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Synthesis and biological evaluation of novel 5-alkyl-2-arylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-ones as potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

A series of novel *S*-DABO analogues of 5-alkyl-2-arylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-ones were synthesized and evaluated as inhibitors of human immunodeficiency virus type-1 (HIV-1). Among them, the most potent HIV-1 inhibitors were compounds **6c1, 6c6**, and **6b1** (EC_{50} = 0.24 ± 0.05, 0.38 ± 0.13, 0.39 ± 0.05 μM, respectively), which possess improved or similar HIV-1 inhibitory activity compared with nevirapine (NVP) (EC_{50} = 0.21 μM) and delavirdine (DLV) (EC_{50} = 0.32 μM). None of these compounds were active against HIV-2 replication. Furthermore, enzyme inhibitory assays were performed with selected derivatives against HIV-1 wtRT, confirming that the main target of these compounds is the HIV-1 RT and these new *S*-DABOs are acting as NNRTIs. The preliminary structure–activity relationship (SAR) of these new congeners is discussed briefly and rationalized by docking studies.

1. Introduction

Since dihydro-alkoxy-benzyl-oxopyrimidines (DABOs) were disclosed as potent HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) in 1992, much effort have been paid for the structural modifications with a view to increase potency and decrease drug resistance. Up to now, three types of DABO analogues have been studied based on the C-2 substitutent features, that is, dihydroalkyloxybenzyloxopyrimidines (*O*-DABOs), dihydroalkylthio benzyloxopyrimidines (*S*-DABOs) and dihydroalkylaminodifluorobenzyloxopyrimidines (*N*-DABOs) (Fig. 1).[1]

Among DABO derivertives, *S*-DABOs were the most potent and selective HIV-1 NNRTIs, with the C-2 alkylthio chain being the peculiar determinant for exhibition of anti-HIV-1 activity. The HNC=O fragment at N3/C4 position of the pyrimidine is a crucial factor for keeping the activity, because the hydrogen bonds are formed by N3-H with the carbonyl group of Lys101 inside the hydrophobic pocket of RT.[2–4] The optimal moieties at positions 5 of the pyrimidine nucleus were dependent on the nature of the C-2 side chain. Both arylcarbonylmethyl and benzyl substituent of *S*-DABOs at the C-2 position of the pyrimidine ring exert their favorable effect on anti-HIV-1 activity by the interaction with the side chain Pro236 of RT binding site.[5,6] Chemical modifications of C-6 substituents led to find numerous compounds as potent HIV-1 NNRTIs by replacement of benzyl moieties with substituted benzyl, 1-naphthylmethyl and 2-naphthylmethyl groups, whereas the introduction of alkyl, phenyl or phenylethyl, phenoxymethyl, and phenylthiomethyl groups to C-6 position resulted in compounds with no or decreased antiviral activity.[7,8]

According to a recent success in the modification of *S*-DABOs with 1-naphthylmethyl at C-6 position (DATNOs) by He et al. (Fig. 1),[7,8] together with the previous SARs studies of DABOs, a novel series of 6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl) substituted *S*-DABOs were designed and synthesized based on the general principle of bioisosterism in medicinal chemistry. We postulated that the newly constructed *S*-DABOs, retaining C-2 preferential active groups and intrducing novel C-6 side chain, might be more favorable to improve a putative π-stacking interaction between the aryl ring of the ligand and Tyr188 or Tyr181 of RT, and further exploration of the structure–activity relationships of *S*-DABOs may yield the discovery of new more potent HIV-1 inhibitors. Here, the synthesis and the anti-HIV evaluation of the designed *S*-DABOs in MT-4 cell culture are reported.

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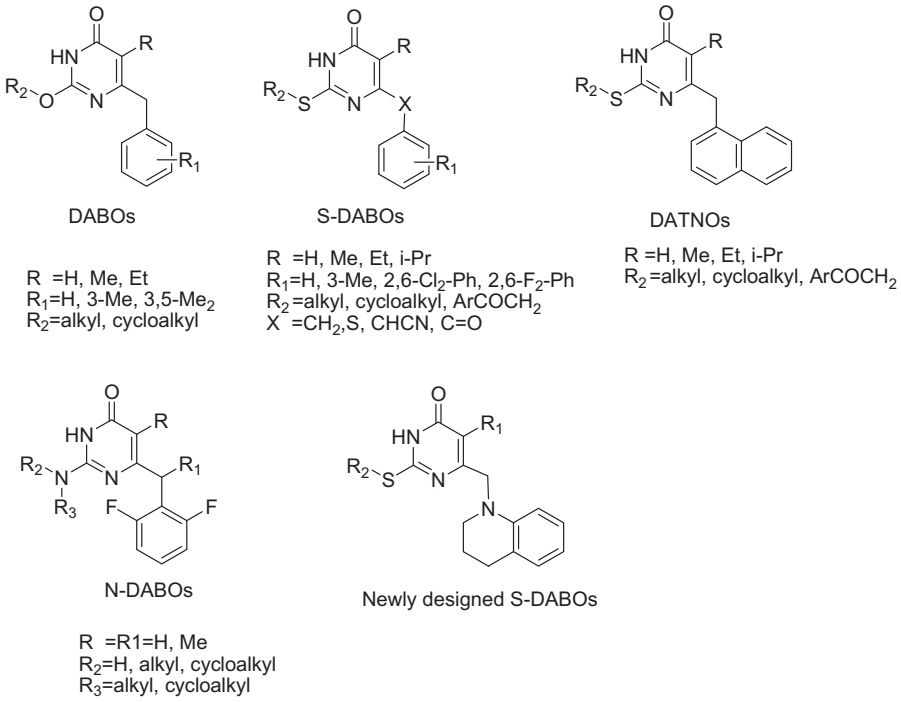


Figure 1. The DABOs families and the newly designed *S*-DABOs.

2. Results and discussion

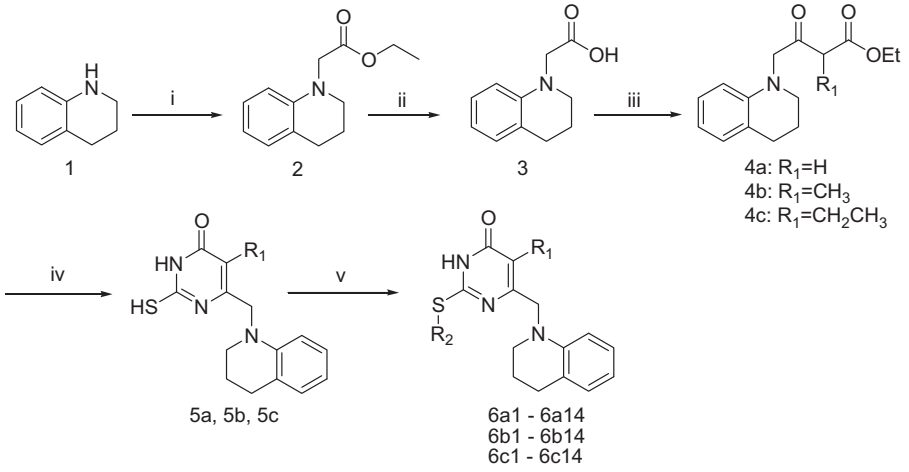
2.1. Chemistry

The synthetic route of the newly designed compounds is described in Scheme 1. The key intermediate β-ketoesters **4** were prepared with a simple method reported by Clay et al.[9] through the reaction of **3** with *N,N′*-carbonyldiimidazole (CDI) followed by treatment with different ethyl potassium malonates in the presence of anhydrous $MgCl_2$ and Et_3N.[10,11] Next, the cyclization reaction of β-ketoesters 4 with thiourea in the presence of EtONa in refluxing EtOH gave the substituted uracil **5.**[12] Treatment of **5** with appropriate arylcarbonylmethyl halides or benzyl halides in the presence of K_2CO_3 in anhydrous DMF afforded the desirable target compounds **6a1–14**, **6b1–14**, **6c1–14**. Both analytical and spectral data of all the compounds are in full agreement with the proposed structures.

2.2. Biological activity

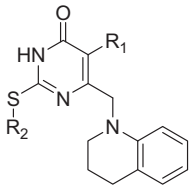
All of the newly designed and synthesized *S*-DABO analogues (compounds **6a1–14, 6b1–14, 6c1–14**) were evaluated for cytotoxicity and anti-HIV activity in MT-4 cells infected with wild-type HIV-1 strain IIIB and HIV-2 strain ROD. The results, expressed as EC_{50}, CC_{50} and SI (selectivity index), are illustrated in Table 1 together with those of nevirapine (NVP), delaviridine (DLV), efavirenz (EFV) and zidovudine (azidothymidine, AZT), which were used as reference drugs.[13–15]

Of the newly synthesized *S*-DABO analogues, compounds **6c1** (EC_{50} = 0.24 μM), **6c6** (EC_{50} = 0.38 μM), **6b1** (EC_{50} = 0.39 μM) and **6b6** (EC_{50} = 0.42 μM) were found to be the active compounds which inhibited HIV-1 replication in cell culture. It should be noted that compound **6c1** (EC_{50} = 0.24 μM) possesses similar HIV-1 inhibitory activity as NVP (EC_{50} = 0.21 μM) and DLV (EC_{50} = 0.32 μM). Comparing the different substitutions at



Scheme 1. Reagents and conditions: (i)K_2CO_3, ethyl 2-bromoacetate, toluene, 24 h, 90 °C; (ii) 15% NaOH, 15 h; (iii) (a) CDI, CH_3CN, rt, 30 min; (b) $MgCl_2$, Et_3N, $R_1CH(CO_2Et)(CO_2K)$, rt, overnight then reflux, 2 h; (iv) thiourea, EtONa, reflux, 6–12 h; (v) arylcarbonylmethyl halides or benzyl halides, K_2CO_3, DMF, rt, 12 h.

Table 1
Anti-HIV-1 activities, cytotoxicities and selectivity indices of new designed *S*-DABO derivatives (**6a1–c14**)



Compound Nos.	R_1	R_2	EC_{50}[a] (μM)		CC_{50}[b] (μM)	SI (IIIB)[c]
			HIV-1 (III B)	HIV-2 (ROD)		
6a1	H	$PhCOCH_2$	24.60 ± 7.48	>152.57	152.57 ± 14.07	6
6a2	H	(4′-CH_3)$PhCOCH_2$	27.99 ± 0.17	>156.05	156.05 ± 0.95	6
6a3	H	(4′-Cl)$PhCOCH_2$	>75.60	>75.60	75.60 ± 46.23	<1
6a4	H	(4′-NO_2)$PhCOCH_2$	>208.78	>208.78	208.78 ± 44.42	<1
6a5	H	(4′-CN)$PhCOCH_2$	>196.88	>196.88	>196.88	< or >1
6a6	H	(4′-OCH_3)$PhCOCH_2$	24.03 ± 0.29	>159.55	159.55 ± 0.29	7
6a7	H	(4′-F)$PhCOCH_2$	29.55 ± 0.17	>135.90	135.90 ± 17.12	5
6a8	H	$PhCH_2$	⩾32.19	>94.17	94.17 ± 46.74	⩽3
6a9	H	(4′-CH_3)$PhCH_2$	>64.32	>64.32	64.32 ± 16.61	<1
6a10	H	(4′-Cl)$PhCH_2$	>53.60	>53.60	>53.60	<1
6a11	H	(4′-NO_2)$PhCH_2$	⩾49.45	>73.89	73.89 ± 36.38	⩽2
6a12	H	(4′-CN)$PhCH_2$	⩾42.99	>86.82	86.82 ± 30.35	⩽2
6a13	H	(4′-OCH_3)$PhCH_2$	25.13 ± 3.28	>180.94	180.94 ± 42.19	7
6a14	H	(4′-F)$PhCH_2$	29.65 ± 6.63	>182.32	182.32 ± 67.66	6
6b1	Me	$PhCOCH_2$	0.39 ± 0.05	>12.18	12.18 ± 0.93	31
6b2	Me	(4′-CH_3)$PhCOCH_2$	1.95 ± 0.07	>62.35	62.35 ± 23.56	32
6b3	Me	(4′-Cl)$PhCOCH_2$	2.16 ± 0.08	>42.10	42.10 ± 34.13	19
6b4	Me	(4′-NO_2)$PhCOCH_2$	⩾3.13	>23.70	23.70 ± 5.14	⩽8
6b5	Me	(4′-CN)$PhCOCH_2$	7.35 ± 6.01	>44.45	44.45 ± 7.96	6
6b6	Me	(4′-OCH_3)$PhCOCH_2$	0.42 ± 0.08	>60.70	60.70 ± 7.54	144
6b7	Me	(4′-F)$PhCOCH_2$	0.46 ± 0.03	>15.43	15.43 ± 5.93	34
6b8	Me	$PhCH_2$	>6.69	>6.69	6.69 ± 4.99	<1
6b9	Me	(4′-CH_3)$PhCH_2$	⩾86.20	>86.20	⩾86.20	<or ×1
6b10	Me	(4′-Cl)$PhCH_2$	⩾2.25	>65.55	65.55 ± 16.78	⩽29
6b11	Me	(4′-NO_2)$PhCH_2$	1.88 ± 0.04	>76.88	76.88 ± 9.91	41
6b12	Me	(4′-CN)$PhCH_2$	1.78 ± 0.18	>12.98	12.98 ± 1.96	7
6b13	Me	(4′-OCH_3)$PhCH_2$	0.64 ± 0.04	>59.55	59.55 ± 9.52	93
6b14	Me	(4′-F)$PhCH_2$	>8.67	>8.67	8.67 ± 4.43	<1
6c1	Et	$PhCOCH_2$	0.24 ± 0.05	>297.95	297.95	1218
6c2	Et	(4′-CH_3)$PhCOCH_2$	1.06 ± 0.67	>288.3	288.3	271
6c3	Et	(4′-Cl)$PhCOCH_2$	1.01 ± 0.07	>275.34	275.34	249
6c4	Et	(4′-NO_2)$PhCOCH_2$	1.98 ± 0.92	>110.99	110.99 ± 35.69	56
6c5	Et	(4′-CN)$PhCOCH_2$	1.06 ± 0.09	>114.86	114.86 ± 67.96	109
6c6	Et	(4′-OCH_3)$PhCOCH_2$	0.38 ± 0.13	>61.84	61.84 ± 23.31	164
6c7	Et	(4′-F)$PhCOCH_2$	0.57 ± 0.053	>156.06	156.06 ± 23.18	273
6c8	Et	$PhCH_2$	>4.80	>4.80	4.80 ± 0.43	<1
6c9	Et	(4′-CH_3)$PhCH_2$	⩾5.42	>159.6	159.6 ± 59.4	29
6c10	Et	(4′-Cl)$PhCH_2$	⩾5.42	>135.22	135.22	⩽25
6c11	Et	(4′-NO_2)$PhCH_2$	1.03 ± 0.25	>194.71	194.71 ± 21.99	188
6c12	Et	(4′-CN)$PhCH_2$	0.48 ± 0.34	>59.68	59.68 ± 44.49	125
6c13	Et	(4′-OCH_3)$PhCH_2$	0.59 ± 0.24	>147.26	147.26 ± 26.82	244
6c14	Et	(4′-F)$PhCH_2$	>133.16	>133.16	133.16	<1
NVP[d]			0.21		>15.02	>72
DLV[d]			0.32		>3.83	>12
EFV[d]			0.0044		>6.34	>1434
AZT[d]			0.015		>93.55	>6192

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-induced cytotoxicity, as determined by the MTT method.
[b] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] SI: selectivity index (CC_{50}/EC_{50}).
[d] The antiviral properties of these compounds were previously described.

C-5 position, the activity of series C5-Et (**6c1–14**) was obviously better than C5-Me series (**6b1–14**), and, followed by C5-H series (**6a1–14**) (Table 1), confirmed that the steric bulkiness of C-5 substituent is favored to maintain inhibitory activity against HIV-1 replication. It is noteworthy that the introduction of a methyl group (series **6b**) maintained the biological activity, but led to increased cytotoxicity, thus providing important information for further design of novel analogues.

Just as SAR of *S*-DABOs made in previous studies, the nature of the substituents at the *para* substitution at the phenyl ring of the C-2 moiety essentially influences the anti-HIV-1 activity. As shown in Table 1, introduction of substituted arylcarbonylmethyl groups at the C-2 side chain led to compounds **6c1–7** with much better activity and selectivity index than the corresponding benzyl substituted compounds **6c8–14**. In addition, when the nitro, cyano and methoxyl group are introduced to the *para*-position of the benzyl

moiety, the bioactivity and selectivity index of these compounds (**6c11–13**) were slightly increased. The experimental results indicated that the majority of the tested series **6b** (**6b1-14**) were found to be active against HIV-1 in the range of 0.39–7.7 μM and agreed with previous SARs findings in **6c** series (**6c1–14**). Thus, low selectivity index of **6b** series may partly due to their high cytotoxicity. Compared to the previous *S*-DABOs with 1-naphthylmethyl at C-6 position, the new compounds showed modest antiviral activity.[8] Based on these results and the fact that charge-transfer interactions between the π-stacking aromatic rings at C-6 position of *S*-DABOs and Tyr188 and Tyr181 in HIV-1 RT probably play an important role in keeping anti-HIV activity, it is suggested that the nature of the 3,4-dihydroquinolin-1(2*H*)-yl)methyl group did not accommodate the chemical environment well in this region of RT. The full description on this issue will be discussed in the following section of molecular simulation analysis.

2.3. Enzyme inhibiting activity against HIV-1 wild-type RT

In order to prove that the newly synthesized compounds were targeted at the HIV-1 RT directly, we chose one compound (**6c1**) to evaluate for HIV-1 RT inhibitory activity, using a poly(rA)/oligo(dT)$_{15}$ homopoly-mer template with the HIV antigen detection ELISA for quantifying expression of HIV-1 RT in culture medium, and nevirapine as a reference compound. The results showed that this new compound inhibited the HIV-1 RT with an IC_{50} value 30.68 μM, with NVP 4.4 μM being the reference, which further indicated that the main target of these compounds is exactly at the HIV-1 RT. However, the IC_{50} value of compound is slightly inconsistent with the result of cell test, which probably because that these new compounds might interfere with another target or act on reverse transcriptase in a different way other than the typical NNRTIs binding pocket. In addition, all of the title compounds were evaluated for their capability to inhibit the HIV-2 (strain ROD) replication in MT-4 cells but no one was active against HIV-2 (ROD). Based on the chemical structure and the general characters that NNRTIs inhibit HIV-1 but not HIV-2 replication, it could be concluded that the new series of *S*-DABOs were specific for HIV-1 and still belonged to HIV-1 NNRTIs.

2.4. Molecular simulation

To investigate the structure–activity relationships of our newly synthesized compounds, **6c1** was docked into the NNRTIs binding pocket (NNIBP) of HIV-1 RT by means of Autodock Vina [http://vina.scripps.edu]. X-ray crystal structure of HIV-1 RT with 6-benzyl-1-(benzyloxymethyl)-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (TNK-651) was taken from PDB (1rt1) and used for docking studies for the high degree of similarity between TNK-651 and *S*-DABOs.[4,16,17] Default parameters were used as described in the Autodock Vina manual unless otherwise specified. The theoretical binding mode of **6c1** to the NNIBP is shown in Figure 2(a).

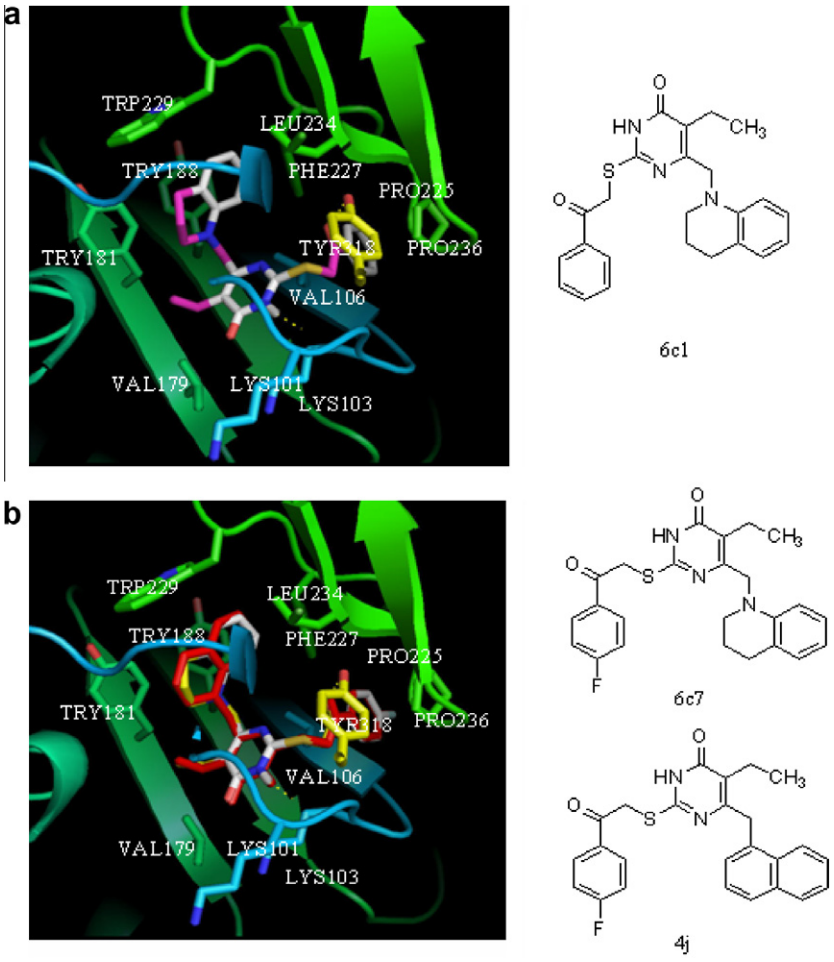


Figure 2. (a) Model of **6c1** docked into the RT non-nucleoside binding site (PDB code: 1rt1) using Autodock Vina [http://vina.scripps.edu]. (b) For comparison purposes, the docked conformation of **6c7** (EC_{50} = 0.57 ± 0.053 μM) and **4j** (red), an earlier 1-naphthylmethyl-*S*-DABO (EC_{50} = 0.078 ± 0.009 μM), are shown in stick representation. The docking result of above is showed by PyMOL [http://pymol.sourceforge.net].

The docking simulation showed the binding mode of the **6c1** into the NNIBP (Fig. 2a). Results showed that the NHC=O fragment at N3/C4 positions of pyrimidine ring was stabilized by a hydrogen bond between the N3-H function of *S*-DABOs and the carbonyl oxygen of Lys101. The extended side chain at C-2 position point toward into a pocket mainly formed by Val106, Pro225, Pro236, and Phe227, with the length and size of the C-2 side chain having only modulator effects on potency. A hydrogen bond is formed between the C=O group of the C-2 side chain and NH group of Lys103 backbone. The ethyl group at C-5 position was positioned in the hydrophobic cavity formed by the Val179 side chain.[15] Moreover, the 3,4-dihydroquinolin-1(2*H*)-yl)methyl substituent at position 6 of the pyrimidinone ring is located in a hydrophobic region defined by Tyr181, Tyr188, Phe227, Trp229 and Leu234. As an additional proof of the effect of the introduced feature, docking experiments were also carried out on the reported 1-naphthylmethyl-*S*-DABO **4j** (EC_{50} = 0.078 ± 0.009 μM, red in Fig. 2b) in comparison with **6c7**. The binding mode of **6c7** displayed a fair superimposition to 1-naphthylmethyl-*S*-DABO **4j** in NNIBP, showing an overall common binding conformation. The superimposed conformation clearly shows that the position of the tetrahydroquinoline ring is somehow slightly tilted in respect to the naphthalene ring of **4j** (Fig. 2b). Conformationnal change of C-6 may lead to the lack of the π-stacking interactions between the aryl ring of the ligand and RT.

In summary, the results of the Auto Docking analysis seem to support the SARs elucidation of our newly designed and synthesized compounds. Further optimization of *S*-DABOs analogues will take into account these aspects in further design attempts.

3. Conclusions

A new series of 5-alkyl-2-arylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-ones (*S*-DABOs) were synthesized and evaluated as potent HIV-1 inhibitors. Among them, the most potent HIV-1 inhibitors were **6c1** (EC_{50} = 0.24 μM), **6c6** (EC_{50} = 0.38 μM), **6b1** (EC_{50} = 0.39 μM) and **6b6** (EC_{50} = 0.42 μM), which possess similar HIV-1 inhibitory activity as NVP (EC_{50} = 0.21 μM) and DLV (EC_{50} = 0.32 μM). The results of enzyme inhibitory assays also showed that these compounds were targeted at the HIV-1 RT, thereby acting as NNRTIs. The preliminary structure–activity relationships and docking studies among the newly disclosed congeners are discussed, which provided useful indications for guiding the further rational design of new *S*-DABO analogues as more active and selective HIV-1 inhibitors.

4. Experimental

All the reactions were monitored by thin-layer chromatography (TLC) on precoated silica gel G plates at 254 nm under a UV lamp. Melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470 FT-IR Spectrometer. Mass spectrometer was taken on Wasters Quattro Micro 2000. ^{1}H-NMR spectra were recorded on a Bruker Avance-600 using TMS as an internal standard and chemical shifts are reported in δ (ppm). Solvents were reagent grade and, when necessary, were purified and dried by standard methods.

4.1. Chemistry

4.1.1. General procedure for the preparation of ethyl-2-(3,4-dihydroquinolin-1(2*H*)-yl) acetate (2)

To a stirred mixture of 1,2,3,4-tetrahydroquinoline (4.04 g, 30.37 mmol), anhydrous K_2CO_3 (8.4 g, 60.78 mmol) in dry toluene (80 mL), ethyl 2-bromoacetate (5.0 g, 29.94 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h (monitored by TLC). Upon completion of the reaction, the reaction mixture was cooled to room temperature, filtered off K_2CO_3 and concentrated in vacuo and the residue was extracted with chloroform (3 × 50 mL). The organic layer was washed with brine, dried over Mg_2SO_4 and evaporated to give a crude compound, which was purified over silica gel column by eluting with ethyl acetate/petroleum ether (1:4) to obtain a brown colored liquid.[18]

4.1.2. General procedure for the preparation of β-ketoesters (4a, 4b, and 4c)

To a well stirred solution of substituted diethyl malonate (500 mmol) in anhydrous EtOH (345 mL) was added dropwise a solution of KOH (28 g, 500 mmol) in EtOH (345 mL) at room temperature over 4 h. The resulting mixture was allowed to stand overnight until the pH of the final mixture had a value between 7 and 8. After removing the solvent, the residue was rinsed with ether and suspended in anhydrous acetonitrile (800 mL), then triethylamine (100 mL, 717 mmol) and magnesium chloride (57 g, 595 mmol) were added. The mixture was continued to stir at room temperature for 2 h, then added a solution of arylacetyl imidazolide which was previously prepared 15 min before by a reaction of 2-[3,4-dihydroquinolin-1(2*H*)-yl]acetic acid (250 mmol) with *N*,*N*′-carbonyldiimidazole (275 mmol) in acetonitrile (300 mL). The reaction mixture was stirred overnight at room temperature and then refluxed for 2 h.[15] After cooling, the reaction mixture was added a solution of 13% HCl (800 mL) and kept stirring for a further 10 min. Finally, the organic layer was separated and concentrated; the residue was extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 × 350 mL) and brine (3 × 350 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the brown oily products **4a–4c**, which is used directly in the next step without further purification. **4a** yield: 15.1%, MS (ESI): *m*/*z* 262.30 (M+1). **4b** yield: 12.1%, MS (ESI): *m*/*z* 276.41 (M+1). **4c** yield: 10.7%, MS (ESI): *m*/*z* 290.33 (M+1).

4.1.3. General procedure for the preparation of 5-alkyl-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (5a, 5b and 5c)

Sodium methoxide was prepared by slow addition of a small piece of sodium metal (6.9 g, 300 mmol), which was previously pealed off and rinsed with the hexane, into 30 mL of anhydrous methanol with vigorous stirring. When the sodium metal was completely dissolved, thiourea (17.1 g, 225 mmol) and the corresponding β-ketoesters **4a**, **4b**, **4c** (150 mmol) were added to the clear solution at room temperature. The reaction mixture was refluxed for 10–12 h (monitored by TLC) under a nitrogen atmosphere. After solvent was evaporated, the residue was dissolved in H_2O (80 mL) acidified by addition of 1 N HCl, and followed by glacial AcOH to adjust to pH = 4. The produced precipitate was collected, washed sequentially with H_2O, EtOH, and Et_2O and then dried to give crude products **5a**, **5b**, and **5c**, which were purified by crystallization to afford the pure compounds.[15] Compound **5a**: Recrystallized from EtOH/DMF as a brown crystal, yield: 67.1%, mp: 238–240 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.41 (s, 1H, N_3H), 12.37 (s, 1H, N_1H), 6.35–6.95 (m, 4H, quinoline-H), 5.41 (s, 1H, C_5-H), 4.19 (s, 2H, NCH_2) 3.31–3.33 (m, 2H, quinoline-H_2), 2.71–2.73 (m, 2H, quinoline-H_4), 1.89–1.90 (m, 2H, quinoline-H_3). ESI-MS: *m*/*z* 274.09 (M+1). $C_{14}H_{15}N_3OS$ (273.35). Compound **5b**: Recrystallized from EtOH/DMF as a brown crystal, yield: 70.3%, mp: 227–228 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.01 (s, 1H, N_3H), 11.99 (s, 1H, N_1H), 6.57–6.99 (m, 4H, quinoline-H), 5.41 (s, 1H, C_5-H), 4.21 (s, 2H, NCH_2) 3.15–3.17 (m, 2H, quinoline-H_2), 2.68–2.70 (m, 2H, quinoline-H_4), 1.86–1.88 (m, 2H, quinoline-H_3),

1.79 (s, 3H, C_5-CH_3). ESI-MS: *m/z* 288.11 (M+1). $C_{15}H_{17}N_3OS$ (287.38). Compound **5c**: Recrystallized from EtOH/DMF as a brown crystal, yield: 69.7%, mp: 210–211 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.03 (s, 1H, N_3H), 12.01 (s, 1H, N_1H), 6.45–6.97 (m, 4H, quinoline-H), 4.20 (s, 2H, NCH_2) 3.30–3.37 (m, 2H, quinoline-H_2), 2.37–2.45 (m, 2H, quinoline-H_4), 2.28–2.31 (q, 2H, *J* = 7.2 Hz, C_5-CH_2CH_3), 1.68–1.79 (m, 2H, quinoline-H_3), 1.79 (s, 3H, C_5-CH_3), 1.62–1.71 (m, 2H, quinoline-H_3), 1.05 (t, 3H, *J* = 7.2 Hz, C_5-CH_2CH_3). ESI-MS: *m/z* 302.12 (M+1). $C_{16}H_{19}N_3OS$ (301.41).

4.1.4. General procedure for the preparation of target compounds (6a1–14, 6b1–14, and 6c1–14)

To a stirred solution of **5a**, **5b**, **5c** (1.5 mmol) in anhydrous DMF (10 mL) was added K_2CO_3 (0.23 g, 1.65 mmol) at room temperature. After stirring for 20 min, arylcarbonylmethyl halides or benzyl halides (3.3 mmol) was added, and stirring was continued at this temperature for other 10 h. The reaction mixture was poured into cold H_2O (100 mL), the resulting precipitate was collected by filtration and washed sequentially with H_2O, EtOH and Et_2O, then dried in vacuo at 40 °C to afford the corresponding crude product, which was purified by crystallization or by flash chromatography to give the pure target compounds **6a1–14, 6b1–14**, and **6c–14**.

4.1.4.1. 2-Phenylcarbonylmethylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a1). Recrystallized from EtOH/DMF as a brown crystal, yield: 48.6%, mp: 160–163 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.72 (s, 1H, NH), 8.04 (d, 2H, *J* = 7.8 Hz, PhH), 7.69 (t, 1H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH),7.58 (t, 2H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH), 6.23–6.86 (m, 4H, quinoline-H), 5.86 (s, 1H, C_5-H), 4.77 (s, 2H, SCH_2), 3.99 (s, 2H, NCH_2), 3.24–3.26 (m, 2H, quinoline-H_2), 2.61–2.63 (m, 2H, quinoline-H_4), 1.75–1.77 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3315 (NH), 2945, 2846 (CH_2), 1703 (C=O), 1654 (C=O), 1598, 1575, 1541, 1499 (aryl), 1239 (C–N), 1204 (C–N). ESI-MS: *m/z* 392.47 (M+1). $C_{22}H_{21}N_3O_2S$ (391.49).

4.1.4.2. 2-(4-Methylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a2). Recrystallized from EtOH/DMF as a brown crystal, yield: 54.1%, mp: 165–166 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.68 (s, 1H, NH), 7.93 (d, 2H, *J* = 7.8 Hz, PhH), 7.36 (d, 2H, *J* = 7.8 Hz, PhH), 6.24–6.87 (m, 4H, quinoline-H), 5.87 (s, 1H, C_5-H), 4.74 (s, 2H, SCH_2), 4.02 (s, 2H, NCH_2), 3.26–3.31 (m, 2H, quinoline-H_2), 2.71–2.73 (m, 2H, quinoline-H_4), 2.39 (s, 3H, $PhCH_3$), 1.76–1.78 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3421 (NH), 2927, 2841 (CH_3, CH_2), 1675 (C=O), 1655 (C=O), 1602, 1538, 1506, 1459 (aryl), 1315 (C–N), 1200 (C–N). ESI-MS: *m/z* 406.47 (M+1). $C_{23}H_{23}N_3O_2S$ (405.51).

4.1.4.3. 2-(4-Chlorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a3). Recrystallized from EtOH/DMF as a brown crystal, yield: 37.9%, mp: 193–195 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.79 (s, 1H, NH), 8.05 (d, 2H, *J* = 8.4 Hz, PhH), 7.64 (d, 2H, *J* = 8.4 Hz, PhH), 6.22–6.86 (m, 4H, quinoline-H), 5.87 (s, 1H, C_5-H), 4.75 (s, 2H, SCH_2), 3.99 (s, 2H, NCH_2), 3.25–3.33 (m, 2H, quinoline-H_2), 2.62–2.64 (m, 2H, quinoline-H_4), 1.76–1.78 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3522 (NH), 2927, 2843 (CH_2), 1690 (C=O), 1656 (C=O), 1600, 1587, 1545, 1503 (aryl), 1310 (C–N), 1195 (C–N). ESI-MS: *m/z* 426.87 (M+1). $C_{22}H_{20}ClN_3O_2S$ (425.93).

4.1.4.4. 2-(4-Nitrophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a4). Recrystallized from EtOH/DMF as an amber crystal, yield: 45.1%, mp: 215–216 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.37 (s, 1H, NH), 8.27 (d, 2H, *J* = 8.4 Hz, PhH), 8.03 (d, 2H, *J* = 8.4 Hz, PhH), 6.35–6.95 (m, 4H, quinoline-H), 5.41 (s, 1H, C_5-H), 4.65 (s, 2H, SCH_2), 4.19 (s, 2H, NCH_2), 3.32–3.34 (m, 2H, quinoline-H_2), 2.71–2.73 (m, 2H, quinoline-H_4), 1.89–1.90 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3405 (NH), 2923, 2856 (CH_2), 1703 (C=O), 1640 (C=O), 1579, 1508, 1458 (aryl), 1308 (C–N), 1185 (C–N). ESI-MS: *m/z* 437.51 (M+1). $C_{22}H_{20}N_4O_4S$ (436.48).

4.1.4.5. 2-(4-Cyanophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a5). Recrystallized from EtOH/DMF as a brown crystal, yield: 43.3%, mp: 174–175 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.73 (s, 1H, NH), 7.67–7.98 (m, 4H, PhH), 6.73–7.01 (m, 4H, quinoline-H), 5.41(s, 1H, C_5-H), 4.66 (s, 2H, SCH_2), 4.12 (s, 2H, NCH_2), 3.27–3.31 (m, 2H, quinoline-H_2), 2.41–2.53 (m, 2H, quinoline-H_4), 1.79–1.90 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3391 (NH), 2925, 2848 (CH_2), 2229 (C≡N), 1662 (C=O), 1601 (C=O), 1581, 1504, 1458 (aryl), 1272 (C–N), 1198 (C–N). ESI-MS: *m/z* 417.52 (M+1). $C_{23}H_{20}N_4O_2S$ (416.50).

4.1.4.6. 2-(4-Methoxylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a6). Recrystallized from EtOH/DMF as a brown crystal, yield: 39.1%, mp: 163–165 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.72 (s, 1H, NH), 8.02 (d, 2H, *J* = 8.4 Hz, PhH), 7.07(d, 2H, *J* = 8.4 Hz, PhH), 6.28–6.87 (m, 4H, quinoline-H), 5.82 (s, 1H, C_5-H), 4.69 (s, 2H, SCH_2), 4.03 (s, 2H, NCH_2), 3.85 (s, 3H, $PhOCH_3$). 3.28–3.38 (m, 2H, quinoline-H_2), 2.63–2.65 (m, 2H, quinoline-H_4), 1.78–1.80 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3416 (NH), 2931, 2838 (CH_3, CH_2), 1656 (C=O), 1610 (C=O), 1599, 1574, 1535, 1507 (aryl), 1259 (C–N), 1172 (C–N). ESI-MS: *m/z* 422.54 (M+1). $C_{23}H_{23}N_3O_3S$ (421.51).

4.1.4.7. 2-(4-Fluorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a7). Recrystallized from EtOH/DMF as a brown needle crystal, yield: 47.1%, mp: 180–181 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.73 (s, 1H, NH), 8.10–8.13 (m, 2H, PhH), 7.37–7.40 (m, 2H, PhH), 6.23–6.86 (m, 4H, quinoline-H), 5.85 (s, 1H, C_5-H), 4.74 (s, 2H, SCH_2), 4.19 (s, 2H, NCH_2) 3.26–3.33 (m, 2H, quinoline-H_2), 2.62–2.64 (m, 2H, quinoline-H_4), 1.77–1.81 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3318 (NH), 2940, 2852 (CH_2), 1702 (C=O), 1655 (C=O), 1597, 1575, 1539, 1502 (aryl), 1220 (C–N), 1154 (C–N). ESI-MS: *m/z* 410.49 (M+1). $C_{22}H_{20}FN_3O_2S$ (409.48).

4.1.4.8. 2-Benzylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl) pyrimidin-4(3*H*)-one (6a8). Recrystallized from EtOH/DMF as a brown crystal, yield: 49.6%, mp: 200–203 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.70 (s, 1H, NH), 7.39 (d, 2H, *J* = 7.2 Hz, PhH), 7.30 (t, 1H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH), 7.26 (t, 2H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH), 6.35–6.90 (m, 4H, quinoline-H), 5.84 (s, 1H, C_5-H), 4.38 (s, 2H, SCH_2), 4.29 (s, 2H, NCH_2), 3.42–3.44 (m, 2H, quinoline-H_2), 2.70–2.72 (m, 2H, quinoline-H_4), 1.90–1.92 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3426 (NH), 2928, 2844 (CH_2), 1656 (C=O), 1602, 1574, 1547, 1508 (aryl), 1310 (C–N), 1246 (C–N). ESI-MS: *m/z* 364.57 (M+1). $C_{21}H_{21}N_3OS$ (363.48).

4.1.4.9. 2-(4-Methylbenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a9). Recrystallized from EtOH/DMF as a brown crystal, yield: 53.2%, mp: 185–186 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.60 (s, 1H, NH), 7.27 (d, 2H, *J* = 7.8 Hz, PhH), 7.10 (d, 2H, *J* = 7.8 Hz, PhH), 6.34–6.91 (m, 4H, quinoline-H), 5.89 (s, 1H, C_5-H), 4.33 (s, 2H, SCH_2), 4.28 (s, 2H, NCH_2), 3.41–3.44 (m, 2H, quinoline-H_2), 2.70-2.73 (m, 2H, quinoline-H_4), 2.27 (s, 3H, $PhCH_3$), 1.90–1.91 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) *v* 3418 (NH), 2921, 2849 (CH_3, CH_2), 1651 (C=O),

1602, 1579, 1534, 1508 (aryl), 1310 (C–N), 1194 (C–N). ESI-MS: *m/z* 378.57 (M+1). $C_{22}H_{23}N_3OS$ (377.50).

4.1.4.10. 2-(4-Chlorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a10). Recrystallized from EtOH/DMF as a brown crystal, yield: 57.9%, mp: 190–193 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.65 (s, 1H, NH), 7.41 (d, 2H, J = 8.4 Hz, PhH), 7.33 (d, 2H, J = 8.4 Hz, PhH), 6.33–6.90 (m, 4H, quinoline-H), 5.90 (s, 1H, C_5-H), 4.36 (s, 2H, SCH_2), 4.28 (s, 2H, NCH_2), 3.41–3.44 (m, 2H, quinoline-H_2), 2.70–2.72 (m, 2H, quinoline-H_4), 1.88–1.92 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3413 (NH), 2921, 2850 (CH_2), 1648 (C=O), 1602, 1581, 1535 (aryl), 1310 (C–N), 1194 (C–N). ESI-MS: *m/z* 398.74 (M+1). $C_{21}H_{20}ClN_3OS$ (397.92).

4.1.4.11. 2-(4-Nitrobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a11). Recrystallized from EtOH/DMF as a amber crystal, yield: 51.5%, mp: 180–182 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.70 (s, 1H, NH), 8.13 (d, 2H, J = 8.4 Hz, PhH), 7.66 (d, 2H, J = 8.4 Hz, PhH), 6.30–6.89 (m, 4H, quinoline-H), 5.90 (s, 1H, C_5-H), 4.47 (s, 2H, SCH_2), 4.28 (s, 2H, NCH_2), 3.39–3.43 (m, 2H, quinoline-H_2), 2.68–2.71 (m, 2H, quinoline-H_4), 1.87–1.91 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3370 (NH), 2930, 2841 (CH_2), 1660 (C=O), 1600, 1583, 1518 (aryl), 1310 (C–N), 1240 (C–N). ESI-MS: *m/z* 409.51 (M+1). $C_{21}H_{20}N_4O_3S$ (408.47).

4.1.4.12. 2-(4-Cyanobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a12). Recrystallized from EtOH/DMF as a brown crystal, yield: 54.0%, mp: 186–188 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.71 (s, 1H, NH), 7.73 (d, 2H, J = 7.8 Hz, PhH), 7.59 (d, 2H, J = 7.8 Hz, PhH), 6.30–6.90 (m, 4H, quinoline-H), 5.90 (s, 1H, C_5-H), 4.43 (s, 2H, SCH_2), 4.27 (s, 2H, NCH_2), 3.38–3.40 (m, 2H, quinoline-H_2), 2.69–2.71 (m, 2H, quinoline-H_4), 1.87–1.91 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3419 (NH), 2927, 2840 (CH_2), 2227 (C≡N), 1656 (C=O), 1601, 1581, 1533, 1504 (aryl), 1311 (C–N), 1235 (C–N). ESI-MS: *m/z* 389.52 (M+1). $C_{22}H_{20}N_4OS$ (388.49).

4.1.4.13. 2-(4-Methoxybenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a13). Recrystallized from EtOH/DMF as a brown crystal, yield: 48.7%, mp: 194–196 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.60 (s, 1H, NH), 7.31 (d, 2H, J = 9.0 Hz, PhH), 6.91 (d, 2H, J = 9.0 Hz, PhH), 6.35–6.84 (m, 4H, quinoline-H), 5.89 (s, 1H, C_5-H), 4.32 (s, 2H, SCH_2), 4.29 (s, 2H, NCH_2), 3.72 (s, 3H, $PhOCH_3$), 3.42–3.45 (m, 2H, quinoline-H_2), 2.70–2.73 (m, 2H, quinoline-H_4), 1.89–1.93 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3426 (NH), 2928, 2835 (CH_3, CH_2), 1649 (C=O), 1603, 1579, 1534, 1510 (aryl), 1311 (C–N), 1246 (C–N). ESI-MS: *m/z* 394.53 (M+1). $C_{22}H_{23}N_3O_2S$ (393.50).

4.1.4.14. 2-(4-Fluorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6a14). Recrystallized from EtOH/DMF as a brown needle crystal, yield: 53.9%, mp: 203–205 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.66 (s, 1H, NH), 7.31 (d, 2H, J = 9.0 Hz, PhH), 6.91 (d, 2H, J = 9.0 Hz, PhH), 6.35–6.84 (m, 4H, quinoline-H), 5.89 (s, 1H, C_5-H), 4.36 (s, 2H, SCH_2), 4.29 (s, 2H, NCH_2), 3.41–3.43 (m, 2H, quinoline-H_2), 2.70–2.72 (m, 2H, quinoline-H_4), 1.90–1.91 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3319 (NH), 2923, 2844 (CH_2), 1649 (C=O), 1600, 1577, 1545, 1508 (aryl), 1313 (C–N), 1229 (C–N). ESI-MS: *m/z* 382.49 (M+1). $C_{21}H_{20}FN_3OS$ (381.47).

4.1.4.15. 5-Methyl-2-phenylcarbonylmethylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b1). Recrystallized from EtOH/DMF as a brown crystal, yield: 45.4%, mp: 188–189 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.77 (s, 1H, NH), 7.86 (d, 2H, J = 7.8 Hz, PhH), 7.69 (t, 1H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH),7.54 (t, 2H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH), 6.29–6.82 (m, 4H, quinoline-H), 4.61 (s, 2H, SCH_2), 4.21 (s, 2H, NCH_2), 3.16–3.18 (m, 2H, quinoline-H_2), 2.43–2.45 (m, 2H, quinoline-H_4), 1.95 (s, 3H, C_5CH_3), 1.60–1.61 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3420 (NH), 2922, 2842 (CH_3, CH_2), 1682 (C=O), 1637 (C=O), 1601, 1572, 1506, 1449 (aryl), 1262 (C–N), 1194 (C–N). ESI-MS: *m/z* 406.47 (M+1). $C_{23}H_{23}N_3O_2S$ (405.51).

4.1.4.16. 5-Methyl-2-(4-methylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b2). Recrystallized from EtOH/DMF as a brown crystal, yield: 52.3%, mp: 203–204 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.71 (s, 1H, NH), 7.76 (d, 2H, J = 8.4 Hz, PhH), 7.31 (d, 2H, J = 8.4 Hz, PhH), 6.31–6.83 (m, 4H, quinoline-H), 4.56 (s, 2H, SCH_2), 4.21 (s, 2H, NCH_2), 3.16–3.18 (m, 2H, quinoline-H_2), 2.39 (s, 3H, PhCH3), 2.47–2.50 (m, 2H, quinoline-H_4), 1.95 (s, 3H, C_5-CH_3), 1.62–1.66 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3428 (NH), 2940, 2920, 2832 (CH_3, CH_2), 1704 (C=O), 1633 (C=O), 1603, 1571, 1550, 1505 (aryl), 1265 (C–N), 1198 (C–N). ESI-MS: *m/z* 420.74 (M+1). $C_{24}H_{25}N_3O_2S$ (419.54).

4.1.4.17. 5-Methyl-2-(4-chlorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b3). Recrystallized from EtOH/DMF as a brown crystal, yield: 36.7%, mp: 187–188 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.79 (s, 1H, NH), 7.85 (d, 2H, J = 8.4 Hz, PhH), 7.58 (d, 2H, J = 8.4 Hz, PhH), 6.28–6.81 (m, 4H, quinoline-H), 4.58 (s, 2H, SCH_2), 4.20 (s, 2H, NCH_2), 3.15–3.17 (m, 2H, quinoline-H_2), 2.43–2.50 (m, 2H, quinoline-H_4), 1.95 (s, 3H, C_5-CH_3), 1.62–1.68 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3434 (NH), 2929, 2842 (CH_3, CH_2), 1681 (C=O), 1637 (C=O), 1600, 1587, 1550, 1504 (aryl), 1256 (C–N), 1193 (C–N). ESI-MS: *m/z* 440.87 (M+1). $C_{23}H_{22}ClN_3O_2S$ (439.96).

4.1.4.18. 5-Methyl-2-(4-nitrophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b4). Recrystallized from EtOH/DMF as a amber crystal, yield: 39.1%, mp: 189–190 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.81 (s, 1H, NH), 8.27 (d, 2H, J = 7.8 Hz, PhH), 8.09 (d, 2H, J = 7.8 Hz, PhH), 6.28–6.79 (m, 4H, quinoline-H), 4.65 (s, 2H, SCH_2), 4.18 (s, 2H, NCH_2), 3.40–3.43 (m, 2H, quinoline-H_2), 2.50–2.51 (m, 2H, quinoline-H_4), 1.89–1.94 (m, 2H, quinoline-H_3), 1.620 (s, 3H, C_5-CH_3). IR (KBr, cm^{-1}) ν 3406 (NH), 2918, 2842 (CH_3, CH_2), 1696 (C=O), 1641 (C=O), 1602, 1524, 1505, 1458 (aryl), 1344 (C–N), 1189 (C–N). ESI-MS: *m/z* 451.43 (M+1). $C_{23}H_{22}N_4O_4S$ (450.51).

4.1.4.19. 5-Methyl-2-(4-cyanophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b5). Recrystallized from EtOH/DMF as a brown crystal, yield: 36.6%, mp: 179–180 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.73 (s, 1H, NH), 7.63–7.96 (m, 4H, PhH), 6.30–6.80 (m, 4H, quinoline-H), 4.62 (s, 2H, SCH_2), 4.19 (s, 2H, NCH_2), 3.14–3.23 (m, 2H, quinoline-H_2), 2.44–2.50 (m, 2H, quinoline-H_4), 1.94 (s, 3H, C_5-CH_3), 1.62–1.78 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3442 (NH), 2919, 2840 (CH_3, CH_2), 2231 (C≡N), 1690 (C=O), 1643 (C=O), 1602, 1544, 1505 (aryl), 1267 (C–N), 1195 (C–N). ESI-MS: *m/z* 431.59 (M+1). $C_{24}H_{22}N_4O_2S$ (430.52).

4.1.4.20. 5-Methyl-2-(4-methoxylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b6). Recrystallized from EtOH/DMF as a brown crystal, yield: 38.9%, mp: 210–211 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.72 (s, 1H, NH), 7.83 (d, 2H, J = 8.4 Hz, PhH), 7.03 (d, 2H, J = 8.4 Hz, PhH), 6.31–6.83 (m, 4H, quinoline-H), 4.54 (s, 2H,

SCH_2), 4.23 (s, 2H, NCH_2), 3.86 (s, 3H, PhOCH3), 3.20–3.22 (m, 2H, quinoline-H_2), 2.47–2.50 (m, 2H, quinoline-H_4), 1.95 (s, 3H, C_5-CH_3), 1.64–1.66 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3225 (NH), 2932, 2839 (CH_3, CH_2), 1667 (C=O), 1646 (C=O), 1599, 1574, 1546, 1502 (aryl), 1266 (C–N), 1165 (C–N). ESI-MS: *m/z* 436.71 (M+1). $C_{24}H_{25}N_3O_3S$ (435.54).

4.1.4.21. 5-Methyl-2-(4-fluorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b7). Recrystallized from EtOH/DMF as a crystal, yield: 47.6%, mp: 179–180 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.73 (s, 1H, NH), 7.90–7.93 (m, 2H, PhH), 7.33–7.36 (m, 2H, PhH), 6.29–6.81 (m, 4H, quinoline-H), 4.58 (s, 2H, SCH_2), 4.21 (s, 2H, NCH_2), 3.16–3.18 (m, 2H, quinoline-H_2), 2.43–2.45 (m, 2H, quinoline-H_4), 1.95 (s, 3H, C_5-CH_3), 1.61–1.63 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3467 (NH), 2951, 2893, 2845 (CH_3, CH_2), 1703 (C=O), 1654 (C=O), 1600, 1575, 1551, 1508 (aryl), 1263 (C–N), 1195 (C–N). ESI-MS: *m/z* 424.56 (M+1). $C_{23}H_{22}FN_3O_2S$ (423.50).

4.1.4.22. 5-Methyl-2-benzylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b8). Recrystallized from EtOH/DMF as a crystal, yield: 51.7%, mp: 224–225 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.63 (s, 1H, NH), 7.20 (d, 2H, J = 7.2 Hz, PhH), 7.17 (t, 1H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH),7.14 (t, 2H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH), 6.42–6.90 (m, 4H, quinoline-H), 4.39 (s, 2H, SCH_2), 4.15 (s, 2H, NCH_2), 3.43–3.45 (m, 2H, quinoline-H_2), 2.61–2.63 (m, 2H, quinoline-H_4), 1.98 (s, 3H, C_5-CH_3), 1.84–1.86 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3427 (NH), 2942, 2838 (CH_3, CH_2), 1640 (C=O), 1602, 1573, 1554, 1508 (aryl), 1264 (C–N), 1197 (C–N). ESI-MS: *m/z* 378.56 (M+1). $C_{22}H_{23}N_3OS$ (377.50).

4.1.4.23. 5-Methyl-2-(4-methylbenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b9). Recrystallized from EtOH/DMF as a crystal, yield: 35.0%, mp: 223–225 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.35 (s, 1H, NH), 7.01 (d, 2H, J = 7.8 Hz, PhH), 6.96 (d, 2H, J = 7.8 Hz, PhH), 6.58–6.61 (m, 4H, quinoline-H), 4.22 (s, 2H, SCH_2), 4.12(s, 2H, NCH_2), 3.43–3.45 (m, 2H, quinolineH_2), 2.61–2.63 (m, 2H, quinoline-H_4), 2.24 (s, 3H, $PhCH_3$), 1.97 (s, 3H, C_5-CH_3), 1.84–1.86 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3334 (NH), 2926, 2853 (CH_3, CH_2), 1683 (C=O), 16001, 1538, 1497 (aryl), 1239 (C–N), 1190 (C–N). ESI-MS: *m/z* 392.53 (M+1). $C_{23}H_{25}N_3OS$ (391.53).

4.1.4.24. 5-Methyl-2-(4-chlorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b10). Recrystallized from EtOH/DMF as a crystal, yield: 56.3%, mp: 220–222 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.64 (s, 1H, NH), 7.15 (d, 2H, J = 7.8 Hz, PhH), 7.11 (d, 2H, J = 7.8 Hz, PhH), 6.40–6.91 (m, 4H, quinolineH), 4.38 (s, 2H, SCH_2), 4.17 (s, 2H, NCH_2), 3.41–3.43 (m, 2H, quinolineH_2), 2.60–2.62 (m, 2H, quinolineH_4), 1.98 (s, 3H, C_5CH_3), 1.82–1.86 (m, 2H, quinolineH_3). IR (KBr, cm^{-1}) ν 3426 (NH), 2941, 2840 (CH_3, CH_2), 1641 (C=O), 1601, 1573, 1554, 1507 (aryl), 1265 (C–N), 1197 (C–N). ESI-MS: *m/z* 412.56 (M+1). $C_{22}H_{22}ClN_3OS$ (411.95).

4.1.4.25. 5-Methyl-2-(4-nitrobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl) pyrimidin-4(3*H*)-one (6b11). Recrystallized from EtOH/DMF as a amber crystal, yield: 52.9%, mp: 209–210 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.69 (s, 1H, NH), 7.93 (d, 2H, J = 8.4 Hz, PhH), 7.34 (d, 2H, J = 8.4 Hz, PhH), 6.39–6.91 (m, 4H, quinoline-H), 4.39 (s, 2H, SCH_2), 4.32 (s, 2H, NCH_2), 3.39–3.40 (m, 2H, quinoline-H_2), 2.57–2.59 (m, 2H, quinoline-H_4), 1.98 (s, 3H, C_5-CH_3), 1.80–1.84 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3334 (NH), 2936, 2842 (CH_3, CH_2), 1643 (C=O), 1599, 1573, 1516 (aryl), 1344 (C–N), 1263 (C–N). ESI-MS: *m/z* 423.57 (M+1). $C_{22}H_{22}N_4O_3S$ (422.50).

4.1.4.26. 5-Methyl-2-(4-cyanobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b12). Recrystallized from EtOH/DMF as a brown crystal, yield: 50.4%, mp: 195–197 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.70(s, 1H, NH), 7.54 (d, 2H, J = 7.8 Hz, PhH), 7.27 (d, 2H, J = 7.8 Hz, PhH), 6.40–6.90 (m, 4H, quinoline-H), 4.38 (s, 2H, SCH_2), 4.25 (s, 2H, NCH_2), 3.37–3.39 (m, 2H, quinoline-H_2), 2.57–2.59 (m, 2H, quinoline-H_4), 1.98 (s, 3H, C_5-CH_3), 1.80–1.84 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3433 (NH), 2935, 2842 (CH_3, CH_2), 2225 (C≡N), 1642 (C=O), 1601, 1572, 1541, 1505 (aryl), 1260 (C–N), 1196 (C–N). ESI-MS: *m/z* 403.57 (M+1). $C_{23}H_{22}N_4OS$ (402.51).

4.1.4.27. 5-Methyl-2-(4-methoxybenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b13). Recrystallized from EtOH/DMF as a brown crystal, yield: 39.8%, mp: 180–182 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.59 (s, 1H, NH), 7.05 (d, 2H, J = 8.4 Hz, PhH), 6.91 (d, 2H, J = 8.4 Hz, PhH), 6.41–6.84 (m, 4H, quinoline-H), 4.38 (s, 2H, SCH_2), 4.10(s, 2H, NCH_2), 3.68 (s, 3H, PhOCH3), 3.44–3.47 (m, 2H, quinoline-H_2), 2.62–2.64 (m, 2H, quinoline-H_4), 1.98 (s, 3H, C_5-CH_3), 1.85–1.89 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3413 (NH), 2928, 2833 (CH_3, CH_2), 1641 (C=O), 1601, 1571, 1545, 1511 (aryl), 1253 (C–N), 1198 (C–N). ESI-MS: *m/z* 408.57 (M+1). $C_{23}H_{25}N_3O_2S$ (407.53).

4.1.4.28. 5-Methyl-2-(4-fluorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6b14). Recrystallized from EtOH/DMF as a brown crystal, yield: 55.8%, mp: 215–217 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.63 (s, 1H, NH), 7.14 (m, 2H, PhH), 6.93 (m, 2H, PhH), 6.41–6.90 (m, 4H, quinoline-H), 4.38 (s, 2H, SCH_2), 4.16 (s, 2H, NCH_2), 3.42–3.44 (m, 2H, quinoline-H_2), 2.61–2.63 (m, 2H, quinoline-H_4), 1.98 (s, 3H, C_5-CH_3), 1.83–1.87 (m, 2H, quinoline-H_3). IR (KBr, cm^{-1}) ν 3459 (NH), 2939, 2840 (CH_3, CH_2), 1641 (C=O), 1601, 1573, 1554, 1508 (aryl), 1265 (C–N), 1222 (C–N). ESI-MS: *m/z* 396.53 (M+1). $C_{22}H_{22}FN_3OS$ (395.49).

4.1.4.29. 5-Ethyl-2-phenylcarbonylmethylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c1). Recrystallized from EtOH/DMF as a brown crystal, yield: 47.5%, mp: 193–194 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.77 (s, 1H, NH),7.85 (d, 2H, J = 7.8 Hz, PhH), 7.68 (t, 1H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH), 7.53 (t, 2H, J_1 = 7.8 Hz, J_2 = 15.6 Hz, PhH), 6.32–6.82 (m, 4H, quinoline-H), 4.60 (s, 2H, SCH_2), 4.24 (s, 2H, NCH_2), 3.16–3.18 (m, 2H, quinoline-H_2), 2.45–2.50 (m, 2H, quinoline-H_4), 2.43 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 1.60–1.63 (m, 2H, quinoline-H_3), 1.05 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3462 (NH), 2931, 2840 (CH_3, CH_2), 1682 (C=O), 1633(C=O), 1599, 1573, 1507, 1448 (aryl), 1254 (C–N), 1195 (C–N). ESI-MS: *m/z* 420.21 (M+1). $C_{24}H_{25}N_3O_2S$ (419.54).

4.1.4.30. 5-Ethyl-2-(4-methylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c2). Recrystallized from EtOH/DMF as a brown crystal, yield: 57.1%, mp: 185–187 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.76 (s, 1H, NH),7.74 (d, 2H, J = 8.4 Hz, PhH), 7.32 (d, 2H, J = 8.4 Hz, PhH), 6.33–6.83 (m, 4H, quinoline-H), 4.56 (s, 2H, SCH_2), 4.24 (s, 2H, NCH_2), 3.16–3.18 (m, 2H, quinoline-H_2), 2.45–2.50 (m, 2H, quinoline-H_4), 2.42 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 2.39 (s, 3H, $PhCH_3$), 1.62–1.65 (m, 2H, quinoline-H_3), 1.02 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3437 (NH), 2929, 2842 (CH_3, CH_2), 1677 (C=O), 1639 (C=O), 1602, 1572, 1505, 1456 (aryl),

1254 (C–N), 1193 (C–N). ESI-MS: *m/z* 434.57 (M+1). $C_{25}H_{27}N_3O_2S$ (433.18).

4.1.4.31. 5-Ethyl-2-(4-chlorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c3). Recrystallized from EtOH/DMF as a brown crystal, yield: 39.2%, mp: 190–192 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.48 (s, 1H, NH), 7.84 (d, 2H, J = 8.4 Hz, PhH), 7.57 (d, 2H, J = 8.4 Hz, PhH), 6.31–7.01 (m, 4H, quinoline-H), 4.57 (s, 2H, SCH_2), 4.24 (s, 2H, NCH_2), 3.24–3.33 (m, 2H, quinoline-H_2), 2.67–2.71 (m, 2H, quinoline-H_4), 2.31 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.87–1.91 (m, 2H, quinoline-H_3), 1.06 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3566 (NH), 2962, 2930, 2868 (CH_3, CH_2), 1686 (C=O), 1640 (C=O), 1601, 1571, 1536, 1506 (aryl), 1254 (C–N), 1191 (C–N). ESI-MS: *m/z* 454.43 (M+1). $C_{24}H_{24}ClN_3O_2S$ (454.13).

4.1.4.32. 5-Ethyl-2-(4-nitrophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c4). Recrystallized from EtOH/DMF as a amber crystal, yield: 43.7%, mp: 191–192 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.78 (s, 1H, NH), 8.27 (d, 2H, J = 7.8 Hz, PhH), 8.04 (d, 2H, J = 7.8 Hz, PhH), 6.32–7.01 (m, 4H, quinoline-H), 4.64 (s, 2H, SCH_2), 4.23 (s, 2H, NCH_2), 3.32–3.34 (m, 2H, quinoline-H_2), 2.71–2.73 (m, 2H, quinoline-H_4), 2.43 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 1.89–1.90 (m, 2H, quinoline-H_3), 1.07 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3467 (NH), 2930, 2869 (CH_3, CH_2), 1693 (C=O), 1640 (C=O), 1601, 1571, 1536, 1506 (aryl), 1344 (C–N), 1189 (C–N). ESI-MS: m/z 465.55 (M+1). $C_{24}H_{24}N_4O_4S$ (464.15).

4.1.4.33. 5-Ethyl-2-(4-cyanophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c5). Recrystallized from EtOH/DMF as a brown crystal, yield: 49.6%, mp: 192–194 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.80 (s, 1H, NH), 7.60–7.96 (m, 4H, PhH), 6.36–6.80 (m, 4H, quinoline-H), 4.61 (s, 2H, SCH_2), 4.31 (s, 2H, NCH_2), 3.33–3.42 (m, 2H, quinoline-H_2), 2.43–2.51 (m, 2H, quinoline-H_4), 2.39 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.60–1.61 (m, 2H, quinoline-H_3), 1.02 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3343 (NH), 2937, 2869 (CH_3, CH_2), 2230 (C≡N), 1688 (C=O), 1640 (C=O), 1601, 1542, 1506 (aryl), 1251 (C–N), 1195 (C–N). ESI-MS: *m/z* 445.54 (M+1). $C_{25}H_{24}N_4O_2S$ (444.16).

4.1.4.34. 5-Ethyl-2-(4-methoxylphenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c6). Recrystallized from EtOH/DMF as a brown crystal, yield: 39.1%, mp: 196–198 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.75 (s, 1H, NH), 7.82 (d, 2H, J = 8.4 Hz, PhH), 7.02(d, 2H, J = 8.4 Hz, PhH), 6.33–6.83 (m, 4H, quinoline-H), 4.53 (s, 2H, SCH_2), 4.24 (s, 2H, NCH_2), 3.86 (s, 3H, $PhOCH_3$). 3.14–3.19 (m, 2H, quinoline-H_2), 2.45–2.50 (m, 2H, quinoline-H_4), 2.27–2.31 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.88–1.90 (m, 2H, quinoline-H_3), 1.05 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3476 (NH), 2932, 2839 (CH_3, CH_2), 1667 (C=O), 1642 (C=O), 1599, 1564, 1541, 1504 (aryl), 1264 (C–N), 1166 (C–N). ESI-MS: *m/z* 450.34 (M+1). $C_{25}H_{27}N_3O_3S$ (449.18).

4.1.4.35. 5-Ethyl-2-(4-fluorophenylcarbonylmethylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c7). Recrystallized from EtOH/DMF as a brown needle crystal, yield: 49.3%, mp: 192–193 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.84(s, 1H, NH), 7.90–7.92 (m, 2H, PhH), 7.32–7.35 (m, 2H, PhH), 6.32–6.82 (m, 4H, quinoline-H), 4.58 (s, 2H, SCH_2), 4.24 (s, 2H, NCH_2), 3.33–3.44 (m, 2H, quinoline-H_2), 2.39–2.50 (m, 2H, quinoline-H_4), 2.28–2.31 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.62–1.71 (m, 2H, quinoline-H_3), 1.05 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3466 (NH), 2933, 2840 (CH_3, CH_2), 1680 (C=O), 1638 (C=O), 1598, 1574, 1547, 1506 (aryl), 1253 (C–N), 1196 (C–N). ESI-MS: *m/z* 438.56 (M+1). $C_{24}H_{24}FN_3O_2S$ (437.16).

4.1.4.36. 5-Ethyl-2-benzylthio-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c8). Recrystallized from EtOH/DMF as a brown crystal, yield: 46.9%, mp: 224–226 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.62 (s, 1H, NH), 7.20 (d, 2H, J = 7.2 Hz, PhH), 7.18 (t, 1H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH),7.14 (t, 2H, J_1 = 7.2 Hz, J_2 = 14.4 Hz, PhH), 6.34–6.91 (m, 4H, quinoline-H), 4.42 (s, 2H, SCH_2), 4.14 (s, 2H, NCH_2), 3.42–3.44 (m, 2H, quinoline-H_2), 2.61–2.63 (m, 2H, quinoline-H_4), 2.46-2.51 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 1.84–1.86 (m, 2H, quinoline-H_3), 1.06 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3361 (NH), 2932, 2840 (CH_3, CH_2), 1635 (C=O), 1600, 1570, 1549, 1508 (aryl), 1252 (C–N), 1197 (C–N). ESI-MS: *m/z* 392.37 (M+1). $C_{23}H_{25}N_3OS$ (391.53).

4.1.4.37. 5-Ethyl-2-(4-methylbenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin -4(3*H*)-one (6c9). Recrystallized from EtOH/DMF as a brown crystal, yield: 47.2%, mp: 218–221 °C(dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.60 (s, 1H, NH), 7.00 (d, 2H, J = 8.4 Hz, PhH), 6.97 (d, 2H, J = 8.4 Hz, PhH), 6.43–6.90 (m, 4H, quinoline-H), 4.41 (s, 2H, SCH_2), 4.09 (s, 2H, NCH_2), 3.42–3.44 (m, 2H, quinoline-H_2), 2.61–2.64 (m, 2H, quinoline-H_4), 2.46–2.51 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 2.22 (s, 3H, PhCH3), 1.84–1.88 (m, 2H, quinoline-H_3), 1.07 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3422 (NH), 2930, 2838, 2796 (CH_3, CH_2), 1634 (C=O), 1600, 1569, 1546, 1507 (aryl), 1251 (C–N), 1197 (C–N). ESI-MS: *m/z* 406.59 (M+1). $C_{24}H_{27}N_3OS$ (405.56).

4.1.4.38. 5-Ethyl-2-(4-chlorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl) pyrimidin-4(3*H*)-one (6c10). Recrystallized from EtOH/DMF as a brown crystal, yield: 49.7%, mp: 227–229 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.65(s, 1H, NH), 7.15 (d, 2H, J = 8.4 Hz, PhH), 7.11 (d, 2H, J = 8.4 Hz, PhH), 6.42–6.91 (m, 4H, quinoline-H), 4.41 (s, 2H, SCH_2), 4.16 (s, 2H, NCH_2), 3.40–3.42 (m, 2H, quinoline-H_2), 2.60–2.62 (m, 2H, quinoline-H_4), 2.47–2.50 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 1.83–1.85 (m, 2H, quinoline-H_3), 1.06 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3326 (NH), 2932, 2840 (CH_3, CH_2), 1636 (C=O), 1600, 1570, 1546, 1507 (aryl), 1251 (C–N), 1197 (C–N). ESI-MS: *m/z* 426.74 (M+1). $C_{23}H_{24}ClN_3OS$ (425. 97).

4.1.4.39. 5-Ethyl-2-(4-nitrobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c11). Recrystallized from EtOH/DMF as a amber crystal, yield: 45.1%, mp: 195–197 °C(dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.71 (s, 1H, NH), 7.93 (d, 2H, J = 8.4 Hz, PhH), 7.34 (d, 2H, J = 8.4 Hz, PhH), 6.42–6.91 (m, 4H, quinoline-H), 4.42 (s, 2H, SCH_2), 4.31 (s, 2H, NCH_2), 3.37–3.39 (m, 2H, quinoline-H_2), 2.56–2.58 (m, 2H, quinoline-H_4), 2.50 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.80–1.82 (m, 2H, quinoline-H_3), 1.07 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3434 (NH), 2931, 2840 (CH_3, CH_2), 1641 (C=O), 1600, 1571, 1519 (aryl), 1250 (C–N), 1196 (C–N). ESI-MS: *m/z* 437.47 (M+1). $C_{23}H_{24}ClN_4O_3S$ (436. 53).

4.1.4.40. 5-Ethyl-2-(4-cyanobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c12). Recrystallized from EtOH/DMF as a brown crystal, yield: 45.2%, mp: 195–196 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.70 (s, 1H, NH), 7.54 (d, 2H, J = 8.4 Hz, PhH), 7.27 (d, 2H, J = 8.4 Hz, PhH), 6.42–6.91 (m, 4H, quinoline-H), 4.41 (s, 2H, SCH_2), 4.24(s, 2H, NCH_2), 3.36–3.38 (m, 2H, quinoline-H_2), 2.57–2.59 (m, 2H, quinoline-H_4), 2.50 (q, 2H, J = 7.8 Hz, C_5-CH_2CH_3), 1.80–1.83 (m, 2H, quinoline-H_3), 1.07 (t, 3H, J = 7.8 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3411 (NH), 2932, 2840 (CH_3, CH_2), 2227 (C≡N), 1640 (C=O), 1601, 1571,

1542, 1503 (aryl), 1251 (C–N), 1195 (C–N). ESI-MS: *m/z* 417.62 (M+1). $C_{24}H_{24}N_4OS$ (416.54).

4.1.4.41. 5-Ethyl-2-(4-methoxybenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c13). Recrystallized from EtOH/DMF as a brown crystal, yield: 48.7%, mp: 187–188 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.58 (s, 1H, NH), 7.04 (d, 2H, J = 8.4 Hz, PhH), 6.91 (d, 2H, J = 8.4 Hz, PhH), 6.43–6.84 (m, 4H, quinoline-H), 4.32 (s, 2H, SCH_2), 4.41 (s, 2H, NCH_2), 3.68 (s, 3H, PhOCH3), 3.45–3.47 (m, 2H, quinoline-H_2), 2.62–2.64 (m, 2H, quinoline-H_4), 2.50 (q, 2H, J = 8.4 Hz, C_5-CH_2CH_3), 1.86–1.88 (m, 2H, quinoline-H_3), 1.06 (t, 3H, J = 8.4 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3434 (NH), 2930, 2833, 2763 (CH_3, CH_2), 1637 (C=O), 1600, 1569, 1544, 1509 (aryl), 1251 (C–N), 1197 (C–N). ESI-MS: *m/z* 422.34 (M+1). $C_{24}H_{27}N_3O_2S$ (421.56).

4.1.4.42. 5-Ethyl-2-(4-fluorobenzylthio)-6-((3,4-dihydroquinolin-1(2*H*)-yl)methyl)pyrimidin-4(3*H*)-one (6c14). Recrystallized from EtOH/DMF as a brown needle crystal, yield: 59.7%, mp: 224–227 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ: 12.63 (s, 1H, NH), 7.12 (d, 2H, J = 8.4 Hz, PhH), 6.93 (d, 2H, J = 8.4 Hz, PhH), 6.43–6.90 (m, 4H, quinoline-H), 4.42 (s, 2H, SCH_2), 4.15 (s, 2H, NCH_2), 3.42–3.44 (m, 2H, quinoline-H_2), 2.60–2.63 (m, 2H, quinoline-H_4), 2.51 (q, 2H, J = 7.2 Hz, C_5-CH_2CH_3), 1.84–1.86 (m, 2H, quinoline-H_3), 1.07 (t, 3H, J = 7.2 Hz, C_5-CH_2CH_3). IR (KBr, cm^{-1}) ν 3359 (NH), 2932, 2841 (CH_3, CH_2), 1636 (C=O), 1600, 1570, 1547, 1508 (aryl), 1251 (C–N), 1221 (C–N). ESI-MS: *m/z* 410.29 (M+1). $C_{23}H_{24}FN_3OS$ (409.52).

4.3. Anti-HIV activity assays

Evaluation of the antiviral activity of the compounds against HIV-1 strain IIIB and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described.[19] Stock solutions (10× final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample. HIV-1(IIIB)[20] or HIV-2 (ROD)[21] stock (50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells[22] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue–purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (MultiscanAscent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of tree wells. The 50% cytotoxic concentration(CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

4.4. RT inhibition assay

Compounds were tested for antiviral activity against HIV-1 wtRT, using a poly(ra)/oligo(dT)$_{15}$ homopolymer template with HIV antigen detection ELISA for quantifying expression of HIV-1 RT in culture medium. Oligo(dT) was immobilized via its 50-terminal phosphate to Covalink-NH microtiter plates. The biotin-dUTP was incorporated by reverse transcriptase. The reaction mixture contained 50 mmol/L Tris-HCl (pH 8.3), 3 mmol/L $MgCl_2$, 75 mmol/L KCl, 5 mmol/L DTT ($_{DL}$-dithiothreitol), 0.13 mg/mL BSA, 10 μg/mL poly (A), 0.75 μM biotin-11-dUTP, and 1.5 μM dTTP. After incubation at 37 °C for 1 h, the plate was washed three times with a wash buffer containing 50 mmol/L Tris-HCl (pH 7.5), 0.15 mol/L NaCl, 0.05 mmol/L $MgCl_2$, and 0.02% Tween-20. After 100 μL of 1% BSA was added to each well and incubated for 30 min at room temperature, the plate was washed with the same buffer. Subsequently 50 μL of SA-ALP (Alkaline Phosphatase Streptavidin) solution (100 ng/mL) was added per well and then incubated for 1 h at 37 °C. The plate was washed as above and to which then was added 50 μL of PNPP (*p*-nitrophenyl phosphate, disodium) (1 mg/mL, pH 9.5), after 30 min at 37 °C, the reaction was stopped by addition of 0.5 M NaOH. The products were detected and quantified using a colorimetric streptavidin alkaline phosphatase reporter system.

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