International Journal of Drug Design and Discovery

Volume 2 ● *Issue* 1 ● *January – March* 2011. 361-368

Synthesis of a Novel Class of Some 1,3,5-Triazine Derivatives and their Anti-HIV Activity

Sweta D. Desai[1] Kishor R. Desai[1] Kishor H. Chikhalia[2]* Christophe Pannecouque[3] and Erik De Clercq[3]

[1]Department of Chemistry, Veer Narmad South Gujarat University, Surat-395 007, Gujarat, India

[2]Department of Chemistry, Gujarat University, Ahemedabad-380 009, Gujarat, India

[3]Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium

ABSTRACT: Novel substituted s-triazine based derivatives were prepared by condensation of 2,4,6-trichloro-1,3,5-s-triazine with anisole, p-trifluoromethyl aniline and amino acid chloride, upon which the compounds were derivatized to their phenyl(thio)urea derivatives, and evaluated for their antiviral activity against human immunodefiency virus HIV-1 (III_B) and HIV-2 (ROD). The structures of novel synthesized compounds have been established on the basis of ^{1}H NMR, FTIR spectral data and elemental analysis.

KEY WORDS: s-triazine; urea; thiourea; anti-HIV activity

Introduction

The pathogenesis of HIV-1 is due to uncontrolled viral replication in CD4+ T cells[1]. Several efforts have been made in the last two decades to understand and control virus replication. In this direction HIV-1 RT has been identified as a prime target for designing inhibitors for the treatment of HIV/AIDS[2-4]. With reference to ITU (Imidoyl Thiourea) and DATAs (Diaryl Triazines), we have found the p-trifluoromethyl aniline and anisole eastern aromatic wing (Wing is the terminology used in describing HIV-1 RT/NNRTI complex structures) was optimal. Also required is formation of intramolecular hydrogen bonding to the basic core s-triazine. Consequently we focused our efforts on varying amino acetyl residue with introducing various aryl ureas and aryl thioureas keeping 2,4-disubstituents unchanged. In continuation of our endeavor to develop new, potent, selective and less toxic anti-HIV agents, we report here the synthesis of some related new substituted s-triazine based compounds and their *in-vitro* anti-HIV activity. Substituted s-triazine derivatives constitute an important class of compounds having anticancer[5], antimicrobial[6], antibacterial[7], or herbicidal activities[8]. They are also used for the treatment of HIV infection[9, 10]. Several investigators found the s-triazine nucleus as a potential scaffold for therapeutic agent against diseases caused by bacteria, malaria and cancer[11]. They may also be valuable leads bases for estrogen receptor modulators[12] and also used as bridging agents to synthesize herbicides[13]. Imidoyl thiourea based diaryl 1,3,5–triazines (DATAs) have been generated with the aim of novel class of potent non-nucleoside reverse transcriptase inhibitors[14]. Some have also been found active against Trypanosomatid Parasites[15]. Several specific synthetic protocols were developed for the preparation of a range of symmetric and non-symmetric di- and tri-substituted 1,3,5 – triazines[16]. All of the s-triazine derivatives that have wide practical applications are 2,4,6-mono, di- or tri-substituted, symmetrical and non-symmetrical compounds bearing different substituents. The most important reagent for obtaining these compounds is cyanuric chloride, because of the reactivity of its chlorine atoms towards nucleophiles[17].

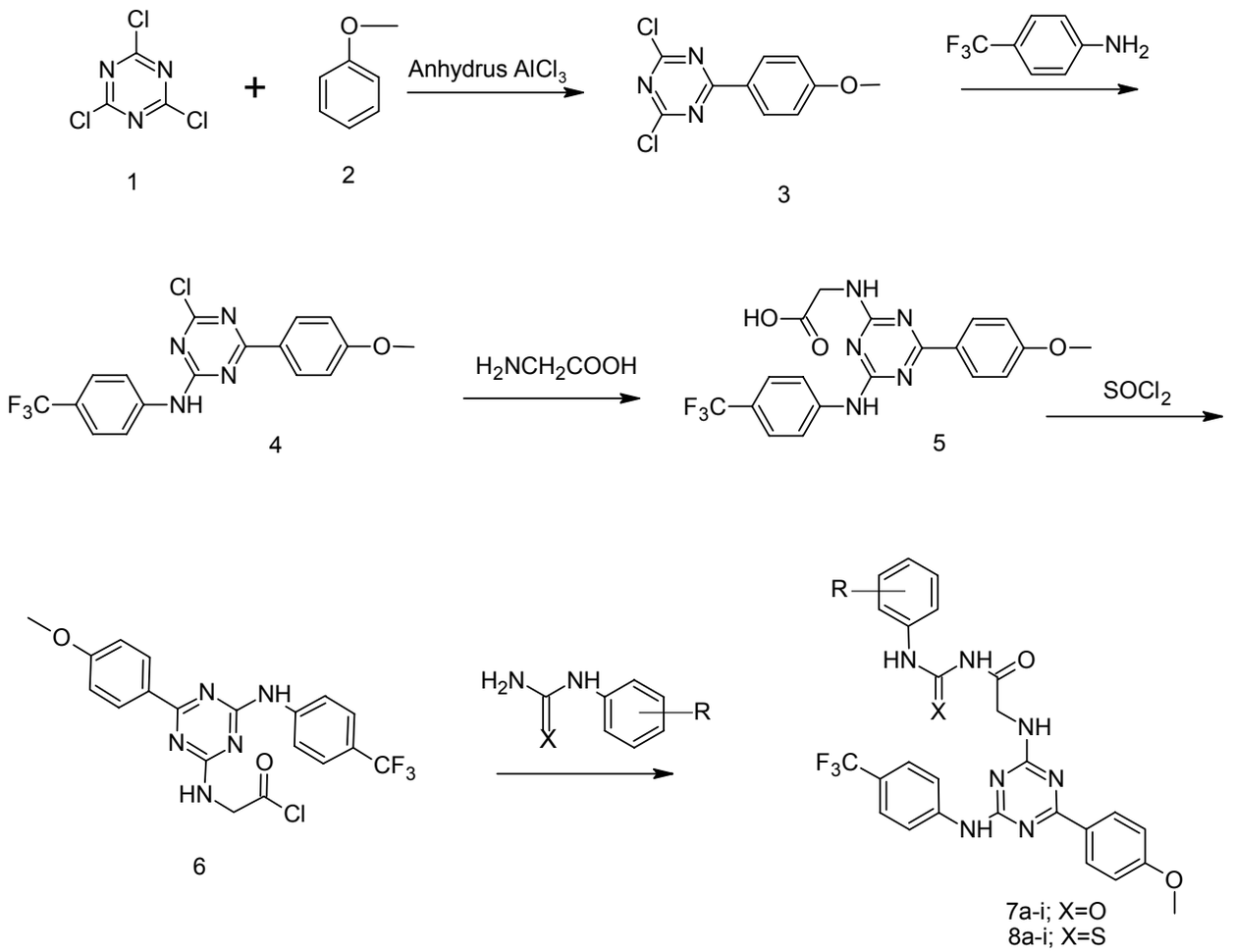
N-Phenyl urea derivatives have been assayed for their possible cytokinin-like activity[18]. Moreover, urea derivatives possess wide therapeutic activities such as hypnotic, antifungal[19], antibacterial[20], diuretic[21] as well as vascular endothelial growth factor receptor (VEGFR)[22].

* *For correspondence:* Kishor H. Chikhalia,

Tel.: 09427155529 Fax : +91 79 263 085 45

E-mail: chikhalia_kh@yahoo.com

Urea derivatives have been used for the treatment of a wide range of solid tumors[23]. Thiourea not only contributes antitubercular or antileprotic activity but also reported to possess antifungal as well as antiviral properties. Recently, the PETT (Phenyl Ethyl Thiazolyl Thiourea)[24] type of reverse transcriptase (RT) inhibitors showing high potency against HIV-1 RT with varying abilities to inhibit HIV-2 RT has been described. These pharmalogical properties drew our interest in synthesizing several new triazine based derivatives containing amino acetyl urea/thiourea moiety to obtain more potent pharmalogically active compounds.



Scheme 1 Synthesis of 7a-i and 8a-i.

Results and Discussion

Considering the versatile chemistry of 2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (aryl ureido/thioureido)}-s-triazine derivatives **(7a i/8a-i)** were prepared by the condensation of 2,4,6-trichloro-1,3,5-s-triazine **1** with anisole **2** and further modification was done by replacing two chlorine atom of the intermediate **3** with p-trifluoromethyl aniline and glycine, the product was then further treated with various substituted phenyl urea/thiourea compounds to get novel **7a-i** and **8a-i,** as shown in **Scheme 1**. The nucleophiles can selectively displace the different chlorines by control of the reaction temperature. In general, the first chlorine can be displaced when the temperature is maintained at 0^{o} C, the second between 25 and 50^{o} C and the third substitution at 65-67^{o} C, due to reactivity the temperature can exceed 80^{o} C. All the compounds were subjected to *in vitro* anti-HIV activity evaluation.

The compounds were screened for their HIV-1 and HIV-2 inhibitory activities described earlier[25]. The synthesized compounds were evaluated for *in vitro* anti-HIV activity by determining their ability to inhibit the replication of HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells in comparison with nevirapine (BIRG587) and efavirenz (DMP-266) used as reference drugs. The cytotoxicity of the compounds was determined in parallel. Comparisons of antivirally effective concentration (EC_{50}), cytotoxic concentration (CC_{50}) and SI (selectivity, given by the CC_{50}/EC_{50} ratio) values for different compounds and their results are depicted in **Table 1**. Amongst the synthesized compounds, **7d**, **7f**, **7h**, **8b**, **8c**, **8e**, **8f** and **8i** were found active against III_B with a selectivity index between 2 to 4, while compounds **7d**, **7f**, **7h**, **8b**, **8e**, **8f** and **8i** were found active against ROD with a selectivity index of 2 to 4. The remaining compounds exhibited an average to poor activity with selective index <1 (Table 1). In particular, a high activity level was observed for compounds substituted with halo and methyl substituents.

Conclusion

In summary, we synthesized and evaluated anti-HIV activity of 2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (aryl ureido/thioureido)}-s-triazine derivatives **(7a-i/8a-i)**. For most of the compounds the CC_{50} values were observed in the equal range as the IC_{50} values, which is responsible for poor potency or selectivity. Only compounds 7d, 7f, 7h, 8b, 8e, 8f, 8i displayed a selective activity against HIV-1(III_B) and HIV-2 (ROD) in MT-4 cells. It was also observed that the anti-HIV activity was enhanced by introducing groups like chloro, methyl and naphthyl ring compared to other substituents.

Table 1 Anti-HIV-1 and HIV-2 activity and cytotoxicity of compounds 7a-i and 8a-i in MT-4 cells.

Compd No.	Ar	HIV-1(III_B) EC_{50}(µg/mL)[a,c]	HIV-2(ROD) EC_{50} (µg/mL)[a c]	CC_{50} (µg/mL)[b,c]	SI (III_B)[d]	SI (ROD)[d]
7a	H	>56.98	>56.98	56.98 ± 1.76	<1	<1
7b	2-Cl	>54.55	>54.55	54.55 ± 2.26	<1	<1
7c	3-Cl	>12.25	>12.25	12.25 ± 0.47	<1	<1
7d	4-Cl	≥16.10	15.65 ± 0.35	53.43 ± 5.01	≤4	3
7e	2-CH_3	>101.37	>101.37	101.37 ± 8.03	<1	<1
7f	3-CH_3	17.90 ± 5.66	16.25 ± 3.75	44.03 ± 6.61	2	3
7g	4-CH_3	>97.43	>97.43	97.43 ± 3.97	<1	<1
7h	$C_{10}H_7$	17.65 ± 1.06	20.75 ± 2.47	53.05 ± 3.26	3	3
7i	4-OCH_3	>61.70	>61.70	61.70 ± 6.68	<1	<1
8a	H	>55.98	>55.98	55.98 ± 7.23	<1	<1
8b	2-Cl	17.70 ± 1.56	19.85 ± 1.20	53.95 ± 1.75	3	3
8c	3-Cl	16.50 ± 2.55	>56.85	56.85 ± 5.03	3	<1
8d	4-Cl	>55.35	>55.35	55.35 ± 3.30	<1	<1
8e	2-CH_3	17.60 ± 6.22	19.65 ± 2.47	61.15 ± 7.96	3	3
8f	3-CH_3	14.35 ± 5.44	16.20 ± 0.57	59.03 ± 10.42	4	4
8g	4-CH_3	>55.95	>55.95	55.95 ± 4.75	<1	<1
8h	$C_{10}H_7$	>13.70	>13.70	13.70 ± 1.37	<1	<1
8i	4-OCH_3	19.85 ± 2.76	21.05 ± 1.06	51.98 ± 3.24	3	2

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells from HIV induced cytopathogenicity, as determined by the MTT method. [b] CC_{50}: concentration of compound that reduces the viability of mock-infected cells by 50%, as determined by the MTT method. [c] All data represent mean values ± standard deviations for at least two separate experiments. [d] SI: ratio of CC_{50}/EC_{50}.

Materials and Methods

All chemicals and solvents were of analytical grade and used directly without further purification unless otherwise stated. All melting points were determined in PMP-DM scientific melting point apparatus and are uncorrected. The completion of reaction was monitored by thin-layer chromatography (TLC) using silica gel-G coated Al-plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation. ^{1}H NMR spectra were acquired on a Bruker Avance-2 model spectrophotometer using DMSO as a solvent and TMS as an internal reference (chemical shifts in δ, ppm). Infra Red spectra were recorded on Perkin Elmer-spectrum BX model spectrophotometer using KBr pallets. Elemental analyses were done on "Haraeus Rapid Analyser" and results were within ± 0.4% of the theoretical value.

Experimental Section

2-(4-Methoxy phenyl)-4,6-dichloro-s-triazine[26] (3).

To a stirred solution of cyanuric chloride (0.1 mole, 18.4 g) in toluene (30 ml) and anisole (0.1 mole, 12 ml) at 0-5 ^{o}C, $AlCl_3$ (0.1 mole, 13.33 g) in toluene (30 ml) was added dropwise in 2 h. The mixture was stirred for 15 h at r.t. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. The progress of reaction was monitored by TLC using hexane: ethyl acetate (6:4) as eluent. After the completion of reaction, stirring was stopped and treated with 50% HCl. The solid product thus obtained was filtered, washed with water and dried. The crude product was purified by recrystallization from ethanol to get the title compound. M. P.110 ^{o}C, yield 76% (Found: N, 16.47 ,$C_{10}H_7ON_3Cl_2$, Anal. calcd: N, 16.37%).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-chloro-s-triazine (4).

p-Trifluoromethyl aniline (0.05 mole, 8.05 g) dissolved in DMF (30ml) was slowly added to a well-stirred slurry of **(3)** (0.05 mole, 12.82 g) in DMF (20 ml), maintaining the temperature at 35- 40 ^{o}C. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. The reaction mixture was stirred for 6 h. The progress of reaction was monitored by TLC using toluene: acetone (2:8) as eluent. After the completion of reaction, the solution was poured into crushed ice. The solid product thus obtained was filtered and dried. The crude product was purified by recrystallization from ethanol to get the title compound. M. P. 85 ^{o}C, yield 72 % (Found: N, 14.65, $C_{17}H_{12}ON_4ClF_3$, Anal. calcd: N, 14.69%).

(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-(amino acetic acid)-s-triazine (5).

A mixture of **(4)** (0.05 mole, 19.05 g) and glycine (0.05 mole, 3.75g) in DMF (30 ml) was refluxed with stirring for 4-5 h. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. The progress of reaction was monitored by TLC using hexane: methanol (2:8) as eluent. After the completion of the reaction, the content was poured into crushed ice, filtered and dried. The solid product thus obtained was purified by recrystallization from absolute alcohol to get the title compound. M. P. 138 ^{o}C, yield 70 % (Found: N, 16.70. $C_{19}H_{16}O_3N_5F_3$, Anal. calcd: N, 16.68%).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-(amino acetyl chloride)-s-triazine (6).

To a stirred solution of **(5)** (0.02 mole, 8.39 g) in chloroform (30 ml) at 0-5 ^{o}C, thionyl chloride (0.05 mole, 3.6 ml) was added. After the complete addition, temperature was gradually raised and refluxed for 2 h. After completion of the reaction, chloroform along with excess of thionyl chloride was distilled off. The product remained was treated with 10% $NaHCO_3$ solution to remove free acid. The acid chloride thus obtained was directly used in the next step.

General procedure for 2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (aryl ureido/thioureido)}-s-triazine (7a-i/8a-i).

To a solution of **(6)** (0.005 mole, 2.19 g) in DMF (30 ml), phenyl urea/thiourea derivatives (0.005 mole, 0.68 g) in DMF (15 ml) was added and refluxed for 5 h. The pH was adjusted to neutral by the addition of 10% $NaHCO_3$ solution. The progress of the reaction was monitored by TLC using hexane: ethyl acetate (8:2) as eluent. After the completion of the reaction, the refluxed content was poured into crushed ice. The solid product thus obtained was filtered and dried. The crude product was purified by recrystallization from absolute alcohol to get the title compound.

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (phenyl ureido)}-s-triazine (7a).

M. P. 150^{o}C, yield 70% (Found: C, 58.14; H, 4.12; N, 18.18, $C_{26}H_{22}O_3N_7F_3$, Anal. calcd: C, 58.10; H, 4.09; N, 18.24%). IR ν max/cm^{-1}: 1543 (>C=O-), 804 (-C=N-), 1606

(-NH-), 1026 (C-O-C (sym)), 1215 (C-O-C (asym)),1253 (-CF_3). ^{1}H NMR δ: 3.40 (s, 3H, Ar-OCH_3), 3.89 (s, 2H, -CH_2), 8.74 (s, 1H, Ar-NH), 9.88 (s, 1H, -NH), 6.93 (s, 1H, -NH), 6.98

(s, 1H, -NH), 7.11-7.49 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (2-chloro phenyl ureido)} -s-triazine (7b).

M. P. 100°C, yield 60% (Found: C, 54.66; H, 3.72; N, 17.08, $C_{26}H_{21}O_3N_7ClF_3$, Anal. calcd: C, 54.59; H, 3.67; N, 17.14%). IR ν max/cm^{-1}: 1549 (>C=O-), 806 (-C=N-), 1612

(-NH-), 1024 (C-O-C (sym)), 1221 (C-O-C (asym)),1251 (-CF_3).^{1}H NMR δ: 3.39 (s, 3H, Ar-OCH_3), 3.87 (s, 2H, -CH_2), 8.78 (s, 1H, Ar-NH), 9.86 (s, 1H, -NH), 6.93 (s, 1H, -NH), 6.97

(s, 1H, -NH), 7.05-7.45 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (3-chloro phenyl ureido)} -s-triazine (7c).

M. P. 120°C, yield 56% (Found: C, 54.64; H, 3.68; N, 17.16, $C_{26}H_{21}O_3N_7ClF_3$, Anal. calcd: C, 54.58; H, 3.66; N, 17.14%). IR ν max/cm^{-1}: 1554 (>C=O-), 808 (-C=N-), 1606

(-NH-), 1020 (C-O-C (sym)), 1215 (C-O-C (asym)),1254 (-CF_3).^{1}H NMR δ: 3.38 (s, 3H, Ar-OCH_3), 3.85 (s, 2H, -CH_2), 8.75 (s, 1H, Ar-NH), 9.87 (s, 1H, -NH), 6.93 (s, 1H, -NH), 6.98

(s, 1H, -NH), 7.12-7.60 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-chloro phenyl ureido)} -s-triazine (7d).

M. P. 131°C, yield 65% (Found: C, 54.52; H, 3.74; N, 17.18, $C_{26}H_{21}O_3N_7ClF_3$, Anal. calcd: C, 54.60; H, 3.68; N, 17.14%). IR ν max/cm^{-1}: 1543 (>C=O-), 804 (-C=N-), 1608

(-NH-), 1025 (C-O-C (sym)), 1220 (C-O-C (asym)), 1253 (-CF_3). ^{1}H NMR δ: 3.39 (s, 3H, Ar-OCH_3), 3.89 (s, 2H, -CH_2), 8.74 (s, 1H, Ar-NH), 9.87 (s, 1H, -NH), 6.93 (s, 1H, -NH), 6.97 (s, 1H, -NH), 7.11-7.49 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (2-tolyl ureido)}-s-triazine (7e).

M. P. 138°C, yield 69% (Found: C, 58.87; H, 4.40; N, 17.69, $C_{27}H_{24}O_3N_7F_3$, Anal. calcd: C, 58.80; H, 4.35; N, 17.78%). IR ν max/cm^{-1}: 1544 (>C=O-), 808 (-C=N-), 1608

(-NH-), 1026 (C-O-C (sym)), 1215 (C-O-C (asym)),1252 (-CF_3).^{1}H NMR δ: 3.42 (s, 3H, Ar-OCH_3), 3.89 (s, 2H, -CH_2), 8.76 (s, 1H, Ar-NH), 9.84 (s, 1H, -NH), 6.92 (s, 1H, -NH), 6.98

(s, 1H, -NH), 7.07-7.48 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (3-tolyl ureido)}-s-triazine (7f).

M. P. 130°C, yield 54% (Found: C, 58.64; H, 4.30; N, 17.84, $C_{27}H_{24}O_3N_7F_3$, Anal. calcd: C, 58.78; H, 4.35; N, 17.78%). IR ν max/cm^{-1}: 1544 (>C=O-), 806 (-C=N-), 1606

(-NH-), 1024 (C-O-C (sym)), 1218 (C-O-C (asym)),1252 (-CF_3).^{1}H NMR δ: 3.44 (s, 3H, Ar-OCH_3), 3.92 (s, 2H, -CH_2), 8.78 (s, 1H, Ar-NH), 9.84 (s, 1H, -NH), 6.94 (s, 1H, -NH), 6.97

(s, 1H, -NH), 7.10-7.54 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-tolyl ureido)}-s-triazine (7g).

M. P. 122°C, yield 68% (Found: C, 58.87; H, 4.31; N, 17.74, $C_{27}H_{24}O_3N_7F_3$, Anal. calcd: C, 58.82; H, 4.36; N, 17.76%). IR ν max/cm^{-1}: 1544 (>C=O-), 806 (-C=N-), 1605

(-NH-), 1022 (C-O-C (sym)), 1180 (C-O-C (asym)),1264 (-CF_3).^{1}H NMR δ: 3.42 (s, 3H, Ar-OCH_3), 3.92 (s, 2H, -CH_2), 8.74 (s, 1H, Ar-NH), 9.86 (s, 1H, -NH), 6.94 (s, 1H, -NH), 6.98

(s, 1H, -NH), 7.09-7.49 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (1-naphthyl ureido)} -s-triazine (7h).

M. P. 120°C, yield 70% (Found: C, 61.34; H, 4.15; N, 16.74, $C_{30}H_{24}O_3N_7F_3$, Anal. calcd: C, 61.32; H, 4.08; N, 16.69%). IR ν max/cm^{-1}: 1543 (>C=O-), 804 (-C=N-), 1612

(-NH-), 1034 (C-O-C (sym)), 1210 (C-O-C (asym)),1266 (-CF_3).^{1}H NMR δ: 3.46 (s, 3H, Ar-OCH_3), 3.89 (s, 2H, -CH_2), 8.76 (s, 1H, Ar-NH), 9.87 (s, 1H, -NH), 6.96 (s, 1H, -NH), 6.98

(s, 1H, -NH), 7.12-7.60 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-methoxy phenyl ureido)} -s-triazine (7i).

M. P. 128°C, yield 65% (Found: C, 57.20; H, 4.32; N, 17.34, $C_{27}H_{24}O_4N_7F_3$, Anal. calcd: C, 57.14; H, 4.23; N, 17.28%). IR ν max/cm^{-1}: 1543 (>C=O-), 804 (-C=N-), 1605

(-NH-), 1024 (C-O-C (sym)), 1215 (C-O-C (asym)), 1253 (-CF_3).^{1}H NMR δ: 3.38 (s, 3H, Ar-OCH_3), 3.86 (s, 2H, -CH_2), 8.75 (s, 1H, Ar-NH), 9.84 (s, 1H, -NH), 6.93 (s, 1H, -NH), 6.97

(s, 1H, -NH), 7.10-7.50 (m, 12H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (phenyl thioureido)} -s-triazine (8a).

M. P. 85°C, yield 66% (Found: C, 56.48; H, 3.93; N, 17.78, $C_{26}H_{22}O_2N_7F_3S$, Anal. calcd: C, 56.41; H, 3.97; N, 17.72%). IR ν max/cm^{-1}: 1530 (>C=S-), 1650 (>C=O-), 826

(-C=N-), 1590 (-NH-), 1024 (C-O-C (sym)), 1209 (C-O-C (asym)), 1250 (-CF_3). ^{1}H NMR δ: 3.36 (s, 3H, Ar-OCH_3), 3.30 (s, 1H, -NH), 3.18 (s, 1H, -NH), 3.86 (s, 2H, -CH_2), 7.09 (s, 1H, Ar-NH), 7.18 (s, 1H, Ar-NH), 8.49 (d, 2H, *J* 7.1 Hz, Ar-H), 8.54 (d, 2H, *J* 8.0 Hz, Ar-H), 7.16-7.60 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluromethyl phenyl amino)-6-{amino acetyl (2-chloro phenyl thioureido)}-s-triazine (8b).

M. P. 125°C, yield 62% (Found: C, 53.02; H, 3.64; N, 16.72, $C_{26}H_{21}O_2N_7ClF_3S$, Anal. calcd: C, 53.10; H, 3.57; N, 16.68%). IR ν max/cm^{-1}: 1530 (>C=S-), 1668 (>C=O-), 806

(-C=N-), 1598 (-NH-), 1024 (C-O-C (sym)), 1215 (C-O-C (asym)), 1250 (-CF_3). ^{1}H NMR δ: 3.38 (s, 3H, Ar-OCH_3), 3.28 (s, 1H, -NH), 3.22 (s, 1H, -NH), 3.82 (s, 2H, -CH_2), 7.07 (s, 1H, Ar-NH), 7.13 (s, 1H, Ar-NH), 8.48 (d, 2H, *J* 7.1 Hz, Ar-H), 8.52 (d, 2H, *J* 8.1 Hz, Ar-H), 7.20-7.80 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (3-chloro phenyl thioureido)}-s-triazine (8c).

M. P. 120°C, yield 55% (Found: C, 53.12; H, 3.65; N, 16.72, $C_{26}H_{21}O_2N_7ClF_3S$, Anal. calcd: C, 53.08; H, 3.57; N, 16.67%). IR ν max/cm^{-1}: 1548 (>C=S-), 1660 (>C=O-), 812

(-C=N-), 1605 (-NH-), 1026 (C-O-C (sym)), 1218 (C-O-C (asym)), 1248 (-CF_3). ^{1}H NMR δ: 3.40 (s, 3H, Ar-OCH_3), 3.32 (s, 1H, -NH), 3.24 (s, 1H, -NH), 3.84 (s, 2H, -CH_2), 7.09 (s, 1H, Ar-NH), 7.16 (s, 1H, Ar-NH), 8.50 (d, 2H, *J* 7.1Hz, Ar-H), 8.56 (d, 2H, *J* 8.1 Hz, Ar-H), 7.18 -7.70 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-chloro phenyl thioureido)} -s-triazine (8d).

M. P. 100°C, yield 60% (Found: C, 53.08; H, 3.57; N, 16.62, $C_{26}H_{21}O_2N_7ClF_3S$, Anal. calcd: C, 53.12; H, 3.58; N, 16.69%). IR ν max/cm^{-1}: 1548 (>C=S-), 1650 (>C=O-), 806

(-C=N-), 1598 (-NH-), 1022 (C-O-C (sym)), 1218 (C-O-C (asym)), 1253 (-CF_3). ^{1}H NMR δ: 3.35 (s, 3H, Ar-OCH_3), 3.32 (s, 1H, -NH), 3.21 (s, 1H, -NH), 3.80 (s, 2H, -CH_2), 7.03 (s, 1H, Ar-NH), 7.13 (s, 1H, Ar-NH), 8.52 (d, 2H, *J* 7.0 Hz, Ar-H), 8.54 (d, 2H, *J* 8.1 Hz, Ar-H), 7.21-7.70 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (2-tolyl thioureido)} -s-triazine (8e).

M. P. 130°C, yield 58% (Found: C, 57.16; H, 4.18; N, 17.33, $C_{27}H_{24}O_2N_7F_3S$, Anal. calcd: C, 57.14; H, 4.23; N, 17.28%). IR ν max/cm^{-1}: 1554 (>C=S-), 1660 (>C=O-), 826

(-C=N-), 1612 (-NH-), 1020 (C-O-C (sym)), 1216 (C-O-C (asym)), 1253 (-CF_3). ^{1}H NMR δ: 3.36 (s, 3H, Ar-OCH_3), 3.32 (s, 1H, -NH), 3.18 (s, 1H, -NH), 3.80 (s, 2H, -CH_2), 7.07 (s, 1H, Ar-NH), 7.16 (s, 1H, Ar-NH), 8.51 (d, 2H, *J* 7.0 Hz, Ar-H), 8.56 (d, 2H, *J* 8.0 Hz, Ar-H), 7.25-7.80 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (3-tolyl thioureido)} -s-triazine (8f).

M. P. 125°C, yield 58% (Found: C, 57.09; H, 4.27; N, 17.20, $C_{27}H_{24}O_2N_7F_3S$, Anal. calcd: C, 57.12; H, 4.24; N, 17.27%). IR ν max/cm^{-1}: 1530 (>C=S-), 1668 (>C=O-), 826

(-C=N-), 1605 (-NH-), 1026 (C-O-C (sym)), 1214 (C-O-C (asym)), 1250 (-CF_3). ^{1}H NMR δ: 3.38 (s, 3H, Ar-OCH_3), 3.30 (s, 1H, -NH), 3.24 (s, 1H, -NH), 3.82 (s, 2H, -CH_2), 7.05 (s, 1H, Ar-NH), 7.15 (s, 1H, Ar-NH), 8.52 (d, 2H, *J* 7.1 Hz, Ar-H), 8.56 (d, 2H, *J* 8.1 Hz, Ar-H), 7.21-7.70 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-tolyl thioureido)} -s-triazine (8g).

M. P. 122°C, yield 60% (Found: C, 57.21; H, 4.32; N, 17.33, $C_{27}H_{24}O_2N_7F_3S$, Anal. calcd: C, 57.15; H, 4.25; N, 17.29%). IR ν max/cm^{-1}: 1548 (>C=S-), 1668 (>C=O-), 833

(-C=N-), 1605 (-NH-), 1022 (C-O-C (sym)), 1216 (C-O-C (asym)), 1265 (-CF_3). ^{1}H NMR δ: 3.35 (s, 3H, Ar-OCH_3), 3.30 (s, 1H, -NH), 3.21 (s, 1H, -NH), 3.86 (s, 2H, -CH_2), 7.07 (s, 1H, Ar-NH), 7.18 (s, 1H, Ar-NH), 8.52 (d, 2H, *J* 7.0 Hz, Ar-H), 8.58 (d, 2H, *J* 8.1 Hz, Ar-H), 7.18-7.60 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (1-naphthyl thioureido)}-s-triazine (8h).

M. P. 120°C, yield 64% (Found: C, 59.72; H, 3.88; N, 16.30, $C_{30}H_{24}O_2N_7F_3S$, Anal. calcd: C, 59.70; H, 3.98; N, 16.25%). IR ν max/cm^{-1}: 1548 (>C=S-), 1650 (>C=O-), 833

(-C=N-), 1590 (-NH-), 1020 (C-O-C (sym)), 1212 (C-O-C (asym)), 1265 (-CF_3). ^{1}H NMR δ: 3.36 (s, 3H, Ar-OCH_3), 3.30 (s, 1H, -NH), 3.18 (s, 1H, -NH), 3.80 (s, 2H, -CH_2), 7.07 (s, 1H, Ar-NH), 7.14 (s, 1H, Ar-NH), 8.48 (d, 2H, *J* 7.1 Hz, Ar-H), 8.56 (d, 2H, *J* 7.9 Hz, Ar-H), 7.21-7.70 (m, 8H, Ar-H).

2-(4-Methoxy phenyl)-4-(4-trifluoromethyl phenyl amino)-6-{amino acetyl (4-methoxy phenyl thioureido)}-s-triazine (8i).

M. P. 130°C, yield 68% (Found: C, 55.50; H, 4.17; N, 16.84, $C_{27}H_{24}O_3N_7F_3S$, Anal. calcd: C, 55.57; H, 4.11; N, 16.80%). IR ν max/cm^{-1}: 1550 (>C=S-), 1654 (>C=O-), 812

(-C=N-), 1598 (-NH-), 1018 (C-O-C (sym)), 1210 (C-O-C (asym)), 1250 (-CF_3). ^{1}H NMR δ: 3.38 (s, 3H, Ar-OCH_3), 3.32 (s, 1H, -NH), 3.16 (s, 1H, -NH), 3.84 (s, 2H, -CH_2), 7.07 (s, 1H, Ar-NH), 7.14 (s, 1H, Ar-NH), 8.50 (d, 2H, *J* 7.1 Hz, Ar-H), 8.54 (d, 2H, *J* 8.0 Hz, Ar-H), 7.18-7.60 (m, 8H, Ar-H).

In vitro anti-HIV assay

Evaluation of the antiviral activity of the test compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells were performed using the MTT assay as previously described. Stock solution (10 × final concentrations) of test compound was 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 5-fold 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(III_B) or HIV-2 (ROD) stock (50µL) at 100-300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium was added to the microtiter tray. Mock-infected cells were used to evaluate the effect of test compounds on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6 x 10^5 cells/mL, and an amount of 50 µL volume was transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells were examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Across 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, Labsystem, Helsinki, Finland) at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells.

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