Design, synthesis, *in vitro* and *in silico* studies of novel 4-oxoquinoline ribonucleoside derivatives as HIV-1 reverse transcriptase inhibitors

Luana da S.M. Forezi, Mariana M.J. Ribeiro, Andressa Marttorelli, Juliana L. Abrantes, Carlos R. Rodrigues, Helena Carla Castro, Thiago Moreno L. Souza, Fernanda da C.S. Boechat, Alessandra M.T. de Souza, Maria Cecília B.V. de Souza

PII: S0223-5234(20)30222-1

DOI: https://doi.org/10.1016/j.ejmech.2020.112255

Reference: EJMECH 112255

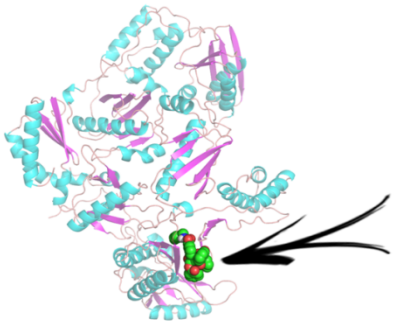
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 5 November 2019

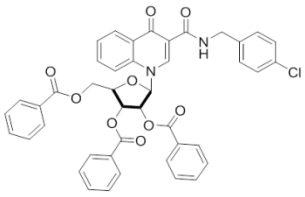
Revised Date: 19 February 2020

Accepted Date: 17 March 2020

Please cite this article as: L.d.S.M. Forezi, M.M.J. Ribeiro, A. Marttorelli, J.L. Abrantes, C.R. Rodrigues, H.C. Castro, T.M.L. Souza, F.d.C.S. Boechat, A.M.T. de Souza, Maria.Cecí.B.V. de Souza, Design, synthesis, *in vitro* and *in silico* studies of novel 4-oxoquinoline ribonucleoside derivatives as HIV-1 reverse transcriptase inhibitors, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112255.



HIV-1 RT



(**9a**)

EC_{50} = 1.4± 0.3 µM

DESIGN, SYNTHESIS, *IN VITRO* AND *IN SILICO* STUDIES OF NOVEL 4-OXOQUINOLINE RIBONUCLEOSIDE DERIVATIVES AS HIV-1 REVERSE TRANSCRIPTASE INHIBITORS

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Abstract

Human immunodeficiency virus type 1 (HIV-1) is a public health problem that affects over 38 million people worldwide. Although there are highly active antiretroviral therapies, emergence of antiviral resistant strains is a problem which leads to almost a million death annually. Thus, the development of new drugs is necessary. The viral enzyme reverse transcriptase (RT) represents a validated therapeutic target. Because the oxoquinolinic scaffold has substantial biological activities, including antiretroviral, a new series of 4-oxoquinoline ribonucleoside derivatives obtained by molecular hybridization were studied here. All synthesized compounds were tested against human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT), and **9a** and **9d** displayed the highest antiviral activities, with IC_{50} values of 1.4 and 1.6 μM, respectively. These compounds were less cytotoxic than AZT and showed CC_{50} values of 1486 and 1394 μM, respectively. Molecular docking studies showed that the most active compounds bound to the allosteric site of the enzyme, suggesting a low susceptibility to the development of antiviral resistance. *In silico* pharmacokinetic and toxicological evaluations reinforced the potential of the active compounds as anti-HIV candidates for further exploration. Overall, this work showed that compounds **9a** and **9d** are promising scaffold for future anti-HIV-1 RT drug design.

Keywords: 4-oxoquinoline; ribonucleoside; antiviral; HIV-1; molecular docking

1. Introduction

Human immunodeficiency virus (HIV) has a global incidence, affecting over 37.9 million people [1]. Highly active antiretroviral therapy (HAART) allows efficient patient management. HAART reduced viremia and HIV-infected individuals may live a normal life. Nevertheless, 770,000 AIDS-related deaths were reported in 2018 [2]. These deaths are associated with the emergence of drug-resistant strains of HIV-1, which jeopardizes the HAART-suppressed viremia. Therefore, the development of new agents is urgently necessary.

HIV-1 enzyme reverse transcriptase (RT) is a validated therapeutic target aimed by Nucleoside and Non-Nucleoside Reverse Transcriptase Inhibitors (acronymed as NRTI and NNRTI, respectively). NRTIs and NNRTIs block RT ability to convert

single-stranded RNA genome to double-stranded DNA during HIV-1replication cycle [3].

NNRTIs are structurally diverse noncompetitive inhibitors that bind onto the hydrophobic pocket located 10 Å from the polymerase active site [4]. Nevirapine, delavirdine, efavirenz, etravirine, rilpivirine and doravirine are exemples of currently approved NNRTIs. Besides these drugs, more than 55 classes of structurally unrelated NNRTIs have already been tested, including clinical trials [5, 6]. Altogether, these information mean the new NNRTIs may pave the way from discovery to clinic.

Previous efforts from our group showed that the chloroxoquinolinic ribonucleoside 6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-quinoline-3-carboxylic acid (**1**) inhibits HIV-1 replication through inhibition of both the wild-type (WT) and antiviral resistant RT [8]. This substance synergizes with both classes of RT inhibitors, represented by the NNRTI and NRTI efavirenz and AZT, respectively [9]. Additionally, the bioisosteric substitution of the carboxylic acid by an amide jointly with molecular simplification of the riboside ring of the 4-oxoquinoline moiety (**2** and **3**) resulted in a lead compound with low toxicity [10]. Thus, our group have demonstrated capacity to discover safe compounds endowed with ability to circumvent antiviral resistance.

The allosteric site of RT was used to rationalize the design of a new series of 4-oxoquinoline derivatives by molecular hybridization (Figure 1). Since this allosteric site is large and hydrophobic, our strategy was to evaluate the increase of the contact surface area within the site by inserting ribofuranosidic units at the N-1 position of 4-oxoquinoline and involving alterations to the type of substituent group at the C-3 position. Toward this end, a new series of 22 4-oxoquinoline ribonucleoside derivatives were synthesized. The anti-HIV activity evaluation demonstrated that derivatives containing the benzoyl substituents showed potent inhibitory activity in the micromolar range against the WT strains of HIV-1. Structure–activity relationship (SAR), molecular docking, *in silico* pharmacokinetics and toxicology studies were also carried out.

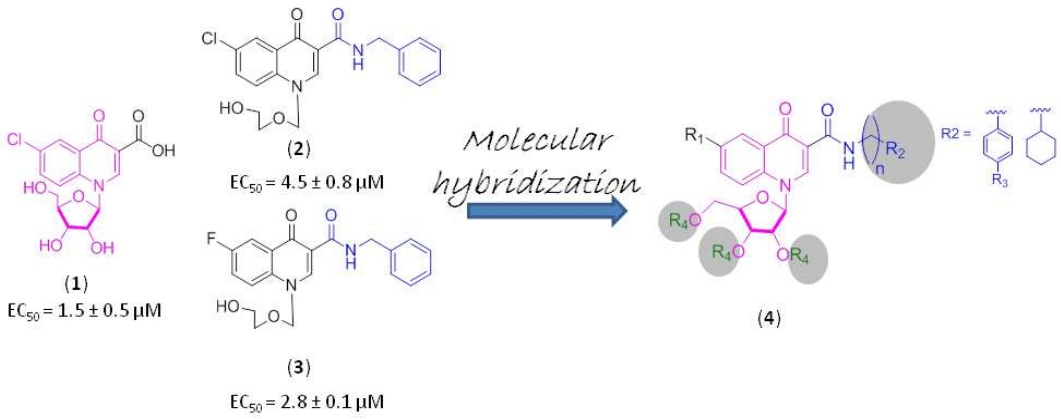


Figure 1. Design strategy of 4-oxoquinoline ribonucleoside 3-carboxamide derivatives by molecular hybridization

2. Experimental Section

2.1. Chemistry

The reagents were purchased from Sigma-Aldrich Brazil and were used without further purification. Analytical thin layer chromatography was performed with silica gel plates (Merck, TLC silica gel 60 F254), and the spots were visualized using UV light. Melting points were obtained on a Fisher-Johns apparatus and were uncorrected. Infrared spectra were measured with KBr pellets on a Perkin-Elmer model 1420 FT-IR spectrophotometer, and the spectra were calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene. NMR spectra were recorded on a Varian Unity Plus VXR (500.00 and 300.00 MHz) instrument in solutions of DMSO-d_6 or $CDCl_3$. The chemical shift data were reported in units of δ (ppm) downfield from the solvent, which was used as the internal standard; coupling constants (*J*) are reported in hertz and refer to apparent peak multiplicities.

2.1.1. General Procedure for the synthesis of 1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-quinoline-3-carboxamides (9a-k).

A stirred solution of carboxamides **8** (1 equiv.), anhydrous acetonitrile (5.0 mL) and BSTFA (1.0 mL) containing 1 % TMCS, was heated at 60-70 °C under nitrogen for 1 h. The resulting mixture was allowed to cool to room temperature, and a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **10** (1 equiv.) in 5 mL of acetonitrile was added, followed by the addition of TMSOTf (0.1 mL). After stirring for 4 h at room

temperature, the solution was poured into ice-cold water (20 g) and neutralized with saturated aqueous sodium bicarbonate solution. The resulting mixture was extracted with methylene chloride (3 × 20 mL), and the combined organic layers were washed with water (3 × 20 mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, and the corresponding products were recrystallized from ethanol.

1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**9a**). White solid, 76 % yield. m.p.: 168-171 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 10.26 (1H, t, *J* 5.5 Hz, C=ONH), 9.29 (1H, s, H-2), 9.51 (1H, dd, *J* 8.1 *and* 1.6 Hz, H-5), 8.10 (2H, d, *J* 7.3 Hz, H-2''' *and* H-6'''), 7.93-7.89 (4H, m, H-2''' *and* H-6'''), 7.75 (1H, d, *J* 8.6 Hz, H-8), 7.60-7.52 (4H, m, H-4''' *and* H-7), 7.46 (1H, t, *J* 7.5 Hz, H-6), 7.42-7.29 (6H, m, H-3''' *and* H-5'''), 7.30 (4H, d, *J* 3.0 Hz, H-2'', H-3'', H-5'' *and* H-6''), 6.51 (1H, d, *J* 4.7 Hz, H-1'), 6.03 (1H, t, *J* 5.3 Hz, H-2'), 5.91-5.88 (1H, m, H-3'), 4.92 (3H, sl, H-4', H-5'a *and* H-5'b), 4.60 (2H, d, *J* 6.0 Hz, CH_2NH). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 177.2 (C=O), 166.2 ($C=O_{OBz}$), 165.1 ($C=O_{OBz}$), 164.8 ($C=O_{OBz}$), 164.5 (C=ONH), 143.3 (C-2), 138.6 (C-8a), 137.6 (C-1''), 134.1 (CH'''), 134.0 (CH'''), 133.6 (CH'''), 133.2 (C-7), 132.9 (C-4''), 130.0 (CH'''), 129.9 (CH'''), 129.8 (CH'''), 129.3 (C-1'''), 129.1 (C-3'' *and* C-5''), 128.8 (CH'''), 128.7 (CH'''), 128.6 (C-2'' *and* C-6''), 128.5 (C-1'''), 128.2 (C-1'''), 127.9 (C-4a), 127.8 (C-5), 125.7 (C-6), 115.5 (C-8), 112.7 (C-3), 92.0 (C-1'), 80.9 (C-4'), 74.0 (C-2'), 70.7 (C-3'), 63.3 (C-5'), 42.7 (CH$_2$NH). **IV (KBr) ν (cm^{-1}):** 3424, 1742, 1728, 1671, 1540. HRMS-ESI Calcd. for $C_{43}H_{33}ClN_2NaO_9^+$: 779,17723. Found for $C_{43}H_{33}ClN_2NaO_9^+$: 779.174003.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**9b**). White solid, 73 % yield. m.p.: 171-173 °C. **^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm):** 10.08 (1H, t, *J* 5.9 Hz, C=ONH), 9.23 (1H, s, H-2), 8.42 (1H, d, *J* 2.5 Hz, H-5), 8.07 (2H, d, *J* 8.4 Hz, H-2''' *and* H-6'''), 7.89 (4H, d, *J* 6.9 Hz, H-2''' *and* H-6'''), 7.70 (1H, d, *J* 9.2 Hz, H-8), 7.59-7.50 (3H, m, H-4'''), 7.41-7.31 (7H, m, H-3''', H-5''' *and* H-7), 7.29 (4H, sl, H-2'', H-3'', H-5''*and* H-6''), 6.42 (1H, d, *J* 4.9 Hz, H-1'), 5.99 (1H, t, *J* 5.7 Hz, H-2'), 5.85 (1H, t, *J* 5.7 Hz, H-3'), 4.87 (1H, sl, H-4', H-5'a *and* H-5'b), 4.57 (2H, d, *J* 6.0 Hz, CH_2NH). **^{13}C/APT NMR (75.0 MHz, $CDCl_3$) δ (ppm):** 175.9 (C=O), 166.2 ($C=O_{OBz}$), 165.2 ($C=O_{OBz}$),

164.8 ($C=O_{OBz}$), 164.1 (C=ONH), 143.6 (C-2), 137.5 (C-1''), 137.0 (C-8a), 134.3 (CH'''), 134.0 (CH'''), 133.6 (CH'''), 133.4 (C-7), 133.0 (C-4''), 132.1 (C-6 *or* C-4a), 130.0 (CH'''), 129.9 (CH'''), 129.2 (C-1'''), 129.1 (C-3'' *and* C-5''), 129.0 (C-6 *or* C-4a), 128.8 (CH'''), 128.7 (CH'''), 128.6 (C-2'' *and* C-6''), 128.4 (C-1'''), 128.1 (C-1'''), 127.2 (C-5), 117.4 (C-8), 113.1 (C-3), 92.2 (C-1'), 81.1 (C-4'), 73.8 (C-2'), 70.7 (C-3'), 63.2 (C-5'), 42.7 ($\underline{C}H_2NH$). **IV (KBr) ν (cm^{-1}):** 3424, 1729, 1714, 1668, 1548. HRMS-ESI Calcd. for $C_{43}H_{32}Cl_2N_2NaO_9^+$: 813,13771. Found for $C_{43}H_{32}Cl_2N_2NaO_9^+$: 813.134898.

6-fluoro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**9c**). White solid, 60 % yield, m.p.: 139-140 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 10.08 (1H, t, *J* 5.5 Hz, C=ON$\underline{H}$), 9.24 (1H, s, H-2), 8.39 (1H, dd, *J* 9.0 *and* 7.0 Hz, H-5), 8.02 (2H, d, *J* 6.5 Hz, H-2''' *and* H-6'''), 7.97 (2H, d, *J* 7.0 Hz, H-2''' *and* H-6'''), 7.91 (2H, d, *J* 7.1 Hz, H-2''' *and* H-6'''), 7.67-7.59 (3H, m, H-4''' *and* H-8), 7.49-7.41 (6H, m, H-3''', H-5''' *and* H-7), 7.38 (2H, d, *J* 8.3 Hz, H-2'' *and* H-6''), 7.33 (2H, d, *J* 8.3 Hz, H-3'' *and* H-5''), 7.07 (1H, d, *J* 5.0 Hz, H-1'), 5.99 (1H, t, *J* 5.5 Hz, H-2'), 5.91 (1H, t, *J* 5.5 Hz, H-3'), 5.01 (1H, dd, *J* 8.0 *and* 4.4 Hz, H-4'), 4.86 (1H, dd, *J* 12.7 *and* 4.4 Hz, H-5'a *or* H-5'b), 4.78 (1H, dd, *J* 12.7 *and* 4.4 Hz, H-5'a *or* H-5'b), 4.49 (2H, dd, *J* 8.0 *and* 2.0 Hz, C$\underline{H}_2$NH). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 175.3 (C=O), 165.6 (d, $^1J_{C\text{-}F}$ = 240.1 Hz, C-6), 165.5 ($C=O_{OBz}$), 164.6 ($C=O_{OBz}$), 164.3 ($C=O_{OBz}$), 163.4 (C=ONH), 143.1 (C-2), 140.2 (d, $^3J_{C\text{-}F}$ = 12.2 Hz, C-4a), 138.5 (C-8a), 134.0 (CH'''), 133.9 (CH'''), 133.4 (CH'''), 131.4 (C-4'' *or* C-1''), 129.6 (d, $^3J_{C\text{-}F}$ = 11.0 Hz, C-8), 129.5 (CH'''), 129.4 (CH'''), 129.3 (CH'''), 129.2 (C-3'' e C-5''), 129.0 (C-1'''), 129.0 (CH'''), 128.7 (CH'''), 128.6 (CH'''), 128.5 (C-1'''), 128.3 (C-2'' *and* C-6''), 128.1 (C-1'''), 123.8 (C-4'' *or* C-1''), 114.0 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, C-7), 112.1 (C-3), 103.3 (d, $^2J_{C\text{-}F}$ = 28.1 Hz, C-5), 89.6 (C-1'), 80.4 (C-4'), 74.4 (C-2'), 70.3 (C-3'), 63.5 (C-5'), 41.6 ($\underline{C}H_2NH$). **IV (KBr) ν (cm^{-1}):** 3424, 1741, 1732, 1721, 1675, 1548. HRMS-ESI Calcd. for $C_{43}H_{32}ClFN_2NaO_9^+$: 797,16726. Found for $C_{43}H_{32}ClFN_2NaO_9^+$: 797.163311.

6-methyl-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**9d**). White solid, 86 % yield, m.p.: 211-212 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 10.31 (1H, t, *J* 5.9 Hz, C=ON$\underline{H}$), 9.25 (1H, s, H-2), 8.29 (1H, sl, H-5), 8.10 (2H, d, *J* 7.3 Hz, H-2''' *and* H-6'''), 7.93 (2H, d, *J*

7.3 Hz, H-2''' *and* H-6'''), 7.89 (2H, d, *J* 7.3 Hz, H-2''' *and* H-6'''), 7.64-7,53 (3H, m, H-4''' *and* H-8), 7.42-7.29 (11H, m, H-3''', H-5''', H-2'', H-3'', H-5'', H-6'' *and* H-7), 6.47 (1H, d, *J* 4.9 Hz, H-1'), 6.02 (1H, t, *J* 4.9 Hz, H-2'), 5.87 (1H, t, *J* 5.9 Hz, H-3'), 4.91 (3H, sl, H-4', H-5'a *and* H-5'b), 4.61 (2H, d, *J* 5.9 Hz, C$\underline{H}_2$NH), 2.44 (C$\underline{H}_3$). **^{13}C/APT NMR (125,00 MHz, $CDCl_3$) δ (ppm):** 177.1 (C=O), 166.2 (C=O_{OBz}), 165.1 (C=O_{OBz}), 164.8 (C=O_{OBz}), 164.7 (C=ONH), 143.9 (C-2), 137.7 (C-8a *or* C-1''), 136.6 (C-8a *or* C-1''), 135.1 (C-4''), 134.6 (CH'''), 134.2 (CH'''), 133.9 (CH'''), 133.5 (C-7), 132.9 (C-6 *or* C-4a), 130.1 (CH'''), 130.0 (CH'''), 129.9 (CH'''), 129.3 (C-6 *or* C-4a), 129.0 (C-3'' *and* C-5''), 128.8 (C-1'''), 128.7 (C-1'''), 128.6 (C-2'' *and* C-6''), 128.5 (CH'''), 128.2 (CH'''), 127.8(CH'''), 127.3 (C-5), 115.4 (C-8), 112.5 (C-3), 92.1 (C-1'), 80.8 (C-4'), 74.0 (C-2'), 70.6 (C-3'), 63.3 (C-5'), 42.7 ($\underline{C}H_2$NH), 21.1 ($\underline{C}H_3$). **IV (KBr) ν (cm^{-1}):** 3424, 1740, 1725, 1655, 1549. HRMS-ESI Calcd. for $C_{44}H_{35}ClN_2NaO_9^+$: 793,19288. Found for $C_{44}H_{35}ClN_2NaO_9^+$: 793.189613.

6-trifluoromethyl-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**9e**). White solid, 74 % yield, m.p.: 208-210 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 10.05 (1H, t, *J* 5.5 Hz, C=ON$\underline{H}$), 9.28 (1H, s, H-2), 8.78 (1H, sl, H-5), 8.10 (2H, d, *J* 7.0 Hz, H-2''' *and* H-6'''), 7.93 (2H, d, *J* 7.3 Hz, H-2''' *and* H-6'''), 7.90 (2H, d, *J* 7.3 Hz, H-2''' *and* H-6'''), 7.64-7.55 (4H, m, H-4''' *and* H-7), 7.43-7.37 (6H, m, H-3''', H-5'''), 7.29 (4H, sl, H-2'', H-3'', H-5'', H-6''), 6.47 (1H, d, *J* 4.9 Hz, H-1'), 6.03 (1H, t, *J* 6.1 Hz, H-2'), 5.90 (1H, t, *J* 5.4 Hz, H-3'), 4.99-4.89 (1H, m, H-4', H-5'a *and* H-5'b), 4.60 (2H, dd, *J* 6.1 *and* 2.4 Hz, C$\underline{H}_2$NH). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 176.4 (C=O), 166.2 (C=O_{OBz}), 165.2 (C=O_{OBz}), 164.9 (C=O_{OBz}), 163.8 (C=ONH), 144.4 (C-2), 140.5 (C-1''), 137.3 (C-8a), 134.3 (CH'''), 134.1 (CH'''), 133.7 (CH'''), 133.1 (C-4''), 130.0 (CH'''), 129.9 (CH'''), 129.4 (q, $^3J_{C\text{-}F}$ = 2.9 Hz, C-7), 129.2 (C-4a), 129.1 (C-3'' *and* C-5''), 128.9 (C-2'' *and* C-6''), 128.8 (CH'''), 128.7 (CH'''), 128.6 (CH'''), 128.4 (C-1'''), 128.0 (C-1'''), 127.8 (q, $^2J_{C\text{-}F}$ = 34.0 Hz, C-6), 127.7 (C-1'''), 125.7 (q, $^3J_{C\text{-}F}$ = 3.8 Hz, C-5), 123.5 (q, $^1J_{C\text{-}F}$ = 273.0 Hz, CF_3), 116.8 (C-8), 113.8 (C-3), 92.5 (C-1'), 81.2 (C-4'), 73.7 (C-2'), 70.7 (C-3'), 63.1 (C-5'), 42.8 ($\underline{C}H_2$NH). **IV (KBr) ν (cm^{-1}):** 3424, 1744, 1727, 1672, 1548. HRMS-ESI Calcd. for $C_{44}H_{32}ClF_3N_2NaO_9^+$: 847,16406. Found for $C_{44}H_{32}ClF_3N_2NaO_9^+$: 847.165322.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-methylphenyl)-quinoline-3-carboxamides (**9f**). White solid, 62 % yield, m.p.: 94-96 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 11.77 (1H, s, C=ONH), 9.32 (1H, s, H-2), 8.50 (1H, d, *J* 2.4 Hz, H-5), 8.11 (2H, d, *J* 7.1 Hz, H-2''' *and* H-6'''), 7.92 (4H, d, *J* 7.2 Hz, H-2''' *and* H-6'''), 7.74 (1H, d, *J* 9.2 Hz, H-8), 7.62-7.52 (5H, m, H-2'', H-6'' *and* H-4'''), 7.44-7.34 (7H, m, H-3''', H-5''' *and* H-7), 7.15 (2H, d, *J* 8.2 Hz, H-3'' *and* H-5''), 6.47 (1H, d, *J* 4.8 Hz, H-1'), 6.04 (1H, t, *J* 5.4 Hz, H-2'), 5.91 (1H, t, *J* 5.4 Hz, H-3'), 4.98-4.88 (3H, m, H-4', H-5'a *and* H-5'b), 2.34 (3H, s, CH_3). **^{13}C/APT NMR (75.0 MHz, $CDCl_3$) δ (ppm):** 176.0 (C=O), 166.3 ($C=O_{OBz}$), 165.2 ($C=O_{OBz}$), 164.8 ($C=O_{OBz}$), 161.7 (C=ONH), 143.7 (C-2), 137.0 (C-8a), 136.1 (C-1''), 134.3 (CH'''), 134.0 (CH'''), 133.7 (CH'''), 133.6 (C-4''), 133.5 (C-7), 132.2 (C-6 *or* C-4a), 130.0 (CH'''), 129.9 (CH'''), 129.5 (C-3'' *and* C-5''), 129.2 (C-1'''), 128.9 (C-1'''), 128.8 (CH'''), 128.7 (CH'''), 128.6 (CH'''), 128.5 (C-6 *or* C-4a), 128.1 (C-1'''), 127.3 (C-5), 120.5 (C-2'' *and* C-6''), 117.5 (C-8), 113.5 (C-3), 92.3 (C-1'), 81.2 (C-4'), 74.0 (C-2'), 70.6 (C-3'), 63.1 (C-5'), 21.1 (CH_3). **IV (KBr) ν (cm^{-1}):** 3424, 1728, 1550. HRMS-ESI Calcd. for $C_{43}H_{33}ClN_2NaO_9^+$: 779,17668. Found for $C_{43}H_{33}ClN_2NaO_9^+$: 779.175082.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-chlorophenyl)-quinoline-3-carboxamides (**9g**). White solid, 46 % yield, m.p.: 184-186 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 11.88 (1H, s, C=ONH), 9.30 (1H, s, H-2), 8.48 (1H, sl, H-5), 8.09 (2H, d, *J* 7.5 Hz, H-2''' *and* H-6'''), 7.93-7.91 (4H, m, H-2''' *and* H-6'''), 7.72 (1H, d, *J* 9.0 Hz, H-8), 7.64 (2H, d, *J* 8.3 Hz, H-2'' *and* H-6''), 7.61-7.51 (3H, m, H-4''' *and* H-7), 7.46-7.35 (7H, m, H-3''', H-5''' *and* H-4'''), 7.28 (2H, d, *J* 8.4 Hz, H-3'' *and* H-5''), 6.48 (1H, d, *J* 3.7 Hz, H-1'), 6.05-6.04 (1H, m, H-2'), 5.91 (1H, t, *J* 5.0 Hz, H-3'), 5.99-4.88 (3H, m, H-4', H-5'a *and* H-5'b). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 175.9 (C=O), 166.2 ($C=O_{OBz}$), 165.2 ($C=O_{OBz}$), 164.8 ($C=O_{OBz}$), 161.0 (C=ONH), 143.5 (C-2), 137.2 (C-4''), 137.0 (C-8a), 134.3 (CH'''), 134.0 (CH'''), 133.6 (CH'''), 133.5 (C-7), 132.3 (C-1''), 130.0 (CH'''), 129.3 (C-6 *or* C-4a), 128.9 (CH'''), 128.8 (C-3'' *and* C-5''), 128.7 (C-1'''), 128.6 (CH'''), 128.4 (C-1'''), 128.0 (C-6 *or* C-4a), 127.2 (C-5), 121.6 (C-2'' *and* C-6''), 117.4 (C-8), 113.0 (C-3), 92.2 (C-1'), 81.3 (C-4'), 74.1 (C-2'), 70.6 (C-3'), 63.1 (C-5'). **IV (KBr) ν (cm^{-1}):** 3420, 1728, 1546. HRMS-ESI Calcd. for $C_{42}H_{30}Cl_2N_2NaO_9^+$: 799,12261. Found for $C_{42}H_{30}Cl_2N_2NaO_9^+$: 799.119327.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-fluorophenyl)-quinoline-3-carboxamides (**9h**). White solid, 35 % yield, m.p.: 174-176 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 11.83 (1H, s, C=ONH), 9.32 (1H, s, H-2), 8.51 (1H, d, *J* 2.5 Hz, H-5), 8.10 (2H, d, *J* 8.2 Hz, H-2''' *and* H-6'''), 7.93-7.91 (4H, m, H-2''' *and* H-6'''), 7.74 (1H, d, *J* 9.2 Hz, H-8), 7.66 (2H, dd, *J* 9.0 *and* 5.0 Hz, H-2'' *and* H-6''), 7.61-7.52 (3H, m, H-4'''), 7.46 (1H, dd, *J* 8.5 *and* 2.5 Hz, H-7), 7.43-7.36 (6H, m, H-3''', H-5'''), 7.03 (2H, t, *J* 9.0 Hz, H-3'' *and* H-5''), 6.47 (1H, d, *J* 4.9 Hz, H-1'), 6.04 (1H, t, *J* 5.9 Hz, H-2'), 5.91 (1H, t, *J* 5.4 Hz, H-3'), 4.98-4.90 (3H, m, H-4', H-5'a *and* H-5'b). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 175.9 (C=O), 166.2 ($C{=}O_{OBz}$), 165.2 ($C{=}O_{OBz}$), 164.8 ($C{=}O_{OBz}$), 161.8 (C=ONH), 159.27 (d, $^{1}J_{C\text{-}F}$ = 243.3 Hz, C-4''), 143.5 (C-2), 137.0 (C-8a), 134.7 (d, $^{4}J_{C\text{-}F}$ = 2.6 Hz, C-1''), 134.3 (CH'''), 134.0 (CH'''), 133.7 (CH'''), 133.5 (C-7), 132.3 (C-6 *or* C-4a), 130.0 (CH'''), 129.9 (CH'''), 129.3 (C-1'''), 128.9 (C-6 *or* C-4a), 128.8 (CH'''), 128.7 (CH'''), 128.4 (C-1'''), 128.1 (C-1'''), 127.3 (C-5), 122.0 (d, $^{3}J_{C\text{-}F}$ = 7.8 Hz, C-2'' *and* C-6''), 117.4 (C-8), 115.5 (d, $^{2}J_{C\text{-}F}$ = 22.4 Hz, C-3'' *and* C-5''),113.2 (C-3), 92.2 (C-1'), 81.2 (C-4'), 74.1 (C-2'), 70.6 (C-3'), 63.1 (C-5'). **IV (KBr) ν (cm^{-1}):** 3420, 1730, 1678, 1558. HRMS-ESI Calcd. for $C_{42}H_{30}ClFN_2NaO_9$: 783,15216. Found for $C_{42}H_{30}ClFN_2NaO_9$: 783.149872

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-(4-methoxyphenyl)-quinoline-3-carboxamides (**9i**). Light purple solid, 72 % yield, m.p.: 156-159 °C. **^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm):** 11.70 (1H, s, C=ONH), 9.31 (1H, s, H-2), 8.47 (1H, d, *J* 2.7 Hz, H-5), 8.10 (2H, dd, *J* 8.4 *and* 1.2 Hz, H-2''' *and* H-6'''), 7.93-7.89 (4H, m, H-2''' *and* H-6'''), 7.72 (1H, d, *J* 9.0 Hz, H-8). 7.62 (2H, d, *J* 9.0 Hz, H-2'' *and* H-6''), 7.57-7.51 (3H, m, H-4'''), 7.43-7.35 (7H, m, H-3''', H-5''' *and* H-7), 6.87 (2H, d, *J* 9.0 Hz, H-3'' *and* H-5''), 6.48 (1H, d, *J* 4.7 Hz, H-1'), 6.04 (1H, dd, *J* 5.7 *and* 4.8 Hz, H-2'), 5.91 (1H, t, *J* 5.5 Hz, H-3'), 4.93-4.86 (3H, m, H-4', H-5'a *and* H-5'b), 3.80 (3H, s, $OC\underline{H}_3$). **^{13}C/APT NMR (75.0 MHz, $CDCl_3$) δ (ppm):** 175.9 (C=O), 166.2 ($C{=}O_{OBz}$), 165.1 ($C{=}O_{OBz}$), 164.8 ($C{=}O_{OBz}$), 161.5 (C=ONH), 156.2 (C-4''), 143.6 (C-2), 137.0 (C-8a), 134.2 (CH'''), 134.0 (CH'''), 133.6 (CH'''), 133.4 (C-7), 132.1 (C-1''), 131.9 (C-6 *or* C-4a), 130.0 (CH'''), 129.2 (C-1'''), 128.8 (CH'''), 128.7 (CH'''), 128.4 (C-6 *or* C-4a), 128.1 (C-1'''), 127.2 (C-5), 121.9 (C-3'' *and* C-5''), 117.5 (C-8), 114.1 (C-2'' *and* C-6''), 113.3 (C-3), 92.3 (C-1'), 81.1 (C-4'), 74.9 (C-2'), 70.6 (C-3'), 63.1 (C-5'), 55.6 ($O\underline{C}H_3$). **IV (KBr) ν (cm^{-1}):** 3420, 1725,

1714, 1668, 1558. HRMS-ESI Calcd. for $C_{43}H_{33}ClN_2NaO_{10}^+$: 795,17214. Found for $C_{43}H_{33}ClN_2NaO_{10}^+$: 795.169872.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-phenyl-quinoline-3-carboxamides (**9j**). White solid, 38 % yield, m.p.: 169-171 °C. **^{1}H NMR (500.00 MHz, $CDCl_3$) δ (ppm):** 11.85 (1H, s, C=ONH), 9.33 (1H, s, H-2), 8.52 (1H, d, *J* 2.5 Hz, H-5), 8.11 (2H, d, *J* 7.4 Hz, H-2''' *and* H-6'''), 7.93 (4H, d, *J* 7.4 Hz, H-2''' *and* H-6'''), 7.74 (1H, d, *J* 8.8 Hz, H-8), 7.71 (2H, d, *J* 7.8 Hz, H-2'' *and* H-6''), 7.60-7.53 (3H, m, H-4'''), 7.46-7.33 (9H, m, H-3''', H-5''', H-3'', H-5'' *and* H-7), 7.11 (1H, t, *J* 7.4 Hz, H-4''), 6.47 (1H, d, *J* 4.4 Hz, H-1'), 6.04 (1H, t, *J* 5.9 Hz, H-2'), 5.91 (1H, t, *J* 5.9 Hz, H-3'), 4.98-4.88 (3H, m, H-4', H-5'a *and* H-5'b). **^{13}C/APT NMR (125.00 MHz, $CDCl_3$) δ (ppm):** 176.6 (C=O), 166.2 ($C=O_{OBz}$), 165.2 ($C=O_{OBz}$), 164.8 ($C=O_{OBz}$), 161.9 (C=ONH), 143.7 (C-2), 138.7 (C-8a *or* C-1''), 138.7 (C-8a *or* C-1''), 134.3 (CH'''), 134.0 (CH'''), 133.7 (CH'''), 133.5 (C-7), 132.3 (C-6 *or* C-4a), 130.1 (CH'''), 130.0 (CH'''), 129.9 (CH'''), 129.3 (C-1'''), 129.0 (CH'''), 128.9 (C-6 *or* C-4a), 128.8 (C-3'' *and* C-5''), 128.7 (CH'''), 128.6 (CH'''), 128.5 (C-1'''), 128.1 (C-1'''), 127.7 (C-4''), 124.0 (C-5), 120.6 (C-2'' *and* C-6''), 117.5 (C-8), 113.4 (C-3), 92.3 (C-1'), 81.2 (C-4'), 74.0 (C-2'), 70.6 (C-3'), 63.1 (C-5'). **IV (KBr) ν (cm^{-1}):** 3420, 1731, 1719, 1679, 1557. HRMS-ESI Calcd. for $C_{42}H_{31}ClN_2NaO_9^+$: 765,16158. Found for $C_{42}H_{31}ClN_2NaO_9^+$: 765.158561.

6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N'-cyclohexyl-quinoline-3-carboxamides (**9k**). White solid, 72 % yield, m.p.: 199-200 °C. **^{1}H NMR (300.00 MHz, $CDCl_3$) δ (ppm):** 9.68 (1H, d, *J* 8.1 Hz, C=ONH), 9.23 (1H, s, H-2), 8.46 (1H, d, *J* 2.4 Hz, H-5), 8.09 (2H, d, *J* 7.2 Hz, H-2''' *and* H-6'''), 7.92 (4H, d, *J* 8.1 Hz, H-2''' *and* H-6'''), 7.72 (1H, d, *J* 9.3 Hz, H-8), 7.60-7,53 (3H, m, H-4'''), 7.46-7.33 (7H, m, H-3''', H-5''' *and* H-7), 6.42 (1H, d, *J* 4.8 Hz, H-1'), 6.02 (1H, t, *J* 5.4 Hz, H-2'), 5.90-5.86 (1H, m, H-3'), 4.92 (3H, sl, H-4, H-5'a *and* 5'b), 3.97 (1H, sl, H-1''), 1.96 (2H, sl, H-2''$_{equatorial}$ *and* H-6''$_{equatorial}$), 1.74 (2H, sl, H-3''$_{equatorial}$ *and* H-5''$_{equatorial}$), 1.61-1.58 (1H, m, H-4''$_{equatorial}$), 1.48-1.25 (5H, m, H-2''$_{axial}$, H-3''$_{axial}$, H-4''$_{axial}$, H-5''$_{axial}$, H-6''$_{axial}$). **^{13}C/APT NMR (75.0 MHz, $CDCl_3$) δ (ppm):** 176.0 (C=O), 166.2 ($C=O_{OBz}$), 165.2 ($C=O_{OBz}$), 164.8 ($C=O_{OBz}$), 162.9 (C=ONH), 143.5 (C-2), 134.2 (C-8a), 134.2 (CH'''), 134.0 (CH'''), 133.6 (CH'''), 133.2 (C-7), 132.0 (C-6 *or* C-4a), 130.0 (CH'''), 129.9 (CH'''), 129.3 (C-1'''), 129.1 (C-6 *or* C-4a), 128.8

(CH’’’), 128.7 (CH’’’), 128.6 (CH’’’), 128.5 (C-1’’’), 128.1 (C-1’’’), 127.2 (C-5), 117.4 (C-8), 113.5 (C-3), 92.3 (C-1’), 81.0 (C-4’), 73.7 (C-2’), 70.7 (C-3’), 63.3 (C-5’), 48.0 (C-1’’), 33.0 (C-2’’ *and* C-6’’), 25.9 (C-4’’), 24.8 (C-3’’ *and* C-5’’). **IV (KBr) ν (cm^{-1}):** 3420, 1727, 1661, 1543. HRMS-ESI Calcd. for $C_{42}H_{37}ClN_2NaO_9^+$: 771,20853. Found for $C_{42}H_{37}ClN_2NaO_9^+$: 771.205347.

2.1.2. General Procedure for the Synthesis of 1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-quinoline-3-carboxamides 4a-j.

A mixture of protected nucleosides **9** (0.13 mmol) in 0.5 M ethanolic sodium carbonate solution (10 mL) was stirred at room temperature for 12-24 h. The resulting solution was neutralized with Dowex 50H$^+$. After filtration and evaporation, the crude ribonucleosides were recrystallized from THF/hexane.

*1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N’-(4-chlorobenzyl)-quinoline-3-carboxamides (**4a**).* White solid, 72 % yield, m.p.: 225-227 °C. **^{1}H NMR (500.00 MHz, DMSO-d_6) δ (ppm):** 10.31 (1H, t, *J* 6.0 Hz, C=ONH), 9.28 (1H, s, H-2), 8.37 (1H, dd, *J* 8.1 e 1.4, H-5), 7.97 (1H, d, *J* 8.7 Hz, H-8), 7.86 (1H, ddd, *J* 8.6, 7.1 *and* 1.6 Hz, H-7), 7.57 (1H, t, *J* 7.6 Hz, H-6), 7.38 (4H, d, *J* 5.0 Hz, H-2’’,H-3’’, H-5’’ *and* H-6’’), 6.16 (1H, d, *J* 4.0 Hz, H-1’), 5.7 (1H, sl, C_2-OH), 5.3 (1H, sl, C_3-OH), 5.1 (1H, sl, C_5-OH), 4.55 (2H, d, *J* 6.0 Hz, CH_2NH), 4.22 (1H, t, *J* 4.5 Hz, H-2’), 4.12 (1H, dd, *J* 8.6 *and* 4.1 Hz, H-4’), 4.01 (1H, t, *J* 5.2 Hz, H-3’), 3.78 (1H, m, H-5’a *or* 5’b), 3.70 (1H, m, H-5’a *or* 5’b). **^{13}C/APT NMR (125.00 MHz, DMSO-d_6) δ (ppm):** 175.8 (C=O), 164.1 (C=ONH), 142.7 (C-2), 138.8 (C-1’’ *or* C-8a), 138.6 (C-1’’ *or* C-8a), 133.0 (C-7), 131.4 (C-4’’), 129.2 (C-3’’ *and* C-5’’), 128.3 (C-2’’ *and* C-6’’), 126.8 (C-4a), 126.1 (C-5), 125.2 (C-6), 116.9 (C-8), 110.8 (C-3), 92.3 (C-1’), 85.4 (C-4’), 74.7 (C-2’), 69.7 (C-3’), 60.8 (C-5’), 41.5 (CH_2NH). **IV (KBr) ν (cm^{-1}):** 3454, 3309, 3100-3200, 1654, 1573. HRMS-ESI Calcd. for $C_{22}H_{21}ClN_2NaO_6^+$: 467,09858. Found for $C_{22}H_{21}ClN_2NaO_6^+$: 467.097519.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N’-(4-chlorobenzyl)-quinoline-3-carboxamides (**4b**).* White solid, 74 % yield, m.p.: 128-130 °C. **^{1}H NMR (500.00 MHz, DMSO-d_6) δ (ppm):** 10.13 (1H, t. *J*= 6.0 Hz, C=ONH), 9.27 (1H, s, H-2), 8.28 (1H, d, *J* 2.6 Hz, H-5), 8.03 (1H, d, *J* 9.3 Hz, H-8), 7.88 (1H, dd, *J* 9.2 *and* 2.6 Hz, H-7), 7.38 (4H, m, H-2’’,H-3’’, H-5’’ *and* H-6’’), 6.14 (1H, d, *J* 4.0 Hz, H-1’), 5.65 (1H, d, *J* 5.0

Hz, C_2-OH), 5.21 (1H, d, *J* 5.1 Hz, C_3-OH), 5.02 (1H, sl, C_5-OH), 4.56 (2H, d, *J* 6.0 Hz, C$\underline{H_2}$NH), 4.23 (1H, dd, *J* 10.1 *and* 5.2 Hz, H-2'), 4.13 (1H, dd, *J* 8.3 *and* 4.0 Hz, H-4'), 4.04 (1H, q, *J* 5.3 Hz, H-3'), 3.78 (1H, m, H-5'a *or* 5'b), 3.70 (1H, m, H-5'a *or* 5'b). **^{13}C/APT NMR (125.00 MHz, DMSO-*d*$_6$) δ (ppm):** 174.5 (C=O), 163.9(C=ONH), 143.0 (C-2), 138.4 (C-1''), 137.4 (C-8a), 132.6 (C-7), 131.3 (C-4''), 130.0 (C-6 *or* C-4a), 129.0 (C-3'' *and* C-5''), 128.1 (C-2'' *and* C-6''), 128.0 (C-6 *or* C-4a), 124.8 (C-5), 119.5 (C-8), 111.2 (C-3), 92.4 (C-1'), 85.7 (C-4'), 74.6 (C-2'), 69.7 (C-3'), 60.7 (C-5'), 41.5 (C$\underline{H_2}$NH). **IV (KBr) ν (cm^{-1}):** 3305, 1651, 1543. HRMS-ESI Calcd. for $C_{22}H_{20}Cl_2N_2NaO_6^+$: 501,05961. Found for $C_{22}H_{20}Cl_2N_2NaO_6^+$: 501.059744.

*6-Fluoro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**4c**).* White solid, 81 % *yield*, m.p.: 222-224 °C. **^{1}H NMR (500.00 MHz, DMSO-*d*$_6$) δ (ppm):** 10.21 (1H, t, *J*= 6.0 Hz, C=ONH), 9.27 (1H, s, H-2), 8.40 (1H, dd, *J* 9.0 *and* 6.6 Hz, H-8), 7.80 (1H, dd, *J* 11.5 *and* 2.1, H-5), 7.43 (1H, td, *J* 8.9 *and* 2.2 Hz, H-7), 7.40-7.35 (4H, m, H-2'',H-3'', H-5'' *and* H-6''), 6.09 (1H, d, *J* 4.7 Hz, H-1'), 5.76 (1H, sl, C_2-OH), 5.28 (1H, sl, C_3-OH), 5.10 (1H, sl, C_5-OH), 4.54 (2H, d, *J* 6.0 Hz, C$\underline{H_2}$NH), 4.21 (1H, d, *J* 4.3 Hz, H-2'), 4.12 (1H, dd, *J* 8.4 *and* 3.9 Hz, H-4'), 4.01 (1H, d, *J* 4.3 Hz, H-3'), 3.78 (1H, m, H-5'a *or* 5'b), 3.70 (1H, m, H-5'a *or* 5'b). **^{13}C/APT NMR (125.00 MHz, DMSO-*d*$_6$) δ (ppm):** 175.3 (C=O), 164.5 (C-6, d, $^1J_{C\text{-}F}$ = 247.9 Hz), 164.0 (C=ONH), 143.5 (C-2), 140.3 (d, $^3J_{C\text{-}F}$ = 12.3 Hz, C-4a), 138.6 (C-8a), 131.5 (C-4'' *or* C-1''), 129.5 (d, $^3J_{C\text{-}F}$ = 10.8 Hz, C-8), 129.2 (C-2' *or* C-3' *or* C-5' or C-6'), 128.4 (C-2' or C-3' *or* C-5' *or* C-6'), 123.8 (C-4'' or C-1''), 113.9 (d, $^2J_{C\text{-}F}$ = 27.7 Hz, C-7), 111.3 (C-3), 103.6 (d, $^2J_{C\text{-}F}$ = 27.7 Hz, C-5), 92.7 (C-1'), 85.8 (C-4'), 74.8 (C-2'), 69.8 (C-3'), 60.8 (C-5'), 41.6 (C$\underline{H_2}$NH). **IV (KBr) ν (cm^{-1}):** 3469, 1657, 1551. HRMS-ESI Calcd. for $C_{22}H_{20}ClFN_2NaO_6^+$: 485,08916. Found for $C_{22}H_{20}ClFN_2NaO_6^+$: 485.087863.

*6-methyl-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**4d**).* White solid, 69 % yield, m.p.: 193-195 °C. **^{1}H NMR (500.00 MHz, DMSO-*d*$_6$) δ (ppm):** 10.42 (1H, t, *J*= 6.0 Hz, C=ONH), 9.30 (1H, s, H-2), 8.21 (1H, sl, H-5), 7.93 (1H, d, *J* 8.9 Hz, H-8), 7.75 (1H, dd, *J* 8.8 *and* 2.0 Hz, H-7), 7.45 (4H, d, *J* 5.3 Hz, H-2'',H-3'', H-5'' *and* H-6''), 6.20 (1H, d, *J* 4.0 Hz, H-1'), 5.82 (1H, d, *J* 5.7 Hz, C_2-OH), 5.37 (1H, d, *J* 5.8 Hz, C_3-OH), 5.18 (1H, t, *J* 4.8 Hz, C_5-OH), 4.62 (2H, d, *J* 6.0 Hz, C$\underline{H_2}$NH), 4.26 (1H, dd, *J* 9.7 *and* 5.2 Hz, H-2'), 4.17 (1H, dd, *J* 8.6 *and* 4.1

Hz, H-4'), 4.06 (1H, dd, *J* 10.8 *and* 5.4 Hz, H-3'), 3.84 (1H, m, H-5'a *or* 5'b), 3.75 (1H, m, H-5'a *or* 5'b), 2.44 (3H, s, C<u>H</u>$_3$). **^{13}C/APT NMR (125.00 MHz, DMSO-*d*$_6$) δ (ppm):** 175.7 (C=O), 164.3(C=ONH), 142.2 (C-2), 138.7 (C-1''), 136.9 (C-8a), 134.9 (C-4''), 134.3 (C-7), 131.4 (C-6 *or* C-4a), 129.2 (C-3'' *and* C-5''), 128.4 (C-2'' *and* C-6''), 126.8 (C-6 or C-4a), 125.5 (C-5), 116.9 (C-8), 110.6 (C-3), 92.3 (C-1'), 85.4 (C-4'), 74.7 (C-2'), 69.8 (C-3'), 60.9 (C-5'), 41.5 (<u>C</u>H$_2$NH), 20.6 (<u>C</u>H$_3$). **IV (KBr) ν (cm^{-1}):** 3476, 1651, 1574. HRMS-ESI Calcd. for $C_{44}H_{32}ClF_3N_2NaO_9^+$: 847,16461. Found for $C_{44}H_{32}ClF_3N_2NaO_9^+$: 847.165322.

*6-trifluoromethyl-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-chlorobenzyl)-quinoline-3-carboxamides (**4e**).* Light yellow solid, 80 % yield, m.p.: 145-147 °C. **^{1}H NMR (500.00 MHz, DMSO-*d*$_6$) δ (ppm):** 10.10 (1H, t, *J*= 6.0 Hz, C=ON<u>H</u>), 9.36 (1H, s, H-2), 8.60 (1H, sl, H-5), 8.22-8.14 (2H, m, H-8 *and* H-7), 7.38 (4H, d, *J* 5.0 Hz, H-2'',H-3'', H-5'' *and* H-6''), 6.21 (1H, d, *J* 4.1 Hz, H-1'), 4.56 (2H, d, *J* 6.0 Hz, C<u>H</u>$_2$NH), 4.24 (1H, t, *J* 4.6 Hz, H-2'), 4.14 (1H, dd, *J* 8.4 *and* 3.8 Hz, H-4'), 4.04 (1H, t, *J* 5.0 Hz, H-3'), 3.79 (1H, dd, *J* 12.0 *and* 3.1 Hz, H-5'a *or* 5'b), 3.70 (1H, dd, *J* 12.0 *and* 4.0 Hz, H-5'a *or* 5'b). **^{13}C/APT NMR (125.00 MHz, DMSO-*d*$_6$) δ (ppm):** 175.2 (C=O), 163.5 (C=ONH), 143.8 (C-2), 141.0 (C-1''), 138.5 (C-8a), 131.4 (C-4''), 129.1 (C-3'' *and* C-5''), 128.8 (C-7), 128.3 (C-2'' *and* C-6''), 126.5 (C-4a), 125.4 (q, $^2J_{C\text{-}F}$ = 32.7 Hz, C-6), 123.4 (q, $^3J_{C\text{-}F}$ = 3.8 Hz, C-5), 118.9 (C-8), 112.0 (C-3), 92.5 (C-1'), 85.8 (C-4'), 74.9 (C-2'), 69.8 (C-3'), 60.7 (C-5'), 41.5 (<u>C</u>H$_2$NH). **IV (KBr) ν (cm^{-1}):** 3262, 1656, 1553. HRMS-ESI Calcd. for $C_{23}H_{20}ClF_3N_2NaO_6^+$: 535,08597. Found for $C_{23}H_{20}ClF_3N_2NaO_6^+$: 535.085623.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-chlorophenyl)-quinoline-3-carboxamides (**4f**).* White solid, 76 % yield, m.p.: 234-236 °C. **^{1}H NMR (300.00 MHz, DMSO-*d*$_6$) δ (ppm):** 12.18 (1H, s, C=ON<u>H</u>), 9.42 (1H, s, H-2), 8.33 (1H, d, *J* 2.5 Hz, H-5), 8.05 (1H, d, *J* 9.3 Hz, H-8), 7.94 (1H, dd, *J* 9.2 *and* 2.6 Hz, H-7), 7.75 (2H, d, *J* 9.0 Hz, H-2'' *and* H-6''), 7.40 (2H, d, *J* 4.5 Hz, H-3''*and* H-5''), 6.19 (1H, d, *J* 3.7 Hz, H-1'), 5.81 (1H, sl, C_2-OH), 5.32 (1H, sl, C_3-OH), 5.14 (1H, sl, C_5-OH), 4.23 (1H, t, *J* 4.2 Hz, H-2'), 4.18-4.13 (1H, m, H-4'), 4.04 (1H, t, *J* 5.2 Hz, H-3'), 3.83 (1H, d, *J* 11.9 Hz, H-5'a *or* 5'b), 3.72 (1H, d, *J* 12.0 Hz, H-5'a *or* 5'b). **^{13}C/APT NMR (75.0 MHz, DMSO-*d*$_6$) δ (ppm):** 174.8 (C=O), 162.1 (C=ONH), 143.4 (C-2), 137.5 (C-1'' *or* C-8a), 137.4 (C-1'' *or* C-8a), 133.1 (C-7), 130.5 (C-6 *or* C-4a *or* C-4''), 128.8 (C-3'' *and*

C-5''), 127.9 (C-6 *or* C-4a *or* C-4''), 127.1 (C-6 *or* C-4a *or* C-4''), 125.0 (C-5), 121.2 (C-2'' *and* C-6''),119.8 (C-8), 110.9 (C-3), 92.8 (C-1'), 85.6 (C-4'), 75.0 (C-2'), 69.6 (C-3'), 60.6 (C-5'). **IV (KBr) ν (cm^{-1}):** 3409, 1674, 1546. HRMS-ESI Calcd. for $C_{21}H_{18}Cl_2N_2NaO_6^+$: 487,04396. Found for $C_{21}H_{18}Cl_2N_2NaO_6^+$: 487.042773.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-fluorophenyl)-quinoline-3-carboxamides (**4g**).* White solid, 82 % yield, m.p.: 232-234 °C. **^{1}H NMR (500.00 MHz, DMSO-d_6) δ (ppm):** 12.11 (1H, s, C=ONH), 9.42 (1H, s, H-2), 8.35 (1H, d, *J* 2.6 Hz, H-5), 8.06 (1H, d, *J* 9.3 Hz, H-8), 7.95 (1H, dd, *J* 9.2 *and* 2,6 Hz, H-7), 7.75 (2H, dd, *J* 9.0 *and* 5.0 Hz, H-2'' *and* H-6''), 7.10 (2H, t, *J* 9.0 Hz, H-3''*and* H-5''), 6.20 (1H, d, *J* 3.8 Hz, H-1'), 5.80 (1H, d, *J* 5.6 Hz, C_2-OH), 5.30 (1H, d, *J* 5.8 Hz, C_3-OH), 5.14 (1H, t, *J* 4.6 Hz, C_5-OH), 4.24 (1H, dd, *J* 9.3 *and* 5.2 Hz, H-2'), 4.17-4.13 (1H, m, H-4'), 4.04 (1H, dd, *J* 10.8 *and* 5.5 Hz, H-3'), 3.82 (1H, m, H-5'a *or* 5'b), 3.73 (1H, m, H-5'a *or* 5'b). **^{13}C/APT NMR (125.00 MHz, DMSO-d_6) δ (ppm):** 174.8 (C=O), 161.9 (C=ONH), 158.15 (d, $^1J_{C\text{-}F}$ = 239.3 Hz, C-4''), 143.3 (C-2), 137.4 (C-8a), 134.9 (C-1''), 133.0 (C-7), 130.4 (C-6 *or* C-4a), 127.9 (C-6 *or* C-4a), 125.0 (C-5), 121.4 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C-2'' *and* C-6''), 119.8 (C-8), 115.5 (d, $^2J_{C\text{-}F}$ = 22.2 Hz, C-3'' *and* C-5''), 111.0 (C-3), 92.8 (C-1'), 85.6 (C-4'), 75.0 (C-2'), 69.6 (C-3'), 60.6 (C-5'). **IV (KBr) ν (cm^{-1}):** 3401, 1669, 1570. HRMS-ESI Calcd. for $C_{21}H_{18}ClFN_2NaO_6^+$: 471,07351. Found for $C_{21}H_{18}ClFN_2NaO_6^+$: 471.072074.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(4-methoxyphenyl)-quinoline-3-carboxamides (**4h**).* Light yellow solid, 83 % yield, m.p.: 193-195 °C. **^{1}H NMR (300.00 MHz, DMSO-d_6) δ (ppm):** 11.95 (1H, s, C=ONH), 9.39 (1H, s, H-2), 8.34 (1H, d, *J* 2.4 Hz, H-5), 8.06 (1H, d, *J* 9.3 Hz, H-8), 7.84 (1H, dd, *J* 9.3 *and* 2.4 Hz, H-7), 7.64 (2H, d, *J* 9.0 Hz, H-3'' *and* H-5''), 6.93 (2H, d, *J* 9.0 Hz, H-2''*and* H-6''), 6.19 (1H, d, *J* 3.9 Hz, H-1'), 5.95 (1H, sl, C_2-OH), 5.51 (1H, sl, C_3-OH), 5.20 (1H, sl, C_5-OH), 4.24 (1H, t, *J* 5.1 Hz, H-2'), 4.15 (1H, m, H-4'), 4.04 (1H, t, *J* 5.4 Hz, H-3'), 3.82 (1H, dd, *J* 11.9 *and* 3.0 Hz, H-5'a *or* 5'b), 3.75 (3H, s, OCH_3), 3.72 (1H, dd, *J* 10.4 *and* 2.4 Hz, H-5'a *or* 5'b). **^{13}C/APT NMR (75.0 MHz, DMSO-d_6) δ (ppm):** 175.7 (C=O), 161.4 (C=ONH), 155.4 (C-4''), 143.1 (C-2), 137.4 (C-8a), 132.9 (C-7), 131.7 (C-6 *or* C-4a *or* C-1''), 130.3 (C-6 *or* C-4a *or* C-1''), 127.9 (C-6 *or* C-4a *or* C-1''), 124.9 (C-5), 121.0 (C-3'' *and* C-5''), 119.7 (C-8), 114.1 (C-2'' *and* C-6''), 111.2 (C-3), 92.7 (C-1'), 85.7 (C-4'), 74.9 (C-2'), 69.7 (C-3'), 60.6 (C-5'), 55.1 (OCH_3). **IV (KBr) ν (cm^{-1}):** 3429,

1670, 1559. HRMS-ESI Calcd. for $C_{22}H_{21}ClN_2NaO_7^+$: 483,09350. Found for $C_{22}H_{21}ClN_2NaO_7^+$: 483.092458.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(phenyl)-quinoline-3-carboxamides (**4i**).* White solid, 79 % yield, m.p.: 188-190 °C. **^{1}H NMR (500.00 MHz, DMSO-d_6) δ (ppm):** 12.12 (1H, s, C=ONH), 9.42 (1H, s, H-2), 8.35 (1H, d, *J* 2.6 Hz, H-5), 8.05 (1H, d, *J* 9.3 Hz, H-8), 7.94 (1H, dd, *J* 9.2 *and* 2.6 Hz, H-7), 7.72 (2H, d, *J* 7.5 Hz, H-2'' *and* H-6''), 7.37 (2H, t, *J* 7.5 Hz, H-3''*and* H-5''), 7.10 (1H, t, *J* 7.4 Hz, H-4''), 6.19 (1H, d, *J* 3.8 Hz, H-1'), 5.78 (1H, d, *J* 5.5 Hz, C_2-OH), 5.29 (1H, d, *J* 5.7 Hz, C_3-OH), 5.14 (1H, t, *J* 4.6 Hz, C_5-OH), 4.24 (1H, dd, *J* 9.2 *and* 5.0 Hz, H-2'), 4.17-4.14 (1H, m, H-4'), 4.04 (1H, dd, *J* 11.0 *and* 5.4 Hz, H-3'), 3.84-3.80 (1H, m, H-5'a *or* 5'b), 3.75-3.71 (1H, m, H-5'a *or* 5'b). **^{13}C/APT NMR (125.00 MHz, DMSO-d_6) δ (ppm):** 174.9 (C=O), 161.9 (C=ONH), 143.3 (C-2), 138.5 (C-1''), 137.4 (C-8a), 133.0 (C-7), 130.5 (C-6 *or* C-4a), 128.9 (C-3'' *and* C-5''), 127.9 (C-6 *or* C-4a), 125.0 (C-5), 123.5 (C-4''), 119.7 (C-8), 119.6 (C-2'' *and* C-6''), 111.2 (C-3), 92.8 (C-1'), 85.7 (C-4'), 75.0 (C-2'), 69.7 (C-3'), 60.6 (C-5'). **IV (KBr) ν (cm^{-1}):** 3400, 1672, 1559. HRMS-ESI Calcd. for $C_{21}H_{19}ClN_2NaO_6^+$: 453,08293. Found for $C_{21}H_{19}ClN_2NaO_6^+$: 453.082354.

*6-chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)-N'-(cyclohexyl)-quinoline-3-carboxamides (**4j**).* Light yellow solid, 86 % yield, m.p.: 198-199 °C. **^{1}H NMR (500.00 MHz, DMSO-d_6) δ (ppm):** 9.79 (1H, d, *J* 7.8 Hz, C=ONH), 9.25 (1H, s, H-2), 8.27 (1H, d, *J* 2.7 Hz, H-5), 8.02 (1H, d, *J* 9.3 Hz, H-8), 7.89 (1H, dd, *J* 9.2 *and* 2.6 Hz, H-7), 6.13 (1H, d, *J* 4.2 Hz, H-1'), 5.94 (1H, sl, C_2-OH), 5.50 (1H, sl, C_3-OH), 5.21 (1H, sl, C_5-OH), 4.21 (1H, t, *J* 4.6 Hz, H-2'), 4.12 (1H, dd, *J* 8.4 *and* 4.0 Hz, H-4'), 4.01 (1H, t, *J* 5.1 Hz, H-3'), 3.84 (1H, m, H-1''), 3.77 (1H, d, *J* 11.8 Hz, H-5'a *or* 5'b), 3.69 (1H, d, *J* 10.5 Hz, H-5'a *or* 5'b), 1.86 (2H, m, H-2''$_{equatorial}$ *and* H-6''$_{equatorial}$), 1.69 (2H, m, H-3''$_{equatorial}$ *and* H-5''$_{equatorial}$), 1.56 (1H, m, H-4''$_{equatorial}$), 1.34 (5H, m, H-2''$_{axial}$, H-3''$_{axial}$, H-4''$_{axial}$, H-5''$_{axial}$, H-6''$_{axial}$). **^{13}C/APT NMR (125.00 MHz, DMSO-d_6) δ (ppm):** 174.6 (C=O), 162.4 (C=ONH), 142.9 (C-2), 137.4 (C-8a), 132.7 (C-7), 131.7 (C-6 *or* C-4a), 130.0 (C-6 *or* C-4a), 128.1 (C-6 *or* C-4a), 124.9 (C-5), 119.5 (C-8), 111.5 (C-3), 92.5 (C-1'), 85.6 (C-4'), 74.7 (C-2'), 69.8 (C-3'), 60.8 (C-5'), 46.8 (C-1''), 32.3 (C-2'' *and* C-6''), 25.1 (C-4''), 24.0 (C-3'' *and* C-5''). **IV (KBr) ν (cm^{-1}):** 3444,

1651, 1563. HRMS-ESI Calcd. for $C_{21}H_{25}ClN_2NaO_6^+$: 459,12988. Found for $C_{21}H_{25}ClN_2NaO_6^+$: 459.128318.

2.2. Biological Assays

2.2.1. Reverse Transcriptase (RT) inhibition assay.

The inhibitory effect of the compounds on the RNA-dependent DNA polymerase (RDDP) activity of the RT clone HXB2 was evaluated using purified recombinant HIV-1 enzyme as reported [10], with modifications. RDDP activity was assayed with a colorimetric assay from Roche Life Sciences. In brief, 3 U of enzyme (one unit is the enzyme concentration that incorporates 1 pmol of dTTP per minute per mg of enzyme at 37 °C under the standard assay conditions) was incubated with different compounds and polirA-oligodU. Incorporation of the incoming free dU labeled with biotin and digoxin (equimolar mixture) was blocked by the drugs. To reveal the incorporated dU, plates were coated with streptavidin and an antibody against digoxin tagged horseradish used. The reactions were initiated at 37 °C, incubated for 30 min, and arrested with 0.5 M EDTA. An automatic ELISA plate reader with a 570 nm test wavelength and 690 nm reference wavelength was used. The 50 % inhibitory concentration (IC_{50}) was calculated by linear regression analysis of the dose-response curves generated from the data.

2.2.2. Cytotoxicity assay

Human primary peripheral blood mononuclear cells (PBMCs), at density of 2×10^4 cell/well in 96-well culture plates, were incubated with the compounds at different concentrations for 72 h. Then, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT; 5 mg/mL) was added to DMEM in the presence of 0.01 % N-methyl-dibenzopyrazine methyl sulfate (PMS). After incubation for 4 h at 37°C, the absorbance of the plates were measured in a spectrophotometer at 492 nm and 620 nm. The 50 % cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose-response curves generated from the data.

2.3. *In silico* studies

2.3.1. Molecular Modeling and SAR analysis

Three-dimensional structures of all compounds (**9a-k** and **4a-j**) were built and optimized using Spartan 10 software. The structures were minimized, and a conformational analysis was performed using molecular mechanics force field (MMFF). Then, the lowest energy conformation obtained was subjected to geometric optimization in a vacuum using the semiempirical RM1 method and single point *ab initio* Hartree-Fock with the 6-31G* basis set. The stereoelectronic properties (HOMO energy, HOMO orbital coefficients distribution, LUMO density, dipole moment, dipole moment vector, lipophilicity (clogP), volume, area, and polarizability) were calculated for all compounds. The theoretical logP (clogP) was calculated at the AM1 semiempirical level using the Villar method included in Spartan.

2.3.2. Molecular docking

To investigate the binding mode of the **4a**, **9a**, **9d** and **9j** compounds, we performed molecular docking studies on the allosteric site of HIV-1 RT. The AutoDock 4.2 [11] program was used on a Windows-based PC.

The three-dimensional structures of these compounds were built and optimized using Spartan 10 software as described above. The coordinates from HIV-1 RT were obtained from the Protein Data Bank (PDB code 1KLM) [12]. The cocrystallized ligand and solvent molecules were removed with PyMOL 1.3 (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC, San Francisco, CA, USA). The ligands were flexibly docked to HIV-1 RT and the docking files were prepared using AutoDock Tools. The protein was treated as rigid, polar hydrogen atoms were added, nonpolar hydrogen atoms were merged and Gasteiger charges were assigned by default. The grid center was established on the allosteric site with 0.375 Å spacing and 52×32×40 points. Docking studies were carried out using the empirical free energy function and the Lamarckian Genetic Algorithm. A total of 50 independent docking runs were carried out using the program's default parameters. The results of the most favorable free energies of binding in the most populated clusters were selected as possible structures of the resultant complex.

2.3.3. Pharmacokinetics and toxicity analyses

ADMET parameters were evaluated using the ADMET Predictor™ version 9.5 (Simulations Plus, Inc., Lancaster, CA, USA) qualitative and quantitative models.

Compounds **4a**, **9a**, **9d**, **9j**, AZT and nevirapine were submitted for *in silico* analysis of their pharmacokinetic and toxicity parameters [13].

3. Results and Discussion

3.1. Chemistry

Initially, we promoted the synthesis of the key intermediates of 4-oxoquinolines **8** using a procedure involving the condensation of anilines **5** with diethyl ethoxymethylenemalonate (EMME) in refluxing ethanol followed by the thermal cyclization of the aniline acrylate intermediates **6** [14]. A nucleophilic substitution reaction between oxoquinolines **7** and the appropriate amines in diphenyl ether afforded the respective carboxamides **8**, as previously reported. Ribonucleosides were prepared according to the Vorbruggen methodology adapted by us for the synthesis of oxoquinoline ribonucleosides [15-17]. Previous silylation of 4-oxoquinoline-3-carbomides **8** with bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1 % trimethylchlorosilane and the subsequent condensation reaction with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **10** in acetonitrile under trimethylsilyl-trifluoromethanesulfonate catalysis led to the corresponding protected ribonucleosides **9**. The treatment of compounds **9** with ethanolic sodium carbonate solution produced the free ribonucleosides **4** in good yields (Figure 2). The structures of the new ribonucleosides **4** and **9** were confirmed by IR, NMR and high-resolution mass spectrometry.

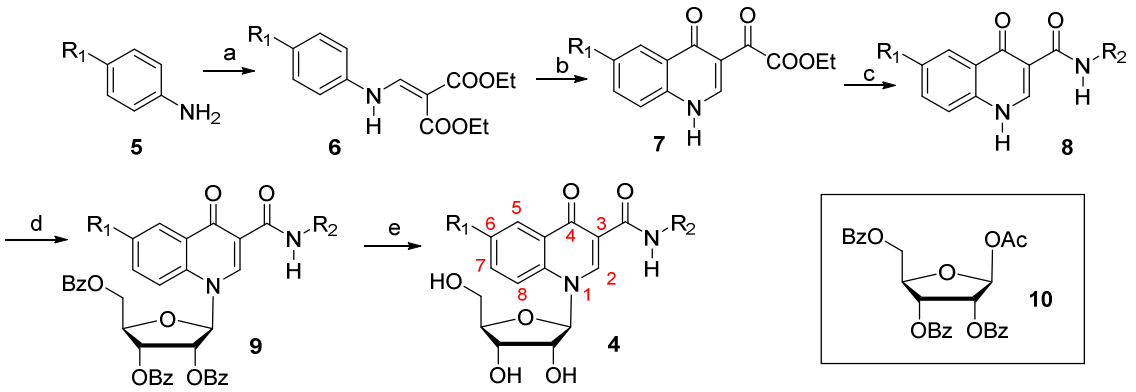


Figure 2. Synthesis of ribonucleosides **9** and **4**. *Reagents and conditions*: (a) EMME, ethanol, reflux, 6 h; (b) diphenyl ether, reflux, 20 min; (c) diphenyl ether, amine, 210°C,

1 h; (d) 1- BSTFA/1 % TMCS, acetonitrile, 60 °C, 2 h; 2- **10**, TMSOTf, rt, 4 h; (e) Na_2CO_3, ethanol, rt, 24 h

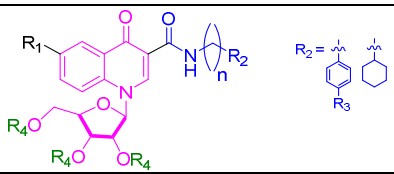
3.2. Biology and Structure-Activity Relationships

The 22 newly synthesized compounds were evaluated for their biological activity against HIV-1 RT. The FDA-approved drug azidothymidine (AZT) was included as the reference (Table 1). All compounds exhibited good anti-HIV activity, ranging from 1.4 to 29 µM, among which compounds **9a** and **9d** displayed the highest antiviral activity profiles, with IC_{50} values of 1.4 and 1.6 µM, respectively.

Previous work from our group showed that chlorine substituents on the 4-oxoquinolic derivatives enhanced the antiviral activity against HIV-1 [8, 9, 10]. Nevertheless, the absence of halogenation at the R1 position rendered **9a** and **9d** with improved antiviral activity when compared to the other compounds (Table 1). Although all tested compounds were less potent than AZT, IC_{50} values in the low micromolar range indicates good candidates for further pharmacological development since resistance is a current problem for HIV treatment that requires new antiviral options.

The compounds were also assayed for cytotoxicity using human primary cells, peripheral blood mononuclear cells (PBMCs). Our compounds were more than 10-fold less cytotoxic than AZT (Table 1), with CC_{50} values above 1000 µM. Most active candidates, compounds **9a** and **9d**, showed CC_{50} values of 1486 and 1394 µM, respectively. Compounds **9a** and **9d** presented selectivity indexes (SI = CC_{50}/EC_{50}) of 998 and 830, respectively, indicating they are as safe as AZT.

Table 1. HIV-1 RT inhibitory effects (IC_{50} µM), cytotoxicity (CC_{50} µM) and volume (**Å³**) of 4-oxoquinoline ribonucleosides (**4a-j** and **9a-k**)



COMPOUND	R1	R2	R3	R4	n	IC_{50} (µM)	CC_{50} (µM)	Volume (Å³)
4a	H	phenyl	Cl	H	1	29.2 ± 4.6	>1000	409.63
4b	Cl	phenyl	Cl	H	1	18.4 ± 6.7	>1000	422.84
4c	F	phenyl	Cl	H	1	3.9 ± 0.3	>1000	414.19
4d	CH_3	phenyl	Cl	H	1	4.3 ± 0.7	>1000	427.59

4e	CF_3	phenyl	Cl	H	1	3.9 ± 1.0	>1000	441.62
4f	Cl	phenyl	Cl	H	0	8.0 ± 0.8	>1000	404.86
4g	Cl	phenyl	F	H	0	5.8 ± 1.3	>1000	396.98
4h	Cl	phenyl	OCH_3	H	0	3.4 ± 0.3	>1000	417.86
4i	Cl	phenyl	H	H	0	2.6 ± 0.3	>1000	390.79
4j	Cl	cyclohexyl	-	H	0	22.4 ± 4.5	>1000	404.33
9a	H	phenyl	Cl	OBz	1	1.4 ± 0.3	1486 ± 203	730.81
9b	Cl	phenyl	Cl	OBz	1	8.8 ± 2.1	>1000	743.56
9c	F	phenyl	Cl	OBz	1	2.8 ± 0.2	>1000	735.63
9d	CH_3	phenyl	Cl	OBz	1	1.6 ± 0.3	1394 ± 127	747.67
9e	CF_3	phenyl	Cl	OBz	1	2.9 ± 0.6	>1000	761.24
9f	Cl	phenyl	CH_3	OBz	0	16.6 ± 3.4	>1000	729.36
9g	Cl	phenyl	Cl	OBz	0	9.9 ± 3.1	>1000	724.27
9h	Cl	phenyl	F	OBz	0	7.3 ± 1.2	>1000	713.66
9i	Cl	phenyl	OCH_3	OBz	0	6.8 ± 0.9	>1000	738.68
9j	Cl	phenyl	H	OBz	0	19.5 ± 5.5	>1000	711.43
9k	Cl	cyclohexyl	-	OBz	0	8.6 ± 2.2	>1000	724.03
AZT	-	-	-	-	-	0.05	126	-

The conformational analysis showed that all derivatives without the methylene spacer presented a phenyl substituent that was planar to the 4-oxoquinoline moiety, whereas insertion of the spacer led to the phenyl group adopting a perpendicular orientation (Figure 3). Afterwards, we have two structural patterns of HIV-1 RT inhibition where the 4-oxoquinoline ribonucleosides with a 2,3,5-tribenzoyl are more active than the 2,3,5-trihydroxyl when R2 contains a methylene spacer (**9a**-**e**/**4a**-**e**), and in the absence of the methylene spacer, the 4-oxoquinolinic β-D-ribonucleosides with the 2,3,5-trihydroxyl presented better activity (**9f**-**k**/**4f**-**j**). This may allow us to infer that in the absence of volumous and flexible substituents, the planarity is important for activity. It is worth mentioning that greater flexibility allows compounds to adopt multiple conformations, support a better fit at the binding site and consequently increasing inhibition potency (Table 1). This structural feature is important for mutant strains that have a resilient profile, where more flexible molecules can be better accommodated at the RT binding site [18]. Also, a larger contact surface area may allow more Van der Waals contacts, contributing to the activity. This is evidenced in the most active compounds (**9a** and **9d**) (Figure 3).

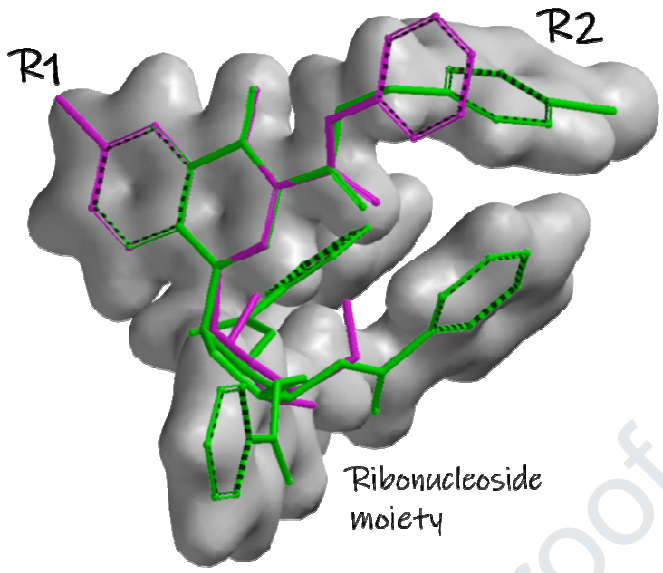


Figure 3. Comparison of the conformational and steric parameters by structural alignment of the 4-oxoquinoline ribonucleosides **9a** (green) and **4i** (magenta). Van der Waals volume of **9a** can be seen in gray

Electronic parameters calculated by quantum chemical methods showed no direct correlation with activity, while the volume and flexibility were key features for the most active compounds (**9a** and **9d**). The allosteric site is largely hydrophobic, includes only a few nonhydrophobic residues, and the size of ligands varies greatly. The calculated volume of the allosteric site HIV-1 RT using the DoGSiteScorer server [19] was approximately 822.01 $Å^3$. Accordingly, this pocket may accommodate bulky molecules as **9a** and **9d** (Table 1).

3.3. Mechanistic studies by a molecular docking approach

To gain a better insight about the mechanism of action, we performed molecular docking studies with the most and the less active compounds **4a**, **9a**, **9d** and **9j** into the allosteric site of HIV-1 RT. Initially, the validation of the docking protocol was carried out through redocking into the HIV-1 RT crystal structure (PDB code 1KLM). Comparison of the redocking results with the cocrystallyzed conformation showed a good success rate with a root-mean-square deviation (RMSD) of 1.08 Å. These data support the hypothesis that the experimental binding mode could be reproduced accurately using this protocol.

Then, the most active compounds (**9a** and **9d**) were investigated. Analysis of the docking complex of **9a** within the allosteric site showed that the R2 group of this compound occupies the subpocket formed by hydrophobic residues Leu100, Tyr181, Tyr188, Phe227, and Trp229 of HIV-1 RT, whereas the 4-oxoquinoline moiety effectively occupies the NNIBP entrance channel. Compound **9a** interacted mainly by Van der Waals interactions and hydrophobic contacts with Leu100, Lys101, Lys102, Lys104, Ser105, Tyr181, Tyr188, Ile195, Glu224, Pro225, Pro226, Phe227, Leu234, His235, Pro236 and Asp237 (Figure 4). The amide moiety of compound **9a** participated in two hydrogen bonds: one with the carbonyl oxygen of Lys103 (distance NH-O of 3.2 Å) and another with the side chain of Tyr318 (O-HO distance of 3.4 Å). Furthermore, a hydrogen bond was observed between the oxygen of the ribofuranose ring and the N from the backbone of Val106 (NH-O distance of 3.2 Å) (Figure 4). The allosteric site of HIV-1 RT is a highly hydrophobic cavity, which may infer that the presence of these hydrogen bonds may contribute to the stabilization of the complex. It is interesting to highlight that no mutations have been described yet for Tyr318, so **9a** could be an inhibitor that is less susceptible to viral resistance [12, 20].

Compound **9d** interacted by Van der Waals interactions and hydrophobic contacts with Leu100, Lys101, Lys102, Lys104, Ser105, Val106, Thr107, Val179, Gly190, Ile195, Glu224, Pro225, Pro226, Phe227, Leu234, His235, Pro236, Asp237 and Tyr318 residues (Figure 4). The amide moiety of compound **9d** participated in an hydrogen bond with N from the backbone of Lys103 (distance NH-O of 3.1 Å) and the oxygen of the ribofuranose ring with N from the backbone of Val106 (NH-O distance of 3.4 Å) (Figure 4). The hydrogen bond with Lys103 or Lys103 together with Val106 seems to be important for the activity.

The less active compounds in each series (**4a** and **9j**) were also investigated. Compound **4a** showed Van der Waals interactions and hydrophobic contacts with Leu100, Lys101, Lys102, Lys103, Lys104, Ser105, Glu224, Pro225, Pro226, Phe227, His235, Pro236, Asp237 and Tyr318 (Figure 4). Hydrogen bond was formed with Val106 (NH-O distance of 2.8Å) and Leu234 (OH-O distance of 2.6Å). Interestingly, due to its conformation in the binding pocket, compound 4a presented intramolecular Hydrogen bonds were also formed (Figure 4).

Compound **9j** interacted by Van der Waals interactions and hydrophobic contacts with Lys101, Lys102, Lys103, Ser105, Val106, Thr107, Ile195, Glu224,

Pro225, Pro226, Phe227, Leu234, His235, Pro236 and Tyr318 residues. One hydrogen bond with Lys104 (NH-O distance of 2.7Å) was formed (Figure 4).

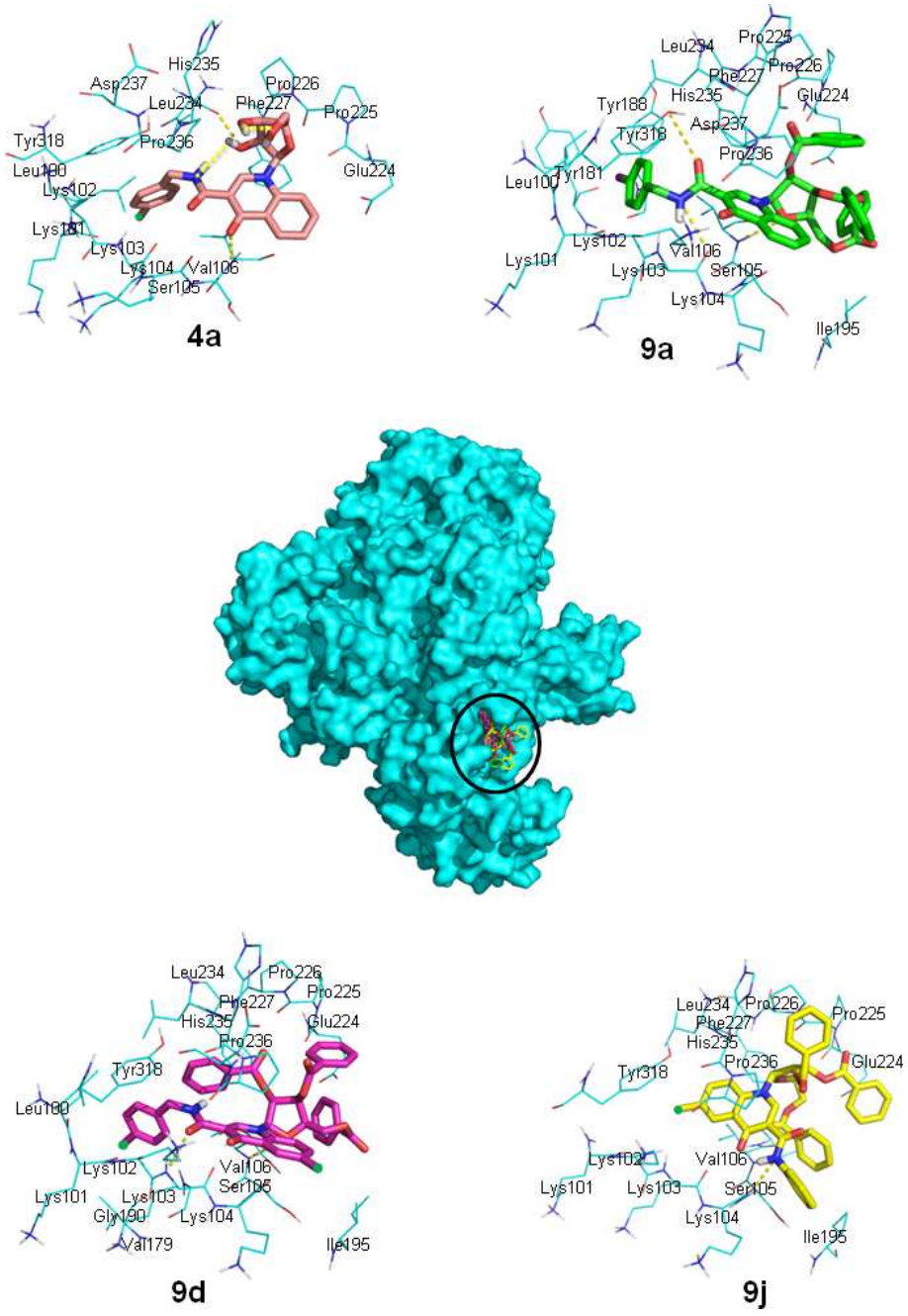


Figure 4. Binding mode analysis of compound **4a** (pink), **9a** (green), **9d** (magenta) and **9j** (yellow) at the HIV-1 RT allosteric site. Residues involved in the interactions are shown in blue and hydrogen bonds are colored in yellow dashed lines.

3.4. *In Silico* Pharmacokinetic and Toxicity Analyses

In this work, we also assessed the pharmacokinetic (PK) and toxicological properties of compounds **4a**, **9a**, **9d**, **9j,** AZT and nevirapine using an *in silico* approach. Considering the PK analysis, all compounds of series **9** presented high flexibility, high numbers of hydrogen bond acceptor groups, high lipophilicity and low solubility, which may lead to absorption issues. AZT is charged and presented low permeability; however, good absorption has been reported in the literature [21]. Problems related to absorption were not observed for nevirapine, which is in accordance with Coffey & Volberding [22]. The evaluation of metabolism showed that series 9 could be metabolized by CYP3A4, whereas AZT and nevirapine are metabolized by glucuronidation [21, 23].

Toxicological evaluations indicated possible carcinogenicity for all compounds and hepatic effects only for **9a** and **9d**. Similar to AZT, mutations were also detected. These results are corroborated by Chiu & Duesberg [24] and by the National Toxicology Program [25]. Finally, nevirapine showed only hepatic problems, which is also corroborated by the literature [22]. These findings highlight the reliability of the *in silico* results herein and, due to the resistance profile of HIV, reinforces the potential of the active compounds as anti-HIV candidates for further exploration.

4. Conclusions

In summary, we synthesized twenty-two 4-oxoquinoline ribonucleoside derivatives with anti-HIV-1 RT activity and low cytotoxicity. Derivatives **9a** and **9d s**howed the highest antiviral activities and SAR studies revealed that volume and flexibility are the key features for for modulating HIV-1 RT. Molecular docking studies showed that the most active compounds bind to the allosteric site of the enzyme, interacting with important residues for the enzymatic activity. Interactions with Tyr318, a conserved residue with no mutations described so far, indicated that **9a** as an inhibitor would be less susceptible to viral resistance. Finally, the *in silico* ADMET results showed that **9a** and **9d** are similar to AZT. Overall, **9a** and **9d** present a promising scaffold for future anti-HIV-1 drug design since their mode of HIV-1 inhibition might differ from the classical NNRTIs and be effective against HIV mutant strains.

Supporting Information

Supporting information includes physical and spectroscopic information for compounds.

Acknowledgments

The authors would like to acknowledge the agencies that funded our research: CNPq, CAPES and FAPERJ. The fellowships granted by CNPq, CAPES and FAPERJ are gratefully acknowledged, as well as and FIOCRUZ for the HRMS analyses.

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HIGHLIGHTS

4-oxoquinoline ribonucleoside with anti-HIV-1 RT activity and low cytotoxicity was prepared

Ribonucleoside derivatives were more than 10-fold less cytotoxic than AZT

SAR studies revealed that volume and flexibility are the key features for modulating HIV-1 RT

Docking studies showed that the most active compounds bind to the allosteric site of the enzyme

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: