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Design and Synthesis of Novel *Bis*-Morpholinotriazine Analogs and their Antibacterial, Antifungal and Antioxidant Studies

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A series of novel analogues of *bis*-morpholino-1,3,5-triazine derivatives are synthesized from cyanuric chloride as starting precursor. The products are characterized by spectral data and their biological evaluation against microbials are reported. Antioxidant properties of these compounds are also studied.

Keywords: *s*-Triazine, Ureido analogs, *Bis*-morpholine, Antioxidative.

INTRODUCTION

Bacterial and fungal infections have great impact on public health. Over the years, using of antimicrobial agents indiscriminately has led to an emergence to resistant strains, which made infections difficult to treat [1]. Multi drug resistance is major common issue now a days and become most commonly caused morbidity and mortality in immuno-compromised patients [2]. Due to limitations of treatments available, fungal infections are also make more impact on global health [3]. There are 1 million or more deaths reported due to antimicrobial resistance and could grow to 10 million by the 2050 [4]. These facts further underscore the urgent necessity to discover and develop new antifungal and antibacterial agents against new or little explored targets.

The 1,3,5-triazine (*s*-triazine) moiety is considered as an important heterocyclic motif in organic chemistry because of its tremendous applications in many different fields. It is the prominent constituent in many pharmaceuticals [5], building blocks for supramolecular chemistry [6], liquid crystals [7], organic light-emitting diodes (OLEDs) [8], reactive dyes [9] and also acts as chemical reagent for selected transformations [10,11]. 1,3,5-Triazines medicinal chemistry is well studied since two decades because of its broad spectrum of biological activities such as antibacterial [12-14], kinase inhibitors in oncology [15,16], antiprotozoal [17], antimalarial [18], antiviral [19], *etc*. Recently several triazine derivatives bearing basic moieties such as piperidine, piperazine and morpholine moieties are characterized by an antibacterial activities with improved pharmacokinetic properties [20]. Based on earlier investigations of *s*-triazine and above these observations, here we are reporting the synthesis of *bis*-morpholino-1,3,5-triazine analogs and evaluated their antibacterial and antifungal activities. In addition, we evaluated antioxidant screening for synthesizing novel analogs. Recent data suggest that many of antibiotic drugs, which are used to control infections in the human body, are causing many side effects, especially increasing reactive oxygen species (ROS) [21,22].

EXPERIMENTAL

All the chemicals were obtained from Sigma-Aldrich Company and used as received. ^{1}H, ^{13}C and DEPT NMR spectra were recorded on Brucker-Avance DPX FT-NMR 500 and 400 MHz instruments. Chemical data for protons are reported in parts per million (ppm) down field from tetramethyl silane and are referenced to the residual proton in the NMR solvent ($CDCl_3$-7.24 and CD_3OD-3.28 ppm). Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz or 100 MHz, chemical data for carbons are reported in parts per million (ppm, δ scale) downfield from tetramethyl silane and are referenced to the carbon resonance of the solvent ($CDCl_3$-77 and CD_3OD-50 ppm). ESI-MS spectra were recorded on

Agilent 1100 LC-Q-TOF machine, *m/z* given for (M+H) or (M+1), M = molecular ion. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. Melting points were recorded on digital melting point apparatus. HPLC analysis was done on Shimadzu HPLC system (model: Shimadzu-LC 10AT) equipped with a PDA detector using Inertsil RP-18 (E-Merck, 5 µ, 4.0 × 250 mm) column. Mobile phase used was water (A) and acetonitrile (B) as gradient as follows: 0.01-10 min 1-60% B, 10-30 min 60-100% B, 23-45 min 100-60% B, at the flow rate of 0.6 mL/min.

Synthesis of 4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)-dimorpholine (2) [16]**:** To a stirred solution of cyanuric chloride (**1**, 9.2g, 49.18 mmol) in 50 mL of acetone and crushed ice (200 g), triethylamine (14.5 g, 147.54 mmol) followed by morpholine (8.5 g, 98.36 mmol) were added slowly at -10 ºC and then the resulted mixture was stirred at room temperature for 2 h. After completion of the reaction, slowly added 130 mL of distilled water, precipitated product was filtered and washed with cold water to obtain 4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)dimorpholine (**2**) as a white solid (yield 82%). The crude product was found to be pure and taken to the next step without further purification (M + H) 286.7.

Synthesis of *tert*-butyl(1-(4,6-dimorpholino-1,3,5-triazin-2-ylpiperidin-4-yl)carbamate (4): To a solution of 4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)dimorpholine (**2**, 2g, 7.01 mmol) in 10 mL of DMF, was added *tert*-butyl piperidin-4-ylcarbamate (**3**, 1.54 g, 7.71 mmol) and K_2CO_3 (1.93 g, 14.035 mmol) subsequently. Then the reaction mixture was heated to 90 ºC and stirred for 4 h. Then the reaction mixture was poured on to ice. The product was precipitated out, filtered washed with cold water and dried to get a white solid (yield 73%). ^{1}H NMR spectrum (400 MHz, DMSO-d_6): δ 3.72-3.60 (m, 17H), 3.12-3.04 (m, 4H), 1.74-1.59 (m, 4H), 1.40 (s, 9H); (M + H) 450.2.

Synthesis of 1-(4,6-dimorpholino-1,3,5-triazin-2-yl)-piperidin-4-amine (5): To a solution of *tert*-butyl (1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)carbamate (**4**, 1 g, 2.22 mmol) in 10 mL of DCM, was added 4 N HCl in dioxane (10 mL, 10 v/v) and stirred for overnight. The reaction mixture was cooled to 0 ºC, neutralized with ammonia solution, the pH was adjusted to 9 and extracted with DCM (3 × 30 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The compound was used directly for next step without purification, yield 62%, (M + H) 350.2.

Synthesis of ethyl 4-(3-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)ureido)benzoate (7a): To a solution of 1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-amine (**5**, 1 mmol) in 8 mL of DCM, was added an isocyanate **6a** (1.1 mmol) and Et_3N (1.5 mmol) subsequently and stirred for overnight. The reaction mixture was diluted with water and DCM, extracted with DCM (3 × 10 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The crude compound was purified in MeOH in DCM (0 to 5%) to afford ethyl 4-(3-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)ureido)benzoate as white solid, yield 55%, m.p. 150-152 ºC; HPLC purity: 97% (t_R = 25.2 min); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 6.34 (d, *J* = 7.6 Hz, 1H), 4.45 (d, *J* = 13.0 Hz, 3H), 4.26 (dd, *J* = 14.1, 7.1 Hz, 3H), 3.62 (dd, *J* = 17.9, 4.3 Hz, 15H), 3.03 (t, *J* = 11.2 Hz, 1H), 1.84 (d, *J* = 10.0 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 176.5, 166.0, 143.1, 129.5, 124.2, 66.5, 63.1, 51.0, 28.3, 15.0; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3403, 2923, 2852, 1648, 1508, 1367, 1154, 1022, 753; LC-ESIMS: $[M+H]^+$; *m/z* 541.3.

Synthesis of 1-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)-piperidin-4-yl)-3-(4-methoxyphenyl)thiourea (7b): Following the same procedure as for **7a** with thioisocyanate **6b** afforded a white solid. Yield 61%, HPLC purity: 96% (t_R = 26.2 min); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40 (s, 1H), 7.11 (d, *J* = 10.1 Hz, 2H), 6.92 (d, *J* = 5.2 Hz, 2H), 5.58 (d, *J* = 5.2Hz, 1H), 4.56 (d, *J* = 15.2Hz, 3H), 3.82 (s, 3H), 3.71-3. (m, 16H), 2.98 (t, *J* = 15.1, 10.2 Hz, 2H), 2.08 (d, *J* = 10.2 Hz, 2H), 1.25 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.0, 175.5, 160.2, 158.0, 119.3, 114.6, 66.2, 52.4, 51.0, 49.2, 29.3; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3418, 2103, 1644, 1534, 1443, 1364, 1118, 752; LC-ESIMS (M+1): *m/z* 515.3.

General procedure for the synthesis of *N*-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)aryl sulfonamides (9a-e): To a solution of 1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-amine (**5**, 1 mmol) in 8 mL of DCM, was added respective aryl sulfonyl chlorides (**8a-e**, 1.1 mmol) and Et_3N (1.5 mmol) subsequently and stirred for overnight. The reaction mixture was diluted with water and DCM, extracted with DCM (3 × 10 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The crude compound was purified by column chromatography using MeOH in DCM (0 to 5%) as eluent, to afford the corresponding *N*-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl) piperidin-4-yl)aryl sulfonamide as white solid.

***N*-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)benzene sulfonamide (9a):** Yield 71%, m.p. 198-200 ºC; HPLC purity: 97% (t_R = 27.2 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, *J* = 10.2 Hz, 2H), 7.62-7.51 (m, 3H), 4.54-4.46 (m, 3H), 3.70-3.64 (m, 16H), 3.40 (t, *J* = 5.2 Hz, 1H), 2.96-2.86 (m, 2H), 1.78 (d, *J* = 10.2 Hz, 2H), 1.33-1.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.2, 176.5, 141.0, 139.2, 129.5, 49.6, 46.1, 28.5; LC-ESIMS (M+1): *m/z* 490.21.

***N*-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-4-methoxybenzene sulfonamide (9b):** Yield 74%, m.p. 236-238 ºC; HPLC purity: 99% (t_R = 25.2 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, *J* = 10.2 Hz, 2H), 6.99 (d, *J* = 15.2 Hz, 2H), 4.54-4.45 (m, 3H), 3.88 (s, 3H), 3.70-3.69 (m, 15H), 3.39-3.34 (m, 1H), 2.90 (t, *J* = 12.2, 8.2 Hz, 2H), 1.77 (d, *J* = 15.2 Hz, 2H), 1.26-1.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.0, 175.8, 142.0, 140.2, 131.5, 51.6, 48.1, 28.1; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3401, 2921, 2852, 1602, 1531, 1444, 1270, 1115, 1023, 770; LC-ESIMS (M+1): *m/z* 520.30.

***N*-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-4-methylbenzene sulfonamide (9c):** Yield 62%, m.p. 210-212 ºC; HPLC purity: 95% (t_R = 25.7 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 10.2 Hz, 2H), 7.31 (d, *J* = 10.2Hz, 2H), 4.49-4.37 (m, 4H), 3.70-3,69 (m, 16H), 3.38 (d, *J* = 10.2 Hz, 2H), 2.92 (t, *J* = 16 Hz, 2H), 1.78 (d, *J* = 10.2 Hz, 2H), 1.33-

1.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 164.9, 162.8, 132.3, 129.0, 114.3, 66.8, 55.6, 51.2, 43.6, 41.6, 32.8, 29.7; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3372, 2920, 2854, 1553, 1530, 1444, 1271, 1115, 981, 756; LC-ESIMS (M+1): *m/z* 504.3.

***N*-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-4-(trifluoromethoxy)benzene sulfonamide (9d):** Yield 69%, m.p. 218-220 ºC; HPLC purity: 97% (t_R = 25.3 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.2Hz, 2H), 4.57-4.49 (m, 3H), 3.70-3.69 (d, 16H), 3.49-3.41 (m, 1H), 2.90 (t, *J* = 12.2 Hz, 2H), 1.81 (d, *J* = 8.2 Hz, 2H), 1.36-1.33 (m, 2H); ^{13}C NMR (100 MHz, CDCl3): δ 165.45, 164.99, 152.10, 139.70, 129.03, 121.52, 121.01, 118.94, 66.86, 51.58, 43.61, 41.56, 32.88; LC-ESIMS (M+1): *m/z* 574.20.

***N*-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-4-nitrobenzene sulfonamide (9e):** Yield 55%, m.p. 238-240 ºC; HPLC purity: 96% (t_R = 26.3 min); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 8.2 Hz, 2H), 8.09 (d, *J* = 8.1 Hz, 2H), 4.68 (d, *J* = 8.1 Hz, 1H), 4.52 (d, *J* = 12.2 Hz, 2H), 3.70-3.69 (m, 16H), 3.49-3.46 (m, 1H), 2.83 (t, *J* = 12.2 Hz, 2H), 1.81 (d, *J* = 12.4 Hz, 2H), 1.40-1.30 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.4, 176.2, 151.0, 142.2, 132.5, 61.6, 52.0, 48.1, 28.1; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3357, 2922, 2853, 1555, 1498, 1325, 1227, 1062, 914; LC-ESIMS: *m/z* 535.5.

4-(3-(1-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)ureido)benzoic acid (10): To a solution of ethyl 4-(3-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-ureido)benzoate (**7a**, 1 mmol) in 10 mL of THF-H_2O (3:1), was added LiOH·H_2O (2 mmol) and stirred for overnight. The reaction mixture was neutralized with 1N HCl solution and pH was adjusted to 6 and extracted with DCM (3 × 30 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The crude compound was purified in MeOH in DCM (0 to 5%) to afford 4-(3-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)ureido)benzoic acid as white solid (yield 70%), m.p. 278-280 ºC; HPLC purity: 96% (t_R = 27.2 min); ^{1}H NMR (400 MHz, DMSO): δ 8.85 (s, 1H), 7.86 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.1Hz, 2H), 6.42 (d, *J* = 8.1Hz, 1H), 4.49 (d, *J* = 16.2 Hz, 2H), 3.70-3.61 (m, 16H), 3.09 (t, *J* = 12.0 Hz, 3H), 1.89 (d, *J* = 12.2 Hz, 2H), 1.38-1.29 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4, 177.2, 159.2, 151.0, 143.2, 132.5, 60.6, 53.0, 48.5, 29.1; IR ($CHCl_3$, ν_{max}, cm^{-1}): 3368, 2921, 2854, 1555, 1445, 1223, 1158, 1016, 874; LC-ESIMS (M+1): *m/z* 513.3

Synthesis of 1-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)-piperidin-4-yl)-3-(4-(4-methylpiperazine-1-carbonyl)phenyl)-urea (12): To a solution of 4-(3-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)ureido)benzoic acid (**10**, 1 mmol) in 6 mL of DMF, was added HBTU (1.5 mmol) and DIPEA (2 mmol) subsequently and stirred for 10 min at room temperature. Then 1-methylpiperazine (**11**, 1.1 mmol) was added to the reaction mixture and stirred for overnight. The reaction mixture was diluted with water and DCM and extracted with DCM (3 × 30 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The crude compound was purified in MeOH in DCM (0 to 5%) to afford 1-(1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)-3-(4-(4-methylpiperazine-1-carbonyl)phenyl) urea as white solid (yield 52%), m.p. 214-216 ºC; HPLC purity: 97% (t_R = 25.3 min); ^{1}H NMR (400 MHz, DMSO): δ 8.78 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 6.41 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 3H), 3.64-3.60 (d, 28H), 3.06 (t, *J* = 12.0 Hz, 3H), 1.83 (d, *J* = 12.0 Hz, 2H), 1.24 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 174.2, 160.2, 153.0, 145.2, 133.5, 61.7, 60.6, 53.4, 48.9, 28.1; IR (KBr, ν_{max}, cm^{-1}): 3405, 2918, 2853, 1553, 1444, 1378, 1115, 1023, 756; LC-ESIMS (M+1): *m/z* 595.4.

Synthesis of *tert*-butyl 4-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperazine-1-carbamate (14): To a solution of 4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)dimorpholine (**2**, 1 mmol) in 10 mL of DMF, was added *tert*-butyl piperazine-1-carboxylate (**13**, 1.1 mmol) and K_2CO_3 (2 mmol) subsequently. Then the reaction mixture was heated to 90 ºC and stirred for 4 h. After confirming with TLC, reaction mixture was poured on to ice. The compound was precipitated out. The compound was filtered, washed with cold water and dried to get white solid (yield 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.71-3.61 (m, 16H), 3.28-3.26 (m, 8H), 1,42 (s, 9H); (M + H) 436.5.

Synthesis of 4,4′-(6-(piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (15): To a solution of *tert*-butyl (1-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperidin-4-yl)carbamate (**14**, 1 mmol) in 10 mL of DCM, was added 4 N HCl in dioxane (10 mL, 10 v/v) and stirred for overnight. The reaction mixture was cooled to 0 ºC, neutralized with ammonia solution. The pH was adjusted to 9 and extracted with DCM (3 × 30 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The compound was used directly for next step without purification (yield 55%); (M + H) 336.5.

General procedure for the synthesis of 16a-c: To a solution of 4,4′-(6-(piperazin-1-yl)-1,3,5-triazine-2,4-diyl)-dimorpholine (**15**, 1 mmol) in 8 mL of DCM, was added sulfonyl chlorides (**8**, 1.1 mmol) and Et_3N (1.5 mmol) subsequently and stirred for overnight. The reaction mixture was diluted with water and DCM, extracted with DCM (3 × 10 mL). The collective organic layer was dried over Na_2SO_4 and concentrated over reduced vapour pressure. The crude compound was purified in MeOH in DCM (0 to 5%) to afford the corresponding 4,4′-(6-(4-((4-aryl)sulfonyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (**16a-c**).

4,4′-(6-(4-((4-Methoxyphenyl)sulfonyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (16a): Yield 70%, m.p. 212-214 ºC; HPLC purity: 97% (t_R = 26.3 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 3,86-3.68 (m, 23H), 2.98 (t, *J* = 4.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 164.8, 163.1, 129.93, 126.8, 114.2, 66.8, 55.6, 46.0, 43.5, 42.3; IR (KBr, ν_{max}, cm^{-1}): 3444, 2961, 2922, 2853, 2571, 2507, 1731, 1596, 1485, 1348, 1307, 1069, 1022, 919, 836, 756, 576; LC-ESIMS (M+1): *m/z* 506.21.

4,4′-(6-(4-((4-Nitrophenyl)sulfonyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (16b): Yield 63%, m.p. 230-232 ºC; HPLC purity: 95% (t_R = 26.4 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 3.88-3.85 (m, 4H), 3.69-3.68 (m, 18H), 2.98 (t, *J* = 4.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2, 166.3, 164.16,

131.2, 125.4, 116.2, 67.8, 56.2, 46.7, 44.0, 41.1; IR (KBr, ν_{max}, cm^{-1}): 3436, 1634, 1536, 1482, 1439, 1349, 1255, 1112, 95, 568; LC-ESIMS (M+1): *m/z* 529.19.

***N*-(4-((4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperazin-1-yl)sulfonyl)phenyl)acetamide (16c):** Yield 59%, m.p. 262-264 ºC; HPLC purity: 99% (t_R = 23.3 min); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H), 3.85-3.81 (m, 4H), 3.68-3.67(m, 18H), 2.99 (t, *J* = 4.2 Hz, 4H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 171.4, 162.2, 155.0, 148.2, 134.2, 61.5, 59.6, 55.4, 48.2, 29.1, 15.2; IR (KBr, ν_{max}, cm^{-1}): 3350, 2958, 2919, 2851, 1679, 1591, 1479, 1366, 1308, 1255, 1111, 1019, 947, 858, 740, 667; LC-ESIMS (M+1): *m/z* 533.20.

Antibacterial activity: The cultures were grown in nutrient agar media and subcultured for log phasic cultures in a liquid nutrient broth medium for minimum inhibitory concentration studies and further subcultured onto media in petri plates for the experimental purposes [23]. The broth cultures were diluted with sterilized saline to bring the final size of inoculum approximately to 10^5–10^6 CFU/mL. The compounds were diluted in acetone, DMSO and diethyl ether for biological assays. Among the three solvents diethyl ether is considered as the best solvent than the remaining two solvents.

The bacterial culture was placed on the media and incubated at 37 ºC for 24 h to 48 h along with the diluted compounds introduced through discs dipped and placed over the nutrient media. The zones of bacterial growth inhibitions were measured using the diameter of the zone as a unit to measure the antibacterial activity. All the results were expressed as zone of inhibition (ZOI) in mm. The results were compared with the activity of the standard antibiotic ciprofloxacin (20 and 40 µg/mL). For disc diffusion method, the diluted test compounds were introduced onto the disc and once the disc was found completely saturated it is immediately transferred on to surface of the medium with bacterial inoculums spreaded by spread plate method evenly. The petri dishes were incubated at 37 ºC for 24 h. Bioactivity was determined by measuring diameter of inhibition zones (DIZ) in mm.

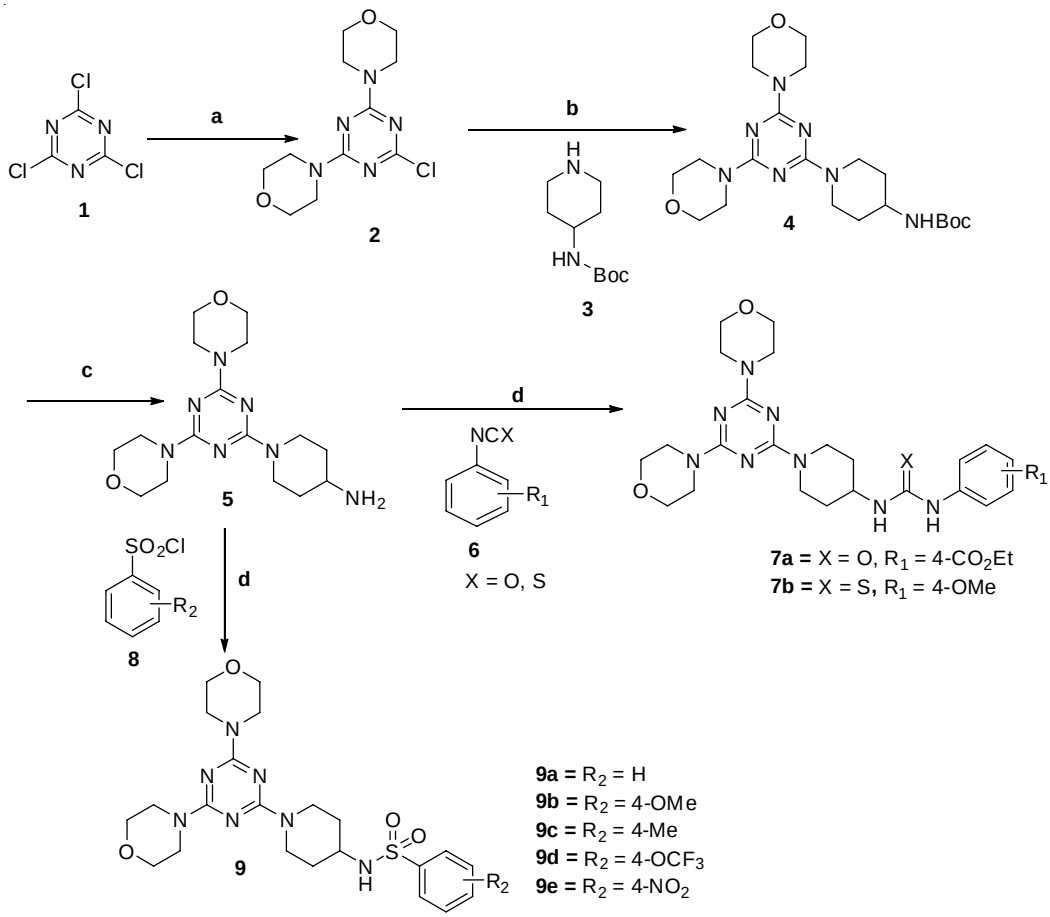
Antifungal bioassays: The antifungal activity of synthesized novel compounds was tested against three pathogenic fungi *viz.*, *Fusarium oxysporum* and *Aspergilus flavus*, by the poison plate technique [24]. Test compounds were dissolved in diethyl ether (10 mL) before mixing with potato dextrose agar medium (PDA, 90 mL). The final concentration of compounds in the medium was maintained to be 500 µg/mL. Above mentioned types of fungi were incubated in PDA at 25 ± 1 ºC for 48-72 h to get long mycelium for antifungal assay, the mycelia disk of approximately 0.45 cm diameters were cut from the PDA medium with a sterilized inoculation needle and inoculated in the center of PDA plate. The inoculated plates were incubated at 27 ± 1 ºC for 3 days. Diethyl ether in sterilized distilled water was taken as control, while hymexazol was used as positive control for all the treatment. The growth of the fungal colonies was measured on the third day and the data were statistically analyzed.

Superoxide anion scavenging activity: This activity was measured following the method of Roback & Gryglewski with slight variations [25] in incubation duration and concentration of PMS taken. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1 mL of nitro blue tetrazolium (NBT, 156 µM), 1 mL of reduced nicotinamide adenine dinucleotide (NADH, 460 µM) 3 mL of synthetic compounds (**5a-j**) of concentrations ranging 50, 100, 200 µg/mL were mixed and the reaction was continued by the addition of 100 µL of phenazine-methosulphate (60 µM PMS). The final mixture was incubated for 5 min at room temperature and spectral measurements were made at 560 nm of visible light region.

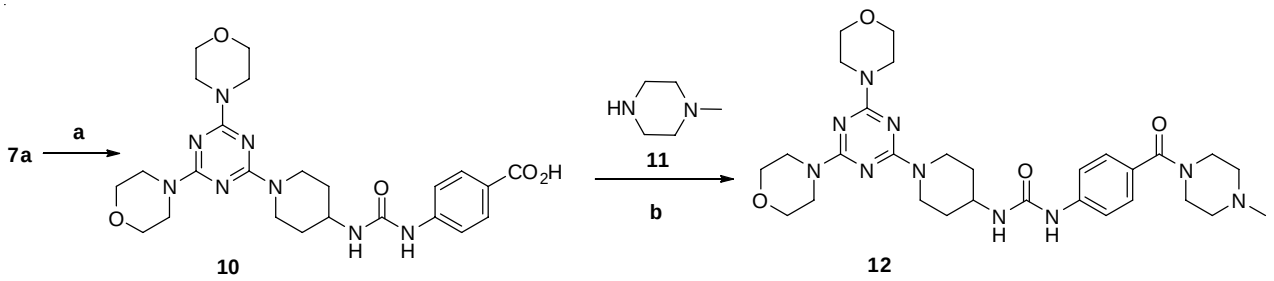
RESULTS AND DISCUSSION

2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride) is an inexpensive reagent and the chemical nature of these chlorine atoms are controlled by temperature, make use of easily derived synthetic libraries. Compound **5** was synthesized as shown in **Scheme-I**. The cyanuric chloride (**1**) was treated with morpholine at controlled temperature with crushed ice to get dimorpholino triazine (**2**) as majorly [16]. Compound **2** was treated with *tert*-butyl piperidin-4-ylcarbamate (**3**) in the presence of K_2CO_3 as base and followed by *Boc*-deprotection with 4 N HCl in dioxane afforded the compound **5**. New urea series **7a**, **7b** were synthesized from **5** using corresponding aryl isocyanate/thioisocyanate **6** while another novel bioactive sulfonamide series **9a**-**e** were obtained in the coupling reaction of various sulfonyl chlorides **8a-e** independently with an amine **5** in basic medium (**Scheme-I**). Further, ester analog **7a** was converted to amide analog **12** by ester hydrolysis of **7a** with aqueous lithium hydroxide in THF, water solvent mixture and further the resulted acid on amine coupling with *N*-methylpiperizine (**11**) in the presence of HBTU as coupling agent and DIPEA as a base (**Scheme-II**). Chloro group in triazine **2** was substituted by *N*-boc piperazine with **13** in the presence of K_2CO_3 afforded **14**, which further on deprotection of BOC group followed by coupling of **15** with sulphonyl chlorides **8** gave the corresponding sulphanilamides **16** (**Scheme-III**).

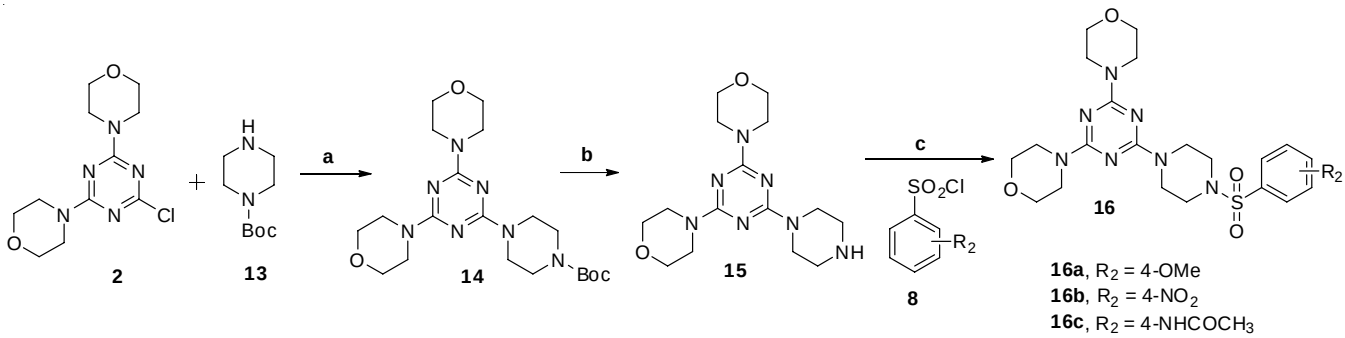
Biological evaluation studies: The synthesized triazine compounds were tested for antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. The bacterial cultures used for activity are Gram-positive species *S. aureus* (ATCC-6538), *B. subtilis* (ATCC-6633) and Gram-negative species *E. coli* (ATCC-25922), *Klebsiella* (ATCC-13883). All the results were expressed as zone of inhibition (ZOI) in diameter, mm and compared with the activity of the standard antibiotic ciprofloxacin (20 and 40 µg/mL). These triazine compounds showed moderate to high activity against bacteria. Compound **7b** exhibited highest bacterial growth inhibition among the series of trisubstituted triazine compounds (Table-1). These new analogs were also screened against pathogenic fungi species *Fusarium oxysporum* and *Aspergilus flavus* using standard drugs amphotericin-B and hymexazol. Compounds **7b** and **9e** showed high antifungal inhibition among these new analogs (Table-2). Further, these compounds are evaluated to antioxidant activity using ascorbic acid as a standard. Almost these new triazine compounds exhibited moderate to high antioxidative activity (Table-3).



Scheme-I: Synthesis of *bis*(morpholino 1,3,5-triazine)piperidin-4-amine derivatives **7a-b**, **9a-e**; Reagents and conditions: (a) **1** (5 mmol), Et_3N (15 mmol), morpholine (10 mmol), 50 mL acetone and 200 g crushed ice, 82%; (b) **2** (1 mmol), **3** (1.1 mmol), K_2CO_3 (2 mmol), 90 °C, DMF, 73%; (c) **4** (1 mmol), 4 N HCl in dioxane (5 w/v), DCM, NH_3 solution, 62%; (d) **5** (1 mmol), **6a/6b/8**, (1.1 mmol), Et_3N (1.5 mmol), DCM, 55-74%



Scheme-II: Synthesis of triazine uridoamide derivative **12**; Reagents and conditions: (a) **7a** (1 mmol), $LiOH{\cdot}H_2O$ (2 mmol), THF-H_2O (3:1), 70%; (b) **10** (1 mmol), **11** (1.1 mmol), HBTU (1.5 mmol), DIPEA (2 mmol), DMF, 52%



Scheme-III: Synthesis of 4-(*bis*morpholino-1,3,5-triazin-2-yl)-piperazin-1-yl analogs **16a-c**; Reagents and conditions: (a) **2** (1 mmol), **13** (1.1 mmol), K_2CO_3 (2 mmol), 90 °C, DMF, 81%; (b) **14** (1 mmol), 4 N HCl in dioxane (10 w/v), DCM, NH_3 solution 55%; (c) **15** (1 mmol), **8**, (1.1 mmol), Et_3N (1.5 mmol), DCM, 59-71%

TABLE-1
ANTIBACTERIAL EVALUATION OF TRISUBSTITUTED TRIAZINE DERIVATIVES AT DIFFERENT CONCENTRATIONS

Compound	Zone of inhibition in diameter (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	Bacillus faecalis		*Staphylococcus aureus*		*Klebsiella pneumoniae*		*Escherichia coli*	
	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL
7a	6.2	9.1	3.5	7.6	10.2	10.4	4.4	11.3
7b	13.5	32.2	15.3	24.7	22.8	37.4	24.3	32.2
9a	8.4	15.4	13.1	10.6	14.3	21.4	18.8	22.8
9b	5.8	9.2	7.4	13.4	6.4	15.8	7.3	17.2
9c	6.7	12.1	7.6	11.2	7.5	17.6	8.5	18.8
9d	3.8	6.2	5.2	10.9	10.3	13.3	6.5	13.8
9e	11.9	28.8	12.3	15.6	15.2	33.8	20.6	28.8
10	4.2	7.2	3.0	8.5	9.4	10.4	4.2	12.7
12	3.2	8.6	3.8	6.4	7.8	9.8	3.1	10.8
16a	9.2	12.4	8.5	14.2	10.2	17.2	13.6	31.4
16b	7.5	13.9	4.6	17.5	14.6	24.2	25.3	28.5
16c	6.2	8.4	15.2	13.5	17.2	26.2	27.5	24.1
Ciprofloxacin	16.8	37.4	18.7	28.5	26.4	42.3	28.3	38.7

TABLE-2
In vitro ANTIFUNGAL EVALUATION
TRISUBSTITUED TRIAZINE DERIVATIVES

Compound	Zone of inhibition in diameter (mm)	
	Aspergillus flavus	*Fusarium oxysporum*
7a	5.3	9.8
7b	16.3	19.9
9a	9.6	12.6
9b	7.3	9.6
9c	8.4	10.7
9d	5.8	7.4
9e	13.3	18.9
10	4.2	11.4
12	3.2	5.4
16a	10.5	13.2
16b	8.2	9.7
16c	10.7	7.4
Amphotericin-B	18.4	24.6
Hymexazol	24.2	26.8

Concentration of the compound is 500 µg/mL

TABLE-3
SUPEROXIDE SCAVENGING ACTIVITY
(ANTIOXIDATIVE EVALUATIONS)

Compound	DPPH (50 µg/mL)	DPPH (100 µg/mL)	DPPH (150 µg/mL)
7a	50.21 ± 0.23	88.21 ± 1.32	93.25 ± 2.32
7b	52.45 ± 0.68	89.47 ± 4.68	95.38 ± 5.25
9a	57.37 ± 2.16	85.34 ± 3.45	92.64 ± 1.65
9b	56.78 ± 3.64	82.77 ± 3.62	89.62 ± 2.25
9c	54.16 ± 1.46	80.64 ± 2.44	86.48 ± 3.34
9d	53.88 ± 1.28	78.46 ± 2.62	82.65 ± 1.46
9e	50.58 ± 1.39	68.74 ± 3.46	78.12 ± 2.35
10	41.31 ± 1.48	72.66 ± 3.31	65.86 ± 1.18
12	44.41 ± 1.48	70.34 ± 1.48	69.26 ± 1.72
16a	62.16 ± 2.13	85.13 ± 3.20	89.21 ± 3.24
16b	67.60 ± 1.51	81.23 ± 2.78	83.25 ± 1.78
16c	55.23 ± 1.28	83.21 ± 2.34	85.21 ± 1.78
Ascorbate	59.25 ± 5.39	92.57 ± 4.42	98.45 ± 4.16

Conclusion

In summary, new *bis*-morpholino trisubstituted (1,3,5)-triazine analogs were synthesized, characterized and evaluated for antibacterial, antifungal and antioxidant activities. The studies showed a moderate to high inhibition towards Gram-positive and Gram-negative bacteria and fungi. These triazine derivatives are also having excellent antioxidative properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. E. Peterson and P. Kaur, *Front. Microbiol.*, **9**, 2928 (2018); https://doi.org/10.3389/fmicb.2018.02928
2. S. Santajit and N. Indrawattana, *BioMed. Res. Int.*, **2016**, 2475067 (2016); https://doi.org/10.1155/2016/2475067
3. A.M. Fuentefria, B. Pippi, D.F. Dalla Lana, K.K. Donato and S.F. de Andrade, *Lett. Appl. Microbiol.*, **66**, 2 (2018); https://doi.org/10.1111/lam.12820
4. E.A. Friedman, Behind the Headlines: 10 Million Deaths From Antimicrobial Resistance by 2050 (or Not?), February, 2020; https://oneill.law.georgetown.edu/behind-the-headlines-10-million-antimicrobial-deaths-by-2050-or-not
5. A. Dhainaut, G. Regnier, A. Tizot, A. Pierre, S. Leonce, N. Guilbaud, L. Kraus-Berthier and G. Atassi, *J. Med. Chem.*, **39**, 4099 (1996); https://doi.org/10.1021/jm9603611i
6. S.W. Kim, S.C. Shim, B.-J. Jung and H.-K. Shim, *Polymer*, **43**, 4297 (2002); https://doi.org/10.1016/S0032-3861(02)00252-5

7. R. Bai, S. Li, Y. Zou, C. Pan, P. Xie, B. Kong, X. Zhou and R. Zhang, *Liq. Cryst.*, **28**, 1873 (2001); https://doi.org/10.1080/02678290110086514
8. J.-W. Kang, D.-S. Lee, H.-D. Park, J.W. Kim, W.-I. Jeong, Y.-S. Park, S.-H. Lee, K. Go, J.-S. Lee and J.-J. Kim, *Org. Electron.*, **9**, 452 (2008); https://doi.org/10.1016/j.orgel.2008.02.002
9. R.N. Butler, A.M. Fahy, A. Fox, J.C. Stephens, P. McArdle, D. Cunningham and A.G. Ryder, *Tetrahedron Lett.*, **47**, 1721 (2006); https://doi.org/10.1016/j.tetlet.2006.01.052
10. N. Karade, P. Thorat and B. Bhong, *Synlett*, **24**, 2061 (2013); https://doi.org/10.1055/s-0033-1339495
11. M. Kitamura, F. Kawasaki, K. Ogawa, S. Nakanishi, H. Tanaka, K. Yamada and M. Kunishima, *J. Org. Chem.*, **79**, 3709 (2014); https://doi.org/10.1021/jo500376m
12. D.H. Patel, K.H. Chikhalia, N.K. Shah, D.P. Patel, P.B. Kaswala and V.M. Buha, *J. Enzyme Inhib. Med. Chem.*, **25**, 121 (2010); https://doi.org/10.3109/14756360903027956
13. A. Solankee, K. Kapadia, Ana Ciriæ, M. Sokovic, I. Doytchinova and A. Geronikaki, *Eur. J. Med. Chem.*, **45**, 510 (2010); https://doi.org/10.1016/j.ejmech.2009.10.037
14. K. Srinivas, U. Srinivas, K. Bhanuprakash, K. Harakishore, U.S.N. Murthy and V. Jayathirtha Rao, *Eur. J. Med. Chem.*, **41**, 1240 (2006); https://doi.org/10.1016/j.ejmech.2006.05.013
15. C. Borsari, D. Rageot, F. Beaufils, T. Bohnacker, E. Keles, I. Buslov, A. Melone, A.M. Sele, P. Hebeisen, D. Fabbro, P. Hillmann and M.P. Wymann, *ACS Med. Chem. Lett.*, **10**, 1473 (2019); https://doi.org/10.1021/acsmedchemlett.9b00333
16. A.M. Venkatesan, C.M. Dehnhardt, E. Delos Santos, Z. Chen, O. Dos Santos, S. Ayral-Kaloustian, G. Khafizova, N. Brooijmans, R. Mallon, I. Hollander, L. Feldberg, J. Lucas, K. Yu, J. Gibbons, R.T. Abraham, I. Chaudhary and T.S. Mansour, *J. Med. Chem.*, **53**, 2636 (2010); https://doi.org/10.1021/jm901830p
17. A. Baliani, G.J. Bueno, M.L. Stewart, V. Yardley, R. Brun, M.P. Barrett and I.H. Gilbert, *J. Med. Chem.*, **48**, 5570 (2005); https://doi.org/10.1021/jm050177+
18. S. Melato, D. Prosperi, P. Coghi, N. Basilico and D. Monti, *ChemMedChem*, **3**, 873 (2008); https://doi.org/10.1002/cmdc.200700344
19. Y.-Z. Xiong, F.-E. Chen, J. Balzarini, E. De Clercq and C. Pannecouque, *Eur. J. Med. Chem.*, **43**, 1230 (2008); https://doi.org/10.1016/j.ejmech.2007.08.001
20. N. Sunduru, L. Gupta, V. Chaturvedi, R. Dwivedi, S. Sinha and P.M. Chauhan, *Eur. J. Med. Chem.*, **45**, 3335 (2010); https://doi.org/10.1016/j.ejmech.2010.04.017
21. A.J. Huh and Y.J. Kwon, *J. Control. Release*, **156**, 128 (2011); https://doi.org/10.1016/j.jconrel.2011.07.002
22. S. Shaikh, N. Nazam, S.M.D. Rizvi, K. Ahmad, M.H. Baig, E.J. Lee and I. Choi, *Int. J. Mol. Sci.*, **20**, 2468 (2019); https://doi.org/10.3390/ijms20102468
23. B. Song, H. Zhang, H. Wang, S. Yang, L. Jin, D. Hu, L. Pang and W. Xue, *J. Agric. Food Chem.*, **53**, 7886 (2005); https://doi.org/10.1021/jf051050w
24. S. Song, L. Zhou, D. Li, D. Tang, J. Li and W. Jiang, *Nat. Prod. Res. Dev.*, **16**, 157 (2004).
25. N. Cotelle, J.-L. Bernier, J.-P. Catteau, J. Pommery, J.-C. Wallet and E.M. Gaydou, *Free Radic. Biol. Med.*, **20**, 35 (1996); https://doi.org/10.1016/0891-5849(95)02014-4