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Research Article

Design, Synthesis, and Biological Evaluation of Novel 4-Aminopiperidinyl-linked 3,5-Disubstituted-1,2,6-thiadiazine-1,1-dione Derivatives as HIV-1 NNRTIs

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Based on the hybridization of the privileged fragments in DABO and DAPY-typed HIV-1 NNRTIs, a novel series of 4-aminopiperidinyl-linked 3,5-disubstituted-1,2,6-thiadiazine-1,1-dione derivatives were designed, synthesized, and evaluated for their *in vitro* anti-HIV activities in MT-4 cells. Most of the target compounds showed weak inhibitory activity against WT HIV-1. In order to confirm the mode of action of the target compounds, representative compounds Ba8 and Bb8 were selected to perform the HIV-1 RT inhibitory assay. In this assay, Ba8 and Bb8 displayed good activity with IC_{50} values of 3.15 and 1.52 μM, respectively. Additionally, preliminary structure–activity relationships (SARs) analysis and molecular docking studies of newly synthesized compounds are also discussed.

Key words: 1,2,6-thiadiazine-1,1-dione, bioactivity, drug design, HIV-1, NNRTIs, RT, synthesis

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As significant and indispensable components of highly active antiretroviral therapy (HAART), HIV-1 non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) possess their unique merits such as high potency, high selectivity, and relatively low toxicity. Unfortunately, the rapid emergence of drug resistance, caused by mutation of the amino acid residues in the binding pocket of HIV-1 RT severely impairs the clinical application of HIV-1 NNRTIs (1). Besides, the clinical advancement of many promising NNRTIs was hindered by poor aqueous solubility, low bioavailability and safety and tolerability issues. In consequence, there is an urgent need to develop novel NNRTIs with high potency, improved resistance profiles, safety, excellent tolerability, and favorable physicochemical properties.

Among the existing HIV-1 NNRTIs, 2-alkoxy-6-benzyl-3,4-dihydro-4-oxopyrimidines (DABOs) and diarylprimidines (DAPYs) analogues represent two highly potent and promising classes (Figure S1). In the DABOs family, F_2-*S*-DABOs (exemplified by **MC1047**) (2,3) and F_2-*N,N*-DABOs (exemplified by **MC1220**) (4) with high potency against both wild-type (WT) and mutant strains have attracted special attention over the past few years. On the other hand, as two DAPY analogues **TMC125** (Etravirine, ETR) and **TMC278** (Rilpivirine, RPV) have been approved by FDA in 2008 and 2011, respectively (5), considerable efforts have been devoted to the structural modification and optimization of DAPYs.

As a part of our continuing efforts directed towards the development of potential DABO and DAPY derivatives (6), herein we designed a novel series of 4-aminopiperidinyl-linked 3,5-disubstituted-1,2,6-thiadiazine-1,1-dione derivatives as DABO–DAPY hybrid based on their structural features, binding mode, and structure–activity relationships (SARs) studies (Figure S2) (7–10).

Concretely, the rational design of 4-aminopiperidinyl-linked 3,5-disubstituted-1,2,6-thiadiazine-1,1-dione (Figure S2) was based on the following considerations:

Nucleus (Domain I): According to the bioisosterism principle of rational drug design, 4-oxopyrimidine in DABOs was replaced by the synthetically accessible 1,2,6-thiadiazine-1, 1-dione heterocyclic motif with an attempt to form additional hydrogen bonds between the ligand and the surrounding amino acids in RT. It should be noted that, previous investigations in our laboratory using structure-based isosterism drug design resulted in the discovery of several novel series of thiadiazine-derived NNRTIs, which proved to be effective in inhibiting HIV-1 replication at micromolar concentration acting as RT inhibitors (6,11–

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13). Thus, this privileged structure should be further exploited for the therapeutic benefits (14).

Substituents at 3-position (Domain II): The privileged substituents of DABO family derivatives, such as 1-naphthylmethyl or 2,4-dichlorobenzyl, were kept to remain the effect of π–π interaction with hydrophobic amino acids Tyr181, Tyr188, and Trp229.

Substituents at 5-position (Domain III): Based on the concept of molecular hybridization, Rotili and his partners have identified novel diarylpyrimidine-dihydrobenzyloxopyrimidine hybrids, which showed characteristic SAR profile and nanomolar bioactivity at both the enzymatic and cellular level (15). Inspired by this successful case of molecular hybridization, the 4-aminopiperidinyl-linked substituted benzyl group which belongs to a typical group of DAPYs was introduced at this position to explore the effects of the linker and steric, electronic, hydrophobic interaction. In addition, the benzyl moiety was substituted by different substituents to explore SARs in this domain.

Consequently, with the aim to achieve our goals described above, novel 4-aminopiperidinyl-linked 3,5-disubstituted-1,2,6-thiadiazine-1,1-dione derivatives were designed, synthesized by a facile synthetic route and evaluated for their anti-HIV activities in MT-4 cells using the MTT assay.

Methods and Materials

Chemistry

All melting points (mp) were determined on a micromelting point apparatus and are uncorrected. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument by electrospray ionization. ^{1}H NMR spectra were obtained on a Bruker Avance-400 NMR-spectrometer in the indicated solvents. Chemical shifts (δ) are reported in parts per million relative to TMS as an internal standard. The ^{13}C NMR spectra were recorded on a Bruker Avance-400 NMR-spectrometer and were reported accordingly. TLC was performed on Silica Gel GF254 for TLC, and spots were visualized by irradiation with UV light (254 nm). Flash column chromatography was performed on a column packed with silica gel 60 (200–300 mesh). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solution involved the use of rotary evaporator at reduced pressure.

General synthetic procedure for compounds 3-(naphthalen-1-ylmethyl)-1, 1-dioxo-2*H*-1,2,6-thiadiazin-5-yl 4-methylbenzenesulfonate (**Bam3**) and 3-(2,4-dichlorobenzyl)-1,1-dioxo-2*H*-1,2,6-thiadiazin-5-yl 4-methylbenzenesulfonate (**Bbm3**).

2,2-dimethyl-5-(2-(naphthalen-1-yl) acetyl)-1,3-dioxane-4,6-dione (**Bam1**) and 5-(2-(2,4-dichlorophenyl) acetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (**Bbm1**) (16).

To a solution of 1-naphthaleneacetic acid (1.86 g, 10 mmol, 1 eqv.) or 2,4-dichlorophenyl acetic acid (2.05 g, 10 mmol, 1 eqv.) in tetrahydrofuran (20 mL), *N*, *N*'-carbonyldiimidazole (CDI) (1.78 g, 11 mmol, 1.1 eqv.) was added, and the solution was heated to 50 °C. Meldrum's acid (1.58 g, 11 mmol, 1.1 eqv.) was added carefully, and the reaction mixture was stirred for another 5 h at 50 °C. The reaction mixture was concentrated under reduced pressure to evaporate the tetrahydrofuran and to obtain a red oil, then 100 mL water and 100 mL dichloromethane (DCM) were added, and the mixture was stirred vigorously. The aqueous phase was adjusted to pH 2 with concentrated hydrochloric acid. The organic phase was washed with 0.1 M hydrochloric acid (30 mL), water (30 mL), 0.1 M hydrochloric acid (30 mL), water (30 mL) successively and was subsequently dried (Na_2SO_4), filtered, and concentrated under reduced pressure to obtain a crude product without further purification.

2,2-dimethyl-5-(2-(naphthalen-1-yl)acetyl)-1,3-dioxane-4,6-dione (**Bam1**): 1.95 g crude yellow residue was obtained with a yield of 62.5%.

5-(2-(2,4-dichlorophenyl)acetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (**Bbm1**): 2.16 g crude yellow–white residue was obtained with a yield of 69.2%.

5-(naphthalen-1-ylmethyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bam2**) and 5-(2,4-dichlorobenzyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bbm2**) (17).

10 mmol (1 eqv.) acylmeldrum's acid and 1.15 g sulfamide (12 mmol, 1.2 eqv.) were added in a mortar. After grinding and mixing, the mixture was transferred into a reaction flask, then heated to 100–110 °C in an oil bath, and further stirred for 1.5 h. After cooling to room temperature, the reaction mixture was dissolved in 30 mL ethyl acetate (EA) and extracted using saturated sodium bicarbonate solution for three times. The aqueous phases were combined, then acidified with 2N diluted hydrochloric acid, adjusted to pH 1, and then extracted with ethyl acetate (30 mL) for three times. Further, organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a crude solid.

5-(naphthalen-1-ylmethyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bam2**): 1.25 g crude yellow residue was obtained with a yield of 43.5%. Mp 165–167 °C. ^{1}H NMR (DMSO-d_6) δ: 9.57 (s, 1H, NH), 7.40-8.06 (m, 7H, naphthalene), 4.37 (s, 1H, C=CH), 3.79 (s, 2H, CH_2). EI-MS: *m/z* 287.2 $[M-H]^-$. $C_{14}H_{12}N_2O_3S$ (288.06).

5-(2,4-dichlorobenzyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bam2**): 1.30 g crude yellow residue was obtained with a yield of 43.5%. Mp 180–183 °C. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 12.85 (s, 1H, NH), 12.12 (s, 1H, NH), 7.69 (d, J = 2.0 Hz, 1H, Ph-H), 7.49 (dd, J = 8.3, 2.0 Hz, 1H, Ph-H), 7.44 (d, J = 8.3 Hz, 1H, Ph-H), 5.01 (s, 1H, C=CH), 3.80 (s, 2H, CH_2). ESI-MS: *m/z* 305.3 $[M-H]^-$, 307.3 $[M-H]^-$. $C_{10}H_8Cl_2N_2O_3S$ (305.96).

3-(naphthalen-1-ylmethyl)-1,1-dioxo-2*H*-1,2,6-thiadiazin -5-yl 4-methylbenzenesulfonate (**Bam3**) and 3-(2,4-dichlorobenzyl)-1,1-dioxo-2*H*-1,2,6-thiadiazin-5-yl 4-methylbenzenesulfonate (**Bbm3**) (18).

19.32 mmol (1 eqv.) 5-(naphthalen-1-ylmethyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bam2**) or 5-(2,4-dichlorobenzyl)-2*H*-1,2,6-thiadiazin-3(6*H*)-one 1,1-dioxide (**Bbm2**) in 50 mL acetonitrile (MeCN) was treated with 2.66 mL triethylamine (TEA) (19.32 mmol, 1 eqv.) at ambient temperature, and the resulting solution was stirred for 1 h. p-toluenesulfonyl chloride (TsCl) 7.38 g (38.64 mmol, 2 eqv.) and 4-dimethylaminopyridine (DMAP) 0.12 g (catalyst) were added into the solution and stirred for another 48 h at ambient temperature. The solution was chromatographed on silica gel (2:1:0.01 DCM/PE/CH_3COOH, eluent) to provide the title product.

3-(naphthalen-1-ylmethyl)-1,1-dioxo-2*H*-1,2,6-thiadiazin-5-yl 4-methylbenzenesulfonate (**Bam3**): 3.96 g white solid was obtained with a yield of 46.3%. Mp 160–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.96 (s, 1H, NH), 7.98–7.93 (m, 2H, Naph-H), 7.90 (d, J = 8.2 Hz, 1H, Naph-H), 7.78 (d, J = 8.4 Hz, 2H, Ph-H), 7.57–7.55 (m, 2H, Naph-H), 7.49 (d, J = 8.2 Hz, 1H, Naph-H), 7.43 (d, J = 8.1 Hz, 2H, Ph-H), 7.40 (d, J = 7.3 Hz, 1H, Naph-H), 5.26 (s, 1H, C=CH), 4.19 (s, 2H, CH_2), 2.41 (s, 3H, CH_3); ESI-MS: *m/z* 441.4 $[M-H]^-$. $C_{21}H_{18}N_2O_5S_2$ (442.07).

3-(2,4-dichlorobenzyl)-1,1-dioxo-2*H*-1,2,6-thiadiazin-5-yl 4-methyl benzenesulfonate (**Bbm3**): 3.96 g white solid was obtained with a yield of 44.4%. Mp 150–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.21 (s, 1H, NH), 7.85 (d, J = 8.3 Hz, 2H, Ph'-H), 7.64 (d, J = 2.1 Hz, 1H, Ph-H), 7.49 (d, J = 8.2 Hz, 2H, Ph'-H), 7.44 (dd, J = 2.1, 8.3 Hz, 1H, Ph-H), 7.36 (d, J = 8.3 Hz, 1H, Ph-H), 5.11 (s, 1H, C=CH), 3.79 (s, 2H, CH_2), 2.43 (s, 3H, CH_3); ESI-MS: *m/z* 459.4 $[M-H]^-$, 461.4 $[M-H]^-$. $C_{17}H_{14}Cl_2N_2O_5S_2$ (459.97).

General synthetic procedure for compound 1-substituted-benzylpiperidin-4-amine (**Bm5**).

To a solution of 0.2 g 4-tert-butoxycarbonylaminopiperidine (1 mmol, 1 eqv.) and substituted benzyl chloride or bromide (1.2 mmol, 1.2 eqv.) in 10 mL dimethyl formamide (DMF), 0.41 g K_2CO_3 (3 mmol, 3 eqv.), catalytic sodium iodide (NaI) and tetrabutylammonium iodide (TBAI) were added, the mixture was then stirred for 12 h at 50 °C. After cooling to room temperature, 100 mL water was added and extracted with ethyl acetate (3 × 40 mL). The organic phase was washed with brine (3 × 40 mL), then dried (Na_2SO_4), filtered, and concentrated to afford crude solid or oil **Bm4** without further purification (19,20).

A solution of **Bm4** in 5 mL dichloromethane, 2 mL trifluoroacetic acid (TFA) was added and stirred for 12 h at room temperature. The reaction mixture was adjusted to pH 8–9 with saturated K_2CO_3 aqueous solution, then 30 mL water was added, and the reaction mixture was extracted with dichloromethane (3 × 40 mL). The organic phase was washed with brine (3 × 40 mL), then dried (Na_2SO_4), filtered, concentrated to afford crude solid or oil **Bm5** without further purification (21,22).

General synthetic procedure for target compounds (Ba1–Ba13, Bb1–Bb13)

4-methylbenzenesulfonate thiadiazine derivatives **Bam3** or **Bbm3** (1 mmol, 1 eqv.) and an excess of 1-substituted-benzylpiperidin-4-amine (**Bm5**) were dissolved in acetonitrile, then TEA (1.5 mmol, 1.5 eqv.) was added, and the mixture was refluxed for 48 h at 90 °C. The solution was chromatographed on silica gel (1:2:0.01 EA/PE/CH_3COOH, eluent) to provide the title target product.

Anti-HIV activity evaluation

In vitro anti-HIV assay

The evaluation of target compounds for their activity against HIV-1 strain (III_B), mutant HIV-1 (RES056: $RT_{K103N/Y181C}$) and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT method as previously described (23). Stock solutions (10 × final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells 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 microtitre trays by adding 100 μL medium to the 25 μL stock solution and transferring 25 μL of this solution to another well that contained 100 μL medium using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA, USA). Untreated control HIV- and mock-infected cell samples were included for each sample. HIV-1(III_B) (24), mutant HIV-1 (RES056) or HIV-2 (ROD) (25) stock (50 μL) at 100-300 $CCID_{50}$ (50% cell culture infectious dose-50%) or culture medium was added in either the HIV-infected or mock-infected wells of the microtitre plate. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g, 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 microtitre tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound

that reduced the viability of the mock-infected MT-4 cells 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}).

In vitro HIV-RT kit assay

Inhibition of HIV-1 RT was performed using homopolymer template/primer (poly(A).oligo$(dT)_{15}$), biotin-dUTP, and RT with detection by ELISA for quantifying polymerase activity. The incorporated quantities of the biotin-dUTP into the template represented the activity of HIV-1 RT. IC_{50} values corresponded to the concentration of target compound required to inhibit biotin-dUTP incorporation by 50%. The procedure for assaying RT inhibition was performed as described in the kit (Roche, Mannheim, Germany) protocol (26).

Results and Discussions

Chemistry

The synthetic route of the 26 target compounds, 5-((1-(substitutedbenzyl)piperidin-4-yl)amino)-3-(naphthalen-1-ylmethyl)-2*H*-1,2,6-thiadiazine 1,1-dioxide (**Ba1**-**Ba13**) and 3-(2,4-dichlorobenzyl)-5-((1-(substitutedbenzyl)piperidin-4-yl)amino)-2*H*-1,2,6-thiadiazine 1,1-dioxide (**Bb1**-**Bb13**), is depicted in Scheme 1.

For the synthesis of these novel derivatives, we choose the readily available Meldrum's acid as starting material. 1-Naphthaleneacetic acid or 2,4-dichlorophenylacetic acid was first reacted with Meldrum's acid followed by cyclization with sulfamide to afford key intermediates 5-substituted-1,2,6-thiadiazine-1,1,3-trione **Bam2** or **Bbm2**, respectively. Then **Bam2** or **Bbm2** was treated with TsCl to yield carbonyl activated thiadiazines **Bam3** or **Bbm3**, respectively. 4-Tert-butoxycarbonylaminopiperidine was substituted in a nucleophilic manner by different substituted benzyls, then the product was treated with TFA to give 1-benzyl-substituted 4-aminopiperidine **Bm5**. Finally, **Bm5** participated in a nucleophilic substitution at the C-5 position of the thiadiazine heterocycle (**Bam3** or **Bbm3**), yielding 26 target compounds. The synthesized compounds were confirmed by physicochemical and spectral means, and the MS, ^{1}H NMR and ^{13}C NMR spectral data were found in agreement with the assigned molecular structures.

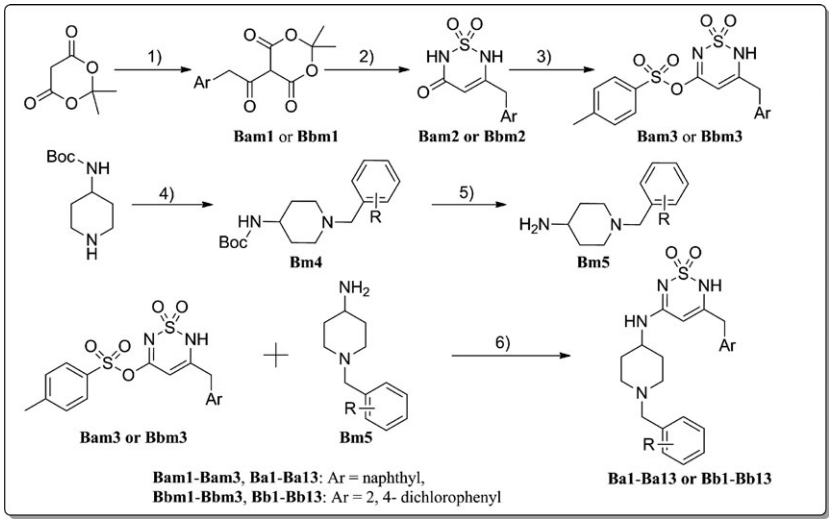
Anti-HIV activity

All the synthesized compounds were evaluated for their anti-HIV activity and cytotoxicity in MT-4 cell cultures using the MTT assay. The used virus strains were WT HIV-1 (III_B), mutant HIV-1 (RES056) and HIV-2 (ROD) (the RES056 strain was prepared by site-directed mutagenesis, provided by the University of Leuven in Belgium, Rega Institute, Institute of Microbiology and Immunology). The FDA approved drugs nevirapine (NVP), delavirdine (DLV), efavirenz (EFV), etravirine (ETR), and zidovudine (AZT) were used as controls. The experimental results of 26 compounds were presented in Table S1.

It was apparent that most of the compounds showed moderate inhibitory activity against WT HIV-1 (III_B) with an EC_{50} value ranging from 22 to 35 μM and low selectivity index (SI) values of 3–5. However, none of the target compounds was actually active against mutant HIV-1 and HIV-2.

Furthermore, the preliminary SARs analysis of these novel thiadiazine derivatives could be summarized as follows:

• On the whole, 2,4-dichlorobenzyl at C-3 position of the thiadiazine heterocycle was more preferable than 1-naphthylmethyl according to the number of active compounds and EC_{50} values.



Scheme 1: Reagents and Conditions: (1) 1-Naphthaleneacetic acid (or 2,4-Dichlorophenylacetic acid), CDI, THF, 0 °C, 20 min, then 50 °C, 5 h; (2) $H_2NSO_2NH_2$, 110 °C, 1 h; (3) TsCl, TEA, DMAP, MeCN, rt, 24 h; (4) K_2CO_3, NaI, TBAI, DMF, 50 °C, 12 h; (5) TFA, DCM, rt, 10 h; (6) MeCN, TEA, reflux, 48 h.

• Both series of compounds had a similar activity profile. For instance, as illustrated by **Ba1**, **Ba7**, **Bb1,** and **Bb7**, the 2,6-dichlorobenzyl or 2,4,6-trimethylbenzyl linked with the 4-aminopiperidine was unfavorable for the activity. In addition, **Ba8** and **Bb8** bearing 4-NO_2 substituted benzyl were the most potent target compounds, with an EC_{50} of 25.26 and 22.94 μM, and SI of 4 and 4, respectively.

• 2-cyano and 4-cyano substituted benzyl were favorable for anti-HIV activity while 3-cyano was unfavorable for the **Ba** series compounds. In contrast, cyano substituted at different positions of benzyl exhibited similar activity in the **Bb** series.

• It is also interesting to note that replacement of 4-bromo on the benzyl present in **Ba10** and **Bb10** with 4-fluoro yielded compounds **Ba9** and **Bb9**, respectively, which showed slightly decreased potency and cytotoxicity.

• 2-Methyl substituted benzyl was preferable, while 2,4,6-trimethyl substituted benzyl resulted in total inactivity.

• Regarding the cytotoxicity, 4-SO_2Me substituted benzyl derivatives always displayed the lowest cytotoxicity.

What not allow to ignore is that, the potencies of bioactive molecules are also closely related to their physicochemical properties including aqueous solubility and cell membrane permeability, which will be fully taken into account in further optimizations.

HIV-1-RT inhibitory activity evaluation

To further define the mechanism of action of our newly designed compounds, **Ba8** and **Bb8**, the best compound from each series were evaluated for their HIV-RT inhibitory activity using the HIV-RT kit assay. As shown in Table S2, compound **Ba8** and **Bb8** exhibited inhibition activity against HIV-1 RT with IC_{50} value of 3.15 and 1.52 μM, respectively, comparable to those of reference drugs NVP (IC_{50} = 2.38 μM) and ETR (IC_{50} = 0.13 μM). It is reasonable that the newly synthesized 1,2,6-thiadiazine NNRTIs probably target HIV-1 RT.

Molecular modeling study

To predict the binding mode and validate the rationalization of novel designed thiadiazine derivatives, representative compounds **Ba8** and **Bb8** were docked into the NNRTIs binding pocket (NNIBP) of WT HIV-1 RT using SYBYL-X 1.1, and the docking results were shown by PyMOL.

The molecular modeling analysis demonstrated that compounds **Ba8** and **Bb8** exhibit similar binding mode with TMC125 and bind to the RT in a 'U-shape' (Figure S3). According to the binding mode, the oxygen of 1-sulfuryl, the nitrogen at the 6 position of 1,2,6-thiadiazine as well as the NH group linked to the 5-position of the heterocycle could form ternary hydrogen bonds with Lys101. In addition, the hydrogen bond between NH at the 2 position of 1,2,6-thiadiazine and Glu138 could also be observed. Furthermore, the aryl 1-naphthyl or 2,4,-dichlorophenyl which may form $\pi-\pi$ interactions with the hydrophobic amino acids locates at the aromatic-rich binding pocket surrounded by the aromatic acids Tyr188, Phe227, and Trp229. The 4-aminopiperidinyl-linked benzyl of the compounds extends to the solvent/protein interface, which was surrounded by Pro235, Pro236, and Val106. The nitryl of compounds **Ba8** and **Bb8** may form additional interactions with surrounding amino acids so that they display the most potent activity against HIV-1. Above all, the binding mode of representative compounds **Ba8** and **Bb8** suggested that they could interact with the key amino acids in a favorable conformation, which is consistent with that of TMC125.

Conclusions

Taken together, based on the bioisosterism and molecular hybridization strategies in drug design, a novel series of 4-aminopiperidinyl-linked 3,5-disubstituted-1,2,6-thiadiazine-1,1-dione derivatives were designed, synthesized and evaluated as HIV-1 NNRTIs. Most of the compounds displayed moderate inhibitory activity against HIV-1 with EC_{50} values ranging from 22 to 35 μM. Furthermore, the preliminary SAR of these compounds based on the anti-HIV-1 activity has been discussed. On the whole, the compounds presented in this paper do not exhibit the desired characteristics as described above, that is, high potency and improved resistance profile. Still, the molecular modeling study further gave insight into important interactions occurring between NNRTIs binding site and thiadiazines which provides useful information for design of potent inhibitors with this heterocyclic motif.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representative analogues of the DABO and DAPY derivatives.

Figure S2. Structure-based design of target compounds.

Figure S3. (A) Molecular docking of **Ba8** (cyan) into the allosteric site of wt HIV-1 RT; (B) The structural overlap of the docking pose of compound **Ba8** (cyan) and TMC125 (yellow); (C) Molecular docking of **Bb8** (orange) into the allosteric site of wt HIV-1 RT; (D) The structural overlap of the docking pose of compound **Bb8** (orange) and TMC125 (yellow). (PDB code: 3MEC. The docking was performed using SYBYL-X 1.1, and the docking results were shown by PYMOL. The hydrogen bonds are shown in red or yellow dashed lines).

Table S1. Anti-HIV activity, cytotoxicity and selectivity indices of target compounds.

Table S2. *In vitro* recombinant HIV-1 RT inhibitory assay of representative compounds **Ba8**, **Bb8** and reference drugs **NVP**, **ETR**.