Synthesis and Antimicrobial Activity of Some 2-aminothiazole and 2-aminothiadiazine Derivatives

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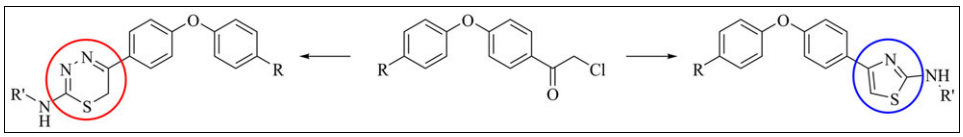

In this study, some thiazole and thiadiazine ring bearing compounds were synthesized, characterized by spectroscopic techniques, and evaluated as potential antimicrobial agents. Their antimicrobial activities evaluated by broth microdilution method and expressed as minimum inhibitory concentration; against *Escherichia coli*, Salmonella typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*. From these compounds, Compounds **2**, **5**, and **9** have been found to selectively inhibit Gram positives.

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INTRODUCTION

Combat against bacterial infections has resulted in the development of a wide variety of antibiotics. Among these antibiotics, thiazole and thiadiazine ring bearing compounds constitute an important class of heterocycles and have a notable pharmaceutical value, because of their potential. Thiazole derivatives are found to be associated with various biological activities such as antimicrobial [1–3], antituberculosis [4], and anti-HIV [5] activities and recently found application in drug development. Also, in recent years, interest in thiadiazine derivatives has increased due to the high biological activity and broad-spectrum action of their derivatives [6]. Many thiadiazine derivatives have been discovered with possible applications in medical practice as sedatives [7], antiasthmatic agents [8], antianxiety agents [9], anticonvulsants [10], and coronary vasodilators [11].

In view of the above-mentioned facts and in continuation of our interest in the synthesis of heterocycles containing sulfur and nitrogen atoms [12], we report in this article the synthesis of some thiazole and thiadiazine derivatives. Their structure characterized by FT-IR and ^{1}H NMR techniques. The antimicrobial activity of the synthesized compounds was evaluated against *Staphylococcus aureus*, *Bacillus cereus*, Salmonella typhimurium, and *Escherichia coli* by broth microdilution method.

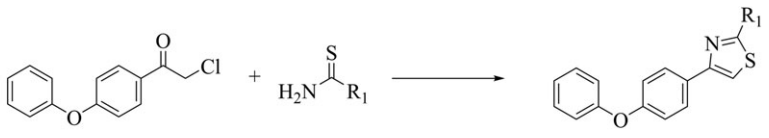
RESULTS AND DISCUSSION

Chemistry. The synthetic procedures adopted to obtain the target thiazole compound are depicted in Scheme 1. The condensation of chloroacetophenone derivatives with appropriate thiourea derivatives in absolute ethanol gave the corresponding thiazole derivatives **1**–**5**. The FT-IR spectra of **1** and **5** showed absorption bands at 3459–3445 cm^{-1} for —NH_2. Compounds **2** and **3** were characterized by the presence of 3244–3203 cm^{-1} bands that indicates —NH— group. The FT-IR spectra of all thiazole derivatives revealed the —C=N— cm^{-1} peaks at 1541–1556 cm^{-1} and —C=C— peaks at 1605–1626 cm^{-1}. The absence of absorption band at 1700–1750 cm^{-1} also confirms the conversion of C=O group to —CHN— group. The ^{1}H NMR spectra ($CDCl_3$) of compounds **1**–**5** displayed singlet signals between δ 6.44 and 6.55 ppm assignable to C—H proton of thiazole ring.

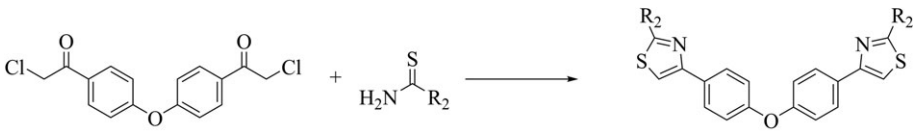
Similarly, the condensation of chloroacetophenone derivatives with appropriate thiosemicarbazide derivatives in absolute ethanol gave the corresponding thiadiazine derivatives **6**–**9** (Scheme 2). FT-IR spectra of compounds **6** and **9** revealed the —NH_2 absorption bands at 3409–3420 cm^{-1}. The presence of absorption bands at 3243 and 3298 cm^{-1} in the FT-IR spectrum of compounds **7** and **8** was attributed to the —NH— group. The FT-IR spectra of all thiadiazine derivatives revealed the —C=N— peaks at 1542–1598 cm^{-1}. Furthermore, the ^{1}H NMR spectra ($CDCl_3$) of compounds **6**–**9** displayed singlet signals between δ 3.51–3.64 ppm which correspond to C—H proton of thiadiazine ring system.

Antimicrobial evaluation. Table 1 summarizes the in vitro susceptibilities of the *E. coli*, S. typhimurium, *B. cereus*, and *S. aureus* to synthesized compounds as determined by the broth microdilution method. Optical densities obtained from control wells indicated

Scheme 1. Reaction of chloroacetophenone with thiourea compounds.

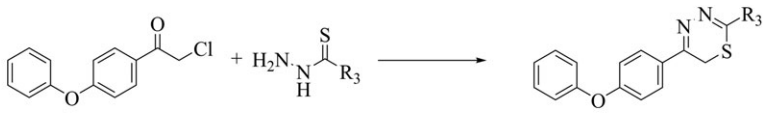


R1= (**1**) -NH_2, (**2**) -NH-CH_3, (**3**) -NH-Ph, (**4**) -N-$(CH_3)_2$

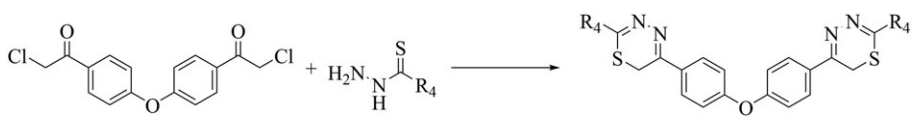


R2= (**5**) -NH_2

Scheme 2. Reaction of chloroacetophenone with thiosemicarbazide compounds.



R3= (**6**) -NH_2, (**7**) -NH-CH_3, (**8**) -NH-Ph



R4= (**9**) -NH_2

Table 1

Minimum inhibitory concentration (μg/mL) of compounds against selected microorganisms

	Microorganism			
Compound	*E. coli*	S. typhimurium	*B. cereus*	*S. aureus*
1	>250	>250	>250	>250
2	>250	>250	125	62.5
3	>250	>250	>250	>250
4	125	>250	62.5	125
5	>250	>250	15.6	31.3
6	>250	>250	>250	>250
7	>250	>250	>250	>250
8	>250	>250	>250	>250
9	>250	>250	62.5	62.5
Gentamycin	7.81	3.91	7.81	7.81

that selected strains were able to tolerate 2.5% DMSO. Minimum inhibitory concentration (MIC) values of compound **4** against four microorganisms were determined in the range of concentration tested. Compounds **2**, **5**, and **9** have been found to selectively inhibit Gram positives. Among all compounds tested, compound **5** exhibited strongest antimicrobial activity with MIC values ranging from 15.6 to 31.3 μg/mL.

CONCLUSION

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some functionalized thiazole and thiadiazine derivatives serving as potent antimicrobial agents. Selective antimicrobial action of compounds **2**, **5**, and **9** can be attributed to differences in the cell envelope of Gram-negative and Gram-positive bacteria. Gram-negative bacteria have a thin peptidoglycan layer that is sandwiched between inner and outer membranes. In Gram positives, in contrast, a thick layer of peptidoglycan lies outside of single bacterial cell membrane [13]. The outer membrane of Gram-negative bacteria acts as a barrier and provides for an increased tolerance to antimicrobial compounds [14].

EXPERIMENTAL

Unless otherwise noted, chemicals were obtained from global suppliers (Merck or Aldrich) and were used without further purification. Solvents were of high performance liquid chromatography (HPLC) or analytical grade and they were dried with molecular sieves (3 Å). All melting points were determined with EZ-Melt Automated Melting Point Apparatus. FT-IR spectra were recorded on Thermo Nicolet İS5. ^{1}H NMR spectra were measured on a Varian 400 MHz in $CDCl_3$ as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ ppm.

Lyophilized cultures of *E. coli* (ATCC 25922), S. typhimurium (ATCC 14028), *B. cereus* (ATCC 11778), and *S. aureus* (ATCC 25923) were supplied from Microbiologics Inc (Saint Cloud, USA). Stock cultures of microorganisms were stored in Nutrient Broth (Lab M, Bury, UK) supplemented with 20% glycerol at −18°C.

Overnight colonies grown on Nutrient Agar (Lab M) were suspended in sterile 0.85% saline, and the cell density of suspension was adjusted to 0.5 McFarland turbidity standard, which represents approximately 1.5×10^8 colony-forming unit (CFU)/mL. Then, these suspensions were diluted by 1/100 with Mueller Hinton Broth (Lab M). The final suspensions were used as inoculum in in vitro antimicrobial activvity assay.

General procedure for the preparation of thiazole derivatives. Thiazole derivatives synthesized according to method described in literature [15]. Chloroacetophenone derivative (8 mmol) and an appropriate thiourea (8 mmol for **1**–**4**, 16 mmol for **5**) in ethanol was refluxed for 3 h. The reaction was followed by thin-layer chromatography. The mixture was then extracted with ethyl acetate (2 × 25 mL). After removing the ethyl acetate, the resulting product was washed with cold ethanol and recrystallized.

4-(4-Phenoxyphenyl)thiazol-2-amine (1): yield (76%); this compound was obtained as white solid, mp 65–67°C; FT-IR: 3459, 3244, 3016, 2202, 2048, 1673, 1626, 1543, 1522, 1491, 1456, 1329, 973, 761 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.12 (br s, 2H, NH2), 6.55 (s, 1H, thiazole H), 7.14–7.72 (m, 9H, Ar—H); ^{13}C NMR (CDCl3, 100 MHz): δ = 98.6, 119.2, 119.7, 124.0, 125.6, 127.9, 130.2, 145.6, 156.4, 158.5, 171.3. *Anal*. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14%; H, 4.51%; N, 10.44%. Found: C, 67.21%; H, 4.72%; N, 10.34%.

N-methyl-4-(4-phenoxyphenyl)thiazol-2-amine (2): yield (74%); this compound was obtained as white needles; melting point 112–114°C; FT-IR: 3244, 3164, 2202, 2048, 1673, 1626, 1544, 1488, 1453, 1400, 1294, 1123, 973, 718 cm^{-1}; ^{1}H NMR (CDCl3, 400 MHz): δ = 3.06 (s, 3H, Me), 5.04 (br s, 1H, NH), 6.55 (s, 1H, thiazole H), 7.03–7.72 (m, 9H, Ar—H) ppm; ^{13}C NMR (CDCl3, 100 MHz): δ = 33.0, 98.5, 119.0, 119.6, 124.1, 125.7, 127.8, 130.1, 145.5, 156.5, 158.6, 171.4 ppm. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06%; H, 5.00%; N, 9.92%. Found: C, 67.89%; H, 4.67%; N, 10.21%.

N-phenyl-4-(4-phenoxyphenyl)thiazol-2-amine (3): yield (69%); this compound was obtained as yellow solid; melting point 127–129°C; FT-IR: 3369, 3203, 3119, 3037 2202, 2048, 1673, 1626, 1588, 1556, 1495, 1454, 1410, 1278, 1123, 1023, 759 cm^{-1}; ^{1}H NMR (CDCl3, 400 MHz): δ = 6.54 (s, 1H, thiazole H), 7.11–7.90 (m, 11H, Ar—H), 9.78 (s, 1H, NH) ppm; ^{13}C NMR (CDCl3, 100 MHz): δ = 102.0, 117.8, 118.7, 121.8, 122.6, 124.1, 125.3, 126.0, 128.9, 137.4, 145.5, 156.2, 165.6 ppm. Calcd for $C_{21}H_{16}N_2OS$: C, 73.23%; H, 4.68%; N, 8.13; S, 9.31%. Found: C, 73.18%; H, 4.76%; N, 8.05; S; 9.22%.

N,*N*-dimethyl-4-(4-phenoxyphenyl)thiazol-2-amine (4): yield (74%); this compound was obtained as yellowish solid; melting point 81–83°C; FT-IR: 3352, 3114, 3028, 2944, 1605, 1486, 1445, 1400, 1294, 1167, 1036, 985, 762 cm^{-1}; ^{1}H NMR (CDCl3, 400 MHz): δ = 3.07 (s, 6H, Me), 6.44 (s, 1H, thiazole H), 7.11–7.90 (m, 11H, Ar—H) ppm; ^{13}CNMR (CDCl3, 100 MHz): δ = 44.0, 101.0, 119.2, 119.7, 124.2, 125.9, 127.6, 146.0, 157.3, 158.2 ppm. Calcd for $C_{17}H_{16}N_2OS$: C, 68.89%; H, 5.44%; N, 9.45; S, 10.82%. Found: C, 68.64%; H, 5.60%; N, 9.38; S, 10.97%.

4,4′-(Oxybis (4,1-phenylene))bis(thiazol-2-amine) (**5**): yield (63%); this compound was obtained as brown solid; melting point 228–230°C; FT-IR: 3445, 3285, 3027, 2202, 2048, 1673, 1626, 1541, 1488, 1456, 1389, 1294, 1123, 973, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.53 (s, 2H, thiazole H) 6.94 (s, 4H, NH2), 6–95–7.79 (m, 8H, Ar—H) ppm; ^{13}CNMR ($CDCl_3$, 100 MHz): δ = 98.5, 118.5, 119.6, 125.7, 126.1, 127.4, 130.1, 145.5, 156.9, 158.6, 171.0 ppm. Calcd for $C_{18}H_{14}N_4OS_2$: C, 59.01%; H, 3.82%; N, 15.30; S, 17.48%. Found: C, 59.19%; H, 3.77%; N, 15.25; S, 17.40%.

General procedure for the preparation of thiadiazine derivatives. Thiadiazine derivatives synthesized according to method described in literature [16]. Chloroacetophenone derivative (8 mmol) and an appropriate thiosemicarbazide (8 mmol for **6**–**8**, 16 mmol for **9**) are taken in 5 mL of anhydrous ethanol, refluxed for about 2 h. The solid obtained on cooling was washed with cold ethanol and recrystallized.

5-(4-Phenoxyphenyl)-6*H*-1,3,4-thiadiazin-2-amine (**6**): yield (82%); this compound was obtained as white crystal; mp 126–128°C; FT-IR: 3409, 3280, 3243, 3151, 3042, 1582, 1504, 1487, 1278,1081, 962, 737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 3.51 (s, 2H, CH_2S), 7.62–7.24 (m, 9H, Ar—H), 8.75 (s, 2H, NH2); ^{13}C NMR (CDCl3, 100 MHz): δ = 13.71, 127.17, 128.27, 129.48, 129.70, 132.80, 133.96, 135.31, 139.93, 147.60, 179.38. *Anal*. Calcd for $C_{15}H_{13}N_3OS$: C, 63.53; H, 4.59; N, 14.82%. Found: C, 63.37; H, 4.46; N, 14.65%.

N-methyl-5-(4-phenoxyphenyl)-6H-1,3,4-thiadiazin-2-amine (**7**): yield (75%); this compound was obtained as yellowish solid; mp 137–139°C; FT-IR: 3366, 3243, 3066, 1602, 1546, 1498, 1288, 1233, 1108, 831, 738 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 2.93 (s, 3H, Me), 3.58 (s, 2H, CH_2S), 5.20 (s, br, NH), 7.68–7.14 (m, Ar—H); ^{13}C NMR (CDCl3, 100 MHz): δ = 13.87, 20.67, 127.17, 128.27, 129.32, 129.54, 132.75, 133.82, 135.47, 139.81, 147.7, 179.5. *Anal*. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; S, 10.78%. Found: C, 64.71; H, 4.94; N, 14.22; S, 10.49%.

N-phenyl-5-(4-phenoxyphenyl)-6*H*-1,3,4-thiadiazin-2-amine (**8**): yield (70%); this compound was obtained as white solid; mp 146–148°C; FT-IR: 3298, 3182, 3050, 1615, 1598, 1516, 1482,1444, 1289, 1189, 971, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 3.62 (s, 2H, CH_2S), 7.75–7.08 (m, Ar—H), 8.91 (s, H, NH); ^{13}C NMR (CDCl3, 100 MHz): δ = 13.9, 125.46, 127.17, 128.27, 128.90, 129.54, 129.72, 132.88, 133.97, 135.34, 139.70, 147.58, 179.46. *Anal*. Calcd for $C_{21}H_{17}N_3OS$: C, 70.19; H, 4.77; N, 11.67; S, 8.93%. Found: C, 70.26; H, 4.60; N, 11.65; S, 8.89%.

5,5′-(Oxybis(4,1-phenylene))bis(6*H*-1,3,4-thiadiazin-2-amine) (**9**): yield (67%); this compound was obtained as white solid; mp 225–227°C; FT-IR: 3420, 3276, 3240, 3018, 2905, 1628, 1588, 1565, 1453, 1323,1084, 906, 707 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 3.64 (s, 4H, CH_2S), 7.60–7.24 (m, 8H, Ar—H), 8.82 (s, 4H, NH2); ^{13}C NMR (CDCl3, 100 MHz): δ = 13.9, 128.27, 129.48, 129.70, 132.80, 133.98, 135.34, 139.89, 147.55, 179.40. *Anal*. Calcd for $C_{18}H_{18}N_6OS_2$: C, 54.53; H, 4.07; N, 21.20; S, 16.17%. Found: C, 54.48; H, 4.01; N, 21.33; S, 16.09%.

Antimicrobial activity assay. Antimicrobial activities of synthesized compounds were evaluated by broth microdilution method and expressed as MIC, in accordance with the procedure outlined by Clinical & Laboratory Standards Institute (CLSI) [17]. The stock solutions of the compounds were prepared in DMSO (10%, Merck, Germany) at a concentration of 1 mg/mL. Twofold dilutions of filter-sterilized stock solutions were used as working solutions. Gentamycin (Sigma-Aldrich, USA) at the same concentration range served as the positive control. Volumes of 130-μL fresh Mueller Hinton Broth and 20-μL inoculum were added to each well of sterile 96-well microplates (Anicrin, Venezia, Italy). Then, 50 μl of the working solutions of compounds were dispensed into the 10 consecutive wells of each microplate row with a final DMSO concentration of 2.5%. Wells 11 and 12 of each row served as the growth control (compound and DMSO-free) and negative control (compound-free), respectively. Contents of each well were mixed thoroughly to give final concentrations of compounds ranging from 0.49 to 250 μg/mL. Then, microplates were sealed and incubated at 35°C. After 24-h incubation, cell growth in each well was determined by measuring optical density with a microplate reader (Biotek, Winooski, VT, USA) at 600 nm. MICs were defined the lowest compound concentrations at which growth was not apparent, as measured by optical density.

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