Research Article

Ultrasonic Irradiation: Synthesis, Characterization, and Preliminary Antimicrobial Activity of Novel Series of 4,6-Disubstituted-1,3,5-triazine Containing Hydrazone Derivatives

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Novel series of 4,6-disubstituted-1,3,5-triazines containing hydrazone derivatives were synthesized employing ultrasonic irradiation and conventional heating. The ultrasonication gave the target products in higher yields and purity in shorter reaction time compared with the conventional method. IR, NMR (^{1}H and ^{13}C), elemental analysis, and LC-MS confirmed the structures of the new products. The antimicrobial and antifungal activities were evaluated for all the prepared compounds against some selected Gram-positive and Gram-negative bacterial strains. The results showed that only two compounds **7i** (pyridine derivative) and **7k** (4-chlorobenzaldehyde derivative) displayed biological activity against some Gram-positive and Gram-negative bacteria, while the rest of the tested compounds did not display any antifungal activity.

1. Introduction

Ultrasound has been employed more and more frequently in organic synthesis [1, 2], because it improved the reaction rate and could adjust the selectivity performance of the reaction [3]. Comparing with traditional methods, ultrasonic irradiation is suitable and simply controlled and it considered as a green powerful synthetic technique in chemical processes. Ultrasonic irradiation has proven to be a particularly important tool for meeting goals of the green chemistry, which is minimization of waste, and decreasing of the energy required for the reaction [4]. Applications of ultrasonic irradiation in organic synthesis are playing important role, especially in cases where traditional methods require drastic conditions or elongated reaction time [5–10].

Richards and Loomis first reported the chemical effects of ultrasound in 1927 [11]. The effect of ultrasound during organic reaction is due to cavitation, which led to separation of molecules of liquids and then the collapse of the bubbles offers strong impulsions that generate short-lived regions with high pressure and temperature. Such localized hot spots act as microreactors in which the sound energy converted into beneficial chemical form [12–14].

Recently Schiff base hydrazone derivatives have attracted great attention in many applications [15–17]. Compounds bearing hydrazone moiety exhibit a broad range of biological activities, including antifungal, antibacterial, antiviral, antimalarial, antiproliferative, anti-inflammatory, and antipyretic properties [18–22].

HNu₁, HNu₂, HNu₃ = N, O, P, S, F nucleophiles
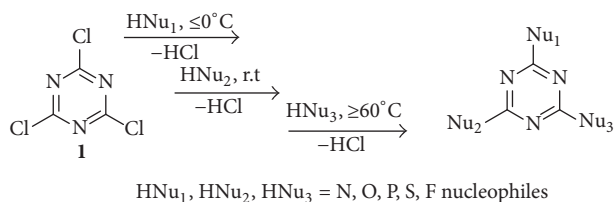
Scheme 1: Nucleophilic substitution reaction of cyanuric chloride.

On the other hand, cyanuric chloride **1** is the most important reagent for s-triazine derivatives, because of the selective reactivity of its chlorine atoms toward nucleophiles. Cyanuric chloride **1** is commercially available and a very cheap reagent, which makes its applications even more attractive [23–29]. The easy displacement of chlorine atoms in cyanuric chloride by various nucleophiles was controlled by temperature to run in a stepwise manner as shown in Scheme 1.

Several derivatives of s-triazine have exhibited antimicrobial [30], antibacterial, [31] antifungal [32], anti-HIV [33], and anticancer [34, 35] properties and a wide array of other biological activities [36, 37]. Recently some of 1,3,5-triazine–Schiff bases have reported a significant activity against Mycobacterium tuberculosis H37Rv [38] and moderate to excellent antiproliferative activity and high selectivity against the human lung cancer cell line H460 [39].

As part of our continuous research on s-triazine derivatives and their biological activities [40, 41], we report here the synthesis and the preliminary antimicrobial activity of novel series of 4,6-disubstituted-s-triazine containing hydrazone derivatives.

2. Experimental Section

2.1. Chemistry

2.1.1. Materials. All solvents were of analytical reagent grade and used without further purification. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL 400 MHz and AVANCE III 400 MHz (Bruker, Germany) spectrometer at room temperature (see Figures S1–S10, S12, and S14–S17 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/3464758) in CDCl₃ and/or DMSO-d₆ using internal standard δ = 0 ppm. Elemental analysis was performed on Perkin-Elmer 2400 elemental analyzer. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on Nicolet 6700 spectrometer from KBr discs. The reaction was follow-up and checks of the purity using TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck) using a mixture of methanol-chloroform (1 : 9) as an eluent. LC-MS (see Figures S11, S13, and S18 in Supplementary Material) was performed on Shimadzu 2020 UFLC-MS using an YMC Triart C₁₈ (5 μm, 4.6×150 mm) column, and data processing was carried out by the LabSolution software. Buffer A: 0.1% formic acid in H₂O and buffer B: 0.1% formic acid in CH₃CN in 30 min at λ_{max} 254 nm were used. High-resolution mass spectrometric data

were obtained using a Bruker micrOTOF-Q II instrument operating at room temperature and a sample concentration of approximately 1 ppm. Ultrasonic bath was purchased from Selecta (Barcelona, Spain). All compounds were named by using ChemBioDraw Ultra version 14.0, Cambridge Soft Corporation (Cambridge, MA, USA).

(i) General Method for the Synthesis of 2-Hydrazino-4,6-dimorpholinio or Dimethoxy-1,3,5-triazine (3 and 5). Hydrazine hydrate (10 mL, 80%) was added dropwise to a solution of 2-chloro-4,6-dimorpholino or dimethoxy-1,3,5-triazine **2** or **4** (20 mmol) in 50 mL ethanol at room temperature and then the reaction mixture was sonicated for 60 min at 60°C. Ethanol and excess hydrazine were removed under vacuum and then excess diethyl ether was added to afford the product as a white solid in yield >90%. The spectral data of the two products **3** and **5** were in good agreement with the reported data [40, 42] and were used directly without further purification.

(ii) General Method for the Synthesis of 1,3,5-Triazine-hydrazone Derivatives.

Method A: Conventional Method. 2-Hydrazino-4,6-disubstituted-1,3,5-triazine **3** or **5** (10 mmol) was added to a solution of aldehyde or ketone **6** (10 mmol) in ethanol (30 mL) containing 2-3 drops of acetic acid and then the reaction mixture was stirred under reflux for 3 hours. After completion of the reaction, the solvent was reduced under vacuum and the precipitated product was filtered off, dried at room temperature, and then recrystallized from ethyl acetate to afford the target product (Table 1).

Method B: Ultrasound Assisted Method. A mixture of aldehyde or ketone **6** (10 mmol) in ethanol (30 mL), 2-hydrazino-4,6-disubstituted-1,3,5-triazine **3** or **5** (10 mmol), and 2-3 drops of acetic acid in a flask was heated into a sonicator at 40°C for 30–60 min. After completion of reaction (TLC, methanol-chloroform 1 : 9) UV lamp was used for spot visualization at λ_{max} 254; the solvent was removed under vacuum and the precipitated product was recrystallized from ethyl acetate to afford the target product (Table 1).

2.1.2. (E)-2-(2-Benzylidenehydrazinyl)-4,6-dimethoxy-1,3,5-triazine (7a, Supporting Information Figure S1 in Supplementary Material). The product was obtained as a yellow solid in yield 74% (A), 93% (B); mp. 235–237°C; IR (KBr, cm^{-1}): 3219 (NH), 1561 (C=N), 1467, 1386 (C=C); ^{1}H NMR (DMSO-d₆): δ = 3.89 (s, 6H, 2 OCH₃), 7.41–7.43 (m, 3H, Ar), 7.66 (d, 2H, J = 6.4 Hz, Ar), 8.17 (s, 1H, CH), 11.68 (s, 1H, NH); ^{13}C NMR (DMSO-d₆): δ = 54.5, 126.8, 128.8, 129.8, 134.4, 144.9, 166.3 ppm; Anal. Calc. for C₁₂H₁₃N₅O₂ (259.27): C, 55.59; H, 5.05; N, 27.01. Found: C, 55.89; H, 5.16; N, 27.23.

2.1.3. (E)-2-(2-(4-Chlorobenzylidene)hydrazinyl)-4,6-dimethoxy-1,3,5-triazine (7b, Supporting Information Figure S2 in Supplementary Material). The product was obtained as a white solid in yield 71% (A), 92% (B); mp. 215–217°C; IR (KBr, cm^{-1}): 3228 (NH), 1614 (C=N), 1577, 1473 (C=C); ^{1}H

TABLE 1: Yield, reaction time, and melting point of **7a**–**o** derivativesusing conventional method and ultrasonic irradiation.

Compd. number	Method A Conventional method		Method B Ultrasonic irradiation		Mp (°C)
	Yield (%)	Time (h)	Yield (%)	Time (min)	
7a	74	4-5	93	30	235–237
7b	71	4-5	92	30	215–217
7c	70	4-5	93	30	204–206
7d	68	4-5	93	30	188–190
7e	75	4-5	95	30	220–222
7f	75	4-5	93	60	225–227
7g	75	4-5	93	60	227–229
7h	71	4-5	92	60	230–232
7i	71	4-5	92	30	212–214
7j	75	4-5	95	30	225–227
7k	70	4-5	94	30	233–235
7l	72	4-5	94	60	236–238
7m	74	4-5	96	60	213–215
7n	70	4-5	92	60	228–230
7o	71	4-5	90	60	232–234

NMR (DMSO-d_6): δ = 3.89 (s, 6H, 2 OCH$_3$), 7.47 (d, 2H, J = 8.8 Hz, Ar), 7.69 (d, 2H, J = 8.8 Hz, Ar), 8.15 (s, 1H, CH), 11.73 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 54.5, 128.4, 128.9, 133.4, 134.1, 143.6, 166.3 ppm; Anal. Calc. for C$_{12}$H$_{12}$ClN$_5$O$_2$ (293.07): C, 49.07; H, 4.12; N, 23.84. Found: C, 48.92; H, 4.22; N, 24.03.

*2.1.4. (E)-2,4-Dimethoxy-6-(2-(4-methylbenzylidene)hydrazinyl)-1,3,5-triazine (**7c**, Supporting Information Figure S3 in Supplementary Material).* The product was obtained as a light yellow solid in yield 70% (A), 93% (B); mp. 204–206°C; IR (KBr, cm^{-1}): 3219 (NH), 1564 (C=N), 1465, 1367 (C=C); ^{1}H NMR (DMSO-d_6): δ = 2.33 (s, 3H, CH$_3$), 3.90 (s, 6H, 2 OCH$_3$), 7.24 (d, 2H, J = 8.0 Hz, Ar), 7.58 (d, 2H, J = 8.08 Hz, Ar), 8.15 (s, 1H, CH), 11.62 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 21.0, 54.5, 126.8, 129.4, 131.7, 139.6, 145.1, 166.2 ppm; Anal. Calc. for: C$_{13}$H$_{15}$N$_5$O$_2$ (273.12): C, 57.13; H, 5.53; N, 25.63. Found: C, 57.33; H, 5.41; N, 25.80.

*2.1.5. (E)-2,4-Dimethoxy-6-(2-(4-methoxybenzylidene)hydrazinyl)-1,3,5-triazine (**7d**, Supporting Information Figure S4 in Supplementary Material).* The product was obtained as a light yellow solid in yield 68% (A), 93% (B); mp. 188–190°C; IR (KBr, cm^{-1}): 3244 (NH), 1570 (C=N), 1479, 1364 (C=C); ^{1}H NMR (DMSO-d_6): δ = 3.80 (s, 3H, OCH$_3$), 3.91 (s, 6H, 2OCH$_3$), 7.04 (d, 2H, J = 8.0 Hz, Ar), 7.62 (d, 2H, J = 8.08 Hz, Ar), 8.12 (s, 1H, CH), 11.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 54.5, 55.3, 114.3, 127.0, 128.5, 130.0, 144.9, 160.6, 166.1 ppm; Anal. Calc for C$_{13}$H$_{15}$N$_5$O$_3$ (289.30): C, 53.97; H, 5.23; N, 24.21. Found: C, 53.73; H, 5.07; N, 24.00.

*2.1.6. (E)-2-(2-(4-Bromobenzylidene)hydrazinyl)-4,6-dimethoxy-1,3,5-triazine (**7e**, Supporting Information Figure S5 in Supplementary Material).* The product was obtained as a white solid in yield 75% (A), 95% (B); mp. 220–222°C; IR (KBr, cm^{-1}): 3217 (NH), 1578 (C=N), 1460, 1368 (C=C); ^{1}H NMR (DMSO-d_6): δ = 3.89 (s, 6H, 2 OCH$_3$), 7.64 (s, 4H, Ar), 8.15 (s, 1H, CH), 11.74 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 54.5, 122.9, 128.7, 131.87, 133.7, 143.8, 166.3 ppm; Anal. Calc. for C$_{12}$H$_{12}$BrN$_5$O$_2$ (338.17): C, 42.62; H, 3.58; N, 20.71. Found: C, 42.88; H, 3.64; N, 20.98.

*2.1.7. (E)-4-((2-(4,6-Dimethoxy-1,3,5-triazin-2-yl)hydrazono)methyl) phenol (**7f**, Supporting Information Figure S6 in Supplementary Material).* The product was obtained as a white solid in yield 75% (A), 93% (B); mp. 225–227°C; IR (KBr, cm^{-1}): 3229 (OH), 3135 (NH), 1609 (C=N), 1567, 1493 (C=C); ^{1}H NMR (DMSO-d_6): δ = 3.88 (s, 3H, OCH$_3$), 3.90 (s, 3H, OCH$_3$), 6.81 (d, 2H, J = 8.0 Hz, Ar), 7.52 (d, 2H, J = 8.0 Hz, Ar), 8.07 (s, 1H, CH), 9.93 (s, 1H, OH), 11.47 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 54.5, 115.8, 125.5, 128.8, 145.5, 159.2, 166.1, 171.8 ppm; Anal. Calc. for C$_{12}$H$_{13}$N$_5$O$_3$ (275.27): C, 52.36; H, 4.76; N, 25.44. Found: C, 52.21; H, 4.89; N, 25.63.

*2.1.8. (E)-4-((2-(4,6-Dimethoxy-1,3,5-triazin-2-yl)hydrazono)methyl)-N,N-dimethylaniline (**7g**, Supporting Information Figure S7 in Supplementary Material).* The product was obtained as a yellow solid in yield 75% (A), 93% (B); mp. 227–229°C; IR (KBr, cm^{-1}): 3211 (NH), 1567 (C=N), 1459, 1362 (C=C); ^{1}H NMR (DMSO-d_6): δ = 2.95 (s, 6H, N(CH$_3$)$_2$), 3.86 (s, 3H, OCH$_3$), 3.89 (s, 3H, OCH$_3$), 6.71 (d, 2H, J = 7.2 Hz, Ar), 7.52 (d, 2H, J = 8.8 Hz, Ar), 8.03 (s, 1H, CH), 11.38 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 40.4, 55.0, 112.5, 122.4, 128.8, 146.6, 151.9, 166.5 ppm; Anal. Calc. for C$_{14}$H$_{18}$N$_6$O$_2$ (302.34): C, 55.62; H, 6.00; N, 27.80. Found: C, 55.88; H, 6.20; N, 28.04.

*2.1.9. (E)-4-(1-(2-(4,6-Dimethoxy-1,3,5-triazin-2-yl)hydrazono)ethyl)phenol (**7h**, Supporting Information Figure S8 in Supplementary Material).* The product was obtained as a yellow solid in yield 71% (A), 92% (B); mp. 230–232°C; IR (KBr, cm^{-1}): 3466 (OH), 3364 (NH), 1576 (C=N), 1475, 1374 (C=C); ^{1}H NMR (DMSO-d_6): δ = 2.26 (s, 3H, CH$_3$), 3.91 (s, 6H, 2OCH$_3$), 6.78 (d, 2H, J = 8.8 Hz, Ar), 7.68 (d, 2H, J = 8.4 Hz, Ar), 9.78 (s, 1H, OH), 10.47 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 13.9, 54.4, 115.2, 127.9, 129.2, 151.3, 158.6, 166.9 ppm; Anal. Calc. for C$_{13}$H$_{15}$N$_5$O$_3$ (289.30): C, 53.97; H, 5.23; N, 24.2. Found: C, 53.77; H, 5.33; N, 24.43.

*2.1.10. (E)-2,4-Dimethoxy-6-(2-(pyridin-2-ylmethylene)hydrazinyl)-1,3,5-triazine (**7i**, Supporting Information Figure S9 in Supplementary Material).* The product was obtained as a pale yellow solid in yield 69% (A), 91% (B); mp. 212–214°C; IR (KBr, cm^{-1}): 3236 (NH), 1572 (C=N), 1467, 1366 (C=C); ^{1}H NMR (DMSO-d_6): δ = 3.92 (s, 6H, 2OCH$_3$), 7.39 (t, 1H, J = 5.6, Ar), 7.88 (t, 1H, J = 7.2 Hz, Ar), 7.96 (d, 1H, J = 8.0 Hz, Ar), 8.22 (s, 1H, CH), 8.59 (d, 1H, J = 5.2 Hz, Ar), 11.38 (s, 1H,

NH); ^{13}C NMR (DMSO-d$_6$): δ = 54.1, 119.7, 123.9, 136.9, 144.4, 149.2, 153.3, 166.5, 171.6 ppm; Anal. Calc. for C$_1$H$_{12}$N$_6$O$_2$ (260.26): C, 50.77; H, 4.65; N, 32.29. Found: C, 50.96; H, 4.73; N, 32.40.

2.1.11. (E)-4,4′-(6-(2-Benzylidenehydrazinyl)-1,3,5-triazine-2,4-diyl)dimorpholine (7j, Supporting Information Figure S10 in Supplementary Material).

The product was obtained as a white solid in yield 75% (A), 95% (B); mp. 215–217°C; IR (KBr, cm^{-1}): 3267 (NH), 1546 (C=N), 1479, 1450 (C=C); ^{1}H NMR (CDCl$_3$): δ = 3.70–3.74 (m, 8H, 4 OCH$_2$-), 3.75–3.84 (m, 8H, 4 NCH$_2$-), 7.35–7.41 (m, 3H, Ar), 7.70–7.73 (m, 2H, Ar), 7.82 (s, 1H, CH), 8.31 (s, 1H, NH); ^{13}C NMR (CDCl$_3$): δ = 43.6, 43.7, 66.8, 66.9, 127.1, 128.6, 129.6, 134.2, 142.5, 165.4 ppm; Anal. Calc. for C$_{18}$H$_{23}$N$_7$O$_2$ (369.43): C, 58.52; H, 6.28; N, 26.54. Found: C, 58.74; H, 6.43; N, 26.79. (m/z) Calcd: 369.43; LC-MS (M+H) Found: 370 at R_t 14.11 min (supporting information Figure S11 in Supplementary Material).

2.1.12. (E)-4,4′-(6-(2-(4-Chlorobenzylidene)hydrazinyl)-1,3,5-triazine-2,4-diyl)dimorpholine (7k, Supporting Information Figure S12 in Supplementary Material).

The product was obtained as a white solid in yield 70% (A), 94% (B); mp. 233–235°C; IR (KBr, cm^{-1}): 3270 (NH), 1516 (C=N), 1480, 1442 (C=C); ^{1}H NMR (CDCl$_3$): δ = 3.71–3.74 (m, 8H, 4 OCH$_2$-), 3.75–3.84 (m, 8H, 4 NCH$_2$-), 7.36 (d, 2H, J = 4.0 Hz, Ar), 7.72 (d, 2H, J = 4.0 Hz, Ar), 7.82 (s, 1H, CH), 8.31 (brs, 1H, NH); ^{13}C NMR (CDCl$_3$): δ = 43.6, 43.7, 66.8, 66.9, 128.2, 128.9, 132.8, 135.4, 141.1, 165.4 ppm; Anal. Calc. for C$_{18}$H$_{22}$ClN$_7$O$_2$ (403.87): C, 53.53; H, 5.49; N, 24.28. Found: C, 53.78; H, 5.60; N, 24.51. (m/z) Calcd: 403.87; LC-MS (M+H) Found: 404 at R_t 15.8 min (supporting information Figure S13 in Supplementary Material)

2.1.13. (E)-4,4′-(6-(2-(4-Bromobenzylidene)hydrazinyl)-1,3,5-triazine-2,4-diyl) dimorpholine (7l, Supporting Information Figure S14 in Supplementary Material).

The product was obtained as a white solid in yield 72% (A), 94% (B); mp 236–238°C; IR (KBr, cm^{-1}): 3273 (NH), 1515 (C=N), 1479, 1441 (C=C); ^{1}H NMR (DMSO-d$_6$): δ = 3.62 (m, 8H, 4 OCH$_2$-), 3.70 (m, 8H, 4 NCH$_2$), 7.59 (s, 4H, Ar), 8.03 (s, 1H, CH), 10.90 (s, 1H, NH); ^{13}C NMR (CDCl$_3$): δ = 43.8, 66.6, 122.6, 128.8, 132.3, 134.9, 141.0, 164.7, 165.3 ppm; Anal. Calc. for C$_{18}$H$_{22}$BrN$_7$O$_2$ (448.33): C, 48.22; H, 4.95; N, 21.87. Found: C, 48.44; H, 5.08; N, 22.03.

2.1.14. (E)-4-((2-(4,6-Dimorpholino-1,3,5-triazin-2-yl)hydrazono)methyl)phenol (7m, Supporting Information Figure S15 in Supplementary Material).

The product was obtained as a white solid in yield 74% (A), 96% (B); mp 213–215°C; IR (KBr, cm^{-1}): 3448 (OH), 3250 (NH), 1543 (C=N), 1493, 1443 (C=C); ^{1}H NMR (CDCl$_3$): δ = 3.62–3.74 (m, 16H, 4 N-CH$_2$-CH$_2$-O), 6.74 (d, 2H, J = 8.56 Hz, Ar), 7.51 (d, 2H, J = 8.60 Hz, Ar), 7.68 (s, 1H, CH), 8.02 (brs, 1H, NH); ^{13}C NMR (CDCl$_3$): δ = 43.6, 43.7, 66.8, 66.9, 115.6, 126.8, 128.9, 157.4, 165.4 ppm; Anal. Calc. for C$_{18}$H$_{23}$N$_7$O$_3$ (385.43): C, 56.09; H, 6.02; N, 25.44. Found: C, 56.23; H, 6.21; N, 25.69.

2.1.15. (E)-4-((2-(4,6-Dimorpholino-1,3,5-triazin-2-yl)hydrazono)methyl)-N,N-dimethylaniline (7n, Supporting Information Figure S16 in Supplementary Material).

The product was obtained as a yellow solid in yield 70% (A), 92% (B); mp 228–230°C; IR (KBr, cm^{-1}): 3277 (NH), 1529 (C=N), 1490, 1441 (C=C); ^{1}H NMR (DMSO-d$_6$): δ = 3.62–3.69 (m, 16H, 4 N-CH$_2$-CH$_2$-O), 6.72 (d, 2H, J = 8.04 Hz, Ar), 7.44 (d, 2H, J = 8.8 Hz, Ar), 7.95 (s, 1H, CH), 10.52 (s, 1H, NH); ^{13}C NMR (DMSO-d$_6$): δ 43.8, 66.6, 112.4, 123.2, 128.2, 143.3, 151.4, 164.5, 165.3 ppm; Anal. Calc. for C$_{20}$H$_{28}$N$_8$O$_2$ (412.50): C, 58.24; H, 6.84; N, 27.17. Found: C, 58.51; H, 6.99; N, 27.36.

2.1.16. (E)-4-(1-(2-(4,6-Dimorpholino-1,3,5-triazin-2-yl)hydrazono)ethyl)phenol (7o, Supporting Information Figure S17 in Supplementary Material).

The product was obtained as a white solid in yield 71% (A), 90% (B); mp.232–234°C; IR (KBr, cm^{-1}): 3452 (OH), 3350 (NH), 1563 (C=N), 1524, 1487 (C=C); ^{1}H NMR (CDCl$_3$): δ = 2.24 (s, 3H, CH$_3$), 3.70–3.83 (m, 16H, 4 N-CH$_2$-CH$_2$-O), 6.80 (d, 2H, J = 8.8 Hz, Ar), 7.69 (d, 2H, J = 8.46 Hz, Ar), 7.91 (s, 1H, CH), 8.01 (brs, 1H, NH); ^{13}C NMR (CDCl$_3$): δ = 12.7, 43.7, 66.9, 115.3, 127.8, 128.2, 130.9, 146.9, 156.8, 164.7, 165.2, 165.4 ppm; Anal. Calc. for C$_{19}$H$_{25}$N$_7$O$_3$ (399.46): C, 57.13; H, 6.31; N, 24.55. Found: C, 57.35; H, 6.44; N, 24.80. (m/z) Calcd: 399.46; LC-MS (M+H) Found: 400 at R_t 13.4 min (supporting information Figure S18 in Supplementary Material)

2.2. Biology

2.2.1. Antimicrobial Activity.

The antimicrobial activities of all compounds 7a–o were evaluated against some selected pathogenic Gram-positive, Gram-negative, and filamentous fungus strains by using the disc diffusion method [43]. *Staphylococcus aureus* (ATCC 29213); *Neisseria meningitides* (ATCC 1302); *Streptococcus mutans* (ATCC 35668); *Escherichia coli* (ATCC 25922); *Pseudomonas aeruginosa* (ATCC 27584); *Salmonella typhimurium* (ATCC 14028); *Brevibacillus laterosporus* Wild strain; *Candida parapsilosis* (ATCC 22019); *Cryptococcus neoformans* Wild strain; *Candida albicans* (ATCC 60193), *Penicillium chrysogenum* (AUMC 9476), *Aspergillus niger* (AUMC 8777), and *Fusarium* sp were used in the evaluation test.

Mueller-Hinton agar (Scharlau Microbiology, Spain) was used and prepared according to manufacturer's guidelines, where 1 L of medium was prepared containing 17.5, 1.5, and 17 gm of peptone, meat infusion solid, starch, and agar, respectively. For preparation of 1 L of medium, 21 gm of powder was dissolved in 1 L of distilled water and sterilized at 121°C for 15 min by an autoclave (HL-321, Taiwan). After sterilization and cooling to 50°C, the medium dispensed in Petri dishes and left to cool down to 25°C. Then, they were inoculated with the bacterial strains by streaking. In the antifungal test, a potato dextrose agar (PDA) (Scharlau, Spain) was used and prepared according to the manufacturer's directions, where 39 gm PDA dissolved in 1 L of distilled water and then followed the previously described method.

The microbial inoculation suspensions were prepared in sterile sodium chloride solution (0.89%) from activated
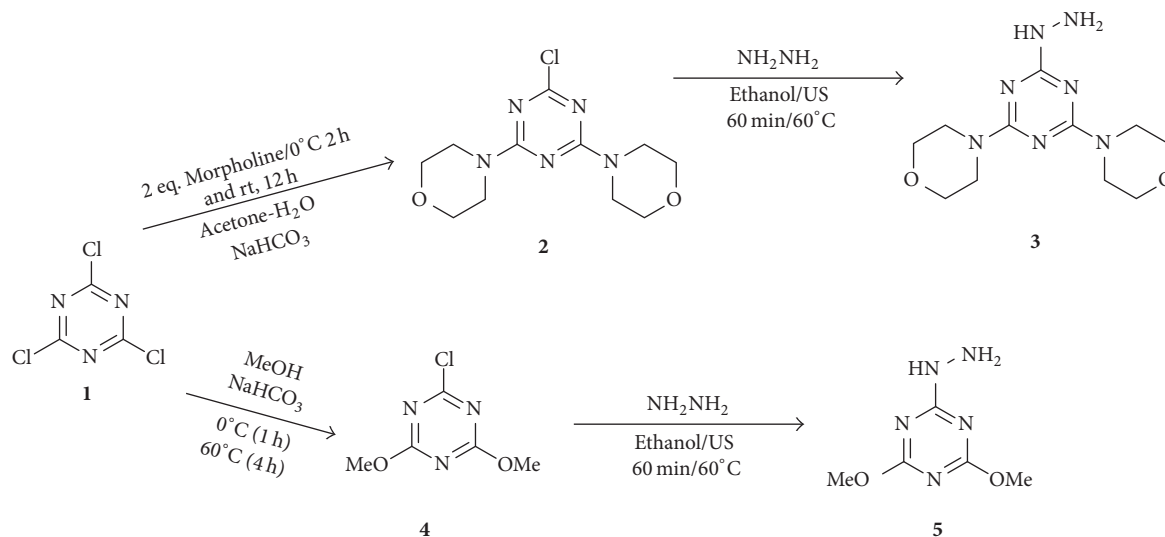
Scheme 2: Synthesis of 2-hydrazino-4,6-disubstituted-1,3,5-triazine derivatives and US: ultrasonic irradiation.

microbial cultures. The optical densities of microbial suspensions were adjusted to 0.64 at 600 nm. A sterile swab was moistened with the microbial suspensions and inoculating the dried surface of the medium in a Petri dish. After inoculation, all Petri dishes were kept at 25°C for 5–10 minutes before dispensing the standard antibiotic agents and the prepared compounds discs on the surface of the media.

In the present work, tobramycin (10 μg/desk), chloramphenicol (30 μg/disk), fusidic acid (10 μg/disk), augmentin (10 μg/disk), cycloheximide (30 μg/disk), canesten (10 μg/disk), and caspofungin (10 μg/disk) were used as standard antibacterial and antifungal agents. To prepare disks (4 mg/disk) from the tested compounds, 200 mg from each compound was dissolved in dimethylsulfoxide (DMSO) and then 20 μL of the solution was added on the sterile filter disk (6 mm) and then allowed to dry at room temperature inside the safety biological cabinet. After 18–24 h of incubation at 37°C for bacteria and 48–72 h at 25°C for fungi, the minimum inhibitory concentration (MIC mg/mL) of the prepared compounds was measured [44]. The microdilution assay was applied in 96-well plate (Corning Incorporated, USA) using twofold serial dilution. The original concentration was 4 mg/mL and the total volume was 200 μL (1:1, chemical suspension: bacterial suspension); 4-iodonitrotetrazolium violet (Sigma, USA) reagent was added after the incubation to measure the bacterial growth through the emergence of violet color.

3. Results and Discussion

3.1. Chemistry. Compounds **2** and **4** were obtained using one-step reaction, where cyanuric chloride **1** was reacted with morpholine (2 equiv.) in acetone-water media or methanol (as a solvent) in the presence of NaHCO$_3$ as hydrogen chloride removal at 0°C for 2 h. In the case of the synthesis of

dimorpholino derivative **2**, the reaction temperature raised gradually to room temperature and kept under stirring for 12 h at the same temperature, while the reported method [40, 42] was used for the preparation of the dimethoxy derivative **4** (Scheme 2). The products **2** and **4** were obtained in good yields and their spectral data agreed with the reported data [40, 42].

The hydrazine derivatives **3** and **5** were obtained by treatment of **2** or **4** with hydrazine hydrate (80%) in ethanol for 60 min at 60°C employing ultrasonic irradiation (Scheme 2) to afford the products in excellent yields (>90%) and purity.

The products **7a–o** were obtained by condensation of the hydrazine derivative **3** or **5** with substituted benzaldehyde or acetophenone **6** in ethanol containing 2-3 drops of glacial acetic acid using ultrasonic irradiation for 30–60 min at 40°C (Scheme 3) to afford the target products in excellent yields and purity as observed from LC-MS (*see Supplementary Figures S11, S13,* and *S18*). Ultrasonic irradiation gave the target products in high yields in shorter reaction time compared with the conventional heating as shown in Table 1.

The ^{1}H NMR spectrum of **7k** as a prototype for the benzaldehyde derivatives showed a multiplet peak in the range at δ 3.71–3.74 ppm related to 4 methylene groups (4 OCH$_2$-), another multiplet peak in the range at δ 3.75–3.84 ppm related to 4 methylene groups (4 NCH$_2$-), doublet at δ 7.36 ppm for the two aromatic protons H-3 and H-5, doublet at δ 7.72 for the twoaromatic protons H-2 and H-6, singlet at δ 7.82 related to the CH (CH=N- group), and a broad singlet at δ 8.31 for the NH. The ^{13}C NMR spectrum of **7k** showed absorption peak for the morpholine residue at δ 43.6, 43.7, 66.8, and 66.9 related to 2 CH$_2$-N-CH$_2$, and 2 CH$_2$-O-CH$_2$ respectively, absorption peaks at δ 128.2, 128.9, 132.8, and 135.4 ppm related to the aromatic carbons, and absorption peaks at δ 142.5 and 165.4 ppm for C=N.

The LC-MS of compound **7k** using buffer A, 0.1% formic acid in H$_2$O, and buffer B, 0.1% formic acid in CH$_3$CN, in
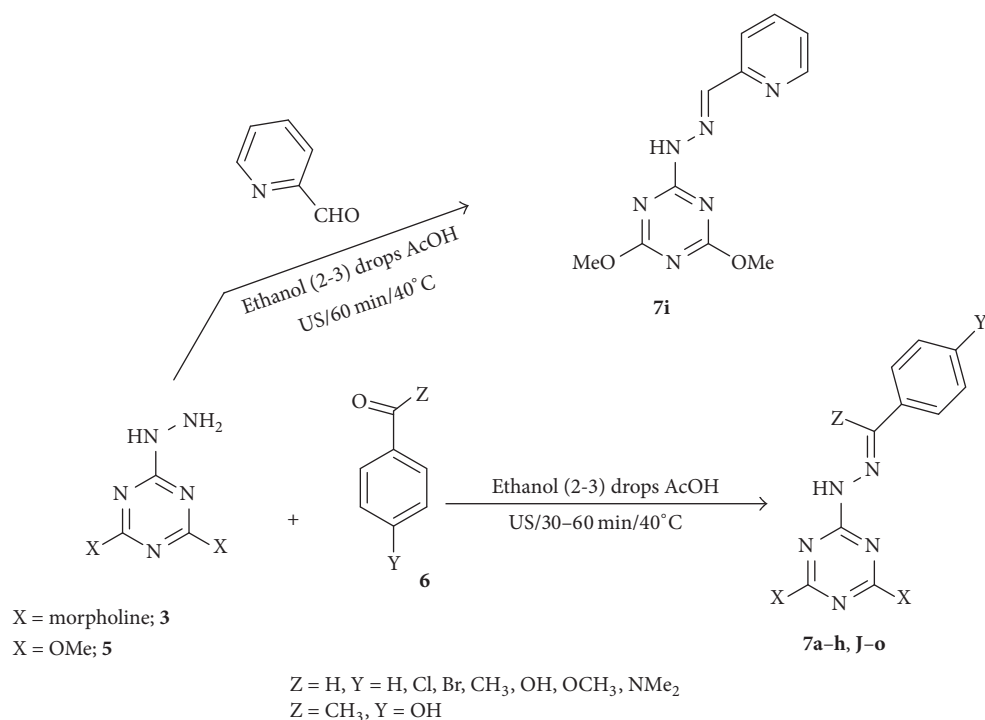
Scheme 3: Synthesis of 4,6-disubstituted-s-triazine-Schiff base derivatives and US: ultrasonic irradiation.
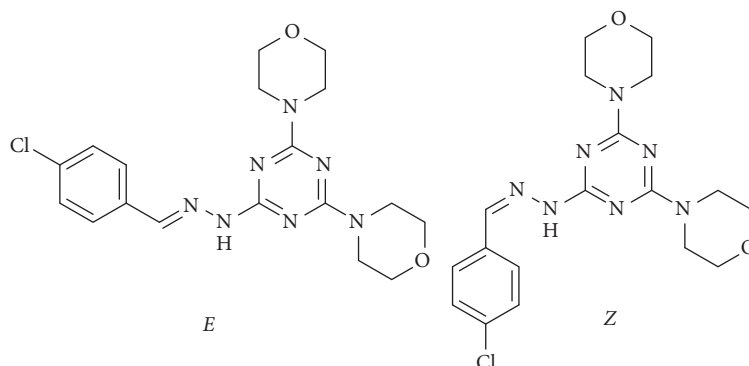
Scheme 4: *E*- and *Z*-isomer of compound **7k**.

30 min showed one peak at R_t 15.8 min with the expected mass [M+H] 404 (*m/z* cacld. 403.87, *Figure S13 in the Supplementary Material*).

Compound **7k** as an example could adopt two different geometrical isomers (*E, Z*; Scheme 4). Therefore, **7k** was demonstrated using molecular mechanics MM2 calculations. In addition, quantum chemical calculations were carried out with the GAUSSIAN 98 suite of programs. Geometry optimization was carried out using the DFT level (B3LYP/6-31G**) of theory to assess the relative stability of the *E-Z* isomeric species. Computed relative energies of **7k** indicated that the *E*-isomer (total energy content 47.7837 kcal/mol) is more stable than the *Z* ones (total energy content 41.7295 kcal/mol) by 6.0542 kcal/mol. This observation agreed with our reported data in which the

s-triazine hydrazone preferred the *E*-isomer rather than the *Z*- ones [41].

As a prototype for acetophenone derivatives, the ^{1}H NMR spectrum of **7o** showed a singlet peak at δ 2.24 ppm for the methyl group of the acetophenone moiety, two multiplet peaks in the range δ 3.70–3.83 ppm related to the 8 methylene groups of the two morpholino rings (4 -N-CH$_2$-CH$_2$-O), and a broad singlet at δ 8.01 ppm related to the NH, beside two doublets forming an AB system at δ 6.80 (*J* = 8.8 Hz) and 7.698 ppm (*J* = 8.46) for the aromatic protons H-3, H-5, and H-2, H-6 respectively. The ^{13}C NMR spectrum showed absorption peak at δ 12.7 related to methyl group and two peaks at δ 43.7 and 66.9 ppm related to the methylene groups (-NCH$_2$ and O-CH$_2$, resp.); the absorption peaks at δ 156.8, 164.7, 165.2, and 165.4 ppm were assigned for carbons of

TABLE 2: Antimicrobial activity (zones of inhibition, mm) and a minimum inhibitory concentration (MIC, mg/ml) of **7i** and **7k** comparing with several standard antimicrobial drugs.

Microorganisms		Antifungal agent			Antibacterial agents			Compounds			
								mM		mg/mL	
		Cy.*	Co.	Ca.	To.	Ch.	Fu.	**7i**	**7k**	**7i**	**7k**
Bacteria	Sa.**	N.T	N.T	N.T	14	30	23	15	—	0.10	—
	Sm.	N.T	N.T	N.T	—	35	20	10	—	0.20	—
	Ec.	N.T	N.T	N.T	20	40	—	—	<8	—	—
	Pa	N.T	N.T	N.T	15	13	—	—	—	—	—
	St.	N.T	N.T	N.T	14	30	—	—	<8	—	—
	Nm	N.T	N.T	N.T	21	34	20	15	17	0.10	0.10
	BL	N.T	N.T	N.T	—	35	20	20	—	0.05	—
Fungi	Cp.	—	—	—	N.T***	N.T	N.T	—	—	—	—
	Cn.	32	25	—	N.T	N.T	N.T	—	—	—	—
	Ca.	—	46	20	N.T	N.T	N.T	—	—	—	—
	Pc.	23	30	—	N.T	N.T	N.T	—	—	—	—
	An.	22	23	20	N.T	N.T	N.T	—	—	—	—
	Fs.	30	15	—	N.T	N.T	N.T	—	—	—	—

*Cy: cycloheximide, Co: canesten, Ca: caspofungin, To: tobramycin, Ch: chloramphenicol, and Fu: fusidic acid. *Sa***: *Staphylococcus aureus* ATCC 29213, Nm: *Neisseria meningitides* ATCC 1302, *Sm*: *Streptococcus mutans* ATCC 35668, *EC*: *Escherichia coli* ATCC 25922, *Pa*: *Pseudomonas aeruginosa* ATCC 27584, *St*: *Salmonella typhimurium* ATCC 14028, *Cp*: *Candida parapsilosis* ATCC 22019, Cn: *Cryptococcus neoformans* Wild strain, *C*: *Candid albicans* ATCC 60193, BL: *Brevibacillus laterosporus* Wild strain, *Pc*: *Penicillium chrysogenum* AUMC 9476, *An*: *Aspergillus niger* AUMC 8777, and *Fs*: *Fusarium* sp. N.T***: not tested.

C=N and the remaining five peaks at δ 115.3, 127.8, 128.2, 130.9, and 146.9 ppm related to the aromatic carbons of the acetophenone moiety.

The LC-MS of compound **7o** using buffer A, 0.1% formic acid in H_2O, and buffer B, 0.1% formic acid in CH_3CN, in 30 min showed one peak at R_t 13.4 min with the expected mass [M+H] 400 (*m/z* calcd 399.46, *Figure S18 in the Supplementary Material*)

3.2. Biology. The antimicrobial activities of the prepared compounds against several pathogenic tested organisms are represented in Table 2. The results revealed that only two compounds **7i** and **7k** from all the tested compounds were biologically active with different spectrum activity; on the other hand, they did not show any antifungal activity (Table 2). The results in Table 2 showed that **7i** had good biological activity against *S. aureus, N. meningitides*, and tobramycin resistant *B. laterosporus,* while it had poor biological activity against *S. mutans* (10 mm). Compound **7k** showed a good biological activity against *N. meningitides* (17 mm) and a very weak activity (<8) against *E. coli* and *S. typhimurium.* The measured minimum inhibitory concentrations (MIC) of **7i** that inhibited *S. aureus, S mutans, N. meningitides*, and *B. laterosporus* were 0.10, 0.20, 0.10 and 0.05 mg/mL, respectively, while the MIC of **7k** that inhibited *N. meningitides* was 0.10 mg/mL.

It is obvious from Table 2 that a small structural variation in s-triazine ring may induce an effect on antibacterial activity. By observing the antibacterial activities of all the derivatives, it is interesting to note that the dimorpholino-4-chlorophenyl-s-triazine derivatives **7k** show good antibacterial activity against *N. meningitides* and weak activity against *E. coli* and *S. typhimurium*. The other derivatives in the same family did not show any biological activity; this observation agreed with the reported data for substituted s-triazine [45]. The presence of pyridine ring (compound **7o**) in place of the benzaldehyde ring (compound **7a**) changed the biological activity significantly as observed from Table 2; on the other hand, all the tested compounds did not show any antifungal activity; this observation also agreed with the reported data for s-triazine derivatives [45].

4. Conclusion

The target products (**7a–o**) were prepared using conventional heating and ultrasonic irradiation. The ultrasonic irradiation affords the products in higher yields and purity in shorter reaction time. The biological activity for the prepared compounds showed that only two compounds **7i** (pyridine derivative) and **7k** (4-chlorobenzaldehyde derivative) have biological activities against some Gram-positive and Gram-negative bacteria, while all tested compounds did not show any antifungal activity. It is obvious that changing the substituent in the s-triazine ring as well as the benzylidene moiety may affect the antimicrobial activity. Further investigations are run in our lab to get insights into the mode of action and the relation between the biological activity and substituent effect in the pattern in s-triazine.

Competing Interests

The authors declare no conflict of interests.

Acknowledgments

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