Full Paper

New 4-[(1-Benzyl-1*H*-indol-3-yl)carbonyl]-3-hydroxyfuran-2(5*H*)-ones, β-Diketo Acid Analogs as HIV-1 Integrase Inhibitors

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In addition to our recent report on a series of rationally designed benzylindolyldiketo acids acting as potent HIV-1 integrase strand transfer inhibitors, we disclose the results obtained with novel compounds chemically modified on the diketo acid moiety in order to investigate its influence on the biological activity and cytotoxicity. The activity of designed and synthesized 4-[(1-benzyl-1*H*-indol-3-yl)carbonyl]-3-hydroxyfuran-2(5*H*)-one derivatives lies in the micromolar range with regard to HIV IN enzymatic activity. The microwave-assisted synthesis was employed in some steps of the chemical procedures.

Keywords: HIV-1 IN inhibitors / Hydroxyfuran-2(5*H*)-ones / Microwave-assisted synthesis / Parallel synthesis / Pharmacophore model

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Introduction

Despite the considerable success of highly antiretroviral therapy (HAART) in western countries, AIDS remains one of the most urgent world health problems. The causative agent, the human immunodeficiency virus type 1 (HIV-1) encodes three enzymes which are required for viral replication: reverse transcriptase (RT), protease (PR), and integrase (IN). Although drugs targeting RT and PR, and recently a viral entry inhibitor (i.e. enfuvirtide), are widely used and show effectiveness particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness [1, 2].

Therefore, there is ever increasing impetus for the discovery of new agents directed against alternative sites in the viral life cycle. IN has thus emerged as an attractive target for anti-HIV therapy, both because it is necessary for stable infection and because known functional analogues are lacking in the human host [3].

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Integrase inserts a double-stranded DNA copy of the viral RNA genome into the chromosomes of an infected cell through two separate reactions: "3′ processing" and "strand transfer".

For the integration divalent cations such as Mn^{2+} or Mg^{2+} are required for the catalytic activity. Although a wide variety of compounds have been reported as IN inhibitors [4], drugs active against this enzyme have not yet been approved by the FDA. One of the major leads in the development of anti-HIV-1 IN drugs is represented by β-diketo acids (DKAs) [5] and their derivatives [4] which selectively inhibit the strand-transfer step by sequestering the divalent cations in the active site of the enzyme [6] and block HIV-1 replication in the infected cells.

In a previous paper, we reported a 3D pharmacophore model for DKAs consisting of four features: two hydrogen bond acceptors (HBA1-2), one hydrogen bond donor (HBD), and one hydrophobic aromatic region (HyAr) [1]. The resulting pharmacophore model guided the rational design of benzylindolyldiketo acids as new IN inhibitors able to map all features of the model (Fig. 1).

The designed derivatives were synthesized and proved to be highly potent IN inhibitors by selectively blocking the strand-transfer process at nanomolar concentrations

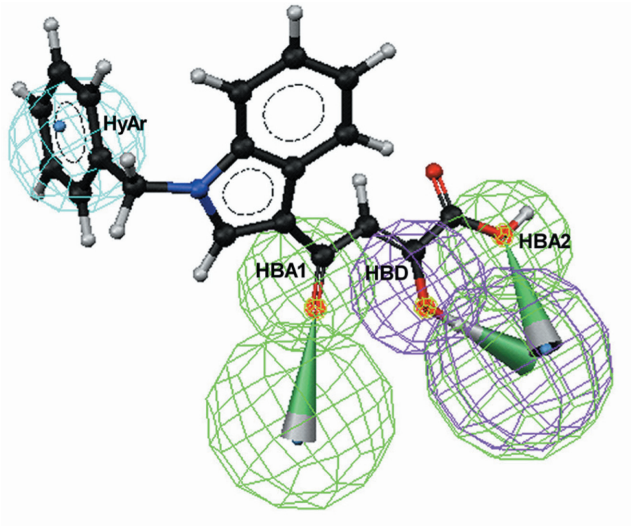


Figure 1. Benzylindolyl diketoacid lead compound **39** aligned on the four-feature pharmacophore model generated for HIV-1 IN DKA inhibitors (HBD, hydrogen bond donor, purple; HBA, hydrogen bond acceptor, green; HyAr, hydrophobic region, cyan). Predicted IC_{50} value = 0.02.

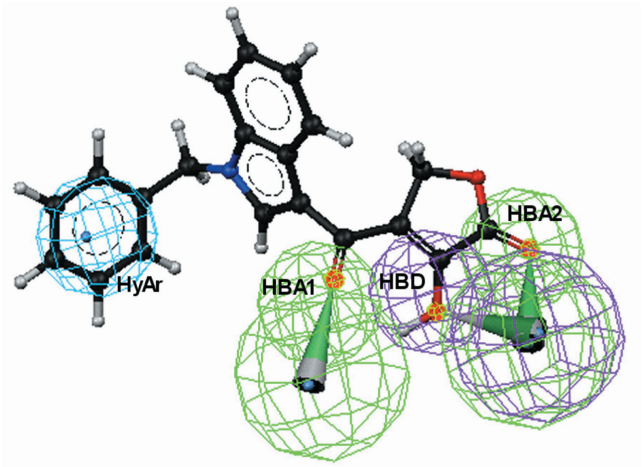


Figure 2. Compound **29** mapped into the four-feature pharmacophore model generated for HIV-1 IN DKA inhibitors (HBD, hydrogen bond donor, purple; HBA, hydrogen bond acceptor, green; HyAr, hydrophobic region, cyan). Predicted IC_{50} value = 0.12.

[1] whereas, generally, they were only slightly inhibiting the replication of HIV-1 in cell culture.

Furthermore, it had been reported both that the presence of the biologically labile 1,3-diketoacid moiety is a concern for the development of these compounds as chemotherapeutic agents [7] and that IN inhibitors of the diketo acid type would affect the activities of other enzymes such as RAG1_2 [8].

On these bases and considering that the 1,3-diketo acid functionality is particularly important for the IN inhibitory activity to capture bivalent cations, our efforts were directed towards seeking viable replacement for this critical moiety in a corresponding closed system.

Results and discussion

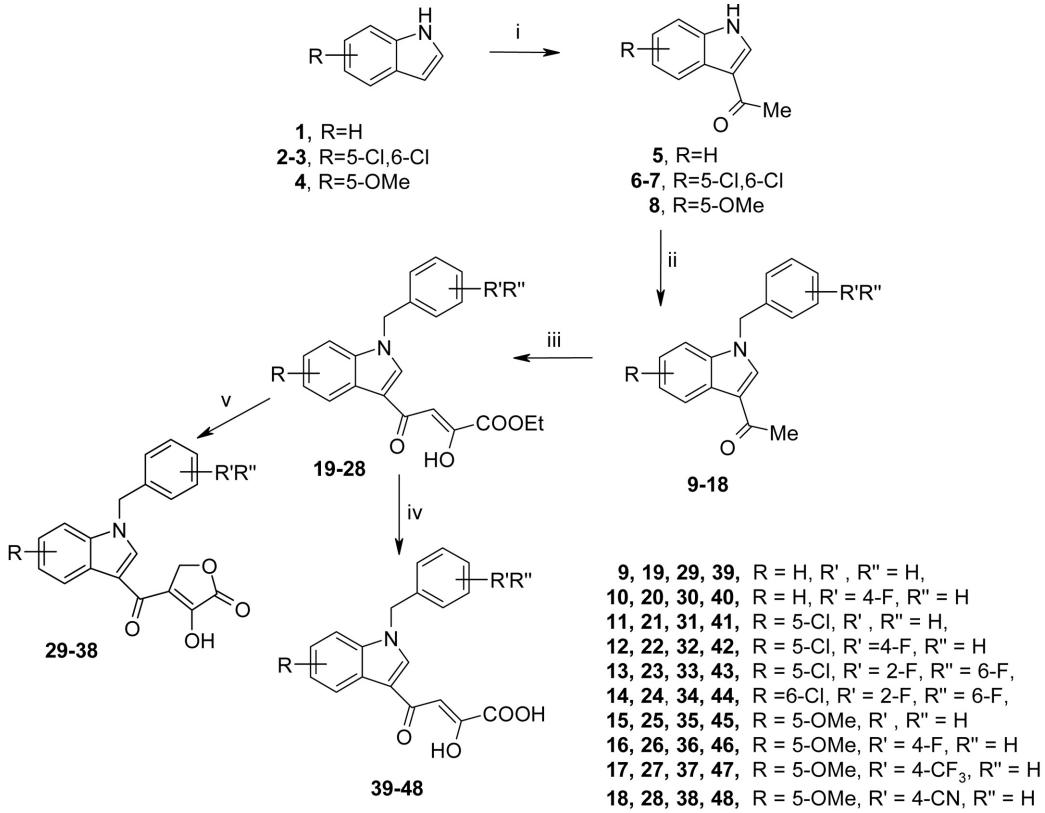
With the aim to replace the 1,3-diketo acid group with a more stable chelating moiety, we describe in this paper the design, synthesis, and biological activity of a set of DKA-analogues in which the 4-carbonyl-3-hydroxyfuran-2(5*H*)-one moiety was introduced as a suitable replacement for the β-diketo acid motif. This idea stems from our previous studies in which we used a qualitative pharmacophore model as a search query to identify structural templates from 3D small molecule databases [9].

Among different identified compounds, 4-(4-chlorobenzoyl)-3-hydroxy-5-phenyl-furan-2(5*H*)-one proved to be an interesting IN inhibitor. We explained this behavior considering that even if this molecule belong to a class of compounds different from previously described DKA analogues, it incorporates the 1,3-diketoacid motif into a closed system [9].

It is well known that the 1,3-diketo acids enolize at the α-position forming 2-hydroxy-4-oxo-2-butanoic acids able to coordinate the two magnesium ions required for IN activity [6]. Analogously, it is possible to suppose that also the 4-carbonyl-3-hydroxyfuran-2-one ring presenting the same chemical functionalities (Fig. 2) could sequester the Mg^{2+} ions.

On this basis, we designed and synthesized a series of 4-[(1-benzyl-1*H*-indol-3-yl)carbonyl]-3-hydroxyfuran-2(5*H*)-ones **29**–**38**, analogues to DKA derivatives **39**–**48**, with the aim to explore the influence of this chemical manipulation on IN inhibitory activity.

The chemistry sequence leading to derivatives **19**–**48** is described in Scheme 1 and was achieved in a multistep reaction, starting from the commercially available 1*H*-indoles **1**–**4** (Scheme 1). At first, they were 3-acetylated by reaction with acetyl chloride and a slight excess of diethylaluminum chloride, then *N*-alkylated by treatment with the suitable benzyl bromide and a small amount of sodium hydride to give intermediates **9**–**18**. Successively, the coupling with diethyl oxalate in the presence of a catalytic amount of sodium methoxide was very efficiently performed under microwave irradiation, to obtain intermediates **19**–**28** with a short reaction time and high yields. Derivatives **19**–**28** were converted to the final γ-lactones **29**–**38** by treatment with water and formaldehyde and successively with hydrochloric acid. From the



Reagents and conditions: i) AcCl, Et_2AlCl, CH_2Cl_2, 0°C, 2 h; ii) appropriate benzyl bromide, NaH, DMF, 0°C, 30 min; iii) diethyl oxalate, dry CH_3ONa, THF, two separated steps in the same conditions: 50°C, 2 min, 250 W, 300 psi; iv) 2 N NaOH, MeOH, rt, 1.5 h; v) CH_2O, Et_2O/H_2O, rt, 2 h.

Scheme 1. Synthesis route of compounds **1–48**.

same derivatives **19–28** the corresponding diketo acids **39–48** were obtained in alkaline medium.

Both analytical and spectral data (^{1}H-NMR) of all synthesized compounds are in full agreement with the proposed structures.

All new 3-hydroxyfuran-2(5*H*)-ones **29–38**, diketo acids **43, 44** and **47**, **48**, and esters **23**, **24**, **27**, **28** were evaluated for their ability to inhibit IN enzymatic activity both against the overall integration reaction and more specifically to the strand-transfer step.

Table 1 shows the biological data of the synthesized compounds in comparison with those of L870-810, a well-known HIV-1 IN inhibitor of the Merck group [7, 10]. As can be seen, derivative **33**, bearing a chlorine atom at C-5 of the benzimidazole system and a 2,6-difluorophenyl group at the 1-position, is a very active compound (IC_{50} = 4 μM). However, even if the furanone derivatives generally exhibited good potency in a micromolar range, the comparison of these derivatives with the corresponding diketo acids and the respective esters pointed out a diminished potency both in enzymatic and cell assays with a low selectivity profile. These results suggest that the replacement of the β-diketo acid motif negatively influences the *in-vitro* biological activity, probably due to a reduced conformational mobility of a closed system with respect to the open form.

However, although their biological profile is far from that of clinically useful chemotherapeutic agents, their activity values, in a micromolar range, are in accordance with the potency required for a new lead and as a starting point for activity optimization. Therefore, in view of the paucity of specific IN inhibitors up to date approved by FDA, we consider it useful to continue the examination of other possible modifications of the labile diketo acid motif and further investigations are in progress.

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Table 1. Inhibition of HIV-1 integrase enzymatic activity, replication of HIV-1, and cytotoxicity in MT-4 cells.

Compound	IN enzymatic activity		Activity in MT-4 cells		
	Overall IC_{50} (μM)[a)]	ST IC_{50} (μM)[b)]	HIV-1 EC_{50} (μM)[c)]	Cytotoxicity CC_{50} (μM)[d)]	SI*
19 £	0.76 ± 0.10	5.40 ± 4.16	14.30 ± 2.33	119.7 ± 66.1	8
20 £	1.20 ± 0.0	1.50 ± 0.57	9.25 ± 3.81	27.2 ± 5.4	3
21 £	0.22 ± 0.04	0.03 ± 0.01	4.50 ± 1.00	34.00 ± 6.78	8
22 £	0.39 ± 0.14	0.07 ± 0.05	3.42 ± 2.23	26.53 ± 4.24	8
23	8.46 ± 0.86	7.03 ± 4.55	4.66 ± 2.43	20.32 ± 3.67	4
24	0.95 ± 0.33	0.38 ± 0.02	21.84 ± 2.54	69.70 ± 12.45	4
25 £	0.15 ± 0.05	0.68 ± 0.42	6.07 ± 0.0	29.04 ± 0.0	5
26 £	0.22 ± 0.08	0.80 ± 0.7	14.34 ± 7.14	127.45 ± 26.55	9
27	≥100	ND	32.46 ± 4.67	32.46 ± 4.67	1
28	≥100	ND	6.87 ± 3.56	6.873.56	1
29	7.5 ± 2.4	276.7 ± 21.7	>81.8 ± 24.2	81.8 ± 24.2	>1
30	20.8 ± 2.9	48.7 ± 0.3	>66.6 ± 40.1	66.6 ± 40.1	>1
31	22.1 ± 5.0	90.8 ± 1.4	>23.4 ± 14.1	23.4 ± 14.1	>1
32	44.7 ± 0.4	46.8 ± 1.3	>30.5	30.5	>1
33	3.3 ± 1.0	4.0 ± 0.3	>10.5 ± 3.1	10.5 ± 3.1	>1
34	71.2 ± 5.5	80.1 ± 9.0	>136.6 ± 12.6	136.6 ± 12.6	>1
35	≥100	ND	>20.5	20.5	>1
36	76.3 ± 0.3	69.0 ± 1.2	>48.8 ± 17.8	48.8 ± 17.8	>1
37	53.7 ± 1.2	≥100	>29.9 ± 10.4	29.9 ± 10.4	>1
38	54.3 ± 18.7	127.0 ± 51.6	>13.6 ± 5.5	13.6 ± 5.5	>1
39 £	0.02 ± 0.0006	0.03 ± 0.0006	19.92 ± 15.25	>78	>4
40 £	0.002 ± 0.001	0.015 ± 0.003	5.31 ± 0.88	>74	>14
41 £	0.010 ± 0.003	0.10 ± 0.01	4.78 ± 0.98	37.24 ± 1.54	8
42 £	0.20 ± 0.08	0.01 ± 0.001	2.78 ± 0.39	53.42 ± 6.69	19
43	0.20 ± 0.10	0.10 ± 0.10	1.84 ± 0.12	25.63 ± 11.01	15
44	0.13 ± 0.03	0.10 ± 0.00	7.89 ± 1.11	52.56 ± 14.24	7
45 £	0.017 ± 0.006	0.021 ± 0.007	5.32 ± 0.24	86.38 ± 52.79	16
46 £	0.019 ± 0.008	0.004 ± 0.001	5.81 ± 1.93	63.54 ± 39.53	11
47	1.81 ± 1.12	4.08 ± 0.55	45.90 ± 8.97	45.90 ± 8.97	1
48	1.51 ± 0.53	3.00 ± 0.48	35.57 ± 26.48	35.57 ± 26.48	1
L-870, 810	0.0005 ± 0.0003	0.0025 ± 0.0007	0.0047 ± 0.0007	2.2 ± 0.2	457

a) Concentration required to inhibit the *in-vitro* overall integrase activity by 50%.
b) Concentration required to inhibit the *in-vitro* strand-transfer step by 50%.
c) Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
d) Cytotoxic concentration to reduce MT-4 cell viability by 50%.
* Selectivity index: ratio CC_{50}/EC_{50}. £ Reference [1].

Experimental

Chemistry

Melting points were determined on a Kofler hot stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. Elemental analyses (C, H, N) were carried out on a C. Erba Model 1106 Elemental Analyzer (Carlo Erba, Milan, Italy) and the results were within ± 0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for TLC (Merck, Darmstadt, Germany). ^{1}H-NMR spectra were measured with a Varian Gemini 300 spectrometer (Varian Inc., Palo Alto, CA, USA) in $CDCl_3$ with TMS as internal standard: chemical shifts are expressed in δ (ppm) and coupling constants (*J*) in Hertz.

3-Acetyl-6-chloro-1H-indole ***7***

According to the procedure reported in reference [1] derivative **7** was obtained starting from 6-chloro-1*H*-indole **3** (152 mg, 0,001 mol). Mp. 209°C dec, yield 97%. ^{1}H-NMR (δ) 2.43 (s, 3H, CH_3), 7.16 – 8.34 (m, 4H, ArH), 12.01 (bs, 1H, NH). Anal. Calcd for $C_{10}H_8ClNO$: C, 62.03; H, 4.16; N, 7.23. Found: C, 62.17; H, 4.00; N, 7.25.

3-Acetyl-1-(2,6-difluorobenzyl)-5-chloro-1H-indole ***13***

According to the procedure reported in reference [1] derivative **13** was obtained starting from 3-acetyl-5-chloro-1*H*-indole **6** (194 mg, 0.001 mol) and 2,6-fluorobenzyl bromide (311 mg, 0.0015 mol). Mp. 123 – 125°C, yield 50%; ^{1}H-NMR (δ) 2.50 (s, 3H, CH_3), 5.37 (s, 2H, CH_2), 6.93 – 8.36 (m, 7H, ArH). Anal. Calcd for $C_{17}H_{12}ClF_2NO$: C, 63.86; H, 3.78; N, 4.38. Found: C, 63.92; H, 3.55; N, 4.60.

3-Acetyl-1-(2,6-difluorobenzyl)-6-chloro-1H-indole ***14***

Accordingly, **14** was obtained starting from 3-acetyl-6-chloro-1*H*-indole **7** (194 mg, 0.001 mol) and 2,6-difluorobenzyl bromide

(311 mg, 0.0015 mol). Mp. 181-183°C, yield 49%; ^{1}H-NMR (δ) 2.51 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 6.96 – 8.30 (m, 7H, ArH). Anal. Calcd for $C_{17}H_{12}ClF_2NO$: C, 63.86; H, 3.78; N, 4.38. Found: C, 63.71; H, 3.90; N, 4.42.

*3-Acetyl-5-methoxy-1-(4-(trifluoromethyl)benzyl)-1H-indole **17***

Accordingly, **17** was obtained starting from 3-acetyl-5-methoxy-1*H*-indole **8** (189 mg, 0.001 mol) and 4-trifluoromethylbenzyl bromide (359 mg, 0.0015 mol). Mp. 111 – 112°C, yield 51%; ^{1}H-NMR (δ) 2.57 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 5.44 (s, 2H, CH_2), 6.92 – 7.98 (m, 8H, ArH). Anal. Calcd for $C_{19}H_{16}F_3NO_2$: C, 65.70; H, 4.64; N, 4.03. Found: C, 65.88; H, 4.71; N, 3.81.

*3-Acetyl-1-(4-cyanobenzyl)-5-methoxy-1H-indole **18***

Accordingly, **18** was obtained starting from 3-acetyl-5-methoxy-1*H*-indole **8** (189 mg, 0.001 mol) and 4-cyanobenzyl bromide (294 mg, 0.0015 mol). Mp. 97-100°C, yield 53%; ^{1}H-NMR (δ) 2.52 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 5.40 (s, 2H, CH_2), 6.87 – 7.93 (m, 8H, ArH). Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 74.87; H, 5.49; N, 9.01.

*Ethyl-4-[1-(2,6-difluorobenzyl)-5-chloro-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate **23***

According to the procedure reported in reference [1] derivative **23** was obtained starting from **13** (320 mg, 0.001 mol). ^{1}H-NMR (δ) 1.45 (t, *J* = 7.1, 3H, CH_3), 3.94 (q, *J* = 7.1, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.12 (bs, 1H, OH), 7.13 – 8.39 (m, 8H, 7ArH and CH). Anal. Calcd for $C_{21}H_{16}CLF_2NO_4$: C, 60.08; H, 3.84; N, 3.34. Found: C, 60.19; H, 3.56; N, 3.48.

*Ethyl-4-[1-(2,6-difluorobenzyl)-6-chloro-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate **24***

Accordingly, **24** was obtained starting from **14** (320 mg, 0.001 mol). Mp. 203°C dec, yield 99%; ^{1}H-NMR (δ) 1.16 (t, *J* = 7.1, 3H, CH_3), 4.10 (q, *J* = 7.1, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.14 (bs, 1H, OH), 7.15 – 8.33 (m, 9H, 8ArH, and CH). Anal. Calcd for $C_{21}H_{16}CLF_2NO_4$: C, 60.08; H, 3.84; N, 3.34. Found: C, 60.01; H, 3.93; N, 3.40.

*Ethyl-4-[5-methoxy-1-(4-(trifluoromethyl)benzyl)-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate **27***

Accordingly, **27** was obtained starting from **17** (347 mg, 0.001 mol). Mp. 268 – 270°C dec, yield 100%; ^{1}H-NMR (δ) 1.24 (t, *J* = 7.1, 3H, CH_3), 3.74 (s, 3H, OCH_3), 4.12 (q, *J* = 7.1, 2H, CH_2), 5.54 (s, 2H, CH_2), 6.28 (bs, 1H, OH), 6.74 – 8.16 (m, 9H, 8ArH, and CH). Anal. Calcd for $C_{23}H_{20}F_3NO_5$: C, 61.75; H, 4.51; N, 3.13. Found: C, 61.88; H, 4.39; N, 3.17.

*Ethyl-4-[1-(4-cyanobenzyl)-5-methoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate **28***

Accordingly, **28** was obtained starting from **18** (304 mg, 0.001 mol). Mp. 248 – 250°C dec, yield 98%; ^{1}H-NMR (δ) 1.24 (t, *J* = 6.8, 3H, CH_3), 3.74 (s, 3H, OCH_3), 4.12 (q, *J* = 6.8, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.23 (bs, 1H, OH), 6.73 – 8.43 (m, 9H, 8ArH and CH). Anal. Calcd for $C_{23}H_{20}N_2O_5$: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.54; H, 5.00; N, 6.71.

*4-(1-Benzyl-1H-indol-3-oyl)-3-hydroxyfuran-2(5H)-one **29***

To a mixture of **19** (349 mg, 0.001 mol) in diethyl ether (5 mL) was added a solution of 40% aqueous formaldehyde in water (4 mL). The stirring was then continued until two clear layers formed (usually within 1 – 2 hours). Sometimes, an additional 4 mL of water was added if the reaction was especially thick, or the solid appeared to react slowly. The clear, aqueous bottom layer was removed and the organic layer was extracted twice with 5 mL of water. The combined aqueous extracts were cooled followed by acidification with 3 mL of concentrated hydrochloric acid. The corresponding furanone precipitated; the solution was then cooled overnight to ensure complete product formation. The resulting solid was collected, dried, and recrystallized from ethanol. Mp. 191 – 193°C, yield 50%; ^{1}H-NMR (δ) 5.13 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 7.22 – 8.65 (m, 10ArH). Anal. Calcd for $C_{20}H_{15}NO_4$: C, 72.06; H, 4.54; N, 4.20. Found: C, 71.98; H, 4.33; N, 4.32.

*4-[1-(4-Fluorobenzyl)-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **30***

Accordingly, **30** was obtained starting from **20** (367 mg, 0.001 mol). Mp. 199 – 201°C, yield 55%; ^{1}H-NMR (δ) 5.13 (s, 2H, CH_2), 5.23 (s, 2H, CH_2), 7.13 – 8.62 (m, 9ArH). Anal. Calcd for $C_{20}H_{14}FNO_4$: C, 68.37; H, 4.02; N, 3.99. Found: C, 68.23; H, 4.33; N, 4.02.

*4-(1-Benzyl-5-chloro-1H-indol-3-oyl)-3-hydroxyfuran-2(5H)-one **31***

Accordingly, **31** was obtained starting from **21** (384 mg, 0.001 mol). Mp. 196 – 198°C, yield 69%; ^{1}H-NMR (δ) 5.15 (s, 2H, CH_2), 5.59 (s, 2H, CH_2), 7.30 – 8.76 (m, 9ArH). Anal. Calcd for $C_{20}H_{14}ClNO_4$: C, 65.31; H, 3.84; N, 3.81. Found: C, 65.49; H, 3.73; N, 3.72.

*4-[1-4-Fluorobenzyl)-5-chloro-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **32***

Accordingly, **32** was obtained starting from **22** (401 mg, 0.001 mol). Mp. 146 – 148°C, yield 25%; ^{1}H-NMR (δ) 5.12 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 7.13 – 8.70 (m, 8H, ArH), 11.80 (bs, 1H, OH). Anal. Calcd for $C_{20}H_{13}ClFNO_4$: C, 62.27; H, 3.40; N, 3.63. Found: C, 62.35; H, 3.28; N, 3.68.

*4-[1-(2,6-Difluorobenzyl)-5-chloro-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **33***

Accordingly, **33** was obtained starting from **23** (420 mg, 0.001 mol). Mp. 182 – 184°C, yield 42%; ^{1}H-NMR (δ) 5.05 (s, 2H, CH_2), 5.60 (s, 2H, CH_2), 7.14 – 8.70 (m, 7ArH). Anal. Calcd for $C_{20}H_{12}ClF_2NO_4$: C, 59.49; H, 3.00; N, 3.47. Found: C, 59.58; H, 3.12; N, 3.61.

*4-[1-(2,6-Difluorobenzyl)-6-chloro-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **34***

Accordingly, **34** was obtained starting from **24** (419 mg, 0.001 mol). Mp. 183-185°C, yield 44%; ^{1}H-NMR (δ) 5.04 (s, 2H, CH_2), 5.60 (s, 2H, CH_2), 7.16 – 8.58 (m, 7ArH). Anal. Calcd for $C_{20}H_{12}ClF_2NO_4$: C, 59.49; H, 3.00; N, 3.47. Found: C, 59.36; H, 3.21; N, 3.35.

*4-(1-Benzyl-5-methoxy-1H-indol-3-oyl)-3-hydroxyfuran-2(5H)-one **35***

Accordingly, **35** was obtained starting from **25** (378 mg, 0.001 mol). Mp. 192°C dec., yield 43%; ^{1}H-NMR (δ) 3.76 (s, 3H, CH_3) 5.13 (s, 2H, CH_2), 5.48 (s, 2H, CH_2), 6.89 – 8.62 (m, 9H, ArH), 11.73 (bs, 1H, OH). Anal. Calcd for $C_{21}H_{17}NO_5$: C, 69.41; H, 4.72; N, 3.85. Found: C, 69.37; H, 4.88; N, 3.77.

*4-[1-(4-Fluorobenzyl)-5-methoxy-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **36***

Accordingly, **36** was obtained starting from **26** (397 mg, 0.001 mol). Mp. 172 – 174°C, yield 52%; ^{1}H-NMR (δ) 3.76 (s, 3H, CH_3) 5.13 (s, 2H, CH_2), 5.48 (s, 2H, CH_2), 6.86 – 8.57 (m, 8ArH). Anal. Calcd for $C_{21}H_{16}FNO_5$: C, 66.14; H, 4.23; N, 3.67. Found: C, 66.25; H, 4.32; N, 3.52.

*4-[5-Methoxy-1-(4-(trifluoromethyl)benzyl)-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **37***

Accordingly, **37** was obtained starting from **27** (447 mg, 0.001 mol). Mp. 189 – 191°C, yield 67%; ^{1}H-NMR (δ) 3.76 (s, 3H, CH_3) 5.14 (s, 2H, CH_2), 5.63 (s, 2H, CH_2), 6.86 – 8.58 (m, 8H, ArH). Anal. Calcd for $C_{22}H_{16}F_3NO_5$: C, 61.26; H, 3.74; N, 3.25. Found: C, 61.06; H, 3.82; N, 3.18.

*4-[1-(4-Cyanobenzyl)-5-methoxy-1H-indol-3-oyl]-3-hydroxyfuran-2(5H)-one **38***

Accordingly, **38** was obtained starting from **28** (404 mg, 0.001 mol). Mp. 168 – 70°C, yield 72%; 1H-NMR (δ) 3.77 (s, 3H, CH_3) 5.13 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 6.86 – 7.81 (m, 8H, ArH). Anal. Calcd for $C_{22}H_{16}N_2O_5$: C, 68.04; H, 4.15; N, 7.21. Found: C, 68.11; H, 4.30; N, 7.09.

*4-[1-(2,6-Difluorobenzyl)-5-chloro-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid **43***

According to the procedure reported in reference [1] derivative **43** was obtained starting from **23** (420 mg, 0.001 mol). Mp. 136 – 138°C, yield 48%; ^{1}H-NMR (δ) 5.41 (s, 2H, CH_2), 6.89 (s, 1H, CH), 6.96 – 8.30 (m, 7H, ArH). Anal. Calcd for $C_{19}H_{12}ClF_2NO_4$: C, 58.25; H, 3.09; N, 3.58. Found: C, 58.03; H, 3.28; N, 3.44.

*4-[1-(2,6-Difluorobenzyl)-6-chloro-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid **44***

Accordingly, **44** was obtained starting from **24** (420 mg, 0.001 mol). Mp. 180 – 182°C, yield 45%; ^{1}H-NMR (δ) 5.40 (s, 2H, CH_2), 6.91 (s, 1H, CH), 7.01 – 8.22 (m, 7H, ArH). Anal. Calcd for $C_{19}H_{12}ClF_2NO_4$: C, 58.25; H, 3.09; N, 3.58. Found: C, 58.41; H, 3.01; N, 3.47.

*4-[1-(4-Trifluoromethylbenzyl)-5-Methoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid **47***

Accordingly, **47** was obtained starting from **27** (447 mg, 0.001 mol). Mp. 183 – 184°C dec., yield 39%; ^{1}H-NMR (δ) 3.92 (s, 3H, OCH_3), 5.42 (s, 2H, CH_2), 6.89 (s, 1H, CH), 7.15 – 7.94 (m, 8H, ArH). Anal. Calcd for $C_{21}H_{16}F_3NO_5$: C, 64.15; H, 3.85; N, 3.39. Found: C, 64.33; H, 4.02; N, 3.17.

*4-[1-(4-Cyanobenzyl)-5-methoxy-1H-indol-3-yl]-2-hydroxy-4-oxobut-2-enoic acid **48***

Accordingly, **48** was obtained starting from **28** (404 mg, 0.001 mol). Mp. 176 – 178°C, yield 57%; ^{1}H-NMR (δ) 3.78 (s, 3H, OCH_3), 5.59 (s, 2H, CH_2), 6.87 – 8.93 (m, 9H, ArH and CH). Anal. Calcd for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44. Found: C, 67.19; H, 4.41; N, 7.12.

Pharmacology

Overall integrase assay using an enzyme-linked immunosorbent assay (ELISA).

To determine the susceptibility of the HIV-1 integrase enzyme towards different compounds, we optimized enzyme-linked immunosorbent assays. These assays use an oligonucleotide substrate of which one oligonucleotide (5′- ACTGCTAGAGATTTTC-CACACTGACTAAAAGGGTC -3′) is labeled with biotin at the 3′-end and the other oligonucleotide is labeled with digoxigenin at the 5′-end. For the overall integration assay the second 5′-digoxigenin labeled oligonucleotide is (5′- GACCCTTTTAGTCAGTGTG-GAAAATCTCTAGCA*GT* -3′). For the strand-transfer assay, the second oligonucleotide lacks GT at the 3′-end. The integrase enzyme was diluted in 750mM NaCl, 10 mM Tris, pH 7.6, 10% glycerol, and 1 mM β-mercapto ethanol. To perform the reaction, 4 μL diluted integrase (corresponding to a concentration of 1.6 μM) and 4 μL of annealed oligonucleotides (7 nM) was added in a final reaction volume of 40 μL containing 10 mM $MgCl_2$, 5 mM DTT, 20 mM HEPES, pH 7.5, 5% PEG, and 15% DMSO. The reaction was carried out for 1 h at 37°C. Reaction products were denatured with 30 mM NaOH and detected by an immunosorbent assay on avidin-coated plates [11].

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

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