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Communication

Fragment-based discovery of sulfur-containing diarylbenzopyrimidines as novel nonnucleoside reverse transcriptase inhibitors

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ABSTRACT

Two series of sulfur-containing diarylbenzopyrimidines are designed by the fragment combination of a thioacetamide with our previous disclosed DABP **3** and further oxidation. The best compound **6e** with a sulfonyl scaffold displayed EC_{50} values of 0.0356 μmol/L against WT and 0.0228 μmol/L against HIV K103N mutant strain. More pronounced, it had a lower cytotoxicity (CC_{50} = 99.6 μmol/L), higher selectivity index (SI_{WT} = 2799, SI_{K103N} = 4375) and better calculated logarithm of the octanol-water partition coefficient (cLog*P*) than the lead compound **3**. Molecular docking and dynamics provided the binding modes of these compounds with reverse transcriptase, explaining their activity. Collectively, the new compounds could be candidates for anti-HIV drug discovery.

Acquired immune deficiency syndrome (AIDS) that has not been eradicated is continued to present a devastating globally health problem. Etiologically, human immunodeficiency virus (HIV) is a main causal agent of AIDS [1]. According to the 2018-WHO report, 37.9 million people were infected with HIV worldwide, 1.7 million people were newly infected, and 0.77 million people died. However, only about 62% of people (23.3 million) living with HIV could receive antiretroviral therapy [2].

Nonnucleoside reverse transcriptase inhibitors (NNRTIs) are important components for the HIV highly active antiretroviral therapy (HAART), leading to remarkable reduction in AIDS-related mortality [3–6]. To date, there are six NNRTIs (Nevirapine, NVP; delavirdine, DLV; efavirenz, EFV; etravirine, ETR; rilpivirine, RPV; and doravirine, DOR) approved by FDA for HIV treatment [7]. However, not all the patients could have response to the therapy and the effectiveness of NNRTIs has been significantly reduced due to the low barrier to genetic resistance [8–10]. For instance, the K103N was one of the most prevalent NNRTI resistance-associated mutations (RAMs) for antiretroviral treatments [11–14]. The early generation NNRTIs (*e.g.*, NVP, DLV and EFV) were reported to be ineffective against the K103N mutant [15–18]. The second generation NNRTIs, FDA-approved ETR and RPV with the diarylpyrimidine (DAPY) scaffold, showed better antiviral activity, especially against HIV mutants, potentially due to a flexible moiety of the DAPYs. However, they presented the high cytotoxicity (CC_{50} < 5 μmol/L) and many serious side effects have been observed, such as skin rash, liver-related, and neuropsychiatric disorders [5,19,20]. Moreover, the absolute oral bioavailability of ETR in human is still unknown due to its bad solubility and RPV is hardly dissolved in water (20 ng/mL at pH 7.0) [4]. The newly approved drug DOR may affect up to 1 in 10 people nausea and headache [21]. More possible side effects of DOR still need further clinical investigations. Thus, exploring new drugs with high potency and safety profile is still in an urgent demand [22–24].

On the basis of the chemical structure of DAPYs, they can be divided into three fragment parts (Fig. 1). They are two diaryl "wings" (Fragment A and Fragment B) to control the flexibility of the compounds and a core pyrimidine (Fragment C). In our previous work, we developed a series of dichloridated

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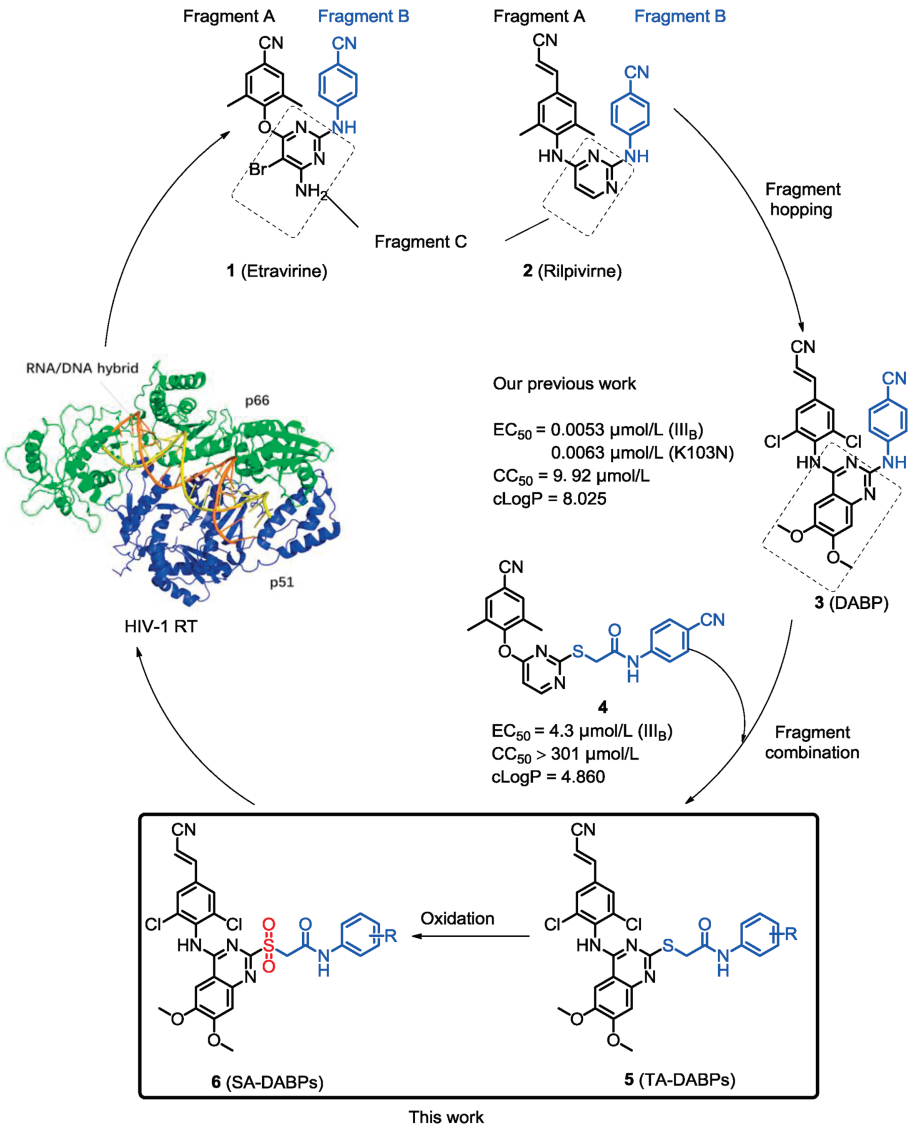


Fig. 1. Design of thioacetamide (TA)- and sulfonylacetamide (SA)-DABPs by the FBDD strategy.

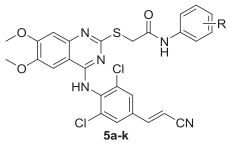
diarylbenzopyrimidines (DABPs) focusing on the core Fragment C and evaluating the SAR of the Fragment A [25]. The DABP **3** displayed promising EC_{50} values of 0.0053 μmol/L against HIV-1 wide type (WT) and 0.0063 μmol/L against the K103N mutant strain. However, the cytotoxicity (CC_{50} = 9.92 μmol/L) and selectivity index were not satisfactory (SI_{WT} = 1889, SI_{K103N} = 1579). Moreover, the cLog*P* of **3** was too high (> 8, Table 1) indicating a non-drug-like property [26].

The impact of sulfur-containing was instrumental to the drug development [27]. Thioacetamide and sulfonyl fragments are widely used in several HIV NNRTIs, such as VRX-480773, RDEA806 and delavirdine. This strategy has been tried in our previous compound **4** [28,29], showing promising activity and especially lower cLog*P* value (4.860). In this study, we hypothesized that introducing the thioacetamide fragment of compound **4** to the lead compound **3** and further oxidation on the sulfur might be favorable to the drug-like property and the antiviral activity.

The general synthetic route of compounds **5a**–**k** and **6a**–**e** is shown in Scheme 1. Detailed procedures and compound characterizations can be found in Supporting information. Briefly, the starting compound **7** was synthesized according to our previous work [25,29]. Compound **7** was reacted with the various substituted α-bromoacetamides in a base condition to give TA-DABPs **5a**–**k**. Then, oxidation on the sulfur by m-CPBA gave corresponding SA-DABPs **6a**–**e**.

At first, we synthesized a series of thioacetamide based on 4-aminobenzonitrile substituted DABP. They decreased the cLog*P* compared to that of **3**, although the antiviral activity was dramatically reduced in more than 200-fold. Among compounds **5a**–**c** with cyano group, compound **5b** with 3-CN showed the best antiviral activity (EC_{50} = 1.36 μmol/L) compared to compound **5c** with 2-CN and compound **5a** with 4-CN. Then, we synthesized a series of TA-DABPs **5d**–**k** in the next round, with substitutions at *meta*- and *ortho*-positions. With electron-donating group methoxy group introduced, the cLog*P* values of **5d** and **5e** were decreased to 6.9 – 7.5. However, the antiviral activity was still unsatisfactory. Compounds **5f**–**k** with electron withdrawing group were synthesized. They were inactive toward viral strains. Compounds **5f** and **5 g** exhibited relatively high cytotoxicity. Molecular docking study revealed that the distance between the amide of TA-DABP **5b** and

Table 1
Activity of **5a**–**l** against HIV-1 wide type (III_B strain), K103N mutant strain and cytotoxicity in MT-4 cells.[a]



5a-k

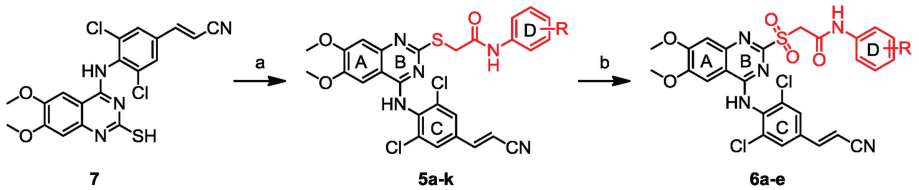
Compd.	R	EC_{50}[b] μmol/L (SI)[c]		CC_{50}[d] (μmol/L)	cLog*P*[e]
		III_B	K103N		
3		0.0053 ± 0.003 (1889)	0.0063 ± 0.0004 (1579)	9.92 ± 0.41	8.025
5a	4-CN	>2.01 (<1)	ND[f] (ND)	2.01 ± 0.61	7.487
5b	3-CN	1.36 ± 0.49 (≥96)	0.357 ± 0.018 (≥290)	>103	7.487
5c	2-CN	>211 (X1)	ND (ND)	>211	6.787
5d	3-OCH_3	>210 (X1)	ND (ND)	>210	7.531
5e	2-OCH_3	>210 (X1[g])	ND (ND)	>210	6.941
5f	3-F	>3.17 (<1)	ND (ND)	3.17 ± 0.93	7.856
5 g	2-F	>3.49 (<1)	ND (ND)	3.49 ± 1.07	7.256
5h	3-CF_3	>197 (X1)	ND (ND)	>197	8.789
5i	2-CF_3	>197 (X1)	ND (ND)	>197	7.339
5j	3-NO_2	>204 (X1)	ND (ND)	>204	7.751
5k	2-NO_2	>204 (X1)	ND (ND)	>204	7.291
NVP		0.235 ± 0.060 (>64)	≥11.2 (X1)	>15.0	–
EFV		0.0038 ± 0.0009 (>1667)	0.124 ± 0.023 (>51)	>6.34	–
ETR		0.0042 ± 0.0007 (>1099)	0.0037 ± 0.0006 (>1250)	>4.59	–
RPV[h]		0.0013 ± 0.0004 (3490)	–	4.38 ± 1.42	–

[a] All data represent the mean values for at least three independent experiments.
[b] EC_{50}: The effective concentration required to protect MT-4 cells against virus-induced cytopathicity by 50%.
[c] SI: selectivity index, ratio of CC_{50}/EC_{50}.
[d] CC_{50}: The cytotoxic concentration of the compound that reduces the normal uninfected MT-4 cell viability by 50%.
[e] cLog*P*: predicted by using software ChemBioDraw Ultra 12.0.
[f] ND: not determined.
[g] X1: ≥1 or <1.
[h] The data were obtained from the same laboratory (Prof. Erik De Clercq, Rega Institute for Medical Research, KU Leuven, Belgium) with the same method [4].

the carbonyl group of K103 of the reverse transcriptase (RT) was 4.4 Å, indicating a weak interaction instead of a hydrogen-bonding interaction (Fig. 2), potentially interpreting the weak antiviral activity.

In the next round, the most potent thio-compound **5b** was selected to be oxidized to SA-DABP **6a**. To our delight, compound **6a** (Table 2) showed better activity against the K103N mutant virus than NVP (EC_{50} ≥ 11.2 μmol/L) and EFV (EC_{50} = 0.124 μmol/L). Moreover, the cytotoxicity was decreased (CC_{50} = 29.8 μmol/L). In addition, compound **6a** displayed an improved lipid-water partition coefficient and the cLog*P* was decreased to 6.6. Encouraged by the results, we synthesized and evaluated a series of SA-DABPs (**6b**–**e**) with 3-substitutions except 3-fluoro (**5f**) with a high cytotoxicity. Compound **6b** with 3-OCH_3 (EC_{50} = 5.12 μmol/L) showed decreased antiviral activity than **6a**, but still exhibited significantly improved antiviral activity than its corresponding TA-DABP **5d** (EC_{50} > 210 μmol/L). Compound **6c** with 3-CF_3 (EC_{50} = 1.08 μmol/L) and compound **6d** (EC_{50} = 0.208 μmol/L) with 3-NO_2 exhibited better activity against the K103N mutant virus than an early generation NNRTI NVP (EC_{50} ≥11.2 μmol/L). Finally, we tried to change the nitro group from meta-position to para-position to obtain compound **6e**. Surprisingly, the activity of compound **6e** was significantly improved while the cytotoxicity was remarkable decreased. Compound **6e** (EC_{50} = 0.0228 μmol/L) showed ~490-fold activity than NVP (EC_{50} ≥ 11.2 μmol/L), ~5-fold than EFV (EC_{50} = 0.124 μmol/L) against the K103N mutant virus, but 10-fold lower than ETR (EC_{50} = 0.0037 μmol/L). Consistent with antiviral activity, compound **6e** showed an IC_{50} of 0.98 μmol/L against the RT enzyme (Table 3), which was lower than those of **3** (IC_{50} = 0.046 μmol/L) and the FDA drugs. However, the CC_{50} value of **6e** (99.6 μmol/L) was much better than them (NVP 15.0 μmol/L, EFV 6.34 μmol/L, ETR 4.59 μmol/L and RPV 4.38 μmol/L), indicating a remarkable decreased cytotoxicity. Moreover, the cLog*P* of **6e** was decreased from 8.025 to 6.900 compared to the lead compound DABP **3**. It suggested that **6e** was more hydrophilic than **3** and a better solubility. Experimentally, the water solubility of **6e** was 300 ng/mL at pH 7, which was higher than RPV (20 ng/mL [4]) and **3** (undetectable at 20 ng/mL).

To better understand the activity, we predicted the binding modes of the compounds with RT by molecular docking and dynamics (Supporting information). Comparing to **5b**, the ligand torsions of **6a** were changed due to the steric hindrance of the newly formed sulfonyl group, making the amide to form a new hydrogen bond with H235 (Fig. 3A). The carbonyl group of **6a** was also pointed to the amide of K103 was 3.8 Å. The activity at the enzyme level also supported our prediction. Compound **6a** had ~4-fold improvement in the anti-RT activity than **5b** (Table 3). In Fig. 3B, the hydrogen of the amide and the nitro group of **6d** could form two hydrogen bonds with K103 and V106, respectively. In Fig. 3C, two hydrogen bonds (between the amide and K103, and the amide and H235) anchored the position of compound **6e** and made the binding more stable than **6d**. Thus, compound **6e** exhibited the 7-fold higher potency (IC_{50} = 0.98 μmol/L) against HIV-1 RT than **6d** (IC_{50} = 7.01 μmol/L). In the K103N RT, besides the two hydrogen bonds similar as WT RT, the distance between the carbonyl group of **6e** and the mutant amide group was 3.5 Å, indicating potential additional interactions (Fig. 3D).



Scheme 1. Synthesis route of compounds **5a**–**k** and **6a**–**e**. Reagents and conditions: (a) substituted α-bromoacetamide, *t*-BuOK, DMF, r.t., 1 h; (b) m-CPBA, CH_2Cl_2/DMF, −78 °C – r.t.

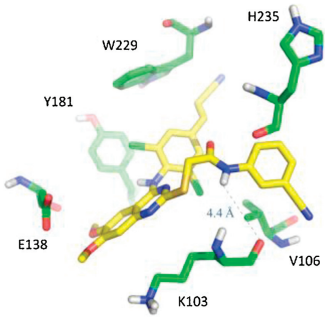
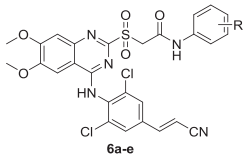


Fig. 2. Predicted binding modes of compound **5b** (carbons in yellow) with the HIV-1 RT (PDB: 6C0N). Residues involved in interactions are shown as green sticks.

Table 2
Activity of **6a**–**f** against HIV-1 III_B, K103N strains and cytotoxicity in MT-4 cells.[a]



Compd.	R	EC_{50} μmol/L (SI)		CC_{50} (μmol/L)	cLog*P*
		III_B	K103N		
3		0.0053 ± 0.003 (1889)	0.0063 ± 0.0004 (1579)	9.92 ± 0.41	8.025
6a	3-CN	0.122 ± 0.040 (244)	0.080 ± 0.037 (373)	29.8 ± 7.9	6.636
6b	3-OCH_3	5.12 ± 1.38 (18)	2.38 ± 0.10 (39)	93.2 ± 47.5	6.681
6c	3-CF_3	7.27 ± 83.9 (1)	1.08 ± 0.08 (8)	8.18 ± 3.51	7.939
6d	3-NO_2	0.286 ± 0.054 (135)	0.208 ± 0.046 (186)	38.6 ± 9.1	6.900
6e	4-NO_2	0.0356 ± 0.0069 (2799)	0.0228 ± 0.0005 (4375)	99.6 ± 32.6	6.900
NVP		0.235 ± 0.060 (>64)	≥11.2 (X1)	>15.0	–
EFV		0.0038 ± 0.0009 (>1667)	0.124 ± 0.023 (>51)	>6.34	–
ETR		0.0042 ± 0.0007 (>1099)	0.0037 ± 0.0006 (>1250)	>4.59	–
RPV		0.0013 ± 0.0004 (3490)	–	4.38 ± 1.42	–

Table 3
The activity of selected compounds against WT HIV-1 RT enzyme.[a]

Compd.	**3**	**5b**	**6a**	**6d**	**6e**	NVP	EFV	ETR
IC_{50} (WT RT, μmol/L)	0.046	23.7	6.74	7.01	0.98	0.75	0.006	0.011

[a] IC_{50}: inhibitory concentration of test compound required to inhibit WT HIV-1 RT polymerase activity by 50%.

In conclusion, we synthesized and evaluated two series of sulfur-containing DABPs (TA-DABPs and SA-DABPs) based on the FBDD strategy. The most potent compound **6e** displayed better antiviral activity against the HIV-1 WT and K103N mutant virus than the early generation NNRTIs, and comparable activity to the second generation NNRTIs. The cytotoxicity and cLog*P* was significantly decreased. Molecular modelling further explained the activity and gave more guidance for further optimizations. Thus, the new sulfur-containing DABPs represent new lead compounds for anti-HIV agents, especially the HIV-1 K103N mutants.

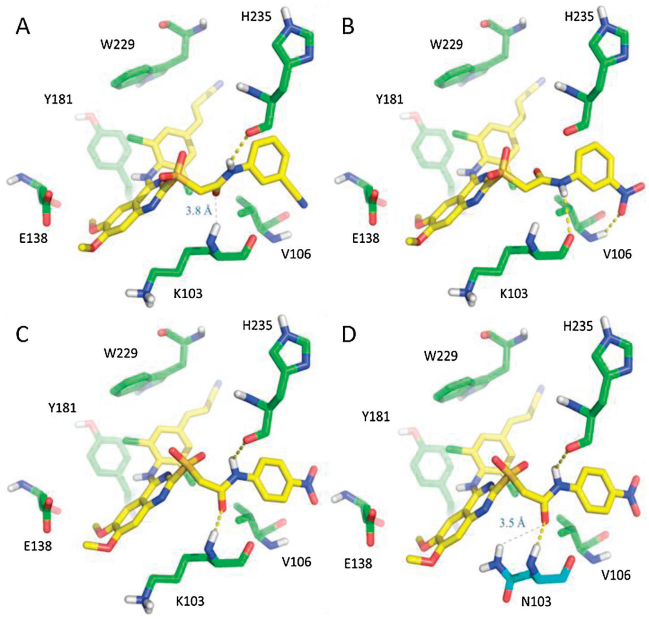


Fig. 3. Predicted binding modes of compounds (carbons in yellow) with the HIV-1 WT and K103N mutant RT (PDB: 6C0N). (A) RT with **6a**; (B) RT with **6d**; (C) RT with **6e**; (D) K103N mutant RT with **6e**. Residues involved in interactions are shown as green sticks. Mutated residues are depicted as cyan sticks. The distances are depicted as black dashed lines.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.11.020.

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