



# Design, synthesis, anti-HIV evaluation and molecular modeling of piperidine-linked amino-triazine derivatives as potent non-nucleoside reverse transcriptase inhibitors

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## ARTICLE INFO

### Article history:

Received 5 March 2012

Revised 11 April 2012

Accepted 16 April 2012

Available online 21 April 2012

### Keywords:

HIV-1  
NNRTIs  
Piperidine  
Triazine  
Antiviral activity  
SAR  
Molecular modeling

## ABSTRACT

A novel series of piperidine-linked amino-triazine derivatives were designed, synthesized and evaluated for in vitro anti-HIV activity as non-nucleoside reverse transcriptase inhibitors on the basis of our previous work. Screening results indicated that most compounds showed excellent activity against wild-type HIV-1 with EC<sub>50</sub> values in low nanomolar concentration range (especially compound **6b3**, EC<sub>50</sub> = 4.61 nM, SI = 5945) and high activity against K103N/Y181C resistant mutant strain of HIV-1 with EC<sub>50</sub> values in low micromolar concentration range. In addition, preliminary structure–activity relationship and molecular modeling of these new analogs were detailed in this manuscript.

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## 1. Introduction

Acquired immune deficiency syndrome (AIDS) mainly caused by human immunodeficiency virus type-1 (HIV-1), is one of the leading pandemic diseases worldwide. Because of the lack of vaccine, the highly active antiretroviral therapy (HAART) regimen plays a crucial role in the treatment of HIV-1 infection, however, drug resistance and side effects often compromise clinical treatment.<sup>1</sup> HIV-1 non-nucleoside reverse transcriptase inhibitors (HIV-1 NNRTIs) have become an indispensable component of HAART, with unique antiviral potency, high specificity and low cytotoxicity.<sup>2</sup> Especially, diarylpyrimidine (DAPY) derivatives, such as Dapivirine (TMC120), Etravirine (TMC125, ETV) and Rilpivirine (TMC278) showed highly potent activity against wild-type and NNRTI-resistant strains of HIV-1 (Fig. 1),<sup>3,4</sup> of which, TMC125 and TMC278 have been approved by FDA, providing an additional treatment option for HAART regimen. Further research of DAPY derivatives was focused on diverse structural modifications, including the left wing,<sup>5–7</sup> the linker between the left benzene ring and the central pyrimidine ring,<sup>8–10</sup> the central pyrimidine core<sup>11–14</sup> and the right wing.<sup>15,16</sup>

Among these structurally modified analogs, piperidine-linked aminopyrimidine derivatives (Fig. 2) displayed promising potency against wild-type and several important resistant mutant strains of HIV-1, as well as enhanced water solubility to potentially meliorate the pharmacokinetic profile, which make it extensive concern in DAPY analogs.

Recently, we designed and synthesized a novel series of DAPY analogs with piperidine-substituted triazine moiety, which are structurally similar to the piperidine-linked aminopyrimidine derivatives. From biological assay, we had discovered some new lead compounds to be potent and selective HIV-1 NNRTIs with IC<sub>50</sub> values at nanomoles ranges.<sup>17</sup> Encouraged by this result and continued to pursue our studies, we undertook a new round of structural modification of piperidine-substituted triazine derivatives, in which, amino group was introduced to the triazine ring, similar to that observed in the structure of Etravirine (Fig. 2), which may improve hydrogen bond interaction with amino acid residues inside the binding pocket of HIV-1 NNRT.<sup>18</sup> In addition, polar hydrophilic substituents and heterocycle were introduced to the right wing (Fig. 2) that is oriented directly into the exterior water through a rather small opening window in the protein/solvent interface, which is thought to be playing an important role in the capacity to inhibit HIV-1 replication and improve pharmaco-

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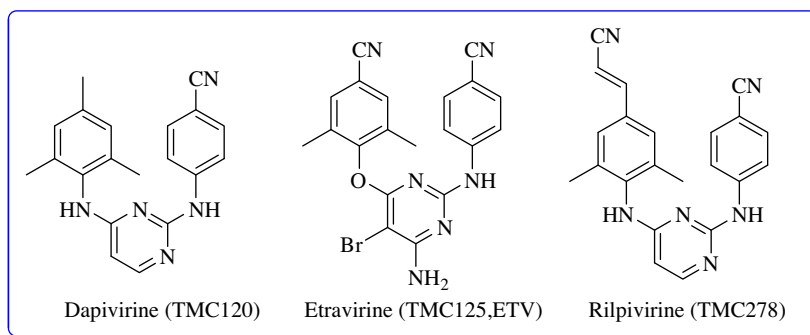


Figure 1. The structures of DAPY derivatives.

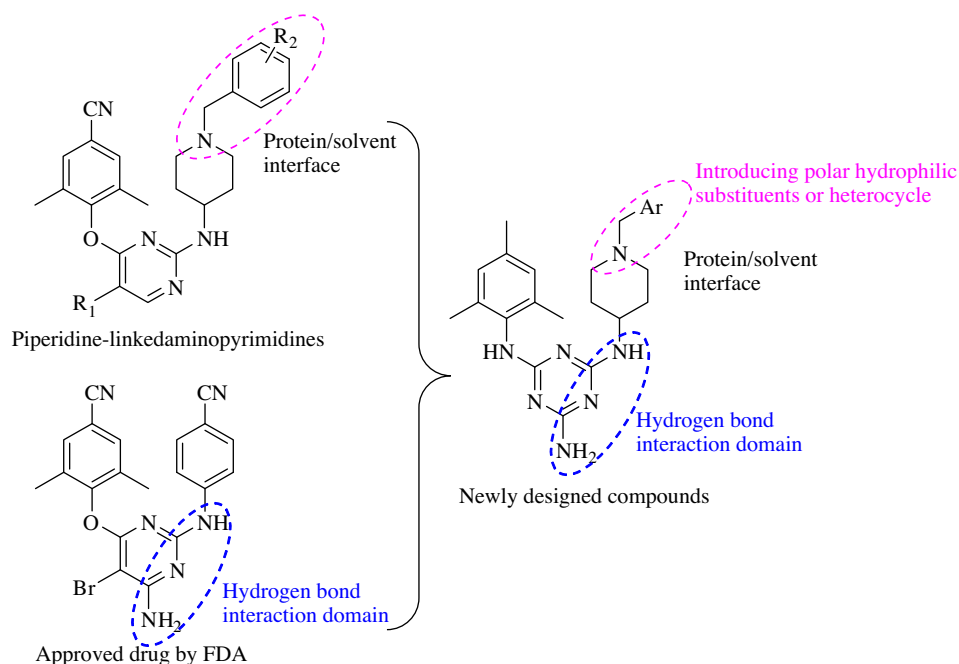


Figure 2. The design of piperidine-linked amino-triazine derivatives.

kinetic properties.<sup>19</sup> Herein, we will detailedly report the synthesis, anti-HIV evaluation, preliminary structure–activity relationship (SAR) and molecular modeling of the newly designed piperidine-linked amino-triazine analogs.

## 2. Results and discussion

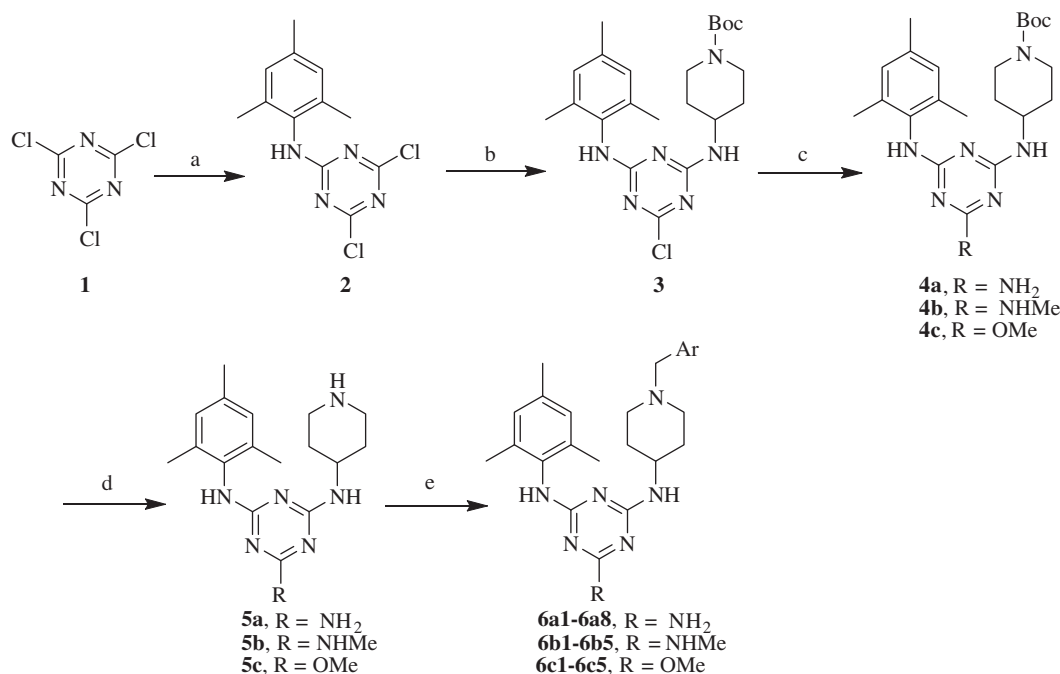
### 2.1. Chemistry

The synthesis route of the target compounds **6a1–6a8**, **6b1–6b5** and **6c1–6c5** was shown in Scheme 1. The intermediate 6-chloro-*N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**3**) was prepared from structurally symmetrical 2,4,6-trichloro-1,3,5-triazine (**1**) by substitution reaction with 2,4,6-trimethylaniline and 4-amino-1-Boc-piperidine successively in mild reaction conditions.<sup>20</sup> Treatment of intermediate **3** with ammonia under high temperature in sealed reactor,<sup>21</sup> aqueous methylamine and sodium methoxide gave the intermediate **4a**, **4b** and **4c**, respectively.<sup>22</sup> Boc group was removed in the presence of trifluoroacetic acid (TFA) at room temperature to afford **5a**, **5b** and **5c**, which were alkylated at the N atom of piperidine, achieving the final compounds **6a1–6a8**, **6b1–6b5** and **6c1–6c5**, respectively. Both analytical and spectral data of all the compounds are in full agreement with the proposed structures.

### 2.2. Biological activity

The newly synthesized piperidine-linked amino-triazine derivatives were tested for their anti-HIV activity and cytotoxicity in MT-4 cells infected by wild-type HIV-1 (IIIB) and the double mutant strains RES056 (K103N+Y181C) along with HIV-2 (ROD) according to the MTT method reported previously.<sup>17,23–25</sup> FDA-approved drugs: Nevirapine (NVP), Delavirdine Mesylate (DLV), Efavirenz (EFV), Zidovudine (Azidothymidine, AZT), Dideoxycytidine (DDC) and Etravirine (ETV)<sup>26</sup> were used as reference drugs. The biological evaluation results were interpreted in EC<sub>50</sub> (anti-HIV activity), CC<sub>50</sub> values (cytotoxicity) and SI (selectivity index, given by the CC<sub>50</sub>/EC<sub>50</sub> ratio) as shown in Tables 1 and 2.

As listed in Table 1, most compounds showed high activity against wild-type HIV-1 with EC<sub>50</sub> values in the range of 4.61–20.43 nM and high SI values ranging from 297 to 5945. Compound **6b3** was the most potent compound (EC<sub>50</sub> = 4.61 nM, SI = 5945), superior to the reference drugs NVP, DLV, EFV, AZT and DDC, but a little inferior to ETV against wild-type HIV-1. Introduction of –NH<sub>2</sub> at the triazine ring (**6a1–6a5**) improved the activity compared to the –NHMe series (**6b1–6b5**) and –OMe series (**6c1–6c5**) except the compound with 4-carbamoylphenyl group (**6a3**). Compared to compound **6a6**, compounds **6a4**, **6a5**, **6a7** and **6a8** showed more potent activity due to the presences of polar hydrophilic substitu-



**Scheme 1.** Reagents and conditions (a) K<sub>2</sub>CO<sub>3</sub>/THF; (b) NaHCO<sub>3</sub> (5%aq)/THF; (c) (c<sub>1</sub>) NH<sub>3</sub> (aq)/THF; (c<sub>2</sub>) NH<sub>2</sub>Me(aq)/NaHCO<sub>3</sub>/THF; (c<sub>3</sub>) Na/MeOH; (d) TFA/CH<sub>2</sub>Cl<sub>2</sub>; (e) K<sub>2</sub>CO<sub>3</sub>/DMF.

ents at the *para* phenyl, such as sulfamoyl, methylsulfonyl, cyano and nitro groups. Additionally, substitution of phenyl group (**6a6**) with polar hydrophilic pyridyl group (**6a1**) was confirmed contributing to the anti-HIV-1 activity. Among the tested compounds, compounds **6a2**, **6b2** and **6c2** with 4-COOEt-Ph displayed reduced potency against wild-type HIV-1 and increased cytotoxicity with lower SI values, because the ethyl group is hydrophobic and could not extend into the exterior water of the opening RT pocket.

In summary, introduction of –NH<sub>2</sub> at the triazine ring and polar hydrophilic groups at the right wing were favorable to improvement of activity against wild-type HIV-1 indeed. Furthermore, none of the newly synthesized compounds were active at subtoxic concentration against HIV-2 (ROD) in MT-4 cells.

In the treatment of HIV-1 infection, drug resistance is still a key cause of failure for their antiviral efficacy. There are many resistant RT mutations of HIV-1, such as L100I, K101E, K103N, V106A, V179F, Y181C, Y188L, G190A, F227C, L234I and Y318F. Among them, K103N and Y181C resistant mutant strains are the most often encountered mutations in the treatment of AIDS with HIV-1 NNRTIs clinically.<sup>27</sup> With the aim to define the resistance profiles of these compounds, they were also assayed against the K103N/Y181C resistant mutant strain of HIV-1 with the results illustrated in Table 2. As the data indicated, both the absolute activity against the HIV-1 mutants (EC<sub>50</sub> values) and the relative activity (fold resistance) needed to be taken into consideration of the potency. Most compounds displayed high activity against K103N/Y181C resistant mutant strain of HIV-1 with EC<sub>50</sub> values in low-micromolar concentration ranges. Compounds **6b1** (EC<sub>50</sub> = 0.55 μM with a 76.4-fold resistance ratio) and **6b4** (EC<sub>50</sub> = 0.51 μM with a 72.7-fold resistance ratio) were more active than reference drugs NVP, DLV and EFV, however, they were less potent than AZT and ETV (EC<sub>50</sub> = 0.034 μM with a 15.5-fold resistance ratio). It is interesting to note that, compounds with 4-SO<sub>2</sub>NH<sub>2</sub>-Ph group (**6a4**, **6b4** and **6c4**) showed better potency against resistant mutant strain of HIV-1 among each series (–NH<sub>2</sub> series, –NHMe series and –OMe series), which could also be observed in NNRTIs of other structural type, such as benzophenone (GW678248)<sup>28</sup> and triazolylthioacetanilide (VRX-480773)<sup>29</sup> NNRTIs. However, compounds with 4-COOEt-Ph group (**6a2**,

**6b2** and **6c2**) displayed no activity against resistant mutant strain of HIV-1.

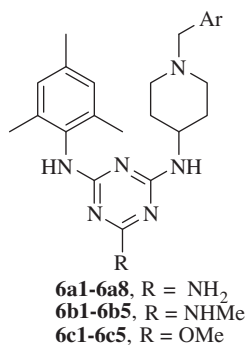
### 2.3. Molecular simulation analysis

In view of piperidine-substituted triazine derivatives and piperidine-linked aminopyrimidine derivatives targeting at the site of RT,<sup>15–17</sup> we decided to investigate the binding mode of the newly synthesized NNRTIs. Potent compounds **6a1** and **6b3** were docked into non-nucleoside inhibitor binding pocket (NNIBP) of wild-type HIV-1 RT (PDB code: 3M8Q) using the software Surflex-Dock SYB-YL-X 1.1 and the docking results were showed by PyMOL 0.99. Default parameters were used as described in the SYBYL-X 1.1 manual unless otherwise specified.

As shown in Figure 3, the new compounds **6a1** and **6b3** could bind into the NNIBP similar with piperidine-linked aminopyrimidine derivatives.<sup>15</sup> Detailed analysis of the docking results revealed the following notable features that may clarify the mechanism of action of piperidine-substituted triazine derivatives. (1) Mesityl moiety of compounds **6a1** and **6b3** fits into the important hydrophobic sub-pocket, which is formed by aromatic amino acid residues Y181, Y188, F227 and W229, exhibiting π–π interaction with these residues. (2) The new compounds form two hydrogen bonds with backbone carbonyl and backbone α-amino of residue K101, similar to that observed in DAPY compounds. Additionally, the –NH<sub>2</sub> and –NHMe groups can form a hydrogen bond with amino acid E138 that may improve stability of RT-inhibitor complex and the affinity between RT and inhibitor. (3) The substituted piperidine sits between amino acid Y318 and V106, and the polar hydrophilic substituents (compound **6a1**) and heterocycle (compound **6b3**) is located toward a solvent exposure region, contacting either side chain of amino acid L234 or P236 (Fig. 3c). This binding model supports our initial design hypothesis that introduction of –NH<sub>2</sub> group at the triazine ring may improve hydrogen bond interaction with amino acid residues of the binding pocket, and polar hydrophilic substituents and heterocycle point directly into the protein/solvent interface.

**Table 1**

Activity and cytotoxicity against HIV-1 (wild-type, IIIB) and HIV-2 (ROD) strains in MT-4 cells



Compds	R	Ar	EC <sub>50</sub> <sup>a</sup>		CC <sub>50</sub> <sup>b</sup> (μM)	SI <sup>c</sup>
			HIV-1 IIIB (nM)	HIV-2 ROD (μM)		
<b>6a1</b>	NH <sub>2</sub>	Pyridin-4-yl	4.90 ± 2.40	>20.12	20.12 ± 8.10	4108
<b>6a2</b>	NH <sub>2</sub>	4-COOEt-Ph	11.55 ± 0.51	>5.91	5.91 ± 1.37	512
<b>6a3</b>	NH <sub>2</sub>	4-CONH <sub>2</sub> -Ph	20.43 ± 19.73	>74.36	74.36 ± 18.94	3640
<b>6a4</b>	NH <sub>2</sub>	4-SO <sub>2</sub> NH <sub>2</sub> -Ph	5.75 ± 2.64	>19.85	19.85 ± 4.52	3451
<b>6a5</b>	NH <sub>2</sub>	4-SO <sub>2</sub> Me-Ph	5.55 ± 1.48	>5.06	5.06 ± 2.49	911
<b>6a6</b>	NH <sub>2</sub>	Phenyl	10.25 ± 0.98	>23.59	23.59 ± 3.40	2302
<b>6a7</b>	NH <sub>2</sub>	4-NO <sub>2</sub> -Ph	7.87 ± 1.41	>3.46	3.46 ± 1.58	439
<b>6a8</b>	NH <sub>2</sub>	4-CN-Ph	5.66 ± 3.13	>3.26	3.26 ± 1.18	576
<b>6b1</b>	NHMe	Pyridin-4-yl	7.25 ± 1.19	>25.80	25.80 ± 1.15	3558
<b>6b2</b>	NHMe	4-COOEt-Ph	16.66 ± 7.94	>4.95	4.95 ± 0.17	297
<b>6b3</b>	NHMe	4-CONH <sub>2</sub> -Ph	4.61 ± 1.90	>27.39	27.39 ± 2.34	5945
<b>6b4</b>	NHMe	4-SO <sub>2</sub> NH <sub>2</sub> -Ph	6.99 ± 1.09	>14.83	14.83 ± 2.49	2122
<b>6b5</b>	NHMe	4-SO <sub>2</sub> Me-Ph	6.06 ± 2.04	>9.64	9.64 ± 3.12	1590
<b>6c1</b>	OMe	Pyridin-4-yl	13.32 ± 2.88	>30.91	30.91 ± 2.23	2320
<b>6c2</b>	OMe	4-COOEt-Ph	14.71 ± 7.04	>8.48	8.48 ± 2.03	577
<b>6c3</b>	OMe	4-CONH <sub>2</sub> -Ph	8.64 ± 1.49	>33.43	33.43 ± 8.98	3869
<b>6c4</b>	OMe	4-SO <sub>2</sub> NH <sub>2</sub> -Ph	10.62 ± 2.58	>19.63	19.63 ± 2.67	1849
<b>6c5</b>	OMe	4-SO <sub>2</sub> Me-Ph	11.90 ± 4.54	>13.45	13.45 ± 3.48	1130
NVP			200.40 ± 105.31		>15.02	>75
DLV			35.01 ± 4.73		>36.19	>1034
EFV			6.57 ± 0.87		>6.34	>964
AZT			6.00 ± 0.57	0.007 ± 0.0008	≥49.77	≥8295
DDC			1394.28 ± 50.22	1.67 ± 0.25	>94.69	>68
ETV <sup>d26</sup>			2.2 ± 0.4	>27.78	27.78 ± 11.53	12884

<sup>a</sup> EC<sub>50</sub>: Concentration of compound required to achieve 50% protection of MT-4 cell against HIV-1-induced cytotoxicity, as determined by the MTT method.<sup>b</sup> CC<sub>50</sub>: Concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.<sup>c</sup> SI: Selectivity index, the ratio of CC<sub>50</sub>/EC<sub>50</sub><sup>HIV-1IIIB</sup>.<sup>d</sup> ETV: Etravirine. The data were obtained from the same laboratory (Rega Institute for Medical Research, K.U. Leuven, Belgium).

Overall, all these analyses were in accordance with the biological data. Apparently, the new compounds showed excellent activity against wild-type HIV-1 as they can bind into the NNIBP of wild-type HIV-1 RT and exhibit interactions with NNIBP. However, the new compounds displayed less potent activity against K103N/Y181C resistant mutant strain of HIV-1 because of the following reasons according to the docking studies. First, the new compounds form hydrogen bonds with amino acid K101 backbone, whereas piperidine-linked aminopyrimidine derivatives form another hydrogen bond with K103 backbone through a water bridge,<sup>15</sup> and this hydrogen bond interaction with main chain is unlikely to be disrupted by side chain mutations. Second, mesityl moiety which fits into the hydrophobic sub-pocket keeps parallel to the side chain phenyl of amino acid Y181 and forms strong  $\pi$ - $\pi$  interaction, when resistant mutation at Y181 side chain emerged the dependent  $\pi$ - $\pi$  interaction lost. Further structural optimization will consider these aspects analyzed above.

### 3. Conclusion

In the present paper, a novel series of piperidine substituted triazine derivatives were designed, synthesized and evaluated in

MT-4 cells along with preliminary SAR analysis. Screening results indicated that most compounds showed excellent activity against wild-type HIV-1 with EC<sub>50</sub> values in low nanomolar concentration range (**6b3**, EC<sub>50</sub> = 4.61 nM, SI = 5945) and high activity against K103N/Y181C resistant mutant strain of HIV-1 with EC<sub>50</sub> values in low micromolar concentration range (**6b1**, EC<sub>50</sub> = 0.55 μM with a 76.4-fold resistance ratio; **6b4** EC<sub>50</sub> = 0.51 μM with a 72.7-fold resistance ratio). In addition, we also built the molecular modes to analyze the interactions between the analogs and wild-type HIV-1 RT. Further studies are ongoing in our laboratories and will be reported in due course.

## 4. Experimental section

### 4.1. Chemistry

All melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. <sup>1</sup>H NMR spectra were obtained on a Bruker Avance-600 NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in  $\delta$  units and TMS as internal reference. Mass spectra were taken on a LC Autosampler

**Table 2**  
Activity against K103N+Y181C mutant strain of HIV-1 (RES056) in MT-4 cells

Compds	EC <sub>50</sub> (μM)	Fold-res. <sup>a</sup>	CC <sub>50</sub> (μM)	SI
<b>6a1</b>	0.97 ± 0.07	198.8	20.12 ± 8.10	21
<b>6a2</b>	>5.91	>511.6	5.91 ± 1.37	<1
<b>6a3</b>	3.33 ± 3.19	162.8	74.36 ± 18.94	22
<b>6a4</b>	0.61 ± 0.01	105.7	19.85 ± 4.52	32
<b>6a5</b>	4.92 ± 4.79	887.5	5.06 ± 2.49	1
<b>6a6</b>	4.93 ± 0.03	481.3	23.59 ± 3.40	5
<b>6a7</b>	>3.46	>439.1	3.46 ± 1.58	<1
<b>6a8</b>	>3.26	>575.8	3.26 ± 1.18	<1
<b>6b1</b>	0.55 ± 0.22	76.4	25.80 ± 1.15	47
<b>6b2</b>	>4.95	>297.0	4.95 ± 0.17	<1
<b>6b3</b>	0.56 ± 0.12	121.6	27.39 ± 2.34	49
<b>6b4</b>	0.51 ± 0.27	72.7	14.83 ± 2.49	29
<b>6b5</b>	3.33 ± 3.33	549.8	9.64 ± 3.12	3
<b>6c1</b>	1.48 ± 0.31	111.1	30.91 ± 2.23	21
<b>6c2</b>	>8.48	>576.6	8.48 ± 2.03	<1
<b>6c3</b>	1.11 ± 0.17	128.3	33.43 ± 8.98	30
<b>6c4</b>	0.94 ± 0.04	88.2	19.63 ± 2.67	21
<b>6c5</b>	≥ 1.59	≥ 133.3	13.45 ± 3.48	≤ 8
NVP	>15.02	>75.0	>15.02	X1 <sup>b</sup>
DLV	>36.19	>1033.6	>36.19	X1
EFV	0.55 ± 0.06	83.6	>6.34	>12
AZT	0.01 ± 0.009	1.9	≥ 49.77	≥ 4397
DDC			>94.69	
ETV <sup>c26</sup>	0.034 ± 0.0046	15.5	27.78 ± 11.53	817

<sup>a</sup> Fold-res: Fold resistance, the ratio of EC<sub>50</sub> value against drug-resistant strain and EC<sub>50</sub> of the wild-type HIV-1 IIIB (EC<sub>50</sub><sup>wt</sup>/EC<sub>50</sub><sup>mutant strains</sup>).

<sup>b</sup> X1: stands for ≥ 1 or <1.

<sup>c</sup> ETV: Etravirine. The data were obtained from the same laboratory (Rega Institute for Medical Research, K.U. Leuven, Belgium).

Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on 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 solutions involved the use of rotary evaporator at reduced pressure.

#### 4.1.1. General procedure for the synthesis of 4,6-dichloro-*N*-mesityl-1,3,5-triazine-2-amine (**2**)

To a solution of cyanuric chloride (**1**, 0.37 g, 2 mmol) in THF (15 mL) at 0 °C was slowly added K<sub>2</sub>CO<sub>3</sub> (0.55 g, 4 mmol) and 2,4,6-trimethylaniline (0.28 g, 2 mmol). The resulting mixture was stirred at 0 °C for 3 h (monitored by TLC). The solvent was removed under reduced pressure and water (20 mL) was added and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 10 mL). Combined organic phase was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. Purification on silica gel gave **2** as an off-white solid. Yield: 91%, mp: 197–199 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, ppm) δ: 10.45 (s, 1H, NH), 6.94 (s, 2H, PhH), 2.25 (s, 3H, CH<sub>3</sub>), 2.08 (s, 6H, 2 × CH<sub>3</sub>). ESI-MS: *m/z* 283.3 (M+1), 285.3 (M+3), 287.3 (M+5). C<sub>12</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>4</sub> [282.04].

#### 4.1.2. General procedure for the synthesis of 6-chloro-*N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**3**)

A solution of crude intermediate **2** (0.56 g, 2 mmol) in THF/acetone/H<sub>2</sub>O (4:1:1, 30 mL) was treated with 4-amino-1-Boc-piperidine (0.4 g, 2 mmol) and 5% w/v aqueous NaHCO<sub>3</sub> (8 mL). The mixture was stirred at 30 °C for 8 h, after that, THF/acetone was evaporated under reduced pressure and extracted with ethyl acetate (2 × 10 mL). Combined extracts were washed with saturated sodium chloride (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo to give the crude product. Purification on silica gel gave **3** as an off-white solid. Yield: 92%, mp: 203–205 °C. <sup>1</sup>H NMR

(DMSO-*d*<sub>6</sub>, ppm) δ: 9.13 (m, 1H), 7.77 (m, 1H), 6.87 (s, 2H, PhH), 3.91 (m, 3H), 2.81 (s, 2H), 2.22 (d, 3H, CH<sub>3</sub>), 2.07 (d, 6H, 2 × CH<sub>3</sub>), 1.37 (s, 9H). ESI-MS: *m/z* 447.5 (M+1), 448.5 (M+2), 449.5 (M+3). C<sub>22</sub>H<sub>31</sub>ClN<sub>6</sub>O<sub>2</sub> [446.22].

#### 4.1.3. General procedure for the synthesis of *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**4a**), *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-methyl-*N*<sup>6</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**4b**) and *N*<sup>2</sup>-mesityl-6-methoxy-*N*<sup>4</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**4c**)

To a solution of solid **3** (7.1 g, 16 mmol) in THF (20 mL) was added ammonia (28% w/v, 100 mL). Then, the mixture was transferred to the reactor, sealed and placed at 100 °C for 5 h with a mechanical stirrer. The reaction solution was left to cool overnight and extracted with ethyl acetate (3 × 30 mL). Combined extracts were washed with 2 N HCl and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to give the crude product. Purification on silica gel gave **4a** as an off-white solid. Yield: 82%, mp: 144–146 °C. ESI-MS: *m/z* 428.3 (M+1), 429.3 (M+2). C<sub>22</sub>H<sub>33</sub>N<sub>7</sub>O<sub>2</sub> [427.27].

Intermediate **3** (0.22 g, 0.5 mmol) was dissolved in THF, followed by addition of saturated NaHCO<sub>3</sub> (2 mL) and 40% w/v aqueous methylamine (0.2 mL). The reaction mixture was stirred at 50 °C for 5 h. THF was removed under reduced pressure and extracted with ethyl acetate (2 × 10 mL). Combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the product **4b** as white solid. Yield: 96%, mp: 131–133 °C, ESI-MS: *m/z* 442.5 (M+1), 443.5 (M+2). C<sub>23</sub>H<sub>35</sub>N<sub>7</sub>O<sub>2</sub> [441.29].

Na (0.35 g, 15 mmol) was dissolved in absolute MeOH (50 mL) followed by addition of 6-chloro-*N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**3**) (4.5 g, 10 mmol). The reaction mixture was stirred at 60 °C for 4 h. After removal of the solvent under reduced pressure, water (20 mL) was added and extracted with ethyl acetate (2 × 20 mL). Combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the product (**4c**) as white solid. Yield: 94%, mp: 190–192 °C, ESI-MS: *m/z* 443.6 (M+1), 444.6 (M+2). C<sub>23</sub>H<sub>34</sub>N<sub>6</sub>O<sub>3</sub> [442.27].

#### 4.1.4. General procedure for the synthesis of *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**5a**), *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-methyl-*N*<sup>6</sup>-(piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**5b**) and *N*<sup>2</sup>-mesityl-6-methoxy-*N*<sup>4</sup>-(piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**5c**)

TFA (4 mL, 54 mmol) was added dropwise under stirring to a solution of intermediate **4a** (3.3 g, 7.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) at room temperature and stirred for 5 h. To the reaction mixture was added water and neutralized with 2 N NaOH to pH8, and the organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the product (**5a**) as white solid. Yield: 91%, mp: 236–238 °C, ESI-MS: *m/z* 328.2 (M+1), 164.7 ((M+2)/2). C<sub>17</sub>H<sub>25</sub>N<sub>7</sub> [327.22].

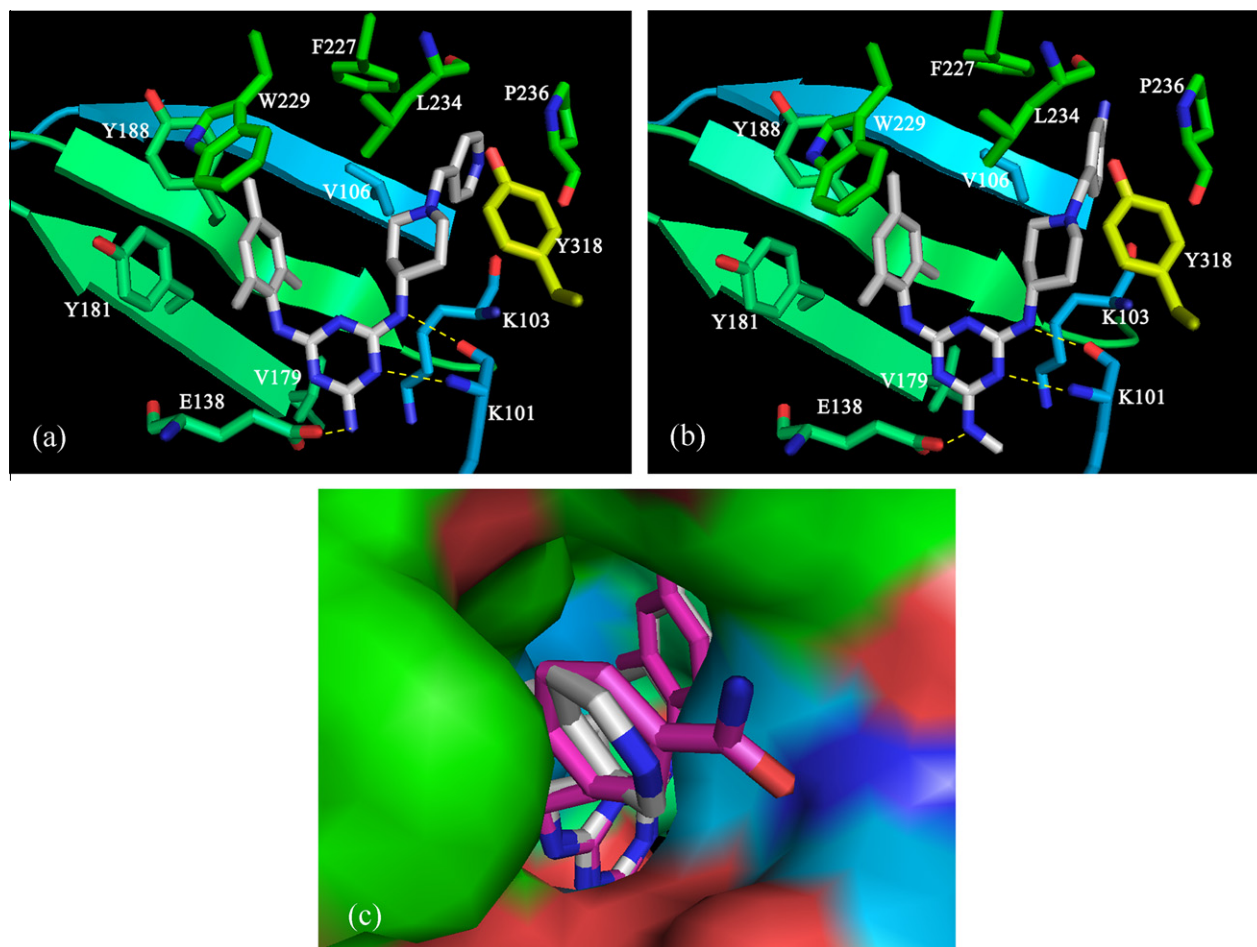
Using intermediate **4b** (0.3 g, 0.67 mmol) and TFA (1 mL, 13.5 mmol) and following the procedure as in the preparation of **5a** to give **5b** as a white solid. Yield: 95%, mp: 139–141 °C, ESI-MS: *m/z* 342.4 (M+1), 171.8 ((M+2)/2). C<sub>18</sub>H<sub>27</sub>N<sub>7</sub> [341.45].

Using intermediate **4c** (0.2 g, 0.45 mmol) and TFA (0.5 mL, 6.7 mmol) and following the procedure as in the preparation of **5a** to give **5c** as a white solid. Yield: 95%, mp: 225–227 °C, ESI-MS: *m/z* 343.5 (M+1), 172.4 ((M+2)/2). C<sub>18</sub>H<sub>26</sub>N<sub>6</sub>O [342.44].

#### 4.1.5. General procedure for the synthesis of target compounds (**6a1**–**6a8**, **6b1**–**6b5** and **6c1**–**6c5**)

Compounds **5a** (**5b** or **5c**) was dissolved in anhydrous DMF (10 mL) in the presence of anhydrous K<sub>2</sub>CO<sub>3</sub> (2 equiv) at 0 °C, fol-





**Figure 3.** Predicted binding modes between the new compounds and NNIBP of wild-type HIV-1 RT (PDB code: 3M8Q): (a) Compound **6a1**; (b) Compound **6b3**; (c) Superimposition of compound **6a1** (white) and compound **6b3** (purple).

lowed by addition of appropriate substituted benzyl chloride (or 4-picolyl chloride hydrochloride) (1.1 equiv). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and water (20 mL) was added. Extracted with ethyl acetate ( $2 \times 10$  mL), and the organic phase was washed with saturated sodium chloride (10 mL), and dried over anhydrous  $\text{Na}_2\text{SO}_4$  to give the corresponding crude products, which were purified by flash column chromatography to afford the target compounds **6a1–6a8** (**6b1–6b5** or **6c1–6c5**), respectively.

**4.1.5.1. *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-(pyridin-4-ylmethyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**6a1**).** Pale yellow powder, yield: 67.6%. Decomposed at 233 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , ppm)  $\delta$ : 8.59 (s, 2H, PhH), 6.97 (s, 2H, PhH), 4.85 (m, 4H), 3.95 (m, 1H), 3.52 (s, 2H,  $\text{CH}_2$ ), 2.81 (s, 2H), 2.35 (s, 3H,  $\text{CH}_3$ ), 2.25 (s, 6H,  $2 \times \text{CH}_3$ ), 2.05 (s, 4H), 1.51 (s, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , ppm)  $\delta$ : 18.59, 20.97, 32.33, 46.91, 52.37, 61.83, 123.75, 128.79, 132.46, 136.59, 147.92, 149.80, 165.72, 167.23. IR (KBr,  $\text{cm}^{-1}$ ): 3460 ( $\nu_{\text{NH}}^{\text{as}}$ ), 3399 ( $\nu_{\text{NH}}$ ), 2936 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1559, 1487 ( $\nu_{\text{C=N}}$ ), 812 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  419.4 ( $M+1$ ), 210.3 ( $(M+2)/2$ ).  $\text{C}_{23}\text{H}_{30}\text{N}_8$  [418.54].

**4.1.5.2. ethyl 4-((4-(4-amino-6-(mesitylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzoate (**6a2**).** White powder, yield: 69.4%. Mp: 141–143 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , ppm)  $\delta$ : 7.99 (d, 2H,  $J = 7.8$  Hz, PhH), 7.39 (s, 2H, PhH), 6.91 (s, 2H, PhH), 4.80 (m, 3H), 4.37 (q, 2H,  $J = 7.2$  Hz,  $\text{CH}_2$ ), 3.80 (m, 1H), 3.55 (s, 2H,  $\text{CH}_2$ ), 2.77 (s, 2H), 2.29 (s, 3H,  $\text{CH}_3$ ), 2.19 (s, 6H,  $2 \times \text{CH}_3$ ),

1.94 (s, 3H), 1.44 (m, 2H), 1.39 (t, 3H,  $\text{CH}_3$ ). IR (KBr,  $\text{cm}^{-1}$ ): 3386 ( $\nu_{\text{NH}}^{\text{as}}$ ), 3316 ( $\nu_{\text{NH}}$ ), 2939 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1718 ( $\nu_{\text{C=O}}$ ), 1560, 1494 ( $\nu_{\text{C=N}}$ ), 1276 ( $\nu_{\text{C-O-C}}^{\text{as}}$ ), 1106 ( $\nu_{\text{C-O-C}}^{\text{s}}$ ), 813 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  490.9 ( $M+1$ ), 246.0 ( $(M+2)/2$ ).  $\text{C}_{27}\text{H}_{35}\text{N}_7\text{O}_2$  [489.61].

**4.1.5.3. 4-((4-(4-amino-6-(mesitylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzamide (**6a3**).** White powder, yield: 71.2%. Decomposed at 266 °C.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , ppm)  $\delta$ : 7.82 (s, 2H, PhH), 7.35 (s, 2H, PhH), 6.82 (s, 2H, PhH), 3.48 (d, 2H,  $J = 24$  Hz), 2.76 (s, 2H), 2.20 (s, 3H,  $\text{CH}_3$ ), 2.08 (s, 6H,  $2 \times \text{CH}_3$ ), 1.88–1.99 (m, 2H), 1.66–1.76 (m, 2H), 1.39–1.49 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ , ppm)  $\delta$ : 18.79, 20.94, 32.31, 46.81, 52.72, 65.49, 127.88, 128.56, 129.01, 133.50, 134.50, 135.09, 136.38, 142.23, 165.95, 167.48, 168.27. IR (KBr,  $\text{cm}^{-1}$ ): 3397 ( $\nu_{\text{NH}}^{\text{as}}$ ), 2924 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1671 ( $\nu_{\text{C=O}}$ ), 1612 ( $\beta_{\text{NH}}$ ), 1568, 1494 ( $\nu_{\text{C=N}}$ ), 813 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  461.6 ( $M+1$ ).  $\text{C}_{25}\text{H}_{32}\text{N}_8\text{O}$  [460.57].

**4.1.5.4. 4-((4-(4-amino-6-(mesitylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzenesulfonamide (**6a4**).** White powder, yield: 70.4%. Decomposed at 242 °C.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , ppm)  $\delta$ : 7.77 (s, 2H, PhH), 7.47 (d, 2H,  $J = 9.6$  Hz, PhH), 7.31 (s, 2H), 6.82 (s, 2H, PhH), 6.11 (d, 1H,  $J = 3.0$  Hz), 3.51 (d, 2H,  $J = 24.6$  Hz), 2.76 (s, 2H), 2.20 (s, 3H,  $\text{CH}_3$ ), 2.08 (s, 6H,  $2 \times \text{CH}_3$ ), 1.83–2.05 (m, 2H), 1.62–1.80 (m, 2H), 1.45–1.55 (m, 2H). IR (KBr,  $\text{cm}^{-1}$ ): 3457 ( $\nu_{\text{NH}}^{\text{as}}$ ), 3420 ( $\nu_{\text{NH}}$ ), 2946 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1556, 1486 ( $\nu_{\text{C=N}}$ ), 1369 ( $\nu_{\text{O=S=O}}^{\text{as}}$ ), 1164 ( $\nu_{\text{O=S=O}}$ ), 1097 ( $\nu_{\text{S=O}}$ ), 813 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  497.6 ( $M+1$ ), 249.5 ( $(M+2)/2$ ).  $\text{C}_{24}\text{H}_{32}\text{N}_8\text{O}_2\text{S}$  [496.63].

**4.1.5.5. *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-(4-(methylsulfonyl)benzyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (6a5).**

White powder, yield: 66.8%. Decomposed at 242 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.89 (d, 2H, *J* = 6.6 Hz, PhH), 7.53 (s, 2H, PhH), 6.91 (s, 2H, PhH), 4.83 (m, 3H), 3.80 (m, 1H), 3.57 (s, 2H, CH<sub>2</sub>), 3.06 (s, 3H, CH<sub>3</sub>), 2.75 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.94 (s, 2H), 1.43 (s, 2H). IR (KBr, cm<sup>-1</sup>): 3422 (ν<sub>NH</sub>), 2943 (ν<sub>CH<sub>3</sub></sub>), 1560, 1513 (ν<sub>C=N</sub>), 1372 (ν<sub>O=S=O</sub>), 1150 (ν<sub>O=S=O</sub>), 1090 (ν<sub>S=O</sub>), 812 (ω<sub>C=N</sub>). ESI-MS: *m/z* 496.6 (M+1). C<sub>25</sub>H<sub>33</sub>N<sub>7</sub>O<sub>2</sub>S [495.64].

**4.1.5.6. *N*<sup>2</sup>-(1-benzylpiperidin-4-yl)-*N*<sup>4</sup>-mesityl-1,3,5-triazine-2,4,6-triamine (6a6).**

White powder, yield: 68.5%. Mp: 253–255 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.31 (s, 4H, PhH), 6.90 (s, 2H, PhH), 4.75 (m, 4H), 3.90 (m, 1H), 3.52 (s, 2H, CH<sub>2</sub>), 2.81 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.96 (s, 4H), 1.46 (s, 2H). IR (KBr, cm<sup>-1</sup>): 3463 (ν<sub>NH</sub>), 3402 (ν<sub>NH</sub>), 3309 (ν<sub>NH</sub>), 2934 (ν<sub>CH<sub>3</sub></sub>), 1557, 1486 (ν<sub>C=N</sub>), 811 (ω<sub>C=N</sub>). ESI-MS: *m/z* 418.4 (M+1), 209.9 ((M+2)/2). C<sub>24</sub>H<sub>31</sub>N<sub>7</sub> [417.55].

**4.1.5.7. *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-(1-(4-nitrobenzyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (6a7).**

White powder, yield: 69.1%. Mp: 248–250 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 8.17 (s, 2H, PhH), 7.50 (s, 2H, PhH), 6.91 (s, 2H, PhH), 4.84 (m, 4H), 3.80 (m, 1H), 3.58 (s, 2H, CH<sub>2</sub>), 2.74 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.94 (s, 2H), 1.43 (s, 2H). IR (KBr, cm<sup>-1</sup>): 3456 (ν<sub>NH</sub>), 3411 (ν<sub>NH</sub>), 3309 (ν<sub>NH</sub>), 2920 (ν<sub>CH<sub>3</sub></sub>), 1557, 1483 (ν<sub>C=N</sub>), 1518 (ν<sub>NO<sub>2</sub></sub>), 813 (ω<sub>C=N</sub>). ESI-MS: *m/z* 463.4 (M+1), 232.3 ((M+2)/2). C<sub>24</sub>H<sub>30</sub>N<sub>8</sub>O<sub>2</sub> [462.55].

**4.1.5.8. 4-((4-(4-amino-6-(mesitylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (6a8).**

White powder, yield: 71.0%. Decomposed at 228 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.60 (d, 2H, *J* = 7.2 Hz, PhH), 7.44 (s, 2H, PhH), 6.91 (s, 2H, PhH), 4.84 (m, 3H), 3.54 (s, 2H, CH<sub>2</sub>), 2.74 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.96 (s, 2H), 1.45 (s, 2H), 1.26 (s, 2H). IR (KBr, cm<sup>-1</sup>): 3397 (ν<sub>NH</sub>), 2923 (ν<sub>CH<sub>3</sub></sub>), 2228 (ν<sub>C≡N</sub>), 1564, 1497 (ν<sub>C=N</sub>), 813 (ω<sub>C=N</sub>). ESI-MS: *m/z* 443.6 (M+1), 222.3 ((M+2)/2). C<sub>25</sub>H<sub>30</sub>N<sub>8</sub> [442.56].

**4.1.5.9. *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-methyl-*N*<sup>6</sup>-(1-(pyridin-4-ylmethyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (6b1).**

Pale yellow powder, yield: 67.7%. Mp: 134–136 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 8.54 (d, 2H, *J* = 3.6 Hz, PhH), 6.90 (s, 2H, PhH), 6.23 (m, 1H), 4.95 (s, 2H), 3.49 (s, 2H, CH<sub>2</sub>), 2.94 (s, 3H, NCH<sub>3</sub>), 2.77 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.20 (s, 6H, 2 × CH<sub>3</sub>), 2.04 (m, 2H), 1.89 (m, 2H), 1.50 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, ppm) δ: 18.59, 20.99, 27.52, 32.28, 52.38, 61.58, 123.79, 128.67, 136.12, 147.88, 149.79. IR (KBr, cm<sup>-1</sup>): 3423 (ν<sub>NH</sub>), 3261 (ν<sub>NH</sub>), 2943 (ν<sub>CH<sub>3</sub></sub>), 1560, 1513 (ν<sub>C=N</sub>), 812 (ω<sub>C=N</sub>). ESI-MS: *m/z* 433.7 (M+1), 217.6 ((M+2)/2). C<sub>24</sub>H<sub>32</sub>N<sub>8</sub> [432.56].

**4.1.5.10. ethyl 4-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzoate (6b2).**

White powder, yield: 70.4%. Mp: 127–129 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.99 (d, 2H, *J* = 8.4 Hz, PhH), 7.38 (d, 2H, *J* = 8.4 Hz, PhH), 6.89 (s, 2H, PhH), 4.84 (s, 2H), 4.37 (q, 2H, *J* = 7.2 Hz, CH<sub>2</sub>), 3.54 (s, 2H, CH<sub>2</sub>), 2.91 (s, 3H, NCH<sub>3</sub>), 2.76 (s, 2H), 2.28 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.97 (m, 2H), 1.46 (m, 2H), 1.39 (t, 3H, *J* = 7.2 Hz, CH<sub>3</sub>). IR (KBr, cm<sup>-1</sup>): 3400 (ν<sub>NH</sub>), 3263 (ν<sub>NH</sub>), 2941 (ν<sub>CH<sub>3</sub></sub>), 1718 (ν<sub>C=O</sub>), 1560, 1513 (ν<sub>C=N</sub>), 1275 (ν<sub>C=O-C</sub>), 1106 (ν<sub>C=O-C</sub>), 812 (ω<sub>C=N</sub>). ESI-MS: *m/z* 504.6 (M+1), C<sub>28</sub>H<sub>37</sub>N<sub>7</sub>O<sub>2</sub> [503.64].

**4.1.5.11. 4-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzamide (6b3).**

White powder, yield: 66.7%. Decomposed at 237 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, ppm) δ: 7.92 (s, 2H, PhH), 7.82 (s, 2H), 7.33 (d, 3H,

*J* = 23.4 Hz), 6.82 (s, 2H, PhH), 6.53 (m, 1H), 3.48 (d, 2H, *J* = 25.2 Hz), 2.74 (s, 3H, NCH<sub>3</sub>), 2.60 (d, 2H, *J* = 9.0 Hz), 2.20 (s, 3H, CH<sub>3</sub>), 2.07 (s, 6H, 2 × CH<sub>3</sub>), 1.91–1.99 (m, 2H), 1.66–1.77 (m, 2H), 1.40–1.50 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm) δ: 18.81, 20.94, 27.59, 32.23, 47.31, 52.81, 62.23, 127.86, 128.51, 128.91, 133.43, 134.59, 135.01, 136.33, 142.52, 165.66, 166.90, 168.31. IR (KBr, cm<sup>-1</sup>): 3402 (ν<sub>NH</sub>), 3271 (ν<sub>NH</sub>), 2923 (ν<sub>CH<sub>3</sub></sub>), 1674 (ν<sub>C=O</sub>), 1613 (β<sub>NH</sub>), 1556, 1516 (ν<sub>C=N</sub>), 812 (ω<sub>C=N</sub>). ESI-MS: *m/z* 475.5 (M+1), C<sub>26</sub>H<sub>34</sub>N<sub>8</sub>O [474.60].

**4.1.5.12. 4-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzenesulfonamide (6b4).**

White powder, yield: 68.3%. Mp: 170–172 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.79 (d, 2H, *J* = 7.2 Hz, PhH), 7.50 (d, 2H, *J* = 7.8 Hz, PhH), 6.79 (s, 2H, PhH), 5.15 (s, 2H, NH<sub>2</sub>), 4.92 (s, 2H), 3.88 (m, 1H), 3.55 (s, 2H, CH<sub>2</sub>), 2.91 (s, 3H, NCH<sub>3</sub>), 2.76 (s, 2H), 2.25 (s, 3H, CH<sub>3</sub>), 2.12 (s, 6H, 2 × CH<sub>3</sub>), 1.82–2.04 (m, 5H), 1.48 (m, 2H). IR (KBr, cm<sup>-1</sup>): 3382 (ν<sub>NH</sub>), 2942 (ν<sub>CH<sub>3</sub></sub>), 1571, 1494 (ν<sub>C=N</sub>), 1371 (ν<sub>O=S=O</sub>), 1160 (ν<sub>O=S=O</sub>), 1097 (ν<sub>S=O</sub>), 811 (ω<sub>C=N</sub>). ESI-MS: *m/z* 511.7 (M+1). C<sub>25</sub>H<sub>34</sub>N<sub>8</sub>O<sub>2</sub>S [510.65].

**4.1.5.13. *N*<sup>2</sup>-mesityl-*N*<sup>4</sup>-methyl-*N*<sup>6</sup>-(1-(4-(methylsulfonyl)benzyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (6b5).**

White powder, yield: 71.4%. Mp: 125–127 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.88 (d, 2H, *J* = 7.8 Hz, PhH), 7.53 (d, 2H, *J* = 7.2 Hz, PhH), 6.89 (s, 2H, PhH), 4.86 (s, 2H), 3.90 (m, 1H), 3.57 (s, 2H, CH<sub>2</sub>), 3.05 (s, 3H, CH<sub>3</sub>), 2.94 (s, 3H, NCH<sub>3</sub>), 2.76 (s, 2H), 2.28 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.90–2.09 (m, 3H), 1.50 (m, 2H). IR (KBr, cm<sup>-1</sup>): 3394 (ν<sub>NH</sub>), 3263 (ν<sub>NH</sub>), 2922 (ν<sub>CH<sub>3</sub></sub>), 1570, 1513 (ν<sub>C=N</sub>), 1371 (ν<sub>O=S=O</sub>), 1150 (ν<sub>O=S=O</sub>), 1089 (ν<sub>S=O</sub>), 812 (ω<sub>C=N</sub>). ESI-MS: *m/z* 510.6 (M+1). C<sub>26</sub>H<sub>35</sub>N<sub>7</sub>O<sub>2</sub>S [509.67].

**4.1.5.14. *N*<sup>2</sup>-mesityl-6-methoxy-*N*<sup>4</sup>-(1-(pyridin-4-ylmethyl)piperidin-4-yl)-1,3,5-triazine-2,4-diamine (6c1).**

Pale yellow powder, yield: 66.9%. Mp: 122–124 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 8.54 (s, 2H, PhH), 7.53 (s, 2H, PhH), 6.90 (d, 2H, *J* = 24 Hz, PhH), 6.23 (m, 1H), 5.06 (m, 1H), 3.93 (s, 3H, OCH<sub>3</sub>), 3.71 (s, 1H), 3.50 (s, 2H, CH<sub>2</sub>), 2.78 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.98 (m, 2H), 1.80 (m, 2H), 1.49 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm) δ: 18.10, 20.41, 31.30, 47.30, 52.24, 53.09, 60.69, 123.55, 128.00, 128.15, 133.29, 135.57, 147.68, 149.40, 166.03, 166.44, 170.58. IR (KBr, cm<sup>-1</sup>): 3392 (ν<sub>NH</sub>), 3249 (ν<sub>NH</sub>), 2948 (ν<sub>CH<sub>3</sub></sub>), 1577, 1499 (ν<sub>C=N</sub>), 1371 (ν<sub>C=O-C</sub>), 815 (ω<sub>C=N</sub>). ESI-MS: *m/z* 434.7 (M+1), 218.1 ((M+2)/2). C<sub>24</sub>H<sub>31</sub>N<sub>7</sub>O [433.55].

**4.1.5.15. ethyl 4-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzoate (6c2).**

White powder, yield: 69.8%. Mp: 142–144 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 8.00 (s, 2H, PhH), 7.40 (s, 2H, PhH), 6.90 (d, 2H, *J* = 22.8 Hz, PhH), 6.29 (m, 1H), 5.05 (m, 1H), 4.37 (q, 2H, *J* = 6.6 Hz, CH<sub>2</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.71 (s, 1H), 3.56 (s, 2H, CH<sub>2</sub>), 2.79 (s, 2H), 2.29 (s, 3H, CH<sub>3</sub>), 2.19 (s, 6H, 2 × CH<sub>3</sub>), 1.97 (m, 3H), 1.43 (m, 2H), 1.39 (t, 3H, *J* = 6.0 Hz, CH<sub>3</sub>). IR (KBr, cm<sup>-1</sup>): 3371 (ν<sub>NH</sub>), 3253 (ν<sub>NH</sub>), 2949 (ν<sub>CH<sub>3</sub></sub>), 1719 (ν<sub>C=O</sub>), 1575, 1499 (ν<sub>C=N</sub>), 1369 (ν<sub>C=O-C</sub>), 1275 (ν<sub>C=O-C</sub>), 1106 (ν<sub>C=O-C</sub>), 815 (ω<sub>C=N</sub>). ESI-MS: *m/z* 505.6 (M+1). C<sub>28</sub>H<sub>36</sub>N<sub>6</sub>O<sub>3</sub> [504.62].

**4.1.5.16. 4-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzamide (6c3).**

White powder, yield: 72.4%. Mp: 155–157 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm) δ: 7.73 (d, 2H, *J* = 7.8 Hz, PhH), 7.39 (d, 2H, *J* = 6.6 Hz, PhH), 6.90 (d, 2H, *J* = 29.4 Hz, PhH), 5.95 (s, 2H, NH<sub>2</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.71 (s, 1H), 3.53 (s, 2H, CH<sub>2</sub>), 2.78 (s, 2H), 2.30 (s, 3H, CH<sub>3</sub>), 2.20 (s, 6H, 2 × CH<sub>3</sub>), 1.96 (m, 2H), 1.77 (s, 2H), 1.48 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>, ppm) δ: 18.10, 20.41, 31.34, 47.42, 52.21,

53.09, 61.65, 127.29, 128.00, 128.15, 128.31, 132.87, 133.29, 135.57, 141.95, 166.03, 166.43, 167.69, 170.58. IR (KBr,  $\text{cm}^{-1}$ ): 3255 ( $\nu_{\text{NH}}$ ), 2923 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1672 ( $\nu_{\text{C=O}}$ ), 1575, 1498 ( $\nu_{\text{C=N}}$ ), 1373 ( $\nu_{\text{C-O-C}}^{\text{as}}$ ), 814 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  476.4 (M+1).  $\text{C}_{26}\text{H}_{33}\text{N}_7\text{O}_2$  [475.59].

#### 4.1.5.17. 4-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzenesulfonamide (6c4).

White powder, yield: 71.1%. Mp: 160–162 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , ppm)  $\delta$ : 7.81 (d, 2H,  $J = 7.2$  Hz, PhH), 7.46 (d, 2H,  $J = 7.2$  Hz, PhH), 6.84 (d, 2H,  $J = 34.8$  Hz, PhH), 6.35 (m, 1H), 5.16 (s, 2H,  $\text{NH}_2$ ), 3.91 (s, 3H,  $\text{OCH}_3$ ), 3.71 (s, 1H), 3.55 (s, 2H,  $\text{CH}_2$ ), 2.78 (s, 2H), 2.25 (s, 3H,  $\text{CH}_3$ ), 2.13 (s, 6H,  $2 \times \text{CH}_3$ ), 1.97 (m, 2H), 1.84 (m, 2H), 1.49 (m, 2H). IR (KBr,  $\text{cm}^{-1}$ ): 3350 ( $\nu_{\text{NH}}$ ), 2924 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1580, 1400 ( $\nu_{\text{C=N}}$ ), 1385 ( $\nu_{\text{O=S=O}}$ ), 1161 ( $\nu_{\text{O=S=O}}$ ), 1098 ( $\nu_{\text{S=O}}$ ), 814 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  512.7 (M+1).  $\text{C}_{25}\text{H}_{33}\text{N}_7\text{O}_3\text{S}$  [511.64].

#### 4.1.5.18. $N^2$ -mesityl-6-methoxy- $N^4$ -(1-(4-(methylsulfonyl)benzyl)piperidin-4-yl)-1,3,5-triazine-2,4-diamine (6c5).

White powder, yield: 72.0%. Mp: 121–123 °C.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , ppm)  $\delta$ : 7.88 (d, 2H,  $J = 7.2$  Hz, PhH), 7.53 (d, 2H,  $J = 7.2$  Hz, PhH), 6.90 (d, 2H,  $J = 22.8$  Hz, PhH), 3.93 (s, 3H,  $\text{OCH}_3$ ), 3.71 (s, 1H), 3.57 (s, 2H,  $\text{CH}_2$ ), 3.05 (s, 3H,  $\text{CH}_3$ ), 2.76 (s, 2H), 2.29 (s, 3H,  $\text{CH}_3$ ), 2.19 (s, 6H,  $2 \times \text{CH}_3$ ), 1.98 (m, 2H), 1.68 (m, 2H), 1.46 (m, 2H). IR (KBr,  $\text{cm}^{-1}$ ): 3365 ( $\nu_{\text{NH}}$ ), 3257 ( $\nu_{\text{NH}}$ ), 2923 ( $\nu_{\text{CH}_3}^{\text{as}}$ ), 1578, 1498 ( $\nu_{\text{C=N}}$ ), 1384 ( $\nu_{\text{O=S=O}}$ ), 1150 ( $\nu_{\text{O=S=O}}$ ), 1089 ( $\nu_{\text{S=O}}$ ), 815 ( $\omega_{\text{C=N}}$ ). ESI-MS:  $m/z$  511.6 (M+1).  $\text{C}_{26}\text{H}_{34}\text{N}_6\text{O}_3\text{S}$  [510.65].

## 4.2. In vitro anti-HIV activity assays

Evaluation of the antiviral activity of the compounds against HIV-1 strain IIB and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described.<sup>24</sup> Stock solutions ( $10 \times$  final concentration) of test compounds were added in 25- $\mu\text{L}$  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(IIB)<sup>30</sup> or HIV-2 (ROD)<sup>31</sup> stock (50  $\mu\text{L}$ ) at 100–300 CCID<sub>50</sub> (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<sup>32</sup> were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at  $6 \times 10^5$  cells/mL, and 50- $\mu\text{L}$  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 (Multiscan Ascent 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 ( $\text{CC}_{50}$ ) was defined as the concentration of the test compound that reduced the absorbance (OD<sub>540</sub>) 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 ( $\text{EC}_{50}$ ).

## Acknowledgments

The financial support from the National Natural Science Foundation of China (NSFC Nos. 81102320, 30873133, 30772629, 30371686), Key Project of NSFC for International Cooperation (No. 30910103908), Research Fund for the Doctoral Program of Higher Education of China (Nos. 20110131130005, 20110131120037), Independent Innovation Foundation of Shandong University (IIFSDU, No. 2010GN044), Shandong Postdoctoral Innovation Science Research Special Program (No.201002023), China Postdoctoral Science Foundation funded project (No.20100481282) and KU Leuven (GOA 10/014) is gratefully acknowledged. We thank K. Erven, K. Uyttersprot and C. Heens for technical assistance with the HIV assays.

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