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Synthesis of Novel MKC-442 Analogues with Potent Activities against HIV-1

Bis(alken-1-yloxy)methanes **2** were synthesized by reacting 2-cyclohexenol, 3-cyclohexenylmethanol, cinnamyl alcohol and its α-methyl analogue with dibromomethane. Condensation of **2** with 5,6-disubstituted uracil derivatives 1 resulted in the desired MKC-442 analogues **3–6**. The most active compounds, N-1 cinnamyloxymethyl- and N-1 2-methyl-3-phenylallyloxymethyl substituted 5-ethyl-6-(3,5-dimethylbenzyl)uracils (**5b** and **6b**), showed activity against wild-type HIV-1 in the nanomolar range, and against Y181C and Y181C+K103N, mutant strains known to be resistant to MKC-442, in the micromolar range.

Keywords: Non-nucleoside reverse transcriptase inhibitors; MKC-442 analogues; Human immunodeficiency virus (HIV-1); HIV-1 mutants; Alkenyloxymethyluracils

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Introduction

The virally encoded reverse transcriptase (RT) of HIV is an important target for the development of anti-AIDS drugs. It plays a multifunctional role in the conversion of the single-stranded RNA viral genome to double-stranded DNA [1]. The non-nucleoside reverse transcriptase inhibitors (NNRTIs), in contrast to nucleoside reverse transcriptase inhibitors (NRTIs) such as AZT [2], ddC [3], ddI [4], 3TC [4], are highly specific since they do not bind to cellular polymerases.

NNRTIs have been found to bind to a specific allosteric site on HIV-1 RT near the polymerase site. Once bound, they interfere with reverse transcription by altering either the conformation or mobility of RT, thereby leading to a non competitive inhibition of the enzyme [5–7]. Since the original synthesis of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) [8], many analogues of it have been developed and investigated for their activity against HIV-1 [9–12]. The analogue 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, also known as Emivirine or Coactinone)[13], showed high activity against HIV-1 but unfortunately in phase III clinical trials it was also found to activate a liver enzyme in the 450 family which metabolizes protease inhibitors [14]. For this reason, and as a part of our continuing interest in the chemistry of NNRTIs [15–17], we are interested in whether alternative drug candidates can be found among the HEPT analogues. Recently, we reported that MKC-442 analogues with a 1-allyloxymethyl substituent showed activity against HIV-1 in the picomolar range [18]. Here we describe the structure activity relationship when the double bond is more heavily substituted.

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Chemistry

The 5,6-disubstituted uracil derivatives **1a–c** were prepared from the corresponding 3-oxoester which was itself prepared by treating arylacetonitrile with ethyl 2-bromobutyrate or methyl 2-bromo-3-methylbutyrate in THF in the presence of activated zinc [19]. The di-(substituted-alkenyloxy)-methane derivatives 2a-d were prepared from the corresponding alcohols, 2-cyclohexen-1-ol, 3-cyclohexene-1-methanol, trans-cinnamyl alcohol, and trans-2-methyl-3-phenyl-2-propen-1-ol, in a reaction with dibromomethane using potassium hydroxide in the presence of tetrabutylammonium bromide in refluxing anhydrous benzene according to the method of Nazaretyan et al [20]. According to NMR data, compound **2a** was a (1:1) diastereomeric mixture, whereas **2b** was a nearly pure diastereomer. ^{13}C-NMR spectra of compounds **2a–d** showed the presence of a O-CH_2-O group at 91–96 ppm.

The uracil derivatives **1b**, **c** were silylated with *N,O*-bis-(trimethylsilyl)acetamide (BSA) [21] in acetonitrile and then treated with bis(2-cyclohexen-1-yloxy)methane (**2a**) as described by Vorbrüggen [22]. This consisted of using trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid catalyst to give 1-[(2-cyclohexen-1-yl)oxy]methyl-6-(3,6-dimethylbenzyl)-5-ethyluracil (**3a**) and 1-[(2-cyclohexen-1-yl)oxy]methyl-6-(3,6-dimethylbenzyl)-5-isopropyluracil (**3b**) in 87 % and 84 % yields, respectively. Using bis(3-cyclohexen-1-yl-methoxy)-methane (**2b**) in a reaction with uracils **1a–c** produced the corresponding N1-(3-cyclohexen-1-yl-methoxy) methyluracil derivatives **4a-c** in 72–79 % yields.

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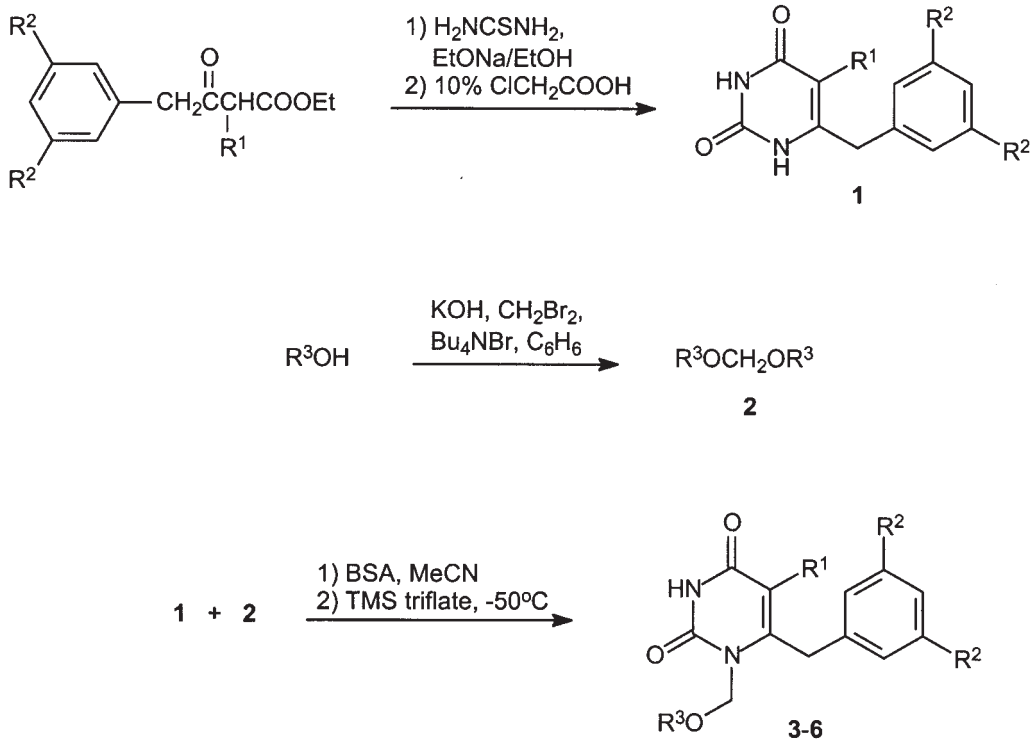
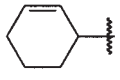
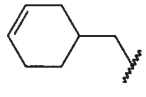
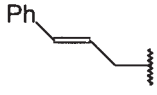
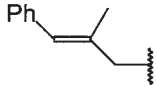


Cpd. **1,3-6**	R^1	R^2	R^3	Cpd. **2**
1a	Pr^i	H		
1b	Et	Me		
1c	Pr^i	Me		
3a	Et	Me		**2a**
3b	Pr^i	Me		
4a	Pr^i	H		**2b**
4b	Et	Me		
4c	Pr^i	Me		
5a	Pr^i	H		**2c**
5b	Et	Me		
5c	Pr^i	Me		
6a	Pr^i	H		**2d**
6b	Et	Me		
6c	Pr^i	Me		

Scheme 1. Synthesis of the MKC-442 analogues **3–6**.

Full Paper

These novel analogues may give improved binding to the allosteric site of HIV-1 RT due to stacking interactions between the alkenyl group and the aromatic amino acid Phe227 in RT. Therefore, we found it of interest to study some alkenyl substituents having a conjugated phenyl group. Using the conditions described above, compounds **1 a–c** were condensed with bis(*trans*-cinnamyloxy)methane (**2 c**) to give the corresponding N1-(*trans*-cinnamyloxy-methyl)-5,6-disubstituted uracil derivatives **5 a–c** in 55–60 % yields. The corresponding condensation with bis((*E*)-2-methyl-3-phenylallyloxy)methane (**2 d**) afforded 5,6-disubstituted-1-((*E*)-2-methyl-3-phenylallyloxy)methyl uracils (**6 a–c**) in 56–59 % yields. The structures of the synthesized compounds were determined by comparison to similar NMR data [15–17, 19, 21].

Biological properties

Compounds 3–6 were tested for their activity against HIV-1 in MT-4 cells infected with the wild-type HIV-1 strain IIIB, the NNRTI resistant strain N119, which contains one mutation (Y181C) or with the NNRTI resistant strain A17, with contains two mutations (K103N + Y181C) (Table 1).

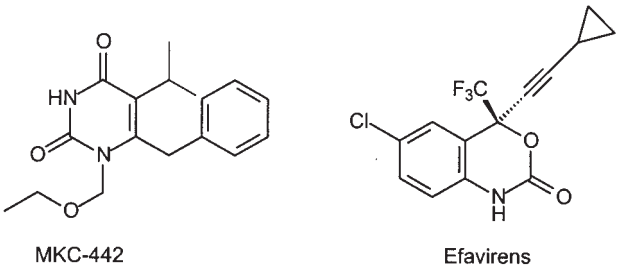


Figure 1. Chemical structure of MKC-442 and Efavirens.

Only compound **5 b,** which contains a N-1 cinnamyl substituent, and compound **6 b,** which contains a homologous substituent, showed marginally higher activity against HIV-1 than MKC-442 and efavirenz and better activity against the N119 strain than that observed with MKC-442. These two compounds also showed activity against the double mutated strain A17 which was comparable to that observed with efavirenz. However, efavirenz displayed higher activity against the N119 strain with one mutation.

Table 1. Antiviral activity of compounds **3–6** against HIV-1 in MT-4 cells[a].

Compound	Wild-type HIV-1 IC_{50}[b] (μM)	Wild-type HIV-1 CC_{50}[c] (μM)	SI[d]	N119 (Y181C) IC_{50}[b] (μM)	A17 (K103N + Y181C) IC_{50}[b] (μM)
3 a	0.04	31	775	>100	>100
3 b	0.03	28	930	>100	>100
4 a	0.30	34	113	ND[e]	ND[e]
4 b	0.02	24	1200	>100	>100
4 c	0.08	30	375	>100	>100
5 a	0.01	29	2900	>100	>100
5 b	0.003	32	10667	4	10
5 c	0.06	31	517	6	>100
6 a	0.04	30	750	>100	>100
6 b	0.003	30	10000	2	4
6 c	0.03	28	933	21	>100
MKC-442	0.02	>100	5000	44	>100
Efavirenz	0.01	>100	>10000	0.3	2.7

[a]All data represent mean values for three separate experiments.
[b]Inhibitory concentration of compound achieving 50 % inhibition of HIV-1 multiplication in MT-4-infected cells.
[c]Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50 %.
[d]Selectivity index: ratio CC_{50}/IC_{50}.
[e]ND = not tested. The symbol (>) indicates that CC_{50} was not reached at the highest concentration tested. For a description of the assay see the Experimental Section.

Experimental

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrophotometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C with TMS as an internal standard. EI mass spectra were recorded on a Finnigan MAT SSQ 710. MALDI spectra were recorded on a 4.7 Tesla Ultima Fourier transform Mass spectrometer (IonSpec, Irvine, CA). Melting points were determined using a Büchi melting point apparatus. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Microanalyses were carried out at the H. C. Ørsted Institute, Copenhagen, Denmark. Solvents were purified using standard procedures [23].

General Procedure for the Synthesis of Bis(alkenyloxy)-methane Derivatives **2 a**–**d**

The appropriate alcohol (100 mmol), dibromomethane (8.79 g, 50.5 mmol) and tetrabutylammonium bromide (1.74 g, 5.35 mmol) were added to potassium hydroxide (5.66 g, 101 mmol) in benzene (30 mL), and the suspension was heated under reflux for 5 hours. After cooling, water (50 mL) was added and the resulting solution extracted with ether (3 × 50 mL). The ether phase was dried with anhydrous magnesium sulphate and evaporated. The residue was purified by distillation under reduced pressure (**2 a** and **2 b**) or chromatographed on a column of silica gel with CHCl3 (**2 c** and **2 d**).

Bis(2-cyclohexen-1-yloxy)methane (**2 a**)

Yield 4.47 g (43 %) as a colourless oil; bp 123 °C/12 mmHg. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.53–2.11 (m, 12 H, 6 × CH_2), 4.17–4.20 (m, 2 H, 2 × CH), 4.82 (s, 2 H, CH_2), 5.74–5.79 (m, 2 H, 2 × CH), 5.8–5.90 (m, 2 H, 2 × CH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 19.04 (CH_2), 25.07 (CH_2), 28.69, 28.91 (CH_2), 69.81, 70.01 (CH), 91.39, 91.65 (CH_2), 127.73, 127.88 (CH), 130.90, 130.96 (CH).

Bis(3-cyclohexen-1-ylmethyloxy)methane (**2 b**)

Yield 5.44 g (46 %) as a colourless oil; bp 135 °C/12 mmHg. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.23–1.36 (m, 2 H, 6a-H, 6′a-H), 1.70–2.16 (m, 12 H, 4 × CH_2, 2 × CH, 6b-H, 6′-H), 3.42 (m, 4 H, 2 × CH_2), 4.68 (s, 2 H, CH_2), 5.62–5.70 (m, 4 H, 4 × CH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 24.57 (CH_2), 25.70 (CH_2), 28.53 (CH_2), 33.90 (CH), 72.60 (CH_2), 95.52 (CH_2), 125.91 (CH), 127.03 (CH).

Bis((E)-cinnamyloxy)methane (**2 c**)

Yield 7.4 g (53 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.27 (dd, 4 H, *J* = 1.4, 6.1 Hz, 2 × CH_2), 4.82 (s, 2 H, CH_2), 6.26 (td, 2 H, *J* = 6.1, 15.9 Hz, 2 × CH), 6.61 (d, 2 H, *J* = 15.9 Hz, 2 × CH), 7.20–7.39 (m, 10 H, H_{arom}). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 68.04 (CH_2), 93.75 (CH_2), 125.53 (CH), 126.44, 127.65, 128.50, 136.60 (C_{arom}), 132.60 (CH).

Bis((E)-2-methyl-3-phenylallyloxy)methane (**2 d**)

Yield 5.9 g (59 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.92 (s, 6 H, 2 × CH_3), 4.18 (s, 4 H, 2 × CH_2), 4.82 (s, 2 H, CH_2), 6.56 (s, 2 H, 2 × CH), 7.19–7.35 (m, 10 H, H_{arom}). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 15.64 (CH_3), 73.58 (CH_2), 93.64 (CH_2), 127.11 (CH), 126.44, 128.06, 128.88, 134.60 (C_{arom}), 137.45 (CH).

General Procedure for the Synthesis of 1-(2-alkenyloxymethyl)uracils **3 a**, **b**, **4 a**–**c**, **5 a**–**c** and **6 a**–**c**

Uracil (**1**, 1 mmol) was stirred in anhydrous CH_3CN (15 mL) under N_2 and *N,O*-bis-(trimethylsilyl)acetamide (BSA) (0.87 mL, 3.5 mmol) was added. After a clear solution was obtained (10 min), the reaction mixture was cooled to –50 °C and trimethylsilyl trifluoromethansulfonate (TMS triflate) (0.18 mL, 1 mmol) was added followed by dropwise addition of bis(2-alkenyloxy)methane (**2**, 0.42 g, 2 mmol). The reaction mixture was stirred at room temperature for 3–4 hours. Cold saturated $NaHCO_3$ solution (5 mL) was added, and the solvent was evaporated under reduced pressure at room temperature. The residue was extracted with ether (3 × 50 mL), dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The product was chromatographed on a column of silica gel using $CHCl_3$ for compounds **3 a**, **b** and **4 a**–**c** and Et_2O:petroleum ether (ν:ν = 1:6) for compounds **5 a–c** and **6 a–c**.

1-(2-Cyclohexen-1-yloxymethyl)-6-(3,5-dimethylbenzyl)-5-ethyluracil (**3 a**)

Yield 0.31 g (87 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.99 (t, 3H, *J* = 7.3 Hz, CH_3), 1.47–1.96 (m, 6 H, 3 × CH_2), 2.21 (s, 6 H, 2 × CH_2), 2.36 (q, 2 H, *J* = 7.3 Hz, CH_2), 4.03 (s, 2 H, CH_2), 4.13 (s, 2 H, CH_2), 5.08–5.11 (m, 1 H, CH), 5.69–5.80 (m, 2 H, 2 × CH), 6.63 (s, 2 H, H_{arom}), 6.82 (s, 1 H, H_{arom}), 9.31 (s, 1 H, NH). NMR ($CDCl_3$): δ (ppm) = 13.75 (CH_3), 18.89 (CH_3), 21.22 (CH_2), 24.99 (CH_2), 28.57 (CH_2), 31.93 (CH_2), 33.19 (CH_2), 65.43 (CH_2), 71.69 (CH), 116.69 (C-5), 124.96, 126.86, 134.99, 138.78 (C_{arom}), 129.83 (CH), 130.44 (CH), 149.48 (C-6), 151.73 (C-2), 163.32 (C-4). HRMS-MALDI: *m*/*z* = 391.1992 (M + Na^+, $C_{22}H_{28}N_2NaO_3$); requires 391.1994.

1-(2-Cyclohexen-1-yloxymethyl)-6-(3,5-dimethylbenzyl)-5-isopropyluracil (**3 b**)

Yield 0.32 g (84 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.28 (d, 6 H, *J* = 7.0 Hz, 2 × CH_3), 1.59–2.02 (m, 6 H, 3 × CH_2), 2.28 (s, 6 H, 2 × CH_3), 2.64 (heptet, 1 H, *J* = 7.0 Hz, CH), 4.13 (s, 4 H, 2 × CH_2), 5.20–5.24 (m, 1 H, CH), 5.82–5.91 (m, 2 H, 2 × CH), 6.71 (s, 2 H. H_{arom}), 6.89 (s, 1 H, H_{arom}), 9.55 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 18.90 (CH_3), 20.40 (CH_3), 21.23 (CH_2), 24.99 (CH_2), 28.28 (CH), 28.57 (CH_2), 33.27 (CH_2), 65.43 (CH_2), 71.66 (CH), 119.54 (C-5), 124.97, 126.91, 135.13, 138.71 (C_{arom}), 128.73 (CH), 131.70 (CH), 148.88 (C-6), 151.91 (C-2), 162.63 (C-4). HRMS-MALDI: *m*/*z* = 405.2149 (M + Na^+, $C_{23}H_{30}N_2NaO_3$); requires 405.2152.

6-Benzyl-1-(3-cyclohexen-1-ylmethoxymethyl)-5-isopropyluracil (**4 a**)

Yield: 0.29 g (79 %) as a white solid; mp 130–131 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.28 (d, 6 H, *J* = 6.9 Hz, 2 × CH_3), 1.73–1.78 (m, 4 H, 2 × CH_2), 2.03–2.06 (m, 3 H, CH, CH_2), 2.88 (heptet, 1 H, *J* = 6.9 Hz, CH), 3.44 (d, 2 H, *J* = 6.4 Hz, CH_2), 4.20 (s, 2 H, CH_2), 5.15 (s, 2 H, CH_2), 5.63–5.66 (m, 2 H, 2 × CH), 7.11–7.37 (m, 5 H, H_{arom}), 9.50 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 20.39 (CH_3), 24.45 (CH_2), 25.37 (CH_2), 28.28 (CH), 33.35 (CH), 33.74 (CH_2), 73.24 (CH_2), 74.10 (CH_2), 119.76 (C-5), 125.68 (CH), 127.01, 127.25, 129.16, 135.39 (C_{arom}), 127.18 (CH), 148.53 (C-6), 151.96 (C-2), 162.50 (C-4). EI MS: *m*/*z* = 368 (M^+). Anal. Calcd for $C_{22}H_{28}N_2O_3$ (368.47): C, 71.71; H, 7.66; N, 7.60. Found: C, 71.62; H, 7.66; N, 7.38 %.

1-(3-Cyclohexen-1-ylmethoxymethyl)-6-(3,5-dimethylbenzyl)-5-ethyluracil (**4 b**)

Yield 0.28 g (74 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.05 (t, 3 H, *J* = 7.4 Hz, CH_3), 1.74–1.78 (m, 4 H, 2 × CH_2), 1.81–1.84 (m, 3 H, CH, CH_2), 2.29 (s, 6 H, 2 × CH_3), 2.44 (q, 2 H, *J* = 7.4 Hz, CH_2), 3.43 (d, 2 H, *J* = 6.2 Hz, CH_2), 4.09 (s, 2 H, CH_2), 5.13 (s, 2 H, CH_2), 5.64–5.66 (m, 2 H, 2 × CH), 6.70 (s, 2 H, H_{arom}), 6.90 (s, 1 H, H_{arom}), 9.41 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.76 (CH_3), 19.11 (CH_2), 21.26 (CH_3), 24.45 (CH_2), 25.38 (CH_2), 28.29 (CH), 33.13 (CH), 33.75 (CH_2),

73.10 (CH_2), 74.09 (CH_2), 116.78 (C-5), 124.99, 127.01, 134.94, 138.84 (C_{arom}), 125.68 (CH), 128.91 (CH), 149.43 (C-6), 151.88 (C-2), 163.37 (C-4). HRMS-MALDI: *m/z* = 405.2149 (M + Na^+, $C_{23}H_{30}N_2NaO_3$); requires 405.2152.

1-(3-Cyclohexen-1-ylmethoxymethyl)-6-(3,5-dimethylbenzyl)-5-isopropyluracil (**4 c**)

Yield: 0.29 g (72 %) as a white solid; mp 149–150 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.29 (d, 6 H, *J* = 7.0 Hz, CH_3), 1.59–1.93 (m, 4H, 2 × CH_2), 2.03–2.17 (m, 3 H, CH, CH_2), 2.23 (s, 6 H, 2 × CH_3), 2.83 (heptet, 1 H, *J* = 7.0 Hz, CH), 3.44 (d, 2 H, *J* = 6.4 Hz, CH_2), 4.11 (s, 2 H, CH_2), 5.15 (s, 2 H, CH_2), 5.64–5.66 (m, 2 H, 2 × CH), 6.71 (s, 2 H, H_{arom}), 6.90 (s, 1 H, H_{arom}), 9.42 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 20.40 (CH_3), 21.25 (CH_3), 24.47 (CH_2), 25.39 (CH_2), 28.30 (CH), 33.17 (CH), 33.76 (CH_2), 73.26 (CH_2), 74.09 (CH_2), 119.52 (C-5), 124.99, 127.02, 135.09, 138.77 (C_{arom}), 125.70 (CH), 128.79 (CH), 148.81 (C-6), 151.99 (C-2), 162.54 (C-4). EI MS: *m/z* = 396 (M^+). Anal. Calcd for $C_{24}H_{32}N_2O_3$ (396.52): C, 72.70; H, 8.13; N, 7.06. Found: C, 72.69; H, 8.18; N, 6.82 %.

6-Benzyl-1-((E)-cinnamyloxymethyl)-5-isopropyluracil (**5 a**)

Yield 0.21 g (55 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.28 (d, 6 H, *J* = 6.8 Hz, 2 × CH_3), 2.85 (heptet, 1 H, *J* = 6.8 Hz, CH), 4.21 (s, 2 H, CH_2), 4.28 (dd, 2 H, *J* = 1.2, 6.2 Hz, CH_2), 5.21 (s, 2 H, CH_2), 6.19 (td, 1 H, *J* = 6.2, 15.9 Hz, CH), 6.60 (d, 1 H, *J* = 15.9 Hz, CH), 7.10–7.38 (m, 10 H, H_{arom}), 9.51 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 20.37 (CH_3), 28.33 (CH), 33.56 (CH_2), 70.23 (CH_2), 72.59 (CH_2), 119.85 (C-5), 124.61 (CH), 126.51, 127.21, 127.24, 127.84, 128.50, 129.17, 135.30, 136.33 (C_{arom}), 133.38 (CH), 148.36 (C-6), 152.04 (C-2), 162.39 (C-4). HRMS-MALDI: *m/z* = 413.1836 (M + Na^+, $C_{24}H_{26}N_2NaO_3$); requires 413.1856.

1-((E)-Cinnamyloxymethyl)-6-(3,5-dimethylbenzyl)-5-ethyluracil (**5 b**)

Yield: 0.23 g (57 %) as a white solid; mp 131–132 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.04 (t, 3H, *J* = 7.3 Hz, CH_3), 2.26 (s, 6 H, 2 × CH_3), 2.43 (q, 2 H, *J* = 7.3 Hz, CH_2), 4.10 (s, 2 H, CH_2), 4.26 (dd, 2 H, *J* = 1.2, 6.2 Hz, CH_2), 5.18 (s, 2 H, CH_2), 6.19 (td, 1 H, *J* = 6.2, 15.9 Hz, CH), 6.60 (d, 1 H, *J* = 15.9 Hz, CH), 6.70 (s, 2 H, H_{arom}), 6.89 (s, 1 H, H_{arom}), 7.26–7.39 (m, 5H, H_{arom}), 8.89 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.75 (CH_3), 19.20 (CH_2), 21.26 (CH_3), 33.35 (CH_2), 70.27 (CH_2), 72.53 (CH_2), 116.83 (C-5), 124.64 (CH), 124.99, 126.53, 127.91, 128.56, 128.99, 134.86, 136.33, 138.91 (C_{arom}), 133.38 (CH), 149.31 (C-6), 151.77 (C-2), 163.05 (C-4). EI MS: *m/z* = 404 (M^+). Anal. Calcd for $C_{25}H_{28}N_2O_3$ (404.50): C, 74.23; H, 6.98; N, 6.93. Found: C, 74.02; H, 6.91; N, 6.86 %.

1-((E)-Cinnamyloxymethyl)-6-(3,5-dimethylbenzyl)-5-isopropyluracil (**5 c**)

Yield: 0.25 g (60 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.29 (d, 6 H, *J* = 6.8 Hz, 2 × CH_3), 2.27 (s, 6 H, 2 × CH_3), 2.86 (heptet, 1 H, *J* = 6.8 Hz, CH), 4.13 (s, 2 H, CH_2), 4.28 (dd, 2 H, *J* = 1.1, 6.1 Hz, CH_2), 5.21 (s, 2 H, CH_2), 6.23 (td, 1 H, *J* = 6.1, 15.8 Hz, CH), 6.61 (d, 1 H, *J* = 15.8 Hz, CH), 6.71 (s, 2 H, H_{arom}), 6.89 (s, 1 H, H_{arom}), 7.22–7.39 (m, 5H, H_{arom}), 9.66 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 20.38 (CH_3), 21.23 (CH_3), 28.34 (CH), 33.39 (CH_2), 70.22 (CH_2), 72.63 (CH_2), 119.70 (C-5), 124.67 (CH), 124.98, 126.51, 127.83, 128.51, 128.83, 134.99, 136.35, 138.78 (C_{arom}), 133.31 (CH), 148.65 (C-6), 152.13 (C-2), 162.55 (C-4). HRMS-MALDI: *m/z* = 441.2149 (M + Na^+, $C_{26}H_{30}N_2NaO_3$); requires 441.2157.

6-Benzyl-5-isopropyl-1-((E)-2-methyl-3-phenylallyloxymethyl)-uracil (**6 a**)

Yield: 0.23 g (57 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.27 (d, 6 H, *J* = 7.0 Hz, 2 × CH_3), 1.86 (s, 3 H, CH_3), 2.84 (heptet, 1 H, *J* = 7.0 Hz, CH), 4.18 (s, 2 H, CH_2), 4.23 (s, 2 H, CH_2), 5.22 (s, 2 H, CH_2), 6.51 (s, 1 H, CH), 7.12–7.37 (m, 10 H, H_{arom}), 9.40 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 15.49 (CH_3), 20.38 (CH_3), 28.32 (CH), 33.51 (CH_2), 72.87 (CH_2), 75.91 (CH_2), 119.80 (C-5), 126.58, 127.26, 128.07, 128.86, 129.21, 133.96, 135.31 (C_{arom}), 127.53 (CH), 137.14 (*C*(Me)=), 148.43 (C-6), 151.91 (C-2), 162.38 (C-4). HRMS-MALDI: *m/z* = 427.1992 (M + Na^+, $C_{25}H_{28}N_2NaO_3$); requires 427.2000.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-((E)-2-methyl-3-phenyl-allyloxymethyl)uracil (**6 b**)

Yield: 0.25 g (59 %) as a white solid; mp 111–113 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.04 (t, 3 H, *J* = 7.3 Hz, CH_3), 1.87 (s, 3 H, CH_3), 2.27 (s, 6 H, 2 × CH_3), 2.44 (q, 2 H, *J* = 7.3 Hz, CH_2), 4.12 (s, 2 H, CH_2), 4.17 (s, 2 H, CH_2), 5.19 (s, 2 H, CH_2), 6.50 (s, 1 H, CH), 6.71 (s, 2 H, H_{arom}), 6.90 (s, 1 H, H_{arom}), 7.21–7.35 (m, 5 H, H_{arom}), 8.99 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.75 (CH_3), 15.52 (CH_3), 19.18 (CH_2), 21.27 (CH_3), 33.30 (CH_2), 72.78 (CH_2), 75.93 (CH_2), 116.79 (C-5), 126.62 (CH), 125.00, 127.51, 128.11, 128.87, 129.00, 134.00, 134.88, 138.92 (C_{arom}), 137.15 (*C*(Me)=), 149.34 (C-6), 151.73 (C-2), 163.13 (C-4). EI MS: *m/z* = 418 (M^+). Anal. Calcd for $C_{26}H_{30}N_2O_3$ (418.53): C, 74.61; H, 7.22; N, 6.69. Found: C, 74.46; H, 7.19; N, 6.66 %.

6-(3,5-Dimethylbenzyl)-5-isopropyl-1-((E)-2-methyl-3-phenyl-allyloxymethyl)uracil (**6 c**)

Yield: 0.24 g (59 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.28 (d, 6 H, *J* = 6.9 Hz, 2 × CH_3), 1.87 (s, 3 H, CH_3), 2.28 (s, 6 H, 2 × CH_3), 2.83 (heptet, 1 H, *J* = 6.9 Hz, CH), 4.15 (s, 2 H, CH_2), 4.19 (s, 2 H, CH_2), 5.22 (s, 2 H, CH_2), 6.51 (s, 1 H, CH), 6.72 (s, 2 H, H_{arom}), 6.90 (s, 1 H, H_{arom}), 7.24–7.33 (m, 5 H, H_{arom}), 9.54 (s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 15.49 (CH_3), 20.39 (CH_3), 21.24 (CH_3), 28.32 (CH), 33.33 (CH_2), 72.90 (CH_2), 75.88 (CH_2), 119.66 (C-5), 126.55 (CH), 125.00, 127.42, 128.06, 128.86, 134.03, 135.02, 128.81 (C_{arom}), 137.17 (*C*(Me)=), 148.69 (C-6), 152.02 (C-2), 162.54 (C-4).HRMS-MALDI: *m/z* = 455.2311 (M + Na^+, $C_{27}H_{32}N_2NaO_3$); requires 455.2305.

Virus and cells

The inhibitory activity of the analogues against HIV-1 infection was evaluated using MT-4 cells [24] as target cells and the HIV-1 strain HTLV-IIIB [25] as infectious virus. The virus was propagated in H9 cells [24] grown at 37 °C, 5 % CO_2 in RPMI 1640 media containing 10 % heat-inactivated fetal calf serum (FCS) and antibiotics. Culture supernatant was filtered (0.45 nm), aliquoted, and stored at –80 °C until use.

Inhibition of HIV-1 replication

Compounds were evaluated for possible antiviral activity against both strains of HIV-1 using MT4 cells as target cells. MT4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compound for six days. Uninfected control and virus infected cultures without compound added were grown in parallel. Expression of HIV in the cultures was quantified indirectly using the MTT assay [26]. Compounds mediating less than 30 % reduction of HIV expression were considered to be biologically inactive. Compounds were tested in parallel for cytotoxic effect in uninfected MT-4

cultures containing the test dilutions of compound as described above. A 30 % inhibition of cell growth relative to control cultures was considered significant. The 50 % inhibitory concentration (IC_{50}) and the 50 % cytotoxic concentration (CC_{50}) were determined by interpolation from the plots of percent inhibition versus concentration of compound. The test for activity against HIV-1 was performed in MT-4 cell cultures infected with either wild-type HIV-1 (strain IIIB [26]) or NNRTI resistant HIV-1 (strain N119 [27], strain A17 [28, 29].

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