Structure–activity relationship studies on new DABOs: effect of substitutions at pyrimidine C-5 and C-6 positions on anti-HIV-1 activity

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Several 5-alkyl, 5-alkenyl, 5-*iso*-alkyl, 5-halo, 5-aminomethyl and 5-carboxy derivatives of *S*-DABOs (dihydro-alkyl (or *cyclo*-alkyl)thio-benzyl-oxopyrimidines), DATNOs (dihydro-alkylthio-naphthylmethyl-oxopyrimidines) and F_2-*S*-DABOs (dihydro-alkyl (or *cyclo*-alkyl)thio-2,6-difluorobenzyl-oxopyrimidines) have been prepared and tested as anti-HIV-1 agents. *S*-DABO derivatives bearing at C-6 position monosubstituted phenylmethyl or heteroarylmethyl units have also been synthesized. 2-Alkylthio-3,4-dihydropyrimidin-4(3*H*)-one derivatives of F_2-*S*-DABO series bearing small alkyl groups at C-5 proved to be potent inhibitors of HIV-1 replication *in vitro* with selectivity indexes ranging from 250 to >2500.

Keywords: HIV, reverse transcriptase inhibitors, NNRTIs, DABOs

Introduction

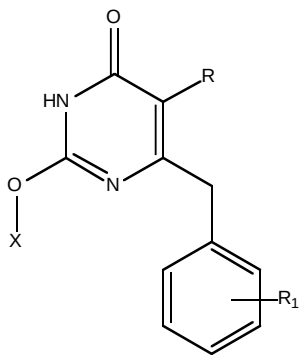
A successful strategy in the development of antiretroviral drugs has been the targetting of the virally encoded reverse transcriptase (RT) (De Clercq, 1998; Artico, 1996). Two classes of compounds potently and selectively inhibit this enzyme: nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively). Both play an important role in the combination therapy for HIV infection/AIDS.

To date, three NNRTIs are available for clinical use: nevirapine, delavirdine and efavirenz. However, new NNRTIs that show better activity against clinically relevant resistant mutants are needed.

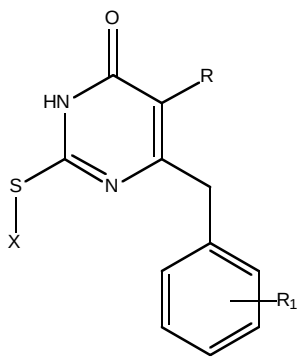
Since 1992, our group has been developing dihydro-alkyloxy (alkylthio)-benzyl-oxopyrimidines (DABOs **1** and *S*-DABOs **2** in Figure 1) (Marongiu *et al.*, 1992; Botta *et al.*, 1992; Artico *et al.*, 1993; Tramontano *et al.*, 1994; Massa *et al.*, 1995; Mai *et al.*, 1995) as a novel class of NNRTIs. The structure–activity relationship studies so far undertaken (Mai *et al.*, 1995; Mai *et al.*, 1997) suggest that structural determinants for the anti-HIV-1 activity in the DABOs pyrimidine ring are: (i) the presence of an alkyloxy (*cyclo*-alkyloxy) or alkylthio (*cyclo*-alkylthio) substituent at the C-2 position; (ii) an aromatic ring linked through a methylene bridge to the C-6 position; and (iii) the unmodified NHCO fragment constituting the N-3 and C-4 positions. Moreover, replacement of the C-6 phenylmethyl moiety with bulkier groups such as 1- (but not 2-) naphthylmethyl (Mai *et al.*, 1997), or the introduction of electron-withdrawing substituents (Cl, F, NO_2) at the *ortho* position of the phenylmethyl moiety (Mai *et al.*, 1999) provided new derivatives active at sub-micromolar (DATNOs, **3**) or nanomolar (F_2-*S*-DABOs, **4**) concentrations (Figure 1).

In this paper we describe novel DABOs designed to explore the anti-HIV-1 effect of derivatives carrying different substitutions at the C-5 position of the pyrimidine ring (compounds **5m–k′** in Tables 1 and 3) or different arylmethyl portions at C-6 (compounds **5a–l** and **6a–w** in Tables 1, 3 and 4). In the first case, various *S*-DABO, DATNO, and F_2-*S*-DABO derivatives (**5m–z**) were synthesized containing a C_{2-4} alkyl, *iso*-alkyl or alkenyl substituent at C-5. Some 5-halo, 5-aminomethyl, 5-carboxy, and 5-carbethoxy derivatives (**5a′–k′**) bearing the *sec*-butylthio side chain at position

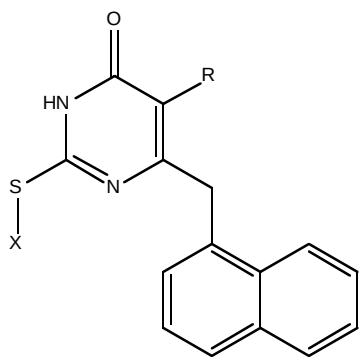
Figure 1. Chemical structures of DABO derivatives



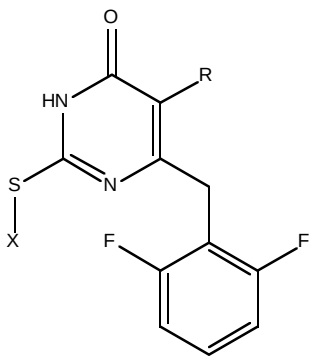
DABOs, **1**
R=H, Me, Et
R_1=H, 3-Me, 3,5-Me_2
X = alkyl, *cyclo*-alkyl



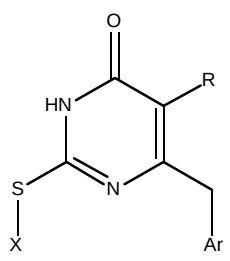
S-DABOs, **2**
R=H, Me
R_1=H, 3-Me
X = alkyl, *cyclo*-alkyl



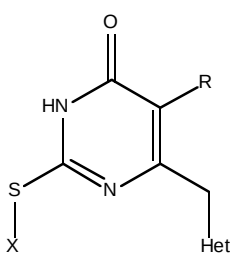
DATNOs, **3**
R=H, Me
X = alkyl, *cyclo*-alkyl



F_2-*S*-DABOs, **4**
R=H, Me
X = alkyl, *cyclo*-alkyl



5
R=H, Me, Et, *n*-Pr, *i*-Pr, allyl, *n*-Bu, I, Br, Cl, CH_2NR_2, COOH, COOEt
Ar = Ph, Me-Ph, MeO-Ph, CF_3-Ph, NH_2-Ph, 1-naphthyl, 2,6-Cl_2-Ph, 2,6-F_2-Ph
X = alkyl, *cyclo*-alkyl



6
R=H, Me
Het = thienyl, pyridyl, pyrryl, indolyl, phthalimido, thianaphthenyl
X = alkyl, *cyclo*-alkyl

2 and phenylmethyl or 1-naphthylmethyl groups at position 6 were also prepared. In the second case, we synthesized novel DABOs featuring various C-6 monosubstituted phenylmethyl (such as CH_3, OCH_3, CF_3 or NH_2 groups, compounds **5a–l**) or heteroarylmethyl (both mono and bicyclic) moieties (compounds of formula **6**).

Materials and Methods: Chemistry

Melting points were determined on a Büchi 530 apparatus and are uncorrected. Infra-red (IR) spectra (KBr pellets) were recorded on a Perkin-Elmer 297 instrument. ^{1}H NMR spectra were recorded at 200 MHz on a Brüker AC 200 spectrometer. Chemical shifts are reported in δ (ppm) units with tetramethylsilane (Me_4Si) as internal reference. All compounds were routinely checked by thin layer chromatography (TLC) and ^{1}H NMR. NMR data were consistent with the designed structures. TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}). Developed plates were visualized by UV light. Chromatographic purifications were performed on Merck silica gel 60. Solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of approximately 20 Torr. Organic solu-

Table 1. Physical and chemical data of compounds **5, 6**

Compound	R	Ar/Het	X	mp,°C	Recrystall solvent	% yield	Formula*
5a	H	2-Me-Ph	*s*-butyl	118–119	*n*-hexane/cyclohexane	67	$C_{16}H_{20}N_2OS$
5b	H	2-Me-Ph	*c*-pentyl	142–144	cyclohexane	61	$C_{17}H_{20}N_2OS$
5c	H	4-Me-Ph	.*s*-butyl	107.5–108.5	*n*-hexane	56	$C_{16}H_{20}N_2OS$
5d	H	2-MeO-Ph	*s*-butyl	123–124	cyclohexane	69	$C_{16}H_{20}N_2O_2S$
5e	H	3-MeO-Ph	*s*-butyl	78–80	*n*-hexane/cyclohexane	71	$C_{16}H_{20}N_2O_2S$
5f	H	4-MeO-Ph	*s*-butyl	112–113	cyclohexane	63	$C_{16}H_{20}N_2O_2S$
5g	H	2-CF_3-Ph	*s*-butyl	125–126	cyclohexane	89	$C_{16}H_{17}F_3N_2OS$
5h	H	4-CF_3-Ph	*s*-butyl	144–145	cyclohexane	75	$C_{16}H_{17}F_3N_2OS$
5i	H	2-NH_2-Ph	*s*-butyl	143–144	benzene/cyclohexane	74	$C_{15}H_{19}N_3OS$
5j	H	4-NH_2-Ph	*s*-butyl	128–130	cyclohexane	77	$C_{15}H_{19}N_3OS$
5k	Me	2-Me-Ph	*s*-butyl	177–178	cyclohexane	55	$C_{17}H_{22}N_2OS$
5l	Me	4-Me-Ph	*s*-butyl	I27–128	*n*-hexane	61	$C_{17}H_{22}N_2OS$
5m	Et	Ph	*s*-butyl	140–141	*n*-hexane	70	$C_{17}H_{22}N_2OS$
5n	allyl	Ph	*s*-butyl	127.5–128.5	cyclohexane	47	$C_{18}H_{22}N_2OS$
5o	*n*-propyl	Ph	*s*-butyl	108–109	*n*-hexane	8	$C_{18}H_{24}N_2OS$
5p	*i*-propyl	Ph	*s*-butyl	125–126	*n*-hexane	42	$C_{18}H_{24}N_2OS$
5q	*n*-hutyl	Ph	*s*-butyl	oil	–	65	$C_{19}H_{26}N_2OS$
5r	Et	1-Naph	*s*-butyl	198.5–199.5	cyclohexane	32	$C_{21}H_{24}N_2OS$
5s	Et	2,6-C1$_2$Ph	*i*-propyl	231–232	benzene/cyclohexane	42	$C_{16}H_{18}Cl_2N_2OS$
5t	Et	2,6-Cl_2Ph	*s*-butyl	209.5–210.5	cyclohexane	62	$C_{17}H_{20}Cl_2N_2OS$
5u	Et	2,6-F_2Ph	*i*-propyl	174–175	benzene	74	$C_{16}H_{18}F_2N_2OS$
5v	Et	2,6-F_2Ph	*s*-butyl	150–151	*n*-hexane/cyclohexane	79	$C_{17}H_{20}F_2N_2OS$
5w	Et	2,6-F_2Ph	*c*-pentyl	165–166.5	cyclohexane	61	$C_{18}H_{20}F_2N_2OS$
5x	*i*-propyl	2,6-F_2Ph	i-propyl	167–168	*n*-hexane	65	$C_{17}H_{20}F_2N_2OS$
5y	*i*-propy!	2,6-F_2Ph	*s*-butyl	149.5–150	*n*-hexane	69	$C_{18}H_{22}F_2N_2OS$
5z	*i*-propyl	2,6-F_2Ph	*c*-pentyl	199.5–200.5	*n*-hexane	66	$C_{19}H_{22}F_2N_2OS$
5a′	I	Ph	*s*-butyl	144–145	cyclohexane	67	$C_{15}H_{17}IN_2OS$
5b′	Br	Ph	*s*-butyl	149–150	benzene/cyclohexane	97	$C_{15}H_{17}BrN_2OS$
5c′	Cl	Ph	*s*-butyl	143–144	cyclohexane	98	$C_{15}H_{17}ClN_2OS$
5d′	I	1-Naph	*s*-butyl	194.5–195.5	methanol/water	71	$C_{19}H_{19}1N_2OS$
5e′	Br	1-Naph	*s*-butyl	215–216	ethanol/water	95	$C_{19}H_{19}BrN_2OS$
5f′	CH_2-NMe_2	Ph	*s*-butyl	110–112	cyclohexane	99	$C_{18}H_{25}N_3OS$
5g′	CH_2-piperidine	Ph	*s*-butyl	125–127	*n*-hexane/cyclohexane	42	$C_{21}H_{29}N_3OS$
5h′	CH_2-piperazNMe	Ph	*s*-butyl	156–157	cyclohexane	75	$C_{21}H_{30}N_4OS$
5i′	CH_2-piperidine	1-Naph	*s*-hutyl	155–156	cyclohexane	45	$C_{25}H_{31}N_3OS$
5j′	COOH	Ph	*s*-butyl	121–122	petr. ether/diethyl ether	54	$C_{16}H_{18}N_2O_3S$
5k′	COOEt	Ph	*s*-butyl	88–89	*n*-hexane	98	$C_{18}H_{22}N_2O_3S$
6a	2-thienyl	H	*s*-butyl	114.5–115.0	*n*-hexane	32	$C_{13}H_{16}N_2OS_2$
6b	2-thienyl	H	*c*-pentyl	154–155	diethyl ether	67	$C_{14}H_{16}N_2OS_2$
6c	2-thienyl	H	*c*-hexyl	210–211	benzene	45	$C_{15}H_{18}N_2OS_2$
6d	3-thienyl	H	*s*-butyl	104.5–105.5	*n*-hexane	82	$C_{13}H_{16}N_2OS_2$
6e	3-thienyl	H	*c*-pentyl	168–169	cyclohexane	80	$C_{14}H_{16}N_2OS_2$
6f	3-thienyl	H	*c*-hexyl	170–171	cyclohexane	50	$C_{15}H_{18}N_2OS_2$
6g	2-pyridy1	H	*s*-butyl	83.5–84.5	*n*-hexane	26	$C_{14}H_{17}N_3OS$
6h	2-pyridyl	H	*c*-pentyl	139–140	ethyl acetate	22	$C_{15}H_{17}N_3OS$
6i	2-pyridyl	H	*c*-hexyl	141–142	*n*-hexane/cyclohexane	10	$C_{16}H_{19}N_3OS$
6j	3-pyridyl	H	*s*-butyl	110–111	benzene/cyclohexane	63	$C_{14}H_{17}N_3OS$
6k	3-pyridyl	H	*c*-pentyl	146.5–147.5	ethyl acetate	54	$C_{15}H_{17}N_3OS$
6l	3-pyridyl	H	*c*-hexyl	152.5–153.5	ethyl acetate	36	$C_{16}H_{19}N_3OS$
6m	4-pyridyl	H	*s*-butyl	148–149	benzene/cyclohexane	15	$C_{14}H_{17}N_3OS$
6n	1-pyrryl	H	*s*-butyl	125–126	cyclohexane	25	$C_{13}H_{17}N_3OS$
6o	phthalimido	H	*s*-butyl	183–184	benzene/cyclohexane	35	$C_{17}H_{17}N_3O_3S$
6p	1-indolyl	H	*s*-butyl	163–164	benzene/cyclohexane	66	$C_{17}H_{19}N_3OS$
6q	3-indolyl	H	*s*-butyl	179–180	benzene	22	$C_{17}H_{19}N_3OS$

(cont. on the following page)

(cont.)

Compound	R	Ar/Het	X	mp,°C	Recrystall solvent	% yield	Formula*
6r	3-thianaph	H	*i*-propyl	166–167	benzene/cyclohexane	47	$C_{16}H_{16}N_2OS_2$
6s	3-thianaph	H	*s*-butyl	147–148	cyclohexane	67	$C_{17}H_{18}N_2OS_2$
6t	3-thianaph	H	*c*-pentyl	183–184	benzene	52	$C_{18}H_{18}N_2OS_2$
6u	3-thianaph	H	*c*-hexyl	176–177	benzene/cyclohexane	55	$C_{19}H_{20}N_2OS_2$
6v	3-thianaph	Me	*i*-propyl	178–179	cyclohexane	58	$C_{17}H_{18}N_2OS_2$
6w	3-thianaph	Me	*s*-butyl	171–172	cyclohexane	54	$C_{18}H_{20}N_2OS_2$

*All compounds were analysed for C, H, N, S and, when required, I, Br, Cl and F; analytical results were within ±0.4% of the theoretical values.

tions were dried over anhydrous sodium sulphate. Analytical results agreed to within ±0.40% of the theoretical values. Specific examples presented below illustrate general synthetic procedures. As a rule, samples prepared for physical (Tables 1 and 2) and biological studies (Tables 3 and 4) were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 90°C, depending on the melting point of the sample considered.

General procedure for the synthesis of ethyl 2-alkyl-4-aryl-3-oxobutyrate derivatives (**7a–i,k,l,n–p,s–w,y,a′–c′**). Procedure A. For example, Ethyl 2-*n*-butyl-4-phenyl-3-oxobutyrate (**7n**)

A solution of potassium hydroxide pellets (1.5 g, 26.6 mmol) in absolute ethanol (30 ml) was added to diethyl 2-*n*-butylmalonate (5.7 g, 5.8 ml, 26.6 mmol) at room temperature during a period of 1 h. The mixture was stirred overnight, and then concentrated under vacuum to remove ethanol. The residue was suspended in dry acetonitrile (100 ml) and, after stirring for 15 min, triethylamine (4.1 g, 5.6 ml, 40.5 mmol) and magnesium chloride (3.0 g, 31.6 mmol) were added, and stirring was continued at room temperature for 2 h. A solution of phenylacetyl imidazolide [prepared from phenylacetic acid (1.7 g, 12.6 mmol) and *N,N*-carbonyldiimidazole (CDI, 2.5 g, 15.2 mmol)] in acetonitrile (50 ml) was added and the reaction mixture was stirred overnight at room temperature. 13% HCl (150 ml) was cautiously added while keeping the temperature below 25°C and the resulting clear mixture was stirred for a further 15 min. The organic layer was separated from aqueous mixture, concentrated, and the residue dissolved in ethyl acetate (100 ml). The aqueous mixture was extracted twice with ethyl acetate (50 ml). The organic extracts were collected, washed with brine (100 ml), dried and evaporated to give **7n** as an oil homogeneous on TLC analysis (SiO_2/$CHCl_3$).

^{1}H NMR ($CDCl_3$) δ 0.80–0.86 (t, 3H, $CH_2CH_2CH_2CH_3$), 1.15–1.25 (m, 7H, OCH_2CH_3 and $CH_2CH_2CH_2CH_3$), 1.77–1.87 (m, 2H, $CH_2CH_2CH_2CH_3$), 3.46–3.56 (t, 1H, CH), 3.77 (s, 2H, CH_2CO), 4.11–4.16 (q, 2H, OCH_2CH_3), 7.16–7.27 (m, 5H, Ph). IR 1730, 1680 cm^{-1}. Anal. calcd for $C_{16}H_{22}O_3$: C, 73.25; H, 8.45. Found: C, 73.47; H, 8.52.

General procedure for the synthesis of ethyl 4-(1*H*-pyrrol-1-yl)- and 4-(1*H*-indol-1-yl)-3-oxobutyrates (**7x,z**). Procedure B. For example, Ethyl 4-(1*H*-indol-1-yl)-3-oxobutyrate (**7z**)

A stirred suspension of sodium hydride (42.7 mmol, 1.9 g of 60% dispersion in mineral oil) in freshly distilled dimethoxyethane (80 ml) was cooled to −30°C. After 10 min, a solution of ethyl 4-chloroacetoacetate (5.8 ml, 42.7 mmol) in the same solvent (40 ml) was added dropwise and stirring was continued for 30 min. Then, a suspension of indole potassium salt [prepared 2 h before by reaction between indole (5.0 g, 42.7 mmol) and a mixture of finely crushed KOH (9.6 g, 170.7 mmol)] in dimethyl sulphoxide (85 ml) was added to the resulting mixture. After stirring at reflux overnight, the mixture was treated with 1N HCl (150 ml), extracted with ethyl acetate (3×70 ml) and dried. Removal of solvent gave a residue, which was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane 1:10).

^{1}H NMR ($CDCl_3$) δ 1.19–1.26 (t, 3H, CH_2CH_3), 3.26 (s, 2H, $COCH_2CO$), 4.07–4.18 (q, 2H, CH_2CH_3), 4.94 (s, 2H, $COCH_2N$), 6.58–6.60 (d, 1H, C-3 indole), 7.02–7.04 (d, 1H, C-5 indole), 7.10–7.23 (m, 3H, C-2,6,7 indole), 7.63–7.66 (d, 1H, C-4 indole). IR 1730, 1710 cm^{-1}. Anal. calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.77; H, 6.19; N, 5.54.

General procedure for the synthesis of ethyl 2-ethyl- and 2-(1-methylethyl)-4-aryl-3-oxobutyrates (**7j,m,q,r**). Procedure C. For example, Ethyl 4-(2,6-difluorophenyl)-2-ethyl-3-oxobutyrate (**7q**)

A suspension of activated zinc dust (4.2 g, 64.1 mmol) in refluxing dry tetrahydrofuran (40 ml) under nitrogen atmosphere was treated with 4–5 drops of ethyl 2-bromobutyrate and stirred at room temperature for 1 h. Then 2,6-difluorophenylacetonitrile (2.0 g, 13.6 mmol) was

Table 2. Physical and chemical data of compounds **7, 8**

Compound	R	Ar/Het	mp, °C	Recrystall solvent	Synthetic procedure*	% yield	Formula†	References
7a	H	2-Me-Ph	oil	–	A	90	$C_{13}H_{16}O_3$	Bellina *et al*. (1977)
7b	H	4-Me-Ph	oil	–	A	100	$C_{13}H_{16}O_3$	Monostory (1952)
7c	H	2-MeO-Ph	oil	–	A	88	$C_{13}H_{16}O_4$	Katagiri *et al*. (1982)
7d	H	3-MeO-Ph	oil	–	A	92	$C_{13}H_{16}O_4$	
7e	H	4-MeO-Ph	oil	–	A	95	$C_{13}H_{16}O_4$	Arovan *et al*. (1971)
7f	H	2-CF_3-Ph	oil	–	A	93	$C_{13}H_{13}F_3O_3$	
7g	H	4-CF_3-Ph	50–51	*n*-hexane/ cyclohexane	A	90	$C_{13}H_{13}F_3O_3$	
7h	Me	2-Me-Ph	oil	–	A	88	$C_{14}H_{18}O_3$	
7i	Me	4-Me-Ph	oil	–	A	92	$C_{14}H_{18}O_3$	Monostory (1952)
7j	Et	Ph	oil	–	C	98	$C_{14}H_{18}O_3$	Danel *et al*. (1995)
7k	allyl	Ph	oil	–	A	95	$C_{15}H_{18}O_3$	
7l	*n*-propyl	Ph	oil	–	A	86	$C_{15}H_{20}O_3$	
7m	*i*-propyl	Ph	oil	–	C	96	$C_{15}H_{20}O_3$	Danel *et al*. (1996)
7n	*n*-butyl	Ph	oil	–	A	99	$C_{16}H_{22}O_3$	
7o	Et	1-Naph	oil	–	A	99	$C_{18}H_{20}O_3$	Danel *et al*. (1997)
7p	Et	2,6-Cl_2Ph	oil	–	A	86	$C_{14}H_{16}C_{l2}O_3$	
7q	Et	2.6-F_2Ph	oil	–	C	58	$C_{14}H_{16}F_2O_3$	
7r	*i*-propyl	2,6-F_2Ph	oil	–	C	47	$C_{15}H_{18}F_2O_3$	
7s	H	2-thienyl	oil	–	A	92	$C_{10}H_{12}O_3S$	Heathcock *et al*. (1983)
7t	H	3-thienyl	oil	–	A	80	$C_{10}H_{12}O_3S$	Keenan *et al*. (1991)
7u	H	2-pyridyl	oil	–	A	75	$C_{11}H_{13}NO_3$	Tani *et al*. (1974)
7v	H	3-pyridyl	oil	–	A	83	$C_{11}H_{13}NO_3$	Bernstein (1955)
7w	H	4-pyridyl	oil	–	A	30	$C_{11}H_{13}NO_3$	
7x	H	1-pyrryl	oil	–	B	75	$C_{10}H_{13}NO_3$	
7y	H	phthalimido	110–111	benzene	A	59	$C_{14}H_{13}NO_5$	
7z	H	1-indolyl	61.5–62.0	cyclohexane	B	21	$C_{14}H_{15}NO_3$	
7a′	H	3-indolyl	111–113 dec	diethyl ether	A	100	C14H15NO3	
7b′	H	3-thianaph	oil	–	A	95	$C_{14}H_{14}O_3S$	
7c′	Me	3-thianaph	oil	–	A	100	$C_{15}H_{16}O_3S$	
8a	H	2-Me-Ph	261–262	ethanol	D	74	$C_{12}H_{12}N_2OS$	
8b	H	4-Me-Ph	233–234	ethanol	D	89	$C_{12}H_{12}N_2OS$	
8c	H	2-MeO-Ph	200–202	ethanol	D	58	$C_{12}H_{12}N_2O_2S$	Monostory (1952)
8d	H	3-MeO-Ph	220–221	ethanol	D	60	$C_{12}H_{12}N_2O_2S$	
8e	H	4-MeO-Ph	205–207	ethanol	D	68	$C_{12}H_{12}N_2O_2S$	Aroyan *et al*. (1971)
8f	H	2-CF_3-Ph	247–249	ethanol	D	41	$C_{12}H_9F_3N_2OS$	
8g	H	4-CF_3-Ph	262–263	acetonitrile	D	69	$C_{12}H_9F_3N_2OS$	
8h	Me	2-Me-Ph	215–216	ethanol	D	50	$C_{13}H_{14}N_2OS$	
8i	Me	4-Me-Ph	229–230	ethanol	D	34	$C_{13}H_{14}N_2OS$	
8j	Et	Ph	191–192	ethyl acetate	D	32	$C_{13}H_{14}N_2OS$	
8k	allyl	Ph	170–171	acetone	D	52	$C_{14}H_{14}N_2OS$	
8l	*n*-propyl	Ph	180–181	diethyl ether	D	32	$C_{14}H_{16}N_2OS$	
8m	*i*-propyl	Ph	201–201.5	acetonitrile	D	74	$C_{14}H_{16}N_2OS$	
8n	*n*-butyl	Ph	144–145	benzene/cyclohexane	D	83	$C_{15}H_{18}N_2OS$	
8o	Et	1-Naph	207–208	benzene	D	22	$C_{17}H_{16}N_2OS$	Danel *et al*. (1997)
8p	Et	2,6-Cl_2Ph	265–266	ethanol	D	36	$C_{13}H_{12}Cl_2N_2OS$	
8q	Et	2,6-F_2Ph	215–217	benzene	D	45	$C_{13}H_{12}F_2N_2OS$	
8r	*i*-propyl	2.6-F_2Ph	236–237	acetonitrile	D	57	$C_{14}H_{14}F_2N_2OS$	
8s	H	2-thienyl	248–250 dec	ethanol	D	67	$C_9H_8N_2OS_2$	Miller *et al*. (1948)
8t	H	3-thienyl	235–236	ethanol	D	77	$C_9H_8N_2OS_2$	
8u	H	2-pyridyl	226–227	ethanol	D	36	$C_{10}H_9N_3OS$	
8v	H	3-pyridyl	275–280 dec	ethanol	D	61	$C_{10}H_9N_3OS$	
8w	H	4-pyridyl	>280 dec	ethanol/water	D	54	$C_{10}H_9N_3OS$	
8x	H	1-pyrryl	>280	acetonitrile	D	48	$C_9H_9N_3OS$	

(cont. on following page)

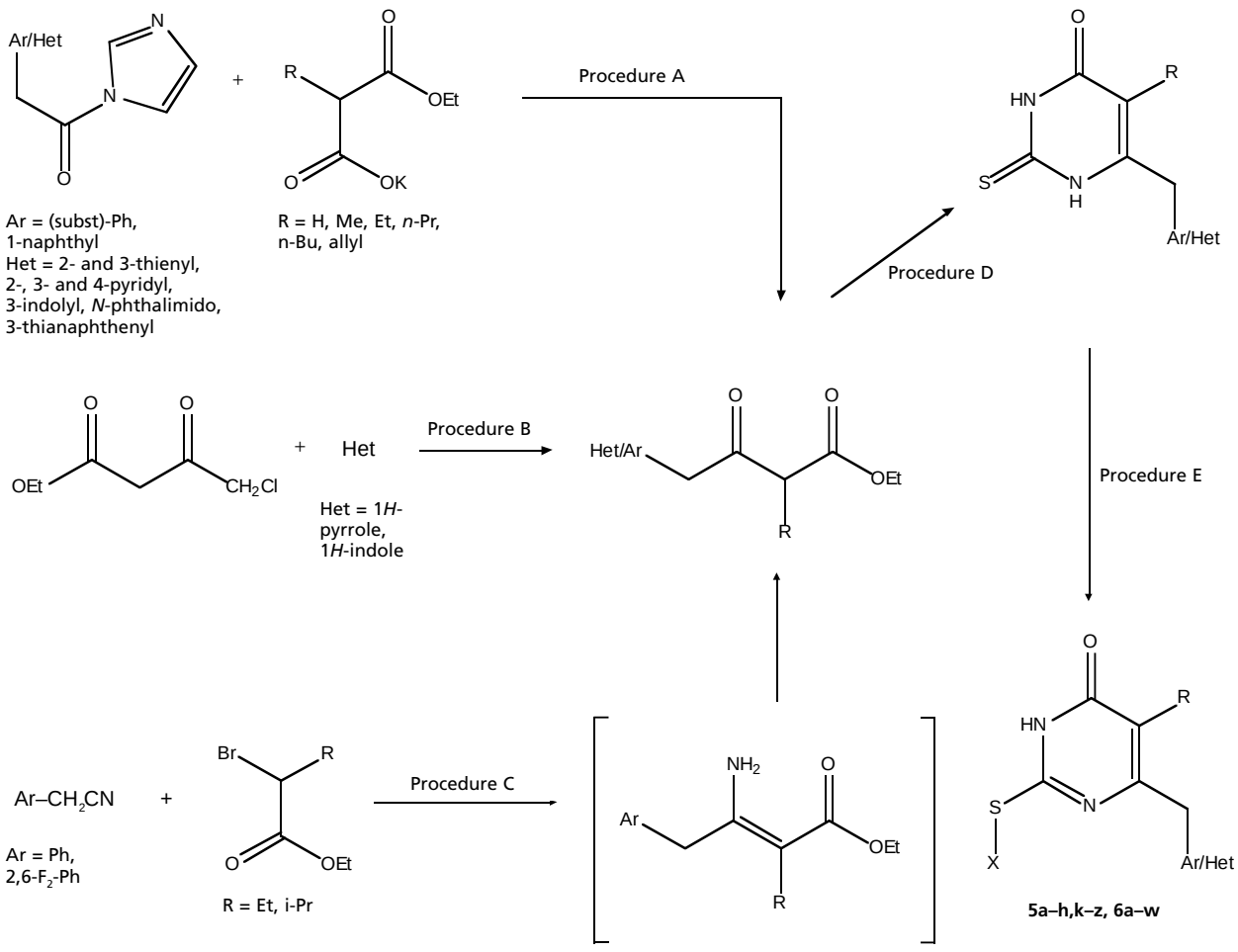
(cont.)

Compound	R	Ar/Het	mp, °C	Recrystall solvent	Synthetic procedure*	% yield	Formula†	References
8y	H	phthalimido	>280	ethanol	D	70	$C_{13}H_9N_3O_3S$	
8z	H	1-indolyl	>280	DMF/water	D	75	$C_{13}H_{11}N_3OS$	
8a′	H	3-indolyl	244–245	ethanol	D	32	$C_{13}H_{11}N_3OS$	
8b′	H	3-thianaph	281–282 dec	ethanol	D	50	$C_{13}H_{10}N_2OS_2$	
8c′	Me	3-thianaph	267–268	ethanol/water	D	68	$C_{14}H_{12}N_2OS_2$	

*See Figure 2. †All compounds were analysed for C, H and when required, N, S, Cl and F; analytical results were within ±0.4% of the theoretical values.

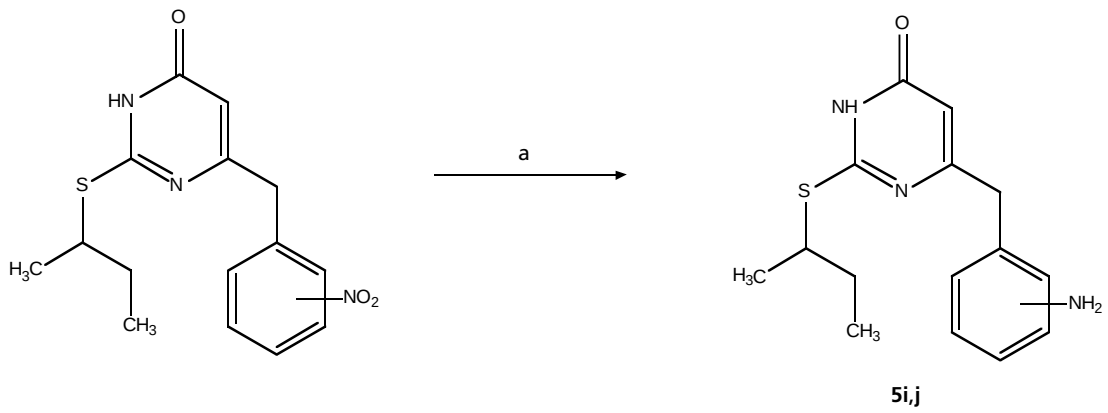
added in one portion followed by addition of ethyl 2-bromobutyrate (6.6 g, 34.0 mmol) drop by drop during 1 h. The resulting mixture was stirred at reflux under nitrogen for 30 min, then was diluted with 100 ml of tetrahydrofuran and quenched with 50% aqueous potassium carbonate (15 ml). After stirring for 30 min the organic layer was separated, evaporated and the residue was dissolved in ethyl acetate (200 ml); the aqueous phase was extracted with ethyl acetate (2×50 ml), and the combined organic extracts were treated with 13 ml of 13% HCl and stirred at room temperature for 45 min. The organic layer was separated, washed with saturated aqueous sodium hydrogen carbon-

Figure 2. Synthetic procedures to prepare new DABO derivatives **5a–h,k–z** and **6a–w***



*Procedure A: (i) $MgCl_2$, Et_3N; (ii) H^+. Procedure B: NaH. Procedure C: (i) Zn; (ii) 50% K_2CO_3, 13% HCl. Procedure D: $(NH_2)_2CS$, EtONa. Procedure E: X-Hal, K_2CO_3

Figure 3. Synthesis of compounds **5i,j***



5i,j

*a: $SnCl_2 \bullet 2H_2O$, 37% HCl.

ate, dried, evaporated and purified by column chromatography on silica gel (eluent: *n*-hexane:ethyl acetate:methanol 12:3:1) to give pure **7q**.

^{1}H NMR ($CDCl_3$) δ 0.92–0.99 (t, 3H, $CHCH_2CH_3$), 1.27–1.32 (t, 3H, OCH_2CH_3), 1.96–2.04 (m, 2H, $CHCH_2CH_3$), 3.48–3.55 (t, 1H, $CHCH_2CH_3$), 3.55–4.04 (d, 2H, CH_2CO), 4.17–4.27 (q, 2H, OCH_2CH_3), 6.90–6.95 (m, 2H, C-3,5 Ar), 7.17–7.36 (m, 1H, C-4 Ar). IR 1730, 1680 cm^{-1}. Anal. calcd for $C_{14}H_{16}F_2O_3$: C, 62.22; H, 5.97; F, 14.06. Found: C, 62.54; H, 6.01; F, 13.87.

General procedure for the synthesis of 5-alkyl-6-arylmethyl-3,4-dihydro-2-thioxopyrimidin-4(3*H*)-ones (**8a–c′**). Procedure D. For example, 3,4-Dihydro-6-phenylmethyl-5-(2-propenyl)-2-thioxopyrimidin-4(3*H*)-one (**8k**)

Sodium metal (0.43 g, 0.019 g-atom) was dissolved in 30 ml of absolute ethanol, then thiourea (1.0 g, 13.0 mmol) and **7k** (2.3 g, 9.3 mmol) were added to the clear solution. The mixture was heated at reflux for 5 h. After cooling, the solvent was removed, the residue was dissolved in water (50 ml) and the aqueous solution was made acid with 2N HCl. Solid precipitates were separated, filtered, washed with diethyl ether and dried *in vacuo* at 50°C for 6 h, whereas oily derivatives were extracted with ethyl acetate (3×50 ml), and the combined organic extracts were washed with brine (100 ml), dried and evaporated to dryness to give solid residues which were purified by crystallization.

^{1}H NMR ($CDCl_3$) δ 3.21–3.24 (d, 2H, CH_2-$CH{=}CH_2$), 3.82 (s, 2H, CH_2Ph), 5.01–5.09 (m, 2H, CH_2-$CH{=}CH_2$), 5.70–5.87 (m, 1H, CH_2-$CH{=}CH_2$), 7.15–7.18 (m, 2H, C-3,5 Ph), 7.34–7.37 (m, 3H, C-2,4,6 Ph), 9.22 (br s, 1H, NH), 9.78 (br s, 1H, NH). IR 1680, 1630 cm^{-1}. Anal. calcd for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84; S, 12.41. Found: C, 65.13; H, 5.45; N, 10.62; S, 12.53.

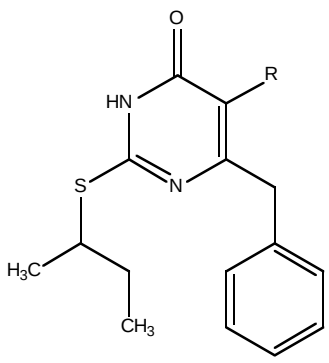
General procedure for the synthesis of 5-alkyl-2-alkylthio-3,4-dihydro-6-(hetero)aryl-methylpyrimidin-4(3*H*)-ones (**5a–h,k–z** and **6a–w**). Procedure E. For example, 3,4-Dihydro-2-(1-methylpropylthio)-6-(3-thianaphthenyl-methyl)pyrimi din-4(3*H*)-one (**6s**)

A mixture of 3,4-dihydro-6-(thianaphthen-3-ylmethyl)-2-thioxopyrimidin-4(3*H*)-one **8b′** (0.5 g, 1.8 mmol), 2-iodobutane (0.3 g, 0.2 ml, 1.8 mmol) and potassium carbonate (0.3 g, 1.8 mmol) in 2 ml of anhydrous *N,N*-dimethylformamide was stirred at room temperature for 8 h. The reaction mixture was poured on cold water (100 ml) and extracted with ethyl acetate (3×50 ml). The organic layers were collected, washed with brine (3×50 ml), dried and evaporated to furnish crude **6s** as a solid, which was purified by column chromatography on silica gel (eluent: *n*-hexane/ethyl acetate/methanol 12/3/1) followed by crystallization.

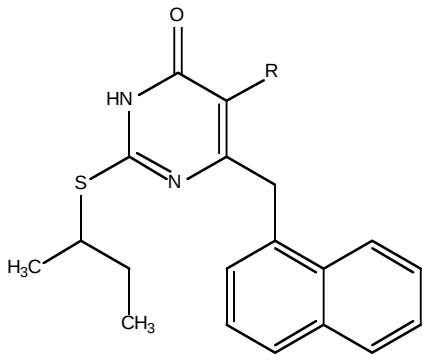
^{1}H NMR ($CDCl_3$) δ 0.93–1.00 (t, 3H, CH_2CH_3), 1.30–1.34 (d, 3H, $CHCH_3$), 1.61–1.74 (m, 2H, CH_2CH_3), 3.82–3.92 (q, 1H, $CHCH_3$), 4.08 (s, 2H, CH_2-Ar), 6.01 (s, 1H, C-5), 7.26 (s, 1H, C-2 thianaph), 7.34–7.39 (m, 2H, C-5,6 thianaph), 7.71–7.76 (m, 1H, C-7 thianaph), 7.86–7.90 (m, 1H, C-4 thianaph), 13.13 (s, 1H, NH exchangeable with D_2O). IR 2930, 1660 cm^{-1}. Anal. calcd for $C_{17}H_{18}N_2OS_2$: C, 61.79; H, 5.49; N, 8.48; S, 19.40. Found: C, 61.85; H, 5.52; N, 8.29; S, 19.22.

Following this procedure 6-arylmethyl-2-cyclohexylthio-3,4-dihydropyrimidin-4(3*H*)-ones **6c,f,i,l,u** were prepared by heating the reaction mixture at 80°C for 8 h.

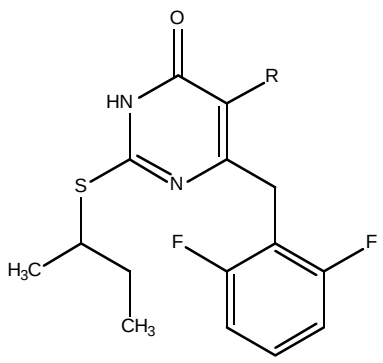
Figure 4. Selected *sec*-butyl-*S*-DABOs



9a,b
9a R = H
9b R = Me



10a,b
10a R = H
10b R = Me



11a,b
11a R = H
11b R = Me

General procedure for the synthesis of 6-(2-aminophenylmethyl or 4-aminophenylmethyl)-3,4-dihydro-2-(1-methylpropylthio)pyrimidin-4(3*H*)-ones (**5i,j**). For example, 6-(4-aminophenylmethyl)-3,4-dihydro-2-(1-methylpropylthio) pyrimidin-4(3*H*)-one (**5j**)

A solution of 3,4-dihydro-2-(1-methylpropylthio)-6-(4-nitrophenylmethyl)pyrimidin-4(3*H*)-one (Mai *et al.*, 1999) (0.94 g, 3.6 mmol) in ethanol (40 ml) was added dropwise to a solution of stannous chloride bihydrate (2.8 g, 12.5 mmol) in 37% HCl (13 ml). After heating at 90°C for 10 min, the reaction was diluted with water (100 ml) and made basic with sodium hydrogencarbonate saturated solution. The product was extracted with chloroform (3×50 ml) and the organic solution was washed with brine (3×50 ml), dried and evaporated. The solid residue was purified by crystallization.

^{1}H NMR ($CDCl_3$) δ 0.93–1.00 (t, 3H, CH_2CH_3), 1.32–1.36 (d, 3H, $CHCH_3$), 1.62–1.72 (m, 2H, CH_2CH_3), 3.71 (s, 2H, CH_2-Ar), 3.86–3.92 (m, 1H, $CHCH_3$), 5.90–5.93 (d, 2H, NH_2 exchangeable with D_2O), 5.96 (s, 1H, C-5), 6.58–6.62 (m, 2H, C-3,5 Ar), 6.98–7.05 (m, 2H, C-2,6 Ar), 12.75 (bs, 1H, NH exchangeable with D_2O). IR 3300, 1680 cm^{-1}. Anal. calcd for $C_{15}H_{19}N_3OS$: C, 62.26; H, 6.62; N, 14.52; S, 11.08. Found: C, 62.54; H, 6.70; N, 14.26; S, 10.97.

General procedure for the synthesis of 6-aryl-methyl-3,4-dihydro-5-iodo-2-(1-methylpropylthio)pyrimidin-4(3*H*)-ones (**5a′,d′**). For example, 3,4-Dihydro-5-iodo-2-(1-methylpropylthio)-6-(1-naphthylmethyl)pyrimidin-4(3*H*)-one (**5d′**)

N-Chlorosuccinimide (0.2 g, 1.3 mmol) in acetone (5 ml) and NaI (0.2 g, 1.3 mmol) in the same solvent (5 ml) were combined, stirred for 10 min, filtered and evaporated under reduced pressure. The resulting *N*-iodosuccinimide (1.3 mmol) was dissolved in dry chloroform (10 ml) and treated with 3,4-dihydro-2-(1-methylpropylthio)-6-(1-naphthylmethyl)pyrimidin-4(3*H*)-one (**10a**, Mai *et al.*, 1997) (0.4 g, 1.2 mmol). The mixture was heated at 70°C for 1 h; after cooling, the solvent was evaporated, water (100 ml) was added to the residue and the aqueous layer was extracted with ethyl acetate (3×50 ml). The organic extracts were collected, washed with brine (3×50 ml), dried and evaporated to furnish crude **5d′**, which was purified by crystallization.

^{1}H NMR (DMSO-d_6) δ 0.58–0.65 (t, 3H, CH_2CH_3), 0.83–0.86 (d, 3H, $CHCH_3$), 1.22–1.30 (m, 2H, CH_2CH_3), 3.10–3.13 (m, 1H, $CHCH_3$), 4.53 (s, 2H, CH_2-naphthyl), 7.43–7.55 (m, 4H, C-2,3,6,7 naphthyl), 7.83–8.04 (m, 3H, C-4,5,8 naphthyl), 12.92 (bs, 1H, NH exchangeable with D_2O). IR 1630 cm^{-1}. Anal. calcd for $C_{19}H_{19}IN_2OS$: C, 50.68; H, 4.25; N, 6.22; S, 7.12; I, 28.18. Found: C, 50.79; H, 4.28; N, 6.29; S, 7.25; I, 28.01.

General procedure for the synthesis of 6-aryl-methyl-5-bromo-3,4-dihydro-2-(1-methylpropylthio)pyrimidin-4(3*H*)-ones (**5b′,e′**). For example, 5-Bromo-3,4-dihydro-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**5b′**)

Benzoyl peroxide (10 mg) was added to a mixture of 3,4-dihydro-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**9a**, Mai *et al.*, 1995) (0.2 g, 0.7 mmol) and *N*-bromosuccinimide (0.13 g, 0.7 mmol) in dry carbon tetrachloride (50 ml). The resulting solution was heated at reflux for 2 h. After cooling, the mixture was diluted with water (100 ml), separated and the aqueous layer was extracted with chloroform (3×20 ml). The organic extracts

Table 3. Cytotoxicity and anti-HIV-1 activity of new DABO derivatives **5***

Compound	R	Ar	X	[μM] CC_{50}†	[μM] EC_{50}‡	SI§
5a	H	2-Me-Ph	*s*-butyl	>200¶	1.8	>111
5b	H	2-Me-Ph	*c*-pentyl	>200	3.4	>59
5c	H	4-Me-Ph	*s*-butyl	200	0.6	333
5d	H	2-MeO-Ph	*s*-butyl	>200	0.96	>208
5e	H	3-MeO-Ph	*s*-butyl	>200	1.2	>166
5f	H	4-MeO-Ph	*s*-butyl	147	14.7	–
5g	H	2-CF_3-Ph	*s*-butyl	200	32	6.2
5h	H	4-CF_3-Ph	*s*-butyl	200	25	8
5i	H	2-NH_2-Ph	*s*-butyl	>200	21.2	>9
5j	H	4-NH_2-Ph	*s*-butyl	>200	23	>8
5k	Me	2-Me-Ph	*s*-butyl	>200	1.7	>118
5l	Me	4-Me-Ph	*s*-butyl	26	0.8	32
5m	Et	Ph	*s*-butyl	>200	1.0	>200
5n	allyl	Ph	*s*-butyl	>200	3	>67
5o	*n*-propyl	Ph	*s*-butyl	190	12	16
5p	*i*-propyl	Ph	*s*-butyl	≥200	2.7	≥74
5q	*n*-butyl	Ph	*s*-butyl	>200	>200	–
5r	Et	1-Naph	*s*-butyl	>200	5.3	>34
5s	Et	2.6-Cl_2Ph	*i*-propyl	>200	0.82	>244
5t	Et	2.6-Cl_2Ph	*s*-butyl	>200	2.1	>95
5u	Et	2,6-F_2Ph	*i*-propyl	65	0.1	650
5v	Et	2,6-F_2Ph	*s*-butyl	>200	0.08	>2500
5w	Et	2,6-F_2Ph	*c*-pentyl	162	0.2	810
5x	*i*-propyl	2,6-F_2Ph	*i*-propyl	>200	0.4	>500
5y	*i*-propyl	2,6-F_2Ph	*s*-butyl	>200	0.1	>2000
5z	*i*-propyl	2,6-F_2Ph	*c*-pentyl	>200	0.8	250
5a′	I	Ph	*s*-butyl	144	4.3	33.5
5b′	Br	Ph	*s*-butyl	153	6.7	23
5c′	Cl	Ph	*s*-butyl	136	16	8.5
5d′	I	1-Naph	*s*-butyl	>200	62	>3.2
5e′	Br	1-Naph	*s*-butyl	>200	>200	–
5f′	CH_2-NMe_2	Ph	*s*-butyl	>200	>200	–
5g′	CH_2-piperidine	Ph	*s*-butyl	>200	>200	–
5h′	CH_2-piperazNMe	Ph	*s*-butyl	>200	>200	–
5i′	CH_2-piperidine	1-Naph	*s*-butyl	26	>26	–
5j′	COOH	Ph	*s*-butyl	>200	>200	–
5k′	COOEt	Ph	*s*-butyl	162	27.8	5.8
9a	H	Ph	*s*-butyl	150	1.2	125
9b	Me	Ph	*s*-butyl	86	0.6	140
10a	H	1-Naph	*s*-butyl	>200	0.33	>606
10b	Me	1-Naph	*s*-butyl	>200	1.0	>200
11a	H	2.6-F_2Ph	*s*-butyl	≥200	0.04	≥5000
11b	Me	2,6-F_2Ph	*s*-butyl	≥200	0.05	≥4000
MKC-442				200	0.03	6666

*Data represent mean values of at least two separate experiments. †Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. ‡Compound dose required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, as determined by the MTT method. §SI: Selectivity index, CC_{50}/EC_{50} ratio. ¶Higher concentrations could not be achieved because of crystallization of compounds in the culture medium.

were collected, washed with brine (3×50 ml), dried and evaporated to furnish crude **5b′**, which was purified by crystallization.

^{1}H NMR ($CDCl_3$) δ 0.89–0.97 (t, 3H, CH_2CH_3), 1.27–1.30 (d, 3H, $CHCH_3$), 1.57–1.68 (m, 2H, CH_2CH_3), 3.70–3.80 (m, 1H, $CHCH_3$), 4.08 (s, 2H, CH_2-Ph), 7.24–7.30 (m, 5H, Ph), 12.41 (bs, 1H, NH exchangeable with D_2O). IR 1640 cm^{-1}. Anal. calcd for $C_{15}H_{17}BrN_2OS$: C, 51.00; H, 4.85; N, 7.93; S, 9.08; Br, 22.62. Found: C, 50.87; H, 4.79; N, 8.02; S, 8.91; Br, 22.74.

5-Chloro-3,4-dihydro-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**5c′**)

A mixture of 3,4-dihydro-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**9a**) (0.2 g, 0.7 mmol) and *N*-chlorosuccinimide (0.16 g, 1.2 mmol) in dry chloroform (10 ml) was heated at reflux for 3 h. After cooling, the mixture was diluted with water (100 ml), separated and the aqueous layer was extracted with chloroform (3×20 ml). The organic extracts were collected, washed with brine (3×50 ml), dried and evaporated to furnish crude **5c′**, which was purified by crystallization.

^{1}H NMR ($CDCl_3$) δ 0.90–0.97 (t, 3H, CH_2CH_3), 1.28–1.31 (d, 3H, $CHCH_3$), 1.58–1.69 (m, 2H, CH_2CH_3), 3.71–3.81 (m, 1H, $CHCH_3$), 4.03 (s, 2H, CH_2-Ph), 7.24–7.29 (m, 5H, Ph), 11.66 (bs, 1H, NH exchangeable with D_2O). IR 1640 cm^{-1}. Anal. calcd for $C_{15}H_{17}ClN_2OS$: C, 58.34; H, 5.55; N, 9.07; S, 10.38; Cl, 11.48. Found: C, 58.55; H, 5.72; N, 9.01; S, 10.46; Cl, 11.34.

General procedure for the synthesis of 5-aminomethyl-6-arylmehtyl-3,4-dihydro-2-(1-methylpropylthio)pyrimidin-4(3*H*)-one derivatives (**5f′–l′**). For example, 3,4-Dihydro-5-(*N*,*N*-dimethylaminomethyl)-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**5f′**)

A mixture of 3,4-dihydro-2-(1-methylpropylthio)-6-(phenylmethyl)pyrimidin-4(3*H*)-one (**9a**) (1.0 g, 3.6 mmol), paraformaldehyde (0.26 g, 8.7 mmol), dimethylamine hydrochloride (0.7 g, 8.7 mmol) and glacial acetic acid (0.05 ml) in absolute ethanol (10 ml) was heated at reflux for 18 h. After cooling, the reaction was quenched with 5% sodium carbonate solution (50 ml) and extracted with ethyl acetate (3×50 ml). The organic extracts were collected, washed with brine (50 ml), dried and evaporated to give a residue (**5f′**), which was purified by passing through a silica gel column eluting with ethyl acetate.

^{1}H NMR ($CDCl_3$) δ 0.95–1.02 (t, 3H, CH_2CH_3), 1.33–1.37 (d, 3H, $CHCH_3$), 1.62–1.73 (m, 2H, CH_2CH_3), 2.26 (s, 6H, $N(CH_3)_2$), 3.49 (s, 2H, CH_2N), 3.79–3.83 (m, 1H, $CHCH_3$), 3.96 (s, 2H, CH_2-Ph), 7.21–7.26 (m, 5H, Ph). IR 1645 cm^{-1}. Anal. calcd for $C_{18}H_{25}N_3OS$: C, 65.22; H, 7.60; N, 12.68; S, 9.67. Found: C, 65.21; H, 7.55; N, 12.73; S, 9.62.

3,4-Dihydro-2-(1-methylpropylthio)-4-oxo-6-(phenylmethyl)pyrimidin-5-carboxylic acid (**5j′**)

A 0.97 M solution of *n*-butyl lithium in *n*-hexane (4.4 ml, 4.2 mmol) was added dropwise to a stirred solution of 5-bromopyrimidin-4(3*H*)-one derivative **5b′** (0.7 g, 1.9 mmol) in dry diethyl ether (100 ml) under nitrogen atmosphere at −70°C. The resulting mixture was stirred for 30 min more, and then poured into diethyl ether (100 ml) saturated with dry ice. The reaction mixture was allowed to reach room temperature, then treated with water (200 ml). The aqueous layer was separated, acidified with 10% HCl, and extracted with ethyl acetate (3×50 ml). The extracts were washed with brine (100 ml), dried and concentrated to give a residue, which was purified by column chromatography on silica gel (eluent: ethyl acetate) followed by crystallization.

^{1}H NMR ($CDCl_3$) δ 0.88–0.96 (t, 3H, CH_2CH_3), 1.25–1.31 (d, 3H, $CHCH_3$), 1.55–1.65 (m, 2H, CH_2CH_3), 3.75–3.89 (m, 1H, $CHCH_3$), 4.65 (s, 2H, CH_2-Ph), 7.24–7.29 (m, 5H, Ph). IR 3400, 1630 cm^{-1}. Anal. calcd for $C_{16}H_{18}N_2O_3S$: C, 60.36; H, 5.70; N, 8.80; S, 10.07. Found: C, 60.45; H, 5.72; N, 8.89; S, 9.89.

Ethyl 3,4-Dihydro-2-(1-methylpropylthio)-4-oxo-6-(phenylmethyl)pyrimidin-5-carboxylate (**5k′**)

96% Sulphuric acid (0.04 ml, 0.8 mmol) was added to a solution of 3,4-dihydro-2-(1-methylpropylthio)-4-oxo-6-(phenylmethyl)pyrimidin-5-carboxylic acid **5j′** (1.26 g, 4.0 mmol) in absolute ethanol (50 ml), and the mixture was heated at reflux overnight. After cooling, the solvent was concentrated under reduced pressure, the residue was partitioned between water (100 ml) and diethyl ether (100 ml) and phases were separated. The aqueous layer was extracted with diethyl ether (2×50 ml), the collected organic extracts (200 ml) were washed with sodium hydrogen carbonate saturated solution (100 ml) and brine (100 ml), dried and evaporated to furnish a residue (**5k′**), which was purified by column chromatography on silica gel (eluent: ethyl acetate/chloroform 1/1) followed by crystallization.

^{1}H NMR ($CDCl_3$) δ 0.86–0.93 (t, 3H, $SCH(CH_3)CH_2CH_3$), 1.27–1.38 (m, 6H, $SCH(CH_3)CH_2CH_3$ and OCH_2CH_3), 1.58–1.71 (m, 2H, $SCH(CH_3)CH_2CH_3$), 3.81–3.88 (m, 1H, $SCH(CH_3)CH_2CH_3$), 4.12 (s, 2H, CH_2-Ph), 4.40–4.47 (q, 2H, OCH_2CH_3), 7.24–7.26 (m, 5H, Ph). IR 1710, 1640 cm^{-1}. Anal. calcd for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; S, 9.25. Found: C, 62.28; H, 6.29; N, 8.17; S, 9.32.

Materials and Methods: Virology

Compounds

Compounds were solubilized in DMSO at 200 μM and then diluted in culture medium.

Cells and viruses

MT-4, C8166, H9/III_B and CEM cells were grown at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin G and 100 μg/ml streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Human immunodeficiency viruses type-1 (HIV-1, III_B strain) and type-2 (HIV-2 ROD strain, kindly provided by Dr L Montagnier,

Table 4. Cytotoxicity and anti-HIV-1 activity of new DABO derivatives **6***

Compound	R	Ar/Het	X	[μM] CC_{50}†	[μM] EC_{50}‡	SI§
6a	H	2-thienyl	*s*-butyl	195¶	17	11.5
6b	H	2-thienyl	*c*-pentyl	129	46	2.8
6c	H	2-thienyl	*c*-hexyl	200	40	5
6d	H	3-thienyl	*s*-butyl	>200	24	>8.3
6e	H	3-thienyl	*c*-pentyl	>200	112	>1.8
6f	H	3-thienyl	*c*-hexyl	>200	33	>6.1
6g	H	2-pyridyl	*s*-butyl	>200	7	>29
6i	H	2-pyridyl	*c*-hexyl	100	7.3	14
6j	H	3-pyridyl	*s*-butyl	>200	59	>3.4
6k	H	3-pyridyl	*c*-pentyl	>200	140	>1.4
6l	H	3-pyridyl	*c*-hexyl	>200	59	>3.4
6m	H	4-pyridyl	*s*-butyl	>200	80	>2.5
6n	H	1-pyrryl	*s*-butyl	>200	>200	–
6o	H	phthalimido	*s*-butyl	>200	35	>5.7
6p	H	1-indolyl	*s*-butyl	>200	29	>6.9
6q	H	3-indolyl	*s*-butyl	200	38	5.3
6r	H	3-thianaph	*i*-propyl	>200	4.4	>45
6s	H	3-thianaph	*s*-butyl	149	3	50
6t	H	3-thianaph	*c*-pentyl	>200	8.8	>22.7
6u	H	3-thianaph	*c*-hexyl	>200	2.7	>74
6v	Me	3-thianpah	*i*-propyl	>200	7.2	>28
6w	Me	3-thianpah	*s*-butyl	>200	1.6	>125
9a	H	Ph	*s*-butyl	150	1.2	125
9b	Me	Ph	*s*-butyl	86	0.6	140
10a	H	1-Naph	*s*-butyl	>200	0.33	>606
10b	Me	1-Naph	*s*-butyl	>200	1.0	>200
MKC-442				200	0.03	6666

*Data represent mean values of at least two separate experiments. †Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. ‡Compound dose required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, as determined by the MTT method. §SI: Selectivity index, CC_{50}/EC_{50} ratio. ¶Higher concentrations could not be achieved because of crystallization of compounds in the culture medium.

Paris, France) were obtained from supernatants of persistently infected $H9/III_B$ and CEM cells, respectively. HIV-1 and HIV-2 stock solutions had titres of 4.5×10^6 and 1.4×10^5 50% cell culture infectious dose ($CCID_{50}$)/ml, respectively.

HIV titration

Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96-well plates. The infectious virus titre was determined by light microscope scoring of cytopathicity after 4 days of incubation, and the virus titres were expressed as $CCID_{50}$/ml.

Anti-HIV assays

Activity of the compounds against HIV-1 and HIV-2 multiplication in acutely infected cells was based on the inhibition of virus-induced cytopathicity in MT-4 and C8166 cells, respectively. In brief, 50 μl of culture medium containing 1×10^4 cells were added to each well of flat-bottom microtiter trays containing 50 μl of culture medium with or without various concentrations of the test compounds. Then 20 μl of an HIV suspension containing 100 (HIV-1) or 1000 (HIV-2) $CCID_{50}$ (50% cell culture infective dose) were added. After a 4-day incubation (5 days for HIV-2) at 37°C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels *et al.*, 1988). Cytotoxicity of the compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by the MTT method.

Results

Chemistry

The majority of ethyl aryl(or heteroaryl)acetylacetates **7**, key intermediates for the synthesis of DABO derivatives **5a–h**,**k–z** and **6a–w**, were prepared by the general procedure A (Figure 2), according to the previously reported simple and high-yield method (Mai *et al.*, 1999), which

involved the reaction of potassium monoethyl 2-alkylmalonate with the proper arylacetyl imidazolide in the presence of magnesium chloride–triethylamine complex (compounds **7a–i**,**k**,**l**,**n–p**,**s–w**,**y**,**a′–c′**).

Ethyl 4-(1-indolyl)acetylacetate **7z** and the pyrrole analogue **7x** were obtained by alkylation of indole (or pyrrole) sodium salt with ethyl 4-chloroacetylacetate (Figure 2; procedure B), while **7j**,**m**,**q**,**r** were prepared by a modified Blaise procedure (Hannick *et al.*, 1983; Danel *et al.*, 1995; Danel *et al.*, 1996; Danel *et al.*, 1997) involving the reaction between arylacetonitriles and ethyl 2-bromobutyrate or -isovalerate in the presence of activated zinc powder, followed by alkaline hydrolysis of the imine intermediate (Figure 2; procedure C).

Condensation of β-oxoesters **7** with thiourea afforded 5-alkyluracil derivatives **8** (Figure 2; procedure D), which were selectively *S*-alkylated in dry DMF with alkyl/*cyclo*-alkyl halides in the presence of potassium carbonate to yield compounds **5a–h**,**k–z** and **6a–w** (Figure 2; procedure E).

The synthesis of 6-(2- and 4- aminophenylmethyl)-2-*sec*-butylthio-3,4-dihydropyrimidin-4(3*H*)-ones **5i**,**j** was accomplished by reduction of the corresponding 6-(2- and 4- nitrophenylmethyl) derivatives (Mai *et al.*, 1999) with stannous chloride dihydrate and 37% HCl (Figure 3).

6-Aryl-2-*sec*-butylthio-5-halopyrimidin-4(3*H*)ones **5a′–e′** were obtained by halogenation of the related 2-*sec*-butylthio-3,4-dihydro-6-phenylmethylpyrimidin-4(3*H*)one **9a** (Mai *et al.*, 1995) and 2-*sec*-butylthio-3,4-dihydro-6-naphthylmethylpyrimidin-4(3*H*)one **10a** (Mai *et al.*, 1997) with *N*-halo succinimide.

5-Aminomethyl-6-arylmethyl-2-*sec*-butylthio-3,4-dihydropyrimidin-4(3*H*)one derivatives **5f′–I′** were prepared by reacting **9a** and **10a** with the proper amine and formaldehyde in acetic acid under Mannich reaction conditions. Reaction of 5-bromoderivative **5b′** with *n*-butyl lithium and dry ice at −70°C followed by acid hydrolysis furnished 5-carboxyderivative **5j′**, which was then esterified with ethanol and sulphuric acid to yield **5k′**.

Antiviral activity

The novel *S*-DABO, DATNO, and F_2-*S*-DABO derivatives were evaluated for cytotoxicity and inhibitory activity against HIV-1-induced cytopathogenicity in MT-4 cells in comparison with selected *S*-DABOs **9a**,**b** (Mai *et al.*, 1995), DATNOs **10a**,**b** (Mai *et al.*, 1997), F_2-*S*-DABOs **11a**,**b** (Mai *et al.*, 1999), and MKC-442 (Baba *et al.*, 1994), used as reference compounds (Figure 4).

In addition, selected new DABOs were tested in cell culture for their ability to inhibit the multiplication of HIV-1 strains carrying the Y181C and Y181C + K103N mutations.

The majority of C-5 and C-6 substituted DABOs were non-cytotoxic for MT-4 cells at the maximum concentration tested (200 μM). Some compounds (**5f**,**o**,**w**,**a′–c′**,**k′**, and **6a**,**b**,**i**,**s**) showed CC_{50} values in the range 100–195 μM or lower (**5l**,**u**,**i′**) (Tables 3 and 4).

As a rule, the introduction of 5-alkyl groups (**5m–z**) at the pyrimidine ring of *S*-DABOs, DATNOs and F_2-*S*-DABOs led to compounds whose potency is inversely related to the substituent size (hydrogen = methyl < ethyl < *iso*-propyl). Among the newly synthesized C-5 DABOs, the most potent is the 2-*sec*-butylthio-5-ethyl-6-(2,6-F_2-phenylmethyl) derivative **5v** showing a EC_{50}=0.08 μM (Table 3). In accordance with previous results (Mai *et al.*, 1999), F_2-*S*-DABOs (**5u–z**) were the most potent and selective among the tested compounds.

When tested against HIV-1 mutant strains, only F_2-S-DABOs (**5u–z**) retained some activity, resulting as 20–60-fold less active against Y181C than against wild-type (wt) HIV-1 [EC_{50} values were as follows: **5v**=0.1 μM (wt), 5.0 μM (Y181C); **5y**=0.18 μM (wt), 3.5 μM (Y181C)]. No activity was found against the double mutant Y181C + K103N (data not shown).

Introduction of iodo, bromo, or chloro substituents at the C-5 position (compounds **5a′–e′**) led sequentially to a progressive loss of antiviral potency. In fact, the 5-iodo-derivatives **5a′** and **5d′** were four- and 188-fold less potent than the corresponding 5-H counterparts, **9a** (Mai *et al.*, 1995) and **10a** (Mai *et al.*, 1997). The 5-bromo analogue **5b'** was sixfold less active than **9a**, while **5e′** was inactive. Finally, 2-*sec*-butylthio-5-chloro-3,4-dihydro-6-phenylmethylpyrimidin-4(3*H*)one **5c′** showed anti-HIV-1 activity 13-fold lower than the related **9a**. Compounds containing at the C-5 position substituents different from halogens (**5f′–k′**), were devoid of anti-HIV-1 activity (Table 3).

The introduction of methyl or methoxy groups at various positions of the *S*-DABO phenylmethyl moiety (compounds **5a–e**,**k**,**l**) led to compounds with a potency comparable to that of the unsubstituted counterparts **9a**,**b**. The sole exception is 2-*sec*-butylthio-3,4-dihydro-6-(4-methoxyphenylmethyl)pyrimidin-4(3*H*)one **5f**, which was found 12-fold less active than **9a** (Table 3).

It is noteworthy that when trifluoromethyl or amino groups were introduced in the phenyl ring (compounds **5g–j**, Table 3), or when the C-6 phenylmethyl portion was replaced by an heteroarylmethyl moiety (both mono- or bicyclic) (compounds **6a–w**, Table 4), large losses of activity were noticed. Compounds **5g–j** are 18–27-fold less potent than **9a**, and 2-*sec*-butylthio-3,4-dihydro-6-heteroarylmethylpyrimidin-4(3*H*)one derivatives **6a**,**d**,**g**,**j**, **m–q**,**s**,**w** are characterized by anti-HIV-1 activity three- to 67-fold lower than the related 6-phenylmethyl counterparts **9a**,**b**.

As far as derivatives carrying mono- and bicyclic 6-heteroarylmethyl substituents are concerned, 2-pyridylmethyl and 3-thianaphthenylmethyl (**6g** versus **9a** and **6s**,**w** versus **10a**,**b**) are the most active derivatives.

Discussion

Recently reported molecular modelling studies of *S*-DABO, DATNO and F_2-*S*-DABO derivatives (Mai *et al.*, 1999) have shown the presence of a favourable effect of 2,6-difluorination related to an attractive π-stacking interaction taking place between the benzene ring of the ligand (made electron-deficient by the two fluorines) and the electron-rich benzene ring of Tyr188 located in the non-nucleoside binding cleft of the RT.

As a matter of fact, among the new C-5 substituted DABO derivatives, the highest anti-HIV-1 potency is shown by compounds belonging to the F_2-*S*-DABO series. This confirms the importance of the 2,6-difluorophenylmethyl moiety as a C-6 substituent. As observed for other DABO series, the potency of F_2-*S*-DABOs is inversely related to the size of the C-5 alkyl substituent, with a trend dissimilar to that observed with the related HEPT derivatives, in which the smaller is the substituent at C-5, the lower is the potency of compounds (Tanaka *et al.*, 1992; Sbardella *et al.*, 2000).

Interestingly, F_2-S-DABOs inhibit the Y181C mutant virus in the low micromolar range. Studies are in progress to find proper substituents to be introduced in the F_2-S-DABO skeleton in order to increase the DABOs anti-HIV activity against clinically relevant HIV-1 mutants.

With the sole exception of small alkyl groups, introduction of substituents (such as halogen atoms, carboxy and carbethoxy, *N,N*-dimethylamino-, piperidino-, and *N*-methyl piperazinomethyl groups) at the C-5 position of the pyrimidine ring yielded considerably less potent (**5a′–d′**,**k′**) or inactive (**5e′–j′**) compounds.

Concerning new C-6 substituted compounds, it is remarkable that a severe decrease of antiviral activity was obtained following both the introduction of an electron-withdrawing group (trifluoromethyl) in the phenylmethyl moiety of the *S*-DABO skeleton and the replacement of the phenyl ring with the electron-deficient pyridyl ring.

The above results suggest that, in addition to electronic effect, other chemico-physical parameters (steric and lipophylic) play a role in the interaction of DABOs with the HIV-1 RT non-nucleoside binding site.

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