Med Chem Res
DOI 10.1007/s00044-013-0521-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Development of 4-aminoquinoline-1,3,5-triazine conjugates as potent antibacterial agent through facile synthetic route

Hans Raj Bhat · Pankaj Kumar Pandey · Surajit Kumar Ghosh · Udaya Pratap Singh

Received: 15 October 2012 / Accepted: 29 January 2013

Abstract A series of novel hybrid 4-aminoquinoline-1,3,5-triazine derivatives were developed and subsequently tested against representative Gram-positive and Gram-negative microorganisms for determination of their antibacterial activity. Screening results indicate that, title molecule exhibit moderate to potent activity in comparison to standard. These hybrid derivatives were synthesized through a facile synthetic routes and structure of reaction intermediates as well as target molecules were recognised with the aid of various spectroscopic techniques viz., FTIR, NMR, mass and elemental analysis.

Keywords 1,3,5-Triazine · 4-Aminoquinoline · Antibacterial

Introduction

The emergence of multi-drug resistance (MDR) pathogens in last decades significantly jeopardises the global health-care system in both poor and developed countries (Alanis, 2005; Shapiro *et al.*, 2011). Whereas, non-judicious use of these drugs has contributed largely among the other factors that brought up the mutation in microbial genome (Martinez and Baquero, 2000). This has put a selective pressure and necessitates the discovery of novel antimicrobial agents that acted via a novel pathway (Davies and Davies, 2010).

The 1,3,5-triazine scaffold has provided the basis for the design of biologically significant molecules with diverse therapeutic profile, e.g. as antifungal (Singh *et al.*, 2012a, 2013a), anticancer (Corbett *et al.*, 1982), antimalarial (Bhat *et al.*, 2011, 2012a, 2013), antiviral (Lozano *et al.*, 2011) and antibacterial activity (Gahtori *et al.*, 2012). In continuation of our project (Junejo *et al.*, 2011), till now, we had reported numerous antibacterial hybrid conjugates of 1,3,5-triazine with thiazole (Singh *et al.*, 2011), piperazine (Ghosh *et al.*, 2012), 4-aminoquinoline (Bhat *et al.*, 2012b) and 1,3,4-thiadiazole (Dubey *et al.*, 2012), thiazolidine-4-one (Kumar *et al.*, 2013) as potent antimicrobial compounds. In our recent communication, inhibition of bacterial translation was identified as mechanism of action for these conjugates (Singh *et al.*, 2012b). Concerning our endeavour on discovery of antibacterial agents (Singh *et al.*, 2013b) and prompted by these results, herein, we disclosed the facile synthesis and antibacterial activity of hybrid conjugates derived from 4-aminoquinoline and 1,3,5-triazine.

Results and discussion

Chemistry

The synthesis of title hybrid 4-aminoquinoline 1,3,5-triazine derivatives **9 (a–i)** were accomplished in five steps. The first

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0521-8) contains supplementary material, which is available to authorized users.

H. R. Bhat (✉) · P. K. Pandey · U. P. Singh
Department of Pharmaceutical Sciences, Sam Higginbottom Institute of Agriculture Technology & Sciences, Formerly Allahabad Agricultural Institute, Deemed to be University, Allahabad 211007, India
e-mail: pharmahans@gmail.com

S. K. Ghosh
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, Assam, India

Present Address:
U. P. Singh
Archimedes DoRa5 Fellow, Division of Bioorganic Chemistry, Institute of Chemistry, University of Tartu, Tartu, Estonia

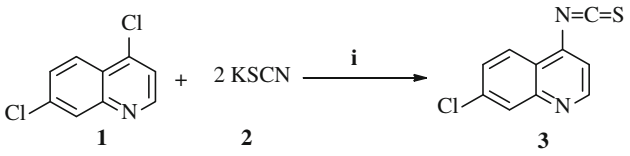
Published online: 06 February 2013

step corresponds to the synthesis 7-chloro-4-isothiocyanato-quinoline (**3**) was achieved by the substitution of potassium thiocynate (**2**) with 4-chloro of 4,7-dichloroquinoline (**1**) in the presences of few pieces of tin granules, Scheme 1. Whereas, in second step, synthesis of mono-substituted 1,3,5-triazines viz., 4,6-dichloro-*N*-(4-nitrophenyl)-1,3,5-triazin-2-amine (**6**) was accomplished by nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (**4**) with *p*-nitro aniline (**5**) in the presence of saturated solution of $NaHCO_3$. The above-obtained compound (**6**) was subsequently treated with different primary and secondary amines (**a**–**i**) as presented in Scheme 2 to afford di-substituted 1,3,5-triazines **7**(**a**–**i**) via nucleophilic substitution at one of Cl atom in the presence of activating base. The synthesis of tri-substituted 1,3,5-triazine **8**(**a**–**i**) was accomplished by the nucleophilic substitution of the remained Cl atom of di-substituted 1,3,5-triazine **7**(**a**–**i**) with piperazine in the presence of saturated solution of $NaHCO_3$. Whereas, title compounds **9**(**a**–**i**) were synthesized by incorporating tri-substituted 1,3,5-triazine moiety **8**(**a**–**i**) with 7-chloro-4-isothiocyanatoquinoline pharmacophore (**3**) and serve as step five as showed in Scheme 3.

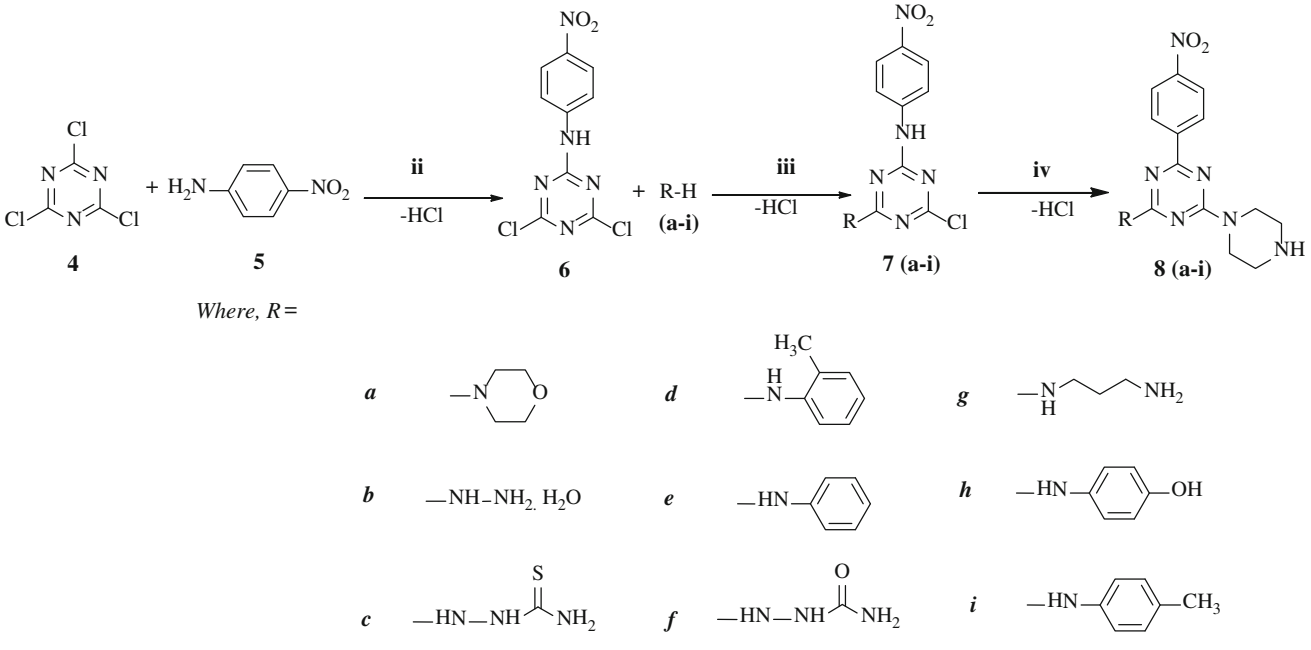
The series of title hybrid analogues were synthesized in moderate to excellent yields. Whereas, structure of the intermediate as well as target molecules were ascertained on the basis of spectroscopic analysis. FTIR spectra of compounds **9**(**a**–**i**) in which aromatic C=N group of 1,3,5-triazine was found in range of 1,679.28–1,548.26 cm^{-1}. Whereas quinoline ring aromatic C=C group appears in range of 1,690–1,640 cm^{-1}, C–N group appear in range of 1,275 cm^{-1} and many strong absorption bands in range of 894–628 cm^{-1} which confirm the existence of aromatic ring. The ^1H NMR spectrums of quinoline expose a signal in the range of 7.27–8.85 ppm. The shielding for bridged NH was usually observed at 3.69–3.59 ppm which is attributable to tri-substituted 1,3,5-triazine but the chemical shift of $–NH_2$ bridge was found in range of 1.93 ppm in case of **8b**. Moreover, all mass spectra and elemental analysis are in agreement of proposed structures.

Antibacterial activity

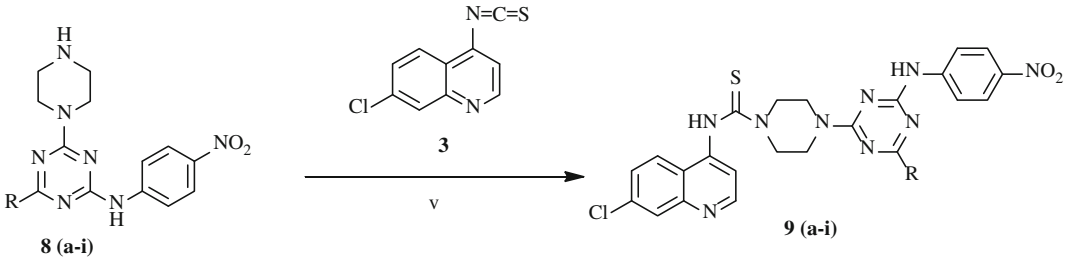
Antibacterial screening of all the synthesized compounds **9(a–i)** (Table 1) revealed moderate to potent activities against the tested gram-positive and gram-negative micro-organisms in comparison to Ofloxacin as a standard drug. The term significant, moderate and no activity were considered for the concentration range of 3.125, 6.25–25 and 50–100 μg mL^{-1}, respectively. Compound with morpholine substitution on 1,3,5-triazine **9a** exhibit significant activity



Scheme 1 *i* Reflux with stirring 18 h at 90–120 °C, anhydrous toluene, tin metal



Scheme 2 Reagents and conditions: *ii* *p*-nitro aniline, 1,4-dioxane 0–5 °C, $KHCO_3$, *iii* R–H (**a**–**i**) various amines, 1,4-dioxane 40–45 °C, 3 h, $KHCO_3$, *iv* piperazine, 1,4-dioxane 120–130 °C, 5–6 h, K_2CO_3



Scheme 3 Reagents and conditions: *v* dry acetone, 40-45 °C, 18 h

Table 1 Antibacterial activity of target hybrid derivatives **9(a–i)**

Compounds	Minimum inhibitory concentration (MIC) (μg mL^{-1})						
	Gram +ve			Gram −ve			
	S. aureus	*B. subtilis*	*B. cereus*	*P. aeruginosa*	*E. coli*	*P. mirabilis*	*P. vulgaris*
9a	3.125	6.25	3.125	12.5	6.25	12.5	3.125
9b	25	6.25	6.25	12.5	25	6.25	12.5
9c	25	6.25	3.125	6.25	6.25	12.5	12.5
9d	25	12.5	12.5	6.25	6.25	12.5	25
9e	12.5	12.5	3.125	6.25	12.5	6.25	12.5
9f	12.5	3.125	6.25	12.5	12.5	6.25	6.25
9g	25	25	12.5	12.5	12.5	12.5	25
9h	100	25	25	25	50	50	50
9i	12.5	6.25	3.125	6.25	12.5	3.125	12.5
Ofloxacin (standard)	6.25	3.125	6.25	6.25	3.125	3.125	3.125

against *Staphlococcos aureus*, *Bacillus cereus*, *Proteus vulgaris* and moderate activity against the *Pseudomonas aeruginosa* and *Bacillus subtilis*. Whereas, on replacement of morpholine to hydrazine in the case of compound **9b**, presented moderate activities against *B. subtilis, S. aureus, P. aeruginosa, Proteus mirabilis, P. vulgaris* and *Escherichia coli*. Compound **9c**, formed on replacement of hydrazine by thiosemicarbazide showed the significant antibacterial activity against *B. cereus*, equipotent to ofloxacin against *P. aeruginosa* and moderate against the *B. subtilis, P. vulgaris* and *S. aureus.* Reasonable activity was observed in case of compound **9d** having *o*-toluidine group as substituent, towards entire microbial strains. Replacement of *o*-toluidine to aromatic amine in the case of compound **9e**, causes significant shift in the activity against *B. cereus* and moderately against the *B. subtilis, S. aureus, P. vulgaris* and *P. aeruginosa.* Compound **9f**, resulted from introduction of semicarbazide in place of aromatic amine disclosed the significant antibacterial activity against *B. subtilis* and moderate activity against the *P. aeruginosa*, *E.coli*, *S. aureus* and *P. vulgaris.* Moderate activity was observed in case of compounds **9g** and **9h** having 1,3-diaminopropane and *p*-aminophenol group as substituent, towards all gram-positive and gram-negative strains. However, substantial-to-significant activity was observed in the case of analogue having *p*-toluidine **9i** against *B. cereus* and moderate activity against *S. aureus*, *E. coli, B. subtilis*, *P. vulgaris* and *P. mirabilis,* respectively.

From the bioactivity profile of the target hybrid derivatives, it was inferred that compounds having aliphatic group at 1,3,5-triazine ring viz., **9b**, **9c**, **9f** and **9g** showed prominent activity against *B. cereus* and *B. subtilis* and no activity against *P. aeruginosa*, *S. aureus*, *E. coli*, *P. vulgaris* and *P. mirabilis*. While, replacement of aliphatic group with aromatic **9a**, **9d**, **9e**, **9h** and **9i** makes the compound prominent active against *B. cereus, P. vulgaris* and no activity against *B. subtilis*, *E. coli, S. aureus*, *P. mirabilis* and *P. aeruginosa*. In addition, it was also corroborated that, replacement of aliphatic by aromatic substituent, was lead to remarkable increase in activity for *B. cereus* and further decrease was reported for rest of the strains.

Experimental

Chemistry

All commercially available solvents and reagents were of analytical grade and used without further purification. Melting points were determined on a Veego, MPI melting point apparatus and FTIR (2.0 cm^{-1}, flat, smooth, abex)

were recorded on Perkin-Elmer RX-I spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance II 400 NMR and ^{13}C NMR spectra on Bruker Avance II 100 NMR spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionisation (ESI) sources. Elemental analysis was carried out on Vario EL-III CHNOS elemental analyser.

*7-Chloro-4-isothiocyanatoquinoline (**3**)*

A solution of 4,7-dichloroquinoline (**1**) (0.01 mol), potassium thiocynate (**2**) (0.02 mol) and few pieces of tin metal in anhydrous toluene was refluxed at 90–120 °C for 18 h. The completion of reaction was monitored by TLC using ethanol:acetone (1:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulted residue was dissolved in dichloromethane, washed with brine and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the title compound (**3**).

Brown crystals, yield: 68 %; m.p.: 197–198 °C; MW: 220.68; R_f: 0.48; FTIR (ν_{max}; cm^{-1} KBr): 1,275 (C–N), 1,630 (C=C), 1,690–1,640 (C=N), 3,000 (C–H), 1,600 (C=C, aromatic ring), 1,470 (C=C, aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.85 (d, 1H J = 4.97 Hz, quinoline ring), 7.27 (d, 1H J = 4.97 Hz, quinoline ring), 7.76 (d, 1H J = 8.60 Hz, quinoline ring), 8.40 (d, 1H J = 8.60 Hz, quinoline ring), 8.01 (d, 1H J = 1.96 Hz, quinoline ring); ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 152.30, 118.30, 138.40, 124.10, 129.80, 128.60, 135.20, 129.10, 137.20; mass: 221.60 $(M+H)^+$; elemental analysis for $C_{10}H_5ClN_2S$: calculated: C, 54.43; H, 2.28; N, 12.69. Found: C, 54.41; H, 2.23; N, 12.58.

*4,6-Dichloro-N-(4-nitrophenyl)-1,3,5-triazin-2-amine (**6**)*

p-Nitro aniline (**5**) (0.1 mol) was added into 100 mL of acetone at temperature 0–5 °C. The solution of 2,4,6-trichloro-1,3,5-triazine (**4**) (0.1 mol) in 25-mL acetone was added to above mixture constantly. The resulting mixture was then stirred for 2 h followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol) taking care that reaction mixture does not become acidic. The completion of reaction was monitored by TLC using benzene:ethyl acetate (9:1) as mobile phase. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products (**6**).

Yellow crystals; yield: 83 %; m.p.: 123–124 °C; MW: 286.07; R_f: 0.48; FTIR (ν_{max}; cm^{-1} KBr): 3,289.56 (N–H secondary), 3,056.73 (C–H broad), 1,548.26– 1,446.16 (aromatic C=N); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 7.63 (d, 2H J = 8.63 Hz, 2× CH, Ar–H), 7.28(d, 2H J = 8.57 Hz, 2× CH, Ar–H), 3.62 (br, s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 118.25, 125.32, 139.26, 144.26, 168.85, 173.56; mass: 287.34 $(M+H)^+$; elemental analysis for $C_9H_5Cl_2N_5O_2$: calculated: C, 37.79; H, 1.76; N, 24.48. Found: C, 36.48; H, 1.75; N, 24.86.

*General procedure for the synthesis of di-substituted 1,3,5-triazine derivatives **7(a–i)***

Various distinguished amines (**a**–**i**) (0.1 mol) were added into 100 mL of acetone maintaining temperature 40–45 °C. The solution of mono substituted-1,3,5-triazine (**6**) (0.1 mol) in 25 mL acetone was added constantly, stirred for 3 h followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol) taking care that reaction mixture does not become acidic. The completion of reaction was monitored by TLC using benzene:ethyl acetate (9:1) as mobile phase. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products **7**(**a**–**i**).

*4-Chloro-6-morpholino-N-(4-nitrophenyl)-1,3,5-triazin-2-amine (**7a**)* Light yellow crystals; yield: 76 %; m.p.: 146–148 °C; MW: 336.73; R_f: 0.56; FTIR (ν_{max}; cm^{-1} KBr): 3,288.57 (N–H secondary), 3,055.79 (C–H broad), 1,679.28–1,638.32 (aromatic C=N), 1,348.23–1,023.12 (aromatic C–N), 1,528.24 (NO_2), 1,662, 786; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 7.28(d, 2H J = 8.57 Hz, 2× CH, Ar–H), 3.58 (br, s, 1H, NH), 3.68 (d, 1H J = 17.31 Hz, 2× CH_2, Ar–H) 3.63 (d, 1H J = 18.37 Hz, 2× CH_2, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 118.21, 124.22, 138.26, 144.25, 168.81, 173.55, 67.34, 46.67; mass: 337.79 $(M+H)^+$; elemental analysis for $C_{13}H_{13}ClN_6O_3$: calculated: C, 46.37; H, 3.89; N, 24.96. Found: C, 46.43; H, 3.97; N, 24.83.

*4-Chloro-6-hydrazinyl-N-(4-nitrophenyl)-1,3,5-triazin-2-amine hydrate (**7b**)* Brown crystals; yield: 86 %; m.p.: 156–158 °C; MW: 299.67; R_f: 0.63; FTIR (ν_{max}; cm^{-1} KBr): 3,315.32 (NH_2), 3,288.53 (N–H secondary), 3,043.79 (C–H broad), 1,676.23–1,639.32 (aromatic C=N), 1,668, 1,347.13–1,002.19 (aromatic C–N), 1,525.27 (NO_2), 757; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.12 (d, 2H J = 8.67 Hz, 2× CH, Ar–H), 7.37 (d, 2H, J = 1.95 Hz 2× CH, Ar–H), 3.67 (br, s, 1H, NH), 1.87 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 183.24, 172.56, 168.37, 143.24, 137.25, 126.75, 117.28; mass: 301.36 $(M+H)^+$; elemental analysis for $C_9H_{10}ClN_7O_3$: calculated: C, 36.07; H, 3.36; N, 32.72. Found: C, 35.96; H, 3.28; N, 32.71.

*2-(4-Chloro-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl) hydrazinecarbothioamide (**7c**)* Yellow crystals; yield: 69 %; m.p.: 169–170 °C; MW:340.75; R_f: 0.68; FTIR (ν_{max}; cm^{-1} KBr): 3,321.35 (NH2), 3,287.47 (N–H secondary), 3,043.76 (C–H broad), 1,676.53–1,639.36 (aromatic C=N),

1,668, 1,525.27 (NO_2), 1,347.13–1,002.19 (aromatic C–N), 1,273.78 (C=S),757; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.08 (d, 2H $J = 8.65$ Hz, 2× CH, Ar–H), 7.48 (d, 2H, $J = 2.96$ Hz, 2× CH, Ar–H), 3.67 (br, s, 1H, NH), 1.87 (s, 1H, NH), 8.77 (s, 1H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 184.52, 180.43, 172.20, 165.73, 152.36, 136.32, 128.36, 117.65; mass: 341.76 $(M+H)^+$; elemental analysis for $C_{10}H_9ClN_8O_2S$: calculated: C, 35.25; H, 2.66; N, 32.88. Found: C, 35.27; H, 2.72; N, 32.86.

*6-Chloro-N^2-(4-nitrophenyl)-N^4-(o-tolyl)-1,3,5-triazine-2,4-diamine (**7d**)* White crystals; yield: 73 %; m.p.: 152–153 °C; MW:356.77; R_f: 0.47; FTIR (ν_{max}; cm^{-1} KBr): 3,285.45 (N–H secondary), 3,045.72 (C–H broad), 1,672.51–1,631.30 (aromatic C=N), 1,667–1,600 (aromatic C=C), 1,528.22 (NO_2), 1,346.12–1,016.12 (aromatic C–N), 894, 635; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.12 (d, 2H $J = 8.66$ Hz, 2× CH, Ar–H), 7.34 (d, 2H, $J = 0.71$ Hz, 2× CH, Ar–H), 3.67 (br, s, 1H, NH), 7.12(d, 1H $J = 8.64$ Hz, 1× CH, Ar–H), 7.03 (d, 1H $J = 7.49$ Hz, 1× CH, Ar–H), 6.72 (d, 1H $J = 1.17$ Hz, 1× CH, Ar–H), 2.21 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 167.32, 164.62, 163.27, 146.23, 141.82, 138.21, 132.82, 129.25, 125.32, 123.73, 122.83, 118.56, 17.63; mass: 357.73 $(M+H)^+$; elemental analysis for $C_{16}H_{13}ClN_6O_2$: calculated: C, 53.86; H, 3.67; N, 23.56. Found: C, 54.02; H, 3.64; N, 23.58.

*6-Chloro-N^2-(4-nitrophenyl)-N^4-phenyl-1,3,5-triazine-2,4-diamine (**7e**)* Light brown crystals; yield: 81 %; m.p.: 182–183 °C; MW:342.74; R_f: 0.52; FTIR (ν_{max}; cm^{-1} KBr): 3,285.45 (N–H secondary), 3,045.78 (C–H broad), 1,672.53–1,631.33 (aromatic C=N), 1,671–1,606 (aromatic C=C), 1,524.32 (NO_2), 1,345.10–1,016.14 (aromatic C–N), 775, 628; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.14 (d, 2H $J = 8.65$ Hz, 2× CH, Ar–H), 7.34 (d, 2H, $J = 0.74$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 7.53(d, 1H $J = 8.29$ Hz, 1× CH, Ar–H), 7.48 (d, 1H $J = 8.15$ Hz, 1× CH, Ar–H), 6.89(d, 1H $J = 1.18$ Hz, 1× CH, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 167.31, 164.52, 164.23, 143.52, 139.39, 137.25, 129.53, 124.73, 122.86, 119.37, 115.84; mass: 343.76 $(M+H)^+$; elemental analysis for $C_{15}H_{11}ClN_6O_2$: calculated: C, 53.86; H, 3.67; N, 23.56. Found: C, 54.02; H, 3.64; N, 23.58.

*2-(4-Chloro-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl) hydrazinecarboxamide (**7f**)* Light yellow crystals; yield: 68 %; m.p.: 156–159 °C; MW:324.68; R_f: 0.46; FTIR (ν_{max}; cm^{-1} KBr): 3,321.35 (NH2), 3,287.47 (N–H secondary), 3,043.76 (C–H broad), 1,676.53–1,639.36 (aromatic C=N), 1,668, 1,525.27 (NO_2), 1,347.13–1,002.19 (aromatic C–N), 1,753.62 (C=O),758; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.05 (d, 2H $J = 8.67$ Hz, 2× CH, Ar–H), 7.65 (d, 2H, $J = 0.74$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 6.77 (s, 1H, NH), 6.87 (s, 1H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 183.35, 171.93, 168.24, 158.42, 147.28, 137.56, 125.35, 118.28; mass: 325.79 $(M+H)^+$; elemental analysis for $C_{10}H_9ClN_8O_3$: calculated: C, 36.99; H, 2.79; N, 34.51. Found: C, 37.02; H, 2.75; N, 34.57.

*N^2-(3-aminopropyl)-6-chloro-N^4-(4-nitrophenyl)-1,3,5-triazine-2,4-diamine (**7g**)* Yellow white crystals; yield: 75 %; m.p.: 172–173 °C; MW:323.74; R_f: 0.54; FTIR (ν_{max}; cm^{-1} KBr): 3,319.56 (NH_2), 3,284.57 (N–H secondary), 3,041.78 (C–H broad), 1,674.45–1,638.12 (aromatic C=N), 1,668, 1,347.13–1,023.19 (aromatic C–N), 1,527.27 (NO_2), 759; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.12 (d, 2H $J = 8.66$ Hz, 2× CH, Ar–H), 7.37 (d, 2H, $J = 2.12$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 3.25 (t, 2H $J = 6.76$ Hz, CH_2,), 2.62 (t, 2H, $J = 6.85$ Hz, CH_2), 1.66 (t, 2H, $J = 6.76$ Hz, CH_2), 6.87 (s, 1H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.25, 171.43, 165.73, 148.12, 136.43, 126.65, 119.28, 38.58, 31.63; mass: 324.83 $(M+H)^+$; elemental analysis for $C12H_{14}ClN_7O_2$: calculated: C, 44.52; H, 4.36; N, 30.29. Found: C, 45.03; H, 4.34; N, 30.28.

*4-((4-Chloro-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl) amino)phenol (**7h**)* Black crystals; yield: 63 %; m.p.: 184–185 °C; MW:358.74; R_f: 0.42; FTIR (ν_{max}; cm^{-1} KBr): 3,416 (OH stretching), 3,286.51 (N–H secondary), 3,046.58 (C–H broad), 2,800–3,012 (CH_2 stretching), 1,672.42–1,637.16 (aromatic C=N), 1,605 (C=C), 1,347.13–1,023.19 (aromatic C–N), 1,527.27 (NO_2), 753; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.14 (d, 2H $J = 8.65$ Hz, 2× CH, Ar–H), 7.34 (d, 2H, $J = 0.73$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 7.41 (d, 1H $J = 8.73$ Hz, Ar–H), 6.82 (d, 1H, $J = 2.26$ Hz, Ar–H), 5.67 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 173.25, 169.54, 167.43, 148.52, 143.85, 139.62, 132.21, 125.73, 122.82, 118.27, 114.75; mass: 359.65 $(M+H)^+$; elemental analysis for $C15H_{11}ClN_6O_3$: calculated: C, 50.22; H, 3.09; N, 23.43. Found: C, 50.32; H, 3.07; N, 23.46.

*6-Chloro-N^2-(4-nitrophenyl)-N^4-(p-tolyl)-1,3,5-triazine-2,4-diamine (**7i**)* Light yellow crystals; yield: 65 %; m.p.: 158–159 °C; MW:356.77; R_f: 0.63; FTIR (ν_{max}; cm^{-1} KBr): 3,285.45 (N–H secondary), 3,045.72 (C–H broad), 1,672.51–1,631.30 (aromatic C=N), 1,667–1,600 (aromatic C=C), 1,528.22 (NO_2), 1,346.12–1,016.12 (aromatic C–N), 894, 635; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.13 (d, 2H $J = 8.64$ Hz, 2× CH, Ar–H), 7.32 (d, 2H, $J = 0.74$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 7.35 (d, 1H $J = 8.27$ Hz, Ar–H), 6.97 (d, 1H $J = 5.49$ Hz, Ar–H), 2.21 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.45, 168.32, 167.36, 152.65, 138.14, 134.53, 130.21, 128.72, 123.87, 118.52, 117.28, 24.37; mass: 357.53 $(M+H)^+$;

elemental analysis for $C_{16}H_{13}ClN_6O_2$: calculated: C, 53.86; H, 3.67; N, 23.56. Found: C, 54.02; H, 3.64; N, 23.58.

*General procedure for the synthesis of tri-substituted 1,3,5-triazine derivatives **8(a–i)***

A solution of di-substituted 1,3,5-triazine compounds **7(a–i)** (0.01 mol), piperazine (0.01 mol) and K_2CO_3 (0.01 mol) in 1,4-dioxane was refluxed for 6–7 h. The completion of reaction was monitored by TLC using benzene:ethyl acetate (9:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by ethanol to afford the desired product **8(a–i)**.

*4-Morpholino-N-(4-nitrophenyl)-6-(piperazin-1-yl)-1,3,5-triazin-2-amine (**8a**)* White crystals; yield: 72 %; m.p.: 216–217 °C; MW: 386.41; R_f: 0.58; FTIR (ν_{max}; cm^{-1} KBr): 3,323.98 (N–H stretching in piperazine), 3,287.37 (N–H secondary), 3,056.78 (C–H broad), 2,813–3,003 (CH_2 stretching), 1,282–1,178 (C–N stretching), 1,676.25–1,637.39 (aromatic C=N), 1,616.15 (N–H bending piperazine), 1,528.53 (NO_2), 1,605 (C=C), 784; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.12 (d, 2H J = 8.66 Hz, 2× CH, Ar–H), 7.32 (d, 2H J = 0.71 Hz, 2× CH, Ar–H), 3.62 (br, s, 1H, NH), 3.69 (d, 1H J = 17.80 Hz, 2× CH_2, Ar–H) 3.65 (d, 1H J = 15.61 Hz, 2× CH_2, Ar–H), 3.12 (d, 4H J = 13.29 Hz, 2× CH_2, Ar–H), 2.73 (d,4H J = 3.21 Hz, 2× CH_2, Ar–H), 1.94 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 183.32, 178.65, 168.86, 147.42, 138.53, 126.21, 119.85, 68.36, 48.73, 46.63, 45.71; mass: 387.63 $(M+H)^+$; elemental analysis for $C_{17}H_{22}N_8O_3$: calculated: C, 52.84; H, 5.74; N, 29.00. Found: C, 52.82; H, 5.71; N, 28.85.

*4-Hydrazinyl-N-(4-nitrophenyl)-6-(piperazin-1-yl)-1,3,5-triazin-2-amine hydrate (**8b**)* Light yellow crystals; yield: 59 %; m.p.: 187–188 °C; MW: 349.35; R_f: 0.46; FTIR (ν_{max}; cm^{-1} KBr): 3,323.98 (N–H stretching in piperazine), 3,312.34 (NH_2), 3,287.56 (N–H secondary), 3,046.72 (C–H broad), 2,803–3,012 (aromatic CH_2) 1,676.23–1,639.32 (aromatic C=N), 1,616.15 (N–H bending piperazine), 1,606 (C=C), 1,349.13-1,008.19 (aromatic C–N), 1,523.27 (NO_2), 767; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.13 (d, 2H J = 8.67 Hz, 2× CH, Ar–H), 7.35 (d, 2H, J = 1.01 Hz 2× CH, Ar–H), 3.65 (br,s 1H, NH), 1.97 (s, 2H, NH_2), 3.15 (d, 4H J = 13.25 Hz, 2× CH_2, Ar–H), 2.76 (d,4H J = 3.11 Hz, 2× CH_2, Ar–H), 1.97 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 184.25, 178.54, 171.75, 152.12, 142.65, 128.73, 121.51, 52.48, 47.14; mass: 351.12 $(M+H)^+$; elemental analysis for $C_{13}H_{19}N_9O_3$: calculated: C, 44.69; H, 5.48; N, 36.08. Found: C, 44.71; H, 5.49; N, 36.12.

*2-(4-((4-Nitrophenyl)amino)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)hydrazinecarbothioamide (**8c**)* Brown crystals; yield: 71 %; m.p.: 197–198 °C; MW: 390.42; R_f: 0.58; FTIR (ν_{max}; cm^{-1} KBr):3,328.98 (N–H stretching in piperazine), 3,321.43 (NH2), 3,286.25 (N–H secondary), 3,048.83 (C–H broad), 1,676.25–1,630.48 (aromatic C=N), 1,617.15 (N–H bending piperazine), 1,525.27 (NO_2), 1,349.13-1,007.19 (aromatic C–N), 1,274.78 (C=S),753; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.05 (d, 2H J = 8.67 Hz, 2× CH, Ar–H), 7.94 (d, 2H, J = 0.96 Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 1.97 (s, 1H, NH), 8.57 (s, 1H, NH_2), 3.18 (d, 4H J = 13.29 Hz, 2× CH_2, Ar–H), 2.74 (d,4H J = 3.29 Hz, 2× CH_2, Ar–H), 1.95 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 185.54, 181.32, 178.53, 186.72, 148,75, 139.36, 127.83, 121.59, 49.13, 44.57; mass: 391.56 $(M+H)^+$; elemental analysis for $C_{14}H_{18}N_{10}O_2S$: calculated: C, 43.07; H, 4.65; N, 35.88. Found: C, 43.11; H, 4.67; N, 35.89.

*N^2-(4-nitrophenyl)-6-(piperazin-1-yl)-N^4-(o-tolyl)-1,3,5-triazine-2,4-diamine (**8d**)* Light yellow crystals; yield: 64 %; m.p.: 212–213 °C; MW:406.44; R_f: 0.47; FTIR (ν_{max}; cm^{-1} KBr): 3,328.98 (N–H stretching in piperazine), 3,286.43 (N–H secondary), 3,043.78 (C–H broad), 1,679.56–1,637.35 (aromatic C=N), 1,663–1,602 (aromatic C=C), 1,617.15 (N–H bending piperazine), 1,525.29 (NO_2), 1,455.38 (C–C stretching aromatic), 1,349.42–1,018.15 (aromatic C–N), 892, 635; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.15 (d, 2H J = 8.62 Hz, 2× CH, Ar–H), 7.38 (d, 2H, J = 1.15 Hz, 2× CH, Ar–H), 3.64 (br,s, 1H, NH), 7.14(d, 1H J = 8.42 Hz, 1× CH, Ar–H), 7.01 (d, 1H J = 7.89 Hz, 1× CH, Ar–H), 6.86 (d, 1H J = 1.18 Hz, 1× CH, Ar–H), 2.16 (s, 3H, CH_3), 3.21 (d, 4H J = 13.26 Hz, 2× CH_2, Ar–H), 2.82 (d,4H J = 3.11 Hz, 2× CH_2, Ar–H), 1.98 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 184.56, 176.73, 168.63, 152.32, 143.21, 139.92, 134.36, 131.42, 128.57, 125.73, 124.83, 119.25, 52.31, 46.82, 18.64; mass: 407.47 $(M+H)^+$; elemental analysis for $C_{20}H_{22}N_8O_2$: calculated: C, 59.10; H, 5.46; N, 27.57. Found: C, 59.13; H, 5.47; N, 27.54.

*N^2-(4-nitrophenyl)-N^4-phenyl-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (**8e**)* Brown crystals; yield: 79 %; m.p.: 217–218 °C; MW: 392.41; R_f: 0.59; FTIR (ν_{max}; cm^{-1} KBr): 3,328.98 (N–H stretching in piperazine), 3,285.45 (N–H secondary), 3,045.78 (C–H broad), 1,672.53–1,631.33 (aromatic C=N), 1,617.15 (N–H bending piperazine), 1,590.27 (C=C stretching aromatic) 1,529.38 (NO_2), 1,349.15–1,015.19 (aromatic C–N), 779, 625; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.13 (d, 2H J = 8.67 Hz, 2× CH, Ar–H), 7.32 (d, 2H, J = 0.69 Hz, 2× CH, Ar–H), 3.68 (br,s, 1H, NH), 7.55 (d, 1H J = 8.15 Hz, 1× CH, Ar–H), 7.46 (d, 1H J = 8.13 Hz, 1× CH, Ar–H), 6.91 (d, 1H J = 1.21 Hz, 1× CH, Ar–H), 3.18 (d, 4H J = 13.29 Hz,

2× CH_2, Ar–H), 2.91 (d,4H $J = 3.16$ Hz, 2× CH_2, Ar–H), 1.97 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 182.35, 173.42, 168.36, 153.63, 142.23, 138.73, 132.46, 128.92, 124.24, 119.53, 52.85, 47.84; mass: 393.49 $(M+H)^+$; elemental analysis for $C_{19}H_{20}N_8O_2$: calculated: C, 58.15; H, 5.14; N, 28.55. Found: C, 58.17; H, 5.13; N, 28.45.

*2-(4-((4-Nitrophenyl)amino)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)hydrazinecarboxamide (**8f**)* Yellow crystals; yield: 64 %; m.p.: 234–235 °C; MW:374.16; R_f: 0.62; FTIR (ν_{max}; cm^{-1} KBr): 3,328.98 (N–H stretching in piperazine), 3,323.38 (NH2), 3,289.42 (N–H secondary), 3,046.72 (C–H broad), 1,672.56–1,634.38 (aromatic C=N), 1,663, 1,617.15 (N–H bending piperazine) 1,527.29 (NO_2), 1,337.17–1,008.29 (aromatic C–N), 1,759.66 (C=O),753; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.08 (d, 2H $J = 8.63$ Hz, 2× CH, Ar–H), 7.48 (d, 2H, $J = 0.78$ Hz, 2× CH, Ar–H), 3.65 (br,s, 1H, NH), 6.73 (s, 1H, NH), 6.82 (s, 1H, NH_2), 3.21 (d, 4H $J = 13.25$ Hz, 2× CH_2, Ar–H), 2.96 (d,4H $J = 3.12$ Hz, 2× CH_2, Ar–H), 1.93 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 185.93, 178.32, 168.34, 158.63, 148.75, 138,72, 128.42, 121.63, 52.36, 45.83; mass: 375.28 $(M+H)^+$; elemental analysis for $C_{14}H_{18}N_{10}O_3$: calculated: C, 44.92; H, 4.85; N, 37.42. Found: C, 44.95; H, 4.82; N, 37.47.

*N^2-(3-aminopropyl)-N^4-(4-nitrophenyl)-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (**8g**)* White crystals; yield: 65 %; m.p.: 241–242 °C; MW: 373.41; R_f: 0.43; FTIR (ν_{max}; cm^{-1} KBr): 3,327.95 (N–H stretching in piperazine), 3,319.56 (NH_2), 3,284.57 (N–H secondary), 3,041.78 (C–H broad), 2,964.65 (C–H stretching in aliphatic chain) 1,674.45–1,638.12 (aromatic C=N), 1,668, 1,618.13 (N–H bending piperazine), 1,347.13–1,023.19 (aromatic C–N), 1,527.27 (NO_2), 1,455.38 (C–C stretching), 756; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.16 (d, 2H $J = 8.68$ Hz, 2× CH, Ar–H), 7.35 (d, 2H, $J = 1.03$ Hz, 2× CH, Ar–H), 3.68 (br,s, 1H, NH), 3.27 (t, 2H $J = 6.78$ Hz, CH_2,), 2.74 (t, 2H, $J = 6.83$ Hz, CH_2), 1.69 (t, 2H, $J = 6.74$ Hz, CH_2), 6.74 (s, 1H, NH_2), 3.19 (d, 4H $J = 13.29$ Hz, 2× CH_2, Ar–H), 2.84 (d,4H $J = 3.14$ Hz, 2× CH_2, Ar–H), 1.96 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 184.52, 172.65, 148.72, 142.83, 128.93, 121.53, 53.42, 46.75, 39.45, 32.21; mass: 374.56 $(M+H)^+$; elemental analysis for $C_{16}H_{23}N_9O_2$: calculated: C, 51.46; H, 6.21; N, 33.76. Found: C, 51.48; H, 6.20; N, 33.76.

*4-((4-((4-Nitrophenyl)amino)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenol (**8h**)* Black crystals; yield: 72 %; m.p.: 236–238 °C; MW:408.41; R_f: 0.59; FTIR (ν_{max}; cm^{-1} KBr): 3,328.93 (N–H stretching in piperazine), 3,416 (OH stretching), 3,286.51 (N–H secondary), 3,046.58 (C–H broad), 2,800–3,012 (CH_2 stretching), 1,672.42–1,637.16 (aromatic C=N), 1,618.13 (N–H bending piperazine), 1,605 (C=C), 1,347.13–1,023.19 (aromatic C–N), 1,527.27 (NO_2), 753; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.13 (d, 2H $J = 8.66$ Hz, 2× CH, Ar–H), 7.37 (d, 2H, $J = 0.68$ Hz, 2× CH, Ar–H), 3.68 (br,s, 1H, NH), 7.37 (d, 1H $J = 8.74$ Hz, Ar–H), 6.87 (d, 1H, $J = 3.22$ Hz, Ar–H), 5.48 (s, 1H, OH), 3.24 (d, 4H $J = 13.24$ Hz, 2× CH_2, Ar–H), 2.91 (d,4H $J = 3.11$ Hz, 2× CH_2, Ar–H), 1.98 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 183.42, 173.23, 167.84, 153.57, 147.32, 139.24, 134.47, 124.63, 123.21, 119.56, 117.82, 52.31, 46.73; mass: 409.45 $(M+H)^+$; elemental analysis for $C_{19}H_{20}N_8O_3$: calculated: C, 55.88; H, 4.94; N, 27.44. Found: C, 55.90; H, 4.92; N, 27.43.

*N^2-(4-nitrophenyl)-6-(piperazin-1-yl)-N^4-(p-tolyl)-1,3,5-triazine-2,4-diamine (**8i**)* Yellow crystals; yield: 73 %; m.p.: 218–219 °C; MW:406.44; R_f: 0.54; FTIR (ν_{max}; cm^{-1} KBr): 3,329.97 (N–H stretching in piperazine), 3,283.48 (N–H secondary), 3,045.71 (C–H broad), 1,674.53–1,633.31 (aromatic C=N), 1,668–1,609 (aromatic C=C), 1,613.19 (N–H bending piperazine), 1,527.28 (NO_2), 1,456.35 (C–C stretching aromatic), 1,344.41–1,015.12 (aromatic C–N), 897, 632; ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.14 (d, 2H $J = 8.66$ Hz, 2× CH, Ar–H), 7.34 (d, 2H, $J = 0.69$ Hz, 2× CH, Ar–H), 3.67 (br,s, 1H, NH), 7.38 (d, 1H $J = 8.09$ Hz, Ar–H), 7.05 (d, 1H $J = 5.42$ Hz, Ar–H), 2.25 (s, 3H, CH_3), 3.28 (d, 4H $J = 13.29$ Hz, 2× CH_2, Ar–H), 2.87 (d,4H $J = 3.08$ Hz, 2× CH_2, Ar–H), 1.97 (s,1H NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 183.42, 172.53, 168.75, 153.47, 142.76, 134.21, 129.72, 126.42, 121.62, 118.82, 52.12, 46.35; mass: 407.47 $(M+H)^+$; elemental analysis for $C_{20}H_{22}N_8O_2$: calculated: C, 59.10; H, 5.46; N, 27.57. Found: C, 59.13; H, 5.47; N, 27.54.

*General procedure for the synthesis of titled compounds **9**(**a**–**i**)*

A solution of compound (**3**) (0.01 mol) and desired tri-substituted 1,3,5-triazine compounds **8**(**a**–**i**) (0.01 mol) in dry acetone was stirred at 40–45 °C for 8–9 h. The completion of reaction was monitored by TLC using ethanol:acetone (1:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine and dried over anhydrous Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the titled compounds **9**(**a**–**i**).

*N-(7-chloroquinolin-4-yl)-4-(4-morpholino-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (**9a**)* Light black crystals; yield: 57 %; m.p: 256–257 °C; MW: 607.09; R_f: 0.42; FTIR (ν_{max}; cm^{-1} KBr): 3,397.12 (C–O stretching), 2,933.32(C–H stretching), 2,344.21 (N–H stretching

secondary amine), 1,602.13(N=O stretching), 1,325.63–1,489.96 (C=C stretching), 1,219.82 (C–N stretching), 771.13 (Cl), 685.7 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.36 (d, 1H $J = 6.60$ Hz, quinoline ring), 7.35 (d, 1H $J = 6.65$ Hz, quinoline ring), 4.07 (m, 4H, 2× CH_2, Ar–H), 2.54 (m, 4H, 2× CH_2, Ar–H), 6.90 (m,2H C–H, Ar–H), 4.13 (br, s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm:185.53, 181.25, 174.31, 168.93, 152.73, 151.26, 145.44, 137.51, 134.83, 129.44, 125.62, 124.74, 121.68, 119.21, 68.35, 58.72, 52.14, 48.94; mass: 608.18 $(M+H)^+$; elemental analysis for $C_{27}H_{28}ClN_{11}O_3S$: calculated: C, 53.42; H, 4.48; N, 23.07. Found: C, 53.46; H, 4.46; N, 23.09.

*N-(7-chloroquinolin-4-yl)-4-(4-hydrazinyl-6-((4-nitrophenyl) amino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide hydrate (**9b**)* Light yellow crystals; yield: 73.32 %; m.p.: 230–236 °C; MW: 570.03; R_f: 0.27; FTIR (ν_{max}; cm^{-1} KBr): 3,396.21 (O–H stretching), 2,372.23 (N–H stretching secondary amine), 1,592.25 (N=O stretching), 1,432.81 (C=N stretching), 1,324.41–1,488.73 (C=C stretching aromatic), 1,220.62 (C–N stretching), 771.73 (C–Cl stretching), 676.32 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.36 (d, 1H $J = 6.60$ Hz, quinoline ring), 7.35 (d, 1H $J = 6.65$ Hz, quinoline ring), 4.07 (m, 4H, 2× CH_2, Ar–H), 6.90 (m,2H C–H, Ar–H), 6.70 (s, 1H, NH), 4.13 (br, s, 1H, NH),; ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 186.73, 181.35, 176.42, 153.92, 151.52, 151.17, 150.06, 139.13, 135.58, 126.83, 124.80, 124.36, 119.39, 117.83, 40.74, 39.94; mass: 572.13 $(M+H)^+$; elemental analysis for $C_{23}H_{24}ClN_{11}O_3S$: calculated: C, 48.46; H, 4.24; N, 27.03. Found: C, 48.50; H, 4.21; N, 27.13.

*4-(4-(2-Carbamothioylhydrazinyl)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)-N-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (**9c**)* Dark yellow crystals; yield: 64.16 %; m.p.: 270–271 °C; MW: 611.10; R_f: 0.49; FTIR (ν_{max}; cm^{-1} KBr): 3,417.21 (N–H stretching of primary amine), 2,373.52 (N–H stretching of secondary amine), 1,595.72 (N–O stretching), 1,429.31 (C=N stretching), 1,330.32–1,485.72 (C=C aromatic), 1,220.12 (C–N stretching), 771.62 (C–Cl stretching), 677.14 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.39 (d, 1H $J = 6.62$ Hz, quinoline ring), 7.32 (d, 1H $J = 6.64$ Hz, quinoline ring), 4.09 (m, 4H, 2× CH_2), 6.88 (m,2H C–H, Ar–H), 6.78 (s, 2H, NH_2), 2.50 (s, 1H NH), 4.19 (br, s, 1H, NH),; ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 185.32, 183.12, 179.72, 175.68, 171.65, 152.81, 146.92, 138.96, 135.32, 126.83, 124.80, 121.47, 120.38, 119.39, 58.94, 54.23; mass: 612.12 $(M+H)^+$; elemental analysis for $C_{24}H_{23}ClN_{12}O_2S_2$: calculated: C, 47.17; H, 3.79; N, 27.50. Found: C, 47.16; H, 3.76; N, 27.53.

*N-(7-chloroquinolin-4-yl)-4-(4-((4-nitrophenyl)amino)-6-(o-tolylamino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (**9d**)* Light yellow crystals; yield: 48.83 %; m.p.: 274–276 °C; MW: 627.12; R_f: 0.46; FTIR (ν_{max}; cm^{-1} KBr): 3,397.83 (N–H stretching), 2,371.13 (N–H stretching in secondary amine), 1,500.63 (N=O stretching), 1,418.14 (C=N stretching), 1,333.82 (C=C stretching aromatic), 1,222.21 (C–N stretching), 771.63 (C–Cl stretching), 662.85 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.81 (d, 1H $J = 8.23$ Hz, quinoline ring), 7.90 (d, 1H $J = 6.78$ Hz, quinoline ring), 4.23 (m, 4H, 2× CH_2), 6.93 (m,2H C–H, Ar–H), 3.66–3.85 (d, 4H $J = 48.6$ Hz, Ar–H), 2.24 (s, 1H NH), 2.51 (s, 3H, CH_3), 4.19 (br, s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 186.83, 178.64, 169.41, 164.62, 154.12, 149.53, 143.55, 142, 137.92, 136.35, 132.32, 131.96, 129.21, 126.83, 126.12, 123.97, 121.63, 119.26, 58.71, 53.37; mass: 628.35 $(M+H)^+$; elemental analysis for $C_{30}H_{27}ClN_{10}O_2S$: calculated: C, 57.46; H, 4.34; N, 22.33. Found: C, 57.49; H, 4.31; N, 22.36.

*N-(7-chloroquinolin-4-yl)-4-(4-((4-nitrophenyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (**9e**)* Brown crystals; yield: 49.23 %; m.p.: 247–248 °C; MW: 613.09; R_f: 0.54; FTIR (ν_{max}; cm^{-1} KBr): 3,020.93 (C–H stretching aromatic), 2,336.21–2,402.35 (N–H stretching secondary amine), 1,500,24 (N=O stretching), 1,417.68 (C=N stretching), 1,330.26–1,493.47 (C=C stretching), 1,217.48 (C–N stretching), 771 (C–Cl stretching), 670.85 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.13 (d, 1H $J = 8.76$ Hz, quinoline ring), 7.78 (d, 1H $J = 6.36$ Hz, quinoline ring), 4.12 (m, 4H, 2× CH_2), 6.85 (m,2H C–H, Ar–H), 3.78 (m. 4H, 4× CH, Ar–H), 2.50 (s, 1H NH), 4.19 (br, s, 1H, NH),; ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 186.85, 181.65, 173.42, 168.96, 158.47, 151.28, 149.36, 146.73, 139.96, 138.21, 136.24, 129.43, 128.94, 126.72, 125.93, 121.67, 119.85, 117.85, 113.38, 57.71, 53.12; mass: 614.17 $(M+H)^+$; elemental analysis for $C_{29}H_{25}ClN_{10}O_2S$: calculated: C, 56.81; H, 4.11; N, 22.85. Found: C, 56.78; H, 4.15; N, 22.87.

*2-(4-(4-((7-Chloroquinolin-4-yl)carbamothioyl)piperazin-1-yl)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)hydrazinecarboxamide (**9f**)* Dark yellow crystals; yield: 46.91 %; m.p.: 214–215 °C; MW: 595.04; R_f: 0.52; FTIR (ν_{max}; cm^{-1} KBr):3,116.63–3,411.87 (N–H stretching of primary amine), 3,020.21 (C–H stretching), 2,402.23 (N–H stretching secondary amine), 1,782.83 (C=O stretching), 1,488.53 (N=O stretching), 1,437.13 (C=N stretching), 1,322.64–1,363.52 (C=C stretching), 1,217.15 (C–N stretching), 770.37 (C–Cl stretching), 670.49 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.47 (d, 1H $J = 6.24$ Hz, quinoline ring), 7.61 (d, 1H $J = 5.12$ Hz, quinoline ring), 4.21 (m, 4H,

2× CH_2), 6.82 (m, 2H C–H, Ar–H), 6.54 (s, 2H, NH_2), 2.60 (s, 1H NH), 4.32 (br, s, 1H, NH),; ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 185.63, 182.36, 176.32, 171.92, 163.41, 154.48, 151.52, 149.32, 146.12, 138.81, 136.94, 129.42, 124.81, 123.42, 121.65, 119.72, 118.54, 57.71, 53.48; mass: 597.07 $(M+H)^+$; elemental analysis for $C_{24}H_{23}ClN_{12}O_3S$: calculated: C, 48.44; H, 3.90; N, 28.25. Found: C, 48.47; H, 3.93; N, 28.24.

*4-(4-((3-Aminopropyl)amino)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)-N-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (**9g**)* Dark brown crystals; yield: 88.83 %; m.p.: 196–197 °C; MW: 594.09; R_f: 0.24; FTIR (ν_{max}; cm^{-1} KBr):3,371.43 (N–H stretching primary amine), 3,020.71 (C–H stretching), 2,369.83 (N–H secondary amine), 1,485.47 (N=O stretching), 1,419.52 (C=N stretching), 1,326.12 (C=C stretching), 1,217.57 (C–N stretching), 768.82 (C–Cl stretching), 671.75 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.67 (d, 1H $J = 5.87$ Hz, quinoline ring), 7.70 (d, 1H $J = 5.37$ Hz, quinoline ring), 4.78 (m, 4H, 2× CH_2), 6.895 (m, 2H C–H, Ar–H), 6.68 (s, 2H, NH_2), 3.67 (m, 2H, CH_2) 2.50 (s, 1H NH), 4.38 (br, s, 1H, NH),; ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 184.53, 178.91, 171.20, 164.52, 154.38, 151.21, 149.62, 148.36, 138.57, 136.29, 131.53, 129.78, 126.85, 123.62, 119.83, 118.38, 58.21, 53.28, 41.81, 32.17; mass: 596.12 $(M+H)^+$; elemental analysis for $C_{26}H_{28}ClN_{11}O_2S$: calculated: C, 52.56; H, 4.75; N, 25.93. Found: C, 52.52; H, 4.77; N, 25.98.

*N-(7-chloroquinolin-4-yl)-4-(4-((4-hydroxyphenyl)amino)-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (**9h**)* Brown yellow crystals; yield: 81.84 %; m.p.: 310–311 °C; MW: 629.09; R_f: 0.46; FTIR (ν_{max}; cm^{-1} KBr): 3,439.83 (O–H stretching), 3,020.73 (C–H stretching), 2,335.52–2,375.81 (N–H stretching in secondary amine), 1,625.38 (C=C stretching), 1,488.57 (N=O stretching), 1,414.32 (C=N stretching), 1,218.31 (C–N stretching), 770.83 (C–Cl stretching), 672.35 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.92 (d, 1H $J = 7.54$ Hz, quinoline ring), 7.45 (d, 1H $J = 5.32$ Hz, quinoline ring), 4.09 (m, 4H, 2×, Ar–H), 7.53 (m, 4H, 4× CH, Ar–H), 6.75 (m, 4H, 4× CH, Ar–H), 5.40 (s, 1H Ar-OH),4.05 (br, s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 185.62, 178.41, 171.28, 167.76, 161.78, 154.53, 149.51, 147.12, 139.96, 134.26, 132.54, 131.29, 128.92, 125.74, 123.15, 121.64, 118.63, 117.28, 115.38, 58.26, 53.42; mass: 630.21 $(M+H)^+$; elemental analysis for $C_{29}H_{25}ClN_{10}O_3S$: calculated: C, 55.37; H, 4.01; N, 22.26. Found: C, 55.38; H, 4.00; N, 22.32.

*N-(7-chloroquinolin-4-yl)-4-(4-((4-nitrophenyl)amino)-6-(p-tolylamino)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (**9i**)* Brown crysals; yield: 78.82 %; m.p.: 145–148 °C; MW: 627.12; R_f: 0.64; FTIR (ν_{max}; cm^{-1} KBr): 3,376.63–3,490.13 (N–H stretching), 2,934.23–3,078.51 (C–H stretching), 2,343.65 (N–H stretching secondary amine), 1,489.73 (N=O stretching), 1,414.57 (C=N stretching), 1,327.12 (C=C stretching), 1,218.57 (C–N stretching), 769.18(C–Cl stretching), 673.37 (C=S stretching); ^{1}H NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 8.84 (d, 1H $J = 7.42$ Hz, quinoline ring), 7.43 (d, 1H $J = 5.38$ Hz, quinoline ring), 4.09 (m, 4H, 2× CH_2, Ar–H), 3.67 (m, 4H, 2× CH_2, Ar–H), 7.08 (m, 2H, 2× CH, Ar–H), 7.43 (m, 2H, 2× CH, Ar–H), 2.49 (t, 3H, CH_3),4.17 (br, s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$); δ ppm: 186.56, 182.38, 172.94, 168.36, 161.24, 153.58, 148.36, 145.58, 141.27, 138.69, 136.94, 132.26, 131.86, 129.47, 126.64, 123.74, 121.87, 118.26, 58.73, 51.54, 23.47; mass: 628.10 $(M+H)^+$; elemental analysis for $C_{30}H_{27}ClN_{10}O_2S$: calculated: C, 57.46; H, 4.34; N, 22.33. Found: C, 57.49; H, 4.32; N, 22.35.

Antibacterial screening

Minimum inhibitory concentration

Entire target compounds were screened for their minimum inhibitory concentration (MIC, μg/mL) against selected gram-positive organisms viz. *B. subtilis* (NCIM-2,063), *B. cereus* (NCIM-2,156), *S. aureus* (NCIM-2079) and gram-negative organism viz. *P. aeruginosa* (NCIM-2036), *E. coli* (NCIM-2065), *P. mirabilis* (NCIM-2241), *P. vulgaris* (NCIM-2027) by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards with minor modifications. Ofloxacin was used as standard antibacterial agent. Solutions of the test compounds and reference drug were prepared in dimethyl sulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 μg mL^{-1}. Eight tubes were prepared in duplicate with the second set being used as MIC reference controls (16–24 h visual). After sample preparation, the controls were placed in a 37 °C incubator and read for macroscopic growth (clear or turbid) the next day. Into each tube, 0.8 mL of nutrient broth was pipette (tubes 2–7), tube 1 (negative control) received 1.0 mL of nutrient broth and tube 8 (positive control) received 0.9 mL of nutrient. Tube 1, the negative control, did not contain bacteria or antibiotic. The positive control, tube 8, received 0.9 mL of nutrient broth since it contained bacteria but not antibiotic. The test compound were dissolved in DMSO (100 μg/mL), 0.1 mL of increasing concentration of the prepared test compounds which are serially diluted from tube 2 to tube 7 from highest (100 μg mL^{-1}) to lowest (3.125 μg mL^{-1}) concentration (tube 2–7 containing 100, 50, 25, 12.5, 6.25, 3.125 μg mL^{-1}). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension, concentration of which corresponded to 0.5 McFarland scale (9×10^8 cells/mL) and each bacterium was incubated at 37 °C for 24 h. The final volume in each tube was 1.0 mL. The incubation

chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, i.e. no growth of inoculated bacteria, (National Committee for Clinical Laboratory Standards, 1982) results were shown in Table 1.

Conclusion

As a concluding remark, we had developed a new series of hybrid 4-aminoquinoline-1,3,5-triazine conjugates as potent antibacterial agents through facile and economical route. In addition, this study suggests the potential utility of this hybrid skeleton to develop newer antibacterial agents. Our studies are in progress towards the development of newer entities of this skeleton and reported subsequently in future.

Acknowledgments Authors are gratified to SAIF, Central Drug Research Institute, Lucknow, India for providing spectral data of compounds synthesized herein and SHIATS for providing basic facilities to carry out the project.

Conflict of interest Authors declare no conflict of interest.

References

Alanis AJ (2005) Resistance to antibiotics: are we in the post-antibiotic era. Arch Med Res 36:697–705

Bhat HR, Singh UP, Yadav PS, Kumar V, Das A, Chetia D, Prakash A, Mahanta J (2011) Synthesis, characterization and antimalarial activity of hybrid 4-aminoquinoline-1,3,5-triazine derivatives. Arabian J Chem. doi:10.1016/j.arabjc.2011.07.001

Bhat HR, Ghosh SK, Prakash A, Gogoi K, Singh UP (2012a) In vitro antimalarial activity and molecular docking analysis of 4-aminoquinoline-clubbed 1,3,5-triazine derivatives. Lett Appl Microbiol 54:483–486

Bhat HR, Gupta SK, Singh UP (2012b) Discovery of potent, novel antibacterial hybrid conjugates from 4-aminoquinoline and 1,3,5-triazine: design, synthesis and antibacterial evaluation. RSC Adv 2:12690–12695

Bhat HR, Singh UP, Gahtori P, Ghosh SK, Gogoi K, Prakash A, Singh RK (2013) Antimalarial activity and docking studies of novel bi-functional hybrids derived from 4-aminoquinoline and 1,3,5-triazine against wild and mutant malaria parasites as *pf*-DHFR inhibitor. RSC Adv. doi:10.1039/C2RA21915H

Corbett TH, Leopold WR, Dykes DJ, Roberts BJ, Griswold DP Jr, Schabel FM Jr (1982) Toxicity and anticancer activity of a new triazine antifolate (NSC 127755). Cancer Res 42:1707–1715

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol 74:417–733

Dubey V, Pathak M, Bhat HR, Singh UP (2012) Design, facile synthesis and antibacterial activity of hybrid 1,3,4-thiadiazole-1,3,5-triazine derivatives tethered via S-bridge. Chem Biol Drug Des 80:598–604

Gahtori P, Ghosh SK, Singh B, Singh UP, Bhat HR, Uppal A (2012) Synthesis, SAR and antibacterial activity of hybrid chloro, dichloro-phenylthiazolyl-s-triazines. Saudi Pharm J 20:35–43

Ghosh SK, Saha A, Hazarika B, Singh UP, Bhat HR, Gahtori P (2012) Design, facile synthesis, antibacterial activity and structure-activity relationship of novel di- and tri-substituted 1,3,5-triazines. Lett Drug Des Dis 9:329–335

Junejo JA, Ghosh SK, Shaikh M, Gahtori P, Singh UP (2011) Facile synthesis, antibacterial activity and molecular properties prediction of Some new 1,3-dihydroimidazol-2-thione derivatives. Lett Drug Des Dis 8:763–768

Kumar S, Bhat HR, Kumawat MK, Singh UP (2013) Design and one-pot synthesis of hybrid thiazolidin-4-one-1,3,5-triazines as potent antibacterial agent against human disease causing pathogens. New J Chem. doi:10.1039/C2NJ41028A

Lozano V, Aguado L, Hoorelbeke B, Renders M, Camarasa MJ, Schols D, Balzarini J, San-Félix A, Pérez-Pérez MJ (2011) Targeting HIV entry through interaction with envelope glycoprotein 120 (gp120): synthesis and antiviral evaluation of 1,3,5-triazines with aromatic amino acids. J Med Chem 54:5335–5348

Martinez JL, Baquero F (2000) Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother 44:1771–1777

National Committee for Clinical Laboratory Standards (1982) Standard methods for dilution antimicrobial susceptibility test for bacteria which grow aerobically. NCCLS, Villanova, p 242

Shapiro RS, Robbins N, Cowen LE (2011) Regulatory circuitry governing fungal development, drug resistance, and disease. Microbiol Mol Biol Rev 75:213–267

Singh UP, Singh RK, Bhat HR, Subhaschandra YP, Kumar V, Kumawat MK, Gahtori P (2011) Synthesis and antibacterial evaluation of a series of novel trisubstituted-s-triazines derivatives. Med Chem Res 20:1603–1610

Singh UP, Bhat HR, Gahtori P (2012a) Antifungal activity, SAR and physicochemical correlation of some thiazole-1,3,5-triazine derivatives. J Mycol Med 22:134–141

Singh UP, Pathak M, Dubey V, Bhat HR, Gahtori P, Singh RK (2012b) Design, synthesis, antibacterial activity and molecular docking studies on novel hybrid 1,3-thiazine-1,3,5-triazine derivatives as potential bacterial translation inhibitor. Chem Biol Drug Des 80:572–583

Singh UP, Bhat HR, Gahtori P, Singh RK (2013) Hybrid thiazole-1,3,5-triazines target cytosolic Leucyl-tRNA synthetase for antifungal action revealed by molecular docking studies. In Silico Pharmacology, In press

Singh UP, Bhat HR, Singh RK (2013b) Ceric ammonium nitrate catalysed expeditious one-pot synthesis of 1,3-thiazine as IspE kinase inhibitor of gram-negative bacteria using polyethylene glycol (PEG-400) as an efficient recyclable reaction medium. C. R. Chime. doi:10.1016/j.crci.2012.11.019