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STRUCTURAL MODIFICATION OF DIKETO ACID PORTION IN 1*H*-BENZYLINDOLE DERIVATIVES HIV-1 INTEGRASE INHIBITORS

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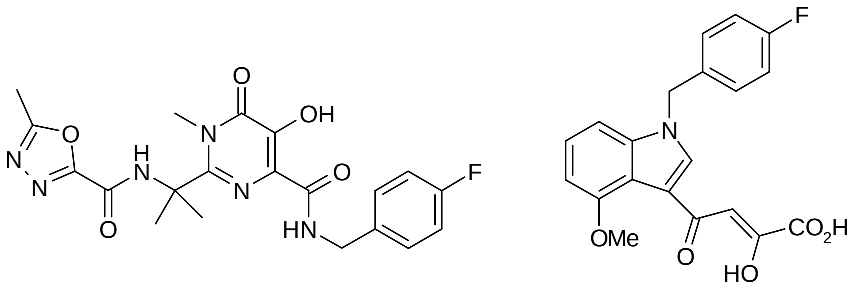
Abstract - Our previous studies led to discovery of a very potent benzylindoldiketo acid (CHI-1043) acting as HIV-1 integrase strand transfer inhibitor. We herein report the synthesis of new structurally different compounds in which the 1,3-diketo acid moiety has been substituted with other functionalities. The synthesized derivatives were evaluated for their activity on the IN enzyme and in MT-4 cells but only 4-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl)-3-hydroxyfuran-2(5*H*)-one (**12**) was able to strongly inhibit HIV-1 probably by biotransformation into CHI-1043.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) resulting from infection with human immunodeficiency virus (HIV) continues to expand worldwide, highlighting the need to develop novel antiviral agents.

Conventional antiretroviral medications consist of different classes of drugs able to inhibit reverse transcriptase (RT), protease, fusion and integrase (IN). Combinations of these agents provide potent suppression of HIV-1 RNA loads and have dramatically improved clinical outcomes for many patients. [1]

Despite the successes with such treatments, the permanent use of anti-HIV drugs induces drug-resistant viral variants and the emergence of unwanted metabolic side effects. Therefore, it is extremely important that new compounds are developed which are able to target other steps of the replication cycle of HIV as well as to overcome the resistance problem. As an spin off of our previous research, in this paper we report the synthesis of new potential inhibitors of IN which constitutes an attractive target for antiviral chemotherapy. [2] IN catalyzes the integration of a DNA copy of the viral genome into the chromosomal DNA through a process consisting of three biochemical steps: (i) assembly, (ii) 3'-processing and (iii) strand-transfer.[3] Integrase strand-transfer inhibitors (INSTIs) representing the major leads in the development of an anti-HIV-1 IN drug, [4] act by sequestering the metal ions bound in the active site of the enzyme. [3] In particular, β-diketo acids (DKAs) and their derivatives were the first INSTIs to display potent antiviral activity via inhibition of IN activity. Recent clinical trials have shown a new INSTI DKA analogue (MK-0518) (Figure 1) to be highly effective and well-tolerated [5] and at the end of 2007, the FDA approved MK-0518 (Raltegravir) as the first active drug (Isentress) against IN enzyme.[6]



MK-0518 CHI-1043

Figure 1. Chemical structures of Integrase Strand Transfer Inhibitors

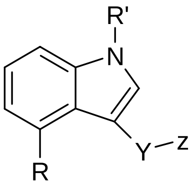
As shown in Figure 1, the diketo acid moiety in MK-0518 has been transformed in an analogue function. In addition it has been reported that the diketo acid portion, even if it seems particularly important to capture bivalent cations for IN inhibitory activity, would also affect the activity of other specific proteins such as RAG (Recombination Activating Gene). For example, the human lymphoid-specific recombinase by activating RAG1 and RAG2 proteins is involved in the V(D)J recombination process, which is an essential step in the development of the specific human immune system.[3]

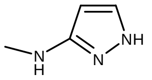
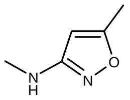
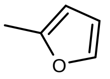
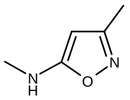
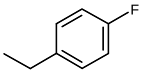
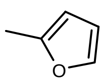
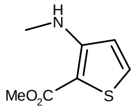
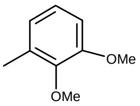
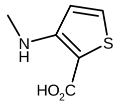
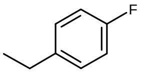
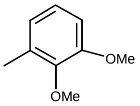
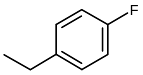
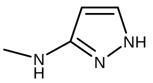
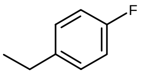
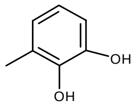
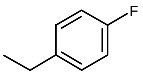
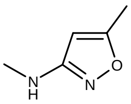
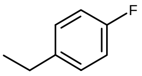
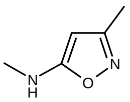
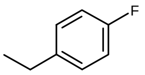
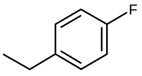
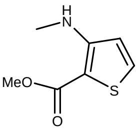
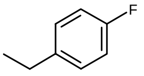
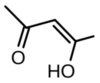
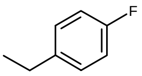
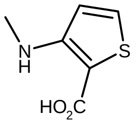
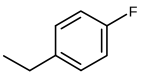
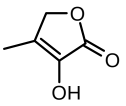
On these bases, we decided to focus our attention on the replacement of the 1,3-diketo acid portion, present in some our potent but not highly selective INSTIs characterized by a 1*H*-benzylindole skeleton.[7-9] Among them CHI-1043 (Figure 1) showed high activity both as IN inhibitor and as an anti-HIV agent in cells.[10]

RESULTS AND DISCUSSION

Using CHI-1043 as a scaffold and in the search for more potent and less toxic IN inhibitors, the aim of this work was the design and synthesis of novel 1*H*-benzylindole derivatives characterized by the presence of new functionalities able to mimic the diketo portion and interact with the enzyme in a more selective manner. Taking into account these considerations, we planned the synthesis of the derivatives, **1a-e**, **2a-e** and **3-12** (Table 1).

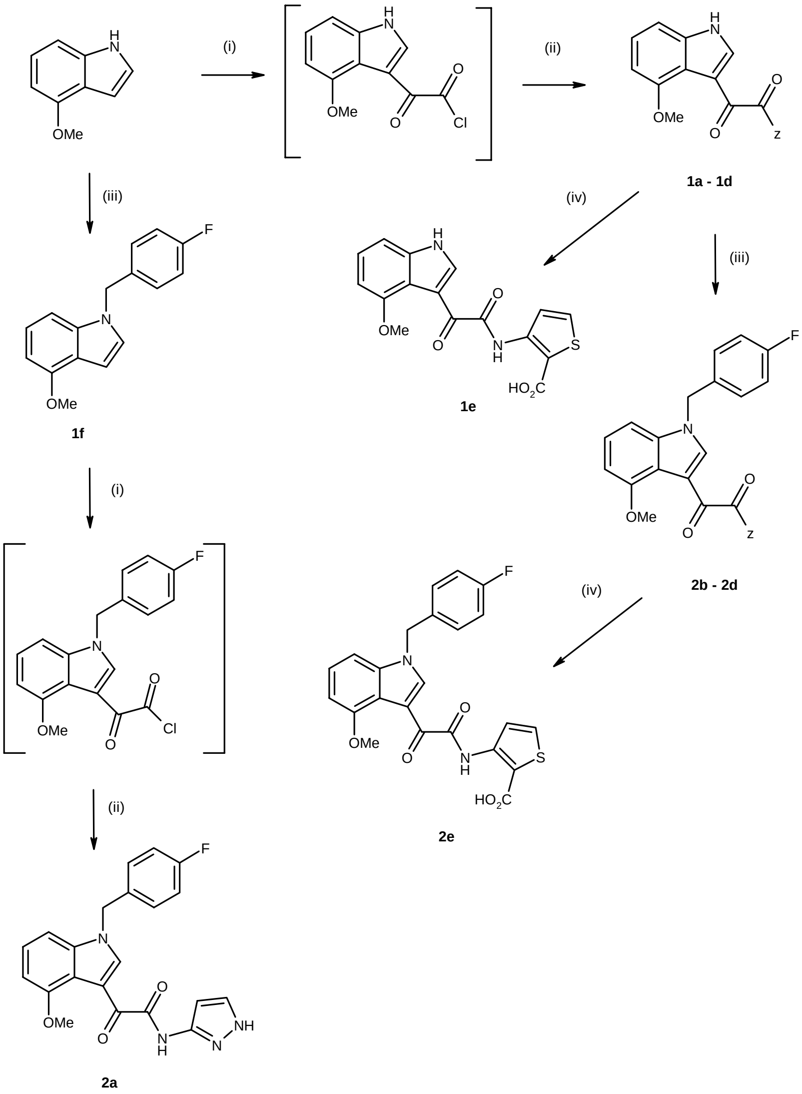
Table 1. Synthesis of the derivatives, **1a-e** and **3-12**



Compd	R	R'	Y	Z	Compd	R	R'	Y	Z
1a	OMe	H	$(CO)_2$		**3**	H	H	CO	NH_2
1b	OMe	H	$(CO)_2$		**4**	H	H	CO-NH-CO	
1c	OMe	H	$(CO)_2$		**5**	H		CO-NH-CO	
1d	OMe	H	$(CO)_2$		**6**	OMe	H	CO	
1e	OMe	H	$(CO)_2$		**7**	OMe		CO	
2a	OMe		$(CO)_2$		**8**	OMe		CO	
2b	OMe		$(CO)_2$		**9**	OMe	H	CO	Me
2c	OMe		$(CO)_2$		**10**	OMe		CO	Me
2d	OMe		$(CO)_2$		**11**	OMe			CO_2-Et
2e	OMe		$(CO)_2$		**12**	OMe		CO	

The newly designed [1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3yl]-glyoxylamides (**2b-2d**) were obtained by the multistep synthetic approaches shown in Scheme 1.

Treatment of commercially available 4-methoxy-1*H*-indole with oxalyl chloride at 0°C produced 2-(1*H*-3-indolyl)-2-oxoacetyl chloride as an inseparable intermediate which gave the compounds **1a-1d** after the reaction with the appropriate arylamine**.** Finally, the *N*-alkylated indoles **2b-2d** were obtained by treatment with 4-fluorobenzyl bromide and a catalytic amount of potassium *t*-butoxide.



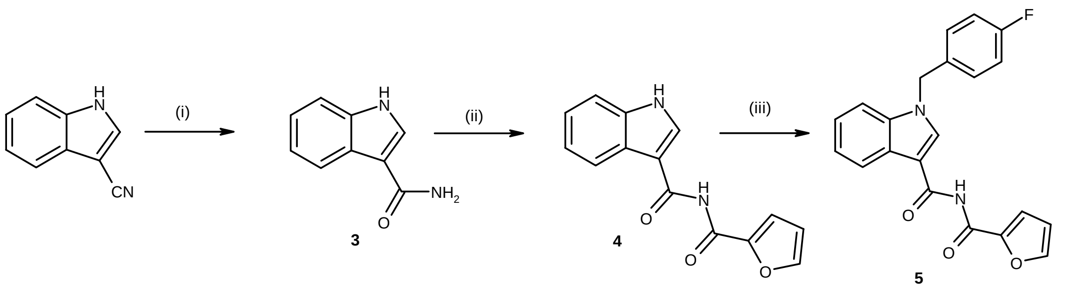
Scheme 1

Reagents and conditions: (i) oxalyl chloride, dry Et_2O, N_2, 0°C, 3 h; (ii) NH_2-Het, dry THF, N_2, rt, 1.5h; (iii) 4-fluorobenzyl bromide, *t*-BuOK, THF, rt, 2h; (iv) 2N NaOH, MeOH, rt, 1.5 h.

In order to avoid the substitution at pyrazole nitrogen, the synthesis of compound **2a** was performed first by the *N*-alkylation of indole to obtain intermediate **1f**, and then by oxyacetylation and condensation in the similar manner with 3-aminopyrazole.

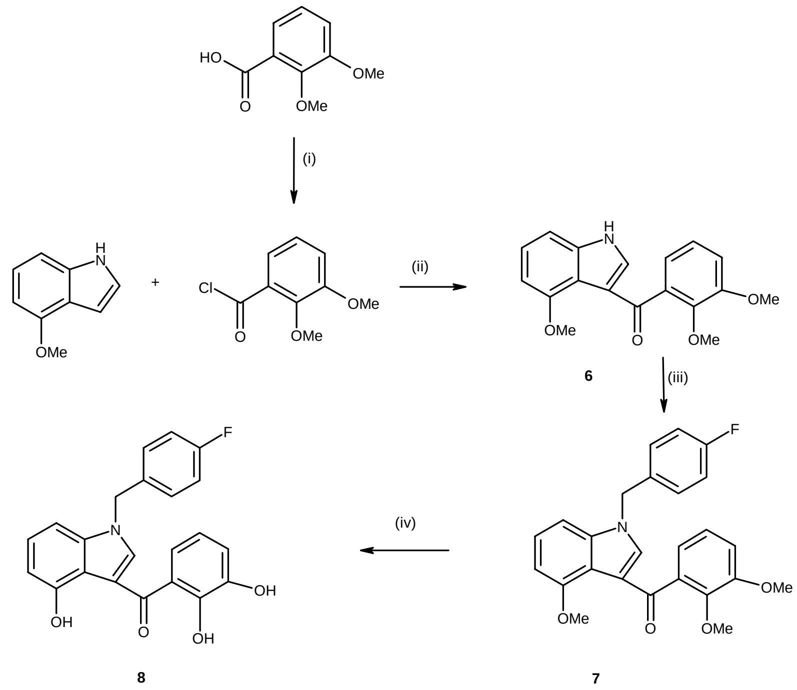
The carboxylic derivatives **1e** and **2e** were obtained by hydrolysis in basic medium from the corresponding esters **1d** and **2d** respectively (Scheme 1).

The synthesis of compound *N*-[1-(4-fluorobenzyl)-1*H*-indol-3-carbonyl]-furan-2-carboxamide (**5**) was performed by starting from 3-cyanoindole (Scheme 2). A methanol solution of 3-cyanoindole was treated with NaOH and H_2O_2 to give derivative **3**, which was directly subjected to condensation with furoyl chloride. Finally, compound **5** was obtained by the reaction of derivative **4** with 4-fluorobenzyl bromide in the presence of an excess of potassium *t*-butoxide.



Scheme 2

Reagents and Conditions: (i) H_2O_2, NaOH, MeOH, rt, 2h; (ii) furoyl chloride, NaH, dry THF, N_2, 0°C, 30 min. (iii) 4-fluorobenzyl bromide, *t*-BuOK, THF, rt, 2h.



Scheme 3

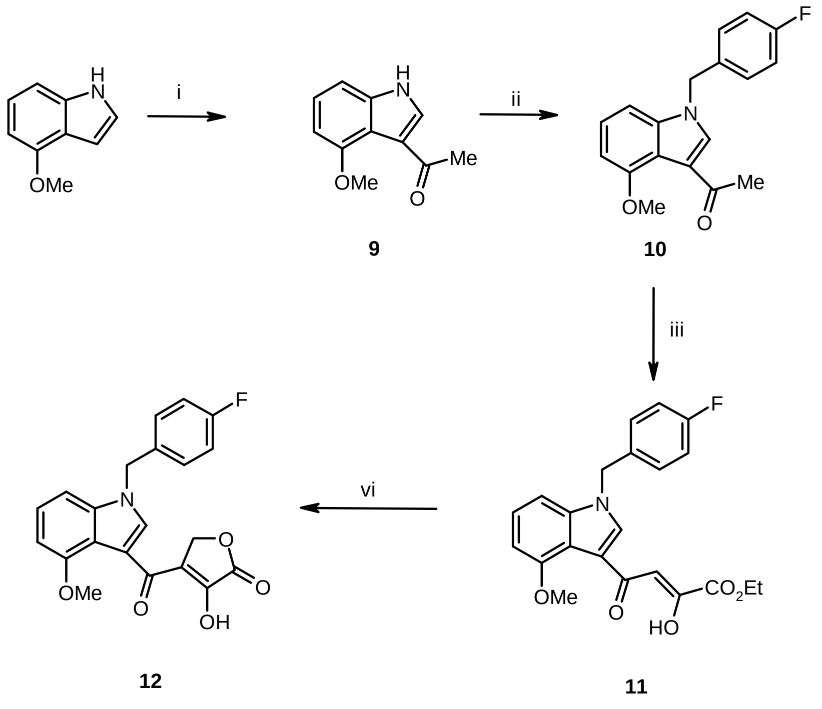
Reagents and Conditions: (i) thionyl chloride, Δ, 2h; (ii) Et_2AlCl, CH_2Cl_2, 0°C, 2 h; (iii) 4-fluorobenzyl bromide, *t*-BuOK, THF, rt, 2h; (iv) BBr_3 1M sol. in CH_2Cl_2, CH_2Cl_2, rt, 16h.

Scheme 3 shows the pathway to synthesize [1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl](2,3-dimethoxyphenyl)methanone (**7**).

The derivative **6** was prepared by 3-acylation of 4-methoxy-1*H*-indole with 2,3-dimethoxybenzoyl chloride that had been obtained from the corresponding acid and thionyl chloride. The intermediate **6** was then alkylated by treatment with 4-fluoro-benzyl bromide using a catalytic amount of potassium *t*-butoxide. Finally, given that some known active IN inhibitors present hydroxylic groups in their structure, the indole derivative **7** was treated with BBr_3 in CH_2Cl_2 to give the corresponding demethylated product **8** (Scheme 3).

The chemistry sequence leading to 4-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-oyl]-3-hydroxyfuran-2(5*H*)-one **12** is described in Scheme 4.

4-Methoxy-1*H*-indole was acetylated by reaction with acetyl chloride and a slight excess of diethylaluminum chloride. After *N*-alkylation by treatment of **9** with 4-fluorobenzyl bromide and a small amount of sodium hydride, the obtained derivative **10** was coupled with diethyl oxalate in the presence of a catalytic amount of sodium methoxide. Derivative **11** was converted to lactone **12** by treatment with formaldehyde.



Scheme 4

Reagents and Conditions: (i) AcCl, Et_2AlCl, CH_2Cl_2, 0°C, 2 h; (ii) 4-fluorobenzyl bromide, NaH, DMF, 0°C, 30 min; (iii) diethyl oxalate, dry MeONa, THF, two separated steps in the same conditions: 50°C, 2 min, 250 W, 300 psi; (iv) CH_2O, Et_2O/H_2O, rt, 2 h.

All the new synthesized compounds were tested for their enzymatic activity. In particular, their ability to inhibit IN, both against the overall integration reaction and more specifically to the strand-transfer step, was evaluated.

The biological results showed that the performed modifications negatively influenced the biological activity. In fact, only 3-hydroxyfuran-2(5*H*)-one **12** showed moderate activity on the strand-transfer step (IC_{50}=72.59±11.64) and thus it was further investigated for anti-HIV activity in MT-4 cells (Table 2).

The obtained results highlighted that derivative **12** showed the same EC_{50} value (0.55 μM) and a better selectivity index (SI=130) than for our diketo acid lead compound (CHI-1043).

Table 2. Anti-HIV activity of **12**

Compound	**IN activity**		**Activity in MT-4 cells**		
	Over-all IC_{50} **[μM]**[a]	**ST** IC_{50} **[μM]**[b]	**HIV-1** EC_{50} **[μM]** [c]	**Cytotoxicity** CC_{50} **[μM]** [d]	**SI** [e]
12	≥100	72.59±11.64	0.55±0.22	71.39±12.15	130
CHI-1043	0.08 ± 0.003	0.14 ± 0.11	0.59 ± 0.38	41.1 ± 16.7	70

All data represent average results ± SD.

[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%.

[e] Selectivity index: ratio CC_{50}/EC_{50}.

On these bases it is possible to suggest a probable pro-drug mechanism of action of the cyclic DKA (**12**) which, because the long incubation period with MT-4 cells, might afford the corresponding DKA CHI-1043 and thus showing the same IC_{50} value. In conclusion, a novel series of 1*H*-indole derivatives were obtained through different synthetic approaches. Biological results demonstrated that the new molecules generally showed a low activity except for compound **12,** a new anti-HIV agent found to be more selective and less toxic than the lead compound CHI-1043.

ACKNOWLEDGMENTS

This work was supported by the European Commission HEALTH-F3-2008-201032 (THINC project).

EXPERIMENTAL

General All microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis

System, Model Discover working at the potency necessary for refluxing under atmospheric conditions (i.e. 100-300W). Melting points were determined on a BUCHI Melting Point B-545 apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ± 0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (230-400 mesh). ^{1}H-NMR spectra were recorded in $CDCl_3$ with TMS as internal standard or DMSO on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm).

General procedure for the synthesis of 2-(4-methoxy-1*H*-indol-3-yl)-*N*-(heterocarbocycle)-2-oxoacetamides (1a-1d)

Oxalyl chloride (0.174 mL, 2 mmol) was added dropwise at 0°C to a solution of 4-methoxy-1*H*-indole (0.147 g, 1 mmol) in anhydrous Et_2O (5 mL) under inert gas conditions. The reaction mixture was stirred at 0°C for 3 h and the solvent was removed under reduced pressure. The residue was dissolved in anhydrous THF (5 mL) and the appropriate heterocyclic amine (1 mmol) was added. The mixture was stirred for 2 h under N_2 atmosphere at rt, quenched with a saturated aqueous $NaHCO_3$ solution (10 mL) and extracted with EtOAc (10 mL x 3). The combined organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (FC) using cyclohexane/EtOAc = 1:1 as eluent.

2-(4-Methoxy-1*H*-indol-3-yl)-*N*-(1*H*-pyrazol-3-yl)-2-oxoacetamide (1a): Oil, yield 55%. ^{1}H-NMR (δ) 3.68 (s, 3H, OCH_3), 6.60-8.22 (m, 6H, ArH), 10.77 (bs, 1H, NH), 12.19 (bs, 1H, NH), 12.43 (bs, 1H, NH). Anal. Calcd for $C_{14}H_{12}N_4O_3$: C, 59.15; H, 4.25; N, 19.71. Found: C, 59.47; H, 4.02; N, 19.84.

2-(4-Methoxy-1*H*-indol-3-yl)-*N*-(5-methylisoxazol-3-yl)-2-oxoacetamide (1b): Mp 233-235°C, yield 38%. ^{1}H-NMR (δ) 2.41 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 6.66-8.21 (m, 5H, ArH), 11.42 (bs, 1H, NH), 12.23 (bs, 1H, NH). Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C,60.42; H, 4.21; N, 14.23.

2-(4-Methoxy-1*H*-indol-3-yl)-*N*-(3-methylisoxazol-5-yl)-2-oxoacetamide (1c): Mp 221-223°C, yield 44%. ^{1}H-NMR (δ) 2.22 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 6.27-8.26 (m, 5H, ArH), 12.23 (bs, 1H, NH), 12.34 (bs, 1H, NH). Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 60.32; H, 4.53; N, 14.22.

2-(4-Methoxy-1*H*-indol-3-yl)-*N*-(thiophen-2-carboxymethyl-3-yl)-2-oxoacetamide (1d): Mp 218-220°C, yield 62%. ^{1}H-NMR (δ) 3.80 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.72-8.63 (m, 6H, ArH), 11.45 (bs, 1H, NH), 12.33 (bs, 1H, NH). Anal. Calcd for $C_{17}H_{14}N_2O_5S$: C, 56.98; H, 3.94; N, 7.82. Found: C, 57.15; H, 3.76; N, 8.01.

General procedure for the synthesis of 2-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(heterocarbocycle)-2-oxoacetamides (2b-2d)

Potassium *tert*-butoxide (0.337 g, 3 mmol) was added at 0°C to a solution of 2-(4-methoxy-1*H*-indol-3-yl)-*N*-(heterocarbocycle)-2-oxoacetamide (**1b-1d**) in tetrahydrofuran (5mL) and the mixture was stirred for 30 min. 4-Fluorobenzyl bromide (0.184 mL, 1.5 mmol) was added dropwise and the reaction was allowed to warm to rt. After 4 h the reaction was quenched by a saturated aqueous NH_4Cl solution (10 mL), the suspension extracted with ethyl acetate (10 mL x 3) and dried over Na_2SO_4. After removal of the solvent the residue was chromatographed on silica gel (cyclohexane/EtOAc = 6:4) to give **2b-2d** as a yellow solid.

2-[1-(4-Fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(5-methylisoxazol-3-yl)-2-oxoacetamide (2b): Mp 182-184°C, yield 35%. ^{1}H-NMR (δ) 2.41 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 5.51 (s, 2H, CH_2), 6.71-8.47 (m, 9H, ArH), 11.48 (bs, 1H, NH). Anal. Calcd for $C_{22}H_{18}FN_3O_4$: C, 64.86; H, 4.45; N, 10.31. Found: C, 65.03; H, 4.32; N, 10.48.

2-[1-(4-Fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(3-methylisoxazol-5-yl)-2-oxoacetamide (2c): Mp 173-175°C, yield 64%. 1H-NMR (δ) 2.22 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 5.51 (s, 2H. CH_2), 6.27-8.51 (m, 9H, ArH), 12.25 (bs, 1H, NH). Anal. Calcd for $C_{22}H_{18}FN_3O_4$: C, 64.86; H, 4.45; N, 10.31. Found: C, 64.72; H, 4.37; N, 10.65.

2-[1-(4-Fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(thiophen-2-carboxymethyl-3-yl)-2-oxoacetamide (2d): Mp 177-179°C, yield 58%. ^{1}H-NMR (δ) 3.96 (s, 3H, CH_3), 3.99 (s, 3H, OCH_3), 5.34 (s, 2H, CH_2), 6.75-8.74 (m, 10H, ArH), 11.72 (bs, 1H, NH). Anal. Calcd for $C_{24}H_{19}FN_2O_5S$: C, 61.79; H, 4.11; N, 6.01. Found: C, 61.98; H, 4.02; N, 6.35.

Procedure for the synthesis of 2-(4-methoxy-1*H*-indol-3-yl)-*N*-(thiophen-2-carboxylic-3-yl)-2-oxoacetamide (1e) and 2-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(thiophen-2-carboxylic-3-yl)-2-oxoacetamide (2e)

A methanol solution (5 mL) of **1d** and **2d** (1 mmol) was treated with 2N NaOH (2 mL, 20 mmol) and stirred at rt for 3.5 h respectively. The reaction mixture was then acidified with conc. HCl to give a solid that was collected and recrystallized from EtOH/Et_2O.

2-(4-Methoxy-1*H*-indol-3-yl)-*N*-(thiophen-2-carboxylic-3-yl)-2-oxoacetamide (1e): Mp 241-243°C decomp., yield 75%. ^{1}H-NMR (δ) 3.79 (s, 3H, OCH_3), 6.72-8.60 (m, 6H, ArH), 11.49 (bs, 1H, NH), 12.40 (bs, 1H, NH). Anal. Calcd for $C_{16}H_{12}N_2O_5S$: C, 55.81; H, 3.51; N, 8.14. Found: C, 56.07; H, 3.73; N, 8.39.

2-[1-(4-Fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(thiophen-2-carboxylic-3-yl)-2-oxoacetamide (2e): Mp 240-242°C dec., yield 65%. ^{1}H-NMR (δ) 3.77 (s, 3H, OCH_3), 5.54 (s, 2H, CH_2), 6.73-8.76 (m, 10H, ArH), 12.28 (bs, 1H, NH). Anal. Calcd for $C_{23}H_{17}FN_2O_5S$: C, 61.06; H, 3.79; N, 6.19. Found: C, 61.42; H, 3.58; N, 6.23.

Procedure for the synthesis of 1-(4-fluorobenzyl)-4-methoxy-1*H*-indole (1f)

1f was obtained using the same procedure for derivatives **2b-2d**, starting from 4-methoxy-1*H*-indole (0.147 g, 1 mmol) and 4-fluorobenzyl bromide (0,283 g, 1,5 mmol).

Oil, yield 68%. ^{1}H-NMR (δ) 3.96 (s, 3H, OCH_3), 5.27 (s, 2H, CH_2), 6.52-7.25 (m, 9H, ArH). Anal. Calcd for $C_{16}H_{14}FNO$: C, 75.28; H, 5.53; N, 5.49. Found: C, 75.33; H, 5.51; N, 5.54.

Procedure for the synthesis of 2-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-*N*-(1*H*-pyrazol-3-yl)-2-oxoacetamide (2a)

2a was obtained using the same procedure for derivatives **1a-1d**, starting from **1f** (0.255 g, 1 mmol), oxalyl chloride (0.174 mL, 2 mmol) and 3-aminopyrazole (0.06 mL, 1 mmol). Mp 198-200°C, yield 45%. ^{1}H-NMR (δ) 4.14 (s, 3H, OCH_3), 5.62 (s, 2H, CH_2), 6.63-8.70 (m, 10H, ArH), 12.60 (bs, 1H, NH), 13.17 (bs, 1H, NH). Anal. Calcd for $C_{21}H_{17}FN_4O_3$: C, 64.28; H, 4.37; N, 14.28. Found: C, 64.39; H, 4.68; N, 14.65.

Procedure for the synthesis of 1*H*-indole-3-carboxamide (3)

3-Cyanoindole (0.142 g, 1 mmol) was added to a methanolic solution of NaOH (1,5 N, 5 mL) and the mixture treated with a solution of hydrogen peroxide (3%, 0.5 mL). The precipitate was filtered out and the solvent removed under vacuo. The resulting residue was purified by silica gel column chromatography ($CHCl_3/CH_3OH$ = 9:1) to give intermediate **3** as a white solid. Mp 197-199°C, yield 55%. ^{1}H-NMR (δ) 6.78 (bs, 2H, NH_2), 7.04-8.13 (m, 5H, ArH), 11.51 (bs, 1H, NH). Anal. Calcd for $C_9H_8N_2O$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.78; H, 5.21; N, 17.34.

Procedure for the synthesis of 1*H*-indol-(3-carbonyl)-furan-2-carboxamide (4)

A suspension of amide **3** (0.160 g, 1 mmol) and sodium hydride (0.120 g, 5 mmol) in THF/DMF = 9:1 (10 mL) was stirred at rt under N_2 atmosphere for 10 min. 2-Furoyl chloride (0.196 g, 1,5 mmol) was added dropwise at 0°C and the reaction mixture was stirred at rt for an additional 30 min. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (10 mL x 3), dried over Na_2SO_4, and concentrated. The resulting residue was crystallized upon trituration with Et_2O and the solid was recrystallized from EtOH to provide derivative **4**. Mp 231-233°C, yield 64%. ^{1}H-NMR (δ) 6.71-8.36 (m, 8H, ArH), 10.54 (bs, 1H, NH), 11.98 (bs, 1H, NH). Anal. Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02. Found: C, 66.28; H, 4.11; N, 11.31

Procedure for the synthesis of *N*-[1-(4-fluorobenzyl)-1*H*-indol-3-carbonyl]-furan-2-carboxamide (5)

5 was obtained using the same procedure for derivatives **(1f, 2b-2d)**, starting from **4** (0.254 g, 1 mmol) and 4-fluorobenzyl bromide (0,283 g, 1,5 mmol). Mp 84-85°C, yield 48%. ^{1}H-NMR (δ) 5.50 (s, 2H, CH_2), 6.72-8.54 (m, 12H, ArH), 10.64 (bs, 1H, NH). Anal. Calcd for $C_{21}H_{15}FN_2O_3$: C, 69.61; H, 4.17; N, 7.73. Found: C, 69.48; H, 4.31; N, 7.56.

Procedure for the synthesis of 2,3-dimethoxybenzoyl chloride

A mixture of 2,3-dimethoxybenzoic acid (0.182 g, 1 mmol) and thionyl chloride (4 mL, 55 mmol) was refluxed under stirring for 2h. The solvent was removed under reduced pressure to give the crude product which was used in the next step without further purification.

Procedure for the synthesis of (4-methoxy-1*H*-indol-3-yl)(2,3-dimethoxyphenyl)methanone (6)

1.5 mL of Et_2AlCl (1 M in hexane) was added at 0°C to a stirred solution of 4-methoxy-1*H* indole (0.147 g, 1 mmol) in CH_2Cl_2 (2 mL), and the mixture was stirred at 0°C for 30 min. CH_2Cl_2 solution (0,5 mL) of 2,3-dimethoxybenzoyl chloride (0.400 g, 2 mmol) was added dropwise and the resulting solution was placed in a cylindrical quartz tube (Ø 2 cm). The reaction mixture was then stirred and irradiated in a microwave oven at 250 Watt, 10 min and 50°C. After cooling, pH 7 aqueous buffer was added and the resulting precipitate was filtered, washed with Et_2O, dried under vacuum and crystallized from EtOH/Et_2O 3:7.Mp 184-186°C, yield 52%. ^{1}H-NMR (δ) 3.59 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.60-7.53 (m, 7H, ArH), 11.91 (bs, 1H, NH). Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.72; H, 5.33; N, 4.74.

Procedure for the synthesis of (1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl)(2,3-dimethoxyphenyl)-methanone (7)

7 was obtained using the same procedure for derivatives **(2a-2d)**, starting from **6** (0,311 g, 1 mmol) and 4-fluorobenzyl bromide (0,283 g, 1.5 mmol). Mp 170-172°C, yield 45%. ^{1}H-NMR (δ) 3.79 (s, 6H, 2-OCH_3), 3.90 (s, 3H, OCH_3), 5.24 (s, 2H, CH_2), 6.64-7.47 (m, 11H, ArH). Anal. Calcd for $C_{25}H_{22}FNO_4$: C, 71.59; H, 5.29; N, 3.34. Found: C, 71.83; H, 5.48; N, 3.19.

Procedure for the synthesis of [1-(4-fluorobenzyl)-4-hydroxy-1*H*-indol-3-yl](2,3-dihydroxyphenyl)-methanone (8)

Derivative **7** (0,419 g, 1 mmol) was dissolved in CH_2Cl_2 (5 mL), treated with BBr3 (1 M in CH_2Cl_2), (6 mL, 6 mmol) and stirred overnight. CH_3OH (7 mL) was carefully added at 0°C and the solvents removed under reduced pressure. The residue was dissolved in EtOAc (10 mL) and washed with water (10 mL x 3). The organic layer was dried (Na_2SO_4) and concentrated. The crude product was crystallized from EtOH. Mp 192-193°C, yield 85%. ^{1}H-NMR (δ) 5.32 (s, 2H, CH_2), 5.79 (s, 1H, OH), 6.84-7.74 (m, 11H, ArH), 10.62 (s, 1H, OH), 10.85 (s, 1H, OH). Anal. Calcd for $C_{22}H_{16}FNO_4$: C, 70.02; H, 4.27; N, 3.71. Found: C, 70.24; H, 4.36; N, 3.59.

For the synthesis of 3-acetyl-4-methoxy-1*H*-indole (9), 3-acetyl-4-methoxy-1-benzyl-1*H*-indole (10) and ethyl 4-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-yl]-2-hydroxy-4-oxobut-2-enoate (11) see experimental part of reference [10]

Procedure for the synthesis of 4-[1-(4-fluorobenzyl)-4-methoxy-1*H*-indol-3-oyl)-3-hydroxyfuran-2(5*H*)-one (12)

A solution of 40% aqueous formaldehyde in water (4 mL) was added to a mixture of **11** (0,397 g, 1 mmol) in Et_2O (5 mL). The stirring was then continued until clear layers were formed (usually within 1-2 hours). Sometimes, an additional 4 mL of water was added if the reaction was especially thick or when the solid appeared to react slowly. The clear, aqueous bottom layer was removed and the organic layer 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 precipitate and the solution was then cooled overnight to ensure complete product formation. The resulting solid was collected, dried and recrystallized from EtOH. Mp 94-96°C, yield 80%; ^{1}H-NMR (δ) 3.76 (s, 3H, OCH_3), 5.03 (s, 2H, CH_2), 5.44 (s, 2H, CH2), 6.66-8.18 (m, 8H, ArH). Anal. Calcd for $C_{21}H_{16}FNO_5$: C, 66.14; H, 4.23; N, 3.67. Found: C, 66.47; H, 4.11; N, 3.43.

Bio-assays. Overall integrase assay using an enzyme-linked immunosorbent assay (ELISA) and Strand-Transfer inhibition. We use enzyme-linked immunosorbent assays to determine the susceptibility of the HIV-1 integrase enzyme towards different compounds. These assays use an oligonucleotide substrate of which one oligonucleotide (5’-ACTGCTAGAGATTTTCCACACTGACTAAAAGGGTC -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’-GACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT -3’). For the Strand Transfer assay the second oligonucleotide lacks GT at the 3’end. The integrase enzyme was diluted in 750 mM 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 MgCl2, 5mM DTT, 20 mM HEPES pH 7.5, 5% PEG and 15% DMSO. The reaction was carried out at 37°C for 1 h. 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-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 (CCID50) of the HIV(IIIB) strain was determined by titration of the virus stock using MT-4 cells. For the drug-susceptibility assays MT-4 cells were infected with 100-300 CCID50 of the virus stock in the presence of five-fold serial dilutions of the antiviral drugs. The number of viable MT-4 cells was determined after 5 days of incubation. 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 (CC_{50}) was determined.

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