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Synthesis of a novel class of some 1,3,4-oxadiazole derivatives as antimicrobial agents

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Abstract

In an effort to discover new candidates with improved antimicrobial activities we report here the synthesis and *in vitro* biological evaluation of various series of 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(phenyl ureido)-s-triazine **(7a–i)** and 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(phenyl thioureido)-s-triazine **(8a-g).** Antimicrobial properties of the title compounds were investigated against two Gram (+ ve) bacteria (*S. aureus*, *B. subtilis*), two Gram (− ve) bacteria (*P. aeruginosa*, *E. coli*) and yeast-like fungi (*C. albicans*) using the broth microdilution method.

Keywords: *1, 3, 4-oxadiazole, s-triazine, thiourea, urea, antimicrobial activity*

Introduction

In recently decades, the problems of multi-drug resistant microorganism have reached on alarming level in many countries around the world. A numbers of recent clinical reports describe the increasing occurrence of meticillin-resistant *S. aureus* and other antibiotic-resistant human pathogenic microorganisms in United State and European countries. Infections caused by those microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to a search for novel antimicrobial agents.

In this work, we report the synthesis & biological activity of some s-triazine based 1,3,4-oxadiazole. 1,3,4-oxadiazole have broad spectrum of medicinal values such as antibacterial [1–4], anti-inflammatory [5], anticonvulsant [6], pesticidal [7], CNS Stimulant [8], antihypertensive [9], hypnotic [10] and sedative activities. Several derivatives of s-triazine show antimicrobial [11], antibacterial [12], and herbicidal activites [13]. They are also used for the treatment of HIV infection.[14,15] Several investigators found s-triazine nucleus as potential therapeutic agents for diseases due to bacteria, malaria and cancer [16].

In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased antimicrobial activity. Therefore, these observations prompted us to synthesize new 1,3,4-oxadiazole derivatives which were attached with s-triazine ring through a sulfur bridge. Then, the synthesized compounds were tested against two Gram (+ve) bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram (− ve) bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and one yeast-like fungi *Candida* using the broth microdilution method.

Experimental section

Chemistry

All chemicals were of analytical grade and use directly. All melting points were determined in PMP-DM

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scientific melting point apparatus and are uncorrected. The completion of reaction checked by thin-layer chromatography (TLC) using silica gel-G coated Al-plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation. Elemental analyses were done on 'Haraeus Rapid Analyzer'. Infra Red spectra were recorde on perkin Elmer-spectrum RX-1 model spectrophotometer using KBr pellets. ^{1}H NMR spectra were acquired on a Bruker Avance-2 model spectrophotometer using $CDCl_3$ as a solvent and TMS as a internal reference (chemical shifts in δ, ppm)

3,4,5-trimethoxy phenyl methyl ester ***(2)***. A mixture of 3,4,5-trimethoxy benzoic acid **(1)** (0.1 mole, 21.2 g.), absolute methanol (80 mL) and con. H_2SO_4 (2 mL) was refluxed for 4 h. The pH was maintained neutral by $NaHCO_3$. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, methanol was distilled out and the product was separated. It was purified by crystallization from absolute alcohol, m.p. 85°C, yield 72%.

3,4,5-trimethoxy benzoicacid hydrazide ***(3)***. The mixture of **(2)** (0.43 mole, 22.6 g.), hydrazine hydrate (0.43 mole, 20.87 mL) and toluene (43 mL) was gently heated in oil-bath for 18 h. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, it was cooled and water (150 mL) was added and stirred for one hour. The crude product was purified by crystallization from absolute alcohol to get title compound, m.p. 141°C, yield 75% (Found: N, 12.28. $C_{10}H_{14}O_4N_2$, requires N, 12.38,%).

2-(3,4,5-trimethoxy phenyl)-5-mercapto-1,3,4-oxadiazole ***(4)***. To a stirred solution of **(3)** (0.1 mole, 22.2 g.), CS_2 (CARE – HIGHLY INFLAMMABLE) (0.1 mole, 7.6 mL) and 15% KOH solution (10 mL) in methanol (82 mL) was added and refluxed for 8 h. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, the resultant mixture was poured in crushed ice. Product was filtered, washed with water and crystallized from ethanol to give white needles, m.p. 146°C, yield 60% (Found: N, 10.38. $C_{11}H_{12}O_4N_2S$, requires N, 10.44,%).

2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4,6-dichloro-s-triazine ***(5)***. To a stirred solution of cyanuric chloride (0.01 mole, 1.84 g.) in D.M.F. (92 mL) at 0-5°C, the solution of oxadiazole **(4)** (0.01 mole, 2.69 g.) in D.M.F. (17 mL) was added and pH was maintained neutral by the addition of 10% sodium carbonate solution. The stirring was continued at 0-5°C for 4 h. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, the resultant mixture was poured in crushed ice. Product was filtered, washed with water and crystallized from ethanol to give **(2)**, m.p. 165°C, yield 60% (Found: N, 16.78%, $C_{14}H_{11}N_5O_4SCl_2$, requires N, 16.83%).

2-{(3,4,5-trimethoxy phenyl-1,3,4- oxadiazolyl)-5-thio}-4-(morpholino)-6-chloro-s-triazine ***(6)***. Morpholine (0.01 mole, 0.87 mL) dissolved in D.M.F. (5 mL) was slowly added to a well-stirred solution of **(5)** (0.01 mole, 4.16 g.) in D.M.F. (10 mL), maintaining the temperature at 35°C. The pH was adjusted neutral by the addition of 10% sodium carbonate solution. The temperature was gradually raised to 45°C during 2 h. Progress of the reaction was monitored by TLC using benzene:acetone (95:5) as eluent. After the completion of reaction, the resultant mixture was poured in to crushed ice. Product was filtered, washed with water and crystallized from ethanol to give solid needles. m.p. 173°C, yield 65% (Found: N, 17.90%, $C_{18}H_{19}N_6O_5SCl$, requires N, 18.00%).

General procedure for 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(phenyl ureido)-s-triazine ***(7a-i)***. To a solution of **(6)** (0.01 mole, 4.65 g.) and appropriate phenyl urea derivatives (0.01 mol) in DMF (18 mL) was refluxed in a water bath at 80–90°C temperature for 3 h. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, the resultant mixture was poured in to crushed ice. Product was filtered, washed with water and crystallized from ethanol.

(7a). m.p. 155°C, yield 60% (Found: C, 52.95; H, 4.59; N, 19.74. $C_{25}H_{26}N_8O_5S$, requires C, 53.00; H, 4.63; N, 19.78%). IR (KBr): 1284 (> C-O-), 815 (-C═N-), 1135 (-CH_2-O- CH_2-), 1558 (-C═O-), 2860 (-NH-), 1245, 1035 (-C-O-C-), 1378 (-C-H-), 2564 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.29 (s, 6H, 2x-OCH_3), 3.92 (s, 3H, -OCH_3), 9.08 (s, 1H, Ar-NH-CO-), 8.14 (s, 1H, -CONH-), 3.60 (m, 4H, -CH_2-O- CH_2-), 3.67 (m, 4H, -CH_2-N-CH_2-), 6.12 (d, 2H, $J = 7$, Ar-H), 6.35 (d, 2H, $J = 8$, Ar-H), 6.78 (s, 1H, Ar-H), 7.12-7.67 (m, 2H, Ar-H).

(7b). m.p. 157°C, yield 70% (Found: C, 53.74; H, 4.81; N, 19.28. $C_{26}H_{22}N_8O_5S$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1270 (> C-O-), 815 (-C═N-), 1126 (-CH_2-O- CH_2-), 1565 (-C═O-), 2854, 1603 (-NH-), 1256, 1032 (-C-O-C-), 1384

(-C-H-), 2564 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.31 (s, 6H, 2x-OCH_3), 3.89 (s, 3H, -OCH_3), 9.06 (s, 1H, Ar-NH-CO-), 8.16 (s, 1H, -CONH-), 3.62 (m, 4H, -CH_2-O- CH_2-), 3.66 (m, 4H, -CH_2-N- CH_2-), 6.23 (dd, 1, 1H, $J = 3$, $J = 7$, Ar-H), 5.18 (s, 1H, Ar-H), 6.80 (d, 2H, $J = 10$, Ar-H), 6.65 (d, 2H, $J = 11$, Ar-H), 7.10-7.51 (m, 2H, Ar-H).

(7c). m.p. 140°C, yield 62% (Found: C, 53.73; H, 4.81; N, 19.26. $C_{26}H_{28}N_8O_5S$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1270 (>C-O-), 815 (-C=N-), 1135 (-CH_2-O- CH_2-), 1558 (-C=O-), 2860, 1610 (-NH-), 1245, 1035 (-C-O-C-), 1378 (-C-H-), 2564 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.33 (s, 6H, 2x-OCH_3), 3.84 (s, 3H, -OCH_3), 9.09 (s, 1H, Ar-NH-CO-), 8.12 (s, 1H, -CONH-), 3.67 (m, 4H, -CH_2-O- CH_2-), 3.67 (m, 4H, -CH_2-N- CH_2-), 6.28 (dd, 1, 1H, $J = 3$, $J = 7$, Ar-H), 5.19 (s, 1H, Ar-H), 6.87 (d, 2H, $J = 10$, Ar-H), 6.64 (d, 2H, $J = 11$, Ar-H), 7.10-7.57 (m, 2H, Ar-H).

(7d). m.p. 170°C, yield 76% (Found: C, 53.74; H, 4.82; N, 19.28. $C_{26}H_{28}N_8O_5S$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1273 (>C-O-), 819 (-C=N-), 1131 (-CH_2-O- CH_2-), 1552 (-C=O-), 2860, 1604 (-NH-), 1242, 1039 (-C-O-C-), 1378 (-C-H-), 2561 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.33 (s, 6H, 2x-OCH_3), 3.84 (s, 3H, -OCH_3), 9.09 (s, 1H, Ar-NH-CO-), 8.12 (s, 1H, -CONH-), 3.67 (m, 4H, -CH_2-O- CH_2-), 3.67 (m, 4H, -CH_2-N- CH_2-), 6.28 (dd, 1, 1H, $J = 3$, $J = 7$, Ar-H), 5.19 (s, 1H, Ar-H), 6.87 (d, 2H, $J = 10$, Ar-H), 6.64 (d, 2H, $J = 11$, Ar-H), 7.10-7.57 (m, 2H, Ar-H).

(7e). m.p. 230°C, yield 65% (Found: C, 49.91; H, 4.16; N, 18.60. $C_{25}H_{25}N_8O_5SCl$, requires C, 49.96; H, 4.19; N, 18.64%). IR (KBr): 1276 (>C-O-), 812 (-C=N-), 1136 (-CH_2-O- CH_2-), 1558 (-C=O-), 2854, 1614 (-NH-), 1246, 1039 (-C-O-C-), 1378 (-C-H-), 2567 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.30 (s, 6H, 2x-OCH_3), 3.85 (s, 3H, -OCH_3), 8.83 (s, 1H, Ar-NH-CO-), 8.14 (s, 1H, -CONH-), 3.69 (m, 4H, -CH_2-O-CH_2-), 3.60 (m, 4H, -CH_2-N- CH_2-), 1.81 (s, 3H, Ar-CH_3), 6.74 (d, 2H, $J = 9$, Ar-H), 6.62 (d, 2H, $J = 11$, Ar-H), 7.18-7.75 (m, 2H, Ar-H).

(7f). m.p. 202°C, yield 80% (Found: C, 49.91; H, 4.16; N, 18.60. $C_{25}H_{25}N_8O_5SCl$, requires C, 49.96; H, 4.19; N, 18.64%). IR (KBr): 1273 (>C-O-), 817 (-C=N-), 1134 (-CH_2-O- CH_2-), 1551 (-C=O-), 2859, 1612 (-NH-), 1249, 1045 (-C-O-C-), 1371 (-C-H-), 2560 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.21 (s, 6H, 2x-OCH_3), 3.88 (s, 3H, -OCH_3), 8.82 (s, 1H, Ar-NH-CO-), 8.14 (s, 1H, -CONH-), 3.66 (m, 4H, -CH_2-O-CH_2-), 3.69 (m, 4H, -CH_2-N- CH_2-), 1.83 (s, 3H, Ar-CH_3), 6.79 (d, 2H, $J = 9$, Ar-H), 6.68 (d, 2H, $J = 11$, Ar-H), 7.18-7.77 (m, 2H, Ar-H).

(7g). m.p. 198°C, yield 68% (Found: C, 49.91; H, 4.16; N, 18.60. $C_{25}H_{25}N_8O_5SCl$, requires C, 49.96; H, 4.19; N, 18.64%). IR (KBr): 1268 (>C-O-), 806 (-C=N-), 1141 (-CH_2-O- CH_2-), 1549 (-C=O-), 2864, 1609 (-NH-), 1256, 1045 (-C-O-C-), 1377 (-C-H-), 2560 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.26 (s, 6H, 2x-OCH_3), 3.89 (s, 3H, -OCH_3), 8.89 (s, 1H, Ar-NH-CO-), 8.11 (s, 1H, -CONH-), 3.62 (m, 4H, -CH_2-O-CH_2-), 3.66 (m, 4H, -CH_2-N- CH_2-), 1.86 (s, 3H, Ar-CH_3), 6.76 (d, 2H, $J = 9$, Ar-H), 6.60 (d, 2H, $J = 11$, Ar-H), 7.18-7.70 (m, 2H, Ar-H).

(7h). m.p. 167°C, yield 72% (Found: C, 49.09; H, 4.11; N, 20.57. $C_{25}H_{25}N_9O_7S$, requires C, 49.09; H, 4.12; N, 20.61%). IR (KBr): 1262 (>C-O-), 812 (-C=N-), 1139 (-CH_2-O- CH_2-), 1541 (-C=O-), 2858, 1611 (-NH-), 1253, 1049 (-C-O-C-), 1371 (-C-H-), 2556 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.36 (s, 6H, 2x-OCH_3), 3.90 (s, 3H, -OCH_3), 8.98 (s, 1H, Ar-NH-CO-), 8.15 (s, 1H, -CONH-), 3.68 (m, 4H, -CH_2-O- CH_2-), 3.63 (m, 4H, -CH_2-N-CH_2-), 6.84 (d, 2H, $J = 7$, Ar-H), 7.08 (d, 2H, $J = 8$, Ar-H), 7.24-7.85 (m, 2H, Ar-H).

(7i). m.p. 162°C, yield 65% (Found: C, 49.09; H, 4.11; N, 20.57. $C_{25}H_{25}N_9O_7S$, requires C, 49.09; H, 4.12; N, 20.61%). IR (KBr): 1262 (>C-O-), 812 (-C=N-), 1139 (-CH_2-O- CH_2-), 1541 (-C=O-), 2858, 1611 (-NH-), 1253, 1049 (-C-O-C-), 1371 (-C-H-), 2556 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.34 (s, 6H, 2x-OCH_3), 3.93 (s, 3H, -OCH_3), 8.91 (s, 1H, Ar-NH-CO-), 8.12 (s, 1H, -CONH-), 3.64 (m, 4H, -CH_2-O- CH_2-), 3.67 (m, 4H, -CH_2-N-CH_2-), 6.80 (d, 2H, $J = 7$, Ar-H), 7.04 (d, 2H, $J = 8$, Ar-H), 7.24-7.82 (m, 2H, Ar-H).

General procedure for 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(phenyl thioureido)-s-triazine ***(8a-g)***. A mixture of **(6)** (0.01 mol, 4.65 g.) and appropriate phenyl thiourea derivatives (0.01 mol) in DMF (15 mL) was refluxed in a water bath at 80–90°C temperature for 3 h. Progress of the reaction was monitored by TLC using toluene:ethylacetate (80:20) as eluent. After the completion of reaction, the resultant mixture was poured in to crushed ice. Product was filtered, washed with water and crystallized from ethanol.

(8a). m.p. 192°C, yield 62% (Found: C, 51.48; H, 4.42; N, 19.15. $C_{25}H_{26}N_8O_5S_2$, requires C, 51.53; H, 4.50; N, 19.23%). IR (KBr): 1275 (>C-O-), 1545, 805 (-C=N-), 1128 (-CH_2-O- CH_2-), 1540 (-C=S-), 3345, 1608 (-NH-), 1235, 1031 (-C-O-C-), 2559 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.32 (s, 6H, 2x-OCH_3), 3.91 (s, 3H, -OCH_3), 8.92 (s, 1H, Ar-NH-CS-), 8.28 (s, 1H, -CSNH-), 3.60 (m, 4H, -CH_2-O- CH_2-), 3.68 (m, 4H, -CH_2-N- CH_2-), 6.69 (d, 2H, $J = 8$, Ar-H), 7.08 (d, 2H, $J = 10$, Ar-H), 6.52 (s, 1H, Ar-H), 7.18-7.78 (m, 2H, Ar-H).

(8b). m.p. 140°C, yield 62% (Found: C, 53.74; H, 4.81; N, 19.28. $C_{26}H_{28}N_8O_5S_2$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1268 (>C-O-), 1541, 811 (-C=N-), 1135 (-CH_2-O- CH_2-), 1548 (-C=S-), 3338, 1614 (-NH-), 1231, 1031 (-C-O-C-), 2563 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.29 (s, 6H, 2x-OCH_3), 3.89 (s, 3H, -OCH_3), 7.98 (s, 1H, Ar-NH-CS-), 8.19 (s, 1H, -CSNH-), 3.62 (m, 4H, -CH_2-O- CH_2-), 3.68 (m, 4H, -CH_2-N- CH_2-), 6.68 (d, 2H, $\mathcal{J} = 7$, Ar-H), 7.11 (d, 2H, $\mathcal{J} = 10$, Ar-H), 7.12-7.66 (m, 2H, Ar-H).

(8c). m.p. 170°C, yield 76% (Found: C, 53.74; H, 4.81; N, 19.28. $C_{26}H_{28}N_8O_5S_2$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1264 (>C-O-), 1541, 816 (-C=N-), 1131 (-CH_2-O- CH_2-), 1567 (-C=S-), 3345, 1620 (-NH-), 1237, 1039 (-C-O-C-), 2561 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.26 (s, 6H, 2x-OCH_3), 3.94 (s, 3H, -OCH_3), 7.98 (s, 1H, Ar-NH-CS-), 8.21 (s, 1H, -CSNH-), 3.59 (m, 4H, -CH_2-O- CH_2-), 3.64 (m, 4H, -CH_2-N- CH_2-), 6.62 (s, 1H, Ar-H), 6.69 (d, 1H, $\mathcal{J} = 8$, Ar-H), 7.16 (d, 1H, Ar-H), 7.26-7.52 (m, 2H, Ar-H).

(8d). m.p. 198°C, yield 68% (Found: C, 53.73; H, 4.82; N, 19.26. $C_{25}H_{26}N_8O_6S_2$, requires C, 53.78; H, 4.86; N, 19.30,%).IR (KBr): 1269 (>C-O-), 1544, 802 (-C=N-), 1126 (-CH_2-O- CH_2-), 1561 (-C=S-), 3341, 1617 (-NH-), 1231, 1031 (-C-O-C-), 2561 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.25 (s, 6H, 2x-OCH_3), 3.85 (s, 3H, -OCH_3), 7.95 (s, 1H, Ar-NH-CS-), 8.13 (s, 1H, -CSNH-), 3.69 (m, 4H, -CH_2-O- CH_2-), 3.68 (m, 4H, -CH_2-N- CH_2-), 6.68 (d, 2H, $\mathcal{J} = 7$, Ar-H), 7.17 (d, 2H, $\mathcal{J} = 8$, Ar-H), 7.21-7.65 (m, 2H, Ar-H).

(8e). m.p. 157°C, yield 70% (Found: C, 53.74; H, 4.81; N, 19.28. $C_{25}H_{26}N_8O_6S_2$, requires C, 53.78; H, 4.86; N, 19.30%). IR (KBr): 1263 (>C-O-), 1548, 814 (-C=N-), 1121 (-CH_2-O- CH_2-), 1566 (-C=S-), 3341, 1613 (-NH-), 1228, 1031 (-C-O-C-), 2567 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.28 (s, 6H, 2x-OCH_3), 3.88 (s, 3H, -OCH_3), 7.94 (s, 1H, Ar-NH-CS-), 8.18 (s, 1H, -CSNH-), 3.60 (m, 4H, -CH_2-O- CH_2-), 3.65 (m, 4H, -CH_2-N- CH_2-), 6.68 (d, 2H, $\mathcal{J} = 7$, Ar-H), 7.10 (d, 2H, $\mathcal{J} = 8$, Ar-H), 7.22-7.68 (m, 2H, Ar-H).

(8f). m.p. 230°C, yield 62% (Found: C, 49.80; H, 4.18; N, 18.60. $C_{25}H_{25}N_8O_6S_2Cl$, requires C, 49.96; H, 4.19; N, 18.64%). IR (KBr): 1261 (>C-O-), 1555, 813 (-C=N-), 1128 (-CH_2-O- CH_2-), 1565 (-C=S-), 3341, 1612 (-NH-), 1228, 1031 (-C-O-C-), 2569 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.28 (s, 6H, 2x-OCH_3), 3.87 (s, 3H, -OCH_3), 7.96 (s, 1H, Ar-NH-CS-), 8.21 (s, 1H, -CSNH-), 3.59 (m, 4H, -CH_2-O- CH_2-), 3.66 (m, 4H, -CH_2-N- CH_2-), 1.89 (s, 3H, Ar-CH_3), 7.88 (d, 1H, $\mathcal{J} = 6$, Ar-H), 7.91 (d, 1H, $\mathcal{J} = 7$, Ar-H), 6.68 (d, 1H, $\mathcal{J} = 9$, Ar-H), 7.12 (d, 1H, $\mathcal{J} = 10$, Ar-H), 7.24-7.52 (m, 2H, Ar-H).

(8g). m.p. 175°C, yield 72% (Found: C, 49.07; H, 4.11; N, 20.57. $C_{25}H_{25}N_8O_6S_2Cl$, requires C, 49.10; H, 4.12; N, 20.61%). IR (KBr): 1271 (>C-O-), 1559, 815 (-C=N-), 1122 (-CH_2-O- CH_2-), 1568 (-C=S-), 334, 1612 (-NH-), 1228, 1037 (-C-O-C-), 2561 (Ar-S-Ar); ^{1}H NMR (DMSO $-d_6$, δ) 3.32 (s, 6H, 2x-OCH_3), 3.95 (s, 3H, -OCH_3), 7.97 (s, 1H, Ar-NH-CS-), 8.23 (s, 1H, -CSNH-), 3.60 (m, 4H, -CH_2-O- CH_2-), 3.65 (m, 4H, -CH_2-N- CH_2-), 6.65 (s, 1H, Ar-H), 7.04 (d, 1H, $\mathcal{J} = 9$, Ar-H), 7.32-7.54 (m, 4H, Ar-H).

Antibacterial activity

The MICs of the chemical compounds assays were carried out as described by Clause [17] with minor modifications. Two Gram-positive (Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633) and two Gram-negative (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) bacteria were used as quality control strains. For determining anti-yeast activities of the compounds, the following reference strains were tested: Candida albicans ATCC 10231. Ampicillin trihydrate and fluconazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds and reference drugs were dissolved in DMSO at a concentration of 20 mg mL^{-1}. The twofold dilution of the compounds and reference drug were prepared (20, 10, 5.0, 2.5, 1.25, 0.625, 0.31, 0.15, 0.07, 0.03, 0.019, 0.01, 0.005 >) mg mL^{-1}. Antibacterial activities of the bacterial strains were carried out in Muller– Hinton broth (Difco) medium, at pH 6.9, with an inoculum of (1–2) × 10^3 cells mL^{-1} by the spectrophotometric method and an aliquot of 100 µl was added to each tube of the serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 24 h at 150 rpm. The minimum inhibitory concentrations of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e. no turbidity) of inoculated bacteria. All fungi were cultivated in Sabouraud Dextrose Agar (Merck). The fungi inoculums were prepared in Sabouraud liquid medium (Oxoid) which had been kept at 36°C overnight and was diluted with RPMI-1640 medium with L-glutamine buffered with 3-[N-morpholino]-propansulfonic acid (MOPS) at pH 7 to give a final concentration of 2.5 × 10^3 cfu/mL. The microplates were incubated at 36°C and read visually after 24 h, except for *Candida* species when it was at 48 h. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentrations of the substances that gave no visible turbidity. The DMSO diluents at a maximum final concentration of 12.5% had no effect on the microorganism's growth.

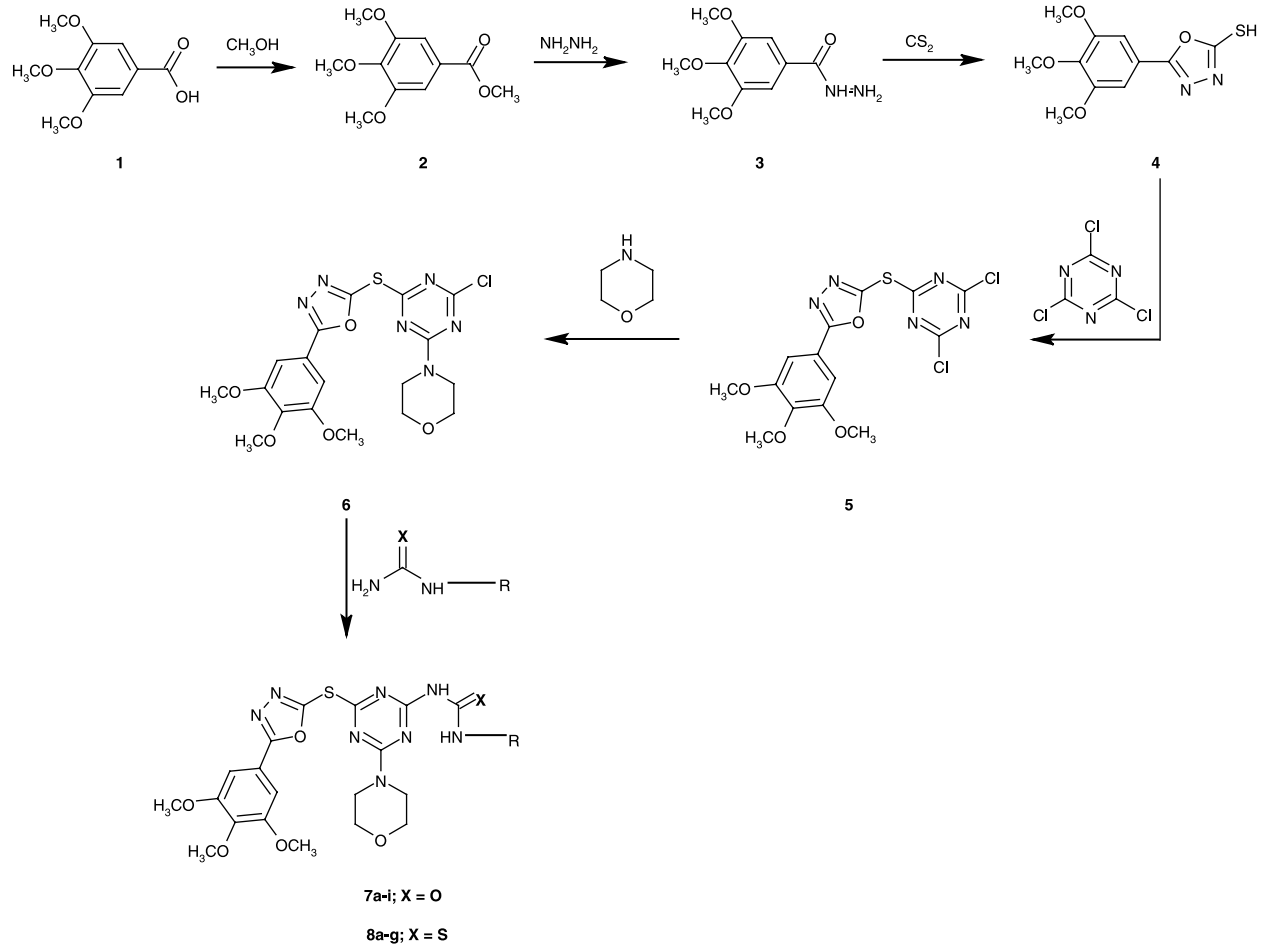
Result and discussion

Chemistry

The commercially available 3,4,5-trimethoxy benzoicacid **1** was converted into 3,4,5-trimethoxy phenyl methyl ester **2** using methanol and sulphuric acid in acceptable yields (Scheme 1). The identity of the product was determined by IR and ^{1}H NMR spectral studies. Ester **2** which on treatment with hydrazine hydrate yielded 3,4,5-trimethoxybenzoicacid hydrazide **3** in good yield. The structure of compound **3** was confirmed by the IR and ^{1}H NMR spectral analysis. The IR spectra of **3** showed the absence of ester stretching frequency, instead in gave a band at 1662 cm^{-1} for carbonyl group and showing two sharp bands in the region of 3300–3400 cm^{-1} due to $-NH_2$ group and 3100–3400 cm^{-1} for –NH frequencies. ^{1}H NMR spectra of compound **3** exhibited no peak corresponds to ester instead it shows signal at δ 10.08 ppm and δ 4.62 ppm for –NH and $-NH_2$ (D_2O exchangeable) of hydrazide respectively. Compound **3** on cyclisation with CS_2 in alcoholic solution of potassium hydroxide gave 2-(3,4,5-trimethoxy phenyl)-5-mercapto-1,3,4-oxadiazole **4**, which on treatment with s-triazine yielded 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4,6-dichloro-s-triazine **5** followed by condensation with morpholine gave 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-chloro-s-triazine **6** were converted into 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(phenyl ureido)-s-triazine **7a–j** and 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino) -6-(phenyl thioureido)-s-triazine **8a-j.** Compound **7a** and **8a** were characterized with the help of elemental analysis, IR and ^{1}H NMR. IR spectra of **7a** compound exhibited absorption bands at 1558 which indicated the presence of carbonyl group. The ^{1}H NMR spectra of compound **7a** shows signal at δ 9.08 of Ar-CO-NH- and δ 8.15 of –CONH- proton was obtained. IR spectra of **8a** compound exhibited absorption bands at 1540 which indicated the presence of > C=S group. The ^{1}H NMR spectra of compound **8a** shows signal at δ 8.92 of Ar-CS-NH- and 8.28 of –CS-NH- proton was obtained.

Antibacterial activity

The present paper is focused on the synthesis of novel heterocyclic compounds as possible antibacterial



Scheme 1. Synthesis of 1,3,4-oxadiazole derivatives.

Table I. Minimum inhibitory concentrations (MICs, mg/mL) for the title compounds.

			Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Compound	R	X	ATCC 25923	ATCC 6633	ATCC 27853	ATCC 27853	ATCC 10231
7a	C_6H_5	O	0.3	0.15	0.15	1.25	2.5
7b	2-CH_3 C_6H_5	O	0.31	0.07	1.25	0.625	5.0
7c	3-CH_3 C_6H_5	O	0.625	0.15	5.0	2.5	10
7d	4-CH_3 C_6H_5	O	2.5	2.5	0.03	5.0	1.25
7e	2-Cl C_6H_5	O	0.15	1.25	0.019	0.019	5.0
7f	3-Cl C_6H_5	O	0.15	0.625	1.25	1.25	2.5
7g	4-Cl C_6H_5	O	0.15	0.3	0.019	0.07	0.15
7h	3-NO_2 C_6H_5	O	–	10	1.25	–	–
7i	4-NO_2 C_6H_5	O	2.5	–	0.625	5.0	10
8a	2-CH_3 C_6H_5	S	1.25	–	2.5	10	–
8b	4-CH_3 C_6H_5	S	1.25	5.0	2.5	1.25	5.0
8c	3-OH C_6H_5	S	2.5	1.25	0.019	2.5	10
8d	4-OH C_6H_5	S	0.15	0.625	2.5	0.625	1.25
8e	4-Cl C_6H_5	S	0.625	0.07	5.0	0.03	0.31
8f	3-NO_2 C_6H_5	S	2.5	2.5	10	1.25	2.5
8g	4-NO_2 C_6H_5	S	2.5	5.0	5.0	0.1	0.15
Ampicillin			0.019	0.005	0.005	0.01	–
Fluconazole							0.01

For antibacterial activity, in present protocol 1.25 mg mL^{-1} is considered as moderate activity, 0.07 mg mL^{-1} is considered as good activity and 0.019 is considered as excellent activity compared to the standard drug ampicillin. For antifungal activity, in present protocol 0.15 mg mL^{-1} is considered as excellent activity compared to the standard drug fluconazole.

agents. The minimal inhibitory concentrations (MICs, mg mL^{-1}) of tested compounds against certain bacteria are shown in Table I. A series of novel compounds **7a-i** and **8a-g** were prepared and tested for their *in vitro* antibacterial activity against the four strains of bacteria (gram + ve, gram −ve). Five compounds of the obtained series showed high *in vitro* antimicrobial activity. 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(2-chloro phenyl ureido)-s-triazine **7e** showed excellent activity against *E. coli* and *P. aeruginosa*, 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(4-chloro phenyl ureido)-s-triazine **7g** showed excellent activity against *P. aeruginosa* indicated *in vitro* antibacterial activity comparable to slightly lower than the ampicilline 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(4-methyl phenyl thioureido)-s-triazine **8c** and 2-{(3,4,5-trimethoxy phenyl-1,3,4-oxadiazolyl)-5-thio}-4-(morpholino)-6-(4-nitro phenyl thioureido)-s-triazine **8g** showed excellent activity against *C. albicans in vitro* antifungal activity comparable or slightly lower than that of Fluconazole. The presence of electron-withdrawing group on the aromatic ring in general increases the antimicrobial activities of the tested compounds compared to compounds having electron donating groups. Based upon the results it will also be necessary to optimize the led compound by substitution in the C_2 and C_4 position in of phenyl ring by chloro and polar group (phenolic or nitro moiety) seem to be very important for antibacterial effect, as well as the presence and the position of –NHCSNH- group in the connecting linker between the aromatic ring seems to be very important for antibacterial effect.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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