

Article

Synthesis and Biological Evaluation of 2-Thioxopyrimidin-4(1*H*)-one Derivatives as Potential Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors

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Abstract: A series of new 5-allyl-6-benzylpyrimidin-4(3*H*)-ones bearing different substituents at the C-2 position of the pyrimidine core have been synthesized and evaluated for their *in vitro* activities against human immunodeficiency virus type 1 (HIV-1) in the human T-lymphotropic type (MT-4 cell cultures). The majority of the title compounds showed moderate to good activities against HIV-1. Amongst them, 5-allyl-6-benzyl-2-(3-hydroxypropylthio)pyrimidin-4(3*H*)-one analogue **11c** exhibited the most potent anti-HIV-1 activity (IC₅₀ 0.32 μM). The biological testing results clearly indicated that the substitution at C-2 position of the pyrimidine ring could increase the anti-HIV-1 reverse transcriptase (RT) activity.

Keywords: AIDS; non-nucleoside reverse transcriptase inhibitors (NNRTIs); 2-alkylthio-6-benzylpyrimidin-4(3*H*)-ones (S-DABOs); anti-HIV-1 activity

1. Introduction

The reverse transcriptase (RT) of the human immunodeficiency virus type 1 (HIV-1 RT) is one of the main targets of drugs used in the treatment of AIDS [1,2]. Several RT inhibitors have been developed and approved by the FDA and are currently in clinical use. Among several kinds of anti-HIV agents, non-nucleoside reverse transcriptase inhibitors (NNRTIs), specifically targeting to reverse transcriptase, have attracted wide attention due to their high specificity, excellent potency and low cytotoxicity [3,4]. NNRTIs are a class of antiretroviral drugs which deactivate HIV-1 reverse transcriptase by inducing conformational change of the enzyme via binding to a hydrophobic site close (approximately 10 Å) to its catalytic site [5]. Since these compounds do not need intracellular metabolic activation, have relatively low cytotoxicity, act on a nanomolar scale and are not active against other retroviruses, they are highly specific HIV-1 inhibitors. Among NNRTIs, dihydro alkoxy benzyl oxopyrimidines (DABOs) are an interesting class of compounds active at nanomolar concentration. This class was first reported in 1992 and further developed during the following years into S-DABOs, N-DABOs and related analogues [6–26]. Over the past decade, 2-alkylthio-6-benzylpyrimidin-4(3*H*)-ones (S-DABOs) have been the subject of great interest and have led to the identification of several new structures [27–29] displaying excellent activities as non-nucleoside inhibitors of HIV-1 reverse transcriptase (RT). In our continuing efforts to find novel effective and selective anti-HIV-1 agents in 2-alkylsulfanyl-6-benzyl-3,4-dihydropyrimidin-4(3*H*)-ones (S-DABOs) type NNRTIs [30–33], herein we present the synthesis and anti-HIV-1 activities of a series of S-DABO analogs.

2. Results and Discussion

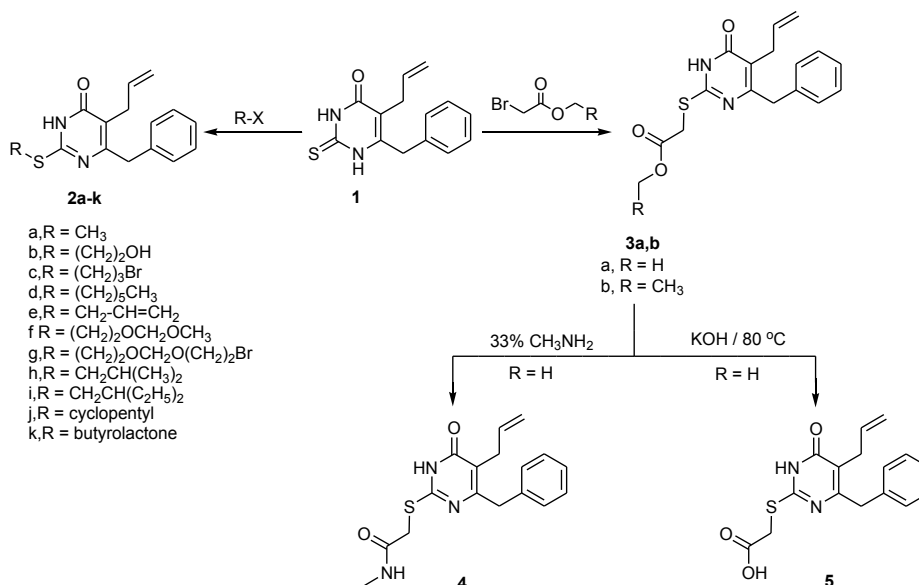
2.1. Chemistry

The synthetic route of the newly designed compounds is described in Schemes 1 and 2. The key intermediate 5-allyl-6-benzyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**1**) was prepared through the method of our previously reported 3-step synthetic strategy [23] through the reaction of β -ketoester with thiourea in the presence of EtONa in refluxing EtOH. Subsequent S-alkylation of **1** with appropriate alkyl halides namely, methyl iodide, 1-bromoethanol, 1,3-dibromopropane, 1-bromohexane, allyl iodide, 1-bromo-2-(methoxymethoxy)ethane, bis(2-bromoethoxy)methane, methyl bromoacetate, ethyl bromoacetate, isobutyl bromide, 1-bromo-2-ethyl butane, cyclopentyl bromide, α -bromo- γ -butyrolactone, chloroethyl methyl ether and 3-bromo-1-propanol in dry dimethylformamide (DMF) in the presence of anhydrous K_2CO_3 afforded the desirable target S-DABO analogues **2a-k**. 5-Allyl-6-benzyl-2-(methyl acetatethio)pyrimidin-4(3*H*)-one **3a** and 5-Allyl-6-benzyl-2-(ethyl acetatethio)pyrimidin-4(3*H*)-one **3b** were obtained when compound **1** was treated with various halo-esters namely, methyl bromoacetate or ethyl bromoacetate in anhydrous DMF in the presence of K_2CO_3 , respectively. The amide **4** was obtained upon treatment of **3a** in 33% ethanolic methylamine. Also, the ester **3a** was hydrolyzed to the corresponding acid **5** in hot aqueous potassium hydroxide (Scheme 1).

However, treatment of compound **1** with cyclohexyl bromide, afforded *S*-cycloalkyl derivative **6**, which upon oxidation with *m*-chloroperbenzoic acid gave the corresponding cyclohexylsulfanyl derivative **7**. Treatment of compound **6** with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetan-2,4-disulfide

(Lawesson's Reagent) in anhydrous benzene afforded 5-allyl-6-benzyl-2-(cyclohexylthio)pyrimidine-4(3*H*)-thione **8** (Scheme 2).

Scheme 1. Synthetic route of *S*-alkyl derivatives **2–5**.



Furthermore, reaction of **1** with 2-bromoacetaldehyde acetal in anhydrous DMF and K₂CO₃ at 65 °C gave the corresponding 5-allyl 6-benzyl-2-(2,2-diethoxyethylthio)pyrimidin-4(3*H*)-one **9** which was then treated with *N,O*-bis(trimethylsilyl) acetamide (BSA) using trimethylsilyl trifluoromethane-sulfonate (TMS triflate) as a catalyst to yield 5-(3,5-dimethylbenzyl)-6-allyl-3-ethoxy-2,3-dihydrothiazolo-[3,2-*a*]pyrimidin-7-one **10**. Acyclic nucleoside Compounds **11a-c** were obtained by *S*-alkylation of the key intermediate 2-thioxopyrimidin-4(1*H*)-one **1** with different acyclic halosugars (Scheme 2). All the target compounds were characterized by nuclear magnetic resonance (NMR), mass spectrum (MS) and elemental analysis. Both analytical and spectral data of all the newly prepared compounds are in full agreement with the proposed structures.

2.2. Pharmacological Screening

The anti-HIV activities and cytotoxicity's of the synthesized S-DABOs analogues are summarized in Table 1. The expression of HIV-1 was quantified by two different methods, either the HIV-1 antigen detection assay enzyme-linked immunosorbent assay (ELISA) [34] or indirectly by the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay [35]. In general, the activity of the tested compounds including S-DABOs derivatives in the side chains at C-2 is to be expected when compared to those previously reported for S-DABOs. As illustrated in Table 1, we could see that most of those compounds showed moderate to potent inhibition of RT activity, suggesting that HIV-1 RT was the target of this series of S-DABO derivatives. The activity and cytotoxicity of these newly synthesized S-DABO analogues were evaluated in MT-4 cells for their ability to inhibit HIV-1 in MT-4 cells, in comparison with (6-benzyl-1-(ethoxymethyl)-5-isopropylpyrimidine-2,4-dione (MKC-442) and azidothymidine (AZT) used as reference drugs. Most of the tested compounds exhibited moderate to good activities against wild-type HIV-1 with EC₅₀ values ranged from 35.00 to 0.32 μM. The most active

compound **11c** showed the highest activity against wild-type HIV-1 with an EC_{50} value of 0.32 μM . The other compounds displayed moderate activity **2e**, **2j**, EC_{50} 2.5 and 2.60 μM , respectively or were inactive **2c**, **2k**, **3b**, **4** and **9**, against HIV-1 replication.

Scheme 2. Synthetic route of *S*-substituted and fused pyrimidin-4(3*H*)-one derivatives **6–11**.

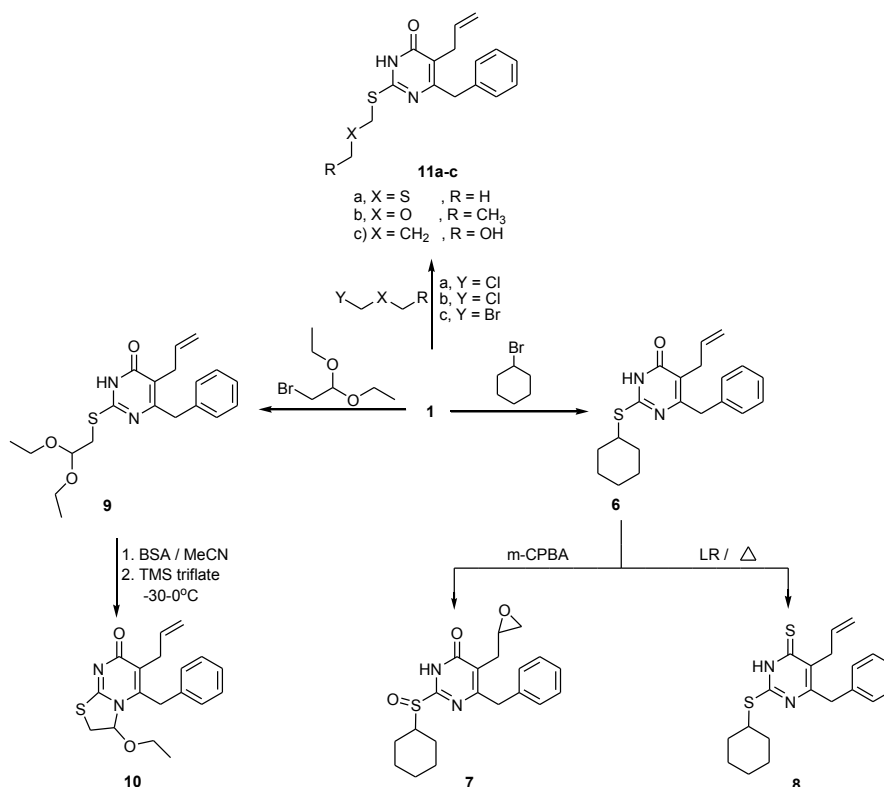


Table 1. Cytotoxicity and anti-HIV-1 activity of newly synthesized compounds in MT-4 cells.

Compound	IC_{50}^a (μM)	CC_{50}^b (μM)	SI^c
1	–	–	–
2a	26.8	>100	>3
2c	–	–	–
2d	7.0	35.0	>5
2e	2.5	25.0	>10
2h	1.0	>100	>100
2j	2.6	>100	>38
2k	–	–	–
3a	35.0	>100	>2
3b	–	–	–
4	–	–	–
8	3.9	>100	>25
9	–	–	–
11a	3.16	>100	>31
11c	0.32	>100	>312
MKC-442	0.005	141	28,000
AZT	0.04	52	1,300

^a IC_{50} , 50% inhibitory concentration; ^b CC_{50} , 50% cytotoxic concentration; ^c Selectivity index (SI), CC_{50}/IC_{50} .

3. Experimental Section

3.1. Chemistry

All melting points were uncorrected and determined on a Büchi melting point apparatus (Hamburg, Germany). NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR using tetramethylsilane (TMS) as internal standard. All chemical shifts are quoted in δ values using parts per million scale (ppm) downfield from TMS at the University of Southern Denmark, Denmark. EI mass spectra were recorded on a Finnigan MAT SSQ 710 spectrometer (Finnigan MAT, Madison, WI, USA) at 70 eV at the University of Southern Denmark, Denmark. Elemental analyses were performed at Atlantic Micro lab, Inc., Atlanta, GA, USA, and the found values agreed favourably with the calculated ones. The progress of reactions was monitored by TLC (analytical silica gel plates 60 F₂₅₄). Merck silica gel (0.040–0.063 mm) was used for column chromatography. The analytical spectral analyses were supported by Erik B. Pedersen, University of Southern Denmark, Institute of Chemistry, and antiviral screening *in vitro* through the Retrovirus Laboratory, Department of Virology, State Serum Institute, Copenhagen, Denmark.

5-Allyl 6-(benzyl)-2-(methylthio)pyrimidin-4(3H)-one (2a). A mixture of compound **1** (1.29 mmol), 10 mL hexamethyldisilazane (HMDS) and 10 mg $(\text{NH}_4)_2\text{SO}_4$ were refluxed for 2 h. The remaining HMDS was removed under reduced pressure. 1 mL of anhydrous DMF and (2.24 mmol) of methyl iodide were added and the solution was stirred at room temperature for 5 h. 100 mL of dichloromethane was added and the solution was washed with 20 mL anhydrous NaHCO_3 . The organic layer was dried over Na_2SO_4 and evaporated till dryness under reduced pressure. The solid was washed with benzene to remove iodide and the solid was dissolved in CH_2Cl_2 , dried and purified by column chromatography (30% EtOAc/pet.ether 60–80 °C) to give compound **2a** in 94% yield, m.p. 214–216 °C; ^1H NMR (DMSO- d_6) δ : 2.38 (s, 3H, CH_3), 3.18 (d, 2H, CH_2), 3.80 (s, 2H, CH_2Ph), 4.94 (m, 2H, $=\text{CH}_2$), 5.75 (m, 1H, CH), 7.15–7.26 (m, 5H, ArH), 12.58 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ : 12.50, 28.49, 39.40, 115.29, 116.05, 126.30, 128.57, 129.10, 134.12, 138.26, 158.39, 160.97, 163.17; MS m/z (%): 272 (M^+). Analytical for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{OS}$ (272.37): calculated C 66.15, H 5.92, N 10.29; found C 66.07, H 5.83, N 10.23.

5-Allyl 6-(benzyl)-2-(alkylthio)pyrimidin-4(3H)-one (2b-k, 11b,c). General procedure: To a stirred solution of compound **1** (3 mmol) in anhydrous DMF (10 mL) was added K_2CO_3 (3.3 mmol) and appropriate alkyl or cycloalkyl halides (3.3 mmol). The stirring was continued at room temperature for 8–12 h. The reaction mixture was poured into cold H_2O (150 mL), the resulting precipitate was collected by filtration and washed sequentially with small portions of H_2O , MeOH, Et_2O and dried *in vacuo* to afford the corresponding crude product, which was purified by flash chromatography to give the pure target compounds.

2-(2-Hydroxyethylthio)-5-allyl-6-benzylpyrimidin-4(3H)-one (2b). Yield 76%, m.p. 164–166 °C (30% EtOAc/pet.ether 60–80 °C); ^1H NMR (DMSO- d_6) δ : 3.01 (s, 2H, CH_2), 3.12 (d, 2H, CH_2), 3.81 (s, 2H, CH_2Ph), 4.28 (t, 2H, CH_2), 4.96 (m, 2H, $=\text{CH}_2$), 5.73 (m, 1H, CH), 7.05–7.19 (m, 5H, ArH), 12.36 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ : 28.51, 32.68, 39.42, 63.22, 115.26, 116.08, 126.34, 128.55, 129.11, 134.16, 138.24, 158.41, 161.03, 163.21; MS m/z (%): 302 (M^+). Analytical for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ (302.39): calculated C 63.55, H 6.00, N 9.26; found C 63.41, H 5.87, N 9.11.

2-(3-Bromopropylthio)-5-allyl-6-benzylpyrimidin-4(3H)-one (2c). Yield 66%, m.p. 138–140 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 2.47 (m, 2H, CH₂), 2.96 (t, 2H, SCH₂), 3.11 (d, 2H, CH₂), 3.45 (t, 2H, SCH₂), 3.82 (s, 2H, CH₂Ph), 4.95 (m, 2H, =CH₂), 5.78 (m, 1H, CH), 7.18–7.25 (m, 5H, ArH); ¹³C NMR (DMSO-d₆) δ: 24.89, 28.15, 31.09, 32.48, 39.40, 115.34, 117.89, 126.36, 128.49, 129.12, 134.28, 137.50, 154.71, 160.83, 163.67; MS *m/z* (%): 298 (M⁺). Analytical for C₁₇H₁₉BrN₂OS (379.31): calculated C 53.83, H 5.05, N 7.39; found C 53.76, H 4.86, N 7.23.

5-Allyl-6-benzyl-2-(hexylthio)pyrimidin-4(3H)-one (2d). Yield 73%, m.p. 153–155 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 0.98 (t, 3H, CH₃), 1.26–1.95 (4m, 8H, 4CH₂), 2.98 (t, 2H, SCH₂), 3.17 (d, 2H, CH₂), 3.86 (s, 2H, CH₂Ph), 5.01 (m, 2H, =CH₂), 5.73 (m, 1H, CH), 7.16–7.24 (m, 5H, ArH), 12.48 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 14.25, 23.46, 26.43, 27.65, 28.41, 30.89, 32.07, 39.42, 115.29, 117.85, 126.37, 128.52, 129.16, 134.22, 137.58, 155.26, 161.12, 163.69; MS *m/z* (%): 342 (M⁺). Analytical for C₂₀H₂₆N₂OS (342.50): calculated C 70.14, H 7.65, N 8.18; found C 70.11, H 7.49, N 8.05.

5-Allyl 6-benzyl-2-(allylthio)pyrimidin-4(3H)-one (2e). Yield 69%, m.p. 114–116 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 3.16 (d, 2H, SCH₂), 3.21 (d, 2H, CH₂), 3.89 (s, 2H, CH₂Ph), 4.91 (m, 2H, =CH₂), 5.04 (m, 2H, =CH₂), 5.76 (m, 1H, CH), 5.05 (m, 1H, CH), 7.21–7.26 (m, 5H, ArH), 12.35 (s, 2H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 28.68, 32.43, 34.15, 39.41, 115.73, 117.90, 126.59, 128.64, 129.41, 134.85, 135.23, 137.82, 154.75, 161.49, 163.90; MS *m/z* (%): 298 (M⁺). Analytical for C₁₇H₁₈N₂OS (298.40): calculated C 68.42, H 6.08, N 9.39; found C 68.29, H 6.01, N 9.28.

5-Allyl-6-benzyl-2-(2-(methoxymethoxy)ethylthio)pyrimidin-4(3H)-one (2f). Yield 69%, m.p. 188–190 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 3.06 (t, 2H, CH₂), 3.21 (d, 2H, CH₂), 3.76 (s, 3H, CH₃), 3.85 (s, 2H, CH₂Ph), 4.27 (t, 2H, CH₂), 5.01 (m, 2H, =CH₂), 5.56 (s, 2H, CH₂), 5.70 (m, 1H, CH), 7.15–7.21 (m, 5H, ArH), 12.47 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 28.33, 31.22, 39.51, 56.01, 67.99, 98.65, 115.32, 117.49, 126.55, 128.61, 129.34, 134.97, 137.62, 154.42, 161.19, 163.52; MS *m/z* (%): 346 (M⁺). Analytical for C₁₈H₂₂N₂O₃S (346.44): calculated C 62.40, H 6.40, N 8.09; found C 62.34, H 6.29, N 7.95.

5-Allyl-6-benzyl-2-(2-((2-bromoethoxy)methoxy)ethylthio)pyrimidin-4(3H)-one (2g). Yield 59%, m.p. 192–194 °C (3% MeOH/CHCl₃); ¹H NMR (DMSO-d₆) δ: 3.10 (t, 2H, CH₂), 3.16 (d, 2H, CH₂), 3.51 (t, 2H, CH₂), 3.84 (s, 2H, CH₂Ph), 4.09, 4.17 (2t, 4H, 2CH₂), 5.05 (m, 2H, =CH₂), 5.60 (s, 2H, CH₂), 5.78 (m, 1H, CH), 7.19–7.24 (m, 5H, ArH), 12.47 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 27.39, 28.35, 30.12, 39.56, 67.92, 69.01, 95.86, 115.30, 117.53, 126.50, 128.67, 129.32, 134.91, 137.59, 154.38, 160.87, 163.41; MS *m/z* (%): 439 (M⁺). Analytical for C₁₉H₂₃BrN₂O₃S (439.37): calculated C 51.94, H 5.28, N 6.38; found C 51.82, H 5.19, N 6.32.

5-Allyl-6-benzyl-2-(isobutylthio)pyrimidin-4(3H)-one (2h). Yield 69%, m.p. 134–136 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 0.98 (d, 6H, 2CH₃), 2.18 (m, 1H, CH), 2.98 (d, 2H, CH₂), 3.17 (d, 2H, CH₂), 3.72 (s, 2H, CH₂Ph), 5.01 (m, 2H, =CH₂), 5.87 (m, 1H, CH), 7.16–7.23 (m, 5H, ArH), 12.58 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 21.69, 30.21, 28.10, 39.44, 40.75, 115.48, 117.91, 126.34, 128.52, 129.17, 134.31, 137.52, 154.69, 161.09, 163.59; MS *m/z* (%): 314 (M⁺). Analytical for C₁₈H₂₂N₂OS (314.45): calculated C 68.75, H 7.05, N 8.91; found C 68.70, H 6.94, N 8.85.

5-Allyl-6-benzyl-2-(2-ethylbutylthio)pyrimidin-4(3H)-one (2i). Yield 72%, m.p. 141–143 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 0.96 (t, 6H, 2CH₃), 1.32 (m, 4H, 2CH₂), 2.05 (m, 1H, CH), 2.95 (d, 2H, CH₂), 3.11 (d, 2H, CH₂), 3.83 (s, 2H, CH₂Ph), 4.99 (m, 2H, =CH₂), 5.82 (m, 1H, CH), 7.19–7.26 (m, 5H, ArH), 12.61 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 11.35, 27.01, 28.19, 34.92, 39.46, 41.76, 115.41, 117.87, 126.30, 128.55, 129.14, 134.28, 137.49, 155.03, 160.78, 163.65; MS *m/z* (%): 342 (M⁺). Analytical for C₂₀H₂₆N₂OS (342.5): calculated C 70.14, H 7.65, N 8.18; found C 70.12, H 7.59, N 8.10.

5-Allyl 6-benzyl-2-(cyclopentylthio)pyrimidin-4(3H)-one (2j). Yield 65%, m.p. 117–119 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 1.53–2.29 (m, 8H, 4CH₂), 3.21 (d, 2H, CH₂), 3.70 (m, 1H, CH), 3.91 (s, 2H, CH₂Ph), 4.95 (m, 2H, =CH₂), 5.74 (m, 1H, CH), 7.12–7.20 (m, 5H, ArH), 12.28 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 26.19, 28.19, 32.21, 34.64, 39.50, 115.62, 117.89, 126.33, 128.52, 129.17, 134.24, 137.64, 154.55, 161.21, 163.75; MS *m/z* (%): 326 (M⁺). Analytical for C₁₉H₂₂N₂OS (326.46): calculated C 69.90, H 6.79, N 8.58; found C 69.84, H 6.70, N 8.49.

5-Allyl-6-benzyl-2-(tetrahydro-2-oxofuran-3-ylthio)pyrimidin-4(3H)-one (2k). Yield 72%, m.p. 153–155 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 2.63 (m, 2H, CH₂), 3.21 (d, 2H, CH₂), 3.83 (s, 2H, CH₂Ph), 4.12 (t, 1H, CH), 4.34 (t, 2H, CH₂), 4.96 (m, 2H, =CH₂), 5.82 (m, 1H, CH), 7.16–7.28 (m, 5H, ArH), 12.54 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 28.73, 29.48, 39.85, 41.32, 66.29, 115.64, 118.24, 126.38, 128.47, 129.10, 134.51, 137.80, 154.42, 163.18, 165.00, 173.73; MS *m/z* (%): 342 (M⁺). Analytical for C₁₈H₁₈N₂O₃S (342.41): calculated C 63.14, H 5.30, N 8.18; found C 63.05, H 5.22, N 8.11.

5-Allyl-6-benzyl-2-(methylacetatethio)pyrimidin-4(3H)-one (3a). Yield 78%, m.p. 117–119 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 3.12 (d, 2H, CH₂), 3.76 (s, 3H, CH₃), 3.80 (s, 2H, CH₂Ph), 3.95 (s, 2H, CH₂), 5.02 (m, 2H, =CH₂), 5.87 (m, 1H, CH), 7.12–7.20 (m, 5H, ArH), 12.51 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 28.16, 32.10, 39.48, 56.14, 115.62, 117.91, 126.38, 128.54, 129.19, 134.31, 137.43, 154.72, 161.17, 163.69, 170.23; MS *m/z* (%): 330 (M⁺). Analytical for C₁₇H₁₈N₂O₃S (330.4): calculated C 61.80, H 5.49, N 8.48; found C 61.68, H 5.41, N 8.36.

5-Allyl-6-benzyl-2-(ethylacetatethio)pyrimidin-4(3H)-one (3b). Yield 61%, m.p. 118–120 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 1.28 (t, 3H, CH₃), 3.09 (d, 2H, CH₂), 3.84 (s, 2H, CH₂), 3.89 (s, 2H, CH₂Ph), 4.27 (q, 2H, CH₂), 5.05 (m, 2H, =CH₂), 5.83 (m, 1H, CH), 7.15–7.22 (m, 5H, ArH), 12.38 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 14.35, 28.19, 31.70, 39.42, 61.48, 115.66, 117.87, 126.32, 128.56, 129.13, 134.26, 137.49, 154.63, 161.22, 163.73, 169.80; MS *m/z* (%): 344 (M⁺). Analytical for C₁₈H₂₀N₂O₃S (344.43): calculated C 62.77, H 5.85, N 8.13; found C 62.65, H 5.74, N 7.96.

2-(5-Allyl-4-benzyl-1,6-dihydro-6-oxopyrimidin-2-ylthio)-N-methylacetamide (4). The ester **3a** (0.60 mmol) was dissolved in 33% methylamine in EtOH (10 mL). When TLC showed no more starting material, the solution was evaporated *in vacuo* to dryness to give the amide derivative **4** in 100% yield, m.p. 167–168 °C; ¹H NMR (DMSO-d₆) δ: 2.51 (s, 2H, CH₃), 3.16 (d, 2H, CH₂), 3.74 (s, 2H, CH₂), 3.78 (s, 2H, CH₂Ph), 4.93 (m, 2H, =CH₂), 5.73 (m, 1H, CH), 7.16–7.25 (m, 5H, ArH), 7.88, 12.46 (2s, 2H, 2NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 25.85, 28.62, 33.39, 39.51, 115.32, 116.87, 126.35,

128.51, 129.16, 135.34, 138.27, 158.34, 161.21, 163.82, 171.85; MS m/z (%): 329 (M^+). Analytical for $C_{17}H_{19}N_3O_2S$ (344.43): calculated C 61.98, H 5.81, N 12.76; found C 61.87, H 5.76, N 12.71.

2-(5-Allyl-4-benzyl-1,6-dihydro-6-oxopyrimidin-2-ylthio)acetic acid (5). The ester **3a** (0.9 mmol) was dissolved in hot (80 °C) aqueous potassium hydroxide (3 mL), and the solution was kept at this temperature for 5 min. After cooling, water was added (3 mL) and the pH adjusted to 7 with 4 M HCl. The precipitate was collected, washed with cold water and dried to give the acid derivative **5** in 61% yield, m.p. 172–173 °C; 1H NMR (DMSO- d_6) δ : 3.23 (d, 2H, CH_2), 3.79 (s, 2H, CH_2), 3.85 (s, 2H, CH_2Ph), 4.97 (m, 2H, = CH_2), 5.83 (m, 1H, CH), 7.17–7.28 (m, 5H, ArH), 12.60, 12.73 (2s, 2H, OH and NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ : 28.09, 31.95, 39.75, 115.98, 117.11, 126.39, 128.47, 129.15, 134.10, 137.20, 156.15, 160.73, 163.75, 169.55; MS m/z (%): 316 (M^+). Analytical for $C_{17}H_{19}N_3O_2S$ (316.37): calculated C 60.74, H 5.10, N 8.85; found C 60.67, H 5.01, N 8.76.

5-Allyl 6-benzyl-2-(cyclohexylthio)pyrimidin-4(3H)-one (6). To a stirred solution of compound **1** (3 mmol) in anhydrous DMF (10 mL) was added K_2CO_3 (3.3 mmol) and cyclohexyl bromide (3.3 mmol). The stirring was continued at 80 °C for 6 h. The reaction mixture was poured into cold H_2O (100 mL), the resulting precipitate was collected by filtration and dried *in vacuo* to afford the corresponding crude product, which was purified by flash chromatography (30% EtOAc/pet.ether 60–80 °C) to give compound **6** in 56% yield, m.p. 138–140 °C; 1H NMR (DMSO- d_6) δ : 1.20–1.84 (m, 10H, 5 CH_2), 3.23 (d, 2H, CH_2), 3.79 (m, 1H, CH), 3.87 (s, 2H, CH_2Ph), 4.98 (m, 2H, = CH_2), 5.76 (m, 1H, CH), 5.85 (m, 1H, CH), 7.14–7.29 (m, 5H, ArH), 12.49 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ : 24.97, 25.55, 28.51, 32.26, 39.43, 43.13, 115.31, 116.84, 126.32, 128.50, 129.14, 135.39, 138.45, 157.13, 161.48, 162.93; MS m/z (%): 340 (M^+). Analytical for $C_{20}H_{24}N_2OS$ (340.48): calculated C 70.55, H 7.10, N 8.23; found C 70.47, H 7.03, N 8.12.

6-Benzyl-2-(cyclohexylsulfinyl)-5-((oxiran-2-yl)methyl)pyrimidin-4(3H)-one (7). A solution of compound **6** (2.5 mmol) and *m*-chloroperbenzoic acid (3 mmol) in dry dichloromethane (20 mL) was allowed to stand at room temperature for 48 h. The reaction mixture was diluted with chloroform (100 mL) and successively washed with 10% sodium bisulfite, 10% sodium thiosulfate, saturated sodium bicarbonate and water. After evaporation, the product was purified by preparative TLC (silica gel, $CH_2Cl_2/MeOH$ 30:1) to give the title compound **7** in 37% yield, m.p. 190–192 °C; 1H NMR (DMSO- d_6) δ : 1.43–2.12 (m, 10H, 5 CH_2), 2.27 (d, 2H, CH_2), 3.07 (m, 1H, CH), 3.28 (m, 1H, CH), 3.76 (d, 2H, CH_2), 3.85 (s, 2H, CH_2Ph), 7.19–7.26 (m, 5H, ArH), 12.58 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ : 21.09, 23.15, 25.44, 25.84, 34.03, 39.93, 46.64, 61.93, 115.13, 126.31, 128.43, 129.14, 137.45, 154.17, 161.59, 164.28; MS m/z (%): 372 (M^+). Analytical for $C_{20}H_{24}N_2O_3S$ (372.48): calculated C 64.49, H 6.49, N 7.52; found C 64.41, H 6.43, N 7.45.

5-Allyl-6-benzyl-2-(cyclohexylthio)pyrimidine-4(3H)-thione (8). A mixture of compound **6** (0.61 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetan-2,4-disulfide (Lawesson's Reagent) (0.92 mmol) in anhydrous benzene 50 mL was refluxed for 1 h. After cooling, the solvent was removed under reduced pressure and the precipitate formed was purified by passing through a silica gel column chromatography eluting with (20% $CHCl_3$ /pet.ether) to afford the title compound **8** in 53% yield, m.p. 143–145 °C; 1H NMR (DMSO- d_6) δ : 1.31–1.98 (m, 10H, 5 CH_2), 3.42 (d, 2H, CH_2), 3.77 (m, 1H, CH), 3.82 (s, 2H, CH_2Ph), 5.05 (m, 2H, = CH_2), 5.91 (m, 1H, CH), 7.21–7.36 (m, 5H, ArH), 12.59 (s, 1H, NH

exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 25.85, 26.32, 29.36, 33.07, 40.63, 44.49, 115.75, 117.89, 126.61, 128.51, 129.62, 135.34, 138.46, 157.73, 163.35, 179.47; MS *m/z* (%): 356 (M⁺). Analytical for C₂₀H₂₄N₂S₂ (356.55): calculated C 67.37, H 6.78, N 7.86; found C 67.28, H 6.781, N 7.79.

5-Allyl 6-benzyl-2-(2,2-diethoxyethylthio)pyrimidin-4(3H)-one (9). The same procedure as mentioned in compound **6**, except the stirring was continued at 65 °C for 8 h. Yield 65%, m.p. 156–158 °C (30% EtOAc/pet.ether 60–80 °C); ¹H NMR (DMSO-d₆) δ: 1.08 (t, 6H, 2CH₃), 3.12 (d, 2H, SCH₂), 3.22 (d, 2H, CH₂), 3.59 (q, 4H, 2 OCH₂), 3.94 (s, 2H, CH₂Ph), 4.52 (t, 1H, CH), 5.03 (m, 2H, =CH₂), 5.78 (m, 1H, CH), 7.18–7.23 (m, 5H, ArH), 12.52 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 15.01, 28.51, 32.47, 39.45, 61.72, 101.35, 115.34, 117.33, 126.51, 128.67, 129.38, 135.36, 137.70, 154.72, 161.28, 163.55; MS *m/z* (%): 374 (M⁺). Analytical for C₂₀H₂₆N₂O₃S (374.5): calculated C 64.14, H 7.00, N 7.48; found C 64.05, H 6.87, N 7.39.

6-Allyl-5-benzyl-3-ethoxy-2,3-dihydrothiazolo[3,2-a]pyrimidin-7-one (10). Compound **9** (4 mmol) was dissolved in dry MeCN (10 mL) under Argon and *N,O*-bis(trimethylsilyl) acetamide (4.4 mmol) was added. The mixture was cooled to –40 °C, and TMS-triflate (4 mmol) was added drop wise. The mixture was allowed to warm to room temperature slowly (typically overnight). Cold saturated aqueous NaHCO₃ was added, and the mixture was extracted with CH₂Cl₂ (3 × 20 mL). The organic phase was washed with saturated aqueous NaCl, dried, evaporated *in vacuo* and purified by column chromatography [CHCl₃/pet.ether (60–80 °C)] to give the title compound **10** in 46% yield, m.p. 162–164 °C; ¹H NMR (DMSO-d₆) δ: 1.12 (t, 3H, CH₃), 3.06 (d, 2H, CH₂), 3.31 (d, 2H, CH₂), 3.54 (q, 2H, CH₂), 3.80 (s, 2H, CH₂Ph), 3.94 (t, 1H, CH), 4.88 (m, 2H, =CH₂), 5.65 (m, 1H, CH), 7.15–7.34 (m, 5H, ArH); ¹³C NMR (DMSO-d₆) δ: 14.38, 29.55, 30.32, 33.30, 39.43, 61.96, 89.74, 115.46, 117.91, 126.59, 128.46, 129.67, 135.68, 146.45, 166.77, 168.28; MS *m/z* (%): 328 (M⁺). Analytical for C₁₈H₂₀N₂O₂S (328.43): calculated C 65.83, H 6.14, N 8.53; found C 65.71, H 6.03, N 8.47.

2-((Methylthio)methylthio)-5-allyl-6-benzylpyrimidin-4(3H)-one (11a). A mixture of compound **1** (1.29 mmol), 10 mL HMDS and 10 mg (NH₄)₂SO₄ were refluxed for 2 h. After evaporation under vacuum, the silylated compound was dissolved in 20 mL anhydrous MeCN and stirred at –40 °C. (4.0 mmol) of chloromethylmethyl sulfide was added, followed by dropwise addition of 0.40 mL TMS-triflate in 10 mL of anhydrous MeCN. The temperature was raised gradually to –15 °C and the mixture was kept at this temperature overnight, then the temperature was raised 5 °C per half hour. The reaction was stopped at 5 °C and 100 mL of CH₂Cl₂ was added. The mixture was washed with 20 mL saturated NaHCO₃ and the organic layer was dried over Na₂SO₄ and evaporated till dryness under reduced pressure. The crude product was purified by flash chromatography (3% MeOH/CHCl₃) to give the title product **11a** in 74% yield, m.p. 218–220 °C; ¹H NMR (DMSO-d₆) δ: 2.11 (s, 3H, CH₃), 3.33 (d, 2H, CH₂), 3.91 (s, 2H, CH₂Ph), 4.25 (s, 2H, CH₂), 5.10 (m, 2H, =CH₂), 5.86 (m, 1H, CH), 7.20–7.31 (m, 5H, ArH), 12.53 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 15.29, 28.97, 36.38, 40.27, 115.73, 118.39, 126.53, 128.61, 129.17, 134.69, 137.87, 156.50, 163.08, 165.22; MS *m/z* (%): 318 (M⁺). Analytical for C₁₆H₁₈N₂OS₂ (318.46): calculated C 60.34, H 5.70, N 8.80; found C 60.28, H 5.62, N 8.69.

5-Allyl-6-benzyl-2-(ethoxymethylthio)pyrimidin-4(3H)-one (11b). Yield 73%, m.p. 142–144 °C (2% MeOH/CHCl₃); ¹H NMR (DMSO-d₆) δ: 1.10 (t, 3H, CH₃), 3.21 (d, 2H, SCH₂), 3.45 (q, 2H, CH₂),

3.90 (s, 2H, CH₂Ph), 5.05 (m, 2H, =CH₂), 5.33 (s, 2H, CH₂), 5.80 (m, 1H, CH) 7.23–7.29 (m, 5H, ArH), 12.31 (s, 2H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 14.50, 29.02, 40.24, 64.92, 72.25, 115.77, 118.93, 126.55, 128.58, 129.32, 135.11, 137.91, 155.75, 161.77, 164.65; MS *m/z* (%): 316 (M⁺). Analytical for C₁₆H₁₈N₂O₂S (316.42): calculated C 64.53, H 6.37, N 8.85; found C 64.45, H 6.29, N 8.76.

2-(3-Hydroxypropylthio)-5-allyl-6-benzylpyrimidin-4(3H)-one (11c). Yield 66%, m.p. 123–125 °C (3% MeOH/CHCl₃); ¹H NMR (DMSO-d₆) δ: 2.17 (m, 2H, CH₂), 2.94 (t, 2H, SCH₂), 3.14 (d, 2H, CH₂), 3.56 (t, 2H, SCH₂), 3.84 (s, 2H, CH₂Ph), 4.58 (s broad, 1H, OH), 4.98 (m, 2H, =CH₂), 5.87 (m, 1H, CH), 7.12–7.21 (m, 5H, ArH), 12.38 (s, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆) δ: 22.65, 28.37, 30.12, 39.46, 62.03, 115.36, 117.93, 126.42, 128.51, 129.15, 134.22, 137.55, 154.70, 161.02, 163.65; MS *m/z* (%): 316 (M⁺). Analytical for C₁₇H₂₀N₂O₂S (316.42): calculated C 64.53, H 6.37, N 8.85; found C 64.37, H 6.325, N 8.73.

3.2. Pharmacological Screening

3.2.1. Virus and cells

The HIV-1 strains HTLV-III_B and NNRTI resistant strain N 119 were propagated in H9 cells at 37 °C, 5% CO₂ using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). The culture supernatant was filtered (0.45 μm), aliquoted and stored at –80 °C until use. Both HIV-1 strains were obtained from the NIH AIDS Research and Reference program.

3.2.2. Inhibition of Human Immunodeficiency Virus Type 1 (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 compounds for six days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was quantitated by the HIV-1 antigen detection assay ELISA [27] or indirectly quantified using the MTT assay [28]. Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT-4 cultures containing the test dilutions of compound as described above. A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentrations (IC₅₀) and the 50% cytotoxic concentrations (CC₅₀) were determined by interpolation from the plots of percent inhibition vs. concentration of compound.

4. Conclusions

Non-nucleoside reverse transcriptase inhibitors (NNRTI) are key components in highly active antiretroviral therapy for treating HIV-1. Herein we present the synthesis for a series of S-DABOs analogues and evaluated as non-nucleoside HIV-1 reverse transcriptase inhibitors for inhibition of HIV-1 replication. Various chemical and spectral data supported the structures of the newly synthesized compounds. The majority of the tested compounds showed moderate to good activities against HIV-1 with an IC₅₀ range from 35.60 to 0.32 μM. These results provided useful indicators for guiding the further rational design of new S-DABO analogues as more active and selective HIV-1 inhibitors.

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Author Contributions

The listed authors contributed to this work as described in the following. Nagy M. Khalifa gave the concepts of the work, interpreted the results and prepared the manuscript and Mohamed A. Al-Omar cooperated in the preparation of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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