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Novel pyrazolyl-s-triazine derivatives, molecular structure and antimicrobial activity

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ABSTRACT

A new series of pyrazole-containing s-triazine derivatives were synthesized by reaction of the corresponding s-triazinyl hydrazine derivatives with acetylacetone in the presence of $HClO_4$ or DMF/TEA. The former method allowed the preparation of the target products with higher yields. All compounds were fully characterized. X-ray single crystal diffraction for two representative compounds (4-(4,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl)morpholine and N-benzyl-4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(piperidin-1-yl)-1,3,5-triazin-2-amine) was studied and the molecular structures were optimized using the DFT/B3LYP method. The structures were found to be in agreement with X-ray structures. The antimicrobial and antifungal activity of the prepared compounds were tested against the growth of several microorganisms.

1. Introduction

1,3,5-Triazine and its derivatives are key moieties in drug discovery due to their wide range of biological activities, such as antibacterial [1], antiprotozoal [2], fungicidal [3], anticancer [1], antiviral [4], cytotoxic [5], sedative and anti-inflammatory [6], herbicide [7], and analgesic [8] effects and hence these moieties have been found to be useful in numerous fields [9–13]. Pyrazole and its derivatives have also been found in numerous biological and pharmaceutical applications, such as hypoglycemic activity inhibitors, deactivators of liver alcohol dehydrogenase and oxidoreductases [14,15] and antihypertensive agents [16]. Therefore, the synthesis of new s-triazine derivatives attached to pyrazolyl rings could be an efficient strategy for the discovery of new drugs.

In literature, s-triazines with a pyrazolyl fragment in their structure can be synthesized by cyclotrimerization of aromatic nitriles [17] or from cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) by direct substitution of first or second chlorine atom by an amine-containing pyrazolyl moiety [18] or by reaction of s-triazine hydrazine derivatives with acetoacetic ester and 3-iminobutyronitrile [19]. More recently, pyrazolyl-s-triazine derivatives have been synthesized by means of reactions of hydrazinyl-s-triazine with 1,3-diketone (aliphatic or aromatic) in the presence of catalytic amount of trimethylamine [20]. These derivatives showed low toxicity towards growth-stimulating activity, antidote activity and also inhibitors of the oil-oxidizing processes [21,22].

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0022-2860/

In our ongoing medicinal chemistry project based on the use of s-triazine as a scaffold [23–25], here we report further modifications of amine-containing s-triazine with various substituents by incorporating mono and dipyrazole derivatives (Fig. 1), along with the X-ray single crystal diffraction of two representative compounds was studied with further validation of molecular structures using the DFT/B3LYP method. The antimicrobial activity of the synthesized compounds was tested in preliminary assays to offer a clear indication regarding effect of substituents on the bioactivity.

2. Experimental section

2.1. Chemistry

Materials and method: The solvents were of analytical reagent grade and were used without further purification. NMR (^{1}H and ^{13}C) spectra were recorded on a JEOL 400 MHz spectrometer at room temperature in $CDCl_3$ using internal standard $\delta = 0$ ppm. Elemental analysis were performed on Perkin-Elmer 2400 elemental analyzer. Melting points were determined on a Mel-Temp apparatus in an open capillary tube and are uncorrected. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on Shimadzu model IRAffinity-1 Spectrometer from KBr discs. The follow-up of the reaction and purity checks were done using silica gel-coated TLC plates (Type 60 GF254, Merck). A mixture of methanol-chloroform (1:9) or ethylacetate-hexane (4:6) was used as eluent.

2.1.1. Synthesis of 2,4-dichloro-6-substituted 1,3,5-triazine derivatives

2,4-Dichloro-6-substituted 1,3,5-triazine derivatives were prepared following the reported method [23–25].

2.1.1.1. 2, 4-dichloro-6-morpholino-1,3,5-triazine ***(2a)***. White solid in 94% yield; mp = 157–158 °C (Lit. [23] mp = 152–153 °C). ^{1}H NMR ($CDCl_3$): δ 3.69 (t, 4H, J = 5.4 Hz, 2-CH_2N), δ 3.73 (t, 4H, J = 5.4 Hz, 2 −CH_2O) ppm.

2.1.1.2. 2,4-dichloro-6-(piperidin-1-yl)-1,3,5-triazine ***(2b)***. White solid in 90% yield; mp = 76 °C (Lit. [25] mp = 72–74 °C); ^{1}H NMR ($CDCl_3$): δ 1.60–1.65 (m, 2H, CH_2), 1.69–1.72 (m, 4H, 2CH_2), 3.80 (t, 4H, J = 6.1 Hz, 2CH_2N) ppm.

2.1.1.3. N-benzyl-4,6-dichloro-1,3,5-triazin-2-amine ***(2c)***. Off white solid in 88% yield; mp = 115–117 °C; ^{1}H NMR ($CDCl_3$): δ 4.65 (d, 2H, J = 6.4 Hz, phC***H$_2$***), 6.61 (bs, 1H, NH), 7.24–7.37 (m, 5H, C_6***H$_5$***) ppm; ^{13}C NMR ($CDCl_3$): δ 45.4, 127.7, 128.1, 128.9, 136.2, 165.8, 169.9, 171.1 ppm. Anal. Calc. for $C_{10}H_8Cl_2N_4$ (255.10): C, 47.08; H, 3.16; N, 21.96. Found: C, 47.19; H, 3.22; N, 22.06.

2.1.2. General method for the synthesis of 2-chloro-4,6-disubstituted 1,3,5-triazine

A solution of amine (20 mmol) in 50 mL DCM was added dropwise at room temperature to a solution of mono substituted-s-triazine (10 mmol) in 50 mL dichloromethane over 10 min. The reaction mixture was stirred for 6–8 h at room temperature and then DCM (100 mL) was added, and the combined organic layer was washed with 1N HCl (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous $MgSO_4$, and after filtration, the solvent was removed under vacuum. The crude product obtained was recrystallized from DCM-hexane (1:2).

2.1.2.1. 4-(4-chloro-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)morpholine ***(5a)***.

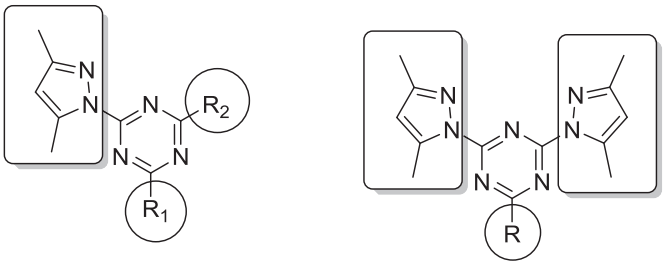
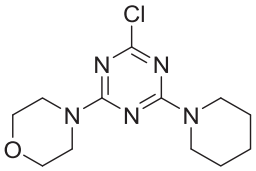
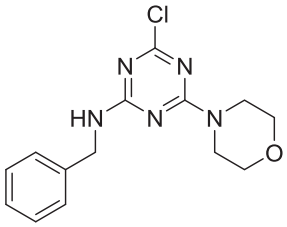


Fig. 1. Structure of mono- and di-pyrazolyl-s-triazine derivatives.



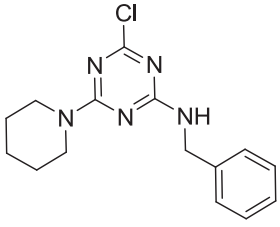
White solid in 87% yield; mp = 125–126 °C; ^{1}H NMR ($CDCl_3$): δ 1.52–1.63 (m, 6H, 3CH_2), 3.68–3.75 (m, 12H, 6CH_2) ppm; ^{13}C NMR ($CDCl_3$): δ 24.6, 25.6, 25.8, 43.6, 43.8, 44.0, 44.5, 66.6, 66.9, 163.9, 164.5, 169.5 ppm. Anal. Calc. for $C_{12}H_{18}ClN_5O$ (283.76): C, 50.79; H, 12.49; N, 24.68. Found: C, 51.01; H, 12.34; N, 24.54.

2.1.2.2. N-benzyl-4-chloro-6-morpholino-1,3,5-triazin-2-amine ***(5b)***.



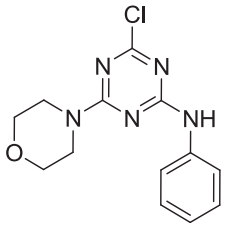
White solid in 85% yield; mp = 158–159 °C; ^{1}H NMR ($CDCl_3$): δ 3.68 (brs, 4H, 2CH_2), 3.77 (brs, 4H, 2CH_2), 4.58 (d, 2H, J = 6.0 Hz, PhCH_2), 6.10 (brs, 1H, NH), 7.26–7.33 (m, 5H, C_6H_5-) ppm; ^{13}C NMR ($CDCl_3$): δ 43.9, 44.8, 66.5, 127.3, 127.4, 127.8, 128.6, 128.7, 163.5, 165.6 ppm. Anal. Calc. for $C_{14}H_{16}ClN_5O$ (305.77): C, 54.99; H, 5.27; N, 22.90. Found: C, 54.76; H, 5.11; N, 23.18.

2.1.2.3. N-benzyl-4-chloro-6-(piperidin-1-yl)-1,3,5-triazin-2-amine ***(5c)***.



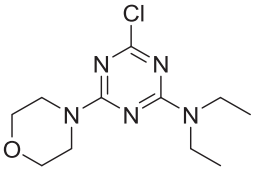
White solid in 85% yield; mp = 151–152 °C; ^{1}H NMR ($CDCl_3$): δ 1.53 (brs, 2H, CH_2), 1.62 (brs, 4H, 2CH_2), 3.71 (brs, 4H, 2CH_2), 4.58 (d, 2H, J = 6.0 Hz, PhCH_2), 6.05 (brs, 1H, NH), 7.25–7.30 (m, 5H, C_6H_5) ppm; ^{13}CNMR ($CDCl_3$): δ 24.4, 25.4, 43.3, 43.4, 126.2, 126.4, 127.1, 127.4, 127.9, 128.2, 140.9, 164.2, 165.6, 168.0 ppm. Anal. Calc. for $C_{15}H_{18}ClN_5$ (303.79): C, 59.30; H, 5.97; N, 23.05. Found: C, 59.11; H, 6.03; N, 23.28.

*2.1.2.4. 4-chloro-6-morpholino-N-phenyl-1,3,5-triazin-2-amine **(5d)**.*



White solid in 85% yield; mp = 125–127 °C; ^{1}H NMR ($CDCl_3$): δ 3.72 (brs, 4H, $2CH_2$), 3.82 (brs, 4H, $2CH_2$), 5.27 (s, 1H, NH), 7.09 (t, 1H, J = 7.6 Hz, Ar), 7.32 (dd, 2H, J = 12.4, Ar), −7.48 (d, 2H, J = 7.2, Ar) ppm; ^{13}C NMR ($CDCl_3$): δ 44.1, 66.5, 66.6, 120.5, 124.0, 128.9, 163.8, 164.6 ppm. Anal. Calc. for $C_{13}H_{14}ClN_5O$ (291.74): C, 53.52; H, 4.84; N, 24.01. Found: C, 53.80; H, 5.04; N, 23.83.

*2.1.2.5. 4-chloro-N,N-diethyl-6-morpholino-1,3,5-triazin-2-amine **(5e)**.*



White solid in 85% yield; mp = 108–109 °C; ^{1}H NMR ($CDCl_3$): δ 1.12 (t, 6H, J = 7.6 Hz, $2CH_3$), 3.46–3.56 (m, 4H, $2CH_2$), 3.66 (t, 4H, J = 3.4 Hz, $2CH_2$), 3.74 (t, 4H, J = 4.4 Hz, $2CH_2$)ppm; ^{13}C NMR ($CDCl_3$): δ 12.5, 13.2, 41.4, 41.6, 43.7, 66.6, 66.8, 163.9, 164.4, 169.2 ppm. Anal. Calc. for $C_{11}H_{18}ClN_5O$ (271.75): C, 48.62; H, 6.68; N, 25.77. Found: C, 48.88; H, 6.74; N, 25.98.

2.1.3. General method for the synthesis of hydrazino −1,3,5-triazine derivatives

The synthesis of hydrazine derivatives was performed following the reported method with slight modifications [26]: Hydrazine hydrate 80% (10 mL) was added dropwise to a solution of chloro-substituted-1,3,5-triazine (20 mmol) in 50 mL ethanol at room temperature. The reaction mixture was then sonicated for 60 min at 60 °C. As a part of the work up, ethanol and excess hydrazine were removed under vacuum, followed by addition of excess diethyl ether to afford the product as a white solid in excellent yield and purities. In the case of the synthesis of the dihydrazine derivatives, 20 mL of hydrazine was used for the substitution process. The mono- and di-hydrazino derivatives were used directly in the next step without further purification.

*2.1.3.1. 4-(4,6-dihydrazinyl-1,3,5-triazin-2-yl)morpholine **(3a)**.* White solid, mp = 215–217 °C in 95% yield.

*2.1.3.2. 2,4-dihydrazinyl-6-(piperidin-1-yl)-1,3,5-triazine **(3b)**.* White solid, mp = 160–162 °C in 93% yield.

*2.1.3.3. N-benzyl-4,6-dihydrazinyl-1,3,5-triazin-2-amine **(3c)**.* White solid; mp = 180–182 °C in 92% yield.

*2.1.3.4. 4-(4-hydrazinyl-6-(piperidin-1-yl)-1,3,5-triazin-2- yl)morpholine (**6a**).* White solid; mp = 140–141 °C in yield 94%.

*2.1.3.5. N-benzyl-4-hydrazinyl-6-morpholino-1,3,5-triazin-2-amine (**6b**).* White solid, mp = 155–156 °C in 90% yield.

*2.1.3.6. N-benzyl-4-hydrazinyl-6-(piperidin-1-yl)-1,3,5-triazin-2-amine (**6c**).* White solid, mp = 163-5 °C in 96% yield.

*2.1.3.7. 4-hydrazinyl-6-morpholino-N-phenyl-1,3,5-triazin-2-amine (**6d**).* White solid, m.p. 145-6 °C in 90% yield.

*2.1.3.8. N,N-diethyl-4-hydrazinyl-6-morpholino-1,3,5-triazin-2-amine (**6e**).* White solid, mp = 163–165 °C in 96% yield.

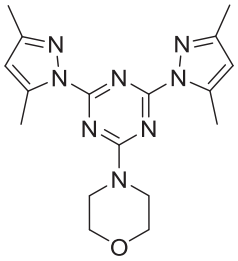
2.1.4. Synthesis of dimethyl(1H-pyrazol-1-yl)-1,3,5-triazine derivatives

Pyrazole-s-triazine derivatives were prepared following the reported method with slight modifications [20,27]. Two methodologies were applied, as explained below.

Method A: [20] 1,3,5-Triazine dihydrazino derivative (10 mmol) was dissolved in 20 mL DMF and then acetylacetone (25 mmol in the case of dihydrazine and 12 mmol in the case of monohydrazine derivatives) was added, followed by addition of triethylamine (8 mmol in the case of the monohydrazine and 16 mmol in the case of dihydrazine derivatives) in 10 mL DMF with stirring at room temperature. The reaction mixture was refluxed for 6–8 h. TLC using ethylacetate-hexane (4:6) monitored the progress of the reaction. The solution was allowed to cool to room temperature, and then ice-cold water was added with continuous stirring. The reaction mixture was kept in an ice bath for 2 h, and the product was collected by filtration, washed with cold water (3 × 20 mL), and then dried under vacuum. The crude product was recrystallized from ethanol to afford the products in 60–75% yields.

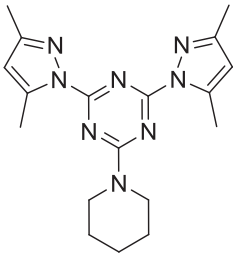
Method B: [27] Acetylacetone was added dropwise (22 mmol) to a cold solution of 1,3,5-triazine dihydrazino or mono hydrazine derivatives (10 mmol) in dilute $HClO_4$ (20 mL). The resulting mixture was stirred at room temperature overnight. The mixture was neutralized with dilute K_2CO_3, and the white precipitate was filtered, washed with water, and crystallized from aqueous ethanol to afford pure products in 90–95% yields. For the reaction of dihydrazine derivatives, double amounts of $HClO_4$, and acetylacetone were used.

*2.1.4.1. 4-(4,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl) morpholine **(4a)**.*



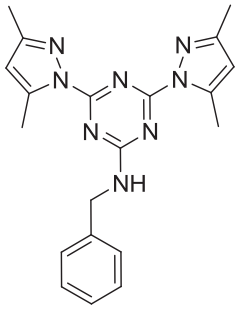
White crystals, mp = 187–188 °C, in yield 92% (**B**); 70% (**A**); IR (KBr, cm^{-1}) 1664, 1620 (C=N), 1595 (C=N, C=C); ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 6H, $2CH_3$),2.632 (s, 6H, $2CH_3$), 3.56 (t, 4H, J = 2.4 Hz, $2CH_2$), 3.86 (t, 4H, J = 4.4 Hz, $2CH_2$), 5.97 (s, 2H, 2CH) ppm; ^{13}C NMR ($CDCl_3$): δ 10.7, 12.9, 41.2, 63.4, 108.1, 140.5, 149.0, 160.5, 162.4 ppm. Anal. Calc. for $C_{17}H_{22}N_8O$ (354.42): C, 57.61; H, 6.26; N, 31.62. Found: C, 57.88; H, 6.35; N, 31.78.

*2.1.4.2. 2,4-bis(3,5-dimethyl-1H-pyrazol-1-yl)-6-(piperidin-1-yl)-1,3,5-triazine **(4b)**.*

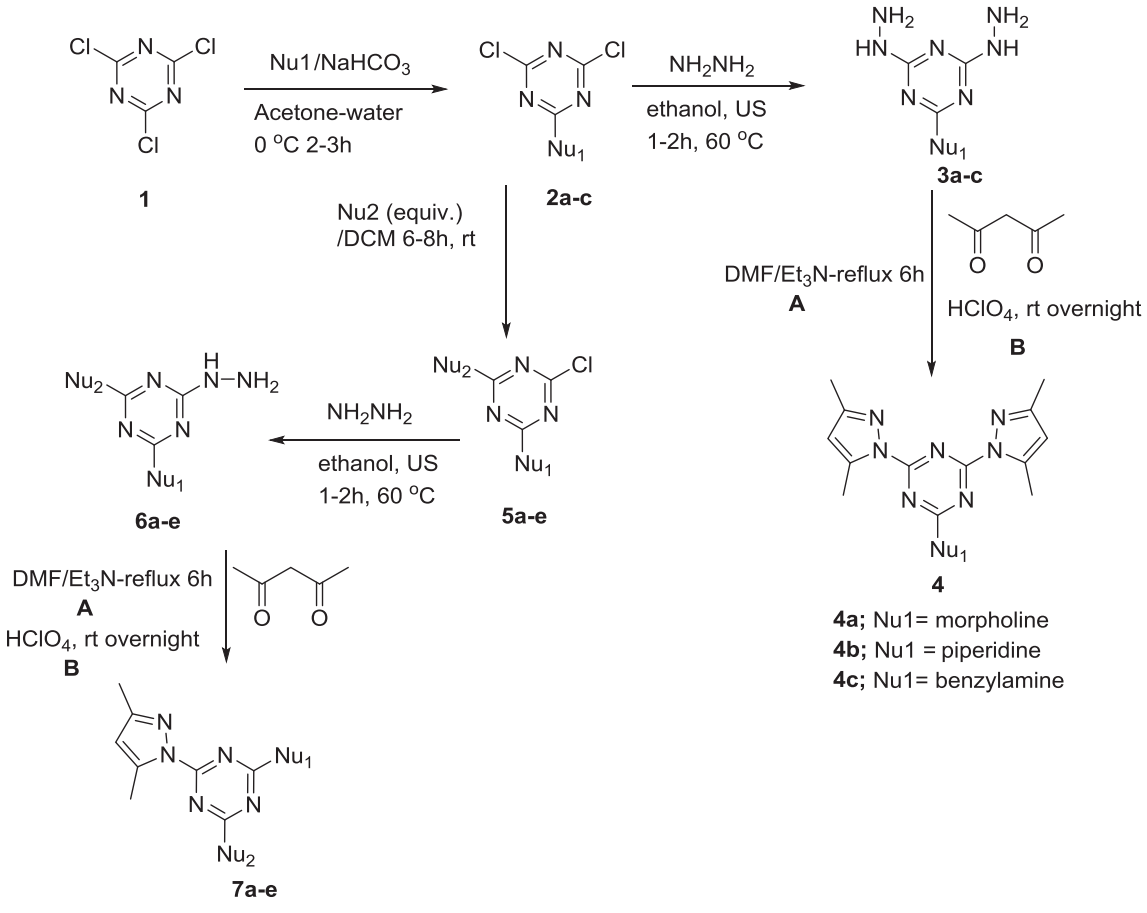


White crystals, mp = 141–142 °C, in 96% yield (**B**); 73% (**A**); IR (KBr, cm^{-1}) 1674, 1624 (C=N), 1595 (C=N, C=C); ^1H NMR ($CDCl_3$): δ 1.60 (m, 6H, $3CH_2$), 2.27 (s, 6H, $2CH_3$),2.62(s, 6H, $2CH_3$), 3.78 (t, 4H,, J = 4.4 Hz, $2CH_2$), 5.97 (s,2H, 2CH) ppm; ^{13}C NMR ($CDCl_3$): δ 13.5, 15.8, 24.3, 25.5, 45.1, 110.8, 143.3, 151.7, 163.5, 164.7 ppm. Anal. Calc. for $C_{18}H_{24}N_8$. (352.45): C, 61.34; H, 6.86; N, 31.79. Found: C, 61.51; H, 6.99; N, 32.00.

*2.1.4.3. N-benzyl-4,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,3,5-triazin-2-amine **(4c)**.*



White crystals, mp = 120–121 °C, in 95% yield (**B**); IR (KBr, cm^{-1}) 3388 (NH), 1634, 1620 (C=N), 1595, 1541 (C=N, C=C); ^1H NMR ($CDCl_3$): δ 2.54 (s, 6H, $2CH_3$), 2.68(s, 6H, $2CH_3$), 4.69 (d, 2H, J = 6.0 Hz, phCH_2), 5.98 (s, 2H, 2CH), 6.45 (t, 1H, NH), 7.24–7.30 (m, 5H, C_6H_5) ppm; ^{13}C NMR ($CDCl_3$): δ 13.7,15.7, 45.2, 110.0, 127.1, 127.5, 128.7, 137.4, 143.9, 144.3, 152.1, 152.3, 163.5, 163.7, 166.7 ppm. Anal. Calc. for $C_{20}H_{22}N8$ (374.45): C, 64.15; H, 5.92; N, 29.93. Found: C, 64.30; H, 6.02; N, 30.04.

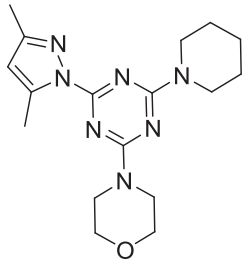


Scheme 1. Synthetic route for the synthesis of pyrazol-s-triazine derivatives.

Table 1
X-ray crystallographic experimental details of **4a** and **7c.**

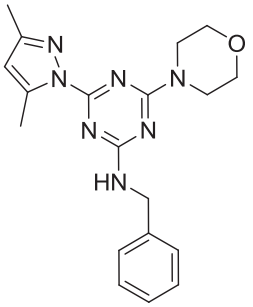
Crystal data	4a	7c
Chemical formula	$C_{17}H_{26}N_8O_3$	$C_{20}H_{25}N_7$
Mr	390.46	363.47
Crystal system, space group	Monoclinic, $P2_1/c$	Triclinic, P-1
Temperature (K)	293	296
a, b, c (Å)	7.7525 (3), 21.5986 (8), 12.3291 (4)	11.1772 (5), 11.9058 (5), 15.3195 (7)
α, β, γ (°)	112.970 (2)	76.367 (2), 79.752 (2), 78.321 (2)
V (Å^3)	1900.73 (12)	1921.93 (15)
Z	4	4
Radiation type	Mo $K\alpha$	Mo $K\alpha$
μ (mm^{-1})	0.10	0.08
Crystal size (mm)	0.57 × 0.15 × 0.11	0.30 × 0.17 × 0.14
Data collection		
Diffractometer	Bruker APEX-II D8 venture diffractometer	Bruker APEX-II D8 venture diffractometer
Absorption correction	Multi-scan SADABS Bruker 2014	Multi-scan SADABS Bruker 2014
T_{min}, T_{max}	0.946, 0.989	0.977, 0.989
No. of measured, independent and observed [I > 2σ(I)] reflections	29796, 4353, 3067	41578, 8821, 5114
R_{int}	0.194	0.113
Refinement		
R[F^2 > 2σ(F^2)], wR(F^2), S	0.059, 0.157, 1.02	0.059, 0.131, 1.03
No. of reflections	4353	8821
No. of parameters	257	499
No. of restraints	0	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.35, −0.45	0.26, −0.33
CDCC numbers	1509638	1509633

*2.1.4.4. 4-(4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)morpholine **(7a)**.*



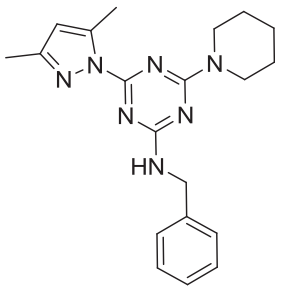
Off white crystals, mp = 123–124 °C, in 96% yield (**B**); 72% (**A**); 75% (A); IR (KBr, cm^{-1}) 1622, 1589 (C═N), 1541 (C═N, C═C); ^{1}H NMR ($CDCl_3$): δ 1.59–1.66 (m, 6H, $3CH_2$), 2.32 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 3.72–3.84 (m, 12H, $6CH_2$), 5.99 (s, H, CH) ppm; ^{13}C NMR ($CDCl_3$): δ 14.0, 16.1, 24.8, 25.9, 43.9, 44.5, 44.7, 66.6, 110.3, 143.4, 150.9, 163.1, 164.9, 165.6 ppm. Anal. Calc. for $C_{17}H_{25}N_7O$. (343.44): C, 59.45; H, 7.34; N, 28.55. Found: C, 59.67; H, 7.50; N, 28.81.

*2.1.4.5. N-benzyl-4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-morpholino-1,3,5-triazin-2-amine **(7b)**.*



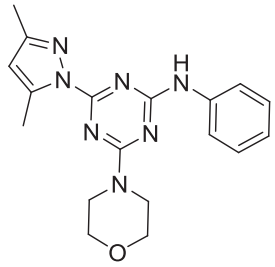
White crystals, mp = 124–125 °C, in 94% yield (**B**), 72% (**A**); IR (KBr, cm^{-1}) 3269 (NH), 1643, 1622 (C═N), 1595, 1553 (C═N, C═C); ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H, CH_3),2.59(s, 3H, CH_3), 3.69 (brs, 4H, $2CH_2$), 3.87 (brs, 4H, $2CH_2$), 4.60 (d, 2H, J = 6.0 Hz), 5.96 (s,1H,CH), 6.20 (brs, 1H, NH), 7.22–7.28 (m, 5H, C_6H_5) ppm; ^{13}C NMR ($CDCl_3$): δ 13.8, 15.9, 43.7, 44.7, 66.5, 116.4, 127.1, 127.2, 128.4, 138.4, 142.9, 165.1, 166.0 ppm. Anal. Calc. for $C_{19}H_{23}N_7O$ (365.44): C, 62.45; H, 6.34; N, 26.83. Found: C, 62.67; H, 6.44; N, 27.05.

*2.1.4.6. N-benzyl-4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(piperidin-1-yl)-1,3,5-triazin-2-amine **(7c)**.*



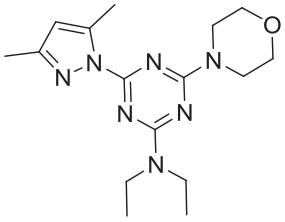
Off white crystals, mp = 142–143 °C, in 92% yield (**B**); IR (KBr, cm^{-1}) 3336 (NH), 1678, 1622 (C═N), 1577, 1529 (C═N, C═C); ^{1}H NMR ($CDCl_3$): δ 1.56–1.65 (m, 6H, $3CH_2$), 2.28 (s, 3H, CH_3),2.62 (s, 3H, CH_3), 3.76 (brs, 4H, CH_2), 4.60 (d, 2H, J = 5.6 Hz, phCH_2), 5.95 (s,1H, CH), 7.23–7.32 (m,5H, C_6H_5) ppm; ^{13}C NMR ($CDCl_3$): δ 13.8, 15.9, 24.6, 25.6, 44.7, 44.8, 110.3, 127.1, 127.3, 127.4, 128.3, 128.4, 138.7, 142.9, 150.4, 162.5, 164.5, 165.9 ppm. Anal. Calc. for $C_{20}H_{25}N_7$ (363.47): C, 66.09; H, 6.93; N, 26.98. Found: C, 66.24; H, 7.10; N, 27.08.

*2.1.4.7. 4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-morpholino-N-phenyl-1,3,5-triazin-2-amine **(7d)**.*



Off white crystals, mp = 213–214 °C, in 95% yield (**B**), 70% (**A**); IR (KBr, cm^{-1}) 3281 (NH), 1633, 1614 (C=N), 1585, 1542 (C=N, C=C); ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3),2.61(s, 3H, CH_3), 3.74 (brs, 4H, $2CH_2$), 3.84 (brs, 4H, $2CH_2$), 5.98 (s,1H,CH), 7.06 (m, 1H, Ar), 7.28–7.32 (m, 3H, Ar), 7.51 (d, 1H, *J* = 8.0 Hz, Ar) ppm; ^{13}C NMR ($CDCl_3$): δ 13.9, 16.1, 44.1, 66.5, 110.9, 120.2, 123.4, 128.8, 138.2, 143.2, 151.8, 163.1, 165.1 ppm. Anal. Calc. for $C_{18}H_{21}N_7O$ (351.41): C, 61.52; H, 6.02; N, 27.90. Found: C, 61.66; H, 6.20; N, 28.18.

2.1.4.8. 4-(3,5-dimethyl-1H-pyrazol-1-yl)-N,N-diethyl-6-morpholino-1,3,5-triazin-2-amine ***(7e)***.



Off white crystals, mp = 94 °C, in 96% yield (**B**) 74% (**A**); IR (KBr, cm^{-1}) 1643, 1612 (C=N), 1541 (C=N, C=C); ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 6H, *J* = 7.6 Hz, $2CH_3$), 2.33 (s, 3H, CH_3), 2.62(s, 3H, CH_3), 3.50–3.60 (m, 4H, $2CH_2$), 3.63–3.86 (m, 8H, $4CH_2$), 5.98 (s, H, CH) ppm; ^{13}C NMR ($CDCl_3$): δ 12.8, 13.3, 13.8, 15.7, 41.5, 43.8, 66.8, 110.2, 143.4, 150.7, 162.7, 164.5, 165.4 ppm. Anal. Calc. for $C_{16}H_{25}N_7O$. (331.42): C, 57.77; H, 7.69; N, 29.83. Found: C, 57.98; H, 7.60; N, 29.58.

2.2. X-ray crystallography

The two compounds **4a** and **7c** were obtained as single crystals by slow evaporation from ethanol solution of the pure compound at room temperature. Data were collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Mo *Kα* radiation, λ = 0.71073 Å at 296 (2) and 293 (2) K. Cell refinement and data reduction were carried out by Bruker SAINT. SHELXT [28,29] was used to solve the structure. The final refinement was carried out using the full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on *F*. CCDC 1509638 and 1509633, respectively, contain the supplementary crystallographic data for these compounds. This information can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

2.3. DFT calculations

Starting geometries were taken from X-ray refined data. The density functional theory (DFT) geometry optimization with the Gaussian09 program package [30] using the B3LYP (Becke three parameters Lee–Yang–Parr) exchange correlation functional, which combines the hybrid exchange functional of Becke [31] with the gradient-correlation functional of Lee, Yang and Parr [32] and the 6-311G++(d,p) basis set, was performed in gas phase. No solvent corrections were made with these calculations as it was reported that gas phase calculations frequently correspond well with crystal structures [33].

2.4. Antimicrobial activity

The antimicrobial activity of synthesized compounds **4a-c** and **7a-e** against bacteria and fungi was determined by modified Kirby-Bauer disk diffusion method [34]. Briefly, cultures of two Gram-negative bacteria (*E. coli* ATCC 25922, *Ps. aeruginosa* ATCC 75853), two Gram-positive bacteria (*M. luteus* ATCC 10240, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300) and *C. albicans* ATCC 10145 (fungi) were grown in Luria broth, nutrient broth, Müller-Hinton broth, nutrient broth, and potato dextrose broth, respectively. These organisms were cultured at their optimal growth temperature i.e., *E. coli* and MRSA at 37 °C, and *P. aeruginosa*, *M. luteus*, MRSA, and *C. albicans* at 30 °C on a rotary shaker at 150 rpm. Agar plates were seeded with a mixture of exponentially growing test organisms containing 10^5-10^6 colony-forming units/ml and molten soft agar (0.7% agar). Plates were allowed to cool and

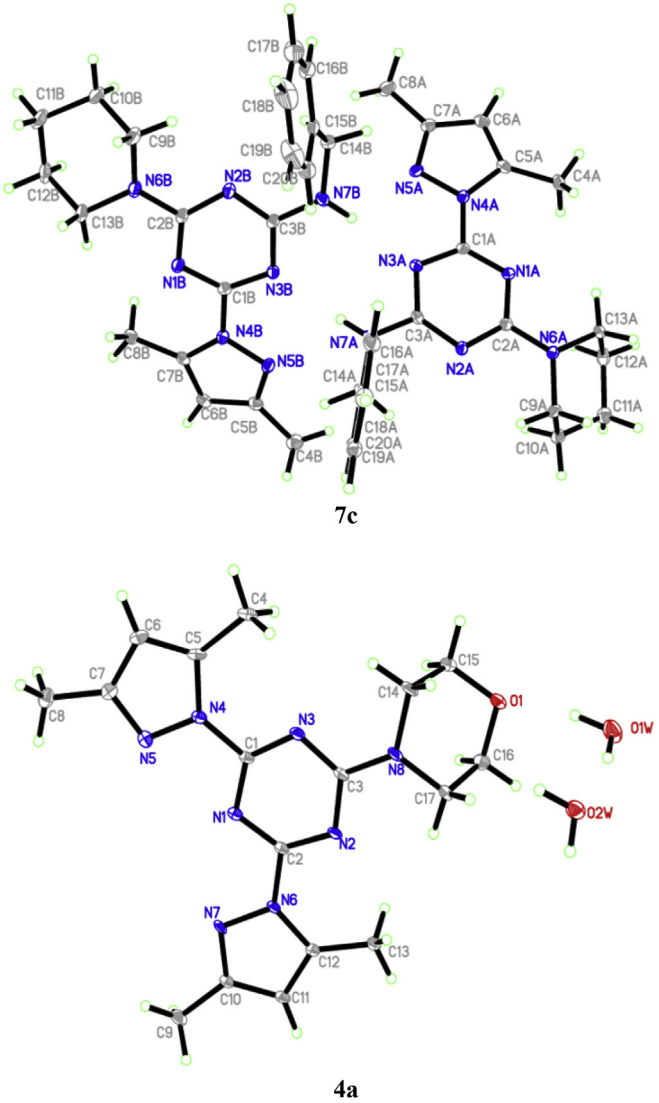


Fig. 2. ORTEP diagram of the titled compounds. Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.

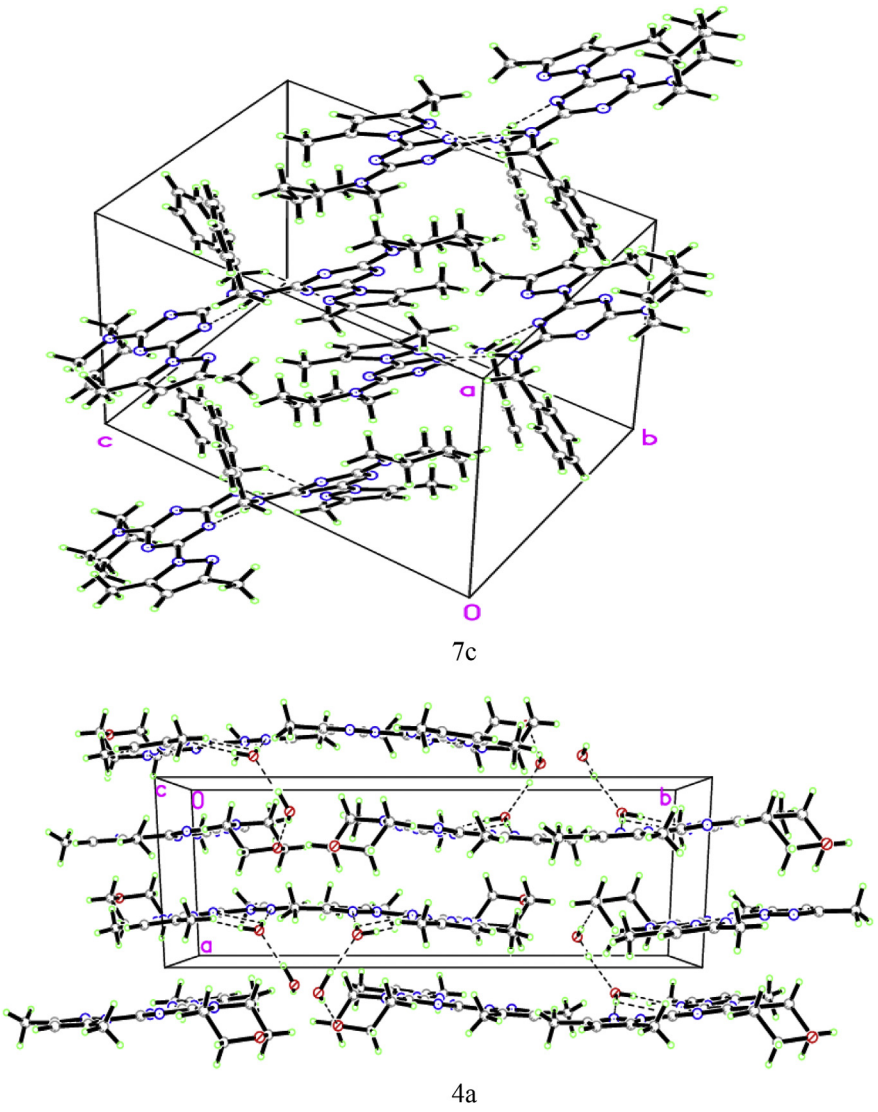


Fig. 3. Molecular packing of titled compounds viewed with hydrogen bonds, which are drawn as dashed lines.

solidify at room temperature for 10–15 min. Wells of 7 mm in diameter were punched into the soft agar layer in order to test the antimicrobial activity of the compounds. Compounds were dissolved in DMSO at 10 mg/mL and then 10 μL of the solution was added to the wells. The plates were incubated overnight at optimal growth temperatures to dry at room temperature inside a biological safety cabinet. Then incubation zones of inhibition were measured and recorded. Tobramycin, fusidic acid and canesten were used as controls.

3. Results and discussion

3.1. Chemistry

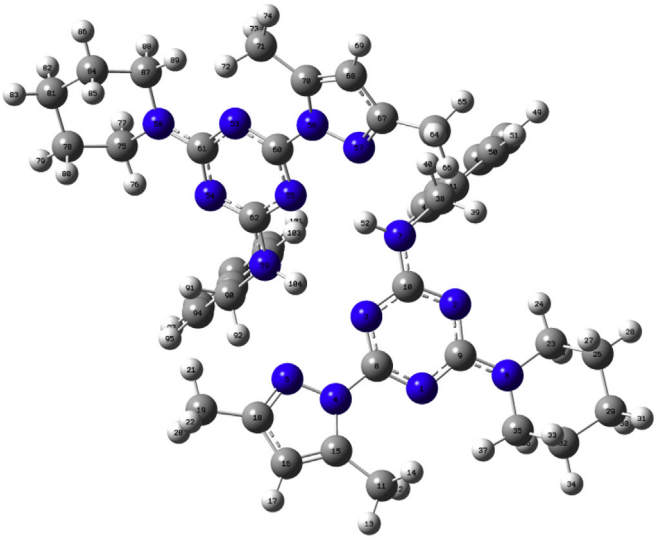
The triazine scaffold of cyanuric chloride was conveniently manipulated by the facile displacement of its chlorine atoms by oxygen- or nitrogen-containing nucleophilic species in the presence of a hydrogen chloride acceptor. These reactions take place with great regioselectivity as a result of the temperature-controlled process.

The mono and dipyrazolyl derivatives were first compounds synthesized following the published method, where the hydrazinyl-s-triazine was reacted with acetylacetone using DMF as a solvent in the presence of trimethylamine (method A) [20]. Alternatively, the method based on the use of $HClO_4$ and then neutralization with K_2CO_3 was also examined (method B) [27].

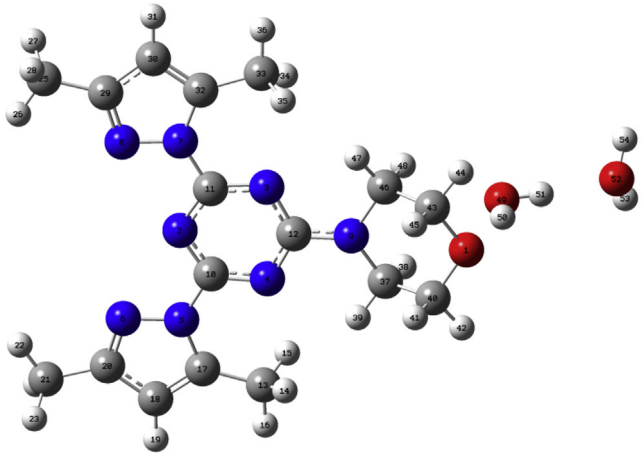
The initial nucleophilic substitution of the first chlorine atom of cyanuric chloride **1** by the first amine (morpholine, piperidine or benzylamine) at 0–5 °C afforded the products **2a-c** (2,4-dichloro-6-substituted-s-triazine derivatives) with excellent yield. The second chlorine of **1** replaced by a second amine at room temperature for 24 h gave the products **5a-e** (2-chloro-4,6-disubstitued-s-triazine derivatives). Products **2a-c** and **5a-e** were treated with hydrazine hydrate (80%) in ethanol for 1–2 h under ultrasonic irradiation (US) [26] to afford the hydrazine derivatives **3a-c** and **6a-e,** respectively. Compounds **3** and **6** were then reacted with acetyl acetone following methods A or B [20,27], as shown in Scheme 1. Method B (using the $HClO_4$) afforded the products in higher yield under moderate conditions (experimental section). The spectral data for the new products confirmed their structure.

3.2. X-ray crystallography

The crystallographic data and refinement information for



7c



4a

Fig. 4. Optimized geometry of **7c** and **4a** using DFT [B3YLP/6-311++(d,p)].

compounds **4a** and **7c** are summarized in Table 1. The selected bond lengths and bond angles are provided in the Supplementary Material (Table S1 and S3). Analysis of the asymmetric unit of **4a** revealed that the molecule crystallized in the P $2_1/c$ space group with two water molecules (Figs. 1 and 2). All the bond lengths and angles were found in normal ranges [35]. Of the four rings present in the unit of **4a**, the morpholine one had a puckered chair conformation. However, no puckered conformation was observed in any other ring. Weak π-π stacking was observed in the crystal structure of **4a** (Cg4-Cg4: 3.6590(10) Å; -1-x,-1-y,-1-z). H-bonding was found between **4a** and the water molecules.

The asymmetric unit of **7c** contained two molecules (Figs. 2 and 3). The molecule crystallized in the P-1 space group. Bond lengths were in the normal range [35]. The piperidine ring present in the two molecules had a puckered chair conformation (Cg5-Cg6: 3.6136(12) Å; 1-x,1-y,1-z), providing extra stability to the crystal structure. No classical H-bonding was observed in the crystal.

3.3. Theoretical calculations (DFT) of 4a and 7c

DFT geometry optimization were made on a crystal unit using Gaussian09 program package. The optimized geometry results in the free molecule state were compared to those in the crystalline state (Table S5 and S6, Supplementary Materials). No negative vibrational modes were obtained. The optimized geometry of **4a** and **7c** revealed P $2_1/c$ and P-1 space group, respectively (Fig. 4).

The optimized geometry of the studied compounds was compared with the structural parameters obtained from the crystallographic information file (CIF). All the bond lengths and angles calculated were in agreement with crystal structure. Among all the four rings present in **4a**, puckering was observed in morpholine ring which is quite similar to that present in the crystal structure. H-bond was found between **4a** and water molecules. The O(1)—H(50) and O(52)—H(51) distance was found to be 1.99 Å (exp. 1.99 Å) and 1.96 Å (exp. 1.89 Å) respectively which indicated the presence of intermolecular H-bond between 4a and water molecules. Torsion angle between O(1)-C(43)-C(46)-N(9) and O(1)-C(40)-C(37)-N(9) was found to be 54.60° (Expt. 56.15°) and −54.61° (Expt. −56.28°) which showed slight deviation from the experimental data but maintaining the puckering for the morpholine ring same as that found in crystal unit. Optimized geometry of **7c** clearly indicates absence of classical H-bonding. Torsion angle between C(29)-C(32)-C(35)-N(6) and C(29)-C(26)-C(23)-N(6) was found to be −53.71° (Expt. −55.04°) and 53.59° (Expt. 55.57°) respectively, with values slightly deviating from the experimental data. The

Table 2
Antimicrobial activities of 4a-c and 7a-e[a,b].

Compounds[c]	Gram-negative		Gram-positive		Fungi
	E. coli	*P. aeruginosa*	*M. luteus*	MRSA	*C. albicans*
4a	–	–	–	–	–
4b	–	13	17	11	wd[d]
4c	–	13[e]	15	7	–
7a	–	–	–	–	9
7b	–	17	9	8	wd[d]
7c	–	19	22	11	–
7d	–	–	–	7	–
7e	–	9	17	8	8

[a] Tobramycin, fusidic acid and canesten were used as controls.
[b] Zone of inhibition in mm.
[c] Compounds were dissolved in DMSO (25% in water) at 10 mg/mL and then 10 μL of the solution was added to the wells.
[d] wd = weak and diffused zones (weak activity and cannot be measured).
[e] 20 μL of the solution was added to the wells.

pyridine ring was found to have chair conformation similar to that present in the crystal.

3.4. Preliminary antimicrobial activity

In the present study, synthesized compounds (**4a**, **4b**, **4c**, **7a**, **7b**, **7c**, **7d** and **7e**) were evaluated for their *in vitro* antibacterial and antifungal activities against microbial strains such as Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 75853), Gram-positive bacteria (*M. luteus* ATCC 10240, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300), and fungi (*C. albicans* ATCC 10145) by the agar-well diffusion method. The results of antimicrobial testing against strains were obtained as zone of inhibition (mm), as tabulated in Table 2.

Compounds **4b**, **4c**, **7b**, **7c** and **7e** exhibited antibacterial activity against bacterial strains, whereas **4a**, **7a** and **7d** did not. However, **7a** showed mild activity against fungi *C. albicans*, but **4a** was inactive against all the strains tested. In addition, **4b** and **7b** also showed weak activity against *C. albicans*.

It is evident from the structure that compounds with one pyrazole molecule (**7a-e**) showed better performance than analogs with two (**4a-c**). Furthermore, presence of piperidine and benzylamine on triazine ring exhibits the highest activity. Comparatively, presence of morpholine moiety appeared to have detrimental effects on activity (**4a**, **7a** and **7d** bear this heterocycle). Compound **7c** which holds pyrazole, piperidine, and benzylamine, exhibited the highest activity against all bacterial strains.

4. Conclusions

In summary, a new series of pyrazole-containing s-triazine derivatives were synthesized by reaction of corresponding s-triazinyl hydrazine derivatives with acetylacetone in presence of $HClO_4$ or DMF/TEA. The $HClO_4$ method allowed the preparation of the target products in higher yields when compared to DMF/TEA approach. X-ray single crystal diffraction of **4a** and **7c** was studied and their molecular geometries were optimized using the DFT/B3LYP 6-311G++(d,p) method. The optimized structures were found to be in good agreement with corresponding X-ray structures. The synthesized compounds were screened for antimicrobial activity. In the view of the results obtained, compounds with one pyrazole molecule showed better performance when compared to analogs with two pyrazole. Presence of piperidine with benzylamine over triazine ring proves to be an optimum factor for enhancing the activity against all bacterial strains. Based on our findings, we conclude that s-triazine with pyrazole and piperidine is a promising scaffold for the development of new active compounds.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2017.05.040.

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