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Synthesis of 5-Alkyl-6-arylmethyl-2-(7-bromo-3,5-dioxaheptylthio)-pyrimidin-4(1*H*)-ones and 7-Oxopyrimidino-1,5,3-oxathiazepines as New S-*DABO* Analogues with Anti-HIV Activity

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Summary. New S-*DABO*s with a long alkylating S-alkyl substituent showing antiretroviral activity against HIV-1 in the micromolar range were prepared from 5,6-disubstituted 4-oxo-2-thiopyrimidines and 1,7-dibromo-3,5-dioxaheptane. The analogues with an ethyl group in position 5 also showed activity in the micromolar range against a Tyr/8/Cys mutant strain of HIV-1. The S-*DABO* analogues showing activity against the HIV-1 RT mutant strain were transformed to the N-3 and N-1 ring closed 7-oxo-pyrimidino-1,3,5-oxathiazepines which surprisingly all showed activity against HIV-1 in the micromolar range, as well as against a Tyr/8/Cys mutant strain of HIV-1. Some analogues of S-*DABO* with a thien-2-ylmethyl residue in position 6 were synthesized and tested against HIV-1 wild type, but they showed less or comparable activities to those of the corresponding 6-benzyl analogues.

Keywords. 1,7-Dibromo-3,5-dioxaheptane; HIV; 7-Oxopyrimidino-1,5,3-oxathiazepines; S-*DABO*; 6-Thienylmethyl-2-thiouracils.

Synthese von 5-Alkyl-6-arylmethyl-2-(7-bromo-3,5-dioxaheptylthio)-pyrimidin-4(1*H*)-onen und 7-Oxopyrimidino-1,5,3-oxathiazepinen als neue S-*DABO*-Analoga mit Anti-HIV-Aktivität

Zusammenfassung. Neue S-*DABO*-Derivate mit langen alkylierten S-Alkylsubstituenten, die antivirale Eigenschaften gegenüber HIV-1 im mikromolaren Bereich zeigen, wurden aus 5,6-disubstituierten 4-Oxo-2-thiopyrimidinen und 1,7-Dibrom-3,5-dioxaheptan dargestellt. Die Analoga mit einer Ethylgruppe in Position 5 zeigten ebenfalls Aktivität im mikromolaren Bereich gegenüber einem Tyr/8/Cys-Mutantenstamm des HIV-1. Die S-*DABO*-Analoga, die Aktivität gegenüber dem HIV-1 RT Mutantenstamm zeigten, wurden zu den über N-3 und N-1 ringverknüpften 7-Oxo-pyrimidino-1,3,5-oxathiazepinen zyklisiert, welche überraschenderweise Aktivität im mikromolaren Bereich gegenüber HIV-1 und dem Tyr/8/Cys-Mutantenstamm des HIV-1 zeigten. Einige S-*DABO*-

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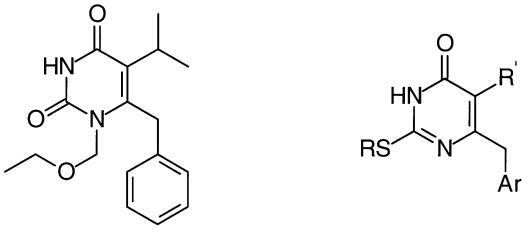
Analoga mit einem Thienyl-2-methylrest in Position 6 wurden ebenfalls dargestellt und gegenüber dem HIV-1-Wildtyp getestet. Sie zeigten jedoch geringere als bzw. vergleichbare Aktivität wie die entsprechenden 6-Benzylanaloga.

Introduction

The virally coded enzyme reverse transcriptase (RT) is an attractive target in the search for new antiviral agents against human immunodeficiency virus type 1 (HIV-1). This enzyme is a pivot in the retroviral cycle of HIV as it synthesizes viral *DNA* for integration in the host genome with viral RNA as template [1]. Several specific inhibitors of HIV-1 RT have been reported; all of them seem to have their effect in a mainly hydrophobic pocket located in the proximity of the catalytic site [2]. Some of these are nevirapine [3], tetrahydroimidazobenzodiazepinethione (*TIBO*) derivatives [4], *bis*-(heteroaryl)-piperazine (*BHAP*) derivatives [5], 1-((2-hydroxyethoxy)-methyl)-6-(phenylthio)-thymine (*HEPT*) derivatives [6], and 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidine (*DABO*) derivatives [7].

One of the *HEPT* derivatives, 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (*MKC-442*) [8] is an especially promising candidate and is now undergoing phase III testing. We have been interested in derivatives of the S-*DABO* class, characterized by an alkyl thio substituent in place of the alkoxy group in *DABO*. The C-6 substituent of S-*DABO* analogues is an aromatic group, *e.g.* phenyl or naphthyl, linked to the pyrimidine ring with a methylene group [9, 10]. In this work we also introduce the thien-2-ylmethyl residue as a possible C-6 substituent of the pyrimidine ring and thus expand the range of possible new S-*DABO* analogues in the future search for anti-HIV active compounds.

The resistance to *MKC-442* for the HIV mutant Tyr181Cys is generally believed to be caused by a lack of π-bond interaction between the 6-benzyl substituent of *MKC-442* and Tyr181Cys which anchors the drug inside the pocket of RT in the case of Tyr181. We are now investigating whether anchoring the drug can be established by formation of a covalent bond to the S-alkyl group of S-*DABO*s. For this purpose we have chosen a bromo substituent on a long 2-alkylthio substituent assuming no change in antiviral activity when compared to a shorter substituent [11]. Taking advantage of crystallographic studies [12] we assumed that proper targets in the RT could be Lys102 and Tyr318 which have an amino group or a hydroxy group, respectively, as close as 4–7 Å to C-2 of *MKC-442*. Proposing



MKC-442 *S-DABOs*

Scheme 1

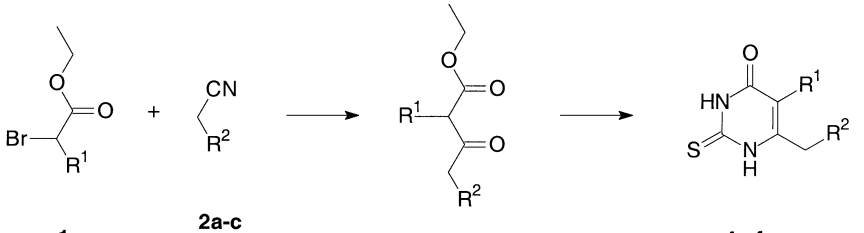
that the S-*DABO*s will be similarly spatially arranged in the RT pocket of *MKC-442*, these amino acids are within reach by using properly chosen bromo substituted S-alkyl derivatized S-*DABO*s. Other target amino acids could be Ser105, Thr107, Ser191, Lys238, and Thr240, although their distances from *MKC-442* are in the range of 10–12 Å.

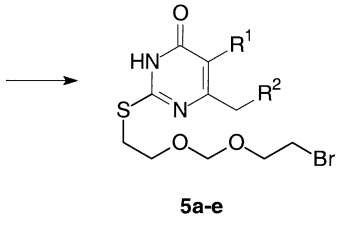
Results and Discussion

Chemistry

The 2-thiopyrimidines **4a–f** were synthesized according to the procedure of *Danel et al*. [13]. Ethyl 2-bromo-3-methylbutanoate was synthesized, using the one-pot procedure of *Berry et al*. [14] starting from valeric acid which was treated with thionyl chloride to afford the acid chloride which in turn was brominated with Br_2 and esterified with ethanol. The proper bromo ester and arylacetonitrile were reacted using zinc in *THF* followed by sequential treatment with aqueous potassium carbonate and hydrochloric acid to afford the proper β-keto ester. This was condensed with thiourea in ethanol and sodium ethoxide to give the 2-thiopyrimidines **4a–f**.

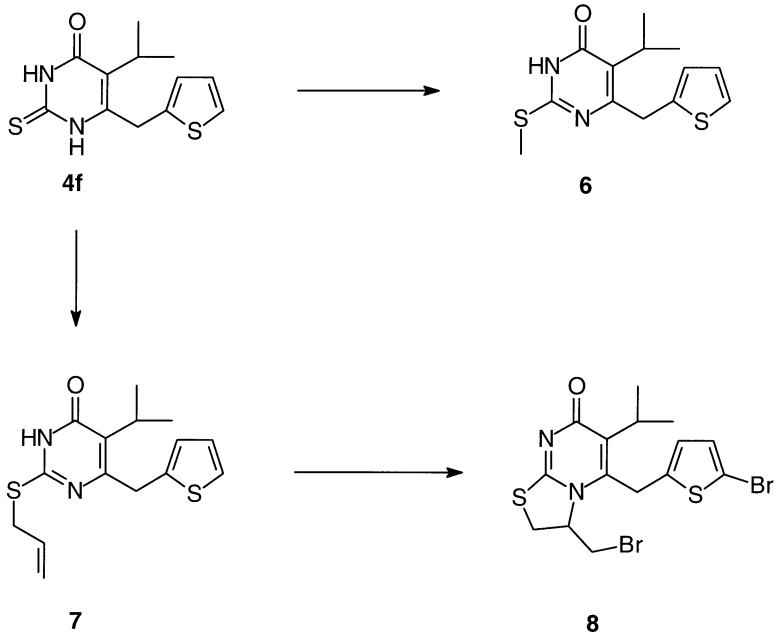
1,7-Dibromo-3,5-dioxaheptane was chosen for S-alkylation of the proper substituted 2-thiouracils because its ability to serve two purposes. It gives a long substituent on C-2 in the pyrimidine ring and it contains oxygen in positions that could be in compliance with one of the hydrophilic regions of the otherwise hydrophobic pocket of RT [15]. After alkylation, the substituent still contains one bromo substituent available for formation of covalent bonding to nucleophilic





	R^1	R^2
3 - 5a	Et	C_6H_5
3 - 5b	Et	3,5-$Me_2C_6H_3$
3 - 5c	Et	2-Thienyl
3 - 5d	*iso*Pr	C_6H_5
3 - 5e	*iso*Pr	3,5-$Me_2C_6H_3$
3 - 5f	*iso*Pr	2-Thienyl

Scheme 2

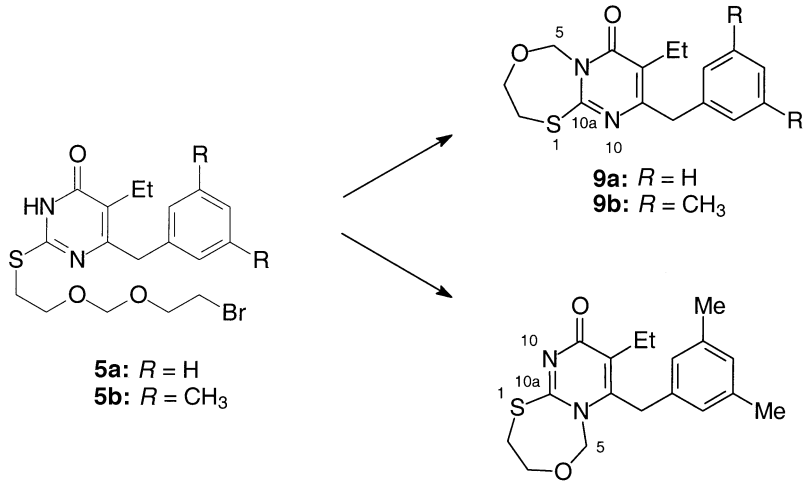


Scheme 3

residues in the hydrophobic pocket. Alkylation of **4** in methanol with methoxide as base afforded the expected S-alkylated products **5a**,**b**,**d**,**e** in 50–75% yield, whereas **5c** was obtained in 35% yield only. The products were isolated using column chromatography followed by recrystallization. Compound **5e** was difficult to crystallize and was obtained as an oil which crystallized slowly on standing.

The synthesis of 1,7-dibromo-3,5-dioxaheptane from 2-bromoethanol, formaldehyde, and calcium chloride has been described previously [16]. In the present work, 1,3,5-trioxane was used as substitute of formaldehyde. It was found to be necessary to replace calcium chloride with sodium sulfate as drying agent in order to avoid impurities of mono and dichloro analogues which were difficult to remove from the desired dibrominated product by distillation. The structure of compounds **5a–e** were confirmed by NMR spectroscopy. A chemical shift value for C-6 of approximately 156 ppm indicates S-alkylation at C-2. This value has been observed in many S-alkylated compounds of the S-*DABO* type [10, 15, 17].

Compound **4f** was alkylated with methyl iodide in methanol with methoxide as base and afforded 5-isopropyl-2-methylthio-6-(thien-2-ylmethyl)-pyrimidine-4(3*H*)-one (**6**) in 75% yield. Alkylation with allyl bromide using the same base/solvent mixture afforded 2-allylthio-5-isopropyl-6-(thien-2-ylmethyl)-pyrimidin-4(3*H*)-one (**7**) in 42% yield. In both alkylation reactions the alkylation reagents were used in 5:1 excess, and as reported by *Danel et al.* [17] only alkylation at the sulfur atom was observed. Ring closing reaction of the allylated S-*DABO* analogue **7** with Br_2 in CH_2Cl_2 according to the procedure of *Danel et al.* [17] gave **8** with the thienyl ring additionally brominated at position 5. The structure was determined by comparing the spectroscopic data with those obtained by *Danel et al.* The methylene protons of the thien-2-ylmethyl group were separated characteristically into two doublets in the ^{1}H NMR spectrum due to the asymmetric position 3.



Scheme 4

Compounds **5a**,**b** were chosen for a ring closure reaction using a modified *Niedballa* and *Vorbrüggen* condensation [18, 19]. Compounds **5a**, **b** were silylated using N,O-*bis*-trimethylsilylacetamide (*BSA*) in dry acetonitrile and treated with trimethylsilyl trifluoromethanesulfonate (*TMS*-triflate) at −40°C to catalyze the ring closure to give **9** and **10**. The product of the reaction seems to be either a thermic or a dynamic product. For a slow reaction with one equivalent of *TMS*-triflate the outcome tends to be a degradation or an N-3 ring closed product (**9a**,**b**), whereas for a fast reaction with two equivalents of *TMS*-triflate the main product will be the N-1 ring closed compound **10**. The 2 equivalents of *TMS*-triflate were added in portions at −40°C until a product appeared on TLC ($MeOH/CH_2Cl_2$) at a lower R_f value than the starting material. During work-up of the N-1 ring closed product **10** it was necessary to avoid any presence of acid contamination in the solvent. Using CH_2Cl_2 untreated with bicarbonate resulted in degradation of the product during work-up and purification. When 1.2 equivalents *TMS*-triflate were added slowly and dropwise at −40°C, no product emerged on TLC within 1–3 hours. The reaction mixture was placed in a −20°C freezer until a product with a high R_f value appeared on TLC ($MeOH/CH_2Cl_2$), the outcome being the N-3 ring closed product **9b**. A similar feature has been observed in the synthesis of N-glycosides [20]. It has been found that when the optimal reaction time was exceeded the yield of the N-1 nucleoside decreased in favour of less polar products. This corresponds well to the formation of the less polar N-3 ring closed products **9a**,**b** with high R_f values compared to the N-1 ring closed product **10** with a lower R_f value. It has also been suggested that if a reaction is not proceeding according to TLC, one should add a further amount of catalyst or raise the temperature [18]. This is also in accordance with our observations, just with the addition that the best result was obtained when additional catalyst was added. The distinction between **9** and **10** was achieved by NOE experiments. For compound **10**, irradiation of H-5 showed a 2.5% NOE effect on the benzyl methylene protons. The irradiation of the

corresponding protons in **9a** showed no NOE effect. The structure of **9a** was instead confirmed by selective decoupling experiments of H-5 and the benzyl protons. C-9 was assigned by decoupling of the phenyl methylene protons. Decoupling of H-5 affected the coupling patterns of C-7 and C-10a, but not the coupling pattern of C-9.

Antiviral activity

The test for activity against HIV-1 was performed in MT-4 cell cultures infected with either wildtype HIV-1 (strain IIIB) or NNRTI resistant HIV-1 (strain N119). The results are presented in Table 1. The HIV-1 strain N119 harbours a substitution of cysteine for the tyrosine at position 181 in the reverse transcriptase enzyme, confering resistance against NNRTI's (Tyr/8/Cys mutant strain) [21].

The benzyl analogues with a dioxaheptane substituent on sulfur in the S-*DABO* class of compounds retained anti-HIV activities showing that long substituents may be an option in the S-*DABO* class. Compounds **5a**,**b**,**d**,**e** were active in the micromolar range against wild type HIV-1 with ED_{50} values ranging from 1 to 10 μM and with CD_{50} values ranging from 28 to 46 μM. Compounds with an ethyl substituent in position 5 of the pyrimidine ring as in **5a** and **5b** also showed activity against the Tyr/8/Cys mutant strain of HIV-1 with an only 3-and 4-fold decrease in the ED_{50} values, respectively. With an isopropyl substituent in position 5 of **5d** and **5e**, the activity was increased by a factor 3 compared to **5a** and **5b** against the wild type HIV-1 strain; however, no activity against the Tyr/8/Cys strain of HIV-1 was observed. Compound **5c** with a 2-thienyl group in place of a phenyl moiety

Table 1. Antiviral activity against HIV-1 in MT-4 cells

	ED_{50} (μM)[a]		CD_{50} (μM)[b]
Compound	HIV-1 III wt	HIV-1 N119 (Tyr/8/Cys)	MT-4
5a	10	31.6	46
5b	2.7	10	42
5c	>37	>37	37
5d	3.6	>31	31
5e	1	>28	28
6	16	ND[c]	100
7	2.2	ND	32
8	>36	ND	36
9a	4.9	ND	>100
9b	1.6	6.3	>100
10	0.6	19	>100
AZT	0.04	0.03	52
MKC-442	0.005	4.2	141

[a] Effective dose of compound achieving 50% inhibition of HIV-1 antigen production in MT-4 cultures; [b]cytoxic dose of compound required to reduce proliferation of normal uninfected MT-4 cells by 50%; [c]not determined (ND)

showed no subtoxic activity against the two strains of HIV-1 in the test. The methylated (**6**) and allylated (**7**) S-*DABO* analogues with 2-thienyl as substitute for phenyl did show activity against HIV-1 wt in the micromolar range. The antiviral activity for the 6-thien-2-ylmethyl analogue **7** was comparable with that found for the 6-benzyl analogue (ED_{50}(**7**): 2.2 μ*M* and ED_{50}(6-benzyl analogue): 1.5 μ*M* [17]), but it was more cytotoxic than the benzyl analogue. Compounds where phenyl is substituted with an aromatic heterocycle may still be an option in the S-*DABO* class in the future search for drug candidates, though substitution of benzyl with thien-2-ylmethyl did not improve the anti-HIV activities. The 6-(thien-2-ylmethyl) analogues had comparable or less activities relative to the benzyl analogues, and for compounds **5c** and **8** the thien-2-ylmethyl analogues were devoid of subtoxic anti-HIV activities.

The 7-oxo-pyrimidino-1,5,3-oxathiazepines **9a**,**b** and **10** showed activity in the micromolar range against HIV-1 wt in MT-4 cells. For compound **10**, the ED_{50} value was as low as 0.6 μ*M*, and the CD_{50} value was higher than 100 μ*M* which was the highest concentration in the test. The activity of **9a** and **9b** is surprising, as previous N-3 ring closed S-*DABO* analogues have been without subtoxic activity against HIV [17]. *HEPT* and S-*DABO* analogues additionally alkylated at N-3 or N-1 have also been found devoid of subtoxic activity [9, 17, 22], probably caused by the lack of N-3 hydrogen which is a prerequisite for hydrogen bonding to the Lys101 carbonyl atom of RT in RT-*HEPT* complexes [12]. Compounds **9b** and **10**, representing the N-3 and N-1 ring closed S-*DABO* analogues with nearly the same activity against the wild type strain of HIV-1, were also tested against a Tyr/8/Cys mutant strain. This also gave ED_{50} values in the micromolar range, surprisingly with the N-3 ring closed compound **9b** as the most active analogue. Compound **10** as the N-1 ring closed analogue and the most potent of these 7-oxopyrimidino-1,5,3-oxathiazepines showed 100-fold less activity than *MKC-442* against HIV-1 wild type but was comparable with *MKC-442* in activity against the mutant strain of HIV-1. The 7-oxopyrimidino-1,5,3-oxathiazepines could serve as new leads in the search for anti-HIV active compounds as they display moderate anti-HIV activities, low cytotoxicity, and may be active against mutant HIV-1 strains that render other NNRTI inactive.

Experimental

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Bruker AC-250 FT spectrometer at 250 MHz for ^{1}H and at 62.9 MHz for ^{13}C with *TMS* as an internal standard. EI mass spectra were recorded on a Finnigan Mat SSQ 710, FAB mass spectra on a Kratos MS50RF instrument. IR spectra were recorded on a Perkin Elmer 1720 FT-IR spectrometer. The progress of the reaction was monitored by TLC (analytical silica gel plates 60 F_{254}). Merck silica gel (0.040–0.063 mm) was used for column chromatography, and Merck silica gel (0.063–0.200 mm) for preparative thin layer chromatography (PTLC). Elemental analyses were performed by the Microanalytical Department, Chemical Laboratory II at The University of Copenhagen, Denmark; the results were in satisfactory agreement with the calculated values.

Typical procedure for the preparation of **3**

Zn (10 g), activated by sequential washing with, 3 *M* HCl, dist. H_2O, abs. EtOH, and dry Et_2O, was suspended in dry *THF* under reflux, and 8 drops of ethyl 2-bromo-3-methylbutanoate and a few

crystals of I_2 were added to initiate the reaction. When the mixture turned green, 2.45 g 3,5-dimethylphenylacetonitrile (16.9 mmol) were added in one portion; subsequently, 9.04 g ethyl 2-bromo-3-methylbutanoate (43.4 mmol) were added dropwise. The mixture was refluxed for further 30 min. After dilution with 150 cm^3 *THF* and cooling to room temperature, the reaction mixture was stirred with 40 cm^3 50% K_2CO_3 for 30 min. The *THF* fraction was decanted and the water fraction was washed with 3×30 cm^3 *THF*. The combined *THF* fractions were stirred with 25 cm^3 10% HCl for 30 min. The solvent was evaporated and the residue was redissolved in 150 cm^3 CH_2Cl_2, washed with $NaHCO_3$, dried over Na_2SO_4, and evaporated *in vacuo* to give **3** quantitatively as an oil that was considered sufficiently pure for the synthesis of **4**.

Ethyl 2-ethyl-3-oxo-4-(2-thienyl)butanoate (**3c**; $C_{12}H_{16}O_3S$)

Yield: 94%; ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 0.88 (t, 3H, $J=7.3$ Hz, CH_2CH_3), 1.26 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 1.90 (quint, 2H, $J=7.4$ Hz, CH_3CH_2CH), 3.51 (t, 1H, $J=7.3$ Hz, C*H*), 4.0 (s, 2H, CH_2), 4.17 (q, 2H, $J=7.1$, CH_2), 6.98–7.24 (m, 3H, *H*-thiophene) ppm; ^{13}C NMR ($CDCl_3$, δ, 750 MHz): 11.7 (CH_3CH_2), 13.9 (OCH_2CH_3), 21.4 (CH_3CH_2), 42.6 (CH_2), 59.2 (CH_2), 61.4 (CH), 125.4, 127.0, 127.3 (C-thiophene), 169.6 (COOEt), 201.5 (CH_2CO) ppm.

Ethyl 4-(3,5-dimethylphenyl)-2-isopropyl-3-oxobutanoate (**3e**; $C_{17}H_{24}O_3$)

$R_f=0.62$ (10% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 0.86 (d, 3H, $J=6.8$ Hz, $CHCH_3$), 0.94 (d, 3H, $J=6.8$ Hz, $CHCH_3$), 1.23 (t, 3H, $J=7.1$ Hz, CH_2CH_3), 2.29 (s, 6H, $PhCH_3$), 2.35–2.52 (m, 1H, $CH(CH_3)_2$), 3.32 (d, 1H, $J=9.4$ Hz, CH), 3.71 (s, 2H, CH_2), 4.13 (q, 2H, $J=7.1$ Hz, CH_2CH_3), 6.80 (s, 2H, H-arom), 6.90 (s, 1H, H-arom) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 13.90 (CH_2CH_3), 20.14, 20.42 ($CHCH_3$), 21.04 ($PhCH_3$), 28.33 ($CHCH_3)_2$), 49.33 (CH_2), 61.07 (OCH_2), 65.60 (CH), 127.50, 128.83, 132.94, 138.17 (C-arom), 169.10 ($CHCO_2$), 202.73 (CH_2CO) ppm.

Ethyl 2-isopropyl-3-oxo-4-(2-thienyl)butanoate (**3f**; $C_{13}H_{18}O_3S$)

Yield: 52% red-brown oil; $R_f=0.49$ (CH_2Cl_2); MS (FAB): $m/z=255$ (M+H); IR (KBr): $\nu=1718$ (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 0.90 (d, 3H, $J=6.8$ Hz, $CHCH_3$), 0.97 (d, 3H, $J=6.7$ Hz, $CHCH_3$), 1.25 (t, 3H, $J=7.1$ Hz, CH_2CH_3), 2.37–2.52 (m, 1H, $CH(CH_3)_2$), 3.36 (d, $J=9.4$ Hz, 1H, CH), 4.01 (s, 2H, CH_2), 4.16 (q, 2H, $J=7.1$ Hz, CH_2), 6.90 (d, 1H, $J=3.5$ Hz, 3-H), 6.97 (dd, 1H, $J=3.3$, 5.1 Hz, 4-H), 7.23 (dd, 1H, $J=1.2$ Hz, 5.1 Hz, 5-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 13.92 (CH_2CH_3), 20.13 ($CHCH_3$), 20.39 (CH_3), 28.46 ($CHCH_3)_2$), 42.83 (CH_2), 61.22 (OCH_2), 65.49 (CH), 125.33, 126.95, 127.25, 134.25 (thiophene), 168.91 ($CHCO_2$), 201.14 (CH_2CO) ppm.

Typical procedure for the preparation of **4**

Na (1.16 g, 50 mg Atom) was dissolved in 25 cm^3 abs. EtOH. Ethyl 4-(3,5-dimethylphenyl)-2-isopropyl-3-oxobutanoate ($\sim$16.9 mmol) and 2.67 g thiourea (34 mmol) were added, and the solution was refluxed for 17 h. The solvent was evaporated and the residue redissolved in 10 cm^3 H_2O. The solution was neutralized with HCl and acidified with CH_3COOH ($pH\sim3$). The solid was filtered off and recrystallized from EtOH to give **4**.

2,3-Dihydro-5-ethyl-6-(thien-2-ylmethyl)-2-thioxopyrimidin-4(1H)-one (**4c**; $C_{11}H_{12}N_2OS_2$)

Yield: 24%; $R_f=0.70$ (10% EtOH in CH_2Cl_2); MS (FAB): $m/z=253$ (M+H); ^{1}H NMR (*DMSO*-d_6, δ, 300 MHz): 0.93 (t, 3H, $J=7.3$ Hz, CH_3) 2.34 (q, 2H, $J=7.3$ Hz, CH_2) 4.04 (s, 2H, CH_2) 6.98–7.44

(m, 3H, H-thiophene) 12.43 (br s, 2H, 2×NH) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 75 MHz): 12.9 (CH_3), 17.7 (CH_2), 29.3 (CH_2), 116.8 (C-5), 125.3, 126.4, 127.1 (C-thiophene), 138.7 (C-2, thiophene), 148.9 (C-6), 161.6 (C-4), 174.5 (C-2) ppm.

2,3-Dihydro-6-(3,5-dimethylphenylmethyl)-5-isopropyl-2-thioxopyrimidin-4(1H)-one (**4e**; $C_{16}H_{20}N_2OS$)

Yield: 1.52 g (32%); $R_f = 0.57$ (6% MeOH in CH_2Cl_2); m.p.: 183°C; MS (FAB): $m/z = 289$ (M+H); ^{1}H NMR (DMSO-d_6, δ, 300 MHz): 1.05 (d, 6H, $J = 6.9$ Hz, CHCH_3), 2.21 (s, 6H, CH_3), 2.78 (sept., 1H, $J = 6.9$ Hz, CH), 3.78 (s, 2H, CH_2), 6.79 (s, 2H, arom), 6.84 (s, 1H, arom), 12.11 (s, 1H, NH), 12.18 (s, 1H, NH) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 75 MHz): 19.46 (CH*C*H$_3$), 20.78 (CH_3), 26.55 (CH), 34.49 (CH_2), 119.64 (C-5), 125.72, 128.15, 136.69, 137.67 (C-arom), 149.13 (C-6), 160.86 (C-4), 174.28 (C-2) ppm.

2,3-Dihydro-5-isopropyl-6-(thien-2-yl-methyl)-2-thioxopyrimidin-4(1H)-one (**4f**; $C_{12}H_{14}N_2OS_2$)

Yield: 24%; $R_f = 0.57$ (10% EtOH in CH_2Cl_2); m.p.: 217–219°C; IR (KBr): $\nu = 1208$ (C=S), 1662 (C=O), 3436 (NH) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, δ, 300 MHz): 1.15 (d, 6H, $J = 6.7$ Hz, CH(CH_3)$_2$), 2.94 (hept, 1H, $J = 6.7$ Hz, CH), 4.05 (s, 2H, CH_2), 6.97–7.0 (m, 2H, 3′-H, 4′-H), 7.4 (dd, 1H, $J = 1.9$ Hz, 4.4 Hz, 5′-H), 12.21 (s, 1H, NH), 12.32 (s, 1H, NH) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 75 MHz): 19.55 (2×CH_3), 26.59 (CH), 29.41 (CH_2), 119.36 (C-5), 125.25, 126.31, 127.05, 138.78 (C-thiophene), 148.53 (C-6), 160.81 (C-4), 174.37 (C-2) ppm.

1,7-Dibromo-3,5-dioxaheptane

1,3,5-Trioxane (3.780 g, 42 mmol) was dissolved in 15 cm^3 2-bromoethanol (212 mmol). Na_2SO_4 (3.370 g, 28 mmol) was added, and the mixture was refluxed for 17 h. After the temperature had reached room temperature the mixture was filtered and washed sequentially with 2×10 cm^3 H_2O, 10 cm^3 0.5 *M* NaOH, 10 cm^3 5% $NaHSO_3$, and 10 cm^3 0.5 *M* NaOH. After drying over Na_2SO_4 the oil was distilled at 130–136°C/20 mbar (Ref. [23]: b.p.$_{0.04}$: 39°C) to afford 5.52 g (10%) 1,7-dibromo-3,5-dioxaheptane as a clear liquid.

^{1}H NMR ($CDCl_3$, δ, 300 MHz): 3.50 (t, 4H, $J = 6.1$ Hz, CH_2Br), 3.90 (t, 4H, $J = 6.1$ Hz, OCH_2), 4.77 (s, 2H, OCH_2O) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 30.62 (CH_2Br), 68.02 (OCH_2), 95.38 (OCH_2O) ppm.

General procedure for the preparation of 2-(7-bromo-3,5-dioxaheptylthio)-pyrimidin-4(1H)-ones **5**

Na (50 mg) was dissolved in 6 cm^3 MeOH. 2-Thiouracil (**4**, 2 mmol) and 1.159 g 1,7-dibromo-3,5-dioxaheptane (4.4 mmol) were added. The mixture was stirred at room temperature until all starting material was consumed according to TLC (6% MeOH in CH_2Cl_2). H_2O (10 cm^3) was added, and the mixture was extracted with 3×25 cm^3 Et_2O. The collected organic phases were dried over Na_2SO_4 and evaporated. The products **5** were purified on a silica column with EtOAc/petroleum ether (60–80°C). The products were further purified by recrystallization from ethyl acetate and petroleum ether.

6-Benzyl-2-(7-bromo-3,5-dioxaheptyl)-thio-5-ethylpyrimidin-4(1H)-one (**5a**; $C_{18}H_{23}BrN_2O_3S$)

Yield: 636 mg (74%); $R_f = 0.39$ (10% MeOH/CH_2Cl_2); m.p.:79–81°C; MS (FAB): $m/z = 429$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.09 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 2.59 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 3.29 (t, 2H, $J = 6.1$ Hz, CH_2-S), 3.45 (t, 2H, $J = 6.1$ Hz, CH_2-Br), 3.72 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2S), 3.85 (t, 2H, $J = 6.1$ Hz,OCH_2CH_2Br), 3.90 (s, 2H, CH_2-Ar), 4.68 (s, 2H, O-CH_2-O), 7.15–7.35 (m, 5H, arom), 12.7 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 13.04 (CH_3CH_2),

18.60 (CH_3CH_2), 30.19 (SCH_2), 30.65 ($BrCH_2$), 40.26 (CH_2Ph), 66.43 (OCH_2CH_2S), 67.97 (OCH_2CH_2Br), 95.35 (OCH_2O), 122.47 (C-5), 126.46, 128.42, 129.04, 138.34 (C-arom), 156.11 (C-6), 161.69 (C-4), 165.24 (C-2) ppm.

2-(7-Bromo-3,5-dioxaheptyl)-thio-6-(3,5-dimethylbenzyl)-5-ethylpyrimidin-4(1H)-one (**5b**; $C_{20}H_{27}BrN_2O_3S$)

Yield: 687 mg (75%); $R_f = 0.30$ (6% MeOH/CH_2Cl_2); m.p.: 116–117°C; MS (FAB): $m/z = 457$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.09 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 2.57 (s, 6H, Ph-CH_3), 2.59 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 3.32 (t, 2H, $J = 6.1$ Hz, CH_2-S), 3.45 (t, 2H, $J = 6.1$ Hz, CH_2-Br), 3.75 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2S), 3.82 (s, 2H, CH_2-Ar), 3.85 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2Br), 4.7 (s, 2H, O-CH_2-O), 6.84 (m, 3H, arom), 12.7 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 Hz): 13.04 (CH_3CH_2), 18.62 (CH_3CH_2), 21.13 (CH_3Ph), 30.27 (SCH_2), 30.61 ($BrCH_2$), 40.13 (CH_2Ph), 66.41 (OCH_2CH_2S), 67.96 (OCH_2CH_2Br), 95.33 (OCH_2O), 122.40 (C-5), 126.82, 128.07, 137.89, 138.11 (C-arom), 156.00 (C-6), 161.91 (C-4), 165.30 (C-2) ppm.

2-(7-Bromo-3,5-dioxaheptyl)-thio-5-ethyl-6-(thien-2-ylmethyl)-pyrimidin-4(1H)-one (**5c**; $C_{16}H_{21}BrN_2O_3S_2$)

Yield: 300 mg (35%); $R_f = 0.38$ (6% MeOH in CH_2Cl_2); m.p.: 80–81°C; MS (FAB): $m/z = 435$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.12 (t, 3H, $J = 7.6$ Hz, CH_2CH_3), 2.60 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 3.39 (t, 2H, $J = 6.1$ Hz, CH_2-S), 3.47 (t, 2H, $J = 6.1$ Hz, CH_2-Br), 3.82 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2S), 3.88 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2Br), 4.06 (s, 2H, CH_2-Ar), 4.73 (s, 2H, OCH_2O), 6.8–7.2 (m, 3H, arom), 12.71 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 13.15 (CH_3CH_2), 18.50 (CH_3CH_2), 30.25 (CH_2S), 30.67 (CH_2Br), 34.71 (CH_2), 66.53 (OCH_2CH_2S), 67.98 (OCH_2CH_2Br), 95.38 (OCH_2O), 122.11 (C-5), 124.41, 125.72, 126.61, 140.03 (C-arom), 156.58 (C-6), 160.61 (C-4), 165.21 (C-2) ppm.

6-Benzyl-2-(7-bromo-3,5-dioxaheptyl)-thio-5-isopropylpyrimidin-4(1H)-one (**5d**; $C_{19}H_{25}BrN_2O_3S$)

Yield: 508 mg (58%); $R_f = 0.64$ (6% MeOH/CH_2Cl_2); m.p.: 75–76.5°C; MS (FAB): $m/z = 443$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.29 (d, 6H, $J = 7.0$ Hz, CH CH_3), 3.10 (sept, 1H, $J = 7.0$ Hz, $CHCH_3$), 3.30 (t, 2H, $J = 6.1$ Hz, CH_2-S), 3.45 (t, 2H, $J = 6.1$ Hz, CH_2-Br), 3.73 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2S), 3.85 (t, 2H, $J = 6.1$ Hz, OCH_2CH_2Br), 3.94 (s, 2H, CH_2-Ar), 4.69 (s, 2H, O-CH_2-O), 7.15–7.35 (m, 5H, arom), 12.88 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 19.59 (CH_3CH), 27.82 (CH_3CH), 30.06 (SCH_2), 30.66 ($BrCH_2$), 40.85 (CH_2Ph), 66.47 (OCH_2CH_2S), 67.94 (OCH_2CH_2Br), 95.34 (OCH_2O), 125.16 (C-5), 126.37, 128.44, 128.83, 138.63 (C-arom), 156.24 (C-6), 161.31 (C-4), 164.80 (C-2) ppm.

2-(7-Bromo-3,5-dioxaheptyl)-thio-6-(3,5-dimethylbenzyl)-5-isopropylpyrimidin-4(1H)-one (**5e**; $C_{21}H_{29}BrN_2O_3S$)

Yield: 466 mg (50%); $R_f = 0.64$ (6% MeOH/CH_2Cl_2); m.p.: 47–48°C; MS (FAB): $m/z = 469$, 471 (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.29 (d, 6H, $J = 6.9$ Hz, 2×$CHCH_3$), 2.27 (s, 6H, $PhCH_3$), 3.09 (sept, 1H, $J = 7.0$ Hz, CH_3CH), 3.32 (t, 2H, $J = 6.1$ Hz, CH_2), 3.44 (t, 2H, $J = 6.1$ Hz, CH_2), 3.76 (t, 2H, $J = 6.1$ Hz, CH_2), 3.85 (t, 3H, $J = 6.1$ Hz, OCH_2), 3.86 (s, 2H, CH_2Ph), 4.69 (s, 2H, OCH_2O), 6.81 (s, 2H, arom), 6.84 (s, 1H, arom), 12.93 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 19.57 (CH_3CH), 21.13 (CH_3Ph), 27.84 (CH_3CH), 30.11 (SCH_2), 30.63 ($BrCH_2$), 40.70 (CH_2Ph), 66.42 (OCH_2CH_2S), 67.92 (OCH_2CH_2Br), 95.30 (OCH_2O), 125.08 (C-5), 126.58, 127.95, 137.88, 138.34 (C-arom), 156.14 (C-6), 161.51 (C-4), 164.85 (C-2) ppm.

5-Isopropyl-2-methylthio-6-(thien-2-ylmethyl)-pyrimidine-4(3H)-one (**6**; $C_{13}H_{16}N_2OS_2$)

4f (266 mg, 1 mmol) was suspended in 6 cm^3 dry MeOH, and sodium methoxide (59 mg, 1.1 mmol) was added. Methyl iodide (710 mg, 5 mmol) was added, and the reaction was monitored by TLC by acidifying a small sample. After 20 min the reaction was stopped by addition 5 cm^3 H_2O to the white suspension. The solid was filtered off, dried and recrystallized from EtOAc and petroleum ether (b.p.: 60–80°C) to give 210 mg (75%) **6**.

$R_f = 0.31$ (30% EtOAc in petroleu mether (60–80°C)); m.p.: 177–179°C (EtOAc in petroleum ether (60–80°C)); MS (FAB): $m/z = 281$ (M+H); IR (KBr): $\nu = 1646$ (C=O), 3436 (NH) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, δ, 300 MHz): 1.21 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 2.49 (s, 3H, SCH_3), 3.13 (hept, 1H, $J = 6.9$ Hz, CH), 4.07 (s, 2H, CH_2), 6.93–6.96 (m, 2H, thiophene), 7.34 (dd, 1H, $J = 1.7$ Hz, 3.1 Hz, thiophene), 12.5 (s, 1H, NH) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 75 MHz): 12.52 (SCH_3), 19.63 ($CH(CH_3)_2$), 26.89 (CH), 34.62 (CH_2), 124.73, 125.64, 126.69, 140.54 (C-thiophene) ppm.

2-Allylthio-5-isopropyl-6-(thien-2-ylmethyl)-pyrimidin-4(3H)-one (**7**; $C_{15}H_{18}N_2OS_2$)

4f (266 mg, 1 mmol) was suspended in 6 cm^3 dry MeOH. Sodium methoxide (59 mg, 1.1 mmol) and 605 mg allyl bromide (5 mmol) were added. After 16.5 h 10 cm^3 H_2O was added, and the mixture was extracted with Et_2O (3×25 cm^3). The organic fractions were dried over Na_2SO_4 and evaporated. The residue was recrystallized from EtOAc and petroleum ether (b.p.: 60–80°C) and then from Et_2O and petroleum ether (b.p.: 60–80°C) to give 130 mg (42%) of **7**.

$R_f = 0.37$ (30% EtOAc in petroleum ether (60–80°C)); m.p.: 99–101°C; MS (FAB): $m/z = 307$ (M+H); IR (KBr): $\nu = 1646$ (C=O), 3437 (NH) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, δ, 300 MHz): 1.18 (d, 6H, $J = 7.0$ Hz, $CH(CH_3)_2$), 3.10 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 3.76 (d, 2H, $J = 6.9$ Hz, SCH_2), 4.04 (s, 2H, CH_2), 5.05 (dd, 1H, $J = 9.9$ Hz, 1.1 Hz, CH=C*H*H), 5.22 (dd, 1H, $J = 15.7$ Hz, 1.1 Hz, CH=CH*H*), 5.86 (m, 1H, C*H*=CH_2), 6.87–6.93 (m, 2H, thiophene), 7.30 (dd, 1H, $J = 1.2$ Hz, 3.7 Hz, thiophene), 12.44 (s, 1H, NH) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 75 MHz): 19.59 ($CH(CH_3)_2$), 26.90 ($CH(CH_3)_2$), 32.10 (CH_2), 34.70 (CH_2), 118.21 (CH=CH_2), 123.54 (C-5), 124.69, 125.67, 126.71 (C-thiophene), 133.64 (CH=CH_2), 140.57 (C-thiophene), 157 (C-6), 159.17 (C-2), 162.54 (C-4) ppm.

3-Bromomethyl-5-((5-bromothien-2-yl)-methyl)-2,3-dihydro-6-isopropyl-7H-thiazolo[3,2-a]pyrimidin-7-one (**8**; $C_{15}H_{16}Br_2N_2OS_2$)

7 (239 mg, 0.78 mmol) was dissolved in 5 cm^3 dry CH_2Cl_2. *Bis*-(trimethylsilyl)-acetamide (160 mg, 0.78 mmol, 0.2 cm^3) was added in one portion, and 310 mg Br_2 (1.94 mmol, 0.1 cm^3) dissolved in 5 cm^3 dry CH_2Cl_2 was added dropwise. During the reaction time the colour of the reaction mixture changed from red-brown to dark green. After 4 h the mixture was evaporated and purified by column chromatography (0.5–1% MeOH in $CHCl_3$) to give 172 mg (47%) of **8**.

$R_f = 0.20$ (5% MeOH in CH_2Cl_2); m.p.: 157–168°C; MS (FAB): $m/z = 465$ (M+H); IR (KBr): $\nu = 1608$ (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.33 (d, 3H, $J = 6.7$ Hz, CH_3), 1.37 (d, 3H, $J = 6.9$ Hz, CH_3), 2.98 (m, 1H, C*H*(CH_3)$_2$), 3.23 (m, 1H, C*H*HBr), 3.42 (d, 1H, $J = 11.9$ Hz, SCH*H*), 3.59 (m, 1H, SC*H*H), 3.71 (t, 1H, $J = 10.6$ Hz, CH*H*Br), 3.91 (d, 1H, $J = 17.2$ Hz,C*H*H-thiophene), 4.32 (d, 1H, $J = 16.9$ Hz, CH*H*-thiophene), 4.84 (m, 1H, C*H*CH_2Br), 6.66 (d, 1H, $J = 3.7$ Hz, thiophene), 6.97 (d, 1H, $J = 3.8$ Hz, thiophene) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 19.35 (CH_3), 20.24 (CH_3), 28.33, 28.40 (*C*H(CH_3)$_2$, *C*HBr), 29.82 (*C*H_2-thiophene), 30.44 (C-2), 63.98 (C-3), 112.04 (C-thiophene), 126.04 (C-6), 126.37, 130.36, 138.67 (C-thiophene), 142.28 (C-5), 164.90, 168.28 (C-7, C-8a) ppm.

9-Benzyl-8-ethyl-7-oxo-2,3,5-trihydropyrimidino[3,2-c]-1,5,3-oxathiazepine (**9a**; $C_{16}H_{18}N_2O_2S$)

5a (407 mg, 0.95 mmol) was suspended in 8 cm^3 dry CH_3CN. *Bis*-(trimethylsilyl)-acetamide (0.50 cm^3, 2.43 mmol) was added, and the mixture was stirred for 4 h at room temperature. The

mixture was cooled to −45°C, and 0.18 cm^3 *TMS*-triflate (0.95 mmol) dissolved in 4 cm^3 dry CH_3CN were added dropwise. The temperature was allowed to raise slowly to room temperature while monitored by TLC (10% MeOH in CH_2Cl_2). After 3 weeks at room temperature the mixture was heated to 40°C for 2 h and then diluted with 50 cm^3 CH_2Cl_2 and washed with sat. $NaHCO_3$ (3×10 cm^3) and H_2O (3×10 cm^3). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to give a yellow oil which was chromatographed on silica (1% MeOH in CH_2Cl_2) to give 54 mg (19%) of **9a** as a foam.

$R_f = 0.79$ (10% MeOH in CH_2Cl_2); MS (FAB): $m/z = 303$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.04 (t, 3H, $J = 7.6$ Hz, CH_2CH_3), 2.56 (q, 2H, $J = 7.6$ Hz, CH_2CH_3), 3.09 (t, 2H, CH_2CH_2S), 3.90 (s, 2H, CH_2-Ph), 4.13 (m, 2H, OCH_2CH_2), 5.80 (s, 2H, H-5), 7.16–7.32 (m, 5H, arom) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 12.54 (CH_2CH_3), 19.74 (CH_2CH_3), 34.14 (C-2), 40.15 (CH_2-Ph), 71.18 (C-3), 77.03 (C-5), 125.80 (C-8), 126.61, 128.57, 128.78, 137.87 (C-arom), 157.20, 163.00 (C-7, C-10a), 158.93 (C-9) ppm.

9-(3,5-Dimethylbenzyl)-8-ethyl-7-oxo-2,3,5-trihydropyrimidino[3,2-c]-1,5,3-oxathiazepine (**9b**; $C_{18}H_{22}N_2OS_2$)

5b (248 mg, 0.6 mmol) was suspended in 8 cm^3 dry CH_3CN, 0.31 cm^3 *bis*-(trimethylsilyl)-acetamide (1.5 mmol) were added slowly, and the solution was stirred at room temperature for 4 h. The mixture was placed under N_2 and cooled to −20°C. 0.12 cm^3 *TMS*-triflate (0.7 mmol) were added dropwise, and the mixture was placed in the freezer (−20°C) for 2 weeks while it was monitored on TLC (10% MeOH in CH_2Cl_2). The mixture was diluted with 50 cm^3 CH_2Cl_2 and washed with sat. $NaHCO_3$ (3×10 cm^3) and H_2O (3×10 cm^3). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica column chromatography (25–50% petroleum ether in EtOAc) to give 35 mg (18%) of **9b** as a white solid.

$R_f = 0.78$ (10% MeOH in CH_2Cl_2); MS (FAB): $m/z = 331$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.06 (t, 3H, $J = 7.4$ Hz, CH_2CH_3), 2.27 (s, 6H, 2×$PhCH_3$), 2.58 (q, 2H, $J = 7.4$ Hz, CH_2CH_3), 3.10 (t, 2H, $J = 4.6$ Hz, CH_2S), 3.83 (s, 2H, CH_2Ph), 4.14 (t, 2H, $J = 4.6$ Hz, CH_2O), 5.81 (s, 2H, OCH_2N), 6.84 (s, 3H, arom) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 12.52 (CH_2CH_3), 19.78 (CH_2CH_3), 21.11 (CH_3Ph), 34.12 (C-2), 40.04 (CH_2-Ph), 71.19 (C-3), 77.02 (C-5), 125.73 (C-8), 126.55, 128.27, 137.63, 138.07 (C-arom), 157.14 (C-9), 159.19 (C-10a), 163.05 (C-7) ppm.

7-(3,5-Dimethylbenzyl)-8-ethyl-2,3,5-trihydro-9-oxo-pyrimidino[1,2-c]-1,5,3-oxathiazepine (**10**; $C_{18}H_{22}N_2OS_2$)

5b (304 mg, 0.66 mmol) was suspended in 8 cm^3 dry CH_3CN, 0.34 cm^3 bis-(trimethylsilyl)-acetamide (1.65 mmol) were added, and the mixture was heated to 50°C overnight protected by a drying tube. The mixture was cooled to −40°C, and 0.24 cm^3 *TMS*-triflate (1.32 mmol) were added in small portions. The temperature was allowed to raise to −20°C, and the mixture was left in a −20°C freezer for two days. After dilution with 50 cm^3 CH_2Cl_2 (washed with $NaHCO_3$ and dried over K_2CO_3) and washing with 3×20 cm^3 sat. $NaHCO_3$ and 3 x 20 cm^3 H_2O, the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford a clear oily residue which was purified by silica column chromatography (25–50% petroleum ether in EtOAc) to give 110 mg (50%) of **10** as a white solid.

$R_f = 0.45$ (10% MeOH in CH_2Cl_2); m.p.: 137–140°C; MS (FAB): $m/z = 331$ (M+H); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 1.04 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 2.24 (s, 6H, 2×$PhCH_3$), 2.49 (q, 2H, $J = 7.5$ Hz, CH_2CH_3), 3.11 (t, 2H, $J = 4.6$ Hz, CH_2S), 4.01 (s, 2H, CH_2Ph), 4.07 (t, 2H, $J = 4.6$ Hz, CH_2O), 5.31 (s, 2H, OCH_2N), 6.66 (s, 2H, arom), 6.87 (s, 1H, arom) ppm; ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 12.52 (CH_2CH_3), 19.69 (CH_2CH_3), 21.08 (CH_3Ph), 33.96, 34.18 (C-2, CH_2-Ph), 70.57 (C-3), 79.84 (C-5), 125.06 (C-arom), 125.58 (C-8), 129.12, 134.75, 139.07 (C-arom), 147.26 (C-7), 163.75 (C-10a), 168.32 (C-9) ppm.

Viruses and cells

The HIV-1 strain HTLV-IIIB [24] and the NNRTI resistant strain N119 [21] were propagated in H9 cells [25] 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), aliquoted, and stored at −80°C until use. Both HIV-1 strains were obtained from the NIH AIDS Research and Reference Program.

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. MT-4 cells were incubated with virus (0.005 MOI) for 2 h, washed, and thereafter added in a proportion of 1:10 to uninfected cells which had been preincubated in growth medium containing the test compound for 6 days in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantitated by the HIV-1 antigen detection assay ELISA [26]. Compounds mediating less than 30% reduction of antigen expression were considered without biological activity. Compounds mediating a reduction of 30% or more were examined for cytotoxic effect using concentration dependent inhibition of MT-4 cell proliferation as measure of cytotoxicity using the MTT assay as previously described [27]. A 30% inhibition of cell growth relative to control cultures was considered significant.

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