Synthesis of 6-(3,5-Dichlorobenzyl) Derivatives as Isosteric Analogues of the HIV Drug 6-(3,5-Dimethylbenzyl)-1-(ethoxymethyl)-5-isopropyluracil (GCA-186)

Esben R. Sørensen[a], Nasser R. El-Brollosy[a], Per T. Jørgensen[a], Erik B. Pedersen[a], Claus Nielsen[b]

[a] Nucleic Acid Center*, Department of Chemistry, University of Southern Denmark, Odense M, Denmark
[b] Retrovirus Laboratory, Department of Virology, State Serum Institute, Copenhagen, Denmark

The HIV-1 inhibitors described in this paper is closely related to 6-(3,5-dimethylbenzyl)-1-(ethoxymethyl)-5-isopropyluracil (GCA-186) an anti-HIV-1 drug that is highly active against both wild type and mutated HIV-1 strains. The two methyl groups on the 6-benzyl moiety have been shown to improve the binding stability of the drug to the NNRTI-binding site in reverse transcriptase of drug mediated mutant HIV-1 viruses. The methyl groups are replaced with isosteric chloro-atoms to avoid metabolism due to the two methyl groups. However, the isosteric chloro derivatives show tenfold less activity against HIV-1 than their corresponding methyl derivatives. The synthesis and the antiviral activities of the corresponding 1-(allyloxy- and indanyloxy)methyl-6-(3,5-dichlorobenzyl)-5-ethyluracil derivatives are also reported.

Keywords: GCA-186; 6-(3,5-Dichlorobenzyl)uracils; Non-nucleoside reverse transcriptase inhibitors; Human immunodeficiency virus (HIV-1); HIV-1 mutants

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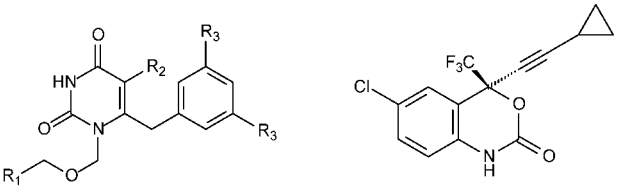
Introduction

In the recent years, the HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been of great interest for the design of drugs against HIV-1 reverse transcriptase (RT) [1], the enzyme being an important factor in the development of the disease Acquired Immuno Deficiency Syndrome (AIDS). Among some of the most common NNRTIs today is 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) [2], 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (Emivirine or MKC-442, **1a**, Figure 1) [3] and its 1-benzyloxymethyl analogue (TNK-651) [4] of which MKC-442 was in clinical trials, but abandoned at phase III studies [5]. When AIDS patients are treated with Emivirine or other HEPT analogues (in general for NNRTIs) a drug resistance mediated by mutations in the binding site for NNRTIs in RT of HIV-1 developes [6]. The corresponding 6-(3,5-dimethylbenzyl) analogue (GCA-186, **1b**, Figure 1) of MKC-442 has also been investigated as a drug candidate [6]. The mutations that appear in the presence of NNRTIs are the Y181C or Y181C+K103N, which are critical for the binding of the NNRTIs to RT [4, 6]. The reason for the interest in GCA-186 is its greater tolerance to the presence of these two mutations. The binding stability with the mutants was due to binding of the two methyl substituents in two hydrophobic pockets in the NNRTI-binding site of RT as revealed by X-ray crystallography.



1a MKC-442 (R_1 = methyl, R_2 = isopropyl, R_3 = H) Efavirenz
1b GCA-186 (R_1 = R_3 = methyl, R_2 = isopropyl)

Figure 1. Chemical structure of MKC-442, GCA-186, and Efavirens.

The two methyl substituents on GCA-186 was thought to undergo metabolism e.g. by the cytochrom P450 system in the liver. Therefore, the idea of the present study is to replace the two methyl substituents with two chloro substituents to prevent rapid drug metabolism in the liver. The chloro group is bioisoster to a methyl group and is believed to be more resistant to degradation. There have been no reports of any studies concerning this type of substitution pattern with chlorine. Therefore, it is of interest to study

Correspondence: Erik B. Pedersen, Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark. Phone: +45 65 50-25-55, Fax: +45 66 15-87-80, e-mail: EBP@Chem.sdu.dk

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whether the 6-(3,5-dichlorobenzyl) analogues of GCA-186 show better or comparable anti-HIV-1 activity than their methyl analogues both towards wild type (wt) and Y181C or Y181C+K103N mutated HIV-1 strains. For the substituents in the N-1 position it has been reported that allyloxymethyl groups stabilizes the binding to both wt and mutated strains [7]. These types of substituents have been included in the present investigation together with indanyloxymethyl substituents. The activities of these compounds against HIV have been compared with those of MKC-442 and Efavirenz.

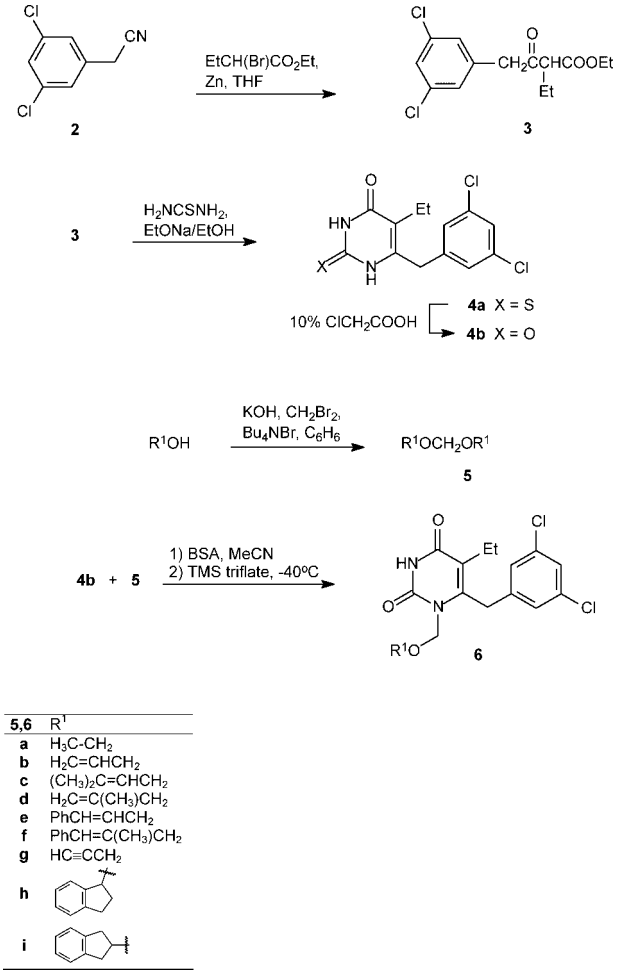
Results and discussion

Chemistry

Since 3,5-dichlorophenylacetonitrile (**2**) is not commercially available, it was necessary to start from 3,5-dichlorobenzoic acid which was reduced to 3,5-dichlorobenzyl alcohol with $LiAlH_4$ in anhydrous THF according to Nishiwaki et al. [8].

The first attempt to convert 3,5-dichlorobenzyl alcohol into **2** was carried out according to Mizuno et al. [9] in an one-pot reaction using KCN, $(Ph)_3P$, CBr_4, and a crown ether. However, this gave the unexpected product 3,5-dichlorobenzylbromid. The attempt to converted this into **2** with KCN in boiling ethanol according to Pitea et al. [10] unfortunately resulted in 3,5-dichlorophenylacetamide instead of **2**. We succeeded to prepare compound **2** in 79% overall yield by treating 3,5-dichlorobenzyl alcohol with boiling $SOCl_2$ to give 3,5-dichlorobenzyl chloride as described by Nishiwaki et al. [8], followed by reaction with NaCN in water under reflux [11]. Reaction of **2** with the zinc organometallic reagent from ethyl 2-bromobutyrate in anhydrous THF gave ethyl 4-(3,5-dichlorophenyl)-2-ethyl-3-oxobutyrate (**3**) which was reacted with thiourea in the presence of sodium ethoxide in boiling ethanol to give the 2-thiouracil **4a** (Scheme 1). Desulfurization of **4a** with aqueous chloroacetic acid afforded 6-(3,5-dichlorobenzyl)-5-ethyluracil (**4b**) according to the procedure described by Danel et al. [12]. The uracil **4b** was silylated with *N,O*-bis-(trimethylsilyl)acetamide (BSA) in acetonitrile and then treated with bis(ethoxy)methane (**5a**), bis(allyloxy)methanes (**5b**–**d**), bis(phenylallyloxy)methanes (**5e**, **f**), bis(propargyloxy)methane (**5g**), bis(indan-1-yloxy)methane (**5h**), and bis(indan-2-yloxy)methane (**6i**) under the Vorbrüggen conditions [13] using trimethylsilyl trifluoromethanesulfonate (TMS-triflate) as a catalyst to give the corresponding GCA-186 analogues **6a**–**i** in 5–98% yields. N-1 alkylations were confirmed by the NOE enhancement on NCH_2O-protons when aryl-CH_2 protons were irradiated and vice versa.

The acetals **5b**–**h** have been developed recently in our group to produce active Emivirine analogues against HIV-1 in the near picomolar range [7, 14, 15]. Bis(indan-1-yloxy)methane (**5h**) was prepared by reacting 1-indanol with dibromomethane in refluxing anhydrous benzene in the presence of potassium hydroxide and tetrabutylammonium bromide.



5,6	R^1
a	H_3C-CH_2
b	H_2C=$CHCH_2$
c	$(CH_3)_2C$=$CHCH_2$
d	H_2C=$C(CH_3)CH_2$
e	PhCH=$CHCH_2$
f	PhCH=$C(CH_3)CH_2$
g	HC≡CCH_2
h	
i	

Scheme 1. Synthesis route for final uracil derivatives.

Biological screening

Table 1 shows the data for the inhibitory activities of compounds **6a**–**i**, against wt HIV-1 and against Y181C (strain N119) and Y181C+K103N (strain A17) HIV-1 resistant strains in MT-4 cells. All the tested compounds **6a**–**i** shows almost identical antiviral activities against the wt HIV-1 IIIB. GCA-186 has not previously been tested on the HIV-1 IIIB MT-4 cell system so it is not possible to make any direct comparison between GCA-186 and the new analogues. One possibility is though to compare the antiviral activities with those reported by Hopkins et al. [6]. When tested on MAGI-CCR5 infected cells, MKC-442 and GCA-186 showed ED_{50}-values of 0.004 μM and 0.001 μM respectively, for the wt HIV-1 IIIB. Assuming GCA-186 4 times more active than MKC-442 we can conclude that the compounds **6a**–**i** are in the range of 10 times less active than

Table 1. Antiviral activity of compounds **6a**−**i** against HIV-1 in MT-4 cells.

Compound	Wild type			N119 (Y181C)	A17 (K103N + Y181C)
	EC_{50}[†] [mM]	CC_{50}[‡] [mM]	SI[§]	EC_{50}[†] [mM]	EC_{50}[†] [mM]
6a	0.04	36	900	> 10	> 10
6b	0.03	23	767	> 10	3
6c	0.05	35	700	> 10	> 10
6d	0.12	25	208	> 10	> 10
6e	0.04	34	850	3	> 10
6f	0.03	32	1057	2	> 10
6g	0.02	27	1350	12	5
6h	0.05	35	700	0.3	10
6i	0.13	30	230	2.1	> 10
MKC-442	0.02	> 100	> 5000	44	> 10
Efavirenz	0.01	> 100	> 10000	0.3	2.7

[†] Effective concentration of compound achieving 50% inhibition of HIV-1 multiplication in MT-4-infected cells.
[‡] Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%.
[§] Selectivity index: ratio CC_{50}/EC_{50}. The symbol (>) indicates that CC_{50} was not reached at the highest concentration tested. For description of assay see Experimental.

GCA-186. The same drop in activity is also noticed comparing other N-1 substituents, where the activities for the methyl analogues already have been published [7]. This indicates that the two chloro groups reduce the binding affinity of the drug to HIV-1 RT.

As mentioned earlier [6], it has been reported that the two methyl groups stabilize the binding of the NNRTI to the HIV-1 RT of the mutated viruses. The compounds **6b, e**−**i** also showed considerable activity towards both the single and double mutated HIV-1 strains with improvements up to 33-fold compared to the activity of MKC-442. The new analogues follow the previous findings of improved activity against the mutated RT when having 3,5 substitutions in the 6-benzyl group. It should be noted that the compounds **6e, f, h, i** showed considerably higher activities against the mutant N119 than the corresponding compounds **6a**−**d, g**. One can speculate whether the aromatic ring in the N-1 substituents of compounds **6e, f, h, i** gives better binding in the hydrophobic region due to stacking intercalations with aromatic amimo acids in N119 reverse transcriptase enzyme. Although the activities were improved against mutants, they are still considerably lower than those reported for recently developed NNRTIs [1].

Compound **6g** with the highest SI-value showed the highest activity in the present investigation against wt HIV-1 and it showed also good activity against the double mutated strain Y181C+K103N, while **6h** exhibited the highest activity against the strain Y181C.

Conclusion

Although a series of 6-(3,5-dichlorobenzyl) analogues of GCA-186 showed significant activities against HIV-1 virus and the drug resistant strains Y181C and Y181C+K103N, the activities for wt virus were in the range of 10 folds lower than the one found for GCA-186. This shows that chlorine is not the substituents of choice for replacing the methyl groups in GCA-186 in order to reduce its biodegradable properties.

Experimental

Chemistry

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer (Varian, Palo Alto, CA, USA) at 300 MHz for ^1H and 75 MHz for ^{13}C with TMS as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). MALDI spectra were recorded on an IonSpec Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (IonSpec Corporation, Lake Forest, CA, USA). Melting points were determined on a Büchi melting point apparatus (Büchi Labortechnik, Flawil, Switzerland). Elemental analyses were performed at H. C. Ørsted Institute, University of Copenhagen; found values agreed favourably with the calculated ones. Thin-layer chromatography was performed on silica gel DC-alufolio 60 F_{254} plates from Merck (Merck, Darmstadt, Germany). The silica gel (0.040−0.063 mm) used for column chromatography was purchased from Merck. HPLC purification were performed on a Waters Prep LC 4000 HPLC with a Waters Prep LC controller, and a Waters 2487 Dual λ absorbance detector on a Waters Delta Pak C18 column, 15 μm (Waters A/S, Hedehusene, Denmark).

*3,5-Dichlorophenylacetonitrile (**2**)*

To a solution of sodium cyanide (3.0 g, 61 mmol) in water (2.6 mL) under reflux a solution of 3,5-dichlorobenzyl chloride (9 g, 46 mmol) in ethanol (6 mL) was added dropwise and the mixture was refluxed overnight. The mixture was allowed to cool to room temperature; after filtration the filtrate was evaporated under reduced pressure. The residue was dissolved in ether and washed with a

$NaHCO_3$ solution (5%, 100 mL). The aqueous phase was extracted with ether (3 × 100 mL). The combined ether phases were dried over anhydrous magnesium sulphate and evaporated under reduced pressure to give **2** after distillation.

Yield 4.87 g (57%) as an oil; bp 102 °C/0.15 mbar, lit. 165–168 °C/17 Torr [22]. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.74 (s, 2H, CH_2CN), 7.26–7.35 (m, 3H, aryl). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 23.04 (CH_2), 116.47 (CN), 126.46, 128.49, 132.92, 135.74 (aryl).

*Ethyl 4-(3,5-dichloropenyl)-2-ethyl-3-oxobutyrate (**3**)*

Zinc dust (53.1 g, 812 mmol) activated by washing with hydrochloric acid (4 M, 3 × 100 mL), H_2O (3 × 100 mL), ethanol (3 · 100 mL) and anhydrous ether (3 × 100 mL) was suspended in anhydrous THF (250 mL) and heated to reflux. Ethyl 2-bromobutyrate (0.1 mL) was added to initiate the reaction and compound **2** (10 g, 54.1 mmol) in 200 mL anhydrous THF was added. Ethyl 2-bromobutyrate (23.4 g, 120 mmol) was added slowly over a period of 1 hour. After the addition was completed, the mixture was refluxed for 20 minutes then cooled to room temperature. The reaction was quenched under ice cooling by adding saturated K_2CO_3 (250 mL). The reaction mixture was stirred overnight. The THF layer was decanted off and the aqueous phase was washed with THF (2 × 100 mL). To the combined THF phases, hydrochloric acid (10%, 100 mL) was added and the mixture was stirred for 45 min. The THF was evaporated under reduced pressure and dichloromethane (300 mL) was added. The organic phase was separated, washed with saturated aqueous $NaHCO_3$ (200 mL) and saturated aqueous NaCl (100 mL). After drying over anhydrous sodium sulphate, evaporation under reduced pressure afforded compound **3** which was used without further purification.

Yield 16 g (98%) as an oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.91 (t, 3H, *J* = 7.3 Hz, CH_3), 1.27 (t, 3H, *J* = 7.2 Hz, CH_3), 1.60, 1.92 (m, 2H, CH_2), 3.45 (t, 1H, *J* = 7.3 Hz, CH), 3.77 (s, 2H, CH_2Ph), 4.18 (q, 2H, *J* = 7.2 Hz, CH_2), 7.09–7.27 (m, 3H, aryl). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.77 (CH_3), 14.04 (CH_3), 21.51 (CH_2), 47.62 (CH_2), 60.09 (CH), 61.53 (CH_2), 127.38, 128.14, 134.94, 136.44 (aryl), 169.36 (C=O), 201.34 (C=O).

*6-(3,5-Dichlorobenzyl)-5-ethyl-2-thiouracil (**4a**)*

Thiourea (40.2 g, 0.53 mol) was dissolved under reflux in a solution of sodium (13.4 g, 0.58 mol) in anhydrous ethanol (300 mL). Compound **3** (16.0 g, 53 mmol) was added slowly over a period of 30 min, and the mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the residue was dissolved in water (400 mL). The solution was acidified with concentrated hydrochloric acid to pH 4. The precipitated 2-thiouracil (**4a**) was filtered off and dried. The raw material was used in the subsequent reaction without further purification.

Yield 2.8 g (18%) as white crystals. ^{1}H-NMR (DMSO): δ (ppm) = 0.70 (t, 3H, *J* = 7.3 Hz, CH_3), 2.13 (q, 2H, *J* = 7.2 Hz, CH_2), 3.73 (s, 2H, CH_2) 7.19–7.36 (m, 3H, aryl), 12.07 (s, 1H, NH), 12.29 (s, 1H, NH). ^{13}C-NMR (DMSO): δ (ppm) = 13.45 (CH_3), 18.25 (CH_2), 34.27 (CH_2), 118.22 (C-5), 127.08, 127.57, 134.64, 141.49 (aryl), 148.07 (C-6), 161.89 (C-4), 174.80 (C-2).

*6-(3,5-Dichlorobenzyl)-5-ethyluracil (**4b**)*

Compound **4a** was suspended in aqueous chloroacetic acid (10%, 250 mL) and refluxed overnight. The reaction mixture was cooled to room temperature and acetic acid (100 mL) was added with subsequent heating for a few minutes. The precipitate thus formed upon cooling was filtered off and dried to give compound **4b**.

Yield 2.5 g (89%) as white crystals; mp 230–232 °C. ^{1}H-NMR (DMSO): δ (ppm) = 1.09 (t, 3H, *J* = 7.3 Hz, CH_3), 2.50 (q, 2H, *J* = 7.3 Hz, CH_2), 4.03 (s, 2H, CH_2) 7.59–7.75 (m, 3H, aryl), 10.97 (s, 1H, NH), 11.30 (s, 1H, NH). ^{13}C-NMR (DMSO): δ (ppm) = 13.50 (CH_3), 17.52 (CH_2), 34.11 (CH_2), 111.83 (C-5), 126.44, 126.99, 134.04, 141.08 (aryl), 147.00 (C-2), 150.81 (C-6), 164.32 (C-4). Anal. calcd. for $C_{13}H_{12}Cl_2N_2O_2$ (299.16): C, 52.19; H, 4.04; N, 9.36. Found: C, 52.16; H, 3.89; N, 8.95.

*Bis(indan-1-yloxy)methane (**5h**)*

A mixture of potassium hydroxide (5.66 g, 101 mmol), 1-indanol (13.4 g, 100 mmol), dibromomethane (8.79 g, 50.5 mmol) and tetrabutylammonium bromide (1.74 g, 5.35 mmol) 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 under reduced pressure. The residue was chromatographed on a silica gel column using $CHCl_3$ to give **5h** as a diastereomeric mixture.

Yield 8.3 g (59%) as a colourless oil. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.11–2.21, 2.36–2.49 (2 × m, 4H, 2-H), 2.78–2.88, 3.05–3.15 (2 × m, 4H, 3-H), 4.98, 4.99 (2 × s, 2H, CH_2), 5.27–5.34 (m, 2H, 1-H), 7.18–7.46 (m, 8H, $H_{arom.}$). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 30.11 (C-3), 32.92, 33.02 (C-2), 80.17, 80.45 (C-1), 92.18, 92.48 (CH_2), 124.94, 124.85, 124.95, 126.34, 126.39, 128.33, 142.68, 142.75, 143.89 ($C_{arom.}$).

*General procedure for the synthesis of non-nucleoside uracil derivatives **6a–i***

The uracil **4b** (250 mg, 0.84 mmol) was suspended in anhydrous acetonitrile (20 mL) under N_2 and *N,O*-bis-(trimethylsilyl)acetamide (BSA) (511 mg, 0.62 mL, 2.52 mmol) was added. After obtaining a clear solution (10 min), the reaction mixture was cooled to −40 °C and trimethylsilyl trifluoromethansulfonate (TMS triflate) (224 mg, 0.18 mL, 1.00 mmol) was added followed by dropwise addition of the appropriate acetals **5a–i** (2,52 mmol). The reaction mixture was stirred at room temperature for 3–5 hours. A saturated $NaHCO_3$ solution (15 mL) was added, and the solution was vigorously stirred for 10 min before the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and washed with a saturated $NaHCO_3$ solution (20 mL). The organic phase was subsequently washed with a saturated NaCl solution (25 mL) and the water phase was extracted with dichloromethane (25 mL). The combined organic phases were dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The product was chromatographed on a column of silica gel with ethylacetate/petroleum ether (v:v = 3:2) for compounds **6a–g** and ether/petroleum ether (v:v = 1:4) for compounds **6h**, **i**. Compounds **6e**, **f** were further purified on HPLC (50% acetonitril in water), t_r 31 and 34 minutes for **6e** and **6f**, respectively.

*6-(3,5-Dichlorobenzyl)-1-(1-ethoxymethyl)-5-ethyluracil (**6a**)*

Yield 184 mg (62%); mp 137–139 °C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.07 (t, 3H, *J* = 7.4 Hz, CH_3), 1.18 (t, 3H, *J* = 7.1 Hz, CH_3), 2.43 (q, 2H, *J* = 7.4 Hz, CH_2), 3.61 (q, 2H, *J* = 7.1 Hz, CH_2), 4.13 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 7.01–7.29 (m, 3H, aryl), 9.62 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.69 (CH_3), 14.95 (CH_3), 19.19 (CH_2), 32.79 (CH_2), 65.14 (CH_2), 72.83 (CH_2), 117.49 (C-5), 125.79, 127.70, 135.84, 138.78 (aryl), 147.33 (C-2), 151.75 (C-6), 163.11 (C-4). Anal. calcd. for $C_{16}H_{18}Cl_2N_2O_3$ (357.24): C, 53.80; H, 5.08; N, 7.84. Found: C, 54.05; H, 5.13; N, 7.64.

*1-(Allyloxymethyl)-6-(3,5-dichlorobenzyl)-5-ethyluracil (**6b**)*

Yield 304 mg (98%); mp 115–117°C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.08 (t, 3H, J = 7.2 Hz, CH_3), 2.44 (q, 2H, J = 7.3 Hz, CH_2), 4.10–4.13 (m, 4H, 2 × CH_2), 5.14 (s, 2H, CH_2), 5.20–5.32 (m, 2H, C=CH_2), 5.86 (m, 1H, CH=C), 7.01–7.29 (m, 3H, aryl), 9.71 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.69 (CH_3), 19.18 (CH_2), 32.84 (CH_2), 70.59 (CH_2), 72.58 (CH_2), 117.59 (C-5), 118.05 (=CH_2), 125.78, 127.75, 135.88, 138.64 (aryl), 133.28 (CH=), 147.23 (C-2), 151.73 (C-6), 163.11 (C-4). Anal. calcd. for $C_{17}H_{18}Cl_2N_2O_3$ (369.25): C, 55.30; H, 4.91; N, 7.59. Found: C, 55.53; H, 4.91; N, 7.59.

*6-(3,5-Dichlorobenzyl)-1-(3-methylbut-2-enyloxymethyl)-5-ethyluracil (**6c**)*

Yield 299 mg (90%); mp 103–105°C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.09 (t, 3H, J = 7.4 Hz, CH_3), 1.66 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.43 (q, 2H, J = 7.4 Hz, CH_2), 4.10 (d, 2H, J = 6.9 Hz, CH_2), 4.12 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 5.27 (tt, 1H, J = 6.9 Hz, J = 1.4 Hz, CH), 7.00–7.29 (m, 3H, aryl), 9.56 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.72 (CH_3), 18.04 (CH_3), 19.16 (CH_2), 25.80 (CH_3), 32.78 (CH_2), 66.10 (CH_2), 72.59 (CH_2), 117.46 (C-5), 119.73 (CH), 125.79, 127.72, 135.85, 138.63 (aryl), 138.73 (C=C), 147.38 (C-2), 151.61 (C-6), 163.07 (C-4). Anal. calcd. for $C_{19}H_{22}Cl_2N_2O_3$ (397.30): C, 57.44; H, 5.58; N, 7.05. Found: C, 57.67; H, 5.66; N, 6.90.

*6-(3,5-Dichlorobenzyl)-1-(2-methylallyloxymethyl)-5-ethyluracil (**6d**)*

Yield 190 mg (59%); mp 116–118°C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.08 (t, 3H, J = 7.1 Hz, CH_3), 1.72 (s, 3H, CH_3), 2.44 (q, 2H, J = 7.2 Hz, CH_2), 4.02 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 4.90, 4.96 (m, 2H, =CH_2), 5.15 (s, 2H, CH_2), 7.02–7.30 (m, 3H, aryl), 9.59 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.70 (CH_3), 19.18 (CH_3), 19.45 (CH_2), 32.80 (CH_2), 72.77 (CH_2), 73.62 (CH_2), 112.79 (=CH_2), 117.56 (C-5), 125.79, 127.78, 135.90, 138.64 (aryl), 140.94 (=C), 147.26 (C-2), 151.68 (C-6), 163.08 (C-4). Anal. calcd. for $C_{18}H_{20}Cl_2N_2O_3$ (383.28): C, 56.41; H, 5.26; N, 7.31. Found: C, 56.59; H, 5.29; N, 7.30.

*1-((E)-Cinnamyloxymethyl)-6-(3,5-dichlorobenzyl)-5-ethyluracil (**6e**)*

Yield 20 mg (5%). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.05 (t, 3H, J = 7.5 Hz, CH_3), 2.41 (q, 2H, J = 7.4 Hz, CH_2), 4.12 (s, 2H, CH_2), 4.23 (d, 2H, J = 6.4 Hz, CH_2), 5.16 (s, 2H, CH_2), 6.15–6.25 (m, 1H, =CHC), 6.56, 6.62 (m, 1H, =CHPh), 7.00–7.21 (m, 3H, aryl), 7.22–7.42 (m, 5H, Ph). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.68 (CH_3), 19.20 (CH_2), 32.90 (CH_2), 70.34 (CH_2), 72.55 (CH_2), 117.50 (C-5), 124.19, 127.77, 135.87, 138.65 (aryl), 125.77, 126.52, 128.55, 136.18 (Ph), 127.99 (=CH), 133.64 (=CHPh), 147.23 (C-2), 151.69 (C-6), 162.99 (C-4). HRMS-MALDI: m/z = 467.0900 (M + Na^+, $C_{23}H_{22}Cl_2N_2NaO_3$); requires 467.0903.

*6-(3,5-Dichlorobenzyl)-1-((E)-2-methyl-3-phenyl-allyloxymethyl)-5-ethyluracil (**6f**)*

Yield 20 mg (5%). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.06 (t, 3H, J = 7.2 Hz, CH_3), 1.86 (s, 3H, CH_3), 2.44 (q, 2H, J = 7.2 Hz, CH_2), 4.15 (s, 2H, CH_2), 4.19 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.49 (=CHPh), 7.02–7.20 (m, 3H, aryl), 7.19–7.40 (m, 5H, Ph). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.70 (CH_3), 15.52 (CH_3), 19.20 (CH_2), 32.85 (CH_2), 72.80 (CH_2), 76.03 (CH_2), 117.56 (C-5), 125.78 127.79, 135.91, 138.64 (aryl), 125.78, 126.68, 128.12, 134.61 (Ph), 128.86 (=CH), 137.00 (=CH), 147.26 (C-2), 151.60 (C-6), 162.98 (C-4). HRMS-MALDI: m/z = 481.1056 (M + Na^+, $C_{24}H_{24}Cl_2N_2NaO_3$); requires 481.1074.

*6-(3,5-Dichlorobenzyl)-1-(prop-2-ynyloxymethyl)-5-ethyluracil (**6g**)*
Yield 81 mg (26%); mp 147–151°C. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.08 (t, 3H, J = 7.4 Hz, CH_3), 2.45 (q, 2H, J = 7.3 Hz, CH_2), 2.4–2.5 (m, 1H, sCH), 4.12 (s, 2H, CH_2), 4.29 (d, 2H, J = 2.3 Hz, CH_2), 5.18 (s, 2H, CH_2), 7.03–7.30 (m, 3H, aryl), 9.62 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.73 (CH_3), 19.19 (CH_2), 32.79 (CH_2), 57.20 (CH_2), 72.56 (CH), 74.98 (CH_2), 78.86 (C=), 117.72 (C-5), 125.84, 127.84, 135.91, 138.47 (aryl), 147.09 (C-2), 151.72 (C-6), 163.01 (C-4). Anal. calcd. for $C_{17}H_{16}Cl_2N_2O_3$ (367.23): C, 55.60; H, 4.39; N, 7.63. Found: C, 55.64; H, 4.40; N, 7.37.

*6-(3,5-Dichlorobenzyl)-5-ethyl-1-(indan-1-yloxy)methyl-uracil (**6h**)*

Yield, 232 mg (62%) as a white foam. ^{1}H-NMR ($CDCl_3$): δ (ppm) =1.05 (t, 3H, J = 7.4 Hz, CH_3), 1.97–2.07 (m, 1H, 2′-H), 2.32–2.48 (m, 3H, 2′-H, CH_2), 2.77–2.87 (m, 1H, 3′-H), 3.03–3.12 (m, 1H, 3'-H), 4.07 (s, 2H, CH_2), 5.08–5.37 (m, 3H, 1′-H, CH_2), 6.91 (s, 2H, $H_{arom.}$), 7.18–7.35 (m. 5H, $H_{arom.}$), 9.45 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): d(ppm) =13.79 (CH_3), 19.21 (CH_2), 30.15 (C-2'), 32.75 (C-3'), 32.83 (CH_2), 71.59 (CH_2), 82.40 (C-1'), 117.60 (C-5), 124.86, 125.04, 125.77, 126.56, 127.73, 128.88, 135.83, 138.59, 141.58, 144.00 ($C_{arom.}$), 147.41 (C-6), 151.61 (C-2), 162.99 (C-4). HRMS-MALDI: m/z = 467.0900 (M + Na^+, $C_{23}H_{22}Cl_2N_2NaO_3$); requires 467.0899.

*6-(3,5-Dichlorobenzyl)-5-ethyl-1-(indan-2-yloxy)methyl-uracil (**6i**)*

Yield 277 mg (74%) as a white solid; mp. 166–167°C. ^{1}H-NMR ($CDCl_3$): δ (ppm) =1.04 (t, 3H, J = 7.4 Hz, CH_3), 2.40 (q, 2H, J = 7.4 Hz, CH_2), 2.89, 3.14 (2 × dd, 4H, J = 3.7, 16.5; 6.1, 16.5 Hz, 1′-H, 3′-H), 4.09 (s, 2H, CH_2), 4.54 (dt, 1H, J = 2.6, 3.7 Hz, 2′-H), 5.18 (s, 2H, CH_2), 6.97–7.28 (m, 7H, $H_{arom.}$), 9.65 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.75 (CH_3), 19.20 (CH_2), 32.80 (CH_2), 39.45 (CH_2), 71.50 (CH_2), 79.11 (CH), 117.63 (C-5), 124.67, 125.77, 126.70, 127.71, 135.83, 138.68, 140.29 ($C_{arom.}$), 147.34 (C-6), 151.73 (C-2), 163.08 (C-4). Anal. calcd. for $C_{23}H_{22}Cl_2N_2O_3$ (445.34): C, 62.03; H, 4.98; N, 6.29. Found: C, 62.03; H, 4.94; N, 6.23. ^{13}C-NMR ($CDCl_3$): δ (ppm) = 13.75 (CH_3), 19.20 (CH_2), 32.80 6.23.

Virus and cells

The inhibitory activity against HIV-1 infection was evaluated using MT-4 cells [16] as target cells and the HIV-1 strain HTLV-IIIB [17] as infectious virus. The virus was propagated in H9 cells [16] at 37°C, 5% CO_2 using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). The culture supernatant was filtered (0.45 nm), aliquotted, 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 MT-4 cells as target cells. MT-4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compounds for six days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was indirectly quantified using the MTT assay [17]. Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT-4 cultures containing the test dilutions of compounds as described above. A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentration (EC_{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 [17]) or NNRTI resistant HIV-1 (strain N119 [19], strain A17 [20, 21]).

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