}).

CONCLUSION

In summary, two series of derivatives based on hydroxypyran-4-one and hydroxypyridine-4-one scaffolds were rationally designed, synthesized and evaluated in cell- and enzyme-based assays as anti-HIV-1 agents targeting the IN enzyme. The compounds are characterized by the presence of an MBG and a specific hydrophobic aromatic moiety. Computational studies allowed to determine a reliable binding mode of these compounds into IN binding site. Molecular docking experiments were conducted using full-length crystal structure of PFV-IN/DNA complex as well as a new modelled HIV-1 IN/DNA complex. As suggested by computational studies, bioassay results supported that most of HP analogues featuring a carboxamide sidechain, **HPa**, **HPb**, **HPc**, **HPd**, **HPe** and **HPg**, showed favorable inhibitory activities against HIV-1 IN spanning from low micromolar to sub-micromolar IC_{50} values. By contrast, all HPO compounds were approximately devoid of any inhibitory activity against HIV-1 IN ($IC_{50} > 250 \mu M$). SAR investigations of the HP series suggested that the antiviral and the IN inhibitory potencies were strongly influenced by the presence of the MBG as a heteroatom triad and the nature of the substituent on the *para*-position of the hydrophobic aromatic ring attached to the amide portion. Moreover, the results also demonstrated that one carbon linker's length (HP derivatives with benzyl moiety) between the amide function and the aromatic ring is more favorable than two carbon atoms (HP derivatives with phenethyl moiety) for achieving both optimal HIV-1 IN and HIV-1 replication inhibitory activities in enzymatic and cellular assays. In this context, the *para*-fluorine and *para*-chlorine derivatives **HPb** and **HPd** showed the best biological activities among the series in terms of reduced toxicity and optimum inhibitory activities against HIV-1 IN and HIV-1 in cell culture. ADME-T properties were investigated *in silico* highlighting a satisfactory drug-like potential. The computational evaluation of potential sites of CYP450-metabolism (soft spots) for HP compounds revealed that the best metabolic stability was achieved by introduction of halogen substituents, Cl and F on the phenyl ring of the studied compounds. Interestingly, two HP analogues bearing halogen groups, **HPb** and **HPd**, exhibited the best biological profiles amongst the series.

Briefly, these findings indicated that hydroxyl-pyran-4-one carboxamide chemotype represents a valuable and valid scaffold for developing effective HIV-1 INIs. Although in all cases, the biological activities were lower than RLIT, further optimization studies on the most potent compounds especially halogenated derivatives, **HPb** and **HPd**, are ongoing in this framework of our research with encouraging results, paving the way to the discovery of more potent antiviral molecules amongst this family of compounds.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material includes Figures S1-S7 (generation of HIV-1 IN model and docking studies) and Tables S1-S7 (details of the computational studies). An additional experimental section, with full details about the materials and methods used in this study is also provided.

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