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Original article

Synthesis and biological evaluation of piperidine-substituted triazine derivatives as HIV-1 non-nucleoside reverse transcriptase inhibitors

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ABSTRACT

A novel series of piperidine-substituted triazine derivatives have been synthesized and evaluated for anti-HIV activities in MT-4 cells. Most compounds displayed extremely promising activity against wild-type HIV-1 with EC_{50} values in low nanomolar concentration, better than that of Nevirapine, Delavirdine, Zidovudine and Dideoxycitidine, and higher potency towards the resistant mutant strain K103N/Y181C than that of Nevirapine and Delavirdine. Selected compounds were also assayed against reverse transcriptase with lower IC_{50} values than that of Nevirapine. The structure-activity relationship (SAR) of these novel structural congeners was also discussed.

1. Introduction

According to the statistic data of AIDS epidemic in 2010 by WHO, there were 33.3 million people living with HIV, 2.6 million new HIV infections and 1.8 million AIDS-related deaths in 2009, therefore, AIDS is still one of the leading pandemic diseases worldwide [1]. Highly active antiretroviral therapy (HAART) provided an effective way to treat AIDS patients by dramatically decreasing the morbidity and mortality from the infection of HIV-1. However, the rapid emergence of drug resistance, serious side effects and poor patient compliance during the long-term in which the drugs are used partly compromise the clinical application of HAART. Therefore, it is an urgent need to design and develop new anti-AIDS drugs with improved potency to halt the spread of HIV.

HIV-1 non-nucleoside reverse transcriptase inhibitors (HIV-1 NNRTIs) with high antiviral potency, high specificity and low cytotoxicity have become an indispensable component in HAART regimen [2]. Among the NNRTIs, diaryltriazine (DATA) and diarylpyrimidine (DAPY) derivatives with superior activity profiles against HIV-1 have attracted considerable attention over the past few years. Etravirine (ETV, TMC125) and Rilpivirine, the representatives of DAPYs, were approved by FDA in January 2008 and May 2011 respectively (Fig. 1) [3,4]. And also, their analogue TMC120 is still under development as a vaginal HIV microbicide [5,6]. However, the pharmacokinetic profiles of most DAPYs are not satisfactory due to the low water solubility [7–9]. Recently, in order to enhance oral bioavailability, a series of piperidine-linked aminopyrimidine derivatives were reported with good potency against wild-type and several important resistant mutant strains of HIV-1 in both enzymic and cellular assays (Fig. 1) [10,11]. Inspired by their good potency and bioavailability profiles, we designed a new series of piperidine-linked triazine derivatives through incorporation of the triazine ring of DATA, a common moiety in HIV-1 NNRTIs [12,13]. Herein, we report the synthesis, anti-HIV evaluation and preliminary structure-activity relationship (SAR) of these hybrid compounds.

2. Chemistry

The synthetic route to the target compounds **6a1**–**7**, **6b1**–**7**, **6a′1**–**4** and **6b′1**–**4** is depicted in Scheme 1. 4,6-dichloro-*N*-mesityl-1,3,5-triazin-2-amine (**2**) was obtained by substitution reaction of commercially available 2,4,6-trichloro-1,3,5-triazine (**1**) with 2,4,6-trimethylaniline in ice bath [14], which was reacted with 4-amino-1-Boc-piperidine to give 6-chloro-N^2-mesityl-N^4-(1-Boc-piperidin-4-yl)- 1,3,5-triazine-2,4-diamine (**3**) [15]. The chlorine atom of intermediate **3** was transformed to methylamino (**4a**) and

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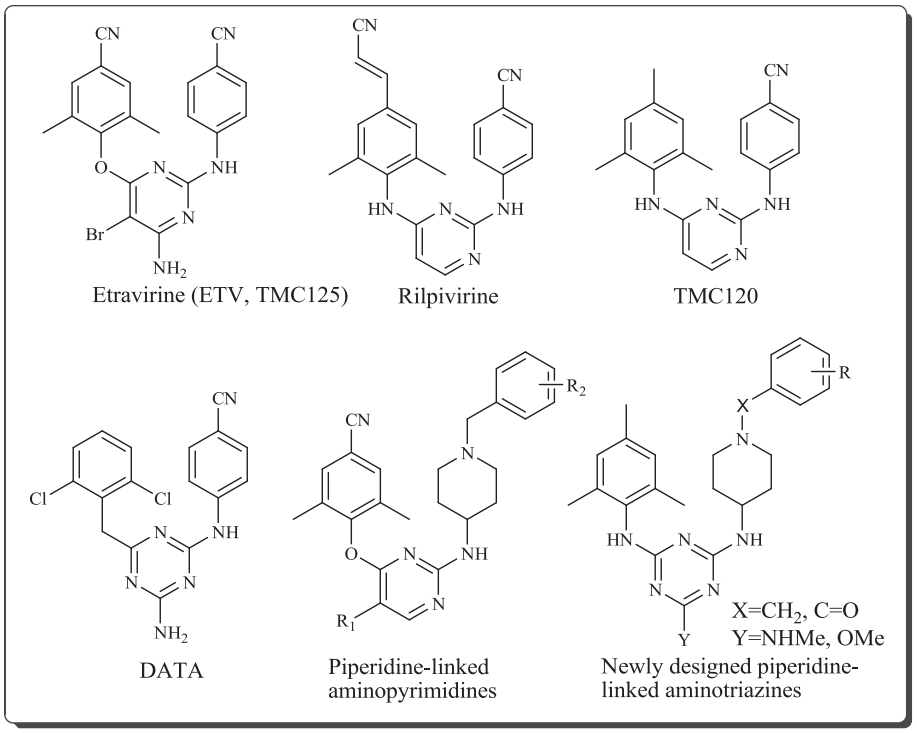


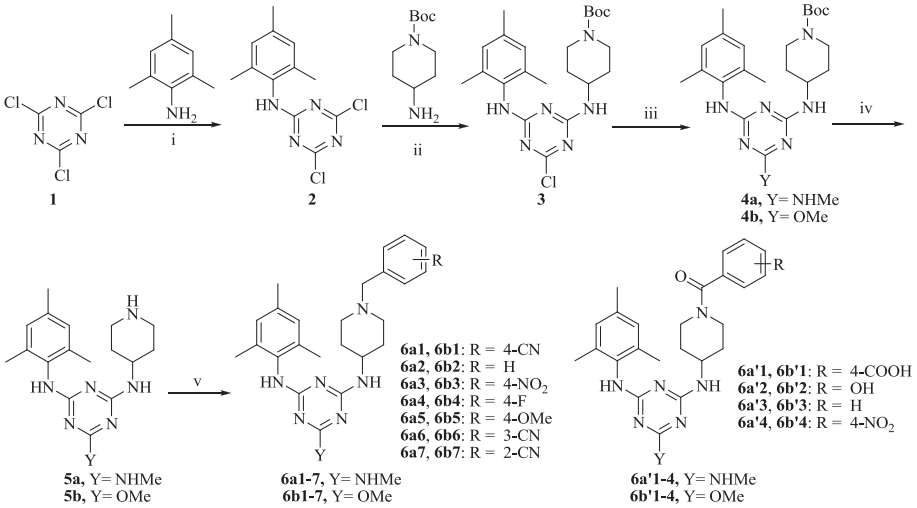
Fig. 1. The structures of DAPYs, DATA and piperidine-linked aminopyrimidine derivative.

methoxyl group (**4b**) under the conditions of NH_2CH_3 (aq)/$NaHCO_3$/THF and Na/CH_3OH respectively [16]. By removing the Boc group of **4a** and **4b** in the presence of trifluoroacetic acid (TFA) at room temperature, compounds **5a** and **5b** were prepared in good yield, which were then reacted with substituted benzyl chloride (bromine) or substituted benzoic acid, achieving the final compounds **6a1**–**7**, **6b1**–**7**, **6a′1**–**4** and **6b′1**–**4** respectively.

3. Results and discussion

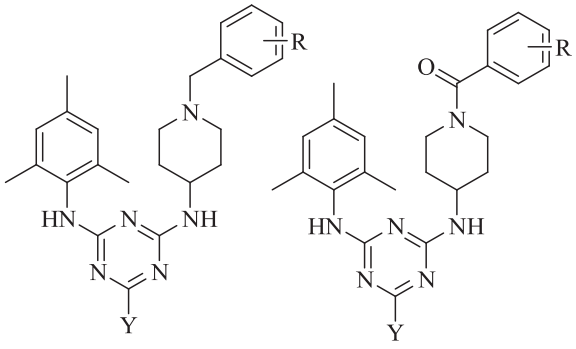
3.1. Anti-HIV activity evaluation

The newly synthesized piperidine-substituted triazine derivatives were evaluated for anti-HIV-1 (IIIB) activity in MT-4 cells using the MTT method [17–20]. Nevirapine (NVP), Delavirdine Mesylate (DLV), Efavirenz (EFV), zidovudine (azidothymidine, AZT), dideoxycitidine (DDC) and ETV were used as reference drugs (Table 1). The results demonstrated that compounds **6a1**–**7** and **6b1**–**7** exhibited higher inhibition activity against HIV-1 than that of NVP, DLV, AZT and DDC, with low EC_{50} values ranging from 7.0 to 19.2 nM, and high selectivity indices ($SI = CC_{50}/EC_{50}$) ranging from 1188 to 3240. Compounds **6a′1**–**4** and **6b′1**–**4** showed moderate activity against HIV-1, of which **6a′2** and **6a′3** were more potent than DDC. However, the new synthesized compounds are less potent than ETV against wild-type HIV-1, which showed EC_{50} value as low as 3.0 nM. Further studies of piperidine-substituted triazine derivatives will assist the design of the next generations of compounds.



Scheme 1. Reagents and conditions i: K_2CO_3, THF; ii: $NaHCO_3$ (5% aq), THF; iii: (a) NH_2CH_3 (aq), $NaHCO_3$, THF; (b) Na/CH_3OH; iv: TFA, CH_2Cl_2; v: substituted benzyl chloride (bromine), K_2CO_3, DMF; or substituted benzoic acid, *N,N′*-Carbonyldiimidazole (CDI),DMF.

Table 1
Activity against wild-type HIV-1 (III_B) in MT-4 cells by MTT method.



6a1-7, Y = NHMe **6a'1-4**, Y = NHMe
6b1-7, Y = OMe **6b'1-4**, Y = OMe

Compd	R	Y	EC_{50} (nM)[a]	CC_{50} (μM)[b]	SI[c]
6a1	4–CN	NHMe	8.5 ± 1.1	10.0 ± 5.6	1188
6a2	H	NHMe	8.8 ± 0.9	28.4 ± 2.5	3187
6a3	4–NO_2	NHMe	8.2 ± 3.1	10.2 ± 3.8	1230
6a4	4-F	NHMe	10.2 ± 0.4	25.6 ± 1.7	2884
6a5	4-OMe	NHMe	9.5 ± 0.2	22.1 ± 3.2	2310
6a6	3–CN	NHMe	7.0 ± 2.2	23.0 ± 2.4	3240
6a7	2–CN	NHMe	9.9 ± 1.3	24.7 ± 2.7	2501
6b1	4–CN	OMe	9.2 ± 2.2	14.6 ± 3.8	1598
6b2	H	OMe	11.3 ± 2.8	28.6 ± 1.8	2512
6b3	4–NO_2	OMe	9.4 ± 2.1	15.4 ± 2.1	1642
6b4	4-F	OMe	11.5 ± 0. 4	24.7 ± 2.0	2140
6b5	4-OMe	OMe	9.7 ± 1.3	19.8 ± 3.2	2043
6b6	3–CN	OMe	9.8 ± 2.2	23.5 ± 2.1	2382
6b7	2–CN	OMe	19.2 ± 7.4	28.0 ± 1.9	1460
6a′1	4–COOH	NHMe	≥127050.2	≥255.3	>orX2[d]
6a′2	4–OH	NHMe	931.6 ± 216.7	55.4 ± 2.2	60
6a′3	H	NHMe	224.4 ± 22.4	30.9 ± 1.8	132
6a′4	4–NO_2	NHMe	1793.9 ± 224.2	33.2 ± 4.5	18
6b′1	4–COOH	OMe	36183.9 ± 2160.8	≥149.2	≥4
6b′2	4–OH	OMe	1664.7 ± 108.1	121.3 ± 30.1	73
6b′3	H	OMe	1791.6 ± 447.9	28.8 ± 1.4	16
6b′4	4–NO_2	OMe	4374.0 ± 915.5	≥25.2	≥6
NVP			90.1 ± 30.0	>15.0	>168
DLV			32.6 ± 3.6	>36.2	>1096
EFV			5.4 ± 0.3	>6.3	>1187
AZT			20.6 ± 23.6	249.5 ± 12.6	12,221
DDC			1041.6 ± 426.0	>94.7	>93
ETV[e] [21]			3.0 ± 0.2	>4.6	>1537

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell against HIV-1-induced cytotoxicity, as determined by the MTT method.
[b] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] SI: selectivity index (CC_{50}/EC_{50}).
[d] X2: stands for ≥2 or <2.
[e] **ETV**: Etravirine. The data were obtained from the same laboratory (Prof. Erik De Clercq, Rega Institute for Medical Research, K.U. Leuven, Belgium).

Based on the results of antiviral assay (Table 1), some important SAR information was summarized as the following: (1) Substituents at the C6 position of triazine ring influenced the activity: *N*-benzyl analogues with Y = NHMe (**6a1**–**7**) and Y = OMe (**6b1**–**7**) showed activities in the similar order of magnitude, but all the compounds with Y = NHMe (**6a1**–**7**) were slightly more potent than the corresponding compounds with Y = OMe (**6b1**–**7**). (2) *N*-benzyl analogues (**6a1-7**, **6b1-7**) showed superior activity to the *N*-benzoyl analogues (**6a'1-4**, **6b'1-4**). (3) In the *N*-benzoyl analogues, the effect of different substituents on the activity was in a clear order: H (**6a′3**, **6b′3**) > (or ≈) 4-hydroxy (**6a′2**, **6b′2**) > 4-nitro (**6a′4**, **6b′4**) > 4-carboxyl (**6a′1**, **6b′1**).

In addition, the compounds **6a1**–**7**, **6b1**–**7**, **6a′1**–**4** and **6b′1**–**4** were also tested their ability to inhibit HIV-2 (ROD) replication in MT-4 cells. But none of them were active at subtoxic concentration,

Table 2
Activity against K103N/Y181C resistant mutant strain of HIV-1 in MT-4 cells by MTT method.

Compd	EC_{50} (μM)[a]	Compd	EC_{50} (μM)
6a1	1.3 ± 0.1	**6b1**	1.6 ± 0.1
6a2	5.0 ± 0.1	**6b2**	4.8 ± 0.02
6a3	≥2.6	**6b3**	≥6.1
6a4	4.9 ± 0.4	**6b4**	6.5 ± 1.0
6a5	5.8 ± 0.7	**6b5**	7.4 ± 0.5
6a6	4.6 ± 0.5	**6b6**	4.6 ± 0.1
6a7	4.6 ± 0.2	**6b7**	6.7 ± 1.6
6a′1	>255.3	**6b'1**	>149.2
6a′2	>55.4	**6b'2**	>121.3
6a′3	>30.9	**6b'3**	>28.8
6a′4	>33.2	**6b'4**	>25.2
NVP	11.2 ± 2.2	**AZT**	0.0086 ± 0.0015
DLV	>36.2	**EFV**	0.57 ± 0.06
ETV [21]	0.026 ± 0.004		

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell against K103N/Y181C resistant mutant strain of HIV-1, as determined by the MTT method.

Table 3
Activity of selected piperidine-substituted triazine derivatives against wild-type HIV-1 RT.

Compd	**6a2**	**6a6**	**6b1**	**NVP**	**ETV**
IC_{50} (μM)[a]	2.46	1.97	1.77	2.74	0.55

[a] IC_{50}: Inhibitory concentration required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50% (RT kit, Roche).

which means the piperidine-substituted triazine derivatives belonged to anti-HIV-1 agents.

Rapid emergence of drug resistance compromise the anti-AIDS therapy clinically, thus, the new analogues were also assayed against the K103N/Y181C resistant mutant strain of HIV-1, which is a frequently encountered double mutant in clinical. The results were illustrated in Table 2 together with NVP, DLV, EFV, AZT and ETV as reference standards. As the data indicated, most *N*-benzyl analogues (**6a1**–**7**, **6b1**–**7**) retained higher activity against the double mutant virus than NVP and DLV, but less than AZT, EFV and ETV. Among the *N*-benzyl analogues, **6a1** (EC_{50} = 1.3 μM) and **6b1** (EC_{50} = 1.6 μM) were the most potent analogues, however, ETV displayed very promising activity (EC_{50} = 0.026 μM) against the resistant mutant strain of HIV-1. All the *N*-benzoyl analogues (**6a′1**–**4** and **6b′1**–**4**) lost their activities against K103N/Y181C resistant mutant strain.

3.2. Inhibition of HIV-1 RT

Meanwhile, for identification of the binding target, some active compounds (**6a2**, **6a6** and **6b1**) were selected to carry out an HIV-1 RT inhibitory assay, using poly(rA)-oligo(dT) as template primer (Table 3) [20,22]. The results indicated that the tested compounds displayed higher inhibitory activity than that of NVP (IC_{50} = 2.74 μM). Though the new compounds were less potent than ETV (IC_{50} = 0.55 μM), it suggests that the newly synthesized piperidine-substituted triazine derivatives bind to HIV-1 RT with good affinity, and belong to HIV-1 NNRTIs.

4. Conclusion

In summary, a series of novel piperidine-substituted triazine derivatives were synthesized *via* an expeditious route, and evaluated for their antiviral activity against of HIV-1 (IIIB strain and K103N/Y181C resistant mutant strains) and HIV-2 (ROD strain) in MT-4 cells, as well as HIV-1 RT inhibitory activity (some selected compounds).

Among them, compound **6a6** was identified as the most promising candidate (EC_{50} = 7.0 nM, SI = 3240), which may contribute to the discovery of potential anti-HIV-1 agents. Further studies are ongoing in our laboratories and will be reported in due course.

5. Experimental Section

5.1. 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. ^{1}H-NMR spectra were obtained on a Brucker Avance-600 NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapours or by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with Silica Gel 60 (230–400 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.

5.1.1. General procedure for the synthesis of N^2-mesityl-N^4-methyl-N^6-(piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**5a**) and N^2-mesityl-6-methoxy-N^4-(piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**5b**)

4,6-dichloro-*N*-mesityl-1,3,5-triazin-2-amine (**2**): To a solution of cyanuric chloride (**1**, 0.37 g, 2 mmol) in THF (15 mL) at 0 °C were slowly added K_2CO_3 (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). After removal of the solvent under reduced pressure, water (20 mL) was added and extracted with CH_2Cl_2 (2 × 10 mL). Combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification on silica gel gave **2** as an off-white solid. Yield: 91%, mp: 197–199 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 10.45 (s, 1H, NH), 6.94 (s, 2H, PhH), 2.25 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3). ESI-MS: *m*/*z* 283.3 (M + 1), 285.3 (M + 3), 287.3 (M + 5).

6-chloro-N^2-mesityl-N^4-(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_2O (4:1:1, 30 mL) was treated with 4-amino-1-Boc-piperidine (0.4 g, 2 mmol) and 5% w/v aqueous $NaHCO_3$ (8 mL). The mixture was stirred at 30 °C for 8 h. 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_2SO_4, 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. ^{1}H-NMR (DMSO-d_6, 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_3), 2.07 (d, 6H,2 × CH_3), 1.37 (s, 9H). ESI-MS: *m*/*z* 447.5 (M + 1), 448.5 (M + 2), 449.5 (M + 3).

N^2-mesityl-N^4-methyl-N^6-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**4a**): Intermediate **3** (0.22 g, 0.5 mmol) was dissolved in THF, followed by addition of saturated $NaHCO_3$ (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_2SO_4, filtered and concentrated under reduced pressure to give the product (**4a**) as white solid. Yield: 96%, mp: 131–133 °C, ESI-MS: *m*/*z* 442.5 (M + 1), 443.5 (M + 2).

N^2-mesityl-6-methoxy-N^4-(1-Boc-piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**4b**): Na (0.35 g, 15 mmol) was dissolved in absolute MeOH (50 mL) followed by addition of 6-chloro-N^2-mesityl-N^4-(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_2SO_4, filtered and concentrated under reduced pressure to give the product (**4b**) as white solid. Yield: 94%, mp: 190–192 °C, ESI-MS: *m*/*z* 443.6 (M + 1), 444.6 (M + 2).

N^2-mesityl-N^4-methyl-N^6-(piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**5a**): TFA (1 mL, 13.5 mmol) was added dropwise under stirring to a solution of intermediate **4a** (0.3 g, 0.67 mmol) in CH_2Cl_2 (10 mL) at room temperature and stirred overnight. The reaction mixture was neutralized with 2N NaOH to pH8, and the organic layer was separated and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the product (**5a**) as white solid. Yield: 95%, mp: 139–141 °C, ESI-MS: *m*/*z* 342.4 (M + 1), 171.8 ((M + 2)/2).

N^2-mesityl-6-methoxy-N^4-(piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**5b**): Using intermediate **4b** (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 **5b** as a white solid. Yield: 95%, mp: 225–227 °C, ESI-MS: *m*/*z* 343.5 (M + 1), 172.4 ((M + 2)/2).

5.1.2. General procedure for the synthesis of target compounds **6a1–7** and **6b1–7**

Compounds **5a** (or **5b**) was dissolved in anhydrous DMF (10 mL) in the presence of anhydrous K_2CO_3 (2 eq) at 0 °C, followed by addition of appropriate substituted benzyl chloride (bromine) (1.1 eq). 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 × 10 mL), and the organic phase was washed with saturated sodium chloride (10 mL), and dried over anhydrous Na_2SO_4 to give the corresponding crude product, which was purified by flash column chromatography to afford compounds **6a1–7** (or **6b1–7**).

5.1.3. 4-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6a1**)

White powder, yield: 68.4%. Mp: 121–123 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.78 (s, 2H, PhH), 7.49 (d, 2H, PhH), 6.82 (s, 2H, PhH), 6.45 (m, 2H), 3.53 (d, 2H, CH_2), 2.71 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.96 (m, 2H, CH_2), 1.78 (m, 2H, CH_2), 1.45 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3416 (υ_{NH}), 2943 (υ^{as}_{CH3}), 2228 ($\upsilon_{C\equiv N}$), 1567, 1513 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 457.5 (M + 1), 229.4 ((M + 2)/2). $C_{26}H_{32}N_8$ (456.27).

5.1.4. N^2-(1-Benzylpiperidin-4-yl)-N^4-mesityl-N^6-methyl-1,3,5-triazine-2,4,6-triamine (**6a2**)

White powder, yield: 53.6%. Mp: 111–113 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.27 (m, 5H, PhH), 6.81 (s, 2H, PhH), 6.53 (m, 2H), 3.43 (d, 2H, CH_2), 2.73 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.94 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.44 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3420 (υ_{NH}), 2941 (υ^{as}_{CH3}), 1563, 1513 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 432.5 (M + 1), 216.9 ((M + 2)/2). $C_{25}H_{33}N_7$ (431.28).

5.1.5. N^2-mesityl-N^4-methyl-N^6-(1-(4-nitrobenzyl)piperidin-4-yl)-1,3,5-triazine-2,4,6-triamine (**6a3**)

White powder, yield: 65.2%. Mp: 127–129 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 8.32 (d, 2H, J = 9.6 Hz, PhH), 7.57 (d, 2H, J = 9.6 Hz, PhH), 6.82 (s, 2H, PhH), 6.52 (m, 2H), 3.58 (d, 2H, CH_2), 2.74 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.01 (s, 6H, 2 × CH_3), 1.79 (m, 2H, CH_2), 1.53 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3415 (υ_{NH}), 2943 (υ^{as}_{CH3}), 1567 ($\upsilon_{C=N}$), 1518 (υ^{as}_{NO2}), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 477.3 (M + 1), 478.3 (M + 2). $C_{25}H_{32}N_8O_2$ (476.26).

*5.1.6. N^2-(1-(4-fluorobenzyl)piperidin-4-yl)-N^4-mesityl-N^6-methyl-1,3,5-triazine-2,4,6-triamine (**6a4**)*

White powder, yield: 55.7%. Mp: 118–120 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.29 (s, 2H, PhH), 7.13 (d, 2H, PhH), 6.81 (s, 2H, PhH), 6.44 (m, 2H), 3.41 (d, 2H, CH_2), 2.74 (s, 3H, N–CH_3), 2.60 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.91 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.43 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3422 (υ_{NH}), 2943 ($\upsilon^{as}{}_{CH3}$), 1570, 1509($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m/z* 450.3 (M + 1), 451.3 (M + 2). $C_{25}H_{32}FN_7$ (449.27).

*5.1.7. N^2-mesityl-N^4-(1-(4-methoxybenzyl)piperidin-4-yl)-N^6-methyl-1,3,5-triazine-2,4,6-triamine (**6a5**)*

White powder, yield: 52.4%. Mp: 155–157 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.18 (d, 2H, PhH), 6.86 (s, 2H, PhH), 6.81 (s, 2H, PhH), 6.43 (m, 2H), 3.73 (s, 3H, O–CH_3), 3.37 (s, 1H), 2.74 (s, 3H, N–CH_3), 2.60 (d, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.90 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.42 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3416 (υ_{NH}), 2938 ($\upsilon^{as}{}_{CH3}$), 1567, 1511 ($\upsilon_{C=N}$), 1246 ($\upsilon^{as}{}_{=C-O-C}$), 812 ($\omega_{C=N}$). ESI-MS: *m/z* 462.3 (M + 1), 463.3 (M + 2) $C_{26}H_{35}N_7O$ (461.29).

*5.1.8. 3-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6a6**)*

White powder, yield: 64.8%. Mp: 112–114 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.72 (m, 2H, PhH), 7.63 (s, 1H, PhH), 7.55 (m, 1H, PhH), 6.81 (s, 2H, PhH), 6.45 (m, 2H), 3.50 (d, 2H, CH_2), 3.17 (d, 1H), 2.74 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.72 (m, 2H, CH_2), 1.46 (m, 2H, CH_2). IR (KBr, cm^{-1}): 3404 (υ_{NH}), 2941 ($\upsilon^{as}{}_{CH3}$), 2229 ($\upsilon_{C\equiv N}$), 1571, 1511 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m/z* 457.3 (M + 1), 458.3 (M + 2). $C_{26}H_{32}N_8$ (456.27).

*5.1.9. 2-((4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6a7**)*

White powder, yield: 67.1%. Mp: 129–131 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.80 (s, H, PhH), 7.67 (s, 1H, PhH), 7.55 (s, 1H, PhH), 7.46 (s, 1H, PhH), 6.82 (s, 2H, PhH), 6.46 (m, 2H), 3.61 (d, 2H, CH_2), 2.75 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.74 (m, 2H, CH_2), 1.45 (m, 4H). IR (KBr, cm^{-1}): 3414 (υ_{NH}), 2943 ($\upsilon^{as}{}_{CH3}$), 2224 ($\upsilon_{C\equiv N}$), 1562, 1513 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m/z* 457.3 (M + 1), 458.3 (M + 2). $C_{26}H_{32}N_8$ (456.27).

*5.1.10. 4-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6b1**)*

White powder, yield: 61.2%. Mp: 106–108 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.80 (s, H, PhH), 7.67 (s, 1H, PhH), 7.55 (s, 1H, PhH), 7.46 (s, 1H, PhH), 6.82 (s, 2H, PhH), 6.46 (m, 2H), 3.61 (d, 2H, CH_2), 2.75 (s, 3H, N–CH_3), 2.61 (s, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.74 (m, 2H, CH_2), 1.45 (m, 4H). IR (KBr, cm^{-1}): 3377 (υ_{NH}), 2948 ($\upsilon^{as}{}_{CH3}$), 2227 ($\upsilon_{C\equiv N}$), 1575, 1498 ($\upsilon_{C=N}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 458.5 (M+1), 459.5 (M + 2), 480.4 (M + Na). $C_{26}H_{31}N_7O$ (457.26).

*5.1.11. N^2-(1-benzylpiperidin-4-yl)-N^4-mesityl-6-methoxy-1,3,5-triazine-2,4-diamine (**6b2**)*

White powder, yield: 55.7%. Mp: 104–106 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.20–7.34 (m, 5H, PhH), 7.13 (d, 1H), 6.84 (s, 2H, PhH), 3.81 (s, 3H, O–CH_3), 3.62 (s, 2H, CH_2), 3.46 (s, 1H), 3.41 (s, 1H), 2.80 (m, 1H), 2.74 (m, 1H), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.68 (m, 2H, CH_2), 1.39 (m, 2H). IR (KBr, cm^{-1}): 3393 (υ_{NH}), 2946 ($\upsilon^{as}{}_{CH3}$), 1574, 1497 ($\upsilon_{C=N}$), 1370 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 433.6 (M + 1), 434.5 (M + 2). $C_{25}H_{32}N_6O$ (432.26).

*5.1.12. N^2-mesityl-6-methoxy-N^4-(1-(4-nitrobenzyl)piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**6b3**)*

White powder, yield: 63.9%. Mp: 119–121 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 8.20 (m, 2H, PhH), 7.60 (m, 2H, PhH), 7.17 (m, 1H), 6.85 (s, 2H, PhH), 3.80 (s, 3H, O–CH_3), 3.60 (d, 2H, CH_2), 3.56 (s, 1H), 2.81 (m, 1H), 2.74 (m, 1H), 2.22 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 2.00 (m, 1H), 1.81 (m, 1H), 1.71 (m, 1H), 1.55 (m, 1H), 1.43 (m, 1H). IR (KBr, cm^{-1}): 3394 (υ_{NH}), 2946 ($\upsilon^{as}{}_{CH3}$), 1575, 1520 ($\upsilon_{C=N}$), 1498 ($\upsilon^{as}{}_{NO2}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 478.3 (M + 1), 479.3 (M + 2). $C_{25}H_{31}N_7O_3$ (477.25).

*5.1.13. N^2-(1-(4-fluorobenzyl)piperidin-4-yl)-N^4-mesityl-6-methoxy-1,3,5-triazine-2,4-diamine (**6b4**)*

White powder, yield: 54.4%. Mp: 123–125 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.30 (m, 2H, PhH), 7.12 (m, 2H, PhH), 7.17 (m, 1H, PhH), 6.84 (s, 2H, PhH), 3.79 (s, 3H, O–CH_3), 3.59 (s, 2H, CH_2), 3.44 (s, 1H), 3.39 (s, 1H), 2.78 (m, 1H), 2.72 (m, 1H), 2.22 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 2.94 (m, 2H), 1.80 (m, 1H), 1.69 (m, 1H), 1.52 (m, 1H), 1.40 (m, 1H). IR (KBr, cm^{-1}): 3391 (υ_{NH}), 2948 ($\upsilon^{as}{}_{CH3}$), 1575, 1507 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 451.3 (M + 1), 452.3 (M + 2). $C_{25}H_{31}FN_6O$ (450.25).

*5.1.14. N^2-mesityl-6-methoxy-N^4-(1-(4-methoxybenzyl)piperidin-4-yl)-1,3,5-triazine-2,4-diamine (**6b5**)*

White powder, yield: 50.2%. Mp: 141–143 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.20 (m, 2H, PhH), 6.88 (d, 2H, J = 8.4 Hz, PhH), 6.84 (s, 2H, PhH), 3.79 (s, 3H, O–CH_3), 3.73 (d, 3H), 3.59 (s, 2H, CH_2), 3.38 (s, 1H), 2.78–2.61 (m, 2H), 2.22 (d, 3H), 2.07 (s, 6H, 2 × CH_3), 1.87–1.96 (m, 2H), 1.78 (m, 1H), 1.68 (m, 1H), 1.49 (m, 1H), 1.37 (m, 1H). IR (KBr, cm^{-1}): 3364 (υ_{NH}), 2944 ($\upsilon^{as}{}_{CH3}$), 1574, 1511 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 463.3 (M + 1), 464.3 (M + 2). $C_{26}H_{34}N_6O_2$ (462.27).

*5.1.15. 3-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6b6**)*

White powder, yield: 59.7%. Mp: 192–194 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.52–7.73 (m, 3H, PhH), 7.15 (d, 1H, J = 7.8Hz, PhH), 6.86 (s, 2H, PhH), 3.77 (s, 3H, O–CH_3), 3.59 (s, 2H, CH_2), 3.38 (s, 1H), 2.71–2.78 (m, 2H), 2.22 (d, 3H), 2.07 (s, 6H, 2 × CH_3), 1.95–2.02 (m, 2H), 1.81 (m, 1H), 1.70 (m, 1H), 1.54 (m, 1H), 1.41 (m, 1H). IR (KBr, cm^{-1}): 3386 (υ_{NH}), 2947 ($\upsilon^{as}{}_{CH3}$), 2229 ($\upsilon_{C\equiv N}$), 1576, 1498 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m/z* 458.3 (M + 1), 459.3 (M + 2). $C_{26}H_{31}N_7O$ (457.26).

*5.1.16. 2-((4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methyl)benzonitrile (**6b7**)*

White powder, yield: 61.2%. Mp: 211–213 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.44–7.82 (m, 4H, PhH), 7.16–7.24 (m, 1H), 6.85 (s, 2H, PhH), 3.80 (s, 3H, O–CH_3), 3.63 (s, 1H), 3.59 (s, 2H, CH_2), 2.74–2.83 (m, 2H), 2.21 (d, 3H), 2.12 (m, 1H), 2.07 (s, 6H, 2 × CH_3), 1.79 (m, 1H), 1.71 (m, 1H), 1.54 (m, 1H), 1.41 (m, 1H). IR (KBr, cm^{-1}): 3391 (υ_{NH}), 2939 ($\upsilon^{as}{}_{CH3}$), 2223 ($\upsilon_{C\equiv N}$), 1581, 1473 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 812 ($\omega_{C=N}$). ESI-MS: *m/z* 458.3 (M + 1), 459.3 (M + 2). $C_{26}H_{31}N_7O$ (457.26).

*5.1.17. General procedure for the synthesis of target compounds **6a′1–4** and **6b′1–4***

N,N′-Carbonyldiimidazole (CDI, 1 eq) and appropriate substituted benzoic acid (1 eq) were added to anhydrous DMF at 0 °C successively, and then stirred for 30 min. Compounds **5a** (or **5b**) (1.1 eq) was added to the mixture slowly and stirred at room temperature overnight. The solvent was removed under reduced pressure, and water (20 mL) was added. Extracted with ethyl acetate (2 × 10 mL), and the organic phase was dried over anhydrous Na_2SO_4 to give the corresponding crude product, which was purified by flash column chromatography to afford compounds **6a′1–4** (or **6b′1–4**).

*5.1.18. 4-(4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidine-1-carbonyl)benzoic acid (**6a′1**)*

White powder, yield: 42.3%. Mp: 187–189 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.99 (s, 2H, PhH), 7.46 (d, 2H, PhH), 6.82 (s, 2H, PhH),

6.52–6.70 (m, 2H), 4.36 (s, 1H), 3.92–4.10 (m, 1H), 2,95–3.12 (m, 2H), 2.76 (s, 1H), 2.61 (s, 1H), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.91 (s, 2H), 1.70–1.80 (m, 2H), 1.29–1.49 (m, 2H). IR (KBr, cm^{-1}): 3401 (υ_{NH}), 2924 ($\upsilon^{as}{}_{CH3}$), 1629 ($\upsilon_{C=O}$), 1571, 1507 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 490.4 (M + 1), 491.4 (M + 2). $C_{26}H_{31}N_7O_3$ (489.25).

*5.1.19. (4-hydroxyphenyl)(4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)methanone (**6a′2**)*

White powder, yield: 44.5%. Mp: 157–159 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.21 (d, 2H, PhH), 6.82 (s, 2H, PhH), 6.78 (s, 2H, PhH), 2.75 (s, 2H), 2.61 (s, 1H), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.91 (s, 2H), 1.33 (m, 3H). IR (KBr, cm^{-1}): 3398 (υ_{NH}), 2924 ($\upsilon^{as}{}_{CH3}$), 1626 ($\upsilon_{C=O}$), 1574, 1514 ($\upsilon_{C=N}$), 811 ($\omega_{C=N}$). ESI-MS: *m*/*z* 462.3 (M + 1), 463.3 (M + 2). $C_{25}H_{31}N_7O_2$ (461.25).

*5.1.20. (4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)(phenyl)methanone (**6a′3**)*

White powder, yield: 49.4%. Mp: 142–144 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.44 (s, 2H, PhH), 7.35 (d, 2H, PhH), 6.82 (s, 2H, PhH), 6.51 (m, 2H), 4.10 (m, 1H), 3.17 (s, 2H), 2.75 (s, 1H), 2.61 (s, 1H), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.80 (m, 2H), 1.32 (m, 2H). IR (KBr, cm^{-1}): 3385 (υ_{NH}), 2921 ($\upsilon^{as}{}_{CH3}$), 1636 ($\upsilon_{C=O}$), 1571, 1496 ($\upsilon_{C=N}$), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 446.5 (M + 1), 447.4 (M + 2). $C_{25}H_{31}N_7O$ (445.26).

*5.1.21. (4-(4-(mesitylamino)-6-(methylamino)-1,3,5-triazin-2-ylamino)piperidin-1-yl)(4-nitrophenyl)methanone (**6a′4**)*

White powder, yield: 50.8%. Mp: 152–154 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 8.29 (d, 2H, PhH), 7.63 (d, 2H, PhH), 6.82 (s, 2H, PhH), 6.51–6.69 (m, 2H), 4.36 (s, 1H), 3.93–4.10 (m, 1H), 3.40–3.47 (m, 1H), 2.89–3.17 (m, 2H), 2.75 (s, 1H), 2.62 (s, 1H), 2.20 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.82 (m, 2H), 1.24–1.51 (m, 3H). IR (KBr, cm^{-1}): 3408 (υ_{NH}), 2945 ($\upsilon^{as}{}_{CH3}$), 1633 ($\upsilon_{C=O}$), 1579, 1520 ($\upsilon_{C=N}$), 1437 ($\upsilon^{as}{}_{NO2}$), 812 ($\omega_{C=N}$). ESI-MS: *m*/*z* 491.5 (M + 1), 492.4 (M + 2). $C_{25}H_{30}N_8O_3$ (490.24).

*5.1.22. 4-(4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidine-1-carbonyl)benzoic acid (**6b′1**)*

White powder, yield: 40.9%. Mp: 147–149 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 8.00 (m, 2H, PhH), 7.46 (m, 2H, PhH), 7.16–7.33 (m, 1H), 6.85 (s, 2H, PhH), 4.39 (m, 1H), 3.93–4.23 (m, 1H), 3.81 (s, 1H), 3.62 (s, 1H), 3.11 (m, 1H), 2.91–2.97 (m, 1H), 2.21 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.91 (s, 1H), 1.84 (m, 1H), 1.72 (m, 1H), 1.36–1.52 (m, 2H). IR (KBr, cm^{-1}): 2924 ($\upsilon^{as}{}_{CH3}$), 1696 ($\upsilon_{C=O}$), 1578, 1505 ($\upsilon_{C=N}$), 1372 ($\upsilon^{as}{}_{=C-O-C}$), 813 ($\omega_{C=N}$). ESI-MS: *m*/*z* 491.4 (M + 1), 492.4 (M + 2). $C_{26}H_{30}N_6O_4$ (490.23).

*5.1.23. (4-hydroxyphenyl)(4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)methanone (**6b′2**)*

White powder, yield: 42.8%. Mp: 154–156 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.18–7.25(m, 2H, PhH), 6.85 (s, 2H, PhH), 6.76–6.81 (m, 2H, PhH), 3.79–3.81 (s, 1H), 3.60 (s, 1H), 2.21 (s, 3H, CH_3), 2.08 (s, 6H, 2 × CH_3), 1.87 (s, 1H), 1.76 (s, 1H), 1.44 (m, 1H), 1.24–1.32 (m, 2H). IR (KBr, cm^{-1}): 3388 (υ_{NH}), 2952 ($\upsilon^{as}{}_{CH3}$), 1578, 1501 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 814 ($\omega_{C=N}$). ESI-MS: *m*/*z* 463.3 (M + 1), 464.3 (M + 2). $C_{25}H_{30}N_6O_3$ (462.24).

*5.1.24. (4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)(phenyl)methanone (**6b′3**)*

White powder, yield: 45.5%. Mp: 106–108 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 7.30–7.46 (m, 5H, PhH), 6.85 (s, 2H, PhH), 4.36–4.43 (m, 1H), 3.93–4.01 (m, 1H), 3.79–3.84 (d, 2H), 3.60 (s, 1H), 3.10 (s, 1H), 2.91 (m, 1H), 2.21 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.71–1.84 (m, 2H), 1.24–1.52 (m, 3H). IR (KBr, cm^{-1}): 2950 ($\upsilon^{as}{}_{CH3}$), 1575, 1497 ($\upsilon_{C=N}$), 1370 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: 447.6 (M + 1), 448.5 (M + 2). $C_{25}H_{30}N_6O_2$ (446.24).

*5.1.25. (4-(4-(mesitylamino)-6-methoxy-1,3,5-triazin-2-ylamino)piperidin-1-yl)(4-nitrophenyl)methanone (**6b′4**)*

White powder, yield: 47.2%. Mp: 123–125 °C. ^{1}H-NMR (DMSO-d_6, ppm) δ: 8.29 (m, 2H, PhH), 7.63 (m, 2H, PhH), 7.19–7.34 (m, 1H), 6.85 (s, 2H, PhH), 4.35–4.43 (m, 1H), 3.79–3.81 (d, 2H), 3.60 (s, 1H), 3.39–3.48 (m, 1H), 3.13 (m, 1H), 2.98 (m, 1H), 2.20 (s, 3H, CH_3), 2.07 (s, 6H, 2 × CH_3), 1.86 (m, 1H), 1.24–1.53 (m, 4H). IR (KBr, cm^{-1}): 3385 (υ_{NH}), 2952 ($\upsilon^{as}{}_{CH3}$), 1633 ($\upsilon_{C=O}$), 1575, 1497 ($\upsilon_{C=N}$), 1371 ($\upsilon^{as}{}_{=C-O-C}$), 815 ($\omega_{C=N}$). ESI-MS: *m*/*z* 492.4 (M + 1), 493.4 (M + 2). $C_{25}H_{29}N_7O_4$ (491.23).

5.2. Biological activity

5.2.1. In vitro anti-HIV activity assays

Evaluation of the antiviral activity of the compounds against HIV-1 strain IIIB and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [18]. Stock solutions (10 × final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(IIIB) [23] or HIV-2 (ROD) [24] stock (50 μL) at 100–300 CCID50 (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 [25] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 10^5 cells/mL, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow coloured 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 (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

5.2.2. HIV-1 RT inhibition assay

Inhibition of HIV-1 RT assay was performed by using homopolymer template/primer linked to microtiter plate, biotin-dUTP and RT with detection of ELISA for quantifying expression [22]. 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 the piperidine-substituted triazine derivatives required to inhibit biotin-dUTP incorporation by 50%.

Conflict of interest

None.

Acknowledgment

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