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Synthesis and evaluation of new potential HIV-1 non-nucleoside reverse transcriptase inhibitors. New analogues of MKC-442 containing Michael acceptors in the C-6 position

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Analogues of MKC-442 capable of undergoing Michael addition reactions were synthesised in order to investigate the activity against the HIV-1 mutant (Y181C). An improved activity was postulated on the basis of a possible covalent binding to the mercapto group of Cys181. Lithiation of the C-6 position of 1-ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (**5**) was followed by reaction with α,β-unsaturated aldehydes and oxidation of the alcohols formed to give the alkenoyl analogues **1a**–**3a**. Analogues **1b**–**3b** containing an allyloxymethyl group in the N-1 position instead of the ethoxymethyl group could not be synthesised due to isomerisation of the allylic group during the metallation reaction. The NMR data for compounds **1a**–**3a** showed a hindered rotation, which was more pronounced for the 6-cyclohexenylcarbonyl derivative **3a** than for the propenyl derivatives **1a** and **2a**. Moderate activity against wild type HIV-1 was observed for the alcohol **8** and the ketones **2a**–**3a**. However, no activity was observed against the Y181C mutant.

Introduction

MKC-442[1] (also known as Emivirine or Coactinon, Fig. 1) belongs to the group of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). This group of compounds inhibits HIV-1 reverse transcriptase (RT) allosterically by binding to a hydrophobic pocket situated approximately 10 Å away from the active site.[2] MKC-442 was selected for clinical trials by Triangle Pharmaceuticals,[3] but the compound was withdrawn from clinical phase III trials in January 2002 as it did not have superior properties compared to Abacavir—a nucleoside reverse transcriptase inhibitor (NRTI) that was used as a comparative drug.[4]

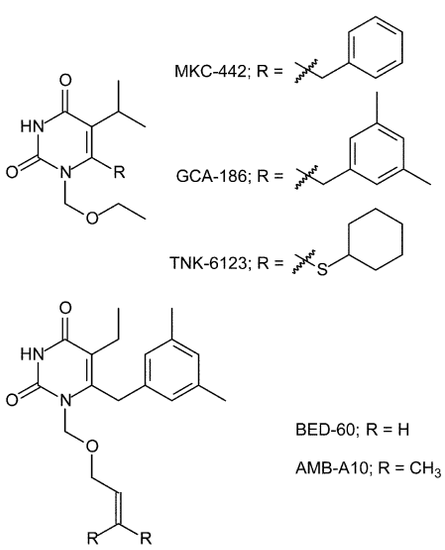

Fig. 1 Structures of the lead molecules MKC-442, GCA-186, TNK-6123, BED-60 and AMB-A10.

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A major problem when using MKC-442 is the rapid selection of resistant viruses, mainly harbouring the RT mutation Y181C.[1] Tyr181 is important for the activity of MKC-442 as the isopropyl group in the C-5 position of MKC-442 induces a shift of Tyr181, which alters the conformation of the enzyme and thereby inactivates it. After the shift Tyr181 is positioned inside a sub-pocket where it can interact with other aromatic amino acids (Tyr183, Tyr188, Phe227, Trp229) and the C-6 substituent of MKC-442.[5]

Analogues of MKC-442 have been developed that show activity against the Y181C mutated virus. TNK-6123 and GCA-186 (Fig. 1) were developed by Hopkins *et al.*[6] and showed activities comparable to MKC-442 against wild-type viruses, whereas they retained some activity against the Y181C mutated virus in contrast to MKC-442 which lost all its activity. It was shown from crystal structures of the two inhibitors in a complex with RT that the two methyl groups of the C-6 substituent of GCA-186 and the flexible C-6 cyclohexyl ring of TNK-6123 had improved contacts to the sub-pocket where the C-6 substituent was positioned.

In our group we developed analogues of GCA-186 that contained unsaturated moieties in the N-1 position (**BED-60** and **AMB-A10**, Fig. 1).[7] These analogues showed excellent activities against wild-type HIV-1 and retained some activity against the Y181C mutant.

We have previously synthesised analogues of MKC-442 containing reactive groups (epoxides or aldehydes) in the C-5 position.[8] The rationale was that the epoxides or aldehydes should react inside the enzyme forming a covalent bond with Cys181 introduced by mutation. Unfortunately, the compounds were not active, either against wild-type or mutant viruses. The epoxides were chemically very reactive and therefore difficult to synthesise. The high reactivity was believed to be unfavourable in the biological perspective under aqueous conditions where hydrolysis could take place before the drugs were positioned inside the enzyme.

In this paper we report the synthesis and activities of new analogues of MKC-442 with potential activity against HIV-1

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containing the RT mutation Y181C. We introduced a Michael acceptor in the C-6 position in the hope that a reaction would take place between Cys181 of the mutated enzyme and the Michael acceptor. To study the effect of bulkiness of the C-6 group we chose the targets **1**–**3** (Fig. 2). In addition to the possible reaction between the Michael acceptor and Cys181 the hope was that the cyclohexene ring of target **3** would fill out the RT sub-pocket in analogy with TNK-6123.

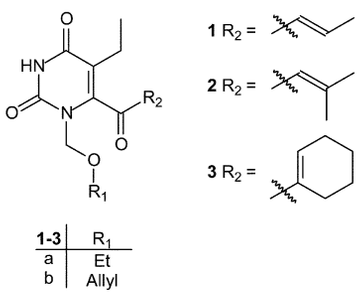


Fig. 2 Target molecules **1**–**3**.

Results and discussion

To visualise the possibility of a reaction taking place between the enzyme and the inhibitor, we did modelling studies on compound **3a** using MacroModel.[9] Because there are no available crystal structures of MKC-442 with the mutated RT (Y181C) the study was based on the crystal structure of TNK-651 (an analogue of MKC-442 where the N-1 ethoxymethyl group was replaced by a N-1 benzyloxymethyl group) in complex with the mutated RT (Y181C).[10] It was believed that the difference in the N-1 substituent would not affect the binding conformation of the C-6 substituent as is the case for the wild-type RT.

A conformational analysis of **3a** was performed based on molecular dynamics[11] followed by multiple minimisations of the generated structures using MMFF.[12] The generated conformations could be divided into two groups. In both groups the uracil ring and the plane of the carbonyl group were placed perpendicular to each other, but for one the carbonyl was pointing out of the page and for the other the carbonyl was pointing into the page (Fig. 3).

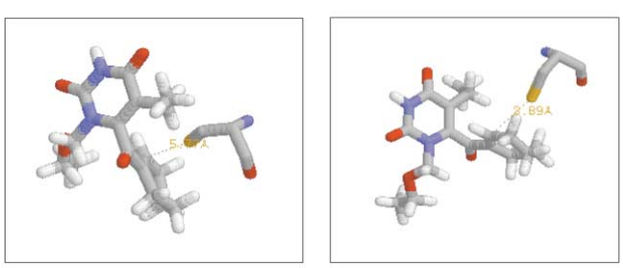
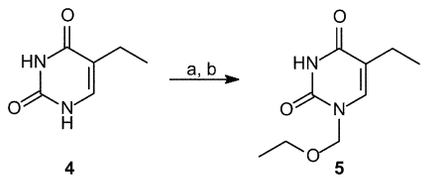


Fig. 3 Models of the two main conformations of compound **3a**. The structures were superimposed into the complex between TNK-651 and the Y181C mutated RT. The distance to Cys181 is illustrated.

The uracil rings of the two main conformations were both superimposed onto the uracil ring of TNK-651 in complex with Y181C mutated RT. This was based on the fact that the uracil rings of different MKC-442 analogues all occupy the same volume inside wild-type RT.[5] Using RasMol[13] the orientation of Cys181 was altered to illustrate the shortest possible distance between the reactive double-bond and the thiol of the cysteine. For the conformation having the carbonyl pointing out of the page (left model, Fig. 3) the distance was measured to 5.8 Å, which is quite large. However, for the conformation having the carbonyl pointing into the page the distance was only 3.9 Å, which made a possible reaction more likely. This structure also resembled the structure of MKC-442 in complex with wild-type RT.[5]

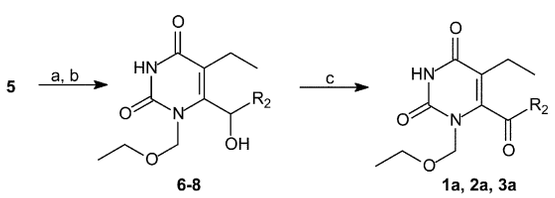
Because of the highly conjugated system of **3a** it was decided to verify the quality of the conformations generated using force field calculations. MP2/6-31g* *ab initio* optimisations of the two chosen conformations were performed and the results corresponded excellently to the force field conformations, verifying that the carbonyl of **3a** is placed perpendicular to the uracil ring in despite of conjugation with the C5–C6 double bond.

5-Ethyl-1*H*-pyrimidine-2,4-dione (**4**) was synthesised on a gram scale in a three step synthesis starting from ethyl butyrate and ethyl formate.[14,15] Compound **4** was silylated using *N,O*-bis(trimethylsilyl)acetamide (BSA) and was reacted with chloromethyl ethyl ether and CsI to give compound **5** in 92% yield (Scheme 1).



Scheme 1 *Reagents and conditions:* a) BSA; b) $ClCH_2OCH_2CH_3$, CsI, 92%.

Lithiation of the C-6 position of compound **5** and reaction of this intermediate directly with the acid chloride (3-methylbut-2-enoyl chloride) to give target compound **2a** was attempted, but only starting material was isolated after work-up. Instead we used aldehydes as electrophiles. The best conditions were found to be the use of 4 eq. of LDA and 6 eq. of the electrophile. Using these conditions we isolated the alcohols **6**–**8** in 50%–61% yield.[16] The alcohols were oxidised using CrO_3, pyridine and Ac_2O and the target compounds **1a**, **2a** and **3a** were isolated in 64%–75% yield, (Scheme 2).[17]



Scheme 2 *Reagents and conditions*: a) DIA, *n*-BuLi; b) R_2–CHO, **6** (50%), **7** (56%), **8** (61%); c) CrO_3, pyridine, Ac_2O, **1a** (69%), **2a** (64%), **3a** (75%). R_2 as defined in Fig. 2.

An interesting feature was observed in the NMR spectra of compounds **1**–**3**. The NCH_2O group of the N-1 substituent was seen as two separate doublets in the spectra of compound **3a** whereas the same CH_2 group appeared as a very broad singlet in the spectra of compounds **1a** and **2a** (Fig. 4).

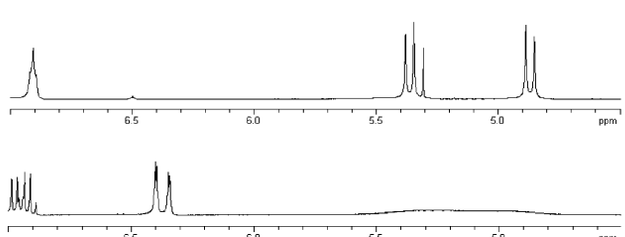


Fig. 4 NMR spectra of compound **3a** (upper) and compound **1a** (lower). The NCH_2O group is seen as two doublets for compound **3a** whereas the same group is seen as a very broad, flat peak for compound **1a**. Vinylic peaks are included for comparison of intensity. The peak at 5.3 (upper spectra) is CH_2Cl_2 impurity.

These observations could be explained by the calculations described above for compound **3a** where the pyrimidine ring and the cyclohexenyl ring were shown to be positioned perpendicular to each other. We believed that this conformation

would be more or less locked depending on the size of the C-6 substituent. For a completely locked conformation the two protons are diastereotopic and can appear as two distinct doublets as observed for compound **3a**. Free rotation around the bond connecting the pyrimidine ring and the carbonyl system would make these two protons appear as a singlet. To prove the hypothesis we heated an NMR sample of compound **1a** to increase the degree of rotation in the molecule. We saw a narrowing of the broad singlet of the CH_2 group when heating to 40 °C and at 60 °C it appeared as a nice singlet (Fig. 5), showing a higher degree of flexibility in the structures of compounds **1a** and **2a**.

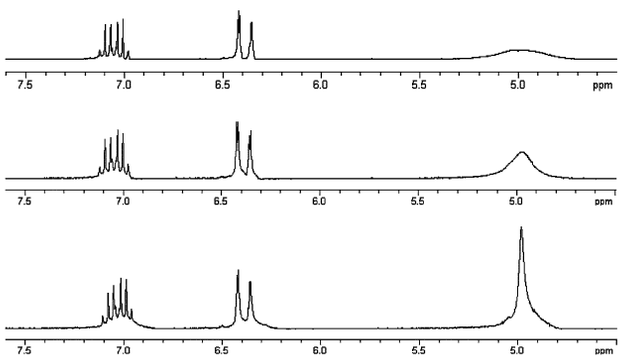
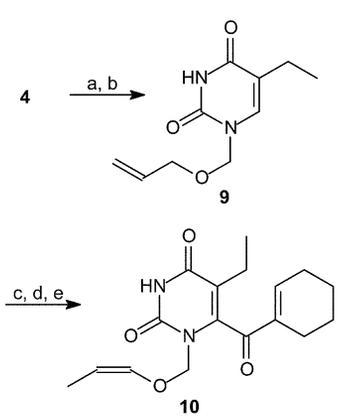


Fig. 5 NMR spectra of compound **1a** at 30 °C (upper), 40 °C (middle) and 60 °C (lower). The NCH_2O peak is narrowing upon heating. The vinylic protons are included for comparison of intensity.

For the synthesis of target compounds **1b**, **2b** and **3b** we needed to introduce an N-1 allyloxymethyl group. The starting compound **4** was silylated using BSA and it was alkylated in the N-1 position using bis(allyloxy)methane[7] under Vorbrüggen conditions.[18] Compound **9** was isolated in 93% yield (Scheme 3). Compound **9** was treated with LDA followed by addition of the aldehydes, but the expected compounds were not isolated. Complex mixtures were formed and it was not possible to identify the main product as it could not be isolated as the pure compound. For the reaction with cyclohex-1-enecarbaldehyde as an electrophile the mixture was directly oxidised in an attempt to isolate a pure compound after this reaction. The only product isolated in 2% yield after HPLC purification was identified as compound **10**[19] (Scheme 3). Apparently the LDA treatment isomerised the allylic group of compound **9**.



Scheme 3 *Reagents and conditions*: a) BSA; b) $(CH_2{=}CHCH_2O)_2CH_2$, TMS–triflate, 93%; c) DIA, *n*-BuLi; d) cyclohex-1-enecarbaldehyde; e) CrO_3, pyridine, Ac_2O.

The test for activity against HIV-1 was performed in MT4 cell cultures infected with either wild-type HIV-1 or the HIV-1 strain N119 harbouring a substitution of cysteine for tyrosine at position 181 (Table 1). For the alcohols **6** and **7** and the ketone **1a** with the least bulky C-6 substituent, no activity against the wild-type virus was observed. The most bulky alcohol **8** and the more bulky ketones **2a** and **3a** showed activities of 3.1–3.9 μM. However, these activities were still a factor of 100 less than the activity of MKC-442. No activity against the Y181C mutant was observed for the three ketones **1a**, **2a** and **3a**. It is therefore unlikely that a reaction has taken place inside the enzyme between Cys181 and the new Michael acceptor analogues.

Table 1

Compound	IC_{50}[a]/μM wild type	IC_{50}[a]/μM Y181C	CC_{50}[b]/μM
6	>100	ND[c]	>100
7	100	ND[c]	>100
8	3.8	ND[c]	>100
1a	>100	>100	30
2a	3.9	>100	>100
3a	3.1	>100	>100
MKC-442	0.031	>100	>100

[a] 50% Inhibitory concentration. [b] 50% Cytotoxic concentration. [c] Not determined.

Experimental

General methods

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz. Internal standards used in ^{1}H NMR spectra were tetramethylsilane (TMS, δ 0.00); in ^{13}C NMR were $CDCl_3$ (δ 77.00), DMSO-d_6 (δ 39.44). MALDI mass spectra were recorded on a 4.7 Tesla Ultima (IonSpec, Irvine, CA) Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Melting points were determined on a Büchi melting point apparatus. Elemental analysis were performed at Mikroanalytisk afdeling, University of Copenhagen, Copenhagen, Denmark. The progress of reactions was monitored by TLC (analytical silica gel plates 60 F_{254}). Merck silica gel (0.040–0.063 mm) was used for column chromatography. Solvents for chromatography were bought as HPLC grade or distilled prior to use. Reactions were in general carried out under a N_2 or Ar atmosphere. Pyridine was dried over KOH. CH_3CN was dried over 3 Å molecular sieves. CH_2Cl_2 and $CHCl_3$ were dried over 4 Å molecular sieves. THF was refluxed over Na using benzophenone as an indicator and distilled immediately before use.

1-Ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (5)

BSA (7.12 g, 35 mmol) was dissolved in dry $CHCl_3$ (100 cm^3) and 5-ethyl-1*H*-pyrimidine-2,4-dione (**4**, 1.40 g, 10 mmol) was added. The mixture was stirred for 10 min and then chloromethyl ethyl ether (1.42 g, 15 mmol) and CsI (2.60 g, 10 mmol) were added to the clear solution. The reaction mixture was stirred for 3 h and then quenched by the addition of sat. aq. $NaHCO_3$ (100 cm^3). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by column chromatography (silica gel, 5% EtOH–CH_2Cl_2) to give compound **5** (1.82 g, 92%) as a white powder; mp = 93–95 °C (lit.[20] mp = 104.8–105.5 °C). NMR data were consistent with those previously reported.[20]

General procedure for the synthesis of alcohols 6–8

DIA (1.01 g, 10 mmol) was dissolved in dry THF (30 cm^3). The solution was cooled to −78 °C and *n*-BuLi (4.44 cm^3, 2.25 M solution in hexanes, 10 mmol) was added. 1-Ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (**5**, 0.50 g, 2.5 mmol) was

dissolved in dry THF (10 cm^3) and added slowly, keeping the temperature below −70 °C. The mixture was stirred for an additional 1 h at −78 °C followed by slow addition of the aldehyde (15 mmol) in dry THF (5 cm^3), still keeping the temperature below −70 °C. The mixture was stirred for 3 h at the same temperature before the reaction was quenched by addition of glacial acetic acid (1.5 cm^3). The solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 (80 cm^3) and the organic phase was washed with H_2O (2 × 50 cm^3). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by column chromatography (silica gel, 2% EtOH–CH_2Cl_2).

1-Ethoxymethyl-5-ethyl-6-(1-hydroxybut-2-enyl)-1*H*-pyrimidine-2,4-dione (6)

Yield: 0.34 g (50%), light-brown glace; R_f 0.31 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.09 (3 H, t, *J* 7.4, $ArCH_2CH_3$), 1.23 (3 H, t, *J* 7.1, OCH_2CH_3), 1.78 (3 H, dt, *J* 6.2, 1.6, $CHCH_3$), 2.48–2.62 (2 H, m, $ArCH_2$), 3.62–3.80 (2 H, m, OCH_2CH_3), 4.53 (1 H, br s, OH), 5.47–5.96 (5 H, m, CH=CH, NCH_2O, CHOH), 9.27 (1 H, br s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 14.3 ($ArCH_2CH_3$), 14.9 (OCH_2CH_3), 17.8 ($CHCH_3$), 18.9 ($ArCH_2$), 65.2 (OCH_2), 69.0 (CHOH), 72.5 (NCH_2O), 117.0 (C-5), 128.3, 130.1 (CH=CH), 150.6, 151.9 (C-2, C-6), 163.3 (C-4); *m*/*z* (MALDI MS (peak matching)) 291.1315 $(M + Na)^+$, calcd. 291.1315.

1-Ethoxymethyl-5-ethyl-6-(1-hydroxy-3-methylbut-2-enyl)-1*H*-pyrimidine-2,4-dione (7)

Yield: 0.40 g (56%), light-brown glace; R_f 0.22 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.10 (3 H, t, *J* 7.4, $ArCH_2CH_3$), 1.23 (3 H, t, *J* 7.1, OCH_2CH_3), 1.78 (3 H, s, CH_3), 1.80 (3 H, s, CH_3), 2.48–2.70 (2 H, m, $ArCH_2$), 3.71 (2 H, q, *J* 7.0, OCH_2CH_3), 3.64 (1 H, br s, OH), 5.52–5.57, 5.68–5.74 (4 H, 2 × m, CH=C, NCH_2O, CHOH), 9.27 (1 H, br s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.9 ($ArCH_2CH_3$), 14.9 (OCH_2CH_3), 18.5 (CH_3-*cis*), 19.0 ($ArCH_2$), 26.1 (CH_3-*trans*), 65.2 (CHOH), 66.5 (OCH_2CH_3), 73.0 (NCH_2O), 116.4 (C-5), 123.4 (CH=C), 139.8 (CH=*C*), 151.5, 151.9 (C-2, C-6), 163.5 (C-4); *m*/*z* (MALDI MS) 305.1 $(M + Na)^+$, 283.2 $(M + H)^+$; $C_{14}H_{22}N_2O_4$; calcd.: C 59.56, H 7.85, N 9.92; found: C 59.72, H 7.91, N 9.57%.

6-(Cyclohex-1-enyl-hydroxymethyl)-1-ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (8)

Yield: 0.47 g (61%), light-yellow foam; R_f 0.29 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.12 (3 H, t, *J* 7.4, $ArCH_2CH_3$), 1.23 (3 H, t, *J* 7.1, OCH_2CH_3), 1.70–2.16 (8 H, m, 4 × CH_2), 2.40–2.52, 2.58–2.72 (2 H, 2 × m, $ArCH_2CH_3$), 3.60–3.80 (2 H, m, OCH_2CH_3), 4.76, 5.36 (2 × 1 H, 2 × d, *J* 9.2, CHOH), 5.42 (1 H, d, *J* 11.1, NCHHO), 5.64 (1 H, d, *J* 11.0, NCHHO), 5.92–6.00 (1H, m, CH=C), 9.49 (1H, s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 14.0 ($ArCH_2CH_3$), 14.8 (OCH_2CH_3), 19.1 ($ArCH_2$), 22.1, 22.4, 24.9, 25.4 (4 × CH_2), 65.3 (OCH_2CH_3), 71.6, 71.9 (NCH_2O, CHOH), 118.0 (C-5), 122.9 (CH=C), 136.1 (CH=*C*), 150.2, 151.9 (C-2, C-6), 163.2 (C-4); *m*/*z* (MALDI MS) 331.2 $(M + Na)^+$, 309.2 $(M + H)^+$; $C_{16}H_{24}N_2O_4$, 0.25 H_2O; calcd.: C 61.42, H 7.73, N 8.95; found: C 61.51, H 7.76, N 8.70%.

General procedure for synthesis of ketones 1a, 2a and 3a

CrO_3 (0.128 g, 1.28 mmol) was pulverised and suspended in dry CH_2Cl_2 (4 cm^3) and the solution was cooled on ice. Anhydrous pyridine (0.228 g, 2.88 mmol) and Ac_2O (0.150 g, 1.47 mmol) were dissolved in dry CH_2Cl_2 (1 cm^3) and added slowly to the cold CrO_3 suspension. The mixture was stirred at rt for 45 min. The alcohols (**6**–**8**, 0.75 mmol) were dissolved in dry CH_2Cl_2 (2 cm^3) and added slowly. The solution was stirred at rt between 4 h and overnight. Then the mixture was poured into EtOAc (25 cm^3) and the mixture was filtered through silica. The silica was rinsed with EtOAc until no more UV-activity was observed. The products were purified by column chromatography (silica gel, 2% EtOH–CH_2Cl_2).

6-But-2-enoyl-1-ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (1a)

Yield: 0.137 g (69%), white powder; mp = 150–153 °C (EtOAc–PE (60–80)); R_f 0.34 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.03 (3 H, t, *J* 7.4, $ArCH_2CH_3$), 1.13 (3 H, t, *J* 7.0, OCH_2CH_3), 2.03 (3 H, dd, *J* 1.3, 6.8, $CHCH_3$), 2.26 (2 H, m, $ArCH_2$), 3.52 (2 H, q, *J* 7.0, OCH_2CH_3), 4.8–5.5 (2 H, br s, NCH_2O), 6.37 (1 H, dd, *J* 1.8, 15.9, COCH), 6.89–7.01 (1 H, m, $CHCH_3$), 9.48 (1 H, s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.4 ($ArCH_2CH_3$), 14.8 (OCH_2CH_3), 18.8 ($CHCH_3$), 19.0 ($ArCH_2$), 64.4 (OCH_2), 73.2 (NCH_2O), 114.3 (C-5), 131.7 (COCH), 146.6 (C-2), 150.7 ($CHCH_3$), 150.8 (C-6), 163.0 (C-4), 189.3 (CO); *m*/*z* (MALDI MS) 289.1 $(M + Na)^+$, 267.1 $(M + H)^+$; $C_{13}H_{18}N_2O_4$; calcd.: C 58.64, H 6.81, N 10.52; found: C 58.39, H 6.79, N 10.28%.

1-Ethoxymethyl-5-ethyl-6-(3-methyl-but-2-enoyl)-1*H*-pyrimidine-2,4-dione (2a)

Yield: 0.135 g (64%), white needles; mp =110–111 °C (Et_2O–PE (60–80)); R_f 0.31 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.06 (3 H, t, *J* 7.4, $ArCH_2CH_3$), 1.14 (3 H, t, *J* 7.0, OCH_2CH_3), 2.02 (3 H, d, *J* 1.1, CH_3), 2.28 (3 H, d, *J* 0.7, CH_3), 2.31 (2 H, q, *J* 7.6, $ArCH_2$), 3.52 (2 H, q, *J* 6.9, OCH_2CH_3), 4.9–5.5 (2 H, br s, NCH_2O), 6.24–6.27 (1 H, m, COCH), 9.28 (1 H, br s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.6 ($ArCH_2CH_3$), 14.7 (OCH_2CH_3), 18.7 (CH_3-*cis*), 21.7 ($ArCH_2$), 28.2 (CH_3-*trans*), 64.4 (OCH_2CH_3), 73.0 (NCH_2O), 113.1 (C-5), 123.8 (COCH), 148.8, 150.8, 161.8, 163.4 (CH=*C*, C-2, C-6, C-4), 187.7 (CO); *m*/*z* (MALDI MS) 303.1 $(M + Na)^+$, 281.1 $(M + H)^+$; $C_{14}H_{20}N_2O_4$; calcd.: C 59.99, H 7.19, N 9.99; found: C 59.67, H 7.18, N 9.89%.

6-(Cyclohex-1-enecarbonyl)-1-ethoxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (3a)

Yield: 0.172 g (75%), light-yellow glace; R_f 0.36 (5% EtOH–CH_2Cl_2); δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.02 (3 H, t, *J* 7.2, $ArCH_2CH_3$), 1.11 (3 H, t, *J* 7.0, OCH_2CH_3), 1.62–1.80 (4 H, m, 2 × CH_2), 2.07–2.30 (2 H, m, $ArCH_2CH_3$), 2.28–2.42 (4 H, m, 2 × CH_2), 3.41–3.59 (2 H, m, OCH_2CH_3), 4.87 (1 H, d, *J* 10.4, NCHHO), 5.36 (1 H, d, *J* 10.4, NCHHO), 6.91 (1 H, t, *J* 3.9, CH=C), 9.28 (1 H, s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 13.3 ($ArCH_2CH_3$), 14.8 (OCH_2CH_3), 19.3 ($ArCH_2$), 21.4, 21.5, 22.1, 26.5 (4 × CH_2), 64.4 (OCH_2CH_3), 73.4 (NCH_2O), 114.3 (C-5), 139.1 (COC), 146.8 (C-2), 148.9 (C=CH), 150.9 (C-6), 162.8 (C-4), 190.6 (CO); *m*/*z* (MALDI MS) 329.1 $(M + Na)^+$, 307.2 $(M + H)^+$; $C_{16}H_{22}N_2O_4$, 0.50 H_2O; calcd.: C 60.94, H 7.03, N 8.88; found: C 61.19, H 7.01, N 8.76%.

1-Allyloxymethyl-5-ethyl-1*H*-pyrimidine-2,4-dione (9)

BSA (5.09 g, 25 mmol) was dissolved in dry CH_3CN (50 cm^3). 5-Ethyl-1*H*-pyrimidine-2,4-dione (**4**, 1.40 g, 10 mmol) was added and the mixture was stirred for 10 min. The resulting clear solution was cooled to −45 °C. TMS–triflate (2.22 g, 10 mmol) was added followed by addition of bis(allyloxy)methane (2.56 g, 20 mmol) dissolved in dry CH_3CN (2 cm^3). The solution was stirred overnight at rt. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (10 cm^3). The solvent was evaporated and the residue was partitioned between CH_2Cl_2 (100 cm^3) and H_2O (50 cm^3). The aqueous phase was further extracted with 2 × 100 cm^3 CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by column chromatography (silica gel,

5% EtOH–CH_2Cl_2) to give **9** (1.96 g, 93%) as a clear oil which crystallised on standing. δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.15 (3 H, t, *J* 7.6, CH_3), 2.38 (2 H, dq, *J* 1.1, 7.5, CH_2CH_3), 4.10 (2 H, dt, *J* 1.4, 5.6, OCH_2CH), 5.18 (2 H, s, NCH_2O), 5.23 (1 H, dq, *J* 1.4, 10.4, CH-*trans* to CH_2), 5.33 (1 H, dq, *J* 1.5, 17.3, CH-*cis* to CH_2), 5.89 (1 H, ddt, *J* 5.7, 10.4, 17.5, CH-*gem* to CH_2), 7.11 (1 H, t, *J* 1.1, H-6), 9.62 (1 H, br s, NH); δ_C(75 MHz; $CDCl_3$; Me_4Si) 12.6 (CH_3), 19.9 (CH_2CH_3), 70.3, 75.8 (OCH_2CH, NCH_2O), 117.4 (C-5), 118.2 ($CH{=}CH_2$), 133.2 ($CH{=}CH_2$), 138.1 (C-6), 151.2 (C-2), 163.9 (C-4); *m*/*z* (MALDI MS (peak matching)) 233.0895 $(M + Na)^+$, calcd. 233.0897.

Viruses and cells

The inhibitory activity against HIV-1 infection was evaluated using MT-4 cells[21] as target cells and either the wild-type HIV-1 strain HTLV-IIIB[22] or the Y181C mutated strain N119[23] as infectious viruses. The virus was propagated in H9 cells[21] at 37 °C, 5% CO_2 using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), separated into aliquots, and stored at −80 °C until use.

Inhibition of HIV-1 replication

Compounds were examined for possible antiviral activity against both strains of HIV-1 using MT-4 cells as target cells. MT4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compound for 6 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.[22] Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT4 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.

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