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Synthesis and biological evaluation of novel 2-arylalkylthio-4-amino-6-benzyl pyrimidines as potent HIV-1 non-nucleoside reverse transcriptase inhibitors

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ABSTRACT

Novel 2-aryalkylthio-4-amino-6-benzylpyrimidines (**3a–i**), which can be considered as S-DABO and TMC-125 analogue hybrid molecules, have been designed and synthesized as inhibitors of HIV-1 RT. The results clearly indicated that the changes at the N_3/C_4 position of pyrimidine ring could affect the hydrogen bonds strength and number between N_3/C_4 and the Lys101 residue which are indispensable for anti-HIV-1 RT activity. The biological activity results are also in accordance with the docking study.

Since the discovery of human immunodeficiency virus (HIV) caused the acquired immunodeficiency syndrome (AIDS),[1] several potential targets for antiviral chemotherapy such as reverse transcriptase (RT), protease enzymes and the fusion process have been found out during the last decade. Among them the non-nucleoside reverse transcriptase inhibitors (NNRTIs) serve as a representative of most frontline HIV combination therapies which are highly effective drugs with less side effects, such as nevirapine and efavirenz.[2–4] But NNRTIs are short for rapidly triggering the emergence of drug resistant HIV-1 variants and the cross-resistance between these structurally unrelated drugs.[5,6] Therefore, one urgent solution is to discover and develop new highly active agents with improved resistance profiles simultaneous.

Following the dihydro-alkoxy-benzyl-oxopyrimidines (DABOs) were reported in 1992 as one of the most representative NNRTIs in the past decade, a number of DABOs were synthesized and tested as anti-HIV-1 agents with the aim to obtain more potent and selective drugs, especially the S-DABOs reported to date (ID_{50} = 26 nM toward the isolated wt enzyme) with subnanomolar activity toward both the wt and the pluriresistant virus (IRLL98) HIV-1 strain (EC_{50} < 0.14 nM and EC_{50} = 0.22 nM, respectively) (Fig. 1).[7–10,16] Studies on crystal structures of S-DABOs and other NNRTIs such as TNK-651 and TMC-125 suggested that these NNRTIs share a common mode of action with the HIV-1 reverse transcriptase. Substituting with aryl moiety at C-2 and C-6 alkyl chains of the pyrimidine ring are structural determinant. The extended side chain at C-2 position point toward into a pocket mainly defined by His235, Pro236 and Val106, and the chain at C-6 position is located in a hydrophobic region delimited by Tyr181, Tyr188 and Trp229. Moreover, the NH–C=O moiety at N_3/C_4 position of pyrim-

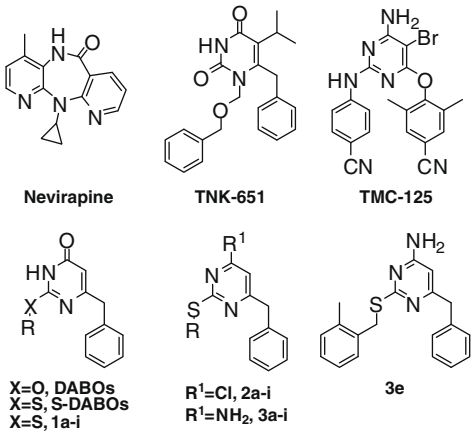

Figure 1. Structures of nevirapine, TNK-651, TMC-125, DABO and designed target compounds.

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idine ring is a crucial factor for the activity because the hydrogen bonds are formed by N_3–H with the carbonyl group of Lys101.[11–18]

Although the structure–activity relationships (SAR) of DABOs have been studied for many years, no or little studies on the effect of varying the hydrogen bond strength and number have been reported. An inspiration prompts us to investigate the importance of hydrogen bond strength and number between the HIV-1 RT and the N_3/C_4 position of the pyrimidine ring. So we designed a series of novel 2-aryalkylthio-4-amino-6-benzyl-pyrimidines **3** which can also be considered as S-DABO and TMC-125 analogue hybrid molecules with two possible orientations in RT as new potential NNRTIs. The N_3H–C_4=O group of pyrimidine ring was changed into N_3=C_4–NH_2 group while the basic structures of the alkyl group chains with an aryl or substituted aryl moiety at the C-2 and C-6 position were kept to verify whether the changes of hydrogen bond strength and number at the C-4 position of pyrimidine ring will effect the activities.

To verify our design, the compound of **3e**[26] was flexibly docked into the binding site of HIV-1 RT (PDB entry 1RT2, complexed with TNK-651) using AUTODOCK 3.05 program.[19] Default parameters were used as described in the AUTODOCK manual unless otherwise specified. The molecule was docked with 100 genetic algorithm runs of up to 250,000 energy evaluations for each run in the docking study of **3e** with a RT non-nucleoside binding site (NNBS). It was predicted that the binding mode of **3e** has approximate conformation as in the TNK-651-RT complex (Fig. 2). The result showed that two chains at C-2 and C-6 of **3e** are located in the HIV-1 RT NNBS as we discussed above. A hydrogen bond is formed between the N_3–H moiety of the TNK-651 and the carbonyl group of Lys101, but there are two hydrogen bonds are formed between the N_3/C_4 positions of **3e** and the carbonyl group of Lys101(Fig. 2) as the tautomerization between N_3H–C_4=NH and N_3=C_4–NH_2. In addition, the hydrogen bonds between **3e** and Lys101 could result in closer interactions (distance C_4–N···O 2.63 Å) than TNK-651 (distance N_3···O 2.76 Å) complex. Based on these results, we hypothesized that the introduction of NH_2 at C-4 position would increase the affinity in the ligand/enzyme interaction and endow the compounds with higher activities against the HIV-1-RT in a broader range. To investigate the role of hydrogen bonds and compare the structure–activity relationship, a Cl substitution was introduced at the C-4 position of pyrimidine ring which can not form a hydrogen bond with Lys101. So we synthesized three series of compounds **1a–i**, **2a–i** and **3a–i** by an efficient route and tested the activity against HIV-1 RT.

The synthesis of the newly designed compounds is described in Scheme 1. The β-ketoesters **5** was prepared by reaction between benzyl cyanide with zinc and ethyl 2-bromoacetate **4** using the method of Hannick and Kishi.[20] Condensation of **5** and thiourea in the presence of sodium ethoxide in boiling ethanol gave the 6-benzyl thiouracil **6**,[21] which was subjected to S-alkylation with appropriate substituted alkyl halide in MeONa to afford the S-DABO analogues **1a–i**.[22] By chlorination of **1a–i** with an excess of $POCl_3$ obtained the products **2a–i**.[23] The replacement of chlorine with amine in dioxane by heated at about 80–90 °C in a sealed tube, gave the desired compounds **3a–i**.[24] The yields of all compounds are showed in Table 1. Both analytical and spectral data of all the target compounds are accordant with the proposed structures.

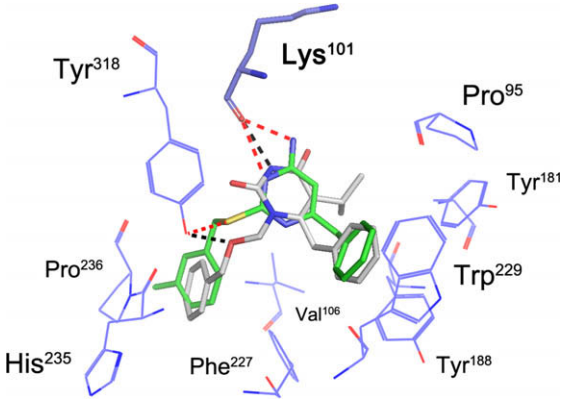
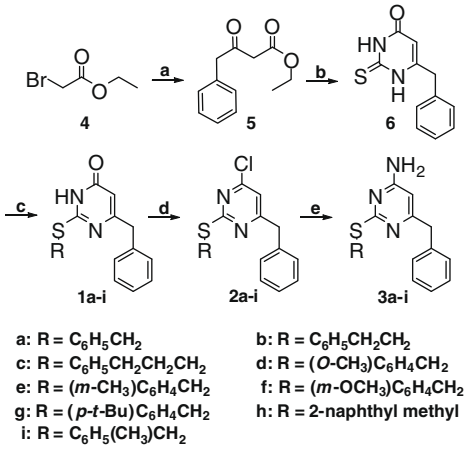


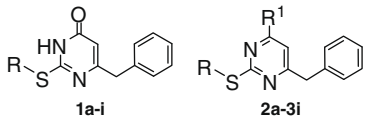
Figure 2. Binding mode of **3e** (green) and TNK-651 (white) with HIV-RT. Hydrogen bonds are shown as red (**3e**) and black (TNK-651) dashed lines.



Scheme 1. Reagents and conditions for the chemical synthesis: (a) benzyl cyanide, Zn/THF, reflux, 95%; (b) thiourea, EtONa, EtOH, reflux, 86%; (c) substituted alkyl halide, Na/MeOH, rt, 65–88%; (d) $POCl_3$, rt, 79–98%; (e) $NH_3{\cdot}H_2O$, dixone, 80–90 °C, 12–24 h, 58–95%.

The compounds **1a–3i** were tested for antiviral activity against HIV-1 RT, using a poly(ra)/oligo(dT)$_{15}$ homopolymer template with HIV antigen detection ELISA for quantifying expression of HIV-1 RT

Table 1
The yields of all target compounds (**1a–3i**)



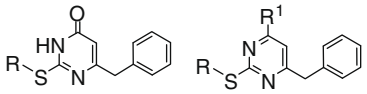
Compds	R	R^1	Yields (%)
1a	$C_6H_5CH_2$	—	80
1b	$C_6H_5CH_2$ CH_2	—	77
1c	$C_6H_5CH_2$ CH_2 CH_2	—	88
1d	$(O\text{-}CH_3)C_6H_4CH_2$	—	88
1e	$(m\text{-}CH_3)C_6H_4CH_2$	—	59
1f	$(m\text{-}OCH_3)C_6H_4CH_2$	—	78
1g	$(p\text{-}t\text{-}Bu)C_6H_4CH_2$	—	79
1h	2-Naphthyl methyl	—	65
1i	$C_6H_5(CH_3)CH$	—	65
2a	$C_6H_5CH_2$	Cl	98
2b	$C_6H_5CH_2$ CH_2	Cl	95
2c	$C_6H_5CH_2$ CH_2 CH_2	Cl	79
2d	$(O\text{-}CH_3)C_6H_4CH_2$	Cl	93
2e	$(m\text{-}CH_3)C_6H_4CH_2$	Cl	94
2f	$(m\text{-}OCH_3)C_6H_4CH_2$	Cl	95
2g	$(p\text{-}t\text{-}Bu)C_6H_4CH_2$	Cl	91
2h	2-naphthyl methyl	Cl	86
2i	$C_6H_5(CH_3)CH$	Cl	95
3a	$C_6H_5CH_2$	NH_2	85
3b	$C_6H_5CH_2$ CH_2	NH_2	83
3c	$C_6H_5CH_2$ CH_2 CH_2	NH_2	79
3d	$(O\text{-}CH_3)C_6H_4CH_2$	NH_2	94
3e	$(m\text{-}CH_3)C_6H_4CH_2$	NH_2	95
3f	$(m\text{-}OCH_3)C_6H_4CH_2$	NH_2	95
3g	$(p\text{-}t\text{-}Bu)C_6H_4CH_2$	NH_2	68
3h	2-Naphthyl methyl	NH_2	70
3i	$C_6H_5(CH_3)CH$	NH_2	58

in culture medium, and nevirapine as a reference compound (Table 2).[25] It is interesting to note that three series of compounds reflect obvious structure–activity relations.

If the C-4 position is substituted by OH or NH_2 group, hydrogen bonds between the compounds and the HIV-1 RT could be formed and obvious activities could be showed. On the contrary, if the C-4 position is substituted by Cl atom, no hydrogen bond and no activity could be observed. So the ability of forming hydrogen bond at the N_3/C_4 position is critical to the potent activity. More compounds in series 3 such as **3d**, **3e**, **3h** and **3i** show higher activity of IC_{50} values than **1d**, **1e**, **1h** and **1i** in series 1, respectively. A preliminary finding could be obtained from the data that the C_4–NH_2 could increase the hydrogen bonds strength and number with Lys101 and increased the activity to some extent. We also noted that some compounds in series 1 such as **1b** and **1c** have obvious activities but the relevant **3b** and **3c** have not any activity. The explanation could be that the other function groups(such as C-2 chains) of compounds **3b** and **3c** take place the conformation change which is different from **1b** and **1c** when the hydrogen bonds between N3/C4 and HIV-RT is stronger and closer. Besides, when we pay attention to the C-2 chain we found that if the size of substituted groups at the benzene is small (H) or big (*t*-Bu) are not beneficial for the activity. More detailed SAR studies on these compounds are under way with a focus on exploring the important role of substituted groups on the aromatic ring at the arylalkylthio substituent of C-2 and C-6 position.

In summary, we have designed three series of novel 2-aryalkylthio-4-amino-6-benzylpyrimidines as inhibitors of HIV-1 RT and described the convenient and efficient method of synthesis for the first time. Although all of the IC_{50} values of the tested compounds were suboptimal comparing to the nevirapine, the SAR exploration encouraged us to the new rational design and the further structure modifications on the N_3/C_4 position and two chains of the pyrimidine ring are underway.

Table 2
Structure and enzymatic activities[a] (IC_{50}) of **1a–3i**



1a-i **2a-3i**

Compds	R	R^1	IC_{50}[b](μM)
1a	$C_6H_5CH_2$	—	NA[c]
2a	$C_6H_5CH_2$	Cl	>100
3a	$C_6H_5CH_2$	NH_2	>100
1b	$C_6H_5CH_2$ CH_2	—	17.45
2b	$C_6H_5CH_2$ CH_2	Cl	NA
3b	$C_6H_5CH_2$ CH_2	NH_2	NA
1c	$C_6H_5CH_2$ CH_2 CH_2	—	8.17
2c	$C_6H_5CH_2$ CH_2 CH_2	Cl	NA
3c	$C_6H_5CH_2$ CH_2 CH_2	NH_2	NA
1d	(*O*-CH_3)$C_6H_4CH_2$	—	47.72
2d	(*O*-CH_3)$C_6H_4CH_2$	Cl	NA
3d	(*O*-CH_3)$C_6H_4CH_2$	NH_2	29.89
1e	(*m*-CH_3)$C_6H_4CH_2$	—	91.14
2e	(*m*-CH_3)$C_6H_4CH_2$	Cl	90.77
3e	(*m*-CH_3)$C_6H_4CH_2$	NH_2	18.03
1f	(*m*-OCH_3)$C_6H_4CH_2$	—	90.85
2f	(*m*-OCH_3)$C_6H_4CH_2$	Cl	NA
3f	(*m*-OCH_3)$C_6H_4CH_2$	NH_2	>100
1g	(*p*-*t*-Bu)$C_6H_4CH_2$	—	NA
2g	(*p*-*t*-Bu)$C_6H_4CH_2$	Cl	NA
3g	(*p*-*t*-Bu)$C_6H_4CH_2$	NH_2	NA
1h	2-Naphthyl methyl	—	NA
2h	2-Naphthyl methyl	Cl	NA
3h	2-Naphthyl methyl	NH_2	17.14
1i	$C_6H_5(CH_3)CH$	—	>100
2i	$C_6H_5(CH_3)CH$	Cl	NA
3i	$C_6H_5(CH_3)CH$	NH_2	63.90

[a] Nevirapine was used as a reference compound here; IC_{50} for nevirapine was 4.12 μM.

[b] Compound dose (μM) required to inhibit the HIV-1 RT activity by 50%; Data represent mean values for three separate experiments, variation among triplicate samples was less than 15%.

[c] No inhibition of reverse transcriptase activity was observed up to a concentration of 100 μM.

Acknowledgments

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26. *Spectral data for compound (**3e**) 2-(3-methylbenzylthio)-6-benzylpyrimidin-4-amine*: White solid; mp 126–128 °C; ^{1}H NMR (500 MHz, CDCl3) δ (ppm): 2.34 (s, 3H, CH_3), 3.92 (s, 2H, $C_6H_5CH_2$), 4.38 (s, 2H, CH_2S), 4.77 (s, 2H, C_4–NH_2), 5.85 (s, 1H, C_5–H), 7.06-7.35 (m, 9H, Ar–H); ^{13}C NMR (125 MHz, CDCl3) δ (ppm): 21.37 (CH_3), 35.00 (CH_2S), 43.78 ($C_6H_5CH_2$), 99.38 (C-5), 126.13, 126.68, 127.71, 128.24, 128.59, 129.44, 129.80, 137.83, 137.96, 138.21 (C-arom), 162.89 (C-4), 168.58 (C-6), 170.63 (C-2); HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{19}H_{20}N_3S$: 322.1378. Found: 322.1373.