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التخليق والتقييم البيولوجي لمكونات عطرية غير متجانسة مبنية على s–ترايزين : التصميم والمبررات والدراسات المقارنة

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الملخص:

إن الاحتياج الملح للبحث عن مكونات بيولوجية جديدة لمكافحة الميكروبات الحديثة المقاومة للأدوية قادنا إلي تقديم تقرير عن مكتبة كاملة من مشتقات ال s– ترايزين. لقد تم تحضير عدة مشتقات ثيوفينولية، فينولية، أنيلينية، ببرازينية، بايبيريدينية ومورفولينية بدأ من 4–((4–كلورو–6–ميثوكسي–1،3،5–ترايزين–2–يل)أمينو) بنزونتريل 3 لتعطي في النهاية 35 من المركبات المستهدفة ألا وهي: (4a–j)، (5a–j)، (6a–g)، (7a–h) على التوالي. لقد تم الفحص المخبري ضد بكتيريا (ستافيلوكوككس اورياس(MTCC96) و (باسيللوس سيركس(MTCC619) و(إسريشيا كولاي(MTCC739) و (سودوموناس ايروجينوسا(MTCC741) وضد فطريات (كانديدا البيكانس(MTCC183) و(اسبيرقيللوس نايقر (MTCC282) و (اسبيرقيللوس كلافاتس(MTCC1323). كما تم كذلك قياس فعالية المركبات المعنية ضد داء السل بإخضاعها لسلالة مايكوباكتيريوم تيوبركيولوسس H37Rv باستعمال طريقة (BACTEC MGIT) وقد وجد عند التقييم البيولوجي بأن مشتقات الثيوفينول هي الاكثر فعالية من الاخرين (ثيوفينول> ببرازين> انيلين> الفينول). لقد تم تحليل المركبات النهائية بواسطة جهاز محول فوريه– طيف الاشعة تحت الحمراء (FT–IR) وجهاز الرنين النووي المغناطيسي (NMR)، وجهازي مطيافية الكتلة (MS) وتحليل العناصر (Corder HCN)

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ORIGINAL ARTICLE

Synthesis and biological evaluation of novel *s*-triazine based aryl/heteroaryl entities: Design, rationale and comparative study

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Piperazine;
s-Triazine;
Thiophenol

Abstract The urgent need in search of new biological entities to fight back with recent drug-resistant microbial flora, has led us report a library of *s*-triazine derivatives. The intermediate 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile 3 was substituted with various thiophenol, phenol, aniline and piperazine/piperidine/morpholine moieties to furnish the final 35 target compounds i.e. (**4a–j**), (**5a–j**), (**6a–g**) and (**7a–h**), respectively. These compounds were screened for in vitro antibacterial evaluation against bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 619, *Escherichia coli* MTCC 739, and *Pseudomonas aeruginosa* MTCC 741) and antifungal activity against fungi (*Candida albicans* MTCC 183, *Aspergillus niger* MTCC 282, and *Aspergillus clavatus* MTCC 1323). The title compounds were further subjected for antituberculosis activity against *Mycobacterium tuberculosis* **H37Rv** strain using the BACTEC MGIT method. In this biological evaluation, thiophenol derivatives were found to be more active than the rest (i.e. -Thiophenol > -piperazine > -Aniline > -phenol). The final compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis.

1. Introduction

Multidrug resistant (MDR) strains, a rapid development of pathogens causing a severe resistance toward currently available standard drugs, pose a frightening threat by increasing severe opportunistic microbial infections in past decades (Gootz, 2010; Niccolai and Tarsi, 1997; Overbye and Barrett, 2005). Such resistant organisms were due for a dramatic and alarming increase in microbial infections which results in pressing problem worldwide. On the other hand, MDR-Tuberculosis (TB) and extensively drug-resistant XDR-TB, caused by some mycobacteria of the *Mycobacterium tuberculosis* complex which most commonly affect the lungs, emerged as one of the most infectious diseases in the recent era (Ducati et al., 2006; Gandhi et al., 2010; Udwadia et al., 2012). The latest statistics of World health Organization (WHO) reported that about one third of the human population were infected with TB which showed the urgent need to combat such dilemma (2012).

Surprisingly, 8.7 million new cases of TB were reported in 2011 from which 13% co-infected with HIV (Human Immuno

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Deficiency); 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 0.4 million people allied to HIV-positive which scores around 25% death due to TB (2013). In view of the above, consequences of these problems highlight the urgent need to develop new agents with specific activity with increased potency to sustain a pool of new bioactive entities. Therefore, design and synthesis of new compounds likely to be unaffected by existing resistance mechanisms are an area of immense significance for medicinal chemists.

Owing to a wide range of biological applications, *s*-triazine nucleus has received an immense attention among chemists through fertile source of pharmacological activities such as antibacterial (Bhushan Singh et al., 2012; Gahtori et al., 2012b; Kumar Ghosh et al., 2012), antimalarial (Gahtori et al., 2012a), antiprotozoal (Baliani et al., 2005), antifungal (Singh et al., 2012), anticancer (Menicagli et al., 2004), antimycobacterial (Patel et al., 2012), and antiviral (Chen et al., 2012). In addition to this several *s*-triazine derivatives bearing p-amino benzonitrile moiety have been found to possess an enhanced antimicrobial profile and improved antitubercular (Patel et al., 2011b) and profound anticancer activity (Patel et al., 2011a) as well. Consequences of such potential effects of triazine and an imperative need in search of new chemical entities lead us to synthesize some biologically efficient molecules.

Recently our research group has reported 2,4,6-trisubstituted triazine derivatives endowing promising biological activity (Modh et al., 2012a,b,c, 2013a,b; Patel et al., 2012, 2011a,b); hence it is worthy to synthesize novel compounds which elicit a series of antimicrobial and anti tuberculosis agents. Recent studies have confirmed that several *s*-triazine derivatives bearing morpholine, piperidine and some piperazine moieties are effective against *M. tuberculosis* H37Rv strain (Sunduru et al., 2010). Prompted by such facts it is worthy to envisage that combination of such bioactive moieties in a compact system may arise with new biologically active agents. We introduced synthetic strategy to acquire triazine nucleus with biolabile derivatives viz. phenol, thiophenol, aniline and piperazine/piperidine/morpholine. Target compounds were rationalized and designed using the hits obtained from the (piperazinyl/piperidinyl)-*s*-triazines derivatives (Patel et al., 2011a), which were previously reported for their antimicrobial, antimycobacterial and anticancer activities besides this, compound R129385 (Das et al., 2004) with *s*-triazine nucleus was reported as an effective antiviral agent (Fig. 1). Adopting all such criteria, herewith, a library of 35 *s*-triazine based compounds were synthesized and evaluated for their biological potential which may lead to future prospects in drug design and discovery.

2. Experimental section

2.1. Materials and methods

All chemicals as well as solvents were procured from sigma Aldrich, Merck and Fluka. Solvents taken were of analytical grade and used without further purification. All reactions were routinely checked by TLC. TLC was performed on aluminum-backed silica gel plates (Silica gel 60 F254 grade, Merck DC) with spots visualized by UV light. Column chromatography was performed on silica gel LC 60A (70–200 μ). Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. FT-IR spectra were recorded on a perkin–Elmer 257 spectrometer using KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz model spectrometer using DMSO-*d6* as a solvent and TMS as an internal standard. The chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. Purity of all tested compounds was ensured on the basis of their elemental analyses (C, H, N) and were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany). The electron spray mass spectra were recorded on a triple quadrupole mass spectrometer with the ionization potential of 70 eV Fig. 2.

2.2. Chemistry: general methods

2.2.1. Synthesis of 2,4-dichloro-6-methoxy-1,3,5-triazine (2)

A mixture of 2,4,6-trichloro-1,3,5-triazine **1** (5.0 g, mol) and sodium bicarbonate (2.5 g, 0.02982 mol) in methanol (10 mL) was stirred at 0–5 °C for 4 h. The progress of the reaction was monitored by TLC using hexane:ethyl acetate (4:1) solvent system as an eluent. After the completion of the reaction, the reaction mass was poured into crushed ice. The solid was separated, washed with cold water, dried and recrystallized from ethanol to give compound **2** (Dudley et al., 1951). Yield: 79%; m.p. 88–90 °C; IR (KBr cm^{-1}): 2815 (–OCH_3), 826 (C_3N_3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 3.77 (s, 3H–Ar–OCH_3); ^{13}C NMR 179.8, 167.5, 58.3; ESI-MS (M + 1): 180.99.

2.2.2. Synthesis of 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (3)

To a stirred solution of compound **2** (5.0 g, 0.02778 mol) and sodium bicarbonate (2.56 g, 0.03056 mol) in THF (20 mL), a solution of 4-amino benzonitrile (3.28 g, 0.02778 mol) was added and stirred for 6 h at room temperature. The progress of the reaction was monitored by TLC using toluene:acetone (4:1) solvent system as an eluent. After the completion of the reaction, resultant mixture was poured into crushed ice. The solid product obtained was filtered, washed with distilled water, dried and purified by column chromatography using toluene:acetone solvent system as an eluent. Yield: 85%; m.p.167 °C; IR (KBr cm^{-1}): 3372 (N–H), 2210 (C═N), 845 (C3N3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*): DMSO-*d6*: δ 2.97 (s, 3H–Ar–OCH_3), 9.8 (s,1H, –NH); ^{13}C NMR 177.1, 169.2, 168.9, 144.6, 135.9, 119.7, 118.5, 105.1, 54.8; ESI-MS (*m*/*z*): 262.67.

2.2.3. General procedure for the preparation of ((4-methoxy-6-(substituted phenylthio)-1,3,5-triazin-2-yl)amino)benzonitrile (4a–j)

A stirred mixture of appropriate thiophenol (0.0191 mol), 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile **3** (5.0 g, 0.0191 mol) and anhydrous K_2CO_3 (2.92 g, 0.0211 mol) in DMF (20 mL) was refluxed for 20 h. The progress of the reaction was monitored by TLC using toluene:acetone (7:3) solvent system as an eluent. After the completion of the reaction, the reaction mass was poured into ice. The

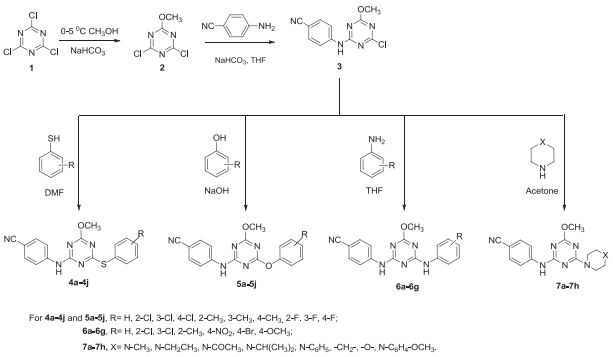

Figure 1 *Scheme*. Synthetic route for novel *s*-triazine based aryl/heteroaryl derivatives.

product was extracted with 25 mL ethyl acetate and then the organic layer was washed with first brine and then with water. The organic layer was separated, dried over Na_2SO_4 and concentrated to dryness to give a yellow solid, which was re-crystallized from n-hexane to give light yellow powder.

*2.2.3.1. 4-((4-Methoxy-6-(phenylthio)-1,3,5-triazin-2-yl)amino) benzonitrile (**4a**)*. Yield: 83%; m.p. 166 °C; IR (KBr cm^{-1}): 3271 (N–H), 2909 (C–H), 2240 (C≡N), 1305 (C–N in 2° aromatic amine), 1210 (C–O), 1100 (C–S), 830 (C_3N_3–*s*-triazine); ^{1}H NMR (400 MHz, DMSO-*d6*): δ ppm 7.54 (d, J = 7.5 Hz, 2H), 7.25–6.89 (m, 7H), 6.33 (s, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.4, 177.9, 166.3, 143.6, 134.4, 132.1, 129.9, 128.1, 126.6, 119.2, 118.1, 103.3, 54.7; Anal. Calcd. for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88; O, 4.77; S, 9.56, Found: C, 60.74; H, 3.79; N, 20.78; O, 4.67; S, 9.45; ESI-MS (M + 1): 336.08.

*2.2.3.2. 4-((4-((2-Chlorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4b**)*. Yield: 79%; m.p. 183 °C; IR (KBr cm^{-1}): 3260 (N–H), 2890 (C–H), 2231 (C≡N), 1309 (C–N in 2° aromatic amine), 1221 (C–O), 1121 (C–S), 841 (C_3N_3–*s*-triazine), 564 (C–Cl); ^{1}H NMR (400 MHz DMSO-*d6*): δ 7.57 (d, J = 7.5 Hz, 2H), 7.34–7.12 (m, 4H), 7.08–7.01 (m, 2H), 6.09 (s, 1H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.4, 179.9, 168.3, 148.6, 137.8, 134.8, 132.4, 130.1, 127.6, 129.9, 126.8, 119.2, 118.2, 103.3, 54.1; Anal. Calcd. for $C_{17}H_{12}ClN_5OS$: C, 55.21; H, 3.27; Cl, 9.59; N, 18.94; O, 4.33; S, 8.67, Found: C, 55.11; H, 3.17; Cl, 9.49; N, 18.83; O, 4.21; S, 8.55; ESI-MS (M + 1): 370.83.

*2.2.3.3. 4-((4-((3-Chlorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4c**)*. Yield: 70%; m.p. 174 °C; IR (KBr cm^{-1}): 3283 (N–H), 2921 (C–H), 2228 (C≡N), 1312 (C–N in 2° aromatic amine), 1215 (C–O), 1110 (C–S) 822 (C_3N_3–*s*-triazine), 594 (C–Cl); ^{1}H NMR (400 MHz DMSO-*d6*): δ 7.51 (d, J = 7.5 Hz, 2H), 7.46 (t, J = 1.3 Hz, 1H), 7.24–7.06 (m, 5H), 6.12 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 193.4, 174.3, 164.8, 149.7, 135.2, 133.7, 131.2, 130.4, 129.6, 127.2, 126.2, 119.2, 118.6, 103.9, 54.1; Anal. Calcd. for $C_{17}H_{12}ClN_5OS$; C, 55.21; H, 3.27; Cl, 9.59; N, 18.94; O, 4.33; S, 8.67, Found: C, 55.10; H, 3.17; Cl, 9.49; N, 18.84; O, 4.23; S, 8.57; ESI-MS (M + 1): 370.83.

*2.2.3.4. 4-((4-((4-Chlorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4d**)*. Yield: 82%; m.p. 180 °C; IR

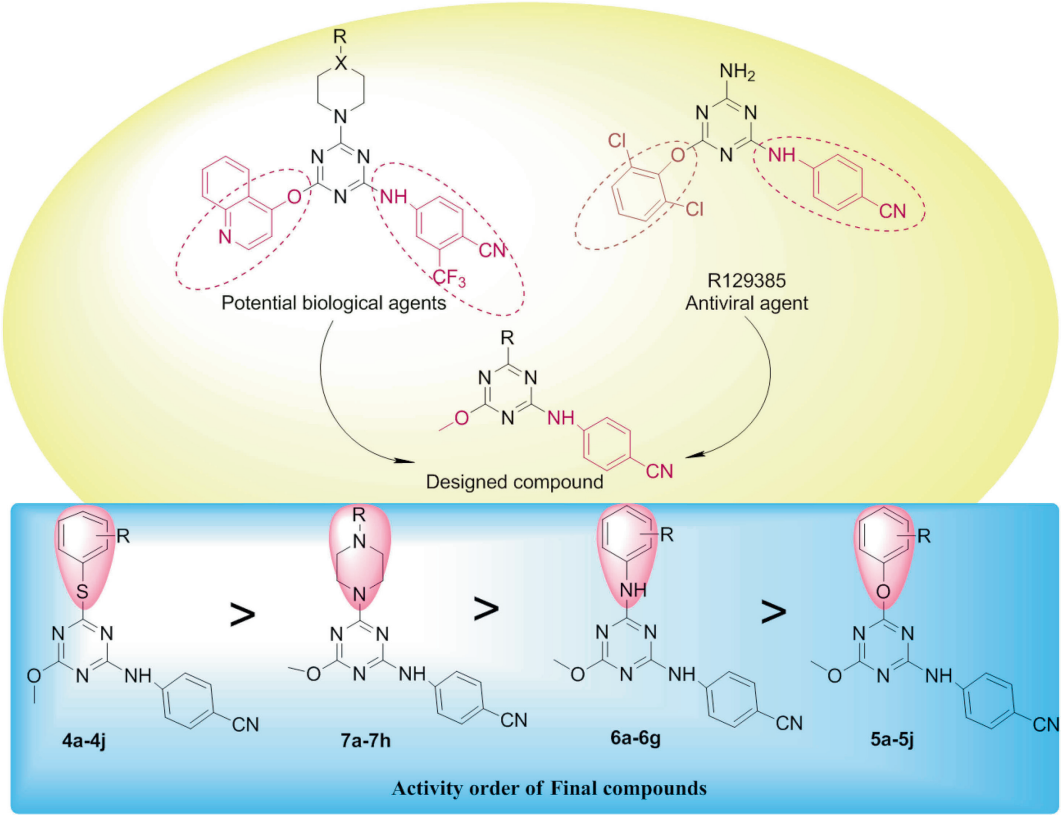

Figure 2 The strategy for design of title compounds.

(KBr cm^{-1}): 3273 (N–H), 2890 (C–H), 2240 (C≡N), 1309 (C–N in 2° aromatic amine), 878 (C_3N_3–*s*-triazine), 1247 (C–O), 580 (C–Cl), 1197 (C–S); ^{1}H NMR (400 MHz DMSO-*d6*): δ 7.41 (d, J = 7.5 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 7.20 (dd, J = 7.5, 4.4 Hz, 4H), 6.29 (s, 1H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 181.1, 172.9, 161.3, 140.6, 135.7, 131.4, 129.4, 127.4, 126.3, 119.8, 118.2, 103.7, 56.9; Anal. Calcd. for $C_{17}H_{12}ClN_5OS$; C, 55.21; H, 3.27; Cl, 9.59; N, 18.94; O, 4.33; S, 8.67, Found: C, 55.21; H, 3.27; Cl, 9.59; N, 18.94; O, 4.33; S, 8.67; ESI-MS (M + 1): 370.83.

*2.2.3.5. 4-((4-Methoxy-6-(o-tolylthio)-1,3,5-triazin-2-yl)amino) benzonitrile (**4e**).* Yield: 77%; m.p. 179 °C; IR (KBr cm^{-1}): 3212 (N–H), 2798 (C–H), 2264 (C≡N), 1397 (C–N in 2° aromatic amine), 812 (C_3N_3–*s*-triazine), 1264 (C–O), 978 (C–S); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.53 (d, J = 7.5 Hz, 2H), 7.50–7.16 (m, 3H), 7.14–6.89 (m, 3H), 6.06 (s, 1H), 3.83 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 195.1, 171.8, 167.3, 156.7, 144.6, 136.9, 133.4, 130.8, 125.6, 123.2, 119.4, 118.6, 115.2, 103.7, 53.9, 20.1; Anal. Calcd. for $C_{17}H_{12}FN_5OS$: C, 57.78; H, 3.42; F, 5.38; N, 19.82; O, 4.53; S, 9.07, Found: C, 57.65; H, 3.31; F, 5.25; N, 19.78; O, 4.41; S, 9.10; ESI-MS (M + 1): 350.41.

*2.2.3.6. 4-((4-Methoxy-6-(m-tolylthio)-1,3,5-triazin-2-yl)amino) benzonitrile (**4f**).* Yield: 62%; m.p. 154 °C; IR (KBr cm^{-1}): 3376 (N–H), 2815 (C–H), 2120 (C≡N), 1264 (C–N in 2° aromatic amine), 892 (C_3N_3–*s*-triazine), 1254 (C–O), 1163 (C–S); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.62–7.47 (m, 2H), 7.30 (s, 1H), 7.22–7.18 (m, 3H), 7.13 (s, 1H), 6.95 (s, 1H), 6.30 (s, 1H), 3.86–3.82 (m, 3H), 2.36–2.32 (m, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.7, 176.1, 163.2, 154.2, 143.1, 137.9, 134.6, 130.9, 128.6, 124.2, 122.6, 119.7, 118.9, 103.1, 59.4, 21.1; Anal. Calcd. for $C_{17}H_{12}FN_5OS$: C, 57.78; H, 3.42; F, 5.38; N, 19.82; O, 4.53; S, 9.07, Found: C, 57.69; H, 3.32; F, 5.28; N, 19.72; O, 4.44; S, 9.05; ESI-MS (M + 1): 350.41.

*2.2.3.7. 4-((4-Methoxy-6-(p-tolylthio)-1,3,5-triazin-2-yl)amino)benzonitrile (**4g**).* Yield: 76%; m.p. 137 °C; IR (KBr cm^{-1}): 3346 (N–H), 2912 (C–H), 2194 (C≡N), 1245 (C–N in 2° aromatic amine), 866 (C_3N_3–*s*-triazine), 1226 (C–O), 1167 (C–S): ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.56 (d, J = 7.5 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 7.5 Hz, 2H), 7.05 (d, J = 7.5 Hz, 2H), 6.30 (s, 1H), 3.78 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.6, 175.3, 166.8, 154.9, 143.6, 142.9, 136.1, 133.4, 129.7, 119.7, 118.2, 103.7, 54.2, 23.9; Anal. Calcd. for $C_{17}H_{12}FN_5OS$: C, 57.78; H, 3.42; F, 5.38; N, 19.82; O, 4.53; S, 9.07, Found: C, 57.66; H, 3.30; F, 5.26; N, 19.70; O, 4.50; S, 9.10; ESI-MS (M + 1): 350.41.

*2.2.3.8. 4-((4-((2-Fluorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4h**).* Yield: 68%; m.p. 146 °C; IR (KBr cm^{-1}): 3243 (N–H), 2887 (C–H), 2251 (C≡N), 1309 (C–N in 2° aromatic amine), 814 (C_3N_3–*s*-triazine), 1210 (C–O), 1320 (C–F), 900 (C–S) ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.61–7.46 (m, 2H), 7.31–7.14 (m, 3H), 7.11 (s, 1H), 6.93 (d, J = 10.7 Hz, 2H), 6.29 (s, 1H), 3.78–3.74 (m, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 188.4, 176.9, 168.9, 158.2, 143.5, 134.6, 128.7, 127.4, 126.1, 124.7, 119.2, 118.7, 115.4, 103.7, 54.8; Anal. Calcd. for $C_{18}H_{15}N_5OS$; C, 61.87; H, 4.33; N, 20.04; O, 4.58; S, 9.18, Found: C, 61.75; H, 4.21; N, 20.10; O, 4.44; S, 9.07; ESI-MS (M + 1): 354.37.

*2.2.3.9. 4-((4-((3-Fluorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4i**).* Yield: 82%; m.p. 164 °C; IR (KBr cm^{-1}): 3273 (N–H), 2840 (C–H), 2240 (C≡N), 1344 (C–N in 2° aromatic amine), 897 (C_3N_3–*s*-triazine), 1364 (C–F), 1296 (C–O), 964 (C–S): ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.53 (d, J = 7.5 Hz, 2H), 8.59–6.81 (m, 8H), 6.15 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 196.3, 177.5, 167.8, 161.9, 145.8, 138.1, 135.2, 128.7, 124.1, 119.2, 118.7, 114.7, 112.3, 101.3, 52.1; Anal. Calcd. For $C_{18}H_{15}N_5OS$ C, 61.87; H, 4.33; N, 20.04; O, 4.58; S, 9.18, Found: C, 61.74; H, 4.23; N, 20.11; O, 4.43; S, 9.05; ESI-MS (M + 1): 354.37.

*2.2.3.10. 4-((4-((4-Fluorophenyl)thio)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**4j**).* Yield: 65%; m.p. 183 °C; IR (KBr cm^{-1}): 3245 (N–H), 2863 (C–H), 2221 (C≡N), 1364 (C–N in 2° aromatic amine), 897 (C_3N_3–*s*-triazine), 1252 (C–O), 1347 (C–F), 946 (C–S): ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.49 (d, J = 7.5 Hz, 2H), 7.37–7.28 (m, 2H), 7.21 (d, J = 7.5 Hz, 2H), 6.91 (t, J = 7.7 Hz, 2H), 6.30 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.6, 178.3, 168.3, 154.1, 141.6, 131.4, 129.2, 127.4, 120.4, 118.4, 114.9, 105.7, 56.5; Anal. Calcd. for $C_{17}H_{12}N_5OS$: C, 61.06; H, 3.62; N, 20.94; O, 4.78; S, 9.59, Found: C, 61.10; H, 3.52; N, 20.83; O, 4.66; S, 9.48; ESI-MS (M + 1): 354.37.

*2.2.4. General procedure for the preparation of 4-((4-(substituted phenoxy)-6-methoxy-1,3,5-triazin-2-yl) amino)benzonitrile (**5a–j**)*

A mixture of 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile **3** (5.0 g, 0.0191 mol), appropriate phenol (0.0191 mol) and sodium hydroxide (0.93 g, 0.0232 mol) in THF (20 mL) was stirred and refluxed for 5 h. The progress of the reaction was monitored by TLC using ethyl acetate:hexane (4:1) solvent system as an eluent. After the completion of the reaction, resultant mixture was poured into crushed ice. The solid product obtained was filtered, washed with distilled water, dried and purified by column chromatography using ethyl acetate:hexane solvent as an eluent.

*2.2.4.1. 4-((4-Methoxy-6-phenoxy-1,3,5-triazin-2-yl)amino) benzonitrile (**5a**).* Yield: 82%; m.p. 140 °C; IR (KBr cm^{-1}): 3397 (N–H), 2788 (C–H), 2164 (C≡N), 1268 (C–N in 2° aromatic amine), 866 (C_3N_3–*s*-triazine), 1234 (C–O); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.55 (d, J = 7.5 Hz, 2H), 7.26–7.12 (m, 4H), 6.98–6.85 (m, 3H), 6.27 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 195.4, 173.9, 167.8, 152.8, 144.1, 136.4, 128.9, 123.6, 122.8, 119.3, 118.7, 104.1, 56.8; Anal. Calcd. for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.93; O, 10.02, Found: C, 63.80; H, 4.00; N, 21.82; O, 10.12; ESI-MS (M + 1): 320.11.

*2.2.4.2. 4-((4-Methoxy-6-(o-tolyloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (**5b**).* Yield: 73%; m.p. 168 °C; IR (KBr cm^{-1}): 3245 (N–H), 2858 (C–H), 2232 (C≡N), 1301 (C–N in 2° aromatic amine), 864 (C_3N_3–*s*-triazine), 1214 (C–O); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.55 (d, J = 7.5 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 7.06 (ddd, J = 14.3, 7.4, 1.5 Hz, 2H), 6.92–6.82 (m, 2H), 6.27 (s, 1H), 3.81 (s, 3H),

2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 188.3, 175.9, 164.3, 157.2, 154.9, 146.1, 134.3, 129.2, 127.6, 124.8, 119.1, 118.8, 114.2, 103.8, 58.1, 18.5; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01; O, 9.60, Found: C, 64.75; H, 4.44; N, 21.11; O, 9.51; ESI-MS (M + 1): 334.12.

*2.2.4.3. 4-((4-Methoxy-6-(m-tolyloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (**5c**)*. Yield: 74%, m.p. 139 °C; IR (KBr cm^{-1}): 3369 (N–H), 2887 (C–H), 2186 (C≡N), 1362 (C–N in 2° aromatic amine), 826 (C_3N_3–*s*-triazine), 1171 (C–O); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.47 (d, *J* = 7.5 Hz, 2H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.12 (t, *J* = 7.4 Hz, 1H), 6.85–6.74 (m, 3H), 6.27 (s, 1H), 3.81 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 186.9, 179.2, 162.8, 158.5, 151.3, 145.7, 133.4, 127.6, 124.9, 121.1, 120.6, 116.8, 112.3, 106.1, 57.4, 22.6; Anal. Calcd. for $C_{18}H_{15}N_5O_2$; C, 64.86; H, 4.54; N, 21.01; O, 9.60, Found: C, 64.73; H, 4.44; N, 21.00; O, 9.58; ESI-MS (M + 1): 334.12.

*2.2.4.4. 4-((4-Methoxy-6-(p-tolyloxy)-1,3,5-triazin-2-yl)amino)benzonitrile (**5d**)*. Yield: 69%; m.p. 161 °C; IR (KBr cm^{-1}): 3361 (N–H), 2859 (C–H), 2114 (C≡N), 1285 (C–N in 2° aromatic amine), 897 (C_3N_3–*s*-triazine), 1216 (C–O); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.22 (d, *J* = 7.5 Hz, 2H), 7.04 (d, *J* = 7.5 Hz, 2H), 6.84 (d, *J* = 7.5 Hz, 2H), 6.27 (s, 1H), 8.35 to −2.37 (m, 15H), 7.50 to −2.37 (m, 13H), 3.81 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.3, 173.9, 162.9, 156.5, 152.3, 147.4, 132.1, 129.4, 121.6, 119.2, 114.1, 104.3, 58.4, 20.4; Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01; O, 9.60, Found: C, 64.76; H, 4.42; N, 20.95; O, 9.50; ESI-MS (M + 1): 334.12.

*2.2.4.5. 4-((4-(2-Fluorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5e**)*. Yield: 68%; m.p. 166 °C; IR (KBr cm^{-1}): 3297 (N–H), 2879 (C–H), 2144 (C≡N), 1321 (C–N in 2° aromatic amine), 844 (C_3N_3–*s*-triazine), 1164 (C–O), 1341 (C–F); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.56 (d, *J* = 7.5 Hz, 2H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.07–6.64 (m, 4H), 6.26 (s, 1H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.9, 178.3, 164.3, 154.8, 152.3, 141.6, 138.2, 127.2, 125.1, 121.4, 119.4, 117.2, 113.7, 104.3, 56.7; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$ C, 60.53; H, 3.59; F, 5.63; N, 20.76; O, 9.49, Found: C, 60.42; H, 3.47; F, 5.53; N, 20.65; O, 9.33; ESI-MS (M + 1): 338.10.

*2.2.4.6. 4-((4-(3-Fluorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5f**)*. Yield: 81%; m.p. 175 °C; IR (KBr cm^{-1}): 3364 (N–H), 2851 (C–H), 2261 (C≡N), 1327 (C–N in 2° aromatic amine), 832 (C_3N_3–*s*-triazine), 1234 (C–O), 1399 (C–F); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.51 (d, *J* = 7.5 Hz, 2H), 7.26–7.12 (m, 3H), 6.68 (ddd, *J* = 14.2, 5.7, 1.3 Hz, 3H), 6.27 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.7, 173.9, 161.3, 154.6, 153.2, 148.6, 134.3, 127.7, 125.1, 123.6, 119.8, 118.4, 115.4, 108.1, 57.2; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$ C, 60.53; H, 3.59; F, 5.63; N, 20.76; O, 9.49, Found: C, 60.41; H, 3.48; F, 5.54; N, 20.64; O, 9.32; ESI-MS (M + 1): 338.10.

*2.2.4.7. 4-((4-(4-Fluorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5g**)*. Yield: 73%; m.p. 175 °C; IR (KBr cm^{-1}): 3313 (N–H), 2812 (C–H), 2245 (C≡N), 1297 (C–N in 2° aromatic amine), 889 (C_3N_3–*s*-triazine), 1145 (C–O), 1362 (C–F); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.57 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 7.5 Hz, 2H), 6.96–6.83 (m, 4H), 6.27 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.9, 174.3, 163.3, 157.1, 151.5, 144.2, 133.7, 125.4, 120.1, 117.8, 114.6, 107.4, 56.9; Anal. Calcd. for $C_{17}H_{12}FN_5O_2$ C, 60.53; H, 3.59; F, 5.63; N, 20.76; O, 9.49, Found: C, 60.45; H, 3.50; F, 5.57; N, 20.66; O, 9.34; ESI-MS (M + 1): 338.10.

*2.2.4.8. 4-((4-(2-Chlorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5h**)*. Yield: 64%; m.p. 136 °C; IR (KBr cm^{-1}): 3293 (N–H), 2866 (C–H), 2164 (C≡N), 1284 (C–N in 2° aromatic amine), 837 (C_3N_3–*s*-triazine), 1164 (C–O), 596 (C–Cl); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.50 (d, *J* = 7.5 Hz, 2H), 7.29–7.11 (m, 3H), 7.08 (td, *J* = 7.5, 1.4 Hz, 1H), 6.91–6.78 (m, 2H), 6.29 (s, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 195.7, 176.3, 164.8, 155.1, 146.8, 133.4, 131.7, 129.2, 127.4, 124.6, 122.9, 120.1, 117.8, 106.3, 57.9; Anal. Calcd. For $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; Cl, 10.02; N, 19.80; O, 9.05, Found: C, 57.61; H, 3.30; Cl, 10.15; N, 19.69; O, 8.96; ESI-MS (M + 1): 354.07.

*2.2.4.9. 4-((4-(3-Chlorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5i**)*. Yield: 68%; m.p. 167 °C; IR (KBr cm^{-1}): 3212 (N–H), 2832 (C–H), 2156 (C≡N), 1244 (C–N in 2° aromatic amine), 846(C_3N_3–*s*-triazine), 1161 (C–O), 569 (C–Cl); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.58 (d, *J* = 7.5 Hz, 2H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.01–6.92 (m, 2H), 6.79 (dt, *J* = 7.3, 1.4 Hz, 1H), 6.27 (s, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.1, 176.9, 164.2, 153.2, 147.2, 139.5, 134.4, 131.9, 126.1, 122.6, 120.2, 119.5, 118.3, 103.2, 56.3; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; Cl, 10.02; N, 19.80; O, 9.05, Found: C, 57.60; H, 3.31; Cl, 10.14; N, 19.71; O, 8.97; ESI-MS (M + 1): 354.07.

*2.2.4.10. 4-((4-(4-Chlorophenoxy)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**5j**)*. Yield: 79%; m.p. 164 °C; IR (KBr cm^{-1}): 3361 (N–H), 2843 (C–H), 2120 (C≡N), 1320 (C–N in 2° aromatic amine), 829 (C_3N_3–*s*-triazine), 1156 (C–O), 561 (C–Cl); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.49 (d, *J* = 7.5 Hz, 2H), 7.21 (dd, *J* = 13.6, 7.5 Hz, 4H), 6.83 (d, *J* = 7.5 Hz, 2H), 6.27 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.2, 177.3, 161.8, 150.9, 145.2, 136.4, 132.9, 130.2, 120.1, 118.6, 114.2, 107.2, 57.4; Anal. Calcd. for $C_{17}H_{12}ClN_5O_2$: C, 57.72; H, 3.42; Cl, 10.02; N, 19.80; O, 9.05, Found: C, 57.62; H, 3.32; Cl, 10.12; N, 19.70; O, 8.95; ESI-MS (M + 1): 354.07.

*2.2.5. General procedure for the synthesis of 4-(4-methoxy-6-phenyl amino-[1,3,5 trazine-2-ylamino)-benzonitrile (**6a**–**g**)*

A stirred solution of 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile **3** (5.0 g, 0.0191 mol), appropriate aniline (0.0191 mol) and sodium bicarbonate (1.77 g, 0.0210 mol) in THF (20 mL) was refluxed for 5 h. The progress of reaction was monitored by TLC using hexane:ethyl acetate (4:1) as an eluent. After the completion of reaction, the refluxed content was poured into crushed ice. The solid product obtained was filtered and dried. The crude product was purified by crystallization from acetone to get the title compound.

*2.2.5.1. 4-((4-Methoxy-6-(phenylamino)-1,3,5-triazin-2-yl) amino)benzonitrile (**6a**)*. Yield: 78%; m.p. 113 °C; IR (KBr cm^{-1}): 3278 (N–H), 1248 (C–O–C), 1310 (CN), 3085 (Aromatic CH str), 836 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.60 (d, J = 7.5 Hz, 2H), 7.30–7.16 (m, 4H), 7.03–6.92 (m, 3H), 4.79 (s, 1H), 4.51 (s, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.7, 172.3, 166.3, 148.8, 136.2, 132.7, 128.7, 122.1, 121.6, 118.4, 113.9, 103.1, 56.8; Anal. Calcd. for $C_{17}H_{14}N_6O$: C, 64.14; H, 4.43; N, 26.40; O, 5.03, Found: C, 62.14; H, 4.33; N, 26.40; O, 5.03; ESI-MS (M + 1): 319.12.

*2.2.5.2. 4-((4-((2-Chlorophenyl)amino)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**6b**)*. Yield: 81%; m.p. 144 °C; IR (KBr cm^{-1}): 3276 (NH), 1245 (C–O–C), 1314 (CN), 3064 (Aromatic CH str), 849 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.60 (d, J = 7.5 Hz, 2H), 9.47–7.22 (m, 3H), 9.47–7.08 (m, 6H), 9.47–7.01 (m, 7H), 9.47–6.94 (m, 7H), 9.47–5.22 (m, 9H), 4.77 (s, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.8, 170.9, 167.7, 145.8, 140.5, 137.6, 135.1, 130.8, 127.6, 122.1, 120.6, 118.5, 112.4, 102.1, 52.7; Anal. Calcd. for $C_{17}H_{13}ClN_6O$: C, 57.88; H, 3.71; Cl, 10.05; N, 23.82; O, 4.54, Found: C, 56.84; H, 3.69; Cl, 10.05; N, 22.82; O, 4.52; ESI-MS (M + 1): 353.08.

*2.2.5.3. 4-((4-((3-Chlorophenyl)amino)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**6c**)*. Yield: 70%; m.p. 138 °C; IR (KBr cm^{-1}): 3259 (NH), 1259 (C–O–C), 1325 (CN), 3059 (Aromatic CH str), 831 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.55 (d, J = 7.5 Hz, 2H), 7.24–7.16 (m, 3H), 7.02–6.92 (m, 3H), 4.70 (s, 1H), 4.60 (s, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 193.3, 174.3, 162.8, 146.3, 143.5, 135.8, 133.1, 128.6, 126.6, 122.1, 118.5, 116.6, 112.3, 106.1, 58.3; Anal. Calcd. for $C_{17}H_{13}ClN_6O$: C, 57.88; H, 3.71; Cl, 10.05; N, 23.82; O, 4.54, Found: C, 57.70; H, 3.55; Cl, 10.05; N, 23.83; O, 4.55; ESI-MS (M + 1): 353.08.

*2.2.5.4. 4-((4-Methoxy-6-(o-tolylamino)-1,3,5-triazin-2-yl) amino)benzonitrile (**6d**)*. Yield: 72%; m.p. 164 °C; IR (KBr cm^{-1}): 3255 (NH), 1256 (C–O–C), 1338 (CN), 3052 (Aromatic CH str), 831 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.84 (t, J = 177.8 Hz, 2H), 9.35–7.07 (m, 6H), 9.35–6.95 (m, 7H), 9.35–4.93 (m, 8H), 8.33–11.38 (m, 16H), 7.65–11.38 (m, 16H), 4.65 (s, 1H), 4.30 (s, 1H), 3.87 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.3, 176.2, 164.4, 146.1, 142.3, 139.8, 135.1, 130.0, 128.2, 126.6, 121.6, 119.3, 118.2, 105.8, 57.6, 18.9; Anal. Calcd. for $C_{18}H_{16}N_6O$: C, 65.05; H, 4.85; N, 25.29; O, 4.81, Found: C, 63.05; H, 3.85; N, 23.29; O, 4.85; ESI-MS (M + 1): 333.14.

*2.2.5.5. 4-((4-Methoxy-6-((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (**6e**)*. Yield: 68%; m.p. 171 °C; IR (KBr cm^{-1}): 3253 (NH), 1239 (C–O–C), 1349 (CN), 3055 (Aromatic CH str), 855 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.16 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 7.18 (d, J = 7.5 Hz, 2H), 4.79 (s, 1H), 4.74 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.0, 177.9, 162.8, 151.9, 144.8, 134.1, 132.5, 126.7, 119.2, 116.8, 113.1, 105.3, 58.2; Anal. Calcd. for $C_{17}H_{13}N_7O_3$: C, 56.20; H, 3.61; N, 26.99; O, 13.21, Found: C, 56.20; H, 3.61; N, 26.99; O, 13.21; ESI-MS (M + 1): 364.11.

*2.2.5.6. 4-((4-((4-Bromophenyl)amino)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**6f**)*. Yield: 73%; m.p. 143 °C; IR (KBr cm^{-1}): 3254 (NH), 1230 (C–O–C), 1339 (CN), 3054 (Aromatic CH str), 850 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.69 (d, J = 7.5 Hz, 2H), 7.42 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 7.5 Hz, 2H), 6.87 (d, J = 7.5 Hz, 2H), 5.65 (s, 1H), 4.46 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.4, 180.7, 169.2, 156.5, 141.9, 136.6, 130.7, 125.1, 120.2, 117.6, 114.9, 107.1, 56.1; Anal. Calcd. for $C_{17}H_{13}BrN_6O$: C, 51.40; H, 3.30; Br, 20.12; N, 21.16; O, 4.03, Found: C, 51.40; H, 3.35; Br, 20.05; N, 21.12; O, 4.04; ESI-MS (M + 1): 397.03.

*2.2.5.7. 4-((4-Methoxy-6-((4-methoxyphenyl)amino)-1,3,5-triazin-2-yl)amino)benzonitrile (**6g**)*. Yield: 82%; m.p. 166 °C; IR (KBr cm^{-1}): 3255 (NH), 1239 (C–O–C), 1347 (CN), 3055 (Aromatic CH str), 856 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.59 (d, J = 7.5 Hz, 2H), 7.17 (d, J = 7.5 Hz, 2H), 6.96 (d, J = 7.5 Hz, 2H), 6.85 (d, J = 7.5 Hz, 2H), 4.58 (s, 1H), 4.31 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 195.1, 171.6, 164.4, 152.9, 144.8, 137.2, 1326, 125.2, 120.8, 118.1, 114.9, 105.5, 59.7, 54.2; Anal. Calcd. for $C_{18}H_{16}N_6O_2$: C, 62.06; H, 4.63; N, 24.12; O, 9.19, Found: C, 61.06; H, 4.60; N, 24.11; O, 9.18; ESI-MS (M + 1): 349.13.

*2.2.6. General procedure of triazine based morpholine piperidine and piperazine derivatives (**7a**–**h**)*

To a stirred solution of 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile **3** (5.0 g, 0.0191 mol) and sodium bicarbonate (1.77 g, .0210 mol) in acetone (10.0 mL), a solution of appropriate piperazines or morpholine or piperidine (0.0191 mol) in 5 mL acetone was added dropwise and refluxed for 4–5 h. The progress of reaction was monitored by TLC using toluene:ethyl acetate (6:4) as an eluent. After the completion of the reaction, the refluxed content was poured into crushed ice. The solid product obtained was filtered and dried. The crude product was purified by crystallization from ethyl acetate to get the title compound.

*2.2.6.1. 4-((4-Methoxy-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)benzonitrile (**7a**)*. Yield: 76%; m.p. 139 °C; IR (KBr cm^{-1}): 3233 (NH), 1274 (C–O–C), 1325 (CN), 3048 (Aromatic CH str), 863 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.81 (d, J = 7.5 Hz, 2H), 7.18 (d, J = 7.5 Hz, 2H), 4.48 (s, 1H), 3.85 (s, 3H), 3.69 (t, J = 5.2 Hz, 2H), 3.55 (t, J = 5.2 Hz, 2H), 2.77 (t, J = 5.1 Hz, 2H), 2.59 (t, J = 5.2 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.5, 172.4, 168.3, 142.7, 134.1, 120.9, 118.4, 105.6, 58.3, 50.8, 49.2, 45.7; Anal. Calcd. for $C_{16}H_{19}N_7O$: C, 59.06; H, 5.89; N, 30.13; O, 4.92, Found: C, 58.06; H, 5.88; N, 30.12; O, 4.92; ESI-MS (M + 1): 326.17.

*2.2.6.2. 4-((4-(4-Ethylpiperazin-1-yl)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**7b**)*. Yield: 69%; m.p. 156 °C; IR (KBr cm^{-1}): 3254 (NH), 1265 (C–O–C), 1339 (CN), 3024 (Aromatic CH str), 844 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.49 (d, J = 7.5 Hz, 2H), 7.14 (d, J = 7.5 Hz, 2H), 4.41 (s, 1H), 3.89 (s, 3H), 3.67 (t, J = 5.1 Hz, 2H), 3.52 (t, J = 5.2 Hz, 2H), 2.81 (t, J = 5.2 Hz, 2H), 2.59–2.49 (m, 4H), 1.08 (t, J = 6.3 Hz,

3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.6, 176.1, 167.7, 145.6, 135.4, 120.2, 114.9, 105.3, 59.3, 55.6, 50.0, 45.1, 13.8; Anal. Calcd. for $C_{17}H_{21}N_7O$: C, 60.16; H, 6.24; N, 28.89; O, 4.71, Found: C, 65.16; H, 6.15; N, 28.89; O, 4.66; ESI-MS (M + 1): 340.18.

*2.2.6.3. 4-((4-(4-Acetylpiperazin-1-yl)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**7c**)*. Yield: 71%; m.p. 136 °C; IR (KBr cm^{-1}): 3240 (NH), 1270 (C–O–C), 1358 (CN), 3076 (Aromatic CH str), 821 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.67 (d, *J* = 7.5 Hz, 2H), 7.12 (d, *J* = 7.5 Hz, 2H), 4.58 (s, 1H), 4.36–3.76 (m, 5H), 4.36–2.26 (m, 11H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 191.1, 177.9, 167.6, 162.9, 145.5, 134.9, 120.3, 115.1, 106.8, 58.4, 52.5, 46.9, 22.7; Anal. Calcd. for $C_{17}H_{19}N_7O_2$: C, 57.78; H, 5.42; N, 27.75; O, 9.06, Found: C, 58.78; H, 5.44; N, 26.75; O, 9.06; ESI-MS (M + 1): 354.16.

*2.2.6.4. 4-((4-(4-Isopropylpiperazin-1-yl)-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**7d**)*. Yield: 84%; m.p. 157 °C; IR (KBr cm^{-1}): 3245 (NH), 1271 (C–O–C), 1355 (CN), 3076 (Aromatic CH str), 850 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.65–7.51 (m, 2H), 7.25–7.10 (m, 2H), 4.48 (s, 1H), 3.87–3.83 (m, 3H), 3.71–3.67 (m, 2H), 3.58–3.54 (m, 2H), 3.16 (s, 1H), 2.79–2.75 (m, 2H), 2.63–2.59 (m, 2H), 1.18 – 1.05 (m, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.1, 172.9, 161.3, 149.4, 135.5, 121.8, 118.1, 104.9, 69.2, 58.4, 54.9, 50.7, 16.5; Anal. Calcd. for $C_{18}H_{23}N_7O$: C, 61.17; H, 6.56; N, 27.74; O, 4.53, Found: C, 62.15; H, 6.56; N, 25.74; O, 4.53; ESI-MS (M + 1): 354.20.

*2.2.6.5. 4-((4-Methoxy-6-(4-phenylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)benzonitrile (**7e**)*. Yield: 67%; m.p. 164 °C; IR (KBr cm^{-1}): 3241 (NH), 1270 (C–O–C), 1358 (CN), 3076 (Aromatic CH str), 844 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.66 (d, *J* = 7.5 Hz, 2H), 7.17 (d, *J* = 7.5 Hz, 2H), 7.11 (dd, *J* = 10.7, 4.4 Hz, 2H), 6.71–6.62 (m, 3H), 4.55 (s, 1H), 3.86 (s, 3H), 4.36–3.37 (m, 11H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 195.8, 176.3, 165.2, 144.9, 141.6, 134.1, 130.9, 125.3, 119.7, 116.6, 112.1, 104.8, 59.5, 47.8, 46.1; Anal. Calcd. for $C_{21}H_{21}N_7O$: C, 65.10; H, 5.46; N, 25.31; O, 4.13, Found: C, 62.10; H, 5.45; N, 24.31; O, 4.10; ESI-MS (M + 1): 388.18.

*2.2.6.6. 4-((4-Methoxy-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)benzonitrile (**7f**)*. Yield: 73%; m.p. 148 °C; IR (KBr cm^{-1}): 3240 (NH), 1272 (C–O–C), 1355 (CN), 3176 (Aromatic CH str), 823 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.58 (d, *J* = 7.5 Hz, 2H), 7.21 (d, *J* = 7.5 Hz, 2H), 4.61 (s, 1H), 3.86 (s, 3H), 3.84–3.39 (m, 2H), 3.31–2.74 (m, 2H), 2.17–1.53 (m, 6H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.1, 177.2, 161.3, 148.9, 135.7, 120.8, 114.6, 104.2, 58.7, 55.1, 28.6, 23.2; Anal. Calcd. for $C_{16}H_{18}N_6O$: C, 61.92; H, 5.85; N, 27.08; O, 5.16, Found: C, 59.92; H, 5.83; N, 27.05; O, 5.14; ESI-MS (M + 1): 311.15.

*2.2.6.7. 4-((4-Methoxy-6-morpholino-1,3,5-triazin-2-yl)amino)benzonitrile (**7g**)*. Yield: 64%; m.p. 152 °C; IR (KBr cm^{-1}): 3249 (NH), 1270 (C–O–C), 1358 (CN), 3077 (Aromatic CH str), 821 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.66 (d, *J* = 7.5 Hz, 2H), 7.16 (d, *J* = 7.5 Hz, 2H), 4.51 (s, 1H), 3.86 (s, 3H), 3.78 (t, *J* = 4.8 Hz, 4H), 3.54 (t, *J* = 4.9 Hz, 2H), 3.43 (t, *J* = 4.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 187.1, 169.4, 162.3, 148.4, 139.6, 121.5, 118.3, 107.6, 67.5, 55.3, 48.2; Anal. Calcd. for $C_{15}H_{16}N_6O_2$: C, 61.92; H, 5.85; N, 27.08; O, 5.16, Found: C, 58.68; H, 5.12; N, 24.91; O, 10.25; ESI-MS (M + 1): 313.13.

*2.2.6.8. 4-((4-Methoxy-6-(4-(4-methoxyphenyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)benzonitrile (**7h**)*. Yield: 78%; m.p. 133 °C; IR (KBr cm^{-1}): 3249 (NH), 1270 (C–O–C), 1358 (CN), 3074 (Aromatic CH str), 825 (s-triazine C–N str.); ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.58 (d, *J* = 7.5 Hz, 2H), 7.19 (d, *J* = 7.3 Hz, 2H), 6.72 (d, *J* = 7.5 Hz, 2H), 6.65 (d, *J* = 7.5 Hz, 2H), 4.54 (s, 1H), 3.86 (s, 3H), 4.36 – 3.52 (m, 14H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 189.9, 173.8, 169.2, 156.4, 146.2, 142.3, 134.5, 127.7, 119.6, 114.9, 112.6, 108.3, 59.1, 56.8, 49.2, 44.5; Anal. Calcd. for $C_{22}H_{23}N_7O_2$: C, 63.30; H, 5.55; N, 23.49; O, 7.67, Found: C, 60.30; H, 5.55; N, 22.49; O, 7.65; ESI-MS (M + 1): 418.19.

2.3. Biological assays

2.3.1. In vitro antimicrobial assays

A stock solution of the final synthesized compounds (200 μg/ml) was prepared in dimethyl sulfoxide and test compounds were taken in a specified quantity of molten sterile agar, i.e., nutrient agar and dextrose agar for antibacterial and for antifungal screening, respectively. Such medium enclosing the test compound was poured into a Petri dish at a depth of 4–5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of 10^5 CFU/ml was prepared and added to plates with serially diluted compounds with concentrations in the range of 3.12–200 μg/ml in dimethyl sulfoxide and incubated at (37 ± 1) °C temperature for 24 h (bacteria) or 48 h (fungi). Minimum concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

2.3.2. In vitro antituberculosis assays

The Mycobacteria Growth Indicator Tubes (MGIT) containing 4 ml of modified Middle brook 7H9 Broth Base were numbered as per the final compounds to be tested for antituberculosis activity by means of various concentrations prepared. The solution was allowed to sit for 20 min, and the tubes were centrifuged at 3000 rpm for 15–20 min. After that about 104–107 CFU/ml of H37RV *M. tuberculosis* strain suspension was added into the medium to be incubated. The MGIT tubes were then closed tightly, stirred well and incubated in a BACTEC MGIT instrument at 37 °C until positivity is observed. The readings were measured from the second day of incubation onwards. Positive cultures were generally detected within 10 days. To observe actual results, the MGIT tubes were removed from incubator and placed under the UV light. Bright fluorescence perceived by the corresponding MGIT tube was noticed in the form of bright orange color in the bottom of the tube showing an orange reflection on the meniscus. The primary screening was carried out at concentration of 12.5 μg/ml against *M. tuberculosis* H37RV in BACTEC MGIT system. Compounds possessing 99% inhibition in the primary screen were described as most active compounds.

3. Results and discussion

3.1. Chemistry

The designed library of target compounds and respective intermediates were synthesized as outlined in Scheme. The first step comprises the nucleophilic substitution of first chlorine atom of cyanuric chloride (**1**) by methanol to give 2,4-dichloro-6-methoxy-1,3,5-triazine (**2**) intermediate with an efficient yield. Appearance of IR absorption peak at 2820 cm^{-1} confirms the presence of the methoxy group in *s*-triazine. The intermediate 4-((4-chloro-6-methoxy-1,3,5-triazin-2-yl)amino)benzonitrile (**3**) was achieved by condensation of compound (**2**) with 4-amino benzonitrile. It displayed absorption band at 2235 cm^{-1} and 3294 cm^{-1} and showed the attachment of cyano and 2° amine group.

The target compound, third chlorine atom of cyanuric chloride was replaced by various substituted phenol, thiophenol, aniline and piperazine/piperidine/morpholine derivatives using appropriate solvents and formed final **4a**–**j**, **5a**–**j, 6a**–**g** and **7a**–**h** compounds respectively which were further characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analyses. Compounds **4a**–**j**'s derivatives endowing thiophenol substituents were confirmed by peaks at 1120 cm^{-1}; phenol substituents of **5a**–**j** were verified by characteristic –C–O– stretching peaks at 1210 cm^{-1}. Additional proton peak of –NH, in ^{1}H NMR, confirmed the substitution with aniline derivatives in the formation of **6a**–**g** compounds whereas besides of $–OCH_3$ peak, ^{1}H NMR spectra of **7a**–**h** compounds appeared with distinguishable $–CH_2–N–CH_2–$ peaks in the range from 3.70 to 3.51 ppm.

3.2. Biological evaluation

All the synthesized compounds (**4a**–**j**, **5a**–**j**, **6a**–**g** and **7a**–**h**) were examined for their antibacterial activities (against four strains of bacteria- *Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430, *Escherichia coli* MTCC 739, and *Pseudomonas aeruginosa* MTCC 741) and antifungal activities (against three strains of fungi- *Candida albicans* MTCC 183, *Aspergillus niger* MTCC 282, and *Aspergillus clavatus* MTCC 1323) using the broth dilution technique (Hawkey and Lewis, 2004) and were also checked for their antituberculosis activity (against *M. tuberculosis* **H37Rv**) using the BACTEC MGIT method as reported earlier (Isenberg and Microbiology, 1992). Ciprofloxacin and ketoconazole were used as standard drugs for antibacterial and antifungal activities, respectively, whereas isoniazid, rifampicin, ethambutol and pyrazinamide are used as standard drugs for antituberculosis activity.

3.2.1. In vitro antibacterial activity

Table 1 shows that all synthesized compounds exhibited well to moderate activity, among them, the chloro group containing moiety **4d** was found to be highly active for the bacterial strain *B. cereus* MTCC 430. The electron donating group i.e. methyl group containing compound **4g** was found superior to others against the bacterial species *E. coli* MTCC 739. Compounds **4i** and **4j** possessing the fluoro group exhibited excellent inhibitory profile against the bacterial strains *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 96 respectively. Compound **4c** having the chloro group showed very good efficacy for the strain *B. cereus* MTCC 430. Another moieties possessing the chloro group i.e. **4c** and **4d** were found to be active for the bacterial strains *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 96 respectively. The fluorinated compounds **4h** and **4i** showed excellent inhibitory profile for the bacterial strains *S. aureus* MTCC 96 and *B. cereus* MTCC 430, respectively. Again compounds **4i** and **4j** also exhibited potent inhibitory profile against the bacterial strains, *E. coli* MTCC 739 and *B. cereus* MTCC 430, respectively. Antibacterial efficacy study showed that out of the compounds **5a**–**j**, compound **5e** endowed with the fluoro group was only found to be highly potent for the bacterial strain *P. aeruginosa* MTCC 741. Compounds **5e** and **5g** possessing the fluoro group displayed potent inhibitory profile for the strains *S. aureus* MTCC 96 and *B. cereus* MTCC 430, respectively. The chloro group containing scaffolds i.e. **5h**, **5i** and **5j** also gave very good antibacterial activity against the strains, *B. cereus* MTCC 430, *E. coli* MTCC 739, and *S. aureus* MTCC 96, respectively. Among the synthesized scaffolds **6a**–**g**, compounds **6c** and **6f** which have the halogen group were found to exhibit excellent inhibitory effect on the bacterial strains *B. cereus* MTCC 430 *and P. aeruginosa* MTCC 741, respectively. The chlorinated moiety **6b** was also found to be active against the bacterial species *B. cereus* MTCC 430. The methyl group containing scaffold i.e. **6d** showed very good antibacterial activity against the bacterium *E. coli* MTCC 739. The brominated compound **6f** exhibited excellent inhibitory effect on the bacterial strain *S. aureus* MTCC 96. Compound **6g** possessing the methoxy group was found to be active for both the bacterial strains, *S. aureus* MTCC 96 and *E. coli* MTCC 739. The antibacterial activity study of compounds **7a**–**h** revealed that there are three triazine moieties which exhibited superior antibacterial efficacy against the specific strain of bacteria. Compound **7c** having the N-acetyl group was found to be highly potent against the bacterial strain *S. aureus* MTCC 96. Compound **7e** endowed with N-phenyl piperazinyl molecule was found superior to other with respect to inhibiting the growth of *P. aeruginosa* MTCC 741. A triazine scaffold **7h** having p-methoxy phenyl piperazinyl entity gave excellent inhibitory effect on the bacterial strain *E. coli* MTCC 739. The *N*-isopropyl piperazinyl triazine moiety **7d** was found as a potent antibacterial agent against both the strains *B. cereus* MTCC 430 and *P. aeruginosa* MTCC 741. A piperidinyl substituted triazine derivative **7f** showed good efficiency against both the bacterial strains *S. aureus* MTCC 96 *and E. coli* MTCC 739. The morpholine substituted triazine scaffold **7g** exhibited potent antibacterial activity for both the strains *S. aureus* MTCC 96 *and P. aeruginosa* MTCC 741. Compound **7h** also showed potency against the strain *P. aeruginosa* MTCC 741.

3.2.2. In vitro antifungal activity

All synthesized triazine scaffolds were examined for their antifungal potency, which is outlined in Table 2 shows that compounds having the halogen group exhibited highly potential antifungal efficacy against specific strain of fungi. The chlorinated moieties **4c** and **4d** displayed excellent inhibitory profile against the fungal strains *C. albicans* MTCC 183 and *A. clavatus* MTCC 1323, respectively. The compound containing the fluoro group i.e. **4i** was found to be highly active for the strain *A. niger* MTCC 282. Another chlorinated moiety **4b** also showed very good antifungal activity against the strain *A. niger* MTCC 282. Compound **4d** was also found to be active against

Table 1 *In vitro* antimicrobial activity of compounds **4a–j**, **5a–j**, **6a–g** and **7a–h**.

Compound	R/X	MIC (μg/mL)			
		Gram +ve		Gram −ve	
		S.a MTCC 96	B.c MTCC 430	E.c MTCC 739	P.a MTCC 741
4a	H	200	50	100	25
4b	2-Cl	25	100	12.5	50
4c	3-Cl	50	6.25	50	12.5
4d	4-Cl	12.5	**3.12**	200	100
4e	2-CH_3	100	200	25	50
4f	3-CH_3	200	200	100	25
4g	4-CH_3	50	25	**3.12**	200
4h	2-F	6.25	100	50	100
4i	3-F	25	6.25	12.5	**3.12**
4j	4-F	3.12	12.5	50	25
5a	H	200	50	200	100
5b	2-CH_3	100	200	25	50
5c	3-CH_3	100	100	50	200
5d	4-CH_3	50	200	100	50
5e	2-F	12.5	25	200	3.12
5f	3-F	50	50	200	100
5g	4-F	25	12.5	50	25
5h	2-Cl	100	12.5	25	100
5i	3-Cl	25	25	12.5	50
5j	4-Cl	12.5	100	50	50
6a	H	200	50	100	200
6b	2-Cl	50	12.5	200	25
6c	3-Cl	100	**3.12**	200	50
6d	2-CH_3	25	50	6.25	100
6e	4-NO_2	200	100	100	50
6f	4-Br	6.25	200	50	**3.12**
6g	4-OCH_3	12.5	200	12.5	25
7a	**X** = N–CH_3	100	50	200	25
7b	N–CH_2CH_3	50	100	200	50
7c	N–$COCH_3$	**3.12**	200	100	100
7d	N–$CH(CH_3)_2$	200	6.25	25	12.5
7e	N–C_6H_5	200	25	50	**3.12**
7f	–CH_2–	12.5	100	6.25	100
7g	–O–	6.25	200	100	12.5
7h	N–C_6H_4–OCH_3	50	200	**3.12**	6.25
Cip.	–	1.72	0.28	1.40	0.62
DMSO	–	–	–	–	–

MIC = Minimum inhibitory concentration, Cip. ciprofloxacin, *S.a Staphylococcus aureus, B.c Bacillus cereus, E.c Escherichia coli, P.a Pseudomonas aeruginosa.*
Bold values indicates superior minimum inhibition concentration against particular microbial strain.

Table 2 *In vitro* antifungal activity of compounds **4a–j**, **5a–j**, **6a–g** and **7a–h**.

Compound	R/X	MIC (μg/mL)		
		C.a MTCC 183	A.n MTCC 282	A.c MTCC 1323
4a	H	100	200	12.5
4b	2-Cl	50	6.25	100
4c	3-Cl	**3.12**	100	50
4d	4-Cl	12.5	50	**3.12**
4e	2-CH_3	25	100	200
4f	3-CH_3	100	25	50
4g	4-CH_3	200	50	100
4h	2-F	50	12.5	100
4i	3-F	25	**3.12**	12.5
4j	4-F	12.5	25	25
5a	H	200	100	25
5b	2-CH_3	50	6.25	200
5c	3-CH_3	100	50	100
5d	4-CH_3	25	12.5	25
5e	2-F	100	100	50
5f	3-F	12.5	25	25
5g	4-F	50	200	100
5h	2-Cl	200	100	200
5i	3-Cl	12.5	50	6.25
5j	4-Cl	6.25	200	25
6a	H	200	100	50
6b	2-Cl	50	200	12.5
6c	3-Cl	6.25	25	200
6d	2-CH_3	100	6.25	200
6e	4-NO_2	25	50	50
6f	4-Br	200	100	**3.12**
6g	4-OCH_3	12.5	200	25
7a	**X** = N–CH_3	100	200	12.5
7b	N–CH_2CH_3	100	100	25
7c	N–$COCH_3$	**3.12**	50	6.25
7d	N–$CH(CH_3)_2$	25	50	50
7e	N–C_6H_5	6.25	12.5	100
7f	–CH_2–	200	**3.12**	200
7g	–O–	200	6.25	12.5
7h	N–C_6H_4–OCH_3	50	100	6.25
Kit.	–	1.56	1.56	0.78
DMSO	–	–	–	–

Kit. ketoconazole, *C.a. Candida albicans, A.n. Aspergillus niger, A.c. Aspergillus clavatus.*
Bold values indicates superior minimum inhibition concentration against particular microbial strain.

the strain *C. albicans* MTCC 183. The fluorinated compounds **4h**, **4i** and **4j** exhibited good antifungal efficacy against the fungal strains *A. niger* MTCC 282, *A. clavatus* MTCC 1323, and *C. albicans* MTCC 183, respectively. Antifungal activity Table 2 revealed that none of the compound of **5a-j** exhibited highly potent activity against the fungal strains. However, some of them were found to be active for the specific fungal species into some extent. Among which, the methyl group containing entities, both **5b** and **5d** showed good potency against the strain *A. niger* MTCC 282. The fluoro group containing scaffold **5f** gave inhibitory effect to the strain *C. albicans* MTCC 183. Compounds **5i** and **5j** were found to be active for the strains *A. clavatus* MTCC 1323 and *C. albicans* MTCC 183, respectively. The chlorinated compound **5i** also gave inhibitory effect on the growth of the fungal strain *C. albicans* MTCC 183. Among the series of compounds **6a–g**, the bromo group containing entity **6f** was found superior to others against *A. clavatus* MTCC 1323. Compounds **6b** and **6c** having the chloro group gave good antifungal activity against the fungal strains *A. clavatus* MTCC 1323 and *C. albicans* MTCC 183, respectively. The electron donating methyl group possessing moiety **6d** was found to be highly active for the strain *A. niger* MTCC 282. The study of compound **7a–h** indicates that the triazine scaffold substituted with N-acetyl piperazine i.e. **7c** exhibited excellent inhibitory profile for the fungal strain *C. albicans* MTCC 183. A piperidinyl derivative of triazine i.e. **7f** displayed a highly potent inhibitory effect on the strain *A. niger* MTCC 282. Compound **7a**, an *N*-methyl piperazine

derivative of triazine was found to be active into some extent against *A. clavatus* MTCC 1323. Again compound **7c** gave very good inhibiting effect on *A. clavatus* MTCC 1323. Compound **7e** endowed with *N*-phenyl piperazinyl molecule showed very high potency to inhibit the growth of both the fungal strains *C. albicans* MTCC 183 and *A. niger* MTCC 282. The morpholine derivative of triazine i.e. **7g** displayed high potency for both the strains *A. niger* MTCC 282 and *A. clavatus* MTCC 1323. The triazine scaffold incorporated with *N*-(4-methoxy phenyl) piperazinyl derivative i.e. **7h** was found to be highly potent against the fungal strain *A. clavatus* MTCC 1323.

3.2.3. In vitro antituberculosis activity

Further all the synthesized triazine scaffolds were examined for their antituberculosis activities against the tubercular strain *M. tuberculosis* **H37Rv** using the BACTEC MGIT method. The results for this study show that the thiophenol substituted triazine scaffolds incorporated with the halogen group i.e. **4b** and **4i** were found to be highly active against the mentioned tubercular strain. The electron donating methyl group containing scaffold **4g** also showed high effectiveness against the mycobacterium species. The study of antituberculosis activity (Table 3) indicates that among the series of compounds **5a-j**, none of the compound was active to inhibit the growth of strain. The chlorinated triazine scaffold **6b** was found to give superior antibacterial efficacy. The triazine molecule endowed with N-isopropyl piperazinyl derivative i.e. **7d** exhibited potent antibacterial profile. The compound containing *N*-(4-methoxy phenyl)piperazinyl moiety i.e. **7h** was also found to be effective in inhibiting the growth of the above mentioned tubercular species.

3.2.4. SAR (structure–activity relationship)

3.2.4.1. Antibacterial activity. Among all synthesized compounds, possessing thiophenol moiety appeared with better antibacterial activity. Interestingly, halogen substitution at para position of thio-phenol ring provides good efficiency against Gram +ve bacteria. When the position of this substituent is replaced with meta, it showed promising activity against Gram −ve bacterial strain. Meanwhile instead of thiophenol ring, incorporation of phenol, amine or piperazine ring deviated the microbial activity but not up to the mark. The presence of two nitrogen atoms in piperazine ring with electron withdrawing acetyl group increases the biological potential than aniline and phenol substituted compounds.

3.2.4.2. Antifungal activity. Only halogen substituted thiophenol compounds provided good inhibition growth of fungal strains. Compared to other compounds, piperazine with acetyl group showed excellent antifungal activity as shown for bacterial strains as well. Phenol substituted derivatives were not as active as arylamino substituted compounds among which 4-fluoro substituent appeared with good inhibition.

3.2.4.3. Anti-tuberculosis activity. Halogen at ortho and meta position of thiophenol ring showed about 95% inhibition of **H37Rv** strain and methyl substituent at para position provided same activity with less MIC values. Isopropyl and anisole substituent in piperazine ring showed better anti-tuberculosis activity than the rest of the compounds. Unfortunately, none of the phenol substituted derivatives showed % inhibition of tuberculosis strain however, ortho substituted chloro group in aniline exhibited excellent inhibition. Nevertheless, rest of the compounds appeared with good to moderate activity due to variation of substituent positions. These positional isomers may affect the potency of titled compounds and hence deviation of biological activity as well.

Table 3 *In vitro* antituberculosis activity of compounds **4a–j**, **5a–j**, **6a–g** and **7a–h**.

Compound	R/X	BACTEC MGIT method [a]	
		MIC μg/ml	% Inhibition
4a	H	>12.5	–
4b	2-Cl	**12.5**	95
4c	3-Cl	>12.5	–
4d	4-Cl	>12.5	–
4e	2-CH_3	>12.5	–
4f	3-CH_3	>12.5	–
4g	4-CH_3	**12.5**	**95**
4h	2-F	>12.5	–
4i	3-F	**12.5**	**95**
4j	4-F	>12.5	–
5a	H	>12.5	–
5b	2-CH_3	>12.5	–
5c	3-CH_3	>12.5	–
5d	4-CH_3	>12.5	–
5e	2-F	>12.5	–
5f	3-F	>12.5	–
5g	4-F	>12.5	–
5h	2-Cl	>12.5	–
5i	3-Cl	>12.5	–
5j	4-Cl	>12.5	–
6a	H	>12.5	90
6b	2-Cl	12.5	95
6c	3-Cl	>12.5	92
6d	2-CH_3	>12.5	90
6e	4-NO_2	>12.5	90
6f	4-Br	>12.5	91
6g	4-OCH_3	>12.5	90
7a	**X** = N–CH_3	>12.5	91
7b	N–CH_2CH_3	>12.5	92
7c	N–$COCH_3$	>12.5	93
7d	N–$CH(CH_3)_2$	12.5	**95**
7e	N–C_6H_5	>12.5	92
7f	–CH_2–	>12.5	93
7g	–O–	>12.5	94
7h	N–C_6H_4–OCH_3	12.5	**95**
Isoniazid	–	0.20	99
Rifampicin	–	0.25	99
Ethambutol	–	3.12	99
Pyrazinamide	–	6.25	99

[a] Each value is the mean of three independent experiments.
Bold values indicates superior minimum inhibition concentration against particular microbial strain.

4. Conclusion

The present work is basically focused on the development of novel s-triazinyl derivatives with wide therapeutic windows. Out of 35 compounds screened, majority of the compounds came out with promising activity against a wide range of pathogenic bacteria, fungi and mycobacteria. From the bioassay results, it was also possible to make a number of correlations

regarding the relationship between the structure of the newer scaffolds and their antimicrobial activities. The thiophenol derivatives bearing chloro and fluoro were found to be the most active among the four series of final *s*-triazine based congeners. Marking on this order, piperazine substituted compounds displayed better activity than aniline and phenol substituted derivatives. *N*-acetyl (**7c**) with 3.12 μg/mL of MIC and *N*-(4-methyl phenoxy) (**7h**) analogous emerged as potential agents against bacteria and *M. tuberculosis* H37Rv strain as well. These privileged structures with enhanced bioactivities lead to provide enough scope to develop new scaffolds for further drug discovery process.

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References

Baliani, A., Bueno, G.J., Stewart, M.L., Yardley, V., Brun, R., Barrett, M.P., Gilbert, I.H., 2005. Design and synthesis of a series of melamine-based nitroheterocycles with activity against trypanosomatid parasites. J. Med. Chem. 48, 5570–5579.

Bhushan Singh, R., Das, N., Jana, S., Das, A., 2012. Synthesis and in vitro antibacterial screening of some new 2,4,6-trisubstituted-1,3,5-triazine derivatives. Lett. Drug Des. Discov. 9, 316–321.

Chen, X., Zhan, P., Liu, X., Cheng, Z., Meng, C., Shao, S., Pannecouque, C., De Clercq, E., 2012. Design, synthesis, anti-HIV evaluation and molecular modeling of piperidine-linked amino-triazine derivatives as potent non-nucleoside reverse transcriptase inhibitors. Bioorg. Med. Chem. 20, 3856–3864.

Das, K., Clark, A.D., Lewi, P.J., Heeres, J., de Jonge, M.R., Koymans, L.M., Vinkers, H.M., Daeyaert, F., Ludovici, D.W., Kukla, M.J., 2004. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J. Med. Chem. 47, 2550–2560.

Ducati, R.G., Ruffino-Netto, A., Basso, L.A., Santos, D.S., 2006. The resumption of consumption: a review on tuberculosis. Mem. Inst. Oswaldo Cruz 101, 697–714.

Dudley, J.R., Thurston, J.T., Schaefer, F.C., Holm-Hansen, D., Hull, C.J., Adams, P., 1951. Cyanuric chloride derivatives. III. Alkoxy-*s*-triazines. J. Am. Chem. Soc. 73, 2986–2990.

Gahtori, P., Ghosh, S.K., Parida, P., Prakash, A., Gogoi, K., Bhat, H.R., Singh, U.P., 2012a. Antimalarial evaluation and docking studies of hybrid phenylthiazolyl-1,3,5-triazine derivatives: a novel and potential antifolate lead for Pf-DHFR-TS inhibition. Exp. Parasitol. 130, 292–299.

Gahtori, P., Ghosh, S.K., Singh, B., Singh, U.P., Bhat, H.R., Uppal, A., 2012b. Synthesis, SAR and antibacterial activity of hybrid chloro, dichloro-phenylthiazolyl-s-triazines. Saudi Pharm. J. 20, 35–43.

Gandhi, N.R., Nunn, P., Dheda, K., Schaaf, H.S., Zignol, M., Van Soolingen, D., Jensen, P., Bayona, J., 2010. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 375, 1830–1843.

Gootz, T.D., 2010. The global problem of antibiotic resistance. Crit. Rev. Immunol. 30, 79–93.

Hawkey, P., Lewis, D.A., 2004. Medical Bacteriology: A Practical Approach. OUP, Oxford.

Isenberg, H.D., Microbiology, A.S.F., 1992. Clinical Microbiology Procedures Handbook. American Society of Microbiology.

Kumar Ghosh, S., Saha, A., Hazarika, B., Pratap Singh, U., Raj Bhat, H., 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. Discov. 9, 329–335.

Menicagli, R., Samaritani, S., Signore, G., Vaglini, F., Dalla Via, L., 2004. In vitro cytotoxic activities of 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines: new molecules in anticancer research. J. Med. Chem. 47, 4649–4652.

Modh, R.P., Patel, A.C., Chikhalia, K.H., 2012a. Design, synthesis biological evaluation of some novel quinazolinone scaffolds. Med. Chem. 8, 182–192.

Modh, R.P., Patel, A.C., Mahajan, D.H., Pannecouque, C., De Clercq, E., Chikhalia, K.H., 2012b. Synthesis and evaluation of novel 4-substituted styryl quinazolines as potential antimicrobial agents. Arch. Pharm. Chem. Life Sci. 345, 964–972.

Modh, R.P., Prasanth Kumar, S., Jasrai, Y.T., Chikhalia, K.H., 2012c. Design, synthesis, biological evaluation, and molecular modeling of coumarin-piperazine derivatives as acetylcholinesterase inhibitors. Arch. Pharm. Chem. Life Sci. 346. http://dx.doi.org/10.1002/ardp.201300242.

Modh, R.P., De Clercq, E., Pannecouque, C., Chikhalia, K.H., 2013a. Design, synthesis, antimicrobial activity and anti-HIV activity evaluation of novel hybrid quinazoline–triazine derivatives. J. Enzyme Inhib. Med. Chem.. http://dx.doi.org/10.3109/14756366.2012.755622.

Modh, R.P., Patel, A.C., Chikhalia, K.H., 2013b. Design, synthesis, antibacterial, and antifungal studies of novel 3-substituted coumarinyl-triazine derivatives. Heterocycl. Commun.. http://dx.doi.org/10.1515/hc-2013-0104.

Niccolai, D., Tarsi, L., Thomas, R.J., 1997. The renewed challenge of antibacterial chemotherapy. Chem. Commun., 2333–2342.

Overbye, K.M., Barrett, J.F., 2005. Antibiotics: where did we go wrong? Drug Discov. Today 10, 45–52.

Patel, R.V., Kumari, P., Rajani, D.P., Chikhalia, K.H., 2011a. Synthesis and studies of novel 2-(4-cyano-3-trifluoromethylphenyl amino)-4-(quinoline-4-yloxy)-6-(piperazinyl/piperidinyl)-*s*-triazines as potential antimicrobial, antimycobacterial and anticancer agents. Eur. J. Med. Chem. 46, 4354–4365.

Patel, R.V., Kumari, P., Rajani, D.P., Chikhalia, K.H., 2011b. Synthesis, characterization and pharmacological activities of 2-[4-cyano-(3-trifluoromethyl)phenyl amino)]-4-(4-quinoline/coumarin-4-yloxy)-6-(fluoropiperazinyl)-*s*-triazines. J. Fluorine Chem. 132, 617–627.

Patel, A.B., Patel, R.V., Kumari, P., Rajani, D.P., Chikhalia, K.H., 2012. Synthesis of potential antitubercular and antimicrobial s-triazine-based scaffolds via Suzuki cross-coupling reaction. Med. Chem. Res. 22, 367–381.

Singh, U.P., Bhat, H.R., Gahtori, P., 2012. Antifungal activity, SAR and physicochemical correlation of some thiazole-1,3,5-triazine derivatives. J. Mycol. Med. 22, 134–141.

Sunduru, N., Gupta, L., Chaturvedi, V., Dwivedi, R., Sinha, S., Chauhan, P.M., 2010. Discovery of new 1,3,5-triazine scaffolds with potent activity against *Mycobacterium tuberculosis* H37Rv. Eur. J. Med. Chem. 45, 3335–3345.

Tuberculosis Fact sheet, 2013. Available from: < http://www.who.int/mediacentre/factsheets/fs104/en/ > (accessed September 05, 2013).

Udwadia, Z.F., Amale, R.A., Ajbani, K.K., Rodrigues, C., 2012. Totally drug-resistant tuberculosis in India. Clin. Infect. Dis. 54, 579–581.

World Health Organization, 2012. Global tuberculosis report 2012. Available from; http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf (accessed September 05, 2013).